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ORIGINAL ARTICLE

Efficacy of *Beauveria Bassiana* and *Bacillus Thuringiensis* Against Maize Stem Borer *Chilo Partellus* (Swinhoe) (Lepidoptera: Pyralidae)

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Abstract

Biocontrol potential of *Beauveria bassiana* (Balsamo) Vuillemin and *Bacillus thuringiensis* (Berliner) was investigated under laboratory conditions against maize stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). Three dose rates of *B. bassiana* (1×10^4, 1×10^6 and 1×10^8 conidia/ml) and one of *B. thuringiensis* (0.75 µg/g) were applied alone and in combination against 2nd and 4th larval instars of *C. partellus*. Larval mortality, pupation, adult emergence, mycosis and sporulation varied against different individual concentrations of *B. bassiana* and its integrated application with *B. thuringiensis*. Results of the experiment revealed that combined application of highest concentration of *B. bassiana* (1×10^8 spores/ml) and *B. thuringiensis* (0.75 µg/g) exhibited highest larval mortality both in 2nd and 4th instars larvae of *C. partellus*. The mortality data also demonstrated that 2nd instar larvae were more susceptible to the tested microbial treatments than 4th larval instars. Moreover, less pupation and adult emergence was observed in combined treatments of entomopathogens rather than their individual applications. Maximum mycosis and sporulation was recorded in the cadavers of *C. partellus* where *B. bassiana* was applied alone at dose rate of 1×10^4 conidia/ml. These outcomes suggest that combined application of *B. bassiana* and the toxic protein produced by *B. thuringiensis* could be a promising ecofriendly approach for the successful management of maize stem borer.

Keywords Biological pest control · Microorganisms · Mycosis · Mortality · Sporulation

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Wirksamkeit von *Beauveria bassiana* und *Bacillus thuringiensis* gegen den Maisstengelbohrer *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae)

Zusammenfassung

Das Potenzial des entomopathogenen Pilzes *Beauveria bassiana* (Balsamo) Vuillemin und des Bakteriums *Bacillus thuringiensis* (Berliner) zur biologischen Kontrolle des Maisstengelbohrers *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) wurde unter Laborbedingungen untersucht. Drei Suspensionskonzentrationen von *B. bassiana* (1×10^4, 1×10^6 und 1×10^8 Konidien/ml) und eine Konzentration von *B. thuringiensis* (0.75 µg/g) wurden allein oder in Kombination gegen Larven (L2 und L4) von *C. partellus* getestet. Die Wirkung von *B. bassiana* auf die Mortalität der Larven, Verpuppung und Entwicklung von Imagines war konzentrationsabhängig. Der höchste Wirkungsgrad (Mortalität) gegen L2 und L4 wurde bei kombinierter Anwendung von *B. bassiana* (1×10^8 Konidien/ml) und *B. thuringiensis* (0.75 µg/g) erzielt; mit deutlich höherer Wirkung gegen L2. Die kombinierte Anwendung beider Mikroorganismen bewirkte eine geringere Verpuppung und Entwicklung von Imagines als die alleinige Anwendung. Der höchste Grad an Verpilzung (Mykose) mit *B. bassiana* an *C. partellus* wurde bei einer Konzentration von 1×10^4 Konidien/ml beobachtet. Die Ergebnisse zeigen, dass durch die kombinierte Nutzung von Mikroorganismen neue Kontrollstrategien gegen den Maisstengelbohrer entwickelt werden können.

Schlüsselwörter Biologische Schädlingsbekämpfung · Mikroorganismen · Mykose · Mortalität · Sporulation

Introduction

In natural and agro-ecosystem plants remain exposed to a range of biotic stresses, of which ubiquitous prevalence of insect herbivores is of prime importance. Maize stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) is one of the major biotic limiting factors of maize and sorghum productivity (Pingali 2001; James 2003). This particular species has also started displacing local stem borers of sorghum and maize, i.e. *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and *Chilo orichalcociliellus* Strand (Lepidoptera: Pyralidae) in Africa by rapidly expanding its geographical ranges from warmer lowlands into higher altitude regions (Kfir 1993; Guofa et al. 2001; Ong'amo et al. 2006). Possible reasons behind this displacement and geographical expansion mainly include its three week shorter life cycle and one month smaller diapause period than *B. fusca* (Dejen et al. 2014).

