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Assortment for host resistance and eco-friendly management of mulberry powdery mildew caused by *Phyllactinia corylea* (Pers.) Karst

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Abstract
Powdery mildew caused by *Phyllactinia corylea* (Pers.) Karst, is an obligate parasitic disease of mulberry inflicting considerable qualitative as well as quantitative losses of leaf yield. Screening of 16 mulberry varieties against powdery mildew disease under field conditions yielded two resistant varieties with the lower powdery mildew disease severity of 2.5 and 5.42 per cent in a variety S-13 and in variety S-34 had 3 and 5.37 per cent after 55 and 70 days after planting respectively. The three varieties *viz*., Kanva-2 / M-5, Mysore local and S-1635 were moderately resistant with the less DS values of 11.43, 11.47 and 11.48 per cent respectively. In management studies, the plant extracts at all concentrations tested were effective in suppressing the disease and at higher concentration of 15%, *Zinger officinale* reduced the disease to 60.31 per cent and *Lantana camara* to 59.04 per cent disease over control and were considered as best for managing powdery mildew disease in mulberry. Among the culture filtrate of different bioagents tested, the *Trichoderma harzianum* reduced the disease to 51.56 per cent and *Pseudomonas fluorescens* to 51.09 per cent over control at 15% concentration and were shown to be potent in fading away the disease.

Keywords: Resistance, Screening, *Zingiber officinale*, *Trichoderma harzianum*, *Pseudomonas fluorescens*

Introduction
India has emerged as a second largest producer of raw silk and contributes about 19 per cent to total global production [4]. In India, West Bengal and Jammu and Kashmir are the oldest silk rearing states. Jammu and Kashmir has been one of the pioneer states for the production of uni\bi voltine silk of international quality. Mulberry, which belongs to family *Moraceae*, genus *Morus*, is a perennial woody plant of considerable economic importance due to its foliage which constitutes the food for mulberry silkworm (*Bombyx mori* L.). About 1100 Kg of mulberry leaves are required for rearing 100 layings of hybrid eggs. One of the major constraints in the production of required quantity and quality of mulberry leaf are diseases. Powdery mildew of mulberry caused by *Phyllactinia corylea* (Pers.) Karst., reduces¸ leaf yield and adversely affects the feeding quality of the leaf due to luxuriant mycelial growth on lower surface [9]. Upon feeding mulberry leaves infected with powdery mildew, the larvae of silkworm remain smaller in size, resulting in smaller sized cocoons as well as poor quality silk [11]. Considerable work has been carried out by a number of workers on the chemical control of this disease. Multiple applications of chemicals are often required to keep the disease under check. Owing to non eco-friendly nature, toxicity to silkworms, high cost of fungicides and reluctance by the farmers to their use and other side effects of chemicals, search for other alternative/eco friendly control strategies devoid of these drawbacks becomes inevitable. Keeping in view the above aspects, present study was initiated for screening of mulberry varieties for resistance and use of plant products and bioagents for eco-friendly management of powdery mildew disease.

Materials & methods
A field experiment was conducted during 2017 at College of Agriculture, V.C. Farm, Mandya to study the efficacy of plant extracts and bio agents against powdery mildew by using the variety V-1 Mulberry in Rabi season. The plot size was 37.2m X 4.2m and treatments maintained in three replications, with the statistical design of RCBD. All the agronomic practices were carried out as per the package of practices recommended for mulberry cultivation by UAS, Bangalore.

Screening of mulberry varieties against Powdery mildew disease

Six month old mulberry saplings of 16 mulberry varieties were planted in microplots with paired row system 150-90 X 60-60 cm spacing at the Central Sericultural Research and Training Institute, Sriramapura, Mysore, India. In screened mulberry varieties, three observations on powdery mildew disease severity were recorded at fortnightly interval under natural infestation. Data on disease severity was recorded by randomly selecting five plants of each variety. In each plant, disease severity was assessed from three randomly selected branches by using the scale 1 [5] and per cent disease index (PDI) was calculated [8].

