EFFICACY OF SOME BIOINSECTICIDES AGAINST *BRUCHIDIUS INCARNATUS* (BOH.) (COLEOPTERA: BRUCHIDAE) INFESTATION DURING STORAGE

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Abstract: Potential activities of three essential oils (cumin, clove and mustard) and of microbial agents (*Paecilomyces fumosoroseus, Nomuraea rileyi, Lecanicillium* (*Verticillium) lecanii* and their combinations against *Bruchidius incarnatus* (Boh.) were evaluated. In choice test, mustard and clove oils revealed a strong repellent activity after 7 days (89% and 71%, respectively) against *B. incarnatus* beetles. Cumin oil showed the lowest repellency (47%). Accumulative mortality of beetles increased gradually with the increase of exposure intervals. Mustard oil treatment gave the highest mortality percentage of 76% after 168 h from treatment followed by clove treatment which amounted to 63% and the lowest percentage of 42.8% was recorded in case of cumin oil. Mustard oil was the most effective in enhancing the potency of *P. fumosoroseus* and *N. rileyi* and decreased LC_{50} of the target insect (100 and 102x10^7, respectively). The persistent effect of formulated mustard oil with either *P. fumosoroseus* or *N. rileyi* fungi on foam covering gunny bags displayed several different modes of action, by reducing oviposition and adult emergence (F1) of *B. incarnatus*. The oviposition was completely inhibited when stored broad bean seeds were treated with mustard oil + *P. fumosoroseus* during 20, 40 and 60 days of storage. Application of mustard oil combined with *P. fumosoroseus* on foam covering gunny bags provided promising oviposition deterrency, toxicity and suppressing *B. incarnatus* infestation, persistence and protecting broad bean seeds from beetles' infestation for 120 days during storage.

Key words: clove, cumin, mustard, *Paecilomyces fumosoroseus, Nomuraea rileyi, Lecanicillium* (*Verticillium*) *lecanii*

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 grains and contaminate the rest with undesirable odours and flavours (Baby 1994). Broad bean plant (*Vicia faba* L.) production was seriously threatened by *Bruchidius incarnatus* (Boh.) infestation under the field and storage conditions. Serious damage is caused to stored dry broad bean on which this pest reproduces rapidly.

The effectiveness of many secondary plant metabolites used against insect pests attacking stored products, was 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; Ketoh *et al.* 2006; Sabbour and Abd El-Aziz 2007) .

There is a growing interest in the use 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 in cases where pests have always developed resistance to conventional pesticides. Currently, many entomopathogens are used for the control of invertebrate pests in glasshouses, row crops, orchards, ornamentals, stored products and forestry (Lacy *et al*. 2001). Būda and Pečiulytė (2008) tested the effect of four fungi species (*Beauveria bassiana*, *Lecanicillium* (*Verticillium*) *lecanii, Metarhizum anisopliae* var. *anisopliae* and *Paecilomyces farinosus*) isolates on adults of Indian meal moth, *Plodia interpunctella* and one species tested on mature larvae of the pest. All the fungal isolates tested were pathogenic, however, with a different effectiveness. During the first three day period after spraying, the highest mortality (35–40% versus control) was caused by *P. farinosus* and *M. anisopliae* var *anisopliae,* and there was no significant difference in the survival as compared to control when *B. bassiana* and *L.* (*Verticillium*) *lecanii* were used.

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 mono-terpenes are considered as effective repellents against insects (Rodriguez and Levin 1975). The LD_{50} for some formulations of *B. bassiana* was reduced to 97% after the addition of coconut oil. It was suggested that the cutinophilic properties of plant oil allowed a greater number of fungal conidia to penetrate the mouth parts of insects. Oil carriers can

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also distribute the inoculum over the thin intersegmental membranes, which are more readily penetrated by entomogenic fungi (Lisansky 1989). Abdel- Gawad and Abdel -Aziz (2004) found that the fungus *B. bassiana* killed the insect pests through the cuticle without a need for the fungus to be consumed by them. The authors also mentioned that the fungus could be used as a biocontrol agent in protection of cowpea. The mineral oil carriers and Silwet L-77 as well as the botanical insecticides Neemix 4.5 and Hexacide were evaluated for their impact on the efficacy of *B. bassiana* (Balsamo) Vuillemin conidia against red flour beetle, *Tribolium castaneum* (Herbst), larvae, (Akbar *et al*. 2005). Hexacide (5% rosemary oil) caused significant mortality when applied without *B. bassiana*, but it did not affect pupation, the germination rate of conidia, or *T. castaneum* mortality when used in combination with the fungus.