Serious economic losses occur in maize crop due to this pest which may reach up to 100%, especially in Asia and Africa (Songa et al. 2002; Bergvinsion et al. 2004; Arabjafari and Jalali 2007). However, the losses may vary in different regions depending on the pest density and phenological stage of the crop infested. The neonates of *C. partellus* prefers feeding on young leaves whorls causing scars and holes; then advance towards growing point of plant by boring into the central whorl (Kfir et al. 2002), which leads to a partial or complete drying of the whole plant, a characteristic symptom known as "dead-heart". However, the older larvae tunnel extensively in maize stem and cobs, resulting in lodging, damaging inflorescence and interference with the grain formation (Kfir et al. 2002).

Traditionally, maize stem borer has been controlled by using insecticides, but the cryptic feeding behavior of this pest makes the timing of insecticide application crucial for success. Moreover, repeated use of insecticides also resulted in some serious ecological backlashes, including negative effects on beneficial insect fauna, environmental pollution, resistance issues in target insects and residue problems (Mostafalou and Abdollahi 2012). These problems prompted the need for exploration of ecologically compatible strategies including application of entomopathogenic viruses, fungi, nematodes, protozoa and bacteria as an alternative to synthetic insecticides for sustainable management of this pest (Castillo et al. 2000).

Among natural enemies, *Beauveria bassiana* (Balsamo) Vuillemin and *Bacillus thuringiensis* (Berliner) are considered the most promising biocontrol agents used to manage maize stem borer populations across the globe (Hajek and St. Leger 1994). There are more than 700 species of fungi that have been reported virulent against different insect pests. Various isolates of *B. bassiana* have been effective against different developmental stages of scores of economically important insect pests under laboratory, greenhouse and field conditions. The fungi usually infect the host by direct contact (primary infection) or through germination of the new propagules from mycosed cadavers (secondary infection) (Zimmermann 2007). Application of conidial suspensions of different isolates of *B. bassiana* and *Metarhizium anisopliae* (Metchnikoff) against *C. partellus* demonstrated a significant reduction in maize stem tunneling (1–5%), deadheart formation (0–33%), number of attacked nodes (0.3–2.5) and holes/plant (0.2–3.3) (Tefera and Pringle 2004).

Similarly, *Bacillus thuringiensis* (Berliner) (*Bt*) is a gram positive sporulating entomopathogenic bacterium that synthesizes different toxic proteins (endotoxins and vegetative insecticidal proteins) active against a wide array of insect pests. Moreover, the *Bt* products have also documented their potential as a biocontrol agent and safety towards natural enemies for several decades (Palma et al. 2014). After entering the insect body, the *B. thuringiensis* toxic crystals solubilize in the alkaline gut of insects, which results in proteolytic activation of the protoxin followed by its binding to the glycoprotein receptors of the midgut epithelial cells, resulting in disruption of cytoplasmic membrane and eventually cell lysis (Vachon et al. 2012). Moreover, intoxication of the commercially available *B. thuringiensis* also increases the efficiency of *B. bassiana* against lepidopterous larvae, when both microbial agents were used in an integrated fashion (Gao et al. 2012; Wakil et al. 2013). The cumulative interest regarding synergistic effects of sub-lethal doses of *B. thuringiensis* with *B. bassaina* has stemmed from their successful control against different insect pests (Furlong and Groden 2003; Ma et al. 2008).

Keeping in mind the significance of these alternative control methods, the current study was planned to evaluate the individual and combined effect of *B. bassiana* and *B. thuriengiensis* against 2nd and 4th larval instars of *C. partellus*. In addition, the post-mortal mycosis and sporulation in *C. partellus* cadavers were also determined.

Materials and Methods

Insect Material

Different growth stages of *C. partellus* were collected from fodder crops of sorghum and maize which were never exposed to any chemical or microbial treatments. All the collected stages were kept in separate plastic jars and brought to Microbial Control Laboratory, Department of Entomology, University of Agriculture, Faisalabad (UAF), Pakistan. The larvae were reared in 32-well plastic trays (6 cm in diameter × 5.5 cm in depth) provided with artificial diet as described by Kfir (1992) until pupation. The emerging adults were kept in plastic jars (15 cm in diameter × 19 cm in depth) lined with wax paper as nappy liner for egg laying. The adults were provided with 10% honey solution through cotton swab kept in 5-ml test tube placed vertically on the top of the jar. The collected eggs were surface-sterilized with 2% formaldehyde for 15 min at room temperature, washed with tap water and finally rinsed with distilled water to remove any potential outside contamination. The eggs were then allowed for air-drying on paper towels and placed in plastic bags for hatching to develop the next generation (Marzban et al. 2009). The rearing conditions were maintained at 26 ± 2 °C, 75 ± 5 R. H. and 12:12 (D: L) photoperiod.

