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Biorational management and mycosis studies of grape thrips, *Rhipiphorothrips cruentatus* H.

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ABSTRACT: Field experiment was conducted during 2017-18 to test the bioefficacy of certain biorational insecticides against thrips *Rhipiphorothrips cruentatus* Hood on grapevine at the Department of Horticulture, MPKV, Rahuri, India. Among botanical insecticides, neem oil 2% (4.09/shoot) was the most effective treatment followed by, karanj oil (4.51/shoot) and commercial azadirachtin formulation (neemazol) (5.08/shoot). The entomopathogenic fungi, *Lecanicillium lecanii* recorded 4.24/shoot followed by *Metarhizium anisopliae* (4.87/shoot) and *Beauveria bassiana* (5.34/shoot). However chlli methanolic extract (6.29/shoot), garlic methanolic extract (6.78/shoot), chilli water extract (6.85/shoot) and garlic water extract (7.08/shoot) were least effective treatments. Incremental Cost Benefit Ratio (ICBR) in respect of different treatments ranged from 1.30 to 7.92. The highest ICBR of 1:7.92 was recorded with emamectin benzoate 5 SG followed by *L. lecanii* (1:6.34) and *M. anisopliae* (1:5.32). Although neem oil and karanj recorded higher reduction of thrips population, they had lower cost benefit ratio due to high dose and its cost. The pathogenesity of *L. lecanii* and *M. anisopliae* was also confirmed through mycosis test.

Keywords: Botanicals, entomofungi, grapes, *Lecanicillium lecanii*, thrips, mycosis

INTRODUCTION

Grapes (*Vitis vinifera* L.) is an important commercially grown fruit crop of India being cultivated in 1,38,000 ha with annual production of 30 lakh MT. Maharashtra is the leading grape growing state covering an area of about 78000 ha with the production of 1.80 lakh MT (Anonymous, 2018). Thrips, once considered to be the insect pests of minor importance in horticultural crops, have gained the paramount importance due to their ability to cause economic losses, to subsist on new hosts and polyphagous nature (Dahiya *et al.*, 1995). *Ripiphorothrips cruentatus* (H.) and *Scirtothrips dorsalis* (H.) are the species recorded infesting the leaves and berries (Butani., 1979) of grapes. Both nymphs and adults of *R. cruentatus* cause damage by rasping the lower surface of the leaf with their stylets and sucking the oozing cell sap. The injured surface is marked by the number of minute spots thereby producing a speckled silvery effect, which can be detected from a distance. They feed in groups, generally on the undersurface of the leaves. Curling of the leaves is observed in case of severe incidence (Kulakarni *et al.*, 2007).

Large number of chemicals are being used on to manage thrips. Chemical control affects the export value of grapes due to pesticide residues. Also some of the results concluded that 27 chemical pesticides out of 171 chemical pesticides can be found usually in grape samples which indicate that the stability of these pesticides is very high or they retain in the grape fruit for a long time after use of them which affect the export value of grapes (Raikwar *et al.*, 2011). Hence it is essential to find effective biorational pesticides like botanicals and entomofungi to have residue free pest management. With this background, the present study was carried out to evaluate the bioefficacy of biorational insecticides and validation of the entomopathegenic fungi growth on grape vine thrips.

MATERIALS AND METHODS

Bioefficacy studies

A field experiment was carried out in a vineyard under All India Co-ordinated Research Project (AICRP) on Fruits at the Department of Horticulture, MPKV., Rahuri, India after October, 2017 pruning to evaluate the bioefficacy of certain bio-rational insecticides against thrips on grapevine. The grape variety 'Flame Seedless' was chosen for the study. Gardens were selected after ensuring that they were totally unprotected after fruit pruning. The trial was laid out in a Randomized Block Design (RBD) with twelve treatments replicated three times containing two vines each.

