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Original Research Paper

Biocompatible Formulation of Potential Fungal Biopesticide *Nomuraea rileyi* (f.) Samson for the Improved Post Treatment Persistence and Biocontrol Potential

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ABSTRACT

The development of pest control measures using microorganisms, especially entomopathogens, has received increasing attention in recent years. Formulation of biological control agent is an important criterion for sustainable agriculture. Fungal biopesticides mainly *Nomuraea rileyi* is widely used to control various economic important insect pests. In the present study, various formulations of *N. rileyi* biogel, oil and hydrogel were prepared, and evaluated for post treatment persistence under different temperatures and the biocontrol potential against groundnut defoliator *Spodoptera litura* (Fab.) (Lepidoptera Noctuidae). Among the formulations, maximum rate of persistence was recorded in biogel. Fungal spores could retain the viability in all the tested temperatures in biogel formulation. Enhanced pesticidal activity was also recorded in the same formulation. The present study suggests the possible utilization of biogel formulation of *N. rileyi* to control economic important pests under field conditions.

INTRODUCTION

The management of insect pests rarely relies on a single management practice, usually a variety of tactics are integrated that are safe to non-target organisms to maintain pests at economic threshold levels (ETL) (Brar et al**.** 2004). The goal of the Integrated Pest Management (IPM) is not to eradicate the pests but to control or manage the pest population. Since, the availability of pests below the ETL is essential to maintain the natural enemy population, the control tactics used in integrated pest management include pest resistance to plants, and cultural, physical, chemical, mechanical and biological control in a compatible manner (Enkerli et al. 2004). Biological control is an important tactic in IPM system. Biological control plays a pro-vital role in the pest management program (Alter & Vandenberg 2000). In several situations, parasitoids (trichogrammatids, encyolids, tochinids and others), predators (coccinelids, lacewings, reduviids, spiders) and entomopathogonic microorganisms (bacteria, fungi, virus, protozoa, nematodes) have been used to keep the population of pests below the damaging level (Sahayaraj & Karthick Raja Namasivayam 2010).

Usage of entomopathogenic fungi as biological control agents of insect pests has been increasing during the last few decade. More than 750 species of fungi, mostly deuteromycetes and entomophthorales, are pathogenic to insects. Species that have been most intensively investigated as mycoinsecticides in the crop pest control include *Beauveria bassiana, B. brongniartrii, Metarhizium anisopliae, Nomuraea rileyi, Paecilomyces fumosoroseus, P. farinosus, Entomophthora* sp., *Fusarium* sp. and *Aspergillus* sp. Entomopathogenic fungi are associated with insects living in diverse habitats such freshwater, soil and aerial location. They are very specific to insects and do not infect host plants. *Nomuraea rileyi* is an important mortality factor for many lipidopteran insects throughout the world. Being developed as a microbial insect control agent, the fungus is capable of causing spectacular epizotic in caterpillar pests of cabbage, clover, soybean, potato, cotton, etc. (Sahayaraj & Karthick Raja Namasivayam 2011).

Formulation of biological control agent is an important criterion for sustainable agriculture (Sharma 2004). Formulation can improve the product stability and viability may result in consistency of field performance of many potential biological control agents. Formulation of biocontrol products has been used against diseases (biofungicides), weeds (bioherbicides) and insect pests (bioinsecticides) (Gopalakrishnana & Mohan 2000). Many of the biocontrol agents have been formulated with dried milk, powdered casein, gelatin, saponins, oils, soaps, etc. In so far as microbial insecticides are concerned, it is essential that the compound used should premature the growth or germination and that it should not inhibit the successful establishment of the pathogens (Tincilley et al. 2000). In the present study, improved persistence and biocontrol potential of *N. rileyi* with biogel, hydrogel and oil formulation was studied.

MATERIALS AND METHODS

Soil sampling: *N. rileyi* was isolated from the soil sample obtained from groundnut field, Chengalpet, Kanchipuram district, Tamil Nadu and processed for the isolation of fungi. Approximately 2 kg of soil was collected from four points, a few meters apart by digging to a depth of 10-15 cm with a small spade. The soil samples were put in plastic bags and taken to the laboratory and stored at 25°C. For processing, the soil was thoroughly mixed and passed through a 0.4 mm mesh sieve to break or separate any coarse lumps of soil or litter. Before microbial analysis, soil aggregates were broken by hands, trays with soil were kept open until moisture was at equilibrium (Asensio et al. 2003). Soil texture pH electrical conductivity organic matter nitrate, phosphorus, potassium, calcium, magnesium, sulphur, sodium, zinc, iron and copper were determined for all the soils collected. These measurements were determined in National Agro Foundation at Taramani, Tamil Nadu.

Isolation of *N. rileyi*: *N. rileyi* SSK 7 strain was isolated from the processed soil sample by the modified method of Clark (1997) using CTC (Chloramphenicol Thiobenzodazole Cyclohexamine) media. The organism was identified based on the morphological and cultural characteristics adopting standard methods and the pure culture was maintained on CTC agar slant. Fungal morphology was confirmed by lacto phenol blue staining (Humber 1997).

