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Field evaluation of EC formulations of *Metarhizium anisopliae* (Meschinikoff) Sorokin and insecticides against groundnut white grub, *Holotrichia fissa* Brenske

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Abstract

A field experiment against groundnut white grubs, *Holotrichia fissa* was laid out in the Hattarawata village of chikkodi taluka, Belgavi district, Karnataka, India during 2013-2014 cropping season. At 30 DAT chlorpyrifos 20 EC @10 l/ha, rynaxypyr 4G @ 20kg/ha, *M. anisopliae* (Novozyme) @ 5×10^9 (3ml/l) and *M. anisopliae* (Novozyme) @ 5×10^9 (2.5 ml/l) cent per cent reduction whereas, *M. anisopliae* (T-stanes) @ 1×10^9 (3ml/l) and *M. anisopliae* (T-stanes) @ 1×10^9 (2.5ml/l) could also produce similar effects among themselves and registered 98.10 and 96.32 % reduction respectively. But, they were significantly superior over *M. anisopliae* (T-stanes) @ 1×10^9 (5 kg/acre), *M. anisopliae* (Novozyme) @ 5×10^9 (2ml/l) and untreated check. Among EC formulations, *M. anisopliae* (Novozyme) @ 5×10^9 (3ml/l) and *M. anisopliae* (T-stanes) @ 1×10^9 (3ml/l) recorded 18.00 and 17.88q/ha yield respectively next to chlorpyrifos 20EC @ 10ml/ l and rynaxypyr 4G @20 kg/ha with 19.17 and 19.16 q/ha respectively. The average benefit for every rupee invested was highest (2.15) in chlorpyrifos 20EC @ 10ml/ l followed by *M. anisopliae* (Novozyme) @ 1×10^9 (3ml/l) with the earnings of Rs. 2.11.

Keywords: Groundnut, EC formulations, *Metarhizium anisopliae* and *Holotrichia fissa*

Introduction

White grubs are one among the five pests of national importance in India and are possessing a serious constraint in production of many annual crops viz., groundnut, pearl millet, sorghum, cowpea, pigeonpea, greengram, clusterbean, chilli and perennial crops like sugarcane, tea, coffee and arecanut. In endemic areas the damage to crops ranges from 20 to 100 per cent. In Karnataka, Groundnut or peanut (*Arachis hypogaea* L.) is growing in an area of 0.658 Mha. With a production of 0.561 MT and a productivity of 898 kg /ha (Anon., 2011) [2]. The major groundnut growing districts in Karnataka are Chitradurga, Dharwad, Belagavi, Vijayapur, Raichur, Ballari and Bidar. About 70 per cent of the crop is grown in black soil and remaining in red soil.

Fifteen species of root grubs viz., *Holotrichia serrata*, *H. rufiflava*, *Sophrops karschi*, *A. ruficapilla*, *Schizonycha ruficollis*, *Anomola bengalensis*, two species of *A. dorsalis*, two species of *Adoratus versutus*, *Apogonia* sp, three species of *Maladera* sp and *Phyllognathus dionysius* were recorded during the study period. *A. ruficapilla* was the dominant species followed by *Sophrops karschi*, *A. bengalensis* and *H. serrata* on neem and ber. The adult beetles were attracted with the help of light source. Adult emergence commenced with the onset of pre-monsoon showers from 10th meteorological standard week (MSW) (March) and continued up to 22nd MSW (June) with peak during 19 to 20th MSW (May) with weekly mean highest rainfall of 67.8mm. Almost all the major *kharif* crops grown in this area like groundnut, soybean, maize, chilly and cucumber were infested with these pests (Vinayaka, and Patil, 2016) [10].

Groundnut is a dietary supplement in developed countries where it is eaten raw, roasted, boiled and as a sauce. Groundnut supplies essential amino acids, lipids, vitamins and minerals to the diets of many in the developing countries. It is the second most important source of vegetable oil in the world. Groundnut contributes 55 per cent of the nation's vegetable oil from 45 per cent of the land devoted to oil seed.

