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Development and evaluation of formulations of *Lecanicillium lecanii* against *Myzus persicae*

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ABSTRACT: Evaluation of oil and talc-based formulations of an indigenous isolate of *Lecanicillium lecanii* (Zimm.) Zare and Games was performed at Kittur Rani Chanamma College of Horticulture (KRCCH), Arabhavi. Different formulations of *Lecanicillium lecanii* were developed and tested against cabbage aphid, *Myzus persicae* (Sulzer) under laboratory and field conditions. Among the various formulations screened, ground nut and sesamum oil-based formulations recorded the highest mean mortality of 96.66 per cent, followed by sunflower oil (95%), coconut oil (94.15%) and mustard oil (93.33%). However, most formulations of *L. lecanii* developed recorded more than 81.50% mean mortality of *M. persicae* under laboratory conditions. Further, based on the efficacy under laboratory conditions, the four best formulations of *L. lecanii* were evaluated against *M. persicae* under field conditions. Among the selected formulations, the maximum per cent reduction *of M. persicae* was recorded in sesamum oil, resulted in 15.14 and 18.60 per cent reductions over control after the first and second sprays.

Keywords: *Lecanicillium lecanii*, *Myzus persicae,* Sesamum oil, Ground nut oil

INTRODUCTION

In agroecosystems, indiscriminate usage of chemical insecticides disturbed the ecosystem's sustainability due to the mortality of predators, parasitoids and pollinators. Therefore, the farmers are inclined towards organic insect pest management (personal communication, while survey), which is valid for the other parts of the country and the world (Parvatha Reddy, 2008). There is a need for ecologically, economically and socially acceptable technology for insect pest management. The biological control of insect pests is a viable partial alternative to insecticides in Integrated Pests Management (IPM). Among the biological control agents, microbial bioagents are considered the best, of which entomopathogenic fungi is the first organism to cause disease in insect and are most important for insect pest management (Fan *et al.,* 2007).

Lecanicillium lecanii is an important entomopathogenic fungus that suppresses sucking insect pests like aphids, leafhoppers, thrips, whitefly, scales, mealy bugs and mites (Goettel *et al.,* 2008; Shinde *et al.*, 2010; Pillai and Visalakshy, 2017; Mani *et al.,* 2016). Cabbage is one of the important crops among the cruciferous vegetables in India. Several insect pests attack this crop, *viz.,* cutworm, diamondback moth, cabbage looper, cabbage leaf webber, tobacco leaf eating caterpillar and green peach aphid. Among the insect pests of cabbage, the cabbage aphid, *Myzus persicae* (Sulzer) is an important sucking pest, which not only reduces the marketable yield but also reduces consumer preference by producing honeydew (Akbar *et al.*, 2010). To keep the menace of the cabbage aphids under control during the seedling and head development stage, the farmers are employing insecticides that result in pesticide residue, pollution to the environment and negatively affecting the non-target organism. Therefore, an attempt was initiated to manage the cabbage aphid with bio-control agents by developing and evaluating formulations of the native isolate of *L. Lecanii*.

MATERIAL AND METHODS

Development of formulations of *Lecanicillium lecanii*

Rearing of cabbage aphid

The wild population of the test insect, the cabbage aphid, *Myzus persicae*, was collected from Kittur Rani Chanamma College of Horticulture (KRCCH) vegetable fields, Arabhavi, during which year. The culture of the test insect was maintained on cabbage seedlings under net house conditions in pots. When plants were 15-20 days old, the field-collected aphids were released,

and the culture of aphids was maintained until all the experiments were completed (Gokak *et al.*, 2017). All the laboratory experiments were conducted in the Biocontrol laboratory, Department of Entomology, K. R.C. College of Horticulture Arabhavi.

Preparation of oil formulations of *Lecanicillium lecanii*

The oil formulations were prepared by following the method of the previously reported procedure with slight modifications (Bhanu Prakash *et al.*, 2015). The different edible oils, such as groundnut, sunflower, coconut, mustard, sesamum and soybean, were sterilized at 121 oC for 15 minutes. They were later cooled under room temperature and utilized for the preparation of oil formulation of *L. lecanii*. Six oil formulations of *L. lecanii* were prepared separately by mixing 20 ml spore suspension of *L. lecanii* with 10 ml of autoclaved cooled oils with tween-20 (0.05 %) and 1 ml of a sticker in one liter of sterilized distilled water and finally filtered through a muslin cloth.

Preparation of powder formulations of *Lecanicillium lecanii*

Different powder formulations of *L. lecanii* were developed by mixing the spore powder of *L. lecanii* obtained from different grains (rice, bajra, wheat and ragi) with talc powder in the ratio of 1:1. Later combination of both spore and talc powder were grinded for 30 seconds to made it as fine dust and finally packed in a sterilized polythene bag.

