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SCREENING OF VEGETABLE POWDERS USED AS A BIO-INSECTICIDE AGAINST *CALLOSOBRUCHUS MACULATUS* F. (CHRYSOMELIDAE: BRUCHINAE)

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ABSTRACT

The beetle *Callosobruchus maculatus* (F. 1775) (Chrysomelidae: Bruchinae) is a destructive pest of stored chickpea seeds. Bio-pesticides are pesticides of animal, plant and bacterial origin. Plant products are among the best known substances tested against insects. These products have an insecticidal and repellent effect on insects and can also affect certain biological parameters such as fecundity, life span and reproduction.

In search of plant bio-pesticides to control *Callosobruchus maculatus* main pest of stored chickpea seeds, 18 plants traditionally used in Morocco to control insect pests have been tested in the laboratory, for their toxic effects against this beetle. A conventional synthetic insecticide was included as a positive control, while untreated seed was used as a control. The toxicity of the powders was assessed by measuring the parameters of the life cycle in a situation of non-choice maintained at a climatic chamber with a temperature of 25 ±1 degrees Celsius, a relative humidity of 75% and a photoperiod of 14h (light) / 10h (darkness) for several successive generations. The powders of *Mentha pulegium* and *Syzygium aromaticum* have completely wiped out the population of the bruches (% IR=100%) 2%, 1% and 0.5% p/p. Similarly, the powders of the two plants retained the weights of the seeds, which remain significantly different (P < 0.01) at the weight of the control. Also *Origanum compactum*, *Mentha officinalis*, *Allium sativum*, *Zingiber officinale*, *Urtica doica* and *Calamintha officinalis* have significantly reduced (P < - 0.01) the population of bruches, the percentage reduction reached (97.5, 89.32, 72.84, 50.3, 46.52 and 39.24% by the highest 2%). The other plants show no significant difference from the control.

The results therefore suggest that *Syzygium aromaticum* powder and *Mentha pulegium* have an insecticide potential similar to those of conventional insecticides and could be a biotechnological alternative against *C. maculatus* infestations and damage in stored products.

Keywords: *Callosobruchus maculatus*; plant; biological control; Morocco

INTRODUCTION

Insect pests are an important problem with stored seeds (Mendoza *et al.* 2004). They can cause significant losses by reducing the quality and quantity of stored products (Allali *et al.* 2020b). In order to control them without using synthetic pesticides, it is interesting to investigate other safer and more effective alternatives in plant protection. Actually, the plants can provide alternative insect control solutions because they are a very rich source of bioactive molecules (Lale 1992; Isman 1995; Qin *et al.* 2010). Many studies have highlighted the bio-insecticidal effects of plants on phytophagous pests (Bruchidae) (Boeke *et al.* 2004; Pourya *et al.* 2018; Neto *et al.* 2019; Ahmad *et al.* 2019; Allali *et al.* 2020c).

In order to preserve their nutritional value and make them fit for human consumption, chickpea seeds must be stored in a suitable place after harvest. However, during the storage period, chickpeas are often infested by insect pests, such as the main pest *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae) (Sharma and Thakur 2014; Boeke *et al.* 2004; Loganathan

et al. 2011; Hamdi *et al.*, 2017). The neonate larvae entered into the seeds and produced important damage, such as loss of seed weight and reductions in germination power and nutritional value (Bamaiyi *et al.* 2006; Hamdi *et al.* 2017; Allali *et al.* 2020a).

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 (Fougrach *et al.* 2007; Khabbach *et al.* 2012). Alongside this particularly promising natural context, Morocco has an ancestral know-how that has been preserved over the centuries (Mehdioui and Kahouadji 2007). 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.

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. 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 (Pannuti *et al.* 2012; Tamgno and Tinkeu 2014; Nenaah 2014; Diouf *et al.* 2016). *Piper nigrum* powder (Piperaceae) has caused a significant reduction in the bruchids population in cowpea stocks (Nwosu *et al.* 2018), and similar results were obtained with Neem powder (*Azadirachta indica* A. Juss)(Neto *et al.* 2019). Essential oils of *Ocimum gratissimum* L, and *Ocimum basilicum* L. have been successfully used against *C. maculatus* (Kéita *et al.* 2001). Some post-harvest storage methods may also be useful to reducing cowpea bruch infestations as part of an integrated pest management approach (Singano *et al.* 2019; Adesina *et al.* 2019). The objective of this study is to evaluate the effect of the application of several Moroccan and imported 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 and then dried in an oven at 60°C for 1 week to ensure the absence of viable insects without the use of chemicals (Sulehrie *et al.* 2003). 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 litre 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.