In recent times *Holotrichia fissa* Br. (Coleoptera: Scarabaeidae) is emerging as a major pest on groundnut as compared to other species of white grubs accounting for 88.00 per cent under

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rain-fed situations of Belagavi district. *H. fissa* Br. is an economically important and found in large numbers as compared to other species of white grubs in the Hattarwat village of Chikkodi taluka on ber and neem. Groundnut, soybean, maize and paddy are infested by *H. fissa* Br. and *H. serrata*. (Tippannavar and Patil 2013)^[9].

To combat this pest usually insecticides are recommended. Prolonged dumping of chemical not only causes soil pollution, but also has a deleterious effect on soil fauna and flora. Application of Entomopathogenic fungi (EPF) *M. anisopliae* when applied on the soil surface are killed by the combination of desiccation and ultra violet light damage (Smith, 1996; Wilson and Gaugler, 2004)^[7, 11]. Thamarai *et al.* (2011)^[8] has indicated that liquid formulation was significantly superior to talc and lignite formulation for enhancing both sugarcane yield and quality by reducing the grub load of *H. serrata* in sugarcane. However, the literature available for utilization of EC formulations of biopesticides especially *M. anisopliae* and *B. bassiana* is very much lacking. So, there is a need to standardise the optimum and effective dose of EC formulation of *M. anisopliae* to get maximum white grub mortality.

Materials and methods

A field experiment against groundnut white grub, *H. fissa* was laid out in the Hattarawata village of chikkodi taluka, Belagavi district during the 2013-2014 cropping season, the groundnut local variety Western-6 was sown during June 2013 with a plot size of 5x5 m² following all the recommended package of practices except white grub management. There were ten treatments laid out in a randomized block design with three replications. The treatments were imposed in the last week of June. *M. anisopliae* (dust) was applied to the root zone by mixing with FYM in 1:1 ratio and *M. anisopliae* (both EC formulations), chlorpyrifos 20 EC @ 10 ml per litre of water prepared and the actual quantity of rynaxypyr 4G @ 20 kg/ha required for the treatment was calculated and applied directly to the soil around the plants by making holes.

Observations were made separately on number of grubs per meter row in the root zone a day before and 7, 15, and 30 days after imposition of treatments. In each treatment five randomly selected spots were observed for the grub population at different intervals. At harvest, pod yield per plot was recorded. The data obtained on different parameters were subjected to $x+0.5$ square root transformation. The transformed data were subjected to Anova (Analysis of Variance) and DMRT (Duncan's Multiple Range Test) was used to determine significance in different treatments.

Results and discussion

Irrespective of time periods *viz.*, 7, 15 and 30 DAT, both Chlorpyrifos 20 EC @10 ml/l and rynaxypyr 4G @ 20kg/ha were recorded significant reduction in the grub load. Particularly, Chlorpyrifos 20 EC @10 l/ha showed cent per cent reduction in grub load at 30 DAT and is significantly superior over all other treatments followed by rynaxypyr 4G @ 20kg/ha by recording 0.92 grubs per meter row at 15 DAT. It was followed by *M. anisopliae* (Novozyme) @5×10⁹ (3ml/l), *M. anisopliae* (Novozyme) @5×10⁹ (2.5ml/l), *M. anisopliae* (T-stanes) @1×10⁹ (2.5ml/l), and *M. anisopliae* (T-stanes) @1×10⁹ (3ml/l) recording 3.16, 3.29, 3.55 and 3.58 grubs per metre row respectively and were on par with each other. Similarly, *M. anisopliae* (T-stanes) @1×10⁹ (2ml/l) and *M. anisopliae* (Novozyme) @5×10⁹ (2ml/l) recorded 6.51 and

6.02 grubs per metre row but were on par with *M. anisopliae* @1×10⁸ (5 kg/acre) which had a grub load of 6.28.

