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Bioassay of *Beauveria bassiana*, three plant oils and five biorational molecules against *Callosobruchus chinensis* L. in chick pea

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Abstract
The present research was conducted to study the toxic effect of entomopathogenic fungus *Beauveria bassiana*, three essential plant oils i.e. neem (*Azadirachta indica* A. Juss), mahogany (*Swietenia mahagoni* L.), and karanja oil (*Millettia pinnata* L.) and five biorational pesticides (spinosad, abamectin, emamectin benzoate, buprofezin and lufenuron) against adult *Callosobruchus chinensis* L. under laboratory conditions from January to October, 2016. *Beauveria bassiana* was found highly infectious and caused significant mortality of adult *Callosobruchus chinensis* populations after 24 hours of treatment application. Among the plant oils, karanja oil was found more toxic (LC_{50} values were 0.139, 0.093 and 0.075% at 24, 48 and 72 hours after treatment application respectively) in laboratory bioassay compared to neem and mahogany oil. In case of biorationals, abamectin showed highest toxicity (LC_{50} values were 0.081, 0.047 and 0.025% at 24, 48 and 72 hours after treatment application respectively) which was followed by emamectin benzoate and spinosad. However, *Callosobruchus chinensis* showed lowest susceptibility to buprofezin and lufenuron, two potential chitin synthesis inhibitors. The results of the present study clearly revealed that all the selected treatments could be successfully used as a grain protectant against *Callosobruchus chinensis* considering their LC_{50} estimates.

Keywords: *Callosobruchus chinensis*, *Beauveria bassiana,* bioassay, plant oils, biorational pesticides

1. Introduction
The pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is a serious storage pest of different pulse grains in the tropics [26] because of the favorable climatic conditions for their proliferation [4]. It is mostly destructive for chickpea, mung, cowpea, lentil and pigeon pea and damage of legume seeds may be up to 100% during storage [7]. The adult female lays eggs on the seed surface and the hatching larvae burrow into the seed, thus lead to damage grains as well as deteriorate nutritional value and germination capacity [26].
Stored grain pests are usually controlled by using different synthetic chemicals specially methyl bromide and aluminum phosphide. However, most of these synthetic pesticides are less biodegradable and they induce resistance development in target pests [28]. Commonly used organophosphorus pesticides such as malathion and dichlorvos have caused residual toxicity and pyrethroids have been reported to have carcinogenic effects [9, 21]. Moreover, synthetic fumigants like methyl bromide and phosphene have been reported to be responsible for ozone layer depletion [13].
The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin has been reported to be effective against many insect pests including aphids, leafhoppers, and whiteflies [8, 24]. However, very little attention has been paid to the use of *B. bassiana* against stored grain pests' specifically *C. chinensis* populations [23].
Different botanicals which are effective, eco-friendly and biodegradable are often inexpensive and have been realized as rational alternatives to synthetic pesticides for controlling pulse beetle [3, 13]. In addition, essential plant oils and their constituents have been shown to be a potent source of botanical pesticide. Many essential plant oils and their chemical constituents have been studied against different bruchid pests [14]. However, the information on bioassay of neem, mahogany and karanja oil against pulse beetle population of Bangladesh was rarely found.
Insect growth regulator (IGR) is a class of bio-rational compounds that adversely affects insect growth and development. Buprofezin and lufenuron, which are potential chitin synthesis inhibitors, were found effective in controlling many insect pests including storage pests [5, 11].

Moreover, some biorational molecules such as spinosad, abamectin and emamectin benzoate are considered as most promising alternatives to currently used pesticides in stored-products' protection [2, 11].

Hence, the present study was aimed to evaluate the toxicity of *B. bassiana*, three essential plant oils and four biorational compounds against *Callosobruchus chinensis* under laboratory conditions.

