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The Use of Entomopathogenic Fungus, *Beauveria bassiana* (Bals.) Vuill. in Assays with Storage Grain Beetles

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ABSTRACT

Chemical insecticides have been widely employed for the control of storage grain pests. This has caused such problems as insecticide resistance along with contamination of foodstuffs with chemical residues. Thus, there is a growing interest in using pathogenic control agents as alternative. In this study, the potential of *Beauveria bassiana* (*Bb*Weevil™, a commercial product containing 2×10^9 conidia g^{-1}) was evaluated against adults of *Tribolium castaneum*, *Sitophilus granarius* and *Oryzaephilus surinamensis*. The experiments were carried out at the rates of 0, 250, 500, 750 and 1,000 mg kg^{-1} and exposure intervals of 5, 10 and 15 days, in 24±2°C and 50±5% r.h. Fifteen 1 kg lots of grain (one lot for each exposure time-rate) were prepared and treated with the appropriate predetermined doses. Four 50 g samples of each were taken as replications and placed in glass vials. Thirty 1-7 day old adults were introduced into each glass vial. Following mortality count in each exposure time, the adults (dead and alive) were removed and the vials left in the same conditions for a further 45 days to have the progeny production assessed. Means were separated by employing Tukey´s Test (P= 0.05). All main effects (dose, exposure time and insect species) as well as associated interactions were significant (P< 0.01), with the exception of exposure time×insect, which was not significant. In all the experiments, mortality increased with increase in dose rates and exposure time with the highest mortality being observed after 15 days of exposure to 1,000 mg kg^{-1} concentration. These amounts were recorded 88.33±3.96, 78.31±2.15 and 64.99±4.4% for *S. granarius*, *O. surinamensis* and *T. castaneum*, respectively. *S. granarius* was more susceptible than the others, because the highest mortalities in each of the three exposure times and for all dose rates were observed in this species. The lowest LC_{50} value within the exposure times was determined 452.855 mg kg^{-1} after 15 days for *S. granarius*. Results achieved from progeny indicate significant differences only between rates and insect species. For all species, the highest progeny production was observed in rate 0 mg kg^{-1}. The results obtained in this research recommend that *Bb*Weevil™ could be used to control different grain storage pests but to find longer exposure intervals and higher rates are subject to further future research.

Keywords: *Beauveria bassiana, Oryzaephilus surinamensis, Sitophilus granarius, Tribolium castaneum.*

INTRODUCTION

The granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the sawtoothed grain beetles, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) cause both quantitative and qualitative damages to stored grain. The main causes are reduction in weight, quality, commercial value and seed viability (Hill, 1990).

Residual insecticides have been employed to control insect pests of stored grains, but

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alternative control strategies are desirable because of the loss of insecticides due to pest resistance and consumer desire for pesticide-free grain (Arthur, 1996). The biggest impetus for the growth of biopesticides comes from the growing awareness by farmers of the value of integrated pest management as a more environmentally sound, economical, safer and a selective approach to crop protection (Menn, 1996).

Entomopathogenic fungi are generally considered to be safe in terms of low risks as compared to chemical pesticides. New areas for use of these fungal biocontrol agents include their use in close proximity to foods and feed, or even applied directly to stored grains as well as to other food commodities (Cox *et al.,* 2003; 2004). The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin bears a considerable potential for the control of the different stored product pests. It is registered by the U.S. Environmental Protection Agency (EPA) for a wide range of insect control applications (Lord, 2001). First Investigation by Ferron (1977) followed by Tanya and Doberski (1984), Adane *et al.* (1996), Hidalgo *et al.* (1998), Rice and Cogburn (1999), Smith *et al.* (1999), Bello *et al*. (2000), Padin *et al.* (2002) and Cherry *et al.* (2005) suggested that *B. bassiana* is a potential microbial control agent against some stored product pests. Nowadays, several *B. bassiana* formulations (Boverosil®, Mycotrol® ES, Mycotrol® 22WP, Naturalis® SC …) are commercially available and are registered for use in storage facilities.

