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Short Paper

Effect of *Verbascum sinuatum* (Scrophulariaceae) on oviposition of *Callosobruchus maculatus* (Bruchidae)

Fatma Demnati* **and Farid Allache**

Faculté des Sciences Exactes et Sciences de la Nature et de la Vie, Département des Sciences Agronomiques, Université Mohamed Khider, 07000 Biskra, Algérie.

Abstract: No doubt, damage and losses inflicted by *Callosobruchus maculatus* to stored products in general and chickpea in particular can be significant. The objective of this work was to evaluate the effect of the *Verbascum sinuatum* powder on *C. maculatus* oviposition behavior in chickpea under laboratory conditions (30 °C, 60% relative humidity). Powder of Mullein plant (*V. sinuatum*) caused reduction in fecundity, fertility, adult emergence from seeds and chickpea weight loss depending on treatment concentration (0.1, 0.05 and 0.025 g/ ml). It reduced the number of eggs laid by the weevil at first concentration to 136 eggs, the egg hatching rate at 52.22% and adult emergence to 34.03%. The percentage of seed weight loss was reduced at all studied concentrations but not significantly different at second and third concentrations compared to control. The lowest weight loss of seeds was registered at first concentration (4.7%). *V. sinuatum*, according to this study, showed a significant potential to reduce the egg deposition and chickpea seed weight loss due to the chickpea beetle.

Keywords: *Verbascum sinuatum*, fecundity; fertility, adult emergence, seed weight loss

Introduction

Leguminous plants are important source of food for humans and animals in many countries including Algeria (Abdelguerfi-Laouar *et al.*, 2000). Leguminous food such as chickpea is attacked in storage environment by various pests, among these, bruchid insects such as *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae) is considered as the main pest (Sharma, 1984). Its larvae survive exclusively inside seeds. It is cosmopolitan and a field to stored insect pest as its infestation often begins in the field as the mature pods dry (Huignard *et al.*, 1985). In addition, the crop can be contaminated with insect body fragments, feces and saprophytic microorganism leading to food quality loss, degrading its economic value and seed germination reduction (Garcia *et al.*, 2003; Paranagama *et al.*, 2003). The insect reproduces very rapidly in storage habits where it causes very high losses (Kshirsagar, 2010). An initial seed infestation rate of 10% by *C. maculatus* larvae was sufficient to destroy in a few months 60 to 70% of the leguminous plants yield (Tanzubil, 1991), and even caused total loss of stored grain (Lienard and Seck, 1994; Ngamo and Hance, 2007).

Chemical control is the main method of combating stored products pests, which their limits are well proven. Therefore, research has increasingly been performed to identify alternative measures to chemical control. Plant-extracted insecticides seem to be one of the alternatives that affect this herbivore damage.

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* **Corresponding author**, e-mail: fat_demnati@yahoo.fr
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Some indigenous plant materials have been known for their effectiveness to reduce oviposition, egg hatchability, adult emergence (Paneru and Shivakoti, 2001) and seed damage (Chinwada and Giga, 1993). The use of botanical pesticides is considered to be the most viable and environmentally safe approach to offset ever increasing danger caused by conventional pesticides (Saxena, 1982).

Wavy leaf Mullein or *Verbascum sinuatum* Linnaeus (Scrophulariaceae) is a large biennial ichtyotoxique plant used as a poison by the ancient Mediterranean peoples to catch fish in rivers (Kerharo *et al.*, 1960) and also used in traditional medicine (Kerharo *et al.*, 1960; Tatli and Akdemir, 2006). The ichtyotoxique power of plants in general is often accompanied by insecticidal properties (Moretti and Grenand, 1982). However, according to Kerharo *et al.* (1960), verbascums are gifted with insecticidal properties against pests such as, aphids and pests of vegetable and flowering plants. *V. sinuatum* occurs in several locations in Algeria (Pers. obs.).

The aim of this study was to assess the effect of vegetable powder extract of *V. sinuatum* on oviposition behavior, seed weight loss and adults emergence of the storage pest, *C. maculatus*, in the laboratory conditions.

Materials and Methods

Plant materials

Chickpea, *Cicer arietinum* Linnaeus, was used to maintain a continuous weevils breeding and for the different tests. Stems of *V. sinuatum* were used to test its efficiency against the bruchid weevil biological parameters.

Rearing procedure

Adults of *C. maculatus* (less than 24 hours old) were obtained from a laboratory culture carried out in the Department of Agricultural Sciences (Biskra). Adults used were aged less than 24 hours. The mass rearing of *C. maculatus* was made in glass jars, they contained a sufficient quantity of healthy and contaminated chickpea seeds covered with fine mesh for ventilation and to prevent adults from escaping. The jars were kept in the dark in a laboratory oven set at 30 °C and humidity of about 60%. Contamination of new quantities of chickpea seeds was carried out regularly to ensure the continuity of breeding.

