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COMPARATIVE EFFECTIVENESS OF ENTOMOPATHOGENIC FUNGI AGAINST OKRA MEALYBUG *PHENACOCCUS SOLENOPSIS*

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Abstract

Microbial formulations prepared from entomopathogenic fungi emerging as the effective alternate of chemical pesticides against different crop pests. They cause lethal infections and regulate insects and mites population in nature by epizootics and considered as primary candidates for mycoinsecticides. In this study, we evaluated five species of entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria farinosa*, *Paecilomyces lilacinus* and *Verticillium lecanii* against *Phenacoccus solenopsis* on okra crop. Three concentrations calculated on the basis of previous study (LD_{50}, ten times higher and ten times lower than LD_{50}) of each fungal biocontrol agent were applied to okra plants infested with mealybugs. In the pot experiment, the higher dose of *M. anisopliae* (7.063×10^8) and *B. bassiana* (1.37×10^8) followed by *P. lilacinus* (6.615×10^7) were highly effective in controlling the pest population, they brought 83.7, 80 and 78.7% reduction, respectively. Second most effective treatments were a higher dose of *V. lecanii* (1.69×10^7) and a medium dose of *P. lilacinus* (6.615×10^6), brought 69.4 and 53% reduction in mealybugs. Among all entomopathogenic fungi, *I. farinosa* was the least effective; its lower, medium and higher doses caused only 20.7, 48.5 and 51.6% reduction, respectively. In field trial, higher doses of all biocontrol agents were more effective than their medium and lower doses. Whereas, maximum yield was recorded in the higher (7.063×10^8) and medium dose of (7.063×10^7) of *M. anisopliae* followed by a higher (1.37×10^8) and medium (1.37×10^7) dose of *B. bassiana.* This study provides new opportunities for effective and eco-friendly control measures of mealybugs.

Key words: *Phenacoccus solenopsis*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria farinosa*, *Paecilomyces lilacinus*, *Verticillium lecanii.*

Introduction

Use of entomopathogenic fungi as natural enemies of insect pests has been getting more attention in present time. Taxonomically, they are a very heterogeneous group belongs to diversified fungal families. Commonly, they are known to cause diseases in different arthropods, including insects and mites, their capability of infecting targeted hosts are now widely utilizing to regulate insect populations in the agro eco-system. In nature, they caused fatal infections and reduced arthropods by epizootics (Burges, 1981; Carruthers & Soper, 1987; McCoy *et al.*, 1988).

More than 750 fungi belonging to 85 genera have been categorized as entomopathogenic fungi (Gillespie & Moorhouse, 1989; McCoy *et al.*, 1988). However, members of order Entomophthorales and Hypocreales are considered the most important entomopathogens, such as *Metarhizium*, *Beauveria*, *Verticillium*, *Nomuraea*, *Entomophthora* and *Neozygites* (Deshpande, 1999). Mostly entomopathogens are host specific, thus the very rare possibility of infecting beneficial insects or other living organisms (Roberts & Humber, 1981). The mode of action of entomopathogenic fungi against targeted insects is contact, do not require ingestion and parasitize their host through the exoskeleton or cuticle (Chandler *et al.*, 2000; Juarez & Fernandez, 2007). They are present in diversified ecological niche as well as their multiplication in controlled conditions is economically feasible (Ferron, 1978; Roberts & Hajek, 1992). After the death of the insect host, the dead body serves as the source of entomopathogns and fungus produces thousands of new spores, which infect new insects (Wraight *et al.*, 2000).

Mealybugs are one of the new emerging threats to different vegetables, field and fruit crops in Pakistan. Mealybug species can infest fairly a large number of crop plants including species of Cucurbitaceae, Fabaceae, Solanaceae and Malvaceae. About 5000 species of mealybug have been recorded from 246 families of plants throughout the world. Mealybug was never considered as major pests of economic importance in Pakistan before 2005. The severe incidence of mealybug (*Phenacoccus solenopsis*) has been reported from Vihari, Punjab (Pakistan) on cotton in 2005 (Abbas *et al.*, 2005; CCRI, 2006; Zaka *et al.*, 2006; Parvez, 2008). Cotton yield in 2005-06 was lower than expected, partly because of the mealybug infestation (CCRI, 2006; USDA, 2008). These significant losses have urged for effective and quick control measures, either through the application of different insecticides or by any other means (Mohyuddin *et al.*, 1997). Chemical pesticides have been in practice since long as the most effective means to control destructive crop pests, but their harmful effects on non-target organisms, increasing ground water pollution, pesticide residues in edibles and the development of resistance in targeted insect pests have forced the industry and scientists to focus alternate control measures. In many cases, the application of the entomopathogenic fungi has also provided an eco-friendly alternative to chemical pesticides. About 171 products consisting of 12 entomopathogenic species and strains prepared by 80 companies have approached to commercial markets as mycoinsecticides (Goettel *et al.*, 2010). However, literature survey indicates the scarcity of information about the use of entomopathogenic fungi for the control of okra mealybug. This study describes the biocontrol potential of different entomopathogens such as *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria farinosa*, *Paecilomyces lilacinus* and *Verticillium lecanii* to okra mealybug *Phenacoccus solenopsis.*

Materials and Methods

Preparation of entomopathogens: The selected fungal biocontrol agents were multiplied on respective suitable substrates for their screen house and field application against mealybug on okra crop. For each biocontrol agent, the most suitable substrate was selected on the basis of the results of initial studies. For this purpose, *B. bassiana* and *M. anisopliae* were multiplied on oat grains; *I. farinosa* on sorghum grains; *P. lilacinus* and *V. lecanii* on millet grains. Each grain substrate was washed thoroughly, soaked in distilled water for 1 hour and strain through a muslin cloth to remove excess of water. The 50 g grains were transferred separately in plastic bags, tied with rubber bands and autoclaved. When the temperatures of substrates become normal, then each bag was inoculated with 1 ml spores suspension of entomopathogenic fungi separately. These were incubated at 25˚C for 20 days and the conidial population per gram of fermented substrates were determined with the help of haemocytometer. On the basis of findings of LD_{50} studies (Khanzada *et al*., 2020), three concentrations of each biocontrol agent were prepared by taking LD_{50} as a base; others two were ten times higher and ten times lower than LD_{50} (Table 1).

Table 1. The final treatments of fungal biocontrol agents used in pot and field experiments.

Doses	Fungal biocontrol agents				
	B. bassiana	*M. anisopliae*	*I. farinosa*	*V. lecanii*	*P. lilacinus*
1	$1.37{\times}10^6$	$7.063{\times}10^5$	$4.173{\times}10^6$	$1.69{\times}10^5$	$6.615{\times}10^5$
2	$1.37{\times}10^7$	$7.063{\times}10^6$	$4.173{\times}10^7$	$1.69{\times}10^6$	$6.615{\times}10^6$
3	$1.37{\times}10^8$	$7.063{\times}10^7$	$4.173{\times}10^8$	$1.69{\times}10^7$	$6.615{\times}10^7$

Pot experiment: The earthen pots were thoroughly washed and sterilized with sprit before use. They were filled with 2 kg of sterilized soil (the mixture of sand, FYM and clay with equal proportions). Five seeds of commonly growing okra variety 'Sabzpari' were sown in each earthen pot. Pots were irrigated regularly to maintain 50% W.H.C. After one month of sowing, each okra plant was artificially infested with 15 females of okra mealybug. After 15 days of mealybug introduction, plants were sprayed with specific concentrations of biocontrol agents. In order to determine the comparative effectiveness of biocontrol agents, the mealybug population was recorded before one day and after 7 days of application. The experiment was arranged as a Randomized Complete Block Design with three replications for each treatment. In total, there were 16 treatments including one untreated control. In control, the plants were sprayed only with distilled sterilized water.

Field experiment: The field experiment was conducted during the month of July-September 2016. Okra seeds of Sabzpari variety were sown on the ridges, with a plant to plant distance of 6 inches and row to row distance of 18 inches. The size of each sub-plot was 5×6 feet. In the month of August, when the mealybug population reached at economic threshold level, the first application of biocontrol agents was sprayed with the help of small hand operated sprayer. Untreated (control) plots received no biocontrol and spayed only with distilled sterilized water. The second spray was applied after the 20 days interval of the first application. Mealybug population was recorded one day before and 7 days after the application. The experiment was arranged as a Randomized Complete Block Design with 3 replications.

Results

Pot experiment

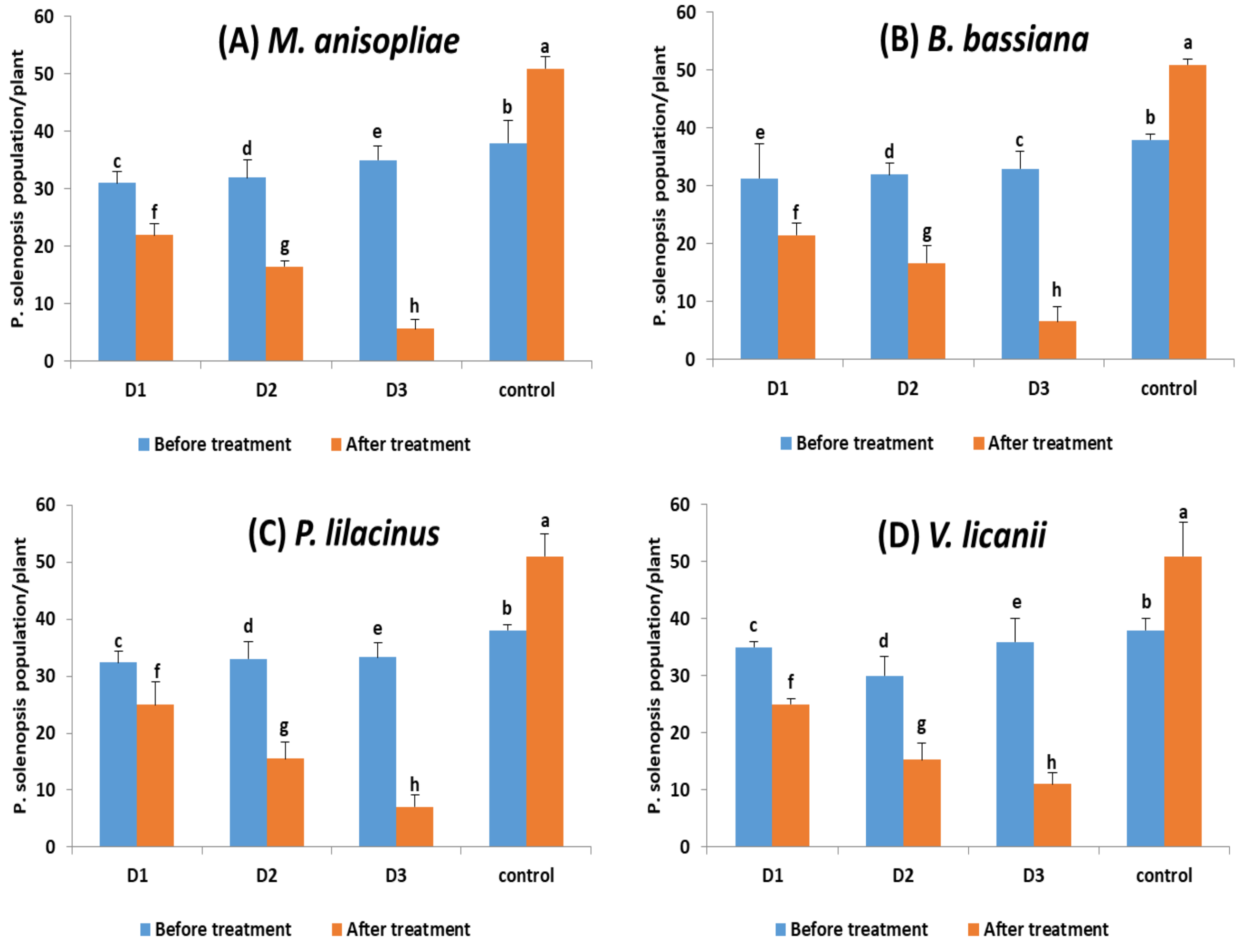
Metarhizium anisopliae: All doses of *M. anisopliae* more or less eliminated the *P. solenopsis* on treated okra plants. However, the lower dose was less effective than the medium and higher doses. The initial dose of *M. anisopliae* ($7.063{\times}10^6$) only reduced the mealybug population from 31 to 21.5, but it was drastically affected with an increasing dose. The medium dose ($7.063{\times}10^{7)}$ of this entomopathogenic fungus reduced the pest population from 32 to 16.5 and a higher dose ($7.063{\times}10^8$) from 35 to 5.7. On the other hand, in untreated (control) plants mealybug population was increased from 38 to 51 (Fig. 1a).

Beauveria bassiana: The almost same pattern was observed in case of *B. bassiana.* Its lower dose ($1.37{\times}10^6$) brought 31% reduction in the pest population. The medium dose ($1.37{\times}10^7$) reduced the mealybug population from 32 to 16.7 (47.8% reduction). While the most remarkable control of *P. solenopsis* was observed in plants sprayed with a higher dose ($1.37{\times}10^8$) of *B. bassiana*, it reduced the pest population from 33 to 6.6 (about 80% reduction) (Fig. 1b).

Paecilomyces lilacinus: A significant control in the mealybug population was observed with different doses of *P. lilacinus* as compared to the control where the pest population has been increased from 38 to 51. The higher dose of *P. lilacinus* provides more promising control than lower and medium doses. The lower dose ($6.615{\times}10^5$) of *P. lilacinus* was comparatively less effective and only reduced mealybugs from 32 to 25; whereas, the medium *P. lilacinus* dose ($6.615{\times}10^6$) minimized about 50% pest population. The higher dose of *P. lilacinus* ($6.615{\times}10^6$) appeared as highly effective, reduced the mealybugs from 33.3 to 7.1 (78.7% reduction) on treated okra plants (Fig. 1c).

Verticillium lecanii: The lower dose ($1.69{\times}10^5$) of *V. lecanii* was least effective reduced the mealybugs from 35 to 25, the medium dose ($1.69{\times}10^6$) appeared as moderately effective reduced about 50% pest population (from 30 to 15.3); while the higher dose ($1.69{\times}10^7$) appeared highly effective, it brought 69% reduction in mealybug population (Fig. 1d).

Isaria farinosa: It was comparatively less effective than other entomopathogenic fungi used. The application of its lower dose ($4.173{\times}10^6$) reduced *P. solenopsis* population from 35.33 to 28 (20.75% reduction). The medium dose was more effective and caused a 48.57% reduction (reduced the mealybugs from 35 to 18). The higher dose ($4.173{\times}10^8$) brought only little increased in the effectiveness of *I. farinosa*, it reduced mealybugs from 31 to 15 (only 51.6% reduction) (Fig. 1e).



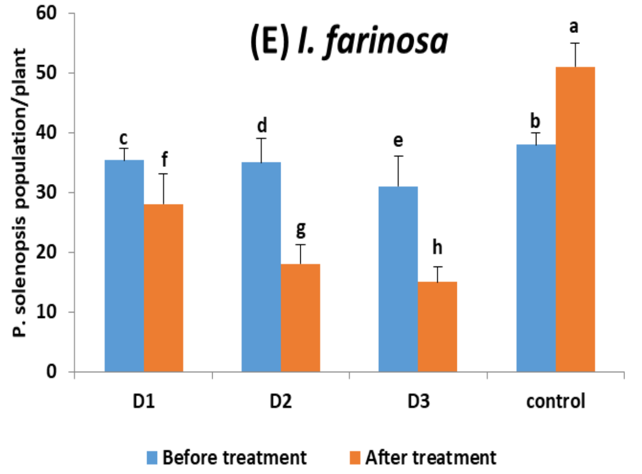


Fig. 1. Mealybug (*P. solenopsis)* population per plant before and after spray and *P. solenopsis* mortality percentage after treatment of okra plant under pot experiment.
A = *M. anisopliae* D1: 7.063×10^6, D2: 7.063×10^7 and D3: 7.063×10^8; B = *B. bassiana* D1: 1.37×10^6, D2: 1.37×10^7 and D3: 1.37×10^8
C = *I. farinosa* D1: 4.173×10^6, D: 24.173×10^7 and D3: 4.173×10^8;
D = *V. lecanii* D1: 1.69×10^5, D2: 1.69×10^6 and D3: 1.69×10^7
E = *P. lilacinus* D1: 6.615×10^5, D2: 6.615×10^6 and D3: 6.615×10^7)

Among five entomopathogenic fungi which applied with three different doses against mealybug infestation, the higher dose of *M. anisopliae* (7.063×10^8) and *B. bassiana* (1.37×10^8) followed by *P. lilacinus* (6.615×10^7) was highly effective in controlling the pest population, bringing 83.7, 80 and 78.7% reduction, respectively. Second most effective treatments included a higher dose of *V. lecanii* (1.69×10^7) and a medium dose of *P. lilacinus* (6.615×10^6), brought 69.4 and 53% reduction in mealybugs, respectively. Among all entomopathogenic fungi, *I. farinosa* was the least effective; its lower, medium and higher doses caused only 20.7, 48.5 and 51.6% reduction, respectively (Fig. 2).

Field experiment: Under field conditions, all biocontrol agents performed well and brought a significant reduction in mealybug infestation on the okra plantation. Generally, their effectiveness was varied with the doses and types of the biocontrol agent. Higher doses of all biocontrol agents were more effective than their medium and lower doses. After 1st spray of different biocontrol agents, a significant maximum reduction in mealybugs was noted in plots sprayed with a higher dose of *B. bassiana* and *M. anisopliae*, brought 62.6% and 60.8% reduction, respectively. Second most effective treatments were a higher dose of *P. lilacinus* and *V. lecanii*, caused 51.9%

and 50.9% reduction in mealybug population on treated okra plants (Table 2).

Collected data also revealed that the application of lower doses of all biocontrol agents killed least mealybugs, but medium dose almost minimized about 50% infestation. After first spray, the lower dose of *M. anisopliae*, *B. bassiana*, *P. lilacinus*, *V. lecanii* and *I. farinosa* caused 26, 22, 24, 20 and 11% reduction in mealybugs, respectively. In all biocontrol agents, *M. anisopliae* and *B. bassiana* performed well against the mealybug infestation under field conditions; while *I. farinosa* was the least effective (Table 2).

The 2nd application of biocontrol agents was done after 20 days interval. It was observed that during this period the mealybug population again increased, which reduced after the first spray. In terms of comparative effectiveness of used biocontrol agents, the trend was similar to the first spray. The remarkable highest reduction in mealybugs was recorded in plants treated with higher doses of *M. anisopliae* (76.6%) followed by *B. bassiana* (70%) (Table 2).

The application of these entomopathogenic fungi enhanced the yield by minimizing the mealybugs in treated plots. Maximum yield was recorded in the higher (7.063×10^8) and medium dose of (7.063×10^7) of *M. anisopliae* followed by a higher (1.37×10^8) and medium (1.37×10^7) dose of *B. bassiana.* Among, five biocontrol agents *M. anisopliae*, *B. bassiana* and *P. lilacinus* performed well in terms of increasing the yield; while *I. farinosa* and *V. lecanii* produced least yield (Fig. 3).

Discussion

All fungal biocontrol agents showed their aggressive activity against okra mealybug, reduced mealybug population and promote plant growth and survival. The biocontrol with suitable concentration, such as *B. bassiana* 1.37×10^8 and *M. anisopliae* 7.063×10^7 appeared as most effective biocontrol agent against mealybug as compared to other entomopathogenic fungi tested. Our findings are closely related to Ujjan *et al*., (2015), who found virulence of *M. anisopliae* in a screen house test on the cotton plants against mealybug. Mohammadbeigi & Port (2013) found that *B. bassiana* (1.5×10^8) and *M. anisopliae* (2×10^7) were caused 100% mortality in direct spray on long-horned grasshopper *Uvarovistia zebra* nymph. *B. bassiana*, *M. anisopliae*, *P. lilacinus*, *V. lecanii* and *I. farinosa* also caused adverse effects on insect growth and development (Wraight *et al*., 2000; Cagan & Sverce, 2001; Carrilloa *et al*., 2015). Herker *et al*., (2010) compared mycosis of *B. bassiana*, *Lecanicillium* (=*Verticillium*) *lecanii*, *P. fumosoroseus* (*Isaria fumosorosea*) and *M. anisopliae* on *Cydia pomonella* and *C. funebrana* (plum fruit moth) and found that *M. anisopliae* and *P. fumosoroseus* produced the highest mycosis rate and mortality of both species. In case of field application, all the above-mentioned fungal biocontrol agents gradually minimized the density of the okra mealybug population (Daniel & Wyss, 2010; Sahayaraj & Namachivayam, 2011). *M. anisopliae* and *B. bassiana* were found more effective than other biocontrol agents, they have strong microbial potential for controlling the okra mealybug. The same findings were recorded by Mohammadbeigi & Port (2013) reported that 1.5×10^8 spores/ml of *B. bassiana* and 2×10^7 spores/ml of *M. anisopliae* caused the highest mortality rate (100-53.3%) of *Uvarovistia zebra*. *Metarhizium* spp. appeared as the best entomopathogenic fungi for controlling *P. solenopsis* under laboratory, greenhouse as well as field condition (Benjamin *et al*., 2002; Ujjan *et al*., 2015). Kaaya & Hedimbi (2012) used *B. bassiana* and *M. anisopliae* against ticks *Rhipicephalus* (*Boophilus*) *appendiculatus*, both entomopathogenic fungi caused 36-64% mortality in the adult tick population.

Table. 2. Effect of fungal biocontrol agents on mealybug population under field condition. ±

Fungal biocontrol agents		Mealybug population					
		Before 1st spray	After 7 days of 1st spray	Reduction %	Before 2nd spray	After 7 days of 2nd spray	Reduction %
	Doses						
M. anisopliae	D1: 7.063×10^6	184 ± 4.56	155 ± 1.19	71 ± 1.47	29 ± 0.9	14 ± 0.93	1 ± 25.2
	D2: 7.063×10^7	173.67 ± 4.56	76 ± 1.19	65.33 ± 1.47	24 ± 0.9	12.33 ± 0.93	0.33 ± 25.2
	D3: 7.063×10^8	166 ± 4.56	40 ± 1.19	51 ± 1.47	23.33 ± 0.9	12 ± 0.93	0 ± 25.2
B. bassiana	D1: 1.37×10^6	170 ± 4.56	104 ± 1.19	61.67 ± 1.47	31 ± 0.9	15.33 ± 0.93	3.33 ± 25.2
	D2: 1.37×10^7	184.33 ± 4.56	105 ± 1.19	65.67 ± 1.47	31.67 ± 0.9	16 ± 0.93	1.67 ± 25.2
	D3: 1.37×10^8	187 ± 4.56	102.33 ± 1.19	73 ± 1.47	36 ± 0.9	18.33 ± 0.93	3 ± 25.2
P. lilacinus	D1: 6.615×10^5	186.33 ± 4.56	117.33 ± 1.19	84.33 ± 1.47	41 ± 0.9	21 ± 0.93	7 ± 25.2
	D2: 6.615×10^6	187.33 ± 4.56	111.67 ± 1.19	83 ± 1.47	41.67 ± 0.9	21.33 ± 0.93	7.33 ± 25.2
	D3: 6.615×10^7	164.33 ± 4.56	108 ± 1.19	81 ± 1.47	40.67 ± 0.9	21 ± 0.93	8 ± 25.2
V. lecanii	D1: 1.69×10^5	170 ± 4.56	112 ± 1.19	94.33 ± 1.47	47 ± 0.9	23.33 ± 0.93	11 ± 25.2
	D2: 1.69×10^6	175.67 ± 4.56	116 ± 1.19	96.33 ± 1.47	47 ± 0.9	23.67 ± 0.93	11 ± 25.2
	D3: 1.69×10^7	181.33 ± 4.56	115 ± 1.19	99.67 ± 1.47	50 ± 0.9	25.33 ± 0.93	12.67 ± 25.2
I. farinosa	D1: 4.173×10^6	163±4.56	120 ± 1.19	97.33 ± 1.47	49 ± 0.9	24.67 ± 0.93	12.33 ± 25.2
	D2: 24.173×10^7	174 ± 4.56	115 ± 1.19	95 ± 1.47	48.33 ± 0.9	24.33 ± 0.93	12.33 ± 25.2
	D3: 4.173×10^8	190.67 ± 4.56	118.33 ± 1.19	96.33 ± 1.47	53 ± 0.9	27.33 ± 0.93	13.67 ± 25.2
Control		186.33 ± 4.56	257.67 ± 1.19	301 ± 1.47	311 ± 0.9	325 ± 0.93	234.67 ± 25.2

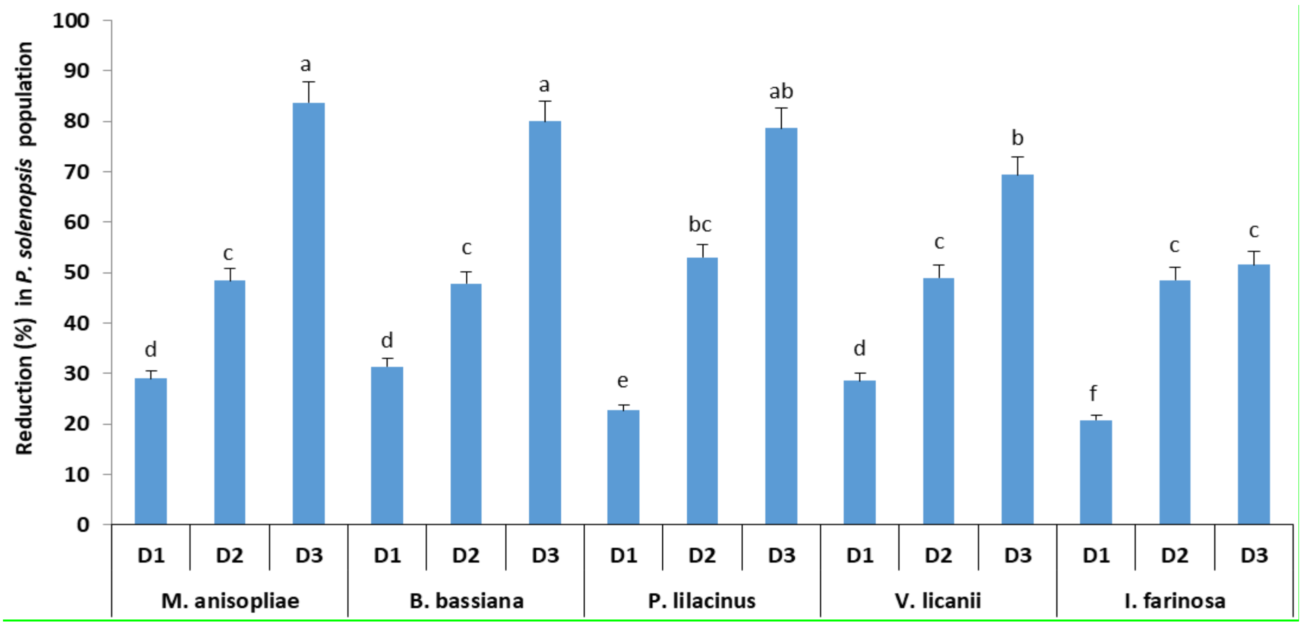


Fig. 2. Reduction in mealybug population due to the application of different doses of entomopathogenic fungi on okra plants grown in pots and artificially infested with *P. solenopsis.*
where;
M. anisopliae D1: 7.063×10^6, D2: 7.063×10^7 and D3: 7.063×10^8; *B. bassiana* D1: 1.37×10^6, D2: 1.37×10^7 and D3: 1.37×10^8; *I. farinosa* D1: 4.173×10^6, D2: 24.173×10^7 and D3: 4.173×10^8; *V. lecanii* D1: 1.69×10^5, D2: 1.69×10^6 and D3: 1.69×10^7; *P. lilacinus* D1: 6.615×10^5, D2: 6.615×10^6 and D3: 6.615×10^7

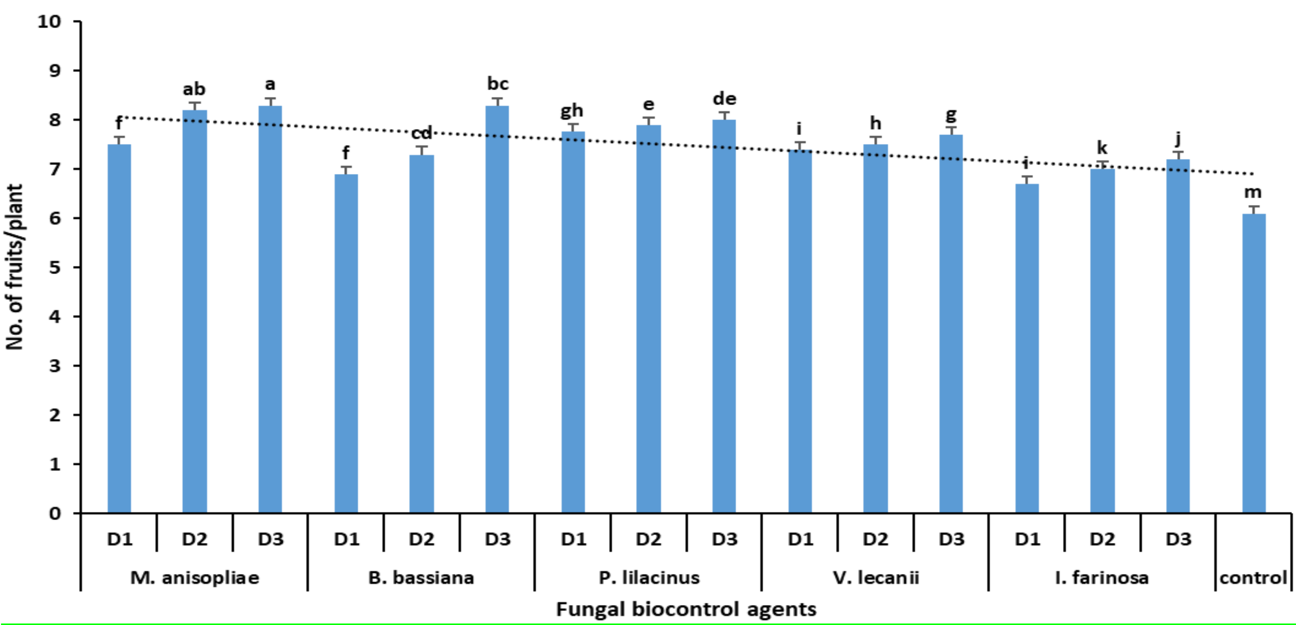


Fig. 3. Effect of fungal bio control agents on okra yield production under field condition.
Where;
M. anisopliae D1: 7.063×10^6, D2: 7.063×10^7 and D3: 7.063×10^8; *B. bassiana* D1: 1.37×10^6, D2: 1.37×10^7 and D3: 1.37×10^8; *I. farinosa* D1: 4.173×10^6, D: 24.173×10^7 and D3: 4.173×10^8; *V. lecanii* D1: 1.69×10^5, D2: 1.69×10^6 and D3: 1.69×10^7; *P. lilacinus* D1: 6.615×10^5, D2: 6.615×10^6 and D3: 6.615×10^7

Conclusions

It is concluded that entomopathogenic fungi (*B. bassiana*, *M. anisopliae*, *I. farinosa*, *P. lilacinus* and *V. lecanii*) have the potential to control the mealybug infestation under field conditions effectively. *Metarhizium anisopliae* and *B. bassiana* appeared highly effective against *P. solenopsis.* Both fungi not only control okra mealybug, but also have a positive effect on okra yield.

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