Tofel Haman Katamssadan
Institut für ökologische Chemie, Pflanzenanalytik und Vorratsschutz

Insecticidal products from local *Azadirachta indica* A. Juss and *Plectranthus glandulosus* Hook for the protection of stored grains against the infestation of *Callosobruchus maculatus* F. and *Sitophilus zeamais* Motschulsky

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Kontakt/Contact:
Tofel Haman Katamssadan, PhD
Entomologist
Ass. Lecturer at the Faculty of Science
The University of Bamenda
P.O Box 39 Bambili.

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A THESIS

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By

TOFEL HAMAN Katamssadan
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Year 2016

DEDICATION

To my parents and siblings

whose faithful support and unfailing encouragements guided me to achieve my study.

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LIST OF ABBREVIATIONS

AGPM : Association Générale des Producteurs de Maïs

CABI: Centre for Agricultural Bioscience International

DDT: Dichloro-Diphényl-Trichloréthane

f.l.: Fiducial limit

FAO: Food and Agricultural Organization

IRAD : Institut de Recherche Agricole pour le Développement

PAN: Pesticide Action Network

r.h. : relative humidity

RNA: Ribonucleus Acid

d: day

ABSTRACT

Callosobruchus maculatus F. (Coleoptera: Chrysomelidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) are very serious storage pests of cowpea and maize, respectively which cause serious losses to during storage. Chemical synthetic residual insecticides, which degrade the environment, are widely used for the control of these pests. Alternative control methods are required to minimize the hazardous effects of such insecticides. Botanical insecticides are more biodegradable and could be a source of more environmental-friendly insecticides. Accordingly, the effectiveness of oils from *Azadirachta indica* A. Juss seeds and pulverized leaves and seeds of this plant and that of *Plectranthus glandulosus* Hook as well as the binary mixtures of the botanical powders were tested against *C. maculatus* on cowpea seeds and *S. zeamais* on maize grains. The azadirachtin A contents of *A. indica* seed oils and powders from sun-dried kernels, shade-dried kernels, sun-dried seeds and shade-dried seeds, and the chemical composition of *P. glandulosus* powders from sun-dried and shade-dried leaves, were determined, before admixing each product with cowpea seeds or maize grains for the different insect bioassay studies. Adult toxicity bioassays involved the introduction of 20 *C. maculatus* to 50 g of treated cowpea and 20 *S. zeamais* to 50 g of treated maize in glass jars at 25ºC and 60% r.h. and also at varying temperatures and relative humidities, and then mortality counts were determined for up to 6 d (*C. maculatus*) or 14 d (*S. zeamais*). After the mortality counts, the grains were kept until all the emerging F_1 progeny were recorded. In separate experiments, cowpea seeds and maize grains were treated with the different botanicals and kept for storage intervals ranging from 1 to 180 d before infesting respectively with adult *C. maculatus* and *S. zeamais*, for mortality determination, similar to that of the toxicity bioassay. From the results, the average content of Azadirachtin A in the seed powder was 1.20 g/kg, and this was not influenced by sun-drying. On the contrary, the oil from the sun-dried seeds (2.89 g/kg) had a lower azadirachtin A content than that from the shade-dried seeds (3.69 g/kg). Sun-drying did not affect the diversity of volatile compounds in the leaves of *P. glandulosus*, as the same 50 compounds were found in the sun-dried and shade-dried leaves, although in different proportions. Generally, *P. glandulosus* powder caused greater mortality to *C. maculatus* and *S. zeamais* than *A. indica* seed powder, but the seed oil was more active towards both insects than the powders. Drying regime had no influence on the toxicity of the botanical powders and oil to both insects, with the recording of 100% mortality for the highest tested dose of each botanical 6 d (*C. maculatus*) or 14 d (*S. zeamais*) post-infestation. The *A. indica* products were more effective in suppressing progeny emergence in both insects than *P. glandulosus* leaf powders. No progeny emerged when the dose was ≥ 2 ml/kg for the seed oil and ≥ 10 g/kg for the seed powder. In line with progeny emergence, *A. indica* products completely prevented grain damage by the two insect species when the dose was ≥ 2 ml/kg for the seed oil and ≥ 10 g/kg for the seed powder. Binary mixtures of the botanicals were antagonistic regarding toxicity to *C. maculatus* and *S. zeamais*. Azadirachtin A content of the seed oil did vary on treated cowpea up to 90 d and on treated maize up to 30 d, but the toxicity of the oil declined greatly after 15 days for *C. maculatus* and 60 days for *S. zeamais*. Whereas variations in temperature and humidity had no effect on the toxicity of *A. indica* seed oil to both insects, the efficacy of the powders from *P. glandulosus* leaves and *A. indica* seeds reduced with increasing relative humidity. Insecticidal products from sun- or shade-dried parts of *A. indica* and *P. glandulosus* could form a major component of the integrated storage protection package for cowpea and maize against beetle infestations.

Key words: *Azadirachta indica*, *Plectranthus glandulosus*, drying regime, *Callosobruchus maculatus*, *Sitophilus zeamais*, cowpea, maize, bioactivity

RESUME

Callosobruchus maculatus F. (Coleoptera: Chrysomelidae) et *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) sont respectivement ravageurs du niébé et du maïs qui causent d'importants dégâts au cours du stockage. Les insecticides chimiques résiduels de synthèse dégradant l'environnement sont largement utilisés pour le contrôle de ces ravageurs. Des méthodes alternatives de contrôle sont nécessaires pour minimiser les effets nocifs de ces insecticides. Les insecticides issus des plantes sont plus biodégradables et peuvent être une source d'insecticides plus respectueuses de l'environnement. A cet effet, l'efficacité des huiles de graines de *Azadirachta indica* et les poudres de feuilles et des graines de cette plante, la poudre des feuilles de *Plectranthus glandulosus* ainsi que le mélange binaire des poudres végétales ont été testés contre *C. maculatus* sur les graines de niébé et contre *S. zeamais* sur les grains de maïs. La teneur en azadirachtine A des poudres et huiles de graines de *A. indica* issues des graines séchées au soleil, des graines séchées à l'ombre, des amandes séchées à l'ombre, des amandes séchées au soleil et la composition chimique des poudres de feuilles de *P. glandulosus* séchées au soleil et séchées à l'ombre ont été déterminées, avant le mélange de chaque produit aux graines de niébé ou aux grains de maïs pour les différents essais biologiques sur les insectes. Le test de la toxicité des adultes consistait en l'introduction de 20 *C. maculatus* à 50 g de niébé traité et 20 *S. zeamais* à 50 g de maïs traité dans un bocal en verre à 25°C et 60% d'humidité relative et également aux températures et humidités relatives variables et le comptage de mortalité a été effectué jusqu'à 6 jours (*C. maculatus*) ou 14 jours (*S. zeamais*). Après le décompte de la mortalité, les grains ont été conservés jusqu'à l'enregistrement de la descendance F_1. Dans des expériences séparées, les graines du niébé et les grains de maïs ont été traités avec les différents produits de plantes et maintenus à des intervalles du temps de stockage allant de 1 à 180 jours avant infestation respectivement par les adultes de *C. maculatus* et *S. zeamais*, afin de déterminer la mortalité, similaires aux test de toxicité. D'après les résultats, la teneur moyenne en azadirachtine A dans la poudre de graines était de 1,20 g /kg, et cela n'a pas été influencé par le séchage au soleil. Au contraire, l'huile issue des graines séchées au soleil (2,89 g /kg) avait une teneur en azadirachtine A inférieure à celle de la graine séchée à l'ombre (3,69 g /kg). Le séchage au soleil n'a pas affecté la diversité des composés volatils des feuilles de *P. glandulosus*, les mêmes 50 composés ont été retrouvés dans les feuilles séchées au soleil et celles séchées à l'ombre, bien que dans des proportions différentes. Généralement, la poudre de *P. glandulosus* a causé une mortalité supérieure à *C. maculatus* et *S. zeamais* que la poudre de graines de *A. indica*, mais l'huile de graines était plus active envers les deux insectes que les poudres. Le mode de séchage n'a eu aucune influence sur la toxicité des poudres et de l'huile végétale à l'égard de deux insectes, avec l'enregistrement de 100% de mortalité à la dose la plus élevée de chaque huile à 6 jours (*C. maculatus*) ou 14 jours (*S. zeamais*) post-infestation. Les produits de *A. indica* ont été plus efficaces dans la suppression de la progéniture chez les deux insectes que les poudres de feuilles de *P. glandulosus*. Aucune descendance n'est apparue lorsque la dose est ≥ 2 ml /kg pour l'huile de graines et ≥ 10 g /kg de la poudre de graines. En concordance avec l'émergence de la progéniture, les produits de *A. indica* ont complètement empêché les dommages de grain par ces deux espèces d'insectes lorsque la dose était ≥ 2 ml/kg pour l'huile de graines et ≥ 10 g/kg pour la poudre de graines. Les mélanges binaires des plantes étaient antagonistes sur la toxicité causée à *C. maculatus* et à *S. zeamais*. La teneur en Azadirachtine de l'huile de graines ne varie pas sur le niébé traité jusqu'à 90 jours et sur le maïs traité jusqu'à 30 jours, mais la toxicité de l'huile a fortement diminué après 15 jours sur *C. maculatus* et 60 jours sur *S. zeamais*. Alors que les variations de température et d'humidité n'ont eu aucun effet sur la toxicité de l'huile de graines de *A. indica* sur les deux insectes, l'efficacité des poudres de feuilles de *P. glandulosus* et des graines de *A. indica* a été réduite avec l'augmentation de humidité relative. Les produits insecticides obtenus a partir des parties de *A. indica* ou de *P. glandulosus* séchées à l'ombre ou au soleil pourraient constituer une composante majeure dans la protection intégrée du niébé et du maïs contre l'infestation des coléoptères pendant le stockage.

Key words: *Azadirachta indica*, *Plectranthus glandulosus*, mode de séchage, *Callosobruchus maculatus*, *Sitophilus zeamais*, niébé, maïs, bioactivité

INTRODUCTION

Millions of people around the world depend on agriculture for their subsistence and the challenge is to feed nine billion people by the year 2050 (Godfray *et al.,* 2010). Paradoxically, many smallholder farmers live on the margins of food insecurity in developing countries. This is because of climate change, absence of food-chain infrastructure and food losses (Beddington *et al.,* 2011; Gustavsson *et al.,* 2011). Food security could be achieved not only by increasing agricultural productivity, but also by reducing pre- and post-harvest crop losses (Tschamtke *et al.,* 2012). In sub-Saharan African countries, crop production is done only within the wet season, which usually spans half or less than half the year, but the produce and products are consumed and marketed all year round (Ngamo *et al.,* 2007a). Proper food storage becomes therefore a matter of survival. Maize (*Zea mays* L.) and cowpea (*Vigna unguiculata* Walp.) are staple foods in many developing countries (Ndjouenkeu *et al.,* 2010; Guèye *et al.,* 2011). Unfortunately, during storage, the crops are heavily damaged by insect pests, especially the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae). *C. maculatus* is responsible for about 30 to 60% weight losses of stored cowpea within six months (Adedire & Ajayi 2003; Ketoh *et al.,* 2005) while 30 to 40% maize weight losses are common with *S. zeamais* infestations (Parugrug & Roxas 2008; Yuya *et al.,* 2009) and thus the fight against stored products insect pests is inevitable.

To reduce post-harvest losses, different methods of grain protection are used by small holder farmers as well as at the industrial level (Isman, 2006). However, over the past decades, synthetic chemical insecticides have played a significant role in modern agricultural pest management (Guo *et al.,* 2014). Their repeated use over the years has led to the evolving of resistance in pest populations and fostered environmental and human health concerns (Ofuya, 2003). These problems have highlighted the need for the development of new types of selective insect-control alternatives (Lee *et al.,* 2001), which combine broad spectrum action against stored product insect pests with low toxicity to non-targeted organisms, but at the same time also readily available and affordable to the small-scale grower (Talukder & Howse, 1995; Nukenine *et al.,* 2007). Currently, research efforts are being intensified on the use of botanicals as alternatives to commonly used insecticides (Poudrox, Malagrain) because many plants demonstrate insecticidal activities against insect pests and plant products are more biodegradable, and thus pose fewer problems to the environment (Boeke *et al.,* 2004; Isman 2008; Jeon *et al.,* 2011).

One of the remarkable plant studied by several researchers for its insecticide activities is *Azadirachta indica* A. Juss (Meliaceae) commonly called neem. The popularity of neem products increased day by day and this plant is known today as village pharmacy or plant of the 21st century (Ilesanmi & Gungula 2013). The plant was introduced in Cameroon in 1947, and it is widely grown in the northern regions and some parts of the southern regions (Yengue´ & Callot 2002). Products from leaves, barks and seeds of this tree have been used for their medicinal properties (Nandagopal & Ghewande, 2004). *A. indica* seed oil is used for soap manufacture (Schmutterer, 1990), motor lubricant and biodiesel (Anya *et al.*, 2012) and as an efficacious insecticide (Girish & Shankara 2008). Barks and leaves of this plant are employed for the treatment of some diseases and are good antidotes against snake bite and scorpion sting (Yengué & Callot, 2002). The twigs of *A. indica* tree are used for dental hygiene (Agrawal, 2002). The plant is toxic to over 500 insect species (Schmutterer, 1990; Athanassiou *et al.,* 2005; Kavallieratos *et al.,* 2007; Roy *et al.*, 2010) including stored product insect pests of cowpea and maize (Bélanger & Musabyinama, 2005; Iloba & Ekrakene 2006; Nukenine *et al.*, 2011a; Debashri & Tamal, 2012). In Cameroon the medicinal uses of *A. indica* by far dominates its insecticidal applications (Tourneux & Yaya, 1998; Noumi & Anguessin, 2010), indicating that more research work is needed in this direction.

Plectranthus glandulosus Hook (syn. *Coleus laxiflorus* (Benth.) Roberty) (Lamiaceae) is an annual, glandular and strongly aromatic herb. The whole leaves of *P. glandulosus* are used by small-scale farmers to protect cereals and pulses during storage against insect infestation in northern Cameroon (Ngamo & Hance, 2007; Ngamo *et al.,* 2007b). The plant is also used locally to treat female infertility (Telefo *et al.,* 2008), colds and sore throat (Ngassoum *et al.*, 2001) and as a spice in some meals (Pele & Berre, 1966). Leaf powders and essential oils of *P. glandulosus* showed greater insecticidal efficacy against adult *S. zeamais* as compared with *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and *Tribolium castaneum* Du Val. (Coleoptera: Tenebrionidae) (Nukenine *et al.,* 2010, 2011a; Goudoum *et al.,* 2012a)**.** To date, there are no scientific publications reporting on *P. glandulosus* powder and *C. maculatus* on cowpea, although the essential oil of the leaves was effective against the beetle on filter paper (Ngamo *et al.,* 2007c). The efficacy of *P. glandulosus* against stored product insect pests is attributable to its richness in terpenoid compounds (Goudoum *et al.,* 2012a).

Azadirachta indica seeds are easily contaminated by aflatoxins (Kaushik *et al.,* 2002) and this is mostly observed during harvesting or drying. In developed countries, where

regulations and facilities about the safety control of plant products exist, it is easy to minimize the risk of neem seed contaminations. In these countries, drying neem seed is therefore not a problem because equipment like oven which could be used to dry the seeds safely is present. This is not the case in developing countries where *A. indica* is wide-spread and fast-drying could mainly be achieved through sun-drying. However, some reports have contended that sun-drying causes photo- and thermo-degradation of *A. indica* which leads to a significant reduction in their bio-activity against pests and in humans (Johnson *et al.*, 2000; Koul & Wahab, 2004). Drying methods could also lead to a decrease in the rate of or the transformation of some pure compounds (Najafian & Agah, 2012; Shahhoseini *et al.*, 2013). In turn, there may be differences in the diversity and quantity of pure compounds between the plant parts dried in sunlight (sun-dried) and those dried in a room (shade-dried), independent of the plant species. Thus, subsistence farmers and traditional doctors are advised to dry their plant materials in shade before mixing with grains in storage and use as medication, respectively, for better efficacy. Shade-drying of *A. indica* seeds may encourage the proliferation of fungi (*Fusarium graminearum*) and the production of aflatoxins in these products, which would in turn attain humans causing serious health hazards (Boeke *et al.*, 2004).

To promote the use of safer *A. indica* seeds or *P. glandulosus* leaf powder combined with good efficacy in stored product protection, the mode of drying of the seeds and leaves need to be reconsidered. Such studies could decipher the better drying regime for botanicals, and thus help growers to obtain more efficient plant-based insecticidal products for stored product protection. Farmers also mix different plant products for stored grain protection. This could have antagonistic, additive or synergistic effects. Scientific experimentation is necessary to determine the insecticidal effect of mixtures of *A. indica* and *P. glandulosus* products. *A. indica* seed products are known to influence adult fecundity and immature stages of *C. maculatus* and *S. zeamais* (Saxena *et al.*, 1988; Isman, 2006), this present study could help to clarify the case of *P. glandulosus* on both insect species as this is the first study reporting the effect of *P. glandulosus* powder on *C. maculatus*. It is also the first research work comparing the plant powder for its efficacy against *C. maculatus* and *S. zeamais*. More so, at the farmer's level temperature and relative humidity are always fluctuating, leading sometimes, to a decrease or an increase of the efficacy of insecticides. As this study is to evaluate the bioactivity of local plants with high potential to fight against insect pests, the present work could determine the best season or period of application of the products for optimum efficacy in different localities. Conducting scientific experimentations for proper use

of botanicals concerned in the present study will lead to the availability of safe and secure food for all population.

The goal of the present research is to enhance food security and safety in Cameroon by reducing grain (maize and cowpea) losses during storage, using better locally formulated products from *A. indica* and *P. glandulosus* as components of integrated control strategies.

The objectives were to:

- evaluate the effect of drying regime on the insecticidal efficacy of local *A. indica* seed oil and powder on adult *C. maculatus* and *S. zeamais*;
- determine the influence of drying regime and particle size on insecticidal efficacy of leaf powder from *A. indica* and *P. glandulosus* against adult *C. maculatus* and *S. zeamais*;
- test the bioefficacy of binary combinations of *A. indica* products and *P. glandulosus* leaf powder on adult *C. maculatus* and *S. zeamais*;
- assess the effect of *A. indica* products and *P. glandulosus* leaf powder on the fecundity and immature stages of *C. maculatus* and *S. zeamais*;
- determine the effect of environmental conditions on the efficacy of *A. indica* products and *P. glandulosus* leaf powder against *C. maculatus* and *S. zeamais*.

CHAPTER 1: LITERATURE REVIEW

1.1 Maize and cowpea

1.1.1 Origin of maize and cowpea

Maize (*Zea mays* L.) is an annual herbaceous tropical plant of the family of Graminaceous (Poaceae) (Anzala, 2006). It is one of the oldest agricultural crops. According to Vavilov's findings, the origin of maize and approximately 49 species are from Mexico and Central America (Serratos –Hernandez, 2009). Mangelsdorf and Reeves, (1938) proposed the foundation for one of the most influential hypothesis on the origin of maize and their hypothesis explicitly stated that teosinte is the ancestor of maize. In 1959, Mangelsdorf and Reeves reviewed their hypothesis and postulated that maize is originated from a cross between perennial teosinte and ancient tunicate-popcorn maize. Other studies have been carried out to determine the exact origin of maize (Serratos – Hernandez, 2009) but the summary of their findings implied that maize came from a wild plant, teosinte (FAO, 2006). It was cultivated in the highlands of Mexico from 7000 years by native people as the basis of their diet (Ognis, 2008). At the time of discovery of the American continent during the 16th century, the plant was already cultivated in the North (Canada) and in the South (Argentina) of the continent. It was introduced to Europe by Christopher Columbus on his return from one of his early expeditions (Farnham *et al.*, 2003; Canadian Food Inspection Agency, 1994). In Cameroon, maize was introduced by Portuguese (Ekobo, 2006).

The precise origin of cultivated cowpea (*Vigna unguiculata* Walp) is not known. However, Asia and Africa were discussed as domestication sites of this crop (Timko *et al.*, 2007). Former research studies showed that the cowpeas present in Asia are very diverse and morphologically different from those growing in Africa, suggesting that both Asia and Africa could be independent centers of origins for the crop (Timko & Sigh, 2008). Nowadays, the wild cowpea, *V. unguiculata* ssp. *unguiculata* var. *spontanea*, is thought to be the likely progenitor of cultivated cowpea (Pasquet & Baudoin 2001). The determination of the origin and domestication of cowpea had been based on botanical and cytological evidence, information on its geographical distribution and cultural practices, and historical records (Steele & Mehra 1980; Ng 1995). While West Africa appears to be the major center of diversity of cultivated forms of cowpea (Ng & Padulosi, 1988) and was probably domesticated by farmers in this region particularly in Nigeria (Ba *et al.*, 2004; Timko & Sigh, 2008), the center of diversity of wild *Vigna* species is southeastern Africa (Padulosi & Ng 1997). Maximum diversity of cultivated cowpea is found in West Africa in an area

encompassing the savanna region of Nigeria, southern Niger, part of Burkina Faso, northern Benin, Togo, and the northwestern part of Cameroon (Ng, 1995; Ng & Marechal, 1985).

1.1.2 Description of maize and cowpea

Maize is a monoecious annual plant whose stem is of variable size, 40 cm to 10 m in height and 3-4 cm in diameter. For the varieties commonly cultivated, the size generally varies from 1 to 3 m (OGTR, 2008; Canadian Food Inspection Agency, 1994). The single erected stem is made up of internodes separated by several nodes. At each node, oppositely fit a leaf (Anzala, 2006). Leaves alternate for a total number of 12 to 20 leaves, issued on the basis of each node. Generally tropical maize plant develops more leaves than temperate cultivars (OGTR, 2008). Leaves limbus are broad with elongate parallel ribs of 35 to 50 cm long and 4 to 10 cm wide (Raillard, 1981). Flowers are a characteristic which distinguish maize from other grasses. They are unisexual and grouped in male and female inflorescences (Raillard, 1981). Sexes are partitioned into separate pistillate (ear), the female flower and staminate (tassel), the male flower (Paliwal *et al.*, 2000). Maize is generally protandrous, the male flower matures earlier than the female flower (OGTR, 2008). The female flower is tightly covered over by several layers of leaves, and so closed in by them to the stem that they do not show themselves easily until emergence of the pale yellow silks from the leaf whorl at the end of the ear (Hitchcock & Chase, 1971). The ears, often one per stem, are formed of a variable number of rows of grains (12 to 16), which will provide from 300 to 1000 grains weighing between 0.19 and 0.3 g each (Canadian Food Inspection Agency, 1994; FAO, 1993). The Maize root system is composed of a large number of adventitious roots on the nodes located at the base of the stem. It is characterized by creeping roots (surface roots), which collect water and nutrients to the plant in the most superficial layers of the soil (Anzala, 2006). This type of exploitation of mineral resources makes that the plant is very demanding in nitrogen and water in proportion to high yields. More soil is rich in nitrogen and water is available, more yield is high (Anzala, 2006).

Cowpea is an annual herbaceous legume that can reach more than 80 cm in height. Some varieties grow upright, while others have procumbent stems often tinged with purple that trail along ground. Cowpea leaves are compound, having two asymmetrical side leaflets and one central terminal leaflet which is symmetrical (Pottorff *et al.*, 2012). The flowers are arranged in racemose or intermediate inflorescence at the distal ends of 5-60 cm long peduncles. Flowers are borne in alternate pairs, with usually two flowers per inflorescence.

Flowers are conspicuous, self-pollinating, borne on short pedicels and the corollas may be white, dirty yellow pink, pale blue or purple in color (Ige *et al.,* 2011). They open in the early morning, close by about midday and then wilt and die. Cultivated cowpea seed weighs between 8 and 32 mg and ranges from round to kidney shaped. Pods are cylindrical and may be curved or straight, with between 8 and 15 seeds per pod (Timko *et al.,* 2007). The seed coat can be either smooth or wrinkled and of various colors including white, cream, green, buff, red, brown, and black (Chevalier, 1944; Timko *et al.,* 2007; Fery, 1985). Cowpea has a strong taproot and many spreading lateral roots in topsoil. Most root growth usually occurs within the topsoil layer, but in times of drought cowpea can grow a taproot to reach moisture deeper in the soil profile (Valenzuela & Smith, 2002). Cowpea is well suited to low rain fall (300-600 mm) in dry tropical zones and is not very demanding in soil, but grows preferably on sandy loam soil well drained (Charrier *et al.,* 1997; Diaw, 1999). In the English speaking parts of Africa it is known as cowpea whereas in the Francophone regions of Africa, the name "niébé" is most often used (Timko & Singh, 2008).

1.1.3 Botanical classification of maize and cowpea

Maize belongs to the class of Monocotyledonea, order of Poales, family of Poaceae (or Gramineae) and the subfamily Panicoideae, tribe of Andropogoneae, Genus of *Zea* and Species *mays*. (Doebley & Iltis, 1980; Eagles & Lothrop, 1994; CABI, 1999). The genus *Zea* consists of four species of which *Zea mays* L. is economically important. The others *Zea* sp., referred to as teosintes, are largely wild grasses native to Mexico and Central America (Doebley *et al.,* 1990; Serratos – Hernandez, 2009).

According to Consoli (2000), maize classification is as follows:

- autotrophic, individuals attached to the soil and having need for light, water and air.……Kingdom: Plantae ;
- Sporophyte differentiated and constitutes the dominant generation, plant bearing grains……..…….....Phylum: Spermatophyta ;
- Plant bears flowers, presence of ovules in an ovary and grains in fruits……...……Sub-phylum :Angiospermatophyta ;
- Presence of nodes and internodes; parallel mode of venation; seeds having just one seed leaf……Class : Monocotyledoneae ;

- Presence of vessels in the stem and leaves; absence of tracheid; nuclear endosperm, large and micro embryos…..……Sub-class: Commelinideae ;
- ……Order : Cyperalidae ;
- Cosmopolitan plant, annual or perennial plant with conspicuous rhizoids. Large, narrow, opposing leaves (about a tenth as wide as they are long), borne alternately along the length of a solid stem …….Family : Poaceae or Graminae ;
- Presence of both the male (tassel) and female (silk) inflorescences on the same plant...…….....Genus : *Zea* ;
- ……..……Species: m*ays*
- Binomial name……..*Zea mays* L. 1753

Cowpea belongs to the class of Dicotyledonea, order of Fabales, family of Fabaceae, subfamily of Faboideae, tribe of Phaseoleae, subtribe of Phaseolinae, and genus of *Vigna* (Padulosi & Ng, 1997). All cultivated cowpeas are grouped under the species *V. unguiculata,* which is subdivided into four cultivar groups: *unguiculata* (the common cowpea), *biflora* (the catjang), *sesquipedalis* (the yard-long bean) and *textilis* (used for fibers) (Marechal *et al.,* 1978; Singh *et al.,* 1997; Reis & Frederico, 2001).

The cowpea classification (Paduli & Ng, 1997) is as follows:

- Autotrophic organism, fixed to the ground through their roots and needs light, water and air ..... Kingdom: Plantae
- Possess seeds ..... Phylum: Spermaphytes
- The wrapped seeds enclosed in the oval pod ..... Subphylum: Angiospermae
- Seeds with two cotyledons, type 5 flowers, leaves with branched ribs..... ..... Class: Dicotyledonea
- An annual or perennial, often alternate and compound leaves; stalked trifoliate; single pistil; single and free ..... Superfamily: Legumes
- The larger upper petals covering two side petals; two lower petals free or partially welded (hull) ... . Family: Fabaceae
- The flowers comprise a petal called standard; two petals called wings and a keel formed by partial melting of the other two petals……..... SubFamily: Papilionaceae
- ..... Genre: *Vigna*
- Each node of the steam carries three axillary buds and two extended insertion under stipules ……..... Species: *unguiculata*

- Binomial name……*Vigna unguiculata*

1. 2 Importance of maize and cowpea

Maize is grown worldwide and is the staple food for a large proportion of humanity. Its production each year is greater than other cereals worldwide and needs to be increased by 60% over the next 40 years to meet the rising demand for food (OECD-FAO, 2012; NCGA, 2013). World production of maize grain has reached 877 million tons in 2012 (International Grains Council, 2013). Maize is used for three main purposes: animal feed, food, and in industry. Animal feed represents 65% of the total world maize production, while 15% is used for food and the remainder 20% has different industrial uses (FAO, 2006; AGPM, 2009). The highest amounts of maize consumed are found in Southern Africa at 85 kg/capita/year as compared to 27% in East Africa and 25% in West and Central Africa (Smale *et al.*, 2011). Maize currently covers 25 million hectares in Sub-Saharan Africa, largely in smallholder systems primarily for food (Smale *et al*., 2011; FAO, 2013). In Cameroon, 37% of the reserved spaces are occupied by maize (Aquino *et al.*, 2001; Minader, 2010). Different varieties are adapted to the country‘s agro-ecological zones (IRAD, 2007). Ndjouenkeu *et al*. (2010) stated that cultivation and production of maize increased for 300% since 1990. According to FAOSTAT (2013), Cameroon production for 2012 was 875 000 tons compared to 531 000 tons in 1992. In Cameroon, maize is characterized by the diversity of its consumption forms (fresh, boiled or roasted corn, foufou) and this could explain its importance among other cereals in daily diet (Ndjouenkeu *et al.,* 2010).

Cowpea is an important crop in many countries of tropical Africa, Asia and South America. Both the grain and leaves are edible products of cowpea that are rich and cheap sources of high-quality protein (25-32%) and vitamins (Duke, 1981; Singh, 2002). Immature pods and peas are used as vegetables while several snacks and main dishes are prepared from the grains (Bittenbender *et al*., 1984). The seed is valued as a nutritional supplement to cereals (Karikari & Molatakgosi, 1999). The freshly harvested leaves are sold in local markets in many parts of Ghana, Mali, Benin, Cameroon, Ethiopia, Uganda, Kenya, Tanzania and Malawi (Barrett, 1987). Cowpea shoots and leaves are rich sources of calcium, phosphorous and vitamin B (Maynard, 2008). The young leaves are especially important in drought-prone regions of Sub-Saharan Africa to tide local populations over during the “hungry period” (Pottorff *et al.,* 2012). Cowpea provides farmers with needed cash income because it is one of

the first agricultural products to reach the market each year (Baoua *et al.,* 2012). Cowpea leaves and stems are also an important source of high-quality hay for livestock feed (Tarawali *et al.*, 2002. The plant fixes atmospheric nitrogen through symbiosis with nodule bacteria (Duke, 1990; Shiringani & Shimeles, 2011). In Cameroon, cowpea production increased from 10 000 tons in 1992 to 155 000 tons in 2012 (FAOSTAT, 2013).

1.3 Necessity of storage

Food security was used to describe whether a country had access to enough food to meet dietary energy requirements (Merino, 2009). Food security exists when all people, at all times, have physical and economic access to sufficient, safe, nutritious food to meet their dietary needs and food preferences for an active life (FAO, 1986). It includes the availability of food, the access to the food from the household production, local markets or public network supports, the quality of food and its stability at the consumer all the year (Parmentier, 1989; Pinstrup-Andersen, 2009). Indeed, due to periodic and sometimes unbalanced rainfall caused by climate variations or changes, agriculture cannot be practiced throughout the year (Hoogland & Holen, 2001). This situation forces the farmer mostly in developing countries to store a large amount of food (Adejumo & Raji, 2007; Adetunji, 2007). Storage is particularly important in agriculture because agricultural production is seasonal while the demands for agricultural commodities are more evenly spread throughout the year. Storage is an art which requires the establishment of an adequate phytosanitary policy to save populations from the risk of food shortage during the agricultural off-season (Adetunji, 2007). In Sub-Sahara Africa, where the dry season lasts most of the year (October to June), crop storage is a matter of survival (Mikolo *et al.*, 2007). The purpose of storage is to maintain the quality and quantity of grain for a long time (Adejumo & Raji, 2007; Godfray *et al.,* 2010). Good management and good preservation of the harvested products are necessary to ensure food safety (Beddington *et al.*, 2011; Gustavsson *et al.,* 2011). The proper management of stocks depends on conservation techniques and storage structures (Iliassa, 2004; Gustavsson *et al.,* 2011). Poor storage facilities, added to insect pests, are the cause postharvest losses (Okonkula *et al.,* 2008; Godfray *et al.*, 2010). There are two main storage structures. Industrial storages whose are usually stores or warehouses (Chicken & Duplantier, 1984; Nukenine, 2010) and traditional storage structures (Seignobos, 2002; Adejumo & Raji, 2007). Nowadays the use of bags is more preferred by small-holder farmers than granaries.

I.4 Post-harvest problems

Storage is successful if, at its term stored product does not present impairment neither of its quality, nor of its quantity (Ngamo & Hance, 2007). Unfortunately, depreciations are always observed during storage in tropical regions. Roughly 30 to 40% of stored products are lost (Godfray *et al.*, 2010). Food losses can be qualitative, such as reduced nutrient value and undesirable changes to taste, texture, or color, or quantitative as measured by decreased weight or volume (Buzby & Hiram, 2012). Food loss is not attributed to exogenous and endogenous factors but also to the absence of food-chain infrastructure and the lack of knowledge or investment in storage technologies on the farm, although data are scarce (Godfray *et al.*, 2010). Endogenous factors are those related to temperature, relative humidity which act indirectly by creating conducive conditions to pests (Walker & Farrell, 2003), the length of storage, the quantity of stored products (Danho *et al*., 2002) and storage of cultivars susceptible to insect pests (Ngamo & Hance, 2007). Exogenous factors refer to pests (insects, fungi, rodents) that directly affect the stored food (Walker & Farrell, 2003). Insects are among the most important pests of stored products. They do not only cause qualitative and quantitative damage but also create favorable conditions for the attack and the proliferation of microorganisms. These fungi (*Aspergillus flavus, Fusarium moniliforme, Monascus ruber*) 3affect stored food by their mycotoxins, thus making the food improper for consumption (De Groot, 1996). Rodents are also an important group of pests. They are not influenced by temperature or humidity. They cause quantitative loss by feeding. They contaminate the food with their droppings making food to lose its aesthetic value and also are vectors of some diseases (plague, typhus, toxoplasmosis, trichinosis or leptospirosis) (Dobigny, 2000).

1.5 Studied insects

1.5.1 *Sitophilus zeamais*

Sitophilus zeamais is an insect of the order Coleoptera found mostly in warm regions infesting maize and in some cases sorghum and rice (Throne, 1994; Danho & Haubruge, 2003). The adult has a size between 2.5 and 5 mm, oval shape with a head extended by a long thin snout. Its color ranges from black to dark brown, usually with two small light spots on each wing (Delobel & Tran, 1993).

The taxonomic position of *S .zeamais* according to Delobel & Tran (1993) is:

- Heterotrophic……Kingdom: Animalia

-Multicellular…….....Sub-Kingdom: Metazoa
- Metameric body, articulated/ jointed appendages, chitinous cuticle, reproduction by molting……..…Phylum: Arthropoda
- Bears a pair of antennae and mandibles……Sub-Phylum: Antennata or Mandibulata
-Body divided into tagmata (head, thorax and abdomen), three pairs of leg…….……..…Class: Insecta
- Anterior wings hard (elytra) and cover membranous posterior wings that are folded at rest……Order: Coleoptera
- Head elongated by a rostrum bearing chewing mouth parts……..Family: Curculionidae
- Two brownish marks on every elytrum…….…..Genus: *Sitophilus*
- Pronotum bearing round punctuations, loves maize…….….Species: *zeamais*
- Binomial name: ……*Sitophilus zeamais* Motschulsky, 1855

1.5.1.1 Distribution of *Sitophilus zeamais*

Sitophilus zeamais is found in tropical and temperate areas (warm humid areas) where corn is grown are favored but can be found in colder climates (Mason & McDonough, 2012). Whereas, the weevil occurs throughout the world, its exact origin is not known (Longstaff, 1981; Ortega, 1987).

1.5.1.2 Biology of *Sitophilus zeamais*

The maize weevil can reproduce in a grain when the moisture content is greater than 10% and the temperature range of 13-35°C (Delobel and Tran, 1993). The female lays about 300 eggs at the rate of two to six per day, depending on the temperature and relative humidity (Delobel & Tran, 1993). Each egg is placed in a small hole in the grain and it is sealed with a mucilaginous saliva cap (Figure 1.1) (Mc Laganet & Dunn, 1935). At 25-27°C and a relative humidity of 70%, eggs hatch within 6-8 days to give small white larvae, legless that feed on the endosperm of the grain. A single larva develops among small grains such as rice, but larger grains such as maize support the development of several individuals (Howe, 1952). Larvae never live outdoors and develop entirely within the grain (Danho & Haubruge, 2003). Larvae molt four times and pupate within the grains, after four to six weeks. Adults emerge after 5-16 additional days and live ca. a year. If disturbed, they feign death by folding their legs over their bodies and remaining in this position (Delobel & Tran, 1993; Danho & Haubruge, 2003).

1.5.1.3 Economic importance of *Sitophilus zeamais*

The maize weevil is one of the most serious pests of stored grain in the world (Corrêa *et al.*, 2013). It is widely spread across countries on imported grains. Between 30 and 40% maize weight losses are common with *S. zeamais* infestations (Parugrug & Roxas, 2008; Yuya *et al.*, 2009). The damage caused by *S. zeamais* is not only the reduction of the grain quantity but also produces a considerable amount of grain dust mixed with frass which affects the quality of maize (Longstaff, 1981). With such high amounts of loss, developing countries stand to suffer substantial economic losses due to *S. zeamais.*

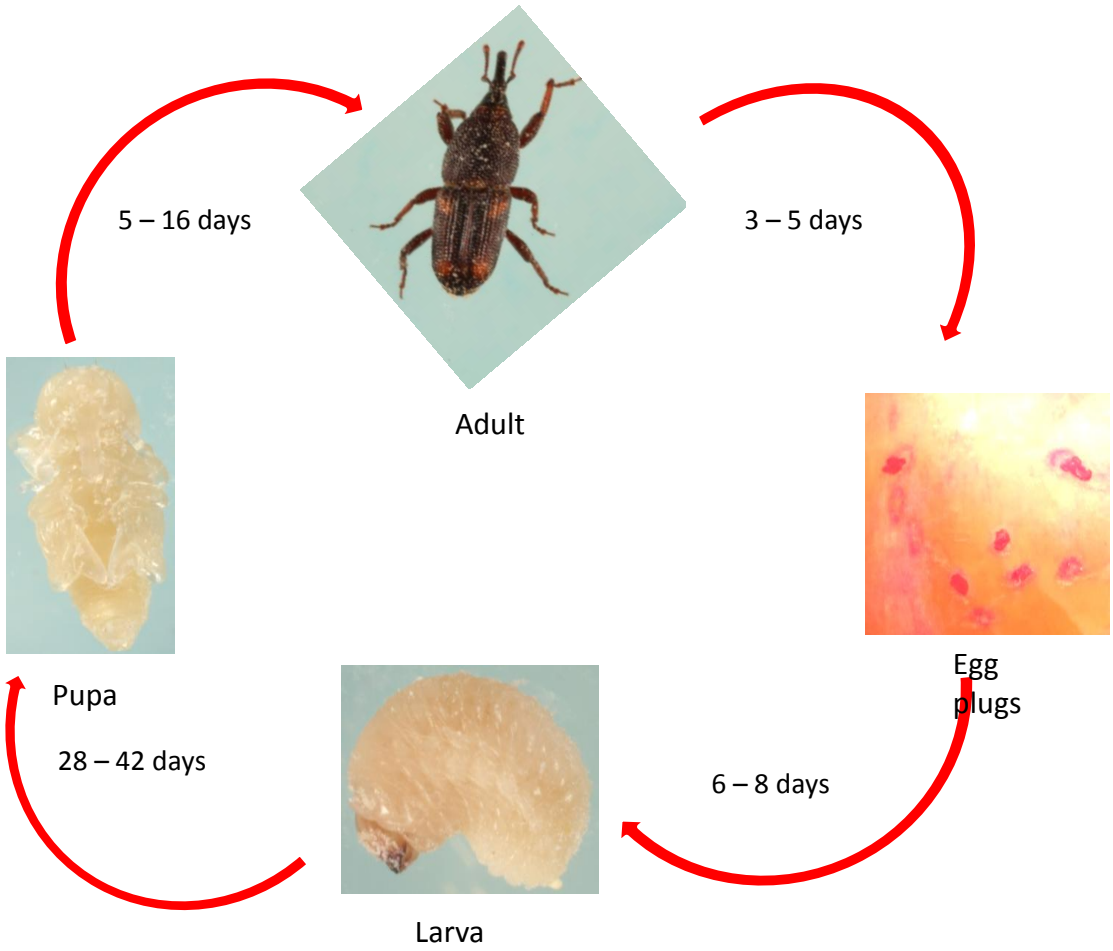


Figure 1.1: Life cycle of *Sitophilus zeamais* (Tofel)

1.5.2 *Callosobruchus maculatus*

The cowpea weevil commonly referred to four-spotted beetle has other synonyms *Bruchus quadrimaculatus, Bruchidius maculatus, B. ornatus, B. ambiguous, B. simatus* (Diaw, 1999). The taxonomic position of *C. maculatus* according to Kergoat *et al.*, (2007) is:

- Heterotrophic……Kingdom: Animalia
-Multicellular…….....Sub-Kingdom: Metazoa
- Metameric body, articulated/ jointed appendages, chitinous cuticle, reproduction by molting……..…Phylum: Arthropoda
- Bears a pair of antennae and mandibles……Sub-Phylum: Antennata or Mandibulata
-Body divided into tagmata (head, thorax and abdomen), three pairs of leg…Class: Insecta
- Anterior wings hard (elytra) and cover membranous posterior wings that are folded at rest……Order: Coleoptera
- Ovoid or elliptical body, concealed and head extended into a "beak" short and wide, no rostrum, antenna without clubs sometimes pectinate, abdomen often discovered, black brown……Family: Chrysomelidae
- Body generally stocky and dull colors, the first three articles and the last of the tarsi are apparent, head elongated well clear of the prothorax……Sub-family: Bruchinae
- Posterior femur with internal and external tooth in the ventral edges, lobed middle part of the posterior edge of the pronotum blistered with a longitudinal groove and a posterior incision; most often covered with hairs much lighter than the rest of the tergit……..Genus: *Callosobruchus*
- Toothless antenna, yellow-brown at their bases, embattled from the fifth article. The elytra are brown with four more or less rounded black spots widespread and located laterally. The pronotum is black with scattered and gold silks. Existence in females of two forms physiologically and morphologically distinct…….Species: *maculatus*
- Binomial name……*Callosobruchus maculatus* (L) Walp, 1843

1.5.2.1 Distribution of *Callosobruchus maculatus*

Callosobruchus maculatus is one of the most widespread species of bruchid beetles, which are distributed throughout the tropics and sub-tropics (CABI, 2014). Its origin is not well known, but Decelle (1981) stated that this species is native to Africa. According to Credland (1990), 20 species thrive at the expense of crops and have become economically important pests.

1.5.2.2 Biology of *Callosobruchus maculatus*

Adult *C. maculatus* does not feed on cowpea seeds and live for a very short period of time generally not more than 12 days (Delobel & Tran, 1993) during favorable conditions. The

developmental cycle depends on the temperature and relative humidity. The optimum temperature for oviposition is between 25 and 35°C and the suitable relative humidity is 60 to 70% (Huignard *et al.*, 2011). Under these conditions a female can lay between 80 and 110 eggs (Huignard *et al.*, 2011) and it has been shown that maximum numbers of eggs are deposited on grain within the four first days after emergence (Credland & Wright, 1989). The eggs are glued on the seed surface and smooth-seeded varieties are preferred for oviposition than rough-seeded varieties (Parr, 1996). Newly laid eggs are small, translucent grey and oval in shape (Figure 1.2) (Prasantha, 2003). Eggs hatch within 5-6 days to give small white legless larvae, which will feed on the endosperm of the cowpea (Lenting, 2000). More than one larva develops in a cowpea beans. Larvae molt four times and pupate within the seeds, after two weeks (Kellouche, 2005). Adults emerge after seven additional days.

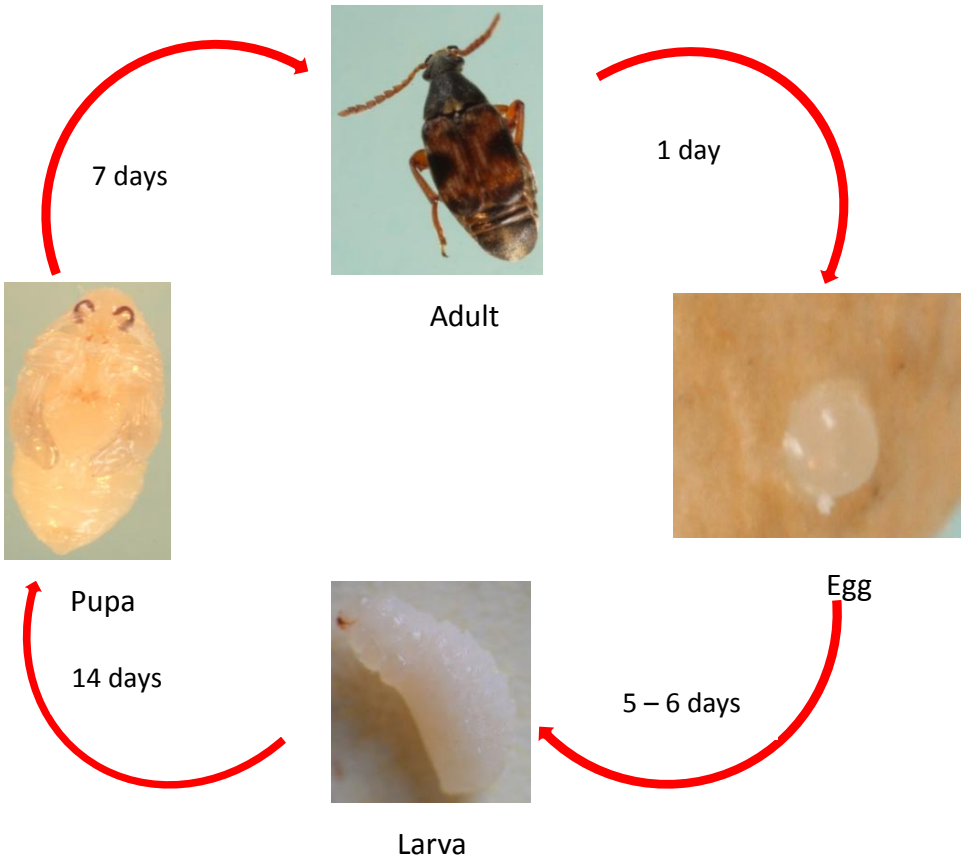


Figure 1.2: Life cycle of *Callosobruchus maculatus* (Tofel)

1.5.2.3 Economic importance of *Callosobruchus maculatus*

This species is a well-known pest of the cultivated cowpea. Amevoin *et al.* (2005) stated that damages caused by cowpea weevils vary according the infestation level and storage time. In

Nigeria, the first producer country of cowpea, the losses occur to grains are around 2 900 tons per year (Alzouma, 1995) and this correspond to $ 30 million of cash losses (Singh *et al.*, 2002). In Senegal, 90% of seeds could be damaged within six months of storage if any protection measurement has been taken (Alzouma, 1995). These losses are high because adults are found regularly in storage places after they emerge from the harvested cowpeas According to Murdock *et al.* (1997) the commercial value of the cowpea decreases if more than one hole is found on seeds.

1.6 Control of storage insect pests

Given the extent of the damage caused by the insect pests to stored products, different control or protection methods have been implemented in order to quantitatively and qualitatively enhance post harvest losses (Cruz *et al.,* 2002).

1.6.1 Physical control

Physical control consists to eliminate insect pests which the creation of hostile environmental conditions for their development (Bell & Posamantier, 1998). It is done by different techniques. Insolation practices before storage of harvest permit the proper drying of the grains as well as the elimination of insects by the heat and rays of the sun (Guèye *et al*., 2011). The yard, where insects present in the grain can be removed by hand. Damaged or infested grains can be removed simultaneously. This method is very accurate but takes a long time (Boeke, 2002). Bagging technique consists to seal the product to store in plastic bags in good storability conditions in order to eliminate pests through anoxia (Singh *et al.,* 1997; Kich & Ntoukam, 2002). This method is popular for low-income farmers. The Purdue Improved Crop Storage (PICS) bags reduces loss of cowpea grain to insect infestation into airtight storage and is extended to others stored cereals and pulses. Reducing the temperature below 10°C or increase beyond 40°C blocks the development of most insects of stored products (Delobel & Tran, 1993; Gwinner *et al.,* 1996). Other traditional practices such fumigation of grains or cobs, and sieving grains from time to time are good ways to fight against insect attack (Stoll, 2002). These protection measures are only short-term because they do not kill insects but repel the stocks (De Groot, 1996).

1.6.2 Biological control

All insects which develop in the grain are not always pests of grain, other are predators or parasites of pests and are useful (De Groot, 1996). Biological control is the use of living called auxiliary organism to prevent or reduce loss or damage caused by pests (Wanjber & Ris, 2007). In nature, every living organism has a range of natural antagonists (Goudoum, 2002). Some of these enemies which are parasitoids develop their larval stage on the body or within the body their host to kill them (Cheyper & Buchmann, 2006).

The most important biological control program conducted to date has been the introduction and release of predatory beetle *Teretrius nigrescens* Lewis against the Larger Grain Borer *P. truncatus* in Africa (Ogemah, 2003). Schöller (2010) reported that females of the Pteromalid wasps Lariophagus distinguendus Foerster, Anisopteromalus calandrae Howard and Theocolax elegans Westwood lay their eggs on host larvae or pupae inside grains or cocoons of Indian meal moth. The ovipositor of the parasitoid is inserted and the host larva is paralysed prior to oviposition. After emergence from the egg, the parasitoid larva feeds on the host larva from the outside, thereby killing it. The adult parasitoid *Dinarmus sp.* controlled the population of *C. maculatus* on stored blackgram and their emergence was the highest in second generation and in subsequent generation the emergence of parasitoid and bruchid was the least (Soundararajan *et al.,* 2012). In the same order Sanon *et al.* (1998) reported that, when *D. basalis* are introduced into the stores at regular intervals, either during the first 2 months of storage or during the entire storage period, the parasitoids reduce the increase in *C. maculatus* numbers and the seed weight losses.

1.6.3 Varietal resistance

Varieties more tolerant to insects have been developed in order to limit losses. The selection of resistant varieties is an interesting method of control for small farmers in the fight against insect pests. Indeed, it replaces chemical control and thus eliminates many disadvantages such as the risks to health and the environment, high cost and problems of acceptance of different products by farmers or, difficulties related to the use of these substances (Francis *et al.,* 1998). Studies were conducted during several years on more than 8 000 varieties of cowpea by IITA (International Institute of Tropical Agriculture) in Nigeria resulted in obtaining three varieties (TVu2027, TVu11952 and TVu11953) showing significant resistance with respect to *C. maculatus* (Singh *et al.,* 1985). According to Ivbiljaro (2009) resistant maize cultivars can

reduce losses due to weevil infestation but no grain was immune to attack by the weevil. The resistance of grains against insect pests is often attributed to the grain hardness (Serratos *et al.,* 1987; Mbata *et al.,* 2009) or the grain size and texture (Koussou *et al.,* 1993). Mostly local varieties are resistant to insects' attacks (Ashamo, 2001). The use of resistant varieties alone may not provide a permanent solution to the problems of maize or cowpea storage but rather may contribute to integrated pest management (Gudrups *et al.,* 2001; Credland *et al.,* 2005).

1.6.4 Chemical control

1.6.4. 1 Use of synthetic insecticides

The use of synthetic pesticides against insect pests of stored grains is the set of the most effective ways to avoid losses during storage. It involves the use of synthetic chemical pesticides (fungicides, nematicides, insecticides) (Park *et al.,* 2002). These are fast-acting toxicants. They act as poisons the nervous system (Scotti, 1978) and the respiratory system (Park *et al.,* 2002).

