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Comparison of *Beauveria bassiana* with IGRs against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae)

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Abstract

In this study the virulence of *Beauveria bassiana* and IGRs (lufenuron, pyriproxyfen and methoxyfenozide) was evaluated against *Tribolium castaneum* and *Trogoderma granarium* at the Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture, Faisalabad from June 2015 to April 2016. Three concentrations of each IGR (1, 5 and 10 ppm) and entomopathogenic fungi (300, 600 and 900 mg per kg of grains) were applied in several bioassays and three replicates were used for each treatment. Data regarding mortality was collected after different intervals 7, 14 and 21 days of the treatment. *Beauveria bassiana* at higher concentration 9×10^7 after 21 days provided 66.80 percent mortality. Among the three IGRs, the pyriproxyfen gave the best result with the 76.66% mortality followed by the methoxyfenozide (73.52%), lufenuron (70.56%). The findings revealed that *Beauveria bassiana* and IGRs are effective for control of grains pests and can be used in the integrated pest management.

Keywords: Entomopathogenic fungi, IGRs, Pyriproxyfen, Stored products, Integrated pest management

1. Introduction

Man is searching for food and has competition with insects as they cause damage by two ways; quantitative and qualitative ^[1]. To face this challenge we should decrease the post harvesting losses in Pakistan which range 5% to 10% ^[2]. It is generally acknowledged that pests cause profound losses to grains countries even affecting their storage ^[3]. The major species of pests affecting grain storage are *Tribolium castaneum*, *Trogoderma granarium*, *Rhizopertha dominica*, and *Sitophilus* ^[4], out of these *Trogoderma granarium* and *Tribolium castaneum*, are regarded as most notorious pest worldwide ^[5]. They inhabitant at high temperature and low humidity ^[6] areas so their larvae cause significant damage ^[7] while *Tribolium castaneum* is also excrete carcinogenic quinones which increased incidence of allergenic diseases ^[8]. In result, affected seed loose ability to germinate as the embryo ^[9].

Several strategies have been adopted to control these pests and chemical control is considered as most excessive and effective but its repeated applications affect adversely ecosystem and human health ^[10, 11]. Moreover, fumigants and synthetic insecticides ^[12], are also used but their accumulation in grains deteriorates grain quality so researchers are investigating a safe biological control ^[13] that replace or enhance current control protocols. The entire procedure of infection by entomopathogenic fungi (*B. bassiana*) generally accomplishes five to six steps including;

1. Adhesion of the fungal conidia to the insect's cuticle,
2. Germination of the conidia,
3. Penetration to host's cuticle,
4. Defeating the host's defense responses,
5. Vegetative growth inside the insect body and
6. Post mortal sporulation ^[14].

Insect growth regulators (IGRs) are commonly used in controlling insect pests. These compounds interfere with insect metabolism and growth and are not neuro toxic hence are harmless to human and vertebrates as compared to other conventional insecticides. They damage the egg, larvae and pupae of insect pests ^[15]. Current research experiments focused on the assessment of entomopathogenic fungus (*Beauveria bassiana*) and different IGR's alone

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and in best possible combinations against *Tribolium castaneum* and *Trogoderma granarium* with these main objectives to evaluate the efficacy of *Beauveria bassiana* against *Tribolium castaneum* (adults) and *Trogoderma granarium* (larvae) as well as to determine the effect of different IGR's formulations against test insects.

2. Materials and Methods

The research was performed at the Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture, Faisalabad from June 2015 to April 2016 and population samples were collected from the grain market of Faisalabad district.

Table 1: Detail of fungus and IGRs used in experimental potential

Active ingredient	Category	Trade name	Formulation
<i>Beauveria bassiana</i>	Biopesticide	Racer™	Wettable Powder
Lufenuron	IGRs	Match®	50%EC
Pyriproxyfen	IGRs	Priority®	1.8%EC
Methoxyfenozide	IGRs	Runner®	240 SC

2.3 Bioassay to evaluate the entomopathogenic impact of *Beauveria bassiana* against *Tribolium castaneum* and *Trogoderma granarium*

Different concentrations of *Beauveria bassiana* 300 mg (3×10^7 conidia), 600 mg (6×10^7 conidia) and 900 mg (9×10^7 conidia) mg/kg of grains were spread against *T. castaneum* and *T. granarium* [16] and three replicates were performed for each treatment. Thereafter, 30 adults of *T. castaneum* and 30 larvae of *T. granarium* were released in each jar and the top of the jars were closed with muslin cloth to maintain the aeration. The treatments were kept in incubator at 30 ± 2 °C temperature and $60 \pm 5\%$ R.H. Mortality was recorded after 7, 14 and 21 days, while dead insects were removed and confirmed for fungal infection. Only those insects showing symptoms of fungal infection as manifested by sporulation of the fungus breaking through the cuticle were considered as killed by the pathogen [17].