Plant material: The choice of plants used for their bio-insecticidal effect was based on the results of a survey conducted in 2019 in two regions of Morocco, Fez-Méknès and Casablanca-Settat (Allali *et al.* 2020c). The identity of the plants used is presented in Table 1. Samples of Moroccan plants are collected from their natural ecosystem dried in the shade, for the imported plants; they were purchased from spice herbalists in Fez. The plant material was ground individually into powder using a clean mortar and pestle. The powders were sieved (mesh size: 1mm²) to produce fine powders (Nwosu *et al.* 2018). The choice of the «test concentration» was made with reference to the study by Lale (2002) 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 were used as a positive control. For each test, 25.0g seeds and 0.50g plant powder (i.e. 2% w/w) were carefully shaken in a petri dish of 9 cm for two minutes. Five males and five females were released into each of the three repeat plates for each plant

species (Boeke *et al.* 2004; Nwosu *et al.* 2018). Plants that showed a 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:

$$(\text{Number of dead individuals} \times 100) / (\text{Total number of individuals of } C. maculatus)$$

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 (1970) 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 *et al.* 2002) as follows: % IR = (Cn-Tn) 100/Cnor:

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 $\alpha = 0.05$.

RESULTS AND DISCUSSION

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

The effect of plant material on mortality, female fertility and emergence rate of *C. maculatus* is presented in Table 2.

The percentage of mortality significantly varies ($P < 0.01$) depending on the treatment. All test materials caused varying degrees of insect mortality after 24 hours of exposure, reaching 100% mortality within 9 days in most tests. *Mentha pulegium* caused the highest mortality of bruchids at all periods of exposure, and this mortality was relatively different from that caused by the rest of the plant material. The chemical control effect (positive control) that caused the adult mortality was similar to that found with *M. pulegium* after 24 hours of treatment. *Syzygium aromaticum* showed a highly significant biocidal effect compared to the control. However, after 3

Scientificname of plants	Family	Local name	Plant type	Method of application
<i>Capsicum frutescens</i> L.	Solanaceae	soudania / al harra /	Cultivated	Integer part and / or powder
<i>Urtica dioica</i> L.	Urticaceae	Al hariga	spontaneous	Integer part
<i>Origanum compactum</i> Benth.	Lamiaceae	zaatar	spontaneous	Integer part
<i>Allium sativum</i> L.	Liliaceae	touma	Cultivated	powder
<i>Inula viscosa</i> (Ait.) L.	Asteraceae	magramane / tirihla	spontaneous	Integer part
<i>Zingiber officinalis</i>	Zingiberaceae	azanjabile	Spontaneous/ Cultivated	powder
<i>Olea europaea</i> L.	Oléaceae	zitoun	Cultivated	Liquid/ Integer part
<i>Rosmarinus officinalis</i> L.	Lamiaceae	azir	spontaneous	Integer part
<i>Pelargonium graveolens</i> L'Hér.	Geraniaceae	laatrcha	Cultivated	Integer part
<i>Syzygium aromaticum</i> L.	Myrtaceae	quoronfl	spontaneous	Integer part
<i>Calamintha officinalis</i> L.	Lamiaceae	manta	spontaneous	Integer part
<i>Myrtus communis</i>	Lamiaceae	Ariihan	spontaneous	Integer part
<i>Mentha officinalis</i> L.	Lamiaceae	Marseta	spontaneous	Integer part
<i>Eucalyptus camaldulensis</i>	Myrtaceae	calytous	Cultivated	Integer part
<i>Artemisia herba Asso.</i>	Asteraceae	chih	spontaneous	Integer part
<i>Mentha pulegium</i> L.	Lamiaceae	fliyoyou	spontaneous	Integer part
<i>Daphne gnidium</i> L.	Thymelaeaceae	alzaz	spontaneous	Integer part
<i>Nerium oleander</i> L.	Apocynaceae	defla	spontaneous	Integer part

days of exposure, *S. aromaticum* and *M. pulegium* showed the same treatment efficacy against adults. Powders of species of *Eucalyptus camaldulensis*, *Inula viscosa*, *Origanum compactum*, *Capsicum frutescens*, *Calamintha officinalis*, *Pelargonium graveolens*, *Myrtus communis*, *Zingiber officinale*, *Daphne gnidium*, *Olea europaea*, *Nerium oleander* and *Rosmarinus officinalis* showed a non-significant difference. However, the powder of *Mentha officinalis*, *Urtica doica*, *Artemisia herba-alba* and *Allium sativum* showed moderate toxicity causing total adult mortality after 6 days.

The number of eggs laid by adult females of *C. maculatus* was significantly different ($P < 0.01$) depending on the treatment. *S. aromaticum* showed a total effect on oviposition 0 eggs/5 females, similar to that of the positive control, followed by *M. pulegium* with a mean oviposition of 0.33 ± 0.58 eggs/5 females, *O. compactum* with a mean of 5.66 ± 8.1 eggs/5 females and *M. officinalis* with a mean of 24 ± 11.79 eggs/5 females. The other plants showed no significant effect compared to the untreated control with the exception of *A. Sativum* which showed an effect on female fertility to a greater or lesser extent with 50.33 ± 5.51 eggs/5 females.

As for the emergence rate, a total absence of emergence was observed in chickpea seeds treated with powders of *S. Aromaticum*, *M. pulegium* and the positive control, it was also significantly lower in seeds protected with powder of *O. compactum*, *M. officinalis* and *A. sativum* with a mean rate of 3 ± 5.97 , 12.33 ± 11.37 and 30.67 ± 11.68 respectively. The other powders did not show significant effects on the rate of emergence.