Preparation of extracts of medicinal plants

Different medicinal plants *viz*; Neem leaf (*Azadirachta indica)*, Onion bulb (*Allium cepa)*, Ginger rhizome (*Zingiber officinale)*, Sweet sledge dried root (*Acorus calamus)*, Garlic bulb (*Allium sativum*) and Lantana leaf (*Lantana camera)* were used for the study. Aqueous extracts of extracts were prepared by using 500g of plant material were taken and cut into small pieces. The sample was put into waring blender containing 500ml sterilized distilled water at a ratio 1:1 (water: plant material). The sample was rotated at low speed for 10-15 minutes in a waring blender till the material formed to fine texture and squeezed through a sterilized muslin cloth so as to get a crude liquid extract. The crude extract was filtered through Whatman no 1 filter paper. The filtrate obtained served as a stock solution of 100 per cent concentration and was further diluted to 5, 10 and 15percent concentrations with water and sprayed twice uniformly at 20 days interval on the mulberry leaves before occurrence of the powdery mildew disease.

Preparation of culture filtrate of bioagents and their evaluation against mulberry powdery mildew

Two each of fungal and bacterial bioagents *viz*: *Trichoderma viride* and *Trichoderma harzianum* and *Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from the NBAIR, Bengaluru were used for the study. The fungal and bacterial bioagents were multiplied on potato dextrose and nutrient broth respectively contained in 2 litre capacity flat bottom round flasks incubated at room temperature by regular shaking. Ten day old fungal bioagent and two days old bacterial bioagent cultures from the broth were filtered through Whatman no.1 filter paper. The filtrate obtained served as a stock solution of 100 per cent concentration and were further diluted to 5, 10 and 15 percent concentrations by adding sterilized distilled water. The different concentrations of culture filtrates of both the bioagents were uniformly sprayed on the surface of mulberry leaves twice at 20 days interval before onset of the disease. The powdery mildew severity was recorded before and after spraying using 0-10 scale. Control treatment was maintained by water spraying. Observations were made one day before and five, ten and fifteen days after spray on disease severity by using scale [5] for all the treatments (Table 3). From each treatment, four plants were selected randomly and from each plant, three branches, each from top, middle and bottom canopy was selected to record disease severity following 0-10 scale. The percent disease control was calculated by the formula:

$$\text{Per cent disease control (\%)} = \frac{\text{PDI in control - PDI in treatment}}{\text{PDI in control}} \times 100$$

Results and discussion

Screening of mulberry varieties against powdery mildew disease

Using of resistant varieties in farming system is the most simple, effective and economical method in the management of powdery mildew disease. Besides this, the resistant varieties conserve natural resources and reduce the cost, time and energy when compared to the other methods of disease management. In the present investigation 16 mulberry varieties were screened against powdery mildew of mulberry under field conditions at during *Rabi* 2017 at Central Sericultural Training and Research Institute, Mysore. When the varieties were grouped into different categories of resistance, it was found that, none of the varieties were found to be highly resistant and only two varieties S-34 and S-13 were found to be resistant, three varieties *viz*., Kanva-2/ M-5, Mysore local and S-1635 was moderately resistant and three varieties *viz.*, S-36, G-2 and MSG-2 were intermediately susceptible and remaining others were susceptible (Table 1). The present studies are in agreement with Jindal and Bhavani (2002) [6] where, they reported that out of 15 varieties screened for powdery mildew disease, only ten varieties were highly resistant and eight were resistant to powdery mildew and also found that the lowest cumulative disease index was observed in *M. multicaulis*, *Thailand lobed* and *Italian mulberry*.

The difference in rate of infection on different mulberry varieties could be attributed to varietal characteristics and their genetic inheritance [2].

Field evaluation of plant extracts against powdery mildew of mulberry

The aqueous extracts of six medicinal plant species *viz*., *Azadirachta indica* (leaf), *Allium cepa* (bulb), *Zingiber officinale* (rhizome) *Acorus calamus* (rhizome), *Allium sativum* (clove) and *Lantana camera* (leaf) were tested to assess their efficacy on management of powdery mildew of mulberry (*Morus indica*) caused by *Phyllactinia corylea* under field condition. All the plant extracts tested at three different concentrations reduced the percent powdery mildew disease of mulberry. In all the cases spraying with higher extract concentrations gave better disease control. Among the plant extracts, the highest value of PDC (60.31%) was obtained with spraying of 15 per cent rhizome extract of *Z. officinale* by reducing the PDI value from 62.85 to 23.99 per cent at six days after second spray. Leaf, rhizome and clove extracts of *L. camera*, *A. calamus* and *A. sativum* at 15 per cent concentrations gave 59.04, 53.32 and 52.19 per cent disease control (PDC) respectively at six days after second spray (Table 2). Thus, the four plant extracts were most effective in the management of powdery mildew of mulberry although the other extracts also reduced the disease. In untreated control plants the PDI was maximum (75.37 to 60.45%) during 14 days compared to all other treatments. Among the treated plants less infected mulberry leaves was observed and gradually turning into healthy appearance after the spraying with effective plant extracts indicating the curative effect in reducing the disease. The present results were confirmatory with Malhotra and Singh (2003) [9], Vadivel and Ebenezer (2006) [14] and Tiken *et al*. (2015) [13].

