Bioefficacy of plant derivatives on the repellency, damage assessment and progeny production of the cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)

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Abstract

A laboratory experiment was conducted to investigate the efficacy of different plant derivatives that affect the development of the cowpea weevil, *Callosobruchus maculatus* fed on cowpea, *Vigna unigulculata* seeds. The leaf extracts of the aromatic plant, *Anisomeles malabarica* and *Azadirachta indica* (neem) were evaluated for their repellency, damage assessment and progeny production of *C. maculatus*. The results revealed that the extracts of the two plant species caused a considerable reduction in the number of weevils. The combination of neem seed kernel extract and leaf extracts of *A. malabarica* was the most effective in checking the insect infestation and allowing the least number of F_1 adults emerging from the seeds over the other treatments. Acetone extracts of leaves of *A. malabarica* were more toxic to adult beetles compared to ethanol plant extracts. It was concluded that the botanical products acted as insect antifeedant and the order of repellency of the two plant leaf and kernel extracts on cowpea weevil were: combination of neem seed kernel extract + *A. malabarica* leaf extract > neem > *A. malabarica.*

Keywords: *Callosobruchus maculatus, Anisomeles malabarica, Azadirachta indica*, Repellency, Damage assessment, Progeny production

1. Introduction

Grain crops are commonly stored on-farm in a small scale due to their valuable nutrient content and relative ease in storage when they are dried after harvest (Duke, 1981). However, storage is one of the most crucial post-harvest operations because insects can infest grain all year round under favorable conditions. All storage insect pests undergo complete metamorphosis, have short developmental periods from egg to imago, and can complete several generations a year (Zakladnoi, 1987). The fast development, high fecundity and fertility of stored grain insects under optimal conditions and their ability to adapt to a range of habitat conditions, i.e. temperature/humidity variations, can lead to very high damage during storage (Zakladnoi, 1987).

The cowpea bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is a cosmopolitan post-harvest pest. It causes quantitative and qualitative losses manifested by seed perforation, reduction in weight, market value and reduced germination of seeds (Anonymous, 1989). About 4% of the total annual production or about 30,000 t values at over 30 million US dollars are lost annually in Nigeria alone to *C. maculatus* (Casewell, 1980).

With the limitations on the use of current pest control methods, there is scope for the discovery of safe, non-polluting, bio-rational pest management technologies for stored products. Extrapolation of the number of plant species studied and the number of compounds known suggests that millions of different compounds possess activity against pests which could be isolated from different plant species (Harborne, 1998).

The use of plant-based pesticides in grain protection has a long history (Chimbe and Galley, 1996) and there are a number of bibliographic databases on the use of different botanicals or parts of plants (leaves, twigs, roots, seeds) or their extracts (hot/cold water) or residues (ash, husk) by farmers in developing countries against stored product insect pests (Dales, 1996; Murugan et al., 1999). These databases provide information on the plant materials, target organism, toxic level of the compound or extract or whole plant material, economic value and active principles when they are known.

Naturally occurring compounds can affect the physiology of insects or they can modify the behavior of insects (Bell et al., 1984). Either the compounds in the vapor phase (volatile) or non-volatile compounds can affect insects to change their behavior. Those compounds can impede development, kill insects or cause losses in fecundity or viability of egg production and, therefore, reduce the number of offspring. They may act by ingestion, cuticle contact or fumigant action (Stadler, 1983). The objectives of the present study was to test the efficacy of Neem seed kernel extract (NSKE) and *Anisomeles malabarica* leaf extracts (AMLE) on repellency, damage assessment and progeny production of *C. maculatus*.

2. Materials and methods

2.1. Rearing of Callosobruchus maculatus

A small population of *C. maculatus* was reared and bred under laboratory conditions on the seeds of cowpea (*Vigna unguiculata*) inside a growth chamber at 30 ± 2^{0}C, 12:12 L: D and with 70% r.h.. Initially, 50 pairs of 1-2 day old adults were placed in a jar containing cowpea seeds. The jars were sealed and a maximum of 7 d were allowed for mating and oviposition. Then parent stocks were removed and cowpea seeds containing eggs was transferred to fresh cowpea seeds in the breeding jars that were covered with pieces of cloth fastened with rubber band to prevent the contamination and escape of beetles. The subsequent progenies of the beetles were used for all experiments.

2.2. Test plant materials

The leaves of *A. malabarica* and *Azadirachta indica* (A. Juss) seeds were collected from plants growing in and around the Bharathiar University Campus, Coimbatore*,* Tamil Nadu, India. The leaves were thoroughly washed and air-dried in the shade; the dried leaves were manually ground into powder with the help of a mortar and pestle.

