

Original Research Article

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Studies on Management of Leaf Spot Disease of Mulberry (*Morus spp.*) through the Use of Botanicals

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ABSTRACT

A study was carried out to evaluate the efficacy of ten plant extracts against *Phleospora maculans* (Bereng) Allesch. both by inhibition zone technique and conidial germination technique on potato dextrose agar (PDA) medium each at three different concentrations viz. 5 percent, 10 percent and 15 percent with carbendazim 50WP (0.02%) as control. Among botanicals tested, the extract of *Allium sativum* (Garlic) has shown maximum inhibition of mycelial growth (66.45mm²), whereas, minimum inhibition was recorded in *Morus alba* (12.87mm²) irrespective of concentrations. The extracts of *Datura stramonium* inhibited the maximum conidial germination of 82.24 percent, whereas, *Urtica dioica* inhibited the minimum conidial germination of 13.85 percent irrespective of concentrations. At 5 percent concentration, *Datura stramonium* showed the highest mycelial inhibition of 48.17mm², whereas, maximum conidial inhibition of 79.16 percent was recorded in *Allium sativum*. At 10 percent concentration, highest mycelial inhibition of 63.50mm² was recorded in case of *Allium sativum*, whereas, highest conidial inhibition of 83.90 percent was recorded in *Datura stramonium*. At 15 percent concentration, *Allium sativum* inhibited highest mycelial growth of 87.97mm², whereas, *Datura stramonium* inhibited highest conidial germination of 86.45 percent.

Keywords

Mulberry,
Leaf spot disease,
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Plant extracts

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Introduction

Mulberry plant is distributed all over the world. It is cultivated both in temperate and tropical regions of the world. Mulberry forms the sole food and the only source of nutrition for silkworm (*Bombyx mori* L.). For the development of silk industry, production of high quality silkworm cocoons is must. To

achieve the goal of production of good quality silkworm cocoon crop, certain factors play an important role. The most important factor is the mulberry leaf, contributing about (38.2%) followed by climate (37%), rearing techniques (9.3%), silkworm race (7.3%), and other factors (6.6%) (Kamili and Masoodi, 2000). Hence, the quality of the mulberry leaf is one of the basic prerequisite for sericulture

and plays a pivotal role in successful silkworm cocoon crop. The growth and development of larvae and subsequent cocoon production are very much influenced by its nutritive value (Krishnaswamy, 1978). Although the leaf quality is a specific character of mulberry variety, it is adversely influenced by the improper soil and climatic conditions, improper agronomic inputs and the outbreak of diseases and pests (Reddy *et al.*, 2001 & 2004; Lakshmi *et al.*, 2001).

Diseases are major limiting factor in mulberry cultivation for production of both quantitative and qualitative leaf. The leaf spot disease of mulberry (*Morus* spp.) caused by *Phleospora maculans* causes serious damage to mulberry leaf yield and its quality under temperate conditions. The disease causes direct leaf yield loss of about 5 per cent due to defoliation, which may, however, reach up to 35 per cent in most severe conditions. In addition, it also causes 20-25 per cent loss due to destruction of leaf lamina (Kausar, 2000).

Various methods for the management of disease have been studied by various workers in other states. There are reports that foliar spray of Carbendazim (Bavistin 0.02%) or Benlate (0.05%) twice at an interval of 15 days after disease appearance were most effective in reducing the disease (Siddaramaiah *et al.*, 1978; Kausar, 2000; Ganga and Chetty, 1997). However, the continued use of synthetic fungicides in sericulture have several limitations *viz.* residual toxicity on silkworm, being costly, elimination of non-target organisms and hazardous to environment. Moreover, overuse of chemical fungicides eliminates natural soil microbial flora (Stangarlin *et al.*, 2011). Increasing conscious about conservation of environment as well as health hazards caused by synthetic chemicals have forced scientists to switch over from chemicals to botanicals. As such exploration of plant resources for

their antifungal potential against the pathogen is quite inevitable for a sustainable management of the pathogen. Further, these plant extracts could be readily used by the farmers to lessen the impact of the pathogen on their mulberry plantation. Using plant resources for its antifungal activity is an attractive avenue for the development of sustainable mode of moriculture in organic system. Hence, new plants especially the one's which are locally available need to be explored for their antifungal property. Keeping in view the commercial and economic value of mulberry, the present study was carried out in order to evaluate the efficacy of different botanicals so that disease could be controlled effectively.