The aim of presented studies was to determine the protective potency of some essential oils and Entomopathogenic fungi and their combinations against the broad bean beetle, *B. incarnatus* during storage.

MATERIALS AND METHODS

Stock culture

The bean bruchids beetle, *B. incarnatus* (Boheman) was reared on broad bean seeds *Vicia faba* (L.) at 28±2°C and 60±5% R.H. under laboratory conditions.

Tested oils

Three essential oils were used in the bioassay tests: 1 – clove buds, *Eugenia caryophyllata* (Fam: *Myrataceae*), 2 – cumin seed, *Cuminum Cyminum* (Fam: *Unibelliferae*), and 3 – black mustard, *Brassica nigra* (Fam: *Cruciferae*). The essential oils were obtained by steam distillation of dried plants (Guenther 1961). The tested oil emulsions were prepared as follows: 5 drops of Triton X-100 as emulsifier were mixed thoroughly with 5 ml of each tested oil, then water was added to obtain the desired concentrations (2%) in percent of (v/v). The emulsifier was mixed at the corresponding concentrations and used as check.

Tested fungi pathogen

Three fungi pathogen were obtained from Florida university: *P. fumosoroseus*; *N. rileyi* and *V. lecanii* were tested against *B. incarnatus* adults. The tested fungi were diluted in distilled water to obtain the desired concentrations (in percent of v/v), (16, 8, 4, 2, 1, 0.5x10^7 spores/ml) were prepared.

Repellency test

The experiments were conducted in an arena based on the choice test (Abd El-Aziz and Ismail 2000). Disc of filter paper (Whatman No. 1) was treated with the tested oil at 1% concentration and placed in the 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)]x100 (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 (24, 48, 96 and 168 h). 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 (100 g/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 (2 g foam/100 g seeds) according to Abd El-Aziz (2001). A pair of newly emerged beetles was placed with treated or untreated broad seeds in glass jars (250 cc capacity) covered with muslin. The number of dead beetles in each jar was counted every day and the percentages of mortality were corrected using the Abbott formula (Abbott 1925).

Insecticidal effects of tested fungi alone and with oils

The tested fungi were diluted in distilled water (v/v) to obtain six concentrations (16, 8, 4, 2, 1 and 0.5x10^7 spores/ml) at the rated 16.5x10^7 spore/ml. While they mixed at 4.25x10^7 spores/ml with the tested oils (0.05% conc.), 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 number of dead beetles in each jar was counted every day and the percentages of mortality were corrected using the Abbott formula (Abbott 1925) and LC_{50} were calculated through the probit analysis (Finney 1964). The experiment was carried under laboratory conditions; 26±2°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 combined with *P. fumosoroseus*; *N. rileyi* (which gave the highest pathogenicity against *B. incarnatus*) 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 (100 g each), fastened, each with a string. The foam granules (about 1 cm 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 (2–3 day) were placed in a jar (2 l capacity with four gunny sacks) and observed for egg laying. The laid eggs were counted on the seeds in the treated and untreated jars. Each experiment was repeated five times, (Abd El-Aziz 2001). The number of deposited eggs was used as a criterion for the evaluation of reduction percentages.

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

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

Dead beetles were removed and the jars were kept under the same experimental conditions until the emergence of F1 progeny adults occurred. Percentage reduction in adult emergence or inhibition rate (% IR) was calculated as:

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

where:
Cn is the number of newly emerged insects in the untreated (control) jar
Tn is the number of insects in the treated jar (Tapondjou *et al.* 2002).

RESULTS AND DISCUSSION

Repellency test

The results of the study on distribution of *B. incarnatus* beetles (in choice test) soon after their placement in the center of arena and for 7 consecutive days were illustrated at figure 1. The majority of beetles moved toward the control cell after a few minutes of exploration in case of mustard and clove oils. Cumin oil gave moderate repellency during the first and second days, respectively and then, the repellency decreased. Mustard oil indicated the more persistent and stable during the whole experimental period (7 days) followed by clove oil and cumin.