Preparation combination of oil and powder formulations of *Lecanicillium lecanii*

Sixteen combinations of oil and spore powder of *L. lecanii* were prepared by mixing different oils, spore dust and spore suspension in a proportion of 10:10:20 with tween 20 (0.05 %) and one ml of sticker in 1 liter of sterilized distilled water and finally filtered through muslin cloth.

Evaluation of developed formulations of *Lecanicillium lecanii* against *Myzus persicae* under laboratory condition

A total of 26 different formulations of *L. lecanii* were developed which includes six oil, four powder based and 16 combination of both oil and powder. All the developed formulations of *L. lecanii* and control (distilled water) were evaluated against *M. persicae* under laboratory conditions for their efficacy.

Bioassay study

About 2.0 ml of each formulations of *L. lecanii* ($2x10^8$ cfu/ml) dissolved in one liter of water and the solution was smeared on the detached fresh cabbage leaf disc (9 cm dia.) and later it was shade dried and placed on Petri dish containing a thin layer of one per cent water agar. A batch of uniform size of 20 net house reared *M. persicae* nymphs were released to each Petri dish. The Petri dish were maintained at room temperature 27.0 ± 1.0 ºC and the relative humidity of 70.0 ± 5.0 per cent. Another group of 20 uniform size of *M. persicae* was released on the leaf smeared with double distilled sterilized water, served as control. Each treatment was replicated twice with a 20 *M. persicae* per replication.

Observations recorded

The mortality of *M. persicae* was recorded after three, five and seven days after treatment (DAT). The moribund aphids were also counted as dead. The per cent aphid mortality was worked out by using following formula.

$$\text{Per cent aphid mortality} = \frac{\text{Number of dead/ moribund aphids}}{\text{Number of live aphids}} \times 100$$

Evaluation of selected formulations of *Lecanicillium lecanii* against cabbage aphid under field condition

Based on laboratory observation, we selected four best formulation of *L. lecanii* to evaluate against *M. persicae* the under field condition. The experiment was conducted in a randomized block design with three replication and eight treatments at College of Horticulture (COH), Bagalkot. The imposition of treatments were made once the infestation of *M. persicae* reached the economic threshold level in field condition at fifteen days interval. The observations were recorded on number of aphids per leaf on one day before and three, five, seven, ten and 15 days after each spray.

$$\text{Reduction over control (\%)} = \frac{\text{(Control – Treatments)}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

A total of 26 different formulations of *L. lecanii* (oil, talc-based and a combination of both) were screened against *M. persicae* under laboratory conditions. The result revealed that the per cent mortality increased with the advancement of the exposure period. The highest mean mortality of *M. persicae* was recorded in groundnut and sesamum oil formulations (96.66 %), followed by sunflower oil (95%), coconut oil (94.16%), and a combination of groundnut oil + rice grains (93.33%)**.** However, most formulations developed

Table 1. Details of the selected formulations of *Lecanicillium lecanii* tested under field condition

Treatments	Formulations	Dosage
T_1	Ground nut oil formulation (GNO) @ 2×10^8cfu/ml	2 ml/l
T_2	Sesamum oil formulation (SEO) @ 2×10^8 cfu/ml	2 ml/l
T_3	Rice grain formulation (RCG) @ 2×10^8 cfu/ml	2 g/l
T_4	Combination of GNO+RCG@ 2×10^8 cfu/ml	2 ml/l
T_5	NSKE	4%
T_6	Commercial formulation of *L. lecanii*@ 2×10^8 cfu/ml	2 g/l
T_7	Imidacloprid 17.8 SL	0.25 ml/l
T_8	Untreated Control	-

Statistical analysis: 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) by using ICAR- Web Agri Stat Package (WASP) software.

recorded more than 81.66 per cent mean morality of *M. persicae,* which was significantly superior over untreated control (Table 2). The reasons for more efficacies of all developed formulations might be due to indigenous isolate of *L. lecanii* and their more virulence, the synergist effect of vegetable oils and grain media and talc powder, and they supply good nutrients, mainly carbohydrates which favour better multiplication of spores. In addition, there was a controlled climatic condition with low temperature and high humidity.

