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SEMI FIELD EVALUATION OF OIL BASED BIO-FORMULATION AND GRANULAR FORMULATION OF ENTOMOPATHOGENIC FUNGI, *Z. RADICANS* AGAINST RICE LEAF FOLDER, *C. MEDINALIS*

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Abstract

Different concentrations of oil and granular formulations were tested against rice leaf folder, *Cnaphalocrocis medinalis*. Among the different treatments of oil formulations, the lowest mean mortality of 18.68% was noticed on *Z. radicans* alone treatment. The highest mean mortality of 49.72% was noticed on *Z. radicans* + Sunflower oil + Glycerol.. Among the different concentrations of granular formulations, average larval mortality of 13.94% was noticed at 10g *Z. radicans*/pot treatment and increased to 47.06% at 40g *Z. radicans*/pot treatment. Larval mortality increases along with increase in the concentration of formulation. Among the oil and granule, oil formulations of *Z. radicans* was found better in causing leaf folder larval mortality when compared to granular formulation of *Z. radicans*.

Key words : *Zoophthora radicans,* rice leaf folder, semi-field, mortality.

Introduction

Rice (*Oryza sativa*) is an important cereal crop and a source of calories for one-third of the world population. It is the most widely consumed staple food for a large part of the world's human population, especially in Asia. It is the agricultural commodity with the third-highest worldwide production, after sugarcane and maize, according to FAO 2014.

Rice leaf folder (RLF), *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyrallidae) is considered as one of the major defoliating insects damaging the rice crop (DOA, 2006). Adults lay eggs either singly or in pairs on the leaf blades of the rice plant infestation (Ranasinghe, 1992). Insecticides are being the main tools in insect pest management strategy. However the indiscriminate use of chemical insecticides also leads to several problems *viz.,* toxicity to non-target organisms (earthworms, parasites, predators and pollinators), pest resistance to pesticides, pest resurgence, intolerable toxic residues in crop plants and produces, user's health hazards and environmental problems. So there is a need for an eco-friendly alternative to chemical insecticides. Among the biocontrol agents, entompathogenic fungi occupy a predominant position (Karthikeyan, 2012). Entomopathogenic fungi may be applied in the form of conidia or mycelium which sporulates after application. Their hosts comprise of numerous pests and its large distinction in virulence towards different insect hosts makes it one of the more resourceful entomophagous fungi for the biological control of insect pests (Shakir *et al.,* 2015).

The entomophthoralean fungus, *Zoophthora radicans* (Brefeld) Batko (Zygomycetes), is a commonly occurring entomopathogen and important natural mortality factor in populations of many insect pests, especially lepidopteran and homopteran species. This fungus has been the subject of much research aimed at harnessing its high-natural epizootic potential for biological control (Leite *et al.,* 1996; Pell and Wilding, 1994). Most of the entomopathogenic fungi including *Z. radicans* (Brefeld) Batko also produce two types of reproductive spores *viz.,* conidia and resting spores. The relatively short lived conidia are actively ejected from cadavers and are normally infectious. Within late instars of larvae killed by *Z. radicans*, azygospores are formed when individual hyphal bodies coil and form a thick double wall. Azygospores are normally dormant after production during early summer but germinate to form infectious

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germ tube during season. The resting spores used for field introductions are collected either through the cadavers or through soil samples containing high load of the resting spores (Senthilkumar *et al.,* 2011). Based on the above literatures, study is conducted to evaluate oil and granular formulations of *Zoophthora radicans* against rice leaf folder*, Cnaphalocrocis medinalis.*

Materials and Methods

Mass culturing of rice leaf folder, *Cnaphalocrocis medinalis*

Cnaphalocrocis medinalis larvae were collected from paddy fields in and around Annamalainagar, Chidambaram. Larvae were reared in a green house on potted rice plants covered with nylon mesh sleeves at 26 ± 2ºC, 70% relative humidity with a 14:10 Light: Dark cycle. Rice plants were grown in cement pots, 15 cm tall with a 10 cm diameter top. Each round pot held 5 plants and gave about 45 tillers. The potted plants were irrigated in about 5 cm of water in everyday. The culture was initiated with partly grown larvae from the field. Thereafter, newly hatched larvae were placed on plants of the rice variety CR-1009, about 50 days old.

