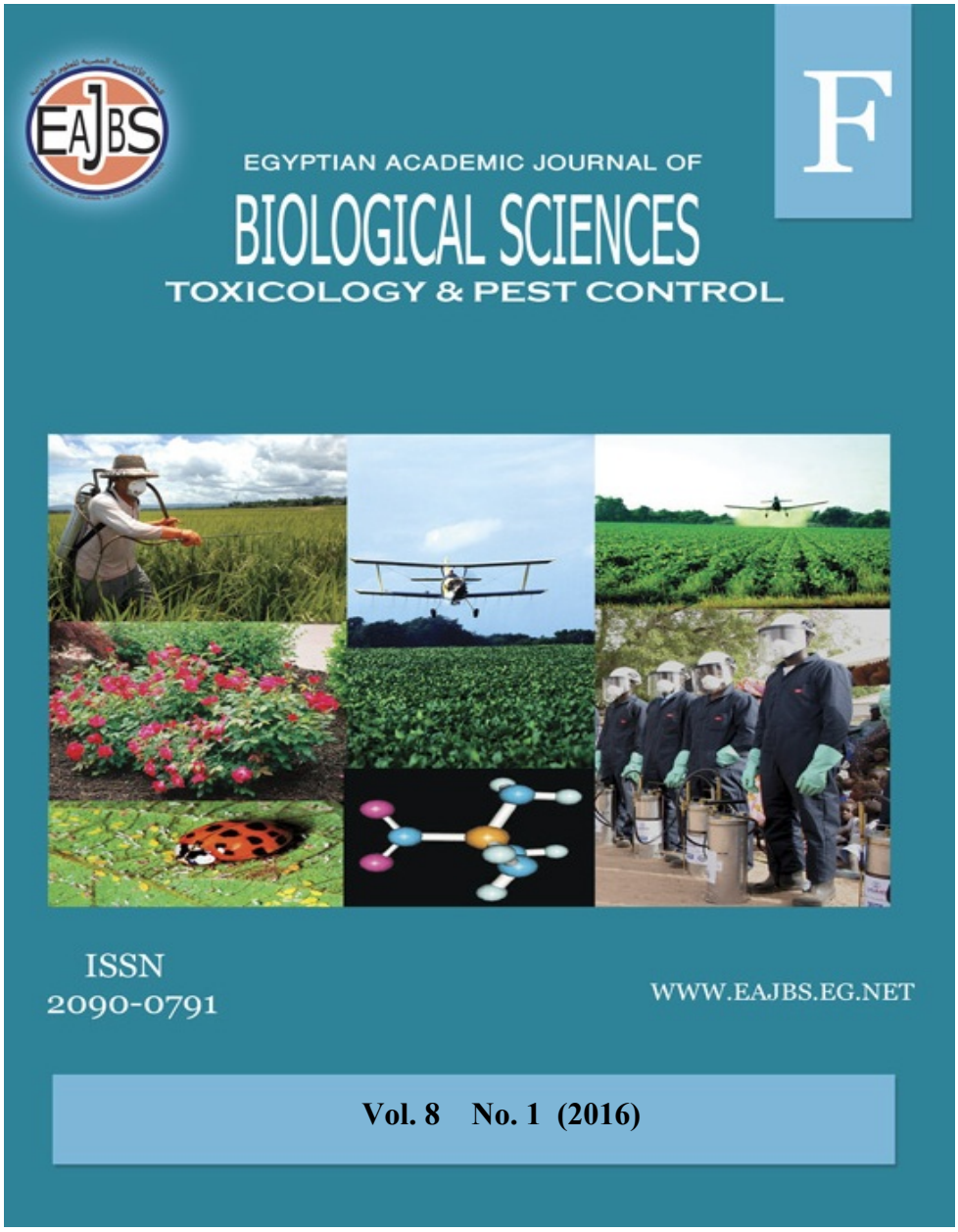

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www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 8(1): 65-72 (2016)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest control
ISSN: 2090 - 0791
www.eajbs.eg.net