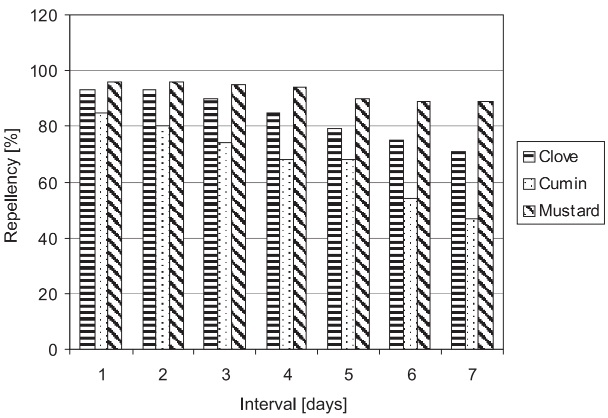

Fig. 1. Repellency activity of oils vapours against *B. incarnatus* beetles during 7 days in choice test

In this respect, in a choice test, filter paper strips treated with *A. calamus* oil at 200, 400 or 800 μg/cm² repelled *Tribolium castaneum* adults during the first 2 weeks, there after repellency decreased more rapidly, than neem oil (Jilani *et al.* 1988).

Abd El-Aziz and Ismail (2000) mentioned that frankincense oil showed strong repellent activity, more persistent and stable than the other tested oils against, *B. incarnatus* beetles. In choice test, mustard and nigella oils had a strong repellent activity after 7 days (51% and 49%, respectively) against *B. rufimanus* beetles (Sabbour and Abd El Aziz 2007)

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). Chaieb *et al.* (2007) mentioned that the main constituents of the clove essential oil are phenylpropanoids such as carvacrol, thymol, eugenol and cinnamaldehyde. The biological activity of *E. caryophyllata* was studied on several microorganisms and parasites, including pathogenic bacteria, *Herpes simplex* and hepatitis C viruses. In addition to its antimicrobial, antioxidant, antifungal and antiviral activity, clove essential oil possesses antiinflammatory, cytotoxic, insect repellent and anaesthetic properties. The toxicity of *E. caryophyllata* bud and leaf oil-derived compounds (acetyleugenol, β-caryophyllene, eugenol, α-humulene, and methyl salicylate) and congeners of eugenol (isoeugenol and methyleugenol) against eggs and females of the head louse, *Pediculus capitis* was examined by Yang *et al.* (2003). The most toxic compound to female *P. capitis* was eugenol followed by methyl salicylate and the effect was largely due to action in the vapor phase.

The insecticidal activity of tested oils

The corrected mortality % of *B. incarnatus* beetles was recorded after 24, 48, 72 and 96 h from the beginning of treatment (Fig. 2). Percentage of accumulative mortality of beetles increased gradually with the increase of the exposure intervals. Mustard oil revealed the highest mortality percentage; 76% after 168 h followed by clove oil of 63% and the lowest percentage was recorded in case of cumin oil and amounted to 42.8%.

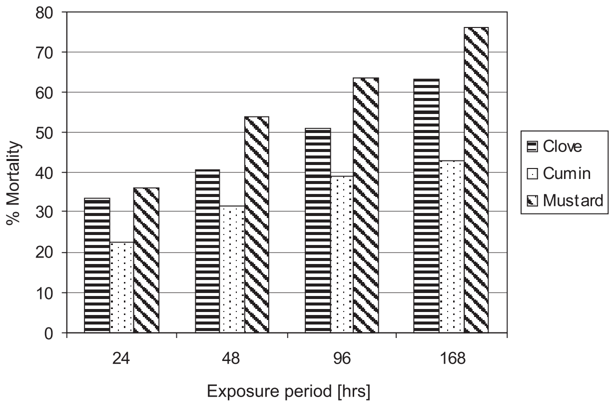

Fig. 2. The insecticidal effect of tested oils against *B. incarnatus* beetles

White mustard oil was found to protect stored pulses, especially the black gram and the green gram against storage insect infestation (Prakash 1982). Kim *et al.* (2003) found that Cinnamon, horseradish and mustard oils, at 0.7 mg/cm², were highly toxic to *Lasioderma serricorne* adult beetles 1 day after treatment. In fumigation test with the beetle adults, insecticidal activity of horseradish oil, mustard oil and *Foeniculum* fruit extract was much more effective in closed cups than in open ones, indicating that the insecticidal activity of these materials was largely attributable to fumigant action. 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 *C. chinensis* infestation was assessed by Khalique *et al.* (1988). The percentage damage decreased with the increase of mustard oil concentration after 5 months of treatment for all varieties of chick pea tested. Das (1986) mentioned that all adults died within 4 days after release, and the oviposition was completely inhibited when stored seeds were treated with the neem, sesame and coconut oils and only a 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 laid eggs, decreased with increasing the oil concentration.

