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Bioactivity of vegetable powders on biological parameters of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae, Bruchinae) in *Vigna unguiculata*

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ABSTRACT

Vigna unguiculata (L.) Walp. is widely distributed in the world, being one of the most important crops in the Brazilian semiarid. Grains, however, are commonly damaged by the larvae of *Callosobruchus maculatus* (Fabr.), causing quantitative and qualitative losses. Thus, the study aimed to determine the potential reduction of eggs, adult emergence and population growth of this pest. The vegetable powders used were: *Eucalyptus urophylla* S T Blake × *E. tereticornis* Smith; *E. brassiana* S T Blake; *E. urophylla* × *E. tereticornis* × *E. pellita*; and of *Azadirachta indica* A. Juss; *Schinnus terebinthifolius* Raddi and *Rosmarinus officinalis* L. All of the powders reduced *C. maculatus* oviposition, adult emergence and the population growth when compared to control at all the concentrations used. The most effective powders were the hybrid of *E. brassiana* (clone 25), *E. urophylla* × *E. tereticornis* (clone 101), *S. terebinthifolius* and *A. indica*.

Key words: Cowpea crop, Cowpea weevil, Pest control, Stored grain insects

The cowpea (*Vigna unguiculata* (L.) Walp.) is a leguminous, annual and herbaceous plant of the family Fabaceae and subfamily Papilionoidea (Almeida *et al*. 2005, Passos *et al*. 2007). It is widely distributed in tropical and subtropical regions (Almeida *et al*. 2009), and tolerant to high temperatures and drought periods (Araujo and Watt 1988). It is grown by small farmers in subsistence agriculture, who market their small surplus stocks, as well as by medium and large producers, aiming the national and international market (Zilli *et al*. 2009). It constitutes a staple food for low-income populations, due to its high nutritional value, consisting of proteins, carbohydrates, soluble and insoluble fiber and lipids (Frota *et al*. 2010).

After harvesting and processing, the grains can be used for human consumption or stored (Quirino 2011). At this stage they are more vulnerable to attack by insect pests (Lopes *et al*. 2000), causing quantitative and qualitative losses (Braccini and Picanço 1995, Pereira *et al*. 2008). The cowpea weevil, *Callosobruchus maculatus* (Fabr.) (Chrysomelidae: Bruchinae) is considered the most important pest of cowpea stored in tropical and subtropical regions (Tanzubil 1991). The insect may present a cross-infestation, starting his attack on the field where it oviposits on dehiscent or defective string beans (Gallo *et al*. 2002), continuing intensively in storage units (Azevedo *et al*. 2007).

The control of *C. maculatus* at the storage units has been done by proper sanitation before the harvest season, insecticides preventive applications with organophosphate or pyrethroids and by curative applications with fumigant phosphine (Pereira *et al*. 2008). Despite the inconveniences related to their costs, their toxicity, their residues, and to the resistance phenomenon tha has appeared in several species of stored product pest insects (Kellouche *et al*. 2010).

Thus, plant extracts, essential oils or powders of different plants with insecticidal activity, has been used for controlling *C. maculatus*, as an alternative to the use of synthetic chemical insecticides (Guerra *et al*. 2009, Oni 2011, Idoko and Adesina 2012, Ileke and Olotuah 2012, Ileke *et al*. 2012). These insecticides act by contact, ingestion or fumigation and are considered promising for the integrated management of *C. maculatus* in storage units (Brito *et al*. 2006). Employing insecticide plants, mainly in the form of dry powders, favors, especially, the small producers for the lowest cost and easily use (Mazzoneto and Verdramim 2003). Thereby, the aim of this work was to evaluate the effects of different vegetable powders on oviposition, adult emergence and population growth of *C. maculatus*.

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MATERIALS AND METHODS

The experiments were performed in the Laboratory of Agricultural Entomology, Federal Rural University of Pernambuco (UFRPE), temperature 28.5 ± 1.6° C, 52.6 ± 7.4% relative humidity and 12 hr photophase.