Effectiveness of *Beauveria bassiana* applied alone or incombination with diatomaceous earth as protectants of wheat grains against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae)

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ARTICLE INFO

Article History
Received:1/3/2016
Accepted:10/4/2016

Key words:
Beauveria bassiana, *Rhyzopertha dominica*, wheat grains

ABSTRACT

Laboratory bioassays were conducted to assess the insecticidal efficacy of the formulated fungi, *Beauveria bassiana* used alone or in combination with a formulated diatomaceous earth (DE) against adults of *Rhyzopertha dominica* on wheat grains. Mortality increased with increasing powder concentrations and DE was more effective than *B. bassiana*. Exposure of *R. dominica* adults to wheat grains treated with mixtures of *B. bassiana* and DE resulted in higher mortality values than each powder alone at various concentrations. When eggs were treated with LC_{50} of each powder alone a significant decrease in adult emergence was achieved. The $LC_{95}$of the two powders caused complete inhibition of adult emergence. The mixture of the two powders caused complete inhibition in adults emergence except in case of LC_{25} mixtures which caused about 80% reduction in progeny production. In most cases, *B. bassiana* and DE treatments did not affect germination of wheat grains as indicated by germination tests.

INTRODUCTION

In Egypt, the percentage of loss of wheat due to stored insect pests was estimated as equivalent to half a million tons per year of which 10% was caused by the lesser grain borer alone (Ministry of agriculture and land reclamation report, Egypt2007). Lesser grain borer, *Rhyzopertha dominica*, is a universal insect. Both adults and larvae feed on whole cereal grains, either externally or inside the grain leaving powdered grains.

One of the basic methods for stored-grain protection is the direct application of contact insecticides. However, insecticidal residues are of concern for human health and their extensive use is hazardous for the environment. This had led to search for a safer and less expensive alternative from various sources.

The entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes) can be used as an alternative to traditional grain protectants.

They have low mammalian toxicity and are very effective against stored-grain insect pests (Wakil and Ghazanfar 2010; Wakil *et al*. 2011). Another potential alternative asgrain protectant is the use of desiccant dusts; particularly diatomaceous earths (DEs).These substances have low mammalian toxicity and can be effective against a wide range of stored-grain insects (Subramanyam and Roesli 2000; Wakil *et al*. 2010). Since both fungi and DEs act on the insect cuticle, their combined use has been proposed as a mean of reducing application rate against certain species of stored-grain insects (Waseem *et al.* 2004; Michalaki *et al.* 2006). The objective of the present study was to evaluate the potential of *B. bassiana* and DE and their combination as protectants to wheat grains against infestation by *R. dominica*.

MATERIALS AND METHODS

Insect, Fungus and DE

All experiments were conducted at the Stored Products Department, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt. A laboratory colony of *Rhyzopertha dominica* was used in this study. Commercially produced conidia of *B. bassiana* strain Botani Gard 22WP, Emerald Bio Agriculture, Butte, MT., USA. The *B. bassiana* technical powder used contained 4.5×10^{10} conidia pergram, DE formulated with 97% silicon dioxide (Insecolo Hedley Pacific Ventures Ltd., Canada), and Egyptian wheat (Sakha 93) with a moisture content of12% were used in all experiments.

Effect of *B. bassiana* and DE on adults of *R. dominica*.

Twenty five adults (1-2 week old) were placedin glass tubes (1x7.5 cm) and exposed to discriminating concentrations of *B. bassiana*, DE. The treatments were mixed thoroughly withten grams of grain by manually shaking the test tubes. Each treatment and the control were replicated3times. The experimental units were placed in an incubator under constant conditions ($28^{o}C\pm2^{o}C$, 60%±5% RH). After 3 d, adult mortality was recorded. Insect mortalities were assessed and corrected by the formula of Abbott (1925). Probit analysis (Finney 1971) was used to estimate the LC_{25}, LC_{50}, LC_{75}, and LC_{95}. Percentage of mortality was plotted versus the corresponding concentrations using LDP line software program to obtain the toxicity regression lines. The previous steps were repeated with DE fungal combinations at concentrations of LC_{25}, LC_{50} and LC_{75} of *B. bassiana* + DE for each of them.