Insects are fought either by increasing the quantities of products used or by applying new active ingredients (Isman, 2008). These substances accumulate in ecosystems and cause an imbalance in food chains and soil contamination can be affected by removal of the Arthropod fauna (Kumar, 1991). This practice although effective in the fight against insects is toxic for consumers, pollutes the environment and induces resistance in pests (Arnaud *et al.,* 2001). Africa uses less than 10% of world production of pesticides but registers 75% of fatal cases due to insecticides (Bambara & Tiemtoré, 2008). The use of increasing amounts of pesticides represents a real danger since it leads to the stage where the insecticide is completely ineffective against the pest, and therefore the resistance occurs. There are three types of resistance mechanisms that result in behavioral changes, physiological and biochemical. (Gwinner *et al.,* 1996; Haubruge & Amichot, 1998; Francis *et al.,* 1998).

In Cameroon for example, prohibited products like dieldrine, lindane and DDT are still in full use (Haile, 2006; Ngamo *et al.,* 2007b). More than 30% of these products sold in Sub-Saharan Africa do not conform to international norms due to the absence of efficient control services (Fleurat-Lessard, 2011). Until today, chemical control is still the most widely practiced despite the risks it causes to fragile ecosystems (Ndiaye, 2000). Farmers are negligent in the use of pesticides, and some seem to be unaware of the dangers they face (PAN, 2003). Some of them also are poor in resources, so that buying the appropriate

chemical seems to be difficult and sometimes not available on the market (Nukenine *et al.*, 2011a). More so obsolete synthetic chemicals are found in our local market which cause serious health hazard (Bambara & Tiemtoré, 2008).

Alternative solutions to the application of synthetic chemicals is the use of phytochemicals (reduced-risk insecticides of plant origin) which is presently being encouraged in stored grain protection because there are more biodegradable, and thus may pose less environmental hazards. *A. indica* and other plant products (Saxena *et al*., 1988; Ogemah *et al*., 2002; Iloba and Ekrakene, 2006, Isman, 2006; Ngamo *et al.,* 2007, Nukenine *et al*., 2007, 2010a, b) stand out as good candidates for physiochemical control of stored product beetles, since their efficacies have been proven.

1.6.4 .2 The use of botanicals

With the increasing concern about the use of synthetic insecticides, the need to find alternatives that are readily available, affordable, less poisonous and less detrimental to the environment cannot be over emphasized (Niber, 1994). The use of plants as protectants of stored foodstuffs or as insecticides is an ancient practice in Asia and Africa (Boeke, 2002; Tapondjou *et al.,* 2002; Aissata, 2009). According to Stoll (2002), almost all plants known as insecticides affect insects in storage by inducing toxicity to adults, larvae, eggs and reduced egg production. Research efforts are being encouraged on the use of botanicals insecticides because they are more biodegradable, and thus pose fewer problems to the environment (Isman 2008; Boeke *et al.,* 2004; Boulogne *et al.,* 2012). Plant products and their secondary metabolites are receiving increasing attention in stored product management (Arthur, 1996; Haque et *al.,* 2000; Zettler & Arther, 2000). The technology is not new as peasant farmers have used it to protect their grains in the small scale and rural settings. Several workers have evaluated the insecticidal, repellent or antifeedant and development inhibiting effects of various plant parts and plant products on *S. zeamais* with varying degrees of success (Belmain *et al.,* 2001; Udo, 2005; Obeng-Ofori *&* Ametiye, 2005; Asawalam *et al.,* 2008; Arannilewa et *al.,* 2006; Nukenine *et al.,* 2011a). Boulogne *et al.,* (2012) mentioned that, 656 plant species worldwide, distributed into 110 families, were identified as to have a significant insecticidal activity. The most cited family is the Lamiaceae, with 181 species distributed into 48 genera, counting for 28 % of the plant families with an insecticidal activity. Botanical insecticides include substances that are potential to control insect. While synthetic chemicals have neurotoxic mode of action and promoted the rapid development of cross-resistance in

insect population, phytochemical insecticides have emphasized non neurotoxic modes of action such as antifeedant action, inhibition of molting, growth reduction, loss of fecundity, respiratory inhibition (Arnason *et al.,* 1993).

One of the plants which was subject of a large number of scientific studies is the neem (*Azadirachta indica*). The vegetable oil of this plant showed insecticidal and repellent to pests foodstuffs (Boeke, 2002). Some plant substances in essential oils occurrence are used for the conservation of grains (Ngamo *et al.,* 2007a; Nukenine *et al.*, 2010a). These plants can be processed into powder and then mixed with the stored grains (Munyuli & Balzi, 2001; Nukenine *et al.*, 2007; Shuka, 2007). In the North Cameroon, Ngamo *et al.* (2007b) identified 27 plants that are used by farmers in the storage structures for the conservation of cereals and legumes among them *P. glandulosus*.

1.6.4 .3 The studied plants

The studied plants in the current research work are *A. indica* family and *P. glandulosus* from the Meliaceae and Lamiaceae family respectively known for their bioactivities.

1.7 Neem tree: *Azadirachta indica*

1.7.1 Origin

The native origin of the neem tree *A. indica* is subject to many controversies. According to Gamble (1902), the center of origin of *A. indica* is in the forests of Karnatka (South India) or the dried inland forests of Burma (Myanmar). National Research council (1992) and Schmutterer (1995) supported the fact that neem is originated from upper Myanmar because of great variety in the shape of the leaves and other morphological features. Other authors were of the opinion that this tree originated in the forests of the Shivalik hills (foothills of the western Himalayas) or on east coast of south India (Puri, 1999) which was widespread in Africa and America (Anonymous, 1963). Above all this diversity in opinion, it is agreed today that *A. indica* is known as “Indian neem tree”.

The *A. indica* called "Ganye" in Cameroon has several local names according to geographical regions (Table 1.1).

Table 1.1: Some local name of *Azadirachta indica*

Geographical location	Local name
Asia- Australia- South Pacific	
India	Limba, Limbo, Neem, Nim, Nimb, Nimba, Vepa, Bery, Roku
Pakistan	Nimmi
Myanmar	Tamarkha
Sri Lanka	Kohomba, Kohunmba
Thailand	Sadao India, Kwinim, Dao
Indonesia	Imba, Mindi, Mimbo, Intran
Malaisia	Mambu
Singapour	Singapour Nimbagaha
Iran	Azad-darakht-i-hindi, Nib
Yemen	Meraimarah
Australia	Neem
Fiji	Neem
Africa	
Nigeria	Babo Yaro, Dongoyaro
Tanzania	Mwarobaini
Cameroon	Ganye, Marrango
Madagascar	Nim
Senegal	Nim, Neem, Nivaquine, Kaaki, Leeki, Nouwakini
America	
U.S.A	Neem
Latin America	Nim
Europe	
Germany	Niembaum, Indischer Zedrach, Nim, Niem, Indischer Flieder
France	Azadira d'Inde, Margousier, Lilas des Indes, Zidirac
Portugal	Margosa, Amargosiera
Spain	Nim, Margosa
England	Neem, Indian Lilac

Source: (Schmutterer, 1995; Puri, 1999; Faye 2010)

1.7.2 Description

The taxonomic position of *A. indica* as described by Adrien Henri Laurent de Jussieu (National research Council, 1992; Schmutterer, 1995; Puri, 1999; Biswas *et al.,* 2002) is as follow:

Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Superorder	Rosanae
Order	Sapindales
Family	Meliaceae
Sub-family	Melioideae
Tribe	Melieae
Genus	*Azadirachta*
Species	*indica*

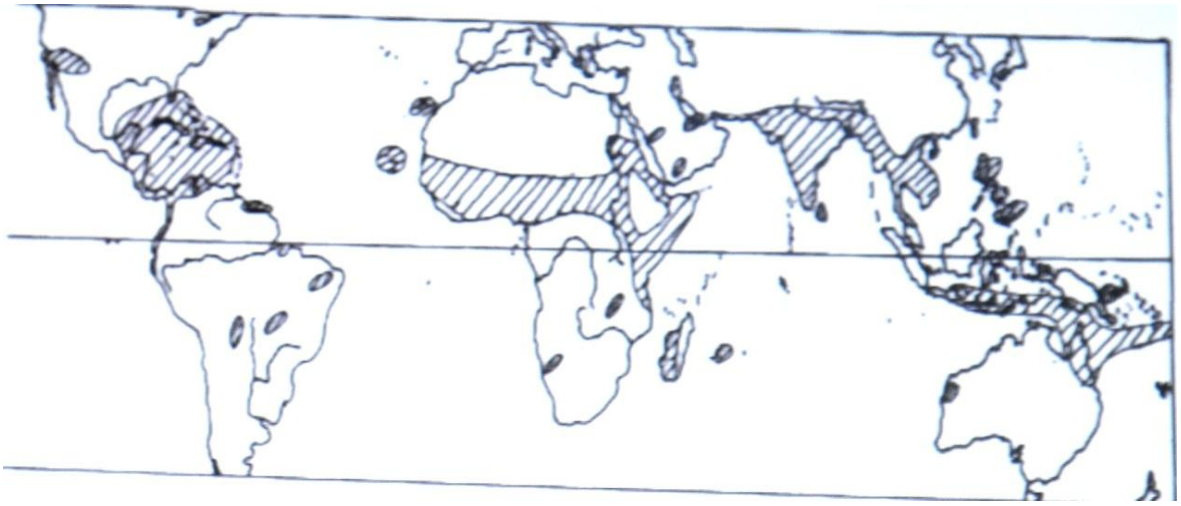
Azadirachta indica is a small to medium-sized tree, usually evergreen, up to 15 (30 max.) m tall (Orwa *et al.*, 2009) (Figure 1.3). Neem leaves are alternately arranged on a long thin stalk (Faye, 2010). The dorsal side of a neem leaf has a dark green color while the ventral side is lighter (Puri, 1999). They are between 20 and 40 cm long and are denser at the ends of branches (Puri, 1999). The youngest leaves have a reddish color. According to Schmutterer (1995) on the same stalk up to 31 can be found. The leaves are smooth and further examination of young leaves located near the shoot apex showed the presence of resin secretory glands (Puri, 1999). The branches are generally large, which explains that the tree produced by these multitudes leaves a large crown, round or oval that can reach 15 to 20 m in diameter for mature trees (National Research Council, 1992; Schmutterer, 1995).

The flowers of the neem are small, white, and supported by an auxiliary beam of up to 25 cm long with form an inflorescence. Its fruit is smooth and ellipsoidal. It measures 1.4 to 2.8 cm long and 1.0 to 1.5 cm (Schmutterer, 1995). Before maturity, it is greenish, and then becomes yellow to greenish-yellow when ripe. It includes a pulp enclosing seed. The exocarp is thin and smooth. The pulp (mesocarp) is sweet, and yellowish-white. It measures 0.3 to 0.5 cm. Inside is the shell (endocarp) which is white, hard enough, and contains within it one and rarely two, or three oval brown kernels (Orwa *et al.*, 2009). The shell is 0.9 to 2.2 cm long and 0.5 to 0.8 cm wide, and its nucleus 0.8 to 1.0 cm long and 0.4 to 0.5 cm wide (Schmutterer, 1995).

Figure 1.3: *Azadirachta indica* trees on both sides of a main street in the city of Maroua, Cameroon

1.7.3 Ecology and distribution

As well adapted in the dry land regions, the neem tree is now widely distributed by introduction in tropical and sub-tropical zones of Asia, Africa, America, Australia and the South pacific Islands (Förster & Moser, 2000) (Figure 1.4). The plant was introduced in Africa through Ghana in the 19th century as an ornamental by a colonial administrator (Tourneux & Yaya, 1998). In Central Africa, neem is present in the Lake Chad Basin (eastern Niger, northeastern and southeastern Nigeria, South-West of Chad, and North Cameroon). It is also common in other parts of Nigeria where it was probably introduced around 1828 (Schmutterer, 1995) mainly in the northern dry regions. It has been present in Cameroon since 1947 (Tourneux & Yaya, 1998; Yengué & Callot, 2002).



Area where neem tree are found

USA	Panama	Surinam	Mali	Egypt	Iraq	Sri Lanka	Philippines
Mexico	Colombia	Brazil	Côte d'Ivoire	Sudan	Saudi Arabia	Myanmar	Indonesia
Guatemala	Ecuador	Bolivia	Burkina Faso	Eritrea	Yemen	Thailand	Papua New
Honduras	Peru	Canary Islands	Ghana	Ethiopia	Qatar	China	Guinea
El Salvador	Puerto Rico	Cape Verde Isl.	Togo	Djibouti	Madagascar	Viet Nam	Australia
Nicaragua	Virgin Isl.	Mauritania	Benin	Somalia	Mauritius	Malaysia	Fiji Islands
Dom. Rep.	Antigua	Senegal	Niger	Kenya	Iran		
Haiti	Montserrat	The Gambia	Nigeria	Uganda	Pakistan		
Cuba	Trinidad-	Guinea Bissau	Cameroon	Tanzania	India		
Jamaica	Tobago	Guinea	Chad	Mozambique	Nepal		
Costa Rica	Venezuela	Sierra Leona	Namibia	Malawi	Bangladesh		
	Guyana	Liberia					

Figure 1.4: Geographical distribution of *Azadirachta indica* tree (Förster & Moser, 2000)

1.7.4 Chemical composition of neem products

Neem is bitter in taste; the bitterness is due to the presence of an array of complex compounds called "triterpenes" or more specifically, limonoids. More than 100 bioactive compounds have been isolated from various parts of the neem tree (Saxena, 2004). Limonoids are a class of highly oxidized triterpenoids and constitutes one third of all compounds isolated and identified from the neem tree. Most of the pesticidal, anti-bacterial, anti-fungal and medicinal properties of *A. indica* are due to limonoids. The main source of limonoids is neem seeds which also are the most important source of neem pesticidal properties (Jianming Dai, 1999; Faye, 2010).

Based on the structure, limonoids from neem can be classified into nine groups: azadirone (from seed oil), amoorastaitin (from fresh leaves), vepinin (from seed oil), vilasinin (from green leaves), gedunin (from seed oil and bark), nimbin (from leaves and seed), nimbolin (from kernel), and salanin (from fresh leaves and seed), and the aza group (from neem seed) (Kraus, 2002; Faye 2010). Azadirachtin, one of the most known and important compounds of neem was isolated by Butterworth and Morgan in 1968.

Azadirachtin (C_{35} H_{14} O_{16}) (Gauvin *et al.*, 2003), a complex tetranortriterpenoid limonoid is the main component responsible for both antifeedant and toxic effects in insects (Luntz & Nisbet, 2000). It was one of the earliest separated compounds from the neem seed. Rembold *et al.* (1984) found that azadirachtin was actually composed of two major compounds, azadirachtin A and azadirachtin B, and two minor compounds, azadirachtin C and D. Today, azadirachtin A-L have already been isolated and identified. Among these azadirachtin, azadirachtin A consist of 85%, where no specification is made, azadirachtin refers to azadirachtin A (Figure 1.5). Azadirachtin content could vary considerably due to edaphic, climatic, or genotype differences. When exposed to light, azadiracthin degrades through a process known as photo-oxidation (Johnson *et al*., 2000). Neem products are also sensitive to high temperatures and should be stored in cool, dark conditions (National Research Council, 1992; Jenkins *et al.*, 2003).

The other compound of neem includes other terpenoïds which are non-limonoïdal compound like diterpenoïds. Four pentatrotriterpenoïd: nimbandiol, 6-acetylnimbandiol, nimbinene and 6-deacetylnimbinene were found in neem seed oil which showed moderate antifeedant, growth inhibiting, and larvicidal effect to some species of pests (Krauss, 1995; Aral *et al.*, 1989, 1990). There are also non- terpenoïdal compound like organic sulfuric

compounds polysaccharide, proteins (amino acids), polyphenolics such as flavonoïds and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds Ogemah, 2003).

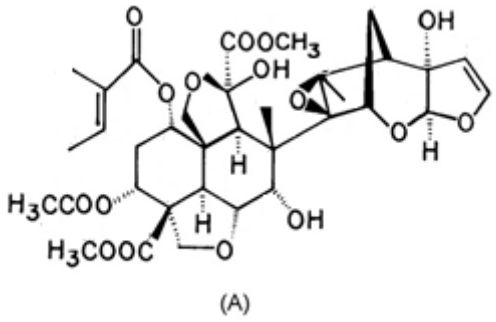


Figure1.5: Azadiracthin A structure

1.7.5 Medicinal properties of neem products

The popularity of neem products increased day by day and this plant is known today as village pharmacy or plant of the 21st century (Ilesanmi & Gungula, 2013). Medical properties of neem have been known among Indians for thousands of years and various part of the tree could be used for various purposes (Warthen *et al.,* 1984; Kausik *et al.*, 2002; Subapriya & Nagini, 2005). The medical properties of neem have been the subject of several researches which demonstrated the efficacy of neem for remedies for intestinal problems, malaria attacks, skin diseases, bacterial infections (Thakurta *et al.,* 2007) . Barks and leaves of this plant are employed for the treatment of syphilis, tuberculosis, rheumatism and are good antidotes against snake bite and scorpion sting (Yengué & Callot, 2002). The twigs of neem tree are use for dental hygiene (Agrawal, 2004).

1.7.6 Insecticidal properties

Azadiractha indica is a wonderful natural insecticide, non toxic for man and other vertebrates when used at recommended dosis. Products extracted from this plant demonstrated their efficacy and are toxic to over 500 insect species (Schmutterer, 1990; Athanassiou *et al.,* 2005; Kavallieratos *et al.,* 2007; Roy *et al.;* 2010) including stored product insect pests of cowpea and maize (Bélanger & Musabyinama, 2005; Iloba & Ekrakene 2006; Debashri & Tamal, 2012). Boeke (2004) demonstrated the insecticidal and repellent properties of *A. indica* seed oils against storage insects. Its compounds, particularly Azadirachtin causes a digestive disturbance, disrupts the metamorphosis of insect larvae by inhibiting molting. This prevents

larvae from developing into pupae and they die without producing new generation (Schmutterer, 1995). According to Belanger & Musabyinama (2005) at least 12 mechanisms of action of neem compounds were reported. Geographical locations, time of harvest, age of plant, environmental conditions, among others are known to greatly influence the activity of neem products against insects (Singh 1986). Several neem-based commercial insecticides are available, including Margosan-O, Neemix 4.5, Azatin-EC, Neem-EC, RH-9999, Agroneem in USA, Neemazal in Germany and Australia, Mubel in Spain, Neemros, Neemroc and Saroneem in Kenya, and more than 12 names in India like Azéri, Margocide, Neemarin, Nimorich (Bélanger & Musabyinama, 2005). NeemAzal powder greatly reduced maize damage caused by *P. truncatus* during storage (Ogemah, 2003). The survey of Ngamo *et al*. (2007b) provided a list of 27 plants used by farmers in northern Cameroon to protect their stocks. Surprisingly, *A. indica* was absent in this list, indicating that it was less being used by farmers in stock protection. However, Tamgno and Ngamo Tinkeu (2014) reported that farmers of the Logone-valley in the Far North region Cameroon are using neem product to protect their stored cereals and pulses.

1.7.7 Mode of action of neem products

Global research on the neem has focused heavily on neem role in crop protection, either in the field or during the storage with over 2 000 papers published in the literature. The last 25 years research indicated that the most important use of neem was as an insecticide (Childs *et al.,* 2000). Neem products are known to affect 400 to 500 species of insects belonging to several orders (Blattodea, Coleoptera, Dermaptera, Diptera) (Koul & Wahab, 2004).

Neem contains several active ingredients, and the most known is azadiracthin which disrupts the metamorphosis of insect larvae by inhibiting molting. This keep larvae from developing into pupae, and they die without producing a new generation (National Research Council, 1992). Products derived from neem seeds act at many levels against insects (Wakil *et al.,* 2008).

a) Antifeedancy

The chemicals which retard or disrupt insect feeding by rendering the treated materials unattractive or unpalatable are known as antifeedant (Saxena *et al.,* 1988). Antifeedant effect of neem products was first demonstrated in 1962 against the desert locust, *Schistocerca gregaria* (Pradha *et al.,* 1962). The compound responsible for antifeedancy is azadiracthin,

other triterpenoïds from neem, like Salannin also have antifeedant properties (National Research Council, 1992). In a warehouse trial conducted in the Philippines, Jilani and Saxena (1988) evaluated the effectiveness of neem oil alone or in combination with fumigation against five species of major stored grains. Rice grain treated with 0.05 to 0.1% neem oil or treated with neem oil after fumigation "phostoxin", and stored for 8 months contained significantly less *Tribolium castaneun* adults than in the untreated control and weevil attacked grains were few(0.2 to 0.4%) in rice grains sampled initially at one months after storage.

b) Repellency

An insect repellent is a chemical stimulus which causes the insect to make oriented movements away from the source of stimulus (Saxena *et al.* 1988). Neem contains several aromatic compounds that can be used to repel insects from biting humans and animals. Neem oil mixed with coconut oil gave up to 98.03% protection against mosquito, *Anopheles culcifacies*, in all-night biting tests conducted in Gujarat, India (Kant & Bhatt, 1994). The efficacy of neem oil against some species of stored grains pests was confirmed in laboratory bioassays. Neem oil mixed with red corn at 1 to 8ml/kg repelled *T. confusum* and *S. zeamais (*Saxena *et al.*, 1988).

c) Reproduction and growth regulation

The treatment of insects or the plants on which they feed with neem products causes insect growth inhibition, malformation and mortality. Azadiracthin, salannin, nimbin and 6-desacetylnimbin disrupt the metamorphosis of insect larvae by inhibiting the activity of ecdysone 20-monooxygenase, a steroid hormone responsible for moulting (National Research Council, 1992; Luntz & Nisbet, 2000; Ukeh *et al.*, 2007). The larvae do not develop into pupae, and the insects die without reproducing. In studies conducted in Kenya, the growth and development of the 1st instars of the maize weevil was completely arrested in maize grain treated with neem oil at 0.02 percent, while the weight loss of treated cobs was less than 1% as compared with a 50% reduction in weight of untreated cobs stored for six months (Kega & saxena, 1996). Neem oil at 0.5% mixed with rice reduced *S. oryzae* and *S. zeamais* populations by almost 90%. Neem treatment did not affect the viability of grain (Saxena *et al.*, 1988).

d) Ovicidal effect

Neem products have also been shown to affect sexual reproduction in female insect by reducing fecundity and fertility. For example, in the treatment of the migratory locust *(Locusta migratoria*) azadiracthin inhibits both oogenis and ovarian ecdysteroid synthesis so preventing oviposition (Luntz & Nisbet, 2000). Male reproduction is also affected by azadiracthin. Injection of male *O. fasciatus* with 0.125 mg of azadiracthin per insect severely reduces male potency as seen by an 80% reduction in the fecundity of normal females when mated with treated males. Azadirachtin also interrupt the meiotic processes which are responsible for the production of mature sperm in locust adult males (Luntz & Nisbet, 2000). Neem seed kernel revealed low ovicidal effects on eggs of *Aedes aegypti* (Umar *et al.,* 2007).

1.7.8 Effects on non-target organisms

There is considerable interest in the effects of neem pesticide on non-target organisms and this is of particular importance when registration is being sought for commercial neem formulations. Effects of neem products on beneficial insects are thought to be relatively minor. A field study in Kenya investigated the effect of using neem seed kernel extracts for controlling insects pests on cowpea, and the effect on beneficial (honey bees) and non-target (spiders and ants) organisms (Childs *et al.,* 1999). Plots sprayed with neem seed kernel extracts received fewer visits from bees than the "no spray" plots, but more visits than the plots sprayed with cypermethrin. Spiders and ants were not significantly affected by neem seeds kernels extracts sprays (Childs *et al.,* 1999). Neem oil does not affect the predatory ability of some non-target species for example *T. nigrescens* a predator of *P. truncatus*, can be effectively used in the control of *P. truncatus* together with neem oil at dosages up to 7.5 ml/kg without any contact toxicity of neem oil on larvae or adults of *T. nigrescens* (Ogemah *et al.,* 2004).

I.8 *Plectranthus glandulosus* Hook

I.8.1 Origin, description and ecology

The genus *Plectranthus* (Lamiaceae family) comprises about 300 species distributed over Tropical Africa, America, Asia and Australia (Retief, 2000; Marques *et al.,* 2012; Soni & Singhai, 2012) *P. glandulosus* (Figure 1.6) (annual herb) is one among species of the genus *Plectranthus* found in the West African flora (Hutchiton & Dalziel, 1958) and in Cameroon Flora (Pele & Berre, 1966; Amvam Zollo *et al.,* 1998). The plant is a coarse, scrambling to

erect, glandular, strongly aromatic herb and up to 3 m high (Poschner, 2013). The leaves are long petioled or cordate-ovate and glabrous, up to 12 cm long and nearly as long as broad. The copious violet inflorescences are ample, decompound, ordered in terminal panicles with slender, glandular-pubescent branchlets (Nduryang, 2006). The upper lip of the flower is unusually four-lobed and the large shoe-shaped lower lip is formed from a single lobe (Collenette, 1985; Pamplona, 1999). The plant is well adapted in the montane forest and amongst scrubs, in areas of higher altitudes (Abdel-Mogib *et al.*, 2002; Poschner, 2013).

Figure 1.6: *Plectranthus glandulosus* plants

1.8.3 Chemical composition of *Plectranthus glandulosus*

Plectranthus glandulosus is known as aromatic plant (Ngassoum *et al.*, 2001). The major phytochemicals found are alkaloids, tannins, anthraquinones, glycosides reducing sugars, saponins, flavonoids, phlobatannins, terpenoids, and steroids (Egwaikhide & Gimba, 2007). The volatile composition of its essential oil reported by different authors is contained in Table 1.2. Ngassoum *et al.*, (2001) collected *P. glandulosus* in the area of the University of Ngaoundere (Adamaoua plateau of Cameroon), in November 1997. Leaf samples were naturally dried at room temperature of the laboratory during one week, before hydro-distillation and the analysis of volatile was done with GC-FID. Nukenine *et al.*, (2007) collected the same plant leaves in October 2004 from Ngaoundere and were dried at room temperature for seven days, and then crushed. The crushed leaves were subjected to steam distillation and the chemical analysis of the oil was achieved by GC-MC. While Goudoum *et*

al., 2013 used the same procedure as Ngassoum *et al.,* (2001) harvested *P. glandulosus* in at the same location but the leaves were cut in pieces and dried for two days.

I.8.4 Insecticidal and medicinal properties

Based on it phytochemicals composition, *P. glandulosus* is reported to be of insecticidal and medicinal interest (Lukhoba *et al.,* 2006; Goudoum *et al.,* 2012a). The plant is used by Baham people in West region of Cameroon to treat female infertility (Telefo *et al.,* 2008). In the Adamawa region, fresh leaves of this plant are used as specie in some meals (Nduryang, 2006). Macerations of the leaves, taken orally, are used to treat colds, sore throat (Ngassoum *et al.,* 2001), malaria, as mosquitoes repellents and for the cure of internal or lower abdominal inflammation (Focho *et al.,* 2009). Leaf powders and essential oils of *P. glandulosus* showed greater insecticidal efficacy against adult *S. zeamais* as compared with *P. truncatus* (Horn) and *T. castaneum* (Nukenine *et al.,* 2010, 2011b, Goudoum *et al.,* 2012a)**.** It is also reported that, *P. glandulosus* inhibit the growth of fungi (Aoudou *et al.,* 2012) and it possesses antimicrobial activity (Egwaikhide & Gimba, 2007). To date, there are no scientific publications reporting on *P. glandulosus* powder against *C. maculatus* on cowpea, although the essential oil of the leaves was effective against the beetle on filter paper (Ngamo *et al.,* 2007c). The efficacy of *P. glandulosus* against stored product insect pests is attributable to its richness in terpenoid compounds (Goudoum *et al.,* 2012b).

Table 1. 2: Chemical composition of the essential oil of *Plectranthus glandulosus* from the Ngaoundere region of Cameroon by different authours

Coumpound	Ngassoum *et al.*, 2001	Nukenine *et al.*, 2007	Goudoum *et al.*, 2013
1-Hexanol	-	-	1.23
α--Pinene	0.2		1.06
A-Fenchene			
Camphene	0.1		
P-Pinene			
Myrcene	1.1		
A-Phellandrene	0.3		
δ -3-Carene	0.6		1.1
A-Terpinene	0.3		
P-Cymene	0.2		
Limonene	1.7		2.7
P-Phellandrene	0.1		
(E)-P-Ocimene	04		
Terpinolene	7.7	3.7	28.29
P-Cymenene	2.2		
Fenchone	21.6	18.3	29.81
Camphor			1.34
Terpinen-4-ol			2.51
Neral	1.5		
P-Cymen-8-ol	0.9		2.8
Piperitone		1.2	
Cispiperitone Oxide	35.1	19.5	2.82
Piperitone epoxide		17.7	
Trans piperitone Oxide	12.6		
Thymol		3.7	
Piperitenone			1.23
Eugenol			
Piperitenone Oxide	6.0	8.9	11.08
Isopulegone 4-methyl			1.11
Diosphenol		2.5	
β -Myrcene			2.32
Germacrene D			1.61

CHAPTER 2: MATERIALS AND METHODS

2.1 Plant materials

Products from *A. indica* and *P. glandulosus* were used as test insecticide materials and maize and cowpea like substrates.

2.1.1 *Azadirachta indica*

2.1.1 Collection and processing of seeds and leaves

Azadirachta indica seeds and leaves were collected at Meskine, Maroua (latitude 10°33' North, longitude 14°15' East, and at an altitude of 356 m a.s.l.), Far-North region of Cameroon in May 2011. The ripe and fresh seeds that had fallen off from the trees were collected on the ground under the *A. indica* trees. Half of the seeds were dehusked manually (Figure 2.1**)**. During seven days, half of the dehusked seeds (kernels) just like the undehusked seeds (Figure 2.2) were sun-dried and the other half, dehusked and undehusked were dried under shade in a room in Maroua. The green leaves close to the lateral buds of the lower branches of the tree were collected. Part of the leaves was sun-dried and the other part was dried under shade for three days in Ngaoundere. The drying temperatures of the seeds and leaves were 27 ± 3° C and 34 ± 4°C in shade and in sunlight, respectively (data collected from the meteological center at the Maroua Salak airport).

All the dry seeds, kernels and leaves were kept in black plastic bags and then stored for four months in a cold room at -14 °C, after which, they were transported to Berlin, Germany. The seeds were dehusked before storage.

Figure 2.1: Neem kernels

Figure 2.2: Neem seeds

In Berlin, the crushed leaves of *A. indica* and part of the dried seeds and kernels of of the plant were ground into powder (Figure 2.3) in a Bosch Universal grinder (model MUM

6012, Remscheid, Germany) (Figure 2.4) until the particles passed through a 0.5- and 1-mm mesh sieve respectively for the leaves and the seeds. The powders obtained were introduced into an opaque glass and stored in a refrigerator until needed for bioassay. NeemAzal powder, a commercial neem product, was provided by Trifolio-M GmbH, Lahnau, Germany.

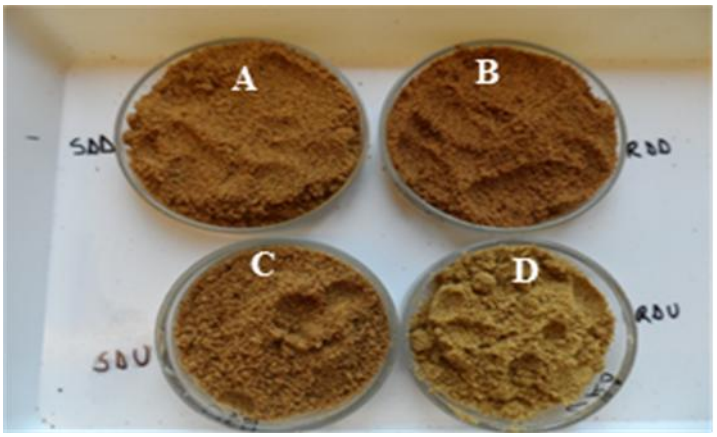


Figure 2.3: Neem seed powder obtained from dehusked and sun-dried seeds (**A**), dehusked and shade-dried seeds (**B**), undehusked and sun-dried seeds (**C**) and undehusked and shade-dried seeds (**D**)

The extraction of *A. indica* seed oil was carried in a mechanical press (model CA59G Komet, Mönchengladbach, Germany) (Figure 2.5). Two kilograms kernels from each drying regime were introduced into the press and crude neem oils were collected, filtered and weighed for the determination of neem oil yields. Oil yield (%) was calculated as weight of oil divided by the weight of the kernel multiplied by 100.

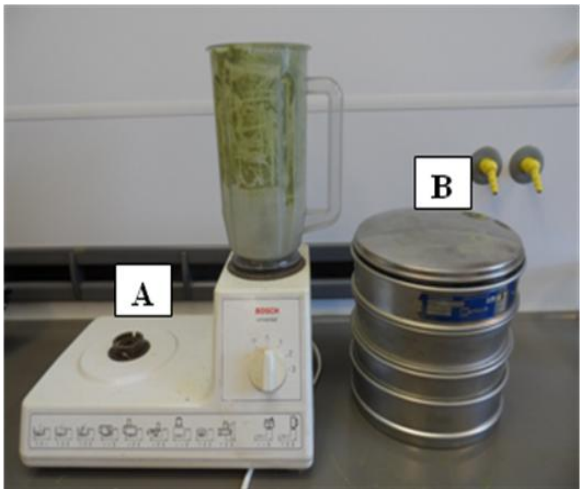


Figure 2.4: Grinder (A) and Sieves (B)

Figure 2.5: Extraction of neem oil using mechanical press

2.1.2 *Plectranthus glandulosus*

The leaves of *P. glandulosus* were collected in October (end of wet season) of 2010 around Ngaoundere (Quartier Champ de prière) (latitude 7°22' North and longitude 13°34' East, altitude of 1,100 m.a.s.l.), located in the Adamawa region of Cameroon. The plants were less than one-year old and only the green leaves were harvested from plants which were yet to attain the flowering stage. Half of the collected leaves were sun-dried and the other part was shade dried in a room until they were crisp dry. The drying temperatures of leaves were 25 ± 1° C and 29 ± 4°C in shade and in sunlight respectively. Dried leaves were hand crushed. The crushed leaves were kept in black plastic bags and were stored for 12 months in a cold room at -14°C, and then transported to Berlin, Germany.

In Berlin, the crushed leaves of *P. glandulosus* was ground into powder using a Bosch Universal grinder (model MUM 6012, Remscheid, Germany) (Figure 2.4) until the particles passed through 0.5, 0.3 and 0.1 mesh sieve, providing three different powders according to the particle sizes.

2.1.3 Cowpea and maize

2.1.3 Origin of maize and cowpea

The maize variety used in this study was yellow Ricardino (KWS) (Figure 2.6A) harvested in an experimental field of the Julius Kühn-Institut (JKI) Braunschweig, Germany in 2012. Cowpea seeds (Black eye beans, Perou variety) (Figure 2.6B) was purchased in a tropical foods store in Berlin, Germany. Maize and cowpea were cleaned by removing broken cobs and grains and kept in a freezer for one week at -15°C to kill any living insects from previous infestation. After this period, the grains were kept in the experimental condition for at least one week before use for bioassay.

2.2 Insects

The parent adults of *S. zeamais* and *C. maculatus* were obtained from colonies maintained at JKI, Institute for Ecological Chemistry, Plant Analysis and Stored Products Protection, Berlin

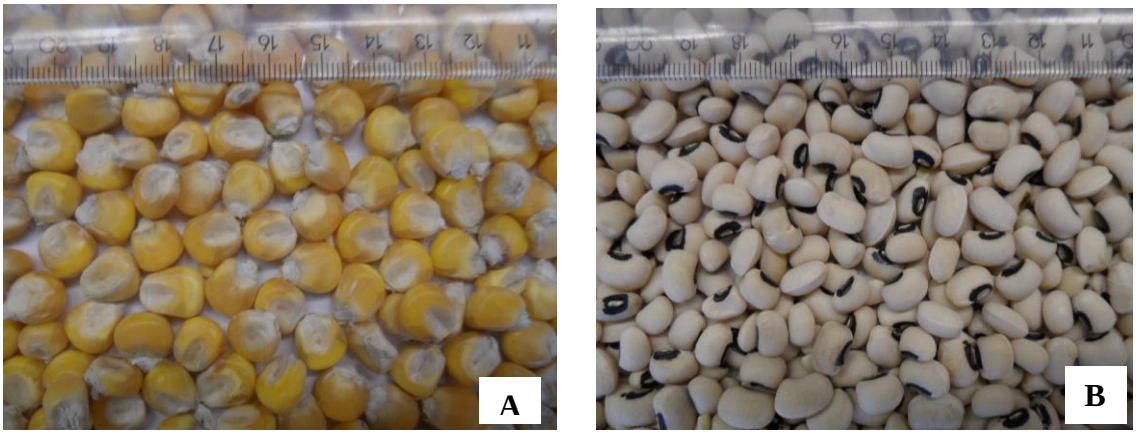


Figure 2.6: A- Yellow Ricardino (KWS) maize variety (**A**) and Cowpea black eye bean, Perou variety (**B**).

since 1968 and 2011, respectively. *S. zeamais* were reared on maize where two-ml (about 250) unsexed adults were introduced in two-liter glass jars containing 500 g seeds, then closed with a muslin cloth and fastened in place with gummy to allow air circulation. After two weeks, parent adults were removed using a 5-mm mesh sieve.

The second sieving was done six weeks after the first one and the obtained progeny was used for bioassays. Three ml (about 300 adults of mixed sex) *C. maculatus* were introduced in one-liter glass jar with 200 g cowpea seeds and closed as described above for *S. zeamais*. After one week, adults were discarded with the help of a 5-mm mesh sieve from jars and 200 g seeds were added. Progeny were obtained four weeks after infestation and used for bioassays.

Every two months, a new experimental culture was established for both species. Unless stated otherwise, insects aged between 7-14 days for *S. zeamais* and 0-1 day for *C. maculatus* were used for bioassay studies involving the adults.

2. 3 Experimental tests

2.3.1 Effect of drying regime on the insecticidal efficacy of local neem seed oil and powder on adult *Sitophilus zeamais* and *Callosobruchus maculatus*

The oils extracted from the *A. indica* kernels and the powders obtained from the same kernels which have been subjected to four different drying regimes, shade-dried kernels, sun-dried kernels, shade-dried seeds and sun-dried seeds were analyzed for their azadirachtin A content

(Section 2.3.1.2 below). The fatty acid content was determined following the different drying regimes for the different oils (Section 2.3.1.1 below).

After a preliminary test, five doses of oils and powders were chosen for bioassays. The oil volumes of 0.1, 0.15, 0.2, 0.25 and 0.3 ml were separately pipetted with a 1 ml syringe to 50 g of maize or cowpea in 250 ml glass jars to give the concentrations of 2, 3, 4, 5 and 6 ml/kg of maize or cowpea. The powder mass of 0.25, 0.5, 1, 1.5 and 2 g were separately added to 50 g of maize or cowpea in 250 ml glass jars to give the doses of 5, 10, 20, 30 and 50 g/kg of maize or cowpea. Controls consisted of grains without the oil or powder. All the powders and oils were tested on *S. zeamais* and *C. maculatus* for adult toxicity, progeny production and grain damage in grains as described in sections 2.4.1-2.4.4 below. The persistence bioassay was carried out only with the oil and powder obtained from the sun-dried kernels. The bioassay for the degradation of azadirachtin A on treated cowpea and maize seeds with *A. indica* oil (sun-dried kernels only) were conducted at 0, 1, 7, 10, 14, 21, 30, 60, 90, 120, 150 and 180 days after treatment.

2.3.1.1 Fatty acids content in oils

The crude *A. indica* seed oils were analyzed as methyl esters to determine the fatty acid composition. Fatty acid methyl esters (FAME) were obtained through a two steps method with sodium methoxyde and HCl as catalysts, and then analyzed by capillary column gas chromatography (GC) (Hewlett Packard HP 6890) equipped with a flame-ionization detector (FID), as described in EN ISO 5509 and EN ISO 5508. 1 ml of the FAME sample was injected and GC separation was carried out in a HP-INNO Wax capillary column.

2.3.1.2 Azadirachtin A quantification in oils and powders alone and in oils on treated grains that were stored at different periods up to 180 days

(a) Sample preparation for *Azadirachta indica* seed oils and powders

Extraction and cleanup of the *A. indica* seed oils and powders from different drying regimes were carried out using QuEChERS (Anastassiades, 2003). 100 µl of oil and 2 g of powder were introduced into a 50 ml polypropylene centrifuge tube and 100 µl of surrogate (Spinosyn 100 g/l) were added. Extraction was performed by adding 10 ml acetonitrile and 10 ml of water in every tube and each tube was shaken using a vortex-mixer (IKA Vortex MS 3 digital

IKA®-Werke, Staufen, Germany) for 45 min and then in an ultrasonic bath for 15 min. To cleanup, anhydrous magnesium sulfate $MgSO_4$ (4 g) and Sodium chloride NaCl (1 g) were added and the tubes were tightly capped and vigorously mixed with vortex for 1 min. Then, the extracts were centrifuged at 3000 g × 5 min. After centrifugation, an aliquot of 100 µl from the upper layer of extract was transferred to an Agilent vial and then dried to evaporate water. The extract was diluted with 1ml of methanol/water 1:1 (v/v) containing an internal standard spinosyn L (used for quantification) at the concentration of 25 pg/µl and subsequently kept in dark at 4°C until analyzed via LC/MS/MS. According to drying method each treatment was replicated thrice and for each tube, two replications were done for a total of six repetitions.

(b) Sample preparation for *Azadirachta indica* seed oils on grains, and then stored for different periods up to 180 days

Cowpea or maize was treated with neem oil from sun-dried kernels at different concentrations (0.1, 0.15, 0.2, 0.25 and 0.3 ml were separately pipetted with a 1 ml syringe to 50 g of maize or cowpea in 250 ml glass jars to give the concentrations of 2; 3; 4; 5 and 6 ml/kg of cowpea or maize). Untreated grains were considered as control. A 5-g sample of grain was taken at 0, 1, 3, 7, 10, 14, 21, 30, 60, 90, 120, 150 and 180 days after treatment for azadirachtin A determination. The 5 g of cowpea or maize were weighed into a 50 ml polypropylene centrifuge tube and 100 µl of surrogate (Spinosyn A 100 g/l) were added. Extraction was performed by adding 25 ml acetone/water in the proportion 80:20 v/v. The mixture was shaken using an ultrasonic bath for 15 min and then vortex-mixer for 45 min. An aliquot of 500 µl from the upper layer of extract was transferred to an Agilent vial and then dried to evaporate water. The extract was diluted with 1ml of methanol/water 1:1 (v/v) containing an internal standard spinosyn L at the concentration of 25 pg/µl and subsequently kept in dark at 4°C until analyzed via LC/MS/MS. According to drying method each treatment was replicated four times and for each tube two replications was done for a total of eight repetitions.

(c) LC–MS/MS analysis

Liquid chromatography–electrospray ionization-tandem mass spectrometry, in positive ion mode, was used to separate, identify, and quantify azadirachtin A. For the LC analysis, a

Shimadzu Prominence UFLCXR HPLC system (Agilent Technologies, Darmstadt Germany) with a binary pump was used. The analytical column employed was a reversed-phase C18 of 50 × 3 mm and 2.6 µm particle sizes. The mobile phase A was methanol-water (90:10, v/v) with 0.1% acetic acid + 5 mmol Ammonium acetate. The mobile phase B was water with 0.1% acetic acid + 5 mmol Ammonium acetate. The gradient program started with 0% of A, constant for 2 min, followed by a linear gradient up to 100% A in 3.5 min, and finishing with 100 % A constant for 3.5 min. After this 5.5 min run time, 3.5 min of post-time followed using the initial 30% of B. The flow rate was set constant at 0.9 ml/min during the whole process, and the injection volume was 5 µl. For the mass spectrometric analysis, a AB SCIEX QTRAP 4000 MS/MS system (AB Sciex Instruments) was used, equipped with a turbo ion spray source operating in positive ionization mode, set with the following parameters: Ion Spray (IS) voltage: 5500 V; curtain gas: 20 psi; nebulizer gas (GS1): 70 psi; auxiliary gas (GS2): 50 psi; source temperature: 550 ◦C. Nitrogen was used as the nebulizer and collision gas. Optimization of the compound was performed by flow injection analysis (FIA), injecting individual standard solutions directly into the source. AB SCIEX Analyst software 1.5.2 was used for data acquisition and processing.

2.3.2 Influence of drying regime and particle size on the insecticidal efficacy of leaf powders from *Azadirachta indica* and *Plectranthus glandulosus* against adult *Sitophilus zeamais* and *Callosobruchus maculatus*

Parts of the leaf powders with particle sizes ≤ 0.5 mm after hand-crushing and passing through a 0.5 mm mesh sieve of the sun-dried and shade-dried leaves of *A. indica* and *P. glandulosus,* were tested on *S. zeamais* and *C. maculatus* for adult toxicity in grains as described in section 2.4.1 below. 0.25, 0.5, 1, 1.5 and 2 g of the powders were separately added to 50 g of maize or cowpea in 250 ml glass jars to give the doses of 5, 10, 20, 30 and 40 g/kg of maize or cowpea. Controls consisted of grains without the plant powders.

After the toxicity tests, part of the sun-dried leaf powders of *P. glandulosus* and *A. indica* (as this tended to be more potent to the beetles) was pulverized, until they passed through sieves with mesh sizes 0.3 and 0.1 mm, respectively (Figure 2.7). Each powder with ≤ 0.5, ≤ 0.3 and ≤ 0.1 mm particle sizes were tested on *S. zeamais* and *C. maculatus* for adult toxicity and progeny production (Olotuah, 2013) as described respectively in sections 2.4.1 and 2.4.2 below. Grain damage was tested only with the different particle sizes of *P.*

glandulosus powder as described in section 2.4.3 below. Persistence bioassays were carried out only for the ≤ 0.1 mm particle size powders of *P. glandulosus* on *S. zeamais* as described in sections 2.4.4 below.

Figure 2.7: The three analytical sieves used to obtain different particle size powders (**A**) and glass jars containing the leaf powders of different particles sizes (≤ 0.1 mm, ≤ 0.3 mm, ≤ 0.5 mm) (**B**)

2.3.2.1 Chemical analysis of *Plectranthus glandulosus* leaf powder

The method of Ulrich & Olbricht (2013) was used for the extraction of powder volatiles by immersion stir bar sorptive extraction (imm-SBSE). 100 µg of each powder were homogenized in 10 ml of a solution of 5 % ethanol by a household mixer for 1 min. The homogenate was centrifuged at 4000 rpm for 30 min. 100 mm of the supernatant were mixed with 10 µl internal standard (0.1 % (v/v) 2,6-dimethyl-5-hepten-2-ol dissolved in ethanol). An aliquot of 8 ml of the saturated homogenate, but without the solid NaCl deposit was transferred in an empty glass vial for volatile isolation by SBSE. A stir bar with 0.5 mm film thickness and 10 mm length coated with polydimethysiloxan (PDMS) was placed in the liquid (Gerstel, Mülheim an der Ruhr, Germany). The stir bar was moved at 350 rotations per minute at room temperature for 45 min. After removal from the leaf extract, the stir bar was rinsed with purified water, gently dried with a lint-free tissue and then transferred into a glass tube for thermal desorption and subsequent GC analysis.

The Gas chromatography – mass spectrometry (GC-MS) was performed. The parameters for the thermal desorption unit (TDU, Gerstel) and the cold injection system (CIS4, Gerstel) were the following: thermal desorption at 250°C, cryo trapping at -150°C. The

TDU-CIS4 unit was used in Gerstel-modus 3: TDU splitless and CIS4 with 15 ml/ min split flow. The analyses were performed with an Agilent Technologies 6890N detector. Compounds were separated on a polar column ZB-Wax plus 30 m length × 0.25 mm ID × 0.5 μm film thickness. Helium was used as a carrier gas with a column flow rate of 1.1 ml/min. Temperature programme: 45ºC (3 min), temperature gradient 3 K/min to 210 ºC (30 min). The mass spectrometer was used with electron ionization at 70 keV in the full scan mode. Compounds were identified by comparing major peak of chromatograph with those of mass spectra database generated from reference substances.

2.3.3 Bioefficacy of binary combinations of *Azadirachta indica* products and *Plectranthus glandulosus* leaf powders on adult *Sitophilus zeamais* and *Callosobruchus maculatus*

The more active powders for each plant from sections 2.3.1 (sun-dried *A. indica* kernel powder) and 2.3.2 (≤ 0.1 mm particle size sun-dried *P. glandulosus* leaf powder) above, according to drying regime and particle size, were considered. NeemAzal powder were mixed with *P. glandulosus* leaf powder, and neem seed powder with *P. glandulosus* leaf powder in the proportions of 100/0, 75/25, 50/50, 25/75 and 0/100% in glass jars. Each glass jar was shaken with a bidimensional mixer (Gerhardt, Dreieich, Germany) for 5 hours to ensure uniform mixture of the powders (Figure 2.8). The masses 0.125, 0.25, 0.5, 0.75 and 1 g were separately introduced to 50 g of maize or cowpea in 250 ml glass jars to give the doses of 2.5, 5, 10, 15 and 20 g/kg of maize or cowpea. Controls consisted of grains devoid of the plant powders. Each binary mixture was tested on *S. zeamais* and *C. maculatus* for adult toxicity, progeny production and grain damage as described in sections 2.4.1-2.4.3 below. The mixture 75 NeemAzal + 25% *P. glandulosus* and 75 neem seed powder + 25% *P. glandulosus* were not considered for grain damage bioassay. The persistence test was performed with 75% *P. glandulosus* + 25% NeemAzal and 50% *P. glandulosus* + 50% *A. indica* powders, respectively, as described in section 2.4.4 below.

The co-toxicity coefficient of powder mixture was used to determine their responses: A co-toxicity coefficient of less than 80 is considered as antagonistic, between 80 and 120 as additive, and higher than 120 as synergistic (Sun & Johnson 1960; Islam *et al.*, 2010). If a mixture (M) compounds of two parts (A and B), and both components have LC_{50}, then the following formulas are used (A serving as standard):

Toxicity index (TI) of A = 100

Toxicity index (TI) of B = $\frac{\text{LC50 of A}}{\text{LC50 of B}} \times 100$

Actual TI of Mixture = $\frac{\text{LC50 of A}}{\text{LC50 of M}} \times 100$

Figure 2.8: Mixing of the different botanical powders for binary bioassays

Theoretical TI of M = TI of A × percentage of A in M + TI of B × percentage of B in M

Co-toxicity coefficient = $\frac{\text{Actual TI of M}}{\text{Theoretical TI of M}} \times 100$

If one component of the mixture alone (for example B) causes low mortality at all doses (< 20%), then the co-toxicity coefficient of the mixture was calculated by the formula: Co-toxicity coefficient = LC_{50} of A alone/LC_{50} of A in the mixture × 100.

2.3.4 Effect of *Azadirachta indica* products and *Plectranthus glandulosus* leaf powder on fecundity and immature stages of *Sitophilus zeamais* and *Callosobruchus maculatus*

2.3.4.1 Fecundity

Callosobruchus maculatus: Five 0 to 1-day-old couples of the beetle species were introduced into 20 grains of cowpea in 5 mm Petri dish. The identification of the insect sex was done by

observation of elytra (Figure 2.9) (Huignard *et al.*, 2011). Two sublethal dosages of each botanical (0.05 and 0.1 ml/kg for oil, 2.5 and 5 g/kg for powder from *A. indica* and powder from *P. glandulosus*, 1 and 2 g/kg for the mixture of 75% *P. glandulosus* -25% NeemAzal and 0.01 g/kg for NeemAzal) were considered. Neem oil was diluted in 0.5 ml acetone. Controls consisted of substrate without botanical products. After three days of exposure to the products, the adult beetles were removed and placed on 50 untreated grains of cowpea for three days to allow oviposition. Each treatment was replicated four times. The number of eggs laid was counted under a stereomicroscope accordingly for the treated and untreated batches of grains.