After pupation, adults emerged on plants in the sleeves. To maintain the culture 12 female and 13 male moths were placed in an oviposition cage containing four potted plants. The moths were fed with 10% sugar solution to enhance oviposition. After two days, the potted plants were removed from the oviposition cage. The leaf portions containing the eggs were clipped and placed on moist filter paper in a Petri plate. The eggs were used to maintain the culture (Senthilnathan, 2005)

Preparation of oil based bio-formulations

Potato Dextrose Agar (PDA) with conidial concentration of 1×10^{6}cfu/ml was prepared. Adjuvant like ten ml of glycerol and one ml of oils like sunflower and mustard were added at different combination to the broth medium containing culture of *Z. radicans* (Boruah *et al.,* 2015).

Preparation of granular formulation

Shelled broomcorn millet (Panivaragu) grains were used as solid substrate to prepare granular cultures of *Z. radicans*. The following procedure was described (Feng and Liang 2003, Hua and Feng 2003) the millet grains (15g per 100ml flask) were soaked in water for 30 min at 80^{0}C. Then after rinsing to remove the excess water and dust, the grains were autoclaved for 15 min at 121^{0}C and cooled to room temperature. Then each flask of the autoclaved grains was inoculated with half a plate colony homogenized in 3ml Potato dextrose broth supplemented with 0.5% (v/v) mustard oil. After plugging with vent stoppers, all flasks were incubated for up to 24 days at 15^{0}C and Light: Dark 12: 12. No agitation measures for aeration were taken during the incubation period.

Semi-field evaluation of oil and granular formulation of *Z. radicans* against mortality of *C. medinalis*

In pot culture conditions, *Z. radicans* were evaluated against third instars larvae. The larvae were released in pots pre-planted with one month rice plants and allowed to settle for 24 hrs. In the test against larvae, the different treatments of oil formulation were directly sprayed on the larvae using a hand atomizer. Water sprayed on the larvae as a control (Agarwal *et al.,* 2012). The different doses of granular formulation were applied at 10, 20, 30 and 40g in each pot. Before applying the granules is thoroughly mixing the product of fine sandy soils, and then evenly distributing the pots. Soil was moist at the time of application (Erler and Ates, 2015). Only soils distribute the pot as a control. Three replications and 10 larvae were used in each replication. The pots were watered immediately. Larvae were examined for infection due to *Z. radicans* at regular intervals and results were tabulated.

Results and Discussion

Among the different treatment of oil based formulations, *Z. radicans* caused the lowest larval mortality of 7.25%, 16.50%, 22.75% and 28.25% after 3, 5, 7 and 10 DAT respectively. *Z. radicans* + Sunflower oil + Glycerol caused the highest larval mortality of 26.25%, 42.15%, 58.25% and 72.25% after 3, 5, 7 and 10 DAT respectively.

Among the different treatment the lowest mean mortality of 18.68% was noticed on *Z. radicans* alone treatment. The highest mean mortality of 49.72% was noticed on *Z. radicans* + Sunflower oil + Glycerol. Because, the combination of sunflower oil and adjuvant (Glycerol) increase the efficiency of the fungus causing mortality of insect.

