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GELATIN CAPSULES TO ENHANCE THE EFFICACY OF *LECANICILLIUM LECANII* AGAINST *MYZUS PERSICAE*

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ABSTRACT

Evaluation of gelatin capsule-based formulations of indigenous isolate of *Lecanicillium lecanii* (Zimm.) Zare and Games was carried out at the College of Horticulture, Bagalkot during 2018-19. The gelatine capsules filled with different ratios of spore and talc powder (1:0, 1:1, 1:2 and 1:3) were evaluated in the laboratory for their efficacy against aphid *Myzus persicae*. The results indicate that equal quantity of spore and talc powder showed better efficacy than other two combinations and next best to spore alone. The gelatine capsules containing the spore powder alone though proved better with maximum mortality when it was used fresh and immediately after their formulation; the efficacy decreased drastically after 30 days after storage. Evaluation of their storability, under varying storage conditions revealed that refrigerated condition is the best in maintaining the virulence of the spore after four months of storage period. The pot culture experiment revealed that significantly more mortality of aphid was obtained with gelatine capsules formulations of local isolate of *L. lecanii* as compared to commercial formulation.

Key words: *Lecanicillium lecanii*, capsule formulations, gelatin, virulence, spore, talc powder, *Myzus persicae*, mortality, storage, period, refrigeration, pot culture

The development of insecticide resistance and their effects on the human kind have forced the scientists for the development of biological based IPM strategies. Biological control is receiving a new thrust and it has become an integral component of IPM in several crops. Due to its host specificity, protect natural enemies, ease in production, farmers and producer's friendly, ecofriendly and better compatibility with other methods, it can be a sustainable option in IPM. Among the entomopathogens, the entomopathogenic fungi (EPF) are currently used in large scale for the management of crop pests. These are having diverse advantages like wider host range, ease of mass production and relatively good environmental tolerance over other entomopathogens. In addition, these occur in frequent epizootics in nature both on chewing and sucking insect pests. Unlike, other entomopathogens such as bacteria and viruses which require ingestion of contaminated food, mere contact of the host is sufficient for the EPF to cause disease. A huge number of myco-insecticides have reached the market and millions of hectares are being treated annually with EPF globally. Though, the efficiency of most of the formulations developed have proved good under laboratory conditions and but are proved ineffective in field trials. The inefficiency in field condition is mainly attributed to loosing virulence by ultraviolet (UV) rays, short shelf life and harsh environmental conditions. Hence, keeping this in view, improving the efficacy is attempted in this study with capsule-based formulations and their evaluation under laboratory and pot culture experiments.

MATERIALS AND METHODS

The study was conducted at the College of Horticulture, Bagalkot during 2018-19 to evaluate the capsule-based formulations of indigenous isolate of *L. lecanii* against *M. persicae* under both laboratory and pot culture experiments. The pure slant cultures of identified virulent local isolate of *L. lecanii* (LL-2) was maintained by subculturing on Sauboured Maltose Agar (SMA) medium and their virulence was retained by inoculation and reisolating on their natural hosts. The spore powder of *L. lecanii* was prepared by growing it on broken grains of rice. About 50 g of broken grains was taken in 250 ml flask containing 50 ml of distilled water and autoclaved at 121˚C for 30 min. After sterilization, broken grains were cooled under room temperature. The cooled flasks containing the broken grains were inoculated with pure culture of *L. lecanii* under aseptic condition in laminar air flow chamber. The inoculated flasks were incubated at room temperature for 15 days under dark condition to harvest the spores. Later, spores were dried and ground for 30 sec to make it as fine powder. Powder formulations were developed by mixing spore powder of *L. lecanii* obtained from rice

grains with talc powder in different ratios (1:1, 1:2 and 1:3) and made into fine dust by grinding for 30 sec to facilitate for easy packing into empty gelatine capsules (2.0x 0.5 cm dia) to hold 2 g powder, obtained from M/s. Amazon Pvt Ltd. Wild population of the test insect, cabbage aphid was collected from vegetable fields of COH, Bagalkot. Its culture was maintained on cabbage seedlings grown under nethouse condition. When plants were 15-20 days old, the field collected aphids were released and the culture of aphids was maintained till completion of the experiment.

The efficacy of gelatine capsules of *L. lecanii* against *M. persicae* was evaluated with leaf dip bioassay. Cabbage leaf disc of 9 cm dia was dipped in gelatine capsule dissolved solution of *L. lecanii* for two min and then air dried and placed onto petridish (10cm dia.). About twenty apterous *M. persicae* were released on to petridish and allowed to feed. The experiment was conducted with five treatments - spore + talc powder (1:1), spore + talc powder (1:2), spore + talc powder (1:3), spore powder alone and control) with $2x10^8$cfu/ ml dosage and three replications at room temperature. Observations on number of moribund and dead aphids recorded at three, five, seven and ten days after treatment (DAT) were made and % mortality of aphid was computed. The spore viability was studied for best gelatine capsule formulations containing spore + talc powder (1:1) and spore powder alone of *L. lecanii* at monthly intervals up to four months. The formulations were stored at different storage conditions- refrigerated condition (4 ^oC), mud pot filled with wet sand (10- 15 ^oC) and ambient condition (28 ^oC)). The bioefficacy study was conducted at monthly intervals up to four months against *M. persicae* with seven replications. The experiment to evaluate the efficacy of capsule formulations of *L. lecanii* against *M. persicae* was conducted on potted cabbage plants with five treatments and four replications under CRD design. The treatments were imposed after uniform natural infestation of *M. persicae* using the hand operated sprayer. Pretreatment observations one day earlier and subsequent observations on one, three, five, seven, ten and fifteen days after treatment were made on number of *M. persicae*/ cm^2 leaf. Data on aphid counts were subjected to square root transformation for reliable analysis and treatments means were compared by Duncan's Multiple Range Test (DMRT, p=0.05%).