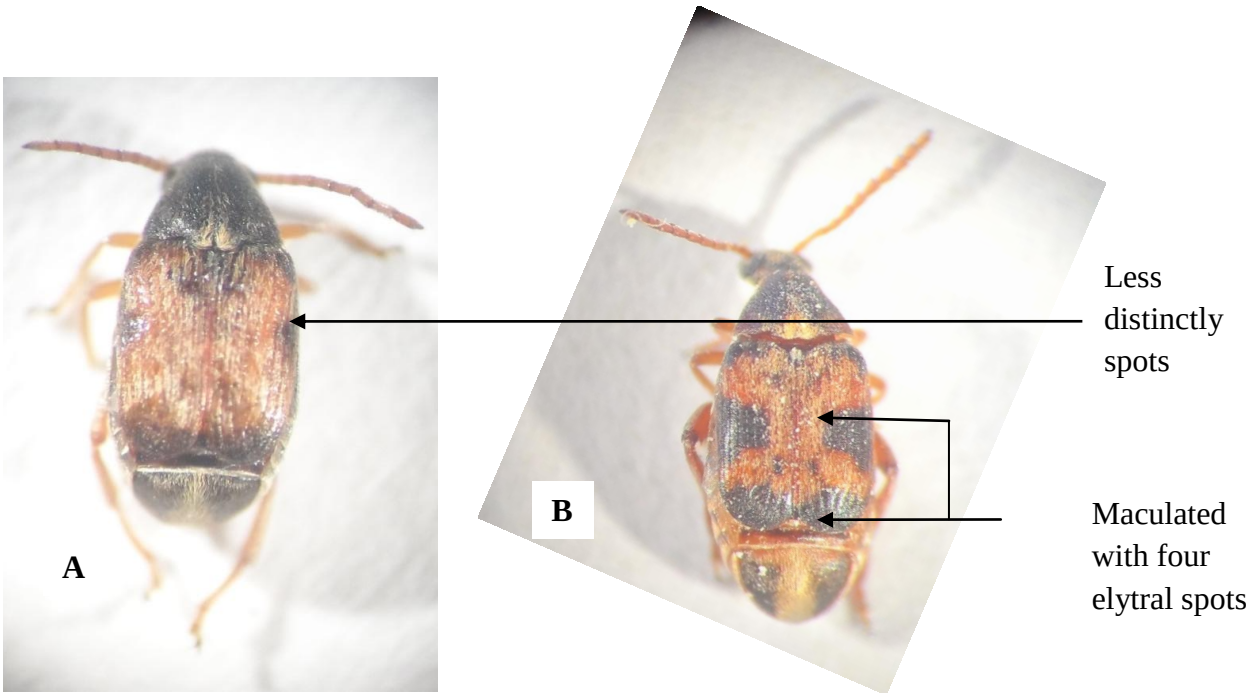


Figure 2.9: Sexual dimorphism difference in *C. maculatus*: male (**A**) and female (**B**) (× 40)

Sitophilus zeamais: Ten 1 to 2-day-old couples of the weevil (identified with the help of a stereo microscope) were introduced to 100 maize grains in jar in 35 ml plastic bottles. The identification of the insect sex was done under stereomicroscope by observing the rostrum (Figure 2.10) (Halstead, 1963). The same sublethal dosages like for *C. maculatus* above were considered. Controls consist of substrate without botanical products. After seven days of exposure to the products, the insects were removed and placed on 100 untreated maize grains and left for seven days for oviposition to occur. The number of eggs laid was counted for both the treated and untreated maize grains. The method described by Holloway (1985) and used by Danho & Haubruge (2000) was applied to count eggs laid by the females.

The grains were introduced first in water for one minute to humidify them and then placed for two minutes in a solution of acid fuchsine 0.5% which colored mucilaginous plugs in red cherry (Figure 2.11). The excess colour was reduced by introducing the grain in clean water for one minute. The grains were then placed on paper to dry them and the count of the eggs was done under a stereomicroscope. Each treatment was replicated four times.

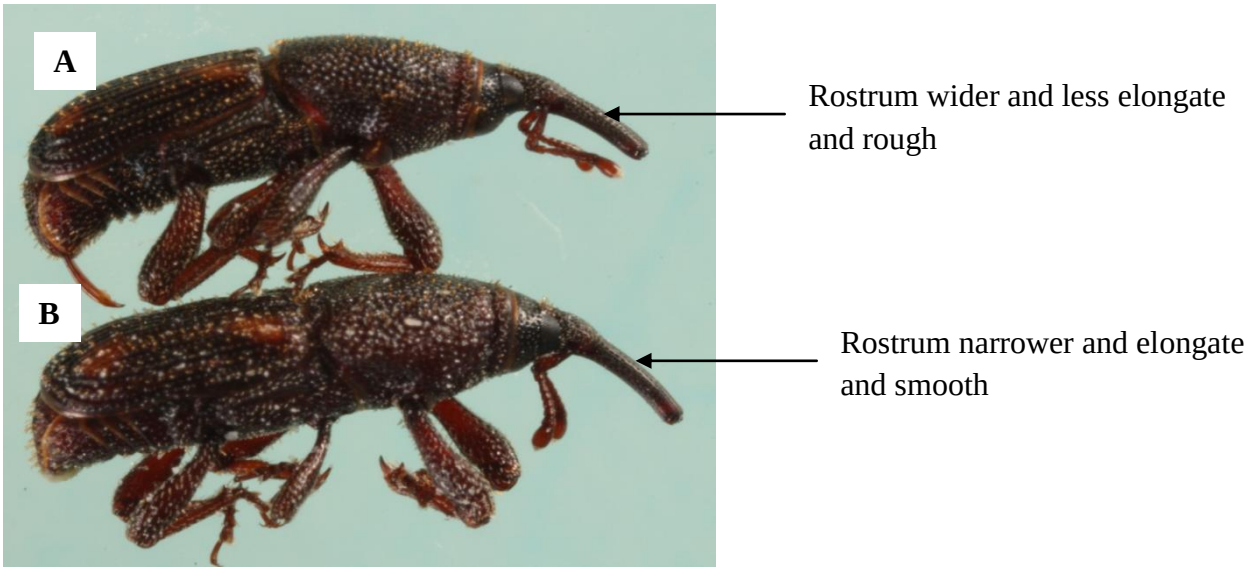


Figure 2.10: Sexual dimorphism in *Sitophilus zeamais*: male (**A**) and female (**B**) (× 40)

Figure 2.11: Maize with *Sitophilus zeamais* egg plugs after treatment with acid fuchsine (× 60)

2.3.4.2 Immature stages

Callosobruchus maculatus: A modified procedure of Obeng-Ofori & Amiteye (2005) was followed here (the number of insects and time for oviposition reduced). One hundred and fifty

C. maculatus adults of mixed sex (1-day old) were introduced onto 2 kg of cowpea for three days to allow for oviposition, after which the parent adults were sieved out. Two days after adult removal, batches of 50 g of cowpea were mixed with the three first dosages of each product (oil and powders from *A. indica* and powder from *P. glandulosus*) found in sections 2.3.1-2.3.3 to evaluate the efficacy of treatments on egg stage. To determine the toxicity of the botanicals on the larval and pupal stages, the experiment was repeated 12 and 18 days after adult removal from the infested grain sample. After 40 days, the number of F_1 progeny emerging was counted. Each treatment was replicated four times.

Sitophilus zeamais: The procedure of Obeng-Ofori & Amiteye (2005) was followed. Two hundred *S. zeamais* adults of mixed sex were placed onto a sample of 2 kg of maize for five days to allow oviposition, after which the parent adults were sieved out. One day after adult removal, batches of 50 g of maize were mixed with the three first dosages of each product (oils and powder from *A. indica* and powder from *P. glandulosus*) found in sections 2.3.1-2.3.3 to determine the efficacy of treatments on egg stage. To determine the toxicity of the botanicals on the early larval, late larval and pupal stages, the experiment was repeated 5, 21 and 28 days after adult removal from the infested grain sample. After seven weeks the number of F_1 progeny emerged was counted. Each treatment was replicated four times.

2.3.5 Effects of environmental condition on the ability of *Azadirachta indica* products and *Plectranthus glandulous* leaf powder to protect grains against the infestation of *Sitophilus zeamais* and *Callosobruchus maculatus*

Oil and powder obtained from sun-dried kernels, ≤ 0.1 mm particle-size *P. glandulosus* leaf powder and powder of the mixture of 75% *P. glandulosus* – 25% NeemAzal were used. The dosages for each product of the sections 2.3.1-2.3.3 were applied for toxicity bioassays (see section 2.4.1 below) which were carried out under two temperature levels (t = 25°C, r.h. = 60%; t = 30 °C, r.h. = 60%) and three relative humidity levels (t = 25 °C, r.h. = 50%; t = 25°C, r.h. = 60%; t = 25 °C, r.h. =70%).

2.4 Bioassays

2.4.1 Toxicity bioassay

Except stated otherwise, all toxicity bioassays were carried out at 25° C and 60% r.h. The five dosages of each treatment mentioned in section 2.3 above for toxicity test were applied

separately onto 50 g of cowpea or maize in 250 ml glass jars. Each jar was shaken with a bidimensional mixer (Gerhardt, Dreieich, Germany) for 4 minutes to ensure uniform coating of the oils or powders on the grain for the entire grain mass (Figure 2.8). Control consisted of grains devoid of insecticidal materials. Groups of 20 *S. zeamais* and 20 *C. maculatus* were added to glass jars containing treated or untreated maize and cowpea, respectively. Glass jars were securely covered with muslin cloth and were tightly held in place with rubber bands to ensure adequate ventilation. All treatments were arranged in a completely randomized design on shelves and each treatment had four replications. Mortality was recorded 1, 3, 7 and 14 days after treatment for *S. zeamais* and 1, 3 and 6 days after treatment for *C. maculatus*. Insects were considered dead when no movement was observed after touching them with forceps twice within two or three minutes.

2.4.2 F_1 Progeny production bioassay

After the 6th-day and 14th-day mortality recordings (section 2.4.1) for *C. maculatus* and *S. zeamais*, respectively, all the insects and insecticide substances were separated from the grains and discarded. The grains were left inside the jars and the F_1 progeny were counted (Nukenine *et al.*, 2011a). To avoid generation overlaps, F_1 progeny were recorded 40 days and 50 days after infestation respectively for *C. maculatus* and *S. zeamais*.

2.4.3 Damage bioassays

Similar dosages of each product as for the toxicity bioassay described above were used for this assay. 100 g grains were considered. A group of 30 adult insects of mixed sex were introduced into each jar containing treated or untreated grains. Untreated control for each set of treatments consisted of grain without plant material. All treatments were replicated four times. After 10 weeks of storage (Figure 2.12), insecticide materials and insects were sieved out. Damage assessment was performed as follows: One hundred grains were randomly selected from each treatment of maize and cowpea (Udo, 2005), and the number of damaged grains (grains with characteristic holes) and undamaged grains were counted and weighed. Percent weight loss (**P**) was computed using FAO (1985) method, thus:

$$P = \frac{U \times Nd - D \times Na}{U(Na + Nd)} \times 100$$

where **U** is the weight of undamaged grain, **D** is the weight of damaged grains, **Na** is the number of undamaged grains, **Nd** is number of damaged grains.

The Percentage damaged grains (PD) was therefore, calculated using the formula:

$$\mathbf{PD = B/A \times 100}$$

Where **B** is number of grains with holes and **A** is total number of grains sampled.

Figure 2.12: Storage of grains on shelves in conditioned laboratory

2.4.4 Persistence bioassay

To assess the persistence of the treatments, each product considered (Oil and powder from sun-dried kernels from *A. indica* and powder from *P. glandulosus*) were tested at five rates following Obeng-Ofori & Amiteye (2005). Twenty adult beetles (*S. zeamais* and *C. maculatus*) were exposed to treated grains (maize and cowpea) which had been stored for 0, 15, 30, 60 and 180 days. Mortality counts were carried out 3 and 5 days after exposure for *C. maculatus* and *S. zeamais*, respectively. All treatments were replicated four times.

2.5 Data analysis

Data on % cumulative corrected mortality, % reduction in F_1 progeny, % damage and % weight loss were arcsine [(square root(x/100)] transformed and the number of F_1 progeny

produced were log (x + 1) transformed to homogenise the variance. The transformed data were subjected to the ANOVA procedure using the Statistical Analysis System (Zar, 1999; SAS Institute, 2008). Tukey (HSD) test (P = 0.05) was applied for mean separation**.** Student's *t*-test was used to compare the effect of drying method (sun-drying and shade-drying) on the insecticidal efficacy of leaf powders of *A. indica* and *P. glandulosus*. Probit analysis (Finney, 1971; SAS institute, 2008) were applied to determine lethal concentrations causing 50% (LC_{50}) and 95% (LC_{95}) mortality of *C. maculatus* and *S. zeamais* at 3 and 7 days, respectively after treatment application. Abbott's formula (Abbott, 1925) were used to correct for control mortality before probit analysis and ANOVA.

CHAPTER 3: RESULTS

3.1 Yield and Azadirachtin A content of *Azadirachta indica* oils and powders from seeds subjected to different drying regimes

The yield of the oils from *A. indica* seeds that were subjected to four drying regimes ranged from 28.30% (sun-dried seeds) to 34.42% (shade-dried kernels), with sun-dried seeds/kernels tending to produce lower quantities of oils than the shade-dried seeds/kernels (Table 3.1). The oil from the sun-dried seeds had lower Azadirachtin A contents compared with the oils from the other three drying regimes (shade-dried seeds, sun-dried kernels and shade-dried kernels), which had similar contents of the substance. The Azadirachtin A content in neem seed powders was similar for the different drying regimes ($P > 0.05$).

Table 3.1: Yield of oils and Azadirachtin A content of *Azadirachta indica* seeds that were subjected to four drying regimes

Drying regime of *A. indica* seeds	Yield (% w/w)	Azadirachtin A in oil (g/kg)[†]	Azarirachtin A in powder (g/kg)
Shade-dried kernels	34.42	3.56 ± 0.14^{a}	1.20 ± 0.02
Sun-dried kernels	28.60	3.09 ± 0.09^{ab}	1.19 ± 0.07
Shade-dried seeds	32.70	3.69 ± 0.16^{a}	1.54 ± 0.26
Sun-dried seeds	30.30	2.89 ± 0.17^{b}	1.05 ± 0.03
$F_{(3,8)}$ [‡]		7.06*	2.54^{ns}

[†] Means ± SE in this column followed by the same letter do not differ significantly at P = 0.05 (Tukey's test)
[‡] Ns $P > 0.05$; * $P < 0.05$

3.2 Fatty acid content of *Azadirachta indica* oils obtained from seeds subjected to four drying regimes

The major fatty acids found in the *A. indica* seed oils in decreasing order were oleic acid >> linoleic, palmitic and stearic acids >>> Arachidic, behenic and lignoceric acids, regardless of

drying regime (Table 3.2). However, the contents of all the fatty acids were similar among the oils of the seeds that were subjected to the four drying regimes.

Table 3.2: Fatty acid contents of *Azadirachta indica* oils from seeds that were subjected to four drying regimes

Fatty acid (%)	Drying regime[†]				
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ [‡]
Palmitic acid	16.00 ± 0.02[c]	15.86 ± 0.04[c]	16.84 ± 0.75[b]	16.41 ± 0.02[b]	1.37 ns
Linoleic acid	16.66 ± 0.03[b]	16.75 ± 0.07[b]	12.21 ± 0.07[b]	16.38 ± 0.08[b]	1.08 ns
Oleic acid	50.03 ± 0.06[a]	51.55 ± 0.25[a]	53.67 ± 0.49[a]	51.82 ± 0.11[a]	1.42 ns
Stearic acid	15.45 ± 0.09[d]	14.48 ± 0.16[d]	15.32 ± 0.66[b]	14.05 ± 0.09[c]	1.07 ns
Arachidic acid	1.53 ± 0.04[e]	1.11± 0.07[e]	1.44 ± 0.07[c]	1.37 ± 0.01[d]	0.84 ns
Behenic acid	0.22 ± 0.07[f]	0.14 ± 0.08[f]	0.30 ± 0.01[c]	0.13 ± 0.08[e]	1.03 ns
Lignoceric acid	0.06± 0.06[f]	0.11± 0.07[f]	0.13 ± 0.08[c]	0.06 ± 0.06[e]	0.33 ns
$F_{(6, 21)}$ [‡]	90544.2***	9522.00***	104.23***	60632.9***	

[†] Means (± SE) in the same column followed by the same letter do not differ significantly at P = 0.05 (Tukey's test).
[‡] ns P > 0.05; *** P < 0.001

3.3 Toxicity of *Azadirachta indica* seed oil and powder against adult *Callosobruchus maculatus* and *Sitophilus zeamais* as influenced by drying regime

3.3.1 Adult mortality caused by *Azadirachta indica* seed oils

All the *A. indica* seed oils generally caused significant mortality to adult *C. maculatus* and *S. zeamais* (Figures 3.1 and 3.2) compared to the control. Mortality increased with ascending dose levels and time, irrespective of drying regime and insect species, but the rate of increase in mortality with days after exposure was lower for *C. maculatus* (Figure 3.1) compared to *S. zeamais* (Figure 3.2). Overall, no significant difference was observed among the oils derived from seeds that were subjected to the four drying regimes, regarding the mortality they caused to *S. zeamais* and *C. maculatus.* Nonetheless, the sun-drying of seeds and kernels led to a higher mortality of *C. maculatus,* three (5 and 6 ml/kg) and six (2 and 5 ml/kg) days post exposure. The oil from the sun-dried kernels of *A. indica* caused greater mortality to *S.*

zeamais than that from the shade-dried kernel only seven days after treatment for the 4 ml/kg dose level. The highest tested dose (6 ml/kg) of *A. indica* oil achieved complete mortality of *C. maculatus* 3 days post-exposure for all the drying regimes, except the shade-dried kernels which caused a maximum mortality of 98.69%, six days after exposure. Oils from the sun-dried kernels and seeds caused total mortality to *S. zeamais* seven days after exposure with the respective doses of 5 and 6 ml/kg. For the shade dried kernels and seeds, the oil respectively caused a maximum mortality of 98.75% (6 ml/kg) and 100% (5 ml/kg) to the weevil, 14 days after exposure.

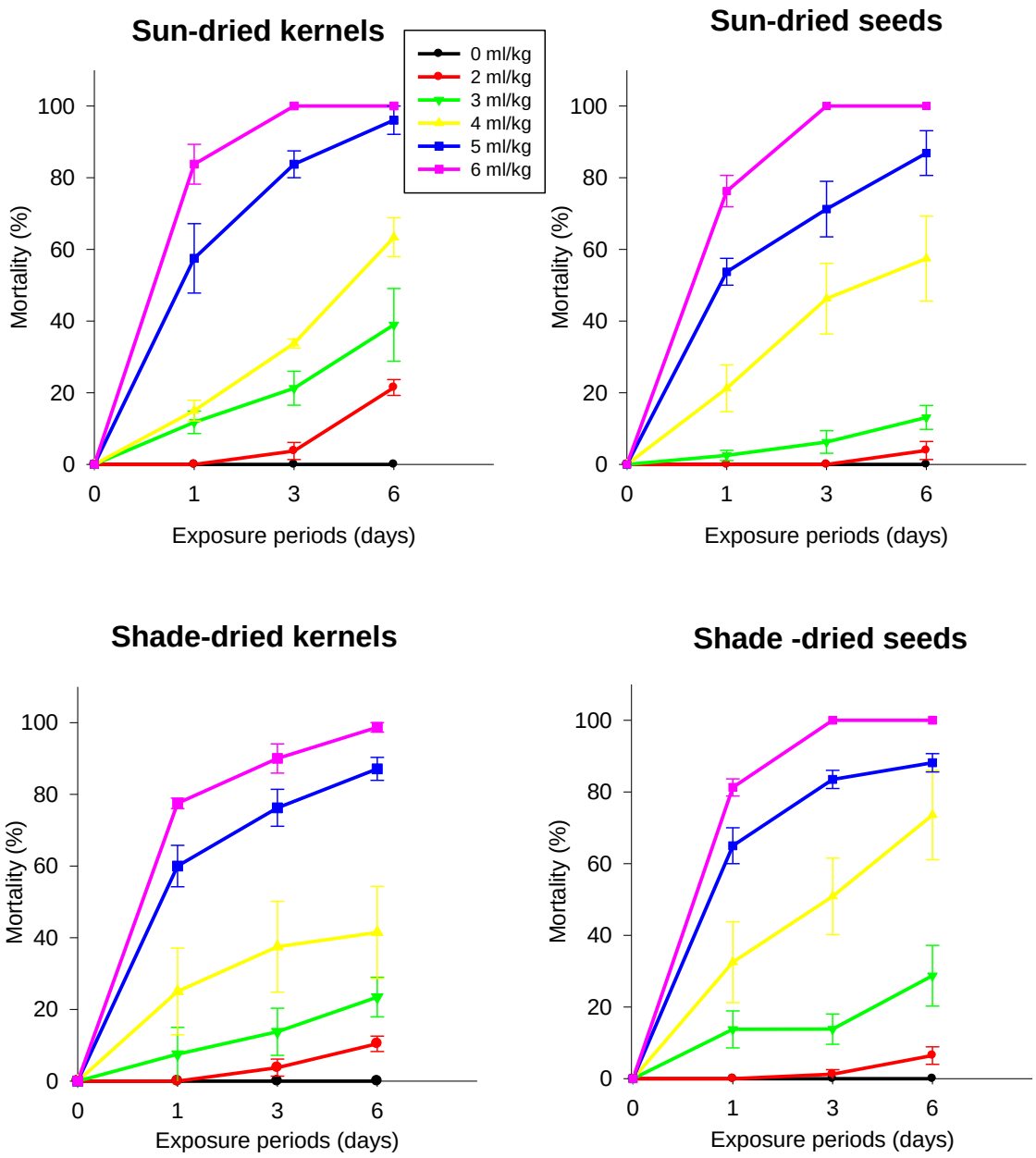


Figure 3.1: Corrected cumulative mortality (mean ± SE) of adult *Callosobruchus maculatus* exposed in grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to four drying regimes

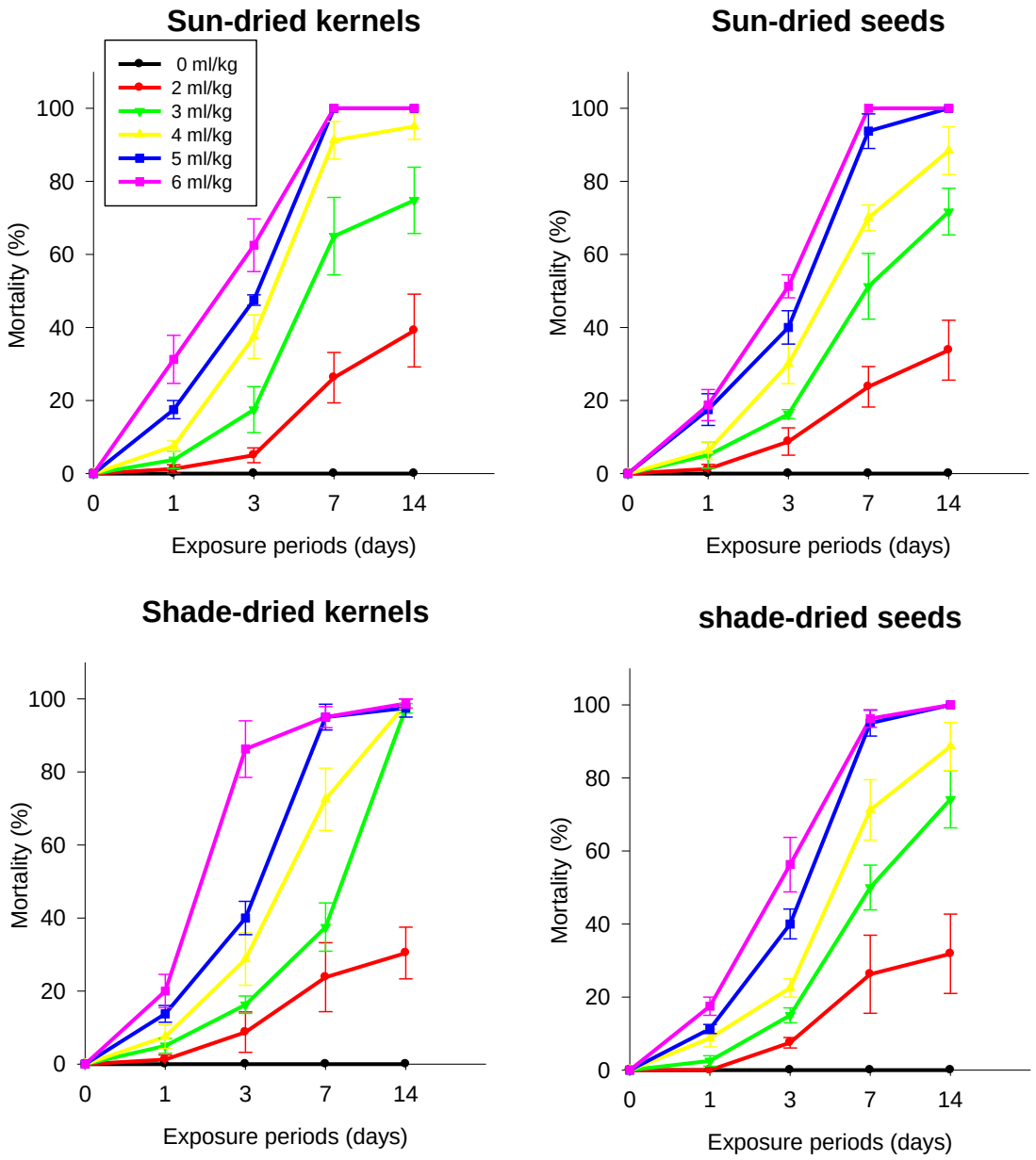


Figure 3.2: Corrected cumulative mortality (means ± SE) of adult *Sitophilus zeamais* exposed in grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to four drying regimes

3.3.2 Dosage-mortality response relationship of the neem seed oils

The results of the toxicity of *A. indica* oils from seeds subjected to four drying regimes on *C. maculatus* and *S. zeamais* are given in Table 3.3. Regardless of drying regime, *A. indica* oil was toxic to *C. maculatus* and *S. zeamais*. LC_{50} and LC_{95} values for the different neem seed oils were similar for the 3-d and 6-d time-points with *C. maculatus* and the 7-d and 14-d time-points with *S. zeamais*.

Table 3.3: Toxicity of *Azadirachta indica* oils extracted from seeds that were subjected to four drying regimes on adult *Callosobruchus maculatus* and *Sitophilus zeamais*

Insect/drying regime	Slope ± S.E	R^2	LC_{50} (95% FL)[a] ml/kg	LC_{95} (95% FL)[a] ml/kg	χ^2 [b]
C. maculatus					
			3 days		
Shade-dried kernels	7.12 ± 1.00	0.92	4.13 (3.57 - 4.76)	7.03 (5.77 - 11.15)	9.81*
Sun-dried kernels	8.03 ± 1.96	0.95	3.89 (2.66 - 5.31)	6.25 (4.81 - 32.27)	31.85***
Shade-dried seeds	9.73 ± 1.25	0.95	3.88 (3.45 - 4.29)	5.73 (5.01 - 7.54)	8.45*
Sun-dried seeds	10.92 ±1.65	0.92	4.16 (3.67 - 4.64)	5.90 (5.15 - 8.18)	10.66*
			6 days		
Shade-dried kernels	6.63 ± 1.03	0.91	3.66 (2.31 - 5.24)	6.48 (4.76 - 12.64)	33.07***
Sun-dried kernels	6.00 ± 1.35	0.96	3.06 (1.88 - 4.02)	5.75 (4.27 - 23.27)	25.47***
Shade-dried seeds	7.79 ± 0.86	0.97	3.37 (2.97 - 3.76)	5.48 (4.75 - 7.13)	7.00[ns]
Sun-dried seeds	9.00 ± 1.72	0.94	3.74 (2.96 – 4.49)	5.70 (4.68 - 10.88)	20.24***
S. zeamais			**7 days**		
Shade-dried kernels	5.44 ± 0.93	0.94	3.00 (2.19 - 3.66)	6.01 (4.65 - 12.64)	14.00***
Sun-dried kernels	7.19 ± 0.60	0.91	2.53 (2.33 - 2.66)	4.28 (3.98 - 4.90)	4.09 [ns]
Shade-dried seeds	5.15 ± 0.70	0.97	2.83 (2.24 - 3.31)	5.90 (4.75 - 9.54)	2.66*
Sun-dried seeds	5.63 ± 0.89	0.99	2.86 (2.17 - 3.41)	5.61 (4.45 - 10.13)	11.52*
			14 days		
Shade-dried kernels	5.89 ± 0.79	0.94	2.61 (2.08 - 3.02)	4.96 (4.10 - 7.40)	7.93*
Sun-dried kernels	6.66 ± 0.63	0.89	2.25 (2.09 - 2.40)	3.99 (3.68 - 4.43)	3.00[ns]
Shade-dried seeds	6.52 ± 0.57	0.90	2.39 (2.23 - 2.54)	4.27 (3.95 - 4.74)	4.92 [ns]
Sun-dried seeds	6.31 ± 0.56	0.92	2.39 (2.22 - 2.53)	4.34 (4.01 - 4.83)	5.15[ns]

[a] FL = Fiducial limits;
[b] ns P > 0.05; *P < 0.05; ***P < 0.001

Comparison of the slopes among the oils from the seeds subjected to the four drying regimes showed no significant differences judging from the standard error values. In general, the slopes were positive. The values of the coefficient of determination R^2 were all significant with R^2 values ranging from 0.89 to 0.99. The values of χ^2 were generally significant for all the oils for *C. maculatus* (days three and six) and *S. zeamais* (day seven).

3.3.3 Adult mortality caused by *A. indica* seed powders

Adult mortality of the insects also increased as the time exposure increased from three days to six days for *C. maculatus* and from one day to 14 days for *S. zeamais*, regardless of drying

regime. *A. indica* seed powder was less effective against *C. maculatus* (Figure 3.3) compared to *S. zeamais* (Figure 3.4). Overall, there was little influence of the drying regime on the mortality of the two insect species caused by the powders. The shade and sun-drying of kernels led to a higher mortality of *C. maculatus,* three (30 and 40 g/kg) and 6 (20 and 30 g/kg) days post exposure. The powder from the shade-dried seeds of *A. indica* caused lower mortality to *S. zeamais* than that from the shade/sun-dried kernels and sun-dried seeds 14 days after treatment for the 5 and 20 g/kg dose levels. No *C. maculatus* mortality was recorded within one day post-exposure for all the drying regimes and doses. Maximum mortality of 34.28%, 30.42%, 23.75% and 22.76% of *C. maculatus* were achieved six days post exposure respectively for sun-dried kernels, shade-dried kernels, shade-dried seeds and sun-dried seeds at the highest tested dose (40 g/kg) of *A. indica* powder. Powders from the sun-dried kernels and seeds caused total mortality to *S. zeamais* 14 days after exposure with the dose of 30 g/kg. For the shade-dried kernels and seeds, the powder respectively caused a maximum mortality of 98.69% and 100% (40 g/kg at 14 days exposure) to the weevils.

3.3.4 Dosage-mortality response relationship of *Azadirachta indica* seed powders

The results of the evaluation of toxicity of powders obtained from the four drying regime of *A.indica* seeds are shown in Table 3.4. All the powders proved to be toxic to adult *C. maculatus* and *S. zeamais* although their bio-efficacy on cowpea weevil was lower compared to that on the maize weevil. The toxic effects of the powder for each of the insects did not differ between times post-exposure, with respect to the LC values. The slopes were similar among the drying regimes for each insect and time post-exposure. In general, the coefficients of determination (R^2) of the powders were ≥ 0.90 for *S. zeamais,* but were between 0.63 and 0.96 for *C. maculatus*. The values of chi-square ($\chi 2$) were not significant for the powders with *C. maculatus* and for shade-dried kernels with *S. zeamais*.

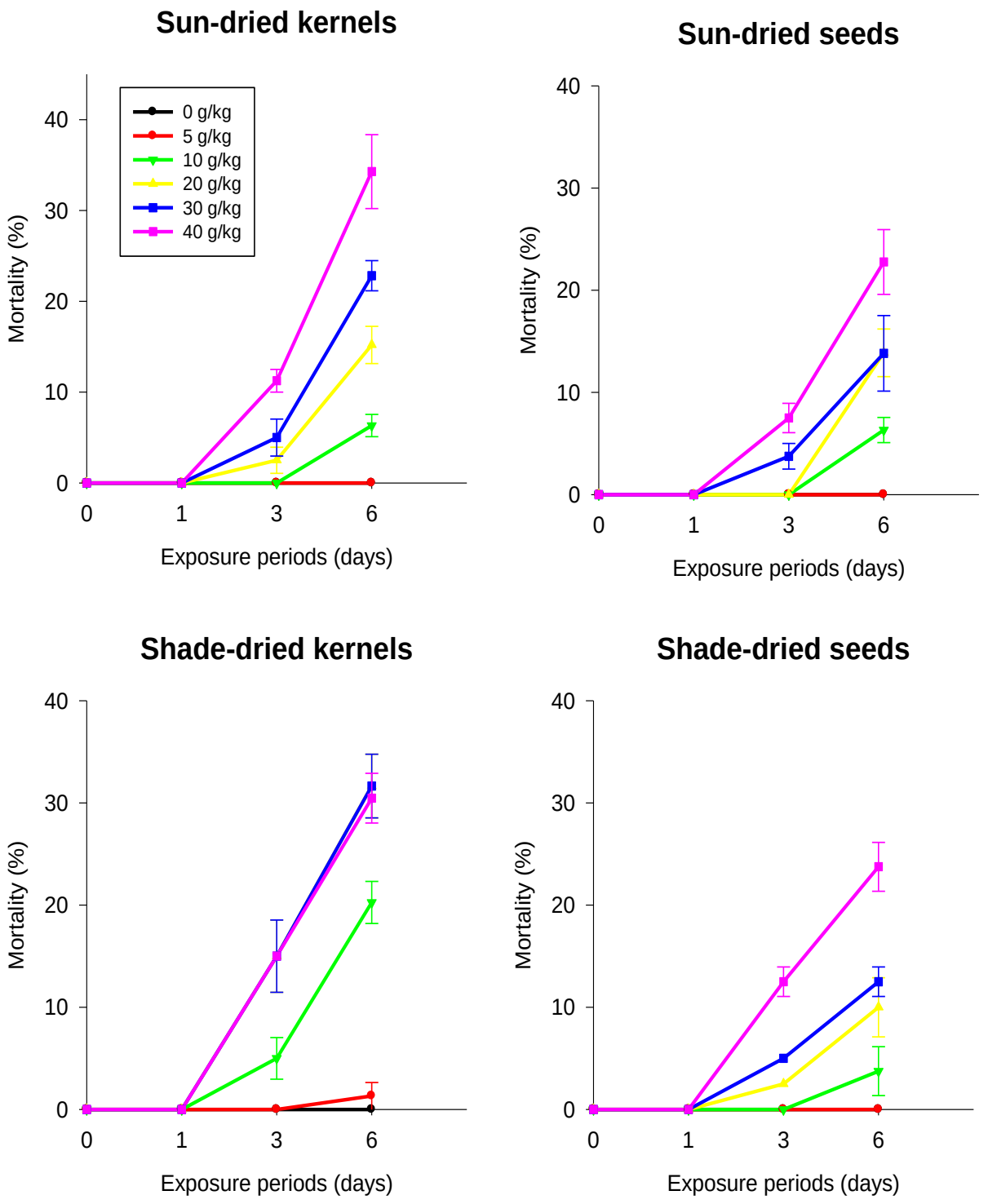


Figure 3.3: Corrected cumulative mortality (mean ± SE) of adult *Callosobruchus maculatus* exposed in grains treated with *Azadirachta indica* seed powders obtained from seeds that were subjected to four drying regimes

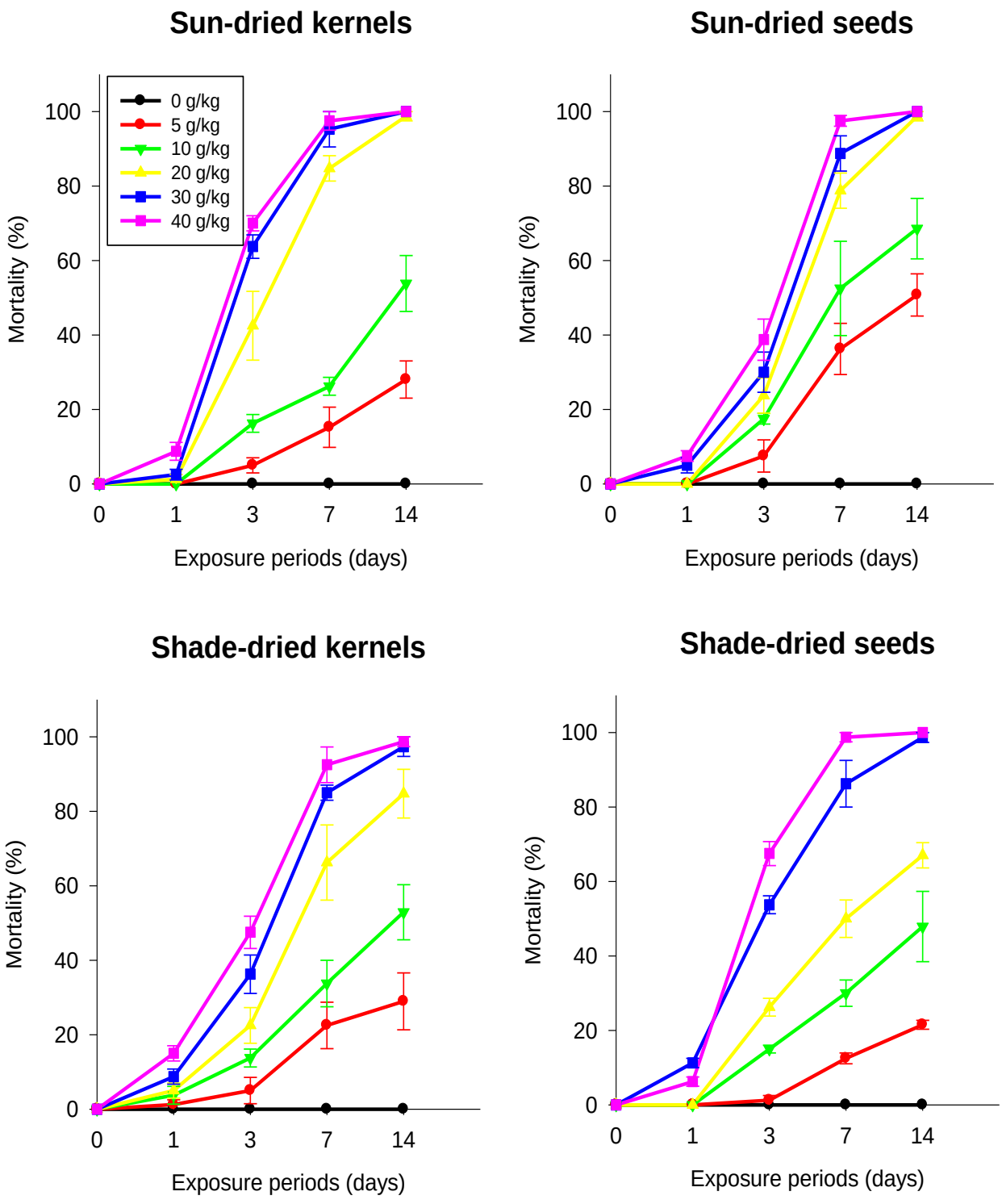


Figure 3.4: Corrected cumulative mortality (mean ± SE) of adult *Sitophilus zeamais* exposed in grains treated with *Azadirachta indica* seed powders obtained from seeds that were subjected to four drying regimes

Table 3.4: Toxicity of *Azadirachta indica* powders obtained from seeds that were subjected to four drying regimes on adult *Callosobruchus maculatus* and *Sitophilus zeamais*

Insect/drying regime	Slope ± S.E	R^2	LC_{50} (95% FL)[a] g/kg	LC_{95} (95% FL)[a] g/kg	χ^{2} [b]
C. maculatus					
			3 days[£]		
Shade-dried kernels	2.66 ± 0.60	0.84	86.27 (60.49 - 207.57)	351.27 (164.46 - 2630)	3.19 [ns]
Sun-dried kernels	2.88 ± 0.88	0.74	105.94 (65.90 -651.15)	393. 26 (151.56 -1672)	0.50 [ns]
Shade-dried seeds	3.07 ± 0.90	0.71	96.07 (62.86 - 436.79)	329.09 (138.93 - 7848)	0.60 [ns]
Sun-dried seeds	4.36 ± 1.68	0.63	83. 27 (56.37 - 1249)	198.53 (93.23 - 43860)	0.88 [ns]
			6 days[£]		
Shade-dried kernels	1.79 ± 0.27	0.96	64.17 (48.38 - 103.78)	529.08 (254.14 - 2010)	2.70[ns]
Sun-dried kernels	2.12 ± 0.32	0.94	62.79 (48.70 - 96.68)	372.87 (197.93 - 1179)	2.00 [ns]
Shade-dried seeds	1.94 ± 0.36	0.87	91.02 (66.13 - 230.68)	696.08 (278.78 - 5119)	2.11[ns]
Sun-dried seeds	1.58 ± 0.31	0.94	118.98 (72.89 -343.20)	1302 (418.68 - 16606)	5.32[ns]
S. zeamais			**7 days**		
Shade-dried kernels	2.45 ± 0.30	0.97	12.23 (8.56 - 16.16)	57.04 (36.69- 141.98)	6.41[ns]
Sun-dried kernels	3.66 ± 0.55	0.93	11.57 (7.58 - 16.12)	32.50 (21.87 - 82.36)	14.02**
Shade-dried seeds	3.08 ± 0.64	0.94	14.46 (7.23 - 24.38)	49.38 (27.82 - 516.10)	25.82***
Sun-dried seeds	2.25 ± 0.21	0.99	8.14 (6.80 - 9.41)	43.89 (35.13 - 59.60)	4.81[ns]
			14 days		
Shade-dried kernels	3.00 ± 0.24	0.97	8.43 (7.40 -9.40)	29.67 (25.18 - 34.70)	4.25 [ns]
Sun-dried kernels	4.13 ± 0.77	0.91	7.80 (3.79 - 17.11)	19.52 (13.10 - 69.11)	14.36**
Shade-dried seeds	3.10 ± 0.68	0.97	10.18 (7.23 - 24.38)	37.37 (20.78 – 567.46)	24.92***
Sun-dried seeds	3.24 ± 0.68	0.91	5.56 (1.93 - 8.41)	17.86 (11.58- 90.78)	12.02**

[a] FL = Fiducial limits;
[b] ns: P > 0.05; **P < 0.001; ***P < 0.0001.
[£] LC values were estimated by extrapolation

3.3.5 Effect of *Azadirachta indica* oils on F_1 progeny production

In all evaluated treatments, the application of *A. indica* seed oils completely suppressed F_1 progeny emergence in *C. maculatus*, regardless of the drying regime to which the seeds were subjected (Table 3.5). Except for maize treated with the lowest dose 2 ml/kg with Sun- and shade-dried kernels and sun-dried seeds, all dose levels of the oils from the seeds dried under the four regimes caused 100% reduction in *S. zeamais* F_1 progeny emergence (Table 3.6). The oils from the seeds tended to reduce F_1 progeny emergence in the weevil than those from the kernels, when maize seeds were treated with the lowest dose 2 ml/kg.

Table 3.5: Progeny production of *Callosobruchus maculatus* exposed in grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to four drying regimes

Dose (ml/kg)	Drying regime				$F_{(3, 12)}$ ‡
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	
	Number (mean ± SE) of F_1 adult progeny †				
0	436.50 ± 22.91[a]	432.25 ± 11.84[a]	460.75 ± 24.08[a]	473.75 ± 20.17[a]	0.94 ns
2	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
3	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
4	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
5	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
6	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
$F_{(5, 18)}$ ‡	362.98***	1332.39***	366.19***	551.81***	
	Percentage (mean ± SE) reduction in adult emergence relative to control †				
0	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
2	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
3	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
4	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
5	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
6	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
$F_{(5, 18)}$ ‡	–	–	–	–	

† Means in the same column followed by the same letter do not differ significantly (Tukey's test; $P < 0.05$)

‡ ns $P > 0.05$; *** $P < 0.001$;

– *F* value estimation is not possible due to equal variance or

3.3.6 Effect of *Azadirachta indica* powders on F_1 progeny production

All the dosages of the *A. indica* seed powders from the four drying regimes completely suppressed F_1 progeny emergence in *C. maculatus* on treated cowpea (Table 3.7). No progeny of *S. zeamais* emerged in maize grains treated with the powders, when the dosage was ≥ 3 ml/kg (Table 3.8).

Table 3.6: Progeny production of *Sitophilus zeamais* in grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to four drying regimes

Dose (ml/kg)	Drying regime				
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
	Number (mean ± SE) of F_1 adult progeny †				
0	48.50 ± 7.35^{a}	46.50 ± 8.87^{a}	44.50 ± 3.69^{a}	42.50 ± 2.33^{a}	0.14 ns
2	7.25 ± 0.48^{bA}	5.25 ± 1.93^{bA}	0.00 ± 0.00^{bB}	3.25 ± 2.29^{bAB}	6.32 **
3	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
4	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
5	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
6	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
$F_{(5, 18)}$ ‡	41.69***	54.28***	61.09***	99.01***	
	Percentage (mean ± SE) reduction in adult emergence relative to control †				
0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
2	84.28 ± 2.15^{bB}	88.71 ± 4.10^{bAB}	100.00 ± 0.00^{bA}	92.75 ± 4.94^{bA}	7.73 **
3	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	–
4	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	–
5	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	–
6	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	–
$F_{(5, 18)}$ ‡	2087.73***	2143.36***	–***	230.11***	

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test at P = 0.05).

‡ ns P > 0.05, ** P < 0.01, *** P < 0.001;

– F value estimation is not possible due to equal variance

Table 3.7: Progeny production of *Callosobruchus maculatus* in grains treated with *Azadirachta indica* seed powders obtained from seeds that were subjected to four drying regimes

Dose (g/kg)	Drying regime				$F_{(3, 12)}$ ‡
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	
	Number (mean ± SE) of F_1 adult progeny †				
0	436.50 ± 22.91[a]	432.25 ± 11.84[a]	460.75 ± 24.08[a]	473.75 ± 20.17[a]	0.95 ns
5	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
10	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
20	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
30	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
40	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
$F_{(5, 18)}$ ‡	362.98***	1332.39***	366.19***	551.81***	
	Percentage (mean ± SE) reduction in adult emergence relative to control †				
0	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
5	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
10	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
20	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
30	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
40	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	100.00 ± 0.00[a]	–
$F_{(5, 18)}$ ‡	–	–	–	–	

† Means in the same column followed by the same letter do not differ significantly (Tukey's test; $P < 0.05$)

‡ ns $P > 0.05$; *** $P < 0.001$;

– F value estimation is not possible due to equal variance

Table 3.8: Progeny production of *Sitophilus zeamais* in grains treated with *Azadirachta indica* seed powders obtained from seeds that were subjected to four drying regimes

Dose (g/kg)	Drying regime				
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
	Number (mean ± SE) of F_1 adult progeny †				
0	39.50 ± 2.66[a]	46.50 ± 8.87[a]	42.25 ± 0.95[a]	42.50 ± 1.94[a]	0.50 ns
5	0.75 ± 0.75[b]	0.00 ± 0.00[b]	0.75 ± 0.48[b]	0.50 ± 0.50[b]	0.48 ns
10	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
20	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
30	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
40	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	–
$F_{(5, 18)}$ ‡	238.85***	891.80***	655.65***	488.88***	
	Percentage (mean ± SE) reduction in adult emergence relative to control †				
0	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	
5	98.37 ± 1.63[a]	100 ± 0.00[a]	98.17 ± 1.17[a]	98.81 ± 1.19[a]	0.60 ns
10	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100.00 ± 0.00[a]	–
20	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100.00 ± 0.00[a]	–
30	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100.00 ± 0.00[a]	–
40	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100.00 ± 0.00[a]	–
$F_{(5, 18)}$ ‡	583.43***	–	759.32***	805.66***	

† Means in the same column followed by the same letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$, *** $P < 0.001$;

– F value estimation not possible due to equal variance

3.3.7 Effect of *A. indica* seed oils on grain damage and weight loss

The infested cowpea (*C. maculatus*) and maize (*S. zeamais*) grains that were previously treated with *A. indica* oils extracted from seeds that were subjected to the four drying regimes had no damaged grains and recorded no weight loss, ten weeks after infestation, when the dose level was ≥ 3 ml/kg (Tables 3.9 and 3.10). When treated with 2 ml/kg of the *A. indica* seed oils, both cowpea and maize grains recorded very little damage and weight losses compared to the control, although the value for these parameters were higher for maize (2.25 – 4.50% damage and 0.33 – 0.75 % weight loss) than cowpea (0.00 – 0.75% grain damage and 0.00 – 0.06% weight loss). For this dosage level, the damage caused by *C. maculatus* to cowpea seeds and *S. zeamais* to maize seeds, as well as the resulting weight losses, were similar across the four drying regimes.

Table 3.9: Grain damage and weight loss of cowpea caused by *Callosobruchus maculatus* in grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to four drying regimes and then stored for 10 weeks

Dose (ml/kg)	Drying regime				$F_{(3, 12)}$ ‡
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	
	Mean (± SE) grain damage (%) †				
0	97.25 ± 0.75^{a}	97.25 ± 0.48^{a}	97.00 ± 0.41^{a}	97.25 ± 0.48^{a}	0.35 ^{ns}
2	0.25 ± 0.25^{b}	0.00 ± 0.00^{b}	0.75 ± 0.00^{b}	0.25 ± 0.25^{b}	0.41 ^{ns}
3	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
4	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
5	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
6	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
$F_{(5, 18)}$ ‡	1594.95***	466.90***	946.09***	2153.73***	
	Mean (± SE) weight loss (%) †				
0	28.52 ± 1.19^{aB}	39.68 ± 1.84^{aA}	36.29 ± 2.59^{aAB}	42.86 ± 2.80^{aA}	8.07 **
2	0.04 ± 0.04^{b}	0.00 ± 0.00^{b}	0.06 ± 0.06^{b}	0.01 ± 0.01^{b}	0.45 ^{ns}
3	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
4	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
5	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
6	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
$F_{(5, 18)}$ ‡	570.00***	95.97***	196.36***	234.48***	

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$;

– *F* value estimation not possible due to equal variance

Table 3.10: Grain damage and weight loss of maize caused by *Sitophilus zeamais* in grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to four drying regimes and then stored for 10 weeks

Doses(ml/kg)	Drying regime †				
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
	Mean (± SE) grain damage (%) †				
0	50.00 ± 1.73^{aA}	45.50 ± 2.84^{aA}	39.75 ± 3.90^{aAB}	37.75 ± 1.93^{aB}	4.13 *
2	3.25 ± 1.25^{b}	4.50 ± 0.29^{b}	2.25 ± 1.03^{b}	2.25 ± 1.44^{b}	1.30 ns
3	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	–
4	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	–
5	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	–
6	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	–
$F_{(5, 18)}$ ‡	458.91***	245.95***	95.10***	97.35***	
	Mean (± SE) weight loss (%) †				
0	17.12± 2.75^{aA}	10.05 ± 0.75^{aB}	12.09 ± 1.50^{aAB}	10.77 ± 1.10^{aB}	3.58 *
2	0.56 ± 0.16^{b}	0.75 ± 0.21^{b}	0.61 ± 0.29^{b}	0.33 ± 0.21^{b}	0.93 ns
3	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
4	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
5	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
6	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
$F_{(5, 18)}$ ‡	38.21***	163.20***	61.78***	91.62***	

† Means in the same column followed by the same lower case letter or in the same line followed by the same uppercase letter do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$, * $P < 0.05$, *** $P < 0.001$;

– F value estimation not possible due to equal variance

3.3.8 Effect of *Azadirachta indica* seed powders on grains damage and weight loss

The damage and weight loss of cowpea and maize grains that were treated with the four drying regimes of *A. indica* seed powder, infested and stored for 10 weeks were statistically different from those of the control ($P = 0.0001$) (Tables 3.11 and 3.12). Generally, the damage caused by *C. maculatus* to cowpea seeds, as well as the resulting weight losses were lower across the four drying regimes compared to that caused on maize by *S. zeamais*. Apart from the cowpea grains treated with the lowest dosage (5 g/kg) of *A. indica* seed powders, which suffered little damage and weight loss caused by *C. maculatus*, the treated grains recorded no grain damage and weight loss (Table 6). Maize grains recorded damage (0.25 – 10.50%) and weight losses (0.02 - 2.78%) when treated with the different dosages of the *A. indica* seed powders depending on the drying regime. Also, maize treated with the powders from the *A.*

indica from the different drying regimes suffered insignificant or no damage and weight loss when the dose level was ≥ 30 g/kg (Table 3.12). Nonetheless, the sun-drying of seeds led to a higher damage and weight loss of cowpea and maize at the lowest tested dosage of 5 g/kg.