Table 1. Bio-efficacy of some biorational insecticides against thrips on grapes (1st spray)

	Treatment	Dose	Number of thrips per shoot						Per cent reduction of thrips over control
			Pre-count	3 DAS	5 DAS	7 DAS	10 DAS	Mean	
T_1	***Beauveria bassiana***	5g/l ($1x10^8$ cfu/g)	8.70 (3.03)	8.13 (2.94)	5.70 (2.49)	3.37 (1.97)	4.47 (2.23)	5.42 (2.43)	38.10
T_2	***Metarhizium anisopliae***	5g/l ($1x10^8$ cfu/g)	8.27 (2.96)	7.43 (2.82)	4.93 (2.33)	3.00 (1.87)	4.20 (2.17)	4.89 (2.32)	44.10
T_3	***Lecanicillium lecanii***	5g/l ($1x10^8$ cfu/g)	8.10 (2.93)	7.10 (2.76)	4.43 (2.22)	2.30 (1.67)	3.90 (2.10)	4.43 (2.22)	49.33
T_4	**Neem oil (2%)**	20 ml/l	8.60 (3.02)	7.03 (2.74)	3.63 (2.03)	2.20 (1.64)	3.97 (2.11)	4.21 (2.17)	51.90
T_5	**Karanj oil (2%)**	20 ml/l	8.50 (3.00)	7.10 (2.76)	3.93 (2.11)	3.07 (1.89)	4.53 (2.24)	4.66 (2.27)	46.76
T_6	**Neemazol (10000ppm)**	3ml/l	8.10 (2.93)	6.93 (2.73)	3.70 (2.05)	2.87 (1.83)	4.23 (2.18)	4.43 (2.22)	49.33
T_7	**Chilli methanolic extract (2%)**	20 ml/l	8.17 (2.94)	7.17 (2.77)	5.37 (2.42)	4.30 (2.19)	8.20 (2.95)	6.26 (2.60)	28.48
T_8	**Garlic methanolic extract (2%)**	20 ml/l	8.27 (2.96)	7.53 (2.83)	5.73 (2.50)	4.90 (2.32)	8.43 (2.99)	6.65 (2.67)	24.00
T_9	**Emamectin benzoate**	0.22g/l	8.30 (2.97)	5.17 (2.38)	2.10 (1.61)	1.73 (1.49)	3.50 (2.00)	3.13 (1.90)	64.29
T_{10}	**Chilli water extract (2%)**	20 ml/l	8.73 (3.04)	8.07 (2.93)	6.47 (2.64)	5.47 (2.44)	8.67 (3.03)	7.17 (2.77)	18.10
T_{11}	**Garlic water extract (2%)**	20 ml/l	8.63 (3.02)	8.00 (2.92)	6.60 (2.66)	5.93 (2.54)	8.60 (3.02)	7.28 (2.79)	16.76
T_{12}	**Untreated Control**	---	8.67 (3.03)	8.83 (3.06)	8.83 (3.06)	8.60 (3.02)	8.73 (3.04)	8.75 (3.04)	0.00
	S. E. m. ±	---	0.117	0.088	0.075	0.065	0.097	0.061	
	CD at 5%	---	NS	0.260	0.220	0.193	0.286	0.181	

* DAS: Days after spraying; NS: Non significant   * Figures in the parenthesis indicate $\sqrt{x+0.5}$ transformed values

Pre-treatment count of thrips, was taken prior to the insecticidal application. Eleven insecticides applications were given in the experimental field with the help of a knapsack sprayer. A total of three sprays were applied at an interval of ten days. The data was recorded on population of thrips by tapping five shoots from each treated vines. Observations on thrips and were taken at 3, 5, 7 and 10 days after spray (DAS), (Duraimurugan and Jagadish, 2004). The insecticidal efficacy was assessed by recording the total number of thrips present on vines as well as bunches on two vines in each treatment. Presence of thrips was recorded on selected shoot and it was expressed as number of thrips per shoot per vine (Kulkarni and Adsule, 2006). On the basis of the absolute counts of the thrips recorded, the population reduction in different treatments over control was calculated by using Modified Abbot's formula given by Fleming and Retnakaran (1985).

