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Identification of pathogen of *Alternaria* leaf spot disease on Asalio and effect of meteorological factors on the development and progression on disease

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Abstract

Studies were undertaken for Management of *Alternaria* leaf spot of Asalio Caused by *Alternaria alternata*. The disease was observed to exhibit an increasing trend in Asalio growing areas. The study was carried out to identification of pathogen and find out the association between weather factors and disease severity. *A. alternata* is a primary pathogen in Asalio, causing a leaf-spot disease. These culture of pathogen was identified by to "Indian Type Culture Collection Identification/Culture supply Services, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012" as *Alternaria alternata* and observed the influence of weather factors on disease development. weather factors (temperature and relative humidity) had direct and significant influence on the disease incidence and disease progress. Maximum incidence of *Alternaria* leaf spot disease was observed at moderate temperature 24.0- 26.9 °C and relative humidity 77.3- 78.7% with AUDPC value (area under disease progressive curve) ranging between 114.73 to 483.39 in pot culture. However, still more epidemiological studies are required to strengthen the forecasting and prediction mechanism of the disease which will ultimately minimize the yield losses caused by the disease.

Keywords: *Alternaria alternata*, temperature, relative humidity, AUDPC value (area under disease progressive curve), *Lepidium sativum*

Introduction

Asalio (*Lepidium sativum* Linn; Family: Brassicaceae) is a medicinal plant. *Lepidium* name derives from Greek word 'lepidion' means small scale probably refers to the form of fruits and *sativum* is derived from 'serere' mean to cultivate, to plant or to sow. It is known as "Common cress", "Land cress", "Haliv", "Garden cress" or "Chandrasur" in some regions of India (Gokavi *et al.*, 2004) [3]. Asalio in local language is known as "Chandrasur" and considered as an important rabi medicinal crop in India (Tiwari and Kulmi, 2004) [27].

Asalio is distributed in various parts of India and Europe including Britain, France, Italy and Germany. Cultivation of *Lepidium sativum* has been taken up in several parts of India *viz.*, states of Madhya Pradesh, Uttar Pradesh, Rajasthan, Gujarat, Maharashtra etc. In Rajasthan more than 8000 ha area is under its cultivation (Paroda *et al.*, 2014) [17]. According to National Horticulture Board, area of aromatic and medicinal plant in 2017-2018 in India is 650 ha with decrease in annual growth rate 2.11 per cent as compared to 2016-2017 and production in India 1037 Million Tonnes with increase in growth rate 6.69 per cent and productivity is 1.6 tonnes/ha in 2017-18.

Asalio is mainly affected by diseases like *Alternaria* leaf spot & white rust. The *Rabi* season Asalio crop in India is commonly affected by *Alternaria* leaf spot. The disease causes extensive damage to the quality and quantity of the foliage and grains. If persistent cloudy and cool weather prevails the blight appears after flowering stage of the crop. *Alternaria* genus is the largest genus. It is distributed worldwide and has been reported on 115 plant genera from 43 plant families that cause blight and leaf spot disease (Neergaard, 1945) [14].

Alternaria genus has a worldwide distribution. Its species have a wide host range fungi and responsible for causing leaf spot diseases in number of economically important crop plants. *Alternaria* causes disease among the most common of many plants throughout the world and the total yield losses caused by the fungus on its wide range of host sometimes exceeds the total yield loss caused by any other pathogen (Agrios, 1997) [5]. A great number of species were recorded for the genus *Alternaria* infecting different crops causing world-wide economic loss (Kirk *et al.*, 2008) [8].

Presently, *Alternaria* leaf spot is the most destructive disease of Asalio. In the year 1967 *Alternaria* blight of Asalio caused by *Alternaria brassicae* was first reported from Kaffa province (Stewart and Dagnalechew, 1967) [26], Ethiopia. In India for the first time Melkania (1980) [12] reported *Alternaria alternata* as instant of *Alternaria* leaf spot on leaves of cress at Almora and in the same year Singh (1980) reported that *A. alternata* as causal agent of *Alternaria* leaf spot on cress at College Farm, Banaras Hindu University, Varanasi (U.P.).