The effectiveness of entomopathogenic fungi after three days of treatment reached a maximum of 90.00 per cent, and after five days of treatment, more than 90.00 per cent, and still it was more after seven days of treatment and reached the peak of a cent per cent mortality of *M. persicae* in all the developed formulations of *L. lecanii*. The efficacy of the entomopathogenic fungi began within 48 h after inoculation, and the hyphae penetrated the integument, epithelial and epidermal cells. After 72h, the fat tissues were damaged, and mortality reached 100 per cent after 96 hrs, as explained by Ei-Sinary (2006) and Quesada- Moraga *et al.* (2006). Among different grains tested, the spores multiplied on rice and sorghum grains recorded the maximum mortality of mealy bugs (96 per cent) after nine days of treatment, as reported by Banu (2013). Similarly, Karthikeyan and Selvanarayanan (2013) reported about a cent per cent morality of *Aphis gossypii and Bemisia tabaci* in a liquid formulation of *L. lecanii* while 93.33 per cent mortality of *A. devastans* was recorded. Three oil and one crude formulation of *Nomuraea (Metarhizium) rileyi* were evaluated against third-instar larvae of *Spodoptera litura* by Sharmila *et al.* (2015). The mean larval mortalities of *S. litura* of 96.67, 93.33, 86.67 and 76.67 per cent were recorded in groundnut, sunflower and coconut oil and crude formulations of *N. rileyi* (1x108 cfu/ml), respectively, at 10 days after treatment. Among the different formulations of *L. lecanii* that were developed and evaluated against *M. persicae,* the oil-based formulations recorded the highest mortality of *M. persicae* compared to using different grains alone and in combination with edible oils. The reason was that oils form a cover over the conidial surface, prevent drying before actual germination and help in more prolonged survival, assist by disrupting the waxy layer of insect cuticle, and better penetration of peg into integument.

Similarly, a field experiment was conducted to evaluate the effect of selected formulations of *L. lecanii* against *M. persicae*. The data on the bio efficacy of various treatments in reducing the aphid population after the first and second sprays are furnished in Table 3. A day before the spray, the population of aphids were uniform across the experimental plot, and it differed significantly after the first spray on different days of treatments. The mean number of aphids per leaf was significantly lowest (3.11 aphids/ leaf) in the imidacloprid treatment, followed by NSKE (5.97 aphids /leaf). Among the selected formulations, sesamum oil recorded the significantly lowest mean aphids (7.45 aphids/ leaf), which was on par with groundnut oil (7.47 aphids/ leaf) and groundnut oil + rice grain (7.67 aphids/ leaf).

Table 2. *In-vitro* evaluation of formulations of *Lecanicillium lecanii* against *Myzus persicae*

Treatments	Per cent mortality of *Myzus persicae*			
	3 DAT	5 DAT	7 DAT	Mean
T_1-Ground nut oil formulation (GNO)	90.00 (71.56) [a]	100 (89.71) [a]	100 (89.71) [a]	96.66 (79.46) [a]
T_2 -Sun flower oil formulation (SFO)	87.50 (69.29) [ab]	97.50 (80.90) [b]	100 (89.71) [a]	95.00 (78.26) [a]
T_3 -Coconut oil formulation (CNO)	85.00 (67.21) [bc]	97.50 (80.90) [b]	100 (89.71) [a]	94.16 (76.01) [b]
T_4 - Soybeanoil formulation (SYO)	82.50 (65.27) [cd]	95.00 (77.07) [c]	100 (89.71) [a]	92.50 (74.10) [bc]
T_5-Mustard oil formulation (MSO)	82.50 (65.27) [cd]	97.50 (80.90) [b]	100 (89.71) [a]	93.33 (75.03) [bc]
T_6 - Sesamum oil formulation (SEO)	90.00 (71.56) [a]	100 (89.71) [a]	100 (89.71) [a]	96.66 (79.46) [a]
T_7 -Rice grain formulation (RCG)	77.50 (61.68) [e]	97.50 (80.90) [b]	100 (89.71) [a]	91.66 (73.21) [c]
T_8 -Ragi grain formulation (RGG)	72.50 (58.37) [f]	92.50 (74.10) [d]	100 (89.71) [a]	88.33 (70.02) [d]
T_9 -wheat grain formulation (WHG)	70.00 (56.78) [fg]	90.00 (71.56) [e]	100 (89.71) [a]	86.66 (68.57) [de]
T_{10} -Bajra grain formulation (BJG)	72.50 (58.37) [f]	92.50 (74.10) [d]	100 (89.71) [a]	88.33 (70.02) [d]
T_{11} -Combination of MSO+RCG	57.50 (49.31) [j]	95.00 (77.07) [c]	100 (89.71) [a]	84.16 (66.54) [e-h]
T_{12} -Combination of MSO+RGG	65.00 (53.72) [ghi]	92.50 (74.56) [d]	100 (89.71) [a]	85.83 (67.88) [def]
T_{13} -Combination of MSO+WHG	65.00 (53.72) [ghi]	90.00 (71.56) [e]	100 (89.71) [a]	85.00 (67.30) [efg]
T_{14} - Combination of MSO+BJG	65.00 (53.72) [ghi]	87.50 (69.29) [f]	100 (89.71) [a]	84.16 (66.54) [e-h]
T_{15} -Combination of SEO+RCG	62.50 (52.23) [hij]	97.50 (80.90) [b]	100 (89.71) [a]	86.66 (68.57) [de]
T_{16} -Combination of SEO+RGG	65.00 (53.72) [ghi]	92.50 (74.10) [d]	100 (89.71) [a]	85.83 (67.88) [def]
T_{17}- Combination of SEO+WHG	62.50 (52.23) [hij]	85.00 (67.21) [g]	95.00 (77.07) [b]	81.66 (64.64) [h]
T_{18} -Combination of SEO+BJG	67.50 (55.24) [fgh]	92.50 (74.10) [d]	100 (89.71) [a]	86.66 (68.57) [de]
T_{19} -Combination of GNO+RCG	80.00 (63.43) [de]	100 (89.71) [a]	100 (89.71) [a]	93.33 (75.03) [bc]
T_{20} -Combination of GNO+RGG	77.50 (61.68) [e]	97.50 (80.90) [b]	100 (89.71) [a]	91.66 (73.21) [c]
T_{21} -Combination of GNO+WHG	70.00 (56.78) [fg]	87.50 (69.29) [f]	100 (89.71) [a]	85.83 (67.88) [def]
T_{22} -Combination of GNO+BJG	57.50 (49.31) [j]	90.00 (71.56) [e]	100 (89.71) [a]	82.50 (65.27) [gh]
T_{23} -Combination of CNO+RCG	70.00 (56.78) [fg]	95.00 (77.07) [c]	100 (89.71) [a]	88.33 (70.02) [d]
T_{24} -Combination of CNO+RGG	60.00 (50.76) [ij]	97.50 (80.90) [b]	100 (89.71) [a]	85.83 (67.88) [def]
T_{25} -Combination of CNO+WHG	70.00 (56.78) [fg]	90.00 (71.56) [e]	100 (89.71) [a]	86.66 (68.57) [de]
T_{26} -Combination of CNO+BJG	57.50 (49.31) [j]	92.50 (74.10) [d]	100 (89.71) [a]	83.33 (65.90) [fgh]
T_{27} -Distilled water as control	0.00 (0.28) [k]	12.50 (20.61) [h]	35.00 (36.22) [c]	15.83 (23.44) [i]
S. Em ±	0.73	0.42	0.57	0.55
C. D. at 1%	2.87	1.64	2.27	1.87
CV (%)	1.85	0.78	0.93	1.83

