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Studies on the effect of storage on viability of wettable powder (WP) formulations of the entomopathogenic fungi *Metarhizium anisopliae* (Metschinikoff) Sorokin

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ABSTRACT

Studies on the effect of storage of developed *Metarhizium anisopliae* (Metschnikoff) Sorokin 5 per cent WP formulation A1($M_{30}S_{1/1}C_{1/2}$) and B1($M_{30}S_{1/1}H_{1/1}$) (comprising adjuvants, fungus and kaolinite) and *M.anisopliae* alone in kaolinite (control) on viability of the fungus are undertaken.At 10 DAI, surface coverage by the fungus varied from 100 to 45.0, 100 to 46.67 and 100 to 0.0 per cent in formulation A1, B1 and control, respectively, when stored for 0 to 300 days. The samples stored upto 150 days showed cent per cent surface coverage in formulation A1 and B1 except control. Significantly higher biomass (9.10 to10.03 g/40ml medium) was produced in samples of formulation A1 and B1 (8.77 to 10.07g) stored upto 210 days as compared to that (3.40g) in formulation A1 and B1 (3.70g) stored for 300 days. The biomass in control was 6.27g in fresh sample against no biomass in sample stored for 300 days. The viability varied from 31.33 to $5.33x10^8$, 30.67 to $6.33x10^8$ and 30.67 to $0.0x10^8$ cfu/ml in formulation A1, B1 and control, respectively, from 0 to 300 days storage. Considering surface coverage (%), biomass produced and viability (cfu/g) the *M. anisopliae* 5 pe cent WP formulation A1, B1 and control could be stored upto 10, 10 and 6 months, respectively for the minimum cfu count of $1x10^8$/g for WP formulations as per norms.

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INTRODUCTION

The green muscardine fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, Moniliales, Moniliaceae is another potential entomopathogenic candidates for biological control. Metschniko (1879) was first to isolate fungus *M. anisopliae* from the larvae of

grain weevil and also first to demonstrate entomopathogenic nature of the fungus against chrysomelid, curculonid and scarabaeid beetles. *M. anisopliae* capable of infecting more than 100 different insect pests belonging to a variety of insect orders *viz*., Orthoptera (Grasshopper and Cockroaches), Homoptera (Spittle bug, *Nilaparvatalugens*) and Lepidoptera (*Helicoverpa armigera, S. litura*) (McCoy *et al*.,1988). Gopalkrishnan and Narayan (1987), reported 80-100 per cent mortality of *H.armigera* by *M.anisopliae*. It also used for the control of *Eariasinsuana* (Aly and Rashad, 1997), cabbage semilooper (Wickramatileke *et al*., 2000), *Marucavitrata* (Ekesi *et al*., 2002) diamond back moth (Silva *et al*., 2003). This fungus is also used for control of sucking pests of important field crops. Virulence of *M. anisopliae* against mustard aphid (Pandey and Kanaujia, 2004), *Aphis gossypii* and *Myzuspersicae* (Loureiro and Moino Junior, 2006) and *Maconellicoccus hirsutus* (Ujjan and SaleemShahzad, 2007) has been documented. *M. anisopliae* is characterized as green muscardine fungus due to green colour of the sporulating colonies. It forms a mycelia mat on cuticle of insects. The infective unit is conidia or blastospores which germinate and forms short germ tube bearing appresoria with infective peg attach to cuticle. The infective peg penetrates in layer of integument by enzymatic dissolution of chitin and protein. It reaches the haemocoel and internal organs and insect is filled with fungus. The death of insects occurs due to obliteration of tissues, also production of toxins (destruxin A,B,C,D,E) and proteolytic enzymes secreted by the fungus. Infected insects show symptoms like loss of appetite, decreased irritability, general or partial paralysis, loss of mobility, discolouration and mummification.

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.

The formulation of the fungi still awaits serious efforts in formulation technology. Exploring formulation of *M. anisopliae* 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 *M. anisopliae* with various adjuvants and vegetable oil alongwith wettable powder formulation of *M. anisopliae* without adjuvant were evaluated for their storage on viability 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, Dept. of Entomology, MPKV, Rahuri during 2009 to 2012.The medium used for multiplication and growth of the fungus was Sabourauds dextrose broth with yeast extract. The pure fungus culture of *M. anisopliae* was made, available from isolates in Biocontrol Laboratory, Department of Entomology, MPKV, Rahuri.

