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RESEARCH **P**APER

Shelf-life and infectivity study of carrier formulations of entomogenous fungus *Nomuraea rileyi* (Farlow) Samson

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N. rileyi formulated in different carriers *viz*., talc, kaoline, charcol, wheat bran, soil and lignite stored at 4°C and room temperature for six months storage to assess viability and infectiveness against third instars larvae of *H. armigera*. All carriers retained the viability in sufficient numbers for a period of 150 days at 4°C temperature. However, after 180 days of storage significant reduction was observed in all carriers. Kaoline supported maximum propagules *i.e.* 14.21 x 10^6 cfu/g followed by lignite (11.95 x 10^6 cfu/g) after 180 days of storage at 4°C temperature. Viability was drastically reduced when carrier's formulations stored at room temperature. Kaoline formulation stored at 4°C was efficient as recorded maximum larval mortality of *H. armigera* at 60, 120 and 180 days followed by lignite. However, at room temperature drastic reduction in per cent larval mortality of *H. armigera* (ranged of 16.67 to 43.33%) was observed.

Key words : *N. rileyi*, Carrier formulations, Viability, Infectivity

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INTRODUCTION

Natural control agents such as entomogenous fungi can be used as a component of integrated pest management of many economically important insect pests. Under natural conditions, these pathogens are a frequent and often cause natural mortalities of insect populations in different crop-system. There is a high potential for the use of *Nomuraea rileyi* (Farlow) Samson for biological control because this fungi can be cost-effectively mass-produced locally, has massive potential for the management of several Lepidopteron pests of economic significance and merits promotion as a myco-insecticide.

The development of suitable formulation is essential to the successful utilization of myco-insecticides and for successful pest control, the infective unit *i.e.* spores must remain viable with high virulence against target pest during storage including package prior to application in the field (Prior *et al.*, 1988). Apart from strain selection, temperature, humidity and light have been shown to affect the stability of conidia of entomopathogenic fungi during production and storage. Meagre information is available on the effect of temperature regimes during storage on viability and virulence of *N. rileyi* in powder formulations. With this view, the different powder based carriers were used for development of formulation and effect of temperature during the storage of carrier formulation on viability and infectivity of *N. rileyi* examined for six months period.

RESEARCH METHODOLOGY

Pure culture of *N. rileyi* isolated from naturally infected cadaver of *Helicoverpa armigera* and maintained in the standard mycological medium SMAY (Saboured's Maltose Agar Yeast extract medium) used

in present studies.

Six carriers' *viz.*, talc, lignite, kaoline (Hi media make), charcoal (200 mech), wheat bran (300 mesh) and soil (200 mech) were used. Spore mass harvested from SMAY media after 25 days of incubation. Fungal biomass mixed with 0.02 per cent tween-80 and blended in an electric mixer for 1-2 min to get homogenous slurry. It was drained through muslin cloth to remove debris under aspectic condition. Spore suspension, 20 ml, was incorporated in 100 g pre-strelized autoclaved talc, lignite, kaoline, charcoal, wheat bran and soil as a carrier. After the addition of spore suspension, the carriers were packed in milky white low density polypropylene bags (size 15x7.5 cm) and sealed with electric sealer. At initial stage moisture level in carrier based formulation was 10 per cent. Four replications were maintained for each carrier to determine the initial number of colony forming unit (Cfu) of *N. rileyi* in formulation. A sample of one gram of the product was drawn from each carrier just before packing, then sealed bags were stored at 4^0C and room temperature for six months and at monthly interval propagules were estimated by serial dilution technique for longevity studies. One gram sample from each carrier at respective interval (30, 60, 90,120,150 and 180 days) were taken and serially diluted up to 10^6 by using sterilized distilled water. An aliquot of 0.1ml was transferred on to each SMAY plate and examined for counting colony forming unit (Cfu).

Virulence of the stored formulation was tested at 0 day and after 60,120 and 180 days of storage against third instars larvae of *H. armigera* following topical application method at 4^0C and room temperature in three replications under laboratory condition and per cent larval mortality was recorded at 10 days after treatment (DAT).