Effect of *B. bassiana* and DE on eggs of *R. dominica*.

Three replicates each of 10 eggs of *R. dominica* were placed in tubes containing 10g of grains treated with LC_{50} and LC_{95} of: (1) *B. bassiana*, (2) DE and (3) *B. bassiana* + DE at concentrations indicated in Table 2; (4)untreated control. All tubes were secured with plastic cover and incubated at $28^{o}C\pm2^{o}C$ and 60% ±5% RH for 8 weeks to determine the number of emerged adults. Percentage reduction in progeny production relative to the control was calculated.

Effect of *B. bassiana* and DE on germination of wheat grains.

Germination test of the treated grains was carried out using the rolled paper towel test, according torules of the Association of Official Seed Analysts test procedures **A**.O.S.A (1981) for small grains. The test was done at initial and after 12 weeks storage interval of grains treated by $LC_{50}$and LC_{95} of *B. bassiana*, DE and their mixtures. 25 wheat grains were taken and four replicates were used. Final germination percentage (FG) was calculated using the following formula:

$$\frac{\text{Number of germinated grains on final day}}{\text{Total number of germinated grains}} \times 100$$

The germination Energy (GE), the percentage of germinated seeds at the 4th day of planting to the total number of tested seeds was calculated according to Ruan *et al*. (2002).

The significance of viability (SV)was calculated using the following formula:

$$SV = \frac{\text{Percent germination in treated grains}}{\text{Percent germination in the control}}$$

The root and shoot lengths were measured at 7th day of germination and expressed in millimeters. Then the root/shoot ratio was calculated.

The vigor index was calculated using the following formula:

(VI) = [FG (%) x seedlings length (mm)]/100.

Where, FG = final germination.

Seedlings length= root length + shoot length (Abdul-Baki and Anderson 1970).

Statistical analysis:

Data were analyzed using one-way analysis of variance (ANOVA), means were compared using the Duncan multiple range tests at 0.05% probability level (Duncan 1955) by SAS software (SASI nstitute 9.1, 2006).

RESULTS AND DISCUSSION

Effect of *B. bassiana* and DE on adults of *R. dominica*.

Results presented in Fig. 1, indicated a gradual increase in the mortality of adults with increasing powder concentrations. Dose-dependent mortality was also observed on *R. dominica* and *Sitophilus oryzae* (Baldassari and Martini 2014) and larvae of *Tribolium castaneum* (Waseem *et al.* 2004).

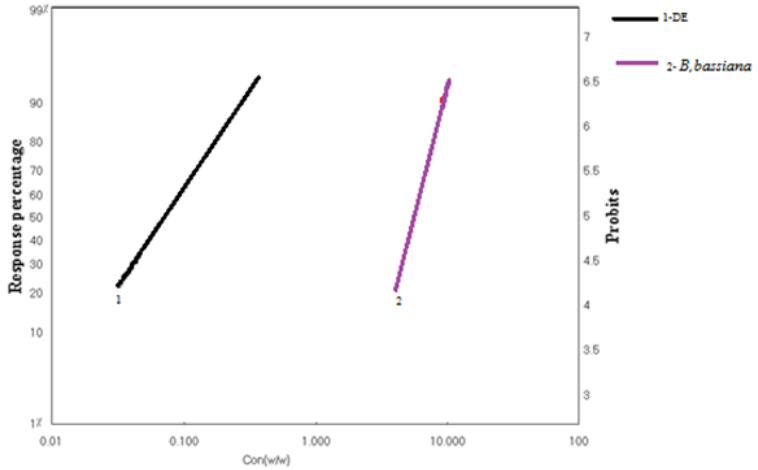

Fig. 1: Log dose-probit mortality lines for *Beauveria bassiana* and diatomaceous earth treatments of *R. dominica* beetles on wheat grains three days post exposure

The calculated LC_{25}, LC_{50}, LC_{75}, and LC_{95}were shown in Fig. 1. After three days of adult exposure, DE was the more effective regarding adult mortality (LC_{50}= 0.08 w/w) than *B. bassiana* (LC_{50}= 5.71 w/w). The highest efficacy of DE is probably due to its silica content. It is known that silica content is one of an array of factors that determine the efficacy of a particular DE (Korunic1997). Rojht *et al*. (2010) reported that silica levels in DE have been found to correlate significantly with efficacy in DE bioassays. Also the small size and the more uniform particles play an important role in the higher efficacy of DE. Smaller particles have greater surface to volume ratio, resulting in increased area of contact between particles and insect cuticle (Korunic1998).

