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Field evaluation of EC formulations of *Metarhizium anisopliae* (Meschinikoff) Sorokin and few insecticides against arecanut white grub, *Leucopholis lepidophora*, Blanchard

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Abstract
A field experiment was laid out at Nevanagere village of Sirsi, Uttar Kannada, Karnataka, India during 2013-2014 cropping season on evaluation of EC formulations against arecanut white grub, *Leucopholis lepidophora* Blanchard. The results revealed that, at 60 days after imposition of treatment chlorpyriphos 20 EC @ 10 ml per palm was the best treatment at all observation periods followed by rynaxypyr 4G @ 25 g per tree. However, among the EC formulations *M. anisopliae* Novozyme @5×10^9 (3ml/lit), *M. anisopliae* T-stanes @1×10^9 (3ml/lit) and *M. anisopliae* Novozyme @5×10^9 (2.5ml/lit) effected highest mortality *viz*., 100.00, 97.87 and 94.44 respectively as compared to lower dose of *M. anisopliae* T-stanes @1×10^9 (2ml/lit), *M. anisopliae* Novozyme @5×10^9 (2ml/lit) and dust formulation of *M. anisopliae* @1×10^8 (5kg/acre) with 84.44 and 83.33 percent mortality.

Keywords: areca nut, white grubs, *Leucopholis lepidophora*, EC formulations and *Metarhizium anisopliae*

Introduction
Karnataka is the major arecanut producing state in India. It is grown in an area of about 119.1 thousand ha (Uttara Kannada 37.5%, Dakhina Kannada 35%, Shimoga 15% and Chickmagalur 12.5%) and more than two lakh farm families are involved in the arecanut production. Arecanut mainly suffers from root grub in Western Ghats and *'malnad'* belt of Karnataka that includes three main species viz., *Leucopholis lepidophora* Blanch. *Leucopholis coniophora* Burn and *Leucopholis burmeisteri* Brenske. *Leucopholis lepidophora* Blanch. in addition to arecanut also infests coconut and cashew (Anon., 1969). In Uttara Kannada and Shimoga districts considerable loss is caused by root grubs in arecanut. Kalleshwaraswamy *et al*., (2015) [7] reported 27.86 to 36.97 percent damage by this species with a yield reduction of 39.79 to 41.60 percent in different districts of hilly and coastal regions of Karnataka. The root grubs cause damage to the arecanut tree by directly feeding on roots resulting in symptoms like yellowing of leaves, stem tapering at the crown region, reduced internode length, nut fall and ultimately leads to reduced vigour, yield and death of plant. Under severe infestation, the palms lose their anchorage due to complete loss of roots and when shaken, moved easily or fell with a little jerk (Kumar, 1997) [10].

Prolonged dumping of chemical not only causes soil pollution but also has deleterious effect on soil fauna and flora. Since the past 5 years dust formulation of *Metarhizium anisopliae has* become very popular with the arecanut farmers of Karnataka as one of the component of IPM. Manual application of *M. anisopliae* is cumbersome. Further, Entomopathogenic fungi (EPF) when applied at the soil surface are killed by combination of desiccation and ultra violet light damage (Smith, 1996; Wilson and Gaugler, 2004) [14, 17]. Eearly instar grubs are distributed throughout the garden (both in between the palms and around the palms) during July to October (rainy season) and later instars are restricted to root zone (only around the palm) from November onwards (when moisture content in the soil gets reduced). With this basic idea of spatial distribution of grubs, where subsurface drippers are going to be installed, an application would yield higher grub mortality when combined with EC formulations of biopesticides especially *M. anisopliae*. Thamarai *et al.*, (2011) [15] has indicated that liquid formulation was significantly superior to talc and lignite formulation for enhancing both sugarcane yield and quality by reducing grub load of *H. serrata* in sugarcane. In recent times sub-surface drip

irrigation system is gaining popularity among Arecanut and coconut farmers of Karnataka. However, the literature available on utilization of EC formulations of biopesticides especially *M. anisopliae* and *B. bassiana* is very much lacking. There is need to standardise the optimum and effective dose of EC formulation of *M. anisopliae* to get maximum white grub mortality before it can be used in sub surface drip irrigation system.

Material and Methods

Selection of arecanut garden for field trials

For conducting field trials, arecanut (tarikere local) garden was selected in a village called Nevanagere which is 12 km away from Agricultural Research Station, Sirsi. Selected garden is spread in an area of 3.5 acre, trees are about eighteen years old and white grub problem is prevailing in the garden for the last seven years.

There were ten treatments laid out in randomized block design with three replications (ten palms per replication). The treatments were imposed during September, 2013. In each treatment three arecanut trees were taken and labelled. Each tree was observed for the grub population in the root zone at 15 cm depth and it was regulated to ten grubs per palm either by releasing if lower or removing if more than ten.

