JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2016. Vol. **10**(2), p. 1485-1490

Laboratory Evaluation of the Bio-Pesticide and Synthetic Insecticides against Sugarcane White Grub *Holotrichia nagpurensis* in Western Plain Zone of Uttar Pradesh

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(Received: 03 March 2016; accepted: 11 April 2016)

Laboratory experiments were conducted in bio-control laboratory, SVPUA&T, Modipuram during 2011-12 and 2012-13 to evaluate the effectiveness of entomopathogenic fungi (EPF) *Beauveria bassiana, Metarhizium anisopliae*, entomopathogenic nematodes(EPN) *Heterorhabditis indica* and one insecticides Chloroyriphos20 EC against white grub *Holotrichia nagpurensis*damaging sugarcane. On the basis of average cumulative grubs mortality during 2011-12 and 2012-13 the treatment *H. indica*1000 IJsml^{-1} (4.0x10^{9}) was found the superior as compared to the other bio agents and recorded 62.78 percent and 62.45 percent decrease in grub population, respectively. The treatment *H. indica* 2000 IJs ml^{-1} (5.0x10^{9})resulted in 61.66 and 61.94 percent, chloropyriphos 20 EC 58.05 and 60.83 percent, *Beauveria bassiana* (2x10^{8}) 44.44 and 47.22, *Metarhizium anisopliae* (2x10^{8}) 34.44 and 32.77 percent, *Beauveria bassiana* x *Metarhizium anisopliae* (2x10^{8}) 29.72 and 28.61, *Metarhizium anisopliae* (2x10^{6}) 21.44 and 21.11 percent, *Beauveria bassiana* x *Metarhizium anisopliae* (2x10^{6}) 13.33 percent decrease in grub population, respectively. The lowest grub mortality was recorded6.11 percent and 7.49 percent decrease in grub population by the treatment *B. bassiana* (2.0x10^{6}sporesg–1). According to the findings the application of *H. indica* 1000 IJsml^{-1} (4.0x10^{9}) is recommend to manage the grub population effectively.

Keywords: *Heterorhabditis, Beauveria, Metarhizium, chloropyriphos, bio-efficacy, Holotrichia nagpurensis, sugarcane.*

In western Uttar Pradesh, sugarcane is important cultivated cash crop, which is being damaged by a grub popularly known as May-June beetle and is menace problem for sugarcane crop. The adults emerge in the month of June following the pre-monsoon rain and feed on the leaves of Neem, Jamun and Poplar plants besides other wild host plants at night thereby defoliating these valuable plants. The grubs of this pest cause severe damage from August to March by feeding on root zone of almost all agricultural crops resulting in symptoms of wilting and drying of plants. Entomopathogenic fungi suchas *Beauveria* and *Metarhizium* have been proved tobe useful microbial agents for the management of *Holotrichia* spp. (Sharma *et al.*, 1998; Bhagat *et al.*, 2003; Gupta *et al.*, 2003; Hiromori*et al.*, 2004)

Several insecticides are recommended for the management of white grubs, but in fact the insecticides do not provide satisfactory control unless used in very high dose, which in turn becomes hazardous and uneconomic besides being unsustainable Santos *et al.* (2008) A satisfactory solution to these conflicting demands can be met through developing safer control methods considering the microbial agents like entomopathogenic fungus (EPF) and

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entomopathogenic nematodes (EPN) against white grub so as to develop a viable, environmentally safe method of management Raid and Cherry (1992)Further, trapping of adult white grub beetles with light source can also be considered economical over pheromones in the context of multi species situation for the management of adults Shah and Garg 1988. There is an ample scope of increasing sugarcane production in the districts like Meerut, Muzaffarnagar, Saharanpur, Bagpat, Ghaziabad and nearby area, as the sugarcane is a main crop of these regions. The present study was done to study the bio-efficacy of bio-pesticide against white grub in laboratory conditions.

