Severe damage of the foliage or seed germination in crucifers including cauliflower (Brassica oleracea L. var. botrytis sub var. cauliflora) occurs due to Alternaria blight caused by Alternaria brassicae (Berk) Sacc. (Kolte, 1985; Tewari, 1991; Verma and Saharan, 1994) and A. brassicicola (Schwein) Wiltshire (Jung et al., 2002). Black spot of different crucifers viz-oil seed rape, cabbage, cauliflower and mustard have been reported in many countries Italy (Tosi and Zazzerini, 1985), USA, UK and several other European countries (Gladders, 1987), Canada (Berkeramp and Kirkham, 1989; Conn and Tewari, 1990), Iran (Nourani et al., 2008) including India (Meena et al., 2010). For infection by Alternaria spp., studies on factors such as concentration of conidia (Humpherson-Jones and Ainsworth, 1982), plant age (Awasthi and Kolte, 1994), temperature, humidity and wetness period (Hong and Fitt, 1995) are reported to influence the severity of the leaf spot diseases in case of mustard (Sharma, 2002). Alternaria is greatly influenced by weather with the highest disease incidence reported in wet seasons and in areas with relatively high rainfall (Humpherson- Jones and Phelps, 1989).

For evaluation of resistance in cabbage and cauliflower against Alternaria black leaf spot, detached leaf inoculation method has been found to be the simple, easy and fast method (Sharma, 2004). To develop precision, sensitivity and for testing different pathogenic isolates of Alternaria brassicae and A. brassicicola the age of the host was used as a basic parameter to distinguish pathogenic levels. Four stages of leaves 15, 30, 45 and 60 days after sowing of the three susceptible cauliflower varieties was used to understand this host pathogen relationship.

MATERIALS AND METHODS

Isolation and maintenance of fungal cultures: Twenty-two isolates of Alternaria species including sixteen Alternaria brassicae and six Alternaria brassicicola isolates were isolated from infected cauliflower leaf samples collected from different cauliflower growing areas of India viz., Uttar Pradesh, Delhi, Rajasthan, Haryana, West Bengal and Tamil Nadu. These isolates were purified and maintained on Potato Dextrose agar (PDA) media at 25°C.

Inoculum preparation: Seven-day old Alternaria cultures grown on PDA plates were flooded with distilled water and spores were released by agitation with a sterile brush. The resulting spore suspension density was adjusted to 4 x 10⁴ spores per ml by haemocytometer. One drop of Tween-20 per one ml suspension was added as a wetting agent.

Plant material: Three cauliflower cultivars namely, Pusa Deepali (early-maturing), Pusa Meghna (early-maturing) and Pusa Sharad (mid-growing) were evaluated against six Alternaria brassicae and six Alternaria brassicicola isolates were isolated from infected cauliflower leaf samples collected from different cauliflower growing areas of India viz., Uttar Pradesh, Delhi, Rajasthan, Haryana, West Bengal and Tamil Nadu. These isolates were purified and maintained on Potato Dextrose agar (PDA) media at 25°C.

ABSTRACT: The age of host leaf and pathogenic nature are very important parameters while evaluating large number of isolates against any crop germplasm. Keeping this in view, three varieties of cauliflower viz., Pusa Deepali, Pusa Meghna and Pusa Sharad were evaluated using detached leaf method, for level of infection by twenty-two isolates of A. brassicae (16) and A. brassicicola (6) collected from different states of India. Four different ages of leaves viz- 15, 30, 45 and 60-day were evaluated for their comparative susceptibility to the pathogens. Percent Disease Index values differed significantly and disease was expressed only in 60-day old leaves with no symptoms in 45-day old leaves, clearly indicating susceptibility of matured leaves. All six A. brassicicola isolates infected both 45 and 60 days old leaves while full infection of Alternaria brassicae was found only on 60 days old leaves.

Key words: Alternaria brassicae, Alternaria brassicicola, black leaf spot, cauliflower

Host age as predisposing factor for incidence of black leaf spot of cauliflower caused by Alternaria brassicae and Alternaria brassicicola

SWATI DEEP and PRATIBHA SHARMA*
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012

*Corresponding author: psharma032003@yahoo.co.in
Detached leaf inoculation: Leaves were detached from three varieties of cauliflower cultivars of four age groups viz. 15, 30, 45 and 60 DAS. Leaves were properly washed under running tap water and then surface wiped off with 70% alcohol and 2 microlitres of $4 \times 10^4$ spores ml$^{-1}$ spores were inoculated with a fine needle (Dispovan, 2.5ml) while sterile distilled water was applied on control. The leaves were placed inside moist chambers in greenhouse conditions and were observed for appearance of disease symptoms on third day after inoculation.

Development of disease symptoms was observed on third day after inoculation in the inoculated leaves while controls remained free from symptoms. Mean values were used in the analysis of data. Disease scores of all isolates on individual leaves were recorded for each treatment using a 1-9 disease rating scale (Sharma, 2004) where, 1= no infection, 9= 81-100% infection. Average sizes of spots (Mean Diameter of spots) were recorded in centimeter depending on the spreading of lesions. Average disease severity value as percent disease index (PDI) was calculated as: PDI = [Σ (Severity grade x Number of Leaves/Plant) / (Maximum grade x Total number of leaves/Plants scored)] x 100. The treatment differences were statistically analyzed by performing two-way ANOVA using PRISM version 3.0 statistical software.