Entomopathogenic fungi within stored food products can be employed to treat empty stores to control residual pests before the new harvest is brought in, or may be applied as direct admixture of conidia to grain, either as preventative or curative treatments of bulk grain. The latter solution is only going to be successful if adding fungus directly to the commodity does not decrease the quality and thus the marketability (Steenberg, 2005).

Recently, most of the research is carried out by formulation of this fungus, alone or in combination with other such alternative material as diatomaceous earth for management of stored product pests (Akbar *et al.,* 2004; Lord, 2007; Vassilakos *et al.,* 2006). In this study, *Bb*Weevil™, a *B. bassiana*-based biopesticide was examined in mixture with stored wheat against adults of three species of grain beetles. This product is an active ingredient for the biological control of certain insect and mite species provided from biostrain PPRI 5339, originally isolated from banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae). The effects of this biopesticide on progeny production of the so called pests were evaluated, too. To our best knowledge, this is the first paper evaluating the potential of this formulation for the control of stored product pest.

MATERIALS AND METHODS

Fungus Formulation

Commercially produced, formulated conidia of *B. bassiana* strain PPRI 5339 (*Bb*Weevil™, Biological Control Products, South Africa) containing 2.9×10^9 conidia per gram of powder was used. The tested batch of "*Bb*Weevil™" was 4 months old and prior to usage it was kept in the dark at 4°C. The germination rate was assessed 18 hours at 25°C. Conidia were spread on Sabouraud Dextrose Agar (SDA) and incubated for 18 hours at 25°C. Two hundred conidia were counted for the presence of visible germ tubes. The germination rate was at least 90%.

Insects

Adults of *T. castaneum*, *S. granarius* and *O. surinamensis* were obtained from cultures maintained at 28°C and 65±5% relative humidity in the dark at the Department of Entomology, Urmia University, Urmia, Iran for at least 5 years, with no history of exposure to insecticides. All adults used in the experiments were 1-7 days old and of mixed sexes.

Commodity

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Whole sample (wheat variety Zarrin) from the Agricultural Research Center of West Azarbaijan, Iran, was used for experimentation. The moisture content of commodities was measured by drying 10-gram samples of each commodity in a ventilated oven at 110°C. The moisture content of kernels was 11.3%. This commodity was stored at -12°C for a week prior to tests to kill any insect at any life stage that may have durated. One percent cracked wheat grain was included in the sample to ensure access to food for the test insects.

Bioassay

Formulation was applied at five rates of: 0, 250, 500, 750 and 1,000 mg kg^{-1}. Fifteen lots of 1 kg of wheat grain (one lot for each exposure time-rate) were prepared and placed in separate cylindrical jars (2 liter capacity with screwed lids) and treated with the appropriate dose. All jars were shaken manually for approximately 2 minutes to achieve uniform distribution of the conidial powder in the entire grain mass. After one day, 4 samples of 50 g each, were taken from each jar as a replication and placed in glass vials (8 cm height and 5 cm diameter). Thirty 1-7 day old adults were introduced into each glass vial, covered with muslin cloth to provide sufficient aeration. All experiments were carried out in a room of stable conditions of 25±2°C and 50±5% r.h. Dead adults were counted after 5, 10 and 15 days of exposure. Dead insects were then incubated in a plastic box with high r.h. (approximately 100%) to observe the outgrowth of fungus. Following mortality count in each exposure time, all the adults (dead and alive) were removed from the vials, and the vials left at the same conditions for a further 50 days to assess progeny production (F_1). The number of emerged individuals of each species was then counted. Only adults were recorded in the case of *S. granarius* and *O. surinamensis*, since its larvae develop inside the grain kernels, while in the case of *T. castaneum*, in addition to the number of adults, the number of immature was also recorded. Progeny mortalities have not been included in data analysis.

Data Analysis

To equalize variances, mortality percentage of adults and number of progeny production were transformed using arcsine $\sqrt{x}$ and *log (x+1),* respectively. The data were analyzed using Analysis of Variance (SAS, 2000) with insect mortality as the response variable and rate, along with the exposure time and insect species as main effects. The same procedure was carried out for progeny production counts. Means were separated by using Tukey´s Test at $P = 0.05$. The concentration required to kill 50% of the insects (LC_{50}) was estimated using probit analysis (SPSS, 1999).

