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Evaluation of botanicals and host infectivity of apple Alternaria leaf blotch pathogen

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ABSTRACT

Apple (*Malus × domestica* Borkh.) is one of the most important fruit crop grown mostly in temperate states of India like Himachal Pradesh and Jammu and Kashmir. Apple orchards are very much prone to diseases like scab, fire blight and leaf blight diseases caused by fungi and bacteria. Among all the diseases, leaf blotch caused by *Alternaria mali* Roberts. has significant effect on the fruit production causing leaf loss and reduction in yield. The experiment was carried out in the state of Meghalaya where Apple varieties are severely attacked by *A. mali.* Host range study of *A. mali* was conducted in different hosts of family Rosaceae and symptoms were noticed in Peach leaves after two days. Various plant extracts were tested against the pathogen under *in-vitro* conditions by using Poisoned food technique. Among all the plant extracts tested garlic clove extract at 10% and 15% gave 100 per cent inhibition and proved as the most effective of all.

1. Introduction

Apple (Malus × domestica Borkh.) belongs to the family Rosaceae which is the most important fruit crop grown extensively in temperate regions. In India, the predominance of apple as a horticultural crop is seen in the Himalayan states viz. Uttaranchal, Himachal Pradesh and Jammu and Kashmir. Apple fruit is found to be highly nutritious with medicinal values. It is rich in vitamin A, B, C and also contains organic acids, proteins and mineral salts such as sodium(Na), potassium(K), calcium (Ca) etc. Consumption of Apple in daily basis provides a number of health benefits that includes weight loss, lowers the risk of heart diseases, improves gut health, lowers risk of cancer and the antioxidants protect the lungs from oxidative damage. Apple is grown in all temperate regions of the world and is ranked second as the most commonly produced fruit.

Apple plantations are subjected to a large number of diseases caused by pathogenic fungi, bacteria, oomycetes and viruses. Fungi cause various diseases, including root rots, leaf spots, leaf blights, blossom blights, fruit decay, fruit spots, defoliation, trunk, branch and twig cankers (Grove et al., 2003). Among them, Alternaria leaf blotch caused by *Alternaria mali*, prevalent in all apple growing areas of the world is an economically important disease. *A. mali* was first described in 1924 in the United States by Roberts. The disease assumed alarming threat to the crop owing to premature defoliation in North Carolina and has potential of becoming threat especially in those apple and loquat producing regions where susceptible cultivars/strains of Delicious are grown (Filajdic and Sutton, 1991). Leaf infection results in 60-85% defoliation and 40 % loss in fruit infection. *A. mali* has attained the status of economically important disease in many Asian countries including Japan and India (Jones and Aldwinckle, 1990). A method for proper management of the disease is needed to reduce the risk of yield loss. An effort was made to study its host range and effect of few botanicals for management of the pathogen.

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2. Materials and methods

Host Range study

This study was undertaken to determine the ability of the test fungus to infect different hosts which belong to the family Rosaceae. The host plants used for the study were peach, plum, pear, rose, cherry blossom. The pathogen *A. mali* was grown on PDA medium for 7 days at 25 ℃ in dark. After 7 to 10 days of incubation, conidia formed by the pathogen were collected in the sterile distilled water and the number of conidia was adjusted to a concentration of 5×10^5 spores/ml. Healthy leaves from each host were collected and washed with sterile distilled water, and blotted dry with paper towels for inoculation. A prepared conidial suspension was sprayed evenly onto the entire lower surface of leaves. Inoculated leaves were kept in Petri plates containing wet filter paper and covered with plastic wrap to maintain high humidity, and the Petri plates were incubated in growth chamber (26℃, in dark).

Six locally available botanicals and were collected from different places of Meghalaya and evaluated for their antifungal potential against the growth of the pathogen by poisoned food technique (Nene and Thapliyal, 1993).