2.4 Impact of different concentrations of various IGRs on the mortality of *Tribolium castaneum* and *Trogoderma granarium*

Three concentrations of each IGRs 1, 5 and 10 ppm were prepared from the stock solution. The determined quantity of formulation of each was mix in water up to required volume to prepare stock solution. The half of this volume was taking into another beaker and volume was made up to original to prepare half percent solution from stock solution. These prepared concentrations of IGRs were thoroughly mixed in the diet of test insects. Then counted member of insects was released in the treated diet which was kept in cooled incubators at optimum growth conditions. The data of the larval mortality, adult mortality was noted at regular intervals 7 days, 14 days and 21 days.

2.5 Combination of *Beauveria bassiana* and IGRs

The investigation was carried out to study the virulence of Racer BB® (*Beauveria bassiana* (Bals) in combination with 3 IGRs against *Tribolium castaneum* and *Trogoderma granarium*. The experiments were performed on sterilized wheat flour at temperature of 30 ± 2 °C and R.H. $60 \pm 5\%$. Three concentrations of one were of 300 mg +1ppm, 600mg +5ppm and 900mg +10 ppm of entomopathogenic fungi and IGRs were applied to assessed percent mortality of the *T. castaneum*. Thirty adults of tested insects were released in

2.1 Insect Rearing

The insect culture was reared in sterilized plastic jars in an incubator (SANYO, MIR-254) at 30 ± 2 °C temperature and $60 \pm 5\%$ relative humidity having respective food commodity. The emerged larvae of *T. granarium* and adults of *T. castaneum* were used for experiments.

2.2 Source of Treatments

Entomopathogenic fungi *Beauveria bassiana* (Racer BB) imported from Agri Life, Medak District. Hyderabad, India and IGRs purchased from local market of district Faisalabad. The trade name, formulation and category of the controlling agent given in table 1.

each treated jars. Completely Randomized Design with three replications of each treatment was used. The data regarding mortality were recorded after 7, 14 and 21 days of exposure period of treatment application.

2.6 Statistical Analysis

Data was analysed using STATISTICA 12 software and significance level was set at $p < 0.05$. Analysis of variance was used and significance level was checked by Tuckey's test. While corrected mortality was calculated using Abbott's formula [18].

3. Results and Discussion

3.1 Mortality effect of Entomopathogenic Fungi

The data regarding different concentrations of *Beauveria bassiana* showed that higher concentration (9×10^7) of *Beauveria bassiana*, after 21 days, produced about 64.28% and 66.80% mortality in *Tribolium castaneum* and *Trogoderma granarium* respectively (Fig 1, 2). Similar findings are already reported that higher concentrations produced significant mortality about 80-100% [19] The finding revealed that entomopathogenic fungi had potential to reduce *Tribolium castaneum* and *Trogoderma granarium* population.

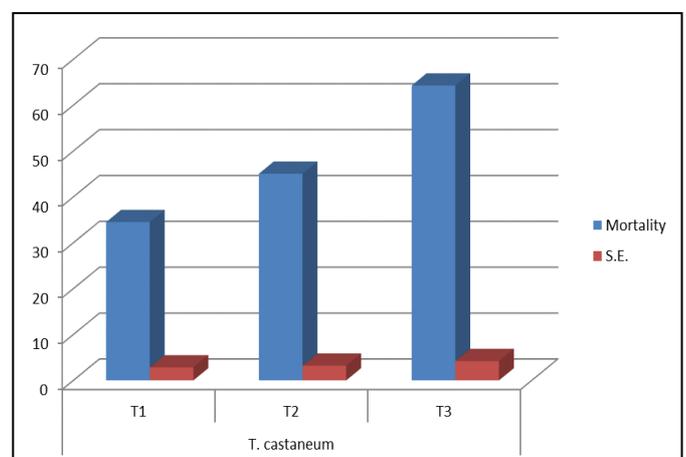


Fig 1: Percentage mortality of *Beauveria bassiana* against *Tribolium castaneum*

