

EVALUATION OF SOME BIOINSECTICIDES AND PACKAGING MATERIALS FOR PROTECTING BROAD BEAN AGAINST *CALLOSOBRUCHUS MACULATUS* (COLEOPTERA: BRUCHIDAE) INFESTATION DURING STORAGE

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ABSTRACT

The potential activities of four essential oils (clove, mustard, nigella and onion) and microbial agents (*B. bassiana*; *M. anisoplia* and *V. lecanii*) and their combinations against *C. maculatus* were evaluated. Gunny sacks followed by paper bags provided more protection against *C. maculatus* infestation than the other tested packing materials.

Accumulative mortality percentage of *C. maculatus* beetles increased gradually by increasing the period of exposure to treated foam with different tested oils. Mustard oil had the highest accumulative mortality, followed by clove, nigella and onion. Mustard and clove oils had strong repellent activity. Mustard oil was the most effective oil in enhancing the potency of *B. bassiana* and *M. anisoplia* and decreased the LC₅₀ of the target insect. While, nigella oil was the most effective oil when combined with *V. lecanii*. Mustard oil combined with *B. bassiana* suppressing *C. maculatus* egg depositing and protecting broad bean seeds from beetles infestation for 5 months during storage .

INTRODUCTION

Almost all the insect pests of stored grains have a remarkably high rate of multiplication and within one season, they may destroy 10-15 % of the grains and contaminate the rest with undesirable odours and flavours (Baby, 1994). Broad bean plant (*Vicia faba* L.) is one of the most important economic crops in Egypt. However, its production has been seriously threatened by infestation of *Callosobruchus maculatus* (F.) in field and store. Serious damage is caused to stored dry broad beans on which this pest reproduces rapidly.

The effectiveness of many secondary plant metabolites for use against insects attacking stored products, had been shown to deter feeding and disturb insects as repellents due to their strong odoriferous nature (Deshpande *et al* 1974 ; Jacobson , 1975; Rodriguez *and* Levin, 1975; Prakash, 1982; Abd El-Aziz and Ismail, 2000; Abd El-Aziz, 2001; Sabbour, 2002 and Ketoh *et al.*, 2006).

There is growing interest in the exploitation of naturally occurring entomopathogenic microorganisms for the control of crop pests. Biological control agents (BCA) may offer more environmentally safe alternatives to chemical pesticides. They could be also used where pests have developed resistance to conventional pesticides. Today a many entomopathogens are used for the control of invertebrate pests in glasshouses, row crops, orchards, ornamentals, stored products and forestry (Lacy *et al.*, 2001). Essential oils may have attractive or repellent effects and in some cases they showed an insecticidal action against insects. Essential oils isolated from plants and consisting of cyclic and monocyclic monoterpenes are effective repellents against insects (Rodriguez and Levin, 1975). The LD₅₀ for some formulations of *Beauveria bassiana* was reduced to 97 % after the addition of coconut oil. It was suggested that the cutinophilic properties of the oil could allow a greater number of fungal conidia to penetrate the mouth parts of insects. Oil carriers can also distribute the inoculum over the thin intersegmental membranes, which are more readily penetrated by entomogenous fungi (Lisansky, 1989). Abdel-Gawad and Abdel-Aziz (2004) found that the fungus *B. bassiana* killed the insect pests through the cuticle and not needed to consume by them, they also mentioned that they caused a protection to the cowpea.

The present work aimed to explore the protective potency of some botanical oils and microbial agents and their combinations against the broad bean beetle, *C. maculatus* during storage.

MATERIAL AND METHODS

The broad bean beetle (cowpea beetle), *Callosobruchus maculatus* (F.) was reared on broad bean seeds *Vigna faba* (L.) at $28 \pm 2^{\circ}$ C and 60 ± 5 %R.H. under laboratory conditions.

Four essential oils were used in bioassay: 1) Clove buds, *Eugenia caryophyllata* (Fam.: Myrataceae); 2) Black-cumin, *Nigella sativa*. (Fam.: Ranunculaceae); 3) Onion, *Allium cepa* (Fam.: Alliaceae); and 4) black mustard, *Brassica nigra* (Fam.: Cruciferae). The essential oils were isolated by steam

distillation of the dried plants (Guenther, 1961). The tested oil emulsions were prepared as follows: 5 drops of "Triton X-100 as emulsifier was mixed thoroughly with 5ml of each tested oil, then water was added to obtain the desired concentrations (0.5, 2 and 3 %) in percent of (v/w). The emulsifier was mixed at the corresponding concentrations and used as check.