DAT-Days after treatment; Figures in the parenthesis are Arc sin transformed values
In a column, means followed by same alphabet(s) do not differ significantly by DMRT (P=0.01)

In contrast, the commercial formulation of *L. lecanii* and rice grain formulation recorded 8.03 and 8.30 aphids per leaf, respectively. After the first spray, the untreated control recorded the maximum mean number of aphids per leaf (8.78 aphids/ leaf). However, after different days of treatments, there was a significant difference recorded with respect to the number of aphids per leaf. The mean number of aphids was significantly lowest in the imidacloprid (3.82 aphids /leaf) treatment, followed by NSKE (12.75 aphids /leaf). Among the selected formulations, the significant lowest mean number of aphids per leaf was recorded in sesamum (18.68 aphids /leaf), groundnut oil (19.15 aphids /leaf) and rice grain in combination with groundnut oil (19.75 aphids/leaf), and they were on par with each other. The untreated control recorded the highest 22.95 mean number of aphids per leaf in the cabbage crop.

The results under field conditions revealed that all the treatments significantly reduced the *M. persicae* over the untreated control. The maximum per cent reduction *in M. persicae* was recorded in imidacloprid followed by NSKE, sesamum oil, groundnut oil, a combination of groundnut oil with rice grain, rice grain formulation and commercial formulations *of L. lecanii* after the first and second spray. The results of the present findings are compared with the earlier workers. Sahayaraj and Namachivayam (2011) reported *L. lecanii* reduced about 62 per cent of *A. craccivora* at 39 days after seedling emergence (DASE). Further, Shivakumar *et al*. (2022) reported that *Verticillium lecani* is effective in reducing the aphid *Aphis nerii* population under laboratory (73.3%) and field conditions (43.84 %). The repeated application of an oil-based formulation of *L. lecanii* at 15 days intervals showed 70 per cent mortality of *P. marginatus* under field conditions by Gulsar and Gopalakrishnan (2012). The effect of all vegetable oils and grain media showed a positive response on the growth and development of *L. lecanii* and increased mortality of *M. persicae.* All the formulations of *L. lecanii* significantly recorded increased mortality with the advancement of the exposure period and reached a cent per cent mortality of *M. persicae* compared to untreated control.

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