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Shelf-life assessment of wettable powder (WP) formulations of the entomopathogenic fungi *Nomuraea rileyi* (Farlow) Samson

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ABSTRACT

Studies on the effect of storage on viability of developed *Nomuraea rileyi* (Farlow) Samson 5 % WP formulation A($N_{30}S_{1/1}$) and B($N_{30}T_{1/2}G_{2/1}H_{1/1}$) (comprising adjuvants, fungus and kaolinite) and *N.rileyi* alone in kaolinite (control) on viability of the fungus are undertaken. At 10 DAI, surface coverage varied from 100 to 36.67, 100 to 33.33 and 100 to 0.0 per cent in formulation A, B and control, respectively from 0 to 300 days storage samples. The formulations A and B stored upto 150 days showed cent per cent surface coverage against the 71.70 per cent in control.Cfu count varied from 24.33 to $1.33x10^8$, 23.67 to $1.33x10^8$ and 23.33 to $0x10^8$ cfu/ml in formulation A, B and control, respectively from 0 to 300 days storage. Formulation A and B maintained their superiority over the control viability of the inoculums, while formulation without adjuvants recorded decline in viability. The reduction in cfu was rapid from 270 to 300 days. Considering surface coverage (%), biomass produced and viability (cfu/g) the *N.rileyi* 5%WP formulation A, B and control could be stored upto 10, 10 and 7 months, respectively for maximum cfu count of $1x10^8$/g for WP formulations as per norms.

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INTRODUCTION

Biological control is an important, effective ecofriendly and economical component of IPM in almost all important crops for the development of sustainable cropping systems. There is ample scope for microbial control of pests of cereals, pulses, vegetables and horticultural crops. So the insect viruses, bacteria, nematodes and fungi are emerging as potential bioagents (Pandey and Kanujia, 2005). At the end of 2001, there were approximately 195 registered biopesticides and 780 formulated products for the control of insects (38.10 %), bacteria (37.00 %), nematodes (15.7 %), fungi (4.7 %), viruses (2.85 %) and protozoa (2.14 %) (Anonymous, 2003). Among the pathogens used in microbial control, entomopathogenic fungi have played an important role in the history of insect pathology and microbial control

of insects. More than 750 species of entomopathogenic fungi, representing 100 genera are currently known (Hajek and Lager, 1994). The entomopathogenic fungi causing diseases to the insects are practically more significant as they are epizootic in nature. Also they have the advantage of ease of production and contact action which allow direct penetration of the host cuticle without ingestion.

Nomuraea rileyi (Farlow) Samson Moniliales, Moniliaceae is a fungus of cosmopolitan nature. *N.rileyi* infects mainly Lepidoptera, particularly economical important and polyphagous noctuid insect pests. *N. rileyi* is an entomopathogen causing natural mortality in as many as 51 Lepidopteran insects throughout the world (Lingappa and Patil, 2002). *N. rileyi* frequently cause epizootics in nature, is one promising because of its wide spread occurrence and relative abundance due to its wide host range which included many catterpiller pests. The pathogenicity of fungi towards insects has been mainly attributed to various hydrolytic enzymes such as chitinase, proteases and lipases. Progress of research on *N. rileyi* in India is slow though the results of the few studies have revealed that *N. rileyi* as a potential mycoinsecticide (Vimladevi *et al.*, 2002).

The formulation of the fungi still awaits a serious efforts in formulation technology. Exploring formulation of *N.rileyi* as a tool in the pest management of Lepidopteran pests is one of requisite mandate.The foregoing problem can largely be overcome by developing suitable formulations. The performance and shelf life can be improved by adding suitable adjuvants subsequently leading to growth, development and viability of the fungus that may act as nutrient, adhesive, UV protectants, wetting agents etc. Presently crude suspensions of the fungi with short shelf life of around one to two months for liquid and 5 to 6 months for WP are marketed. For developing wettable powder formulation, basic research on standardization of bioactive ingredient and suitable adjuvants is necessary before the formulation. There are many examples were fungi have been formulated with various adjuvants. The addition of nutrients to a spore spray did improve control of aphids and white flies in green house cucumber, compared with spores applied in water alone (Hall and Bell, 1961). Humectants prolong the viability of *Alternaria cassia* (Shabana *et al*., 1977).

