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Original Research Article

Efficacy of Aromatic and Medicinal Plant Powders against *Callosobruchus maculatus* F. (Chrysomelidae: Bruchinae)

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ABSTRACT

The cowpea bruchid *Callossobruchus maculatus* Fab. (Coleoptera: Chrysomelidae: Bruchinae) is the major pest of chickpea in storage. Thus, the present study aimed to evaluate the insecticidal properties of powders of 8 Moroccan aromatic plants on biological parameters of *C. maculatus*. The toxicity of the powders was assessed by measuring the parameters of the life cycle in a situation of non-choice under laboratory conditions (25 ± 1°C, 70–75% HR and a photoperiod of 14h (light) / 10h (darkness). The powder of *Mentha pulegium* has completely wiped out the population of the *Callosobruchus maculatus* (% IR=100%) 2%, 1% and 0.5% W/w. Similarly, *Mentha pulegium* powder retained seed weights, which remained significantly different (P < 0.01) from the control weight. Also, *Origanum compactum, Mentha rotundifolia* and *Inula viscosa* have significantly reduced (P < 0.01) the population of bruchids, the percentage reduction reached (% IR= 97.5, % IR = 89.32, and % IR = 27, 38% by the highest 2%). The other plants show no significant difference from the control. The results therefore suggest that *Mentha pulegium* powder has an insecticide potential similar to those of conventional insecticides and could be an alternative method against infestations and damage caused by *C. maculatus* in stored products.

Keywords: *Callosobruchus maculatus*, Plant, Biological control, Morocco.

Introduction

In Morocco, legumes have always played an important role in different aspects, namely food security and nutrition, agriculture and environment, and socio-economics. Indeed, in addition to ensuring the balance of human and animal nutrition, food legumes contribute strongly to regenerate soil fertility and ensure the sustainability of production systems. Economically and socially, food legumes are grown by smallholder farmers, which guarantee workdays and income for rural families.[1,2]

Callosobruchus maculatus is a cosmopolitan pest of food legume seeds in many parts of Africa where a large proportion of the rural population lives on vegetable crops.[3] This pest causes significant damage and induces losses in quality and quantity of stored legumes, especially a deterioration of its ability of germination.[4-6] Morocco is a biogeographical unit whose characteristics shape a completely original natural setting. Through its geographical contrasts, it offers a varied range of bioclimates allowing the installation of a rich flora with marked endemism.[7,8] Alongside this particularly promising natural context, Morocco has an ancestral know-how that has been preserved over the centuries.[9] Farmers have been using plants for a long time, some parts of which, such as leaves, flowers, fruits, etc., have insect-repellent and/or insecticide potential.[10,11]

Natural compounds of plant origin are biodegradable, often of low toxicity to mammals, and represent a low hazard to the environment if used in small quantities.

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Recent research has focused on alternatives to chemicals for pest control in developing countries. Various studies have demonstrated the efficacy of several plants as protective agents.[12–15]

Piper nigrum powder (Piperaceae) has caused a significant reduction in the bruchids population in cowpea stocks,[16] and similar results were obtained with Neem powder (*Azadirachta indica* A. Juss).[17] Essential oils of *Ocimum gratissimum* L, and *Ocimum basilicum* L. have been successfully used against C. *maculatus*.[18] Some post-harvest storage methods may also be useful to reducing cowpea bruch infestations as part of an integrated pest management approach.[19,20] The objective of this study is to evaluate the effect of the application of several Moroccan plants in powder form on the longevity, fecundity, emergence rate and duration of the larval phase of *C. maculatus.*

Materials and Methods

Chickpea used

Chickpea (*Cicer arietinum*) seeds were cleaned and frozen at -18°C for 1 week, then dried in an oven at 60°C for 1 week to ensure the absence of viable insects inside the seeds without the use of chemicals.[21] Seeds were stored in airtight plastic jars at room temperature before use.

Mass rearing of insects

The species studied is *Callosobruchus maculatus*, it was obtained from a sample of chickpea from a stock in the city of Fez. It is maintained by mass rearing at laboratory level in 1.5 liter glass jars in the presence of chickpea seeds (*Cicer arietinum*). The jars are kept in a climatic chamber at a temperature of 25 ± 1°C, a relative humidity of 70 ± 5% and a photoperiod of 14h (light) / 10h (dark) for several successive generations.