Aqueous leaf extracts of the botanicals were prepared by taking 100 grams of the desired plant material. They were washed twice in running tap water and allowed them to dry for some time. Then subjected to washing again thrice with sterile distilled water and crushed in a surface sterilized pestle and mortar by adding equal amount of sterile distilled water. After proper grinding, the extract was squeezed through three layers of muslin cloth for extracting the juice. The juice was filtered through a Whatmann No. 42 filter paper. The plant extracts were boiling at the temperature of 50°C in water bath to avoid contamination (Mishra and Gupta,2012). The final extracts prepared were considered as the standard plant extracts of 100% concentration. These were evaluated at different concentrations i.e, 5, 10 and 15 % by adding required amount of extract to PDA medium. The plates containing PDA medium without any plant extract was considered as untreated control. After solidification of medium, all the plates were aseptically inoculated by placing a 5 mm mycelial disc of the pathogen in the centre from four day old actively growing pure culture and were incubated at 25± 1 ℃. Each treatment was replicated thrice. The observations on linear growth of fungus were recorded till the entire plate in control was completely covered with mycelium. The per cent inhibition (I) of the pathogen over control was worked out by using formula given by Vincent (1947) as mentioned below:

$$I = \frac{C - T}{T} \times 100$$

Where, I =per cent inhibition of mycelial growth

C = growth in control

T = growth in treatment

3. Results

Host range study

Five hosts belonging to family Rosaceae were used for the host range study under *in-vitro* conditions. Since *Alternaria* causes leaf spots in most cases, leaves were used and inoculation was done as explained in the chapter materials and methods. Positive reaction was seen on peach leaves after 2 days of inoculation. On the leaves, purple brown to black spots were noticed and in later stage spots coalesced causing necrosis of entire leaf. The results were shown in table 1.

Table1: Results of host range study conducted for the apple leaf blotch pathogen, *A. mali.*

S.No	Botanical name	Common name	Reaction
1	*Prunus persica*	Peach	+
2	*Rosa chinensis*	Rose	-
3	*Prunus domestica*	Plum	-
4	*Pyrus communis*	Pear	-
5	*Prunus cerasoides*	Cherry blossom	-

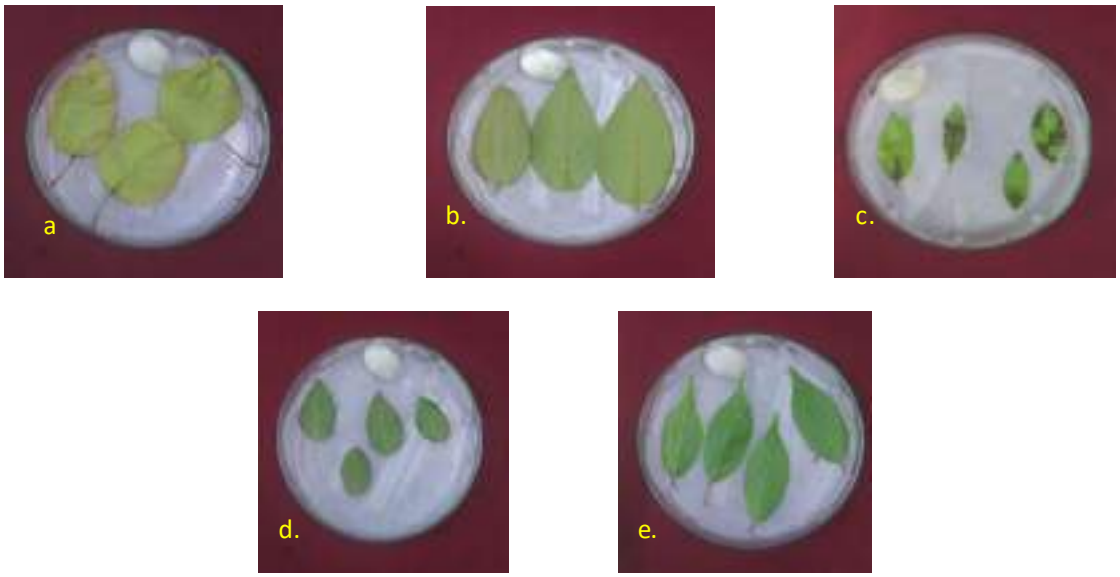

Figure: Host plants used for the test, a.Plum, b.Pear, c.Peach, d.Rose, e.Cherry blossom