Insecticidal effects of tested fungi alone and with oils

Mustard oil was the most effective oil in enhancing the potency of *P. fumosoroseus* and *N. rileyi* and decreased LC_{50} of the target insect (100 and $102x10^7$), respectively (Table 1). The increase in the pathogenicity of both *P. fumosoroseus* and *N. rileyi* combined with mustard oil to *B. incarnatus* 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 case of *Manduca sexata* treated with *M. anisopliae* and the chitin-synthesis inhibitor dimilin. Synergistic effects of a combined application of *B. bassiana* and the chloronicotinyl insecticide imidiaclopride on the curculionid *Diaprepes abbreviatus* were reported by Quintela and McCoy (1998). Also, Ibrahim *et al*. (1999) formulated Conidia of *M. anisopliae* in an aqueous or oil carrier. Oils appeared to extract fungistatic and stimulatory compounds from the insect cuticle. The conidia were probably deposited at sites which are conducive for germination and infection and consequently increased the overall mortality of insects. Destruxins and other toxins include the cyclic depsipeptides beauvericin (*V. lecanii*) and bassianolide (*B. bassiana*), which may function principally as endocellular ionophores, leucinostatins and efrapeptins (linear peptides from *Paecilomyces* and *Tolypocladium* spp., respectively) with antimicrobial activity and cytochalasins (*M. anisopliae*), which may paralyze host cells, Butter (1999). Lisansky (1989) mentioned that the LD_{50} for some formulations of *B. 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 inter segmental membranes, which are more readily penetrated by entomogenous fungi. Ali *et al.* (2005) found out dipicolinic acid as an insecticidal toxin from *P. fumosoroseus* and tested against whiteflies *Bemisia tabaci* and *B. argentifolii.*

Table 1. Probit analysis for estimating LC_{50} values of tested fungi alone and with tested oils

Microbial agent	Tested oil	LC_{50} [x 10^7]	Slope	Variance	95% confidence limits
	clove	143	1.4	0.001	121–166
	cumin	154	1.2	0.001	120–176
	mustard	100	1.3	0.002	88–156
P. fumosoroseus	alone	188	1.2	0.001	160–210
	clove	155	1.3	0.002	132–188
	cumin	166	1.2	0.001	189–210
	mustard	102	1.3	0.002	100–143
N. rileyi	alone	210	1.2	0.001	188–243
	clove	178	1.4	0.002	132–188
	cumin	186	1.3	0.003	123–177
	mustard	149	1.4	0.003	99–156
V. lecanii	alone	232	1.1	0.001	200–256

The persistence of mustard oil combined with tested fungi during storage

The persistent effect of formulated mustard oil with either *P. fumosoroseus* or *N. rileyi* fungi on foam covering gunny bags displayed several different modes of action by reducing oviposition and adult emergence (F1) of *B. incarnatus* (Table 2). The oviposition was completely inhibited when stored broad bean seeds were treated with mustard oil + *P. fumosoroseus* during 20, 40 and 60 days of storage. The % reduction values in the number of laid eggs and adult emergence after 120 days were 74.5 and 93%, respectively in case of mustard oil + *P. fumosoroseus.* Application of mustard oil combined with *P. fumosoroseus* on foam covering gunny bags provided promising oviposition deterrency, toxicity and suppressing *B. incarnatus* infestation, persistence and protecting broad bean seeds from beetles' infestation for 120 days during storage. Abd El-Aziz and Sharaby (1997) tested the effects of

Table 2. Effect of mustard oil combined with fungus on number of laid eggs/female and % of adult emergence (F1) of *B. incarnatus* beetles during storage periods of broad bean seeds

Storage Interval [days]	Check		Mustard + *P. fumosoroseus*		Mustard + *N. rileyi*	
	no. of eggs /♀±S.E.	% adult emergence (F1)	no. of eggs /♀±S.E.	% of adult emergence (F1)	no. of eggs /♀±S.E.	% of adult emergence (F1)
20	87.8±1.56	81.0	0.0±0.0 (100)*	0.0 (100)**	0.0±0.0 (100)	0.0 (100)
40	90.2±1.39	85.0	0.0±0.0 (100)	0.0 (100)	4.6±0.81 (94.9)	8 (99.52)
60	85.0±1.84	83	0.0±0.0 (100)	0.0±0.0 (100)	13.8±1.36 (83.8)	12 (97.65)
80	92.0±1.42	90	6±1.0 (92.9)	5 (99.6)	19.8±1.22 (78.5)	20 (95.2)
100	98.0±1.44	84	15±0.71 (83.7)	14 (97.5)	30.4±2.12 (69.4)	42 (84.49)
120	91.4±1.81	89	25 ± 1.3 (74.5)	23 (93)	42.2±1.43 (53.8)	60 (68.88)

