

Use of vegetable powders as alternative to control of *Callosobruchus maculatus*

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The weevil *Callosobruchus maculatus* Fabr. (Coleoptera: Bruchidae), is the most important pest in stored cowpea, it requires the use of control methods. The chemical control of cowpea weevil is one of the most commonly used methods nowadays, nevertheless, their repeated can bring about number of problems, encouraging the use of alternative controls methods. This work evaluate the effects of eleven vegetal species powder on individuals of weevils in laboratory conditions. Six non-sexed adults of cowpea weevil were kept for seven days in plots containing 0.3g of the powders of each treatment mixed to 10g of cowpea beans. The trials were set up in a completely randomized design, with six replicates per treatment. After 20 days of infestation, oviposition was evaluated and emerged adults were counted. After that, the dry weight of the insects, the weight of the consumed grains and the development period from egg to adult were evaluated. The powders of *Chenopodium ambrosioides* L. and *Piper nigrum* L. reduced both oviposition and adult emergence of *C. maculatus*, revealing great possibility as an alternative insect control.

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INTRODUCTION

Cowpea beans, *Vigna unguiculata* L. Walp., were introduced in Brazil in the 16th century in Bahia State by Portuguese settlers. This legume occupies 60% of the total area cultivated with beans in the Northeast region of Brazil (ANDRADE JÚNIOR *et al.*, 2007), serving as a staple food for low-income populations (LIMA *et al.*, 2011). They are currently grown throughout the country and have substantial social and economical value, in addition to its great agricultural potential (FREIRE FILHO *et al.*, 2005).

Callosobruchus maculatus Fabr. (Coleoptera: Bruchidae) is a cosmopolitan field-to-store pest ranked as the principal post-har-

vest pest of several pulses including cowpea and chickpea (*Cicer arietinum* L.). The adults lay their eggs on the seeds in the storage; larval feeding in the cotyledons causes substantial quantitative and qualitative losses (OGUNWOLU & ODUNLAMI, 1996, PAS-CUAL-VILLALOBOS & BALLESTA-ACOSTA, 2003, ALMEIDA *et al.*, 2005, PEREIRA *et al.*, 2008). Its attack starts in the field, but it is intensified in the storage phase, which may lead to total loss of the grains after five months of infestation (MESSINA & RENWICK, 1985, MBATA, 1993). Over 90% of the insect damage to cowpea seeds is caused by *C. maculatus* (CASWELL, 1981).

The chemical control of cowpea weevil through the fumigation of grains has been

one of the most commonly used methods up to this moment (HASAN & REICHMUTH, 2004). Although effective, their repeated use has disrupted biological equilibrium and led to develop of resistance against important insecticides including phosphine (LORINI, 2003). Furthermore, many synthetic insecticides have undesirable effects on non-target organisms and foster environmental and human health concerns (BHUPINDER *et al.*, 2003). These aspects have triggered the studies of alternative approaches to pest control, with the use of vegetable origin pesticides (ALMEIDA *et al.*, 2004, TAVARES & VENDRAMIM, 2005).

Studies concerning plants applied with powder, aqueous and organic extracts, and oils, have increased significantly in pest management (BRITO *et al.*, 2006). These substances may cause mortality, repellence, oviposition inhibition and also contribute to the alteration of the biological development of the insects (BOEKE *et al.*, 2004, KETOH *et al.*, 2005). In addition, such formulations can show advantage like significant efficiency, fast degradation and low toxicity level to the people applying it (SOUSA *et al.*, 2005).

Therefore, the present research has the objective to evaluate the effects of powders of different species and vegetable structures concerning biological aspects of *C. maculatus*.

MATERIAL AND METHODS

The research was carried out in the LARESPI (Laboratory of Plant Resistance to Insects and Pesticide Plants) of the Department of Plant Production - Crop Protection of FCA/UNESP in Botucatu, during the year of 2010.