2.3. Soxhlet extraction

Fifty g of leaves were extracted in 500 ml acetone, ethanol by ***soxhlation*** for 48 h. The extracts were filtered and filtrate was evaporated under reduced pressure to obtain crude. After complete solvent evaporation, one gram of each concentrated solvent extract was dissolved in 9 ml of acetone and used in repellent bioassays and respective concentrations were prepared by dilution.

2.4. Bioassays

2.4.1. Repellency bioassay

An olfactometer was constructed from a large (19 cm diameter) plastic Petri dish. Ten small (5 cm diameter) Petri dishes were attached to the central dish around its circumference and a small hole was made to allow free passage between each small Petri-dish and the large central dish. In the lid of the large dish, a small hole was made to allow the release of insects into the chamber. This hole was closed during the experimental tests preventing insect escape. Twenty seeds were thoroughly mixed with 2 mL of each plant extract. Residual extract was allowed to evaporate from the seeds. This experimental procedure was repeated for plant extract in each of the solvent. Each experimental test was replicated 4 times. For each replicate, fifty *C. maculatus* adults were released into the large dish and twenty grains, soaked in individual plant extract were placed in the small peripheral dishes. The direction of movement of beetles was recorded at 15 min, 30 min, 1 hr, 2 hr and 24 hr intervals.

2.4.2. Damage assessment

Damage assessment was carried out on treated and untreated grains. Samples of 100 g of grains were taken from each jar and the number of damaged grains was counted and weighed.

Percentage seed weight loss = UNd – Dnu

$$\frac{\text{X } 100}{\text{U (Nd + Nu)}}$$

Where U = weight of undamaged grain
D = weight of damaged grain
Nd = number of damaged grains
Nu = number of undamaged grains

2.4.3. Progeny production

Twenty pairs of beetles were introduced into treated and control grains and after 30, 15 and 7 d after the oviposition period for *C. maculatus* respectively, the parent adults were removed. Insect subsequently emerging were counted to estimate the F1 progeny production. Counting was stopped after 63, 42 and 42 days *C. maculatus*, respectively to avoid overlapping of generation (Mian and Mulla, 1982).

2.5. Statistical Analyses

All data were subjected to analysis of variance (ANOVA) and the means were separated using Duncan's multiple range test (Duncan, 1955).

3. Results

3.1. Repellency bioassay

The repellent activity of extracts of neem seed kernel (NSKE) and AMLE at three concentrations against *C. maculatus* at one-hour intervals showed that maximum repellent activity (81%) was observed for NSKE at 2% concentration after 1 h. (Table 1) with increasing time, the repellent activity was decreased for all the other extracts. When the plant powders of neem seed kernel and powdered leaves of *A. malabarica* at three different concentrations against *C. maculatus* at one-hour intervals were treated, maximum repellent activity (76%) was observed for NSKP (2%) after 1 h, followed by 62% repellency with AMLP (Table 2).

Table 1 Repellent activity of different plant extracts on *Callosobruchus maculatus*

Treatment	Conc. (%)	Repellency (%) 1 HAT	2 HAT	3 HAT	4 HAT
NSKE	0.5	61 c	55 d	50 d	47 c
	1	72 ab	64 ab	60 ab	55 b
	2	81 a	72 a	67 a	64 a
AMLE	0.5	52 bc	50 cd	46 cd	43 cd
	1	61 c	56 c	51 c	46 d
	2	73 b	65 b	62 b	54 ab
Control	0	0	0	0	0

Within a column means followed by a same letter is not significantly different 5% level of DMRT. HAT: Hours After Treatment. *NSKE* = Neem Seed Kernel Extract, *AMLE* = *Anisomeles malabarica* Leaf Extract.

Table 2 Repellent activity of different plant powders on *Callosobruchus maculatus*.

