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Evaluation of different plant extracts for management of powdery mildew of *Quercus serrata* Thunb. caused by *Phyllactinia corylea* (Pers.) Karst.

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ABSTRACT

Aqueous extracts of seven medicinally valued plant species viz. Acorus calamus (rhizome), Azadirachta indica (leaf), Melia azedarach (leaf), Melothria perpusilla (leaf), Phlogacanthus thyrsiflorus (leaf), Vitex trifolia (leaf) and Zingiber officinale (rhizome) were tested to assess their efficacy on management of powdery mildew of oak tree (Quercus serrata) caused by Phyllactinia corylea under field condition. The efficacies of the plant extracts were also compared by testing their effect on conidial germination of the pathogen in vitro. Foliar spray with plant extracts significantly reduced the percent disease index (PDI). Application of higher concentrations of the extracts showed better disease control. Among the plant species, rhizome extract of Zingiber officinale at 15% concentration was found to be most effective by showing the highest value (73.72%) of percent disease control (PDC) in comparison to untreated plants. All the plant extracts were also found to have inhibitory effect on conidial germination at all the concentrations tested. However, the maximum inhibitory effect of 73.40% was shown by rhizome extract of Z. officinale at 15% concentration.

Key words: *Quercus serrata*, powdery mildew, plant extracts, disease severity, conidial germination

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INTRODUCTION

Leaf of *Quercus serrata* Thunb. is used as primary food for the silkworm *Antheraea proylei* Jolly which produces the temperate tasar silk. Powdery mildew caused by *Phyllactinia corylea* (Pers.) Karst. is one of the major foliar diseases of *Q. serrata* in Manipur [1]. The occurrence of this leaf disease is reported from America, Europe, Australia and Asia and it is the most common disease on oaks in Europe where it was recorded at the beginning of 20th century [2]. The pathogen rapidly colonizes green tissues via asexual reproduction and can negatively affect host physiology. Immature conidia germinate on the leave while mature conidia remain attached to the conidiophores [3]. The disease affects most parts of the leaf and reduces leaf quality. Severe infection leads to premature senescence of the injured leaves. Reduction of leaf yield and adverse effect on feeding of leaf by silkworm due to luxuriant growth of mycelia on lower leaf surface were also reported [4]. The size of silkworm larvae was reduced on feeding with infected leaves resulting in smaller sized cocoons as well as poor quality silk [5]. Not only poor silk quality but also higher mortality of silkworms and reduction of other economic parameters such as larval weight, cocoon weight, shell weight and shell ratio were also found to be associated with the feeding of powdery mildew infected leaves [6]. Although several chemical fungicides are available for management of powdery mildew of oak and their use in silkworm culture is very limited considering the toxic effect on the feeding larvae. Several plant extracts had been tested for the control of powdery mildew diseases of different host plants [7-12] and also assessed their effect on conidial germination [13-15]. Previous *in vitro* studies had reported inhibitory effect of plant extracts on conidial germination of powdery mildew of pea [13] and mulberry [10, 15]. The present study was made for evaluation of aqueous extracts of seven plant species for management of powdery mildew of oak under field conditions and their effect on conidial germination of the pathogen in the context of temperate tasar silk cocoon production under subtropical climatic conditions.

MATERIALS AND METHODS

The experiments were conducted at the oak tasar farm of Regional Tasar Research Station, Imphal, India (latitude-$93^0 56^/$ E; longitude-$24^0 50^/$N; altitude-786.38m above msl) repeating twice during the year 2010 and 2011. The oak tree (*Quercus serrata*) being deciduous, new flushing of leaf started from the month of February every year. The trees were pruned in the last week of December to get new leaf flushing next year. Appearance of powdery mildew on the leaves was normally recorded from the month of April and continued till November every year.