Results in Table 1 indicated that mixing wheat grains with the tested powders resulted in pronounced potentiating effects on adult mortality after three days of exposure. The mortality increased by increasing the concentration of DE in the mixtures, accordingly the mortality ranged from 89.3% to 99%.A synergistic interaction between the effect of *B. bassiana* and DE was observed. These results are in agreement with those obtained on different insect species (e.g. Lord 2001; Waseem *et al.* 2004; Batta 2008).

Table 1: Mortality of *R. dominica* adults at various concentrations of *B. bassiana* and DE three days post exposure.

Treatments	% Adults mortality
LC_{25} *B. bassiana* + LC_{25} DE	89.3
LC_{25} *B. bassiana* + LC_{75} DE	99
LC_{50}*B. bassiana* + LC_{50} DE	93.3
LC_{75} *B. bassiana* + LC_{25} DE	89.3

The suggested mode of action of diatomaceous earth on target insects is the desiccation effect by abrading the cuticle due to contact with the sharp-edged silica (silicon dioxide) particles of the dusts following application, so that the wax layers of insect cuticle is destroyed, leading to desiccation(water loss) then death of treated insects (Mewis and Ulrichs 2001). Moreover, the fundamental reasoning of enhanced action of both diatomaceous earth and *B. bassiana*is due to their physical mode of action against *R. dominica*. In this work the efficacy of *B. bassiana* was enhanced by the addition of diatomaceous earth, which facilitated penetration into the abraded cuticle of *R. dominica*. This was also indicated by Lord (2005) for *R. dominica* and Michalaki *et al.* (2006) for *Tribolium confusum* working at similar experimental conditions.

Effect of *B. bassiana* and DE on eggs of *R. dominica*.

Results in Table 2 showed the extended effect of DE and *B. bassiana* for the control of the *R. dominica* eggs expressed as number of adult emerged. A high significant difference between the untreated control and the treated samples in the mean number of adult emergence (at LC_{50} and LC_{95}) and their mixtures. A negative correlation between the concentrations of the tested powders and the number of emerged adults at LC_{50} and LC_{95} was also observed. At LC_{50}, *B. bassiana* and DE yielded 0.17 and 0.03adults, respectively, compared to 0.67 progeny for the control. DE caused the highest reduction (95.5%) in the progeny and *B. bassiana* caused 74.6% reduction (Table 2). Similar results were reported by Athanassiou *et al*. (2003). Furthermore, both *B. bassiana* and DE caused complete inhibition in adult emergence at the LC_{95} level.

All tested mixtures were able to prevent adult emergence completely, except the mixtures of the LC_{25} of both *B. bassiana* and DE (Table 2). The present results indicate that, in most cases, progeny production was not totally suppressed, but was notably reduced three days after exposure. A possible explanation might be due to that DE and *B. bassiana* have ovicidal effects. This findingis in agreement with El Halfawy *et al.* (1977) who reported reduced hatchability of eggs of *R. dominica*, and *Callosobruchus chinensis* when the eggs were exposed to lightly inert dusts. The reduced progeny production was due to eggs failing to hatch on inert dust-treated grains. Tested powders probably affected the eggs by penetration of the egg integument before hatching. These findings are in agreement with those obtained by Rodriguez-rueda and Fargues (1980) whore ported that the contamination of newly hatched larvae

might be caused either by fungal penetration of the egg integument before hatching, or by the conidia on the egg cuticle serving as an inoculums for emerging larvae, which feed upon the chorion.

