The effectiveness of some plant powders on the egg lying and development of the cowpea beetle *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae) in stored chickpeas

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Abstract

There is a growing interest in the use of natural products derived from plants as an alternative to conventional insecticides for insects' control. This is due to the fact that they contain a range of bioactive chemicals, many of which are effective with little or no harmful effect to humans or non targeted organisms and the environment. Therefore, this study was conducted to assess the bio-efficacies of four plant powders on the development of the cowpea beetle, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae) fed on chickpeas (*Cicer arietinum* L.). The plant powders used were; fruit of black pepper *Piper nigrum* L., rhizomes of ginger *Zingiber offiicinale*, leaf powders of rosemary *Rosmarinus officinalis* and retem *Retama raetam* for the following concentrations 0.0, 1, 2, 3 and 4% w/w to evaluate their oviposition inhibition, reproduction and F1 adult deterrence of *C. maculatus*. The results showed that a maximum percentage of oviposition deterrent (%OD) (92.90%) was observed with powder of black pepper *P. nigrum*, followed by ginger *Z. offiicinale* (77.40%), retem *R. raetam* (76.80%) and rosemary *R. officinalis* (63.10%) at concentration of 4% w/w. Also, adult emergence reduction was highest from black pepper *P. nigrum* (74.32%), followed by *R. raetam* (62.49%), *Z. offiicinale* (60.81%) and *R. officinalis* (57.15%). The results showed there was a significant oviposition deterrence and inhibition of F1 progeny emergence of *C. maculatus* in treated chickpeas. This study therefore, suggest the need for more research for plant derived insecticides that will control the cowpea weevil *C. maculatus* in stored pulses.

Key words: *Callosobruchus maculatus*, plant powders, chickpeas, oviposition deterrence, adult emergence

Introduction

Chickpeas, beans, lentils, peas, broad beans, and cowpea are important pulses around the world for human nutrition. They are low in fat and high in carbohydrate and have a nutritious feature [1, 2]. However, they suffer heavy qualitative and quantitative losses from the attack of *Callosobruchus maculatus* during storage [3]. The adult beetles do not require food or water and spend their limited lifespan in mating and laying eggs on the seeds which later hatch and penetrate into the seed cotyledons [4, 5], where the larvae and pupae develop and could completely damage seed viability and nutritional quality [6]. The adults emerge out leaving behind holed seeds. They can mate and oviposit within hours after eclosion [7]. Infestation begins in the field, but most of the damage is done during storage and may cause total loss within three months [8-10].
Generally, management of pests in stored products is done through fumigation [11], and controlled by synthetic insecticides [12]. Although chemical control is a limited choice due to the strict guidelines for the safe use of insecticides on / near stored food, the use of pesticides prevents some

losses in grain during storage. However, the chemicals should have low toxicity to humans, minimum residue levels and they should not affect the quality of food products [13, 14].
Currently, there is growing interest in the use of natural products derived from plants as an alternative to conventional insecticides for insects' control [15, 16]. Several studies have been conducted on the effects of plants with insecticidal potential to control the cowpea beetle *C. maculatus* [17-20], and different types of aromatic plant preparations such as powders, solvent extracts, essential oils and whole plants are being investigated for their insecticidal activity including their effect as repellents, anti-feedants and insect growth regulators [21-24]. Thus, the use of plant products may offer a sustainable, environmentally friendly and safer alternative to synthetic insecticides.
The cowpea beetle *C. maculatus* has economical importance in stored pulses in Libya. The present study was conducted to assess the efficacy of four plant powders on the oviposition and F1 adult deterrence of *C. maculatus* on stored chickpeas. These were; fruit of black pepper *Piper nigrum* L., rhizomes of ginger *Zingiber offiicinale* Roscoe, leaf powders of rosemary *Rosmarinus officinalis* L. and retem *Retama raetam* (Forssk.).