In the present study wettable powder formulations of *N. rileyi* with various adjuvants and vegetable oil alongwith wettable powder formulation of *N. rileyi* without adjuvant were evaluated for their shelf-life under ambient conditions with an object to find out suitable viable formulations.

MATERIAL AND METHODS

Laboratory studies in Completely Randomized Design with three replications were carried out in the biological control laboratory, Deparment of Entomology, MPKV, Rahuri during 2009 to 2012.

Fungus culture:

The pure fungus culture of *N. rileyi* was made, available from isolates in Biocontrol Lab of Entomological centre, College of Agriculture, Pune.

Medium:

The medium used for multiplication and growth of the fungus was Sabourauds dextrose broth with yeast extract.

Shelf-life assessment of WP formulations at ambient conditions:

The samples of the WP formulations of *N. rileyi* with adjuvants alongwith WP formulation of *N. rileyi* without adjuvants were evaluated for their shelf-life. The sample was drawn each of the formulations at 30 days interval upto 10 months and evaluated for growth, development and viability of the entomopathogen immediately after each of the sampling at 28 ± 2^0C. One gram of the WP formulation was added to 40ml Sabouraud's dextrose broth medium in glass bottle and closed with cotton wool. The whole process was carried out in laminar flow cabinet. The observations on per cent surface coverage and biomass developed by fungus on 10^{th} days after inoculation were noted. The experimental data were subjected to statistical analysis.

The drawn samples were also tested for its colony forming unit (cfu) per gram simuntaneously with growth and biomass development. One gram of each of the product was drawn for monthly cfu count estimation by serial dilution technique for viability studies. The experiment was carried with 3 replications in Completely Randomized Design.

Testing cfu count of formulations:

The method suggested by Ming-Guang Feng *et al.*

(1990) was used. The autoclaved Sabouraud's dextrose agar with yeast extract (SDA) medium in petridishes, (100 mm diameter) was inoculated with the help of micropipette by releasing 1 ml *N.rileyi* suspension prepared in the distilled water in laminar flow cabinet. Other petridishes with the medium were prepared in similar manner and inoculated with various dilutions in the series (10^1 to 10^{10}cfu/ ml) at 27±1^{0}C. After 48 hrs from the 10 samples in each Petridishes the numbers of colonies/petrideshes were counted and cfu/ml was calculated. The period for shelf-life assessment of WP formulations of *N.rileyi* was from April to Dec. 2011 and Jan., Feb. 2012 when average maximum 33 ±1 and minimum 17 ±1^{0}Ctemperature. The average humidity for morning and evening were 91 and 38.

RESULTS AND DISCUSSION

Data on effect of storage on viability of developed *N. rileyi* 5 per cent WP formulation A ($N_{30}S_{1/1}$) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) (comprising adjuvants, fungus and kaolinite) and *N. rileyi* alone in kaolinite (control) on viability of the fungus are presented in Table 1. At 10 DAI, surface coverage varied from 100 to 36.67, 100 to 33.33 and 100 to 0.0 per cent in formulation A, B and control, respectively from 0 to 300 days storage samples. The formulations A and B stored upto 150 days showed cent per cent surface coverage against the 71.70 per cent in control. Significantly higher biomass (10.40 to 11.10g/40ml medium) was produced by the inoculums in formulation A and B (9.67 to 10.67g) stored upto 180 days as compared to that (1.90g) in formulation A and B (1.77g) stored for 300 days. The biomass produced in

Table 1: Effect of storage of *N.rileyi* 5 per cent WP formulations on growth, biomass and viability under ambient conditions