Table 3.11: Grain damage and weight loss of cowpea caused by *Callosobruchus maculatus* in grains treated with *Azadirachta indica* seed powder obtained from seeds that were subjected to four drying regimes and then stored for 10 weeks

Doses (g/kg)	Drying regime				$F_{(3, 12)}$ ‡
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	
	Mean (± SE) grain damage (%) †				
0	98.25 ± 0.25^{a}	97.75 ± 0.63^{a}	98.50 ± 0.29^{a}	98.00 ± 0.41^{a}	0.50 ns
5	0.50 ± 0.50^{bB}	0.00 ± 0.00^{bB}	5.75 ± 2.25^{bAB}	9.00 ± 2.48^{bA}	6.52**
10	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
20	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
30	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
40	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
$F_{(5, 18)}$ ‡	1501.19***	1858***	858.80***	301.51***	
	Mean (± SE) weight loss (%) †				
0	48.05 ± 1.54^{aAB}	38.42 ± 4.61^{aB}	51.52 ± 1.57^{aAB}	52.57 ± 3.66^{aA}	4.20*
5	0.05 ± 0.05^{bB}	0.00 ± 0.00^{bB}	1.24 ± 0.42^{bA}	0.85 ± 0.28^{bAB}	5.79 **
10	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
20	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
30	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
40	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	–
$F_{(5, 18)}$ ‡	1580.47***	201.31***	1053.75***	353.83***	

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$; – F value estimation not possible due to equal variance

Table 3.12: Grain damage and weight loss of maize caused by *Sitophilus zeamais* in grains treated with *Azadirachta indica* seed powders obtained from seeds that were subjected to different drying regimes and then stored for 10 weeks

Doses (g/kg)	Drying regime †				
	Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
	Mean (± SE) grain damage (%) †				
0	49.75 ± 1.03^{a}	52.00 ± 3.03^{a}	45.25 ± 3.28^{a}	46.00 ± 2.20^{a}	1.55 ns
5	2.75 ± 1.03^{bB}	3.25 ± 1.60^{bcB}	3.25 ± 0.75^{bB}	10.50 ± 1.26^{bA}	9.57**
10	2.50 ± 0.29^{c}	4.25 ± 0.95^{b}	2.25 ± 0.48^{b}	4.75 ± 1.70^{bc}	1.52 ns
20	0.75 ± 0.48^{c}	2.00 ± 0.71^{bcd}	1.50 ± 1.50^{b}	2.00 ± 0.91^{cd}	0.37 ns
30	0.50 ± 0.29^{c}	0.75 ± 0.48^{cd}	1.75 ± 0.85^{b}	0.00 ± 0.00^{d}	2.07 ns
40	0.75 ± 0.48^{c}	0.00 ± 0.00^{d}	0.25 ± 0.25^{b}	0.00 ± 0.00^{d}	1.71 ns
$F_{(5, 18)}$ ‡	102.03***	83.87***	51.82***	103.66***	
	Mean (± SE) weight loss (%) †				
0	12.40 ± 0.30^{a}	11.41 ± 1.41^{a}	12.33 ± 1.30^{a}	8.93 ± 2.95^{a}	0.84 ns
5	0.53 ± 0.18^{bB}	0.60 ± 0.17^{bB}	0.72 ± 0.24^{bB}	2.78 ± 0.23^{bA}	27.70***
10	0.32 ± 0.29^{b}	0.95 ± 0.28^{b}	0.38 ± 0.07^{b}	1.56 ± 0.62^{bc}	2.82 ns
20	0.23 ± 0.16^{b}	0.32 ± 0.18^{bc}	0.08 ± 0.08^{b}	0.35 ± 0.17^{bc}	0.62 ns
30	0.21 ± 0.12^{b}	0.02 ± 0.02^{d}	0.64 ± 0.32^{b}	0.00 ± 0.00^{c}	2.92 ns
40	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.08 ± 0.08^{b}	0.00 ± 0.00^{c}	1.58 ns
$F_{(5, 18)}$ ‡	66.25***	71.20***	59.14***	11.44***	

† Means in the same column followed by the same lower case letter or in the same line followed by the same uppercase letter do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$, * $P < 0.05$, *** $P < 0.001$

3.3.9 Persistence of *Azadirachta indica* seed oil and powder in cowpea and maize grains

Data on the effectiveness of *A. indica* oil from sun-dried kernels showed that, the insecticidal efficacy on treated grains decreased significantly with storage interval (Figure 3.5). At the highest dose (6 ml/kg) when the storage interval of the treated grains was 15 days, adult mortality of *C. maculatus* decreased from 100% to 38.29%. No adult mortality was observed when the storage interval of the treated grains was 180 days. The mortality caused to *S. zeamais* 60 days after treatment of maize did not differ from the observed mortality at 0 day ($P > 0.05$), but drastically decreased after 180 days of storage of the treated grains.

Data on the effectiveness of *A. indica* powder from sun-dried kernels showed that, the insecticidal efficacy on treated grains decreased significantly with storage interval (Table 3.13). The mortality caused to *C. maculatus* ≥ 30 days after treatment differed from those

registered at 0 day ($P < 0.05$) when the dose level was ≥ 20 g/kg. In *S. zeamais*, except the doses 30 and 40 g/kg, the efficacy of sun-dried neem seed powder persisted at 60 days storage interval of treated maize and then greatly decreased by the 180 days storage interval. At the highest dose (40 g/kg), mortality of *C. maculatus* on cowpea decreased from 17.50% (0 day) to 0.00% (60 days storage interval). Maximum mortality of 26.25% (40 g/kg) in *S. zeamais* was recorded at 180 days storage interval of treated maize grains.

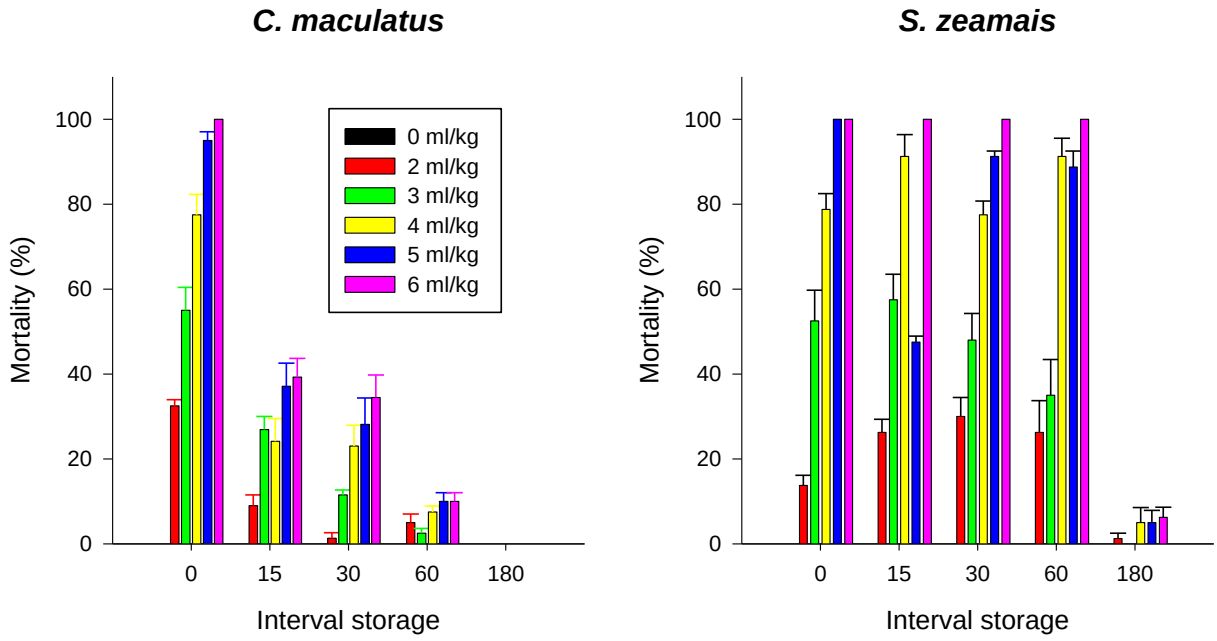


Figure 3.5: Residual toxicity *Azadirachta indica* seed oil obtained from sun-dried kernels after different storage intervals in *Callosobruchus maculatus* and *Sitophilus zeamais* on treated cowpea and maize grains

Table: 3.13: Residual toxicity *Azadirachta indica* seed powder obtained from sun-dried kernels after different storage intervals in *Callosobruchus maculatus* and *Sitophilus zeamais* on treated cowpea and maize grains

Insects / doses (g/kg)	Storage intervals (days)[†]					
	0	15	30	60	180	F (5, 15)[‡]
C. maculatus						
0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00	
5	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00	$-^{ns}$
10	3.75 ± 3.75^{bc}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00	1^{ns}
20	5.00 ± 3.02^{abcA}	2.50 ± 1.44^{bcAB}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{B}	0.00 ± 0.00	4.40^{*}
30	12.50 ± 3.23^{abA}	3.75 ± 1.25^{abAB}	1.25 ± 1.25^{abB}	0.00 ± 0.00^{B}	0.00 ± 0.00	12.40^{***}
40	17.50 ± 3.23^{aA}	8.75 ± 1.25^{aAB}	3.75 ± 1.25^{aB}	0.00 ± 0.00^{C}	0.00 ± 0.00	30.93^{***}
F(5, 18)[‡]	10.20***	11.10***	4.40**	– ns	– ns	
S. zeamais						
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
5	10.00 ± 2.04^{dA}	2.50 ± 1.44^{cdAB}	7.50 ± 2.50^{bAB}	10.00 ± 4.08^{bcAB}	0.00 ± 0.00^{cB}	3.95^{*}
10	$25.00 \pm 4.56_{cAB}$	10.00 ± 2.89^{cAB}	36.25 ± 12.48^{aA}	31.25 ± 10.87^{abAB}	5.00 ± 3.54^{bcB}	3.96^{*}
20	50.00 ± 3.54^{bA}	35.00 ± 8.66^{bA}	45.00 ± 3.54^{aA}	47.50 ± 5.95^{aA}	5.00 ± 2.04^{bc}	15.38^{***}
30	73.75 ± 2.39^{aA}	58.75 ± 3.75^{aAB}	52.50 ± 3.23^{aB}	41.25 ± 6.88^{aB}	18.75 ± 5.15^{abC}	19.21^{***}
40	82.50 ± 4.79^{aA}	62.50 ± 1.44^{aAB}	61.25 ± 4.27^{aAB}	46.25 ± 8.98^{aBC}	26.25 ± 10.68^{aC}	8.99^{***}
F(5, 18)[‡]	106.34***	48.08 ***	25.18***	13.40***	8.39**	

[†] Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).
[‡] ns $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$;
– F value estimation of is not possible due to equal variance

3.3.10 Degradation of Azadirachtin A on cowpea and maize treated with *Azadirachta indica* seed oil after different storage intervals

Data on the degradation on Azadirachtin A contained in *A. indica* oil on treated cowpea and maize showed that the active ingredient known for its efficacy against insect pests decreased with ascending storage intervals ranging from 1 – 180 days, irrespective of the dose level ($P < 0.001$) (Figure 3.6). The data indicated that Azadirachtin A was relatively stable on maize up to the 21 days storage interval with a content of 1.30 mg/kg (0 day) and 1.28 mg/kg (21 days) when treated with 6 ml/kg neem oil, whereas on cowpea by the 14 days storage interval, less than 1 mg/kg of Azadirachtin A was left. At the 180 days storage interval and with the lowest

dose of 2 ml/kg roughly 0.10 mg/kg of Azadirachtin A remained on the treated maize and cowpea grains.

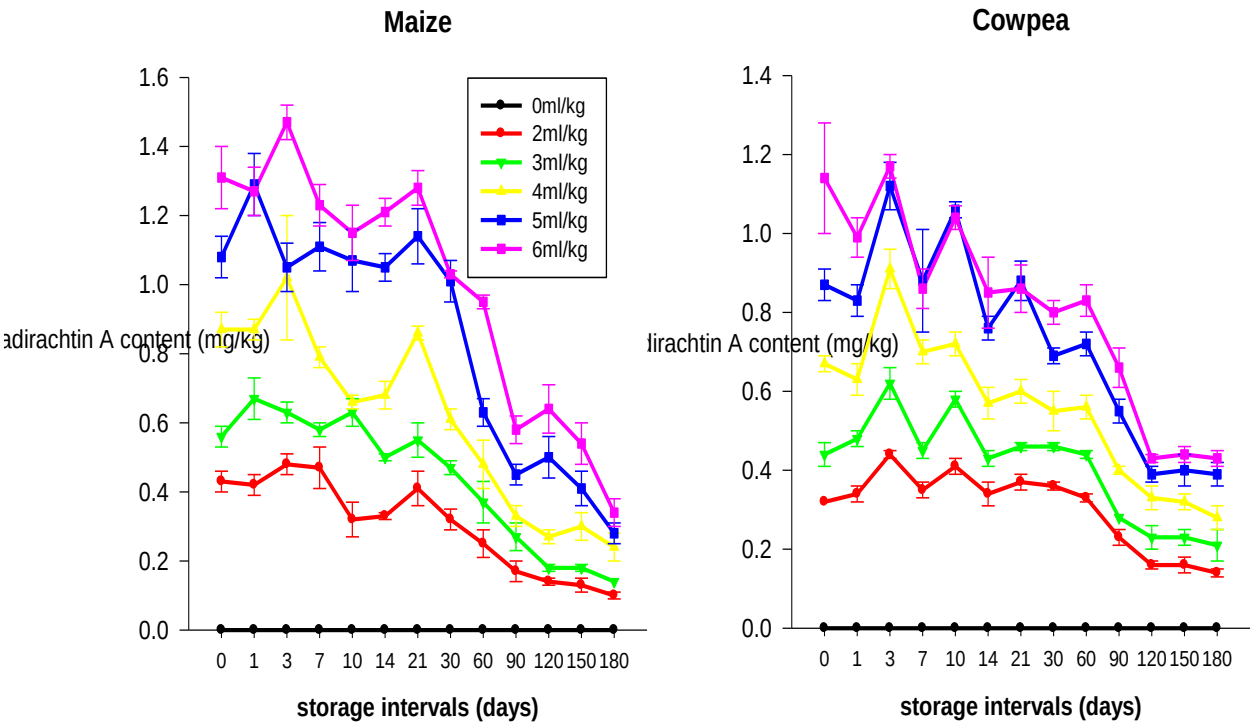


Figure 3.6: Degradation of Azadirachtin A in maize and cowpea treated with *Azadirachta indica* oil after different storage intervals.

3.4 Influence of drying regime and particle size of leaf powders from *Azadirachta indica* and *Plectranthus glandulosus* leaves on adult *Callosobruchus maculatus* and *Sitophilus zeamais*

3.4.1 Chemical constituents of shade- and sun-dried *Plectranthus glandulosus* leaf powder

The trend of the semi-quantitative analysis of the chemical composition of *P. glandulosus* leaf powders showed that a total of the same 50 compounds with variable proportions were found in the sun-dried and shade-dried leaves, respectively (Table 3.14). Thus, the drying method had less effect on the diversity of the volatile compounds of the leaves. However, the overall tendency was lower rates of volatiles in the sun-dried compared to the shade-dried leaves. Eighteen compounds had similar rates (proportions) in the shade- and sun-dried leaf powders.

Twenty four other compounds were higher in proportion in the shade-dried leaves compared to the sun-dried ones, with three (terpinolene, germacrene D and piperitone oxide) of them were particularly abundant in the shade-dried leaves. Only eight compounds were more abundant in the sun-dried than the shade-dried leaves. The compounds found in higher proportion in sun-dried leaves were oxygenated terpenes among which seven of them were oxygenated monoterpenes. All monoterpene hydrocarbons and more than half of sesquiterpene hydrocarbons were found abundantly in the shade-dried leaves.

Table 3.14: Comparison of the chemical constituents of the powders from shade- and sun-dried leaves of *Plectranthus glandulosus* collected at Ngaoundere, Cameroon

Retention time	Compound	shade	sun
Monoterpene hydrocarbons			
7.21	α-Pinene	X	
8.77	Camphene	X	
10.26	β-Pinene	*	*
12.15	3-Carene	X	
12.91	Sabinene	X	
13.53	α-Terpinene	X	
14.40	Limonene	X	
16.16	R-α-Pinene	X	
16.57	γ-Terpinene	*	*
16.91	Ocimene (Z)-β or α	X	
17.68	Cymene	X	
18.31	Terpinolene	X XX	
24.62	bis(1-Methylethylidene)-cyclobutene	X	
Oxygenated monoterpenes			
14.94	1,8-Cineole	X	
23.44	Fenchone	*	*
25.11	Dehydro-para-Cymene		X
26.55	(Z)-Sabinene hydrate	X	
28.42	Camphor		X
29.93	Linalool		X
31.38	(+)-Fenchol		X
32.68	β-Cyclocitral	*	*
36.83	Piperitone oxide	XXX	
37.63	(E)-Piperitol	X	
39.76	Diosphenol		X
41.27	p-Cymene-8-ol		X
41.35	Geranylacetone	*	*
43.73	Chrysanthenone	*	*
44.29	β-Ionone, (E)-	*	*
51.62	Eugenol	*	*
52.22	Thymol		X
58.78	(E)-Carveole	*	*

Table 3.14 Cont'd

Retention time	Compound	Shade	sund
Sesquiterpene hydrocarbons			
27.30	α-Cubebene	*	*
30.69	β-Cubebene	X	
33.25	γ -Elemene	X	
34.42	α-Caryophyllene	*	*
35.28	2-Carene	*	*
35.94	(E)-Germacrene D	XXX	
38.31	Δ-Cadinene	X	
53.65	γ -Gurjunene	*	*
Oxygenated sesquiterpenes			
47.62	Nerolidol, (E) or (Z)	*	*
54.23	Ledol	*	*
61.71	Solavetivone	X	
57.36	Ledene oxide		X
Fatty acids			
54.08	Ethylpalmitate	X	
61.95	Ethyl linoleate	X	
64.30	Ethyl linolenate	X	
68.89	Myristic acid	*	*
82.78	Palmitic acid	X	
Aromatic compounds			
48.75	7-Methoxy-2,2-dimethyl-3-chromene	*	*
61.30	1.2.3-Trimethylindene	*	*

XXX The peak height of the compound was far much higher in this drying regime than the other with an empty space; X The peak height of the compound was higher in this drying regime than the other with an empty space, * Equal peak height of the compound was observed in both drying regimes

3.4.2 Effect of drying regime of leaf powders from *Azadirachta indica* and *Plectranthus glandulosus* on adult mortality of *Callosubruchus maculatus* and *Sitophilus zeamais*

The powders from the shade- and sun-dried leaves of *P. glandulosus* and *A. indica* generally caused significant adult mortality to *S. zeamais* and *C. maculatus* relative to the control, although the mortality caused by *A. indica* for both insect species and *P. glandulosus* for *C. maculatus* were rather low (Tables 3.15 and 3.16).

The *P. glandulosus* powders from the sun-dried leaves caused higher (t = -1.29; P < 0.001) mortality to *S. zeamais* than those from the shade-dried ones (Table 3.16), but the mortality with *C. maculatus* was similar (t = 0.34; P > 0.05) for the two drying regimes (Table 3.15). That the moisture contents of the powder from the sun- (8.7%) and shade-dried (8.9%) leaves were similar. The powders from sun-dried leaves of *A. indica* caused comparable adult mortality in *C. maculatus* (t = 0.36; P > 0.001) and *S. zeamais* (t = - 0.22; P

> 0.001) like those dried in shade. Percentage mortality increased with increasing powder contents and days post-exposure for both insect pests. Within 6 days of exposure and at the powder content of 40 g/kg ca. 50% mortality was recorded in *C. maculatus,* while *S. zeamais* registered 100% mortality within 7 days when the grains were treated with *P. glandulosus* leaf powders. *A. indica* caused less than 25% mortality in both insects as they were exposed to the highest content (40 g/kg) of powders at the maximum exposure time. The two insects showed

Table 3.15: Corrected cumulative mortality of *Callosobruchus maculatus* exposed in grains treated with leaf powders of *Azadirachta indica* and *Plectranthus glandulosus* obtained from sun-dried and shade-dried leaves

Exposure period/doses (g/kg)	*P. glandulosus*/ Mean (± SE) (%)			*A. indica* /Mean (± SE) (%)		
	Sun-dried	Shade-dried	*t* value	Sun-dried	Shade-dried	*t* value
1 day						
0	0.00 ± 0.00	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	
5	0.00 ± 0.00	0.00 ± 0.00	–	0.00 ± 0.00	0.00 ± 0.00	–
10	0.00 ± 0.00	0.00 ± 0.00	–	0.00 ± 0.00	0.00 ± 0.00	–
20	0.00 ± 0.00	0.00 ± 0.00	–	0.00 ± 0.00	0.00 ± 0.00	–
30	0.00 ± 0.00	1.25 ± 1.25	1^{ns}	0.00 ± 0.00	0.00 ± 0.00	–
40	2.50 ± 1.44	1.25 ± 1.25	$- 0.65^{ns}$	0.00 ± 0.00	2.50 ± 1.44	–
$F_{(5, 66)}$	3.00^{ns}	0.80^{ns}			3.00^{ns}	
3 days						
0	0.00 ± 0.00^{c}	0.00 ± 0.00		0.00 ± 0.00	0.00 ± 0.00	
5	0.00 ± 0.00^{c}	0.00 ± 0.00	–	0.00 ± 0.00	0.00 ± 0.00	–
10	0.00 ± 0.00^{c}	0.00 ± 0.00	-1^{ns}	0.00 ± 0.00	1.25 ± 1.25	–
20	1.25 ± 1.25^{c}	0.00 ± 0.00	0^{ns}	0.00 ± 0.00	2.50 ± 5.50	1.00^{ns}
30	6.25 ± 1.15^{b}	0.00 ± 0.00	0.93^{ns}	3.75 ± 1.25	2.50 ± 1.44	$- 0.65^{ns}$
40	20.00 ± 2.04^{a}	10.00 ± 6.12	2.50^{ns}	3.75 ± 2.39	3.75 ± 1.25	3.67^{ns}
$F_{(5, 66)}$	49.08***	3.00 ns		3.88^{ns}	1.53^{ns}	
LC_{50} (g/kg)	59.95^{β}	42.57^{β}		141.08^{β}	1970^{β}	
6 days						
0	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}		0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	
5	18.46 ± 7.83^{b}	10.27 ± 2.27^{c}	0.38^{ns}	5.00 ± 2.04^{ab}	3.88 ± 1.29^{bc}	$- 0.46^{ns}$
10	31.84 ± 2.03^{bc}	24.66 ± 1.43^{bc}	$- 1.76^{ns}$	7.57 ± 1.14^{a}	5.34 ± 2.27^{b}	$- 83^{ns}$
20	36.03 ± 5.42^{bc}	32.82 ± 1.87^{ab}	- 0.19*	8.88 ± 2.42^{a}	7.84 ± 1.41^{ab}	$- 37^{ns}$
30	34.64 ± 5.82^{ab}	32.68 ± 7.75^{ab}	$- 6.63^{ns}$	10.07 ± 3.5^{a}	15.81 ± 0.34^{a}	1.63^{ns}
40	54.11 ± 4.23^{a}	49.34 ± 2.36^{a}	5.69^{***}	14.01 ± 2.63^{a}	15.74 ± 2.04^{a}	1.65^{ns}
$F_{(5, 66)}$	16.96***	41.28***		9.12*	14.71***	
LC_{50} (g/kg)	47.37^{β}	51.29^{β}		4518^{β}	436.17^{β}	

Means ± SE in the same column followed by the same lowercase letter within the same group of treatments do not differ significantly at P = 0.05 (Tukey's test).

^{ns} non-significant; * P<0.05; *** P<0.001;

– *t*-test is impossible due to absence of mortality; $^{\beta}$ LC_{50} values were estimated by extrapolation

Table 3.16: Corrected cumulative mortality of *Sitophilus zeamais* exposed in grains treated with leaf powders of *Azadirachta indica* and *Plectranthus glandulosus* obtained from sun-dried and shade-dried leaves

Exposure period/doses (g/kg)	*P. glandulosus*/Mean (± SE) (%)			*A. indica*/Mean (± SE) (%)		
	Sun-dried	Shade-dried	*t* value	Sun-dried	Shade-dried	*t* value
1 day						
0	0.00 ± 0.00	0.00 ± 0.00^{c}		0.00 ± 0.00	0.00 ± 0.00	
5	0.00 ± 0.00	0.00 ± 0.00^{c}	–	0.00 ± 0.00	0.00 ± 0.00	–
10	0.00 ± 0.00	0.00 ± 0.00^{c}	–	0.00 ± 0.00	0.00 ± 0.00	–
20	0.00 ± 0.00	1.25 ± 1.25^{c}	1 ^{ns}	0.00 ± 0.00	0.00 ± 0.00	–
30	0.00 ± 0.00	8.75 ± 4.27^{b}	2.05 ^{ns}	1.25 ± 1.25	0.00 ± 0.00	- 1.00^{ns}
40	2.50 ± 1.44	22.50 ± 4.33^{a}	4.38 ^{ns}	5.00 ± 3.54	2.50 ± 1.44	- 0.65^{ns}
$F_{(5, 90)}$	3.00 ns	15.01***		1.91^{ns}	3.00^{ns}	
3 days						
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{a}		0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	
5	0.00 ± 0.00^{d}	0.00 ± 0.00^{a}	–	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1.02^{ns}
10	2.50 ± 2.50^{cd}	0.00 ± 0.00^{a}	-1 ^{ns}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1.02^{ns}
20	6.25 ± 1.25^{bc}	6.25 ± 2.30^{b}	0 ^{ns}	1.25 ± 1.25^{b}	0.00 ± 0.00^{b}	- 1.00^{ns}
30	13.75 ± 2.39^{ab}	17.50 ± 3.23^{c}	0.93 ^{ns}	1.25 ± 1.25^{b}	5.00 ± 2.04^{a}	1.57^{ns}
40	22.50 ± 1.44^{a}	33.75 ± 4.27^{d}	2.50 ^{ns}	10.00 ± 3.54^{a}	7.50 ± 1.44^{a}	- 0.65^{ns}
$F_{(5, 90)}$	30.15***	42.88***		9.10***	16.76***	
LC_{50} (g/kg)	88.84$^{\beta}$	50.87$^{\beta}$		89.24$^{\beta}$	88.89$^{\beta}$	
7 days						
0	0.00 ± 0.00^{d}	0.00 ± 0.00 ^{d}		0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	
5	13.75 ± 6.88^{cd}	17.50 ± 7.22^{c}	0.38^{ns}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	--
10	28.75 ± 4.27^{bc}	18.75 ± 3.75^{bc}	- 1.76^{ns}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	--
20	42.50 ± 6.61^{b}	41.25 ± 1.25^{ab}	- 0.19*	2.50 ± 1.44^{ab}	0.00 ± 0.00^{b}	- 1.73^{ns}
30	97.50 ± 2.50^{a}	45.00 ± 7.91^{ab}	- 6.63^{ns}	6.25 ± 2.39^{ab}	5.00 ± 2.04^{a}	- 40^{ns}
40	100 ± 0.00^{a}	55.00 ± 7.91^{a}	5.69 ***	11.25 ± 5.15^{a}	10.00 ± 2.04^{a}	- 0.23^{ns}
$F_{(5, 90)}$	80.13***	16.91***		4.45**	19.16^{ns}	
LC_{50} (g /kg)	14.04	34.51		104.82$^{\beta}$	73.87$^{\beta}$	
14 days						
0	0.00 ± 0.00^{c}	0.00 ± 0.00 ^{d}		0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
5	37.04 ± 10.09^{b}	22.83 ± 7.28^{c}	-1.08 ^{ns}	3.82 ± 2.41^{bc}	8.88 ± 1.30^{b}	1.85^{ns}
10	56.84 ± 8.75^{b}	40.26 ± 2.28^{b}	- 1.10 ^{ns}	3.82 ± 2.41^{bc}	10.20 ± 3.63^{b}	1.46^{ns}
20	89.87 ± 3.54^{a}	69.47 ± 5.74^{ab}	- 3.02*	16.38 ± 7.97^{ab}	10.13 ± 0.13^{ab}	- 0.78^{ns}
30	100 ± 0.00^{a}	79.80 ± 2.78^{a}	-7.27***	20.20 ± 3.45^{ab}	17.76 ± 2.60^{ab}	- 0.56^{ns}
40	100 ± 0.00^{a}	83.43 ± 2.54^{a}	- 6.59^{ns}	24.01 ± 3.67^{a}	24.15 ± 2.74^{a}	0.97^{ns}
$F_{(5, 90)}$	69.17***	36.32***		9.60***	15.04***	
LC_{50} (g /kg)	7.28	12.12		124.95$^{\beta}$	600.24$^{\beta}$	

Means ± SE in the same column followed by the same lowercase letter within the same group of treatments do not differ significantly at P = 0.05 (Tukey's test). ns non-significant; * P<0.05; *** P<0.001;

– *t*-test is impossible due to absence of mortality; $^{\beta}$ LC_{50} values were estimated by extrapolation

similar susceptibility (P > 0.05; *t*-test) to the shade-dried leaves of both plant powders. *S. zeamais* (7-d) was more susceptible to the *P. glandulosus* powders from the sun-dried leaves

than *C. maculatus* (6-d), with LC_{50} values of respectively 14.04 and 47.37 g/kg. Similar effect was observed with sun-dried leaves powders of *A. indica.*

3.4.3 Effect of particle size of *Azadirachta indica* and *Plectranthus glandulosus* leaf powders on adult mortality of *Callosobruchus maculatus* and *Sitophilus zeamais*

Various levels of insecticidal efficacy of *P. glandulosus* and *A. indica* against *C. maculatus* and *S. zeamais* were recorded when ≤ 0.1 mm, > 0.1 ≤ 0.3 mm and > 0.3 ≤ 0.5 mm particle size of leaf powders were applied (Figures 3.7 and 3.8). The mean adult mortality of the insects on grains treated with the leaf powders was significantly ($P < 0.05$) influenced by the particle sizes except *P. glandulosus* on *C. maculatus*. Overall, adult mortality of the insects increased as the time post-exposure increased from three days to six days for *C. maculatus* and from three days to 14 days for *S. zeamais*, regardless of particle size. *P. glandulosus* leaf powder was less effective against *C. maculatus* compared to *S. zeamais* (Figure 3.7), while the opposite result was observed with *A. indica* leaf powders (Figure 3.8). The ≤ 0.1 mm particle size of *P. glandulosus* led to a higher mortality (100%) of *S. zeamais*, seven days (40 g/kg) post-exposure, whereas with > 0.1 ≤ 0.3 mm and > 0.3 ≤ 0.5 mm particle size, 100% mortality was achieved within 14 days infestation at the dose level of 30 g/kg and 40 g/kg respectively. For *C. maculatus*, maximum mortality of 70.87 ± 3.27% was recorded (≤ 0.1 mm particle size) six days post-exposure when the cowpea was treated with the highest content of *A. indica* leaf powder (40 g/kg). Maximum mortality of 15.00%, 2.50% and 2.50% of *S. zeamais* were achieved 14 days post exposure respectively for ≤ 0.1 mm, > 0.1 ≤ 0.3 mm and > 0.3 ≤ 0.5 mm particle size at highest tested content (40 g/kg) of *A. indica* powder. No adult mortality was recorded within one day time exposure for all particle size and doses when *A. indica* leaf powders were used, but with *P. glandulosus*, a maximum mortality of 3.75% were obtained with the ≤ 0.1 mm particle size powder, within the same exposure period on maize weevil. For *P. glandulosus*, the ≤ 0.1 mm, > 0.1 ≤ 0.3 mm and > 0.3 ≤ 0.5 mm particle size powders respectively caused a maximum mortality of 45.83%, 50.00% and 47.79% (40 g/kg at 6 d post-exposure) to the cowpea weevil.

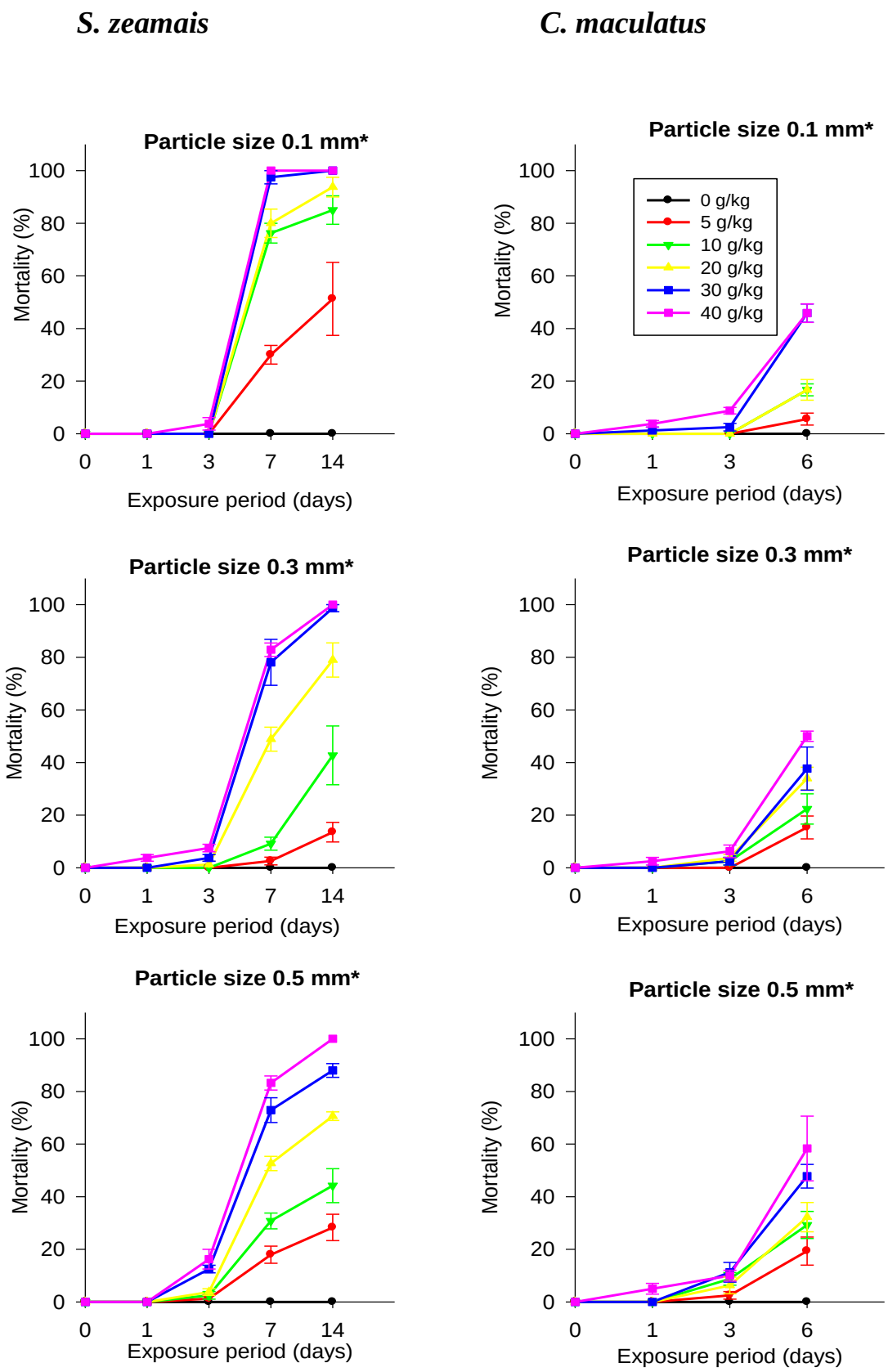


Figure 3.7: Corrected cumulative mortality (mean ± SE) of *Callosobruchus maculatus* and *Sitophilus zeamais* exposed to *Plectranthus glandulosus* leaf powder of three particle sizes
* 0.1 mm = ≤ 0.1 mm; 0.3 mm = > 0.1 ≤ 0.3 mm; 0.5 mm = 0 > 0.3 ≤ 0.5 mm

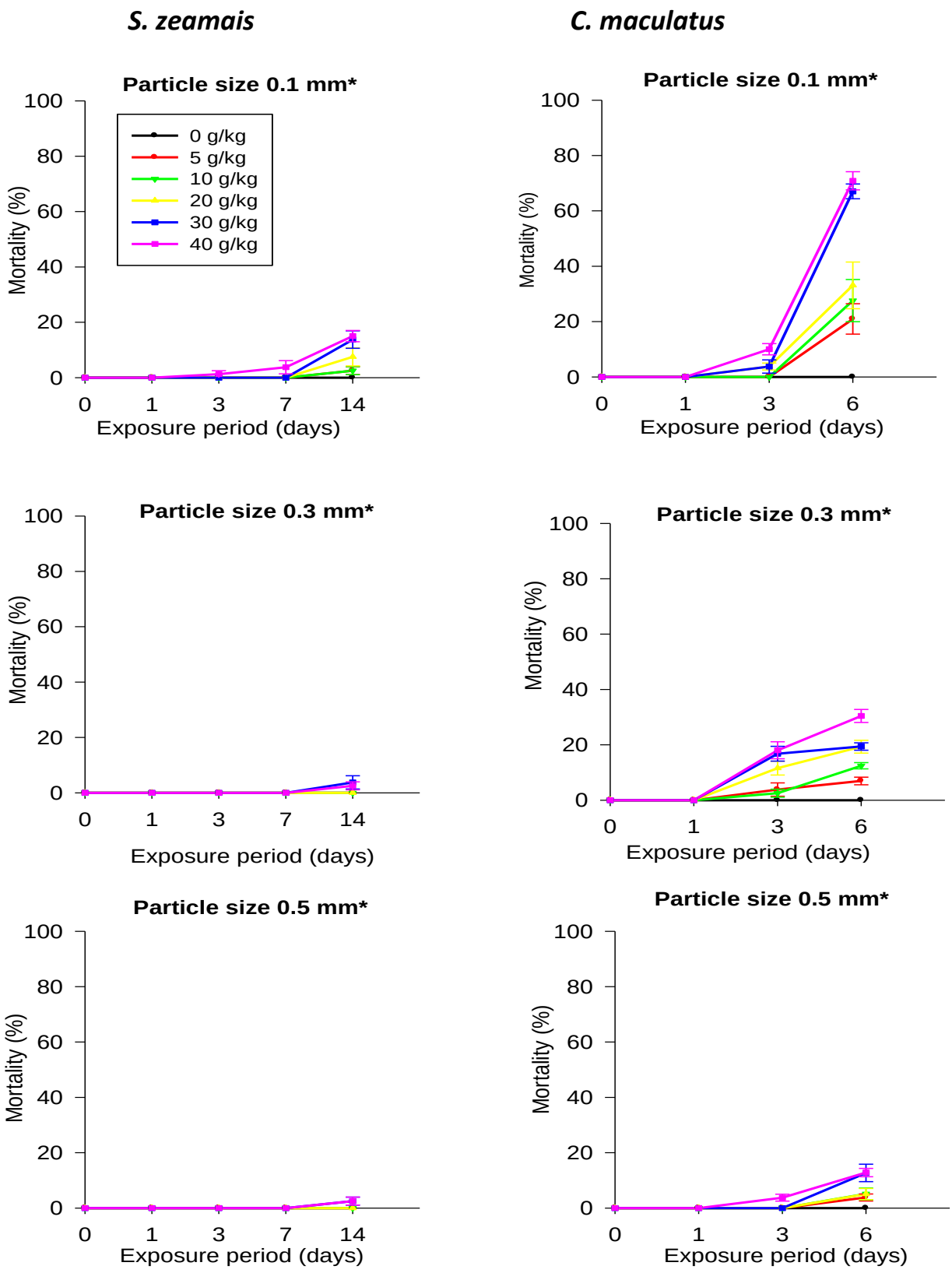


Figure 3.8: Corrected cumulative mortality (mean ± SE) y of *Callosobruchus maculatus* and *Sitophilus zeamais* exposed to *Azadirachta indica* leaf powder of three particle sizes
* 0.1 mm = ≤ 0.1 mm; 0.3 mm = > 0.1 ≤ 0.3 mm; 0.5 mm = 0 > 0.3 ≤ 0.5 mm

3.4.4 Dosage-mortality response relationship of different particle sizes of *Plectranthus glandulosus* leaf powder

The results of the evaluation of the toxicity of powders of three particle sizes of *P. glandulosus* are shown in Table 3.17. The powders were more toxic to *S. zeamais* than *C. maculatus*, irrespective of particle size, and for both insect species, the LC values did not differ between exposure periods. *S. zeamais* was generally more susceptible to the *P. glandulosus* powder with particle size of ≤ 0.1 mm compared to > 0.1 ≤ 0.3 mm and > 0.3 ≤ 0.5 mm particle sizes. The slope of the ≤ 0.1 mm (5.50 ± 2.08) particle size powder with *C. maculatus* was larger than those of the > 0.1 ≤ 0.3 mm (0.98 ± 0.44) and > 0.3 ≤ 0.5 mm (0.59 ± 0.27) particle sizes, 3-d post-exposure, but for *S. zeamais*, larger slopes were obtained with the > 0.1 ≤ 0.3 mm particle size 7-d (3.65 ± 0.28) and 14-d (3.83 ± 0.46) time post-exposure. The coefficients of determination (R^2) of the powders ranged from 0.85 to 0.99 for *S. zeamais* and between 0.54 and 0.95 for *C. maculatus.* The values of $\chi 2$ were significant for the ≤ 0.1 mm particle size powders for *C. maculatus* (six days) and ≤ 0.1 mm (seven days), > 0.1 ≤ 0.3 mm and > 0.3 ≤ 0.5 mm (14 days) particle size for *S. zeamais.*

3.4.5 Dosage-mortality response relationship of three particle sizes of *Azadirachta indica* leaf powder

The *A. indica* powders did not show any clear-cut trend linked to exposure interval and differential toxicity to *S. zeamais* and *C. maculatus* (Table 3.18). However, the ≤ 0.1 mm particle size powder was generally more toxic to *C. maculatus* than the > 0.1 ≤ 0.3 mm and > 0.3 ≤ 0.5 mm particle sizes. The coefficients of determination (R^2) of the powders ranged from 0.35 to 0.89. Generally, the values of $\chi 2$ were not significant.

Table 3.17: Toxicity of *P. glandulosus* leaf powders of three particle sizes on adult *Callosobruchus maculatus* and *Sitophilus zeamais*

Insects/ particle size[§]	Slope ± S.E	R^2	LC_{50} (95% FL)[a] g/kg	LC_{95} (95% FL)[a] g/kg	$\chi^{2\,b}$
C. maculatus					
			3 days[£]		
0.1 mm	5.58 ± 2.08	0.54	69.42 (51.98 - 416.11)	136.74 (77.45 – 5192)	0.22^{ns}
0.3 mm	0.98 ± 0.44	0.72	1630 (215.10 – $3.43E^{16}$)	75608 (1689 – $1.60E^{16}$)	2.54^{ns}
0.5 mm	0.59 ± 0.27	0.66	4861(331.73 - $1.80E^{30}$)	$2.19E^{6}$ (82.08 – $1.77E^{62}$)	3.05^{ns}
			6 days[£]		
0.1 mm	1.37 ± 0.40	0.74	69.68 (32.57 - $6.31E^{8}$)	1090 (152.23 – $2.17E^{25}$)	9.63^{*}
0.3 mm	1.08 ± 0.19	0.95	48.04 (34.44 - 87.37)	1600 (495.05 – 17647)	1.09^{ns}
0.5 mm	1.11 ± 0.18	0. 89	33.49 (25.69 – 50.74)	992.99 (362.17 - 7051)	3.79^{ns}
S. zeamais			**7 days**		
0.1 mm	2.82 ± 0.60	0.88	7.90 (2.08 – 11.32)	27.25 (16.10 – 215.85)	17.54^{***}
0.3 mm	3.65 ± 0.28	0.94	20.41 (18.69 - 22.24)	51.52 (49.28 – 70.40)[£]	4.55^{ns}
0.5 mm	2.08 ± 0.00	0.98	15.90 (13.87 - 18.15)	97.72 (72.42 – 149.29)[£]	3.28^{ns}
			14 days		
0.1 mm	2.97 ± 0.32	0.85	4.82 (3.89 – 5.66)	17.25 (14.51- 21.96)	4.31^{ns}
0.3 mm	3.83 ± 0.46	0.98	10.66 (7.95 – 13.59)	28.66 (20.98 – 51.86)	8.09^{*}
0.5 mm	2.55 ± 0.45	0.99	9.99 (5.00 – 14.95)	43.96 (25.71- 223.15)[£]	13.36^{**}

[a] FL = Fiducial limits;

[b] ns: P>0.05, *P<0.05**P<0.001, ***P<0.0001.

[£] The LC values obtained by extrapolation

[§] 0.1 mm = ≤ 0.1 mm; 0.3 mm = > 0.1 ≤ 0.3 mm; 0.5 mm = 0 > 0.3 ≤ 0.5 mm

Table 3.18: Toxicity of *Azadirachta indica* leaf powders of three particle sizes on adult *Callosobruchus maculatus* and *Sitophilus zeamais*

Insects/ particle size[§]	Slope ± S.E	R^2	LC_{50} (95% FL)[a] g/kg	LC_{95} (95% FL)[a] g/kg	$\chi^{2\,b}$
C. maculatus					
			3 days[£]		
0.1 mm	2.34 ± 0.74	0.75	144.31 (77.14 - 1953)	723.59 (211.12 - 1.4E^5)	2.08 ^{ns}
0.3 mm	1.20 ± 0.28	0.88	216.35 (101.31 - 1545)	5066 (903.62 - 5.0E^5)	2.77^{ns}
0.5 mm	43.35 ±0.00	0.35	43.97 (42.91 - 45.04)	47.98 (46.83- 49.15)	0^{ns}
			6 days		
0.1 mm	1.58 ± 0.42	0.88	21.31 (9.83 - 115.09)	233.23 (65.97 - 1.45E^8)[£]	14.63**
0.3 mm	0.96 ± 0.22	0.89	166.05 (81.34 - 1006) [£]	8279 (1244 - 1.19E^6) [£]	1.47 ^{ns}
0.5 mm	0.78 ± 0.28	0.75	15.90 (13.87 - 18.15)	1.41E^5 (3703 -7.98E^{13})[£]	1.99^{ns}
S. zeamais			**7 days**[£]		
0.1 mm	43.35 ±0.00	0.35	43.96 (42.91– 45.04)	47.98 (46.83- 49.15)	0^{ns}
0.3 mm	-	-	-	-	-
0.5 mm	-	-	-	-	-
			14 days[£]		
0.1 mm	1.23 ± 0.31	0.87	268.11 (113.96 – 3117)	5785 (909.09 -1325746)	0^{ns}
0.3 mm	2.53 ± 1.39	0.57	199.34	885.61	3.12^{ns}
0.5 mm	2.84 ± 1.72	0.66	177.65	671.66	1.44^{ns}

[a] FL = Fiducial limit;
[b] ns: P>0.05, **P<0.001.
[£] The LC values obtained by extrapolation
[§] 0.1 mm = ≤ 0.1 mm; 0.3 mm = > 0.1 ≤ 0.3 mm; 0.5 mm = 0 > 0.3 ≤ 0.5 mm

3.4.6 Effect of particle size of *Azadirachta indica* and *Plectranthus glandulosus* leaf powder on F_1 progeny production

Tables 3.19 and 3.20 show the number of F_1 progeny and percentage of progeny inhibition of *C. maculatus* and *S. zeamais* that emerged from grains treated with powders of *A. indica* or *P. glandulosus* of different particle sizes, applied at different doses. In general, the powders significantly reduced the emergence of progeny relative to the control in a dose-dependent manner for both insect species.

Overall, the rate of progeny inhibition reduced with increase in particle size for *C. maculatus*, but this was noticeable at higher powder contents (≥ 20 g/kg) with *P. glandulosus* and at lower powder contents with *A. indica* (Table 3.19). However, the rate of progeny inhibition was generally similar between the > 0.1 ≤ 0.3 mm and > 0.3 ≤ 0.5 mm particle powders, irrespective of the plant species. The *A. indica* powder was more efficient at inhibiting progeny production compared with that of *P. glandulosus* for all the particle sizes, although latter plant tended to be more efficient at higher powder contents and smallest particle size. For the powder content of 5 g/kg, the 0.1, 0.3 and 0.5 mm particle-size powder of *A. indica* reduced progeny emergence by respectively 48.10%, 33.68% and 21.78%, but these reductions were respectively 15.68%, 9.25% and 5.51% with *P. glandulosus* powders. The 40 g/kg powder contents of *A. indica* inhibited progeny production by 65.81%, 52.74% and 67.07% compared with 95.67%, 38.72% and 35.23% for *P. glandulosus* powder, respectively with the particle sizes 0.1 , 0.3 and 0.5 mm.

Similarly, with *S. zeamais*, the rate of progeny inhibition generally reduced with ascending particle size of the powders (Table 3.20). This reduction was evident for all the dosage levels of *A. indica*, but only at the 20 g/kg powder content with *P. glandulosus*, although the 0.3 and 0.5 mm particle-size powders inhibited progeny production in a similar manner. *P. glandulosus* powder was more potent in inhibiting progeny production in *S. zeamais* than *A. indica*, regardless of particle size. The highest tested powder content of 40 g/kg suppressed progeny production by 99.65%, 97.55% and 96.93% for the particles size ≤ 0.1, > 0.1 ≤ 0.3 mm and > 0.3 ≤ 0.5 mm of *P. glandulosus*, respectively, but only respectively 53.61%, 31.58% and 38.20% with *A. indica*.