The insects were reared on *V. unguiculata* beans cv. Sempre verde, packed in glass containers closed with perforated lids and covered with voile fabric to provide aeration. The adults were confined for three days in the containers, for the oviposition and subsequently sieved and kept in the laboratory until the emergence of new adults. This procedure was performed for many generations.

Clean and dry grains, used for experiments, were placed in plastic bags and kept in a freezer at 10°C for seven days, to eliminate possible insect infestation from the field. Then, the grains were transferred to glass flasks and kept in the laboratory for 10 days in order to reach the grains moisture equilibrium.

Initially, preliminary tests were performed, aiming to define the concentrations of powders to be tested. *Eucalyptus* hybrids were obtained from the Experimental Station of the IPA-Araripina/PE, the *Azadirachta indica* from Cruangi sugarcane mill, Timbaúba - PE, *Schinnus terebinthifolius* and *Rosmarinus officinalis* from the company Flores e ervas com. Farmac. LTDA, Piracicaba – SP. Further information of plant materials and concentrations appear, respectively, in Tables 1 and 2.

The experiments were performed in a completely randomized design with four replicates. The powders were mixed with the grain inside plastic recipients by manual agitation for two minutes. Each replicate was composed of 20g of beans, packed in a plastic container with perforated lid to allow gas exchanges with the outside and infested with 10 *C. maculatus* females 0-48 hr old. After 48 hr, the insects were removed and discarded. The number of eggs was counted 12 days after infestation with the aid of a stereomicroscope. Then, the grains were again placed in plastic containers and kept in the laboratory. After 32 days of infestation, the number of emerged insects in each replicate was evaluated.

Table 1 Vegetable material and portion used to obtain the powders

Material	Common name	Portion used
Eucalyptus brassiana S T Blake Hybrid	Clone 25	Leaves
Eucalyptus urophylla S T Blake × *Eucalyptus tereticornis* Smith Hybrid	Clone 51	Leaves
Eucalyptus urophylla S T Blake × *Eucalyptus tereticornis* Smith Hybrid	Clone 101	Leaves
Eucalyptus urophylla S T Blake × *Eucalyptus tereticornis* Smith × *Eucalyptus pellita* F. Muell. Hybrid	Clone 156	Leaves
Eucalyptus urophylla S T Blake × *Eucalyptus tereticornis* Smith Hybrid	Clone 158	Leaves
Azadirachta indica A. Juss	Neem	Cake
Schinnus terebinthifolius Raddi.	Brazilian pepper	Bark
Rosmarinus officinalis L.	Rosemary	Leaves

Table 2 Powder concentrations used in *C. maculatus* bioassays

Powders	Concentrations (g/kg of cowpea grains)
Clone 25 (*Eucalyptus brassiana* hybrid)	0; 25; 50; 75; 100; 125
Clone 51 (*Eucalyptus urophylla* × *E. tereticornis* hybrid)	0; 25; 50; 100; 150
Clone 101 (*Eucalyptus urophylla* × *E. tereticornis* hybrid)	0; 25; 50; 100; 150
Clone 156 (*Eucalyptus urophylla* × *E. tereticornis* × *E. pellita* hybrid)	0; 25; 50; 100; 150
Clone 158 (*Eucalyptus urophylla* × *E. tereticornis* hybrid)	0; 25; 50; 100; 150
Azadirachta indica	0; 25; 50; 100; 150
Schinus terebinthifolius	0; 25; 50; 75; 100
Rosmarinus officinalis	0; 25; 50; 100; 150

From the number of insects emerged in each replicate, after 32 days of infestation, it was calculated the instantaneous rate of growth (r_i) according to the equation:

$r_i = \ln(N_f/N_o)/\Delta t$

Where: N_f is the number of *C. maculatus* adults present in the final evaluation, 32 days after infestation; N_o is the initial number of adults transferred to each replicate at the beginning of the bioassay and Δt is the bioassay period. According to the equation, if the estimated value of $r_i = 0$, there is a balance in the population growth, on the other hand, if $r_i > 0$, the population growth remains increasing and if $r_i < 0$, the population is suffering a decline, which could take it to extinction when $N_f = 0$ (Stark and Banks 1997).