Materials and methods

Insect rearing

Adults of *C. maculatus* used to establish the culture were obtained from already existing culture in the entomology laboratory of the Plant Protection Department, Faculty of Agriculture, Tripoli University, Tripoli/ Libya. They were subsequently reared on whole, undamaged, and disinfested chickpeas (*Cicer arietinum* L.) in 2 litre glass jars. The substrate jars were infested with 50 pairs unsexed adults, which were allowed to feed and lay eggs for seven to ten days, after which they were separated and discarded. The cultures were incubated at 28°C ± 2°C and 60% ± 5% relative humidity (r.h.) and darkness. Newly emerged adults of 1 - 2 days old were then used for the experiment.

Preparation of plant material powders:

The botanicals used in this study were fruit of black pepper *P. nigrum*, rhizomes of ginger *Z. offiicinale*, leaf powders of rosemary *R. officinalis* and retem *R. raetam*. The fresh leaves of *R. raetam* and *R. officinalis* used for this study were collected fresh from farms in Tarhuna city, whilst, the fruit of black pepper *P. nigrum* and rhizomes of ginger *Z. offiicinale* were obtained from a local market in Tripoli. These plant materials were dried in the laboratory at room temperature (25-28°C) until they became completely dry, and then ground into very fine powder using an electric blender. The powder was then used immediately for the experiment.

Effect of plant powders on adult *C. maculatus* oviposition:

Five pairs of 1-2 days old male and female adults of *C. maculatus* were added in glass jars (100 ml) containing 20 g of chickpeas. Adult sexes can be distinguished by means of readily observed morphological differences. Females have dark stripes on each side of the posterior dorsal abdomen that are not found in males (25). Quantities of 0.2, 0.4, 0.6 and 0.8 g powder of each of the plant materials: *S. rosmarinus*, *R. raetam*, *P. nigrum* and *Z. offiicinale* were added respectively to each jar to give 1.0, 2.0, 3.0 and 4.0% w/w. The chickpeas in the control contained no plant powders (0.0% w/w). The jars containing the insects were sealed and left for 5 days for mating and oviposition. Each jar was then covered with muslin cloth and rubber to prevent the contamination and escape of beetles. Each treatment and control was replicated four times. Temperature and relative humidity (r.h.) ranges between 28°C ± 2°C and 60% ± 5% respectively. Five days after treatment with plant powders, all dead and live adults of *C. maculatus* were sieved out from each jar and ovipositions were noted before returning the chickpeas to their jars. To determine the oviposition, ten chickpeas were selected randomly from each jar and examined with a dissecting microscope (Zeiss, Germany). The eggs were counted before returning them to the same jar. The number of eggs laid on treated chickpeas (Ts) and control chickpeas (Cs) were recorded and the percentage of oviposition deterrence (% OD) was calculated by the following formulae given by Singh and Jakhmola (2011) [26].

%OD = Cs-Ts / Cs × 100

Ts = number of eggs laid on treated chickpeas; **Cs** = number of eggs laid on control chickpeas.

Jars containing treated and untreated chickpeas were kept in the same laboratory conditions. A month later, progeny emergence (F1) was recorded until there was no emergence for three consecutive days. The percentage reduction in F1 adult emergence (% RA) was calculated by the following formulae given by Singh and Jakhmola [2011] (26).

% RA= Ac-At / Ac × 100

Ac = number of F1 adults emerged from the control chickpeas; **At** = number of F1 adults emerged from the treated chickpeas.

Statistical analysis:

Mean number of eggs laid on treated and control chickpeas and F1 adult emergence were calculated using the above formula. The data obtained from the experiments were subjected to analysis of variance (ANOVA) (GENSTAT DISCOVERY EDITION 4) Completely Randomized Design (CRD) and mean Separation Test = Least Significant Difference (LSD).

Results:

The effect of *P. nigrum*, *Z. offiicinale*, *R. raetam* and *R. officinalis* powders on oviposition of *C. maculates*

All plant powders used effectively reduced oviposition of *C. maculatus* (Figure 1). The number of eggs laid by *C. maculatus* on treated chickpeas was significantly lower (P>0.05) than the number of eggs laid on the control. *P. nigrum* deterrence oviposition was the highest especially at 3% and 4% w/w concentrations, followed by *Z. officinale* (Figure 1). The maximum percentage of oviposition deterrence (%OD) (92.90%) was observed with *P. nigrum*, followed by *Z. offiicinale* (77.40%), *R. raetam* (76.80%) and *R. officinalis* (63.10%) at 4% w/w concentration (Figure 2). However, there was no significant difference (P<0.05) in the mean number of eggs laid on the treated chickpeas with *Z. offiicinale*, *R. raetam* and *R. officinalis* powders (Figure 2).