Preparation of plant extracts

Seven medicinal plants viz., *Azadirachta indica* A. Juss. (Verbanaceae), *Zingiber officinale* Rosc. (Zingiberaceae), *Vitex trifolia* L. (Verbanaceae), *Acorus calamus* L. (Acoraceae), *Phlogacanthus thyrsiflorus* Nees. (Acanthaceae), *Melothria perpusilla* (Blume) Cogn. (Cucurbitaceae) and *Melia azedarach* L. (Meliaceae) were selected for the present study.

Freshly collected 100 g each of healthy plant parts (leaf or rhizome) was washed thoroughly in running tap water, surface sterilized with 70% alcohol then washed in sterile distilled water and macerated with sterile distilled water at 1:1 (w/v) ratio using mortar and pestle. The plant extract was filtered through Whatman no. 1 filter paper. The filtrate thus obtained was considered as 100% concentration of plant extract [10, 15]. From this concentrated extract desired concentrations of 5%, 10% and 15% plant extracts were prepared by adding sterile distilled water.

Field application of plant extracts

The experiment was conducted on randomized oak bushes grown in ½ acre of plot under conditions of natural infestation with seven treatments. The host leaves were checked at 9 -10 am for disease incidence during the first week of April and tagged with replication numbers on the infected branches. Branches having 15-25 leaves each were selected for treatment. Spraying of plant extracts was carried out using an atomizer in the evening hours (4-5 pm) when there is no wind blow to avoid extract drift from the spraying leaves. Care was taken while spraying not to cross to other branches. For each treatment 3 branches from 1 bush were randomly selected and each branch was treated as a replicate. The control branches were spray with sterilized distilled water. To prevent washing of spores from infected leaves by rainfall, transparent polythene sheets were stretched above the bushes. Second spraying of the selected branches was performed after 7 days of the first spray. The method of Gunashekar and Govindaiah [16] was followed for assessment of disease rating. Disease severity was recorded 1day before spray and 1 day after each of the two sprays following the formula of disease rating scale [15] and percent disease control was calculated using another formula [17]

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of all numerical ratings (Grade)}}{\text{Total no. of leaves X Maximum ratings (Grades) examined}} \text{X100}$$

$$\text{Percent disease control (PDC)} = \frac{\text{Control PDI} - \text{Treatment PDI}}{\text{Control PDI}} \text{ X100}$$

Effect of plant extracts on conidial germination

Fresh plant extracts were prepared to examine their effect on conidial germination of *P. corylea* at three concentrations (5, 10 and 15%). The double moist blotter method [15] was followed for the experiment. One circular blotting paper moistened with sterile distilled water was placed in a Petri plate. Two glass slides with proper spacing were placed on the moist blotting paper to support another glass slide on which a rectangular blotting paper moistened with plant extract was placed. In the control set the blotting paper placed on the glass slide was moistened with sterile distilled water. Three replicated Petri plates were maintained for each treatment. A cellophane paper measuring 18 mm X 18 mm was placed carefully on the blotting paper above the slide and freshly collected matured conidia of *P. corylea* were dusted on it. The Petri plates were incubated at 25^0C. The cellophane strips were removed after 24 hr incubation period and examined under microscope to record the total number of germinated conidia. Percent conidia germination was assessed by counting germinated and un-germinated spores in sufficient number of microscopic fields to give a minimum count of 500 spores per slide [15]. Only the single spores were counted while double or clump of three or more spores were ignored [18]. The percent germination of conidia was calculated by the formula [15]:

$$\text{Percent conidial germination} = \frac{\text{Total number of conidia germinated}}{\text{Total number of conidia observed}} \text{ X100}$$