At 30 days after treatment it was quite interesting to observe that chlorpyrifos 20 EC @10 l/ha, rynaxypyr 4G @ 20kg/ha, *M. anisopliae* (Novozyme) @5×10⁹ (3ml/l), *M. anisopliae* (Novozyme) @5×10⁹ (2.5 ml/l), *M. anisopliae* (T-stanes) @1×10⁹ (3ml/l) and *M. anisopliae* (T-stanes) @1×10⁹ (2.5ml/l) could produce similar effects among themselves but significantly superior to *M. anisopliae* (T-stanes) @1×10⁹ (5 kg/acre), *M. anisopliae* (Novozyme) @5×10⁹ (2ml/l) and untreated check (Table 1).

The highest pod yield was recorded in chlorpyrifos 20EC @ 10ml/ l and rynaxypyr 4G @20 kg/ha with 19.17 and 19.16 q/ha respectively and were at par and significantly superior to other treatments. Among EC formulations, *M. anisopliae* (Novozyme) @5×10⁹ (3ml/l) and *M. anisopliae* (T-stanes) @1×10⁹ (3ml/l) recorded 18.00 and 17.88q/ha respectively which were on par with each other followed by *M. anisopliae* (Novozyme) @5×10⁹ (2.5ml/l) and dust formulation, *M. anisopliae* @1×10⁸ (5kg/acre) had 17.14 and 16.88 q/ha respectively and were behaving similarly. *M. anisopliae* (T-stanes) @1×10⁹ (2.5ml/l) and *M. anisopliae* (Novozyme) @5×10⁹ (2ml/l) recorded 15.84 and 15.57 q/ha respectively and were on par with each other but *M. anisopliae* (T-stanes) @1×10⁹ (2ml/l) recorded Rs 14.82 q/ha and was superior to the untreated check (Table 2).

The average benefit for every rupee invested was highest (2.15) in chlorpyrifos 20EC @ 10ml/ l followed by *M. anisopliae* (Novozyme) @1×10⁹ (3ml/l) with the earnings of 2.11. The next best treatments were rynaxypyr 4G @20 kg/ha and *M. anisopliae* (T-stanes) @5×10⁹ (3ml/l) with the earnings of 2.08 and 2.03 respectively. This was followed by the *M. anisopliae* (Novozyme) @5×10⁹ (2.5ml/l) and dust formulation, *M. anisopliae* @1×10⁸ (5kg/acre) recorded Rs.1.96 and 1.90 respectively. Whereas *M. anisopliae* (T-stanes) @1×10⁹ (2.5ml/l) recorded Rs 1.88. The lower dosage of *M. anisopliae* (Novozyme) @5×10⁹ (2ml/l) and *M. anisopliae* (T-stanes) @1×10⁹ (2ml/l) recorded Rs 1.80 and 1.76 respectively (Table 2).

At 30 DAT chlorpyrifos @ 10ml/l, rynaxypyr 4G @ 20kg/ha, *M. anisopliae* Novozyme @5×10⁹ (3ml/lit) and *M. anisopliae* T-stanes @1×10⁹ (3ml/lit) resulted in a lower number of grubs/ meter row length and higher pod yield/ha. (Pandey, 2010) reported that the chemical insecticides *i.e.* imidacloprid (0.08 kg a.i. ha⁻¹) and chlorpyrifos (0.8 kg a.i./ha) were most effective and significantly superior to all the treatments as well as control. Both the treatments, *i.e.* imidacloprid (0.08 kg a.i. ha⁻¹) and chlorpyrifos (0.8 kg a.i. ha⁻¹) reduced 82.28 per cent and 75.9 per cent grub population, due to infestation of *H. consanguinea* to soybean respectively, over control, but were at par with each other. The chemical control of groundnut white grubs, *Holotrichia serrata* F. and *H. reynaudi* Blanchard (Coleoptera: Scarabaeidae), was studied in south-central India. Microplot trials demonstrated that chlorpyrifos and imidacloprid seed-dressings were effective against *H. serrata* at rates as low as 0.6 and 3.5 g a.i. /kg, respectively, while microplot and on-farm trials showed that 1.2 and 3.5 g a.i./kg of chlorpyrifos and imidacloprid, respectively, were required for *H. reynaudi*. (Anitha *et al.*, 2005)^[11] and this report fully justify the superior effect of chlorpyrifos against *H. fissa*, though the method of application is soil drenching and the species studied is *H.fissa*.