Materials and Methods

The entire research programme was conducted at Temperate Sericulture Research Institute, SKUAST-K, Mirgund.

Sample collection

Samples of the leaf spot infested leaves were collected from Kokuso-21 variety of mulberry (*Morus* spp.) from the mulberry farm of Temperate Sericulture Research Institute, Mirgund in clean polythene bags for the purpose of isolation of pathogen in culture.

Preparation of Potato Dextrose Agar medium

Potato	: 200g
Dextrose	: 20g
Agar	: 20g
Distilled water	: 1 litre

Preparation

The potatoes were peeled, washed, diced and boiled with minimum quantity of distilled water until it became soft. The boiled potatoes

were filtered through a single layer of muslin cloth. The filtrate and the requisite quantity of other ingredients of the medium were mixed and made up to one litre with distilled water. The medium was sterilised by autoclaving at 121°C for 15 minutes at a pressure of 15 psi and 18 to 20 ml of medium was poured aseptically into sterile Petri plates.

Isolation, purification and maintenance of the pathogen

The isolation of the pathogen was made from the diseased samples of the susceptible mulberry cultivar “Kokuso-21”. The selected leaves along with infection were plucked and after the macroscopic and microscopic examination, were thoroughly washed first in tap water and then in distilled water. The infected portions of the leaves were cut with the help of sterilised blade in a closed chamber into small bits of size 3-5mm² with a healthy leaf tissue around. These bits were then surface sterilised with 0.1 per cent mercuric chloride solution for 30 seconds. The tissues were then again rinsed thrice in distilled water to remove the traces of mercuric chloride solution and dried with the help of blotting paper. The bits were transferred aseptically on to potato dextrose agar medium (PDA) in sterilised Petri plates. Three pieces of sterilised specimen were placed in each 9cm diameter Petri plate and incubated at 25±1°C. After 72 hours of incubation, radiating mycelial growth was observed from the edges of the infected bits.

The culture thus obtained was purified by fungal tip as well as by single spore method, maintained on PDA slants. The pure culture was then sub cultured after every 20 days on freshly prepared PDA slants to retain the vigour of the fungus; it was isolated repeatedly and purified by method described earlier.

Identification of the pathogen

The cultural and morphological characteristics of the causal organism were compared with the authentic description (Kausar, 2000).

Preparation of aqueous plant extracts

The test plants were collected from different regions of Kashmir and the test botanicals were prepared from different plant parts *viz.* roots, seeds, rhizome, leaves, bulbs, cuttings and hull (Table 1). The fresh botanicals were washed with tap water followed by washing with distilled water and were dried under shade by spreading over the newspapers inside the laboratory till they becomes brittle. The semi dried botanicals were crushed with the help of pestle and mortar at the rate of one gram of plant tissue per ten ml of distilled water (1:1 W/V) (Vinod *et al.*, 2008; Reddy *et al.*, 2009; Ganie *et al.*, 2013b; Haq *et al.*, 2014) . The extracts were purified by passing through double layered cheese cloth and finally through Whatman’s No. 1 filter paper. The plant extracts so obtained were taken as standard (100%) stock solutions and were used to prepare further dilutions of desired concentrations (Plate 2). 5 ml, 10 ml and 15 ml of stock solutions were mixed with 95, 90 and 85 ml of distilled water separately so as to get 5, 10 and 15 per cent concentrations.