The number of eggs and emerged adults were subjected to analysis of variance (ANOVA) and regression. The instantaneous rate of population growth was subjected to analysis of variance and means were compared by Tukey test at 5% probability. Both analyzes were performed using the computer program SAS version 8.02 (SAS Institute 2001).

RESULTS AND DISCUSSION

Powders effects on the oviposition and emergence of C. maculatus

The number of eggs and adult emergence of *C. maculatus* varied according to the concentrations of each vegetable powder. All of the powders reduced the oviposition and emergence when compared to control at all the concentrations tested. In general, the higher the powder concentration, the lower the number of emerged adults and eggs (Fig 1 and 2).

The linear regression model was the better adjusted to the results of the number of eggs and emerged insects. The equations for oviposition and emergence of *C. maculatus* and their respective parameter settings are found in Table 3.

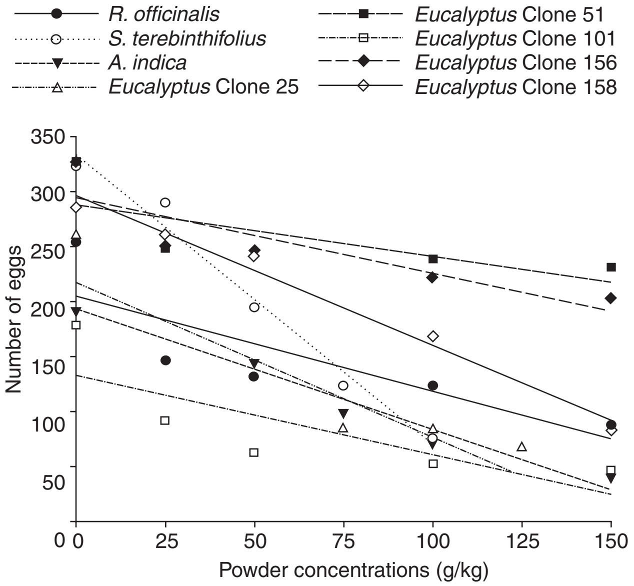

Fig 1 Number of *Callosobruchus maculatus* eggs in cowpea grains treated with different concentrations of vegetable powders

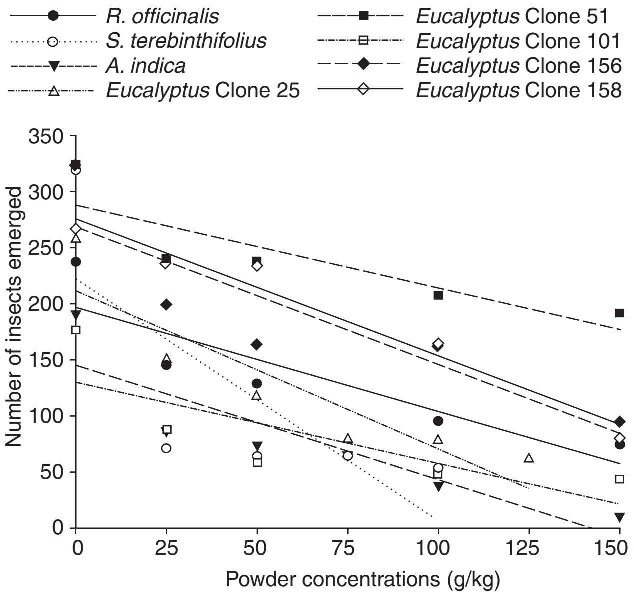

Fig 2 Number of *Callosobruchus maculatus* adults emerged in cowpea grains treated with different concentrations of vegetable powders.