Fungal and *B. Thuringiensis* Culture

Morphologically identified (Barnett and Hunter 1998) *Beauveria bassiana* strain used in the present study was obtained from the culture collection of the Microbial Control Laboratory. The fungal strain was originally isolated from infected pupae of *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) by single spore method (Choi et al. 1999). The fungi were further sub-cultured on Sabouraud Dextrose Agar (SDA) with 0.5% yeast in glass petri dishes. The petri dishes were incubated for fungal growth and sporulation at 25 ± 1 °C; 75 ± 5% R. H for two weeks. After 14 days conidia were harvested using a sterilized scalpel, suspended in sterile 0.01% Tween-80 (Merck, KGaA, Darmstadt, Germany), enumerated with a hemocytometer and adjusted to achieve the required concentrations. Similarly, Dipel®, a commercial wettable powder (WP) formulation of *Bt* having subspecies kurstaki with a potency of 16,000 i.u., bearing a density of 3.2% (active toxin) and 96.8% (inert material) was provided by BioSciences Corporation, (Libertyville, IL, USA). One gram of Dipel powder was liquefied in 2 ml of sterile distilled water and gradually streaked on the nutrient agar media (5 g peptone, 5 g NaCl, 1.5 g beef extract, 1.5 g yeast extract, 15 g agar and 1000 ml distilled water) amplified with suitable antibiotic. For the extraction of *Bt* toxin, the spores and crystals were first collected by centrifugation at 16,000 rpm for 15 min at 4 °C temperature (Crecchio and Stotzky 2001; Hernández et al. 2005). The remaining pellet was washed three times with cold 1 M NaCl and re-suspended in 1 M NaCl. Assessment of spore-crystal concentration was carried out in 1:100 dilutions by measuring the optical density at 600 nm (Hernández et al. 2005) and the samples were stored in the refrigerator until used.

Bioassay

In a laboratory bioassay, the fungal treatments were applied by immersing each 2nd and 4th larval instars of *C. partellus* in conidial suspension of 1×10^4, 1×10^6 and 1×10^8 spores/ml for 10 s. The treated larvae were then air-dried for 10 min in sterile Petri dishes (9 cm diameter). The formulation of *B. thuringiensis* (0.75 µg/g) was applied by exposing the both larval instars to *Bt* incorporated diet until pupation. Moreover, the combined effect of *B. bassiana* and *B. thuringiensis* was evaluated by dipping the larvae in fungal suspension for 10 s followed by air drying with immediate provision of *Bt* incorporated diet. The larvae were allowed to feed on *Bt* incorporated diet until pupation. The larvae immersed in aqueous solution containing

0.01% Tween-80 only served as control. The experiment was carried out in a completely randomized design using 15 larvae of each instar of *C. partellus* per replicate. The same procedure was carried out for both larval instars and each treatment was replicated six times using new material and insect batches each time.

Mycosis and Sporulation

For mycosis and sporulation, the cadavers of *C. partellus* from fungal treatments were collected, placed on a sterile petri dish and refrigerated at 4 °C in plastic vials. After collection all the cadavers were surface sterilized for 2–3 min with 0.05% sodium hypochlorite solution followed 2–3 washing with distilled water. The cadavers were then placed on Potato Dextrose Agar (PDA) plates and incubated at 25 ± 1 °C; 75 ± 5% R. H. for one week. The cadavers showing the external fungal growth were observed under the microscope. For determining the sporulation, the mycosed cadavers from each replication were mixed in 20 ml distilled water with a drop of Tween-80 and stirred for 10 min. The total number of conidia/ml was enumerated with the help of hemocytometer under microscope (Riasat et al. 2011).

Statistical Analysis

The mortality means were corrected by using Abbott's (1925) formula and the data was analyzed with the MINITAB 13.2 statistical package (Steel et al. 1997) using one way analysis of variance (ANOVA). However, throughout the experiment, control mortality was very low, therefore excluded from the analysis. Moreover, the treatment means were separated by LSD test at 5% significance level.

Results

Mortality of *C. Partellus*

Significant differences were detected in the larval mortality of 2nd and 4th instars of *C. partellus* when subjected to individual or combined treatments of *B. bassiana* (*Bb*) and *B. thuringiensis* (*Bt*). Moreover, the tested instars (2nd and 4th) of *C. partellus* exhibited higher percent mortality towards integrated use of both bio-control agents compared to their individual applications (Table 1).