Pre count (1 DBS) and post count (mean of 3, 5, 7 and 10 DAS) population and per cent reduction over control were calculated after each spray. Cumulative mean of three sprays is analysed in order to get the best treatment.

$$\text{Per cent reduction over control} = \frac{\text{Population in control - population in treatment}}{\text{Population in control}} \times 100$$

Incremental cost benefit ratio and yield data

The incremental cost benefit ratio of each insecticide was calculated by taking into account of the prevailing market price of input, produce and labour charges. Grape bunches were harvested from each treatment separately and yield was recorded. Total yield was calculated by adding the yield from different treatments. The per treatment yield was then converted to tonnes per ha.

Mycosis Studies

Three mycoinsecticides like *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii* were studied for mycosis test on grape thrips. The fungal suspension of three mycoinsecticides are prepared separately in beakers by mixing 5 g of each mycoinsecticide in 100 ml of water in beaker. All the three mycoinsecticides suspensions are prepared in three separate beakers. The young grape leaves are collected from field and their surface is cleaned with mercuric chloride by using cotton, in order to remove fungal spores present on the leaves. Later on the leaves are rinsed with the distilled water to remove the chemical on leaves. These grape leaves were smeared with the fungal suspension prepared and placed in the petri plates. For each mycoinsecticide three petri plates were prepared for mycosis test. Thrips nymphs were collected from the field and released into each petri plates in numbers of 10. These petri plates were packed with the polythene stripe in order to avoid the escape of thrips from petri plates. These petri plates were incubated in cool place for seven days to promote the infection of fungus on the thrips (Latha *et al.* 2010). Detailed microscopic examination of thrips samples collected from the petri plates of different treatments were observed after seven days and ten days of treatment under the stereo microscope with various resolutions like 10 and 40X for the growth of different fungus on various body parts of the thrips. These microscopic photographs are clearly mentioned in the results.

RESULTS AND DISCUSSION

Bioefficacy of biorational insecticides against grape thrips

The thrips population recorded, a day before spraying (PTC) varied from 8.10 to 8.73 thrips per shoot, which showed non significant difference among treatments indicating homogenous distribution of thrips population in the experimental area (Table 1). There was significant difference among the treatments after 3, 5, 7 and 10 days of first spraying. Considering the mean population of thrips after first spray, it was found that biorational insecticides neem oil (4.21/shoot) and *L. lecanii* (4.43/ shoot) was the most effective treatment with least population of thrips. Whereas, chilli water extract and garlic water extract was least effective with 7.17 and 7.28 thrips per shoot, respectively. However standard check emamectin benzoate 5 SG @ 11 g a.i.ha^{-1} proved to be significantly superior recording minimum thrips population (3.13/shoot). The data also indicated that higher reduction of population over control was observed in plots treated with standard check emamectin benzoate (64.29%). Among biorational insecticides neem oil (51.90%). *Lecanicillium lecanii* and neemazol had same per cent reduction over control *i.e* 49.33%. Next in order of effectiveness were karanj oil (46.76%), *M. anisopliae* (44.10%) and *B. bassiana* (38.10%).

Second Spraying

The results on efficacy of insecticides on population of thrips after second spray were presented in (Table 2).The data on thrips population collected at 10 DAS after I spray was considered as pre count for second spray. Considering the mean population of thrips after second spray, it was found that standard check emamectin benzoate was the most effective treatment with least population of thrips (3.06/shoot). Among biorational insecticides neem oil (4.07/shoot) and *L. lecanii* (4.16/ shoot) proved as effective treatments. Whereas, chilli

Table 2. Bio-efficacy of some biorational insecticides against thrips on grapes (2nd spray)