MATERIALS AND METHODS

The experiment was laid out in the bio-control laboratory, S.V.P.U.A.T, Modipuram, Meerut in the last week of March, 2011-12 and 2012-013. In this evaluation, the bio-pesticides and one synthetic insecticide were used on grubs of white grub. The late instar grubs of white grubs were collected from the infested sugarcane field by digging. They were released into autoclaved mixture of F.Y.M and fine sand in the ratio of 1:1 in plastic box and required moisture level was maintained by adding water to the mixture of FYM and fine sand. Half decay potato tubers were placed in each box for the feeding of grubs. A separate untreated control was also maintained without any treatment. Applications of entomopathogenic fungi were done by mixing their powder formulation in the soil before release of grubs. Also, aqueous solution of entomopathogenic nematodes and chlorpyriphos 20 EC were drenched into soil and mixed thoroughly. For each treatment 20 grubs were released and replicated thrice. Trial was conducted at room temperature. All the boxes were secured with a lid on them. All the treatments were applied on grubs of white grub with a dosage mentioned against them. Observations on larval mortality were recorded at 7, 14, 30, 45 and 60 days after treatment imposition and per cent larval mortality was calculated by the formula. (Rakesh H.S 2007) studies on arecanut root grub, Leucopholislepidophora Blanch. and its management by entomopathogenic fungi and plant products.

The data recorded from different treatments were subjected to statistical analysis through OPSTAT computer program.

$$\text{Percent reduction} = \frac{\text{Infestation in control plot} - \text{Infestation in treated plot}}{\text{Infestation in control plot}} \times 10$$

RESULTS AND DISCUSSION

Mortality after seven days

The mortality of grubs after seven days of treatment ranged from 0.00 to 47.22 per cent among all the treatments, the highest mortality of 47.22 per cent was recorded in treatment with chlorpyriphos 20 EC and it was significantly superior over all other treatments. This was followed by *Hetrorhabditis indica* 2000, 1000 and 500 IJs/ per cent with 36.11, 30.55 and 22.22 per cent mortality during 2011- 12 (Table 1). Present findings are in conformity with the finding of (Gupta *et al.* 1998, Sharma *et al*. 2009 and Prasad *et al.* 2012).

The mortality of grubs after seven days of treatment ranged from 0.00 to 45.83 per cent among all the treatments, the highest mortality of 45.83 per cent was recorded in treatment with chlorpyriphos 20 EC and it was significantly superior over all other treatments. This was followed by *Hetrorhabditis indica* 2000, 1000 and 500 IJs with 33.33, 20.67 and 13.89 per cent mortality during 2012- 13. (Table 1) Present findings are in conformity with the finding of (Gupta *et al*. 1998, Sharma *et al*. 2009 and Prasad *et al*.2012).

Among two fungal pathogens, *M. anisopliae* with 2 x 10^8 and 2 x 10^6 conidial doses srecorded no mortality of root grubs at 7 days after treatment. *B. bassiana* with 2 x $10^8$and 2 x 10^6 conidial doses also recorded no mortality of root

Treatment	Dose/ ha
$T_{1:}$ *Beauveria bassiana*	(2x10^8 CFU/g)
$T_{2:}$ *Metarhizium anisopliae*	(2x10^8 CFU/g)
$T_{3:}$ *Beauveria bassiana*	(2x10^6 CFU/g)
$T_{4:}$ *Metarhizium anisopliae*	(2x10^6 CFU/g)
$T_{5:}$ *B. bassiana* x *M. anisopliae*	(2x10^6 CFU/g)
$T_{6:}$ *B. bassiana* x *M. anisopliae*	(2x10^8 CFU/g)
$T_{7:}$ *Heterorhabditis indica*	(1000 IJs /ml)
$T_{8:}$ *Heterorhabditis indica*	(2000 IJs/ml)
$T_{9:}$ *Heterorhabditis indica*	(500 IJs/ml)
$T_{10:}$ Chlorpyrifos	20 EC
$T_{11:}$ Control	Untreated

Table 1. Evaluation of bio-agents and synthetic insecticides against sugarcane white grub, under laboratory condition during 2011-12 and 2012-13.