Collection and preparation of plant material

The identity of the plants used is presented in Table 1. Samples plants are collected from their natural ecosystem dried in the shade, the powder were obtained by grinding with an electric mill.

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Table 1: List of plants tested for their bio-insecticidal effects against *C. maculatus*

Scientific name of plants	Family	Local name	Plant type	Harvesting site	Harvest period
Origanum compactum	Lamiaceae	zaatar	spontaneous	Taounate	June 2019
Inula viscosa	Asteraceae	magramane / tirihla	spontaneous	Fez	June 2019
Rosmarinus officinalis	Lamiaceae	azir	spontaneous	Boulemane	April 2019
Calamintha officinalis	Lamiaceae	minta	spontaneous	Taounate	April 2019
Myrtus communis	Lamiaceae	Ariihan	spontaneous	Taounate	April 2019
Mentha rotundifolia	Lamiaceae	Marseta	spontaneous	Taounate	April 2019
Artemisia herba alba	Asteraceae	chih	spontaneous	Boulemane	July 2019
Mentha pulegium	Lamiaceae	fliyou	spontaneous	Taounate	April 2019

The powders were sifted (mesh size: 1mm2) to get fine powders.[16] The choice of the "test concentration" was made with reference to the study by Lale[22] who reported that the concentration of plant powder should not be higher than 2.0% w/w to be economically justified.

Biological tests

All the tests were carried out under the same conditions. Untreated seeds were used as controls for each experiment and seeds treated with a commercial chemical insecticide (permethrin) were used as a positive control. For each test, 25.0 g of seeds and 0.50 g of plant powder (i.e., 2% w/w) were carefully agitated in a 9-cm Petri plate for two minutes. Five males and five females were released into each of three replicate plates for each plant species 6.23. Plants that showed remarkable biocidal effect against the insect C. *maculatus* were selected and tested at several doses of 2%, 1%, 0.5%, and 0.25%.

Daily observation was carried out for 9 days and mortality data on adult bruchids were collected and recorded every 24 hours. The percentage of mortality was calculated using the standard formula:

$$\frac{\text{Number of dead individuals x 100}}{\text{Total number of individuals of C.}\mathit{maculautus}}$$

The adult survival time in days was recorded and the total number of eggs was counted. Petri dishes were incubated under standard conditions to allow the eggs to develop into adults. Emerging F1 adults were counted, sexed according to the method of Raina [23] and removed from the seeds each day, and the seeds were weighed. Thus, information on the lifetime fecundity of females and the survival of the larval and pupal life of the beetle was collected. Two to five treatment sets (6 to 15 Petri dishes) were tested simultaneously with two sets of six dishes from the two controls.

The percentage reduction in adult emergence or inhibition rate (% IR) was determined by Tapondjou [24] as follows:

$\% IR = (Cn - Tn)\,100/Cn$ or:

Cn is the number of newly emerged insects in the untreated (control) jar.

Tn is the number of insects newly emerged in the treatments

Statistical analysis

The statistical software SPSS for Windows® (version 21.0) was used. The data were subjected to an analysis of unidirectional variance (ANOVA) to determine the difference between the extreme values of the group. Fisher's Least Significant Difference (LSD) test was used to separate significant from non-significant means at α = 0.05.

Results and Discussion

Effect of plant material on the biological parameters of C. maculatus

The effect of plant powders on mortality, female fecundity, and emergence rate of *C. maculatus* is shown in Table 2 and 3.