Plant extract

The Mullein was harvested at Tizi-Ouzou region (100 km east of Algiers) in July 2009 at flowering period. It was washed, shade dried then grinded until fine powder. The active ingredients of the *V. sinuatum* powder were extracted from plants using methanol. Based on our primary study, three different concentrations of the plant powder were used.

We tried to extract the active ingredients of *V. sinuatum* plant by conventional method as follows: a quantity of 10 g of plant powder was mixed with 100 ml of methanol. The resulting solution represents the first concentration (D_1). Similarly, the second ($D_{2)}$ and third concentration ($D_{3)}$ were prepared based on the amounts of 5 and 2.5 g of powder, respectively. The mixtures were left to rest for seven days in the dark at laboratory ambient temperature (25°), and then the contents were filtered.

The dose of 1 ml of methanol solution containing an amount of 0.1g/ml of the insecticidal extract for the first concentration, 0.05g / ml for the second and 0.025g / ml for the third concentration were applied on seeds.

Treatment procedure

The different extracted concentrations (0.1, 0.05 and 0.025 g/ ml) were applied on healthy chickpea seeds, and then offered to weevils (less than 24 hours old). The control seeds were treated with a mixture of distilled water-methanol.

Fecundity and fertility

Ten pairs of weevils aged less than 24 hours were placed in Petri dishes (9cm diameter) with 10 seeds; for fecundity, the females were left to lay their eggs on seeds treated with the different concentrations of plant powder until their death. For fertility, females were removed after a sufficient number of eggs were laid, approximately 30 eggs per dish. The control

was treated with distilled water-methanol mixture. The test was repeated three times. The weevils were subjected to the same environmental conditions as that of breeding. The number of eggs, the number of hatched eggs and adult emergence was recorded.

Estimate of weight loss of chickpea

Ten pairs of weevils (less than 24 hours old) were placed in glass jars (7cm diameter, 14cm high) containing 100g of healthy chickpea seeds. The seeds were treated with the three prepared concentrations and the control with the mixture of distilled water-methanol. The females laying their eggs were left until they died. After laying eggs, the dead adults were removed from jars. Hatched eggs were allowed to develop until adult emergence. After about less than a month the newly emerged adults were systematically eliminated to avoid re-laying eggs or re-infestation. Then, the seeds were weighed again. According to Shaheen and Abdul (2005), the weight loss was calculated using the following formula:

$WL\ (\%) = [(W_{bef.\ inf.} - W_{aft.\ inf.})/W_{bef.\ inf.}] \times 100$

WL: Weight loss in percent

$W_{bef.\ inf.}$: Weight before infestation

$W_{aft.\ inf.}$: Weight after infestation

Statistical analysis

Means were compared using LSD test at 5% level of significance (Statistica 6.1 Statsoft, Inc.1984–2003) and the graph was drawn with Excel software.

Results

Fecundity

The number of eggs laid by females on treated chickpea seeds were significantly less than that of control (Table 1). The lowest number of eggs were laid on the seeds treated with the first concentration (0.1 g/ ml); it was significantly different from seeds treated with the second concentration (0.05 g/ ml) ($p = 0.001$), third concentration (0.025 g/ ml) ($p = 0.011$) and control seeds ($p = 0.011$).

Fertility

Regarding the fertility of *C. maculatus*, the rate of hatched eggs was significantly affected by the treatments; it ranged from 52.22% to 62.23%. The treatment effect was significantly different only at the first concentration as compared to the control ($p= 0.021$) (Table 1).

Adult emergence

The results showed that the different concentrations of the Mullein powder decreased significantly adult emergence depending on the concentration (Table 1). Total emergence of adults in control was approximately 69.47%. Emergence from seeds treated with the first concentration was the least with an average percentage of 34.03% followed by the second concentration (40.42%). These two rates were significantly different from control adult emergence ($p = 0.008$; $p = 0.022$ respectively).

Weight loss of seeds

The loss in chickpea seed weight among different concentration of the Mullein powder ranged from 4.70 to 5.63% (Fig. 1). The loss rate in seeds weight decreased with the increasing of the concentration level. There was a significant difference between control and the first concentration ($p = 0.038$); however, no difference was recorded for the second and third concentration ($p = 0.148$ and 0.197, respectively).