Tr. No.	Treatment Age in days	Surface coverage (%) at 10 DAI			Biomass g/40ml medium			Cfu/g (x 10^8)		
		Form. A**	Form. B***	Form. Control (*N.r.* alone)	Form. A	Form.B	Form. control (*N.r.* alone)	Form.A	Form. B	Form. control (*N.r.* alone)
1	00	100.0 (90.00)*	100.0 (90.00)	100.0 (90.00)	11.10	10.67	5.47	24.33	23.67	23.33
2	30	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	10.90	10.57	5.43	23.67	23.00	21.67
3	60	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	10.90	10.37	5.27	22.00	20.67	20.33
4	90	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	10.70	10.17	5.27	18.67	17.67	17.00
5	120	100.0 (90.00)	100.0 (90.00)	90.0 (71.56)	10.60	10.10	5.07	17.67	16.67	14.33
6	150	100.0 (90.00)	100.0 (90.00)	71.70 (57.86)	10.40	9.68	3.43	16.33	15.67	11.67
7	180	85.00 (67.21)	88.30 (70.00)	55.00 (47.87)	10.40	9.67	2.23	12.67	11.33	8.67
8	210	70.00 (56.79)	70.00 (56.79)	45.00 (42.13)	7.20	7.10	1.33	8.67	6.67	3.67
9	240	55.00 (47.87)	56.67 (48.79)	30.00 (33.21)	5.90	5.77	0.30	5.67	4.33	0.67
10	270	43.33 (41.15)	50.00 (45.00)	15.00 (22.79)	5.20	5.10	0.0	3.67	2.33	0.00
11	300	36.67 (37.29)	33.33 (35.24)	0.00 (0.0)	1.90	1.77	0.0	1.33	1.33	0.00
	S.E. ±	1.78	1.14	1.41	0.14	0.08	0.07	0.33	0.33	0.60
	C.D.(P=0.05)	5.34	3.41	4.23	0.42	0.24	0.21	0.99	0.99	1.79

*Figures in parentheses are arcsin values DAI= Days after inoculation **A= (N_{30} $S_{1/1}$) ***B = ($N_{30}T_{1/2}$ $G_{2/1}$ $H_{1/1}$)

control was 5.47g in fresh sample against no biomass production after 270 and 300 days storage.

Cfu count varied from 24.33 to $1.33x10^8$, 23.67 to $1.33x10^8$ and 23.33 to $0x10^8$ cfu/ml in formulation A, B and control, respectively from 0 to 300 days storage (Fig.1). At 0 day age, formulation A recorded highest ($24.33x10^8$ ml) cfu count. It was at par with formulation stored at 30 days. In case of formulation B samples stored at 0 days recorded maximum ($23.67x10^8$ cfu/ml) viability. However, it was at par with the viability (23.10^8cfu/ml) at 30 days of storage. Formulation A and B maintained their superiority over the control viability of the inoculums, while formulation without adjuvants recorded decline in viability. The reduction in cfu was rapid from 270 to 300 days. There was complete loss of viability of the inoculums in control at 270 days of storage.

Considering surface coverage (%), biomass produced and viability (cfu/g) the *N.rileyi* 5%WP formulation A, B and control could be stored upto 10, 10 and 7 months, respectively for maximum cfu count of $1x10^8$/g for WP formulations as per norms of Central Insecticide Board and Registration Committee, Faridabad, Haryana.

The present findings are in conformity with those reported by Chaudhari (2010) that *N.rileyi* formulated as wettable powder was better in shelf-life with $5.50x10^6$cfu/ml after 180 days of storage at 27^0C. The formulation of *N.rileyi* inoculated after 15 months resulted in growth and development of the mycoagent. Nahar *et al*. (2004) recorded greater than 80 per cent conidial germination of *N. rileyi* after 36 hrs of storage in sunflower oil, diesel and sunflower oil mixture 7:3 and tween-80. Ramegowda (2005) reported that the rice flour, talc and sorghum flour was the best carrier for *N. rileyi*. The vability of conidia after one year of storage was 22.21 per cent in refrigerated condition while it was 15.64 per cent at ambient condition. But the literature on shelf-life of wettable powder and sprayable formulation was not available.