RESULTS AND DISCUSSION

All the sixteen *Alternaria brassicaceae* and six *Alternaria brassicicola* isolates were evaluated in the form of size of lesions and percent disease incidence on three different varieties viz., Pusa Deepali, Pusa Meghna and Pusa Sharad. None of the above isolates could produce any symptom in 15 and 30 days. The symptoms were initiated in the form of dot spot 45 DAS which intensified on 60-day old leaves (Fig 1). Each variety showed a consistent reaction to both *Alternaria brassicaceae* and *Alternaria brassicicola* in both age groups. Variable sizes of spots appear though small in size. The isolates can be grouped based on the sizes of the visible spots viz., no spot (mean diameter is zero cm), small spot (mean diameter <60cm) and big spots (mean diameter >60cm). Variable reactions in size of spots (Table 1a) and PDI values (Fig. 2a, b, c) were observed in *A. brassicaceae* isolates on all three cultivars of cauliflower. In 45-day old Pusa Deepali leaves only seven out of sixteen *A. brassicaceae* isolates (U8, U9, D1, D4, R3, W3 and T5) were able to produce leaf spots of size 0.05-0.63cm. The disease severity (PDI) value ranged 5.0%-43.2%. In Pusa Meghna (45-day old) five isolates (U5, U8, U9, D1 and T5) produced spots of size 0.20-0.52cm with PDI value 7.4%-59.2% while in Pusa Sharad (45 days old) only U8, D5 and T5 could produce symptoms of 0.40, 0.37 and 1.16cm in diameter with PDI value of 15.5, 22.2 and 100.0%, respectively. Among sixteen isolates, seven (U3, U4, U7, D2, D6, H1 and W2) could not produce symptoms of black leaf spot on 45-day old leaves though infection by all isolates could be observed on 60-day old leaves. In Pusa Deepali, diameter of spots varied 0.03 cm -0.92 cm with PDI value ranging 5.0%-83.3%. In Pusa Meghna and Pusa Sharad the lesion size varied from 0.10-1.15 cm and 0.13-1.16 cm, respectively with PDI of 5.0-100.0%. The isolates which are not able to produce any symptoms in 45-day old leaves produced noticeable lesions of 0.67-2.67 cm with PDI value of 5.0%-34.6% in 60-day old leaves of all three cultivars. Among all *A. brassicaceae* isolates, two (U8 and T5) produced maximum size of spot with highest disease severity. Significant differences were found among all sixteen isolates of *A. brassicaceae* and all three cultivars 45 and 60 DAS at p<0.0001 significance level.

Similarly, when six *Alternaria brassicicola* isolates were inoculated on the same three cultivars of two age groups of 45 and 60-day old, all were found to produce lesions of 0.36-1.15 cm with PDI value ranging 38.9 - 100.0% (Table 1b and Fig 4 d, e, f). The size of lesions in each variety of cauliflower were found to be 0.36-0.73 cm (Pusa Deepali), 0.43-0.80 cm (Pusa Meghna) and 0.43-0.60 cm (Pusa Sharad) in 45-day old leaves and 0.48-1.15 cm (Pusa Deepali), 0.78-1.08 cm (Pusa Meghna) and 0.83-1.04 cm (Pusa Sharad) in 60-day old leaves, respectively. The disease severity value of all six *A. brassicicola* isolates was found to be more than 50% infecting all three cultivars at both 45 and 60 days.

The results showed that the appearance of symptoms varying in all the three cultivars of two age groups depend on the aggressiveness of isolates. Two *Alternaria brassicaceae* isolates are highly aggressive and produces large necrotic lesion with high PDI values like 88.8% (U8) and 100% (T5) in 60 DAS Pusa Sharad, some isolates were moderately aggressive as they were effective in producing more than...
Table 1a. Size of spots (mean diameter in cm) of sixteen Alternaria brassicae isolates on 45 and 60 day old leaves of three cauliflower cultivars

<table>
<thead>
<tr>
<th>Alternaria brassicae isolates</th>
<th>Pusa Deepali (45 DAS)</th>
<th>Pusa Meghna (45 DAS)</th>
<th>Pusa Sharad (45 DAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U3</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>U4</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>U5</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>U7</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>U8</td>
<td>0.17</td>
<td>0.35</td>
<td>0.00</td>
</tr>
<tr>
<td>U9</td>
<td>0.23</td>
<td>0.35</td>
<td>0.00</td>
</tr>
<tr>
<td>D1</td>
<td>0.13</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
<td>D2</td>
<td>0.00</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
<td>D4</td>
<td>0.20</td>
<td>0.52</td>
<td>0.00</td>
</tr>
<tr>
<td>D5</td>
<td>0.00</td>
<td>0.52</td>
<td>0.00</td>
</tr>
<tr>
<td>R3</td>
<td>0.05</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>H1</td>
<td>0.00</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>W2</td>
<td>0.00</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>W3</td>
<td>0.29</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>T5</td>
<td>0.63</td>
<td>0.20</td>
<td>0.00</td>
</tr>
</tbody>
</table>