Discussion

The use of plant parts or plant extracts to protect stored products from insect damage is an ancient practice (Akinyemi *et al*., 2005; Regnault-Roger and Philogène, 2008). Insecticidal properties and biological parameters of various plant extracts have been evaluated against stored product insects (Adedire and Ajayi, 2003; Ebadollahi, 2011). According to our results, the average number of eggs deposited by the females decreased due to the *V. sinuatum* powder effect; it varied according to concentration. In the same way, Al-Lawati *et al.* (2002) announced that the number of eggs deposited by *C. chinensis* Linnaeus decreased after treated by *Annona*

squamosa Linnaeus (Annonaceae). According to Al-Moajel (2006) no eggs laid by *Sitophilus granarius* Linnaeus treated with seed extract of *Sesbania sesban* Linnaeus at the lethal dose of 95. In addition, the egg laying was reduced by 85.44 to 90% in *C. subinnotatus* Pic treated with *Piper guineense* Schumacher & Thonnig powder (Oparaeke and Bunmi, 2006) and in *Sitophilus zeamais* Motschulsky treated with essential oils of *Annona senegalensis* Persoon (Annonaceae), *Hyptis spicigera* Lamarck (Lamiaceae) and *Lippia rugosa* A. Chevalier (Verbenaceae) (Ngamo *et al.*, 2007). An egg laying inhibition or an ovicidal action was observed in *C. maculatus* beetles treated with leaf powders of *Nicotiana tabacum* Linnaeus (Solanaceae), *Erythrophleum suaveolus* Guillemin & Perrottet (Caesalpiniaceae) (Ofuya, 1990) or neem powders *Azadirachta indica* A. Jussieu (Seck *et al.*, 1991), *Boscia senegalensis* Persoon (Khaire et *al.*, 1992; Seck *et al.*, 1993) and many essential oils of *Citrus* (Don-Pedro, 1996).

In the present study, the fertility of *C. maculatus* females decreased with increasing of the concentrations of *V. sinuatum*. Zidan *et al.* (1993) also highlighted the action of various plant extracts, such as *Synzygium aromaticum* Linnaeus on the fertility of *S. oryzae* and *C. maculatus.* The fertility of *C. chinensis* was 3.33% when treated with 1g powder of Santolina (Singh, 2011). Whereas, Kachare *et al.* (1994) found that egg hatching of the same species was null during storage of pigeonpea seeds treated by neem essential oil.

Table 1 Effect of *Verbascum sinuatum* powder on egg laying, egg hatching and adult emergence of *Callosobruchus maculatus.*

Treatment	No. of eggs per 10 beetles	Eegg hatch (%)	Emerged adults (%)
Control	279.00 ± 3.47[a]	72.00 ± 4.80[a]	69.47 ± 20.02[a]
V. sinuatum (D_1 = 0.1g/ml)	136.00 ± 37.64[b]	52.22 ± 1.92[b]	34.03 ± 3.18[b]
V. sinuatum (D_2 = 0.05g/ml)	238.66 ± 17.04[a]	60.22 ± 5.88[a]	40.42 ± 19.17[b]
V. sinuatum (D_3 = 0.025g/ml)	239.67 ± 65.77[a]	62.22 ± 15.03[a]	46.91 ± 5.51[a]

Values are means (± SD) of three replications.
Means followed by same letter in a column are not significantly different at 5% level by LSD test.

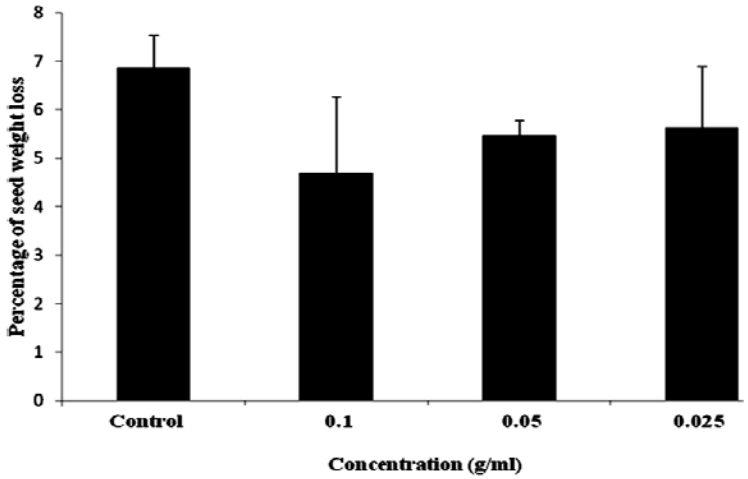

Figure 1 Effect of the different concentrations of *Verbascum sinuatum* powder on chickpea seed weight loss.