Table 3.19: Progeny production of *Callosobruchus maculatus* in grains treated with *Plectranthus glandulosus* and *Azadirachta indica* leaf powders of three particle sizes

Products and doses (g/kg)	Particle sizes			
	≤ 0.1 mm	> 0.1 ≤ 0.3 mm	> 0.1 ≤ 0.3 mm	$F_{(2, 9)}$ ‡
	Number (mean ± SE) of F_1 adult progeny †			
P. glandulosus **leaf powder**				
0	440.00 ± 10.75^{a}	475.25 ± 25.49^{a}	466.00 ± 17.23^{a}	0.94^{ns}
5	469.25 ± 40.47^{a}	430.75 ± 22.57^{ab}	439.50 ± 13.45^{ab}	1.86^{ns}
10	315.25 ± 34.97^{ab}	395.25 ± 48.60^{abc}	414.00 ± 5.82^{ab}	2.50^{ns}
20	223.50 ± 12.52^{bB}	335.50 ± 20.44^{bcA}	383.00 ± 25.99^{bcA}	18.73***
30	119.00 ± 10.34^{cB}	330.50 ± 22.93^{bcA}	331.50 ± 13.30^{cdA}	70.95***
40	18.75 ± 6.28^{dB}	293.25 ± 31.50^{cA}	300.75 ± 6.28^{dA}	72.30***
$F_{(5, 18)}$ ‡	55.26***	5.48**	18.11***	
A. indica **leaf powder**				
0	409.00 ± 11.25^{a}	407.75 ± 14.56^{a}	390.75 ± 28.63^{a}	0.31^{ns}
5	211.25 ± 12.96^{bB}	270.50 ± 21.90^{bAB}	302.00 ± 8.90^{aA}	9.18**
10	212.00 ± 11.71^{bB}	233.00 ± 8.53^{bcB}	312.25 ± 16.62^{aA}	17.55***
20	183.75 ± 28.58^{b}	178.50 ± 27.17^{bc}	207.75 ± 5.74^{b}	0.52^{ns}
30	169.50 ± 38.26^{b}	175.25 ± 14.37^{c}	178.50 ± 10.60^{bc}	0.11^{ns}
40	138.50 ± 28.61^{b}	192.00 ± 26.69^{c}	130.50 ± 24.43^{c}	1.44^{ns}
$F_{(5, 18)}$ ‡	8.60***	15.07***	29.39***	
	Percentage (mean ± SE) reduction in adult emergence relative to control †			
P. glandulosus **leaf powder**				
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	
5	15.68 ± 10.16^{de}	9.25 ± 2.22^{c}	5.51 ± 2.53^{bc}	0.38^{ns}.
10	10.84 ± 3.08^{cd}	17.42 ± 6.52^{bc}	10.84 ± 3.08^{b}	1.25^{ns}
20	49.22 ± 2.57^{bcA}	29.36 ± 2.31^{abB}	17.77 ± 4.94^{bB}	16.96***
30	73.00 ± 2.07^{abA}	30.55 ± 2.09^{abB}	28.65 ± 3.35^{abB}	83.55***
40	95.69 ± 2.19^{aA}	38.72 ± 3.44^{aB}	35.23 ± 2.39^{aB}	81.40***
$F_{(5, 18)}$ ‡	34.76***	23.22***	22.88**	
A. indica **leaf powder**				
0	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	
5	48.10 ± 4.16^{aA}	33.68 ± 4.61^{bAB}	21.78± 4.68^{cB}	8.45**
10	47.98 ± 3.66^{aA}	42.58 ± 3.19^{abA}	19.57 ± 3.63^{cB}	17.35***
20	54.86 ± 7.34^{a}	56.11 ± 6.37^{a}	45.99 ± 4.13^{b}	0.82^{ns}
30	58.09 ± 9.95^{a}	56.83 ± 14.10^{a}	53.73 ± 3.88^{ab}	0.14^{ns}
40	65.81 ± 7.44^{a}	52.74 ± 6.75^{ab}	67.07 ± 4.17^{a}	1.52^{ns}
$F_{(5, 18)}$ ‡	26.30***	45.43***	65.98***	

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 3.20: Progeny production of *Sitophilus zeamais* in grains treated with *Plectranthus glandulosus* and *Azadirachta indica* leaf powders of three particle sizes

products and doses (g/kg)	Particle size			
	≤ 0.1 mm	> 0.1 ≤ 0.3 mm	> 0.3 ≤ 0.5 mm	$F_{(2, 9)}$ ‡
Number (mean ± SE) of F_1 adult progeny †				
***P. glandulosus* leaf powder**				
0	50.75 ± 1.25^{a}	51.25 ± 1.31^{a}	56.25 ± 1.80^{a}	4.24^{ns}
5	38.00 ± 1.87^{aB}	40.25 ± 4.77^{aAB}	51.75 ± 0.85^{aA}	5.65^{*}
10	20.00 ± 4.98^{b}	18.50 ± 1.50^{b}	13.75 ± 0.75^{b}	1.14^{ns}
20	4.75 ± 0.85^{cB}	13.50 ± 2.66^{bA}	13.25 ± 0.75^{bA}	11.64^{**}
30	2.75 ± 0.75^{c}	2.50 ± 0.87^{c}	2.50 ± 0.29^{c}	0.07^{ns}
40	1.75 ± 1.03^{c}	1.25 ± 1.25^{c}	1.75 ± 0.48^{c}	0.30^{ns}
$F_{(5, 18)}$ ‡	62.48^{***}	62.48^{***}	594.45^{***}	
***A. indica* leaf powder**				
0	59.00 ± 4.04^{a}	65.25 ± 3.07^{a}	73.75 ± 6.37	2.77^{ns}
5	36.75 ± 4.77^{bB}	63.25 ± 3.17^{aA}	67.50 ± 4.12^{A}	16.37^{***}
10	32.00 ± 6.12^{bB}	59.00 ± 1.22^{abA}	54.00 ± 0.00^{A}	10.93^{**}
20	28.00 ± 4.12^{bB}	56.75 ± 2.84^{abA}	56.75 ± 7.93^{A}	10.34^{**}
30	25.75 ± 1.80^{bB}	50.5 ± 4.92^{abA}	57.50 ± 5.74^{A}	16.82^{***}
40	27.00 ± 3.03^{bB}	44.75 ± 3.33^{bA}	49.75 ± 3.77^{A}	12.86^{**}
$F_{(5, 18)}$ ‡	7.62^{***}	5.44^{**}	2.43^{ns}	
Percentage (mean ± SE) reduction in adult emergence relative to control †				
***P. glandulosus* leaf powder**				
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	
5	24.84 ± 4.77^{c}	21.97 ± 7.34^{c}	7.80 ± 2.43^{c}	3.57^{ns}
10	60.60 ± 9.86^{b}	63.77 ± 3.37^{b}	75.46 ± 1.66^{b}	1.68^{ns}
20	90.74 ± 1.45^{aA}	73.43 ± 5.60^{bB}	76.45 ± 1.07^{bB}	9.46^{**}
30	94.74 ± 1.49^{a}	95.10 ± 1.74^{a}	95.56 ± 0.48^{a}	0.16^{ns}
40	96.65 ± 1.96^{a}	97.55 ± 2.45^{a}	96.93 ± 0.77^{a}	0.51^{ns}
$F_{(5, 18)}$ ‡	89.27^{***}	75.68^{***}	671.24^{***}	
***A. indica* leaf powder**				
0	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	473.7
5	36.12 ± 11.18^{aA}	3.09 ± 1.06^{cdB}	16.18 ± 5.90^{abAB}	5.76^{*}
10	45.76 ± 8.78^{aA}	9.23 ± 2.55^{bcB}	33.10 ± 5.02^{aA}	12.19^{**}
20	50.98 ± 10.06^{aA}	12.70 ± 4.72^{bcB}	30.06 ± 9.07^{abAB}	5.65^{*}
30	55.54 ± 4.81^{aA}	22.96 ± 5.60^{abB}	28.72 ± 7.05^{abB}	8.27^{**}
40	53.61 ± 5.63^{aA}	31.58 ± 2.91^{aB}	38.20 ± 5.26^{aAB}	5.67^{*}
$F_{(5, 18)}$ ‡	14.51^{***}	18.78^{***}	3.85^{*}	

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

3.4.7 Dosage-F_1 progeny inhibition response relationship of different particle sizes of *P. glandulosus* and *A. indica* leaf powders

Table 3.21 shows that the EC_{50} values were smaller for the ≤ 0.1 mm particle-size powder of *P. glandulosus* on *C. maculatus* and *A. indica* on *S. zeamais* as compared with the other particle sizes. With *P. glandulosus* powder, the EC_{50} values were smaller for *S. zeamais* than *C. maculatus*, while the reverse trend was recorded with *A. indica* powder. The *P. glandulosus* powder slopes, which ranged from 1.09 to 3.25 were higher than those of *A. indica* (0.47 -1.47). R^2 value varied from 0.60 (> 0.3 ≤ 0.5 mm *A. indica* powders on *S. zeamais*) to 0.97 (> 0.1 ≤ 0.3 mm *P. glandulosus* powders on *C. maculatus*).The χ^2 values were not significant for all *A. indica* particle-size powders but significant for *P. glandulosus* (≤ 0.1 mm on *C maculatus* and > 0.1 ≤ 0.3 mm and > 0.3 ≤ 0.5 mm on *S. zeamais*).

Table 3.21: Effective concentration resulting in 50% (EC_{50}) and 95% (EC_{95}) reduction of *Callosobruchus maculatus* and *Sitophilus zeamais* F_1 emergence in grains treated with *Plectranthus glandulosus* and *Azadirachta indica* leaf powders of three particle sizes

Product/ particle size[§]	Slope ± S.E	R^2	EC_{50} (95% FL)[a]	EC_{95} (95% FL)[a]	χ^2 [b]
***P. glandulosus* leaf powder**					
			C. maculatus		
0.1 mm	2.56 ± 0.57	0.94	15.65 (8.11 - 27.23)	68.78 (35.31 - 1134) [£]	18.75***
0.3 mm	1.09 ± 0.20	0.97	74.48 (48.49 - 175.26) [£]	2376 (636.91 - 40209) [£]	0.80[ns]
0.5 mm	1.37 ± 0.23	0.94	80.00 (54.31 - 164.47) [£]	1263 (446.15 - 9869) [£]	0.62[ns]
			S. zeamais		
0.1 mm	2.96 ± 0.24	0.92	8.19 (7.15 - 9.22)	32.00 (26.94 - 40.07)	2.01[ns]
0.3 mm	2.75 ± 0.44	0.93	8.95 (4.86 - 12.81)	35.40 (22.38 - 115.19)	11.36**
0.5 mm	3.25 ± 0.85	0.81	9.48 (1.32 - 17.68)	30.38 (16.63 - 3004)	34.65 ***
***A. indica* leaf powder**					
			C. maculatus		
0.1 mm	0.47 ± 0.17	0.85	8.90 (1.49 - 15.27)	26448 (1188 - 2.46E[12])[£]	1.50 [ns]
0.3 mm	0.63 ± 0.17	0.82	19.16 (12.45 - 33.08)	7553 (870.05 - 9.24E[6])[£]	2.73 [ns]
0.5 mm	1.46 ± 0.30	0. 89	24.02 (14.70 - 62.08)	321.99 (96.26 - 86654)[£]	7.74 [ns]
			S. zeamais		
0.1 mm	0.51 ± 0.17	0.92	9.73 (11.41 - 44.22)	29361 (1493 - 3.86 E[10])[£]	0.72[ns]
0.3 mm	1.47 ± 0.25	0.90	93.67 (61.75 - 209.19)[£]	1220 (432.58 - 1015)[£]	1.51[ns]
0.5 mm	0.52 ± 0.19	0.60	188.80 (65.501-5053)[£]	2.64E[6] (4943 - 1.24E[15])[£]	5.75[ns]

[a] FL = Fiducial limit;
[b] ns P>0.05, **P<0.001, ***P<0.0001.
[£] The EC values were given by extrapolation
[§] 0.1 mm = ≤ 0.1 mm; 0.3 mm = > 0.1 ≤ 0.3 mm; 0.5 mm = 0 > 0.3 ≤ 0.5 mm

3.4.8 Effect of particle size of *Plectranthus glandulosus* leaf powder on grain damage and weight loss caused by *Callosobruchus maculatus*

The damage and weight loss of cowpea grains that were treated with the different particle sizes of *P. glandulosus* leaf powder, then infested with *C. maculatus*, and stored for 10 weeks were generally either statistically similar or higher than those of the control, irrespective of dose (Table 3.22). However, with the particle size of ≤ 0.1 (87.25%) and > 0.1 ≤ 0.3 mm (91.25%) and for the highest tested powder content 40 g/kg, grain damage was lower than those of the control (97.00% and 97.75%, respectively). Five g/kg content of the ≤ 0.1 mm particle-size powder caused a greater weight loss (55.05%) of cowpea than the control (40.83%).

Table 3.22: Grain damage and weight loss of cowpea caused by *Callosobruchus maculatus* in grains treated with *Plectranthus glandulosus* leaf powders of different particle sizes and then stored for 10 weeks

Doses (g/kg)	Particle size			
	≤ 0.1 mm	> 0.1 ≤ 0.3 mm	> 0.3 ≤ 0.5 mm	$F_{(2, 9)}$ ‡
	Mean (± SE) grain damage (%) †			
0	97.00 ± 0.91^{a}	97.75 ± 0.34^{a}	98.50 ± 0.29	1.29^{ns}
5	99.00 ± 0.00^{a}	98.50 ± 0.50^{a}	98.25 ± 0.48	0.97^{ns}
10	98.75 ± 0.25^{a}	98.50 ± 0.29^{a}	99.00 ± 0.00	1.29^{ns}
20	97.00 ± 0.82^{a}	95.50 ± 0.50^{ab}	97.00 ± 0.82	1.42^{ns}
30	95.50 ± 1.19^{a}	94.75 ± 1.11^{ab}	98.00 ± 0.41	2.52^{ns}
40	87.25 ± 2.78^{bB}	91.25 ± 2.50^{bAB}	98.25 ± 0.25^{A}	8.52**
$F_{(5, 18)}$ ‡	9.54*	6.32**	2.19^{ns}	
	Mean (± SE) weight loss (%) †			
0	40.83 ± 3.33^{b}	47.66 ± 2.28	50.51 ± 5.08	1.77^{ns}
5	55.05 ± 3.28^{a}	54.22 ± 3.49	51.01 ± 2.22	0.49^{ns}
10	44.95 ± 1.34^{abB}	53.39 ± 4.58^{AB}	61.54 ± 3.02^{A}	6.40*
20	41.57 ± 2.21^{ab}	49.87 ± 5.05	46.59 ± 10.24	0.34^{ns}
30	49.54 ± 2.76^{ab}	47.73 ± 1.90	54.08 ± 0.91	2.66^{ns}
40	42.12 ± 4.76^{ab}	47.69 ± 4.32	53.66 ± 3.55	1.85^{ns}
$F_{(5, 18)}$ ‡	3.19*	0.63^{ns}	0.94^{ns}	

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; P < 0.05).
‡ ns P > 0.05, * P < 0.05, ** P < 0.01;

3.4.9 Effect of particle size of *Plectranthus glandulosus* and *Azadirachta indica* leaf powder on grain damage and weight loss caused by *Sitophilus zeamais*

Data on grain weight loss and number of grains damaged by *S. zeamais* for the different particle-size powders of *P. glandulosus* and *A. indica* and doses are given in Table 3.23. Both grain damage and weight loss were significantly ($P \leq 0.05$) reduced by treatments with the botanicals apart from the $> 0.3 \leq 0.5$ mm particle-size powder of *A. indica* which did not show any difference among the contents applied and the control concerning weight loss. But for the powder contents of 10 and 40 g/kg, grain damage varied with the particle size of *P. glandulosus* powder, although there was a linear trend only with the dose of 20 g/kg, with increase in damage as the particle size increased. With *A. indica* powder, grain damage increased with ascending powder particle size only for the 30 and 40 g/kg dose levels. For the two botanical powders, particle size did not influence weight loss in the stored maize grains.

3.4.10 Persistence of 0.1 mm particle-size leaf powder of *Plectranthus glandulosus* in treated maize grains

The lethal effect of ≤ 0.1 mm particle-size leaf powder of *P. glandulosus* on *S. zeamais* in treated maize grains over a six-month storage interval is given in Table 3.24. For the lower dose levels 5, 10 and 20 g/kg, the mortality caused by the powder to *S. zeamais* dis not vary with storage intervals up to 180 days. In the contrary, at the higher dose level of 30g/kg, the mortality reduced with increasing storage intervals from 15 – 180 days. For the highest dose level of 40 g/kg, the mortality of S. zeamais decreased with storage intervals up to 15 days, there after remained constant up to the 180-day storage interval

3.5 Effect of binary combinations of *Azadirachta indica* products and *Plectranthus glandulosus* leaf powder on adult *Sitophilus zeamais* and *Callosubruchus maculatus*

3.5.1 Effect of binary combinations of powders from *Plectranthus glandulosus* leaves and *Azadirachta indica* seeds

The powders of *P. glandulosus* and *A. indica* in isolation as well as the different proportions of their binary combinations generally caused significant mortality to adult *C. maculatus* and *S. zeamais* compared to the controls (Figures 3.9), although the mortality caused to both insects by the binary powder combinations was lower than those of the individual powders.

Table 3.23: Grain damage and weight loss of maize caused by *Sitophilus zeamais* in grains treated with *Plectranthus glandulosus* and *Azadirachta indica* leaf powders of different particle sizes and then stored for 10 weeks

Products and doses (g/kg)	Particle size			
	≤ 0.1 mm	> 0.1 ≤ 0.3 mm	> 0.3 ≤ 0.5 mm	$F_{(2, 9)}$ ‡
	Mean (± SE) grain damage (%) †			
***P. glandulosus* leaf powder**				
0	42.75 ± 5.45^{ab}	52.25 ± 4.80^{a}	39.75 ± 1.55^{ab}	2.31^{ns}
5	55.50 ± 1.44^{aA}	29.75 ± 3.92^{bB}	52.50 ± 0.09^{aA}	16.98 ***
10	35.25 ± 8.40^{b}	24.00 ± 2.27^{bc}	33.75 ± 6.49^{abc}	0.87^{ns}
20	13.25 ± 2.78^{cB}	16.50 ± 2.72^{bcAB}	25.00 ± 0.71^{bcdA}	5.82*
30	3.50 ± 0.65^{cB}	16.50 ± 3.71^{bcA}	8.00 ± 2.86^{cdAB}	6.01*
40	2.75 ± 0.65^{c}	9.25 ± 2.02^{c}	14.00 ± 9.03^{d}	1.69^{ns}
$F_{(5, 18)}$ ‡	26.40***	20.08**	10.88**	
***A. indica* leaf powder**				
0	54.75 ± 4.39^{a}	57.75 ± 3.28^{a}	55.00 ± 1.47^{a}	0.26^{ns}
5	37.75 ± 2.59^{abB}	52.50 ± 2.33^{aA}	46.25 ± 2.56^{abA}	8.68**
10	33.50 ± 7.03^{bcB}	61.50 ± 1.49^{aA}	52.75 ± 5.34^{aAB}	7.95 *
20	29.50 ± 4.63^{bc}	36.25 ± 2.72^{b}	33.25 ± 2.25^{bc}	1.05^{ns}
30	21.00 ± 2.16^{bc}	30.00 ± 5.79^{b}	20.75 ± 5.31^{b}	1.58^{ns}
40	18.75 ± 2.87^{c}	19.50 ± 1.19^{c}	24.25 ± 0.00^{c}	0.65^{ns}
$F_{(5, 18)}$ ‡	9.87*	25.17***	15.03**	
	Mean (± SE) weight loss (%) †			
***P. glandulosus* leaf powder**				
0	9.41 ± 3.02^{ab}	13.90 ± 0.75^{a}	12.52 ± 0.69^{ab}	
5	10.33 ± 2.55^{a}	8.91 ± 1.63^{b}	14.42 ± 1.80^{a}	0.38^{ns}.
10	5.28 ± 0.93^{abc}	4.85 ± 1.18^{c}	9.36 ± 2.03^{abc}	1.94^{ns}
20	2.23 ± 0.57^{bc}	3.97 ± 0.88^{c}	5.75 ± 0.96^{bc}	4.62^{ns}
30	0.84 ± 0.38^{c}	3.93 ± 1.27^{c}	2.47 ± 0.80^{c}	4.08^{ns}
40	0.71 ± 0.18^{c}	1.40 ± 0.25^{c}	3.65 ± 2.60^{c}	1.23^{ns}
$F_{(5, 18)}$ ‡	6.45**	17.32***	8.76**	
***A. indica* leaf powder**				
0	15.85 ± 1.30^{a}	16.23 ± 0.78^{a}	14.69 ± 2.39	0.26^{ns}
5	10.88 ± 1.01^{ab}	14.54 ± 1.37^{a}	13.12 ± 1.72	1.79^{ns}
10	11.50 ± 3.37^{ab}	18.52 ± 1.41^{a}	10.90 ± 2.21	2.52^{ns}
20	8.27 ± 1.95^{ab}	8.19 ± 0.53^{b}	8.80 ± 1.44	0.06^{ns}
30	8.66 ± 1.44^{ab}	8.96 ± 1.14^{b}	5.86 ± 2.66	0.31^{ns}
40	4.03 ± 1.03^{b}	5.42 ± 0.74^{b}	7.26 ± 3.67	0.45^{ns}
$F_{(5, 18)}$ ‡	4.44**	24.51***	1.89^{ns}	

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$; * $P < 0.05$** $P < 0.01$;*** $P < 0.001$

Table 3.24: Corrected cumulative mortality (mean ± SE) of adult *Sitophilus zeamais* in grains treated with *P. glandulosus* leaf powder of particle size ≤ 0.1 mm after different storage intervals

Doses (g/kg)	Storage intervals (days)/ Mortality (%)[†]					
	0	15	30	60	180	$F_{(4,15)}$ [‡]
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	
5	6.25 ± 2.39^{de}	2.50 ± 2.50^{cd}	1.25 ± 1.25^{d}	3.75 ± 1.25^{cd}	1.25 ± 1.25^{c}	1.23^{ns}
10	13.75 ± 4.27^{cd}	10.00 ± 0.00^{cd}	5.00 ± 2.04^{cd}	11.25 ± 1.25^{bc}	15.00 ± 4.08^{b}	2.27^{ns}
20	22.50 ± 3.23^{c}	15.00 ± 5.00^{c}	7.50 ± 1.44^{c}	15.00 ± 3.54^{ab}	18.75 ± 4.27^{ab}	2.79^{ns}
30	38.75 ± 2.39^{bA}	30.00 ± 2.04^{bAB}	20.00 ± 2.04^{bBC}	13.75 ± 2.39^{abC}	23.75 ± 3.75^{abBC}	13.55**
40	60.00 ± 3.54^{aA}	45.00 ± 5.40^{aAB}	37.50 ± 3.23^{aB}	28.75 ± 5.15^{aB}	32.50 ± 2.50^{aB}	9.00**
$F_{(5,18)}$ [‡]	57.27***	27.97***	55.88***	12.65***	17.14***	

[†] Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).
[‡] ns $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$

The mortality generally increased with ascending dose levels and time post-exposure. There were no differences in the mortality among the mixed proportions for the same insect species ($P > 0.05$). Within one day of exposure, and at all doses of combined botanicals, no adult mortality was recorded in *C. maculatus* and *S. zeamais*. Within 14 days of exposure, maximum mortality of 68.75, 57.50 and 45.50% were achieved for the combinations 75% *P. glandulosus* + 25% *A. indica*, 50% *P. glandulosus* + 50% *A. indica* and 25% *P. glandulosus* + 75% *A. indica* (20 g/kg), respectively for *S. zeamais* while with *C. maculatus*, maximum mortality of 43.75, 37.50 and 31.25 % were registered for the combinations of 50% *P. glandulosus* + 50% *A. indica*, 75% *P. glandulosus* + 25% *A. indica* and 25% *P. glandulosus* + 75% *A. indica* (20 g/kg), respectively within six days of exposure. When applied alone and at the highest content (20 g/kg), NeemAzal, *P. glandulosus* and *A. indica* respectively caused maximum mortality of 100% (three days), 42.50% (six days) and 1.13% (six days), respectively to *C. maculatus*. At the same dose level (20 g/kg) and within 14 days, 100% mortality in maize weevil was recorded when treated with NeemAzal and *P. glandulosus* powders and 96.25% mortality with the neem seed powder.

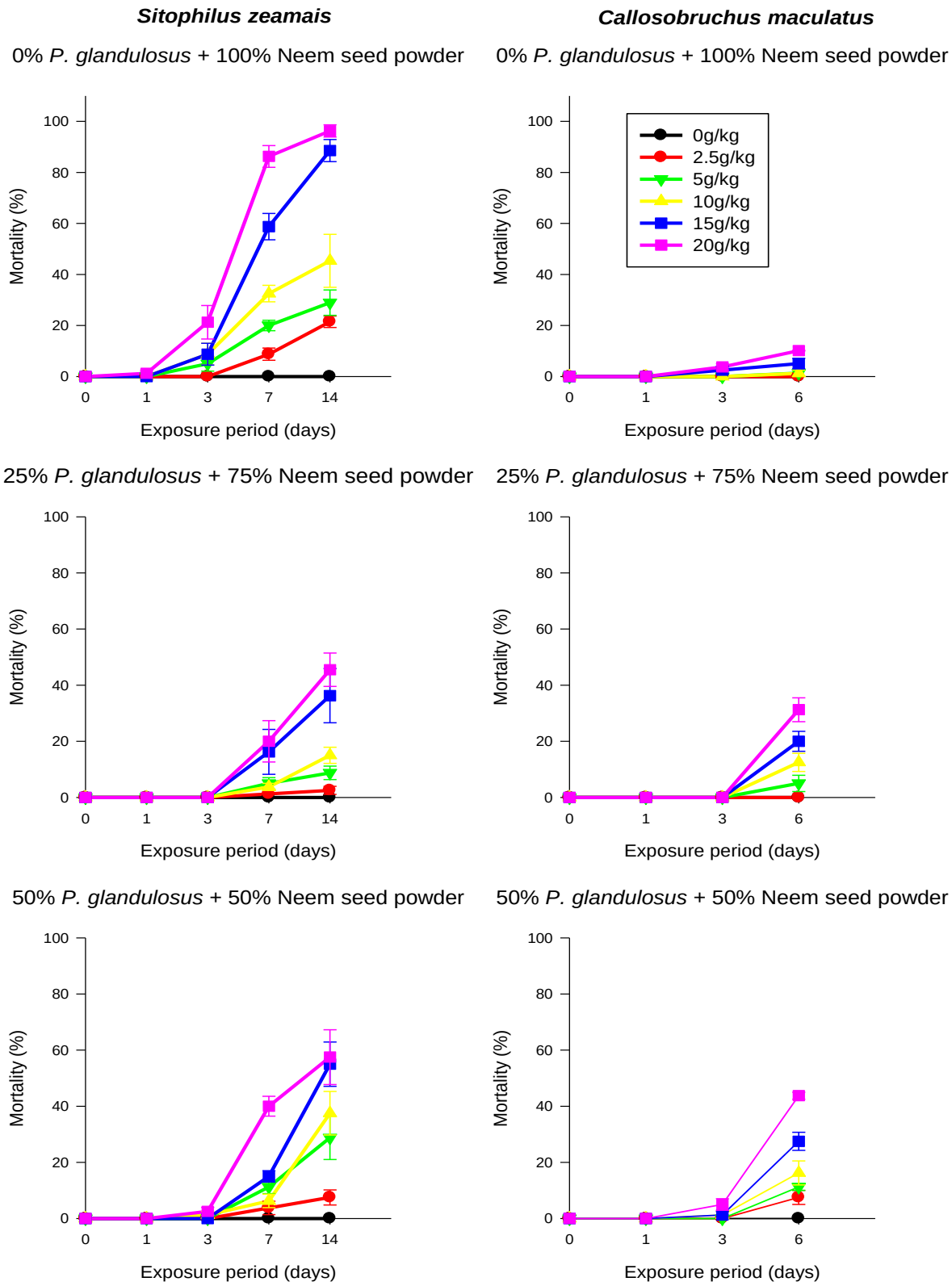


Figure 3.9: Corrected cumulative mortality (mean ± SE) of *Callosobruchus maculatus* and *Sitophilus zeamais* exposed to binary combinations of powders from *Plectranthus glandulous* leaves and *Azadirachta indica* seeds

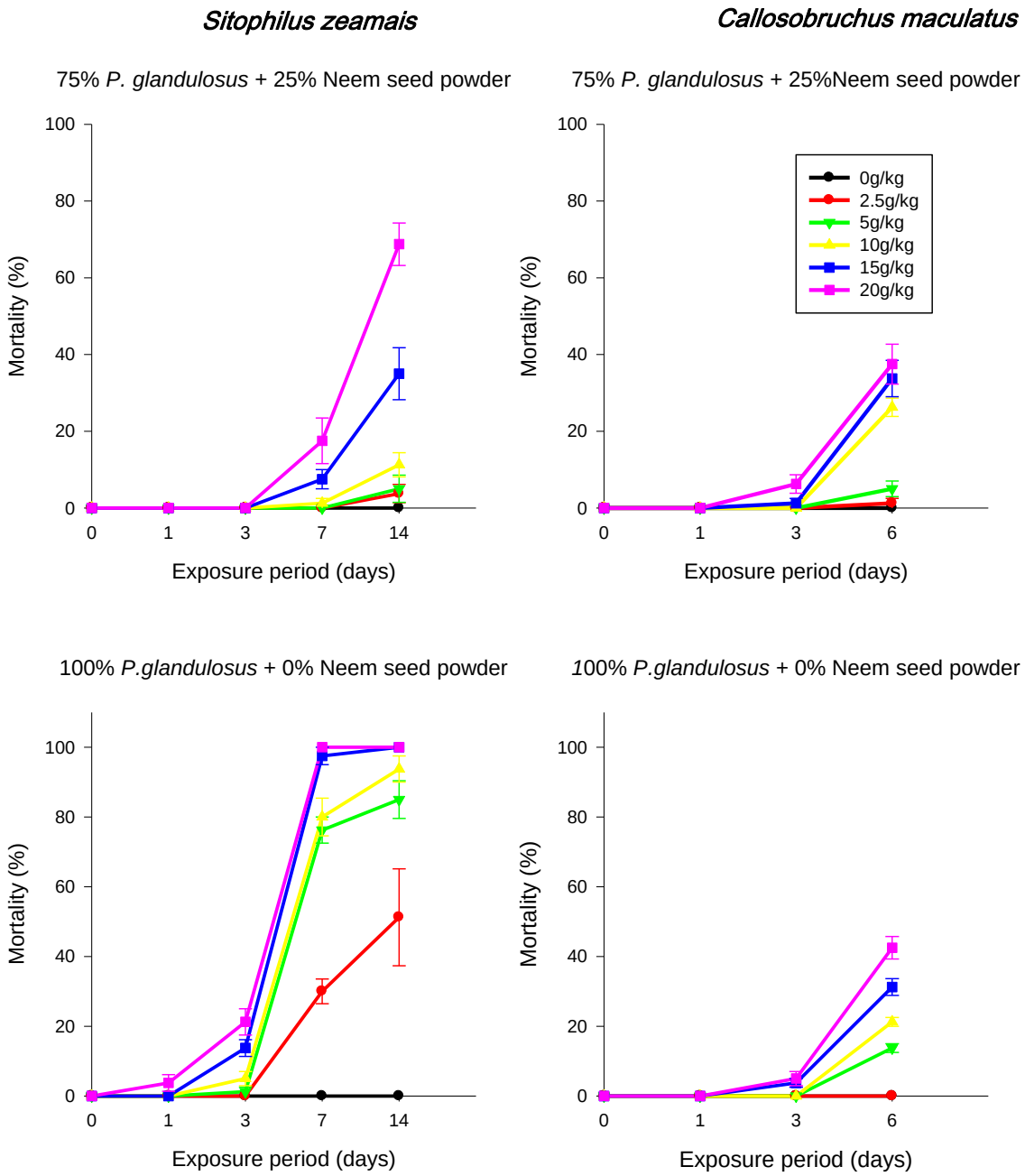


Figure 3.9 cont'd

3.5.2 Dosage-mortality response relationships of the binary combinations of powders *from Plectranthus glandulosus* leaves and *Azadirachta indica* seeds

Regardless of the mixture proportion, the binary combinations of *P. glandulosus* and *A. indica* powders were toxic to *C. maculatus* and *S. zeamais* (Table 3.25). LC_{50} and LC_{95} values for the different mixtures reduced with time post-exposure. The LC_{50} and LC_{95} values of 75% *P. glandulosus* + 25% *A. indica* combination appeared to be more effective than those of the

other mixed proportions. The 3-d LC_{50} value (35.70 g/kg) of 75% *P. glandulosus* and 25% *A. indica* on *C. maculatus* was similar to that of seven days (32.71 g/kg) on *S. zeamais*. All the LC_{95} values were estimated by extrapolation. The coefficients of determination (R^2) of the plant powder combinations ranged from 0.50 to 0.97. All the co-toxicity coefficients were less than 80. Overall the values of chi-square ($\chi 2$) were not significant.

3.5.3 Effect of binary combinations of NeemAzal and *Plectranthus glandulosus* leaf powder on the mortality of adult *Callosobruchus maculatus* and *Sitophilus zeamais*

All the different combinations of NeemAzal and *P. glandulosus* generally caused significant mortality to adult *C. maculatus* and *S. zeamais* compared to the control (Figures 3.10). The increase in mortality with ascending dose levels and time exposure was much more pronounced within three days post exposure than thereafter, irrespective of mixture proportions and insect species. Overall, mixture proportions generally had no effect on the mortality of the two insect species caused by the mixed *P. glandulosus* leaf powder and NeemAzal. However, the combination 25% NeemAzal + 75% *P .glandulosus* powder tended to be less potent to both insect species, since the lowest tested powder dose of 2.5 g/kg caused lower than 100% mortality to *C. maculatus* (6-d) and *S. zeamais* (7-d) for this combination while the other two combination proportions caused complete mortality . The highest tested dose (20 ml/kg) achieved complete mortality of both weevils three days post exposure for all mixtures, except the 25% NeemAzal + 75% *P. glandulosus* leaf powder which caused a maximum mortality of 87.50% in *S. zeamais*.

3.5.4 Dosage-mortality response relationships of binary combinations of NeemAzal and *Plectranthus glandulosus* leaf powder

The toxicity parameters of the binary mixture of *P. glandulosus* and NeemAzal powders to *C. maculatus* and *S. zeamais* are given in (Table 3.26). The 3-d LC_{50} values decreased with ascending proportion of NeemAzal in the mixture from 3.21 g/kg (25% NeemAzal + 75% *P. glandulosus*) to 0.24 g/kg (75% NeemAzal + 25% *P. glandulosus*) for *S. zeamais*. With *C. maculatus*, the opposite effect was observed, the LC_{50} values increased as the quantity of NeemAzal in the mixture increased. When the proportion of NeemAzal was $\geq$ 50% the LC_{50} and LC_{95} LC were not estimated due to complete adult mortality. The slopes seemed similar (1.24 – 1.51) for all the combinations of the powders in *S. zeamais* while they decreased (18.82 – 1.45) with increase in the quantity of *P. glandulosus* in the mixture. The coefficients

Table 3.25a: Toxicity of binary combinations of powders from *Plectranthus glandulosus* leaves and *Azadirachta indica* seeds at different proportions to adult *Callosobruchus maculatus*

Insects/ product proportion	Slope ± S.E	R^2	LC_{50} (95% FL)[c]	LC_{95} (95% FL)[a £]	Co-toxicity coefficient	χ^{2} [b]
C. maculatus						
			3 days			
100% *P.gland*. + 0% *A. indica*	3.49 ± 1.57	0.69	55.80 (31.40 - 4.43E^{5})	11.00 (8.22 -17.78)		1.55^{ns}
75% *P.gland*. + 25% *A. indica*	6.06 ± 2.77	0.50	35.70 (25.84 - 9887.30)	66.66 (36.16 - 3.84E^{6})	1.62	0.06^{ns}
50% *P.gland*. + 50% *A. indica*	2.50 ± 1.20	0.64	95.06(39.19 - 1.59E^{14}) [£]	430.96 (86.35 - 1.17E^{25})	0.62	0.97^{ns}
25% *P.gland*. + 75% *A. indica*	-	-	-	-	-	-
0% *P.gland*. + 100 % *A. indica*	3.48 ± 1.84	0.68	61.93	183.75		0.90^{ns}
			6 days			
100% *P.gland*. + 0% *A. indica*	1.97 ± 0.26	0.97	25.01 (20.11 – 35.01)	173.84 (96.60 -471.19)		6.17^{ns}
75% *P.gland*. + 25% *A. indica*	2.13 ± 0.28	0.96	24.63 (20.14 - 33.53)	145.44 (85.28 - 356.53)	1.24	4.07^{ns}
50% *P.gland*. + 50% *A. indica*	1.41 ± 0.22	0.81	34.63 (24.55 - 63.49)	503.58 (195.95 - 2998)	1.20	5.52^{ns}
25% *P.gland*. + 75% *A. indica*	2.16 ± 0.34	0. 92	34.48 (26.14 - 56.48)	199.56 (102.01 – 709.58)	1.61	1.56^{ns}
0% *P.gland*. + 100% *A. indica*	2.00 ± 0.61	0.72	93.54 (44.80 - 1723)	919.34 (147.48 - 2.06E^{5})		2.11^{ns}

[a] FL = Fiducial limit;

[b] ns: P>0.05, ***P<0.0001.

[£] The LC values were given by extrapolation

- LC values not estimated because of the absence of mortality

Table 3.25b: Toxicity of binary combinations of powders from *Plectranthus glandulosus* leaves and *Azadirachta indica* seeds at different proportions to adult *Sitophilus zeamais*

Insects/ product proportion	Slope ± S.E	R^2	LC_{50} (95% FL)[c]	LC_{95} (95% FL)[a £]	Co-toxicity coefficient	χ^{2} [b]
S. zeamais						
			7 days			
100% *P.gland*. + 0% *A. indica*	2.82 ± 0.54	0.87	3.56 (1.04 – 5.66)	13.02 (8.05 – 107.92)		17.54***
75% *P.gland*. + 25% *A. indica*	4.32 ± 1.08	0.67	32.71 (25.76 - 63.32)	78.51 (46.87 - 349.31)	0.13	0.04ns
50% *P.gland*. + 50% *A. indica*	1.46 ± 0.64	0.55	49.47	660.73	0.11	19.08 ***
25% *P.gland*. + 75% *A. indica*	1.55 ± 0.32	0.77	73.80 (41.80 - 275.27) £	839.59 (238.57 - 16996)	0.10	4.44ns
0% *P.gland*. + 100% *A. indica*	2.59 ± 0.54	0.88	11.00 (6.49 – 23.63)	49.01 (23.28 - 2068)		17.94***
			14 days			
100 % *P.gland*. + 0% *A. indica*	2.97 ± 0.33	0.85	2.41 (1.73 – 2.60)	8.62 (7.26 – 10.78)		4.01ns
75% *P.gland*. + 25% *A. indica*	2.88 ± 0.97	0.71	18.05	67.37	0.16	30.89***
50% *P.gland*. + 50% *A. indica*	1.67 ± 0.20	0.97	10.66 (7.95 - 13.59)	134.94 (79.49 - 379.72)	0.25	4.04ns
25% *P.gland*. + 75% *A. indica*	2.17 ± 0.27	0.88	22.93 (19.03 - 30.11)	130.77 (79.98 - 290.40)	0.20	3.71ns
0% *P.gland*. + 100% *A. indica*	2.60 ± 0.75	0.88	6.91 (0.60 – 21.10)	29.64 (13.25 – 1.1.50E^{8})		38.34***

[a] FL = Fiducial limit;

[b] ns: P>0.05, ***P<0.0001.

[£] The LC values were given by extrapolation

- LC values not estimated because of the absence of mortality

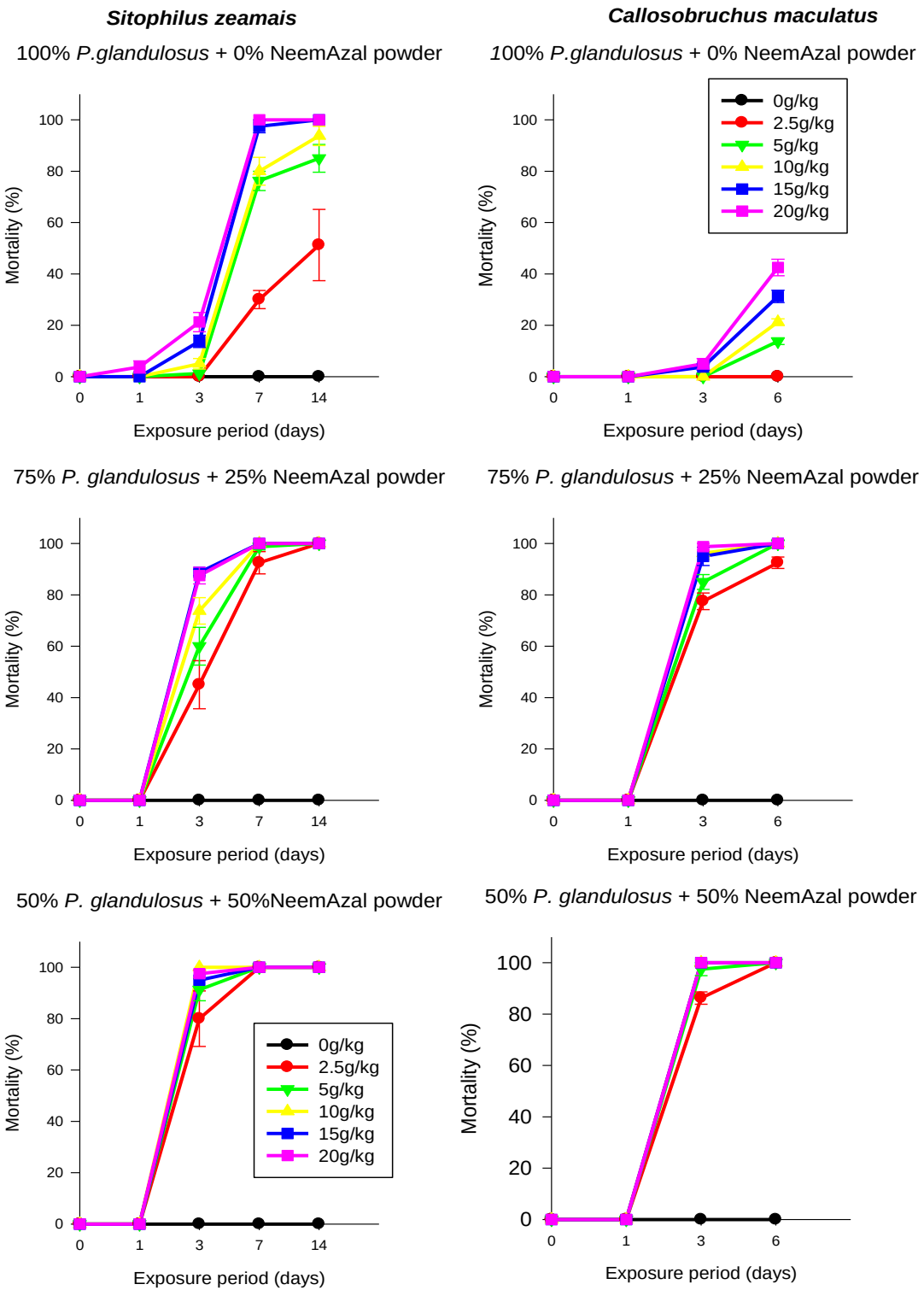


Figure 3.10: Corrected cumulative mortality (mean ± SE) of *Callosobruchus maculatus* and *Sitophilus zeamais* exposed to binary combinations of NeemAzal and *Plectranthus glandulous* leaf powder

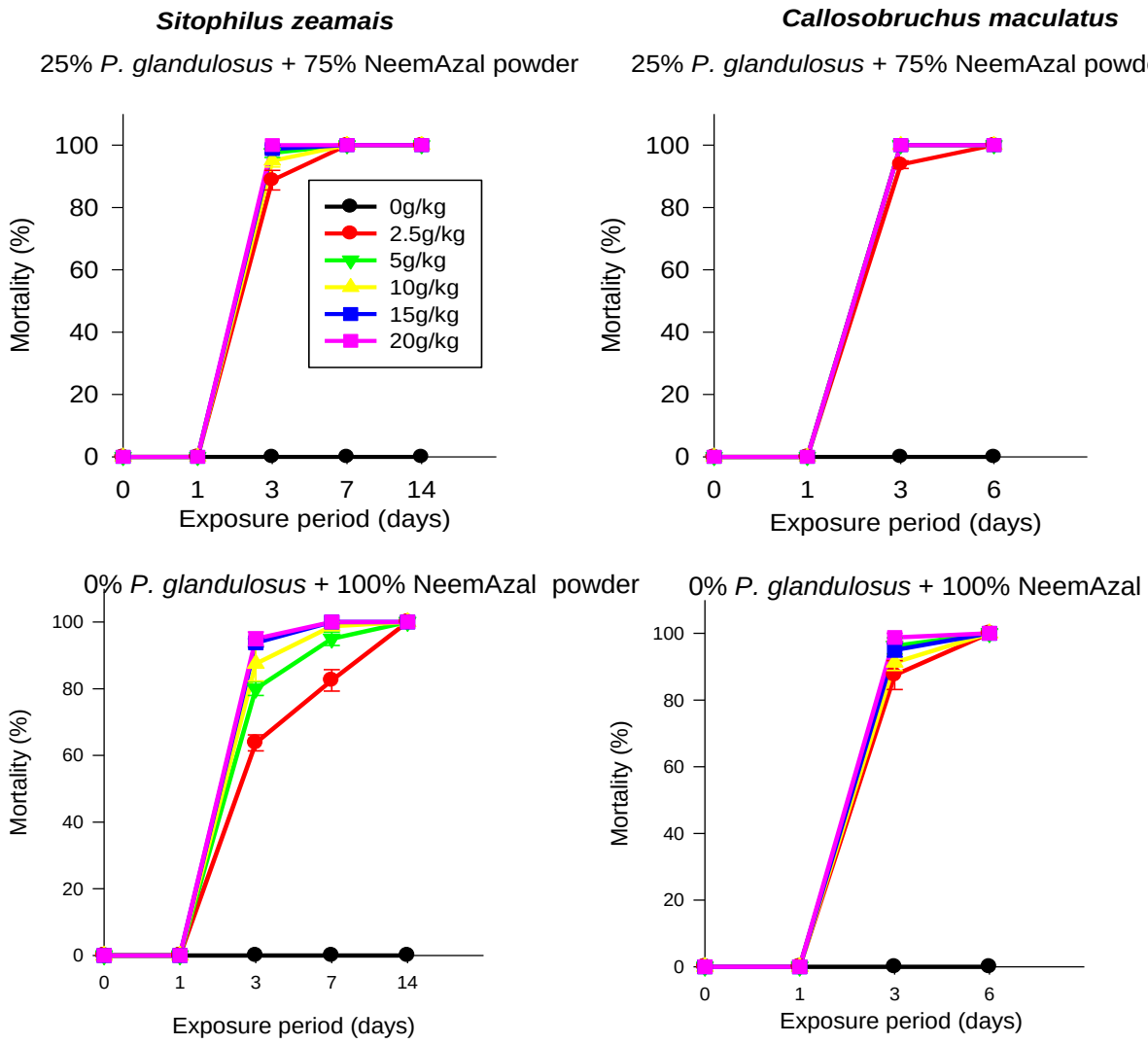


Figure 3.10 cont'd

of determination (R^2) of the powder combinations ranged from 0.58 to 0.97. All the estimated co-toxicity coefficients were less than 80. The values of chi-square ($\chi 2$) were not significant.

Table 3.26a: Toxicity of binary combinations of NeemAzal and *Plectranthus glandulosus* leaf powder at different proportions to adult *Callosobruchus maculatus*

Insects/ product proportion	Slope ± S.E	R^2	LC_{50} (95% FL)[a]	LC_{95} (95% FL)[a]	Co-toxicity coefficient	$\chi^{2\,b}$
C. maculatus						
			3 days			
100% *P.gland*. + 0% NeemAzal	3.49 ± 1.57	0.69	55.80 (31.40 - 4.43E^5)	11.00 (8.22 -17.78)		1.55^{ns}
75% *P.gland*. + 25% NeemAzal	1.45 ± 0.25	0.94	0.81 (0.27 - 1.39)	11.00 (8.22 -17.78)	0.20	2.64^{ns}
50% *P.gland*. + 50% NeemAzal	3.20 ± 0.84	0.73	1.15 (0.39 -1.66)	3.76 (3.14 - 5.29)	0.07	0.25^{ns}
25% *P.gland*. + 75% NeemAzal	18.82 ± -1.62E^9	0.58	2.07	2.53	0.17	3.81^{ns}
0% *P.gland*. + 100% NeemAzal	0.70 ± 0.40	0.52	0.04	10.34		6.68^{ns}
			6 days			
100% *P.gland*. + 0% NeemAzal	1.97 ± 0.26	0.97	25.01 (20.11 – 35.01)	173.84 (96.60 -471.19)		6.17^{ns}
75% *P.gland*. + 25% NeemAzal	19.13 ± -1.06E^9	0.58	0.10	2.56	0	0.00^{ns}
50% *P.gland*. + 50% NeemAzal[£]	-	-	-	-	-	
25% *P.gland*. + 75% NeemAzal[£]	-	-	-	-	-	
0% *P.gland*. + 100% NeemAzal[£]	-	-	-	-	-	

[a] FL = Fiducial limits;

[b] ns: P>0.05;

[£] Toxicity parameters were not determinate due to 100% mortality

Table 3.26b: Toxicity of binary combinations of NeemAzal and *Plectranthus glandulosus* leaf powder at different proportions to adult *Sitophilus zeamais*

Insects/ product proportion	Slope ± S.E	R^2	LC_{50} (95% FL)[a]	LC_{95} (95% FL)[a]	Co-toxicity coefficient	$\chi^{2\,b}$
S . zeamais						
			3 days			
100% *P.gland*. + 0% NeemAzal	2.84 ± 0.52	0.84	40.23 (29.25 – 80.77)	171.52 (84.11 -872.97)		0.34^{ns}
75% *P.gland*. + 25% NeemAzal	1.51 ± 0.19	0.97	3.21 (2.30 - 4.05)	39.22 (26.90 - 71.36)	1.57	2.38^{ns}
50% *P.gland*. + 50% NeemAzal	1.33 ± 0.43	0.72	0.51	8.90	5.17	7.51^{ns}
25% *P.gland*. + 75% NeemAzal	1.24 ± 0.34	0.72	0.24 (0.01 - 0.74)	5.13 (3.20 - 8.13)	7.63	5.80^{ns}
0% *P.gland*. + 100% NeemAzal	1.42 ± 0.31	0.96	1.39 (0.69 – 2.07)	19.68 (14.14 -34.30)		0.42^{ns}
			7 days			
100% *P.gland*. + 0% NeemAzal	1.45 ± 0.25	0.94	3.56 (1.04 – 5.66)	13.02 (8.05 -107.92)		17.54^{***}
75% *P.gland*. + 25% NeemAzal	2.95 ± 1.08	0.72	0.81 (0.03 - 1.45)	2.96 (2.05 - 4.20)	2.75	0.13^{ns}
50% *P.gland*. + 50% NeemAzal[£]	-	-	-	-	-	
25% *P.gland*. + 75% NeemAzal[£]	-	-	-	-	-	
0% *P.gland*. + 100% NeemAzal[£]	2.45± 0.49	0.82	1.05 (0.45 – 1.56)	4.92 (3.97- 6.75)	-	0.55^{ns}

[a] FL = Fiducial limits;

[b] ns: P>0.05;

[£] Toxicity parameters were not determinate due to 100% mortality

3.5.5 Effect of binary combinations of powders from *Azadirachta indica* seeds and *Plectranthus glandulosus* leaves on F_1 progeny production

Table 3.27 shows the number of F_1 progeny and percentage of progeny inhibition of *C. maculatus* and *S. zeamais* that emerged from grains treated with different combinations of *A. indica* and *P. glandulosus* at different doses. The number of F_1 progeny produced reduced significantly in both insect species with increase in doses. The inhibitory potential on progeny production for *C. maculatus* decreased in the order: 100% *A. indica* > 25% *P. glandulosus* + 75% *A. indica* > 50% *P. glandulosus* + 50% *A. indica*, 75% *P. glandulosus* + 25% *A. indica* > 100% *P. glandulosus*, and for *S. zeamais*: 100% *A. indica* > 25% *P. glandulosus* + 75 *A. indica* > 50% *P. glandulosus* + 50% *A. indica*, 100% *P. glandulosus* > 75% *P. glandulosus* + 25% *A. indica*. Only ≥ 10 g/kg of *A. indica* completely suppressed progeny emergence in *C. maculatus* and *S. zeamais*. The minimum number of emerged adults found in cowpea and maize treated with the binary combinations of the powders was 2.25 *C. maculatus* and 0.75 *S. zeamais* (20 g/kg of 25% *P. glandulosus* + 75% *A. indica*), respectively. Overall, the percentage reduction of F_1 progeny was above 50% in all mixed proportions at the dose ≥ 10 g/kg excluding the mixed powder of 75% *P. glandulosus* + 25% *A. indica* on maize where only 38.28% of F_1 offspring emergence was recorded.

