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Effect of drying and formulation of conidia on virulence of the entomofungal pathogen *Nomuraea rileyi* (F) Samson

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ABSTRACT: **Drying *Nomuraea rileyi* conidia along with the solid substrate in an air stream gave a virulent product that gave high mortality of *Spodoptera litura* larvae in a laboratory bioassay whereas drying over silica gel or calcium chloride resulted in loss of efficacy. Bioassays with oil formulations of spores dried in an air stream resulted in comparable mortality of third instar *S. litura* (Fabr.) larvae with that of unformulated conidia. The oil formulation with Triton-X-100 caused significant reduction of *S. litura* populations feeding on groundnut and castor in field experiments. Natural parasitisation levels of two larval parasitoids on *S. litura* were similar in the fungus treatments and untreated controls.**

KEY WORDS: Castor, drying, field-tests, formulation, groundnut, *Nomuraea rileyi*, *Spodoptera litura*

The entomofungal pathogen, *Nomuraea rileyi* (Farlow) Samson, infects several lepidopteran pests. These include the tobacco caterpillar, *Spodoptera litura* (Fabricius) (Rao and Phadke, 1977), gram caterpillar, *Helicoverpa armigera* (Hubner.) (Gopalakrishnan and Narayanan, 1988), *Spilarctia obliqua* Walker (Singh and Gangrade, 1975) and *Thysanoplusia orichalcea* (Fabricius) (Agarwal and Rajak,1985). Field applications of *N. rileyi* have generally been as foliar sprays of conidia, soil application of conidia along with the solid substrate (Vimala Devi, 1994; 1995), dusting of dry formulations (Ignoffo, 1981) and field distribution of diseased cadavers (Sprenkel and Brooks, 1975). Information on formulations of *Nomuraea rileyi* is scanty. Formulating entomofungal pathogens in oils increases their effectiveness (Prior *et al.*, 1988) and lipophilic conidia showed superior performance when formulated in oils compared to aqueous suspensions (Bateman, 1997). Oil-based formulations are most effectively delivered at ultra-low volumes (ULV) (Mathews, 1992). Although ULV formulations of lipophilic conidia greatly enhance infectivity (Prior and Greathead, 1989), the method is currently not in use in India as many farmers do not own such equipment. An alternative then is to use an oil-in-water emulsion which may give the benefits of an oil-based formulation but enables farmers to use conventional sprayers namely hydraulic high volume sprayers. Conidia of *N. rileyi* suspend readily in oil (Vimala Devi and Prasad, 1996). This paper discusses the effect of drying *N. rileyi* conidia on infectivity, formulation and field performance of the oil-in-water emulsion spray.

MATERIALS AND METHODS

A. Laboratory testing of *N. rileyi*

Multiplication and drying of conidia

Three isolates of the entomofungal pathogen *N. rileyi* were multiplied on crushed sorghum grains (Vimala Devi, 1994). The substrate overgrown with the sporulated fungus was placed in a Petri-plate and dried overnight at 26±1°C over silica gel, calcium chloride in a desiccator and in an air stream in the laminar flow (MICRO-FILT, India). The conidia were obtained as a loose powder without any clumps after sieving the dried substrate through a fine mesh (200μ)/ single layered muslin cloth.

Bioassay with dried conidia

Spodoptera. litura was reared on castor leaves in the laboratory (27±1°C). Ten third instar larvae of *S. litura* (7-8 day old) were released on castor leaves with stalks wrapped in wet cotton and the inoculum containing $2x10^8$ conidia ml^{-1} conidia in 0.02% Tween-80 water (single distilled water) was then sprayed. Three such replicates were maintained for each treatment. Untreated controls were maintained by feeding the larvae on castor leaves with Tween-80 (.02%) water. Observations of larval mortality were recorded daily.

Preparation of formulations

Three oils *viz.*, sunflower, groundnut and mineral oil and five emulsifiers *viz.*, Tween-20, Tween-40, Tween-80, Triton X-100 and Labogent (a neutral soap) were used to identify the best combination of spore, oil and emulsifier. Conidia of isolate no.2 dried in an air stream (1g) were mixed with emulsifier 10 percent and oil 90 percent. Oil-emulsifier combinations with and without spores were rated visually in terms of miscibility before and after diluting with water.

Efficacy of oil-in-water emulsion spray

Freshly prepared conidial formulation (0.25g) was suspended in sterile distilled water and the suspension was adjusted to a final concentration of $2x\ 10^8$ conidia ml^{-1}. The suspension was then sprayed on to castor leaves for bioassays against *S. litura*. Freshly harvested conidia without formulation were suspended in Tween-80 (0.02%) water and tested at a similar concentration for comparison.