Silva *et al*. (1993) found that the dead larvae of velvet bean caterpillar *Anticarsia gemmatalis* covered with sopres of *N.rileyi* were kept their viability and pathogenicity for upto 6 years of storage. The highest conidial viability after 45 days was recorded by Hidalgo *et al*. (1998) with the conidia in fat formulation. It was (84.70% at 25^0C and 91.30% at 4^0C). Faria *et al*. (1999) experienced decrease in germination of conidia in 16 to 84 months old of *N. rileyi*, *M. anisopliae* and *B. bassiana* culture stored in liquid nitrogen. Greater conidial viability and better survivability of talc based formulation

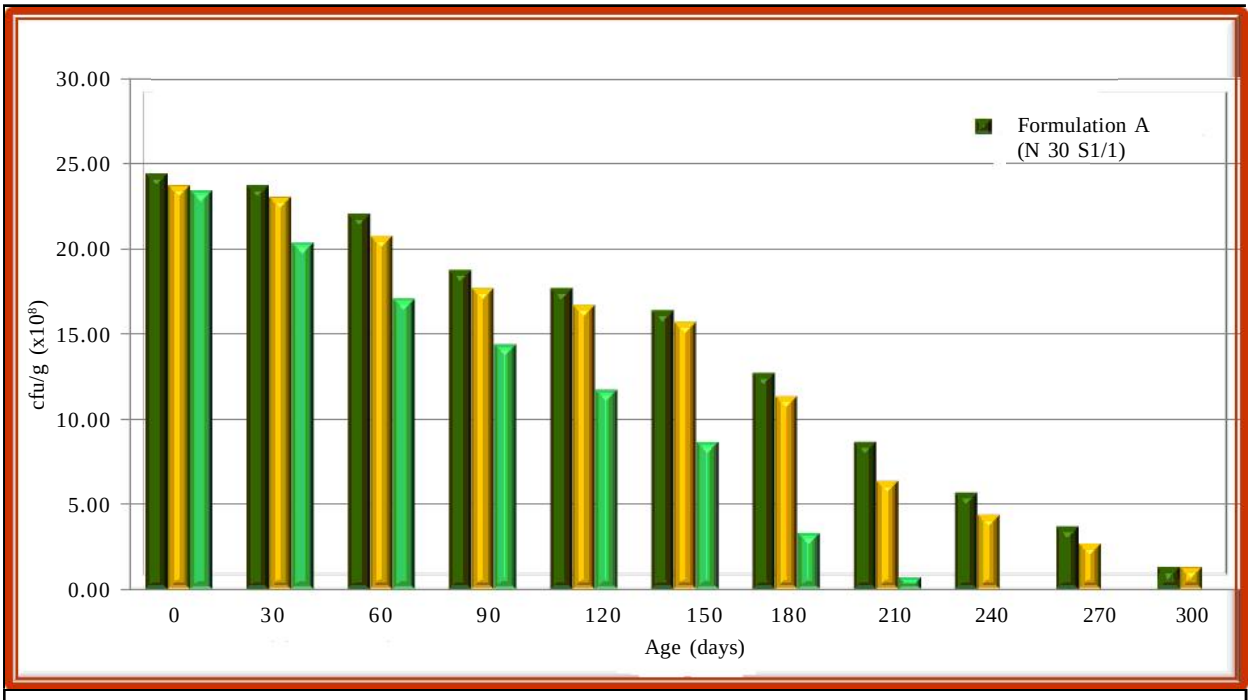

Fig. 1: Shelf-life assessment of *N.rileyi* 5 per cent WP formulations on the basis of cfu/g

of *B. bassiana* was obtained in upto 11 months where average temperature ranged from 5 to 25^{0}C. Alves *et al*. (2001) reported the better viability of *M. anisopliae* in medium term storage of 40 weeks at 10^{0}C and 27^{0}C for oil based formulation. Peanut oil maintained viability of conidia greater than 90 per cent.