3.5.6 Effect of binary combinations of NeemAzal and *Plectranthus glandulosus* leaf powder on F_1 progeny production

The number of F_1 progeny and the percentage inhibition of the progeny of *C. maculatus* and *S. zeamais* that emerging from grains treated with different combinations of NeemAzal and *P. glandulosus* at different doses are shown in Table 3.28. The number of emerging F_1 progeny reduced significantly ($P \leq 0.01$) in both insect species with ascending of the botanicals. The binary combinations of the powders reduced progeny emergency more than each botanical applied alone, with NeemAzal being more potent than *P. glandulosus*. All the binary combinations of the powders completely suppressed progeny production in *S. zeamais*. On cowpea 97.77%, 97.15% and 66.78% inhibition of *C. maculatus* emergence were recorded respectively with the combinations 25% *P. glandulosus* + 75% NeemAzal, 50% *P. glandulosus* + 50% NeemAzal and 75% *P. glandulosus* + 25% NeemAzal at the highest tested dose of 20 g/kg.

Table 3.27: Progeny production of *Callosobruchus maculatus* and *Sitophilus zeamais* in grains treated with binary combinations of powders from *Plectranthus glandulosus* leaves and *Azadirachta indica* seeds

Insects/ doses (g/kg)	Proportion of powders in mixture					
	100% *P. glandulosus*	75% *P. gland* + 25% *A. indica*	50% *P. gland* + 50% *A. indica*	25% *P. gland* + 75% *A. indica*	100% *A. indica*	$F_{(4, 15)}$ ‡
	Number (mean ± SE) of F_1 adult progeny †					
C. maculatus						
0	443.50 ± 15.61^{a}	403.25 ± 8.89^{a}	403.25 ± 8.89^{a}	403.25 ± 8.89^{a}	439.50± 13.99^{a}	3.25^{ns}
2.5	409.50 ± 3.01^{aA}	311.75 ± 34.92^{abA}	307.75 ± 35.24^{abA}	171 ± 22.69^{bB}	16.25 ± 1.70^{bC}	69.38***
5	355.75 ± 13.77^{abA}	206.50 ± 43.81^{bcA}	280.25 ± 45.96^{bA}	75.75 ± 2.48^{cB}	1.25 ± 0.75^{cC}	40.23***
10	283.75 ± 31.76^{abA}	168.25 ± 24.68^{cB}	211.50± 18.42^{bcAB}	29.75 ± 4.17^{cdC}	0.00 ± 0.00^{cD}	88.91***
15	260.25 ± 50.34^{abA}	174.25 ± 7.19^{cA}	162.50 ± 7.19^{cdA}	5.50 ± 1.94^{dB}	0.00 ± 0.00^{cB}	67.47***
20	206.50 ± 56.16^{bA}	162.00 ± 10.51^{cA}	114.00 ± 10.93^{dAB}	2.25 ± 0.63^{eBC}	0.00 ± 0.00^{cC}	29.38***
$F_{(5,18)}$ ‡	4.43**	12.35***	19.67***	87.43***	1611.21***	
S. zeamais						
0	51.25 ± 0.95^{aBC}	57.50 ± 2.99^{aAB}	57.75 ± 2.39^{aAB}	63.50 ± 2.78^{aA}	42.50 ±1.66^{aC}	13.32***
2.5	36.75 ± 2.25$^{a\ A}$	43.75 ± 6.03^{aA}	11.25 ± 2.17^{bB}	19.00 ± 4.06^{bB}	8.00 ± 0.91^{bB}	20.97***
5	22.00 ± 4.71^{bAB}	35.50 ± 5.84^{abA}	8.00 ± 1.00^{bcB}	11.00 ± 0.00^{bcB}	9.00 ± 2.21^{bB}	5.70**
10	6.00 ± 0.91^{cB}	35.25 ± 2.84^{abAB}	5.00 ± 2.27^{bcBC}	4.75 ± 0.00^{cdBC}	0.00 ± 0.00^{cC}	29.03***
15	3.00 ± 0.71^{cB}	21.00 ± 5.80^{bA}	3.50 ± 1.44^{cB}	2.75 ± 0.63^{cdB}	0.00 ± 0.00^{cB}	34.59***
20	1.75 ± 1.03^{cB}	16.50 ± 3.84^{bA}	3.25 ± 0.85^{cB}	0.75 ± 0.75^{dB}	0.00 ± 0.00^{cB}	19.04***
$F_{(5,18)}$ ‡	63.83***	8.89***	46.24***	48.58***	30.97***	
	Percentage (mean ± SE) reduction in adult emergence relative to control †					
C. maculatus						
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	
2.5	7.88 ± 3.21^{cdC}	22.46 ± 8.89^{bC}	23.91 ± 7.74^{cC}	57.23 ± 6.36^{cB}	96.26 ± 0.51^{bA}	30.63***
5	19.35 ± 4.99^{bcC}	48.40 ± 11.82^{abBC}	31.00 ± 10.06^{bcC}	81.08 ± 6.80^{bAB}	99.71 ± 0.18^{aA}	20.29***
10	36.24 ± 6.45^{abD}	58.02 ± 6.55^{aC}	47.45 ± 4.76^{abcCD}	92.680± 0.88^{abB}	100 ± 0.00^{aA}	65.47***
15	41.93 ± 10.52^{abB}	56.67 ± 2.42^{aB}	59.70 ± 2.41^{abB}	98.64 ± 0.49^{aA}	100 ± 0.00^{aA}	56.71***
20	54.18 ± 12.18^{aB}	59.87 ± 2.14^{aB}	71.55 ± 3.23^{aB}	99.44 ± 0.16^{aA}	100 ± 0.00^{aA}	28.49
$F_{(5, 18)}$ ‡	13.11***	22.31***	21.17***	150.01***	3514.10***	
S. zeamais						
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	
2.5	28.14 ± 4.90^{cB}	23.25 ± 11.19^{bB}	80.80 ± 3.02^{bA}	88.81 ± 1.19^{bcA}	81.25 ± 1.78^{bA}	17.08***
5	56.71± 9.53^{bAB}	38.86 ± 7.76^{abB}	86.03 ± 1.96^{abA}	82.40±4.69^{cAB}	76.85 ± 8.60^{bAB}	3.66*
10	88.30 ± 1.78^{aB}	38.28 ± 5.75^{abC}	90.81 ± 4.45^{abB}	92.96 ± 3.93^{abAB}	100 ± 0.00^{aA}	35.45***
15	94.08 ± 1.43^{aA}	63.29 ± 10.21^{aB}	94.05 ± 2.34^{aA}	95.72 ± 0.90^{abA}	100 ± 0.00^{aA}	14.12***
20	96.65 ± 1.96^{aA}	70.88 ± 7.30^{aB}	94.65 ± 3.23^{aA}	98.84 ± 1.06^{aA}	100 ± 0.00^{aA}	16.09***
$F_{(5, 18)}$ ‡	86.50***	17.40***	109.11***	1245.03***	45.54***	

† Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ** $P < 0.01$; *** $P < 0.001$.

P. gland = Plectranthus glandulosus.

Table 3.28: Progeny production of *Callosobruchus maculatus* and *Sitophilus zeamais* in grains treated with binary combinations of *Plectranthus glandulosus* leaf powder and NeemAzal

Insects/ doses (g/kg)	Proportion of powders in mixture					
	100% *P. glandulosus*	75% *P. gland* + 25% NeemAzal	50% *P. gland* + 50% NeemAzal	25% *P. gland* + 75% NeemAzal	100% NeemAzal	$F_{(5, 19)}$ ‡
Number (mean ± SE) of F_1 adult progeny †						
C. maculatus						
0	443.50 ± 15.61^{a}	439.50 ± 13.36^{a}	439.50 ± 13.36^{a}	439.50 ± 13.36^{a}	439.50 ± 13.36^{a}	0.02^{ns}
2.5	409.50 ± 3.01^{aA}	299.75 ± 36.67^{abB}	35.25 ± 1.65^{bD}	30.75 ± 3.88^{bD}	97.00 ± 8.60^{bC}	146.42***
5	355.75 ± 13.77^{abA}	250.50 ± 43.84^{bcA}	27.50 ± 1.71^{bcB}	20.50 ± 3.77^{bcB}	58.00 ± 12.46^{bcB}	68.11***
10	283.75 ± 31.76^{abA}	201.50 ± 23.06^{bcA}	18.00 ± 4.04^{cdB}	18.00 ± 2.08^{bcB}	35.50 ± 8.53^{cdB}	69.85***
15	260.25 ± 50.34^{abA}	144.50 ± 13.32^{cB}	12.75 ± 1.55^{dC}	11.00 ± 2.27^{cC}	17.25 ± 4.17^{dC}	44.10***
20	206.50 ± 56.16^{bA}	146.00 ± 5.48^{cA}	12.50 ± 1.55^{dB}	9.75 ± 2.17^{cB}	11.75 ± 1.49^{dB}	21.26***
$F_{(5,18)}$ ‡	4.43**	15.84***	647.93***	407.35***	143.42***	
S. zeamais						
0	51.25 ± 0.95^{aB}	69.25 ± 2.10^{aA}	62.75 ± 1.65^{aA}	63.75 ± 2.95^{aA}	65.25 ± 2.53^{aA}	9.79**
2.5	36.75 ± 2.25$^{a\ A}$	0.75 ± 0.75^{bC}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	6.00 ± 0.71^{bB}	194.11***
5	22.00 ± 4.71^{bA}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	4.50 ± 1.32^{bcB}	40.93***
10	6.00 ± 0.91^{cA}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bC}	1.25 ± 0.48^{bcB}	41.44***
15	3.00 ± 0.71^{cA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{cB}	28.37***
20	1.75 ± 1.03^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	2.92^{ns}
$F_{(5,18)}$ ‡	63.83***	570.87***	1442.77***	465.57***	277.34***	
Percentage (mean ± SE) reduction in adult emergence relative to control †						
C. maculatus						
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	
2.5	7.88 ± 3.21^{cdC}	31.84 ± 8.08^{cB}	91.97 ± 0.35^{cA}	92.99± 0.93^{bA}	77.84 ± 2.26^{cA}	73.68***
5	19.35 ± 4.99^{bcC}	43.00 ± 9.96^{bcB}	93.71± 0.51^{bcA}	95.34 ± 0.85^{abA}	86.76 ± 2.96^{bA}	47.23***
10	36.24 ± 6.45^{abC}	56.16 ± 5.17^{abA}	95.83 ± 1.04^{abB}	95.93 ± 0.37^{abB}	91.75 ± 2.17^{abB}	60.26***
15	41.93 ± 10.52^{abC}	67.28 ± 2.01^{aB}	97.11 ± 0.24^{aA}	97.46 ± 2.27^{abA}	96.00 ± 1.07^{aA}	38.86***
20	54.18 ± 12.18^{aB}	66.78 ± 0.74^{aB}	97.15 ± 0.35^{aA}	97.77 ± 0.58^{abA}	97.31 ± 0.37^{aA}	20.07***
$F_{(5, 18)}$ ‡	13.11***	35.05***	1947.02***	1245.03***	338.90***	
S. zeamais						
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00d^{d}	
2.5	28.14 ± 4.90^{cC}	98.96 ± 1.04^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	90.68 ± 1.43^{cB}	148.34***
5	56.71 ± 9.53^{bC}	100 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{a}	93.21 ± 1.89^{cB}	41.78***
10	88.30 ± 1.78^{aC}	100 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	98.05 ± 0.78^{bB}	44.25***
15	94.08 ± 1.43^{aB}	100 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00± 0.00^{aA}	45.92***
20	96.65 ± 1.96^{a}	100 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	2.98^{ns}
$F_{(5,18)}$ ‡	86.50***	922.23***	∞***	∞ ***	579.71***	

† Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$; *** $P < 0.001$.

P. gland = Plectranthus glandulosus.

3.5.7 Dosage-F_1 progeny inhibition response relationship of binary combinations of *Plecthranthus glandulosus* with NeemAzal and *Azadirachta indica* powders

The results of evaluation of the ability of binary combinations of powders to inhibit F_1 progeny production are shown in Table 3.29. The EC_{50} and EC_{95} values appeared to be lower for the *P. glandulosus* –NeemAzal mixture than those of the *P. glandulosus – A. indica*. Due to total emergence inhibition of *S. zeamais*, the estimation of the EC values were not possible when the mixtures of the 50% *P. glandulosus* + 50% NeemAzal and 25% *P. glandulosus* + 75% NeemAzal were applied on maize. The 50% *P. glandulosus* + 50% *A. indica* EC_{50} and EC_{95} (0.24 and 21.72 g/kg, respectively) were smaller on *S. zeamais* than on *C. maculatus* (9.61 and 142.14 g/kg respectively). The 75% *P. glandulosus* + 25 NeemAzal powder slope (16.70) were higher compared to other mixtures where the slopes were ranged between 0.57 and 2.39. Except the R^2 value for the combination 75% *P. glandulosus* + 25 NeemAzal on maize (0.58) all R^2 values were greater than 0.80. In general, the χ^2 values were not significant.

3.5.8 Effect of the binary combinations of powders from *Plectranthus glandulosus* leaves and *Azadirachta indica* seeds on grain damage and weight loss caused by *Callosobruchus maculatus* and *Sitophilus zeamais*

Generally, damage caused by *C. maculatus* to treated cowpea seeds, as well as the resulting weight losses were higher than those caused to maize by *S. zeamais*, irrespective of the proportion of each powder in the binary combinations (Tables 3.30). *A. indica* powder alone was more efficient in reducing damage and weight loss in maize and cowpea caused their respective insect pests than *P. glandulosus* powder alone and the two binary combinations of the botanicals. The grains treated with *A. indica* powder alone incurred little or no damage from both insect species. Although smaller than that of the control, the damage caused by C. *maculatus* to the cowpea grains that were treated by *P. glandulosus* alone or the two binary combinations, were heavy, with a range of between 58.75% (20 g/kg 75% *P. glandulosus* + 25% *A. indica*) and 98.75 (2.5 g/kg *P. glandulosus* alone) for grain damage, and with similar trends for weight loss. However, *P. glandulosus* alone and the two binary combinations of the two botanicals greatly reduced the damage caused by *S. zeamais* to maize, especially when the dose level was $\geq$ 10 g/kg.

Table 3.29: Effective concentration resulting in 50% (EC_{50}) and 95% (EC_{95}) reduction of *Callosobruchus maculatus* and *Sitophilus zeamais* F_1 progeny emergence in grains treated with binary combinations of *Plectranthus glandulosus* with *A. indica* and NeemAzal powders

Product/ particle size	Slope ± S.E	R^2	EC_{50} (95% FL)[a]	EC_{95} (95% FL)[a]	χ^{2} [b]
***P. glandulosus* + NeemAzal powder**					
			C. maculatus		
100% *P.g.* + 0% Nz	1.63 ± 0.21	0.97	17.81 (14.63 – 23.48)	180.00 (99.56 – 485.44)	0.82^{ns}
75% *P.g.* + 25% Nz	1.07 ± 0.17	0.98	7.11 (5.39 – 9.09)	245.83 (104.17 - 1290)	0.96^{ns}
50% *P.g.* + 50% Nz	0.59 ± 0.28	0.98	0.01 (1.13E^{-7} - 0.27)	6.84 (0.69 - 103.68)	0. 08^{ns}
25% *P.g.* + 75% Nz	0.57 ± 0.30	0.95	0.01	4.95	0.16^{ns}
0% *P.g.* + 100% Nz	1.22 ± 0.24	0.94	0.01 (0.13 – 1.21)	13.47 (9.53 – 25.47)	0.44^{ns}
			S. zeamais		
100% *P.g.* + 0% Nz	2.78 ± 0.23	0.95	4.10 (3.58 – 4.64)	15.97 (13.42 - 20.03)	0.88 ^{ns}
75% *P.g.* + 25% Nz	16.70 ± 0.24	0.58	1.81	2.28	0.00^{ns}
50% *P.g.* + 50% Nz	-	-	-	-	-
25% *P.g.* + 75% Nz	-	-	-	-	-
0% *P.g.* + 100% Nz	1.63 ± 0.41	0.97	0.45 (0.04 – 1.00)	4.60 (2.71 – 5.70)	2.93^{ns}
***P. glandulosus* + *A. indica* seed powder**					
			C. maculatus		
100% *P.g.* + 0% *A. i.*	1.63 ± 0.21	0.97	17.81 (14.63 – 23.48)	180.00 (99.56 – 485.44)	0.82^{ns}
75% *P.g.* + 25% *A. i.*	1.00 ± 0.27	0.81	8.76 (2.59 - 38.63)	378.11 (60.10 -1.72E^{14})	7.27 ^{ns}
50% *P.g.* + 50% *A. i.*	1.40 ± 0.18	0.97	9.61 (7.98 - 11.79)	142.14 (78.26 - 382.87)	2.60 ^{ns}
25% *P.g.* + 75% *A. i.*	2.39 ± 0.28	0. 92	2.13 (1.59 – 2.63)	10.37 (8.52 - 13.71)	1.21 ^{ns}
0 % *P.g.* +100% *A. i.*	3.32 ± 1.99	0.79	0.72	2.27	0.01 ^{ns}
			S. zeamais		
100% *P.g.* + 0% *A. i.*	2.78 ± 0.23	0.95	4.10 (3.58 – 4.64)	15.97 (13.42 - 20.03)	0.88 ^{ns}
75% *P.g.* + 25% *A. i.*	1.33 ± 0.50	0.87	9.73 (11.41 - 44.22)	166.40 (44.37 -5.92E^{5})	8.37 *
50% *P.g.* + 50% *A. i.*	0.84 ± 0.23	0.99	0.24 (0.05 - 0.80)	21.72 (12.72 - 94.64)	0.13^{ns}
25% *P.g.* + 75% *A. i.*	1.71 ± 0.25	0.96	1.33 (0.72 -1.92)	12.13 (9.37 – 18.02)	0.98^{ns}
0% *P.g.* + 100% *A. i.*	2.17 ± 1.00	0.76	1.31	7.49	25.73 ***

a FL = Fiducial limit;

b ns: P>0.05, * P<0.05.

£ The LC values were given by extrapolation

P.g = Plectranthus glandulosus, Nz = NeemAzal, *A. i = Azadirachta indica*

- no adult emerged

3.5.9 Effect of the binary combinations of *Plectranthus glandulosus* leaf powder and NeemAzal on grain damage and weight loss caused by *Callosobruchus maculatus* and *Sitophilus zeamais*

As expected, like for the case in section 3.5.8 involving *A. indica* seed powder, damage caused by *C. maculatus* to treated cowpea seeds, as well as the resulting weight losses were higher than those caused to maize by *S. zeamais*, irrespective of the proportion of each powder in the binary combinations (Tables 3.31). NeemAzal alone and the binary combination 50% *P. glandulosus* + 50% A. indica were more efficient in reducing damage and weight loss in cowpea caused by *C. maculatus* than *P. glandulosus* powder alone and the combination 75% *P. glandulosus* + 25% NeemAzal. *P. glandulosus* alone was less efficient in reducing maize damage due to *S. zeamais* infestations compared with NeemAzal and the two binary combinations of the botanical powders, in which little or no grain damage and weight loss were recorded with the different doses. On the contrary, cowpea grains treated even with the highest dose of *P. glandulosus* alone and the combination 75% *P. glandulosus* + 25 NeemAzal suffered serious grain damage (range 92.25% - 98.75%) and weight loss (range 35.72% - 55.94%) from *C. maculatus* infestations. No maize grains were damage with the combinations 75% *P. glandulosus* and 25% NeemAzal when the dose was ≥ 15 g/kg and 50% *P. glandulosus* and 50% Neemazal, when the dose was ≥ 10 g/kg.

3.5.10 Persistence of the binary combinations of *Plectranthus glandulosus* with *Azadirachta indica* and NeemAzal powders on adult *Callosobruchus maculatus* and *Sitophilus zeamais*

Figure 3.11 shows the results of the persistence of the mixture of 75% *P. glandulosus* + 25% NeemAzal and 50% *P. glandulosus* + 50% *A. indica* powders on *C. maculatus* and *S. zeamais*. The efficacy of the mixture varied significantly ($P < 0.001$) with the ascending dose and also with the storage interval of the treated grains except the 75% *P. glandulosus* + 25% NeemAzal powder on cowpea in which the efficiency persisted up to the 180-d storage interval. For this binary combination, the mortality caused to *S. zeamais* and *C. maculatus* at the 180-d storage interval did not differ from the observed mortality at the 0-d storage interval ($P > 0.05$) for all the dose levels. The bioactivity of the 50% *P. glandulosus* + 50% *A. indica* powder decreased significantly after 15 days of storage in both insect species and then became inactive. At the highest dose (20 g/kg), 0 day storage interval of the treated grains, adult mortality of cowpea or maize weevils decreased from 16.25% and 28.75% respectively to no adult mortality at the 60-d storage interval.

Table 3.30: Grain damage and weight loss caused by *Callosobruchus maculatus* and *Sitophilus zeamais* in grains treated with binary combinations of powders from *Plectranthus glandulosus* leaves and *Azadirachta indica* seeds, and then stored for 10 weeks

Insects/ Doses (g/kg)	Proportion of powders in mixture				
	100% *P. glandulosus*	75% *P. gland* + 25% *A. indica*	50% *P. gland* + 50% *A. indica*	100% *A. indica*	$F_{(3, 15)}$ ‡
	Mean (± SE) grain damage (%) †				
C. maculatus					
0	98.00 ± 1.00^{a}	98.00 ± 1.00^{a}	98.00 ± 1.00^{a}	98.00 ± 1.00^{a}	
2.5	98.75 ± 0.25^{aA}	96.00 ± 1.08^{abA}	97.00 ± 0.41^{aA}	7.25 ± 1.89^{bB}	483.84^{***}
5	98.50 ± 0.50^{abA}	92.50 ± 0.65^{abcB}	97.98 ± 0.73^{aA}	2.25 ± 1.11^{cC}	467.60^{***}
10	98.75 ± 0.25^{abA}	92.00 ± 0.71^{bcC}	95.50 ± 0.50^{aB}	0.00 ± 0.00^{cD}	4336.51^{***}
15	96.50 ± 0.95^{abA}	86.25 ± 2.43^{cB}	96.75 ± 0.63^{aA}	0.00 ± 0.00^{cC}	750.00^{***}
20	95.50 ± 0.96^{bA}	58.75 ± 5.30^{dB}	95.75 ± 1.44^{aA}	0.00 ± 0.00^{cC}	358.94^{***}
$F_{(5,18)}$ ‡	3.64^{*}	37.07^{***}	1.87^{ns}	400.91^{***}	
S. zeamais					
0	50.00 ± 1.73^{a}	50.00 ± 1.73^{a}	50.00 ± 1.73^{a}	50.00 ± 1.73^{a}	
2.5	$47.50 \pm 6.38^{a\,A}$	25.50 ± 4.50^{abAB}	25.75 ± 2.02^{bB}	4.00 ± 0.71^{bC}	30.57^{***}
5	55.50 ± 1.44^{aA}	18.50 ± 4.84^{bB}	23.00 ± 2.48^{bB}	2.25 ± 0.48^{bcC}	67.84^{***}
10	35.25 ± 8.40^{abA}	12.00 ± 5.77^{bAB}	16.50 ± 2.72^{bcA}	1.50 ± 1.50^{bcB}	9.37^{**}
15	11.75 ± 4.03^{cAB}	7.50 ± 4.50^{bAB}	14.75 ± 2.43^{bcA}	1.75 ± 0.85^{bcB}	3.89^{*}
20	13.25 ± 2.78^{bcA}	6.50 ± 2.53^{bB}	10.75 ± 2.46^{cA}	0.25 ± 0.25^{cB}	14.92^{***}
$F_{(5, 18)}$ ‡	11.92^{***}	11.75^{***}	26.17^{***}	66.31^{***}	
	Mean (± SE) weight loss (%) †				
C. maculatus					
0	47.36 ± 3.08^{ab}	47.36 ± 3.08^{a}	47.36 ± 3.08^{a}	47.36 ± 3.08^{a}	
2.5	55.94 ± 2.14^{aA}	25.22 ± 8.40^{abB}	46.01 ± 5.50^{aAB}	1.57 ± 0.34^{bC}	22.01^{***}
5	54.20 ± 4.04^{abA}	28.91 ± 3.14^{abB}	53.97 ± 8.27^{aA}	0.41 ± 0.22^{bcC}	50.97^{***}
10	44.52 ± 2.49^{aA}	37.54 ± 3.72^{aA}	49.47 ± 7.33^{aA}	0.00 ± 0.00^{cB}	67.71^{***}
15	46.95 ± 3.11^{abA}	25.05 ± 4.41^{abB}	44.79 ± 4.66^{aA}	0.00 ± 0.00^{cC}	82.39^{***}
20	42.99 ± 1.76^{bA}	14.49 ± 2.33^{bB}	48.47 ± 6.85^{aA}	0.00 ± 0.00^{cC}	79.49^{***}
$F_{(5, 18)}$ ‡	3.33^{*}	4.92^{**}	0.26^{ns}	329.43^{***}	
S. zeamais					
0	14.04 ± 1.81^{a}	14.04 ± 1.81^{a}	14.04 ± 1.81^{a}	14.04 ± 1.81^{a}	
2.5	13.06 ± 1.49^{aA}	5.97 ± 0.37^{abB}	7.99 ± 1.02^{abAB}	1.49 ± 0.76^{bC}	21.71^{***}
5	10.33 ± 2.55^{abA}	4.62 ± 1.67^{bcA}	4.96 ± 1.16^{bcA}	0.38 ± 0.07^{bcB}	12.07^{***}
10	5.28 ± 0.93^{bc}	2.88 ± 1.51^{bcAB}	3.63 ± 0.97^{bcA}	0.08 ± 0.08^{cB}	7.83^{**}
15	2.11 ± 0.66^{c}	1.80 ± 1.35^{c}	3.37 ± 1.08^{bc}	0.64 ± 0.32^{bc}	2.14^{ns}
20	2.23 ± 0.53^{cA}	1.30 ± 0.58^{cA}	2.82 ± 0.83^{cB}	0.08 ± 0.08^{cB}	9.71^{**}
$F_{(5, 18)}$ ‡	14.94^{***}	8.11^{***}	11.27^{***}	42.55^{***}	

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

P. gland = Plectranthus glandulosus

Table 3.31: Grain damage and weight loss caused by *Callosobruchus maculatus* and *Sitophilus zeamais* in grains treated with binary combinations of *Plectranthus glandulosus* leaf powder and NeemAzal, and then stored for 10 weeks

Insects/ Doses (g/kg)	Proportion of powders in the mixture				
	100% *P. gland*	75% *P. gland* + 25% NeemAzal	50% *P. gland* + 50% NeemAzal	100% NeemAzal	$F_{(3,12)}$ ‡
			Mean (± SE) grain damage (%) †		
C. maculatus					
0	98.00 ± 1.00^{a}	98.00 ± 1.00^{a}	98.00 ± 1.00^{a}	98.00 ± 1.00^{a}	
2.5	98.75 ± 0.25^{aA}	97.50 ± 0.87^{a}	95.50 ± 1.32^{a}	95.50 ± 1.19^{aA}	3.39^{ns}
5	98.50 ± 0.50^{abA}	96.75 ± 1.11^{abA}	79.00 ± 6.10^{bB}	95.98 ± 0.41^{aA}	16.03***
10	98.75 ± 0.25^{abA}	96.00 ± 0.58^{abB}	66.75 ± 1.84^{bC}	66.00 ± 1.96^{bC}	254.52***
15	96.50 ± 0.95^{abA}	95.50 ± 0.29^{abA}	36.00 ± 4.60^{cB}	38.50 ± 5.55^{cB}	105.90***
20	95.50 ± 0.96^{bA}	92.25 ± 1.93^{bA}	28.50 ± 6.86^{cB}	11.75 ± 1.49^{cB}	53.29***
$F_{(5, 18)}$ ‡	3.64*	3.62*	51.14***	70.44***	
S. zeamais					
0	39.75 ± 3.90^{a}	39.75 ± 3.90^{a}	39.75 ± 3.90^{a}	39.75 ± 3.90^{a}	
2.5	47.50 ± 6.38$^{a\ A}$	1.75 ± 0.85^{bB}	1.00 ± 0.71^{bB}	2.75 ± 1.18^{bB}	46.86***
5	55.50 ± 1.44^{aA}	1.00 ± 0.58^{bB}	0.25 ± 0.25^{bB}	2.00 ± 0.71^{bB}	140.46***
10	35.25 ± 8.40^{abA}	0.25 ± 0.25^{bB}	0.00 ± 0.00^{bB}	0.75 ± 0.48^{bB}	35.75***
15	11.75 ± 4.03^{cA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}	0.50 ± 0.29^{bB}	13.68***
20	13.25 ± 2.78^{bcA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}	0.75 ± 0.78^{bB}	40.58***
$F_{(5, 18)}$ ‡	11.92***	74.20***	111.06***	45.25***	
			Mean (± SE) weight loss (%) †		
C. maculatus					
0	47.36 ± 3.08^{ab}	47.36 ± 3.08^{a}	47.36 ± 3.08^{a}	47.36 ± 3.08^{a}	
2.5	55.94 ± 2.14^{a}	44.28 ± 8.49^{a}	40.41 ± 1.54^{a}	46.49 ± 5.79^{a}	1.51^{ns}
5	54.20 ± 4.04^{abA}	39.80 ± 3.84^{aA}	24.17 ± 2.94^{bB}	40.03 ± 3.31^{aA}	12.10***
10	44.52 ± 2.49^{aA}	35.72 ± 3.94^{aA}	15.29 ± 3.11^{bB}	11.81 ± 1.61^{bB}	29.08***
15	46.95 ± 3.11^{abA}	38.53 ± 5.86^{aA}	6.92 ± 1.90^{cB}	8.16 ± 1.89 ^{bcB}	37.22***
20	42.99 ± 1.76^{bA}	40.05 ± 5.38^{aA}	2.51 ± 0.35^{cB}	3.32 ± 0.90^{cB}	91.10***
$F_{(5, 18)}$ ‡	3.33*	0.60^{ns}	64.35***	48.75***	
S. zeamais					
0	12.09 ± 1.50^{a}	10.77 ± 1.10^{a}	12.09 ± 1.50^{a}	12.09 ± 1.50^{a}	
2.5	13.06 ± 1.49^{aA}	0.33 ± 0.12^{bB}	0.26 ± 0.15^{bB}	0.74 ± 0.43^{bB}	59.64***
5	10.33 ± 2.55^{abA}	0.40 ± 0.33^{bB}	0.06 ± 0.06^{bB}	0.31 ± 0.14^{bB}	28.60***
10	5.28 ± 0.93^{bcA}	0.06 ± 0.06^{bB}	0.00 ± 0.00^{bB}	0.23 ± 0.16^{bB}	50.45***
15	2.11 ± 0.66^{cA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}	0.21 ± 0.12^{bB}	16.63***
20	2.23 ± 0.53^{cA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{bB}	0.26 ± 0.18^{bB}	22.06***
$F_{(5, 18)}$ ‡	14.94***	71.28***	111.28***	39.19***	

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$; * $P < 0.05$; *** $P < 0.001$

P. gland = Plectranthus glandulosus

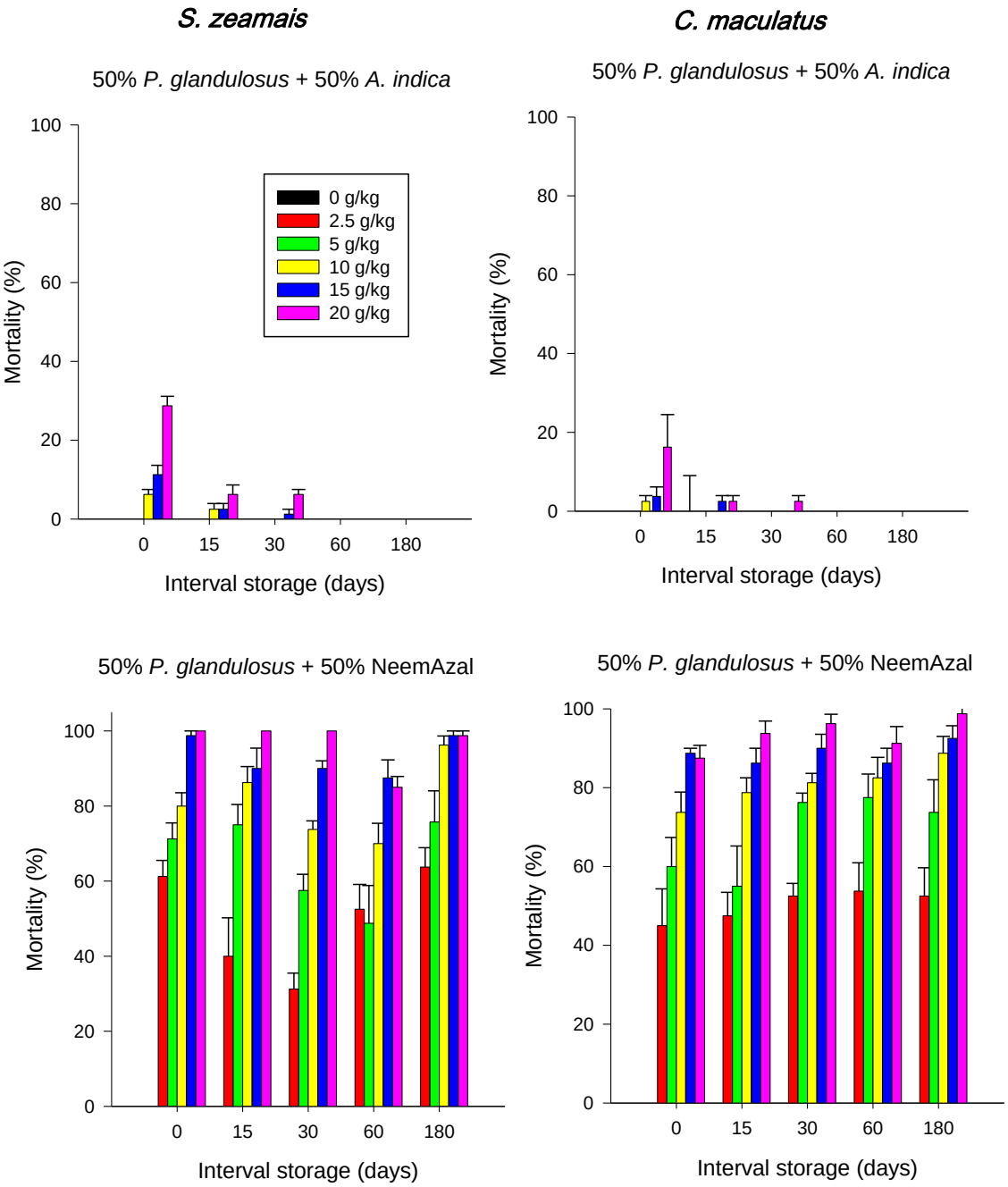


Figure 3.11: Corrected cumulative mortality of *Callosobruchus maculatus* and *Sitophilus zeamais* exposed in grains treated with the combinations of *Plectranthus glandulosus* with NeemAzal and *Azadirachta indica* seed powders at different storage intervals

3.6 Efficacy of *Azadirachta indica* products and *Plectranthus glandulosus* leaf powder on female fecundity and immature stages of *Callosobruchus maculatus* and *Sitophilus zeamais*

3.6.1 Influence of neem products and *P. glandulosus* leaf powder on female fecundity

The mean number of eggs laid by *C. maculatus* on grains varied widely on treated and untreated cowpea with various plant products (t = -13.81 to -2.48) while on maize the number of eggs oviposited by *S. zeamais* did not vary between the treated and untreated grains, except when *A. indica* seed powder was used (Table 3.32).

Table 3.32: Mean (± SE) number of eggs laid by females of *Callosobruchus maculatus* and *Sitophilus zeamais* on treated vs. untreated grains with *Azadirachta indica* products and *Plectranthus glandulosus* leaf powder

Products and doses	Mean number of eggs laid by females[†]					
	C. maculatus			*S. zeamais*		
	Treated grains	Untreated grains	*t*-value	Treated grains	Untreated grains	*t*-value
A. indica seed oil (ml/kg)						
0	122.50 ± 4.65 ^{a}	119.00 ± 4.38^{a}	0.55^{ns}	62.75 ± 2.95^{a}	65.00 ± 2.42^{a}	0.59^{ns}
0.05	46.00 ± 5.61^{b}	95.50 ± 10.63^{a}	- 4.12**	12.25 ± 2.20^{b}	15.75 ± 1.11^{b}	0.57^{ns}
0.1	14.75 ± 1.55^{c}	102.75 ± 8.42^{a}	-10.28***	6.25 ± 1.03^{b}	12.25 ± 2.59^{b}	-2.15^{ns}
$F_{(2, 9)}$ [‡]	166.18***	2.09^{ns}		180.34***	120.64***	
A. indica seed powder (g/kg)						
0	122.50 ± 4.65^{a}	119.00 ± 4.38	0.55^{ns}	62.75 ± 2.95^{a}	65.00 ± 2.42^{a}	0.59^{ns}
2.5	55.50 ± 5.52^{b}	136.00 ± 9.26	-7.47**	16.25 ± 1.25^{b}	29.25 ± 2.56^{b}	-4.56**
5	18.25 ± 1.03^{c}	126.00 ± 7.74	-13.81***	11.00 ± 1.22^{b}	23.00 ± 1.68^{b}	-5.76**
$F_{(2, 9)}$ [‡]	125.55***	1.28 ^{ns}		206.41***	78.31**	
P. glandulosus leaf powder (g/kg)						
0	122.50 ± 4.65	119.00 ± 4.38^{a}	0.55^{ns}	62.75 ± 2.95^{a}	65.00 ± 2.42^{a}	0.59^{ns}
2.5	141.25 ± 5.68	165.25 ± 7.81^{b}	-2.48**	40.25 ± 2.95^{b}	36.25 ± 3.15^{b}	0.93^{ns}
5	133.75 ±12.72	218. 00 ± 8.38^{c}	-5.53**	18.50 ± 1.32^{c}	26.75 ± 3.57^{b}	-2.17^{ns}
$F_{(2, 9)}$ [‡]	1.24^{ns}	49.05***		76.46***	33.33***	
75% *P. glandulosus* + 25% NeemAzal (g/kg)						
0	122.50 ± 4.65^{a}	119.00 ± 4.38	0.55^{ns}	62.75 ± 2.95^{a}	65.00 ± 2.42^{a}	0.59^{ns}
1	112.50 ± 9.68^{a}	118.00 ± 9.44	- 0.41^{ns}	20.00 ± 2.35^{b}	30.00 ± 3.42^{b}	-2.41^{ns}
2	57.75 ± 4.11^{b}	106.25 ± 7.95	- 5.42**	15.00 ± 1.83^{b}	22.50 ± 4.80^{b}	-1.46^{ns}
$F_{(2, 9)}$ [‡]	27.57***	0.88 ^{ns}		117.65***	22.47***	
NeemAzal (g/kg)						
0.01	105.25 ± 4.65	108.75 ± 9.79	- 0.34^{ns}	40.25± 2.21	53.25 ± 2.84	-3.61*

[†] Means in the same column followed by the same letter do not differ significantly (Tukey's test; P < 0.05)

[‡] ns P > 0.05; * P < 0.05; ** P < 0.01*** P < 0.001

This number of eggs deposited by female *S. zeamais* on treated and untreated grains varied significantly ($P \leq 0.01$) with the ascending doses and considerably reduced compared to the control. On cowpea, apart *P. glandulosus* where the number of eggs laid on untreated seeds was statistically different from the treated ones ($P \leq 0.001$) and increased with the rising dose. All the used botanicals did no differ with the increase of product contents. The result also showed that despite the reduced number eggs recorded on treated cowpea, the use of applied products was far less effective in reducing the number of eggs laid by *C. maculatus* compared to *S. zeamais*. However, the neem products except NeemAzal were the more effective to inhibit the lay of maize weevil eggs on both treated and untreated grains compared to *P. glandulosus* and the binary mixture of *P. glandulosus* and NeemAzal. The lowest numbers of egg deposited on untreated grains by female of *S. zeamais* and *C. maculatus* were 12.25 and 102.75 (0.1 ml/kg *A. indica* seed oil) respectively while a maximum egg number (218) was recorded on untreated cowpea as the female was first exposed on treated seeds with *P. glandulosus* leaf powder (5 g/kg).

3.6.2 Effect of *Azadirachta indica* products and *Plectranthus glandulosus* leaf powder on eggs and immature stages of *Callosobruchus maculatus*

All the tested botanicals significantly influenced the development of the eggs and immature stages of *C. maculatus* ($P < 0.05$) (Table 3.33), although their effectiveness decreased with the evolution of the developmental stages. But for where *P. glandulosus* leaf powder was present at the larval stage, the progeny that emerged from seeds treated for each stage decreased with ascending contents, regardless of the botanical product. Their ability to inhibit the development of eggs in decreasing order was: *A. indica* seed oil, NeemAzal > *A. indica* seed powder >> 75% *P. glandulosus* + 25% NeemAzal, *P. glandulosus* leaf powder. The 75% *P. glandulosus* + 25% NeemAzal and *P. glandulosus* powders were more potent on the egg stage than the larval and nymph stages. For the treated nymphal stages, the number of progeny produced was generally similar among treatments, regardless of the botanical product. No adults emerged from the grains on which the eggs were treated with *A. indica* seed oil (2 - 4 ml/kg), neem seed powder (20 g/kg) and NeemAzal powder (5 g/kg).

3.6.3 Effect of *Azadirachta indica* products and *Plectranthus glandulosus* leaf powder on eggs and immature stages of *Sitophilus zeamais*

Table 3.34 shows the result of *A. indica* seed oil and powder, *P. glandulosus* leaf powder, NeemAzal and 75% *P. glandulosus* and 25% NeemAzal on eggs the immature stages of *S. zeamais*. All the botanical products significantly influenced the development of the immature stages of the weevil ($P < 0.05$). Overall, the bioactivity of these products on the eggs and immature stages was dose-dependent. *A. indica* seed oil was more efficient in inhibiting the development of egg and early larval stages (2.5 and 0, respectively at 4 ml/kg) compared with the late larval and nymphal stages (23 and 30.50 adults, respectively at 4 ml/kg). Neem seed powder affected *S. zeamais* similarly until the late larval stage regardless of doses. Contrariwise, NeemAzal and *P. glandulosus* leaf powder performed better on larval stages than on egg and nymphal stages. The mixed powder of 75% *P. glandulosus* + 25% NeemAzal gave modest efficiency on egg stage where an average of 10 adults emerged independently of contents.

Table 3.33: Effect of *Azadirachta indica* products and *Plectranthus glandulosus* leaf powder on eggs and immature stages of *Callosobruchus maculatus*

Products and doses	Treated insect stage / Number of progeny emerged (mean ± SE) †			
	Egg	Larva	Nymph	$F_{(2, 9)}$
A. indica seed oil (ml/kg)				
0	42.25 ± 0.48^{aB}	51.50 ± 1.19^{aA}	58.50 ± 2.80^{aA}	13.30^{**}
2	0.00 ± 0.00^{bB}	20.50 ± 5.19^{bA}	35.00 ± 4.06^{bA}	21.37^{***}
3	0.00 ± 0.00^{bB}	8.75 ± 1.49^{cB}	34.50 ± 3.37^{bA}	45.28^{***}
4	0.00 ± 0.00^{bB}	4.00 ± 0.71^{cB}	33.25 ± 2.95^{bA}	107.11^{***}
$F_{(3, 12)}$ ‡	858.64^{***}	58.78^{**}	10.88^{***}	
A. indica seed powder (g/kg)				
0	42.25 ± 0.48^{aB}	51.50 ± 1.19^{aA}	58.50 ± 2.80^{aA}	13.30^{**}
5	11.75 ± 1.03^{bC}	41.25 ± 2.72^{abB}	51.50 ± 2.18^{abA}	156.73^{***}
10	0.50 ± 0.50^{bC}	30.75 ± 3.79^{bB}	48.00 ± 5.37^{abA}	39.89^{***}
20	0.00 ± 0.00^{bC}	8.00 ± 0.91^{cB}	39.50 ± 2.90^{bA}	131.43^{***}
$F_{(3, 12)}$ ‡	160.39^{***}	74.71^{***}	4.71^{*}	

Table 3.33 Cont'd

P. glandulosus leaf powder (g/kg)				
0	42.25 ± 0.48^{aB}	51.50 ± 1.19^{aA}	58.50 ± 2.80^{aA}	13.30**
5	11.50 ± 1.85^{bB}	47.50 ± 2.33^{aA}	47.50 ± 1.55^{bA}	115.20***
10	9.00 ± 1.91^{bB}	48.25 ± 1.93^{aA}	44.75 ± 1.03^{bA}	167.34***
20	11.25 ± 2.84^{cB}	44.00 ± 2.34^{aA}	49.25 ±1.49^{abA}	48.69***
$F_{(3, 12)}$ ‡	32.78 ***	2.36 ^{ns}	5.49*	
75% *P. glandulosus* + 25% NeemAzal (g/kg)				
0	42.25 ± 0.48^{aB}	51.50 ± 1.19^{aA}	58.50 ± 2.80^{aA}	13.30**
2.5	12.50 ± 0.87^{bC}	49.00 ±1.29^{aB}	54.75 ± 0.85^{aA}	500.72***
5	10.00 ± 1.17^{bB}	44.75 ± 2.87^{aA}	43.75 ± 1.65^{bA}	89.43***
10	8.75 ± 1.44^{bB}	42.50 ± 2.66^{aA}	46.25 ± 1.11^{bA}	123.34***
$F_{(3, 12)}$ ‡	84.6 1 ***	3.58^{ns}	15.54**	
NeemAzal (g/kg)				
5	0.00 ± 0.00^{C}	49.50 ± 0.65^{A}	35.15 ± 4.09^{B}	165.22***

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).
‡ ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

3.7 Influence of environmental condition on the insecticidal efficacy of *Azadirachta indica* products and *Plectranthus glandulosus* leaf powder on *Callosobruchus maculatus* and *Sitophilus zeamais*

3.7.1 Effect of relative humidity on the insecticidal efficacy of *Azadirachta indica* products and *Plectranthus glandulosus* leaf powder on *Callosobruchus maculatus* and *Sitophilus zeamais*

All the products generally caused significant mortality to adult *C. maculatus* and *S. zeamais* at the various tested relative humidity (Tables 3.35 and 3.36) compared to the control. Mortality increased with ascending time exposure, irrespective of products and insect species but the rate of increase in mortality with days after exposure was lower for *C. maculatus* (Table 3.35) compared to *S. zeamais* (Figure 3.36). Except neem seed powder and *P. glandulosus* leaf powder (on both insect species), no significant difference was observed among the relative humidity on where treated and infested grains were exposed regarding the mortality they caused to *S. zeamais* and *C. maculatus.* However, where there was difference (neem seed powder and *P. glandulosus*), the 70% r.h. led to a lower mortality of *C. maculatus* and *S. zeamais* at all days post exposure. Six days after exposition at 70% r.h. 2.75 and 8.25% *C. maculatus* mortality were recorded respectively with neem seed powder and *P. glandulosus*. At the maximum tested day (14 days) 75% *P. glandulosus* + 25% NeemAzal achieved complete mortality of *S. zeamais* for all the r.h., except the 70% r.h. which caused a maximum

mortality of 98.75%. Seen the mortality observed the 60 r.h. seems suitable to maximize the bioactivity on *P. glandulosus* and neem seed powder to cause adult cowpea or maize weevils mortality.