***In vitro* evaluation of botanicals**

For the management of the leaf spot disease of mulberry (*Morus* spp.), ten locally available botanicals (Plate 3 & 4) belonging to various families were evaluated for their antifungal activity against leaf spot disease of mulberry.

In vitro, efficacy of 10 botanicals was studied against leaf spot of mulberry both by inhibition zone technique as well as conidial germination technique.

Inhibition zone technique

Ten plants were evaluated *in vitro* at 5 per cent, 10 per cent, and 15 per cent concentrations besides 50WP (carbendazim) @ 0.02 per cent were included in the experiment as check. To study the antifungal mechanism of plant extracts/fungicide in inhibiting mycelial growth of the fungus, inhibition zone technique was used.

Twenty millilitres of PDA medium was poured into each of 9cm diameter sterile Petri plate. Two millilitres of spore suspension was spread over the surface of culture medium. Discs of 5mm diameter were cut from Whatman's No. 1 filter paper, using a punching machine. The discs were dipped for 5 minutes in selected botanicals of different concentrations besides carbendazim 0.02 per cent which was used as control. The discs were placed individually in each Petri plate as per the treatment. Three discs were placed in each Petri plate and each disc representing one replication. The Petri plates were incubated at 25±5°C for 10 days and observations regarding inhibition zone were recorded. The area of inhibition zone developed by the botanicals against the test fungus was calculated by the formula:

$$I = \pi(R^2 - r^2)$$

Where,

I = Inhibition zone area

R = Radius of inhibition zone in mm

r = Radius of paper disc in mm

Conidial germination technique

Ten botanicals were selected and used against conidial germination of leaf spot pathogen of mulberry (*Morus* spp.). Each botanical at three different concentrations was used in this study. For each treatment three replications were maintained. The design of the

experiment was completely randomized design (CRD). Slide germination technique as described by Wellmann and Macallan (1943) was adopted by using cavity slides, where desired concentration of botanicals was obtained. A spore suspension from 20 days old culture was prepared and its concentration was maintained at 5×10^4 spores/ml. One drop of each botanical was mixed with a drop of spore suspension on a cavity slide. The slides so prepared were placed in Petri plates lined with moist filter paper. The plates were incubated at 25±5°C for 24 hours. A control was also maintained by adding a drop of fungicide to a drop of spore suspension. Spores were observed for each replicated treatment and average germination inhibition was calculated by using the formula suggested by Vincent (1947).

Statistical analysis

The minitab-17 statistic quality analysis was used to calculate means, standard errors and standard deviations. The data obtained was analysed using technique of ANOVA as given by Walpole (1982) to test the effectiveness of plant extracts and to check whether there is any significant difference in the antifungal properties of plant extracts.

Results and Discussion

Inhibition zone technique

Inhibition of mycelial growth varied significantly with different concentrations viz. 5%, 10% and 15% concentrations (Table-1). Among all the treatments tested, the highest area of inhibition zone (96.83mm²) was recorded in case of control (Carbendazim 0.02%). Among the botanicals tested, highest mycelial inhibition was recorded in Garlic (66.45mm²) followed by Datura (59.46mm²), Onion (51.21mm²), and all of them were found significantly different from each other. Mulberry (12.87mm²) was least effective

among all the plant extracts tested. Among all the concentrations tested, highest mycelial inhibition was recorded in 15 per cent concentration (48.42mm²) followed by 10 per cent concentration (37.38mm²) and 5 per cent concentration (28.33mm²) and all of them were found significantly different from each other. Data further revealed significant interaction between botanical and concentration (Graph 1). At 5 per cent concentration, highest mycelial inhibition was recorded in Datura (48.17mm²) followed by Garlic (47.87mm²) but both of them were at par with each other. The lowest inhibition was found in case of mulberry (8.44mm²) followed by Urtica (8.89mm²) and they were also found at par with each other. At 10 per cent concentration, maximum mycelial growth inhibition was recorded in Garlic (63.50mm²) and minimum in case of Mulberry (13.36mm²). At 15 per cent concentration, highest mycelial growth inhibition was recorded in Garlic (87.97mm²) while lowest inhibition was recorded in case of Mulberry (16.81mm²).