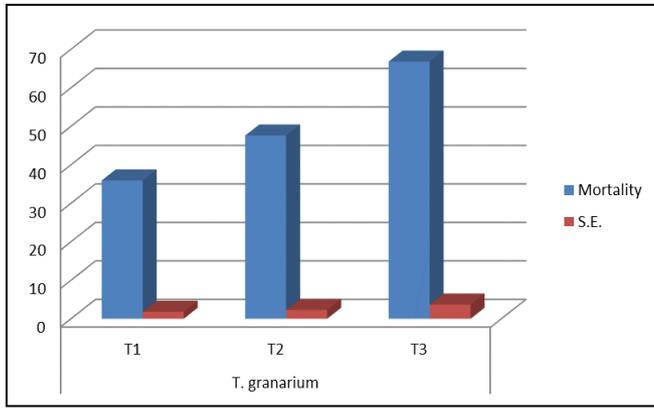


Fig 2: Percentage mortality of *Beauveria bassiana* against *Trogoderma granarium*

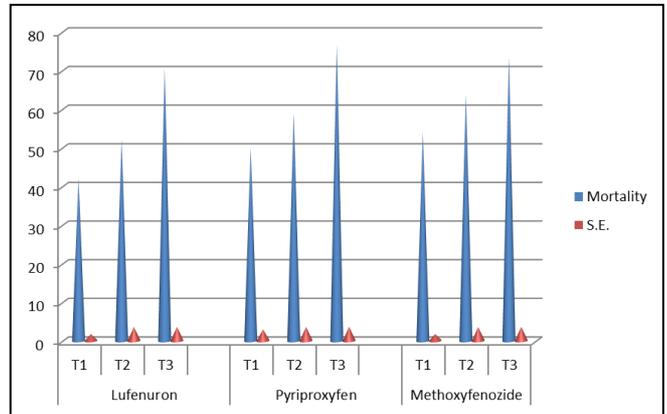


Fig 4: Percentage mortality of Lufenuron, Pyriproxyfen and Methoxyfenozide against *Trogoderma granarium*

3.2 Mortality effect of IGRs

The data regarding mortality effect of different IGRs, pyriproxyfen, methoxyfenozide and lufenuron at three different concentrations 1, 5 and 10 ppm showed that all the IGRs at higher concentration (10 ppm) after 21 days provided the maximum mean percent mortality (Fig 3, 4). These findings were similar to previous report Besheli (2010) [20], who evaluated the toxicity of different IGRs against the *Phyllocnistis citrella* *in vitro*. Moreover, another study Elghar *et al.*, (2004) [21] reported that the pyriproxyfen was found active after 8 month and 100% suppression was found. Furthermore, Kostyukovsky *et al.*, (2000) [22] studied the effect of the two juvenoids and two ecdysteroids against the *Tribolium castaneum*, *Rahizopertha dominica* and *Sitophilus oryzae*. He revealed that at very low dose 0.1ppm pyriproxyfen did not kill the larvae of *Tribolium castaneum* but at the higher doses it was found effect against the *Tribolium castaneum*. At the dose rate of the 2 ppm, 95% reduction was found in the F₁ generation. Arthur *et al.*, (2009) [23] studied the effect of pyriproxyfen on wood concrete and metal media and concluded that adult emergence was suppressed to the 95.8%. Kavallieratos *et al.*, (2012) [24] Conducted the study against the stored grain insect to evaluate the IGRs fenoxycarb, pyriproxyfen, diflubenzuron, flufenoxuron, lufenuron, triflumuron and methoxyfenozide against adults of *Prostephanus truncatus* and *Rhizopertha dominica*. The experiment was conducted on the three different temperature condition 20 °C, 25 °C and 300 °C and doses 1ppm, 5ppm and 10ppm. They concluded that all IGRs were effective which suppressed the population up to 88% which is more than the current study which may differ due to type of insect or difference in the other physical conditions.

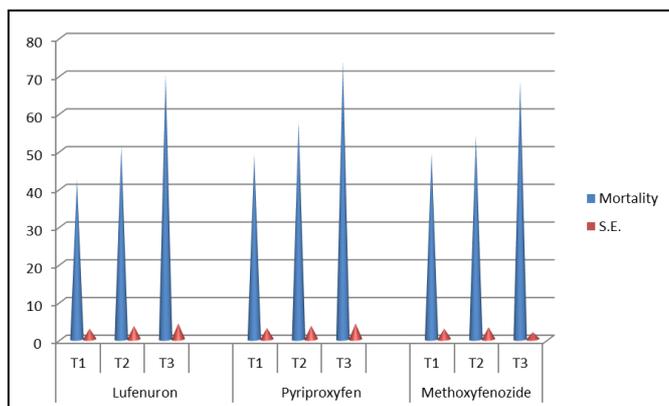


Fig 3: Percentage mortality of Lufenuron, Pyriproxyfen and Methoxyfenozide against *Tribolium castaneum*