The maximum larval mortality of 2nd (96.58%) and 4th instar (90.87%) of *C. partellus* was recorded in treatment containing highest dose rate of *B. bassiana* (1×10^8 conidia/ml) and *B. thuringiensis* (0.75 µg g^{-1}). Moreover, the highest individual concentration of *B. bassiana* (1×10^8 conidia/ml) consistently killed more larve in both 2nd (58.01%) and 4th (51.03%) instar of *C. partellus* as compared to *B. thuringiensis* (0.75 µg g^{-1}) treatment (2nd: 44.20% and 4th: 37.46%). The results further revealed that the susceptibility of 2nd instar *C. paretllus* larvae was found more than its 4th larval instar in all the tested entomopthogen treatments (Table 1).

Pupation and Adult Emergence

A significant reduction in pupation and adult emergence was observed in tested larval instars (2nd and 4th) of *C. partellus* when subjected to combined application of *B. bassiana* and *B. thuringiensis* as compared to their individual treatments (Table 1).

The lowest pupation and adult emergence in 2nd (0% and 0%) and 4th (5.55% and 0%) larval instar of *C. partellus* was observed in treatment where highest concentration of *B. bassina* (1×10^8 conidia/ml) was applied in combination

Table 1 Mean effect of *Beauveria bassiana* (*Bb*) and *Bacillus thuringiensis* (*Bt*) on mortality, pupation, and adult emergence (% ± SE) of 2nd and 4th larval instars of *Chilo partellus*

Treatments	Mortality (%)		Pupation (%)		Adult emergence (%)	
	2nd Instar	4th Instar	2nd Instar	4th Instar	2nd Instar	4th Instar
*Bb*1[a]	27.14 ± 1.99f	19.28 ± 2.41f	67.42 ± 1.89b	73.33 ± 2.89b	55.25 ± 2.67b	64.94 ± 2.26b
*Bb*2[b]	39.84 ± 2.64e	33.01 ± 2.61e	57.93 ± 2.33bc	64.40 ± 2.50bc	38.65 ± 2.95c	45.49 ± 2.89c
*Bb*3[c]	58.01 ± 1.60d	51.03 ± 2.88d	37.46 ± 2.97d	47.48 ± 2.35d	18.05 ± 2.01de	26.38 ± 2.16de
Bt[d]	44.20 ± 2.45e	37.46 ± 2.00e	48.81 ± 3.09 cd	59.67 ± 2.21 cd	23.60 ± 2.82 cd	32.63 ± 3.12 cd
*Bb*1 × *Bt*	72.70 ± 2.22c	62.38 ± 2.85c	23.05 ± 1.96e	32.81 ± 2.33e	7.50 ± 2.74ef	17.50 ± 3.57def
*Bb*2 × *Bt*	85.08 ± 3.06b	76.19 ± 2.97b	11.11 ± 2.81ef	17.77 ± 2.66f	0.00 ± 0.00f	8.33 ± 2.93ef
*Bb*3 × *Bt*	96.58 ± 1.39a	90.87 ± 2.60a	0.00 ± 0.00f	5.55 ± 1.75f	0.00 ± 0.00f	0.00 ± 0.00f
Control	–	–	93.09 ± 1.69a	97.69 ± 1.33a	86.60 ± 1.98a	93.09 ± 2.30a

Means sharing with same lower case letters are not significantly different from each other at 5% significance level
[a] 1×10^4 conidia/ml
[b] 1×10^6 conidia/ml
[c] 1×10^8 conidia/ml
[d] 0.75 µg/g

with *B. thuringiensis* (0.75 µg g^{-1}). However, maximum pupation and adult emergence in 2nd (67.42% and 55.25%) and 4th instar (73.33% and 64.94%) of *C. paretllus* was observed in treatment where larvae were exposed to lowest individual fungal concentration (1×10^4 conidia/ml). Overall, highest pupation and adult emergence was observed in treatments with least mortality (Table 1).

Mycosis and Sporulation

The applied treatments also significantly affected the mycosis and sporulation in the cadavers of 2nd and 4th instar larvae of *C. partellus.* A significantly higher rate of mycosis and sporulation was observed in both larval instars of *C. partellus* when they were subjected to individual treatments of *B. bassiana* rather than its integrated application with *B. thuringiensis* (Figs. 1 and 2).