S. No.	Treatments	Dose / ha	Per cent mortality 2012					Average	Per cent mortality 2013					Average
			7 DAT	14 DAT	30 DAT	45 DAT	60 DAT		7 DAT	14 DAT	30 DAT	45 DAT	60 DAT	
1	*Hetrorhabditis indica*	5 x 10^9	36.11	48.61	62.50	77.77	83.33	61.664	33.33	51.39	66.67	72.22	86.11	61.944
	2000 IJs		(36.92)	(44.18)	(52.24)	(61.86)	(65.98)	(52.236)	(35.23)	(45.78)	(54.74)	(58.18)	(68.32)	(52.45)
2	*H.indica* 1000 IJs	4 x 10^9	30.55	45.83	63.89	84.73	88.89	62.788	20.67	59.83	65.28	75.22	90.27	62.454
			(33.53)	(42.58)	(53.05)	(67.02)	(70.57)	(53.350)	(26.95)	(43.29)	(53.88)	(60.18)	(71.87)	(51.234)
3	*H.indica* 500 IJs	2.5 x 10^9	22.22	36.11	52.00	59.72	72.22	48.454	13.89	34.73	55.55	59.75	70.83	46.95
			(28.09)	(34.39)	(46.13)	(50.58)	(58.18)	(43.474)	(21.83)	(36.92)	(48.17)	(50.89)	(57.28)	(43.018)
4	Chiloropyriphos	500 g *a.i*	47.22	51.39	59.72	63.88	68.06	58.054	45.83	59.72	62.50	66.67	69.44	60.832
	20 EC		(43.38)	(45.77)	(50.58)	(53.05)	(55.57)	(49.670)	(42.58)	(50.58)	(52.24)	(54.72)	(56.43)	(51.31)
5	*Metarhizium anisopliae*	5 Kg	0.00	13.89	29.17	51.39	77.77	34.444	0.00	16.67	30.55	37.50	79.16	32.776
	2x10^8		(0.00)	(21.82)	(32.64)	(45.77)	(61.86)	(32.418)	(0.00)	(23.97)	(33.53)	(37.73)	(62.81)	(31.608)
6	*B. bassiana* x	5 Kg	0.00	9.72	26.39	43.05	69.44	29.72	0.00	9.72	26.39	34.72	72.22	28.61
	M. anisopliae 2x10^8		(0.00)	(18.07)	(30.88)	(40.98)	(56.43)	(29.272)	(0.00)	(16.42)	(30.88)	(36.09)	(58.18)	(28.314)
7	*M.anisopliae*2x10^6	5 Kg	0.00	5.55	19.44	31.95	52.77	21.942	0.00	6.94	15.28	27.77	55.55	21.108
			(0.00)	(13.43)	(26.13)	(34.39)	(46.57)	(24.104)	(0.00)	(15.10)	(22.95)	(31.78)	(48.17)	(23.600)
8	*Beauveria bassiana*	5 Kg	0.00	4.16	18.06	27.77	44.44	18.886	0.00	5.55	13.89	25.00	47.22	18.332
	2x 10^8		(0.00)	(11.76)	(25.12)	(31.77)	(41.78)	(22.086)	(0.00)	(13.43)	(21.83)	(29.98)	(43.38)	(21.724)
9	*B. bassiana* x	5 Kg	0.00	2.77	15.28	22.22	26.39	13.332	0.00	4.16	8.33	22.22	31.95	13.332
	M. anisopliae 2x10^6		(0.00)	(7.84)	(22.96)	(28.09)	(30.88)	(17.954)	(0.00)	(11.76)	(16.78)	(28.09)	(34.39)	(18.204)
10	*B. bassiana* 2x10^6	5 Kg	0.00	0.00	5.55	9.72	15.28	6.11	0.00	1.39	4.16	11.11	20.83	7.498
			(0.00)	(0.00)	(13.43)	(18.08)	(22.96)	(10.894)	(0.00)	(3.92)	(11.76)	(19.21)	(27.14)	(12.406)
11	Control	-	0.00	0.00	1.39	4.16	4.16	1.942	0.00	0.00	1.39	5.55	5.55	2.498
			(0.00)	(0.00)	(3.93)	(11.76)	(11.76)	(5.490)	(0.00)	(0.00)	(3.92)	(13.44)	(13.43)	(6.158)
CD at 5%			1.533	4.457	4.974	2.663	3.044	3.3342	2.557	5.174	4.449	3.737	3.327	3.8488

DAT- days after treatment.
Values with in parentheses are angular transformed.

grubs at 7 days after treatment during 2011-12 and 2012-13 (Table 1). These findings are in conformity with the finding of (Gupta *et al.* 1998 and Prasad *et al.* 2012).

Mortality after fourteendays

After 14 days of treatment, the mortality of grubs among all the treatments ranged from 0.00 to maximum of 51.39 per cent. The highest per cent mortality was recorded in treatment with chloropyriphos 20 EC. This treatment was significantly superior over all other treatments. This was followed by *Hetrorhabditis indica* 2000, 1000, 500 IJs with 48.61, 45.83 and 36.11 per cent mortality (Table 1). These findings are in conformity with the finding of (Sharma *et al*. 2009 and Prasad *et al.*2012).