RESULTS

Insect Mortality

All main effects as well as associated interactions were significant at $P< 0.01$

Table 1. ANOVA parameters for main effects and associated interactions for adults mortality counts (Total df= 179).

Source	*df*	*F*	*P*
Treatment	4	458.97	< 0.0001
Exposure time	2	300.21	< 0.0001
Insect	2	40.25	< 0.0001
Treatment×Exposure time	8	16.96	< 0.0001
Treatment×Insect	8	3.02	< 0.0037
Exposure time×Insect	4	1.87	0.1190

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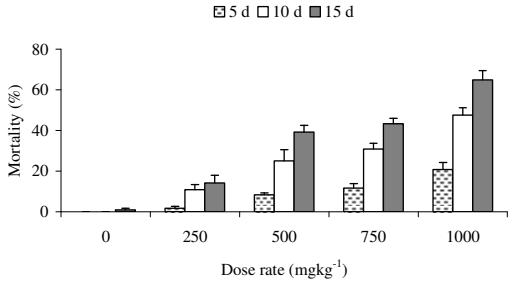

Figure 1. Mean mortality (%+SE) of *T. castaneum* adults, in wheat treated with *Bb*Weevil™ after 5, 10 and 15 days of

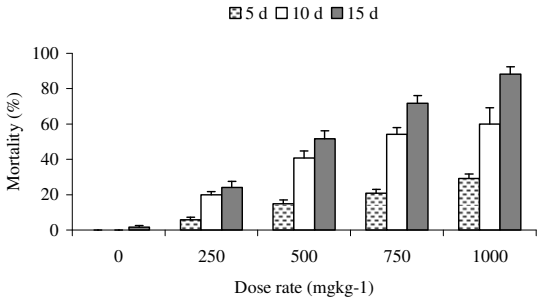

Figure 2- Mean mortality (%+SE) of *S. granarius* adults, in wheat treated with *Bb*Weevil™ after 5, 10 and 15 days of exposure.

level, with the exception of exposure time×insect, which was not (Table 1). In all the experiments, mortality increased with increase in dose rates and exposure time while the highest mortality was observed after 15 days of exposure to 1,000 mg kg^{-1} of *Bb*Weevil™. These figures were recorded as 88.33±3.96, 78.31±2.15 and 64.99±4.4 percent for *S. granarius*, *O. surinamensis* and *T. castaneum*, respectively (Figures 1, 2 and 3). Within the group of insects, *S. granarius* was more susceptible to *B. bassiana* because the highest mortalities of adults in all the exposure times and dose rates were observed for this species (Figure 2).

Probit analysis was carried out to determine LC_{50} and LC_{95} for each insect in three exposure times. The parameters of the probit analysis and LC_{50} are given in Table 2. The lowest LC_{50} and LC_{95} in all the exposure times were observed in *S. granarius* with the exception of LC_{95} in 5 days after exposure that was recorded for *T. castaneum*. The lowest LC_{50} figure was noted 452.855 mg kg^{-1} for *S. granarius* after 15 days exposure.

Progeny Production (F_1)

For progeny production, significant differences were noted between among rates at $P< 0.01$ level whereas exposure interval did not have significant effect on progeny production. Also, none of the associated interactions were significant, with the exception of dose rate×insect which was significant at the $P< 0.01$ (Table 3). In all the three species of insects, the highest

Table 2. The lethal concentration for the 50% (LC_{50}) in wheat treated with *Bb*Weevil™.