RESULTS AND DISCUSSION

The results reveal that the mortality of *M. persicae* increased with advancement of 10 days exposure period. Gelatin capsule formulation of *L. lecanii* spore alone recorded significantly high mortality (90.00%) which was followed by spores mixed with talc powder in the ratio of 1:1 (82.50%). No mortality of aphid was recorded in untreated control (Table 1). The mortality of nymphs of *M. persicae* was noticed after three days of treatment, and this reached 100% after five to seven days after treatments. Similarly, Ei-Sinary and Quesada-Moraga (2006) observed that the efficacy of the EPF began clearly after 48 hr after inoculation and during which hyphae penetrated the integument, epithelial and epidermal cells. After 72 hr, fungi damage the fat tissues and finally, mortality reached 100% after 96 hr.

Table 1. Efficacy of gelatine capsule formulations of *L. lecanii* against *M. persicae* under laboratory conditions

Treatments	Formulations	Cumulative mortality (%)				
		3 DAT	5 DAT	7 DAT	10 DAS	Mean
T_1	Spore + talc powder (1:1)	65.00 (53.73)^b	77.50 (61.68)^b	87.50 (69.30)^b	100 (90.00)^a	82.50 (65.27)^b
T_2	Spore + talc powder (1:2)	47.50 (43.57)^c	52.50 (46.43)^c	67.50 (55.24)^c	75.00 (60.00)^b	60.62 (51.13)^c
T_3	Spore + talc powder (1:3)	32.50 (34.76)^d	37.50 (37.76)^d	47.50 (43.57)^d	57.50 (49.31)^c	43.75 (41.40)^d
T_4	Spore powder alone	75.00 (60.00)^a	85.00 (67.21)^a	100.00 (90.00)^a	100.00 (90.00)^a	90.00 (71.56)^a
T_5	Control	0.00 (0.29)^e	0.00 (0.29)^e	0.00 (0.29)^e	0.00 (0.29)^d	0.00 (0.29)^e
S. Em.±		0.26	0.24	0.59	0.32	0.36
CD (p=0.05)		0.81	0.74	0.19	0.12	0.47
CV (%)		3.12	2.73	2.05	2.5	2.6

DAT-Days After Treatment; Figures in the parentheses arcsine transformed values; In a column, means followed by same alphabet (s) do not differ significantly by DMRT (p=0.05); Dosage $2x10^8$ cfu/ ml

Among the eight wettable powder (WP) formulations evaluated, the crude WP registered the least LC_{50} value of 80.09 x 10^3 conidia ml^{-1} followed by talc-based WP and rice flour. Among the WP formulations crude formulation recorded 82% mortality followed by talc and rice flour. Similarly, Mallikarjuna et al. (2010) formulated *M. rileyi* as WP using different carrier materials viz*.,* bentonite + glucose (7:1), bentonite + sucrose (7:1), talc + glucose (7:1) and talc + sucrose (7:1); these were evaluated against *Spodoptera litura* and *Helicoverpa armigera* resulting in mortalities of 72-87% in the former and 66- 88% in the latter. Hence, the present study indicated that efficacy increases with incorporating the spores in the gelatine capsules with equal proportion of talc. This can be attributed to the retention of moisture inside the capsule.

The pot culture experiment revealed reduction in counts of *M. persicae* with capusule formulation over commercial formulations of *L. lecanii*; maximum reduction being with standard check, chlorantraniliprole 18.5 SC followed by NSKE 5%. Six isolates of *L. lecanii* screened against *A. craccivora* revealed that, *L. lecanii*-3 as most virulent EPF isolate 1x10^8 cfu/ ml (Table 2). Naik and Shekharappa (2009) evaluated fungal formulations against sucking pests of okra, and oil based formulations were observed to be superior in reducing the pests giving increased fruit yield. The study conducted by Gulsar and Gopalakrishnan (2012) on management of *Paracoccous marginatus* in potted papaya plant revealed that the oil-based formulations of local isolate of *L. lecanii* was more efficient compared to talc-based formulations. The efficacy of *B. bassiana* and *L. lecanii* against whitefly under polyhouse condition showed 69.64 to 85.65% mortality. Rashmi (2018) conducted the semi-field experiment to evaluate the effectiveness of oil formulations of *M. rileyi* against *S. litura* damaging cabbage. Among the tested formulations of *M. rileyi*, the groundnut oil formulation was efficient (31.73%) as compared to the rest of the formulations. Similarly, Varun (2018) evaluated the effectiveness of *L. lecanii* oil formulations against *M. persicae*. Among the formulations, sesamum oil was superior. The effectiveness of formulated products depends mainly on external factors. Thus, there was a significant difference in suppressing the *M. persicae* in tested gelatine capsules over commercial formulation available in the market.