2. Materials and methods

2.1 Test insect rearing

Callosobruchus chinensis adults were collected from naturally infested chickpea seeds and reared on uninfested chickpea (*Cicer arietinum* L.) grains. The cultures were maintained in a controlled environment chamber under a 12/12 h light: dark photoperiod at 27±2 ºC and 80±5% relative humidity (RH). All experiments were carried out under the same environmental conditions in the Department of Entomology, Bangladesh Agricultural University, Mymensingh from January to October, 2016. Adult beetles (males and females), 2–3 days old were used for bioassay.

2.2 Specification of the treatments

The entomopathogenic fungus, *Beauveria bassiana* 1.15 WP (1×10^8 conidia/ml) was supplied by Ispahani Biotech Ltd., Bangladesh. Commercially available formulations of three essential plant oils namely neem oil (*Azadirachta indica* A. Juss), mahogany oil (*Swietenia mahagoni* L.) and karanja oil (*Millettia pinnata* L.) were collected from a local company. Five bio-rational pesticides were selected and collected from the dealer shop of which, spinosad (Libsen 45 SC) and abamectin (Benten 1.8 EC) were marketed by Asia Trade International, lufenuron (Heron 5 EC) and emamectin benzoate (Suspend 5 SG) were marketed by Haychem (Bangladesh) Ltd., and buprofezin (Award 40 SC) was marketed by Square Pharmaceuticals Ltd.

2.3 Bioassay procedures

Five concentrations of each of the selected treatments (*Beauveria bassiana,* three essential plant oils and five biorational pesticides) were sprayed thoroughly in 100 g of chickpea seeds using a hand sprayer. The control group was treated with distilled water. For all treatments, preliminary concentration-mortality experiments were conducted to select the concentration range producing 5-95% mortalities (data not shown). After spraying, these treated seeds were allowed to air dry and finally kept at 25 ± 2°C temperatures, 80 ± 5% RH and 16L: 8D photoperiod in an incubator. Ten 2-3 days old adults were used in each replicate and released into Petri dish (90 mm diameter) with treated pulse seeds. There were three replicates for each of the treatments and the number of dead beetles was recorded after 24, 48 and 72 hours of treatment application respectively. Mortality was considered when the beetle did not respond to gentle pressure using a fingertip. After each counting, dead pulse beetles were removed.

2.4 Statistical analysis

The percentage of mortalities was corrected using Abbott's formula [1]. The median lethal values (LC_{50}) values were determined by Probit analysis. Statistical differences between LC_{50} estimates were determined by using a 95% CI for the ratio of two estimates.

3. Results

3.1 Toxicity of *Beauveria bassiana*

The response of *Callosobruchus chinensis* adults to *B. bassiana* is presented in the Table 1. The LC_{50} values of *B. bassiana* against *Callosobruchus chinensis* were 0.356, 0.211 and 0.189% after 24, 48 and 72 hours of treatment (HAT) respectively (Table 1). The death of *Callosobruchus chinensis* caused by the infection of *B. bassiana* initiated after 24 hours of fungal application and the morality was completely concentration dependent i.e. mortality increased with the increase in conidial concentration, being highest at the highest dose. It was observed that the infected adults were inoperative having less movement and showed mycotic symptoms with slight body discoloration (data not shown).

Table 1: LC_{50} values (%) of *Beauveria bassiana* against adult *Callosobruchus chinensis*

Test Materials	24 Hours				48 Hours				72 Hours			
	LC_{50} 95% CI	Slope±SE	χ^2	df	LC_{50} 95%CI	Slope± SE	χ^2	df	LC_{50} 95%CI	Slope±SE	χ^2	df
Beauveria bassiana	0.356 (0.213-0.471)	1.737±0.439	0.213	3	0.211 (0.081-0.307)	1.723±0.455	0.267	3	0.189 (0.076-0.272)	1.929±0.477	1.823	3

Different letters within a column indicate significant difference (based on overlapping confidence intervals; $P<0.05$); df stands for degrees of freedom

3.2 Toxicity of plant oils

The toxicity of three essential plant oils against adult *C. chinensis* is presented in Table 2. The results revealed that one day after treatment application, karanja oil showed highest toxicity and resulted in lowest LC_{50} value (0.139%) which was followed by neem (0.156%) and mahogany oil (0.174%) respectively. However, with increasing time length, both neem and mahogany oil showed better toxicity and the LC_{50} value became close to karanja oil. After 72 hours of treatment application, there were no significant differences among the toxicity of all three plant oils and the LC_{50} values were almost similar (Table 2).