Field evaluation of bioagents against powdery mildew of mulberry

Biological control, as a crop protection strategy has emerged as a response to the search for a safe, effective and

environmentally friendly approach to replace or supplement the use of chemical pesticides. Biological control of plant diseases involves the use of antagonistic microorganisms to control a pathogen. Over the past three decades, research has repeatedly demonstrated that many microorganisms can act as natural antagonists to plant pathogens [3]. Powdery mildew fungi are prime targets for biocontrol agents because of their superficial growth [1]. This has attracted many researchers to conduct intensive investigations in order to find antagonists that can provide acceptable levels of disease control.

In the present study it was found that all the biocontrol agents have reduced the disease at higher concentration of 15 per cent. The foliar sprays of *T. harzianum* at higher concentration have reduced the maximum disease when compared to other three bio control agents. The maximum per cent disease control of 51.56 per cent was observed in *T. harzianum* and the next best biocontrol agent observed for their efficacy against powdery mildew disease was *P. fluorescens* with 51.09 per cent PDC followed by *T. viridae* with 45.90 per cent disease control (Table 3). The highest per cent disease control by *T. harzianum* this might be due to antagonistic ability against the pathogen which inhibit the spore germination by producing hydrolytic enzymes. The present findings were in close conformity with [11] who reported that at higher filtrate concentration of *T. harzianum* and *T. viridae* had reduced the disease intensity of powdery mildew of mulberry. The lowest per cent disease control of 21.58 per cent was recorded in *B. subtilis* this might be due to less favourable climate to show its antagonistic ability against the pathogen in the prevailing mulberry ecosystem. The results are in correlating with Kikkort *et al*., (2000) [7].

The present investigation identified resistant Mulberry varieties, Excavated potential botanicals and bio agents which were effective in suppressing the mulberry powdery mildew pathogen. The finding could be exploited to formulate ecofriendly cost effective integrated management strategy for the management of powdery mildew disease of mulberry to get quality leaf yield and enhance the silkworm yield and it is helpful for the doubling of yield and income level of the sericulture farming community.

Table 1: Screening of mulberry varieties against mulberry powdery mildew disease

S. N	Name of a variety	Disease severity (%)			Disease reaction
		40 DAP	55 DAP	70 DAP	
1	Mysore Local	9.25	10.31	11.47	MR
2	KANVA-2	6.25	9.06	11.43	MR
3	S-36	10.00	17.43	21.27	MS
4	G-2	8.75	17.18	21.52	MS
5	G-4	11.25	22.81	26.75	S
6	V-1	20.00	36.25	37.27	S
7	S-1635	8.28	10.72	11.48	MR
8	MSG-2	5.00	10.00	13.66	MS
9	Sahana	22.50	37.45	44.28	S
10	AR-11	15.00	27.41	33.72	S
11	AR-12	43.75	62.93	74.57	HS
12	S-13	0.00	2.50	5.42	R
13	AGB-8	10.00	16.25	25.64	S
14	S-34	0.00	3.00	5.37	R
15	RC-1	15.00	28.75	32.24	S
16	RC-2	13.75	27.18	35.61	S

DAP= Days after pruning; PDI =per cent disease index;
Disease reaction: Highly resistant (0%); Resistant (0 to 3%); Moderately resistant (>3-6%); Moderately susceptible (>12-25%); Susceptible (>25-50%); Highly Susceptible (>50)

Table 2: Evaluation of plant extracts against powdery mildew of mulberry under field condition

