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Influence of Inoculum Density of *Alternaria brassicae* on Development of Alternaria leaf Spot in Cauliflower

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ABSTRACT: Cauliflower belongs to the family Brassicaceae and is one of the most important winter vegetable after cabbage in terms of area and production across the globe. Alternaria leaf spot is the most destructive disease of cauliflower crop at vegetable curd stage and seed setting stage. It has been attributed to cause 30-50% losses and in most severe cases, the losses in yield may reach up to 98% in seed crop. The pathogen *Alternaria brassicae* was found to produce typical symptoms of the disease as small, irregular brown spots with concentric rings, also surrounded by yellow chlorotic halo. Severely affected seedlings showed dark brown spots with damping off symptoms. During the present investigation, different inoculum levels of the pathogen- *Alternaria brassicae* were evaluated to assess their effect on development of Alternaria leaf spot disease on cauliflower. The lowest Percent Disease Index (7.41%) was noticed with 5g of inoculum load whereas 40g of inoculum load caused maximum disease development with Percent Disease Index (50.81%).

Keywords: *Alternaria brassicae*, Cauliflower, Disease severity, Inoculum load, Percent Disease Index.

INTRODUCTION

The cauliflower, an important vegetable crop, is cultivated in different climatic conditions, from temperate to tropics, and available in the market round the year. The major cauliflower growing countries in the world are China, India, United States of America, Bangladesh, Australia, England and Germany. *Alternaria* leaf spot is incited by *Alternaria-Alternaria brassicae* and *Alternaria brassicicola*. The *A. brassicicola* produces dark coloured, zonated leaf spots, whereas *A. brassicae* develops light brown or grey colored leaf spots on cruciferous vegetables.The pathogen - *A. brassicae* was reported as the causal agent of dark leaf spot of brassicas, for the first time in Thailand in 1983; However, its pathogenicity was not established (Sontirat *et al.*, 1983); the disease was reported to inflict considerable loss to the crop in the country (Visethsung and Saranak 1988). Brassicas are reported to be susceptible to a dark leaf spot, often known as black spot (Brazauskiene *et al*., 2011) or Alternaria blight (Kumar *et al*., 2014). At least 20% of agricultural waste is attributed to *Alternaria* spp. Infection of seeds and seedlings, as well as edible produce, causes losses of 15 to 70% (Kumar *et al.*, 2014). In severe cases, the yield losses may go as high as 80%. In India, the loss due to disease in cauliflower vegetable crops is about 30-50%, whereas 5-30% loss is caused by *A. brassicae* alone (Mishra *et al.*, 2012). The loss up to 98 per cent was reported in cauliflower seed crop due to *Alternaria* spp. from Germany (Nowakowska *et al.*, 2019). A better understanding of the Influence of inoculum load on disease, as related to pathogens, may lead to better prediction of disease development and more efficient disease management strategies. The present study was undertaken to determine the effects of different inoculum load of *Alternaria brassicae* inducing leaf spot with concentric rings in cauliflower on the development of Alternaria Leaf Spot on cauliflower crop.

MATERIALS AND METHODS

Pot Experiment. To ascertain the impacts of *Alternaria brassicae* inoculum load under pots, a pot culture experiment was carried out in the Department of Plant Pathology's greenhouse during the *Rabi* season of 2020–21.

Multiplication of pathogen on Sand Maize media (9:1). The pathogen *A. brassicae* was multiplied on the Sand Maize media in 9:1 ratio. For this, 90g of Sand was mixed with 10g of Maize and this mixture was taken in conical flask and then added 20ml of sterile distilled water. Then, conical flasks were plugged with cotton and sterilized in an Autoclave at 15psi for 20 min. at 121ºC. Five millimeters mycelial discs were cut with the help of cork borer from seven day-old culture of pathogen and transferred to the Sand Maize media under aseptic conditions. These flasks were plugged

with cotton and incubated in BOD at 28 ± 2 ºC for 15 days. The 15 day-old-fungal culture was used for inoculation in pot.