SEM± 0.147 0.175 0.139
CD (p<0.0001) 3.328 3.952 3.168

*Means of three replications

DAS: days after sowing

Table 1b. Size of spots (mean diameter in cm) of six Alternaria brassicicola isolates on 45 and 60 day old leaves of three cauliflower cultivars

<table>
<thead>
<tr>
<th>Alternaria brassicicola isolates</th>
<th>Pusa Deepali (45 DAS)</th>
<th>Pusa Meghna (45 DAS)</th>
<th>Pusa Sharad (45 DAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R5</td>
<td>0.36</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>R6</td>
<td>0.55</td>
<td>0.69</td>
<td>0.43</td>
</tr>
<tr>
<td>T1</td>
<td>0.63</td>
<td>0.63</td>
<td>0.85</td>
</tr>
<tr>
<td>T2</td>
<td>0.52</td>
<td>0.52</td>
<td>1.08</td>
</tr>
<tr>
<td>T3</td>
<td>0.50</td>
<td>0.57</td>
<td>1.00</td>
</tr>
<tr>
<td>T4</td>
<td>0.37</td>
<td>0.86</td>
<td>0.95</td>
</tr>
</tbody>
</table>

SEM± 0.221 0.155 0.107
CD (p<0.0001) 1.878 1.314 0.912

*Means of three replications

DAS: days after sowing

50% disease severity index value of PDI like U5 (54.4%), D4 (59.2%, 60 DAS Pusa Meghna) and D5 (64.1%, 60 DAS Pusa Sharad) while others were found to be less aggressive as they could produce less than 50% PDI in leaves of all the cultivars at both 45 and 60 DAS.

In this study, age of plants played a significant role in disease development. It was observed that the younger plants at 15 and 30 DAS were not susceptible to infection for all three cultivars as they could not produce any leaf spot symptoms. The disease was initiated on crop at 45 DAS, which expressed fully when the crop was 60-day old with high PDI values. Similar observations have been reported in white rust development among leaves of different ages of Brassica campestris (Verma et al., 1983) who also have reported that the age of the leaves does not greatly affect the initiation of disease, but it affects the subsequent development of the pathogen in the host tissue. Older sunflower leaves were found to be more susceptible to infection by Alternaria helianthi than younger leaves at both optimal and suboptimal temperatures. The number of lesions per square centimeter of leaves was approximately double the number that formed on the younger leaves (Allen, 1983). Depending on the particular plant- pathogen combination, the age of the host plant at the time of arrival of the pathogen may affect the development of infection and of an epidemic...
Fig. 2. Disease Severity (Mean PDI value) appeared on both 45 and 60 DAS leaves of Pusa Deepali, Pusa Meghna and Pusa Sharad respectively caused by Alternaria brassicae (a, b, c) and Alternaria brassicicola (d, e, f) isolates.

considerably. In a similar situation it was reported that younger leaves of sesame are more susceptible to infection due to more stomata (Shukla and Chand, 1975), higher nitrogen and moisture content than in older leaves (Mamza et al., 2008). Alternaria brassicae and A. brassicicola usually infects cauliflower and mustard leaves by producing an appresorium before directly penetrating the cuticle and epidermis. It may be possible, therefore, that all these physiological factors play an important role in disease development, which need to be further evaluated. Older age susceptibility to Alternaria infection has been discussed by Horsefall and Dimond (1957) who introduced the concept that the low sugar levels in plants make them susceptible to necrotrophic pathogens such as Alternaria. The Alternaria infection at the late vegetative to budding stage caused greatest losses in yield was reported by Chattopadhyay (1999), which was further supported by Meena et al. (2004) who found the Alternaria blight severity 45 d.a.s. in Mustard.

The three cultivars used in this study namely Pusa Deepali, Pusa Meghna and Pusa Sharad belonging to different maturity groups are similar in expression of the disease symptoms. The age of the leaves was observed to be the common factor. The results clearly indicate the significant difference (at p<0.0001) among the average size of spots (Table 1) and PDI value (Fig. 2) of A. brassicae and A. brassicicola isolates, respectively causing spot on leaves both at 45 and 60 days in all three varieties of cauliflower namely Pusa Deepali, Pusa Meghna and Pusa Sharad. But the appearances of spots were found to be higher in 60-day old leaves. Alternaria brassicicola isolates are highly aggressive than Alternaria brassicae in producing black leaf spot in cauliflower leaves. It is imperative to keep the age of
the host as a priority parameter in resistance breeding programme so as to avoid encountering the young leaves resistance in cauliflower crop against both the pathogens.

ACKNOWLEDGEMENTS

Authors are grateful to Indian Council of Agricultural Research for providing funds under Outreach Programme on “Diagnosis and management of leaf spot diseases of field and horticultural crops” and Head, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi for providing facilities.

REFERENCES


Received for publication: September 24, 2011
Accepted for publication: February 23, 2012