Among the entomopathogenic fungi *Metarhizium anisopliae* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 13.89 and 5.55 percent mortality of grubs at 14 days after treatment. *Beauveria bassiana* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 4.16 per cent mortality and no mortality found. The combination of the entomopathogenic fungi *M. anisopliae* X *B. bassiana* 2 x 10^8 and 2 x 10^6 conidial doses recorded 9.72 and 2.77 percent mortality of grubs of white grub at 14 days after treatment during 2011-12 (Table 1). These findings are in conformity with the finding of (Sharma *et al*. 2009 and Prasad *et al.* 2012).

During 2012 – 13, after 14 days of treatment, the mortality of grubs, among all the treatments ranged from 0.00 to maximum of 59.72 per cent. The highest per cent mortality was recorded in treatment with chloropyriphos 20 EC. This treatment was significantly superior over all other treatments. This was followed by *Hetrorhabditis indica* 2000, 1000, 500 IJs with 51.39, 59.83 and 34.73 per cent mortality (Table 1). These findings are in conformity with the finding of (Hajeri 2003 and Prasad *et al.* 2012).

Among the entomopathogenic fungi *M. anisopliae* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 16.67 and 6.94 percent mortality of grubs at 14 days after treatment. *B. bassiana* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 5.55 and 1.39 per cent mortality. The combination of the entomopathogenic fungi *M. anisopliae* X *B. bassiana* 2 x 10^8 and 2 x 10^6 conidial doses recorded 9.72 and 4.16 percent mortality of grubs at 14 days after treatment (Table 1). These findings are in conformity with the finding of (Sharma *et al*. 2009 and Prasad *et al.*2012).

Mortality after thirty days

After 30 days of treatment, the mortality of grubs, among all the treatments ranged from 1.39 to maximum of 62.50 per cent. The highest per cent mortality was recorded in treatment with *H. indica* 2000 IJs. This treatment was significantly superior over all other treatments. This was followed by *H. indica* 1000, 500 IJs with 63.89 and 52.00 per cent mortality. The chloropyriphos 20 EC was recorded 59.72 per cent mortality of grubs of white grub (Table 1). These findings are in conformity with the finding of (Sharma *et al*. 2009 and Prasad *et al.* 2012).

Among the entomopathogenic fungi *M. anisopliae* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 29.17 and 19.44 percent mortality of grubs at 30 days after treatment. *B. bassiana* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 18.06 per cent mortality and 5.55 mortality found. The combination of the entomopathogenic fungi *M. anisopliae* X *B. bassiana* 2 x 10^8 and 2 x 10^6 conidial doses recorded 26.39 and 15.28 percent mortality of grubs of white grub. The chloropyriphos 20 EC was recorded 59.72 per cent mortality of grubs of white grub at 30 days after treatment during, 2011-12 (Table 1). These findings are in conformity with the finding of (Milner 1990, Sharma *et al*. 2009 and Prasad *et al.* 2012).

During 2012 – 13, after 30 days of treatment, the mortality of grubs of white grub, among all the treatments ranged from 1.39 to maximum of 66.67 per cent. The highest per cent mortality was recorded in treatment with *Hetrorhabditis indica* 2000 IJs. This treatment was significantly superior over all other treatments. This was followed by *H. indica* , 1000, 500 IJs with 65.28 and 55.55 per cent mortality. The chloropyriphos 20 EC was recorded 62.50 per cent mortality of grubs of white grub at 30 days after treatment (Table 1). Present findings are in conformity with the finding of (Sharma *et al*. 2009 and Prasad *et al.*2012).

Among the entomopathogenic fungi *M. anisopliae* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 30.55 and 15.28 percent mortality of grubs at 30 days after treatment. *B. bassiana* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 13.89 and 4.16 per cent mortality. The combination of the

entomopathogenic fungi *M. anisopliae* X *B. bassiana* 2 x 10^8 and 2 x 10^6 conidial doses recorded 26.39 and 8.33 percent mortality of grubs of white grub at 30 days after treatment (Table 1). Present findings are in conformity with the finding of (Sharma *et al*. 2009 and Prasad *et al.* 2012).

Mortality after forty five

After 45 days of treatment, the mortality of grubs, among all the treatments ranged from 4.16 to maximum of 84.73 per cent. The highest per cent mortality was recorded in treatment with *H. indica* 1000 IJs. This treatment was significantly superior over all other treatments. This was followed by *H. indica* 2000, 500 IJs with 77.77 and 59.72 per cent mortality. The chloropyriphos 20 EC was recorded 63.88 per cent mortality of grubs of white grub (Table 1). Present findings are in conformity with the finding of (Sharma *et al*. 2009 and Prasad *et al.*2012).