Mortality rate varied significantly (P < 0.01) with treatment. All powders tested caused varying degrees of insect mortality after 1 day of exposure, reaching 100% mortality in 9 days in most tests. *Mentha pulegium* caused the highest mortality of bruchids at all exposure periods, and this mortality was relatively different from that caused by the rest of the powders. The effect of permethrin (positive control) which caused adult mortality was similar to that obtained with M. pulegium after 1 day of treatment. However, the powder *of Inula viscosa, Origanum compactum, Calamintha officinalis, A. herba-alba Myrtus communis*, and *Rosmarinus officinalis* showed moderate toxicity causing total adult mortality after 6 days. The fecundity of adult females of C. maculatus was significantly different (P < 0.01) according to the treatment. *M. pulegium* showed a total effect on oviposition of 0.33 ± 0.58 eggs/5 females, similar to that of the positive control, followed by *O. compactum* with a mean of 5.66 ± 8.1 eggs/5 females and *M. rotundifolia* with a mean of 24 ± 11.79 eggs/5 females. The other plants showed an average effect, but still significant compared to the untreated control. Concerning the emergence rate, a total absence of emergence was registered in chickpea seeds treated with *M. pulegium* powders and the positive control; it was also significantly lower in the seeds protected with *O. compactum*, *M. rotundifolia* and *I. viscosa* powders, with a mean level of 3 ± 5.97, 12.33 ± 11.37 and 61.67 ± 27.43 respectively. The other powders did not show significant effects on emergence rate. Through optimizing the doses of the plants that showed a very high biocidal effect at 2% (w/w), we were able to determine the optimal dose for toxicity. Figure 2 shows that the plant powders affect the longevity of the adults very significantly, *M. pulegium* caused an average total mortality of 100% at 1 day of exposure for the doses of 2%, 1% and 0.5%, for *O. compactum* and *I. viscosa* the total mortality was recorded after 6 days of exposure for the doses 2% and 1% and *O. compactum* after 9 days of exposure (Figure 2). The mortality decreased with the decrease of the doses. While for all control batches (untreated batches); an average mortality of 93.33% was recorded after 9 days of exposure. The numbers of eggs laid by *C. maculatus* females were also significantly different (P < 0.01) according to the treatment (Figure 3). The optimum dose that caused a total reduction in the number of eggs laid by *C. maculatus* in *M. pulegium* was 0.5% (w/w), followed by *M. rotundifolia* which significantly reduced fecundity by an average of 55 eggs/5 females and finally *O. compactum* and *I. viscosa* with an average fecundity of 105 and 112 eggs/5 females. Thus, the oviposition recorded in the control batches was on average 142 eggs/5 females. Regarding the rate of emergence (Figure 3), no individuals emerged in chickpea lots protected with 0.5% (w/w) *M. pulegium*. Emergence was also significantly low (3 ± 1 individuals) in chickpea seeds protected with 2% (w/w) *O. compactum* and (12.33 ± 1.7) in chickpea seeds protected with 2% (w/w) *M. rotundifolia*. In the control plots, the average number of emerging individuals reached a value of 123 ± 7.55 individuals. The presence of toxic activity against insects is one of the most important values in plants, these insecticidal potentialities can be a real solution for pest control in storage systems to replace or even minimize the application

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of chemical pesticides. In the present study, the plant species tested when applied as contact powders showed adulticidal, ovicidal and larvicidal activity against C. *maculatus*. The toxicity of the powder varies according to the species tested, the dose used and the exposure time; this toxicity is much greater at high doses. M. *pulegium* showed the greatest insecticidal activity against *C. maculatus* at an optimum dose of 0.5% (w/w). Their toxic effect on the insect did not differ significantly from that of the positive control throughout the exposure period. A complete reduction in fecundity and emergence confirms that *M. pulegium* has good bio-insecticidal potential for controlling C. *maculatus* beetle infestations. These results are in agreement with those obtained by Kumar et al[25] who evaluated the effect of species of the *mentha* genus. This study showed an insecticidal activity mainly due to pulegone and menthone, major components of essential oils of M. pulegium[26] which are highly insecticidal against various crop pests. These terpene compounds play a repellent role at low concentrations and a lethal role at high concentrations.[27] Traditionally, plant powders have been mixed with stored seeds in Morocco since ancient times and could be used as a natural, safe and less expensive strategy to protect stored seeds from insect infestation.[28] The strategy used varies from region to another and seems to depend in part on the type and effectiveness of the flora available in the different regions.[29–31]