The fungi (*Beauveria bassiana*), *Metarhizium anisopliae* and *Verticillium lecanii* were isolated from infested larvae of *Callosobruchus maculatus* (F.) and identified at the Plant Pathology Department, NRC. The fungi were diluted in distilled water to obtain the desired concentrations (in percent of v/v). Six concentrations (16, 8, 4, 2, 1 and 0.5×10^7 spores /ml) were prepared of *B. bassiana*, *M. anisopliae* and *V. lecanii* at the rated 16.5×10^7 spore/ml.

Tested Packaging materials

To screen the most suitable packaging materials for prevention of broad bean beetles infestation, the following packaging materials were tested: muslin, paper (multiwall paper (3 layers)), cheesecloth, wax paper, gunny bags and polypropylene. All packaging materials were provided with heat sterilized broad bean seeds (25 seeds each), fastened each with a string. Twenty pairs of newly emerged beetles were introduced to a jar (1L capacity with one package). Ten replicates were used for each packaging materials. The numbers of penetrated insects on each tested packaging material was counted after one week. The experiment was repeated four times.

Repellency test

The experiments were conducted in an arena in choice test (Abd El-Aziz and Ismail, 2000). Disc of filter paper (Whatman No. 1) was treated with the tested oil at 1 %conc. and placed in cell A. While filter paper treated with distilled water and emulsifier only as control was placed in the cell B. Twenty newly emerged beetles were introduced into each arena. After 1, 2, 3, 4, 5, 6 and 7 days, the number of beetles present in the cells A and B was recorded. The percentages of repellency values were calculated using the equation: $D = (1 - T/C) \times 100$ (Lwande *et al.*, 1985) where T and C represent the mean number of beetles in cells A and B (Treated and untreated), respectively.

The insecticidal activity of tested oils

Experiment was designed to test the initial as well as the persistent effect of the tested oils on beetles as cumulative mortality during successive intervals (0, 2, 4,

and 7 days). Foam granules about 1cm in diameter were treated at time (zero time) with tested oils (2% conc.), dried and provided with heat sterilized bean seeds (100g/each) fastened each with a string. Then all treatments were used immediately as non-choice test. The foam granules treated with the tested oils were mixed with bean seeds (2g foam/100g seeds) according to Abd El-Aziz (2001).

Ovipositional deterrent effect of tested oils (no choice test)

To evaluate the oviposition deterrent of the tested oils, a pair of newly emerged beetles, was placed with treated or untreated broad seeds in glass jars (250 cc capacity) covered with muslin. The beetles were left to lay eggs, and then the deposited eggs were counted on the seeds in the treated and untreated jars. Each experiment was repeated five times, (Abd El-Aziz and Ismail, 2000). The number of deposited eggs was used as a criterion for the evaluation of reduction percentages.

$$\text{Reduction \%} = \left[100 - \frac{\text{No. of deposited eggs in treatment}}{\text{No. of deposited eggs in control}} \right] \times 100$$

The percent reduction is an index of effectiveness of the applied oils in reducing infestation and was calculated according to Su (1989).

Insecticidal effects of a mixture of microbial agents and botanical oils

The tested fungi were mixed at 4.25×10^7 spores/ml with the plant oils tested at 0.05% then sprayed to the seeds. A pair of newly emerged beetles was placed with treated or untreated broad seeds in glass jars (250 cc capacity) covered with muslin. The percentages of mortality were counted and calculated according to Abbott (1925) while LC_{50} were calculated through probit analysis (Finney, 1964). The experiments were carried under laboratory conditions; $26 \pm 2^\circ$ C and 60-70% R.H. the experiment was replicated 4 times.

The persistence of mustard oil combined with tested fungi during storage

Experiment was designed to test the persistent effect of mustard oil alone and combined with tested fungus on foam as surface protectant at 20- day intervals over 120 days. All gunny sacks (20x20 cm each) were full of heat sterilized broad bean seeds (100g each), fastened, each with a string. The foam granules (about 1cm in diameter) were sprayed with treatments, dried and provided as a layer between sacks. Following exposing to those treatments, two pairs of newly emerged beetles were placed in a jar (2L capacity with four gunny sacks) and observed for egg laying. Eggs on broad bean seeds/female were counted using a laboratory microscope and then, all treatments were used as non-choice test.