***In-vitro* efficacy of plant extracts against the pathogen**

Six plant extracts were tested for their efficacy on growth of the pathogen at three different concentrations at 5, 10 and 15 per cent. The experiment was conducted by following poisoned food technique. The per cent inhibition was worked out based on growth of the pathogen in culture plates.

The results are presented in figure and significant difference of per cent inhibition was seen among all plant extracts. The study revealed that among six extracts evaluated, Garlic at 10 and 15 per cent gave the highest per cent inhibition of 100 per cent of mycelial growth and the lowest per cent inhibition of 21.48 per cent was recorded in *Ageratum sp.*

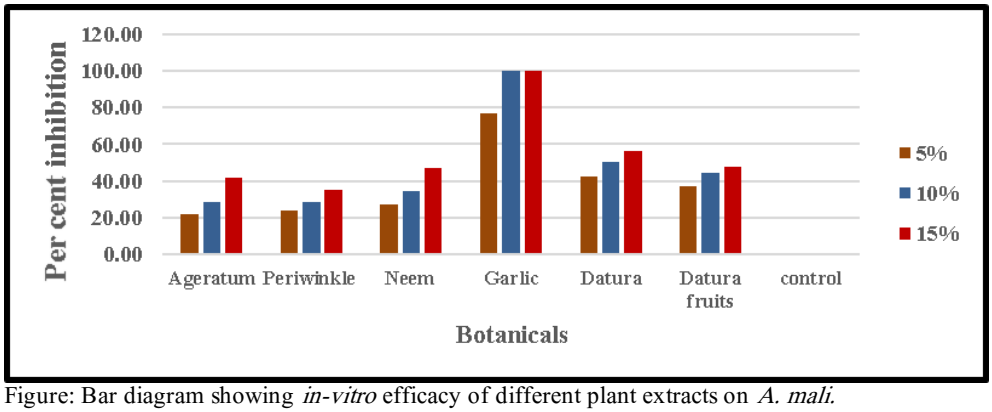

Figure: Bar diagram showing *in-vitro* efficacy of different plant extracts on *A. mali.*

Discussion

The study revealed that among the five different host selected from family Rosaceae for their reaction to *A. mali*, only peach leaves showed the characteristic symptoms after three days of inoculation. Inoue and Hideo (2000) cross-inoculated *Alternaria* isolates among apple, peach and pear and found that the isolates were host-specific . However, in the current study the *Alternaria* isolate from apple also caused infection in Peach. Tanahashi *et al.* (2016) conducted similar study and found that *A. mali* induced infection in European pear. This finding contradicted the current study as there were no symptoms were observed in pear leaves and it shows that pear varieties grown in Meghalaya might be resistant to the pathogen.

Garlic clove extract at 10 and 15 % concentration gave the highest per cent inhibition of 100 per cent and the lowest per cent inhibition of 21.48 per cent was recorded in the treatment of Ageratum leaf extracts. Datura at 15 % concentration was found effective in controlling the pathogen with a per cent inhibition of 56.3 per cent. Thaware *et al.* (2010) performed in-vitro screening of plant extracts and proved garlic clove extract to be the most effective one against *A. alternata* in cowpea which was similar to the current study. Zade *et al.* (2018) also obtained similar results and found that garlic extract at 10% concentration as the most effective against *A. alternata*. Ranaware *et al.* (2010) also studied antifungal activity of some botanicals and obtained that garlic extract was the most effective one followed by

Datura which was at par with the current study. The results of the present research work are in agreement with the earlier reports.

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