Application of $5.1 \pm 0.7 \times 10^4$ *M. anisopliae* (DAT F-00 1) spores per g of soil (2 cm below the soil surface) during mid-

winter resulted in 30.3 per cent fewer larvae in treating plots by 21 weeks and 57.8 per cent less pupae by 27 weeks. In the two subsequent generations of primary population, there were 63.2 per cent and 45.0 per cent fewer larvae in the treated plots before the damaging third instar stage. They also found that the level of the fungus in the soil was still twice than the originally applied concentration at the conclusion sampling (Rath *et al.* 1995) [6]. The best control of *M. melolontha* was obtained by the soil injection of *B. brongniartii* spore suspension at 20×10^9 conidia per m^2 . Epizootic was developed in the field 16 months after soil inoculation and the disease persisted into the next generation (Ferron *et al.*, 1975) [3]. Soil inoculation with *B. brongniartii* at 3×10^{14} conidia per ha has given satisfactory results against *M. melolontha* (Martynenko, 1975) [4]. (Xu, 1988) reported that furrow application of *B. bassiana* conidia (1: 30 India soil mixture) when seeding or weeding resulted in 60 - 70 per cent control of white grub, *Holotrichia* sp. Yokoyama *et al.* (1998) [13] reported that, application of granules (2 mm dia) of *M.*

anisopliae in a ridge or over the entire area of experimental plot at 100 g per m^2 prior to planting sweet potato. The percentage of undamaged roots were significantly higher in the plots with application over the entire area than in the control plots on the day of harvest.

In the present investigation highest cost benefit ratio of Rs 2.15 was obtained in both chlorpyrifos 20EC @ 10ml/l. The next best treatment was *M. anisopliae* Novozyme @ 1×10^9 (3ml/l) and rynaxypyr 4G @ 20kg/ha respectively. The Cost economics of use of Entomopathogens against *H. fissa* is being reported for the first time.

It may be concluded that, under field condition *M. anisopliae* (Novozyme) @ 5×10^9 (3ml/l) and *M. anisopliae* (T-stanes) @ 1×10^9 (3ml/l) were superior to all other entomopathogenic fungi and found an alternative to the hazardous chemical pesticides. Hence the EC formulations of biopesticide *M. anisopliae* of both Novozyme and T-stanes were found to be effective in the control of ground nut white grub *H. fissa*.

Table 1: Field evaluation of EC formulations of *M. anisopliae* on white grub, *H. fissa* in groundnut at Hattarwata (Kharif-2013)