Table 2: LC_{50} values (%) of essential plant oils against adult *Callosobruchus chinensis*

Test Materials	24 Hours				48 Hours				72 Hours			
	LC_{50} 95%CI	Slope± SE	χ^2	df	LC_{50} 95%CI	Slope± SE	χ^2	df	LC_{50} 95%CI	Slope± SE	χ^2	df
Neem oil	0.156 a (0.117-0.325)	1.661±0.510	0.453	3	0.105 a (0.075-0.152)	1.653±0.500	0.468	3	0.078 a (0.043-0.105)	1.624± 0.501	0.207	3
Mahogany oil	0.174 a (0.128-0.412)	1.664±0.515	0.546	3	0.113 a (0.082-0.176)	1.600±0.499	0.125	3	0.079 a (0.042-0.107)	1.552± 0.499	0.419	3
Karanja oil	0.139 a (0.106-0.236)	1.764±0.508	0.577	3	0.093 a (0.060-0.128)	1.605±0.498	0.029	3	0.075 a (0.044-0.099)	1.757± 0.506	1.093	3

Different letters within a column indicate significant difference (based on overlapping confidence intervals; $P<0.05$); df stands for degrees of freedom

3.3 Toxicity of biorational pesticides

The bioassay revealed that there were significant differences in the toxicity of biorational pesticides against adult *C. chinensis* (Table 3). After 24 hrs of treatment application, abamectin showed highest toxicity (0.081% LC_{50}) which was consistent after 48 hrs (0.047% LC_{50}) and 72 hrs (0.025% LC_{50}) of treatment application. Emamectin benzoate and abamectin were moderately toxic against *C. chinensis* adult in all three days after treatment application. However, two insect growth regulators, buprofezin and lufenuron showed comparatively lower toxicity having highest LC_{50} estimates. Although buprofezin showed slightly more toxicity than lufenuron, they were statistically indifferent.

Table 3: LC_{50} values (%) of biorational pesticides against adult *Callosobruchus chinensis*

Test Materials	24 Hours				48 Hours				72 Hours			
	LC_{50} 95%CI	Slope± SE	χ^2	df	LC_{50} 95%CI	Slope±SE	χ^2	df	LC_{50} 95%CI	Slope±SE	χ^2	df
Spinosad	0.163 ab (0.124-0.335)	1.897±0.566	0.036	3	0.095 bc (0.074-0.118)	2.310±0.550	0.495	3	0.055 ab (0.029-0.071)	2.187±0.572	1.033	3
Abamectin	0.081 a (0.059-0.127)	1.603±0.447	0.677	3	0.047 a (0.031-0.062)	1.842±0.442	0.511	3	0.025 a (0.009-0.037)	1.724±0.459	3.862	3
Emamectin benzoate	0.107 ab (0.085-0.151)	2.169±0.642	0.617	3	0.072 ab (0.048-0.089)	2.350±0.640	0.873	3	0.052 ab (0.024-0.068)	2.233±0.656	1.873	3
Buprofezin	0.180 b 0.132-0.479	1.788±0.567	0.080	3	0.123 c 0.094-0.202	1.753±0.541	0.553	3	0.078 b 0.049-0.101	1.857±0.539	2.899	3
Lufenuron	0.194 b 0.140-0.595	1.787±0.574	0.220	3	0.129 c 0.092-0.186	1.813±0.542	0.306	3	0.091 b 0.062-0.122	1.780±0.536	0.435	3

