Insecticidal properties of whole meal or protein extracts of the bean seeds *Phaseolus vulgaris* L. on juvenile stages of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)

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Abstract

Callosobruchus maculatus is a pest that causes serious damage to *Cicer arietinum* (chickpea) stored seeds, but that does not develop in seeds of other legumes such as *Phaseolus vulgaris* or *Pisum sativum*. The bean seed is rich in antinutritional compounds known to inhibit the development of *C. maculatus*. In an integrated approach to protect stocks of *Cicer arietinum* against attacks of this weevil, this study had the main objective to assess the potential of using bean flours from a wild bean *Vigna caracalla*, four varieties of *P. vulgaris*, and of a crude extract from *P. vulgaris* lectins seed. The extraction method was chosen to extract lectin-like protein compounds. The biological effects of bean flour or protein extracts were observed on artificial seeds composed from *C. arietinum* flour enriched with *P. vulgaris* whole flour or extracts incorporated at different percentages. The antinutritional activity either of bean-seed whole meal or of lectin-like extracts was determined by the analysis of different biological parameters. Incorporation of bean flour mixed with chickpeas decreased fertility and fecundity of female *C. maculatus* and caused longer development times of juvenile stages. Peptide extracts of the *P. vulgaris* reduced fecundity and survival of *C. maculatus*.

Keywords: *Callosobruchus maculatus, Phaseolus vulgaris*, Lectin-like extract, Insecticidal properties, Artificial seed

1. Introduction

Large-seed legume cultivation is an important crop in Algeria (Anonymous, 2006), but these plants are exposed to many post-harvest pests, the most serious damage being caused by the cowpea weevil, *Callosobruchus maculatus* (F.) (Coleloptera: Bruchidae). This insect is an excellent disperser, and it is capable of laying eggs in cultivated fields and in storage. Each year in tropical countries, weight losses of pulses seeds may reach 800 g kg-1 in only a few months (Ouedraogo et al., 1996). Unsubstantiated estimates claim that 30% weight loss is due to infestation of legume seeds by weevils in Africa (Rodrigues Macedo et al., 2000).

Synthetic insecticides are widely used to control and prevent infestation after the harvest. However, the use of insecticides has several disadvantages: residues on the seed, availability, costs, resistant insect populations, worker safety and consumer concerns. Therefore, there is a growing interest in finding alternatives to chemical control for legume seed weevils. Pulses have evolved a large array of antinutritional compounds to protect their seeds against insects (Janzen, 1976). Chickpea, *Cicer arietinum* (L.) (Fabaceae), have endogenous natural insecticides produced in the seed that are active against *C. maculates*, (Mouhouche and Fleurat-Lessard, 2004). There are several examples of legumes being a source of natural insecticides against other stored-product insects (Jouvensal et al., 2003; Louis et al., 2004; Taylor et al., 2004).

Sales et al. (2000) and Carlini and Grossi-de-Sá (2002) have demonstrated feeding inhibition by legume seed vicilin compounds and arcelin that may be used for the defense of legumes against bruchid beetles. From a nutritional point of view, the legume lectins are part of the human diet. Surprisingly, these highly antinutritional compounds are resistant to proteolytic degradation during their transit through the human gut (Vasconcelos and Oliveira, 2004).

Earlier work had also identified the lectins as biochemical factors in plant resistance to insects, including mainly coleopteran species. Janzen (1976) showed that larvae of *C. maculatus* are unable to attack the seeds of the common bean *Phaseolus vulgaris* (L.) (Fabaceae) seeds, because there are a number of defensive compounds: lectins or phyto-hemagglutinins (PHA). This work focused on *C. maculatus* with a prime objective to determine the effect of insecticidal lectins present in bean seeds on *C. maculatus* developmental biology. Two specific objectives were to study the insecticidal activity of five bean flours after their incorporation into artificial chickpea seeds, and study insecticidal activity of lectin-like compounds extracted from P. vulgaris in artificial chickpea seeds.

2. Materials and methods

2.1. Insects

Callosobruchus maculatus was reared in the laboratory since 1998 at the laboratory of Zoology at the Institute National Agronomique (INA) at Algiers El Harrach, (Algeria). The food consisted in 100 g organic chickpea purchased on the market that was infested with 20 pairs of *C. maculatus*. Food and insects were held in jars in an incubator at 28± 2° C and 70± 5% r.h. in the dark.