Table 3.34: Effect of *Azadirachta indica* products and *Plectranthus glandulosus* leaf powder on eggs and immature stages of *Sitophilus zeamais*

Products and doses	Treated insect stage / Number of progeny emerged (mean ± SE) †				
	Egg	Early larva	Late larva	Nymph	$F_{(3, 12)}$
A. indica seed oil (ml/kg)					
0	41.25 ± 2.02^{aA}	38.00 ± 1.08^{aA}	38.50 ± 2.25^{aA}	38.50 ± 1.66^{aA}	0.67^{ns}
2	10.05 ± 0.65^{bB}	5.75 ± 0.95^{bB}	26.25 ± 2.25^{bA}	27.00 ± 1.83^{bA}	48.59^{***}
3	5.00 ± 2.12^{bcB}	3.25 ± 0.48^{cB}	24.50 ± 1.26^{bA}	25.00 ± 2.86^{bA}	39.33^{***}
4	2.50 ± 0.87^{cC}	0.00 ± 0.00^{dC}	23.00 ± 0.41^{bB}	30.50 ± 1.85^{abA}	209.69^{***}
$F_{(3, 12)}$ ‡	42.95***	337.77***	16.13 ***	7.33**	
A. indica seed powder (g/kg)					
0	41.25 ± 2.02^{aA}	38.00 ± 1.08^{aA}	38.50 ± 2.25^{aA}	38.50 ± 1.66^{aA}	0.67^{ns}
5	16.00 ± 2.52^{bB}	14.00 ± 2.16^{bB}	8.50 ± 2.22^{bB}	29.00 ± 1.68^{abA}	17.23^{***}
10	14.33 ± 1.86^{bB}	8.25 ± 2.06^{bB}	10.00 ± 1.91^{bB}	26.75 ± 1.11^{bA}	23.40^{***}
20	2.67 ± 0.33^{cB}	0.50 ± 0.50^{cB}	7.00 ± 1.78^{bB}	23.25 ± 2.75^{bA}	30.54^{***}
$F_{(3, 12)}$ ‡	58.92 ***	66.61***	31.89***	9.00**	
P. glandulosus leaf powder (g/kg)					
0	41.25 ± 2.02^{aA}	38.00 ± 1.08^{aA}	38.50 ± 2.25^{aA}	38.50 ± 1.66^{aA}	0.67^{ns}
5	36.50 ± 1.19^{aA}	15.00 ± 1.47^{bB}	18.25 ± 1.31^{bB}	31.50 ± 2.72^{abA}	33.48^{***}
10	34.50 ± 1.26^{aA}	9.25 ± 1.31^{cC}	9.50 ± 0.96^{cC}	24.75 ± 2.02^{bB}	73.59^{***}
20	20.00 ± 2.12^{bA}	8.50 ± 1.19^{cB}	9.25 ± 1.65^{cB}	23.50 ± 1.99^{bA}	22.81^{***}
$F_{(3, 12)}$ ‡	28.53***	73.37***	58.52***	12.03***	
75% *P. glandulosus* + 25% NeemAzal (g/kg)					
0	41.25 ± 2.02^{aA}	38.00 ± 1.08^{A}	38.50 ± 2.25^{aA}	38.50 ± 1.66^{aA}	0.67^{ns}
2.5	9.75 ± 0.85^{bB}	30.25 ± 3.47^{A}	23.75 ± 2.25^{bA}	23.25 ± 0.63^{bA}	16.26^{***}
5	11.75 ± 0.85^{bC}	32.75 ± 1.44^{A}	20.50 ± 1.71^{bcB}	23.25 ± 1.38^{bB}	39.35^{***}
10	10.00 ± 0.82^{bD}	32.25 ± 1.03^{A}	15.50 ± 1.19^{cC}	22.00 ± 1.87^{bB}	55.03^{***}
$F_{(3, 12)}$ ‡	139.52***	2.36^{ns}	26.24***	24.42***	
NeemAzal (g/kg)					
5	20.75 ± 0.85^{CA}	11.50 ± 1.66^{B}	8.25 ± 0.85^{B}	24.75 ± 3.33^{A}	15.63^{***}

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).
‡ ns $P > 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 3.35: Comparison of adult mortality of *C. maculatus* caused by Neem products and *P. glandulosus* leaf powder at different relative humidity

Products and days post infestation	Relative humidity / % Mortality (mean ± SE)[†]			
	r.h. = 50%	r.h. = 60%	r.h. = 70%	$F_{(2, 69)}$
A. indica seed oil				
1	38.75 ± 8.91^{A}	33.50 ± 7.60^{bA}	23.00 ± 7.76^{A}	0.86^{ns}
3	43.00 ± 8.87^{A}	48.50 ± 8.58^{abA}	38.00 ± 8.79^{A}	0.30^{ns}
6	55.25 ± 8.16^{A}	64.50 ± 7.28^{aA}	45.25 ± 8.03^{A}	1.09^{ns}
$F_{(2, 69)}$ [‡]	0.79^{ns}	3.52*	1.64^{ns}	
A. indica seed powder				
1	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	-
3	1.00 ± 0.46^{bB}	3.75 ± 1.08^{bA}	0.00 ± 0.00^{bB}	7.56^{**}
6	13.50 ± 4.84^{aA}	14.50 ± 3.13^{aA}	2.75 ± 0.77^{aB}	3.30^{*}
$F_{(2, 69)}$ [‡]	22.11***	12.17***	13.24***	
P. glandulosus leaf powder				
1	0.00 ± 0.00^{cB}	1.00 ± 0.46^{bA}	0.00 ± 0.00^{bB}	4.60^{*}
3	6.00 ± 1.39^{bA}	2.25 ± 0.85^{bB}	0.00 ± 0.00^{bB}	9.29^{***}
6	18.25 ± 3.25^{aB}	29.50 ± 2.99^{aA}	8.25 ± 5.60^{aB}	14.65^{***}
$F_{(2, 69)}$ [‡]	31.02***	68.02***	29.29***	
3/4 *P. glandulosus* leaf powder + 1/4 NeemAzal				
1	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	-
3	69.50 ± 5.39^{bA}	71.00 ± 4.51^{bA}	54.00 ± 6.74^{bA}	1.29^{ns}
6	99.25 ± 0.55^{a}	98.25 ± 1.04^{a}	98.75 ± 0.62^{a}	0.001^{ns}
$F_{(2, 69)}$ [‡]	49.65***	51.59***	47.14 ***	

[†] Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).
[‡] ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 3.36: Comparison of adult mortality of *S. zeamais* caused by Neem products and *P. glandulosus* leaf powder at different relative humidity

Products and days post infestation	Relative humidity / % Mortality (mean ± SE) [†]			
	r.h. = 50%	r.h. = 60%	r.h. = 70%	$F_{(2, 69)}$
A. indica seed oil				
1	49.25 ± 8.62^{bA}	12.25 ± 2.87^{bB}	34.50 ± 5.34^{AB}	5.59^{**}
3	56.75 ± 8.30^{bAB}	34.00 ± 5.15^{bB}	44.50 ± 5.80^{B}	2.12^{ns}
7	80.50 ± 6.40^{aA}	76.50 ± 6.91^{aA}	57.75 ± 7.60^{A}	1.64^{ns}
14	81.00 ± 6.90^{aA}	81.79 ± 5.88^{aA}	60.00 ± 7.57^{A}	1.61^{ns}
$F_{(3, 92)}$ [‡]	2.71^{*}	16.42^{***}	2.17^{ns}	
A. indica seed powder				
1	0.00 ± 0.00^{cB}	2.50 ± 0.92^{cA}	0.00 ± 0.00^{bB}	8.75^{***}
3	5.00 ± 1.66^{bB}	39.50 ± 6.14^{bA}	0.00 ± 0.00^{bB}	31.72^{***}
7	22.75 ± 4.49^{abB}	64.87 ± 8.44^{aA}	5.50 ± 2.20^{abB}	19.73^{***}
14	40.50 ± 6.48^{aB}	76.12 ± 7.04^{aA}	12.25 ± 3.64^{aC}	18.05^{***}
$F_{(3, 92)}$ [‡]	16.29^{***}	16.49^{***}	6.95^{***}	

Table 3.36: Cont'd

P. glandulosus leaf powder				
1	0.00 ± 0.00^{bA}	0.75 ± 0.55^{bA}	0.00 ± 0.00^{bA}	1.86^{ns}
3	10.25 ± 2.13^{bA}	8.25 ± 2.06^{bA}	4.25 ± 0.75^{bA}	2.56^{ns}
7	65.00 ± 6.46^{aAB}	76.75 ± 5.94^{aA}	37.50 ± 5.32^{aB}	3.63*
14	69.25 ± 6.56^{aAB}	86.00 ± 4.99^{aA}	56.50 ± 5.60^{aB}	3.07*
$F_{(3, 92)}$ ‡	31.02***	44.21***	29.00***	
75% *P. glandulosus* + 25% NeemAzal				
1	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	-
3	58.50 ± 6.86^{bA}	71.00 ± 4.51^{bA}	57.50 ± 6.71^{bA}	0.60^{ns}
7	98.50 ± 1.03^{aA}	98.25 ± 1.04^{aA}	89.75 ± 3.76^{aA}	0.42^{ns}
14	100 ± 0.00^{aA}	100 ± 0.00^{aA}	98.75 ± 1.02^{aA}	0.02^{ns}
$F_{(3, 92)}$ ‡	35.43***	39.32***	32.44***	

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; P < 0.05).
‡ ns P > 0.05; * P < 0.05; ** P < 0.01; *** P < 0.001

3.7.2 Effect of temperature on the efficacy of neem products and *P. glandulosus* leaf powder on adult mortality of *C. maculatus* and *S. zeamais*

The result of the influence of temperature on the bioactivity of neem products and *P. glandulosus* on cowpea or maize weevils are given in Tables 3.37 and 3.38 respectively. Data showed that adult mortality differed significantly (P≤ 0.05) with time post exposure but not with temperature except neem seed oil one and three days after infestation. At both tested temperature, products reacted better on *S. zeamais* than on *C. maculatus*. Within one day exposure period and at 25°C the neem seed powder and mixed 75% *P. glandulosus* leaf powder + 25% NeemAzal recorded no adult cowpea bruchid mortality. The similar result was observed with *S. zeamais* as the late product was applied on maize. Lowest adult mortality (14.50 and 9.25% at respectively 25°C and 30°C) was registered with neem seed powder six days in *C. maculatus.* Complete adult mortality (100%) was recorded at 25°C 14 days after exposure while at 30°C 99.74% *S. zeamais* mortality observed as 75% *P. glandulosus* leaf powder + 25% NeemAzal was used on maize.

Table 3.37: Comparison of the effect of temperature on neem products and *Plectranthus glandulosus* leaf powder on *C. maculatus* mortality

Products and days post-infestation	Temperature/ % Mortality (mean ± SE) †		*t* value
	T = 25 °C	T = 30°C	
Neem seed oil (ml/kg)			
1	33.50 ± 7.60^{b}	29.25 ± 6.55^{b}	0.39^{ns}
3	48.50 ± 8.58^{ab}	33.75 ± 7.42^{b}	1.17^{ns}
6	64.50 ± 7.28^{a}	54.03 ± 7.58^{a}	0.82^{ns}
$F_{(2, 69)}$) ‡	3.52*	3.22*	
Neem seed powder (g/kg)			
1	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	-
3	3.75 ± 1.08^{b}	1.25 ± 0.62^{b}	1.94^{ns}
6	14.50 ± 3.13^{a}	9.25 ± 1.51^{a}	1.28^{ns}
$F_{(2, 69)}$ ‡	12.17***	22.32***	
P. glandulosus leaf powder (g/kg)			
1	1.00 ± 0.46^{b}	0.75 ± 0.55^{b}	0.35^{ns}
3	2.25 ± 0.85^{b}	4.75 ± 1.47^{b}	- 1.42^{ns}
6	29.50 ± 2.99^{a}	33.00 ± 3.56^{a}	- 0.03^{ns}
$F_{(2, 69)}$ ‡	68.02***	40.88***	
75% *P. glandulosus* + 25% NeemAzal (g/kg)			
1	0.00 ± 0.00^{c}	3.50 ± 0.73^{c}	- 4.37***
3	71.00 ± 4.51^{b}	79.50 ± 5.95^{b}	- 0.68^{ns}
6	98.25 ± 1.04^{a}	98.68 ± 0.75^{a}	- 0.26^{ns}
$F_{(2, 69)}$ ‡	51.59***	43.21***	

† Means in the same column followed by the same lower case do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 3.38: Comparison of the effect of temperature on neem products and *Plectranthus glandulosus* leaf powder on *S. zeamais* mortality

Products and days post-infestation	Temperature/ % Mortality (mean ± SE) †		*t* value
	T = 25 °C	T = 30°C	
A. indica oil (ml/kg)			
1	12.25 ± 2.87^{b}	75.75 ± 5.72	- 6.63***
3	34.00 ± 5.15^{b}	79.00 ± 5.42	- 4.11***
7	76.50 ± 6.91^{a}	81.00 ± 4.81	- 0.34^{ns}
14	81.79 ± 5.88^{a}	83.39 ± 4.37	- 0.12^{ns}
$F_{(3, 92)}$ ‡	16.42***	0.13^{ns}	
A. indica seed powder (g/kg)			
1	2.50 ± 0.92^{c}	2.50 ± 0.92^{c}	0.19^{ns}
3	39.50 ± 6.14^{b}	25.00 ± 4.93^{b}	1.17^{ns}
7	64.87 ± 8.44^{ab}	49.50 ± 6.64^{ab}	2.74*
14	76.12 ± 7.04^{a}	60.86 ± 7.56^{a}	1.12^{ns}
$F_{(3, 92)}$ ‡	16.49***	15.56***	
P. glandulosus leaf powder (g/kg)			
1	0.75 ± 0.55^{b}	0.25 ± 0.25^{b}	0.82^{ns}
3	8.25 ± 2.06^{b}	15.25 ± 5.88^{b}	-1.09^{ns}
7	76.75 ± 5.94^{a}	60.50 ± 7.07^{a}	1.26^{ns}
14	86.00 ± 4.99^{a}	66.75 ± 6.78^{a}	1.46^{ns}
$F_{(3, 92)}$ ‡	44.21***	21.48***	
75% *P. glandulosus* powder + 25% NeemAzal (g/kg			
1	0.00 ± 0.00^{a}	0.00 ± 0.00^{c}	-
3	71.00 ± 4.51^{b}	89.75 ± 3.33^{a}	- 1.56^{ns}
7	98.25 ± 1.04^{a}	97.75 ± 0.99^{a}	0.04
14	100 ± 0.00^{a}	99.74 ± 0.26^{a}	0.02^{ns}
$F_{(3, 92)}$ ‡	39.32***	36.72***	

† Means in the same column followed by the same lower case letter do not differ significantly (Tukey's test; $P < 0.05$).
‡ ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.00$

CHAPTER 4: DISCUSSION

The results of *A. indica* oil yield in the present study showed that sun-dried kernels produced lower quantity of oil (28.60% w/w) than the other drying regimes. Faye (2010) reported that dehusked neem seeds (kernels) gave lower oil quantity than undehusked seeds. In the same line, Soetaredjo *et al.* (2008) observed that when the exposure temperature of neem seeds increased, the yield of oil decreased from 32% at room temperature to 18% at 80°C. They noticed that drying seeds in sunlight reduces their moisture contents and leads to the attachment of the oil to the proteins within the seed structures. Kumar & Parmar (1996), Munoz-Valenzuela *et al.* (2007) and Jadega *et al.* (2011), screened *A. indica* seeds from different regions in India and Mexico and found that the yield of the oil ranged from 15.4 to 54%, the range of 28.60% to 34.42% for the present study is in accordance with their findings. These authors found that variation in yield of the oil was independent on the age of the trees and the origin of the seeds but dependent on rainfall, humidity and temperature of the area.

Unsaturated fatty acids (oleic acid and linoleic acid) were higher (68%) than saturated fatty acids (palmitic acid, stearic acid, arachidic acid, behenic acid and lignoceric acid) in our *A. indica* seeds for all the four drying regimes. The presence of unsaturated fatty acids in *A. indica* seed oil is an important indicator of the quality of the oil (Kaushik, 2002) and it reduces the degradation rate of azadirachtin A (Johnson et *al.*, 2000), which is the main compound in *A. indica* oil reputed for insecticidal efficiency. Kaushik & Vir (2002), Djenontin *et al.* (2012) and Tomar *et al.* (2012), recorded similar results to that of the present study, with respect to the type of fatty acids and the patterns of the saturated and unsaturated fatty acids found in *A. indica* seed oils from India and Nigeria. Also, the diversity and quantity of the fatty acids in this study are close to those obtained with the edible oils of the oleic type such as that extracted from groundnut (Kapseu & Parmentier 1999).

It is widely reported that the sun-drying of plant materials has an effect on their chemical composition and therefore reduced their efficacy when used as medications or insecticides (Caboni *et al.*, 2009; Najafian & Agah 2012; Shahhoseini *et al.*, 2013). Johnson *et al.* (2003), Rembold (2004) reported that Azadirachtin is extremely labile in light with photolysis half lives ranging from 48 min to 3.98 days in thin films, under UV light. The Azadirachtin A content in the oil obtained from the sun-dried seeds in the present study was less compared to other drying regimes. Sidhu *et al.* (2003) studied the variation of

Azadirachtin A of *A. indica* oil of 43 provenances in India. They recorded a range from 0.55 to 3.03 g/kg of Azadirachtin A with only those from four provenances reaching the rate 2.00 g/kg, thus even the sun-dried kernels and sun-dried seeds oil in the present study had higher Azadirachtin A contents compared to theirs. In neem seed powders, there was no significant difference in Azadirachtin A amounts among the seeds or kernels shade/sun-dried. Drying regime did not affect this limonoid content. Overall about 1.2 g/kg Azadirachtin A was recovered in powder and this is consistent with the study of Barrek *et al.,* (2004) where no notable variations in the reduction of Azadirachtin A level were observed as the *A. indica* products were kept in daylight or in darkness. The obtained amount of Azadirachtin A in powders in this study is lower than those obtained by other workers. Gruber (1991), Boursier *et al.* (2011) and Faye (2010) respectively analyzed the neem seeds from Nicaragua, Mali and Senegal and correspondingly recorded 4.0 g/kg, 3.5 g/kg and 2.0 g/kg Azadirachtin A. This difference in Azadirachtin content may be explained by the variation of the geographical locations (Ermel *et al.,* 1986). Soils and climate may influence the Azadirachtin A contents in plants (Sidhu *et al.*, 2003; Gupta *et al.,* 2010).

Factors such as the pH, temperature, relative humidity, daylight, ultra violet lights and carriers (Barreck *et al.,* 2004; El Shafie *et al.*, 2012) affect the degradation of Azadirachtin A. Barrack *et al*. (2004) reported that the disappearance of Azadirachtin A in daylight was faster than in dark. Radwan and El-Shiekh (2012) stated that some neem formulations retain their Azadirachtin A content for at least one year when stored in the dark. however, information on the degradation of Azadirachtin A on cowpea seeds or maize grains treated with *A. indica* oil are lacking. Notwithstanding, the results of the present study showed that on grains treated with *A. indica* seed oil, Azadirachtin A, the main insecticidal component in the oil, degraded slowly and reduced four-folds within six months of storage in the dark.

With *P. glandulosus* leaf powders, the drying method had less effect on the diversity of the volatile compounds of the leaves, but the sun-dried leaves had a harboured lower rates of volatiles compared to the shade-dried leaves. Sellami *et al.* (2011) reported that the increase of temperature during the drying process leads to a rapid release of monoterpenes, which results in the loss of most monoterpene hydrocarbons (Pirbalouti *et al.,* 2013). Some compounds seem to have more affinity to the water fraction in the leaves and thereby are lost during drying (Pirbalouti *et al.,* 2013). Plants which belong to the Lamiaceae family as *P. glandulousus* are known to keep their volatile compounds on or near the leaf surfaces and then easily lose such compounds when the temperature increases (Sellami *et al.,* 2011).This

might explain the loss of hydrocarbon constituents of sun-dried leaves. Essential oils obtained from room dried leaves of *P. glandulousus* collected from the same location like that in the present study, showed a higher percentage of piperitone oxide (Ngassoum *et al.,* 2001), when the leaves were shade-dried. This confirms the higher peak of piperitone oxide with the powder from the shade-dried leaves in the present study. Sun-drying effect is not only a consequence of the disappearance of some compounds but may also result in the appearance of others, which were absent or found in smaller quantities in the fresh or shade-dried leaves (Pirbalouti *et al.,* 2013). The increase of temperature may trigger oxidation processes and chemical reorganization, which leads to the appearance of some new molecules or the rapid release of others (Asekun *et al.,* 2007; Pirbalouti *et al.,* 2013). This might justify the higher proportion of oxygenated volatiles in the sun-dried leaves than in the shade-dried ones. Hassanpouraghdam *et al*. (2010) compared the effect of drying method on chemical composition of *Ocimum basilicum,* a plant in the same family of Lamiaceae like *P. glandulousus*. These authors observed that the concentration of compounds like linalool and camphor is higher in sun-dried than shade-dried leaves, which is consistent with higher levels of these substances in the sun-dried leaves in the present study. It could be asserted that the monoterpenes γ-terpinene, fenchone, β-pinene and eugenol which were found in equal proportions in the sun- and shade-dried leaves are less photodegradable. Díaz-Maroto *et al.* (2003) demonstrated sesquiterpenes, which are relatively less volatile are released more slowly than the other compounds. This contention corroborates the similar rates of sesquiterpenes between the sun- and shade-dried leaves in our study. Changes in the concentration of chemicals during drying are related to the drying regime/method (solar energy, oven temperatures, etc.) (Hassanpouraghdam *et al.,* 2010; Najafian & Agah, 2012; Shahhoseini *et al.,* 2013). Therefore, the drying regime/method determines the final chemical composition of dried plant materials.

The increase in adult mortality of *C. maculatus* and *S. zeamais* with increasing dose and time post exposure, irrespective of the drying regime suggests that the toxicity of the botanicals to the insects depends on the quantity of the active ingredients, which were not generally related to the drying regime. Mbaiguinam *et al.* (2006) obtained 100% mortality of *C. maculatus* with 5 ml/kg of *A. indica* seed oils from Chad, while Wadehi *et al.* (2013) reported that A. indica seed oil from Egypt the same rate caused 100% mortality to *S. zeamais.* The complete mortality of *C. maculatus* and *S. zeamais* achieved in our study when cowpea and maize were treated with *A. indica* seed oil from the sun-dried kernels (6 ml/kg)

within 3 and 7 days after exposure, respectively is similar to those of the previous authors. However, Obeng-Ofori & Amiteye (2005) obtained better efficacy with groundnut and soybean oil from Ghana at the rate of 5 g/kg, which caused 93% mortality to *S. zeamais* within 24 h of exposure. This difference in results for *S. zeamais* mortality among the vegetable oils may highlight the fact that neem oil as opposed to other vegetable oils has antifeedant properties, caused by its limonoids constituents like azadirachtin, nimbin, salanin, nimbidin and meliantriol (Schumutterer, 1990; Addea-Mensah, 1998). Antifeedanacy leads to a slower rate in mortality. Azadirachtin activates deterrent cells in the chemoreceptors of the mouthparts, interferes with other taste chemoreceptors, and blocks firing of "sugar" receptor cells which are responsible for stimulating feeding. These combined effects result in death by anorexia (primary antifeedancy) (Rukmini, 1987; Schmutterer, 1990; Petit, 2008; Anuradha & Annadurai, 2008). Thes limonoid compounds also inhibit peristalsis, reduces the production of digestive enzymes as food moves through the gut, restrain mid-gut cell replacement and food intake (secondary antifeedancy) (Mordue & Blackwell, 1993; Koul *et al.*, 2004; Pamela, 2009).

Like all bruchids, adult *C. maculatus* does not feed, while adult *S. zeamais* feeds on maize grains, but the *A. indica* seed oil caused greater mortality to *C. maculatus* than *S. zeamais*, and this was remarkable from the first day after infestation. This supports the antifeedant mechanism of *A. indica* oil against *S. zeamais*. Vegetable oils are known to penetrate the cuticle of insects (Ibrahim *et al.* 1999) and also block the spiracles, which will in turn prevent respiration, leading to the death of the insect by asphysiation (Don-Pedro 1989; Iloba & Ekrakene, 2006). The sclerotization of insect cuticles increase with age, whereby the cuticle becomes hardened and darkened, having addition waxe layers, leading to less permeability with age (Odeyemi et al., 2010). The 1-d old *C. maculatus* were much younger than the 7 to 14-d old *S. zeamais* in the present study. The elytras of *C. maculatus* partially covers the dorsal abdomen, while with *S. zeamais*, the dorsal abdomen is completely covered by the elytras. More so, *C. maculatus* is more mobile than *S. zeamais*, which could lead to a greater contact of the oil with the former than the latter. Therefore, because of the preceding reasons, more *A. indica* oil may have penetrated the body of *C. maculatus* than *S. zeamais* and the blocking of spiracles would have been more evident with *C. maculatus*, which could explain the higher susceptibility of *C. maculatus* than *S. zeamais* to the *A. indica* seed oils.

The similarity in the insecticidal effectiveness of the oils from the sun-dried kernels and seeds, as well as the shade-dried kernels and seeds against *C. maculatus* and *S. zeamais* is

at variance with the findings of Radwan and El-Shiekh (2012) where *A. indica* oil from seeds that were exposed to sunlight compared to those indoors, caused less mortality to the cotton leafworm, *Spodoptera littoralis* Boisduval. (Lepidoptera: Noctuidae). The similarity in the fatty acid composition among the oils from seeds that were subjected to the different drying regimes in the present study could explain why sundrying had no influence on insecticidal efficacy. Lienard *et al.* (1993) reported that oils with higher contents of fatty acids are more toxic to insects than those with lower levels of the acids. Notwithstanding, further studies are needed to clarify the relationship among fatty acids, limonoid componds and insecticidal efficacy of *A. indica* seed oil (Gauvin *et al.*, 2004).

The present study revealed also that, contrary to the seed oil, *S. zeamais* was more susceptible than *C. maculatus* to *A. indica* seed powder, regardless of the drying regime. It could be speculated that since the powder was oily with large particle sizes (1 mm), the concentration of the active ingredient was low on the treated grains, leading to a limited antifeedant effect. Thus *S. zeamais* was able to feed more on the A. indica seed powder treated grains than the seed oil treated grains, and thus ingested a significant amount of the active principle. *C. maculatus* did not ingest the active principle in the seed powder since adults do not feed, and had limited contact with the active principle in the very small amount of oil in the seed powder. Fritzsch & Cleffmann (1984) reported when ingested, the Azadirachtin in A. indica powder may inhibit cell proliferation and RNA synthesis, which results to direct cell death, and thus the death of insect. Neem seed powder is oily, and when in contact with insect may obstruct some spiracles of the insect and thus with time lead to asphyxiation and death ensues (Reuben *et al.,* 2006). This is thought to be one of the mechanism in which neem seed powder caused the death of both insects. This present work concerning neem powder corroborates with the findings of Bamaiyi *et al.* (2007), who recorded lower mortality in *C. maculatus* than *S. zeamais* with *Khaya senegalensis* seed powder. Contrarily, Kosma *et al.* (2014) used *Melia azedarach* seed powder, a plant from the same family like *A. indica*, against *C. maculatus* and noticed higher adult mortality (80 to 100% at 1 to 2 g/100 g of grains rate) in the bruchid. This difference could be attributed to experimental conditions. Their work was carried out at 32° C with insect aged three days old, while in the present case only cowpea weevils aged one day old were used under fixed laboratory conditions (25°C and 60% r.h.) The increase of temperature to over 30°C could by itself be detrimental for the survival of *C. maculatus* (Delobel & Tran, 1993).

The drying regime did not influence the potency of A. indica sed powder towards *C. maculatus* and *S. zeamais,* which is consistent with the similarly of Azadirachtin A contents in the seeds that were subjected to the different drying regimes. *A. indica* seeds from Sudan that were stored under sunlight compared to those in a room had the same insecticidal efficacy against *Tribolium castaneum* Du Val (El Shafie & Almahy, 2012), and they concluded that sun- or shade-drying of *A. indica* seeds does not affect their effectiveness against *C. maculatus* and *S. zeamais*.

As expected, both the powders from the shade- and sun-dried leaves of *P. glandulosus* and *A. indica* generally caused significant adult mortality to *S. zeamais* and *C. maculatus* relative to the control, although the mortality caused by neem leaf powders was rather low. For the *P. glandulosus* powders from the sun-dried leaves, the 6-d and 7-d LC_{50} values were 47.37 and 14.04 g/kg respectively for *S. zeamais* and *C. maculatus,* indicating that the former insect was more susceptible to the leaf powder than the later. This could be attributed to the fact that adult *S. zeamais* fed on the treated grains while *C. maculatus,* as all other bruchids, did not, and thus did not ingest the plant powders. The intake of powder during feeding might act as stomach poison which led to the higher death rate of the adult insects in the case of *S. zeamais* (Mulungu *et al.*, 2007). Mulungu *et al.* (2010) reported that *S. zeamais* was more susceptible to botanicals than *P. truncatus*. These authors demonstrated that since adult *S. zeamais* spend more time feeding on the surface of grains while *P. truncatus* is found most of the time within the grain, the former insect usually ingest more surface insecticides than the latter. *P. glandulosus* being an aromatic plant, might have released toxic volatiles from the powders which contributed to the death of the two insect species. Adult *C. maculatus* was more susceptible to the leaf powder of another aromatic plant (*Dracaena arborea*), than adult *S. zeamais* (Udo *et al.,* 2011), probably because the volatile compounds from the plant were more toxic to the former than the latter insect.

Contrary to *P. glandulosus* leaf powder, the neem leaf powder caused less than 25% mortality to both insect species. This result deviates from those of other studies where leaf powders were applied (Boeke *et al.,* 2001; Iloba & Ekrakene, 2006). Ojo *et al.* (2013) applied *Moringa oleifera* leaf powder to *C. maculatus* and recorded more than 80% mortality after 6 d post-exposure. Iloba & Ekrakene (2006) used neem leaf powders against *C. maculatus* and *S. zeamais* and found that higher adult mortality was observed in the cowpea bruchid. Other factors like the origin of the leaves, the climate and the soil may influence the effectiveness of the neem leaf powder. Generally neem leaves contain less Azadirachtin A and more nimbin

and therefore the leaf and not the seed powders were less efficacious towards insects, since the efficacy of neem products is based mainly on its Azadirachtin A amount (Ghimeray *et al.*, 2009).

It is widely reported that the direct exposure of plants to sunlight or increasing temperatures has an effect on the sensitive compounds, leading to photodegradation or thermodegradation (Müller & Heindl, 2006; Ngamo *et al.,* 2007c). In addition, plant materials for insect bioassays studies are generally dried under shade conditions (Arannilewa *et al.,* 2006; Ngamo *et al.,* 2007c; Goudoum *et al.,* 2012a). Nukenine *et al.* (2013) revealed that the powder from the shade-dried leaves of *P. glandulosus*, with unknown chemical composition, was more effective against *S. zeamais* compared to the sun-dried under fluctuating laboratory conditions. However, the present study indicated that under controlled laboratory conditions, mortality of *S. zeamais* was higher with the powders from the leaves of the same plant dried in sun light compared to those from shade-dried leaves. A mixture with higher levels of camphor and other phytochemicals like linalool was highly toxic to *S. zeamais* while phytochemical mixtures lacking camphor was more or less inactive against this insect (Bekele & Hassanali, 2001). In this line, the higher levels of camphor and thymol in the sun-dried leaves might have been responsible for the higher potency of the sun- compared to the shade-dried powders against adult *S. zeamais*. More so, the rate of linalool was higher in the sun- than the shade-dried leaves, and this compound was reported to act on the nervous system of insects, affecting ion transport and the release of acetylcholinesterase, which results in total breakdown of the nervous system (López & Pascual-Villabolos, 2010; Shukla *et al.,* 2011; Yeom *et al.,* 2012). As a corollary, as has been the practice, shade-drying of plant leaves may not improve their toxicity towards insects. Therefore, there is a need to intensify efforts towards studies involving different drying regimes of plant materials and bioactivity against several species of insects, since photodegradation and thermodegradation may not always correlate directly with insecticidal efficacy.

No difference in adult mortality was observed when the grains were treated with *A. indica* powders from leaves that were shade- or sun-dried. This might be the resultant of the low levels of the active ingredients responsible for the death of weevils. The similarity in adult mortality could be due to the physical action of powders to kill stored product insect pests. Unfortunately, there is little or no research work concerning the influence of drying regime on the efficacy of A. indica leaf powders and extracts. The analysis of the

Azadirachtin content in neem leaves according to drying regimes or methods merits investigation.

Finer particle-size powders (0.1 mm) of *A. indica* and *P. glandulosus* were more active against *C. maculatus* and *S. zeamais*, respectively, than the coarse ones (0.5 mm), confirms the findings of Vayias *et al.* (2009) that the efficacy of powders is inversely related to their particle size. This indicates the superiority of finer particle size over the coarser ones to protect grains against the infestations of weevils (Asawalam *et al.*, 2007). Particle size affects distribution and the finer the particles, the more uniformly the dusts will coat treated grains, and storage containers, thus enhancing contact with the target insects (Olotuah, 2013; Zibaee *et al.*, 2013). Olotuah (2013) similarly reported that the most finely ground seed powders (particle size 0.15 mm) of *Piper guineense* and *Eugenia aromatica* were more active insecticidally to *S. zeamais* than the most coarse (particle size of 0.5 mm). With *C. maculatus*, the 0.212 mm particle-size powder of *P. guineense* caused higher mortality compared to the 1 mm particle-size ones (Ofuya & Dawodu, 2002). In a related study carried out under fluctuating laboratory conditions, 0.2 mm particle-size leaf powders of *P. glandulosus* caused 68% mortality to *S. zeamais* at the dose of 40 g/kg after 30 days of exposure (Nukenine *et al.*, 2013), while in the present study at the same dose level, 100% mortality of *S. zeamais* was recorded, 7 d with the 0.1 mm particle-size powder. The inconsistency in results could be related to the storage conditions. In the current study, the environmental conditions were fixed, but it was not the case in the previous work. Also Nukenine *et al.* (2013) harvested *P. glandulosus* leaves in 2006 and those from the present work in 2010. This could lead to speculate that the the potency of *P. glandulosus* varies with the year of harvest. In future studies, it will be recommendable to carry out research on the effect of harvesting time/year on the insecticidal efficacy of this botanical.

The proportion of each botanical had no influence on the effectiveness of the binary combinations in protecting maize and cowpea against the beetle infestations. While we hypothesized higher adult mortality when powders of *A. indica* seeds and *P. glandulosus* powders were combined, instead, adverse results were recorded, as the mixture was antagonistic. The lower mortality caused by the binary mixtures to *C. maculatus* and *S. zeamais* may be attributed to the different mode of action of each powder. *A. indica* seed powder is oily and acts on insect by antifeedency and blocking respiration (Don- Pedro, 1989; Schumuterrer, 1995) and *P. glandulosus* might act by the release of volatiles (López & Pascual-Villabolos, 2010). When combined, the leaf powder could absorb the oil contained in

the seed powder and, therefore, it becomes impossible for *P. glandulosus* to release its insecticidal compounds and for *A.indica* seed powder to coat well the insect body to block respiration, thus antagonism. These results do not conform to the previous studies (Musa *et al.*, 2009; Idoko & Adesina, 2012; Mwangi & Mutisya, 2013). The mixture of *P. guineense* and Pirimiphos methyl caused chronic toxicity to *C. maculatus* (Idoko & Adesina, 2012). The combination of neem seed powder and Malathion at the proportions of 40%+20% and 50%+10% on maize were additive with respect to the mortality caused to *Sitotroga cerealella* Olivier (Yuya, 2014). Binary mixtures of Pirimiphos methyl with groundnut, coconut or soybean oil registered higher mortality of adult *S. zeamais* (Obeng-ofori & Ametiye, 2005). These combined botanicals with synthetic chemicals, while in the present study considered only plant powders.

The mixture of NeemAzal and *P. glandulosus* leaf powder was also antagonistic regarding the mortality they caused to *C. maculatus* and *S. zeamais*. In isolation, NeemAzal caused greater mortality to boh insects than *P. glandulosus*. The mixture of *Vernonia amygdalina* and neem powder was antagonistic with respect to insecticidal efficacy (Akunne *et al.*, 2013). The NeemAzal used in the present study was produced by incorporating Azadirachtin into silica gel. The mortality observed with NeemAzal could largely be due to the presence of silica gel compared to that of Azadirachtin (Ogemah, 2003). Silica gel acts by desiccation, as the insects move through grains, they pick up the powder on their cuticle which leads to the absorption of the cuticular waxes from the epicuticule surface of the insect, thus enhancing the rate of desiccation (Prasantha, 2003). Ulrich & Mewis (2000) showed that combinations of diatomaceous earth (Fossil shield (1 gm/kg) and a commercial neem product NeemAzal (1 gm/kg) resulted in higher mortality of the weevils. Since NeemAzal contains silica gel, the mixture of this powder with Fossil shield implies the doubling of the concentration of diatomaceous earths, which resulted in higher mortality in the study of Ulrich and Mewis (2000).

Mortality of *C. maculatus* and *S. zeamais* decreased as the relative humidity increased from 60% to 70%, but did not vary as the temperature increased from 25°C to 30°C particularly for powders from *A. indica* seeds and *P. glandulosus* leaves. Ttored product insects' mortality increase with the augmentation of temperature and the reduction of relative humidity or grain moisture content (Kuronic, 1998; Fields & Kuronic, 2000; Arthur, 2002; Baldassari *et al.*, 2008). As relative humidity increased, the neem seed and *P. glandulosus* powders became less effective because the powders absorbed water from the surrounding

environment, reducing thir concentrations. Mewis and Ulrich (2001) observed that the efficacy of diatomaceous earth decreased when the relative humidity got higher. Like with the present study, Athanassiou *et al.* (2005) revealed that temperature had no influence on the toxicity of NeemAzal to *S. oryzae* on oats.

Faraway (2002) reported that in the Biological Sciences when the coefficient of determination, $R^2 \geq 0.6$, then the favorable results are attributable to the products used. In the present study most of the $R^2 \geq 0.8$. The few smaller values for the coefficient of determination are linked to high doses of applied substances, which lead to complete or alamost complete efficacy, with no variation in the insct responses (mortality, progeny inhibition, grain damage). Therefore, the botanicals were greatly responsible for the responses of *C. maculatus* and *S. zeamais* on the treated commodities. The chi-square values ($\chi 2$) were generally not significant for all products, implying that the obtained regression models approximate the theoretical model, concerning the toxicity of the used substances to both insect species (Finney, 1971).

One of the basic characteristics of an effective grain protectant is its ability to reduce progeny production in treated grains (Khoshnoud *et al.,* 2008). Results of inhibition of progeny production showed that oils extracted from *A. indica* seeds that were subjected to the four drying regimes completely inhibited progeny emergence of *C. maculatus and S. zeamais,* showing their enormous ability to control both insects. The neem oils might have acted physically or chemically on eggs or immature stages, depending on the insect species. Suppression of emergence in *C. maculatus* could be related to physical action of the neem seed oil. The couting of the seeds by *A. indica* oil, prevents the eggs from adhering unto the seeds. Therefore, it is not possible for the eggs to hatch in the grains and death ensues. Similar explanation was advanced by others researchers, where *A. indica* seed oil completely inhibited the progeny production of *S. oryzae* and *C. maculatus* (Bamaiyi *et al.,* 2007; Kemabonta & Falodu, 2013; Ilesanmi & Gundula, 2013). In addition, *A. indica* oil, like other vegetable oils, penetrates the chorion of bruchid eggs via the micropyle and the oil might occlude the egg funnel, which blocks exchange with outside, leading to the asphyxiation of the developing insect, then death (Copping & Menn, 2000).

Neem seed oils could also inhibit progeny production by non mechanical mechanisms, especially with *S. zeamais*. Female maize weevil lay eggs inside the grain. If, on treated grains oviposition is not deterred by the presence of the oil, then the development of immature stages

could be affect chemically by limonoids. As the oil has the ability to infiltrate the grains, the larvae of *S. zeamais*, which feed inside the grains would ingest some quantity of azadirachtin and other compounds like nimbin and salanin in neem oil. These compounds have growth regulatory effects on larvae, such as, disruption of moulting, growth inhibition, malformation, which may block the developmental stages of the weevils or cause mortality of immature stages (Isman, 2006). Udo (2005) stated that, there is a relationship between F_1 progeny emergence and adult mortality. His statement is confirmed by the report of Fekalu *et al.* (2012) who found that *Gossypium hirsitum* and *Brassica carinata* seed oils reduced adult emergence of *S. zeamais*. But it was not the case in the present work, since there was living *S. zeamais* 14 days (5 ml/kg) after infestation and offspring was recorded at the dosage level of 3 ml/kg.

No progeny emerged when powders obtained from the neem seeds that were subjected to the four drying regimes where applied on grains, except the lowest dose level of 5 g/kg on maize, where not more than one adult *S. zeamais* emerged when treated with shade-dried kernel, shade and sun-dried seeds. Our result is in accordance with those of other workers. Neem seed powder inhibited progeny production of *C. maculatus* (Lale & Abdulrahman, 1999). The powder and the oil reduced adult emergence of *S. zeamais* (Nukenine *et al.*, 2011a, b). Powders of *Calotropis procera* AIT and *Senna occidentalis* L. reduced by 99%, the F_1 progeny production of *Caryedon serratus* (OL.) on groundnut (Thiaw *et al.*, 2007). Suppression of progenies may have been achieved through a combination of oviposition deterrence, high mortality of eggs, larvae and nymphs (Lale & Adulrahman, 1999). Neem seed powder inhibited progeny production through similar mechanism like the neem oil.

The leaf powders from *P. glandulosus* and *A. indica* also greatly inhibited progeny production of *C. maculatus* and *S. zeamais* and the inhibition rate increased was decreasing particle size of the powders from 0.5 mm to 0.1 mm. One of the problems posed by powders to inhibit insect progeny production is that the developmental stage of most stored product insects is inside the grain. Powders can only coat the outer part of the grain and the active ingredients would not penetrate the grains (Adedire & Ajayi, 1996; Ukeh *et al.*, 2010). Therefore, the growth and development of the insect is not hampered inside grains by powders or dust. The reduction in adult emergence could be related in this case to adult mortality rather than the toxicity to the immature stages. Before dying, insects did not have time to lay eggs due to toxicity of botanicals or physiological dysfunction. It could be assumed that the test powders did not affect directly the insect development (Akob & Ewete

2007; Mwangangi & Mutisya, 2012). The lower ability of neem leaf or *P. glandulosus* powders to reduce the progeny emergence in the present study could be attributed to the low mortality recorded. Other research works showed that *P. glandulosus* reduced the production of *S. zeamais* progeny (Nukenine *et al.* 2007, 2011a) and this is in accordance with the findings of the preseny study.

Particle size affects distribution of powders and the finer the particles, the more uniformly the powder would coat treated grains and storage containers, thus enhancing contact with the target insects, limiting the insects' movement and reducing their ability to deposit eggs (Ivbijaro & Agbaje 1986). Adler *et al.* (2002) reported that, when applied as ground powder, *P. guineense* was more active to inhibit progeny production of *S. zeamais*. Ogunwolu & Idowu (1994) also stated that the most finely ground root bark of *Zanthoxylum zanthoxyloides* was more active to *C. maculatus* than the coarse (particle size of 2 mm) ones..

It could also be concluded that the binary mixtures at different proportion levels of the powders from *A. indica* seeds and *P. glandulosus* leaves or NeemAzal and *P. glandulosus* has various effects on adult emergence. As stated above, because of lower adult mortality, the mixture of *A. indica* seed and *P. glandulosus* leaf powders was not efficient in suppressing the F_1 progeny in *C. maculatus*, while the mixture of NeemAzal and *P. glandulosus* reduced almost completely the emergence of adult insects when the rate of NeemAzal $\geq$ 50%. Nukenine *et al.* (2011a) and Tofel *et al.* (2012) reported that under fluctuating conditions, NeemAzal powder registered similar results on *S. zeamais* and *C. maculatus.* It seems that the silica gel absorbed the water contained in grains which affected the development of the weevils. Before treatment the moisture content of the grains was above 12% and after F_1 progeny evaluations, this value decreased to less than 10%. When the moisture content of the grains is less than 10%, the development of immature stages of both insect species is hindered.

Cowpea and maize suffer heavy damage and losses during storage due to *C. maculatus* and *S. zeamais,* respectively. In the control treatment, within 10 weeks of storage, 98% and 45% of cowpea and maize, respectively, were damaged. *A. indica* oil or powder protected well maize and cowpea from the damage and the consequent weight loss caused respectively by *S. zeamais* and *C. maculatus*. Adult mortality and the inhibition of progeny emergence must, at least in parts, be responsible for the little or no damage on the commodities. Neem seed oil and *Moringa* seed oil protected cowpea for 60 days without damage (Ilesanmi &

Gundula, 2013). Cashew kernel oil offered 100% protection of maize grains against *S. zeamais* after 90 days (Adedire *et al.*, 2012). Niber (1995) concluded that the action of neem oil to reduce seed damage was chemical rather physical. Ogemah (2003) observed also reduced seed damage on neem seed oil treated maize against *Prostephanus truncatus* Horn.

The present investigation concerning neem seed powders substantiates the findings of Gueye *et al.* (2012), who demonstrated that maize cob dust reduced weight loss and grain damage of maize after four months storage with minor weight losses. Similar pattern of low seed damages was noticed as rubber seed oil, palm oil and palm seed oil were used on *C. maculatus* (Law-Ogbomo, 2007). Our results for cowpea and neem seed powder differs from those of other works (Brisibe *et al*., 2011; Udo *et al*, 2011), where plant powders were applied. Their experiments were carried out with neem seeds from other origin (Nigeria), which may have different rates of insecticidal compounds. The constituents of neem seed powder, like Azadirachtin A, persisted on treated seeds in the present study, which affected insect at different developmental stages. Thus, the reduction of damage is the consequence of adult mortality, oviposition deterrence, ovicidal, larval and nymphal mortality or blockage.

Except *P. glandulosus* powder on *C. maculatus*, NeemAzal, *P. glandulosus* and the mixture of both reduced grain damage and weight loss caused by *C. maculatus* and *S. zeamais* on the commodities. This is a consequence of adult mortality and speciesspecific behavior. Adult bruchids do not feed on stored cowpea seeds but only deposit their eggs which continued their development by damaging seeds. Nukenine *et al.,* (2010) revealed similar result that *P. glandulosus* leaf powder protected maize from *S. zeamais* damage. The present findings are in discordance with the study of Islam *et al.* (2013) for *C. maculatus,* who reported that black cumin (*Nigella sativa*), methi (*Trigonella foenum-graecum*) and garlic (*Allium sativum*) reduced damaged on gram (*Cicer arietinum*) *by C. chinensis.*

The activity of the neem seed oil from the sun-dried kernels remained high up to the 60-d storage interval (100% mortality at 6 ml/kg) for *S. zeamais*, but drop drastically between the 0-d (100% mortality at 6 ml/kg) and 15-d (< 20% at 6 ml/kg) storage interval for *C. maculatus*. This difference in persistence of the oil towards the two insect species could be due to the variation in the seed coat of the treated grains. Cowpea seed coat is thinner and more oil penetrated into the seed than that of maize which is thicker and retained more oils on the grains. Through this mechanism of permeability, the physical contact between insect and oil is reduced and limited mortality of *C maculatus* by anoxia insues. More so, *S. zeamais*

adults feed on grains, during food intake took some triterpenoid compounds of neem oil which could lead to the death of the adult insect. Similar results were registered with *Jatropha* seed oil on cowpea with *C. maculatus*, within the same period of storage, by Boateng and Kusi (2008). The persistence trends for the two insect species with neem seed powder were similar to those with the seed oils. The decrease of persistence of sun-dried kernel powder over time could be attributed to the degradation of its main compound Azadirachtin A, as observed in the present study. Boursier *et al.* (2011) mentioned that, if Azadirachtin A is stored at 25°C, its content can stay stable at least between seven and 14 days. So, as the efficacy of the powder persisted up to more than two months, it means that Azadirachtin A could stay stable for more than one month or neem powder may contain some other molecules which caused maize weevil mortality after degradation of its main insecticidal constituent. NeemAzal contains silica gel and for this reason, the activity of its mixture with *P. glandulosus* was more or less constant up to 180 d compared to the reduction 70% reduction in the efficacy of *P. glandulosus* alone. Silica gel is an inert durst and does not contain volatiles like *P. glandulosus*, which loses its active ingrediants with time. The activity of *Ocimum basilicum*, an aromatic plant of the Lamiaceae family like *P. glandulosus on S. zeamais* mortality declined most 0 (80% mortality) and 28 d (15% moratlity) (Mwangangi & Mutisya, 2013), which is in conformity with the results of the presentt work.

CONCLUSION

The task of the present study consisted mainly in investigating the influence of drying regime of local *P. glandulosus* leaves and *A. indica* seeds and leaves as well as the mixture of these two plant products for their various bioactivities against *C. maculatus* and *S. zeamais,* major storage insect pests respectively of cowpea and maize. This is a contribution to the search of less hazardous botanicals, which could be cheaper insecticides, accessible to local farmers for the enhancement of food security and safety by reducing grain losses during storage using better locally formulated products from *A. indica* and *P. glandulosus* as components of integrated stored product protection strategies.

The sun-drying of *A. indica* seeds led to a smaller quantity of oil in the seeds (28.68% w/w) compared to that in the shade-dried seeds (34.42% w/w). The oil from the sun-dried seeds (2.89 g/kg) also contained smaller amounts of Azadirachtin A than the shade-dried ones (3.69 g/kg). The rate of Azadirachtin A in *A.indica* powders from the leaves that was subject to different drying regimes did not vary in function of the drying regime, with an average of 1.20 g/kg of powder.

The major fatty acids found in the neem oils, in the range 0.06% - 53.67%, were oleic acid > palmitic acid, linoleic acid, stearic acid >> arachidic acid, behenic acid and lignoceric acid, with no variations among the four drying regimes (sun- and shade-dried seeds and sun- and shade-dried kernels).

The sun-drying of *P. glandulosus* leaves had little or no effect on the diversity of the volatile compounds of the leaves, and a total of the same 50 compounds were found respectively in the sun-dried and shade-dried leaves. Of the 50 compounds 18 were similar, 24 higher and eight lower in the shade-dried than the sun-dried leaves. The rate of the three compounds, terpinolene, piperitone oxide and (E)-germacrene D, were far much higher in the shade- than the sun-dried leaves.

All the tested products (*A. indica* seed powder and oils, powders from the leaves of *A. indica* and *P. glandulosus* and NeemAzal powder, as well as the binary combinations of the powders) caused significant mortality to *C. maculatus* and *S. zeamais*, relative to the control, irrespective of the drying regime

The seed oil of *A. indica* was more active towards both insect species than the powder, with no significant influence of the drying regime on the bioactivity. The potency of the oil was generally similar towards both insect species, causing > 90% mortality at the highest tested dose of 6 ml/kg. *S. zeamais* was more susceptible to the seed powder than *C. maculatus*, attaining > 90% mortality 7-d after infestation and *C. maculatus* attaining < 40% mortality 6-d after infestation, with the highest tested dose of 40g/kg. 6-d LC_{50} was 64.17 (48.38 - 103.78) for *C. maculatus* and 7-d LC_{50} for *S. zeamais* was 12.23 (8.56 - 16.16), with the powders from the shade-dried kernels.

The insecticidal efficacy of the *A. indica* oil was more or less stable for 60 days on *S. zeamais*, but declined roughly three-folds within 15 days with *C. maculatus*. However, when uninfested cowpea seeds and maize grains were coated with the oil, the degradation rate of Azadirachtin A showed a similar trend for both commodities, with roughly 0.2 g/kg remain with the smallest dose of 2 ml/kg after 60 d, for an initial rate of roughly 0.4 g/kg, and roughly 0.6 g/kg for the highest dose of 6 ml/kg, for an initial rate of 1.1 - 1.3 g/kg.

Progeny emergence was totally supressed when the *A. indica* oil concentration was ≥ 3 ml/kg and powder ≥ 10 g/kg, with no resultant grain damage and weight loss.

The powders from *P. glandulosus* leaves were more effective against *C. maculatus* and *S. zeamais* than those from *A. indica* leaves, regardless of the drying regime. 3 d after infestation, *P. glandulosus* caused 20% mortality to *C. maculatus* and 22.50% to *S. zeamais*, *A. indica* caused 3.75% mortality to *C. maculatus* and 11.25% to *S. zeamais*. The fine particle-size powders (0.1 mm) of both plant species were more active against *C. maculatus* and *S. zeamais* than the coarse particle-size powders (≥ 0.5 mm), with respect to adult mortality, F_1 progeny production and grain damage, irrespective of the drying regime.

Generally, the binary combinations of *P. glandulosus* and *A. indica* seed powder on the one hand, and *P. glandulosus* and NeemAzal in the other hand, were antagonistic, regarding their toxicity to *C. maculatus* and *S. zeamais*. For the two insect species, the binary mixtures of the powders caused lower mortality, produced more progeny and incurred more grain damage, compared to the cases with the individual powders.

The treatment of cowpea seeds with *A. indica* seed oil, *A indica* seed powder, *P. glandulosus* leaf powder, NeemAzal and a combination of 75% *P. glandulosus* + 25% NeemAzal greatly reduced fecundity in *C. maculatus*. Only treatments of maize grains with *A.*

indica seed powder decreased fecundity in *S. zeamais*. Treatment of the pupal stages of both insect species had less effect on the insects than the treatment of the egg and larval stages.

Considering the tested range of relative humidity 60-70% and temperature 25ºC – 30ºC in the present study, temperature had no influence on the efficacy of the botanicals against *C. maculatus* and *S. zeamais*, but their activity against the insects declines with increasing relative humidity.

That the bioactivity of products from sun-dried *A. indica* parts were generally similar to those of the shade-dried ones, could speed up processing of seeds by farmers and minimize attacks by fungi which may produce aflatoxins on treated grains. Since neem products taste bitter, they may be recommended more for long term grains storage (≥ 6 months), during which the bitter taste may reduce as the Azadirachtin level would drop to close to zero. *P. glandulosus* could be also easily sun-dried and 0.1 mm particle-size suitable for the protection of maize, but not cowpea, against the infestation of its major insect pest. The mixing of neem products with other botanicals in stored grains should be discouraged. Even *P. glandulosus* leaf powder with modest efficacy against *S. zeamais* could be adopted by growers for the protection of maize and cowpea stocks. Finally, insecticidal products from sun- or shade-dried parts of *A. indica* and *P. glandulosus* could form a major component of the integrated storage protection package for cowpea and maize against beetle infestations.