Treatment	Conc. (%)	Repellency (%) 1 HAT	2 HAT	3 HAT	4 HAT
NSKE	0.5	52 d	41 d	34 cd	27 cd
	1	68 b	53 c	40 c	35 c
	2	76 a	64 a	59 a	42 ab
AMLE	0.5	46 cd	40 cd	37 d	32 d
	1	54 c	53 c	50 ab	45 b
	2	62 ab	58 b	54 b	49 a
Control	0	0	0	0	0

Within a column means followed by a same letter is not significantly different 5% level of DMRT. HAT: Hours After Treatment. *NSKE* = Neem Seed Kernel Extract, *AMLE* = *Anisomeles malabarica* Leaf Extract

3.2. Damage assessment

The results of damage assessment of *C.maculatus* on cowpea after the treatment of acetone plant extracts were shown in Table 3. Damage decreased with increasing concentration of extract. Among the acetonic extracts studied, NSKE showed better activity (14%) than AMLE (19%). The damage assessment of *C. maculatus* on cowpea after the treatment of ethanol plant extracts showed that increase in concentration causes a decrease in damage of the seeds (Table 4). Among the ethanol extracts tested, NSKE (18%) showed higher activity than AMLE (23%). However, the acetone extracts of NSKE showed maximum protection than the ethanol extracts of NSKE. The damage assessment of *C. maculatus* on cowpea after the treatment of plant powders of neem seed kernel and powdered leaves of *A. malabarica* showed that a decrease in percentage of damage was observed while increasing the concentration of the powder. Among the treatments, NSKP exhibited better activity (12%) than AMLP (33%), but the NSKE treated grains caused less seed damage than NSKP treated grains (Table 5).

Table 3 Damage assessment of *Callosobruchus maculatus* on cowpea after the treatment of acetone plant extracts.

Treatment	Conc. (%)	Damage (%)
NSKE	2	35 b
	4	27 c
	6	20 d
	8	14 e
AMLE	2	64 b
	4	48 c
	6	33 d
	8	19 e
Control	0	97 a

Within a column means followed by a same letter is not significantly different 5% level of DMRT. *NSKE* = Neem Seed Kernel Extract, *AMLE* = *Anisomeles malabarica* Leaf Extract.

Table 4 Damage assessment of *Callosobruchus maculatus* on cowpea after the treatment of ethanol plant extracts.

Treatment	Conc. (%)	Damage (%)
NSKE	2	42 c
	4	36 d
	6	27 cd
	8	18 f
AMLE	2	72 b
	4	51ab
	6	42 c
	8	23 e
Control	0	97 a

Within a column means followed by a same letter is not significantly different 5% level of DMRT. *NSKE* = Neem Seed Kernel Extract, *AMLE* = *Anisomeles malabarica* Leaf Extract.

3.3. Progeny production

The various plant powders caused a significant reduction of progeny of *C. maculatus*. NSKP significantly reduced the progeny production of. *C. maculatus* compared to AMLP, but the combined treatment of NSKP and AMLP showed maximum progeny reduction (Table 6). The acetone plant extracts of NSKE was more effective in reducing the F_1 progeny than the ethanol extracts (Table 7). Among the extracts tested, the combinations of acetone extracts of NSKE and AMLE proved to be the best plant materials in controlling the emergence of F_1 individuals.

4. Discussion

In the present investigation, the efficacy of neem seed kernel and *A. malabarica* afford better protection to the infestation of the cowpea weevil, *Callosobruchus maculatus*. Pradhan et al. (1963) reported that neem seed kernel possess an extra ordinary gustatory repellent properties, much higher than neem leaf powder against the desert and migratory locusts. Rouf et al. (1996) showed that mixing of neem leaf powder with lentil seeds resulted in reduced oviposition and adult emergence of the pulse beetle, *Callosobruchus chinensis* (Linnaeus) Pandey et al. (1986) reported that a petroleum ether extract of neem leaves and twigs mixed with green gram seeds inhibited the oviposition of *C. chinensis*. Butterworth and Morgan (1971) revealed that the most active antifeedant is reported to occur in neem seed kernel powder, further, the results were confirmed by who also reported that azadirachtin is a major compound in the seed kernel responsible for the reduced oviposition and adult emergence in beetles. Neem has many other activities against insects disrupting or inhibiting development of eggs, larvae or pupae, preventing the molting of larvae or nymphs, disrupting mating and sexual communication, repelling larvae and adults, deterring females from laying eggs, sterilizing adults, poisoning larvae and adults, feeding deterrent, blocking the ability to swallow by reducing the motility of the gut preventing metamorphosis, thus preventing adult maturation, inhibiting the formation of chitin, the substance essential for the insect to form an exoskeleton. This huge array of insecticidal properties of neem is thought to be due to it's adversely affecting the insect's hormone system (Joshi and Sitaramaiah, 1979; Murugan et al., 2009).

Table 5 Damage assessment of *Callosobruchus maculatus* on cowpea after the treatment of certain plant powders.