Among the different concentration of granular formulation, 10g *Z. radicnas*/pot caused the lowest larval mortality of 4.50, 13.25, 16.50 and 21.50 after 3, 5, 7 and 10 DAT respectively. 40g *Z. radicnas*/pot caused the highest larval mortality of 22.50, 41.00, 56.25 and 68.50 after 3, 5, 7 and 10 DAT respectively. Among the different concentrations average larval mortality of 13.94% was noticed at 10g *Z. radicans*/pot treatment and increased to 47.06% at 40g *Z. radicans*/pot treatment. Larval mortality increases along with increase in the concentration of formulation. Among the oil and granule, oil formulations of *Z. radicans* was found better in leaf

Table 1: Semi-field evaluation of oil based bio-formulation of *Z. radicans* against rice leaf folder, *C. medinalis.*

Sl. No	Treatment	Per cent larval mortality				Mean
		3 DAT	5 DAT	7 DAT	10 DAT	
1	*Z. radicans* alone	7.25(15.61)^{f}	16.50(23.95)^{f}	22.75(28.48)^{e}	28.25(32.09)^{e}	18.68(25.03)^{e}
2	*Z. radicans* + Glycerol	12.50(20.69)^{e}	21.50(27.61)^{e}	29.75(33.04)^{d}	36.50(37.15)^{d}	25.06(29.62)^{d}
3	*Z. radicans* + Sunflower oil	21.25(27.44)^{c}	35.50(36.56)^{c}	44.00(41.53)^{bc}	57.50(49.29)^{b}	39.56(38.71)^{b}
4	*Z. radicans* + Sunflower oil+ Glycerol	26.25(30.81)^{a}	42.15(40.47)^{a}	58.25(49.73)^{a}	72.25(58.19)^{a}	49.72(44.80)^{a}
5	*Z. radicans* + Mustard oil	18.50(25.46)^{d}	33.00(35.05)^{d}	42.50(40.67)^{c}	50.00(44.98)^{c}	36.00(36.54)^{c}
6	*Z. radicans* + Mustard oil + Glycerol	23.25(28.81)^{b}	38.25(38.19)^{b}	44.75(41.97)^{b}	58.25(49.73)^{b}	41.12(39.68)^{b}
7	Control	0.00(0.28)^{g}	0.00(0.28)^{g}	0.00(0.28)^{f}	0.00(0.28)^{f}	0.00(0.28)^{f}
C. D (p= 0.05)		0.33	0.55	1.09	0.88	5.56
SE(d)		0.15	0.25	0.49	0.40	1.86

Each value is mean of three replications. Figures in parentheses are arc sine transformed values. In a column means followed by a common letter are not significantly different (P=0.05) by DMRT. DAT = Days after treatment.

Table 2: Semi -field evaluation of granular formulation of *Zoophthora radicans* against rice leaf folder, *C. medinalis.*

Sl. No	Treatment (gram/pot)	Per cent larval mortality				Mean
		3 DAT	5 DAT	7 DAT	10 DAT	
1	10	4.50(12.23)^{d}	13.25(21.34)^{d}	16.50(23.95)^{d}	21.50(27.61)^{d}	13.94(21.29)^{d}
2	20	9.25(17.70)^{c}	18.75(25.65)^{c}	24.25(29.49)^{c}	32.25(34.59)^{c}	21.12(26.86)^{c}
3	30	15.50(23.17)^{b}	29.50(32.88)^{b}	36.50(37.15)^{b}	43.25(41.10)^{b}	31.18(33.58)^{b}
4	40	22.50(28.30)^{a}	41.00(39.79)^{a}	56.25(48.57)^{a}	68.50(55.84)^{a}	47.06(43.13)^{a}
5	Control	0.00(0.28)^{e}	0.00(0.28)^{e}	0.00(0.28)^{e}	0.00(0.28)^{e}	0.00(0.28)^{e}
C. D (p= 0.05)		0.78	0.86	0.64	0.62	6.70
SE(d)		0.34	0.37	0.27	0.26	3.04

Each value is mean of three replications. Figures in parentheses are arc sine transformed values. In a column means followed by a common letter are not significantly different (P=0.05) by DMRT. DAT = Days after treatment.

folder larval mortality when compared to granular formulation of *Z. radicans*.