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Shelf-life of *N. rileyi* in different carrier based formulations stored at 4°C temperature :

Data on viability of *N. rileyi* in different carrier at 4°C storage temperature till 180 days period are presented in Table 1. Initially (0 days) all the carriers revealed non-significant differences in colony forming unit (cfu) and cfu ranged from 18.38 to 20.50 x 10^6 /g in six different carriers. Spores of *N. rileyi* remained viable in sufficient numbers in all carriers for a period of 150 days (five months) at 4°C temperature. However, after 180 days of storage (six months later) the significant reduction in conidial viability was observed in all carriers. However, maximum number of cfu were recorded in kaoline (14.21 x 10^6 /g) followed by lignite (11.95 x 10^6/g). Charcoal, talc, soil and wheat bran formulations were found equally effective for retaining the viability while the minimum population *i.e.* 9.53 x 10^6 cfu/g was recorded in wheat bran.

Shelf-life of *N. rileyi* in different carrier based formulations stored at room temperature :

Conidia were viable in sufficient in minimum number and ranges from 14.15 and 18.60 x 10^6 cfu/g for a period of one month at room temperature (Table 2). After two months (60 days) of storage, the colony forming unit

Table 1 : Shelf-life of *N. rileyi* in different carrier based formulations for six months stored at 4°C

Sr. No.	Carrier	Initial	Cfu x 10^6 /g after days *					
		(0 days)	30	60	90	120	150	180
1.	Talc	19.95	19.08	18.00	16.53	14.90	12.98	11.18
2.	Kaoline	20.50	20.10	19.60	18.73	17.55	16.00	14.21
3.	Charcoal	18.38	18.13	17.20	16.28	14.75	12.98	11.23
4.	Wheat bran	18.45	17.50	16.43	15.05	13.55	11.53	9.53
5.	Soil	19.45	18.68	17.60	16.13	14.40	12.55	10.63
6.	Lignite	20.25	19.68	18.65	17.45	15.50	13.95	11.95
	Average	19.49	18.87	17.91	16.69	15.10	13.34	11.45
	F' test	NS	NS	NS	NS	NS	NS	Sig
	S.E.±	1.06	0.84	1.01	0.81	0.99	0.83	0.68
	C.D. (P=0.01)	--	--	--	--	--	--	2.80

*Average of four replication NS= Non-significant

reduced drastically in all the carriers. However, viability was considerably more in kaoline at monthly interval followed by lignite and charcoal while wheat bran recorded less number of colony forming unit after 180 days.

Viability was drastically reduced when carrier based formulation stored at room temperature as compared to 4°C temperature. However, conidial viability in kaoline and lignite formulations was higher at each monthly interval even at room temperature. The present study confirms that viability of *N. rileyi* declined rapidly after six months of storage at 4°C and room temperature and kaoline is the best carrier for making the formulations. Further the product should be stored at low temperature *i.e.* 4°C temperature to retain viability of spores. The present results correlates the findings of Walstad *et al.* (1970); Daoust *et al*. (1983) and Moore and Prior (1993). Physical properties of kaoline *viz.*, proper water retention capacity, regulation in pH and electrostatic charge, fine structure might be responsible for retention of viability of *N. rileyi* spores for longer period followed by lignite and charcoal powder.

Virulence of *N. rileyi* stored at 4°C and room temperature in different carrier based formulations:

At initial level *i.e.* (at 0 days) mortality of *H. armigera* ranges from 70.0 to 86.67 per cent (Table 3). Infectivity of the spores of *N. rileyi* at 4°C in different carrier based formulations reveals that there was a significant variation in per cent larval mortality. At 60 days, mortality ranges from 63.33 to 83.33 per cent whereas after 120 days of interval mortality ranged from

Table 2 : Shelf-life of *N. rileyi* in different carrier based formulations for six months stored at room temperature