	Treatment	Dose	Number of thrips per shoot					Per cent reduction of thrips over control
			3 DAS	5 DAS	7 DAS	10 DAS	Mean	
T_1	*Beauveria bassiana*	5g/l ($1x10^8$ cfu/g)	8.06 (3.02)	6.00 (2.55)	3.27 (1.94)	4.07 (2.14)	5.48 (2.45)	37.45
T_2	*Metarhizium anisopliae*	5g/l ($1x10^8$ cfu/g)	8.03 (2.92)	5.47 (2.44)	2.80 (1.82)	3.50 (2.00)	4.95 (2.33)	43.54
T_3	*Lecanicillium lecanii*	5g/l ($1x10^8$ cfu/g)	7.20 (2.77)	4.83 (2.31)	1.97 (1.57)	2.63 (1.77)	4.16 (2.16)	52.57
T_4	Neem oil (2%)	20 ml/l	6.70 (2.68)	4.67 (2.27)	1.77 (1.51)	3.13 (1.91)	4.07 (2.14)	53.61
T_5	Karanj oil (2%)	20 ml/l	5.93 (2.54)	4.87 (2.32)	3.27 (1.94)	3.80 (2.07)	4.47 (2.23)	49.05
T_6	Neemazol (10000ppm)	3ml/l	7.23 (2.78)	6.00 (2.55)	5.00 (2.35)	4.80 (2.30)	5.76 (2.50)	34.32
T_7	Chilli methanolic extract (2%)	20 ml/l	7.40 (2.81)	6.53 (2.65)	5.03 (2.35)	6.27 (2.60)	6.31 (2.61)	28.04
T_8	Garlic methanolic extract (2%)	20 ml/l	8.10 (2.93)	7.07 (2.75)	6.07 (2.56)	6.97 (2.73)	7.05 (2.75)	19.58
T_9	Emamectin benzoate	0.22g/l	4.30 (2.19)	3.67 (2.04)	1.63 (1.46)	2.63 (1.77)	3.06 (1.89)	65.11
T_{10}	Chilli water extract (2%)	20 ml/l	7.80 (2.88)	6.73 (2.69)	5.67 (2.48)	7.20 (2.77)	6.85 (2.71)	21.86
T_{11}	Garlic water extract (2%)	20 ml/l	7.40 (2.81)	7.07 (2.75)	6.33 (2.61)	7.60 (2.85)	7.10 (2.76)	19.01
T_{12}	Untreated Control	---	8.63 (3.02)	8.67 (3.03)	8.87 (3.06)	8.90 (3.07)	8.77 (3.04)	0.00
	S. E. m. ±	---	0.100	0.082	0.067	0.074	0.060	
	CD at 5%	---	0.295	0.241	0.197	0.217	0.176	

* DAS: Days after spraying; NS: Non significant   * Figures in the parenthesis indicate $\sqrt{x+0.5}$ transformed values

Table 3. Bio-efficacy of some biorational insecticides against thrips on grapes (3rd spray)

	Treatment	Dose	Number of thrips per shoot					reduction of thrips over control (%)	ICBR
			3 DAS	5 DAS	7 DAS	10 DAS	Mean		
T_1	***Beauveria bassiana***	5g/l (1x10^8 cfu/g)	8.20 (2.95)	5.17 (2.38)	3.13 (1.91)	4.03 (2.13)	5.13 (2.37)	39.19	5.13
T_2	***Metarhizium anisopliae***	5g/l (1x10^8 cfu/g)	7.63 (2.85)	5.20 (2.39)	2.80 (1.82)	3.40 (1.97)	4.76 (2.29)	43.63	5.32
T_3	***Lecanicillium lecanii***	5g/l (1x10^8 cfu/g)	7.17 (2.77)	4.90 (2.32)	2.10 (1.61)	2.30 (1.67)	4.12 (2.15)	51.23	6.34
T_4	**Neem oil (2%)**	20 ml/l	6.40 (2.63)	4.73 (2.29)	1.97 (1.57)	2.90 (1.84)	4.00 (2.12)	52.62	2.81
T_5	**Karanj oil (2%)**	20 ml/l	6.13 (2.58)	4.80 (2.30)	2.93 (1.85)	3.80 (2.07)	4.42 (2.22)	47.68	3.04
T_6	**Neemazol (10000 ppm)**	3ml/l	7.23 (2.78)	4.80 (2.30)	3.10 (1.90)	5.10 (2.37)	5.06 (2.36)	40.08	1.98
T_7	**Chilli methanolic extract (2%)**	20 ml/l	8.10 (2.93)	6.00 (2.55)	4.80 (2.30)	6.27 (2.60)	6.29 (2.61)	25.47	2.19
T_8	**Garlic methanolic extract (2%)**	20 ml/l	8.27 (2.96)	6.23 (2.59)	5.10 (2.37)	6.97 (2.73)	6.64 (2.67)	21.32	1.30
T_9	**Emamectin benzoate**	0.22g/l	4.40 (2.21)	3.77 (2.07)	1.73 (1.49)	2.53 (1.74)	3.11 (1.90)	63.18	7.92
T_{10}	**Chilli water extract (2%)**	20 ml/l	7.60 (2.85)	6.23 (2.59)	5.20 (2.39)	7.10 (2.76)	6.53 (2.65)	22.61	2.19
T_{11}	**Garlic water extract (2%)**	20 ml/l	7.80 (2.98)	6.73 (2.99)	5.67 (2.99)	7.20 (3.00)	6.85 (2.99)	18.85	1.48
T_{12}	**Untreated Control**	---	8.37 (2.88)	8.43 (2.69)	8.47 (2.48)	8.50 (2.77)	8.44 (2.71)	0.00	
	S. E. m. ±	---	0.097	0.084	0.072	0.093	0.063	---	
	CD at 5%	---	0.286	0.249	0.214	0.274	0.186	---	