2.2. Source of Seed

Four *P. vulgaris* varieties were tested, as well as a wild bean from India, *Vigna caracalla* (L.) (formerly Phaseolus caracalla). Four of these were supplied by the Institut Technique des Grandes Cultures of Oued Smar El Harrach. These varieties were selected for their properties of resistance to fungal diseases such as rust and anthracnose. They were introduced to Algeria in the 1990's to study their behaviour in relation to agronomical performance: variety S102 (B15V2); variety Terga (V2B2); variety Pinto (V1B2); and variety Cotender. The V. caracalla was grown and harvested at the horticultural station of INA. As a control, a *C. arietinum* a variety of Algerian commercial grade, with wrinkled external seed coat (widely consumed in Algeria, and imported from Mexico) was used.

2.3. Fecundity and longevity

Fecundity was determined as the number of eggs laid by a female during her life. Fecundity was studied in 25 pairs of adults aged 0-24 h, distributed in five replicates. Five pairs of *C. maculatus* were placed on 10 g of artificial chickpea seed in 190 mL/bottles. The number of eggs laid was counted daily using a binocular microscope. Any mortality of females and males was noted. These and other tests were run at 30±1°C and 70±5% r.h. in a dark incubator.

2.4. Adult emergence and development duration

To determine the adult emergence and development time of *C. maculatus*, 150 eggs aged 0-48 h were recovered during the study of fecundity (section 2.3). These eggs were distributed into jars, 30 eggs each containing, 15 artificial seeds with varying proportions of bean and chickpea flour or bean peptide extract and chickpea flour. There were five replicates per treatment. The duration of development was calculated as the time elapsed from the middle of the egg laying period until 50% adult emergence (Haryadi, 1994). This important parameter allowed an overall assessment of the nutritional quality of food for *C. maculatus* (ensuring the nutritional needs of juvenile stages during their active growth).

2.5. Index of Susceptibility

Index of Susceptibility (IS) was used to determine the sensitivity of artificial seeds to stored-product-insect attack (Dobie, 1974). It is based on two factors important for population dynamics: total number of emerging adults (NE) and duration of mean development (DMD). Index of Susceptibility = (Loge Yield of emerging adults)/duration of mean development.

2.6. Bean flour mixed with chickpea flour

Chickpea flour that was used as the basis of chick-pea artificial seeds was obtained from the milling of a chickpea variety imported from Turkey available on the market. Chickpeas or beans were ground using a hard seed grinder type (IKA-Werk, Germany). The seeds were ground three times to obtain a fine flour. Additionally, the particle size was homogenized by sieving flour with 0.5 mm mesh sieve to eliminate the large particles. Then, the fine flour was used to make chickpea artificial seeds enriched with various amounts of bean flour obtained from the five beans. Flour of each bean variety was added to chickpea flour in proportions of 0, 10, 20, 40, 80 or 100%. After the blending the two flours, the mixture was placed in a centrifuge mixer for 60 seconds.

To obtain a firm dough, 45 mL of water was added to 100 g of the flour mixture. The hydrated flours mixture formed a paste that was spread with a rolling pin, and cut into 1 x 1 cm squares. A spherical artificial seed from each square of paste was manually made similar in size to a chickpea seed. These artificial chickpea seeds were dried for 48 h in a dark oven set at 20°C to avoid denaturing the proteins. After drying, 20 g of seed was placed into incubation jars and insects added on the seed. The number of live and dead *C. maculatus* was counted after 3 d. There were five replicates for each treatment.

2.7. Lectin-rich bean extracts mixed with chickpea flour

The wild bean species *V. caracalla* was most toxic to inserts but due to in sufficient quantities, *P. vulgaris* variety S102, the second most toxic seed was used.The method to extract truncated lectins from beans was that described by Moreira and Perrone (1977). This method involved mixing 80 g bean flour with 800 mL distilled water. The extraction of bean flour produced 420 mL of extract in distilled water. This extract is supposed to contain lectin-like compounds which are toxic to *C. maculatus*. The pH of the solution was adjusted to 2.4 with hydrochloric acid. This solution was mixed for 4 h to obtain a homogeneous solution. After centrifugation at 2000 g-force for 20 min at 4°C, the supernatant was recovered. Toxicity tests were performed with doses of 50, 100 and 200 mL of supernatant mixed with 100 g chickpea flour; artificial seeds were made from this, and tested with insects as above.

3. Results

3.1. Fecundity

All bean varieties reduced the fecundity of *C. maculatus*. At 10% bean flour, the lowest concentration tested, fecundity dropped to below 25% of the pure chickpea control seed. At 100% bean flour, less than three eggs were laid by the five females. There were significant differences (Tukey's Multiple Range Test, $P<0.05$) between the bean flours. At 10%, *V. caracalla* and S102 had the lowest egg load, and Pinto, Cotender and Terga were not significantly different and had a higher egg load than *V. caracalla* and S102.