Dose optimization: By 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 of

toxicity. The figures 1, 2, 3 and 4 shows that plant powders affect the longevity of adults in a very significant way. *M. pulegium* and *S. aromaticum* caused a mean total mortality of 100% at 1 day exposure for the 2%, 1% and 0.5% doses (Fig 2, 3), for *A. sativum* total mortality was recorded after 6 days of exposure and *O. compactum* after 9 days of exposure to a 2% powder dose (Fig 1, 4).

This mortality decreases with decreasing doses. While for all control batches (untreated batches); an average mortality of 93.33% were recorded after 9 days of exposure. Also the number of eggs laid by *C. maculatus* females is also significantly different ($P < 0.01$) depending on the treatment (Fig 5). The optimum dose that caused a total reduction in the number of eggs laid by *C. maculatus* in *S. aromaticum* and *M. pulegium* was 0.5% (w/w), followed by *O. compactum* which significantly reduced fecundity with an average of 5.67 eggs/5 females and finally *A. sativum* with an average fecundity of 50.33 eggs/5 females, where the optimum dose was 2%. Thus the oviposition registered in the control batches is on average 142 eggs/5 females.

As for the emergence rate (Fig 6), no individuals emerged in the chickpea lots protected with just 0.5% (w/w) powder of *S. aromaticum* and *M. pulegium*. The emergence is also significantly lower (3 ± 1 individuals) in chickpea seeds protected with 2% (w/w) *O. compactum* and (48.67 ± 17.16) in chickpea seeds protected with 2% (w/w) *A. sativum*. In the control lots, the mean of emerged individuals reached a value of 123 ± 7.55 individuals.

Means in a column followed by the same letter are not significantly different ($\alpha = 0.05$)

Mortality is significant at $P < 0.01$ according to LSD test.

Table 3 shows the effect of plant powders on the larval phase of *C. maculatus* and the reduction of seed

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

	Mortality (% Mean \pm SD)				Number of eggs (Mean \pm SD)	Emergence (Mean \pm SD)	Mean life cycle duration (Days)	% IR
	1days	3 days	6 days	9 days				
Control*	0 \pm 0.0a	3,33 \pm 5.77 a	56,67 \pm 11.55a	96,67 \pm 5.77a	132 \pm 12a	114 \pm 6.57a	29.33 \pm 1.15a	-
Positive control**	100 \pm 0.0e	100 \pm 0.0k	100 \pm 0.0b	100 \pm 0.0 a	0 \pm 0.0 c	0 \pm 0.0c	-	100
<i>E.camaldulensis</i>	3,33 \pm 5.77 ab	16,67 \pm 5.77 ab	60 \pm 10ac	100 \pm 0.0 a	83,33 \pm 3.51b	64 \pm 10.15b	38.33 \pm 2.31b	43.79
<i>I.viscosa</i>	23,33 \pm 15.27bc	30 \pm 10bc	93,33 \pm 5.77b	100 \pm 0.0 a	111.33 \pm 36.56ab	81.67 \pm 27.43ab	42.67 \pm 4.16bc	27.38
<i>M.officinalis</i>	43,33 \pm 15.27cd	56,67 \pm 5.77d	93,33 \pm 5.77b	100 \pm 0.0 a	24 \pm 11.79c	12.33 \pm 11.37c	46.67 \pm 4.62cd	89.32
<i>O.compactum</i>	10 \pm 17.32abc	26,67 \pm 15.27bce	73,33 \pm 15.27c	100 \pm 0.0 a	5.66 \pm 8.1 c	3 \pm 5.97c	53 \pm 0.00e	97.5
<i>M.pulegium</i>	100 \pm 0.0e	100 \pm 0.0k	100 \pm 0.0b	100 \pm 0.0 a	0.33 \pm 0.58 c	0 \pm 0.0c	-	100
<i>C.frutescens</i>	3,33 \pm 5.77 abc	16,67 \pm 5.77abce	100 \pm 0.0b	100 \pm 0.0 a	92,67 \pm 12.42 ab	77 \pm 12.17 b	42 \pm 1.73bdf	32.6
<i>C.officinalis</i>	3,33 \pm 5.77abc	30 \pm 10,0bce	73,33 \pm 5.77c	100 \pm 0.0 a	82.33 \pm 33.31b	68.67 \pm 26.76 b	43 \pm 3.61cdf	39.24
<i>P.graveolens</i>	0 \pm 0.0ab	73,33 \pm 5.77f	93,33 \pm 5.77b	100 \pm 0.0 a	96.61 \pm 20.21 ab	85 \pm 19.19 ab	37 \pm 0.0bh	25.85
<i>M.communis</i>	0 \pm 0.0ab	3,33 \pm 5.77ab	86,67 \pm 5.77bc	100 \pm 0.0 a	93.67 \pm 30.53ab	83.67 \pm 32.01ab	40.67 \pm 2.52bcfgh	26.85
<i>Z.officinale</i>	3,33 \pm 5.77abc	23,33 \pm 5.77bce	66,67 \pm 15.77ac	96,67 \pm 5.77a	104.33 \pm 20,84 ab	56 \pm 39.67 bd	45.67 \pm 4.51cdf	50.3
<i>U.dioica</i>	23,33 \pm 15.27bcd	56,67 \pm 5.77d	96,67 \pm 5.77b	100 \pm 0.0 a	78,67 \pm 46 b	61 \pm 31.76 b	41 \pm 3bcghi	46.52
<i>A.herba-alba</i>	23,33 \pm 5.77bcd	40 \pm 17.32bdeg	100 \pm 0.0b	100 \pm 0.0 a	88.33 \pm 61.85 ab	79.33 \pm 23.03b	39.33 \pm 2.31bcfgh	30.37
<i>D.gnidium</i>	10 \pm 0.0abc	53,33 \pm 23.09dgh	100 \pm 0.0b	100 \pm 0.0 a	106,33 \pm 32.02ab	89 \pm 8.66ab	36.33 \pm 3.51bcg	22.03
<i>O.europaea</i>	0 \pm 0.0ab	26,67 \pm 5.77bcegi	60 \pm 17.32ac	100 \pm 0.0 a	112.33 \pm 40.41 ab	96.67 \pm 7.57 ab	42.67 \pm 1.15bcdfh	14.94
<i>N.oleander</i>	3,33 \pm 5.77abc	30 \pm 10.0bcegi	56,67 \pm 11.55b	100 \pm 0.0 a	103,33 \pm 6.69ab	77.33 \pm 22.14b	39.67 \pm 0.57bcfgh	31.79
<i>R.officinalis</i>	10 \pm 17.32abc	16,67 \pm 11.55bceci	50 \pm 0.0a	100 \pm 0.0 a	117 \pm 26,46 ab	102 \pm 27.87 a	41 \pm 1.73bcfgh	11.09
<i>A.sativum</i>	46,67 \pm 32.15d	66,67 \pm 15.77dfh	100 \pm 0.0b	100 \pm 0.0 a	50,33 \pm 5.51bc	30.67 \pm 11.68bcd	36.67 \pm 1.15bgh	72.84
<i>S.aromaticum</i>	86,67 \pm 23.09 e	100 \pm 0.0k	100 \pm 0.0b	100 \pm 0.0 a	0 \pm 0.0 c	0 \pm 0.0c	-	100