Z. radicans + Glycerol 10% + Sunflower oil 1% were caused the highest (49.72%) larval mortality of *C. medinalis* followed by *Z. radicans* + Glycerol 10% + Mustard oil 1% with the mortality up to 41.12%. Our results also coincided with the results of Boruah *et al*. (2015) who also stated that liquid formulation amended with Glycerol 10 per cent + Sunflower oil 0.5 per cent was highly pathogenic to aphid and killed 80 per cent of the test population at 30 days after spraying.

This might be due to the reason that addition of oils and adjuvants increases infectivity of entomopathogenic fungi by enhancing conidial adherence and prolonged persistence (Meyer *et al.,* 2002; Visalaksy *et al.,* 2006). Somervaille *et al*., (2012) reported that adjuvants lower the surface tension of spray droplet which will help in better retention of spray droplet on the plant surface. The present study also revealed that *Z. radicans* + Glycerol + Sunflower oil were better in causing larval, pupal and adult mortalities of *C. medinalis*.

Thompson *et al*., (2006) reported that the adjuvant Tinopal has been found to be effective UV protectant for viruses and fungi including *B. bassiana*. Tinopal was found to confer total protection (95% conidial germination) by recording quicker and higher mortality of *H. armigera*.

Among the different concentration of granular formulation of *Z. radicans* tested, larval, pupal and adult mortality were high at higher dose. In a similar work, Hua and Feng (2005) reported *P. xylostella* larval mortalities were 36.9 – 91% at 15.1 – 81.1 spores mm^{-2} in assay 1, 33.6 – 91.5% at 15.6 – 87.8 spores mm^{-2} in assay 2 and 11.3 – 93.6% at 4.2 – 91.0 spores mm^{-2} in assay 3, respectively.

Jyothi *et al*. (2014) also reported that 7 Days after treatment (DAT), highest adult mortality percentage of lesser grain borer was recorded with *Metarhizium* + groundnut oil (59.35%) followed by *Beauveria* + groundnut oil (56.02%), *Beauveria* + sunflower oil (48.51%) and groundnut oil (43.15%). At 15 DAT, the highest per cent adult mortality was noticed on *Metarhizium* + groundnut oil (81.57%) followed by *Beauveria* + groundnut oil (78.54%), *Beauveria* + sunflower oil (73.67%), *Lecanicillium* + sunflower oil

(68.96%).

Inglis *et al*., (1996) reported that the vegetable oils allow the entomopathogenic fungi to penetrate into the host cuticle by replacing epicuticular lipids by aqueous phase, followed by aqueous cuticular fluids covering the surface with water droplets which enhances conidial germination. The present results are in agreement with Hidalgo *et al*., (1998) who reported that rape seed oil containing 1×10^{10} conidia of *B. bassiana* gave 100% mortality of *Sitophilus zeamais* in maize. Smith *et al*., (1999) reported that 96 – 100% mortality of larger grainborer, *Prostephanus truncates* Horn with *B. bassiana* + Rapeseed oil. Malsame *et al*. (2002) reported that 100% mortality of whitefly, *Trialeurodes vaporariorum* (Westwood) with *Metarhizium* + sunflower oil. Sabbour and Shadia (2007) reported that 74.2% and 60.1% mortality of broad bean beetle, *Bruchus rufimanus* (Boheman) with mustard oil and nigella oil, respectively in cowpea.

Among the oil and granular, oil formulation of *Z. radicans* were found better in causing mortality of different life stages of leaf folder when compared to granular formulation of *Z. radicans*. Burges (1988) reported that higher efficiency of oil based formulation compared to other formulation might be due to oil prevents the desiccation of the conidia and helps in longer survival period and better penetration of peg into integuments. These results are in agreement with Prior *et al*., (1988) who also concluded that higher mortality of grasshopper, noticed in oil formulation of *Metarhizium anisopliae* than aqueous formulation.

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