Conidial germination technique

Inhibition of mycelial growth varied significantly with different concentrations viz. 5%, 10% and 15% concentrations (Table 2). Among all the treatments tested, the highest conidial inhibition (97.41%) was recorded by control (Carbendazim 0.02%).

Among the botanicals tested, Datura was found to be the best treatment, which recorded the highest conidial inhibition percentage (82.24%) followed by Garlic (81.86%) but they were at par with each other. Urtica (13.85%) and Walnut (13.96%) were found least effective among all the botanicals tested and both of them were at par with each other. Among all the concentrations tested, highest conidial germination inhibition was recorded in 15 per cent concentration (44.42%) followed by 10 per cent concentration (38.74%) and 5 per cent concentration (33.18%) and all of them were found significantly different from each other.

Table.1A Botanicals and their parts used for the preparation of plant extracts for the management of leaf spot disease of mulberry

Local name	English name	Botanical name	Family	Plant part used
Koth	Costus	<i>Saussurea lappa</i> Clarke	Asteraceae	Root
Tul	Mulberry	<i>Morus alba</i> L.	Moraceae	Root
Datur	Datura	<i>Datura stramonium</i> L.	Solicaceae	Seeds
Rohan	Garlic	<i>Allium sativum</i> L.	Amaryllidaceae	Rhizome
Soi	Stinging nettle	<i>Urtica dioica</i> L.	Urticaceae	Leaves
Dach	Grape vine	<i>Vitis vinifera</i> L.	Vitaceae	Cuttings
Ghande	Onion	<i>Allium cepa</i> L.	Amaryllidaceae	Bulb
Tethwan	Artemesia	<i>Artemisia absinthium</i> L.	Asteraceae	Leaves
Pran	Shallot	<i>Allium porrum</i> Leek	Amaryllidaceae	Bulb
Doon	Walnut	<i>Juglans regia</i> L.	Juglandaceae	Hull

Table.1B *In vitro* efficacy of aqueous extracts of various botanicals at different concentrations in inhibiting mycelial growth of *Phleospora maculans*

Area of inhibition zone (mm ²)				
Conc. (%) Treatment	5	10	15	Mean
Garlic	47.87	63.50	87.97	66.45 ^a
Datura	48.17	54.84	75.36	59.46 ^b
Mulberry	8.44	13.36	16.81	12.87 ^j
Urtica	8.89	17.00	20.18	15.36 ⁱ
Costus	22.66	29.85	38.23	30.26 ^g
Artemesia	21.37	32.62	51.74	35.24 ^f
Onion	38.07	52.28	63.29	51.21 ^c
Grape vine	32.65	38.16	45.78	38.86 ^e
Walnut	15.96	21.99	29.20	22.38 ^h
Shallot	39.26	50.14	55.67	48.25 ^d
Mean	28.33 ^c	37.38 ^b	48.42 ^a	
Carbendazim	0.02			96.83

C.D 0.05

Botanical

2.17

Concentration

1.18

Botanical x Concentration

3.75

Table.2 *In vitro* efficacy of aqueous extracts of various botanicals at different concentrations for per cent inhibition of conidial germination of *Phleospora maculans*

Per cent inhibition of conidial germination				
Conc. (%) treatment	5	10	15	Mean
Datura	76.39	83.90	86.45	82.24 ^a
Mulberry	12.14	16.02	21.56	16.57 ^g
Urtica	9.14	14.96	17.19	13.85 ^h
Costus	27.51	35.72	50.29	37.84 ^c
Artemesia	17.36	24.00	30.21	23.85 ^f
Garlic	79.16	81.18	85.25	81.86 ^a
Onion	29.79	34.62	40.57	34.99 ^d
Grapevine	23.77	29.55	37.04	30.12 ^e
Walnut	11.00	14.83	15.96	13.96 ^h
Shallot	45.36	52.65	59.74	52.58 ^b
Mean	33.18 ^c	38.74 ^b	44.42 ^a	
Carbendazim	0.02			97.41