*Untreated seed

**Seed treated with a conventional synthetic insecticide

% IR: percentage of population reduction

 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 ($\alpha = 0.05$).

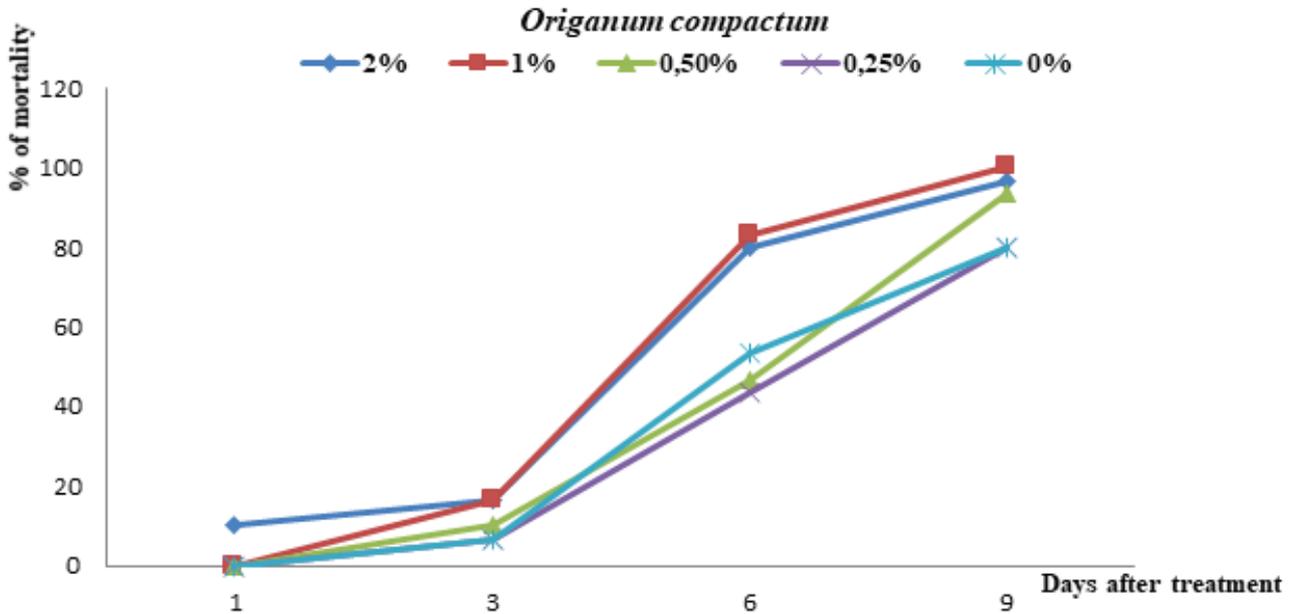


Fig 1: Mortality rate of adults of *C. Maculatus* in the presence of chickpea seeds treated with different concentrations of powders of *O. compactum*