Insect Exposure time	LC_{50} (mg kg^{-1})	LC_{95} (mg kg^{-1})	χ^2	*P*	Slop (b)	Intercept (a)
T. castaneum						
5 d	2568.11	16149.5	0.69	0.70	2.06	-2.02
10 d	1166.47	10055.4	3.74	0.15	1.75	-0.39
15 d	747.74	4186.8	3.66	0.16	2.19	-1.31
O. surinamensis						
5 d	4124.94	59182.8	0.21	0.89	1.42	-0.14
10 d	878.41	5451.56	0.21	0.90	2.07	-1.1
15 d	562.71	2832.22	0.22	0.89	2.34	-1.44
S. granarius						
5 d	2198.33	25134.7	0.15	0.92	1.65	-0.52
10 d	653.54	4253.45	0.3	0.98	2.02	-0.69
15 d	452.855	1606.58	2.51	0.28	2.99	-2.94

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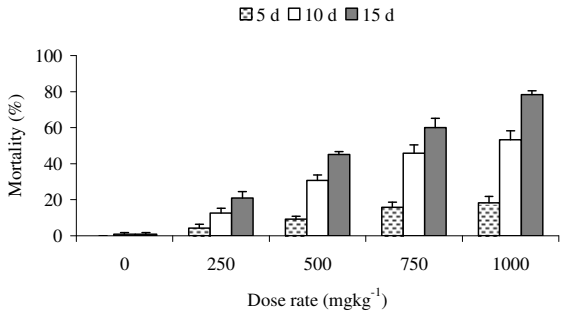

Figure 3. Mean mortality (%+SE) of *O. surinamensis* adults, in wheat treated with *Bb*Weevil™ after 5, 10 and 15 days of exposure.

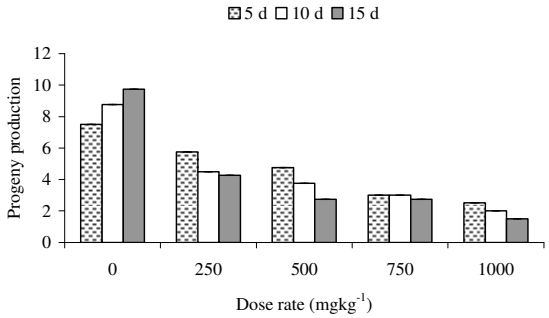

Figure 4. Progeny production (Mean number of adults/Vial±SE) of *T. castaneum*, in wheat treated with *Bb*Weevil™.

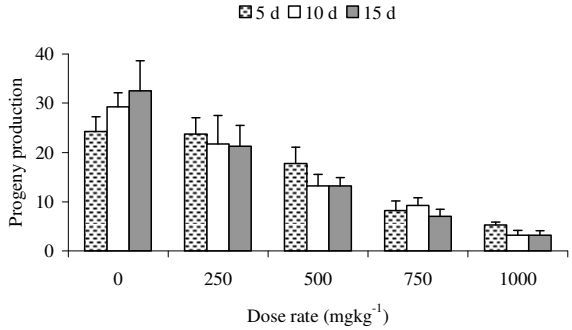

Figure 5. Progeny production (Mean number of adults/Vial ±SE) of *S. granarius*, in wheat treated with *Bb*Weevil™.

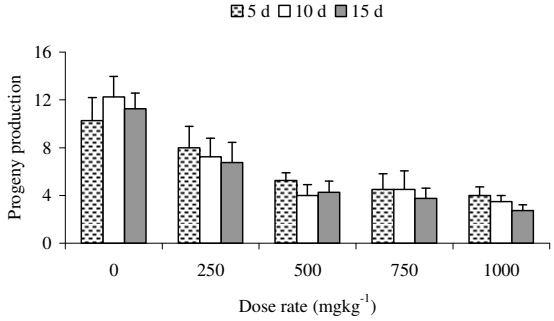

Figure 6. Progeny production (Mean number of adults/Vial±SE) of *O. surinamensis*, in wheat treated with *Bb*Weevil™.

number of progeny production was recorded at 0 mg kg^{-1} and increase in dose rate significantly rebated the progeny production (Figures 4, 5 and 6).

DISCUSSION

Post-mortem mycelial and conidial growth demonstrated that most insects had died due to the presence of the fungus. The formulation of *B. bassiana* at 1,000 mg kg^{-1} provided an effective control of the three species mentioned, although *S. granarius* was the most susceptible of the species studied. Nowadays, it is revealed that isolates recovered from a target host and closely related species are generally more virulent than isolates from non-related species (Inglis *et al.,* 2001). Because the Strain PPRI 5339 was originally isolated from banana weevil (*C. sordidus*), its potential for the control of

Table 3. ANOVA parameters for main effects and associated interactions for progeny production counts (Total df= 179).