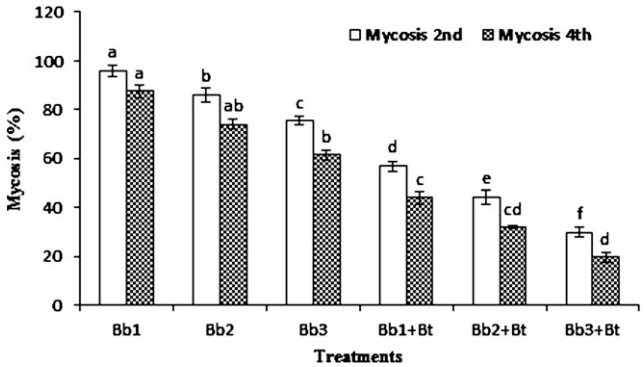

Fig. 1 Mean effect of *Beauveria bassiana* (*Bb*) and *Bacillus thuringiensis* (*Bt*) concentrations on mycosis in cadavers of 2nd and 4th instars of *Chilo partellus* larvae (*Bars* with the same letters are not significantly different at 5% significance level. *Vertical bars* indicate SE. Bb1: 1×10^4 conidia/ml, Bb2: 1×10^6 conidia/ml, Bb3: 1×10^8 conidia/ml, Bt: 0.75 µg/g)

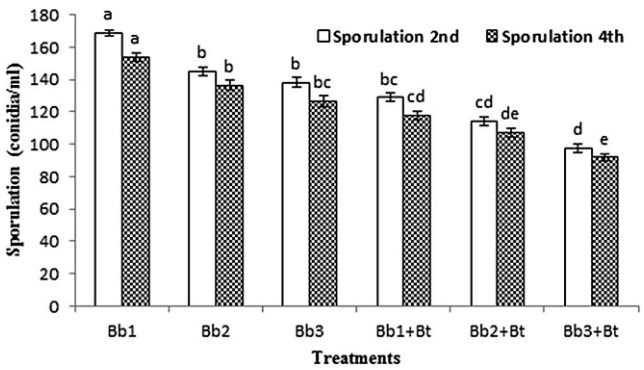

Fig. 2 Mean effect of *Beauveria bassiana* (*Bb*) and *Bacillus thuringiensis* (*Bt*) concentrations on sporulation in cadavers of 2nd and 4th instars of *Chilo partellus* larvae (*Bars* with the same letters are not significantly different at 5% significance level. *Vertical bars* indicate SE. Bb1: 1×10^4 conidia/ml, Bb2: 1×10^6 conidia/ml, Bb3: 1×10^8 conidia/ml, Bt: 0.75 µg/g)

Maximum mycosis (2nd: 95.83% and 4th: 87.50%) and sporulation (2nd: 169.00 conidia/ml and 4th: 154.00 conidia/ml) was observed in treatment where lowest concentration of *B. bassiana* (1×10^4 conidia/ml) was applied alone. Contrary to dose effect for larval mortality, the highest fungal concentration rendered lowest mycosis and sporulation in both tested instars of *C. partellus* (Figs. 1 and 2).

Discussion

The 2nd and 4th larval instars of *C. partellus* showed slightly different developmental and mortality responses to bio-control agents used in this study. A significantly higher larval mortality was observed in both instars against combined application of *B. bassiana* and *B. thuringiensis* as compared to their individual treatments. These findings are in line with Lacey et al. (1999), who reported lowest number of hibernating adult population of *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) in plots treated simultaneously with *B. bassiana* and *B. thuringiensis*. Similarly Wraight and Ramos (2005) also witnessed that integrated applications of *B. thuringiensis* and *B. bassiana* caused higher larval mortality of Colorado potato beetle than their individual treatments. Moreover, Lewis et al. (1996) also reported that *B. bassiana* reduced the larval population of *Ostrinia nubilalis* (Lepidoptera: Crambidae), but a significant increase in the mortality was observed in those plants where fungus was applied with the combination of *B. thuringiensis.* Similarly, laboratory bioassays demonstrating the effectiveness of integrated application of *B. thuringiensis* and *M. anisopliae* against various larval instars of *H. armigera* (Lawo et al. 2008; Wakil et al. 2013) gave further confirmation of the present findings. Furthermore, the pretreatment of *Crioceris quatuordecimpunctata* larvae with sublethal dose of *B. thuringiensis* toxin suffered significantly higher mortality when exposed to *B. bassiana* infection (Gao et al. 2012).