Alternaria is included in order Hyphomycetes, family Dematiaceae, genus dictyosporic and it is a Fungi Imperfecti. *Alternaria* genus was first reported by Nees (1816). *A. alternata* belongs to Longicatenatae according to Neergaard (1945) [15]. Melkania (1980) [13] reported that *Alternaria* leaf spot on leaves of Asalio was caused by *A. alternata* and Utikar and Padule (1980) [28] described its morphology. He reported that conidiophores of *A. alternata* were simple, light brown, variable in length ranging from 17.10 to 61.56 µm and mostly 2-3 septate rarely 4-5 septate. Conidia were found light to dark brown in colour, uniform with 0-2 longitudinal septa and 1-6 transverse septa, and variable in shape and size, mostly oval shape with rudimentary beak and in size measuring about 10.26-77.52 x 4.56-14.82 µm. Simmons and Roberts (1993) observed three-dimensional sporulation patterns of *A. alternata* in electron microscope at 50 magnification.

The symptoms produced by *Alternaria brassicae* on *Lepidium sativum* L. are given in detail by (Singh and Upadhyaya, 1971) [23]. The initiation of disease symptom is from basal leaves in the form of small, yellow, circular patches which become necrotic, having 2-5 concentric rings and light brown in colour (1-7 mm in diameter). Sometimes these spots coalesce with each other and occupy large blighted area. In later stage, spots become larger in size with distinct concentric rings and dark in colour. The severity of the disease increases upto mid January. At this stage, the stem and floral parts of plant also become diseased. Finally, whole plant shows typical blight symptoms. Fungal colonizes the in xylem of the host plant, and as a result, blockage and breakdown of the xylem lead to wilt disease symptoms such as, leaf wilting, yellowing and eventually the death of the plant.

Asalio is an upright, smooth herb, upto 45 cm in height, leaves are pinnatisect and wholly lobed. (Prajapati *et al.*, 2003) [19]. It is usually cultivated for its leaves, which are used in salad, sandwiches and seeds with high nutritional value can be exploited as a functional food ingredient (Eddouks *et al.*, 2005) [2]. It is an annual, erect, glabrous, herb growing up to the height of about 20-45 cm. It has raceme inflorescence, white flowers and the pods are round, elliptic, emarginated notched at apex and winged (Kumar, 2006) [11]. It contains good amount of vitamin E which is responsible for reducing risks of infertility, inflammation, cardiovascular diseases, neurological disorders, diabetes and certain types of cancers in humans (Kulie *et al.*, 2009) [10]. Hence, its commercial demand has increased many folds. November to January are the most suitable months of the year for sowing in a North Indian climate.

Asalio crop is grown world wide. Asalio gets infected by *Alternaria* species. *Alternaria* is the largest genus. It is distributed worldwide and is ubiquitously present in almost all the crops. It has been reported on 115 plant genera from 43 plant families that cause blight and leaf spot disease

(Neergaard, 1945) [15]. A great number of species were recorded for the genus *Alternaria* infecting different crops causing world-wide economic loss (Kirk *et al.*, 2008) [8].

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As discussed earlier there is another pathogen *A. brassicae* that also has been reported to cause *Alternaria* leaf spot in Asalio. Its epidemiology also is narrated here. According to Singh and Upadhyaya (1971) [23] the symptoms were produced by *A. brassicae* on Asalio are initiation of yellow, small, circular patches which later become necrotic, light brown in colour and having 2-6 concentric rings (1-6 mm in diameter) on basal leaves. In later stage, spots cover larger area of leaf with distinct dark brown concentric rings. The severity of the disease gradually increases with times and reach maximum upto mid January. By the time, floral parts of plant and the stem also become infected. Finally, all aerial parts of plant show symptoms of the disease. *Alternaria* colonizes the xylem of plant, and finally, breaks down and blocks the xylem and produces leaf wilting, yellowing symptoms and finally the death of the plant. Humperson *et al.* (1983) [7] observed that *A. brassicae* needed free water with 15 °C optimum temperature for infection on cabbage plants. This temperature is required for 16 hours minimum for initiation of infection and 50 to 70 hours for disease development. The fungus produces typical lesions at 100 C. While, (Sinha *et al.*, 1987) [24] reported that maximum 30 °C temperature and minimum 1.5 to 4.2 °C with 94.75 per cent relative humidity are favorable for disease to occur. Maximum disease incidence occurs at minimum temperature 8.0 to 12.670 C and maximum temperature 21.87 to 25.10 °C, with 100 per cent relative humidity and about 51 mm average rainfall. Weather factors that favour the development and spread of the disease are essential to pinpoint the crucial contributing to development of disease epidemics Guyota *et al.* (2005) [4]. So, research was initiated on these lines and the results of which are reported here under.