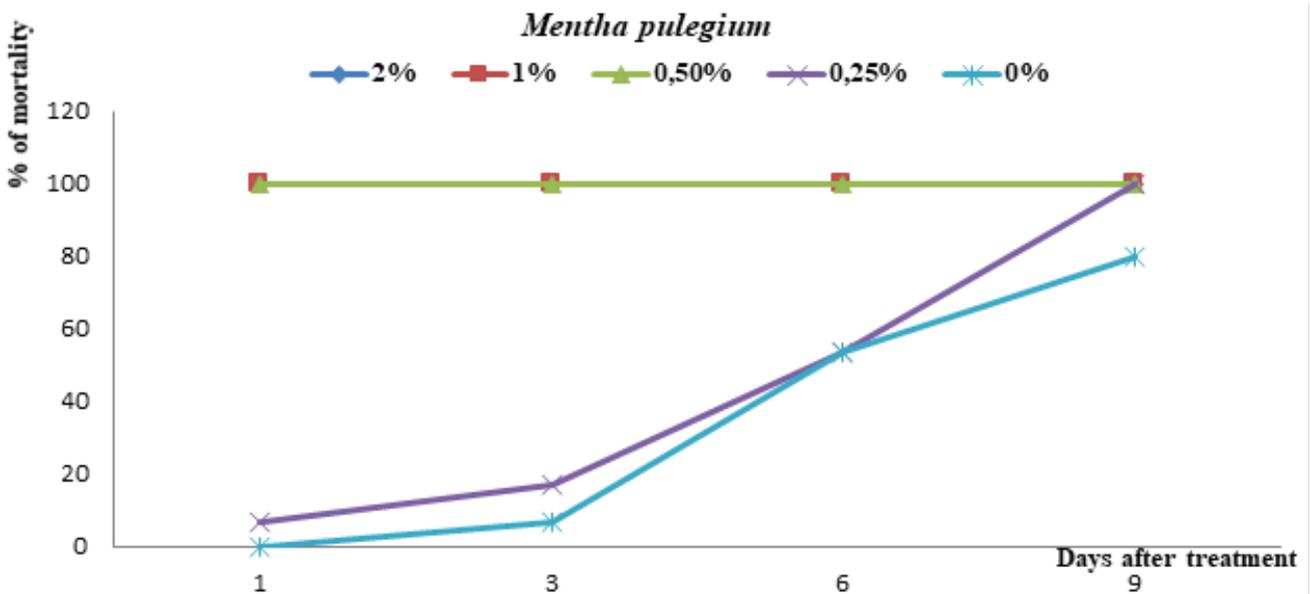


Fig 2: Mortality rate of adults of *C. maculatus* in the presence of chickpea seeds treated with different concentrations of *M. pulegium* powders

weights after treatment. The results of the experiments showed that there is no significant difference between control lots and lots treated with powders of *S. aromaticum*, *M. pulegium* and *A. sativum* concerning the larval stage. On the other hand, tests treated with *O. compactum* significantly ($p=0.005$) increased the duration of the larval phase.

Measurement of seed weights showed that treatment with powders of the two species *S. aromaticum* and *M. pulegium* protected chickpea seeds and significantly reduced damage by weight for all doses ($P=0.0018$). The same results were obtained when treated with a 2% dose of *O. compactum*. For *A. sativum* powders the differences in the means of all the doses were not significant in comparison with the results obtained in the control batches.

Discussion: One of the most important values

in plants is the presence of toxic activity against insects, these insecticidal potentialities can be a real solution for the control of pests in storage systems to replace or even minimize the application 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 powders varies according to the species tested, the dose used and the time of exposure; this toxicity is much greater at high doses. *M. pulegium* and *S. aromaticum* showed the highest insecticidal activity against *C. maculatus* at an optimal 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 total reduction in fecundity and emergence confirms that both plants have good bio-insecticidal potential to control beetle infestations. These results are in agreement with