Sr. No.	Carrier	Initial	Cfu x 10^6 /g after days*					
		(0 days)	30	60	90	120	150	180
1.	Talc	19.95	15.85	13.65	11.90	8.83	4.95	1.90
2.	Kaoline	20.50	18.60	16.55	13.65	12.93	9.25	4.70
3.	Charcoal	18.38	16.70	14.90	12.10	9.60	5.35	2.10
4.	Wheat bran	18.45	14.15	12.48	9.93	7.63	2.63	1.00
5.	Soil	19.45	16.33	13.25	10.75	8.73	4.65	1.60
6.	Lignite	20.25	17.25	14.85	12.40	9.45	7.00	3.10
	Average	19.49	16.48	14.28	11.79	9.53	5.64	2.40
	F' Test	NS	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	S.E.±	1.06	0.43	0.46	0.48	0.44	0.37	0.36
	C.D. (P=0.01)	--	1.78	1.89	1.96	1.79	1.54	1.46

*Average of four replication NS= Non-significant

Table 3 : Infectivity of the spores of *N. rileyi* stored at different temperature regimes in different carrier based formulations

Sr. No.	Formulation	Initial Mortality 0 days	4°C			Room temperature		
			60	120	180	60	120	180
1.	Talc	73.33 (59.00)**	66.67 (54.7)	60.00 (50.7)	50.00 (45.0)	56.67 (48.8)	46.67 (43.0)	33.33 (35.2)
2.	Kaoline	86.67 (68.86)	83.33 (66.1)	76.67 (61.2)	66.67 (54.7)	63.33 (52.7)	53.33 (46.9)	43.33 (41.1)
3.	Charcoal	73.33 (59.00)	70.00 (56.79	63.33 (52.7)	53.33 (46.9)	56.67 (48.8)	50.00 (45.0)	36.67 (37.2)
4.	Wheat bran	70.00 (59.79)	63.33 (52.7)	56.67 (48.8)	46.61 (43.0)	53.33 (46.9)	40.00 (39.2)	16.67 (24.0)
5.	Soil	76.67 (61.12)	70.00 (56.7)	60.00 (50.7)	53.33 (46.9)	60.00 (50.7)	43.33 (41.1)	30.00 (33.2)
6.	Lignite	80.00 (63.43)	76.67 (61.2)	70.00 (56.7)	60.00 (50.7)	60.00 (50.7)	50.00 (45.0)	36.67 (37.2)
7.	Control	00.00 (00.00)	00.00 (00.0)	00.00 (00.0)	00.00 (00.0)	00.00 (00.0)	00.00 (00.0)	00.00 (00.0)
	F' test	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	S.E.±	1.77	1.70	1.52	1.90	1.46	1.78	1.81
	C.D. (P= 0.01)	7.47	7.17	6.42	8.03	6.18	7.49	7.65

*Avg. of three replication ** Figures in parenthesis are arc sin transformed mean value NS= Non-significant

56.67 to 76.67 per cent in different treatments. The efficiency of *N. rileyi* was found to decrease after 180 days even stored at 4°C storage temperature. Kaoline and lignite as carrier proved to be very effective in retaining the virulence resulted in highest per cent mortality till 180 days.

Storage at room temperature, drastic reduction in virulence of *N. rilyi* spore observed. However, maximum percent larval mortality was observed in kaoline formulation at 60, 120 and 180 days and registered 63.33, 53.33 and 43.33 per cent mortality, respectively. Lowest per cent mean mortality was recorded in wheat bran formulation *i.e.* 16.67 per cent after 180 days of storage period at room temperature condition. Results in the similar pattern reported by Gupta *et al*. (2000).

The result indicated that for retaining higher efficiency of *N. rileyi* against its target pest it should be stored under low temperature (4°C) upto 180 days in kaoline. Drastic reduction in the virulence of *N. rileyi* was observed in all carriers when stored at room temperature for a period of 180 days. Therefore, it advocates that carriers based formulation of *N. rileyi* must be kept at low temperature.

No related information could be traced during literature hunt on infectivity of *N. rileyi* conidia stored in different carrier at different temperature regimes and hence, it provides useful information.

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