Different letters within a column indicate significant difference (based on overlapping confidence intervals; $P<0.05$); df stands for degrees of freedom

4. Discussion

4.1 Effects of *Beauveria bassiana*

Microbial pathogen *Beauveria bassiana* was found highly infectious against adult *C. chinensis* and causing significant mortality. Several previous studies also documented that *B. bassiana* is capable of penetrating through the insect cuticle, secreting cuticle degrading enzymes [6, 20], being effective towards several bruchid pests [27]. Therefore, fungal pathogen *Beauveria bassiana* could be considered for protecting stored grains from *C. chinensis* populations and adoption of this safe and nonchemical method can potentially benefit the environment.

4.2 Effects of plant oils

All the plant oils (neem, mahogany and karanja) were found effective in suppressing *C. chinensis* adults and they significantly protected chickpea grains from infestation. The death of insects might be recognized in regard to the contact toxicity or to the abrasive effect on the pest cuticle which might also interfere with the respiratory mechanism of insects [16]. The mode of action studies on natural plant oils in insects has largely focused on monoterpenoid and several reports indicated that monoterpenoids cause insect mortality by inhibiting acetylcholinesterase enzyme (AChE) activity [10].

In the present study, the exposure period appeared to be one of the most important factors affecting the toxic effects of the tested oils other than the dosages. Several previous studies also revealed that exposure period and dosages are two crucial factors for the activity of plant oils [18, 22]. Among three essential plant oils, karanja and neem oil showed comparatively better efficacy than mahogany oil up to 96 hrs after treatment application. This difference of toxicity among the oils may be due to differences in the chemical composition of the oil, which in turn depends on the source, season and ecological conditions, method of extraction, time of extraction and plant part used [17].Our result clearly revealed that all three essential oils (neem, mahogany and karanja) could be used as an alternative to develop less toxic treatment systems in protecting chickpea seeds from *C. chinensis* invasion.

4.3 Effects of biorationals

Among the biorationals, abamectin, emamectin benzoate and spinosad showed more toxicity compared to two insect growth regulators, buprofezin and lufenuron. Rao and Subbaratnam had reported that the toxicity of chitin synthesis inhibitors increases with the period of exposure [25] but as the present study was confined to only 72 hours of exposure, the toxicity was found lower in case of both buprofezin and lufenuron. Although most of the IGRs are safe for the storage systems, they have not been widely adopted for stored grain when compared with traditional residual contact insecticides and fumigants, probably because of cost and lack of such immediate knockdown properties. In this study, abamectin was the most toxic among the biorational pesticides tested against the adults of *C. chinensis*. Abamectin attacks the nervous system of insects causing paralysis within hours and the paralysis cannot be reversed. Hussain and Ashfaq also reported that abamectin was comparatively more toxic than spinosad and indoxacarb to both malathion-resistant and organophosphates-susceptible strains of *T. castaneum* after 48 hours of treatment through residual film method [12].

However, effectiveness of emamectin benzoate and spinosad observed in the present investigation are in agreement with the findings of several previous studies which showed that different formulations of spinosad (liquid or dust) and emamectin benzoate could be effective against several stored-product pests especially on resistant strains of the pests [15, 19, 29]. However, our results indicated that effectiveness of biorational molecules against *C. chinensis* depends on treatment rates and intervals exposed to it [29].

5. Conclusion

The findings of the present study showed that *Beauveria bassiana* was highly effective in controlling *Callosobruchus chinensis* population however it requires a higher conidial concentration for the development of mycosis and causing significant mortality. Among the selected plant oils karanja oil was found most effective which was closely followed by neem and mahogany oils. Moreover, abamectin was the best treatment among the biorational molecules which was followed by emamectin benzoate, spinosad, buprofezin and lufenuron respectively.

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