Treatment	Conc. (%)	Damage (%)
NSKP	2	45 b
	4	31c
	6	20 d
	8	12 e
AMLP	2	75 b
	4	56 c
	6	42 d
	8	33 e
NSKP+AMLP	2+2	36 b
	4+4	24 c
	6+6	15 d
	8+8	9 e
Control	0	95 a

Within a column means followed by a same letter is not significantly different 5% level of DMRT. *NSKP*= Neem Seed Kernel Powder, *AMLP* = *Anisomeles malabarica* Leaf Powder

Table 6 Emergence of F_1 progeny of *Callosobruchus maculatus* on cowpea after the treatment of certain plant powders.

Treatment	Number of F_1 adults
NSKP	7 c
AMLP	9 b
NSKP+AMLP	6 ab
Control	18 a

Within a column means followed by a same letter is not significantly different 5% level of DMRT. *NSKP*= Neem Seed Kernel Powder, *AMLP* = *Anisomeles malabarica* Leaf Powder.

This study also demonstrated the potential of using *A. malabarica* to control *C. maculatus* in stored cowpea. *A. malabarica*, commonly called as Malabar catmint is a highly aromatic plant belonging to the family Lamiaceae (Joshi, 2000). The plant powder obtained from this plant in this study can be used in the same manner as most conventional insecticides, and could be less toxic to humans (Duke, 1985). This may be due to the presence of volatile compounds such as anisomelic acid, betulinic acid, citral, gerainic acid and ovatodiolide in the plant extract (Guha Bakshi et al., 1999). Of the above phytochemicals, it is supposed that citral plays a key role as an insecticide in controlling the bruchid population and disrupting the physiology of *C. maculatus* (Prajapati and Kumar, 2003). Our treatments of various plant extracts and powders were repellent to *C. maculatus*. The phytochemicals like azadirachtin present in neem extract and citral present in *A. malabarica* plant extract may suppress the phagostimulation and arrest the physiological events of the beetle. The antifeedant effects of azadirachtin are well known (Jacobson, 1989; Schmutterer, 1990; Ascher, 1993; Mordue and Blackwell, 1993; Murugan et al., 1988). Both primary and secondary antifeedant effects have been observed with azadirachtin (Ascher, 1993).

Table 7 Emergence of F_1 progeny of *Callosobruchus maculatus* on cowpea after the treatment of certain plant extracts.

Treatment	**Number of F_1 adults**	
	Acetone extract	**Ethanol extract**
NSKE	5 ab	6 ab
AMLE	7 b	8 b
NSKE+AMLE	4 c	5 c
Control	15 a	15 a

Within a column means followed by a same letter is not significantly different 5% level of DMRT. *NSKE* = Neem Seed Kernel Extract, *AMLE* = *Anisomeles malabarica* Leaf Extract.

Among the treatments, NSKE and AMLE showed higher activity than other combinations. This clearly suggests that the plant extracts contain powerful phytochemicals, which suppress the chemoreceptors in the mouthparts of the beetle and reduced the feeding in *C. maculatus*. Neem's efficacy to non-target and beneficial organisms has been documented (Schmutterer, 1995; Ascher, 1993; Murugan *et al*., 1999). Many biologically active compounds can be extracted from neem, including triterpenoids, phenolic compounds, carotenoids, steroids and ketones. The tetranortriterpenoid azadirachtin has received the most attention as a pesticide, because it is relatively abundant in neem kernels, and has shown biological activity on a wide range of insects. Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin-A to azadirachtin-G with azadirachtin-A being present in the highest quantity and azadirachtin-E regarded as the most effective insect growth regulator (Verkerk et al., 1993). Many other compounds have been isolated that shows antifeedant activity as well as growth regulating activity on insects. This cocktail of compounds significantly reduces the chances of tolerance or resistance developing in any of the affected organisms. However, only four of the compounds in neem have been shown to be highly effective in their activity as pesticides: azadirachtin, salannin, meliantriol, and nimbin (Jacobson, 1990; National Research Council, 1992; Murugan et al., 1998.).

The emergence of F_1 progeny of *C. maculatus* on cowpea after the treatment of NSKE, AMLE, NSKP and AMLP suggests that the acetone plant extracts of NSKE was more effective in reducing the F_1 progeny than the ethanol extracts. The insect growth regulatory effect of azadiracthin and citral causes various developmental, post-embryonic, reproductive and growth inhibitory affects in insects so that the emergence of F_1 generation is prevented.

Botanical materials tested here could be useful and further studies are recommended to determine if these plant species control other storage pests through direct effects and also indirect effects. The plants tested in our study are a possible source of natural products that could be used as an alternative to synthetic insecticides (Zebitz, 1987; Murugan et al., 2004).

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