M. anisopliae (dust) 5 kg/acre was applied to the root zone by mixing with FYM in 1:1 ratio and different dosage of *M. anisopliae* (both EC formulations) were applied around the tree trunk according to the dosage mentioned in Table 1. In case of chlorpyriphos 20EC, 10 ml of insecticide formulation in one litre of water was prepared and two litres solution was drenched to the soil around the tree. Rynaxypyr 4G granules were applied @ 25g per palm by broadcasting at the base of the tree.

Grub mortality was recorded by digging the soil at the base of tree and counting the number of live grubs. Observations were recorded on 15, 30, 45 and 60 days after treatment imposition. The data obtained on different days were subjected to suitable statistical analysis using DMRT.

Result and discussion

The results on field evaluation of EC formulation of *M. anisopliae* on Arecanut white grub are presented in Table 1.

15 DAT: After 15 days of treatment, grub mortality ranged from zero to maximum of 13.33 per cent. Highest mortality (13.33) was recorded with chlorpyriphos 20 EC @10 l/ha and was significantly superior over all other treatments. This was followed by rynaxypyr 4G @ 20kg/ha recording grub mortality of 5.56 per cent mortality and was significantly superior to all other treatments. Among the EC formulations, *M. anisopliae* (Novozyme) @5×10^9 (3ml/l), *M. anisopliae* (T-stanes) @1×10^9 (3ml/l), *and M. anisopliae* (T-stanes) @1×10^9 (2.5ml/l) recorded 3.33 per cent grub mortality and were on par with each other. Whereas *M. anisopliae* (Novozyme) @5×10^9 (2.5ml/l) and *M. anisopliae* (Novozyme) @5×10^9 (2ml/l) had 2.22 per cent mortality and were on par with each other. *M. anisopliae* @1×10^8 (5kg/acre) recorded 1.11 per cent mortality and was superior to *M. anisopliae* (T-stanes) @1×10^9 (2ml/l) that failed to effect any mortality.

30 DAT: Thirty days after imposition of treatment, the mortality ranged from 20.00 to 86.67 per cent. The highest mortality was in chlorpyriphos 20 EC@ 10 l/ha and stood significantly superior to all other treatments. This was followed by rynaxypyr 4G @ 20kg/ha which was on par with *M. anisopliae* (Novozyme) @5×10^9 (3ml/l). *M. anisopliae* (T-stanes) @1×10^9 (3ml/l) recorded 52.22 and 48.89 per cent grub mortality respectively and were on par with each other. *M. anisopliae* (Novozyme) @5×10^9 (2.5ml/l), *M. anisopliae* (T-stanes) @1×10^9 (2.5ml/l) effected 35.56 and 28.89 per cent mortality and both the treatments were statistically at par with each other. *M. anisopliae* (Novozyme) @5×10^9 (2ml/l) and *M. anisopliae* (T-stanes) @1×10^9 (2ml/l) recorded 21.11 and 20.00 mortality respectively and produced similar results of *M. anisopliae* @1×10^8 (5kg/acre) but superior over untreated check (0.00).

45 DAT: At 45 days after treatment, the mean per cent grub mortality ranged from 55.56 to 100 per cent. The highest mortality (100%) was achieved by chlorpyriphos 20 EC@ 10 l/ha and was significantly superior to all other treatments. This was followed by rynaxypyr 4G @ 20kg/ha and was on par with *M. anisopliae* (Novozyme) @5×10^9 (3ml/l) and *M. anisopliae* (T-stanes) @1×10^9 (3ml/l). This was followed by *M. anisopliae* (Novozyme) @5×10^9 (2.5ml/l) showing 76.67 per cent mortality but significantly differed with other treatments. *M. anisopliae* (T-stanes) @1×10^9 (2.5ml/l) and *M. anisopliae* @1×10^8 (5kg/acre) recorded 73.33 and 63.33 per cent mortality and both the treatments were statistically on par with each other. *M. anisopliae* (Novozyme) @5×10^9 (2ml/l) and *M. anisopliae* (T-stanes) @1×10^9 (2ml/l) recorded 58.89 and 55.56 respectively and were less effective at both the levels of conidial concentration recording lower grub mortality even at 45 days after treatment but superior over untreated check.