C.D 0.05

Botanical

1.54

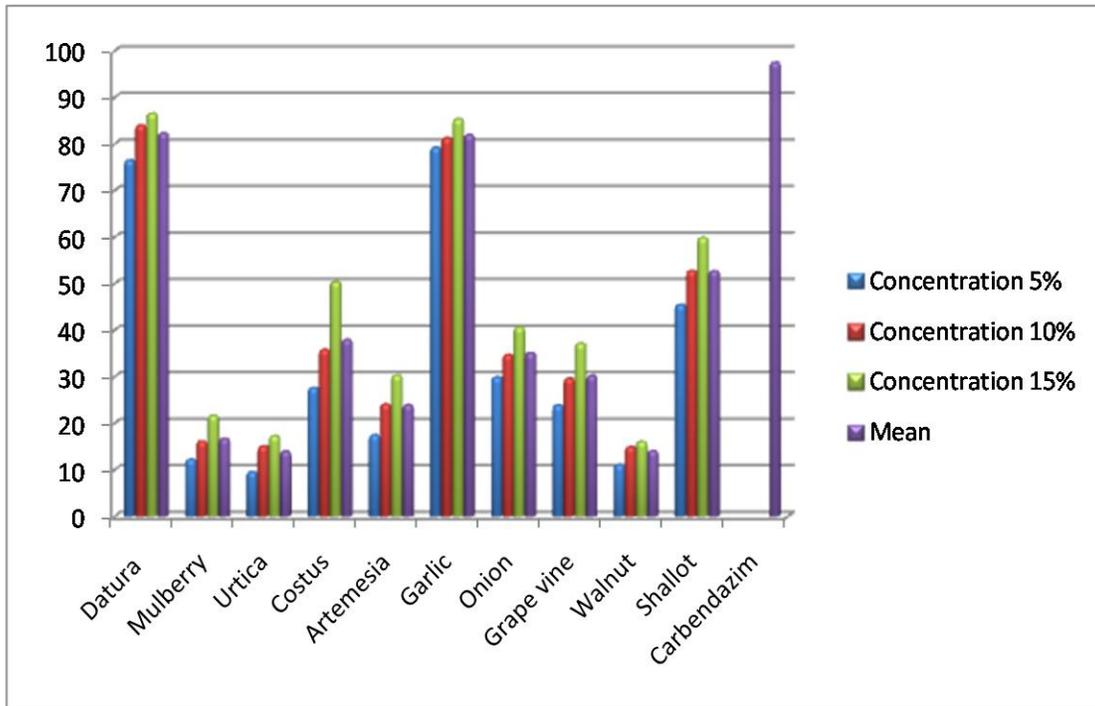
Concentration

0.84

Botanical x Concentration

2.68

Graph.1 Effect of different botanicals used in inhibiting mycelial growth of *Phleospora maculans*



Graph.2 Effect of different botanicals used in per cent inhibition of conidial germination of *Phleospora maculans*