Preparation of fungal inocula: Fungal inocula was obtained from 15 days old CTC slant culture by scrapping slant with a sterilized glass rod. A homogenous conidial suspension was prepared in sterile distilled water by adding a few drops of the wetting agent Tween 80(0.01%). The conidial concentration of the suspension was determined using an improved Neubauer haemocytometer.

Dry granular biogel formulation: One gram of rice and soya bean powder were mixed with sterile distilled water in a beaker and sterilized by autoclaving. After sterilization the mixture was allowed to cool and 1mL of fungal inoculum was added to the mixture and incubated at 40°C for overnight. After drying the mixture was grinded into granules and transferred to the sterile screw cap vials.

Hydrogel formulation: Hydrogel was prepared with alginate + agar + starch (1%), agar (1%), alginate + agar (1%) and starch + agar (1%). The contents were boiled in microwave oven for the complete dispersion, transferred to the sterile Petri plates and 1 mL of fungal spore inoculum was added. The above prepared plates were kept in hot air oven at 40°C for 24 hours and the dried gel was collected in screw cap vial and used for further studies.

Oil formulation: Oil formulation was prepared with the following composition. 45 mL of sterile distilled water, 4mL of glycerine, 750µL of liquid paraffin, 2.5 mL of Tween 20 and 10 mL of coconut oil. The ingredients were mixed well, transferred to the 100 mL of conical flask and sterilized by autoclaving. After the sterilization, 1 mL of fungal inoculum was added. The mixture was kept for stirring for 1 hour at room temperature and the homogenous mixture was used for further studies.

Effect of temperature on the viability of different formulation of *N. rileyi*: Effect of temperature on the viability of respective formulation on *N. rileyi* was studied under *in vitro* condition. 1gram of dry granular and hydrogel and 1 mL of oil formulation was suspended in 99 mL sterile distilled water separately. The mixture was heated at 30°C, 40°C and 50°C for 10 minutes, after the heat treatment the suspension was serially diluted and 1 mL of aliquot was transferred to the Petri plate. 20 mL of sterile molten CTC media was poured. After the incubation period the colonies where counted.

Effect of formulation on post treatment persistence of *N. rileyi* under pot assay: Effect of the respective formulation on the post treatment persistence of *N. rileyi* was also studied under pot condition. One kg of fertile loam soil was sterilized by autoclaving. After sterilization, 1g of the dry biogel, hydrogel and 1mL of the oil formulation were mixed, transferred to the pots (14cm in height and 12cm) and the seeded pots were incubated at 25°C, 30°C, 35°C for 20 days in the environmental chamber incubator (Make: Remi, India). After the incubation period, 10 g of soil was taken from the respective formulation incubated at the respective temperature and suspended in 990 mL of sterile distilled water followed by successive dilution in 9mL of sterile distilled water and the respective aliquot was plated on sterile molten CTC medium.

Evaluation of biocontrol potential against *Spodoptera litura*: Evaluation of biocontrol potential of respective formulation of *N. rileyi* was studied under laboratory condition. Laboratory stock culture of III and IV instars of *S. litura* was used in this study. One g of dry granular, hydrogel and 1mL of oil formulated *N. rileyi* were suspended in 100 mL of sterile distilled water and the suspended respective formulation was sprayed on the 20 larvae of the each instars, transferred to the plastic container provided with moist cotton swap and covered with tissue paper at the bottom of the container to provide humidity. The containers were covered with meshed lid to provide aeration to the larvae. For control category, another 20 larvae of each instar were treated with distilled water only. The containers were incubated at room temperature 28 ± 0.5°C in an incubator. Daily observation on larval mortality was recorded. The total larval and pupal durations, adult longevity and the adult

Table 1: Physico-chemical parameters of soil samples collected from Chengalpet groundnut field.

S.No	Parameters	
1	pH	7.95
2	Electrical conductivity (ms/cm)	0.600
3	Organic matter (%)	2.33
4	Nitrate nitrogen (ppm)	24.9
5	Available phosphorus (ppm)	237.7
6	Potassium exchangeable, (ppm)	93
7	Calcium exchangeable (ppm)	1932
8	Magnesium exchangeable (ppm)	511
9	Sulphur available s as SO_4 ((ppm)	49.3
10	Sodium exchangeable (ppm)	302
11	Zinc available (ppm)	2.15
12	Manganese available (ppm)	4.72
13	Iron available (ppm)	1.36
14	Copper available (ppm)	1.84

Table 2: Effect of formulation on the viability of *N. rileyi* at different temeperatures (°C).

S.No.	Formulation	Colony count (CFU/mL)		
		30°C	40°C	50°C
1	Dry biogel	63.1×10^7	50.0×10^7	41.2×10^7
2	Oil	45.0×10^5	31.0×10^4	,30.0×10^4
3	Hydrogel	31.2×10^3	67.1×10^3	41.0×10^3
4	Control	30.0×10^4	41.2×10^3	31.2×10^3

Table 3: Radial mycelial growth of *N. rileyi* formulation at different temperatures.