3.3 Combine effect of Beauveria bassiana and IGRs

Interaction effect of the *Beauveria bassiana* with the 3 IGRs (Pyriproxyfen, Lufenuron, and Methoxyfenozide) was also measured against the *Tribolium castaneum* and *Trogoderma granarium*. The data revealed that all of the three IGRs were found compatible with the entomopathogenic fungi *Beauveria bassiana* and comparatively higher mortality rate were observed then alone applications. The result revealed that the combination of *Beauveria bassiana* and Pyriproxyfen gave the 81.04% and 77.69% mortality at the highest dose 900mg +10ppm against the *Tribolium castaneum* and *Trogoderma granarium* respectively. From the combination of *Beauveria bassiana* and Lufenuron the mortality in the *Tribolium castaneum* and *Trogoderma granarium* was 73.58% and 74.29%, respectively. The combination of the *Beauveria bassiana* and Methoxyfenozide provided the 82.53% and 79.18% mortality in *Tribolium castaneum* and *Trogoderma granarium*, respectively (Fig 5, 6). Hadi *et al.*, (2013) [25] checked the synergistic effect of *Beauveria bassiana* with the lufenuron and revealed that higher mortality was observed when lufenuron was added to the *Beauveria bassiana* the spore formation was increased and in the time interval 15 hours. In another studied Pelizza *et al.*, (2015) [26], combined effect of the two entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* was studied with lufenuron and methoxyfenozide they concluded that higher mortality was found in the case of combination of IGRs and entomopathogenic fungi and mortality was less when both of these were applied alone. This study confirms the result of the current study.

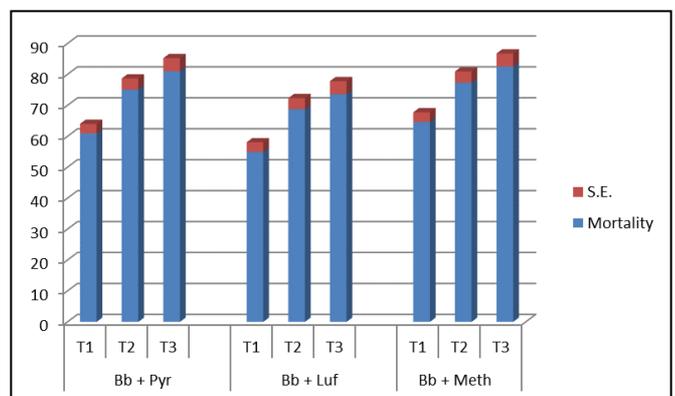


Fig 5: Percentage mortality of combination *Beauveria bassiana* with IGRs against *Tribolium castaneum*

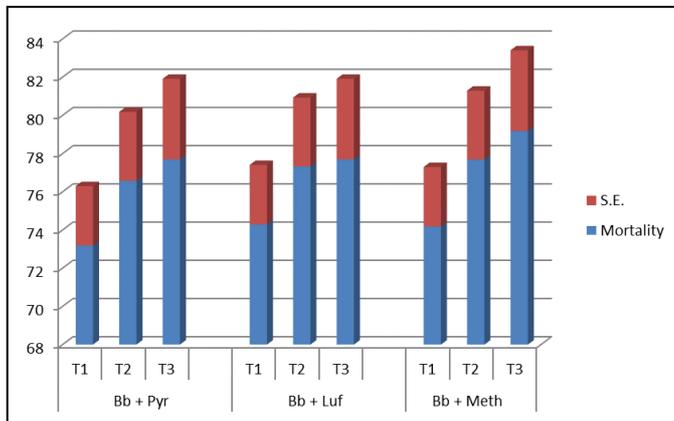


Fig 6: Percentage mortality of combination *Beauveria bassiana* with IGRs against *Trogoderma granarium*

4. Conclusion

The present study revealed that we should prefer the pyriproxyfen for the control stored grain pest particularly *Tribolium castaneum* and *Trogoderma granarium*. Other two IGRs and *Beauveria bassiana* may also be used as a tool in the integrated stored grain pest management. These are preferred because they are bio pesticides and they have less resistance, less harmful for humans and ecofriendly.

5. Acknowledgement

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