Source	*df*	*F*	*P*
Treatment	4	73.08	<0.0001
Exposure time	2	2.36	0.0983
Insect	2	120.73	<0.0001
Treatment×Exposure time	8	1.30	0.2471
Treatment×Insect	8	4.55	<0.0001
Exposure time×Insect	4	0.06	0.9929

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the curculionid *S. granarius* was greater than that of the other species.

The first study on application of entomopathogenic fungi as formulated materials for control of storage pests was carried out by Hluchi and Samsinakova (1989). They used Boverosil®, a formulation as wettable powder (containing 5.92×10^9 conidia g^{-1} powder) from *B. bassiana* for control of *S. granarius* adults. They noted that Boverosil® can cause mortality in this insect, since 90% mortality was recorded at 5.92×10^8 conidia ml^{-1}. But, they added, effective treatment requires a period of high humidity at dew point that is a critical parameter in use of entomopathogenic fungi in storage facilities and it is not accessible. There is a predominant perception that fungi require a moist atmosphere. While conidiation requires atmospheric moisture near saturation, conidial germination and initiation of the process of insect infection is less demanding. Studies on the relationships between moisture and fungal efficacy for insects show great variation. Certainly the architecture and physiology of the target species are the major factors. Convoluted cuticles with favorable microclimates are most conductive to efficacy (Lord, 2005a; b). It was recently demonstrated that reduced atmospheric and grain moisture, could increase the efficacy of entomopathogenic fungi especially *B. bassiana* in storage facilities (Lord, 2005a; Athanassiou and Steenberg, 2007). The longevity of conidia of *B. bassiana* is generally more stable at cool and dry conditions (Hong *et al.,* 1997). It was also proved that this fungus is more effective at moderate temperatures with an optimum around 25ºC (Walstad *et al.,* 1970; Ekesi *et al.,* 1999; Lord, 2005a). For an assessment of potential of this formulation, all experiments were carried out at a room with the stable conditions of 25±2°C and 50±5% r.h.

Akbar *et al.* (2004) in their investigations demonstrated that adults of *T. castaneum* exhibited very little susceptibility to *B. bassiana*. They showed that in commercial products of this fungus, technical powder contained 9.4×10^{10} conidia per gram (strain GHA, Emerald BioAgriculture, Butte, MT) even in 2,000 mg kg^{-1} could only control 8.3±2.5% of this pest after 7 days of treatment. This result is in contrast with ours that indicate 64.99±4.4% mortality of adults *T. castaneum* occurred in 1,000 mg kg^{-1} (corresponding to 2.9×10^9). In rationalization of this difference, it could be said that the used strain of this species is relatively susceptible to used strain of *B. bassiana* or this commercial product possessed a suitable potential for control of this pest. Lord (2001) indicated that adults of *O. surinamensis* had an apt susceptibility to strain GHA of *B. bassiana*, commercially produced while containing 6.3×10^{10} conidia per gram (Mycotech, Butte, MT). They recorded 71.5±8.17% mortality at rate of 300 mg kg^{-1} (corresponding to 1.89×10^{10} conidia per gram) which is similar to the present results that indicate 78.33±2.15% mortality for adults of this pest at 1,000 mg kg^{-1}.

Reduced progeny production in the treated substrate is considered as equal or even more important than parental mortality. Unfortunately, a few published data exist concerning the effects of entomopathogenic fungi on progeny production in either storage commodities or facilities. Athanassiou and Steenberg (2007) used *B. bassiana* against *S. granarius* in stored wheat. They indicated that this fungus at rate of 0.72×10^{12} spores per kg grain can cause 52% mortality 7 days after treatment and 64% reduction in progeny production (143.8 insect/vial for control group and 52.5 insect/vial for treated one) 65 days after treatment at 55% of r.h. and 25ºC. The results achieved from the present experiment demonstrated that *B. bassiana* at 1,000 mg conidia kg^{-1} grain can reduce the production of progeny of *S. granarius* and while increase in rate can raise the degree of reduction. Also Throne and Lord (2004) showed that adding 150 mg of conidia per kilogram of commercially produced, unformulated conidia of *B. bassiana* isolate GHA (Emerald BioAgriculture, Butte, MT) to cracked or whole "Paul" oats resulted in a

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70 and 98% reduction, respectively, in the number of progeny produced by *O. surinamensis*.