The powders of *S. terebinthifolius*, Eucalyptus clone 25, Eucalyptus clone 101 and *A. indica* provided the greatest reduction in the number of eggs at the highest concentration, with 75.0, 66.5, 46.25 and 39.5 eggs, respectively (Fig 1). When the highest concentration was compared to the control treatment, the powders of *S. terebinthifolius* and *A. indica* promoted a reduction of 76.8 and 79.4% in the number of *C. maculatus* eggs. The results of this study corroborate with Medeiros *et al*. (2007) who observed a reduction in the average number of *C. maculatus* eggs of 118.37 and 105.25 for powders of dried neem leaves at the concentrations 0.75 and 1.0 g, respectively. These authors also observed that the powders

Table 3 Equations for *Callosobruchus maculatus* oviposition and emergence in grains of *Vigna unguiculata* beans treated with different vegetable powders and their respective parameter settings (F, Fisher test, P significance value; R^2, coefficient of determination)

Treatment		Equations	F^P	R^2
Clone 25 (*Eucalyptus brassiana* hybrid)	Oviposition	y=216.35-1.39x	$36.05^{<0.0001}$	0.62
	Emergence	y=211.92-1.41x	$33.24^{<0.0001}$	0.60
Clone 51 (*Eucalyptus urophylla*×*E. tereticornis* hybrid)	Oviposition	y=287.61-0.46x	$4.67^{0.0443}$	0.61
	Emergence	y=288.09-0.74x	$12.13^{0.0027}$	0.70
Clone 101 (*Eucalyptus urophylla*×*E. tereticornis* hybrid)	Oviposition	y=132.83-0.72x	$27.30^{<0.0001}$	0.60
	Emergence	y=130.32-0.72x	$27.01^{<0.0001}$	0.60
Clone 156 (*Eucalyptus urophylla*×*E. tereticornis*× *E. pellita* hybrid)	Oviposition	y=293.93-0.68x	$16.41^{0.0007}$	0.68
	Emergence	y=268.67-1.23x	$29.72^{<0.0001}$	0.62
Clone 158 (*Eucalyptus urophylla*×*E. tereticornis* hybrid)	Oviposition	y=295.87-1.36x	$33.89^{<0.0001}$	0.65
	Emergence	y=276.10-1.22x	$34.92^{<0.0001}$	0.66
Azadiractha indica	Oviposition	y=192.76-1.09x	$278.68^{<0.0001}$	0.92
	Emergence	y=145.40-1.02x	$83.47^{<0.0001}$	0.79
Schinus terebinthi-folius	Oviposition	y=333.40-2.65x	$64.94^{<0.0001}$	0.78
	Emergence	y=222.25-2.15x	$18.91^{0.0004}$	0.51
Rosmarinus officinalis	Oviposition	y=204.91-0.87x	$23.67^{0.0001}$	0.57
	Emergence	y=196.93-0.93x	$34.55^{<0.0001}$	0.66

of neem leaves induced the reduction of *C. maculatus* adult emergence at different concentrations, obtaining an average of 69.50 emerged adults for the powder of dried neem leaves and 85.62 for powders of green neem leaves at the concentration 1.0 g.

Papachristos and Stamopoulos (2002) highlighted the insecticide potential of *R. officinalis* for the management of stored grain pests as an oil vapor, noting that *R. officinalis* reduced *Acanthoscelides obtectus* oviposition from 42.3 in control to 16.6 eggs per female in the treatment. In the present study, *R. officinalis*, despite not having been one of the most promising vegetable powders among the tested ones, negatively affected *C. maculatus*, reducing oviposition, the number of emerged insects and thus, the population growth.