Shelf-life assessment of WP formulations at ambient conditions:

The samples of the WP formulations of *M. anisopliae* with adjuvants along with WP formulation of *M. anisopliae* 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^{0}C. 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 10th 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.

Testing cfu count of formulation:

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 *M. anisopliae* 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 (storage) assessment of WP formulations of *M. anisopliae* was from April to Dec. 2011 and Jan., Feb. 2012 when average maximum

Table 1 : Effect of storage of *M.anisopliae* 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. A1**	Form. B1***	Form. Control (*M.a.* alone)	Form. A1**	Form. B1***	Form. Control (*M.a.* alone)	Form. A1**	Form. B1***	Form. Control (*M.a.* alone)
1	00	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)	10.03	10.07	6.27	31.33	30.67	30.67
2	30	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	9.87	9.97	6.20	30.33	29.67	29.33
3	60	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	9.67	9.77	6.00	29.33	28.33	25.67
4	90	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	9.70	9.80	5.90	27.33	26.33	23.33
5	120	100.00 (90.00)	100.00 (90.00)	88.33 (70.00)	9.63	9.73	5.93	25.33	24.33	20.33
6	150	100.00 (90.00)	100.00 (90.00)	86.67 (68.61)	9.40	9.50	5.77	24.33	22.67	18.67
7	180	95.00 (73.08)	100.00 (90.00)	55.00 (47.87)	9.20	9.30	2.97	22.33	21.33	6.33
8	210	91.67 (73.26)	96.67 (79.53)	28.33 (32.14)	9.10	8.77	2.13	19.33	18.33	0.67
9	240	73.33 (58.89)	85.00 (67.21)	0.0 (0.00)	6.83	6.97	0.0	17.00	16.33	0.00
10	270	55.00 (47.87)	60.00 (50.77)	0.00 (0.00)	4.60	5.20	0.00	12.00	13.00	0.00
11	300	45.00 (42.13)	46.67 (43.11)	0.00 (0.00)	3.40	3.70	0.00	5.67	6.67	0.00
	S.E ±	2.94	1.64	1.20	0.15	0.17	0.07	0.26	0.26	0.33
	C.D.(P=0.05)	8.83	4.91	3.59	0.45	0.50	0.22	0.78	0.76	0.99

Figures in parentheses are arcsin values, DAI = Days after inoculation , *M.a = Metarhizium anisopliae*, **A1= ($M_{30}S_{1/1}C_{1/2}$) *** B1= ($M_{30}S_{1/1}H_{1/1}$)

33 ±1 and minimum 17 ±1^{0}C temperature. The average humidity for morning and evening were 91 and 38.

RESULTS AND DISCUSSION

Data on effect of storage on viability of developed *M. anisopliae* 5 per cent WP formulation A1 ($M_{30}S_{1/1}C_{1/2}$) and B1 ($M_{30}S_{1/1}H_{1/1}$) (comprising adjuvants, fungus and kaolinite) and *M. anisopliae* alone in kaolinite (control) on viability of the fungus are presented in Table 1. At 10 DAI, surface coverage by the fungus varied from 100 to 45.0, 100 to 46.67 and 100 to 0.0 per cent in formulation A1, B1 and control, respectively, when stored for 0 to 300 days. The samples stored upto 150 days showed cent per cent surface coverage in formulation A1 and B1 except control. Significantly higher biomass (9.10 to10.03 g/40ml medium) was produced in samples of formulation A1 and B1 (8.77 to 10.07g) stored upto 210 days as compared to that (3.40g) in formulation A1 and B1 (3.70g) stored for 300 days. The biomass in control was 6.27g in fresh sample against no biomass in sample stored for 300 days.

The viability varied from 31.33 to 5.33x10^8, 30.67 to 6.33x10^8 and 30.67 to 0.0x10^8 cfu/ml in formulation A1, B1 and control, respectively, from 0 to 300 days storage (Fig. 1). The formulation A1 registered substantially highest (31.33x10^8/ml) cfu count. Formulation A1 and B1 maintained its superiority for the viability (5.67 to 6.67x10^8 cfu/ml) upto 300 days, while it declined substantially (6.33 to 0x10^{7}cfu/ml) in control formulation after 180 days of storage.