Table 2: Effect of various concentrations of *B. bassiana* and DE on the eggs of *R. dominica.*

Treatments	Mean no. of emerged adults	%Reduction in progeny
B. bassiana LC_{50}	0.17^{b}	74.6
LC_{95}	0	100
LC_{25} *B. bassiana* + LC_{25} DE	0.13^{b}	80.6
LC_{25} *B. bassiana* + LC_{75} DE	0	100
LC_{50} *B. bassiana* + LC_{50} DE	0	100
LC_{75} *B. bassiana*+ LC_{25} DE	0	100
Control	6.5^{a}	0

Means with the same letter are not significantly different (Duncan's multiple test at $p<0.05$).

Effect of *B. bassiana* and DE on germination of wheat grains.

To observe the adverse effect of tested powders on the quality of wheat grains, germination test of the treated grains was carried out separately at initial and after 12 weeks storage (Table 3). Generally, *B. bassiana* and DE treatments did not show significant interaction in germination tests of wheat grains. The reduced viability observed at initial application never exceeded 0.01% of the control. In consistence with these results, El-Khayat (2000) stated that *B. bassiana* showed no effect on wheat grains germination. In this study no treatments were deleterious to plant growth (root length, shoot length, and vigor index); with exceptions of stunting of root length was significant in grains treated previously with LC_{50} of *B. bassiana* and DE at the initial application (Table 3). Insignificant increase in germination parameters based on shoot length, root /shoot ratio and the vigor index compared to control were observed at initial application.

Table 3: Effect of various concentrations of *B. bassiana* and DE on germination parameters of wheat grains at the initial time andafter the storage periods.

Treatments	Germination parameters													
	Initial time							After storage						
	FG (%)	GE (%)	SV	RL mm	SHL mm	R/SH ratio	VI	FG (%)	GE (%)	SV	RL mm	SHL mm	R/SH ratio	VI
B. bassiana LC_{50}	95^{ab}	87^{ab}	0.99^{b}	1986.3^{b}	1501.5^{bc}	1.3^{b}	827.5^{c}	NA	NA	NA	NA	NA	NA	NA
LC_{95}	99^{a}	93^{ab}	1.03^{b}	3110^{a}	2372.3^{a}	1.3^{b}	1357.5^{a}	100^{a}	71^{b}	1.14^{a}	2051^{a}	1783^{a}	1.2^{b}	95.9^{a}
DE LC_{50}	98^{a}	92^{ab}	1.02^{b}	2110^{b}	1761.5^{bc}	1.2^{bc}	948^{bc}	NA	NA	NA	NA	NA	NA	NA
LC_{95}	96^{ab}	92^{ab}	1^{b}	3019.3^{a}	2392.5^{a}	1.3^{bc}	1300.2^{a}	99^{ab}	96^{a}	1.13^{a}	2209.5^{a}	1575.3^{b}	1.4^{ab}	93.8^{a}
Control	79.8^{b}	1.5^{a}	1294.8^{c}	1929.8^{a}	1.13^{a}	88^{a}	99^{ab}	420.7^{e}	0.23^{a}	1431^{c}	3197.5^{c}	1^{a}	89^{ab}	96^{ab}
LC_{25} *B. bassiana* + LC_{75} DE	95^{ab}	92^{a}	0.99^{a}	1693.5^{ad}	1173^{b}	1.2^{a}	763.3^{cd}	95^{a}	91^{a}	0.99^{a}	3092.3^{ab}	1774.8^{cd}	1.4^{a}	870.2^{bc}
LC_{50} *B. bassiana* + LC_{50} DE	94^{ab}	91^{a}	0.93^{a}	1818.5^{ad}	1045.5^{b}	1.1^{a}	635.6^{cd}	98^{a}	96^{a}	0.98^{a}	3116.8^{ab}	1946.5^{bc}	1.5^{abc}	983.05^{ab}
LC_{75} *B. bassiana* + LC_{25} DE	95^{ab}	94^{a}	0.96^{a}	2113.3^{a}	1357.3^{ab}	1.7^{a}	665.9^{c}	98^{a}	92^{a}	0.99^{a}	3572.5^{a}	2128.3^{b}	1.2^{ab}	966.2^{a}
Control	98^{a}	93^{a}	1^{a}	1445.5^{d}	1398^{ab}	1.05^{a}	696.5^{cd}	97^{a}	96^{a}	1^{a}	2885^{b}	1828^{bcd}	1.6^{abc}	1146.3^{bc}

* Storage period: 12 weeks.