The lowest emergence of *C. maculatus* occurred when the vegetable powders of Eucalyptus clone 25, Eucalyptus clone 101, *S. terebinthifolius* and *A. indica* were used, which

Table 4 *Callosobruchus maculatus* instantaneous rate of population growth (r_i) in cowpea grains treated with different concentrations of vegetable powders

Vegetable powders	Concentrations (g/kg of cowpea grains)	Instantaneous rate of population growth (r_i)
Clone 25	0	0.100852 B
(*Eucalyptus*	25	0.082135 B
brassiana hybrid)	50	0.076271 B
	75	0.062848 AB
	100	0.060871 AB
	125	0.054497 A
Clone 51 (*Eucalyptus*	0	0.107899 B
urophylla × E,	25	0.099286 AB
tereticornis hybrid)	50	0.098900 AB
	100	0.093020 AB
	150	0.092183 A
Clone 101 (*Eucalyptus*	0	0.089617 C
urophylla × E,	25	0.067832 C
tereticornis hybrid)	50	0.055022 C
	100	0.048695 B
	150	0.045688 A
Clone 156 (*Eucalyptus*	0	0.107899 C
urophylla × E,	25	0.093577 B
tereticornis × E,	50	0.087117 B
pellita hybrid)	100	0.086182 B
	150	0.070093 A
Clone 158 (*Eucalyptus*	0	0.102042 B
urophylla × E,	25	0.098291 A
tereticornis hybrid)	50	0.098096 A
	100	0.085274 A
	150	0.064998 A
Azadiractha indica	0	0.09210 F
	25	0.06733 E
	50	0.06265 D
	75	0.05873 C
	100	0.04130 B
	150	0.00077 A
Schinus terebinthifolius	0	0.1083 E
	25	0.0614 D
	50	0.0581 C
	75	0.0580 B
	100	0.0526 A
Rosmarinus officinalis	0	0.09895 E
	25	0.08378 D
	50	0.07985 C
	100	0.07068 B
	150	0.06296 A

Means followed by the same letter do not differ significantly by Tukey test at 5% probability.

enabled a number of insects emerged of 61.5, 43.5, 53.7 and 10.25, respectively (Fig. 2). Regarding the different genotypes of *Eucalyptus, sp*. Souza *et al*. (2005) testing leaf extracts of *Eucalyptus citriodora* at a concentration of 2.5% (v/v) observed a reduction of 62.05% and 43.36% in *C. maculatus* oviposition and adult emergence, confirming the deleterious effect of secondary compounds from the genus *Eucalyptus* upon this pest.

At the highest concentration of *S. terebinthifolius* and *A. indica* there was a drastic reduction in the number of emerged insects when compared to control. These powders decreased in 83.2 and 94.6% the number of insects in the F1 generation. There were no reports in literature of *S. Terebinthifolius* insecticidal activity on stored grain pests, however, powders of several other species have shown a proven effect upon *C. maculatus*. Castro *et al*. (2010) reported an inhibition of *C. maculatus* oviposition using the powders of *Piper tuberculatum* (fruit), *Lippia sidoides* (leaves) and *Sapinus saponaria* (leaves and seeds). In these tests, the control had an average of 74.5 eggs while the treatments had 1.7, 0 and 8.5 eggs for *P. tuberculatum*, *L. sidoides* and *S. saponaria*, respectively.

Effect of vegetable powders on the instantaneous rate of population growth (r_i)

The instantaneous rates of population growth (r_i) decreased with the increasing concentrations. However, none of the tested concentrations caused negatives r_i (Table 4).

The lowest r_i values were observed for the vegetable powders of Eucalyptus clone 101, *S. terebinthifolius* and *A. indica*. In this last one, the concentration 150g/kg showed the lowest value of r_i (0.00077) among all the vegetable powders tested.

The results achieved in our study laud the possibility of using vegetable powders for *C. maculatus* management in storage units, especially to small farmers from subsistence agriculture, as well as confirm the relevance of continuous research in pursuit of new species and secondary compounds with insecticidal bioactivity.

The powders of *Eucalyptus* clone 25, *Eucalyptus* clone 101, *S. terebinthifolius* and *A. indica* were the most effective against *C. maculatus*.

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