Among the entomopathogenic fungi *M. anisopliae* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 51.39 and 31.95 percent mortality of grubs at 45 days after treatment. *B. bassiana* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 27.77 per cent mortality and 9.72 mortality found. The combination of the entomopathogenic fungi *M. anisopliae* X *B. bassiana* 2 x 10^8 and 2 x 10^6 conidial doses recorded 43.05 and 22.22 percent mortality of grubs of white grub. The chloropyriphos 20 EC was recorded 59.72 per cent mortality of grubs of white grub at 45 days after treatment during 2011-12 (Table 1). Present findings are in conformity with the finding of (Sharma *et al*. 2009 and Prasad *et al.* 2012).

During 2012 – 13, After 45 days of treatment, the mortality of grubs, among all the treatments ranged from 5.55 to maximum of 75.22 per cent. The highest per cent mortality was recorded in treatment with *Hetrorhabditis indica* 1000 IJs. This treatment was significantly superior over all other treatments. This was followed by *H. indica*, 2000, 500 IJs with 72.22 and 59.75 per cent mortality. The chloropyriphos 20 EC was recorded 66.67 per cent mortality of grubs of white grub at 45 days after treatment (Table 1). Present findings are in conformity with the finding of (Sharma *et al*. 2009 and Prasad *et al.*2012).

Among the entomopathogenic fungi *M. anisopliae* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 37.50 and 27.77 percent mortality of grubs at 45 days after treatment. *B. bassiana* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 25.00 and 11.11 per cent mortality. The combination of the entomopathogenic fungi *M. anisopliae* X *B. bassiana* 2 x 10^8 and 2 x 10^6 conidial doses recorded 34.72 and 22.22 percent mortality of grubs of white grub at 45 days after treatment (Table 1). Present findings are in conformity with the finding of (Sharma *et al*. 2009 and Prasad *et al.* 2012).

Mortality after sixty days

After 60 days of treatment, the mortality of grubs, among all the treatments ranged from 4.16 to maximum of 88.89 per cent. The highest per cent mortality was recorded in treatment with *H. indica* 1000 IJs. This treatment was significantly superior over all other treatments. This was followed by *H. indica* 2000, 500 IJs with 83.33 and 72.22 per cent mortality. The chloropyriphos 20 EC was recorded 68.06 per cent mortality of grubs of white grub (Table 1). These findings are in conformity with the finding of(Braza 1990, Sharma *et al*. 2009and Prasad *et al.* 2012).

Among the entomopathogenic fungi *M. anisopliae* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 77.77 and 52.77 percent mortality of grubs at 60 days after treatment. *B. bassiana* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 44.44 per cent mortality and 15.28 mortality found. The combination of the entomopathogenic fungi *M. anisopliae* X *B. bassiana* 2 x 10^8 and 2 x 10^6 conidial doses recorded 69.44 and 26.39 percent mortality of grubs of white grub during 2011-12 (Table 1). Present findings are in conformity with the finding of (Sharma *et al*. 2009 and Prasad *et al.*2012).

During 2012 – 13, after 60 days of treatment, the mortality of grubs, among all the treatments ranged from 5.55 to maximum of 90.27 per cent. The highest per cent mortality was recorded in treatment with *Hetrorhabditis indica* 1000 IJs. This treatment was significantly superior over all other treatments. This was followed by *H. indica*, 2000, 500 IJs with 86.11 and 70.83 per cent mortality. The chloropyriphos 20 EC was recorded 69.44 per cent mortality of grubs of white grub at 60 days after treatment (Table 1). These findings are in conformity with the finding of (Braza 1990, Sharma *et al*. 2009 and Prasad *et al.*2012).

Among the entomopathogenic fungi *M. anisopliae* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 79.16 and 55.55 percent mortality of grubs

at 60 days after treatment. *B. bassiana* with 2 x 10^8 and 2 x 10^6 conidial doses recorded 47.22 and 20.83 per cent mortality. The combination of the entomopathogenic fungi *M. anisopliae* X *B. bassiana* 2 x 10^8 and 2 x 10^6 conidial doses recorded 72.22 and 31.95 percent mortality of grubs at 60 days after treatment (Table 1). These findings are in conformity with the finding of (Sharma *et al*. 2009 and Prasad *et al.*, 2012).

It may be concluded that entomopathogenic nematodes *H. indica* 1000 IJs ml^{-1} and *H. indica* 2000 IJs ml^{-1} are effective bio-agents in controlling the white grub as compared other bio-agents. It is ecofriendly management and decreases the discriminate use of pesticide.

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