S.No	Formulation	Radial mycelial growth (mm)		
		30°C	40°C	50°C
1	Dry biogel	25.0	19.0	19.0
2	Oil	21.0	17.0	14.0
3	Hydrogel	15.0	12.0	11.0
4	Control	15.0	11.0	10.0

emergence were recorded.

RESULTS AND DISCUSSION

N. rileyi was isolated from the groundnut field soil adopting culture dependent method and the isolated fungi was identified based on the cultural characteristics on the CTC medium, which revealed brilliant green aerial mycelium and the microscopic examination of fungal spore by lactophenol cotton blue showed spherical conidia. Soil physico-chemical characteristics highly influenced the natural occurrence of *Nomurea rileyi*. *Nomurea rileyi* isolated from the respective soil sample reveals high organic matter, available nitrogen and phosphorus (Table 1). This may favour the viability of the fungal spore and thus improved the natural occurrence of *Nomurea rileyi.*

Among the different formulations, dry granular biogel retained maximum viability of *N. rileyi* in all the tested temperatures. 63.1×10^7, 50.0×10^7 and 41.2×10^7CFU/g of colony count was recorded at the respective temperatures (Table 2). Least count was recorded in unformulated *N. rileyi* free spores (control) at all the respective temperatures, 30.0×10^4, 41.2×10^3 and 31.2×10^3 CFU/g. Biogel oil formulation supported maximum viability at all the tested temperatures, which revealed 45.0×10^5, 31.0×10^4, 30.0×10^4CFU/g at the respective temperatures. Hydrogel formulation revealed least count in all the temperatures. Radial growth of *N. rileyi* formulation at the respective temperatures was also higher than the unformulated *N. rileyi.* 25.0, 19.0 and 19.0 mm of mycelial radial growth was recorded in biogel formulation at the respective temperatures, whereas 15.0,11.0 and 10.0mm of radial mycelial growth was recorded in free *N. rileyi* free spores. Increase in radial mycelial growth was also observed in oil formulation as in dry granular biogel formulation. Mycelial growth was also reduced in the same treatment (Table 3).

Effect of the formulation on the viability of *N. rileyi* was also studied under pot assay, which revealed similar status as in laboratory study as described above (Table 4). In the case of pot assay, maximum rate of persistence was recorded in dry granular biogel formulation incubated at all the tested temperatures. Oil formulation also supported maximum viability. Least viability was reported in hydrogel formulation. Similar finding were also recorded in radial growth (Table 5). Biocontrol potential of respective formulations against larval instars of *S. litura* showed both the dry biogel and oil formulations maximum mortality to the both larval instars (Table 6). Cumulative mortality (%) of the III and IV instars of *S. litura* with dry granular formulation was found to be 90.5 and 89.0 %. 89.0 and 87.0 % mortality was recorded in oil formulation against III and IV instars.

The successful use of biological control agents depends on the suitable formulation technique. This formulation technique increases their persistence and their dependability on the prevailing environment. They also revealed that the infective propagules of fungus, either in the form of blastospore or conidiospores, showed better activity than they were mixed with suitable ingredients, which may act as nutrients, additives or wettable agents. These agents provide protection to the propagules against the unfavourable environment condition. In the present study, biogel formulation of *N. rileyi* supported maximum viability which might be due to the natural polymers present in the substrate (rice flour and soya bean powder) forming a protective layer around the fungal spores. The lipophilic conidia of deuteromycete fungi readily suspend in oil uniformly and dispersed its conidia might

Table 4: Effect of formulation on *N. rileyi* viability under pot assay.

S. No.	Formulation	Colony count (CFU/mL)		
		30°C	40°C	50°C
1	Dry biogel	65.1×10^{7}	53.0×10^{7}	42.2×10^{7}
2	Oil	46.0×10^{5}	33.0×10^{4}	33.0×10^{4}
3	Hydrogel	34.2×10^{3}	69.1×10^{3}	44.0×10^{3}
4	Control	32.0×10^{4}	43.2×10^{3}	34.2×10^{3}

Table 5: Radial mycelial growth of *N. rileyi* formulation at different temperatures.

S.No.	Formulation	Radial mycelial growth (mm)		
		30°C	40°C	50°C
1	Dry biogel	26.0	21.0	20.0
2	Oil	22.0	18.0	15.0
3	Hydrogel	16.0	13.0	12.0
4	Control	15.0	11.0	10.0

Table 6: Effect of *N. rileyi* formulation on the biocontrol potential of *S. litura.*

S.No.	Formulation	Cumulative mortality (%)	
		Instar III	Instar IV
1	Dry biogel	90.5	89.0
2	Oil	89.0	87.0
3	Hydrrogel	63.0	59.5
4	Control	62.0	60.0

favour and uniform coverage of plant surfaces. Oil based formulation effect of *Nomuraea rileyi* and *B. bassiana* on *S. litura* and *Myzus persicae* was reported by Vimala Devi et al. (2000) and Sahayaraj & Karthick Raja Namasivayam (2011) respectively. They observed that oil formulations reduced the pest populations distinctly than other formulations. Further study with the bioencapsulated fungal biocontrol agent under field condition is now in progress.

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