Pot Experiment. To ascertain the impacts of *Alternaria brassicae* inoculum load under pots, the experiment was carried out in the Green house of Department of Plant Pathology, RPCAU Pusa during the *Rabi* season of 2020–21. This experiment was carried out in completely randomized design in pots, maintaining four replications for each treatment. Pots were inoculated with different inoculum load as mentioned below.

Table 1: Different level of inoculum load.

Sr. No.	Treatments (g/kg of soil)
1.	5 g
2.	10 g
3.	20 g
4.	30 g
5.	40 g
6.	0 g (control)

Healthy cauliflower seedlings were planted in the inoculated pots (only one plant in each pot). Intensity of the disease was determined in all the treatments based on the standard 0-9 grade disease rating scale (Mayee and Datar 1986) as detailed below in Table 2.

Table 2: Standard disease rating scale (0-9) for accessing Percent Disease Index (PDI) of Alternaria leaf spot of cauliflower

Rating scale	Percentage area of leaf infected
0	No symptoms on leaves
1	Small, irregular brown spots covering 1 percent or less of the leaf area
3	Small, irregular, brown spots with concentric rings covering 1-10 percent of the leaf area.
5	Lesions enlarging, irregular, brown with concentric rings covering 11-25 percent of the leaf area.
7	Lesions coalescing to form irregular brown patches with concentric rings. Covering 26-50 percent of the leaf area. Lesions also on stem and petioles.
9	Lesions coalescing to form irregular, dark brown patches with concentric rings covering 51 percent or more of the leaf area. Lesions on stem and petioles.

Per cent disease Index was calculated by using formulae:

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of individual ratings}}{\text{Total number of plants/Leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

Statistical analysis. The data generated during present investigation were presented in tabular form and analyzed using the statistical method applicable under Completely Randomized Design as per procedure given by Gomez and Gomez (1983).

RESULTS AND DISCUSSION

Standardization of inoculum for disease development under pot conditions. Sick pots were prepared by using the method described in previous chapter. The cauliflower plants were grown in sick pots in four replications for 30 days and observations on disease development (PDI) were taken at 15 days and 30 days; and presented in Table 3.

It is clear from the data presented in table that, at fifteen days after transplantation, 5g of inoculum load showed small, irregular brown spots with PDI of 2.75%. There was increase in disease development with increase in the load of inoculum. The inoculum level of 10g recorded small, irregular brown spots with concentric rings with 8.35 PDI and that of 20g recorded enlarged lesions, irregular, brown spots with concentric rings with PDI of 13.82%. When inoculum level was increased to 30g, irregular brown patches with concentric rings developed and disease development was registered up to 19.49% (PDI). 40g of inoculum was found to form irregular, dark brown patches with concentric rings with PDI upto 25.12%. There was further increase in disease development when examined after 30 days. There was maximum disease development with highest PDI (50.81%) after 30 days at the inoculum level of 40g and comparatively less PDI was observed in treatments at lower inoculum level as evident from the result that, after 30 days of transplantation, 5g of inoculum showed PDI of 7.41. 10g of inoculum recorded 25.06 PDI. 20g of inoculum showed PDI of 36.04, whereas 30g of inoculum registered PDI of 47.05 per cent.

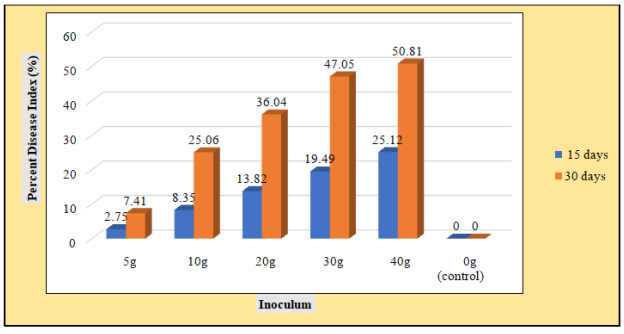

Fig. 1. Effect of different level of inoculum on disease development under pot condition.

Table 3: Effect of different level of inoculum on disease development under pot condition.