It appears from tests of adult emergence of *C. maculatus* that emergence varied according to the concentration used and was reduced compared to control. This can be explained by the effect of the *V. sinuatum* extract on the immature stages of the pest. This was in accordance with Musa *et al*. (2009). However, Oparaeke and Bunme (2006) noted that the emergence of the adults of *C. subinnotatus* treated with *P. guineense* powder was reduced up to 100%. A complete suppression of the progeny was observed in *S. oryzae* population treated with *V. cheiranthifolium* Boisduval and *V. speciosum* Schrader (Khoshnoud *et al*., 2008). A 50% reduction rate of *C. maculatus* emerging from seeds treated by the extract of *Striga hermonthica* Delile was noted which was attributed to its ovicidal action (Kiendrebeogo *et al*., 2006). Furthermore, other plant extracts like *Vitex grandifolia* Gürke have been reported to inhibit the emergence of *C. maculatus* adults (Epidi *et al*., 2008).

According to this study, the loss in weight of seeds of chickpea due to *C. maculatus* decreased as the concentration of the plant extract increased. The loss reduction in seed weight can be explained by the decrease of fertility and the mortality of *C. maculatus* larvae. Al-Moajel (2006) also stated a reduction in the weight loss of corn seeds affected by *S. granarius* treated by the extract of *S. sesban*. The damage due to *C. maculatus* was significantly reduced when seed oil of *Khaya senegalensis* A. Jussieu was used for stored seeds (Bamaiyi *et al.*, 2006). In certain countries of Africa, palm or groundnut oils were used to impregnate seeds of cowpea in order to preserve them against larvae and eggs of *C. maculatus* during storage (Osekre *et al.*, 2002). Modgil and Mehta (1997) revealed that leaf powders of mint and eucalyptus protect stored corn in the various structures of storage for five to six months. The damage caused by *C. subinnotatus* was reduced up to 100% on seeds treated by *P. guineense* powder (Oparaeke and Bunmi, 2006). In the same way, oil based treatment extracted from seeds of *A. indica* in laboratory allowed to reduce the rate of seeds destroyed to less than 4% during three months of storage (Ivbijaro, 1990).

According to this study, the wavy leaf Mullein *V. sinuatum* exhibited an effect on *C. maculatus* behavior. Thus, this plant is promising bio-insecticide that can be used to control this insect and even as a part in efficient integrated management program.

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تأثیر گیاه *Verbascum sinuatum* (Scrophulariaceae) روی تخم‌ریزی *Callosobruchus maculatus* (Coleoptera: Bruchidae)

فاطمه دمناتی* و فرید علاش

Exactes دانشکده علوم تجربی محض و علوم طبیعی و زیستی - بخش علوم زراعی، دانشگاه محمد خیدر ۰۷۰۰۰ بیسکرا، الجزیره.
* پست الکترونیکی نویسنده مسئول مکاتبه: fat_demnati@yahoo.fr
دریافت: ۱۵ مرداد ۱۳۹۲؛ پذیرش: ۹ بهمن ۱۳۹۲

چکیده: بدون شک زیان و خسارت ناشی از *Callosobruchus maculatus* به فرآورده‌های انباری به‌طور کلی و به نخود به‌طور اخص قابل توجه است. هدف از انجام این تحقیق ارزیابی از پودر *Verbascum sinuatum* روی رفتار تخم‌ریزی *C. maculatus* روی نخود در شرایط آزمایشگاهی (۳۰ °C، ۶۰٪ رطوبت نسبی) بود. پودر گیاه (*V. sinuatum*) باعث کاهش باروری زادآمدی، ظهور حشرات کامل از بذور و کاهش وزن دانه‌های نخود شد که میزان کاهش بستگی به غلظت تیمار داشت (۰/۱، ۰/۰۵ و ۰/۰۲۵ گرم/میلی‌لیتر). این ترکیب باعث کاهش تعداد تخم‌های گذاشته شده توسط آفت، در غلظت اول تا میزان ۱۳۶ تخم، کاهش تفریخ تخم تا ۵۲/۲۲٪ و ظهور حشرات کامل تا ۳۴/۰۳٪ گردید. درصد وزن دانه‌ها در همه غلظت‌های مورد مطالعه کاهش یافت ولی در غلظت‌های دوم (۵٪) و سوم (۲۵٪) در مقایسه با شاهد تفاوت معنی‌دار نداشت. کمترین کاهش وزن دانه‌ها در غلظت اول (۰/۰۱) به ثبت رسید (۴/۷٪).

براساس این تحقیق گیاه *Verbascum sinuatum* تأثیر بالقوه معنی‌داری در کاهش تعداد تخم‌های گذاشته شده و کاهش وزن دانه در اثر تغذیه سوسک نخود داشت.

واژگان کلیدی: کاهش وزن دانه، ظهور حشرات کامل، باروری، جفت‌گیری (زادآوری)، *Verbascum sinuatum*