RESULTS AND DISCUSSION

All the seven plant extracts at the three tested concentrations could reduce the percent disease index of powdery mildew of *Q. serrata* (Table 1). In all cases spraying with higher extract concentrations gave better disease control. Among the plant extracts the highest value of PDC (73.7%) was obtained with spraying of 15% rhizome extract of *Z. officinale* by reducing the PDI value at 12.0% after the second spray schedule. Leaf extracts of *V. trifolia* and *A. indica* at 15% concentrations also gave 70.0% and 64.9% PDC values, respectively after the second spray. Thus, the three plant extracts were most effective in the management of powdery mildew of oak although the other extracts also reduced the disease severity. Leaf extracts of *P. thyrsiflorus* and rhizome extract of *A. calamus* appeared to be least effective in reducing the PDI values. The untreated control plants showed an increase in PDI from 21.0% to 45.6% during 14 days while among the treated plants, less infected oak leaves were observed gradually turning into healthy appearance after the spraying of the effective plant extracts indicating the curative effect of the extracts. None of the phytotoxic symptoms like injury on leaf tips and leaf surface, necrosis, wilting and vein clearing were observed after spraying of the plant extracts. The results suggested that the effective plant species, especially those which are cheaply available like *V. trifolia* and *A. indica*, could be effectively and safely used for the management of powdery mildew of oak.

All the seven plant extracts also showed inhibitory effect on conidial germination of *P. corylea* (Table 2). The extracts of *Z. officinale*, *V. trifolia* and *A. indica* were again found to be most effective in inhibiting the conidial germination of the pathogen, the percent germination inhibition values being 73.40%, 64.06% and 64.86%, respectively. As found in disease control experiment, treatment with higher extract concentrations showed greater inhibitory effect on conidial germination. Leaf extract of *M. purpusilla* was least effective in inhibiting conidial germination of the powdery mildew pathogen.

Table 1. Effect of plant extracts on disease severity (percent disease index) of powdery mildew of oak

Extract conc.	5%					10%					15%				
Plant sp.	DI			DC		DI			DC		DI			DC	
	S0	S1	S2	S1	S2	S0	S1	S2	S1	S2	S0	S1	S2	S1	S2
Z. officinale	30.3 ±3.2	22.0 ±1.9	18.3 ±1.6	47.4	60.0	20.6 ±3.5	17.0 ±3.9	14.6 ±3.6	59.5	67.8	33.6 ±2.9	15.3 ±2.1	12.0 ±2.9	63.5	73.7
V. trifolia	36.3 ±2.5	31.6 ±0.8	25.3 ±0.9	24.3	44.7	25.3 ±3.0	24.0 ±3.0	17.0 ±2.9	42.8	62.7	24.6 ±3.7	18.6 ±2.1	13.6 ±1.4	55.5	70.0
A. indica	40.3 ±2.6	30.3 ±4.5	28.6 ±4.9	27.5	37.4	37.0 ±5.3	23.3 ±0.4	21.3 ±3.8	44.4	53.3	31.0 ±7.5	19.0 ±2.9	16.0 ±2.5	54.7	64.9
P. thyrsiflorus	43.3 ±1.9	36.6 ±1.1	30.6 ±1.5	12.3	33.1	39.6 ±1.8	34.0 ±1.0	27.3 ±1.3	19.5	40.1	37.0 ±2.4	29.6 ±1.8	26.6 ±1.2	29.3	41.6
M. azedarach	31.6 ±1.8	30.6 ±0.9	28.6 ±1.7	26.7	37.4	32.0 ±1.3	27.0 ±0.8	23.6 ±1.4	35.7	48.1	29.3 ±1.2	23.0 ±1.3	20.6 ±2.4	45.2	54.7
A. calamus	40.0 ±2.4	36.3 ±1.7	35.0 ±0.4	13.2	23.6	41.0 ±3.0	32.3 ±1.4	28.3 ±1.0	23.0	37.9	40.6 ±2.6	31.0 ±3.6	24.0 ±2.3	26.1	47.5
M. perpusilla	38.0 ±2.7	35.0 ±2.6	33.0 ±2.8	16.3	28.0	29.0 ±2.2	26.3 ±4.3	25.0 ±1.9	37.3	45.2	28.0 ±3.0	24.3 ±2.5	19.0 ±2.2	42.0	58.4
Control	21.0 ±2.8	42.0 ±0.8	45.6 ±0.8			21.0 ±2.8	42.0 ±0.8	45.6 ±0.8			21.0 ±2.8	42.0 ±0.8	45.6 ±0.8		
SEM(±)	3.59	3.18	3.42			3.59	3.18	3.42			5.60	3.45	3.02		
CD (p=0.05)	7.33	6.48	6.97			7.33	6.48	6.97			11.43	7.04	6.17		

DI = % disease index; DC = % disease control; S0 = before first spray; S1= after first spray; S2 = after second spray; ± = S.E.