In a related study, Johnson *et al*.[32] reported that powder from the dry leaves of *M. piperita* at 0.4% (w/w) significantly reduced the fecundity of *C. maculatus* by more than 60% and the rate of emergence by more than 80%. In the same sense, the work of Kumar *et al*.[5] on the genus of *Mentha* reports that the insecticidal properties of the different species of *Mentha* are generally related to its essential oils or plant extracts, which is correlated to their chemical composition. These results are more marked compared to those obtained by other authors who have worked on *C. maculatus* or other insects. *O. compactum*, *M. rotundifolia* and *I. viscosa*, are ranked second with significant biocidal activity achieving a percentage reduction of 97.5%, 89.32% and 27.38% respectively by a 2% w/w dose (Table 2). Our results are consistent with many other studies. Khalfi et al.[33] reported that *O. compactum* showed insecticidal activity against *Rhizopertha dominica* and that this activity increases with increasing dose. Benelli et *al*.,[43] added that the toxicity of *Origanum syriacum* tested against several insect pests is mainly due to its majority compound carvacrol which is the same majority compound in *O. compactum*. The vegetal materials' mechanisms of action are contact and fumigation[34–38]. The plant powders effectively protect the seeds against bruchid infestation and therefore do not present any risk to human health and the environment, unlike conventional insecticides.

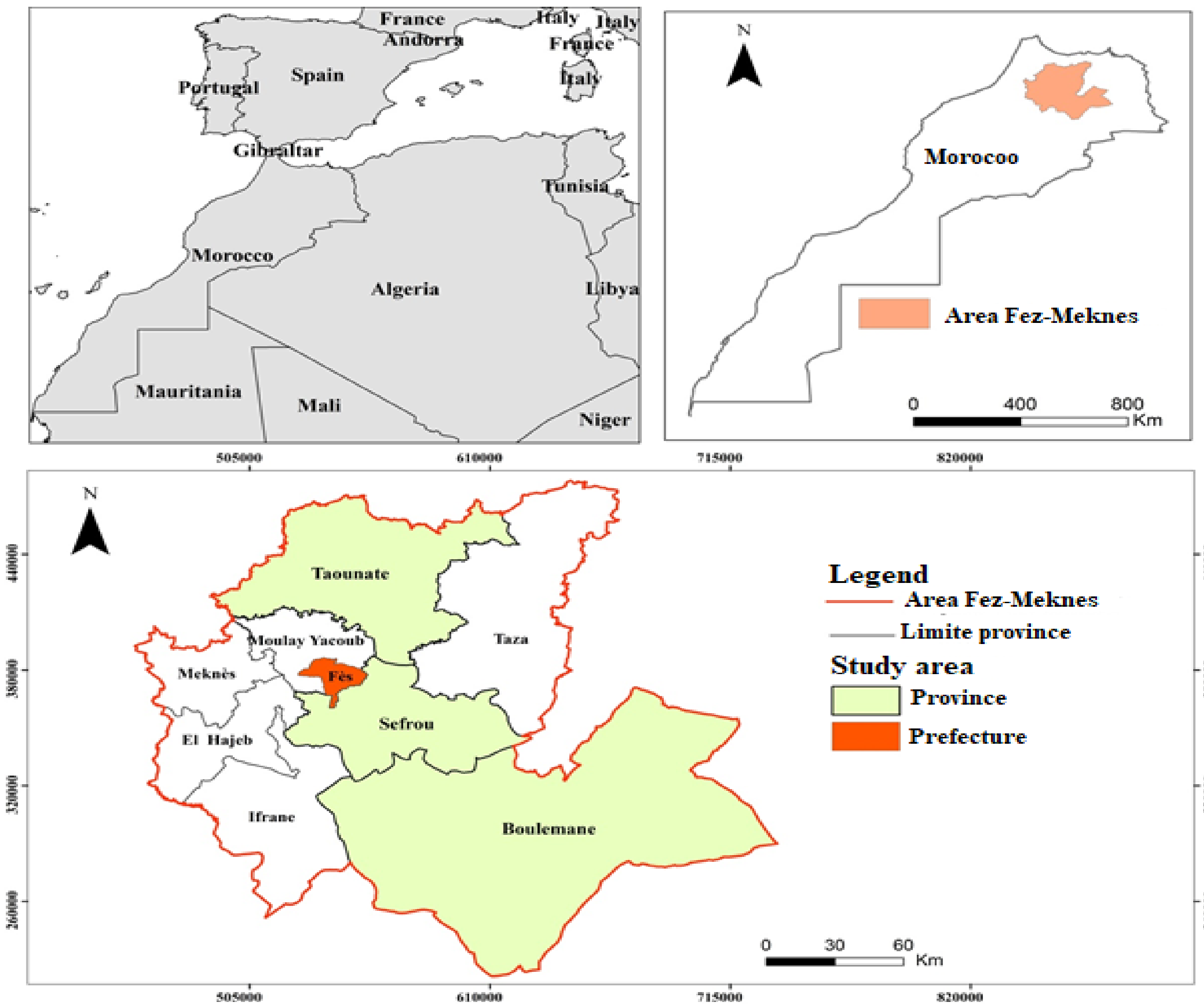

Figure 1: Location of harvesting sites in different study areas

Table 2: Effect of plant powders [2% (w/w)] on the longevity of C. *maculatus* adults