Laboratory Insect Colony *C. Maculatus*

A laboratory insect rearing-stock of *C. maculatus* was kept inside climatic cham-

bers type B.O.D. ($T = 25 \pm 2^\circ\text{C}$; R.H. = $70 \pm 10\%$; 12:12-L:D) during the experiment.

One-liter transparent glass containers (10.0 cm in diameter \times 13.0 cm long) were used in the rearing of the insects, sealed on the upper part with a screwable lid, in which a round opening (6.0 cm in diameter) was made, where a screen (30 mesh) was adapted promoting internal ventilation. 0.3 kg of freshly picked cowpea-beans were placed in each flask (Paulistinha genotype). They were then infested with approximately 300 adults of *C. maculatus*. Every 30 days, the emerged adults were removed from these containers and used to start the infestations in new containers.

Collection of vegetable species

Eleven treatments started from the following species were prepared: *Coriandrum sativum* L. (leaves), *Ruta graveolens* L. (leaves + stems), *Trichilia pallida* Sw. (leaves), *T. pallida* Sw. (stems), *Azadirachta indica* A. Juss. (leaves), *A. indica* A. Juss. (almonds), *Tagetes erecta* L. (leaves + stems), *Corymbia citriodora* Hook. (leaves), *Mentha pulegium* L. (leaves), *Piper nigrum* L. (seeds) and *Chenopodium ambrosioides* L. (leaves + stems).

After the collection in the field (January to March, 2010), the vegetable structures were transferred to green-house, where they were maintained for about four to six days (depending of plant species) for drying with air circulating at 40°C . The dry materials were then ground in knife mills in order to obtain the respective powders (BALDIN *et al.*, 2009). The powder of each plant structure was stored in plastic containers and tightly sealed for up to three months. Apart from *A. indica* and *T. pallida* species, from ESALQ/USP, all the other vegetable structures were obtained at FCA/UNESP in Botucatu City, where collection was always carried out in the morning.

Bioassays

For the bioassays, each part was made of 10 g of cowpea-bean grains, mixed to 0.3 g of the respective powders, according to MAZZONETTO & VENDRAMIM (2003) and BALDIN *et al.* (2009). The weight of grains and powders were done using an analytical balance (0,0001 g) and subsequently placed in plastic recipients (4.0 cm in diameter × 5.0 cm long), sealed on the upper part with a screwable lid. Firstly it was added the grains and then the powders were placed in recipients. One week before the test setup, the containers were screened each day and the hatched insects were separated into different containers, according with the day, ensuring the same age.

Six replicates were performed in a completely randomized design. The experimental plots were infested with six adults (non-sexed) of *C. maculatus* (48 h of age) for a period of seven days, after which the insects were removed. According to Brito *et al.* (2006), the sex ratio of *C. maculatus* is presented in 1:1, being dispensable to determine the sex before infestation. Twenty days after infestation, the total number of eggs per recipient was counted, using magnifying glass Ramsor model LL-24. It was released 20 days to ensure the oviposition period ended and did not occur the eggs hatch, then it was obtained the percentage of viable eggs and the oviposition preference index (BALDIN *et al.*, 2009), through the following formula: $OPI = [(T-S)/(T+S)] \times 100$, where T represents the number of eggs counted in the evaluated treatment (powder) and S represents the number of eggs counted in the witness standard (without powder). The index ranged between 100 (very stimulating), zero (to neutral), up to -100 (total deterrence). The classification was determined based on the comparison of the mean of the eggs treated to the witness standard mean, taking into consideration the mean standard deviation error of the assay for their differentiation.

Twenty days after initial infestation, the experimental plots started to be evaluated daily in order to determine the number of emerged adults, the development period (egg - adult), dry weight of insects and the weight of consumed grains. For the determination of the development period, it was considered the interval between the egg stage and adult emergence.