The gelatine capsule formulations stored under refrigerator condition recorded the significantly more mortality with >30% in all the formulations even at

Table 2. Evaluation of selected capsule formulations of *L. lecanii* against *M. persicae* under pot culture experiment

Tr. No.	Treatments	Dosage	I Spray						II Spray					
			Number of aphids / 5 cm^2 leaf area						Number of aphids / 5 cm^2 leaf area					
			DBS	3 DAS	5 DAS	7 DAS	10 DAS	15 DAS	Mean	DBS	3 DAS	5 DAS	7 DAS	10 DAS	15 DAS	Mean
T_1	Gelatine capsule formulation	2 capsules/1	22.21 (4.71)	16.85 (4.16)^c	15.85 (4.04)^c	16.42 (4.05)^b	19.57 (4.36)^b	22.28 (4.71)^a	18.19	15.74 (3.96)	12.14 (3.55)^e	11.42 (3.45)^e	12.14 (3.55)^b	12.71 (3.63)^e	13.78 (3.77)^a	12.43
T_2	Chlorantraniliprole 18.5 SC	0.2ml/1	23.42 (4.83)	6.28 (2.60)^e	3.85 (2.08)^e	0.28 (0.52)^d	2.28 (1.66)^e	4.14 (4.09)^d	3.36	15.57 (3.94)	4.53 (3.28)^d	2.71 (1.79)^e	0.85 (1.16)^d	2.64 (1.77)^e	4.14 (2.15)^b	2.97
T_3	NSKE	5%	22.85 (4.78)	14.42 (3.86)^d	14.00 (3.80)^d	13.28 (3.64)^e	14.57 (3.86)^d	16.28 (3.36)^e	14.51	15.64 (3.95)	10.28 (2.24)^e	9.42 (3.14)^d	8.85 (3.05)^e	9.56 (3.17)^d	14.67 (3.86)^a	10.55
T_4	Commercial formulation of *L. lecanii* @ 2 X 10^8 cfu/ ml	2 g/ 1	20.81 (4.56)	17.57 (4.25)^b	16.42 (4.11)^b	15.85 (3.98)^b	17.28 (4.21)^c	18.42 (4.34)^c	17.10	15.57 (3.94)	13.28 (3.71)^b	12.53 (3.60)^b	11.85 (3.51)^b	13.42 (3.73)^b	14.56 (3.88)^a	13.13
T_5	Control	-	22.22 (4.71)	21.22 (4.66)^a	22.65 (4.81)^a	22.5 (4.74)^a	21.50 (4.69)^a	21.60 (4.70)^b	21.89	15.64 (3.95)	15.71 (4.01)^a	15.03 (3.94)^a	14.42 (3.86)^a	14.99 (3.93)^a	15.24 (3.96)^a	15.08
S. Em.±				0.022	0.022	0.038	0.022	0.015	-		0.038	0.031	0.038	0.027	0.122	-
C.D.(5%)				NS	0.024	0.023	0.076	0.028	0.016	-	NS	0.039	0.038	0.066	0.029	0.374	-
C.V. (%)					0.386	0.394	1.527	0.435	0.275	-		0.766	0.682	1.340	0.544	7.059	-

DBS- Days before spray; DAS- Days After Spraying; Figures in parentheses square root transformed value. In a column, means followed by same alphabet (s) do not differ significantly by DMRT (p=0.05)

120 days of storage. Similarly, the formulations stored under mud pot filled with wet sand also recorded the desirable mortality of *M. persicae.* The formulation stored under room temperature gave less mortality. The reason for the higher mortality of *M. persicae* in above storage condition might be due to low temperature which retained the viability of fungal spore and lead to maximum mortality. Among the capsule formulations of *L. lecanii*, the ones filled with spore and talc resulted in maximum mortality of *M. persicae* for a prolonged period of storage. It might be attributed to the talc which served a good carbon source (35-100 %) and mineral composition. While spore powder alone maintained the superiority only short storage period (<30 days). The least mortality was observed in the formulations stored under room temperature during storage period. These results are in confirmation with the studies conducted by Simkova (2009) that conidial germination of *B. bassiana* was maximum (97.33 %) at 4˚C after 90 days of treatment compared to 22˚C. Viability of *M. anisopliae* was found better at 10˚C than 27˚C for all tested oil-based formulations when stored for 40 weeks. Viability of *M. rileyi* conidia was about 50% even after six months of storage as reported by Swetha (2011) and Ramegowda (2005) had reported 82.47% conidial viability of *M. rileyi* formulations stored for 180 days in refrigerated condition compared to only 63.23% per cent under room temperature with talc. The present studies revealed that, carrier material, temperature and storage duration had significant effect on *M. persicae* mortality. Virulence decreased over storage time in all the formulations across different storage conditions.

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