Sl. No	Treatments	Dosage	White grubs/m row				% decrease over untreated check at 30 DAT
			DBT	7 DAT	15 DAT	30 DAT	
1	<i>M.anisopliae</i> @ 1×10^8 (Dust)	5 kg/acre	6.95 (2.72) ^a	6.58 (2.66) ^b	6.28 (2.60) ^d	1.03 (1.23) ^{cd}	86.95
2	<i>M.anisopliae</i> @ 1×10^9 (T-stanes)	2 ml/l	8.04 (2.92) ^a	7.98 (2.91) ^b	6.51 (2.64) ^d	1.61 (1.42) ^d	79.59
3	<i>M.anisopliae</i> @ 1×10^9 (T-stanes)	2.5 ml/l	7.86 (2.86) ^a	7.27 (2.78) ^b	3.55 (2.00) ^c	0.29 (0.87) ^{ab}	96.32
4	<i>M.anisopliae</i> @ 1×10^9 (T-stanes)	3 ml/l	7.97 (2.91) ^a	6.95 (2.72) ^b	3.58 (2.01) ^c	0.15 (0.80) ^{ab}	98.10
5	<i>M.anisopliae</i> (Novozyme) 5×10^9	2 ml/l	7.75 (2.86) ^a	7.64 (2.84) ^b	6.02 (2.54) ^d	0.61 (1.05) ^{bc}	92.27
6	<i>M.anisopliae</i> (Novozyme) 5×10^9	2.5 ml/l	7.83 (2.88) ^a	6.98 (2.73) ^b	3.29 (1.95) ^c	0.00 (0.71) ^a	100.00
7	<i>M.anisopliae</i> (Novozyme) 5×10^9	3 ml/l	7.83 (2.88) ^a	6.77 (2.69) ^b	3.16 (1.90) ^c	0.01 (0.72) ^a	99.87
8	Chlorpyrifos 20EC	10 ml/l	8.08 (2.93) ^a	2.81 (1.81) ^a	0.08 (0.76) ^a	0.00 (0.71) ^a	100.00
9	Rynaxypyr 4G	20 kg/ha	8.01 (2.92) ^a	3.12 (1.90) ^a	0.92 (1.18) ^b	0.00 (0.71) ^a	100.00
10	Untreated check	-	7.97 (2.91) ^a	7.93 (2.90) ^b	7.90 (2.90) ^c	7.89 (2.89) ^c	0.00
	SE.m		0.13	0.09	0.13	0.09	
	C.D. (5%)		NS	0.28	0.39	0.27	
	C.V.		7.60	6.27	11.12	14.02	

DBT=Days before treatment, DAT=Days after treatment, Figures in the parenthesis are $\sqrt{x+0.5}$ transformed values, means followed by the same alphabets in columns do not differ significantly ($p = 0.05$) by DMRT

Table 2: Cost economics in groundnut against *H. fissa* as influenced by different formulations of Metarhizium and insecticides

Sl. No.	Treatments	Dosage	Pod yield (q/ha)	Total cost of cultivation(Rs/ha)	Gross returns	Net returns	B:C
1	<i>M.anisopliae</i> @ 1×10^8 (Dust)	5 kg/acre	16.88 ^c	33768.79	64144.00	30375.21	1.90
2	<i>M.anisopliae</i> @ 1×10^9 (T-stanes)	2 ml/l	14.82 ^c	31918.60	56328.67	24660.07	1.76
3	<i>M.anisopliae</i> @ 1×10^9 (T-stanes)	2.5 ml/l	15.84 ^d	32081.10	60204.67	28436.07	1.88
4	<i>M.anisopliae</i> @ 1×10^9 (T-stanes)	3 ml/l	17.88 ^b	32243.60	67944.00	36075.40	2.11
5	<i>M. anisopliae</i> (Novozyme) 5×10^9	2 ml/l	15.57 ^d	32868.60	59166.00	27497.40	1.80
6	<i>M. anisopliae</i> (Novozyme) 5×10^9	2.5 ml/l	17.14 ^c	33268.60	65144.67	33376.07	1.96
7	<i>M. anisopliae</i> (Novozyme) 5×10^9	3 ml/l	18.00 ^b	33668.60	68412.67	36544.07	2.03
8	Chlorpyrifos 20EC	10 ml/l	19.17 ^a	33868.60	72833.33	38964.74	2.15
9	Rynaxypyr 4G	20 kg/ha	19.16 ^a	35018.60	72820.67	37802.07	2.08
10	Untreated check	-	13.17 ^f	30954.60	50046.00	19091.40	1.62

B: C ratio: Benefit cost ratio, UTC = untreated check;

Means followed by the same alphabets do not differ significantly ($p = 0.05$) by DMRT

Market price of groundnut = 3800/q,

Price of *M. anisopliae* (Novozyme) 5×10^9 = Rs 800 /l; *M. anisopliae* @ 1×10^9 (T-stanes) = Rs325/l

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