B. Field testing

The formulation of isolate no.2 containing spores, sunflower oil and emulsifier (Triton-X-100) was mixed with water to give a final concentration of 2×10^{11} conidia l^{-1}. Sprays were undertaken in the evening just before sunset using a 3 litre capacity knapsack sprayer @ 500 litre/ha.

Test on groundnut

Field testing of the oil-emulsion formulation was carried out both during rainy and post-rainy seasons of 1997. Groundnut (cvTMV-2) was sown in 2x2m plots at a spacing of 30x10cm in a completely randomised design. Three replicates were included for each treatment. One metre inter-plot distance was maintained all around. Larval releases were made @ 100 third instar *S. litura* larvae $plot^{-1}$. Due to the migratory nature of *S. litura* larvae, the experimental plots were fenced on all four sides with 30cm high aluminium sheets. After spray the plots were covered with nylon net to prevent predation of larvae by birds. Presence of fungus infected larvae on all plants and soil was observed daily. Natural larval parasitisation due to *Cotesia* spp. (solitary) and *Apanteles* spp. (gregarious) was recorded. Either the solitary pupa or a mass of pupae (gregarious) was considered as parasitizing one larva. Cumulative percentage mortality was computed from the total number of larvae released.

Test on castor

Field testing of *N. rileyi* against *S. litura* was undertaken on castor during rainy- season. Castor (cv VP-1) was sown in 5x5m plots at a spacing of 60x30cm in a completely randomised design. Each treatment was maintained in three replications. Spraying was done on larval releases made @ 100 third instar *S. litura* larvae $plot^{-1}$. Percentage mortality was worked-out from pre-treatment counts of larval population.

Analysis of variance was performed to test the differences in larval mortality due to fungus and natural parasitisation levels due to parasitoids between treatments.

RESULTS AND DISCUSSSION

Conidia obtained by drying along with the substrate over silica gel or calcium chloride failed to cause any mortality in *S. litura* larvae. Conidia dried in an air stream in a laminar flow cabinet alone resulted in significant mortality comparable to freshly harvested conidia without drying (Table 1). The drying process was rapid (12h) with calcium chloride or silica gel due to their anhydrous and hygroscopic nature. Rapid drying might impart greater physiological stress and lead to desiccation of the spores with resultant loss in virulence. The time taken for drying of conidia in an air stream in the laminar flow cabinet was much longer (2 days). Slow and gradual drying of substrate in an air stream perhaps caused less physiological stress with little effect on virulence. Moisture content of the conidia after drying and storage temperatures greatly influences conidial viability (Hedgecock *et al.*, 1995). In this study the source of conidia for the three methods of drying was from the same production batch in order to ensure that the initial moisture content was similar. Differences in virulence can therefore be attributed to the drying method as larval bioassays were conducted immediately after drying. Of the combinations of three oils and five emulsifiers tested, sunflower oil gave good miscibility with Tween-80 and Triton X-100 resulting in a uniform emulsion when mixed with water for preparing the spray fluid. All other combinations resulted in separation of the solution into oil and water layers. These two combinations namely conidia + sunflower oil + Triton X-100 and conidia + sunflower oil + Tween-80 were therefore used for laboratory bioassays against third instar larvae of *S. litura*. Both the formulations resulted in similar mortality at 9 days after exposure with that of unformulated conidia (Table 2). *N. rileyi* conidia suspend readily in oil. Uniform dispersion of conidia for good coverage on plant surfaces when the formulation is mixed with water depends mainly on the oil-emulsifier combination used to develop oil-in-water emulsion formulations. Tween-80 and Triton X-100 were the best in terms of miscibility with sunflower oil as well as resulting in a spray fluid with uniform dispersion of conidia in water. Spore-oil-emulsifier-water combinations with these two emulsifiers resulted in optimum mortality of the larvae in the laboratory bioassay and field tests with Triton X-100 as emulsifier. Oil formulations of conidia are ideal for use in ultra low

Table 1. Effect of flow drying on efficacy of *N. rileyi* on *S. litura*

Treatment[b]	Mortality due to fungus (%)	Live larvae (%)	Mortality due to bacteria/virus (%)
Isolate 1	70.53 (57.5)[a]	29.5 (32.9)[a]	0.0
Isolate 2	87.1 (72.6)	0.0 (0.0)	12.9
Isolate 3	63.3 (52.7)	26.5 (31.0)	10.2
Unsprayed	0.0 (0. 0)	95.8 (83.1)	4.2
CV %	17.49	15.57	-
SEM±	3.99	2.86	-
CD (P=0.01)	20.97	15.01	-

[a]Figures in parentheses are angular transformed values.