The effect of *P. nigrum*, *Z. offiicinale*, *R. raetam* and *R. officinalis* powders on F1 adult deterrence of *C. maculates*

The emergence of F1 progeny was significantly affected by the plant powders at all concentrations compared with the control. It was found that all different concentrations reduced significantly the level of adult *C. maculates* that emerged. The number of *C. maculates* progeny emerged in the control was significantly higher (37) than in treated chickpeas (one adult for *P. nigrum*, 4.75 adults for *Z. offiicinale*, 3.50 adults for *R. raetam* and 8.00 adults for *R. officinalis* at 4% w/w concentration (Figure 3). Considering the results had been seen with oviposition, the fewest progeny was produced on the *P. nigrum* treatment, which was significantly lower than the other plant powders at all concentrations. Generally, the higher the concentration of the powder used, the lower the number of insects that emerged (Figure 3).

Figure 4 presents the reduction rate of F1 adult emergence (% RA) on chickpeas with different plant powders at different concentrations. The emergence of F1 progeny was significantly affected by plant powders compared with the control (P>0.05). Overall, the highest percentage of adult emergence reduction was found in *P. nigrum* (74.32%), followed by *R. raetam* (62.49%), *Z. offiicinale* (60.81%), *R. raetam* and *R. officinalis* (57.15%).

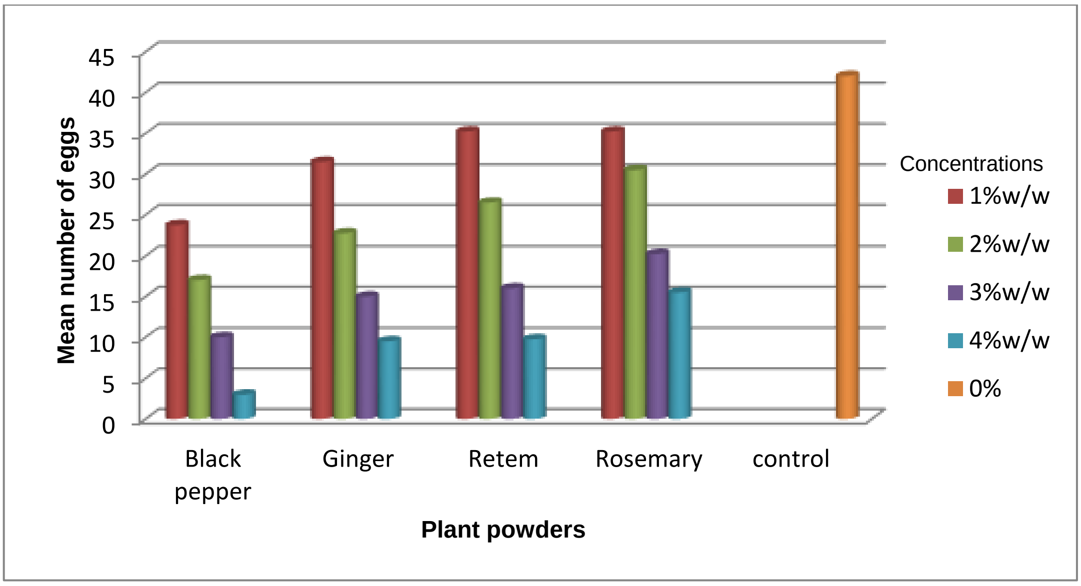

Figure 1. Effect of some plant powders on oviposition of *Callosobruchus maculates*.