Previous workers had reported the antifungal activity of rhizome extract of ginger against the powdery mildew pathogen *P. corylea* (10) and its efficacy for the control of powdery mildew of pea under field conditions (13). Gingerol, the active constituent of ginger had been isolated and studied for its pharmacological properties and toxic effects (19). Neem extract is widely known for its antifungal activity (15, 20) and also used against many human diseases (21). Aqueous extract *A. indica* and *M. azedarach* could significantly reduce the biomass production of *Macrophomina phaseolina* (22). Various workers had observed the antifungal activities of the other tested plant species like *V. trifolia* (23), *A. calamus* (24) and *P. thyrsiflorus* (25). In conformity with the present findings, better survival of powdery mildew infected cucumber plants was observed when sprayed with higher concentrations of effective plant extracts (26) which was due to availability of more amount of active principles.

Many plant extracts are known to inhibit fungal spore germination. Extracts of *Excoecaria agallocha* (leaf, stem and roots) at various concentrations inhibited the spore germination of *Heminthosporium oryzae*, *Fusarium oxysporum*, *Alternaria tennuis* and *Sachharomyces cerevisiae*. Maximum inhibition of 76.8% was observed in *H. oryzae* by 20% leaf extract of the plant (27). Aqueous extracts of *A. indica* and *Z. officinale* showed 80% germination inhibition against powdery mildew conidia (10). In another study, 500ppm concentration of bark extract of *A. indica* showed

more than 80% inhibition on conidial germination of *Erysiphe pisi* which was followed by rhizome extract of ginger (28). Active principle isolated from ginger was found to be fungitoxic against spore germination of many other fungi (29). The differences in activity among the leaf extracts were due to variations in concentration and composition of antifungal compounds in different plants [30] but the difference may also be due to specificity of antifungal compounds towards pathogens [10]. The inhibitory effect of the extracts is also depended on the type of plant species used, method of extraction and time of application of the extracts [30]. Some workers suggested that the inhibitory effects of plant extracts are due to hydrophobic characters of the plant extracts and their components. This enables them to partition in the lipids of the fungal cell wall membrane and mitochondria disturbing their structure and rendering them more permeable. Leaking of ions and other cell contents can occur causing cell death [31].

Table 2. Effect of plant extracts on conidial germination (%) of *Phyllactinia corylea*

Plant extract	Extract concentration (%)		
	5	10	15
Z. officinale	26.67 (59.38)	21.00 (68.02)	17.47 (73.40)
V. trifolia	31.07 (52.68)	23.73 (63.86)	23.60 (64.06)
A. indica	33.73 (48.63)	27.33 (58.38)	23.07 (64.86)
P. thyrsiflorus	35.67 (45.68)	32.58 (50.38)	25.20 (61.63)
M. azedarach	36.42 (44.54)	33.20 (49.44)	25.87 (60.61)
A. calamus	38.07 (42.02)	36.27 (44.76)	29.80 (54.62)
M. purpusilla	43.40 (33.91)	39.13 (40.41)	34.40 (47.62)
Control	65.67	65.67	65.67
SEM (±)	1.93	2.52	1.22
CD (P=0.05)	4.21	5.50	2.65

Figures in parentheses are percent inhibition of conidial germination

CONCLUSION

Spraying with 7 test plant extracts for two times significantly reduced the PDI of powdery mildew of *Q. serrata* as compared to untreated control plants without showing any phytotoxic symptoms. These plant extracts also showed inhibitory effect on conidial germination of the pathogen *P. corylea*. It can be concluded that the effective plant extracts especially which are cheaply available like *V. trifolia* and *A. indica* can be used for management of powdery mildew of oak for avoiding toxic residual effect of chemical fungicides.

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