The enhanced synergisitic effect of the entomopathogens used in this study could be due to the fact that *B. thuringiensis* intoxication induced starvation stress in insects which increased their larval development period and this may increase their susceptibility to fungal infection due to prolonged inter molt periods. These results were in line with Furlong and Groden (2003), who reported that starvation stress increased the infectivity of *B. bassiana* against *Leptinotarsa decemlineata* (Say). Similarly, Kryukov et al. (2009) also reported maximum larval mortality of *L. decemlineata* (Say) with synchronous coinfection of *B. thuringiensis* and *B. bassiana.* The bacteria seized the nutrition of insects, while the fungal spores rapidly kill the weakened larvae. Moreover, feeding restrictions due to fungal infection has also been reported in several insects (Arthurs

and Thomas 2000; Ekesi and Maniania 2000; Tefera and Pringle 2003; Maehara et al. 2007; Mohammadbeigi and Port 2015).

Entomopathogenic fungi also have a great potential to suppress lepidopterous insect pests (Vega-Aquino et al. 2010) whenever applied alone having confirmations in the present findings in which *B. bassiana* caused significant mortality in both larval instars at highest dose rate. In agreement with this srudy, outcomes relating effectiveness of entomopathogenic fungi against various larval instars of *H. armigera* were also observed by Nguyen et al. (2007). A positive correlation between fungal concentration and larval mortality was also observed in the current study, previously reported by various scientists (Sasidharan and Varma 2005; Malarvannan et al. 2010).

The current results further revealed that early (2nd) larval instar of *C. partellus* was more susceptible to infection caused by tested bio-agents than its later (4th) instar. These results are in accordance with the findings of Inglis et al. (2001), who reported that different growth stages of insects vary in their susceptibility to fungal infection. Similarly, Shefik (2010) also reported that the early larval instars (1st and 2nd) of *Phthorimaea operculella* were more susceptible to *B. bassiana* infection as compared to their later (3rd and 4th) instars. Moreover, Pandey and Kanaujia (2004) documented an increase of 1.96 and 3.02 times in LC_{50} value for 3rd and 4th larval instar respectively over 2nd instar larvae of *S. litura* when treated with *M. anisopliae*. The toxicity of *B. thuringiensis* toxin decreased with the development of *C. partellus* larvae, and this decline in the insecticidal activity of *Bt* with the growth and development of lepidopterous larvae is consistent with the previous findings of Herbert and Harper (1985). Similarly, Zehnder and Gelernter (1989) also reported higher larval mortality (40–98%) of 2nd instar of Colorado potato beetle as compared to its 3rd instar (52%) when treated with *B. thuringiensis* var. *san diego* (M-ONE). The possible reason behind this could be the upsurge of melanin contents in the mid gut and cuticle of insects (late instars) which impedes the penetration of the fungal germ tube (Wilson et al. 2001). Moreover, enzymatic activity is also the prime culprit for differences in mortality of different larval instars. It has been stated that the action of detoxification enzymes changes significantly within and among different developmental stages of insects. This action is minor in egg stage, amplifies with each nymphal or larval stage and then again reduces to zero at pupal stage (Ahmad 1986; Mullin 1988).

The persistence of a microbial pesticide in an agro-ecosystem depends not only on its primary inoculum but also relies partly on the secondary infection from mycosed cadavers (Thomas et al. 1995; Wood and Thomas 1996). In our studies, percent mycosis and sporulation (conidia per ml) achieved the maximum in treatment where *B. bassiana* alone was applied at its lowest concentration. These results were supported by Tefera and Pringle (2003), who reported higher percent mycosis and sporulation in *C. partellus* cadavers treated with lower conidial concentration of *B. bassiana* as compared to its higher concentrations. Similarly, Riasat et al. (2011) also noted higher mycosis and sporulation in the cadavers of *R. dominica* at lower conidial concentration of *B. bassiana*. The lower percent mycosis and sporulation at high conidial concentrations may be attributed to the self-inhibiting mechanism of fungal spores at higher concentrations (Garraway and Evans 1984; Tefera and Pringle 2003).

Conclusion

Our results indicated that the combination of *B. thuringiensis* and *B. bassiana* could be a cost effective strategy against *C. partellus* population. Moreover, the chances of resistance development in insects against integrated approaches like use of different entomopathogens is also very low. This approach of combined applications of entomopathogens promises to provide better efficacy than previous attempts to control the insect pest with singly applied treatments but further research is still needed under field conditions for confirmation of the method's success in maize crop.

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Conflict of interest M. Sufyan, A. Abbasi, W. Wakil, M.D. Gogi, M. Arshad, A. Nawaz andZ. Shabbir declare that they have no competing interests.

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