[b] Spore concentration at 2×10^8 ml^{-1}

Table 2. Laboratory testing of formulations of *N. rileyi* against *S. litura*

Treatment	Mortality 9 days after exposure (%)
Conidia + sunflower oil + Tween-80	65.7 (54.3)[a]
Conidia + sunflower oil + Triton-X-100	83.9 (66.6)
Fungus alone	70.2 (57.2)
Untreated control	0.0 (0.0)
CV (%)	12.63
SEM ±	2.81
CD (P=0.01)	14.76

[a]Figures in parentheses are angular transformed values.

[b] Spore concentration at 2×10^8 ml^{-1}

volume applications in areas of low humidity (Bateman *et al.*, 1993). However, opportunities for such applications in India are less as the ULV sprayers are not available with farmers. Oil-in-water emulsions, therefore, are an attractive alternative.

In the field test on rainy-season groundnut, cumulative larval mortality was 44.3 per cent with the oil emulsion formulation while it was 65.9 per cent on post-rainy groundnut 8 days after spray (Table 3). Initial mortality was observed 6 days after spray. Natural parasitisation due to *Cotesia* spp. and *Apanteles* spp. was non-significant between the treated and untreated plots during both the seasons. Larvae (late fifth instar) observed in the control plots were uninfected.

On castor, mortality of *S. litura* larvae due to oil-in-water emulsion spray was 62.7 per cent at the end of 8 days (Table 3). Initial mortality was observed starting 5 days after spray. Natural parasitisation levels were similar in the fungus treated and untreated plots. A small percentage of fungus infected larvae in the controls plots was probably due to either spray drift or larval dispersal.

Nomuraea rileyi has been field tested mainly as foliar sprays (Ignoffo *et al.*, 1976; Mohammed *et al.*, 1978) with only one instance of testing as a dust with pyrophyllite as the carrier (Ignoffo, 1981). Soil treatment of conidia along with the solid substrate has shown promise for the control of *S. litura* on groundnut, which is a close-canopy crop (Vimala Devi, 1995). However, greater temperature tolerance during storage prior to actual field-use was achieved with conidia in oil formulations (McClatchie *et al.*, 1994). Field trials under various conditions have demonstrated the practical efficacy of experimental oil-based formulations of *Metarhizium flavoviridae* Gams and Rozsypal (Lomer *et al.*, 1993; Milner *et al.*, 1994). The present study is the first report on field efficacy of *N. rileyi* conidia formulated in oil and applied as an oil-in-water emulsion spray. Due to the lipophilic nature of *N. rileyi* conidia, in the formulation oil forms a thin layer around the spore thus protecting it from dessication as well as ensuring a better spread over the larval surface. Tween and Triton aid in the formation of a stable, uniform suspension of spray fluid when diluted in water without any need for constant agitation during spraying.

The spectrum of *N. rileyi* is primarily limited to lepidopteran insects (Ignoffo, 1981). Natural incidence of two larval parasitoids on *S. litura* both on groundnut and castor was similar in fungus treated and untreated plots indicating that

Table 3. Field testing of *N. rileyi* formulation in sunflower oil against *S. litura* on groundnut and castor

Treatment	Cumulative mortality due to fungus[a] (%)	Parasitisation[a] (%)
Rainy-season groundnut[a]		
Oil-in-water emulsion spray	44.3 (41.69)	18.00 (25.03)
Control	0.67 (2.71)	18.33 (25.30)
CD (P=0.05)	23.48	NS
Post-rainy season groundnut[b]		
Oil-in-water emulsion spray	65.9 (54.3)	26.9 (31.3)
Control	3.1 (8.2)	22.6 (28.2)
CD (P=0.01)	20.81	NS
Rainy-season castor[c]		
Oil-in-water emulsion spray	62.8 (52.5)	12.4 (20.4)
Control	3.6 (10.54)	18.2 (24.9)
CD (P=0.05)	10.67	NS

[a] Figures in parentheses are angular transformed values.

[b] Mortality at 8 days spray (2×10^{11} conidia l^{-1}) computed from pre-treatment release of larvae

[c] Mortality at 8 days after spray (2×10^{11} conidia l^{-1}) computed over pre-treatment counts

application of *N. rileyi* oil-in-water emulsion sprays do not adversely affect parasitoid activity.

In the present study, drying of fungal conidia in an air stream along with the substrate, formulation with sunflower oil and Triton X-100 and field testing on groundnut and castor showed that the formulation caused significant mortality of *S. litura* larvae 8 days after spraying. It was safe to the natural parasites *Cotesia* spp. and *Apanteles* spp.

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