Efficacy of oil and granular based formulations of entomopathogenic fungi, *Zoophthora radicans* against the biology of rice leaf folder, *Cnaphalocrocis medinalis*

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ABSTRACT

Experiment was conducted to know the effect of oil and granular based formulations of entomopathogenic fungi, *Zoophthora radicans* against the biology of rice leaf folder, *Cnaphalocrocis medinalis.* Among the different formulations of *Zoophthora radicans,* the highest larval mortality, pupal mortality, adult mortality and the lowest pupal formation and adult emergence was noticed in *Z. radicans* + Sunflower oil + Glycerol treatment in oil formulation and 81.3 spores/mm^{-2} concentration of granular formulation. The lowest larval mortality, pupal mortality and adult mortality were noticed in *Z. radicans* alone treatment and 12.2 spores/mm^{-2} concentration of granular formulation. It was concluded that oil formulations with *Z. radicans* + Sunflower oil + Glycerol were found better in causing mortality of life stages of leaf folder when compared to granular formulation with concentration of 81.3 spores/mm^{-2}

Keywords: The Oil formulation, granular formulation, *Zoophthora radicans*, rice leaf folder

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INTRODUCTION

Rice leaf folder (RLF), *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyrallidae) is considered as one of the major defoliating insects damaging rice crop. The rice leaf folder (RLF), *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae), is a predominant foliage feeder and one of the most destructive pests, affecting all the rice ecosystems in Asia (Luo, 2010) Insecticides are 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, 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 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 formulation of entomopathogenic fungi is essential to increase the shelf life of the fungus. Liquid bio-formulation of *M. anisopliae* amended with Liquid formulation + Glycerol + Sunflower oil was found significantly effective which showed higher surface area covered and biomass respectively (Boruah *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 (Torres Acosta, 2016). This fungus has been the subject of much research aimed at harnessing its high-natural epizootic potential for biological control. In this scenario, present study aims at formulating entomopathogenic fungi, *Zoophthoa radicans* to evaluate against biology of rice leaf folder, *Cnaphalocrocis medinalis.*

Materials and methods

Mass culturing of *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 every day. 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)

Collection of cadavers and identification

As the natural incidence of the entomopathogenic fungi was aided by the humid climate coinciding with the winter season of the year, field visits were undertaken during samba season (October-February) of 2018-2019 from faculty of agriculture farm, Annamalai University. Cadavers encountered were preserved in sterile petriplates and glass vials, brought to laboratory and data like stage of the host, date and place of collection were recorded. The mycosed leaf folder larvae collected from the rice fields were surface sterilized in 70% ethanol for 10 seconds followed by 2% sodium-hypochlorite solution for 2 min. and two washes with sterile distilled water for 2 min. each. Then the cadavers were transferred to synthetic medium, Sabouraud maltose Agar with Yeast extract (SMAY). The fungi isolated were subjected to identification. Fresh cadavers of various pathogenic forms bearing characteristics of the original specimens were gathered either from the rice fields or from glasshouse and each specimen was kept in clear and sterile plastic vial and tightly packed following usual precaution to avoid contamination. Besides, fungal cultures in slides, plastic petridishes and bottle with broth were also sent. Identity of the specimen or culture was ascertained by United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Entomopathogenic Fungal Museum, New York.

Preparation of oil formulation

Rice leaf folder, *Cnaphalocrocis medinalis* larvae were collected from experimental farm, Faculty of Agriculture, Annamalai University. *Zoophthora radicans* was isolated from cadavers of rice leaf folder. Potato Dextrose Agar (PDA) with conidial concentration of 1×10^6cfu/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). Newly moulted second instar larvae of *C. medinalis* were bioassayed for their susceptibility to fungal pathogens. The larvae were air – dried by keeping them in laminar air flow for 5 min and carefully transferred the larvae to individual clean sterile petriplate which contains the moistened tissue paper. The different treatments of oil formulation were directly sprayed on the larvae using a hand atomizer. Water sprayed on the larvae as a control. Six replications and five larvae were used in each replication. The number of larvae died (larval mortality), number of larvae pupating (Pupal percentage), number of pupa died after pupation (Pupal mortality), number of adult emerged from pupa(adult emergence) and number of adults died (adult mortality percentage) due to mycosis were calculated and the results were tabulated (Sivasundaram *et al.,* 2007).

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 followed (Feng and Liang

2003; Hua and Feng, 2003) the millet grains (Panivaragu) were taken at the rate of 15g in 100 ml conical flask, after adding distilled water the grains were autoclaved for 15 min at 121^0C 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 15^0C and Light: Dark 12: 12. No agitation measures for aeration were taken during the incubation period. Millet grains cultured with *Z. radicans* for 21 days were uniformly distributed on 2% agar in a 90 mm diameter petri dish and incubated at 15^0C and Light/Dark 12/12 hrs for 24-48 hrs for abundant sporulation. The larvae of *C. medinalis* were taken in the petriplates containing millet grain culture (*Z. radicans*). For inoculation, second instars *C. medinalis* larvae on detached rice leaves in Petridishes were exposed to spore showers containing different concentrations (12.2±2.5, 34.6±3.1, 54.3±3.5 and 81.6±4.8 spores/mm^{-2}). Different concentrations of *Z. radicans* in the grain were pre assessed using haemocytometer. Each spore concentration included six replications (5 larvae/ replicate). Larvae in six Petri dishes unexposed to spore showers were included as blank control (Hua and Feng, 2005). All the results were analysed in the completely randomized block design.