Materials and Methods

At the Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, the study on "Management of *Alternaria* leaf spot of Asalio Caused by *Alternaria alternata*." was undertaken during Rabi 2018-2019. The laboratory experiments were carried out in Department of Plant Pathology and the pot experiments were carried out at cage house in Department of Plant Pathology. The details of used materials and the methodology followed in conducting the experiments are described in this chapter.

The infected samples of Asalio showing symptoms of *Alternaria* leaf spot disease were collected from the farmer's fields around Udaipur. Fungal pathogen was isolated from diseased samples of Asalio using standard methodology on potato dextrose agar (PDA) medium. Small bits of infected portions were surface sterilized for 1 minute in mercuric chloride solution (0.1%) and washed thrice in sterilized

distilled water under totally aseptic conditions in a laminar air flow. These were then dried by keeping in two folds of sterilized filter papers then aseptically transferred to PDA in Petri plates. The plates were incubated at 27 ± 1 °C for 7-8 days. For Sub-culturing 5 mm bit of the culture were cut from the periphery of the mycelial growth of 6-7 days old colonies and transferred on to the (PDA) slants. The cultures were incubated at 27 ± 1 °C for growth and sporulation and further purified by single spore/hyphal tip method. Pure cultures were maintained on PDA slants. The culture was sent for final identification to "Indian Type Culture Collection Identification/Culture supply Services, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012".

A pot experiment was laid out with five replications of each treatment following completely randomized design (CRD). A soil mixture containing soil from fields of RCA, Udaipur and FYM (3:1) was used to fill 30 cm earthen pots. Seeds of susceptible local landrace of Asalio were sown in these pots. Five pots for each plant age group (having 10 plants each) were maintained in cage house of Department of Plant Pathology, RCA, Udaipur. The conidial suspension (1×10^3 conidia ml^{-1}) of pathogen was used for inoculation of different age groups of plants. Inoculation was made by spray inoculation technique using a hand held atomizer. Disease severity of diseases from initiation and at interval of seven days was recorded following the disease rating scales. Weather variables viz., Temperature and Relative Humidity. Were also recorded for crop season and correlation was worked out.

Per cent disease intensity (PDI) was calculated based on each reading till physiological maturity of crop. Weekly meteorological data on maximum and minimum temperature, morning and evening relative humidity were obtained from agro met observatory, Agronomy farm, RCA, Udaipur for the period between disease recordings to establish their correlation with disease development. The rating scale was using 0 to 5 scales which given by Gawande and Patil (2003) as in Table 3.6.

Area under disease progress curve (AUDPC) values was calculated for different recording by the formula given by Campbell and Madden (1990) [1] as follows:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where,

Y_i = The cumulative disease index expressed as a proportion at the i th observation

T_i = Time (days after planting) at the i th observations.

N = Total number of observations

Result

Dr. T. Prameela Devi and Dr. Deeba Kamil identified these cultures as *Alternaria alternata*. The identification number is 10,856.18 is allotted by ITCC. These cultures were used further research of *Alternaria alternata*.



Plate 1: Pathogenicity of *A. alternata* on Asalio
 a. Field view of disease
 b. Diseased plant
 c. Typical symptom generated by *A. alternata*
 d. Close view of disease symptoms
 e. Pure culture of *A. alternata*
 f. Conidia at 40X

A pot experiment was laid out with five replications of each treatment following completely randomized design (CRD). A soil mixture containing soil from fields of RCA, Udaipur and FYM (3:1) was used to fill 30 cm earthen pots. Seeds of susceptible local landrace of Asalio were sown in these pots. Five pots for each plant age group (having 10 plants each) were maintained in cage house. Observations for disease severity for respective diseases were recorded (as per details given in Materials and Methods).

Alternaria leaf spot inoculated plants were periodically observed for disease severity and area under disease progress curve (AUDPC) calculation. Considerable variations were observed in AUDPC from sowing date and correlation with weather factors on disease development. To know the relationship between the dependent variable *i.e.*, disease severity (s) and four independent variables weather factors (max. temp., min. temp., max. RH, min. RH) multiple regression analysis was done starting with 22nd October 2018. By fitting equations, the contribution of weather factors in the development of *Alternaria* leaf spot was observed.