60 DAT: At 60 days after treatment the grub mortality ranged from 83.33 to 100 per cent. Cent per cent mortality was produced in chlorpyriphos 20 EC@ 10 ml /l and was followed by *M. anisopliae* (Novozyme) @5×10^9 (3ml/l) and *M. anisopliae* (T-stanes) @1×10^9 (3ml/l), *M. anisopliae* (Novozyme) @5×10^9 (2.5ml/l) and rynaxypyr 4G @ 20kg/ha all were on par with each other. *M. anisopliae* (Novozyme) @5×10^9 (2ml/l) effected 92.22 per cent mortality and were significantly superior to both *M. anisopliae* @1×10^8 (5kg/acre) and *M. anisopliae* (T-stanes) @1×10^9 (2ml/l) with 84.44 and 83.33 per cent mortality which were statistically at par with each other but superior to untreated check.

At the final observation (60 DAT) the higher concentration of *M. anisopliae* (Novozyme) @5×10^9 (3ml/l) and *M. anisopliae* (T-stanes) @1×10^9 (3ml/l), and recommended dose *M. anisopliae* (Novozyme) @5×10^9 (2.5ml/l) proved at par with chlorpyriphos 20 EC @ 10 ml per palm and rynaxypyr 4G @ 25 g per tree and were significantly superior to both *M. anisopliae* @1×10^8 (5kg/acre) and *M. anisopliae* (T-stanes) @1×10^9 (2ml/l) with 84.44 and 83.33 per cent mortality which were statistically at par with each other but superior to untreated check. The superiority of higher dose EC formulations of fungal pathogens is because of the moisture content present in the EC formulations over dust formulation and also the higher concentration of spore suspension as well as mycilium present in the concentrated form. Efficacy of EC formulation of *M. anisopliae* against *L. lepidophora* is being reported for the first time. The present findings are in complete collaboration with Thamarai *et al*., (2011) [15] who reported that the liquid formulation of the microbial insecticide is effective in controlling sugarcane white grub. The formulation, application and selection of bio pesticide strain is one of the key steps for field trials. It has been suggested that oil formulation can prevent conidial

desiccation and improve adhesion of conidia to the hydrophobic surface of insect cuticle (Inyang *et al*, 2000, Vimala devi and Hari, 1999) [6, 16]. Keller (1998) [8] suggested that repeated application of the entomopathogenic fungal (granular) formulations enhance the pest control process and white grubs could be controlled in field situations in various crops, like *H. consanguinea* infesting potatoes were controlled by *M. anisopliae* (Kulye and Pokharkhar, 2009) [9] or high virulence has been reported against *H. serrata* using *B. brongniarti* as lignite or press mud formulations (Eswaramoorthy *et al*., 2005). Glare and Milner, 1991 reported that high dosages of 10^8 to 10^9 conidia per ml causes normally the higher mortality of white grub larvae.

Rakesha *et al.* (2012) [13] reported that *M. anisopliae* at higher dosage of 4 x 10^8 conidia/g recorded higher per cent mortality of *L. lepidophora* grubs (33.33) as compared to lower dosage of 2 x 10^8 conidia/g resulting in 14.81 per cent mortality and is in collaboration with the present findings. Among the two mycopathogens, *viz., Metarhizium* and *Beauveria* under study at two different dosages, except *M. anisopliae* with 2 x 10^8 conidia per gram @ 20 g per tree no other treatment caused mortality of root grub till 30 days after imposition of treatment, but as time advanced all mycopathogens treatment showed improvement in their performance. At last observation *M. anisopliae* with 2 x 10^8 conidia per g @ 20 g per palm recorded the mortality similar to that of lower dose. Prabhu *et al.* (2011) [12] studied the bioefficacy of bioagents and plant products against arecanut white grub, *Leucopholis lepidophora*. The results revealed that chlorpyriphos 20 EC @ 6 ml per palm was found effective by recording 77.36 per cent grub mortality. The entomopathogens studied at two different dosages *viz., M. anisopliae* and *B bassiana,* with 2 × 10^8 conidia per g @ 10 and 20 g per palm, wherein *M. anisopliae* with 2 x 10^8 conidia per g @ 20 g per palm recorded 31.38 per cent grub mortality as against aqueous mixture of soapnut and neem oil @ 5 per cent performing superior by recording 53.55 per cent grub mortality and similar findings observed in the present study with respect to chlorpyriphos 20 EC @ 10 ml per palm and also the high dosages caused the higher mortality of white grub larvae in EC formulations of *M. anisopliae* (Novozyme) @5×10^9 (3ml/l) and *M. anisopliae* (T-stanes) @1×10^9 (3ml/l) against *M.anisopliae*@1×10^8(Dust) 5 kg/acre.

Hajeri (2003) [5] has reported that dust formulation of *M. anisopliae* @ 2 x 10^{13} conidia per ha recorded 60.06 per cent reduction in III instar grub population (60 DAT) of *L. coneophora* in arecanut ecosystem and was next best to chlorpyriphos 20 EC drenching @ 5 l/ha and is more or less in confirmation with present finding on the efficacy of *M. anisopliae* even though it is EC formulation. Further, the present study is also in agreement with Channakeshava (2006) [2] who reported that *M. anisopliae* @2 x 10^8 conidia per gram @ 1.1 kg per acre gave 50.97 per cent mortality of *L. lepidophora* in arecanut ecosystem though the formulation used is dust.