Recently-emerged adults of cowpea weevils were kept in small glass flasks (2.2 cm × 5.0 cm) and immediately placed in a freezer in order to rapidly interrupt the vital cycle, thus avoiding weight loss and maintaining them in perfect conservation state. At the end of the emergences, these flasks were opened and placed in greenhouses at 45°C for 48 h to determine the dry weight of the insects. The consumption of bean grains by the weevil larvae was determined by comparing the dry weight of the infested experimental plots to the dry weight of the non-infested experimental plots.

All the parameters obtained underwent variability analyses, where the means were compared by using the Tukey test ($P \leq 0,05$). The data related to the number of eggs and emerged adults were transformed in $(x + 0,5)^{1/2}$; the data related to the percentage of viable eggs were transformed in $\arcsin [(x + 0,5)/100]^{1/2}$; other data were not transformed. In the data presented were used non-transformed values. The statistic software Stat Plus 2007 was used.

RESULTS AND DISCUSSION

The grains impregnated to the powders of *C. ambrosioides* (F+R) (64.33 eggs) and *P. nigrum* (S) (52.83 eggs) were less oviposited by *C. maculatus* when compared to the other treatments, which suggests the volatilization of the components that affected negatively the oviposition of the cowpea weevil (Table 1).

Table 1. Means (\pm SE) of number of eggs and percentage of viable eggs of *C. maculatus* in cowpea-grains impregnated with powders of different vegetable species

Treatments ¹	N.º of eggs ²	Percentage of viable eggs ³
<i>Coriandrum sativum</i> (L)	195.50 \pm 19.32 a	87.67 \pm 1.45 c
<i>Ruta graveolens</i> (L+ST)	188.33 \pm 12.12 a	94.97 \pm 0.74 ab
<i>Trichilia pallida</i> (L)	172.67 \pm 14.33 a	91.48 \pm 1.05 abc
<i>Azadirachta indica</i> (L)	172.50 \pm 24.71 a	92.03 \pm 1.34 abc
<i>Tagetes erecta</i> (L+ST)	169.33 \pm 34.47 a	91.02 \pm 0.82 bc
<i>Corymbia citriodora</i> (L)	167.83 \pm 18.38 a	90.93 \pm 1.26 bc
<i>Trichilia pallida</i> (ST)	152.67 \pm 12.96 a	94.12 \pm 1.12 ab
<i>Mentha pulegium</i> (L)	149.83 \pm 11.22 a	93.12 \pm 0.50 abc
Control	147.33 \pm 12.21 a	92.83 \pm 0.95 abc
<i>Azadirachta indica</i> (A)	135.50 \pm 6.26 a	91.23 \pm 1.79 abc
<i>Chenopodium ambrosioides</i> (L+ST)	64.33 \pm 4.98 b	91.83 \pm 0.85 abc
<i>Piper nigrum</i> (S)	52.83 \pm 5.64 b	96.08 \pm 1.37 a
F	8.77*	3.44*
CV (%)	14.58	4.81

¹ Legend: A = almonds; L = leaves; ST = stems; S = seeds.

² Means within columns followed by the same letter are not significantly different by Tukey test ($P \leq 0.05$). Data transformed in $(x + 0.5)^{1/2}$.

³ Means within columns followed by the same letter are not significantly different by Tukey test ($P \leq 0.05$). Data transformed in $\arcsin [(x + 0.5)/100]^{1/2}$.

Similar results were found by BALDIN *et al.* (2008), where *C. ambrosioides* reduced significantly the number of eggs deposited by adults of *Zabrotes subfasciatus* Bohemann, 1833, with powders in the concentration of 30.000 ppm w/w, in research carried out with common beans. In addition, it observed that the concentration used by the same authors for *Z. subfasciatus*, MAZZONETTO & VENDRAMIM (2003) and BALDIN *et al.* (2009) for *A. obtectus* proved to be efficient to use in tests with *C. maculatus*.