Treatments (g/kg of soil)	Percentage area of leaf infected	Percent disease index after 15 days	30 days
5g	Small, irregular brown spots covering 1 percent or less of the leaf area	2.75	7.41
10g	Small, irregular, brown spots with concentric rings covering almost 10 percent of the leaf area	8.35	25.06
20g	Lesions enlarging, irregular, brown with concentric rings covering almost 25% of leaf area.	13.82	36.04
30g	Lesions coalescing to form irregular brown patches with concentric rings. Covering almost 50% of leaf area. Stem also affected.	19.49	47.05
40g	Lesions coalescing to form irregular, dark brown patches with concentric rings covering more than 50% of leaf area.	25.12	50.81
0g (control)	No symptoms	00.00	00.00
CD at 5%		0.59	1.28
SE(m)±		0.20	0.42
C.V.		3.32	2.98

CD: Critical difference; CV: Coefficient of variation

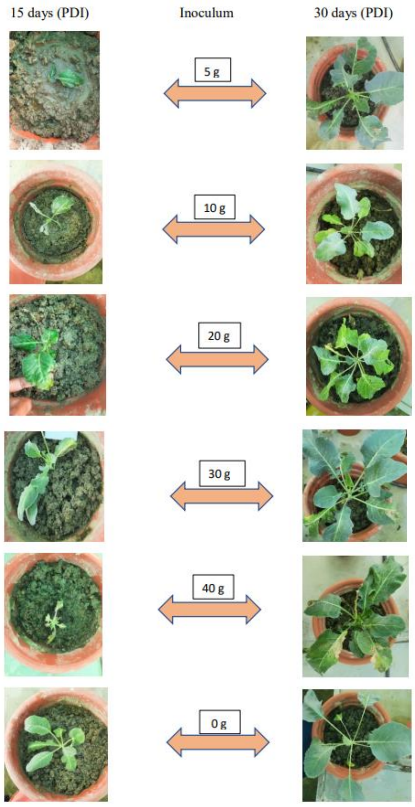

Plate I. Effect of different level of inoculum on disease development.

Various levels of inoculum of the pathogen - *Alternaria brassicae* were evaluated to identify the effective amount of inoculum in order to develop a sick pot for successful disease development. The cauliflower plants were grown in inoculated (sick) pots and observations were taken for 30 days. The inoculum load of 5g/pot showed the least disease development (PDI). The trend of disease development was found to increase with increasing inoculum levels. When the inoculum load was increased to 40g/pot, there was maximum disease development (50.81% PDI).

In present study, the disease first appeared at 9-11 days after inoculation. The spots of characteristic concentric circumferences were occasionally found to be surrounded by yellow chlorotic halo. Later lesions coalesce to form irregular, brown to dark brown patches with concentric rings, it was also found to affect petiole, stem and curd at later stages. Severely affected seedlings showed dark brown spots with damping off symptoms. The disease development in the present study were in accordance with those described by earlier workers on various crucifer crops (Kadian and Saharan 1983; Verma and Saharan 1994). The need of standardizing the inoculum density in the soil-fungicide evaluation test was highlighted by Richardson and Munnecke (2011). According to Siddiqui *et al.* (2011), the two formulations had a negative effect on *Chenopodium album* shoot biomass, and this effect was exacerbated by an increase in *Alternaria alternata* inoculum concentration. Ahmad and Siddiqui (2019) revealed that plant length, plant fresh weight, shoot and root dry weight, chlorophyll and carotenoid decreased progressively with the corresponding increase in the inoculum levels of *Alternaria dauci* on carrot. The increase in the disease intensity by increasing the level of inoculum conforms to the basic principle of disease development due to host-pathogen interaction.

CONCLUSIONS

The current research finding showed the effect of various inoculum level of the *Alternaria brassicae* on the development of Alternaria leaf spot on cauliflower. The disease development was markedly influenced by the concentration of inoculum as evident from increasing intensity of disease with increase in the inoculum load. Soil application of 40g inoculum of pathogen caused maximum disease development (50.81%) under pot condition. Thus, inoculum level largely determine the quantum of disease development. Thus, the present finding will be helpful in predicting the disease development based on availability of inoculum level, thereby deciding the management strategy to prevent the disease development.

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Conflict of Interest. None.

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