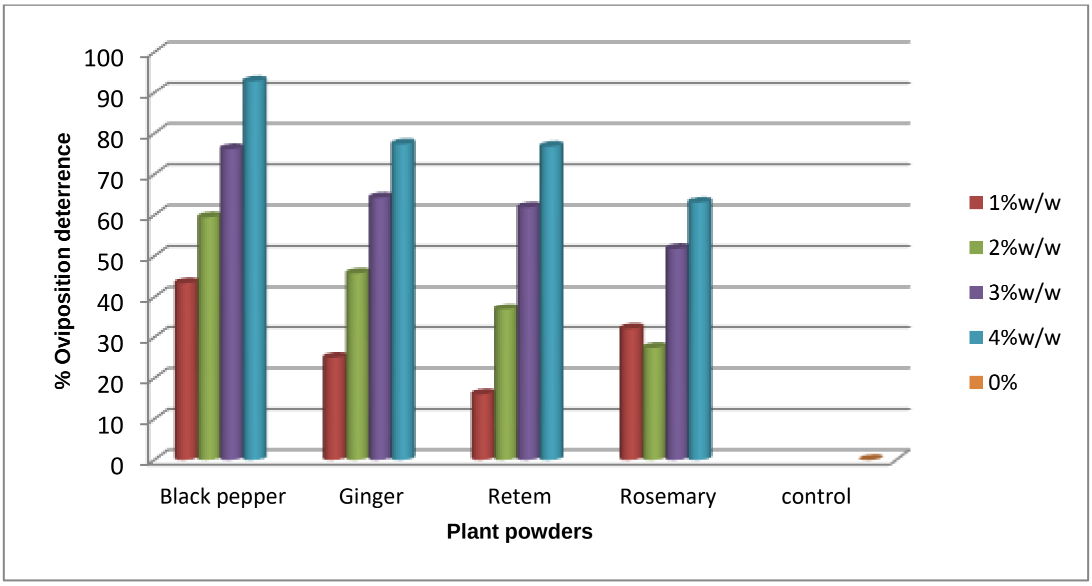

Figure 2. Effect of some plant powders on the percentage of eggs reduction at different concentrations.

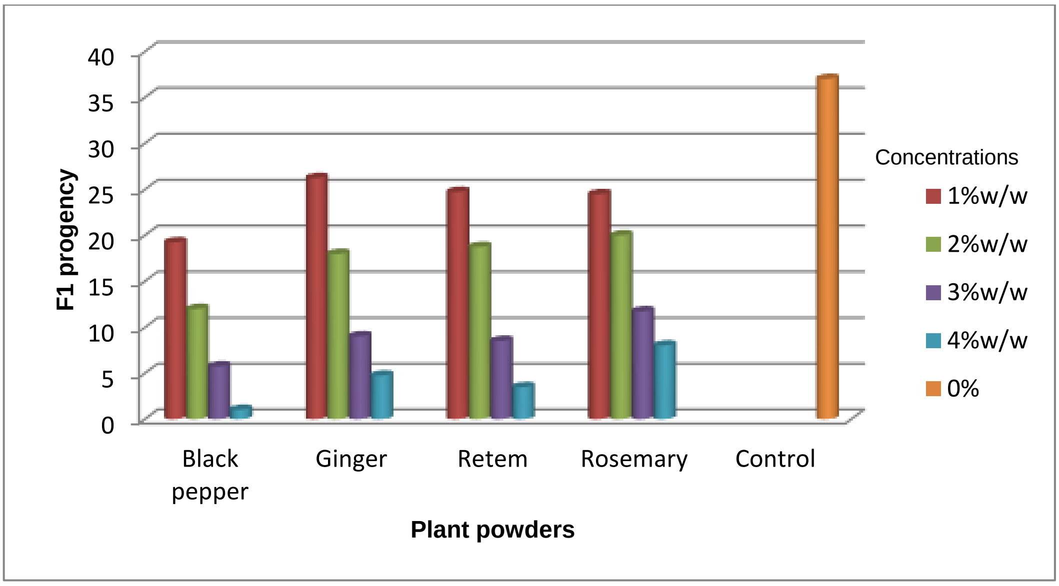

Figure 3. Effect of some plant powders on adult emergence of *Callosobruchus maculates*.