The grains mixed to the powder of *C. sativum* (L) were more oviposited (195.50 eggs) by *C. maculatus*, contrasting with the results of BALDIN *et al.* (2009), where this treatment stood out among the ones that reduced most the oviposition of *Acanthoscelides obtectus* Say, 1831 in common beans, using the same concentration of the powders. It is possible that vegetable powder affects weevil species differently, which may justify the variations observed in treatments with *C. sativum* (L). Moreover, the

fact that the mixture of the volatiles of the powders with that of the grains (common beans and cowpea beans) may produce behavioral variations in the weevils should be taken into account.

The viability of the eggs varied between 87.67 and 96.08%, being higher in *P. nigrum* (S) and lower in *C. sativum* (L), *C. citriodora* (L) and *T. erecta* (L+ST). Based on the oviposition preference index (Figure 1), the treatments with *C. ambrosioides* (L+ST) and *P. nigrum* (S) were classified as deterrents to insect oviposition when compared to the control. On the other hand, *C. sativum* (L), *R. graveolens* (L+ST) and *T. pallida* (L) stimulated the oviposition of weevils, whereas the others were considered neutral.

The treatments with *C. ambrosioides* (L+ST) and *P. nigrum* (S) also showed low means for the emergence of weevil adults (Table 2), which may suggest a larvicidal activity over *C. maculatus*. However, the means of larval viability for all treatments were around 90%, without significant differ-

ences, indicating that the low emergence of adults of *C. maculatus* is a consequence of

low oviposition (Table 1), which was verified in treatments with these powders.

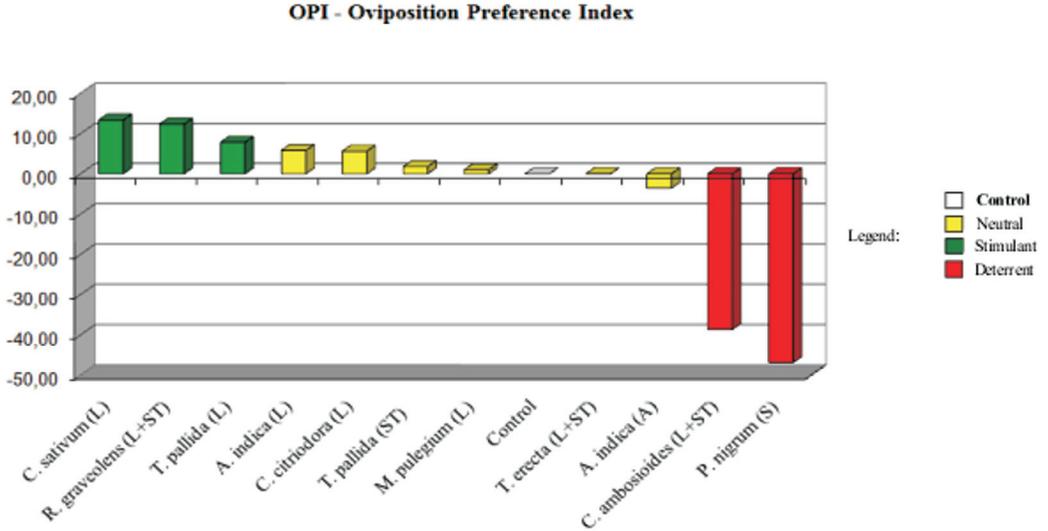


Figure 1. Index and classification of the materials concerning oviposition preference of *C. maculatus*. IPO = $[(T-P/T+P)] \times 100$; T = # of eggs in witness. IPO varies from + 100 (stimulating) to -100 (deterrence). A = almonds; L = leaves; ST = stems; S = seeds

Table 2. Means (\pm SE) of emergence, dry weight of adults and consumed weight by *C. maculatus* in cowpea-grains impregnated with powders in different vegetable species