* DAS: Days after spraying; NS: Non significant   * Figures in the parenthesis indicate $\sqrt{x+0.5}$ transformed values.

water extract and garlic water extract was least effective with 6.85 and 7.10 thrips per shoot, respectively. The cumulative effect of treatments indicated that higher reduction of population over control was observed in plots treated with standard check emamectin benzoate (65.11%). Among biorational imsecticides neem oil (53.61%) emerged as best treatment over control. Next in order of effectiveness were *L. lecanii* (52.57%), karanj oil (49.05%), *M. anisopliae* (43.54%) and *B. bassiana* (37.45%).

Third Spraying

The results with regard to the efficacy of treatments after third spray were presented in (Table 3). Considering the mean population of thrips after third spray, it was found that standard check emamectin benzoate was the most effective treatment with least population of thrips (3.11/shoot). Among biorational insecticides neem oil (4.00/shoot), *Lecanicillium lecanii* (4.12/shoot) and karanj oil (4.42/shoot) were the best treatments. Whereas, garlic methanolic extract and garlic water extract was least effective with 6.64 and 6.85 thrips per shoot respectively. The cumulative effect of treatments indicated that higher reduction of population over control was observed in plots treated with standard check emamectin benzoate (63.18%). Among biorational insecticides neem oil is the best treatment with 52.62% reduction over control. Next in order of effectiveness were *L. lecanii* (51.23%), karanj oil (47.68%), *M. anisopilae* (43.63%), neemazol (40.08%) and *B. bassiana* (39.19%).

Pooled data

The data pertaining to efficacy of insecticides against thrips during first, second and third spray are pooled and presented in Fig. 1. It could be seen that all the insecticidal treatments were significantly superior over untreated control. The pooled data of three sprays revealed that standard check emamectin benzoate 5 SG consistently proved to be the most promising by recording the least population (3.10/shoot). Among biorational insecticides neem oil 2% (4.09/shoot), karanj oil (4.51/shoot) and neemazol (5.08/shoot). While entomopathogenic fungi *L. lecanii* recorded less population (4.24/shoot) as compared to the *M. anisopliae* (4.87/shoot) and *B. bassiana* (5.34/shoot). The data also indicated that higher per cent reduction over control of population was observed in plots treated with standard check emamectin benzoate 5 SG (64.21%). Among biorational insecticides neem oil (52.71%) and *L. lecanii* (51.04%). Next in order of effectiveness were karanj oil (47.83%), *M. anisopliae* (43.76%), neemazol (41.26%), *B. bassiana* (38.23%), chlli methanolic extract (27.35%), garlic methanolic extract (21.63%), chilli water extract (20.83%) and garlic water extract (18.20%).