Results were subjected to statistical analysis according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Penetration of *C. maculatus* through various types of packaging test

The heavier the package material, the fewer *C. maculatus* beetles that penetrated the package. Gunny bags and paper packages were more resistant than all other packaging materials with highly significance difference, and there was no significant difference between gunny and paper bags (Table 1). Only 4.9 ± 0.8 beetles penetrated the gunny bags with 24.5% penetration after one week of releasing beetles. Similar results were found by Leelavathi *et al.* (1984) and Upadhyay *et al.* (1994). Both muslin bags and cheesecloth gave very little protection to broad bean seeds with 79 and 73% penetration after one week.

We chose gunny bags for further experiments because of their resistant than all other packaging materials.

Repellency test

The study of distribution of the beetles (in choice test) soon after their placement in the center of arena and for 7 consecutive days was illustrated at (Fig 1). The majority of beetles moved toward the control cell after a few minutes of exploration in case of mustard and clove oils. Nigella and onion oils gave moderate repellency during the first and second days, respectively and once there, the repellency decreased sharply. Mustard oil indicated the more persistent and stable during all the experimental period (7days) followed by clove oil comparing with other oils.

In this respect, Deshpande *et al.* (1974) reported that oleic and linoleic acid as insecticidal components from *Nigella sativa* which were found to be toxic to the pulse beetle, *C. chinensis*. In a choice test, filter paper strips treated with *Acorus calamus* oil at 200,400 or 800 $\mu\text{g}/\text{cm}^2$ repelled *Tribolium castaneum* adults during the first 2 weeks, there after repellency decreased (Jilani *et al.*, 1988). Abd El-Aziz and Ismail (2000) mentioned that Nigella oil gave 45.5 and 40.2 % repellency during the first and second days, respectively. Nigella oil became attractive to *Bruchidius incarnatus* beetles and had little persistent. Pumpkin oil at 1 % conc., had strong repellent activity (88%) during the first day of observation and then decreased gradually to reach (0.0%) repellency during the last day of experiment. Frankincense oil indicated the more persistent. White Mustard oil was found to protect storage insects infesting stored

pulses, especially the black gram and the green gram, (Prakash, 1982). Black mustard seeds contain sinigrin and myrosin and yield after maceration with water 0.7–1.3% of volatile oil. The latter contains over 90% of allylisothiocyanate (Olivier *et al.*, 1999). The main chemical components of clove oil are eugenol, eugenol acetate, iso-eugenol and caryophyllene (Olivier *et al.*, 1999).

The insecticidal activity of tested oils

Data in Table (2) indicated that accumulative mortality (%) of *C. maculatus* beetles increased gradually by increasing the period of exposure in case of treated foam with different tested oils. Mustard oil had the highest cumulative mortality (73%) followed by clove with (59 %). While the cumulative mortality (%) in case of nigella and onion oils were almost equal (43 and 39.9%), respectively. There was no obvious effect on adult mortality during the next storage intervals. But the same oils had significantly reduced the beetle's fecundity. In this respect, Chander and Ahmed (1986) applied different doses of the essential oil of *Acorus calamus* to seeds of green gram *Vigna radiata* (Wilcz) to protect them against *Callosobruchus chinensis* and found that 1ml/Kg offered a high degree of protection up to a period of 135 days. Prolonged protection of the seeds was mainly due to a high adult mortality besides reduced oviposition and low hatching. Abd El-Aziz, 2001 reported that foam sprayed with clove oil (5%) and placed between sacks caused the highest mortality (66.6%) of *C. maculatus* as compared with treated sacks or foam inside sacks (63.3% and 42%, respectively) after 6 days of storage. The same results were obtained by Chander and Ahmed (1986); Saxena *et al.*, (1976), Surabaya *et al.*, (1994) and Maheshwari *et al.*, (1998).

Ovipositional deterrent effect

The effect of tested plant oil vapors on the reproduction of the *C. maculatus* beetles were studied using the no-choice test (Table 3). The reproduction of beetles was reduced by the treatments with mustard and clove vapors, but not inhibited completely. Mustard oil acted not only as oviposition deterrents but also adversely influence fecundity. Beetles oviposited eggs on treated seeds with mustard oil but the numbers of eggs is always lower in treated seeds than in the control. Shaaya *et al.*, (1997) reported that edible oils are potential control agents against *C. maculatus* and can play an important role in stored-grain protection. Abd El-Aziz (2001) mentioned that clove and eucalyptus oil vapors impaired the fecundity of the bruchid beetles, *Callosobruchus maculatus*. Data proved promising oviposition deterrence, toxicity and suppressing egg deposition and adult emergence.