3.2. Adult emergence

There was high adult emergence (97%) with pure chickpea flour (Fig. 1). All five bean flours at 10% of the artificial seed significantly reduced adult emergence to 30 - 90%. There was no adult emergence at 40% *V. caracalla*, 80% S102 and Pinto, and 100% Cotender and Terga varieties. Similar trends were seen with the differences between bean flours as were observed with the fecundity. The wild bean, *V. caracalla*, S102 were significantly lower than control and dose 10% (Tukey's multiple range test, $P<0.05$).

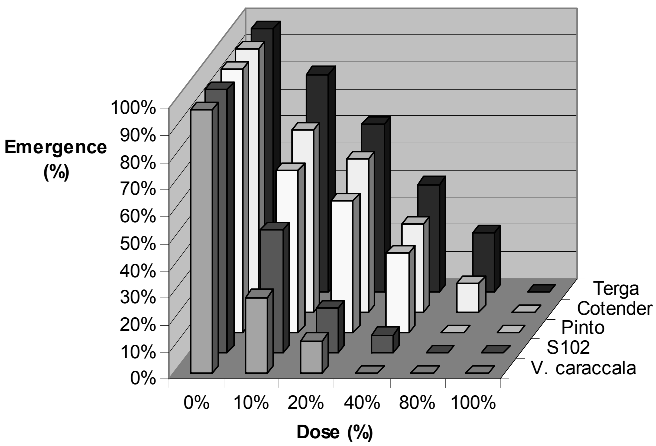

Figure 1 Emergence (%) of C. maculatus that developed on artificial chickpea seeds mixed with flour of five beans with different rates of incorporation.

3.3. Development time

There was a progressive increase in the total duration of development of *C. maculatus* from egg to adult with increasing concentrations of bean flour in the artificial see. Similar trends were seen with the differences between bean flours in development time as were observed with the fecundity and adult emergence.

Table 1 Fecundity of five *Callosobruchus maculatus* females on artificial seeds made with chickpea flour and varying amounts of five beans.

	Eggs five females (Mean + SE) *V. caracalla* and *P. vulgarius* varieties				
Bean Flour (%)	***V. caracalla***	**S102**	**Pinto**	**Cotender**	**Terga**
0	52.00 ± 0.01 a	52.04 ± 0,06 a	52.04 ± 0.06	52.04 ± 0,06	52.04 ± 0.06
10	3.60 ± 0.09 bA	4.72 ± 0.01 bA	12.56 ± 0.05 B	12.52 ± 0.03 B	13.60 ± 0.02 B
20	2.76 ± 0.05 b	4.00 ± 0.02 b	6.76 ± 0.04	8.60 ± 0.09	11.80 ± 0.02
40	1.24 ± 0.03 b	3.00 ± 0.07 b	5.20 ± 0.01	6.24 ± 0.03	8.48 ± 0.05
80	0.40 ± 0.01 b	0.72 ± 0.02 b	1.72 ± 0.04	3.52 ± 0.07	5.56 ± 0.08
100	0.36 ± 0.09 b	0.56 ± 0.07 b	0.68 ± 0.05	1.20 ± 0.06	2.24 ± 0.03

For a given bean (columns), means followed by a different small letter are significantly different, for 10% bean flour (row), means followed by a different large letter are significantly different (Tukey's multiple range test, $P<0.05$).

Table 2 The development time of *Callosobruchus maculatus* from egg to 50% adult emergence on artificial seeds made with chickpea flour and varying amounts of five beans.

	Development time (d) *V. caracalla* and *P. vulgarius* varieties				
Bean Flour (%)	***V. caracalla***	**S102**	**Pinto**	**Cotender**	**Terga**
0	29.00	29.00	29.00	29.00	29.00
10	80.40	60.93	39.03	38.59	33.25
20	145.80	86.80	47.23	41.98	38.10
40	NA	88.80	53.93	48.10	42.84
80	NA	NA	NA	45.00	47.96
100	NA	NA	NA	NA	NA

NA= no adults

3.4. Index of Susceptibility

Similar trends were seen with the Index of Susceptibility as were seen with the previous biological parameters. This is not surprising given the Index of Susceptibility is calculated from survival to adult and mean development time. Increasing proportions of bean flour caused decreases in IS (Table 3). The wild bean, *V. caracalla*, S102 were significantly lower than the other beans.

Table 3 Index of Susceptibility for *Callosobruchus maculatus* on artificial seeds made with chickpea flour and varying amounts of five beans.

	Index of Susceptibility *V. caracalla* and *P. vulgarius* varieties				
Bean Flour (%)	***V. caracalla***	**S102**	**Pinto**	**Cotender**	**Terga**
10	2.01	3.00	5.00	5.19	6.26
20	0.86	1.61	3.93	4.59	5.16
40	NA	1.12	3.04	3.51	4.15
80	NA	NA	NA	2.67	3.16
100	NA	NA	NA	NA	NA

NA= no adults; Index of Susceptibility = (Loge percentage of emerging adults)/average duration of development.