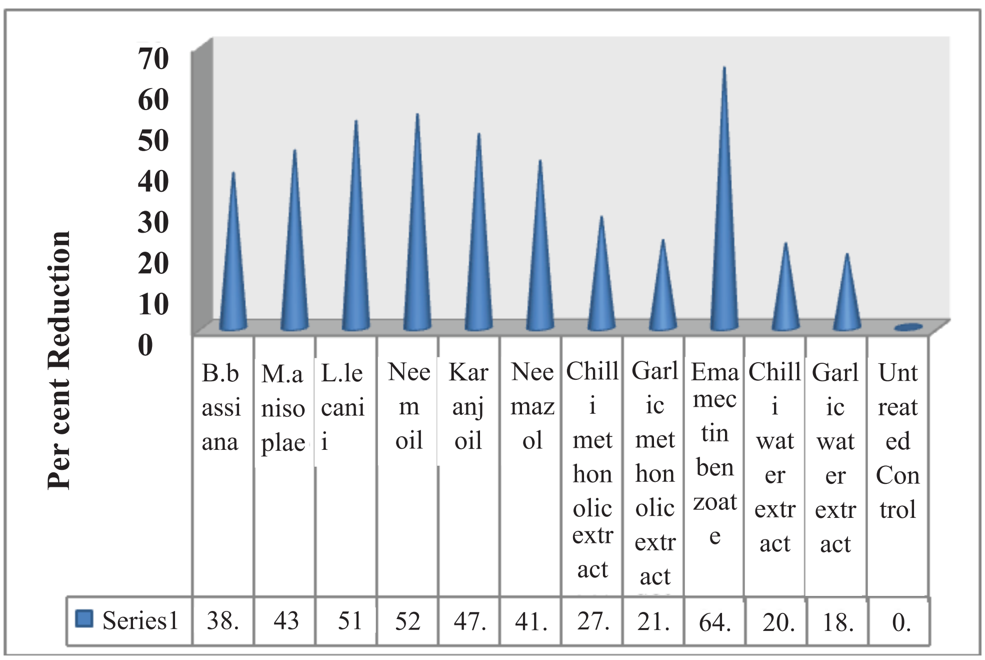

Fig 1. Per cent Reduction over Control of biorational insecticides against thrips on grapes (pooled data)

Cost economics of grapes

The cost effectiveness of the different insecticides used during study was assessed and presented in the (Table 3). The ICBR in respect of different treatments ranged between 1.30 to 7.92. The highest C:B ratio in *L. lecanii* (1:6.34) and *M. anisopliae* (1:5.32). Although neem oil and karanj oil has great reduction of thrips population, but has less cost benefit ratio *i.e* 2.81 and 3.04, respectively due to high cost of the insecticide.

Mycosis test of mycoinsecticides on grape vine thrips

The fungal suspension of three mycoinsecticides *viz. B. bassiana, M. anisopliae* and *L. lecanii* were studied for mycosis test on grape thrips. Detailed microscopic examination of the thrips samples collected from the petri plates showed that all the test entomopathogenic fungi were found growing in the body of the thrips. The moribund adult thrips showed profuse fungal growth in the body cavity. The microscopic photographs in the plates clearly indicated the mycosis by *B. bassiana* was predominant behind compound eyes, prothorax, near fore coxa, stomach portion and between inter segmental spaces. Close up view showed clear growth of fungus in thorax and abdominal portion. In advanced stages after tight filling the body cavity the fungus outgrowth was observed on head, legs and posterior part of abdomen. Highly pronounced mycosis by *M. anisopliae* was observed in the thrips which shrunken and hardened its body. The growth was observed in almost all body parts. The growth was observed along inter segmental joints around genital parts, tergo- sternum joint, head and prothorax and inter wings. *L. lecanii* soften the body of the thrips and growth was observed on antennary tips, around compound eyes, legs and tissues in different part of the body and alimentary canal in mid infestation (Plate 3 – a), in advanced stages whole body was captured by the fungus and growth was also vivid on surface of the body (Plate 1).

Beauveria bassiana ***Metarhizium anisopliae*** ***Lecanicillium lecanii***

Plate 1. Mycosis of thrips by three species of entomofungi

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