This study also indicates some potentially fruitful directions for future research that may eventually lead to the protection of stored maize and cowpea against their major pests and enhance food security and safety. These include:

- country-wide survey to investigate the effectiveness of the use of neem products and other plant substances in stored product protection;
- investigation on the acceptability of neem products as an insecticide by the rural masses;
- influence of treating stored grains with *A. indica* and *P. glandulosus* products on the quality characteristics of the grains;
- toxicity of neem products to *C. maculatus* and *S. zeamais* under different environmental conditions in Cameroon;

- stability of azadarachtin on treated grains as influence by Cameroonian environmental conditions;
- Azadirachtin contents and insecticidal efficacy of different *A. indica* cultivars or neem plants from a wide range of localities in Cameroon against *C. maculatus* and *S. zeamais.*

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APPENDIX

1: Corrected cumulative mortality of adult *Callosobruchus maculatus* exposed in cowpea grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to different drying regimes

Exposure period (days)	Dose (ml/kg)	Drying regime / % Mortality (mean ± SE) †				
		Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
1	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	
	2	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	–
	3	7.50 ± 7.50^{bc}	11.75 ± 3.13^{c}	13.75 ± 5.15^{b}	2.50 ± 1.44^{d}	1.90 ns
	4	25.00 ± 12.08^{b}	15.00 ± 2.89^{c}	32.50 ± 11.27^{b}	21.25 ± 6.57^{c}	0.53 ns
	5	60.00 ± 5.77^{a}	57.50 ± 9.68^{b}	65.00 ± 5.00^{a}	53.75 ± 3.75^{b}	0.49 ns
	6	77.50 ± 1.44^{a}	83.75 ± 5.54^{a}	81.25 ± 2.39^{a}	76.25 ± 4.37^{a}	0.84 ns
	$F_{(5, 18)}$ ‡	27.64***	50.42***	32.37***	79.92***	
3	0	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	$0.00 \pm 0,00^{e}$	0.00 ± 0.00^{c}	
	2	3.75 ± 2.39^{d}	3.75 ± 2.39^{e}	1.25 ± 1.25^{e}	0.00 ± 0.00^{c}	1.58 ns
	3	13.75 ± 6.57^{cd}	21.25 ± 4.73^{d}	13.82 ± 4.23^{d}	6.25 ± 3.15^{c}	1.09 ns
	4	37.50 ± 12.67^{bcAB}	50.86 ± 10.65^{cA}	33.75 ± 1.25^{cB}	46.25 ± 9.87^{bA}	2.65*
	5	76.25 ± 5.15^{ab}	83.75 ± 3.75^{b}	83.49 ± 2.54^{b}	71.25 ± 7.74^{b}	1.34 ns
	6	90.00 ± 4.08^{aB}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	7.95*
	$F_{(5, 18)}$ ‡	34.99***	175.51***	80.81***	63.96***	
6	0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	
	2	10.40 ± 2.15^{cB}	21.45 ± 2.22^{cA}	6.45 ± 2.45^{cdB}	3.88 ± 2.51^{deB}	5.79*
	3	23.42 ± 5.53^{bc}	38.95 ± 10.13^{bc}	28.73 ± 8.48^{c}	13.11 ± 3.32^{d}	2.22 ns
	4	41.84 ± 12.86^{b}	63.36 ± 5.41^{b}	73.54 ± 12.47^{b}	57.41 ± 11.87^{c}	1.54 ns
	5	87.11 ± 3.20^{aB}	96.05 ± 3.95^{aA}	88.14 ± 2.55^{abB}	86.86 ± 6.25^{bB}	2.78*
	6	98.69 ± 1.32^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	1.00 ns
	$F_{(5, 18)}$ ‡	47.10***	41.35***	46.76***	58.71***	

† Means in the same column followed by the same lowercase letter within the same exposure period or in the same line followed by the same uppercase letter, do not differ significantly (Tukey's test; $P < 0.05$)

‡ ns $P > 0.05$; * $P < 0.05$; *** $P < 0.001$; – F value estimation is not possible due to equal variance

2: Corrected cumulative mortality of adult *Sitophilus zeamais* exposed in maize grains treated with *Azadirachta indica* oils extracted from seeds that were subjected to different drying regimes

Exposure period (days)	Dose (ml/kg)	Drying regime / % Mortality (mean ± SE)				
		Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
1	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	
	2	1.25 ± 1.25^{c}	1.25 ± 1.25^{c}	0.00 ± 0.00^{d}	1.25 ± 1.25^{b}	0.33 ^{ns}
	3	5.00 ± 2.04^{bc}	3.75 ± 2.39^{bc}	2.50 ± 1.44^{cd}	5.00 ± 3.54^{ab}	0.19 ^{ns}
	4	7.50 ± 3.23^{bc}	7.50 ± 1.44^{bc}	8.75 ± 2.39^{bc}	6.25 ± 2.39^{ab}	0.30 ^{ns}
	5	13.75 ± 2.30^{ab}	17.50 ± 2.50^{ab}	11.25 ± 1.25^{ab}	17.50 ± 4.33^{a}	1.20 ^{ns}
	6	20.00 ± 4.56^{a}	31.25 ± 6.57^{a}	17.50 ± 2.50^{a}	18.75 ± 4.27^{a}	1.75 ^{ns}
	$F_{(5, 18)}$ ‡	8.31***	14.87***	19.12 ***	6.92**	
3	0	0.00 ± 0 .00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	
	2	8.75 ± 5.54^{cd}	5.00 ± 2.04^{d}	7.50 ± 1.44^{cd}	8.75 ± 3.75^{de}	0.24 ^{ns}
	3	16.25 ± 2.39^{bc}	17.50 ± 6.29^{c}	15.00 ± 2.04^{cd}	16.25 ± 1.25 ^{cd}	0.10 ^{ns}
	4	28.75 ± 7.18^{bc}	37.50 ± 5.95^{bc}	22.50 ± 2.50^{bc}	30.00 ± 5.40 ^{bc}	1.29 ^{ns}
	5	40.00 ± 4.56^{ab}	47.50 ± 1.44^{ab}	40.00 ± 4.08^{ab}	40.00 ± 4.56 ^{ab}	0.99 ^{ns}
	6	86.25 ± 7.74^{a}	62.50 ± 7.22^{a}	56.25 ± 7.47^{a}	51.25 ± 3.15^{a}	0.46 ^{ns}
	$F_{(5, 18)}$ ‡	15.27***	27.75***	24.91***	30.23 ***	
7	0	0.00 ± 0.00 ^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	
	2	23.79 ± 9.44^{c}	26.25 ± 6.88^{c}	26.25 ± 10.68^{cd}	23.75 ± 5.54^{c}	0.03 ^{ns}
	3	37.50 ± 6.61^{bc}	65.00 ± 10.61^{b}	50.00 ± 6.12^{bc}	51.25 ± 9.00^{b}	1.79 ^{ns}
	4	72.50 ± 8.54^{abB}	91.25 ± 5.15^{aA}	71.25 ± 8.26^{abB}	70.00 ± 3.54^{bB}	3.02 *
	5	95.00 ± 3.54^{a}	100 ± 0.00^{a}	95.00 ± 3.54^{a}	93.75 ± 4.73^{a}	0.85 ^{ns}
	6	95.00 ± 2.89^{a}	100 ± 0.00^{a}	96.25 ± 2.39^{a}	100.00 ± 0.00^{a}	1.96 ^{ns}
	$F_{(5, 18)}$ ‡	41.35***	57.00***	37.46***	58.58*	
14	0	0.00 ± 0.00 ^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00 ^{d}	0.00 ± 0.00^{d}	
	2	30.40 ± 7.08^{c}	39.15 ± 9.97^{b}	31.84 ± 10.85^{c}	33.78 ± 8.20^{c}	0.17 ns
	3	77.38 ± 1.31^{b}	74.80 ± 9.06^{a}	74.15 ± 7.84^{b}	71.70 ± 6.40^{b}	1.73 ^{ns}
	4	98.60 ± 1.40^{a}	95.00 ± 3.54^{a}	88.62 ± 6.57^{ab}	88.41 ± 6.54^{ab}	0.40 ^{ns}
	5	97.50 ± 2.50^{a}	100 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	1.00 ^{ns}
	6	98.75 ± 1.25^{a}	100 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	1.00 ^{ns}
	$F_{(5, 18)}$ ‡	66.42***	51.22***	45.49***	65.49***	

† Means in the same column followed by the same lowercase letter within the same exposure period or in the same line followed by the same uppercase letter, do not differ significantly (Tukey's test; P < 0.05))

‡ ns P > 0.05, * P < 0.05; ** P < 0.01, *** P<0.001; – F value estimation is not possible due to equal variance

3: Corrected cumulative mortality of adult *Callosobruchus maculatus* exposed in grains treated with *Azadirachta indica* seed powders obtained from seeds that were subjected to different drying regimes

Exposure period (days)	Dose (g/kg)	Drying regime / % Mortality (mean ± SE) †				
		Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
1	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
	20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
	30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
	40	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
	$F_{(5, 18)}$ ‡	–	–	–	–	
3	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	
	5	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	–
	10	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	–
	20	5.00 ± 2.04^{bc}	2.50 ± 1.44^{bc}	2.50 ± 1.44^{bc}	0.00 ± 0.00^{b}	2.00 ns
	30	15.00 ± 3.54^{aA}	5.00 ± 2.04^{abB}	5.00 ± 0.00^{bB}	3.75 ± 1.25^{aB}	6.03*
	40	15.00 ± 00^{aA}	11.25 ± 1.25^{aAB}	12.50 ± 1.44^{aAB}	7.50 ± 1.44^{aB}	6.82*
	$F_{(5, 18)}$ ‡	32.52**	12.50***	28.42***	21.51***	
6	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	–
	5	1.32 ± 1.32^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	1.00 ns
	10	7.57 ± 1.41^{bc}	6.32 ± 1.23^{c}	3.75 ± 2.39^{cd}	6.32 ± 1.23^{b}	0.96 ns
	20	20.26 ± 2.06^{bA}	15.20 ± 2.05^{bAB}	10.00 ± 2.89^{bcB}	13.88 ± 2.33^{abAB}	3.24*
	30	31.65 ± 3.12^{aA}	22.83 ± 1.66^{bA}	12.50 ± 1.44^{abB}	13.82 ± 3.70^{abB}	11.24***
	40	30.46 ± 2.43^{a}	34.28 ± 4.07^{a}	23.75 ± 2.39^{a}	22.76 ± 3.17^{a}	3.17 ns
	$F_{(5, 18)}$ ‡	62.13***	109.59***	24.85***	40.49***	

† Means in the same column followed by the same lowercase letter within the same exposure period or in the same line followed by the same uppercase letter, do not differ significantly (Tukey's test; $P < 0.05$)

‡ ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; – F value estimation is not possible due to equal variance

4: Corrected cumulative mortality of adult *Sitophilus zeamais* exposed in grains treated with *Azadirachta indica* seed powders obtained from seeds that were subjected to different drying regimes

Exposure period (days)	Dose (g/kg)	Drying regime / % Mortality (mean ± SE)				
		Shade-dried kernels	Sun-dried kernels	Shade-dried seeds	Sun-dried seeds	$F_{(3, 12)}$ ‡
1	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	
	5	1.25 ± 1.25^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1 ns
	10	3.75 ± 2.39^{bc}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	2.45 ns
	20	5.00 ± 2.04^{abcA}	1.25 ± 1.25^{bAB}	0.00 ± 0.0^{bB}	0.00 ± 0.00^{bB}	3.91 *
	30	8.75 ± 1.25^{abA}	2.50 ± 1.44^{bAB}	1.25 ± 1.25^{bB}	5.00 ± 2.04^{aAB}	4.67 *
	40	15.00 ± 2.04^{aA}	8.75 ± 2.39^{aAB}	6.25 ± 1.25^{aB}	7.50 ± 1.44^{aAB}	4.46 *
	$F_{(5, 18)}$ ‡	8.30***	8.67***	15.93 ***	16.76*	
3	0	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
	5	5.00 ± 3.54^{de}	5.00 ± 2.04^{cd}	1.25 ± 1.25^{c}	7.50 ± 4.33^{bc}	0.72 ns
	10	13.75 ± 2.39^{bc}	16.25 ± 2.39^{c}	15.00 ± 1.09^{b}	17.50 ± 1.44 ^{ab}	0.59ns
	20	22.50 ± 4.79^{bc}	42.50 ± 9.24^{b}	26.25 ± 2.39^{b}	23.75 ± 4.73 ^{ab}	2.53 ns
	30	36.25 ± 5.15^{abBC}	63.75 ± 3.15^{abA}	53.75 ± 2.39^{aAB}	30.00 ± 5.40 ^{aC}	13.57***
	40	47.50 ± 4.33^{aB}	70.00 ± 2.04^{aA}	67.50 ± 3.23^{aA}	38.75 ± 5.54^{aB}	14.53***
	$F_{(5, 18)}$ ‡	26.21***	54.65***	141.06***	10.93 ***	
7	0	0.00 ± 0.00 ^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	
	5	22.50 ± 7.46^{d}	15.20 ± 5.40^{b}	12.50 ± 1.44^{c}	36.25 ± 6.88^{d}	2.69 ns
	10	33.75 ± 6.25^{cd}	26.91 ± 2.40^{b}	30.00 ± 3.54^{bc}	52.50 ± 12.67^{cd}	2.42 ns
	20	66.25 ± 10.08^{bcAB}	84.74 ± 3.41^{aA}	50.00 ± 5.40^{bB}	78.75 ± 4.73^{bcA}	5.73*
	30	85.00 ± 2.04^{ab}	95.25 ± 4.75^{a}	86.25 ± 6.25^{a}	88.75 ± 4.73^{ab}	0.95 ns
	40	92.50 ± 4.79^{a}	97.50 ± 2.50^{a}	98.75 ± 1.25^{a}	97.50 ± 1.44^{a}	0.94 ns
	$F_{(5, 18)}$ ‡	32.54***	64.89***	82.63***	44.61*	
14	0	0.00 ± 0.00 ^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	
	5	28.95 ± 7.62^{cAB}	28.03 ± 5.00^{cAB}	21.51 ± 1.19^{dB}	50.72 ± 5.67^{bA}	5.33*
	10	52.90 ± 7.39^{b}	53.82 ± 7.51^{b}	47.90 ± 9.45^{c}	68.55 ± 8.13^{b}	1.19 ns
	20	84.74 ± 6.53^{aA}	98.75 ± 1.25^{aA}	67.04 ± 3.38^{bB}	98.75 ± 1.25^{aA}	15.85 ***
	30	97.37 ± 2.63^{a}	100 ± 0.00^{a}	98.69 ± 1.32^{a}	100.00 ± 0.00^{a}	0.73 ns
	40	98.69 ± 1.32^{a}	100 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	1.00 ns
	$F_{(5, 18)}$ ‡	54.28***	209.69***	151.80***	160.04***	

† Means in the same column followed by the same lowercase letter within the same exposure period or in the same line followed by the same uppercase letter, do not differ significantly (Tukey's test; P < 0.05)

‡ ns P > 0.05, * P < 0.05; *** P<0.001

5: Residual toxicity *Azadirachta indica* seed oil obtained from sun-dried kernels after different storage intervals in *Callosobruchus maculatus* and *Sitophilus zeamais* on treated cowpea and maize

Insects /doses (ml/kg)	Storage intervals (days)/ % mean mortality[†]					
	0	15	30	60	180	$F_{(4, 15)}$[‡]
C. maculatus						
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00	
2	32.50 ± 1.44^{dA}	9.01 ± 2.53^{bB}	1.32 ± 1.32^{cC}	5.00 ± 2.04^{abcBC}	0.00 ± 0.00^{C}	28.41***
3	55.00 ± 5.40^{cA}	26.91 ± 3.08^{aB}	11.52 ± 1.17^{bC}	2.50 ± 1.14^{bcD}	0.00 ± 0.00^{D}	64.04***
4	77.50 ± 4.79^{bA}	24.15 ± 5.38^{aB}	23.03 ± 4.89^{abB}	7.50 ± 1.44^{abC}	0.00 ± 0.00^{D}	62.12***
5	95.00 ± 2.04^{aA}	37.11 ± 5.46^{aB}	28.16 ± 6.22^{abB}	10.00 ± 2.04^{aC}	0.00 ± 0.00^{D}	93.98***
6	100 ± 0.00^{aA}	38.29 ± 4.38^{aB}	34.48 ± 5.29^{aB}	10.00 ± 2.04^{aC}	0.00 ± 0.00^{D}	275.71***
$F_{(5, 18)}$	172.87***	30.57***	27.10***	7.92***	–	
S. zeamais						
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}	0.00 ± 0.00^{d}	0.00 ± 0.00	
2	13.75 ± 2.39^{dA}	26.25 ± 6.88^{cA}	30.00 ± 4.46^{eA}	26.25 ± 7.47^{cA}	1.25 ± 1.25^{B}	9.39***
3	52.50 ± 7.22^{cA}	57.50 ± 6.01^{bA}	48.00 ± 6.25^{dA}	35.00 ± 8.42^{cA}	0.00 ± 0.00^{B}	27.76***
4	78.75 ± 3.75^{bA}	91.25 ± 5.15^{aA}	77.50 ± 3.23^{cA}	91.25 ± 4.27^{abA}	5.00 ± 3.54^{B}	31.37***
5	100 ± 0.00^{aA}	100 ± 0.00^{aA}	91.25 ± 1.25^{bB}	88.75 ± 3.75^{bB}	5.00 ± 2.89^{C}	131.39***
6	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	6.25 ± 2.39^{B}	318.13***
$F_{(5, 18)}$	273.33**	89.01***	214.45**	67.03***	1.89^{ns}	

[†] Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

[‡] ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;

– F value estimation is not possible due to equal variance.

6: Degradation of Azadirachtin A in maize and cowpea treated with *Azadirachta indica* oil after different storage intervals

Com§/Content (ml/kg)	Storage intervals (days) / Azadirachtin A content (g/kg)†													
	0	1	3	7	10	14	21	30	60	90	120	150	180	$F_{(12,39)}$‡
Cowpea														
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
2	0.32± 0.03^{dC}	0.34± 0.02^{dBC}	0.42± 0.01^{dA}	0.35± 0.02^{cBC}	0.41± 0.02^{dAB}	0.34± 0.03^{dBC}	0.37± 0.02^{cABC}	0.36± 0.01^{cABC}	0.33± 0.01^{cBC}	0.23± 0.02^{cD}	0.16± 0.01^{dDE}	0.16± 0.02^{dDE}	0.14± 0.01^{dE}	34.54***
3	0.44± 0.03^{cdC}	0.48± 0.02^{cdBC}	0.62± 0.04^{cA}	0.45± 0.02^{bcC}	0.58± 0.03^{cAB}	0·02^{cdC}	0.46± 0.01^{bcBC}	0.46± 0.01^{bcC}	0.44± 0.01^{cC}	0.28± 0.00^{bcD}	0.23± 0.03^{cdD}	0.23± 0.02^{cdD}	0.21± 0.04^{cdD}	33.83***
4	0.67± 0.02^{bcBC}	0.63± 0.04^{cBD}	0.91± 0.05^{bA}	0.70 ± 0.03^{abBC}	0.72 ± 0.03^{bB}	0.57± 0.04^{bcBC}	0.60± 0.03^{bBC}	0.55± 0.05^{bCD}	0.56± 0.03^{bBCD}	0.40± 0.01^{bDE}	0.33± 0.03^{bcE}	0.32± 0.02^{bcE}	0.28± 0.03^{bcE}	29.17***
5	0.87± 0.04^{abBC}	0.83± 0.04^{bBC}	1.12± 0.06^{aA}	0.88± 0.13^{aABC}	1.06± 0.02^{aAB}	0.76± 0.03^{abCD}	0.88± 0.05^{aABC}	0.69± 0.02^{aCD}	0.72± 0.03^{aCD}	0.55± 0.02^{aDE}	0.39± 0.02^{abE}	0.40± 0.03^{abE}	0.39± 0.03^{abE}	23.72***
6	1.14± 0.04^{aAB}	0.99± 0.05^{aABC}	1.17± 0.03^{aA}	0.86± 0.01^{aBDC}	1.04± 0.03^{aABC}	0.85± 0.09^{aBCD}	0.86± 0.06^{aBCD}	0.80± 0.03^{aCD}	0.83± 0.04 ^{aCD}	0.66± 0.05^{aDE}	0.43± 0.01^{aE}	0.44± 0.02^{aE}	0.43± 0.02 ^{aE}	19.45***
$F_{(4,15)}$‡	22.31***	54.07***	60.27***	14.84***	113.84***	19.70***	36.60***	40.91***	50.47***	43.59***	24.23***	19.20***	19.88***	-
Maize														
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
2	0.43± 0.03^{cA}	0.42± 0.03^{cAB}	0.48± 0.03^{dA}	0.47± 0.06^{cA}	0.32± 0.05^{cABC}	0.33± 0.01^{eABC}	0.41± 0.05^{bAB}	0.32± 0.03^{cABC}	0.25± 0.04^{dBC}	0.17± 0.03^{dCD}	0.14± 0.01^{bD}	0.13± 0.02^{cD}	0.10± 0.01^{dD}	15.39***
3	0.56± 0.03^{cAB}	0.67± 0.06^{bcA}	0.63± 0.03^{cdAB}	0.58± 0.02^{bcAB}	0.63± 0.04^{bAB}	0.50± 0.01^{dABC}	0.55± 0.05^{bAB}	0.47± 0.02^{bcBC}	0.37± 0.06^{cdCD}	0.27± 0.04^{cdDE}	0.18± 0.01^{bE}	0.18± 0.01^{cE}	0.14± 0.00^{cdE}	27.32***
4	0.87± 0.05^{bAB}	0.87± 0.03^{bAB}	1.02± 0.18^{bcA}	0.79± 0.03^{bAB}	0.66± 0.02^{bABCD}	0.68± 0.04^{cABC}	0.86± 0.20^{abAB}	0.61± 0.03^{bBCDE}	0.48± 0.07^{bcBCDE}	0.33± 0.03^{bcCDE}	0.27±0.02^{bD} E	0.30± 0.04^{bcCDE}	0.24± 0.04^{bcE}	10.57***
5	1.08± 0.06^{abA}	1.29± 0.09^{aA}	1.05± 0.07^{bA}	1.11± 0.07^{aA}	1.07± 0.09^{aA}	1.05± 0.04^{bA}	1.14± 0.18^{aA}	1.01± 0.06^{aA}	0.63± 0.04^{bB}	0.46± 0.03^{abB}	0.50± 0.06^{aB}	0.41± 0.05^{abB}	0.28± 0.03^{abB}	20.33***
6	1.31± 0.09^{aAB}	1.27± 0.0^{aAB}	1.47± 0.05^{aA}	1.23± 0.06^{aAB}	1.15± 0.08^{aABC}	1.21± 0.04^{aABC}	1.28± 0.05^{aAB}	1.03± 0.01^{aBC}	0.95± 0.02^{aC}	0.64± 0.07^{aD}	0.53± 0.00^{aD}	0.54± 0.06^{aD}	0.38± 0.04^{aD}	38.60***
$F_{(4,15)}$‡	40.68***	36.38***	18.06***	38.25***	31.19***	136.35***	8.88***	80.24***	30.91***	23.00***	27.84***	17.11***	13.61***	

§Com: commodity.

† Means in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ *** $P < 0.001$;

7: Corrected cumulative mortality (mean ± SE) of *Callosobruchus maculatus* exposed to *Plectranthus glandulosus* leaf powder of three particle sizes

Exposure period (days)	Doses (g/kg)	Particle size/Mortality (mean ± SE)†			
		≤ 0.1 mm	> 0.1 ≤ 0.3 mm	> 0.3 ≤ 0.5 mm	$F_{(2, 9)}$ ‡
1	0	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
	5	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
	10	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
	20	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
	30	1.25 ± 1.25^{ab}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1^{ns}
	40	3.75 ± 1.25^{a}	2.50 ± 1.44^{a}	5.00 ± 2.04^{a}	0.60^{ns}
	$F_{(5, 18)}$ ‡	4.40^{*}	3.00^{ns}	6.00^{**}	
3	0	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	–
	5	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	2.50 ± 1.44^{ab}	3.00^{ns}
	10	0.00 ± 0.00^{bB}	2.50 ± 1.44^{abB}	8.75 ± 2.39^{aA}	10.93^{*}
	20	0.00 ± 0.00^{b}	3.75 ± 1.25^{ab}	6.25 ± 3.15^{ab}	2.59^{ns}
	30	2.50 ± 1.44^{b}	2.50 ± 1.44^{ab}	11.25 ± 3.75^{a}	4.20^{ns}
	40	8.75 ± 1.25^{a}	6.25 ± 2.39^{a}	10.00 ± 2.04^{a}	0.95^{ns}
	$F_{(5, 18)}$ ‡	20.31^{***}	2.95^{*}	5.24^{*}	
6	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	–
	5	5.56 ± 2.27^{c}	15.33 ± 4.34^{cd}	19.33 ± 5.34^{b}	2.87^{ns}
	10	16.67 ± 2.27^{b}	22.36 ± 5.75^{bc}	29.23 ± 5.15^{ab}	1.83^{ns}
	20	16.67 ± 3.93^{b}	33.92 ± 4.27^{abc}	32.24 ± 5.56^{ab}	4.20^{ns}
	30	23.61 ± 3.49^{bB}	37.70 ± 8.18^{abAB}	47.79 ± 4.50^{abA}	4.46^{*}
	40	45.83 ± 3.50^{a}	50.00 ± 1.97^{a}	58.32 ± 12.29^{a}	0.72^{ns}
	$F_{(5, 18)}$ ‡	31.00^{***}	13.43^{***}	9.93^{***}	

† Means in the same column followed by the same letter do not differ significantly (Tukey's test; $P < 0.05$)

‡ ns $P > 0.05$; *** $P < 0.001$; – F value estimation is not possible due to equal variance

8: Corrected cumulative mortality (mean ± SE) of *Callosobruchus maculatus* exposed to *Azadirachta indica* leaf powder of three particle sizes

Exposure period (days)	Doses (g/kg)	Particle size/Mortality (mean ± SE)[†]			
		≤ 0.1 mm	> 0.1 ≤ 0.3 mm	> 0.3 ≤ 0.5 mm	$F_{(2, 9)}$ [‡]
1	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	40	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	$F_{(5, 18)}$ [‡]	-	-	-	
3	0	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	_
	5	0.00 ± 0.00^{b}	3.82 ± 2.41^{b}	0.00 ± 0.00^{b}	2.85^{ns}
	10	0.00 ± 0.00^{b}	2.50 ± 1.44^{b}	0.00 ± 0.00^{b}	3.00^{ns}
	20	3.75 ± 1.25^{bB}	11.58 ± 2.48^{abA}	0.00 ± 0.00^{bC}	13.58^{**}
	30	3.75 ± 2.39^{bB}	16.78 ± 2.67^{aA}	0.00 ± 0.00^{bB}	17.04^{***}
	40	10.00 ± 2.04^{aAB}	18.03 ± 5.07^{aA}	3.75 ± 1.25^{aB}	5.87^{***}
	$F_{(5, 18)}$ [‡]	8.07^{***}	7.65^{***}	9.00^{***}	
6	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	_
	5	20.95 ± 5.47^{bA}	6.95 ± 1.39^{cdB}	3.82 ± 1.27^{bB}	7.41^{*}
	10	27.68 ± 7.62^{bA}	12.44 ± 1.13^{bcAB}	5.13 ± 2.15^{abB}	6.53^{*}
	20	33.09 ± 8.42^{bA}	19.33 ± 2.34^{bAB}	5.07 ± 2.04^{abB}	7.31^{*}
	30	67.05 ± 2.67^{aA}	19.40 ± 1.33^{bB}	12.71 ± 3.14^{aB}	88.56^{***}
	40	70.87 ± 3.27^{aA}	30.44 ± 2.34^{aB}	12.83 ± 1.49^{aC}	130.39^{***}
	$F_{(5, 18)}$ [‡]	25.66^{***}	43.11^{***}	7.02^{***}	

[†] Means in the same column followed by the same letter do not differ significantly (Tukey's test; $P < 0.05$)

[‡] ns $P > 0.05$; *** $P < 0.001$; – F value estimation is not possible due to equal variance

9: Corrected cumulative mortality (mean ± SE) of *Sitophilus zeamais* exposed to *Plectranthus glandulosus* leaf powder of three particle sizes

Exposure period (days)	Doses (g/kg)	Particle size/Mortality (mean ± SE)†			
		≤ 0.1 mm	> 0.1 ≤ 0.3 mm	> 0.3 ≤ 0.5 mm	$F_{(2, 9)}$ ‡
1	0	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00	_
	5	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00	_
	10	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00	_
	20	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00	_
	30	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00	_
	40	3.75 ± 2.39^{a}	3.75 ± 1.25^{a}	0.00 ± 0.00	1.69 ^{ns}
	$F_{(5, 18)}$ ‡	2.45 ^{ns}	9.00*	_	
3	0	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	_
	5	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	1.25 ± 1.25^{b}	1^{ns}
	10	1.25 ± 1.25^{c}	0.00 ± 0.00^{c}	2.50 ± 1.44^{b}	1.29 ^{ns}
	20	5.00 ± 2.04^{bc}	1.25 ± 1.25^{bc}	3.75 ± 1.25^{b}	1.50 ^{ns}
	30	13.75 ± 2.39^{abA}	3.75 ± 1.25^{abB}	12.50 ± 1.44^{aA}	9.50 *
	40	21.25 ± 3.75^{aA}	7.50 ± 1.44^{aB}	16.25 ± 3.75^{aBC}	4.81 *
	$F_{(5, 18)}$ ‡	18.04***	10.56***	12.54***	
7	0	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	
	5	30.00 ± 3.54^{cA}	2.57 ± 1.48^{cC}	17.96 ± 3.26^{cB}	22.43***
	10	76.25 ± 3.75^{bA}	9.15 ± 2.46^{cC}	30.79 ± 3.00^{cB}	120.23 ***
	20	80.00 ± 5.40^{bA}	48.89 ± 4.57^{bB}	52.63 ± 2.76^{bB}	15.01*
	30	97.50 ± 2.50^{aA}	78.09 ± 8.72^{aAB}	72.90 ± 4.71^{aB}	4.83*
	40	100 ± 0.00^{aA}	82.87 ± 2.57^{aB}	83.23 ± 2.67^{aB}	20.90**
	$F_{(5, 18)}$ ‡	156.37***	78.00***	111.09***	
14	0	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	
	5	51.25 ± 3.90^{cA}	13.54 ± 3.72^{dB}	28.31 ± 5.00^{dAB}	4.67*
	10	85.00 ± 5.40^{bA}	42.73 ± 11.18^{cB}	44.20 ± 6.45^{dB}	9.48 *
	20	93.75± 3.75^{abA}	78.97 ± 6.50^{bAB}	70.64 ± 1.53^{cB}	7.00*
	30	100 ± 0.00^{aA}	98.69 ± 1.32^{aA}	88.00 ± 2.61^{bB}	20.14**
	40	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	_
	$F_{(5, 18)}$ ‡	39.71***	86.56***	109.89***	

† Means in the same column followed by the same letter do not differ significantly (Tukey's test; P < 0.05)

‡ ns P > 0.05; *** P < 0.001; – F value estimation is not possible due to equal variance

10: Corrected cumulative mortality (mean ± SE) of *Sitophilus zeamais* exposed to *Azadirachta indica* leaf powder of three particle sizes

Exposure period (days)	Doses (g/kg)	Particle size/Mortality (mean ± SE)†			
		≤ 0.1 mm	> 0.1 ≤ 0.3 mm	> 0.3 ≤ 0.5 mm	$F_{(2, 9)}$ ‡
1	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	40	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	$F_{(5, 18)}$ ‡	_	_	_	
3	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00^{b}	_
	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00^{b}	_
	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00^{b}	_
	20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00^{b}	_
	30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00^{b}	_
	40	1.25 ± 1.25	0.00 ± 0.00	0.00 ± 0.00^{b}	1 ^{ns}
	$F_{(5, 18)}$ ‡	1 ^{ns}	_	_	
7	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	30	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	_
	40	3.75 ± 2.39	0.00 ± 0.00	0.00 ± 0.00	2.83^{ns}
	$F_{(5, 18)}$ ‡	2.45^{ns}	_	_	
14	0	0.00 ± 0.00^{c}	0.00 ± 0.00	0.00 ± 0.00	_
	5	2.50 ± 1.44^{b}	0.00 ± 0.00	0.00 ± 0.00	3.00^{ns}
	10	2.50 ± 1.44^{b}	0.00 ± 0.00	0.00 ± 0.00	3.00 ^{ns}
	20	7.50 ± 2.23^{abA}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	5.40*
	30	13.75 ± 3.15^{aA}	3.75 ± 2.39^{B}	2.50 ± 1.44^{B}	6.44*
	40	15.00 ± 2.04^{aA}	2.50 ± 1.44^{B}	2.50 ± 1.44^{B}	18.75 ^{ns}
	$F_{(5, 18)}$ ‡	8.35***	2.12 ^{ns}	2.40 ^{ns}	

† Means in the same column followed by the same letter do not differ significantly (Tukey's test; $P < 0.05$)

‡ ns $P > 0.05$; *** $P < 0.001$;

– F value estimation is not possible due to equal variance

11: Corrected cumulative mortality (mean ± SE) of *Callosobruchus maculatus* exposed to binary combinations of powders from *Plectranthus glandulous* leaves and *Azadirachta indica* seeds

Insects/ doses (g/kg)	Proportion of powders in mixture/Mortality (mean ± SE)[†]					
	100% *P. gland*	75% *P. gland* + 25% *A. indica*	50% *P. gland* + 50% *A. indica*	25% *P. gland* + 75% *A. indica*	100% *A. indica*	$F_{(4, 15)}$ [‡]
			1-d			
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
2.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
$F_{(5,18)}$ ‡	–	–	–	–	–	
			3-d			
0	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00^{b}	–
2.5	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00^{b}	–
5	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00^{b}	–
10	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1.25 ± 1.25^{ab}	0.00 ± 0.00	0.00 ± 0.00^{b}	1^{ns}
15	3.75 ± 1.25^{a}	1.25 ± 1.25^{b}	1.25 ± 1.25^{ab}	0.00 ± 0.00	2.50 ± 1.44^{ab}	1.50^{ns}
20	5.00 ± 2.04^{a}	6.25 ± 2.39^{a}	5.04 ± 0.85^{a}	0.00 ± 0.00	3.75 ± 1.25^{a}	1.85^{ns}
$F_{(5,18)}$ ‡	5.51^{**}	5.14^{**}	3.06^{*}	–	4.54^{*}	
			6-d			
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	
2.5	0.00 ± 0.00^{eB}	1.25 ± 1.25^{bAB}	7.50 ± 2.50^{cA}	0.00 ± 0.00^{dB}	0.00 ± 0.00^{cB}	5.66^{**}
5	13.75 ± 1.25^{dA}	5.00 ± 2.04^{bBC}	11.25 ± 1.25^{cAB}	5.00 ± 2.89^{cdBC}	1.32 ± 1.32^{bcC}	7.46^{**}
10	21.25 ± 1.25^{cAB}	26.25 ± 2.39^{aA}	16.25 ± 4.27^{bcAB}	12.50 ± 3.23^{bcBC}	1.25 ± 1.25^{bcC}	12.04^{***}
15	31.25 ± 2.39^{bA}	33.75 ± 4.73^{aA}	27.50 ± 3.23^{bA}	20.00 ± 3.54^{abA}	5.07 ± 2.04^{bB}	12.07^{***}
20	42.50 ± 3.23^{aA}	37.50 ± 5.20^{aA}	43.75 ± 1.25^{aA}	31.25 ± 4.27^{aA}	10.13 ± 0.13^{aB}	16.40^{***}
$F_{(5, 18)}$ ‡	230.45^{***}	28.89^{***}	39.00^{***}	18.68^{***}	12.68^{***}	

[†] Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

[‡] ** $P < 0.01$; *** $P < 0.001$.

P. gland = Plectranthus glandulosus.

12: Corrected cumulative mortality (mean ± SE) of *Sitophilus zeamais* exposed to binary combinations of powders from *Plectranthus glandulous* leaves and *Azadirachta indica* seeds

Insects/ doses (g/kg)	Proportion of powders in mixture/Mortality (mean ± SE)[†]					
	100% *P. gland*	75% *P. gland* + 25% *A. indica*	50% *P. gland* + 50% *A. indica*	25% *P. gland* + 75% *A. indica*	100% *A. indica*	$F_{(4, 15)}$ [‡]
			1-d			
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
2.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
20	3.75 ± 2.39	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.25 ± 1.25	1.82^{ns}
$F_{(5,18)}$ ‡	2.45^{ns}	–	–	–	–	
			3-d			
0	0.00 ± 0.00^{c}	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ± 0.00^{b}	–
2.5	0.00 ± 0.00^{c}	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ± 0.00^{b}	–
5	1.25 ± 1.25^{c}	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	5.00 ± 2.89^{ab}	2.37^{ns}
10	5.00 ± 0.91^{bc}	0.00 ±0.00	1.25 ± 1.25	0.00 ±0.00	8.75 ± 4.27^{abC}	3.03^{ns}
15	13.75 ± 2.39^{abA}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	8.75 ± 4.27^{abA}	8.59^{**}
20	21.25 ± 3.75^{aA}	0.00 ± 0.00^{B}	2.50 ± 1.44^{B}	0.00 ± 0.00^{B}	21.25 ± 6.57^{aA}	10.62^{***}
$F_{(5, 18)}$ ‡	18.04^{***}	–	1.80^{ns}	–	4.24^{*}	
			7-d			
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{e}	
2.5	30.00 ± 3.54^{cA}	0.00 ± 0.00^{bC}	3.75 ± 2.39^{cdBC}	1.25 ± 1.25^{bC}	8.75 ± 2.39^{deB}	29.85^{***}
5	76.25 ± 3.75^{bA}	0.00 ± 0.00^{bD}	11.25 ± 2.39^{bcBC}	5.00 ± 2.04^{abC}	20.00 ± 2.04^{cdB}	90.50^{***}
10	80.00 ± 5.40^{bA}	1.25 ± 1.25^{bC}	6.25 ± 2.39^{cdC}	3.75 ± 1.25^{abC}	32.50 ± 3.23^{cB}	49.23^{***}
15	97.50 ± 2.50^{aA}	7.50 ± 2.50^{aC}	15.00 ± 2.04^{bC}	16.25 ± 8.00^{aC}	58.75 ± 5.15^{bB}	57.94^{***}
20	100 ± 0.00^{aA}	17.50 ± 5.95^{aC}	40.00 ± 3.54^{aB}	20.00 ± 7.36^{aBC}	86.25 ± 4.27^{aA}	60.60^{***}
$F_{(5, 18)}$ ‡	156.37^{***}	18.16^{***}	20.76^{***}	6.17^{**}	98.31^{***}	
			14-d			
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	
2.5	51.25 ± 13.90^{cA}	3.75 ± 2.39^{cdB}	7.50 ± 2.50^{dB}	2.50 ± 1.44^{deB}	21.25 ± 2.22^{cdAB}	9.42^{***}
5	85.00 ± 5.40^{bA}	5.00 ± 3.54^{cdC}	28.75 ± 7.74^{cB}	8.75 ± 2.39^{cdBC}	28.95 ± 4.98^{bcB}	29.01^{***}
10	93.75 ± 3.75^{abA}	11.25 ± 3.15^{bcC}	37.50 ± 7.77^{abBC}	15.00 ± 2.89^{bcC}	45.33 ± 10.40^{bB}	26.85^{***}
15	100 ± 0.00^{aA}	35.00 ± 6.77^{bB}	55.00 ± 7.91^{aB}	36.25 ± 9.66^{abB}	88.55 ± 4.33^{aA}	25.45^{***}
20	100 ± 0.00^{aB}	68.75 ± 5.54^{aB}	57.50 ± 9.47^{aB}	47.50 ± 5.95^{aB}	96.25 ± 2.39^{aA}	27.68^{***}
$F_{(5, 18)}$ ‡	39.71^{***}	28.74^{***}	30.42^{***}	23.31^{***}	54.24^{***}	

[†] Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ** $P < 0.01$; *** $P < 0.001$.

P. gland = Plectranthus glandulosus

13: Corrected cumulative mortality (mean ± SE) of *Callosobruchus maculatus* exposed to binary combinations of NeemAzal and *Plectranthus glandulous* leaf powder

Doses (g/kg)	Proportion of powders in mixture/Mortality (mean ± SE)[†]					
	100% *P. gland*	75% *P. gland* + 25% NeemAzal	50% *P. gland* + 50% NeemAzal	25% *P. gland* + 75% NeemAzal	100% NeemAzal	$F_{(4, 15)}$ [‡]
			1-d			
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
2.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
$F_{(5,18)}$ [‡]	–	–	–	–	–	
			3-d			
0	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
2.5	0.00 ± 0.00^{bC}	77.50 ± 2.23^{bB}	86.25 ± 2.39^{bAB}	93.75 ± 1.25^{bA}	87.50 ± 4.33^{bAB}	208.65^{***}
5	0.00 ± 0.00^{bC}	85.00 ± 2.89^{bcB}	97.50 ± 2.50^{aA}	100 ± 0.00^{aA}	96.25 ± 2.39^{abA}	143.47^{***}
10	0.00 ± 0.00^{bC}	96.25 ± 2.39^{abAB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	91.25 ± 2.39^{abB}	264.67^{***}
15	3.75 ± 1.25^{abB}	95.00 ± 3.54^{abA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	95.00 ± 2.04^{abA}	102.92^{***}
20	5.00 ± 2.04^{aB}	98.75 ± 1.25^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	98.75 ± 1.25^{aA}	165.62^{***}
$F_{(5, 18)}$ [‡]	5.51^{**}	222.49^{***}	795.68^{***}	6265.00^{***}	247.17^{***}	
			6-d			
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	
2.5	0.00 ± 0.00^{eC}	92.50 ± 3.23^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	314.11^{***}
5	13.75 ± 1.25^{dB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	3941^{***}
10	21.25 ± 1.25^{cB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	5310.1^{***}
15	31.25 ± 2.39^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	825^{***}
20	42.50 ± 3.23^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	690.67^{***}
$F_{(5, 18)}$ [‡]	230.45^{***}	936.60^{***}	$-^{***}$	$-^{***}$	$-^{***}$	

[†] Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

[‡] ** $P < 0.01$; *** $P < 0.001$.

P. gland = Plectranthus glandulosus.

14: Corrected cumulative mortality (mean ± SE) of *Sitophilus zeamais* exposed to binary combinations of NeemAzal and *Plectranthus glandulous* leaf powder

Insects/ doses (g/kg)	Proportion of powders in mixture/Mortality (mean ± SE)[†]					
	100% *P. gland*	75% *P. gland* + 25% NeemAzal	50% *P. gland* + 50% NeemAzal	25% *P. gland* + 75% NeemAzal	100% NeemAzal	$F_{(4, 15)}$ [‡]
			1-d			
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
2.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
20	3.75 ± 2.39	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
$F_{(5,18)}$ ‡	2.45^{ns}	–	–	–	–	
			3-d			
0	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	
2.5	0.00 ± 0.00^{cC}	45.00 ± 9.35^{cB}	80.00 ± 10.80^{bA}	88.75 ± 3.15^{bA}	63.75 ± 2.39^{cB}	28.23^{***}
5	1.25 ± 1.25^{cD}	60.00 ± 7.36^{bcC}	91.25 ± 4.27^{abAB}	97.50 ± 1.44^{aA}	80.00 ± 2.04^{bB}	65.36^{***}
10	5.00 ± 0.91^{bcD}	73.75 ± 5.15^{abC}	100 ± 0.00^{aA}	95.00 ± 2.04^{abAB}	87.50 ± 5.20^{abBC}	56.93^{***}
15	13.75 ± 2.39^{abC}	88.75 ± 1.25^{aB}	95.00 ± 0.00^{abAB}	98.75 ± 1.25^{aA}	93.75 ± 1.25^{aAB}	190.33^{***}
20	21.25 ± 3.75^{aC}	87.50 ± 3.23^{aB}	97.50 ± 1.44^{abAB}	100 ± 0.00^{aA}	95.00 ± 2.04^{aAB}	96.72^{***}
$F_{(5,18)}$ ‡	18.04^{***}	37.24^{***}	64.95^{***}	525.81^{***}	183.60^{***}	
			7-d			
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	
2.5	30.00 ± 3.54^{cC}	92.50 ± 4.33^{aAB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	82.50 ± 2.23^{cb}	51.35^{***}
5	76.25 ± 3.75^{bB}	98.75 ± 1.2^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	95.00 ± 2.04^{bA}	25.93^{***}
10	80.00 ± 5.40^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	98.75 ± 1.25^{abA}	20.70^{***}
15	97.50 ± 2.50^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	1^{ns}
20	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	–
$F_{(5, 18)}$ ‡	156.37^{***}	477.74^{***}	$-^{***}$	$-^{***}$	578.26^{***}	
			14-d			
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	
2.5	51.25 ± 13.90^{cB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	12.30^{***}
5	85.00 ± 5.40^{bAB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	7.71^{**}
10	93.75 ± 3.75^{ab}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	2.93ns
15	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	–
20	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	–
$F_{(5, 18)}$ ‡	39.71^{***}	$-^{***}$	$-^{***}$	$-^{***}$	$-^{***}$	

[†] Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

[‡] ** $P < 0.01$; *** $P < 0.001$.

P. gland = Plectranthus glandulosus.

15: Residual toxicity of the mixture of *Plectranthus glandulosus* *Azadirachta indica* seed powders after different storage intervals in *Callosobruchus maculatus* and *Sitophilus zeamais* on treated cowpea and maize grains

Insects / doses (g/kg)	Storage intervals (days)/ Mortality (mean ± SE)[††]					
	0	15	30	60	180	$F_{(4, 15)}$[‡]
C. maculatus						
0	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
2.5	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
5	0.00 ± 0.00^{b}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
10	2.50 ± 1.44^{abc}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.00^{ns}
15	3.75 ± 2.39^{ab}	2.50 ± 1.44	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.18^{ns}
20	16.25 ± 8.26^{aA}	2.50 ± 1.44^{AB}	2.50 ± 1.44^{AB}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	3.41^{*}
$F(5, 18)$[‡]	3.16^{*}	2.40^{ns}	3.00^{ns}	–	–	
S. zeamais						
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
2.5	00.00 ± 0.00^{dA}	0.00 ± 0.00^{cdAB}	7.50 ± 2.50^{bAB}	10.00 ± 4.08^{bcAB}	0.00 ± 0.00^{cB}	3.95^{*}
5	$0.00 \pm 0.00_{cAB}$	0.00 ± 2.89^{cAB}	36.25 ± 12.48^{aA}	31.25 ± 10.87^{abAB}	5.00 ± 3.54^{bcB}	3.96^{*}
10	6.25 ± 1.25^{bA}	2.5 ± 8.66^{bA}	45.00 ± 3.54^{aA}	47.50 ± 5.95^{aA}	5.00 ± 2.04^{bc}	15.38^{***}
15	11.25 ± 2.39^{aA}	58.75 ± 3.75^{aAB}	52.50 ± 3.23^{aB}	41.25 ± 6.88^{aB}	18.75 ± 5.15^{abC}	19.21^{***}
20	28.75 ± 2.39^{aA}	62.50 ± 1.44^{aAB}	61.25 ± 4.27^{aAB}	46.25 ± 8.98^{aBC}	26.25 ± 10.68^{aC}	8.99^{***}
$F_{(5, 18)}$[‡]	106.34***	48.08 ***	25.18***	13.40***	8.39**	

[†] Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

[‡] ns $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$;

– *F* value estimation of is not possible due to equal variance

16: Residual toxicity of the mixture of *Plectranthus glandulosus* with NeemAzal powders after different storage intervals in *Callosobruchus maculatus* and *Sitophilus zeamais* on treated cowpea and maize grains

Insects / doses (g/kg)	Storage intervals (days)/Mortality (mean ± SE)†					
	0	15	30	60	180	$F_{(4, 15)}$‡
C. maculatus						
0	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	
2.5	45.00 ± 9.35^{c}	47.50 ± 5.95^{c}	52.50 ± 3.23^{d}	53.75 ± 7.18^{b}	52.50 ± 3.23^{c}	0.37^{ns}
5	60.00 ± 7.36^{bc}	55.00 ± 10.21^{bc}	76.25 ± 2.39^{c}	77.50 ± 5.95^{ab}	73.75 ± 8.26^{bc}	1.84^{ns}
10	73.75 ± 5.15^{ab}	78.75 ± 3.75^{ab}	81.25 ± 2.39^{bc}	82.50 ± 5.20^{a}	88.75 ± 4.27^{ab}	1.64^{ns}
15	88.75 ± 1.25^{a}	86.25 ± 3.75^{a}	90.00 ± 3.54^{ab}	86.25 ± 3.75^{a}	92.50 ± 3.23^{ab}	0.67^{ns}
20	87.50 ± 3.23^{a}	93.75 ± 3.15^{a}	96.25 ± 2.39^{a}	91.25 ± 4.27^{a}	98.75 ± 1.25^{a}	2.08^{ns}
$F(5, 18)$‡	37.24^{***}	40.27^{***}	190.51^{***}	48.82^{***}	75.77^{***}	
S. zeamais						
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	
5	10.00 ± 2.04^{dA}	2.50 ± 1.44^{cdAB}	7.50 ± 2.50^{bAB}	10.00 ± 4.08^{bcAB}	0.00 ± 0.00^{cB}	3.95^{*}
10	$25.00 \pm 4.56_{cAB}$	10.00 ± 2.89^{cAB}	36.25 ± 12.48^{aA}	31.25 ± 10.87^{abAB}	5.00 ± 3.54^{bcB}	3.96^{*}
20	50.00 ± 3.54^{bA}	35.00 ± 8.66^{bA}	45.00 ± 3.54^{aA}	47.50 ± 5.95^{aA}	5.00 ± 2.04^{bc}	15.38^{***}
30	73.75 ± 2.39^{aA}	58.75 ± 3.75^{aAB}	52.50 ± 3.23^{aB}	41.25 ± 6.88^{aB}	18.75 ± 5.15^{abC}	19.21^{***}
40	82.50 ± 4.79^{aA}	62.50 ± 1.44^{aAB}	61.25 ± 4.27^{aAB}	46.25 ± 8.98^{aBC}	26.25 ± 10.68^{aC}	8.99^{***}
$F_{(5, 18)}$‡	106.34***	48.08 ***	25.18***	13.40***	8.39**	

† Means ± SE in the same column followed by the same lower case letter or in the same line followed by the same upper case letter, do not differ significantly (Tukey's test; $P < 0.05$).

‡ ns $P > 0.05$, ** $P < 0.01$, *** $P < 0.001$;

– *F* value estimation of is not possible due to equal variance

17: List of articles published or in the process of publication with data from this thesis

A. **Tofel H.K, Nukenine E.N., Ulrich D. and Adler C. 2014.** Effect of drying regime on the chemical constituents of *Plectranthus glandulosus* leaf powder and its efficacy against *Callosobruchus maculatus* and *Sitophilus zeamais. International Journal of Agronomy and Agricultural Research* Vol. 5 (1), 80-91.

B. **Tofel H.K, Nukenine E.N., Stahler, C. and Adler C. 2015.** Insecticidal efficacy of *Azadirachta indica* powders from sun- and shade-dried seeds against *Sitophilus zeamais* and *Callosobruchus maculatus*. *Journal of Entomology and Zoology studies* Vol. 3 (1), 100-108.

C. **Tofel H.K., Nukenine E.N., Stähler M., Adler C. 2015.** Bio-efficacy of *Azadirachta indica* A. Juss oil extracted from sun- and shade-dried seeds against two stored-products beetles. *International Journal of Biosciences* Vol.7 (2) 135-151.


JKI
Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen