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Efficacy of Entomopathogenic Fungus *Metarhizium anisopliae* and *Beauveria bassiana* as Bio-control Agent Against Sugarcane White Grubs in Bangladesh

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Abstract

The study was conducted at Regional sugarcrop research station farm, Thakurgaon during the cropping season of 2013-14, 2014-15 and 2015-16 with a view to find out the effective bio-insecticides against white grubs, *Holotrichia seticollis* in sugarcane cultivation. The experiment was laid out in a RCBD with three replications and comprised of seven treatments. Cropping season 2013-14, the highest efficacy 45.86% was observed in T4 (42.41%) and the lowest 24.25% efficacy was found in T1. Cropping season 2014-15, the highest efficacy 41.28% was observed in T6 (41.26%) and the lowest 16.57% efficacy was found in T1. Cropping season 2015-16, the highest efficacy 50.41% was observed in T3 followed by T6 (48.36%) and the lowest 19.11% efficacy was found in T1. In case of Brix% increase over control, the highest 18.14% increase was found in T3 followed by T6 13.78% and the lowest 9.47% was found in T4. Yield increase over control, the highest yield increase 48.83% was found in T6 followed by T3 which was 47.36% and the lowest 30.89% yield increase was found in T1. Among the two bio-pesticides viz., *Metarhizium anisopliae* and *Beauveria bassiana* @ 5.0 kg ha-1 were provided effective control against sugarcane white grubs.

Keywords: Bio-insecticides, *Metarhizium anisoplia*, *Beauveria bassiana* and sugarcane white grub.

Introduction

White grubs, *Holotrichia seticollis* (Coleoptera: Scarabaeidae) are soil-inhabiting and root-feeding immature stages of scarab beetles. The white grub family, Scarabaeidae is the second largest and omnipresent family within the order Coleoptera. The world fauna of white grub exceeds 30,000 species (Mittal, 2000), and there are about 1300 North American species (Borror *et al.*, 1975). The maximum number occurs in the tropical areas of the world, particularly in African and Oriental regions. The fauna of the Indian sub-region is very rich and diverse, but it is yet to be fully explored (Mishra and Singh, 1999). White grubs have become serious pests of sugarcane (Potter *et al.*, 1992). White grubs are polyphagous pests with a wide range of hosts, damaging both on

adult and larval stages; however, the larvae are a greater nuisance. Beetles are defoliating pests and damage many sweet, fruit crops and forest trees as a result of feeding on apical buds and tender leaves, whereas the grubs feed on plant roots, causing yellowing. They cause wilting, which is characterized by an initial purpling of the leaves, followed by the death of small plants and reduced vigor or lodging of larger ones. Sometimes affected plants produce dead hearts. Some plants wilt and ultimately die; such plants can be easily pulled out. The extent of damage caused by white grubs depends on the species involved, the numbers present, and the host crop. In India, white grub is one of the five pests of national importance (Yadava and Vijayavergia, 1994). In many crops, white grubs cause losses to the extent of 40-80 % (Prasad and Thakur, 1959). In Bangladesh, white grub is one of the seven pests of national importance (Miah *et. al.*, 1983). Its attack is mainly confined to the non-flooded sandy loam soil areas in the north and north-western parts of Bangladesh. So far, 17 species of white grubs have been identified in sugarcane (Annon, 1981 and 1984).

Infestation started from February and continued up to August/ September ranging from 20-100% (*Biswas et al.*, 2008.). The grubs feed on the roots and underground portions of stalk rendering the plants looking pale and sickly; ultimately the affected shoot /cane dries up. The affected clumps showing the symptom of wilting. These clumps can easily be pulled out. The canes in the affected clumps fall down and gradually dry up. The cane from affected clumps become useless for crushing as well as seed materials. The yield loss due to white grubs was found to be 23.07-38.17 t ha^{-1} (Miah *et al.*, 1986).

Implementation of microbial control includes several components that require an understanding of host insects and pathogens and the host and pathogenicity behavior. At the same time, farmers' knowledge is of prime important for any scientific study (Gurung, 1985). The severity of white grubs' problem is also due to the involvement within the sandy and sandy loam non-flooded area. The white grub species are both useful and harmful. The beneficial ones include the coprophagous or saprophytes which play a significant role in nutrient cycling as scavengers (Mittal and Vadhara, 1998). Therefore, correct identification of a pest species and understanding of their life cycle is essential before initiating any control measures. Therefore, focused on exploring the indigenous fungal antagonists, method of production and use against damaging species of white grubs in particular and soil pests in general.

Insect pathogenic fungi, *Metarhizium anisopliae* (green muscardine fungus) and *Beauveria bassiana* (white muscardine fungus) were identified by a Swiss scientist, Dr Siegfried Keller for the first time in Nepal. The insect pathogenic fungi from the soils and insects mainly from white grub prone areas of Nepal. The potential of fungal entomopathogens for insect pest control was recognized at the time of the description of *B. bassiana* and already in 1884 E. Metchnikoff had put in place a small plant for the production of fungal inoculums to control the sugar beet weevil. Research in the second half of the last century favored the development of *B. thuringiensis*, which was the first microbial insecticide to obtain practical and economical significance. Microbial control strategies are valuable components in integrated pest management and have advantages over chemical pesticides (Rosset and Moore, 1997). Meanwhile, a number of fungal species have been investigated, undergone commercial development and reached registration. Products, based on *B. bassiana* and *M. anisopliae* are applied in a number of countries namely in Europe (Switzerland, Austria) New Zealand and Australia (Keller, 2000; Rath *et al.*, 1995). Microbial control is compatible with biological, toxicological, environmental and social requirements (Pereira and Roberts, 1991). The sustainability and economics of production of microbial agents is very important which also the efficacy of target organisms and at the same time the non-target organisms (Burges and Hussey, 1971). Therefore, the selection of effective strains of entomopathogens is essential for the development of microbial insecticides.

Modern approaches to crop protection rely on management rather than control or eradication. In this endeavor, a pest species is considered a pest only when its population reaches levels that can cause yield reduction. Pesticides are used only as a last resort to bring pest densities down when crop loss is expected to exceed the cost of treatment. Novel pest control emphasises the use of biological control and other control measures, and especially the chemicals must play a supportive, rather than disruptive role. Chemicals should not be used on a 'calendar' basis but strictly when needed, as defined by systematic pest monitoring. Selective rather than broad-spectrum chemicals should take preference. The aim is to produce high-quality, marketable produce at minimal cost by intelligently using the various control options

to target pests. The present study was undertaken to evaluate the indigenous species of fungi that are pathogenic to white grubs in Bangladesh's sugarcane farmers' fields; to evaluate the entomopathogenic fungus *M. anisopliae* and *B. bassiana* against white grubs in sugarcane fields and to develop a suitable recommendation for eco-friendly control of white grubs to various counterparts in Bangladesh.

Materials and Methods

The field experiment was conducted at the experimental farm of Regional Sugarcrop Research Station (BSRS), Thakurgaon during the cropping seasons of 2013-14, 2014-15 and 2015-16. The experiment consisted of seven treatments in cropping season of 2013-16 including one control. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The individual plot size was 6m × 6m. Block to Block distance was 2m apart with a border of 1.5 m. The variety Isd 37 was used as planting material and planting was done through conventional sett placement In the trenches. The test insecticides were entopathogenic fungus *Metarhizium anisopliae* and *Beauveria bassiana* as bio-control agent. The application schedules of bio-insecticides were such that it was planting, March, May and July. Bio-insecticides application at each time was followed by irrigation. Before irrigation, soil ridges surrounding individual plots were made to protect the flow of water from one plot to another. The fertilizer application, weeding, mulching and earthing up were done as normal cultural practices. For application of bio-insecticides furrows were made on both sides of cane rows and then insecticides were applied at base of the plants and in the space between furrows and mixed with soils followed by irrigation and then covered with soils. The experiment was located in Old Himalayan Piedmont Plain Soil under Agro-ecological Zone-1 with high land of typical sandy loam soil having 4.5 to 5.5 pH.

The experiment was comprised of seven treatments were:

T1: *Metarhizium anisopliae* @ 3.0 kg ha-^{1} applied in Planting + March + May + July

T2: *Metarhizium anisopliae* @ 4.0 kg ha^{-1} applied in Planting + March + May + July

T3: *Metarhizium anisopliae* @ 5.0 kg ha^{-1} applied in Planting + March + May + July

T4: *Beauveria bassiana* @ 3.0 kg ha^{-1} applied in Planting + March + May + July

T5: *Beauveria bassiana* @ 4.0 kg ha^{-1} applied in Planting + March + May + July

T6: *Beauveria bassiana* @ 5.0 kg ha^{-1} applied in Planting + March + May + July

T0: Control (untreated)

The experimental plot was prepared with plowing thoroughly because sugarcane being deep-rooted crops requires a good tilth of soil free from clods for its proper growth. Total fertilizers were applied at the time of final land preparation. TSP, Gypsum, Zinc sulphate, Boron and ⅓ Urea and ⅓ MOP were applied in the trenches. Rest amount of Urea and MOP were applied in two installments at March and June months. *Metarhizium anisopliae* and *Beauveria bassiana* are slow growing fungus. It used as a solution made of powder with water in the sugarcane field. Sugarcane field must be moist condition when sprayed this solution. For counting the population of white grubs, data were taken from 5 randomly selected clumps per plot. An area of 60 cm × 60 cm was dug up from a depth of 40 cm with spade. Population of larvae and adult (if any) was counted in roots and in soils of these pits. Data were taken thrice, 40 days after each application. Total weight was calculated (t ha-1) from each plot and statistically analyzed with the help of Statistic-10 program with LSD test at 5% level of significance (Gomez and Gomez, 1984).

Results and Discussion

Table 1 revealed that in April and June months of the cropping season 2013-14, statistical significance was observed in control plot and other bio-insecticidal treatments among them. From pool data, the highest efficacy 45.86% was observed in *Beauveria bassiana* @ 5.0 kg ha^{-1} followed by *Metarhizium anisopliae* @ 5.0 kg ha^{-1} (42.41%) applied in Planting + March + May + July and the lowest 24.25% efficacy was found in *Metarhizium anisopliae* @ 3.0 kg ha^{-1} in Planting + March + May + July. Also, the bio-insecticidal treated plots showed more than 40% infestation reduction over control which was satisfactory. Raid and Cherry (1992) stated that bio-insecticidal effects of entomopathogenic fungus *Metarhizium anisopliae* could be reduced the infestation of sugarcane grubs in commercial cultivation of sugarcane growing areas of Bangladesh.

From the Table 2, it was observed that in May, June, August and November months of the cropping season 2014-15, statistical significant difference was observed in control plot and other bio-insecticidal treatments in May month. But no significant difference was observed in

August and November months. From pool data, the highest efficacy 41.28% was observed in *Beauveria bassiana* @ 5.0 kg ha^{-1} followed by *Metarhizium anisopliae* @ 5.0 kg ha^{-1} (41.26%) applied in Planting + March + May + July and the lowest 16.57% efficacy was found in *Metarhizium anisopliae* @ 3.0 kg ha^{-1} in Planting + March + May + July. Also the bio-insecticidal treated plots showed more than 40% infestation reduction over control which was satisfactory. Zimmermann (1993) reported that bio-insecticidal effects of entomopathogenic fungus *Metarhizium anisopliae* as a potential bio-control agent on sugarcane white grubs, *Holotrichia serrata* F which was reduced the infestation of sugarcane grubs in commercial cultivation of sugarcane growing areas of Bangladesh.

Table 1. Efficacy of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* on sugarcane white grubs, RSRS, Thakurgaon, 2013-14.

Treatments	white grubs (5 clumps uprooted/plot) Mean		Pool data
	April' 14	June' 14	
T1	9.25bc	4.50ab	7.00
	(24.49)	(28.00)	(24.25)
T2	8.75bcd	4.00b	6.38
	(28.57)	(36.00)	(32.28)
T3	7.25cd	3.50b	5.38
	(40.82)	(44.00)	(42.41)
T4	4.75ab	8.75bcd	6.75
	(24.00)	(28.57)	(26.29)
T5	6.50d	3.00b	5.25
	(42.94)	(41.00)	(41.97)
T6	7.25cd	3.50b	5.12
	(46.82)	(44.90)	(45.86)
T0	12.25a	6.25a	9.25
LSD (0.05)	2.5085	2.0603	-

T1 = Metarhizium anisopliae @ 3.0 kg ha-1 in Planting + March + May + July, T2 = *Metarhizium anisopliae* @ 4.0 kg ha^{-1} in Planting + March + May + July, T3 = *Metarhizium anisopliae* @ 5.0 kg ha^{-1} in Planting + March + May + July, T_4 = *Beauveria bassiana* @ 3.0 kg ha^{-1} in Planting + March + May + July, T_5 = *Beauveria bassiana* @ 4.0 kg ha^{-1} in Planting + March + May + July, T_6 = *Beauveria bassiana* @ 5.0 kg ha^{-1} in Planting + March + May + July T0=Control (untreated)

From the Table 2 it was observed that in May, June, August and November months of the cropping season 2014-15, statistical significant difference was observed in control plot and other bio-insecticidal treatments in May month. But no significant difference was observed in August and November months. From pool data, the highest efficacy 41.28% was observed in *Beauveria bassiana* @ 5.0 kg ha^{-1} followed by *Metarhizium anisopliae* @ 5.0 kg ha^{-1} (41.26%) applied in Planting + March + May + July and the lowest 16.57% efficacy was found in *Metarhizium anisopliae* @ 3.0 kg ha^{-1} in Planting + March + May + July. Also the bio-insecticidal treated plots showed more than 40% infestation reduction over control which was satisfactory. Zimmermann (1993) reported that bio-insecticidal effects of entomopathogenic fungus *Metarhizium anisopliae* as a potential bio-control agent on sugarcane white grubs, *Holotrichia serrata* F which was reduced the infestation of sugarcane grubs in commercial cultivation of sugarcane growing areas of Bangladesh.

Table 2. Efficacy of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* on sugarcane white grubs, RSRS, Thakurgaon, 2014-15.

Treatments	White grubs populations (5 clumps uprooted/plot) Mean				Pool Data
	May'15	Jun'15	Aug'15	Nov'15	
T1	3.67 ab	6.67	2.33	3.33	4.00
	(35.27)	(9.00)	(12.73)	(9.26)	(16.57)
T2	2.67 b	6.00	2.33	3.00	3.50
	(52.91)	(18.14)	(12.73)	(18.26)	(25.51)
T3	2.33 b	4.33	1.78	2.50	2.74
	(58.91)	(40.93)	(33.33)	(31.88)	(41.26)
T4	3.67 ab	6.33	2.00	3.67	3.92
	(35.27)	(13.64)	(25.09)	(9.26)	(20.82)
T5	3.00 ab	7.00	1.67	2.67	3.59
	(47.09)	(4.50)	(37.45)	(27.24)	(29.07)
T6	3.00 ab	4.10	1.67	2.33	2.78
	(47.09)	(44.07)	(37.45)	(36.51)	(41.28)
T0	5.67 a	7.33	2.67	3.67	4.84
LSD (0.05)	2.9395	ns	ns	ns	-

T1 = Metarhizium anisopliae @ 3.0 kg ha-1 in Planting + March + May + July, T2 = *Metarhizium anisopliae* @ 4.0 kg ha^{-1} in Planting + March + May + July, T3 = *Metarhizium anisopliae* @ 5.0 kg ha^{-1} in Planting + March + May + July, T_4 = *Beauveria bassiana* @ 3.0 kg ha^{-1} in Planting + March + May + July, T_5 = *Beauveria bassiana* @ 4.0 kg ha^{-1} in Planting + March + May + July, T_6 = *Beauveria bassiana* @ 5.0 kg ha^{-1} in Planting + March + May + July T0=Control (untreated)

From the Table 3, it was found that in May, June and August months of the cropping season 2015-16, statistically significant difference was observed in control plot and other bio-insecticidal treatments in August month. But no significant difference was observed in May and June months. From pool data, the highest efficacy 50.41% was observed in *Metarhizium anisopliae* @ 5.0 kg ha^{-1} followed by *Beauveria bassiana* @ 5.0 kg ha^{-1} (48.36%) applied in Planting + March + May + July and the lowest

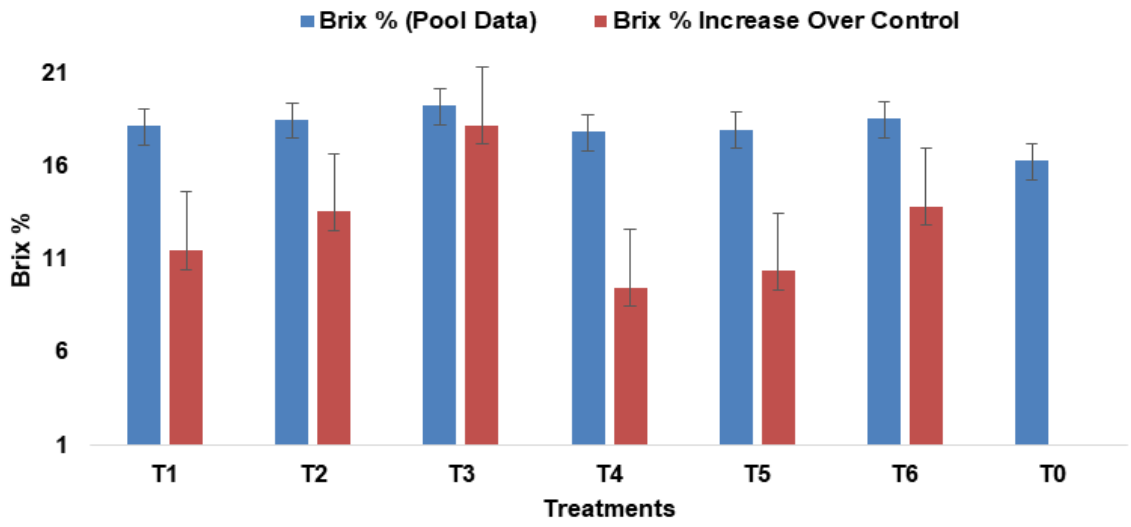

Figure 1. Effects of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* on Brix% of sugarcane against white grubs, RSRS, Thakurgaon, 2013-16.

T1 = Metarhizium anisopliae @ 3.0 kg ha-1 in Planting + March + May + July, T2 = *Metarhizium anisopliae* @ 4.0 kg ha^{-1} in Planting + March + May + July, T3 = *Metarhizium anisopliae* @ 5.0 kg ha^{-1} in Planting + March + May + July, T_4 = *Beauveria bassiana* @ 3.0 kg ha^{-1} in Planting + March + May + July, T_5 = *Beauveria bassiana* @ 4.0 kg ha^{-1} in Planting + March + May + July, T_6 = *Beauveria bassiana* @ 5.0 kg ha^{-1} in Planting + March + May + July T0=Control (untreated)

19.11% efficacy was found in *Metarhizium anisopliae* @ 3.0 kg ha^{-1} in Planting + March + May + July. Also the bio-insecticidal treated plots showed more than 40% infestation reduction over control which was satisfactory. David *et al.*, (1997) found that bio-insecticidal effects of entomopathogenic fungus *Metarhizium anisopliae* as a potential agent of integrated control on sugarcane white grubs, *Holotrichia serrata* F which was reduced the infestation of sugarcane grubs in commercial cultivation of sugarcane growing areas of Bangladesh.

From the Figure 1 it was observed that Brix% (pool/mean data) of the three cropping season 2013-16, the highest Brix 19.21% was observed in *Metarhizium anisopliae* @ 5.0 kg ha^{-1} followed by *Beauveria bassiana* @ 5.0 kg ha^{-1} (18.50%) applied in Planting + March + May + July and the lowest 16.26% Brix was found in control plot. In case of Brix% increase over control, the highest Brix increase over control 18.14% was found in *Metarhizium anisopliae* @ 5.0 kg ha^{-1} followed by *Beauveria bassiana* @ 5.0 kg ha^{-1} 13.78% applied in Planting + March + May + July and the lowest 9.47% Brix increase over control was found in *Beauveria bassiana* @ 3.0 kg ha^{-1} treated plot applied in Planting + March + May + July. Butt *et al.*, (2000)

Table 3. Efficacy of Entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* on sugarcane white grubs, RSRS Thakurgaon, 2015-2016

Treatments	Percent infestation Mean (5 clumps/plot)			Pool data
	May' 16	Jun' 16	Aug' 16	
T1	3.00 (18.25)	5.20 (13.33)	7.67ab (25.75)	5.29 (19.11)
T2	2.47 (32.69)	4.33 (24.83)	6.97b (32.53)	4.59 (33.67)
T3	1.67 (54.49)	3.10 (48.33)	5.33b (48.40)	3.37 (50.41)
T4	3.33 (9.26)	4.52 (24.67)	7.57ab (26.72)	10.10 (20.22)
T5	2.88 (21.53)	3.93 (34.50)	6.67ab (35.43)	4.49 (30.49)
T6	1.73 (52.86)	3.37 (43.83)	5.33b (48.40)	3.48 (48.36)
T0	3.67	6.00	10.33a	-
LSD(0.05)	ns	ns	2.95	

T1 = Metarhizium anisopliae @ 3.0 kg ha-1 in Planting + March + May + July, T2 = *Metarhizium anisopliae* @ 4.0 kg ha^{-1} in Planting + March + May + July, T3 = *Metarhizium anisopliae* @ 5.0 kg ha^{-1} in Planting + March + May + July, T_4 = *Beauveria bassiana* @ 3.0 kg ha^{-1} in Planting + March + May + July, T_5 = *Beauveria bassiana* @ 4.0 kg ha^{-1} in Planting + March + May + July, T_6 = *Beauveria bassiana* @ 5.0 kg ha^{-1} in Planting + March + May + July T0=Control (untreated)

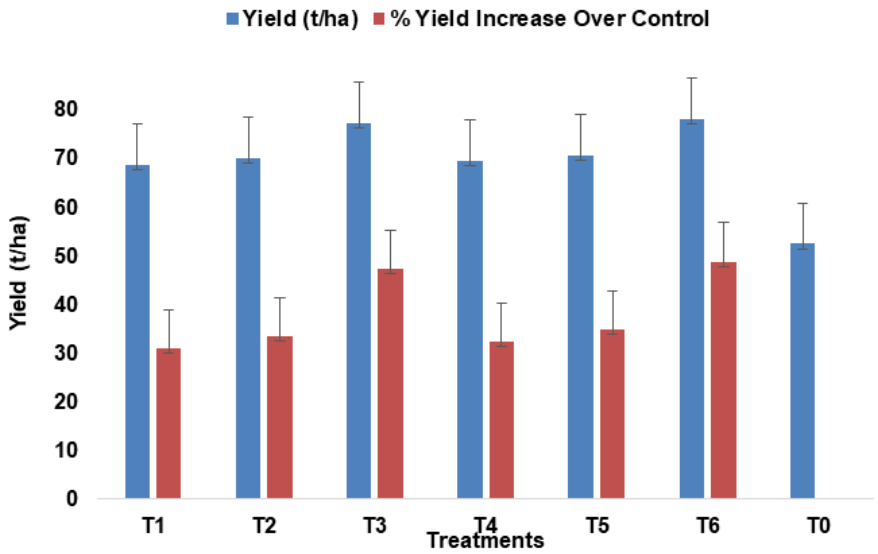

Figure 2. Effects of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* on yield of sugarcane against white grubs, RSRS, Thakurgaon, 2013-16

T1 = Metarhizium anisopliae @ 3.0 kg ha-1 in Planting + March + May + July, T2 = *Metarhizium anisopliae* @ 4.0 kg ha^{-1} in Planting + March + May + July, T3 = *Metarhizium anisopliae* @ 5.0 kg ha^{-1} in Planting + March + May + July, T_4 = *Beauveria bassiana* @ 3.0 kg ha^{-1} in Planting + March + May + July, T_5 = *Beauveria bassiana* @ 4.0 kg ha^{-1} in Planting + March + May + July, T_6 = *Beauveria bassiana* @ 5.0 kg ha^{-1} in Planting + March + May + July T0=Control (untreated)

observed that bio-insecticidal effects of entomopathogenic fungus *Metarhizium anisopliae* as a potential agent of integrated control on sugarcane white grubs, *Holotrichia serrata* F which was reduced the infestation of sugarcane grubs in commercial cultivation of sugarcane growing areas of Bangladesh.

From the Figure 2 reaveled thar yield tha^{-1} (pool/mean data) of the three cropping season 2013-16, the highest yield 78.09 was observed in *Beauveria bassiana* @ 5.0 kg ha^{-1} followed by *Metarhizium anisopliae* @ 5.0 kg ha^{-1} which was 77.32 tha^{-1} applied in Planting + March + May + July and the lowest 52.47 yield was found in control plot. In case of yield increase over control, the highest yield increase over control 48.83% was found in *Beauveria bassiana* @ 5.0 kg ha^{-1} followed by *Metarhizium anisopliae* @ 5.0 kg ha^{-1} which was 47.36% applied in Planting + March + May + July and the lowest 30.89% yield increase over control was found in *Metarhizium anisopliae* @ 3.0 kg ha^{-1} treated plot applied in Planting + March + May + July. Keller *et al.* (2003) stated that effects of entomopathogenic fungus *Metarhizium anisopliae* as a potential bio-agent of sugarcane white grubs, *Holotrichia serrata* F control which was reduced the infestation of sugarcane grubs in commercial cultivation of sugarcane growing North-western regions of Bangladesh.

Conclusions

The following conclusions are drawn from experiments conducted under the studies:

- Bio-pesticides *Metarhizium anisopliae* and *Beauveria bassiana* might be used alternatively to chemical control as an environmental friendly component which might reduce the use of chemical insecticides against sugarcane white grubs and management cost and ensure environmental safety.
- Among the two bio-pesticides viz., *Metarhizium anisopliae* and *Beauveria bassiana* @ 5.0 kg ha^{-1} were provided effective control against sugarcane white grubs in commercial cultivation of sugarcane growing North-Western regions of Bangladesh.

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