	Mortality (% Mean ± SD)			
	1day	3 days	6 days	9 days
Control	0 ± 0.0[a]	3,33 ± 5.77[a]	56,67 ± 11.55[a]	96,67 ± 5.77[a]
permethrin	100 ± 0.0[e]	100 ± 0.0[k]	100 ± 0.0[b]	100 ± 0.0 [a]
I. viscosa	23,33 ± 15.27[bc]	30 ± 10[bc]	93,33 ± 5.77 [b]	100 ± 0.0 [a]
M. rotundifolia	43,33 ± 15.27[cd]	56,67 ± 5.77[d]	93,33 ± 5.77 [b]	100 ± 0.0 [a]
O. compactum	10 ± 17.32[abc]	26, 67 ± 15.27[bce]	73,33 ± 15.27 [c]	100 ± 0.0 [a]
M.pulegium	100 ± 0.0[e]	100 ± 0.0[k]	100 ± 0.0[b]	100 ± 0.0 [a]
C. officinalis	3,33 ± 5.77[abc]	30 ± 10,0[bce]	73,33 ± 5.77 [c]	100 ± 0.0 [a]
M. communis	0 ± 0.0[ab]	3,33 ± 5.77[ab]	86, 67 ± 5.77 [bc]	100 ± 0.0 [a]
A. herba-alba	23,33 ± 5.77 [bcd]	40 ± 17.32[bdeg]	100 ± 0.0 [b]	100 ± 0.0 [a]
R. officinalis	10 ± 17.32[abc]	16, 67 ± 11.55[bcei]	50 ± 0.0 [a]	100 ± 0.0 [a]

Means in a column followed by the same letter are not significantly different and means followed by different letters are significant at P < 0.01 in the LSD test (α = 0.05).

Table 3: Effect of plant powders [2% (w/w)] on the biological parameters of *C. maculates* % IR: percentage of population reduction

	Number of eggs (Mean ± SD)	**Emergence (Mean ± SD)**	**Mean life cycle duration (Days)**	**% IR**
Control	132 ± 12[a]	114 ± 6.57[a]	29.33 ± 1.15[a]	-
permethrin	0 ± 0.0 [c]	0 ± 0.0[c]	-	100
I. viscosa	86.33 ± 16.56 [ab]	61.67 ± 27.43 [b]	42.67 ± 4.16[bc]	27.38
M. rotundifolia	24 ± 11.79[c]	12.33 ± 11.37[c]	46.67 ± 4.62[cd]	89.32
O. compactum	5.66 ± 8.1 [c]	3 ± 5.97[c]	53 ± 0.00[e]	97.5
M.pulegium	0.33 ± 0.58 [c]	0 ± 0.0[c]	-	100
C. officinalis	82.33 ± 33.31[b]	68.67 ± 26.76 [b]	43 ± 3.61[cdf]	39.24
M. communis	93.67 ± 30.53 [ab]	83.67 ± 32.01 [ab]	40.67 ± 2.52[bcfgh]	26.85
A. herba-alba	88.33 ± 61.85 [ab]	79.33 ± 23.03[b]	39.33 ± 2.31[bcfgh]	30.37
R. officinalis	117 ± 26,46 [ab]	102 ± 27.87 [a]	41 ± 1.73[bcfgh]	11.09

Means in a column followed by the same letter are not significantly different and means followed by different letters are significant at P < 0.01 in the LSD test (α = 0.05).