Treatments ¹	N.º of emerged adults ²	Dry weight of adults (mg) ³	Weight of grains consumed (g) ³
<i>Ruta graveolens</i> (L+ST)	162.50 \pm 8.37 a	1.9 \pm 0.06 a	2.11 \pm 0.28 a
<i>Coriandrum sativum</i> (L)	148.83 \pm 14.23 a	1.8 \pm 0.12 a	1.90 \pm 0.41 ab
<i>Tagetes erecta</i> (L+ST)	142.00 \pm 27.15 a	1.9 \pm 0.10 a	1.86 \pm 0.87 ab
<i>Trichilia pallida</i> (L)	138.67 \pm 12.07 a	1.8 \pm 0.05 a	1.78 \pm 0.41 ab
<i>Corymbia citriodora</i> (L)	138.50 \pm 13.35 a	1.9 \pm 0.08 a	1.63 \pm 0.63 ab
<i>Azadirachta indica</i> (L)	135.33 \pm 15.24 a	1.9 \pm 0.05 a	1.64 \pm 0.61 ab
<i>Trichilia pallida</i> (ST)	134.83 \pm 10.87 a	1.8 \pm 0.13 a	1.67 \pm 0.34 ab
<i>Mentha pulegium</i> (L)	127.33 \pm 8.61 a	1.8 \pm 0.07 a	1.56 \pm 0.36 ab
Control	115.00 \pm 11.03 a	1.8 \pm 0.10 a	1.39 \pm 0.35 abc
<i>Azadirachta indica</i> (A)	109.33 \pm 7.67 ab	1.9 \pm 0.08 a	1.14 \pm 0.42 bc
<i>Chenopodium ambrosioides</i> (L+ST)	58.00 \pm 4.35 bc	2.0 \pm 0.07 a	0.56 \pm 0.18 c
<i>Piper nigrum</i> (S)	47.67 \pm 5.26 c	2.0 \pm 0.12 a	0.51 \pm 0.20 c
F	8.50*	2.47 ^{ns}	7.30*
CV (%)	13.81	4.89	31.25

¹ A = almonds; L = leaves; ST = stems; S = seeds.

² Means within columns followed by the same letter are not significantly different by Tukey test ($P \leq 0.05$). Data transformed in $(x + 0.5)^2$.

³ Means within columns followed by the same letter are not significantly different by Tukey test ($P \leq 0.05$). Original data.

TAVARES & VENDRAMIM (2005) also verified substantial reduction in the emergence of the adults of *Sitophilus zeamais* (Mots.) in maize grains impregnated with fruits and whole plants of *C. ambrosioides*, in the proportion of 0.3 g of powder 20 g⁻¹ of maize grains; according to these authors, the low emergence rate is due to high mortality level and low oviposition in weevils. Silva *et al.* (2003) also obtained favorable results in the application of *C. ambrosioides* powder in order to control of *S. zeamais*, with 100% of mortality in the concentration of 1% (w/w). Mortality tests of *C. maculatus* were not carried out in this paper and should be studied in future researches.

Low emergence of adults of *C. maculatus* in cowpea grains containing powder of *P. nigrum* seeds (Table 2) corroborates with the results of SOUSA *et al.* (2005), using 2,5% (w/w) of powder concentration, who found 100% of reduction in oviposition and emergence of adults of this weevil using this very same plant species. In studies aiming at verifying the effect of aqueous extracts from five botanic species, ALMEIDA *et al.* (2004) found that the treatment with *P. nigrum* was the most efficient, in the extract form, lead-

ing to 100% of mortality of the adults of *C. maculatus* after a ten-minute exposition.

The means of the treatments for dry weight of adults (Table 2) did not differ significantly between treatments, corroborating that the powders of the vegetable species used did not affect the development of the larvae.