Present findings are analogous with Kumar and Daniel (1981) [11] reported that *L. burmeisteri* infesting arecanut roots can be managed effectively by using dimethoate 5 per cent granules at 30 kg/ha, chlordane 5 per cent dust at 90 and 120 kg/ha and quinalphos 1.5 per cent dust at 90 and 120 kg/ha and are in line with the present report with respect to efficacy of granular insecticide rynaxypyr used @ 25 kg/ha to manage *L.lepidophora*.

Field evaluation of EC formulations of *M. anisopliae* against arecanut white grubs (late second/ early third) revealed that 60 days after application, higher concentration of *M. anisopliae* (Novozyme) @5×10^9 (3ml/l) and *M. anisopliae* (T-stanes) @1×10^9 (3ml/l), and recommended dose *M. anisopliae* (Novozyme) @5×10^9 (2.5ml/l) registered 100.00, 97.78 and 94.44 per cent population reduction in white grubs as compared to *M. anisopliae* dust application. The higher mortality of white grubs in recommended and higher doses of EC formulations is due to the presence of higher conidial or spore suspension to produce maximum infection in arecanutfield though there is a lack of soil moisture as compared to use of dust formulation of *M. anisopliae*. The lower efficacy of Metarhizium formulation immediately after imposition of treatment is due to the fact that the mycopathogens require time for conidial germination, pegging of the mycelium into the host and development of the fungus on the host to cause mycosis and death of the white grubs. Use of Metarhizium is an effective ecofriendly approach especially the use of EC formulations through drip has lot of potential in arecanut and coconut ecosystem. So, there is need to popularise this technology in the farmer's field.

Table 1: Field evaluation of EC formulations of *Metarhizium anisopliae* on white grub, *Leucopholis lepidophora* in arecanut at Nevanagere

Sl. No	Treatments	Dosage	White grub/m row			
			15 DAT	30 DAT	45 DAT	60 DAT
1	*M.anisopliae*@1×10^8(Dust)	5 kg/acre	1.11 (3.51)^{e}	22.22 (28.10)^{d}	63.33 (52.71)^{de}	84.44 (66.77)^{c}
2	*M.anisopliae*@1×10^9 (T-stanes)	2 ml/l	0.00 (0.00)^{f}	20.00 (26.50)^{d}	55.56 (48.23)^{e}	83.33 (65.95)^{c}
3	*M.anisopliae*@1×10^9 (T-stanes)	2.5 ml/l	3.33 (10.52)^{c}	28.89 (32.33)^{cd}	73.33 (58.91)^{de}	94.44 (76.49)^{bc}
4	*M.anisopliae*@1×10^9 (T-stanes)	3 ml/l	3.33 (10.52)^{c}	52.22 (46.26)^{b}	87.78 (69.55)^{bc}	97.78 (82.95)^{ab}
5	*M.anisopliae* (Novozyme) 5×10^9	2 ml/l	2.22 (7.01)^{d}	21.11 (26.91)^{d}	58.89 (50.15)^{e}	92.22 (74.17)^{bc}
6	*M.anisopliae* (Novozyme) 5×10^9	2.5 ml/l	2.22 (7.01)^{d}	35.56 (36.58)^{c}	76.67 (61.56)^{cd}	94.44 (79.32)^{ab}
7	*M.anisopliae* (Novozyme) 5×10^9	3 ml/l	3.33 (10.52)^{c}	48.89 (44.35)^{b}	88.89 (70.81)^{bc}	100.00 (89.96)^{a}
8	Chlorpyriphos 20EC	10 ml/l	13.33 (21.41)^{a}	86.67 (68.66)^{a}	100.00 (89.96)^{a}	100.00 (89.96)^{a}
9	Rynaxypyr 4G	20 kg/ha	5.56 (13.15)^{b}	58.89 (50.25)^{b}	94.44 (78.84)^{b}	94.44 (78.84)^{ab}
10	Untreated check	-	0.00 (0.00)^{f}	0.00 (0.00)^{e}	0.00 (0.00)^{f}	0.00 (0.00)^{d}
	SE.m		0.62	2.44	3.39	3.46
	C.D. (5%)		1.84	7.24	10.08	10.28
	C.V.		12.87	11.72	10.03	8.38

DBT=Days before treatment, DAT=Days after treatment, Figures in the parenthesis are arc sine transformed values, means followed by the same alphabets in columns do not differ significantly (p = 0.05) by DMRT

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