Considering surface coverage (%), biomass produced and viability (cfu/g) the *M. anisopliae* 5 per cent WP formulation A1, B1 and control could be stored upto 10, 10 and 6 months, respectively for the minimum 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 corroboration with those reported by Daust *et al*. (1983) the conidia stored in 20 per cent dust retained high viabilities over 12 months. Moore *et al*. (1993) reported that dried conidia stored as powder retained germination level of 95 per cent at 10-14^{0}C but the germination was upto 27 per cent at 28 to 32^{0}C. Batt (2003) concluded that the fungal conidia of *Metarhizium anisopliae* formulated in invert emulsion (water in oil formulation) remained viable for 30.8 months with 50 per cent reduction in conidial viability after 4 to 6 months at 20 $\pm$ 1^{0}C.

Rachappa *et al*. (2007) observed that storing the conidia of *M. anisopliae* under refrigeration provided

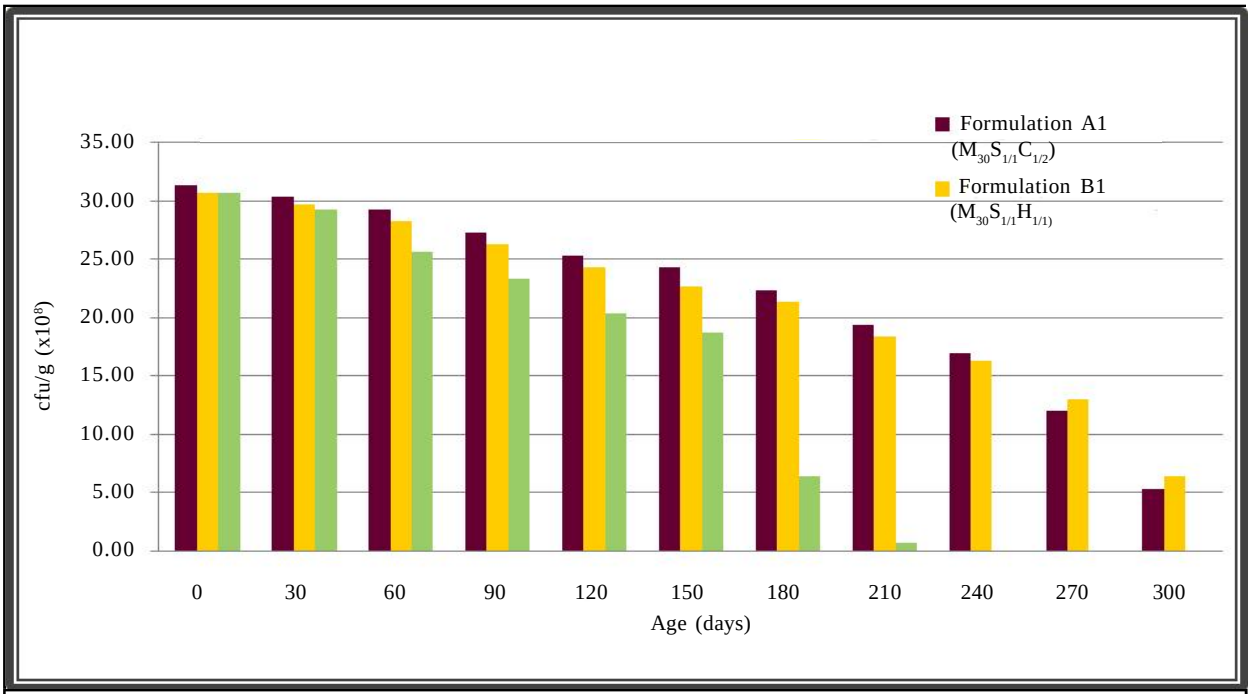

Fig. 1: Viability of *M. anisopliae* 5 per cent WP formulations on the basis of cfu/g

longer life compared to ambient temperature. After 180 days of the storage, the cfu reduced from 250 to 176.50x10^6/g. The cfu count was least affected by kaolinite. The wettable powder formulation, attapulgite and kaolinite retained viability of conidia (33.5 and 31.9%), respectively, after one year. It was followed by sorghum flour (27.9%) and talc (26.90%).

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