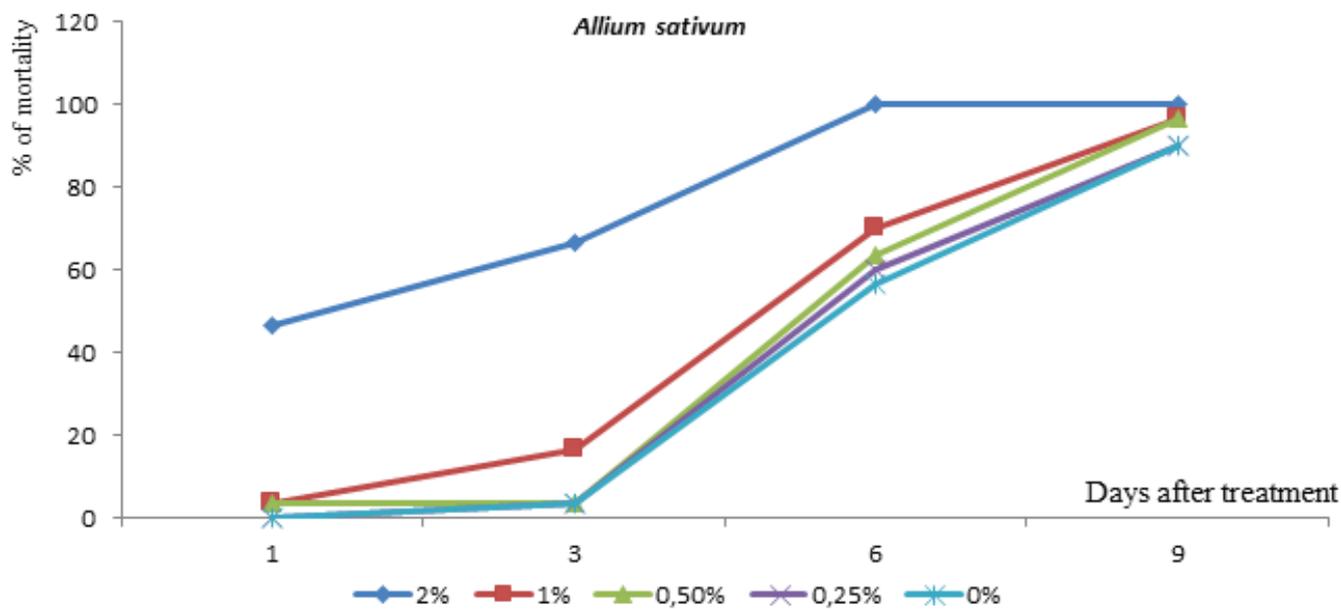


Fig 3: Mortality rate of adults of *C. maculatus* in the presence of chickpea seeds treated with different concentrations of *S. aromaticum* powders

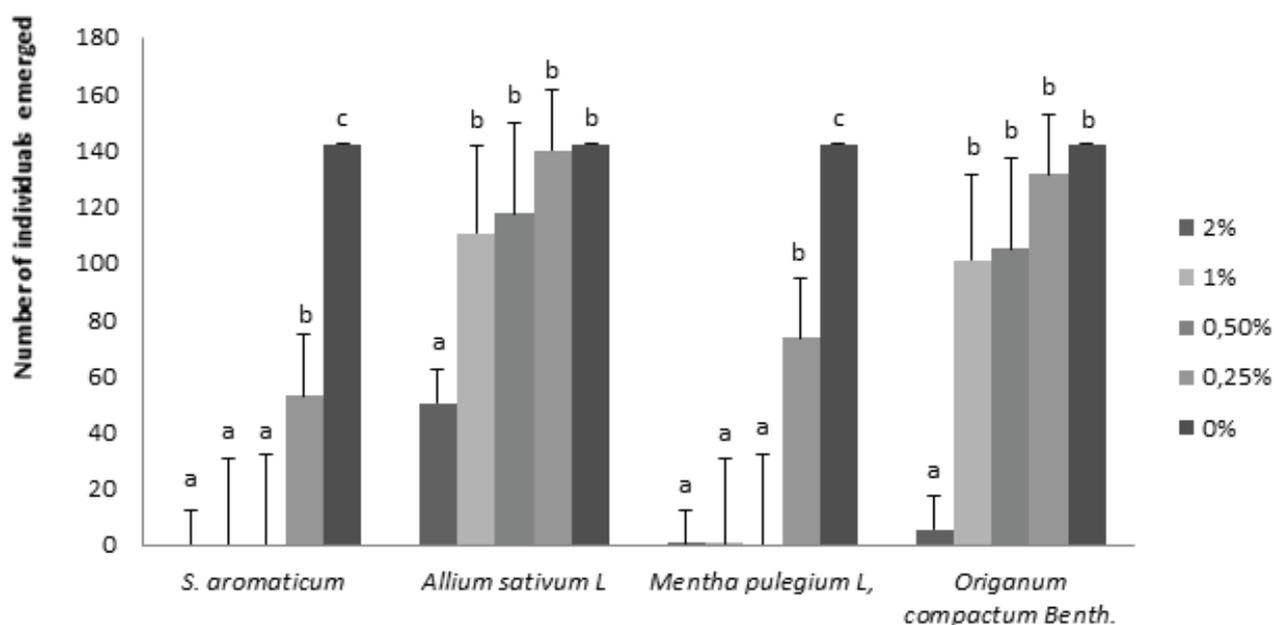


Fig 4: Mortality rate of adults of *C. maculatus* in the presence of chickpea seeds treated with different concentrations of *A. sativum* powders

those obtained by Kumar *et al.* (2011) who evaluated the effect of species of the genus mentha and Lawal *et al.* (2014) who worked on *Syzygium*. These two studies showed insecticidal activity mainly due to pulegone and menthone, major components of the essential oil of *M. pulegium* (Domingues and Santos 2019) and eugenol, major component of the essential oil of *S. aromaticum* (Fayemiwo *et al.* 2014) 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 (Picimbon 2002).