FG, final germination.GE, germination energy. SV, Significance of viability.RL, root length.SHL, shoot length.

R/SH, root and shoot ratio.VI,vigor index.NA, Not applicable.

Means with the same letter are not significantly different (Duncan's multiple test at $p<0.05$).

In general, *B. bassiana* and DE treatments did not show significant decrease in germination tests of wheat grains; *B. bassiana* at LC_{95} showed a significant reduced level in the germination energy (71%) and root/shoot

ratio compared to control (88%), after 12 weeks storage. Significant increase in shoot length and vigor index was observed. These results corroborates those obtained by Michelle *et al.* (2011) who found that, fungi improved wheat grains germination and energy of germination. Mazzuferi *et al.* (2006) and Simioni *et al.* (2008) reported that, no adverse effect due to DE powder on the germination, quality or vigor of maize and wheat grains was observed.

Effective levels of the mixtures of *B. bassiana* with DE generally (Table 3) improved all germination parameters. However, *B. bassiana* at LC_{75} and DE at LC_{25} showed a significant reduced level in the vigor index compared to control after 12 weeks storage. In general, the results of the present study on germination parameters and plant growth demonstrate that *B. bassiana* and diatomaceous earth used in this investigation can effectively safe guard early growth stages of wheat grains from *R. dominica*. This was also indicated by Savitri *et al.* (1994) when they used the combination strategy of fungicides and insecticides.

It is noteworthy to mention that the combination of *B. Bassiana* and DE enhanced their performance as insect control agents by reducing the application rate of each one when applied in a combined form for the control of insects.

The current study demonstrates the potential and future perspective for inert dusts and bioproducts in IPM strategies for the control of *R. dominica* on wheat.

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ARABIC SUMMERY

فاعلية تطبيق بوفاريا باسيانا بمفردها او بالخلط مع التربة الدياتومية كمواد واقية لحبوب القمح ضد حشرة ريزوبرثا دومينيكا

عبير عمر بيومى أبوطالب[١] ،سلوى مصطفى سيد أحمد[١] ،رجاء قطب عبد الجابر حامد[٢] ،عبد الموجود عبد الله عسران[١] , بهيرة محمود الصواف[٢]

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تم اختبار فاعلية كلا من فطر بوفاريا باسيانا والتربة الدياتومية كل علي حدا او بخلطهما بتركيزات مختلفة ضد الحشرة الكاملة لثاقبة الحبوب الصغر يريزوبرثا دومينيكا. واظهرت النتائج الي وجود علاقة طردية بين النسبة المئوية لاماتة الآفة وكلا من تركيز المادتين ومخاليطهما. واظهرت الدراسة ان المخاليط قد اعطت نسب اماتة تفوق نسب الاماتة الناتجة عن كل منهما منفردافي جميع التركيزات المستعملة.

وتم عمل دراسة علي البيض لدراسة تأثير التركيز نصف المميت ٥٠ % (LC_{50}) والمميت ٩٥% (LC_{95}) للمواد منفردة ومخاليطهما علي عدد الخلفة فى الجيل الأول.وقد أظهرت النتائج إنخفاض معنوى ملحوظ فى خروج الحشرات البالغة من الجيل الأول عند استخدام المخاليط. وأظهرت النتائج عدم وجود تأثير يذكر لكل من التركيز نصف المميت ٥٠ % (LC_{50}) والمميت ٩٥% (LC_{95}) للمواد ومخاليطهما علي عوامل الانبات ونمو القمح باستثناء المخلوط المكون من التركيز LC_{75} لفطر البوفاريا والتركيز LC_{25} للتربة الدياتوميه.