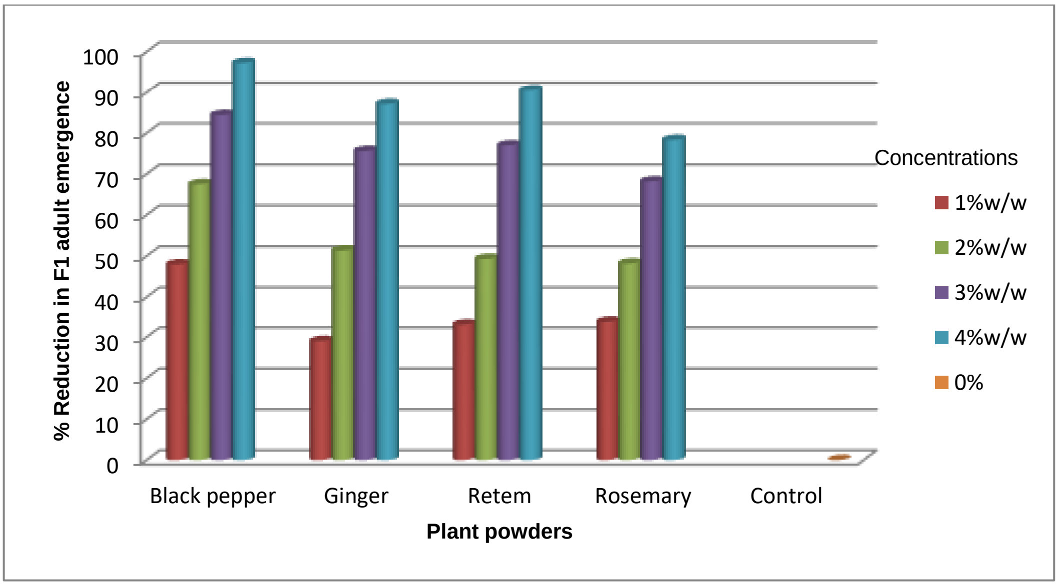

Figure 4. Effect of some plant powders on the percentage of reduction F1 adult emergence at different concentrations.

Discussion:

Previous studies reported that the use of different plant parts such as: leaf, bark, seed powder, or oil extracts as mixture to the stored grains have resulted into reduced rates of grain damage and insect oviposition as well as suppression of adult emergence in different stored grain insect pests [27- 29]. Oviposition deterrent and adult emergence reduction observed in all treated chickpeas with plant powders could be a result of high adult mortality of *C. maculatus*. Some previous studies have reported that the cloves and black peppers treated *Callosobruchus* spp. weevils on pulse grains produced less number of adults [30]. The reason for that is probably the affect of components of both spices on the physiological behaviour of *Callosobruchus* spp. weevils, such as adult mortality, ovipositional activity and growth inhibition [31]. Sadeghi *et al.* (2006) who tested plant lectins for inhibitory activity on *C. maculatus* for oviposition found that coating of chickpeas caused a significant reduction in egg laying. It is possible that large numbers of *C. maculatus* adults physically removed some of the plant powder layer when exploring the grains and this may have resulted in repellent effect of the plant materials [32]. Musenga *et al*. (2007) reported that the black pepper *P. nigrum* contains the following bioactive components; essential oils, piperine, eugenol, the enzyme lipase, and minerals [33]. Okonkwo and Okoye (1996) reported that the bioactive agents in some members of Piperaceae are piperine and chavicine and these are highly insecticidal to various stored pests [34]. This can explain the reason why *P. nigrum* was significantly better than the other plant powders at evoking insecticidal activity.

The ginger *Z. offiicinale* rhizom powder also reduced significantly the production and inhibited F1 progeny emergence of *C. maculatus*. According to Grøntved *et al*. (1988), *Z. officinale* has alpha-zingiberine as the major phytochemical which is believed to be toxic [35]. The toxicity of ginger in the present study is somewhat confirmed by the report of Bandara *et al*. (2006) that the ginger species *Z. purpureum*, was ovicidal against *C. maculatus* [36]. It has been reported that *Z. offiicinale* is toxic to storage insect pests [37]. Also, the results obtained in this study were in agreement with the findings of Ali and Mohammed (2013) who reported that *Z. officinale* proved to be the most effective of the six test plants materials against the larvae of *Tribolium confusum* [38].