Insecticidal effects of a mixture of microbial agents and botanical oils

Mustard oil was the most effective oil in enhancing the potency of *B. bassiana* and *M. anisoplia* and decreased the LC_{50} of the target insect (101 and 115 $LC_{50} \times 10^7$), respectively (Table 4). While, nigella oil was the most effective oil when combined with *V. lecanii* (128 LC_{50}). The LD_{50} for some formulations of *Beauveria bassiana* was reduced to 97 % after the addition of coconut oil. It was suggested that the cutinophilic properties of the oil could allow a greater number of fungal conidia to penetrate the mouth parts of insects. Oil carriers can also distribute the inoculum over the thin intersegmental membranes, which are more readily penetrated by entomogenous fungi (Lisansky, 1989).

The increase in the pathogenicity of *B. bassiana* combined with mustard oil to *C. maculatus* beetles may be attributed to some degradation occurring at the structural level of the integument, which could have facilitated the penetration of the cuticle by the germ tube of the fungus. Similar results were obtained by Hassan and Charnley (1989) in *Manduca sexta* treated with *Metarhizium anisopliae* and the chitin-synthesis inhibitor dimilin. Synergistic effects of a combined application of *B. bassiana* and the chloronicotinyl insecticide imidaclopride on the curculionid *Diaprepes abbreviatus* were reported by Quintela and McCoy (1998).

The persistence of mustard oil combined with tested fungi during storage

The persistent effect of mustard oil alone and combined with tested fungus on foam were tested as surface protectant at 20- day intervals over 120 days (Fig 2) . Application of Mustard oil combined with *B. bassiana* on foam covering gunny bags provided promising oviposition deterrency, toxicity and suppressing *C. maculatus* infestation, persistence and protecting broad bean seeds from beetles infestation for 4 months during storage .Abd El-Aziz (2001) mentioned that the treated foam with clove and eucalyptus oil vapours covering gunny sacks was the most significantly effective against *C. maculatus* infestation after 90 days from storage copared with the other applications (treated sacks or foam inside sacks) .

The foregoing results indicate that the mustard and clove essential oils have properties which cause adult mortality, repellency of *C. maculatus* and this may be correlated to the chemical constituents of these oils. For instance, black mustard seeds contain sinigrin and myrosin and yield after maceration with water 0.7–1.3% of volatile oil. The latter contains over 90% of allylisothiocyanate (Olivier *et al.*, 1999). Also, The main chemical components of clove oil are eugenol, eugenol acetate, iso-eugenol and caryophyllene (Olivier *et al.*, 1999). Glucosinolates or their

breakdown products are powerful allomones and have a marked antibiotic effect (Van Etten, 1969). The efficacy of mustard oil at (5.0, 7.5 or 10 ml/kg) for protecting the seeds of 9 varieties of chick pea *Cicer arietinum* against attack by *C. chinensis* was assessed by Khalique *et al.*, (1988). The percentage damage decreased with increase in concentration of mustard oil after 5 months of treatment for all the varieties of chick pea tested. Das (1986) mentioned that all adults died within 4 days of release, and the oviposition was completely inhibited when stored seeds were treated with the neem, sesame and coconut oils and very few eggs were found on seeds treated with soyabean and mustard oils. Parsia *et al.*, (1990) studied the efficacy of groundnut or mustard oil against *C. chinensis* on urd bean. The development period, adult emergence of the pest and numbers of eggs laid decreased with increasing oil concentration.

TABLE (I)

Number of penetrating beetles and % of penetration of *C. maculatus* through various types of packaging

Packaging material	Number of penetrating beetles (\pm SE) (% penetration)
Cheesecloth	14.6 \pm 0.763 d (73)
Gunny bags	4.9 \pm 0.809 a (24.5)
Muslin	15.8 \pm 0.841 d (79%)
Paper	5.4 \pm 0.702 a (27)
Polypropylene	8.8 \pm 0.879 b (44)
Wax paper	11.2 \pm 0.629 c (56)

TABLE (II)

Accumulative mortality of *C. maculatus* adults during the first week of broad bean seeds exposed to treated foam with different oils

Time(days)	Accumulative mortality of tested oils				
	Clove	Mustard	Nigella	Onion	untreated
0.0	19.3	25.7	23.3	15.3	0.0
2	32.6	47.8	34.6	23.5	0.0
4	41.7	61.4	38.9	26.8	0.0
7	59.7	73.1	43.7	39.9	2.1

TABLE (III)

Ovipositional deterrent effect of tested oils against *C. maculatus* beetles.