RESULTS AND DISCUSSION

Among the different treatment of oil based formulate of *Z. radicans*, the highest larval mortality, pupal mortality, adult mortality and also the lowest pupal formation and adult emergence was noticed in *Z. radicans* + Sunflower oil + Glycerol. The lowest larval mortality, pupal mortality and adult mortality were noticed in *Z. radicans* alone. Among the different concentration of granular formulation of *Z. radicans*, the highest larval mortality, pupal mortality, adult mortality and The lowest larval mortality, pupal mortality and adult mortality noticed on 12.2 spores/mm^{-2} concentration. Pupation percentage and adult emergence percentage decreases with increase in the concentration of Spores. Among the oil and granular, oil formulation of *Z. radicans* were found better in causing mortality of life stages of leaf folder when compared to granular formulation of *Z. radicans*. The present study revealed that *Z. radicans* + Glycerol+ Sunflower oil were better in causing larval, pupal and adult mortalities of *C. medinalis*. Adjuvant glycerol may increase the sticking ability of the entomopathogenic fungi and increase the mortality of rice leaf folder larvae. Our results coincided with those of Williams *et al*. (2000) who also stated that *V. lecanii* with adjuvant like glycerol, cutinol reduced the progeny of *Myzus persicae*. Glycerol is also found to confer protection by recording higher spore germination. Our results are also similar to those of with Thompson *et al*. (2006) who also reported that the adjuvant Tinopal found to confer total protection (95% conidial germination) by recording quicker and higher mortality of *H. armigera* Oil and adjuvant reduces evaporation of spray droplets and extends the active life of the bioagent (Kaaya *et al.*, 2011). Adjuvant and oil may also affect the uptake of biopesticides across insect cuticle, physically disrupting the surface by dissolving waxy deposits (Somervaille, *et al*., 2012) resulting in the death of the insect. Mallikarjuna *et al.* (2010) also reported that the highest mortality was recorded in the formulation with sunflower oil ten days after treatment, followed by groundnut oil and sesame oil. Our results are also in line with Malsame *et al*., (2002), who reported 100% mortality of whitefly, reported that 100% mortality of whitefly. *Trialeurodes vaporariorum* (Westwood) with *M.* + sunfloweroil. Jyothi *et al*. (2014) also reported that 7 days after treatment (DAT), the highest adult mortality percentage of lesser grain borer was recorded with *Metarhizium* + sunflower oil followed by *Beauveria* + groundnut oil. Our results also coincided with Hedimbi *et al.* (2008) who also reported that utilizing the sunflower oil formulation at a higher concentration increases the adhesion of spores to the surface of the tick's cuticles which would be very beneficial in a field setting.

Table1. Efficacy of oil and granular based formulations of entomopathogenic fungi, *Zoophthora radicans* against the biology of rice leaf folder, *Cnaphalocrocis medinalis*

Treatments	Larval mortality (%)	Pupal formation (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)
Z. radicans alone	11.25	88.75	8.25	80.50	8.25
Z. radicans + Glycerol	17.50	82.50	13.78	68.65	9.25
Z. radicans + Sunflower oil	31.25	68.75	24.25	44.50	22.25
Z. radicans + Sunflower oil+ Glycerol	38.75	61.25	26.25	35.00	22.50
Z. radicans + Mustard oil	21.25	78.75	17.25	61.50	12.75
Z. radicans + Mustard oil + Glycerol	28.50	71.50	19.75	51.75	15.25
Control	0.00	100	0.00	100	0.00
C. D (p= 0.05)	0.76	0.63	0.59	0.72	0.85
SE(d)	0.35	0.29	0.27	0.33	0.39

Each value is mean of six replications. In a column means followed by a common letter are not significantly different (P=0.05) by DMRT

Table 2. Efficacy of granular based formulations of entomopathogenic fungi, *Zoophthora radicans* against the biology of rice leaf folder, *Cnaphalocrocis medinalis*

Spore concentration (spores/mm^{-2})	Larval mortality (%)	Pupal formation (%)	Pupal mortality (%)	Adult emergence (%)	Adult mortality (%)
12.2	14.25^{d}	85.75^{b}	8.25^{d}	77.50^{b}	5.75^{d}
34.6	16.50^{c}	83.50^{c}	9.25^{c}	74.25^{c}	7.75^{c}
54.3	23.25^{b}	76.75^{d}	13.25^{b}	63.50^{d}	13.75^{b}
81.3	27.50^{a}	72.50^{e}	19.25^{a}	53.25^{e}	16.50^{a}
Control	0.00^{e}	100	0.00^{e}	100^{a}	0.00^{e}
C. D (p= 0.05)	1.38	1.17	0.72	0.73	0.85
SE(d)	0.61	0.52	0.32	0.33	0.37

In a column means followed by a common letter are not significantly different (P=0.05) by DMR.

Additionally, oil formulations protect the spores Oil formulation enhances fungal virulence toward insect. Since insect cuticle especially epicuticle (lipid layer) is the primary site of establishment of mycosis, oil formulation increase the adhesion of spore to the insect cuticle through hydrophobic interaction between the spore and cuticle surface (Bandani and Esmailpour, 2006). The application of oil based formulation of *M. anisopliae, B. bassiana* and *V. lecanii* resulted in reduction of hoppers and maximization of yield of okra (Harischandra Naik and Shekharappa, 2008). Vimaladevi and Prashanth (2009) showed that the performance of ITCC 4513 and HaBb DOR the two isolates 73 of *B. bassiana* when formulated in oils was superior to that of unformulated conidia as reflected by the higher mortality of *H. armigera* larvae in laboratory sunflower. It was concluded that oil formulations of *Zoophthora radicans* was found better in causing mortality of different life stages of rice.

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