In summary, the present results demonstrate that *B. Bassiana* can be used with success against pests injurious stored wheat. A long term use of this formulation as well as other formulations of entomopathogenic fungi are recommended in conditions of grain storage.

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بررسی استفاده از قارچ *Beauveria bassiana* (Bals.) Vuill. برای کنترل آفات انباری غلات

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چکیده

حشره‌کش‌های شیمیایی به طور گسترده بر علیه آفات انباری استفاده شده و خطرات زیادی از جمله باقیمانده سموم در مواد غذایی و مقاومت حشرات را به همراه دارند. در سال‌های اخیر توجه زیادی به کنترل این آفات به وسیله عوامل بیماریزا شده است. در این طرح، فرمولاسیون تجاری قارچ *Beauveria bassiana* (*Bb*Weevil™-۲×۱۰^{۹} conidia/g) بر علیه حشرات بالغ *Tribolium castaneum*، *Sitophilus granarius* و *Oryzaephilus surinamensis* مورد ارزیابی قرار گرفت. آزمایشات با استفاده از غلظت‌های ۰، ۲۵۰، ۵۰۰، ۷۵۰ و ۱۰۰۰mg/kg و در فواصل زمانی ۵، ۱۰ و ۱۵ روز به صورت مستقل انجام گرفت. ۱۵ واحد یک کیلوگرمی از گندم با غلظت‌های مورد نظر از فرمولاسیون مخلوط شدند (برای هر غلظت و فاصله زمانی یک واحد). ۴ نمونه ۵۰ گرمی از هر واحد به عنوان تکرار برداشته شدند. برای هر تکرار ۳۰ حشره ۷-۱ روزه مورد استفاده قرار گرفت. آزمایشات در شرایط دمایی ۲۴±۲°C و رطوبت نسبی ٪۵۰±۵ انجام گرفت. پس از شمارش تلفات در هر فاصله زمانی، حشرات زنده و مرده از تکرارها دور ریخته شده و تیمارها در همان شرایط برای ارزیابی نتاج حاصل به مدت ۴۵ روز قرار داده شدند. مقایسه میانگین‌ها با استفاده از آزمون توکی ($P<۰/۰۵$) انجام گرفت. نتایج نشان داد بین غلظت‌ها، فواصل زمانی و گونه حشرات و اثرات متقابلشان به جز در مورد اثر متقابل گونه حشره × فاصله زمانی اختلاف معناداری ($P<۰/۰۱$) وجود دارد. تلفات با افزایش غلظت و فاصله زمانی افزایش یافته، بیشترین میزان تلفات در غلظت ۱۰۰۰mg/kg و در فاصله زمانی ۱۵ روز مشاهده گردید. بیشترین میزان تلفات ۸۸/۳۳±۳/۹۶، ۷۸/۳۱±۲/۱۵ و ۶۴/۹۹±۴/۴ درصد به ترتیب برای *S. granarius*، *O. surinamensis* و *T. castaneum* به ثبت رسید. بالاترین میزان تلفات در تمامی فواصل زمانی و غلظت‌ها و همچنین کمترین میزان LC۵۰ در تمامی فواصل زمانی مربوط به گونه حساس *S. granarius* بود که برای میزان ۴۵۲/۸۵ mg/kg و در فاصله زمانی ۱۵ روز محاسبه گردید. در تولید نتاج، اختلاف معنادار فقط بین غلظت‌ها و گونه حشره مشاهده گردیده و در تمامی موارد بیشترین تولید نتاج در غلظت ۰ mg/kg مشاهده گردید. نتایج حاصل، استفاده از این فرمولاسیون را برای کنترل آفات انباری توصیه می‌کند.

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