Traditionally, plant powders have been mixed with seeds stored in Morocco since ancient times and could be used as a natural, safe and less expensive strategy to protect stored seeds from insect infestation (Allali *et al.* 2020c). The strategy used varies from one region to another and

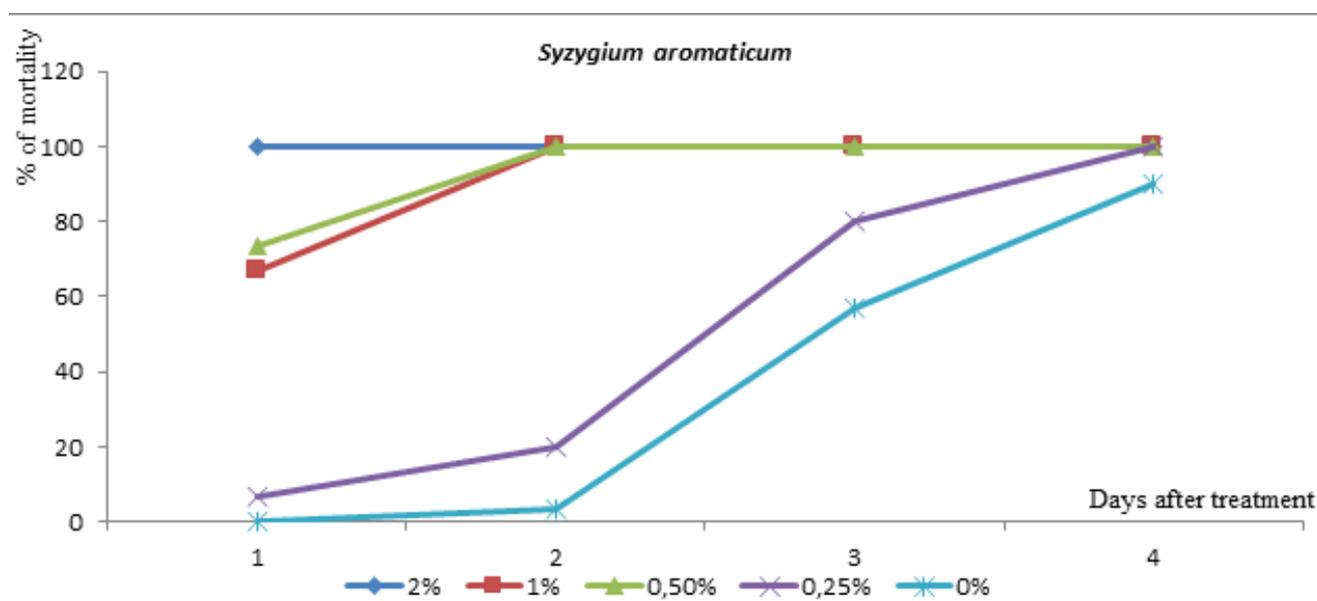
seems to depend in part on the type and effectiveness of the flora available in the different regions (Levinson and Levinson 1998; Golob 1999; Nenaah and Ibrahim 2011).

In a related study, Johnson *et al.* (2006) reported that powder from the dry leaves of *M. piperita* (species of the same genus) 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.* 2011) 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. Tripathi *et al.* (2009) reported that *S. aromaticum* powder caused 100% adult repulsion

Table 3: Effect of plant powder on larval phase and seed weight

Dose	<i>S.aromaticum</i>	<i>M.pulegium</i>	<i>O.compactum</i>	<i>A.sativum</i>
Duration of the larval phase in days				
2%	-	-	55±1.15 e	34.33±0.57a
1%	-	-	43.67±3.66d	35±2.65a
0.5%	-	-	38.67±1.5bc	36.33±2.05a
0.25%	29±2a	28.67±1.53 a	35±0.57bc	34±2a
0%	29.33±1.15a	29.33±1.15 a	29.33±1.15 a	29.33±2.15a
Seedweightloss in %.				
2%	0±0.0a	0±0.0 a	0,04±0.06 a	1.33±0.31 a
1%	0±0.0 a	0±0.0 a	1,4±0.25 ab	1.97±0.21 a
0.5%	0,03±0.06 a	0±0.0 a	2,67±0.12bc	2.47±0.75 a
0.25%	0,11±0.1 a	0,76±0.13 a	2,84±0.15bc	2.62±0.29 a
0%	3,26±0.64 b	3,26±0.64 b	3,26±0.64 c	3.26±0.64 a

Column means followed by the same letters are not significantly different and means followed by different letters are significant at $P < 0.01$ on the LSD test ($\alpha = 0.05$).

**Fig 5:** Number of eggs laid/5 female *C. maculatus* released on chickpea seeds treated with different rates of plant powders

of *T. castaneum* at a dose of 1.5 g / 50 g or 3% w/w. Also AL and Albandari (2015) reported that *S. aromaticum* oil caused 63.333% mortality two days after exposure to the highest concentration (5mg/L), rising to 96.667% four days after treatment at the same concentration.

O. compactum, *M. officinalis* and *A. sativum*, are ranked second with significant biocidal activity achieving a percentage reduction of 97.5%, 89.32% and 72.84% respectively by a 2% w/w dose (Table 2). Our results are consistent with many other studies. Ahmad *et al.* (2019) reported that *A. sativum* is an effective control against *T. castaneum* insect pests of stored products, Khalfi *et al.* (2008) reported that *O. compactum* showed insecticidal activity against *Rhizoperthadominica* and that this activity increases with increasing dose. Benelli *et al.* (2019) 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*.