3.5. Lectin-rich bean extract mixed with chickpea flour

Given that there was insufficient *V. caracalla* and S102 was the most toxic of the *P. vulgaris* varieties, this variety was chosen for the extraction of lectin-like proteins needed for the second part of the study. Placing *C. maculatus* adults on artificial seed for 3 d caused 71, 79, and 100% mortality for 50, 100 and 200 mL of extract respectively (Fig. 2), whereas the untreated seed had 4% mortality. The lethal dose to kill 50% of the population was estimated at 35 mL of extract per 100 g of chickpea flour using probit analysis. In addition to reducing the survival, the extracts also reduced the number of eggs laid on artificial seeds. Females laid over 50 eggs on untreated seed, but only 50 mL of the extract reduce the number of eggs by almost 90% (Table 4).

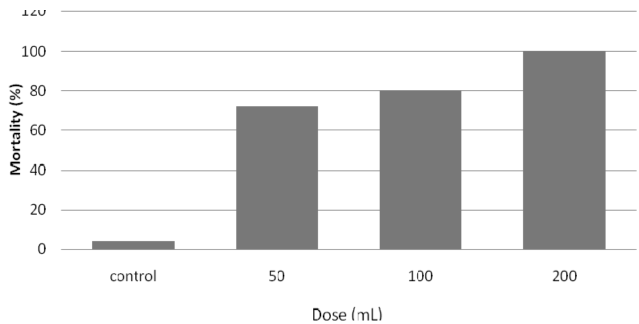

Figure 2 Effect of different doses of the water-soluble extract containing lectin-like compounds from bean seeds of S102 variety on mortality of adult Callosobruchus. maculatus.

Table 4 Effect of *P. vulagris* S102 flour extract incorporated in artificial chickpea seed on *C. maculatus* fecundity.

Age of female (d)	Eggs/female Bean flour extract with lectins (mL) 0	50	100	200
2	16.80	4.08	1.64	1.04
4	14.60	2.12	0.96	0.08
6	9.60	0.00	0.00	0.00
8	6.56	0.00	0.00	0.00
10	2.88	0.00	0.00	0.00
12	1.04	0.00	0.00	0.00
14	0.20	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00
Total eggs/female	51.7 ± 6.6	6.2 ± 1.5	2.6 ± 0.6	1.1 ± 0.4

4. Discussion

Our results show that the wild bean *V. caracalla* and *P. vulgaris* variety S102 were potent inhibitors for egg laying of *C. maculatus* when incorporated at 10% into artificial chickpea seeds. *C. maculatus* prefer to lay on smooth varieties (Lepesme, 1944). However, our results showed a much lower rate of eggs laid by this pest species on artificial chickpea with a smooth surface when these seeds are enriched with a small amount of bean flour or bean flour water-soluble protein extract. This means that the texture of the seed coat is not the only factor influencing the egg laying behavior of female *C. maculatus*. This work suggests behavior is also related to the biochemical composition of seeds perceived by females, which refused to lay eggs and which were rapidly killed after a few days in contact with “toxic” artificial chickpea seeds.

The high percentage of eggs failing to hatch on bean flour enriched chickpea artificial seeds showed that insecticidal potential of certain bean varieties may be related to the presence of hydrophilic protein such as lectins that could prevent egg hatching and larval development of *C. maculates* as previously observed by Janzen et al. (1976), Gatehouse et al. (1995), Gatehouse and Gatehouse (1998), Okeola et al. (2002), and Boleti et al. (2007). These authors found that high rates of lectins in certain species of legume such as Dolichos lablab (L.) and Rhycocea saucia prevent the development of *C. maculatus*. However, according to Goossens et al. (2000), with lower incorporation level of an extract containing glycoproteins isolated from bean at about 5% in weight, did not reduce fertility or development time of *C. maculatus*. The limit of the content of these insecticidal compounds in chickpea could be estimated between 5 and 10 % to expect a control of *C. maculatus* and the reduction of damage on stored chickpeas.

Our results were in agreement with those obtained in earlier studies by other authors: Hamelryck et al. (1996), Louis (2004), Brinda et al., (2004) and Zambre et al., (2005) have already reported the resistance of bean seeds of Phaseolus toward *C. maculatus*. Among resistant varieties, we found variety referenced G02771, and two other species of Phaseolus Genus: *P. calcaratus* (L.) and *P. lathyroides* (L.).We deduced that the extracts toxicity was probably related to the presence of “reserve glycoproteins” (Hamelryck et al., 1996) whose role is to protect bean seeds against attack by non-adapted bruchid species to antinutritional compounds present in bean seeds, such as the chickpea weevil *C. maculatus*.

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