The quantity of grains consumed by the insects was lower for the treatment with powders of *C. ambrosioides* (0.56 g) and *P. nigrum* (0.51 g), which is the consequence for lower quantity of emerged weevil in grains impregnated with these treatments (Table 2). In these two treatments shorter periods of development were also verified (Figure 2), with means of 32.15 and 31.25 days, respectively. Even though an increase in the susceptibility of grains by using these two powders may be suggested, probably, this reduction in the cycle is a consequence of fast mortality of weevils in the experimental plots experimental plots, which may have ended the oviposition in the first days. Considering that the grains remain under infestation up to seven days, it is possible that the superior cycle in the other materials may be a consequence of greater activity in the weevils.

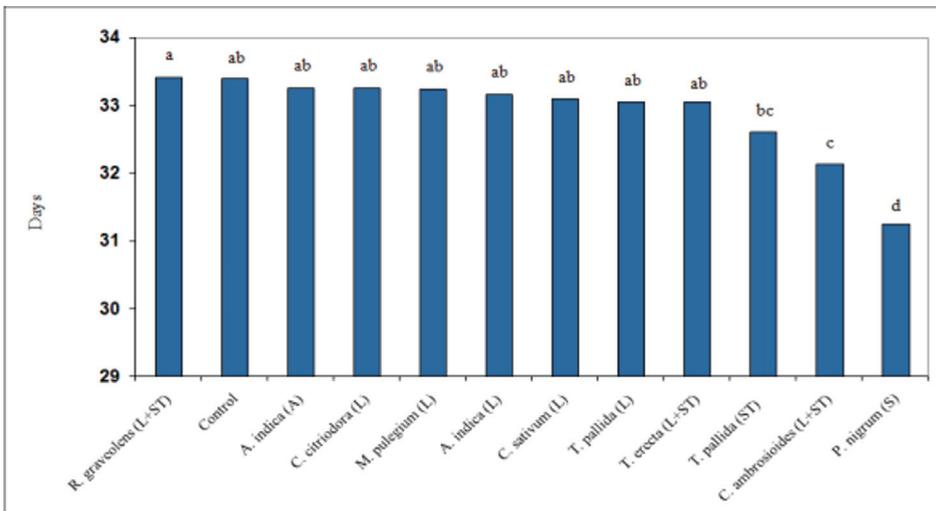


Figure 2. Mean Period (\pm SE) of development (egg-adult) of *C. maculatus* in cowpea-bean grains impregnated with powders of different vegetable species. A = almonds; L = leaves; ST = stems; S = seeds

RESUMEN

PANNUTI, L. E. R., L. S. MARCHI, E. L. L. BALDIN. 2012. Uso de polvos de origen vegetal como alternativa para el control de *Callosobruchus maculatus*. Bol. San. Veg. Plagas, **38**: 33-40.

El gorgojo *Callosobruchus maculatus* Fabr. (Coleoptera: Bruchidae) es la plaga más importante de frijoles almacenados, exigiendo el uso de métodos de control. El control químico del gorgojo es uno de los métodos más utilizado actualmente, pero su uso continuado puede causar problemas, incentivando la búsqueda de métodos de control alternativos. Este trabajo evalúa los efectos de once especies vegetales en forma de polvo sobre individuos del gorgojo en laboratorio. Se mantuvieron seis adultos no sexados del gorgojo durante siete días en recipientes que contenían 0,3 g del polvo de cada tratamiento mezclados con 10 g de frijoles. Se empleó un diseño completamente aleatorizado con seis repeticiones por tratamiento. Después de 20 días de la infestación, se evaluó la ovoposición y se inició el conteo de adultos emergidos. Posteriormente se evaluó el peso seco de los insectos, el peso de los granos consumidos y el período de desarrollo del huevo a adulto. Los polvos de *Chenopodium ambrosioides* L. y *Piper nigrum* L. redujeron la ovoposición y la emergencia de adultos de *C. maculatus*, revelando su potencial para el control alternativo del insecto.

Palabras clave: gorgojo, plantas insecticidas, polvo vegetal.

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