Z. officinale, *E. camaldulensis* and *U. dioica* significantly reduced ($p \leq 0.001$) the population of *C. maculatus* with 50.3%, 46.52% and 43.79% respectively by 2% w/w dose (Table 2). These results are similar to those obtained by Al Qahtani *et al.* (2012) who reported that *Zingiber officinale* caused 63.2% mortality of *Oryza ephilussurinamensis* at the same 2% w/w dose. In addition to secondary metabolites, plants possess other direct defense responses against attack by phytophagous insects such as production of insecticidal peptides or proteins. A particular class of entomotoxic proteins found in many plant species is the carbohydrate-binding lectin protein group. A type of lectin called UDA (*Urtica dioica agglutinin*) is found in *Urtica dioica* causing varying degrees of mortality against *C. maculatus* (Murdock *et al.* 1990; Vandenborre *et al.* 2011). For *eucalyptus*, Prates *et al.* (1998) showed that *E. camaldulensis* showed biocidal activity against *Rhizopertha dominica* and *Tribolium castaneum*, which are important pests of stored seeds, and this could be due to two major compounds present in their essential oils.

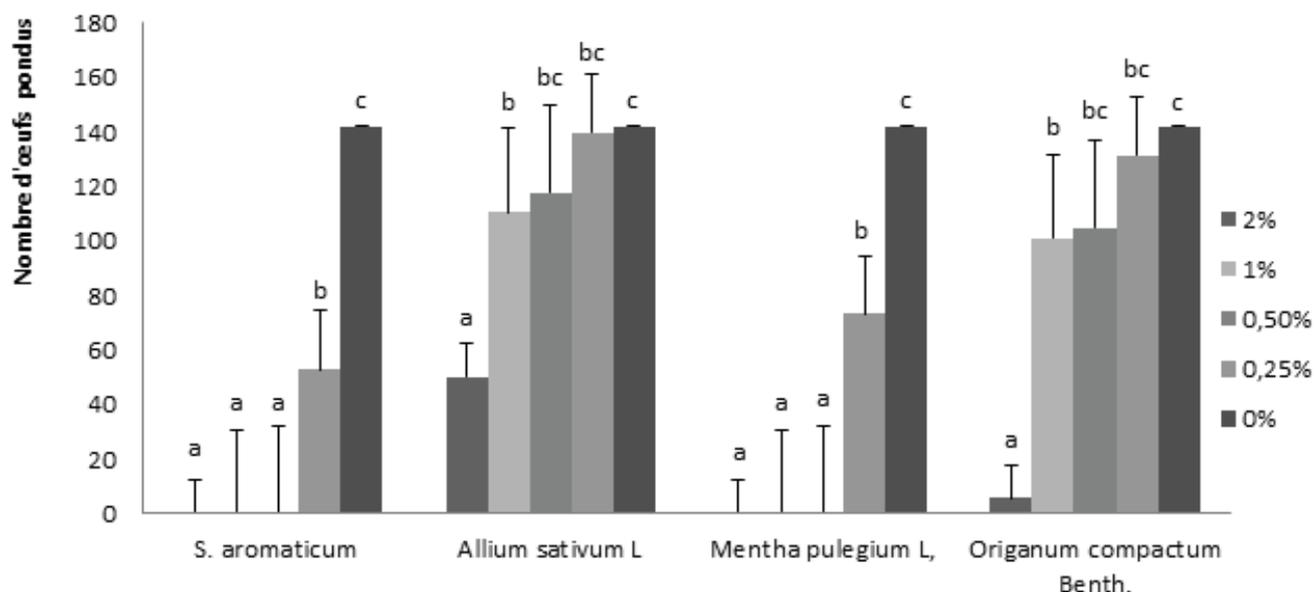


Fig 6: Average number of individuals emerged in the batches treated with different vegetable powders.

The other plants tested caused a small and non-significant percentage reduction of *C. maculatus* compared to the untreated control (Table 2).

The vegetal materials' mechanisms of action are contact and fumigation (Adedire and Lajide 1999; Asawalam and Emosairue 2006; Asawalam *et al.* 2006; Franccedil *et al.* 2009; Ukeh *et al.* 2010). The plant powders effectively protect the seeds against bruch infestation and therefore do not present any risk to human health and the environment, unlike conventional insecticides.

CONCLUSION

The plant species tested showed considerable toxicity against *C. maculatus* from stored chickpeas, when applied as a powder. Farmers were able to introduce these herbs into storage systems that release toxic volatile compounds into the storage space. 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 phytotoxicity on crops. Studies should be expanded to evaluate their mammalian safety, insecticidal mode of action and formulations for use in seed stores.

List of abbreviations

W/w :Weight/weight

% IR :The percentage reduction in adult emergence

ANOVA: analysis of variance

LSD: Fisher's Least Significant Difference

UDA: Urticadioïca agglutinin

IPM: integrated pest management programs

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