Tr	Conc. (%)	Before spray	PDI after 1st spray	PDI after 2nd spray	
			At 6 days	At 6 days	PDC
T1	5	56.78(48.89)	51.18(45.67)	36.43(37.12)	39.74(39.07)
T2	10	62.56(52.27)	50.26(45.14)	32.77(34.92)	45.79(42.58)
T3	15	60.67(51.15)	46.45(42.96)	30.79(33.70)	49.07(44.46)
T4	5	66.67(54.73)	54.78(47.74)	36.05(36.89)	40.36(39.44)
T5	10	63.33(52.72)	49.25(44.56)	33.99(35.66)	43.77(41.42)
T6	15	69.89(56.71)	47.54(43.58)	31.95(34.41)	47.15(43.36)
T7	5	56.67(48.83)	48.36(44.05)	33.71(35.49)	44.23(41.68)
T8	10	57.71(49.43)	43.47(41.24)	25.22(30.14)	58.28(49.76)
T9	15	62.85(52.44)	40.40(39.46)	23.99(29.32)	60.31(50.94)
T10	5	67.33(55.13)	51.81(46.03)	34.49(35.96)	42.94(40.94)
T11	10	63.33(52.72)	50.39(45.22)	30.94(33.79)	48.82(44.32)
T12	15	62.19(52.05)	46.72(43.11)	28.22(32.08)	53.32(46.90)
T13	5	65.20(53.84)	49.18(44.52)	34.48(35.95)	42.96(40.95)
T14	10	64.09(53.18)	50.33(45.18)	30.03(33.23)	50.32(45.18)
T15	15	70.00(56.78)	46.64(43.07)	28.90(32.52)	52.19(46.25)
T16	5	64.39(53.36)	49.15(44.51)	30.42(33.47)	49.68(44.81)
T17	10	52.12(46.21)	46.23(42.83)	26.89(31.23)	55.52(48.16)
T18	15	63.93(53.08)	44.87(42.05)	24.76(29.84)	59.04(50.20)
T19	-	75.37(60.24)	71.02(57.42)	60.45(51.03)	0.00 (0.00)
S. Em±		NS	1.45	1.12	1.82

CD @ P=0.05		4.16	3.20	5.22

(Figures in the parenthesis are Arcsine transformed values)
Tr= Treatment; PDI= per cent disease index; PDC= Per cent disease control;
NS= Non-significant;
Treatment details: T_1, T_2 and T_3 = *Azadirachta indica* T_4, T_5 and T_6 = *Allium cepa;* T_7, T_8, and T_9 = *Zingiber officinale* T_{10}, T_{11} and T_{12}= *Acorus calamus;* T_{13}, T_{14} and T_{15} = *Allium sativum* T_{16}, T_{17} and T_{18} = *Lantana camara;* T_{19} = Control (Water spray)

Table 3: Evaluaton of bioagents against powdery mildew of mulberry under field condition

Tr	Conc. (%)	Before	PDI after 1st spray	PDI after 2nd spray	PDC
			At 6 days	At 6 days	
T1	5	58.89 (50.11)	50.05 (45.02)	34.40 (35.91)	31.88 (34.37)
T2	10	56.67 (48.83)	45.00 (42.12)	34.51 (35.97)	31.66 (34.24)
T3	15	56.67 (48.83)	45.37 (42.34)	27.32 (31.51)	45.90 (42.64)
T4	5	65.56 (54.06)	54.10 (47.35)	29.36 (32.81)	41.86 (40.31)
T5	10	53.40 (46.94)	42.22 (40.52)	24.70 (29.80)	51.09 (45.62)
T6	15	55.56 (48.19)	40.30 (39.40)	24.46 (29.64)	51.56 (45.89)
T7	5	60.00 (50.76)	52.41 (46.38)	36.43 (37.12)	27.86 (31.85)
T8	10	55.56 (48.19)	46.96 (43.25)	28.28 (32.12)	44.00 (41.55)
T9	15	57.78 (49.47)	44.44 (41.80)	27.68 (31.74)	45.19 (42.23)
T10	5	62.22 (52.07)	55.13 (47.94)	39.60 (38.99)	21.58 (27.68)
T11	10	60.00 (50.76)	51.11 (45.63)	33.34 (35.26)	33.98 (35.65)
T12	15	63.33 (52.72)	50.22 (45.12)	33.62 (35.43)	33.43 (35.32)
T13	-	67.78 (55.41)	62.60 (52.29)	50.50 (45.28)	0.00 (0.00)
S.Em±		NS	1.15	1.14	1.72
CD @ P=0.05			3.35	3.31	5.02

(Figures in the parenthesis are Arc sine transformed values)
Tr= Treatment; PDI= per cent disease index; PDC= Per cent disease control; NS= Non-significant;
Treatment details: T_1, T_2 and T_3 = *Trichoderma viridae;* T_4, T_5 and T_6 = *Trichoderma harzianum;* T_7, T_8 and T_9 = *Pseudomonas fluorescencs;* T_{10}, T_{11} and T_{12} = *Bacillus subtilis;* T_{13} = Control (Water spray)

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