*numbers between brackets represent percent reduction than control
**numbers between brackets represent percent reduction in adult emergence than control

white mustard oil on egg lying and egg masses viability of *Spodoptera littoralis*. Spraying cotton plants with 2.5% of oil caused reduction in egg laying. The moths laid only 7% of their egg masses and the percentage of repellency was 89.4%. At 2.5% conc., egg masses of different ages (24, 48 and 72 h old) were highly affected and the reductions were 66.6, 45 and 92%, respectively compared to the control. Compared with the investigation of Prakash (1982), white mustard oil was found to protect stored pulses against storage insects' infestation, especially the black gram and the green gram. Regnault-Roger and Hamraoui (1995) reported that eugenol, the main constituent of the essential oil of clove, also produced a strong inhibition of larval penetration of *Acanthoscelides obtectus* (Say) and finally a complete inhibition of emergence. Turcani (2001) experimented combinations of neemazal and Btk products against gypsy moth in oak stands. All combinations gave 100% mortality after three weeks of exposure.

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 of storage compared 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 *B. incarnatus* and this may be correlated to the chemical constituents of these oils. Application of mustard oil formulated with *P. fumosoroseus* on foam covering gunny bags provided promising oviposition deterrency, toxicity and suppressing *B. incarnatus* infestation, persistence and protecting broad bean seeds from beetles' infestation for 120 days during storage.

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POLISH SUMMARY

SKUTECZNOŚĆ WYBRANYCH BIOINSEKTYCYDÓW DO ZWALCZNIA *BRUCHIDIUS INCARNATUS* (BOH.) (COLEOPTERA: BRUCHIDAE) NA NASIONACH BOBU W OKRESIE PRZECHOWYWANIA

Przedstawiono ocenę działania roślinnych olejków eterycznych (kmin rzymski, goździkowiec korzenny, gorczyca) i grzybów (*Paecilomyces fumosoroseus*, *Nomurea rileyi*, *Lecanicillium* (*Verticilium*) *lecanii*) zastosowanych indywidualnie oraz jako formulacje przeciwko *Bruchidius incarnatus*. Na podstawie wyników testu wyboru stwierdzono, że olejki roślinne pochodzące z gorczycy i goździkowca korzennego wykazały silne repelentne działanie (odpowiednio 89 i 71%) na chrząszcze *B. incarnatus*, po upływie 7 dni. Olejek z kminu rzymskiego wykazał najsłabsze działanie, a procent śmiertelności był najniższy i wynosił 47%. Śmiertelność chrząszczy zwiększała się wraz ze wzrostem okresu ekspozycji. Najwyższy procent śmiertelności uzyskano w przypadku zastosowania olejku z gorczycy (76%) po 168 godzinach, a w dalszej kolejności olejku z goździkowca korzennego (63%). Najniższa śmiertelność chrząszczy (48%) wystąpiła po zasto-

sowaniu olejku z kminu rzymskiego. Olejek z gorczycy pozytywnie wpływał na żywotność grzybów *P. fumosoroseus* i *N. rileyi*, a jednocześnie powodował obniżenie wartości LC_{50} dla szkodnika (odpowiednio 100 i 102x10^7). Trwały skutek działania formulacji olejku gorczycowego z grzybem *P. fumosoroseus* lub *N. rileyi* zastosowanej na granule steropianowe pokrywające worki jutowe polegał zarówno na ograniczaniu procesu składania jaj, jak też pojawu osobników dorosłych (F1) chrząszczy *B. incarnatus*. Składanie jaj zostało całkowicie zahamowane kiedy przechowywane nasiona bobu traktowano olejkiem gorczycy + grzyb *P. fumosoroseus* w okresie 20, 40 i 60 dni przechowywania. Zastosowanie olejku gorczycowego w kombinacji z grzybem *P. fumosoroseus* na granule styropianowe spowodowało ograniczenie składania jaj i nasilenia występowania szkodnika oraz zapewniło trwałą ochronę nasion bobu przed *B. incarnatus* w ciągu 120 dni przechowywania.