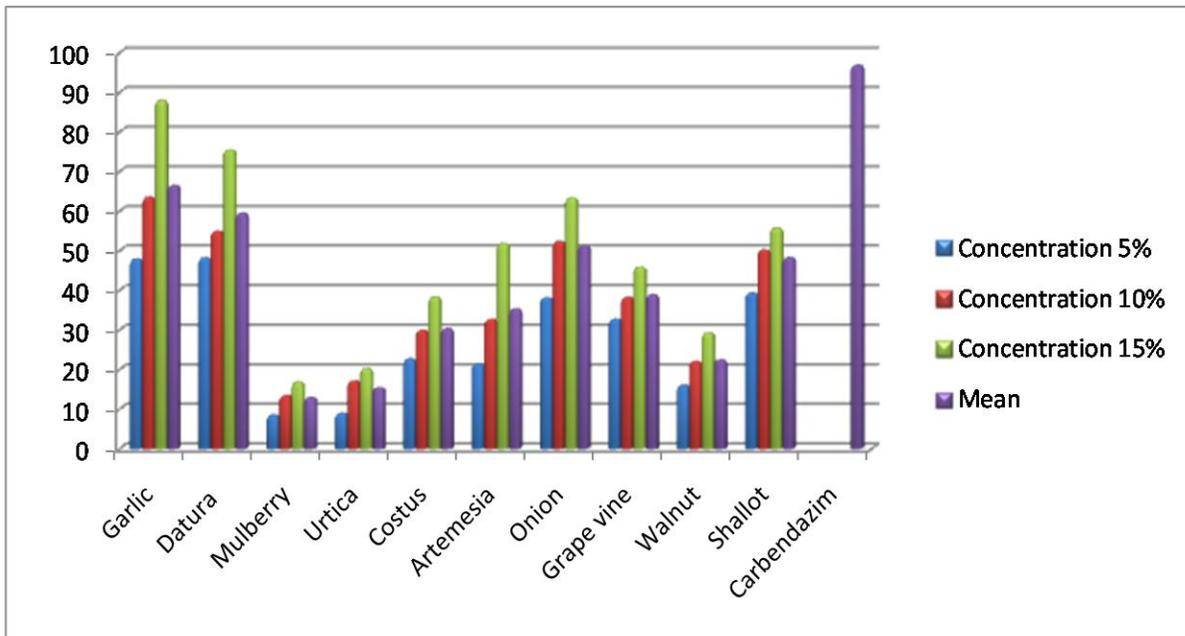


Plate.1 Area of inhibition zone formed by *Allium sativum* against *Phleospora maculans*



Plate.2 Area of inhibition zone formed by *Allium cepa* against *Phleospora maculans*



Data further revealed significant interaction between botanical and concentration. At 5 per cent concentration, highest conidial germination inhibition was recorded in Garlic (79.16%) and lowest in case of Urtica (9.41%). At 10 percent concentration, maximum conidial germination inhibition was recorded in Datura (83.90%), while minimum conidial germination inhibition was recorded in case of Walnut (14.83%), Urtica (14.96%) and Mulberry (16.02%) but all the three were found at par with each other. At 15 per cent concentration, highest conidial germination inhibition was recorded in Datura (86.45%) and Garlic (85.25%) but both of them were at par with each other while lowest inhibition was recorded in Walnut (15.96%) and Urtica (17.19%) and they were also found at par with each other.

From the *in vitro* results, it can be safely concluded that the aqueous extracts of the *Allium sativum* and *Datura stramonium* could be used in the organic farming environment to lessen the impact of the pathogens on mulberry leaf crop, although complete control could not be attained. Yet based on their wide availability and ease of application, it could be used on a wide scale in the moriculture.

Even though more useful oils and other components could be extracted through the use of other synthetic solvent and refined techniques, yet their use by the marginal farmers in the organic environment is limited. Hence the use of aqueous extracts has merits and is simple and could be easily followed even by a layman. This study would benefit the farmers who wish to lessen the impact of leaf spot disease and enhance the cocoon crop. More novel plants need to be explored to increase the resource base for use in eco-organic moriculture in a sustainable mode. They are widely available in the state. Hence these plants could be used in the organic farming environment to lessen the impact of the leaf spot disease at global level and mostly in the temperate region of Jammu and Kashmir. Under *in vitro* conditions, the disease was well controlled by *Allium sativum* and *Datura stramonium*. These need to be further evaluated under *in vivo* conditions so as to make minimum use of chemicals.

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