Type of oils	Mean number of eggs/female \pm S.E.		
	0.5%	2%	3%
Clove	44.4 \pm 4.4 (67.56)*	39.5 \pm 4.5 (73.51)	35.3 \pm 5.3 (78.61)
Mustard	39.4 \pm 4.4 (73.63)	29.5 \pm 3.3 (85.66)	21.4 \pm 4.4 (95.50)
Nigella	58.4 \pm 4.4 (50.55)	54.4 \pm 5.7 (55.41)	59.1 \pm 3.6 (49.70)
Onion	74.6 \pm 5.8 (30.86)	69.5 \pm 4.5 (37.06)	61.5 \pm 3.2 (46.78)
Control	82.3 \pm 3.4		
F value	27.54		
LSD	10.18		

* Numbers between brackets represent percent reduction than control

TABLE (IV)

Efficacy of microbial agents combined with tested botanical oils at 0.5% against *C. maculatus* under laboratory conditions

Microbial agent	Tested oil	Lc_{50} $\times 10^7$ ()	Slope	Variance	95% confidence limits
	clove	120	1.19	0.01	132 --77
	mustard	101	1.23	0.02	188-99
	onion	130	1.33	0.01	168 -100
	nigella	113	1.28	0.02	189-110
<i>B. bassiana</i>	alone	135	1.11	0.01	199-134
	clove	133	1.12	0.02	170-111
	mustard	115	1.11	0.02	155-99
	onion	141	1.10	0.01	168-129
	nigella	125	1.12	0.01	167-101
<i>M. anisopliae</i>	alone	140	1.12	0.02	166-120
	clove	166	1.11	0.02	187-144
	mustard	139	1.20	0.02	167-101
	onion	165	1.22	0.01	178-144
	nigella	128	1.11	0.01	147-100
<i>V. lecanii</i>	alone	198	1.11	0.02	233-177

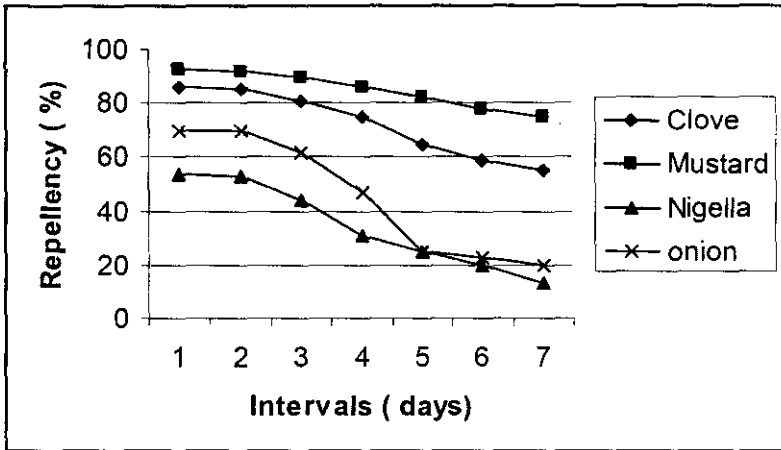


Fig. (1): Repellency activity of oils vapor against *C. maculatus* beetles during 7 days in (choice test).

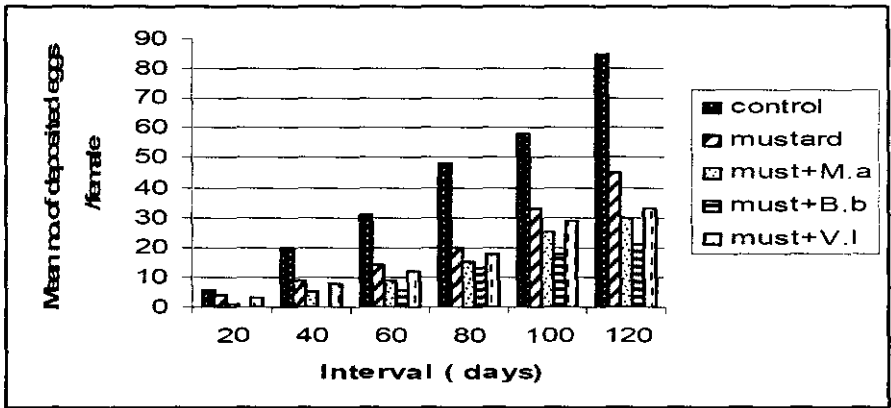


Fig. (2): Effect of mustard oil combined with fungus on number of deposited eggs/female of *C. maculatus* beetles during storage periods of broad bean seeds

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