R. raetam and *R. officinalis* powders caused significantly high mortality rate of adults and reduction in both, oviposition and adult’s emergence. Benelli *et al*. (2012) reported that *R. officinalis* essential oils caused mortality rate higher than 70% of *Ceratitis capitata* at 24 h [39]. The longevity of *C. maculatus* adults when in contact with different concentrations of rosemary’s essential oils is short compared to the untreated weevils and rosemary’s essential oils proved to be toxic to the cowpea weevils [40]. The effectiveness of *R. raetam* and *R. officinalis* powders in causing mortality, reduced oviposition and adult’s emergence could be attributed to the presence of some chemical compounds in their leaves. However, the results showed that the increase in concentrations is directly proportional with the mortality rate and inversely proportional with oviposition and percentage reduction in F1 adult emergence this is consistent with other studies [41, 42].

Conclusion:

Based on these results, it can be concluded that all the plant powders effectively reduced oviposition of *C. maculatus*. The results showed there was a significant oviposition deterrence and inhibition of F1 progeny emergence in treated chickpeas especially at 3% and 4% w/w concentrations. Among the plant powders tested, black pepper *P. nigrum* which showed best results as insecticidal product on reduction in stored chickpeas damage caused by *C. maculatus*. Ginger *Z. officinale* rhizomes and retem *R. raetam* leaves as well as *R. officinalis* leaves powders were found to be effective to some degree in reducing the ovipositional preferences and increasing the inhibition rates. Significantly fewer F1 adults emerged from chickpeas treated with plant powders. Thus, the results of this study strongly suggest the need for more research for plant derived insecticides that will control the cowpea weevil *C. maculatus* in stored pulses.

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تأثير بعض المساحيق النباتية على معدل وضع البيض و تطور حشرة خنفساء اللوبياء (Coleoptera: Chrysomelidae) *Callsobruchus maculates* Fab.

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قسم وقاية النبات، كلية الزراعة، جامعة طرابلس، طرابلس، ليبيا

الملخص/

هناك إهتمام متزايداً من الباحثين بالتفكير و البحث عن وسائل بديلة للمبيدات الكيميائية لمكافحة الحشرات، و منها إستعمال المنتجات الطبيعية للنباتات و ذلك لإحتوائها على مواد فعالة ضد الحشرات الضارة، و غير ضارة أو ضررها قليل على الإنسان و الحيوان و البيئة. و عليه، تم أجرى الدراسة الحالية لدراسة الفعالية الحيوية لأربعة مساحيق نباتية (الفلفل الأسود *Piper nigrum*، الزنجبيل *Zingiber offiicinale*، الرتم *Retama raetam* و إكليل الجبل *Rosmarinus officinalis*) ضد حشرة خنفساء اللوبيا (.Callosobruchus maculatus (Fab في الحمص، عند التراكيز 0.0، 1، 2، 3 و 4 % وزن/وزن. قيم تأثير هذه المساحيق النباتية كمواد مانعة لوضع البيض، عدد البالغات الخارجة، تثبيط ظهور أفراد جديدة للجيل الأول. أعلى نسبة سجلت في خفض معدل البيض (92.90%) شوهدت عند معاملة الفلفل الأسود *P. nigrum* ، يليه الزنجبيل *Z. offiicinale* بنسبة (77.40%) ، ثم الرتم *R. raetam* (76.80%)، و الإكليل *R. officinalis* (63.10%) عند تركيز 4% (وزن/وزن). أيضاً أعلى نسبة لنقصان افراد الجيل الاول سجلت في مسحوق الفلفل الأسود (74.32%)، يليه الرتم (62.49%)، ثم الزنجبيل (60.81%) و إكليل الجبل (57.15%). أوضحت النتائج أن هناك تأثير معنوي على تثبيط وضع البيض، و منع ظهور أفراد الجيل الأول (F1) لحشرة *C. maculatus* في الحمص المعامل. و بناء عليه هذه الدراسة تشدد على الحاجة إلى إجراء المزيد من البحث بإستخدام المنتجات الطبيعية للنباتات لمكافحة خنفساء اللوبياء *C. maculatus* في مخازن البقوليات.

الكلمات المفتاحية: *Callosobruchus maculatus* ، المساحيق النباتية، الحمص، منع وضع البيض، خروج البالغات.