REFERENCES:

Alves, R.T., Oliveira, M.A.S., Batenan, R.P., Prior, C. and Leather, S.R. (2001). Spreading and effectiveness of an emulsifiable adjuvant oil fungal formulation. *Bulletin de Pesquisa-e Desenvolvimento Embrapa Cerrados*, **6**: 14.

Anonymous (2003). Biopesticide, *Agrolook*, **4**(1) : 4-5.

Chaudhari, C.S. (2010). Isolation, production and evaluation of *Nomuraea rileyi* (Farlow) Samson against *Spodopteralitura* (Fabricius). Ph. D. Thesis, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmed Nagar, M.S.(India).

Faria, M.R., Martins, I., Mello, R., Tigano, M.S. and Faria, M.R. (1999). Entomopathogenic fungal (hyphomycetes) collection: assessment of conidial viability. Colecao de fungos entomopatogenicos (Hyphomycetes): avaliacao da viabilidade conidial. *Pesquisa Agropecuaria Brasileira*, **34** (8) : 1497-1503.

Hajek, A.E. and Lager, R.J. (1994). Interactions between fungal pathogen and insect hosts. *Annu.Rev. Entomol.*, **39**: 293-322.

Hall, I.M. and Bell, I.V. (1961). Further studies on the effect of temperature on the growth of some entomophthoraceous fungi. *J. Insect Pathol.*, **3**: 289-296.

Hidalgo, E., Moore, D. and Patourel, G.L. (1998). The effect of different formulations of *Beauveria bassiana* on *Sitophilus zeamais*in stored maize. *J. Stored Prod. Res.*, **34** (2-3) : 171-179.

Lingappa, S. and Patil, R.K. (2002). *Nomuraea rileyi –A Potential Mycoinsecticide*.University of Agricultural Sciences, Dharwad, 30 pp.

Ming Guang Feng, L.R., Johnso, J.B. and Kish, L.P. (1990). The virulence of *Verticillium lecanii* and an aphid derived isolate of *Beauveria bassiana* for six species of cereal infecting aphids. *J. Environ. Entom.*, **19** (3): 815-820.

Nahar, P., Yadav, P., Kulye, M., Hadapad, A., Hassani, M., Tuor, U., Keller, S.,Chandele, A.G., Thomas, B. and Deshpande, M.V. (2004). Evaluation of indigenous fungal isolates, *Metarhiziumanisopliae* M 34412, *Beauveria bassiana* B3301 and *Nomuraea rileyi* N 812 for the control of *Helicover paarmigera* (Hub.) in pigeonpea field. *J. Biological Control*, **18** (1) : 1-8.

Pandey, K.A. and Kanujia, K. (2005). Effect of different grain media on sporulation, germination and virulence of *Beauveria bassiana* (Balsamo) Vuillemin against *Spodoptera* (Fab.) larvae. *J. Biological Control*, **19** : 129-133.

Ramegowda, G.K. (2005). Aerobiology, Epizootiology and Utilization of *Nomuraea rileyi* (Farlow) Samson. Ph. D. Thesis, University of Agricultural Sciences, Dharwad, Karnataka (India).

Shabana,YM., Charudattan, R., Devalerio, J.T. and Elawakil, M.A. (1977). An evaluation of Hydrophilic polimars for formulating the bioherbicide agents *Aternaria cassia* and *A. eichhorniae*. *Weed Technology*, **11** : 212-220.

Silva, L.D.A., Silva, R.F.P., Heineck, M.A. and Silva, L. (1993). Parasites, predators anddiseases of larvae of *Diacrisia oblique* (Lepidoptera: Arctidae) on soybean, *Current Sci.*, **44**: 481-482.

Vimladevi, P.S.V., Anitha Chowdary, Prasad, Y.G. and Chowdary, A. (2002). Compatibility of entomopathogenic fungus *Nomuraea rileyi* with commonly used pesticides. *Indian J. Agric. Sci.*, **72** (6) : 370-372.