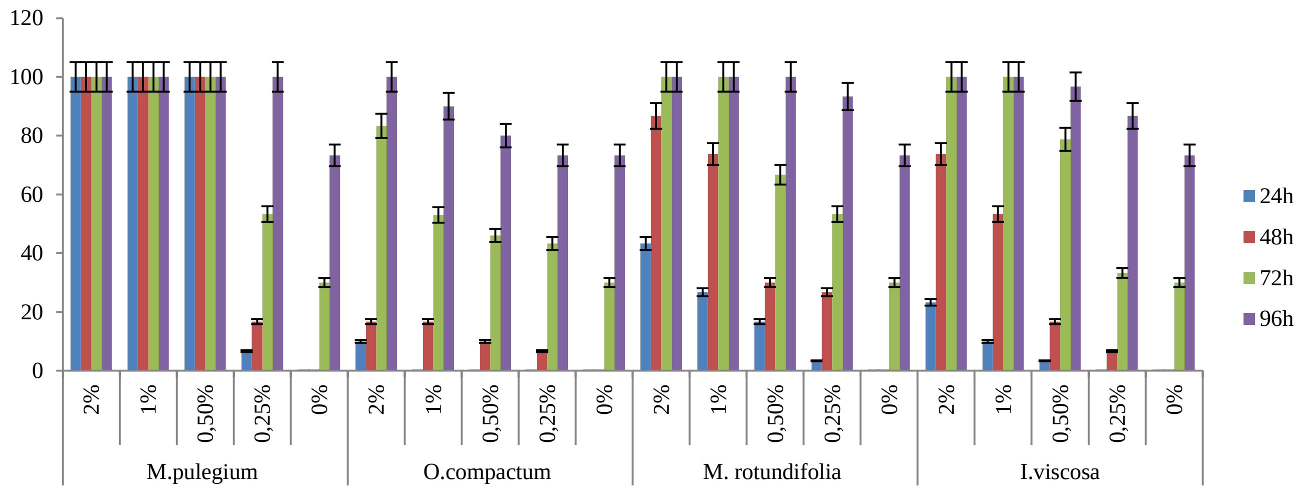

Figure 2: Effect of *M.pulegium, O. compactum, M. rotundifolia and I. viscosa* on the longevity of *C. maculatus* adults

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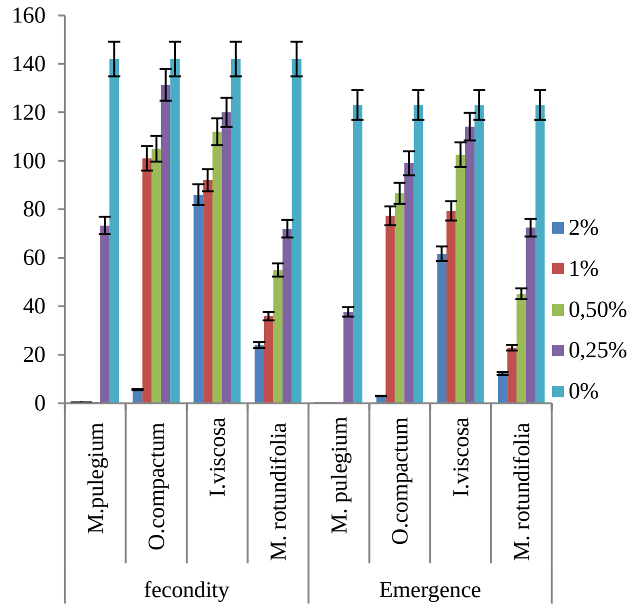

Figure 3: Effect of *M.pulegium, O. compactum, M. rotundifolia and I. viscosa* on the longevity of *C. maculatus* adults

Conclusion

The plant powders tested and in particular that of *mentha pulegium* have great potential to protect chickpea seeds from damage by *C. maculatus.* Farmers could use these locally available plant powders to preserve their chickpea seeds from *C. maculatus* attacks at stores. Given the well-documented difficulties associated with the design of synthetic chemicals, in addition to the hazardous effects, the costs associated with the use of synthetic insecticides, and the pest resistance problems of these chemicals, these traditional control methods may play a wiser role in the future of IPM programs. Although test plants are used in folk medicine and also in many pharmaceutical preparations and are probably relatively safe, experiments should be conducted to assess their phyto-toxicity on crops. Studies should be expanded to evaluate their mammalian safety, insecticidal mode of action and formulations for use in seed stores.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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