The analysis(s) is as under:

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4$$

Thus, the equation comes to:

$$a = (-)1, 137.422, b_1 = (-) 0.364, b_2 = (-) 0.752, b_3 = 0.660, b_4 = 0.358$$

$$= -1, 137.422 - 0.364X_1 - 0.752X_2^{**} + 0.660X_3^{**} + 0.358X_4$$

Where,

R² = multiple correlation coefficient

Y = per cent disease index (dependent variable)

** = significant at 1% level

a = constant (intercept)

b₁, b₂, b₃ and b₄ = partial regression coefficients

X₁ = maximum temperature

X₂ = minimum temperature

X₃ = maximum relative humidity

X₄ = minimum relative humidity

The effect on per cent disease index, constant in 1 °C maximum temperature resulted in - 0.36 change in disease. With a unit change in 1 °C minimum temperature disease will be decreased by - 0.75. In case of maximum and minimum relative humidity, 1% increase in maximum RH gave 0.66% increase in disease index. However, 1% decreases in minimum RH resulted in 0.36% increase in disease (Table-3).

Multiple correlation coefficients (R²) were 0.47 (47%). It indicated that there was 68% influence of four meteorological factors and the remaining 53% variations were unexplained.

Asalio was sowing on 22nd October. The plants were inoculated with an inoculum concentration of 1×10³ conidia ml⁻¹ of *A. alternata* on 15th November. The disease appeared on 4th week of observations (21st November) with AUDPC 32.65, in 5th week of observations it increased to 77. In 6th week of observations it increased to 98. In 7th week of observations it increased to 114.73. In 8th to 12th weeks after inoculation due to dry spell with moderate in temperature and increase in relative humidity, there was an abrupt increase in AUDPC range from 114.73 - 442.58. Followed by 13th to 15th weeks after inoculation the AUDPC was observed to be constantly increasing ranging from 483.39 – 507.54, reaching to its maximum (Table – 3 and Fig- 3).

The results show that the disease development had three distinct phases *i.e.*, establishment, progress and decline phases. The rise in infection index in the initial stage of *Alternaria* leaf spot was very slow. This was followed by rapid progress of the diseases due to favorable weather conditions and reached maximum but not decline later, it was constant due to moderate temperature, rise in RH (Plate- 4).

Table 3: Evaluation of different weather parameters (Temperature and Relative Humidity) against the disease development of *Alternaria* leaf spot on pot grown Asalio plants

S. No.	Duration	SNW	Temperature		Humidity		PDI	AUDPC
			Max.	Min.	Morning	Evening		
1	22 - 28 Oct.	43	33.5	13.7	64.1	19.6	0.00	0.00
2	29 Oct.- 4 Nov.	44	32.0	12.6	67.4	27.3	0.00	0.00
3	5 - 11 Nov.	45	30.6	9.8	57.4	20.7	0.00	0.00
4	12 - 18 Nov.	46	31.1	11.3	69.9	23.9	9.33	32.65
5	19 - 25 Nov.	47	30.9	11.0	69.7	26.4	12.67	77
6	26 Nov. - 2 Dec.	48	27.6	8.3	77.0	27.9	15.33	98
7	3 - 9 Dec.	49	26.0	7.9	80.9	33.6	17.45	114.73
8	10 - 16 Dec.	50	23.9	7.0	77.3	30.0	25.88	151.66
9	17 - 23 Dec.	51	24.0	4.1	77.3	26.3	43.16	241.64
10	24 - 31 Dec.	52	23.8	4.1	78.8	23.1	50.10	326.41
11	1 - 7 Jan.	1	25.7	5.8	75.7	20.0	58.34	379.54
12	8 - 14 Jan.	2	23.4	4.9	83.4	29.1	68.11	442.58
13	15 - 21 Jan.	3	26.9	5.6	78.7	29.7	70.00	483.39
14	22 - 28 Jan.	4	21.6	5.6	80.1	32.7	72.01	497.04
15	29 - 4 Feb.	5	37.1	8.2	73.1	28.7	73.00	507.54

* Mean of five replications

Parameters	Observation
Maximum temp.	-0.402 ^{NS}
Minimum temp	-0.786 ^{**}
Morning relative humidity	0.685 ^{**}
Evening relative humidity	0.346 ^{NS}
Constant	-171.100
R ² -value	0.4688

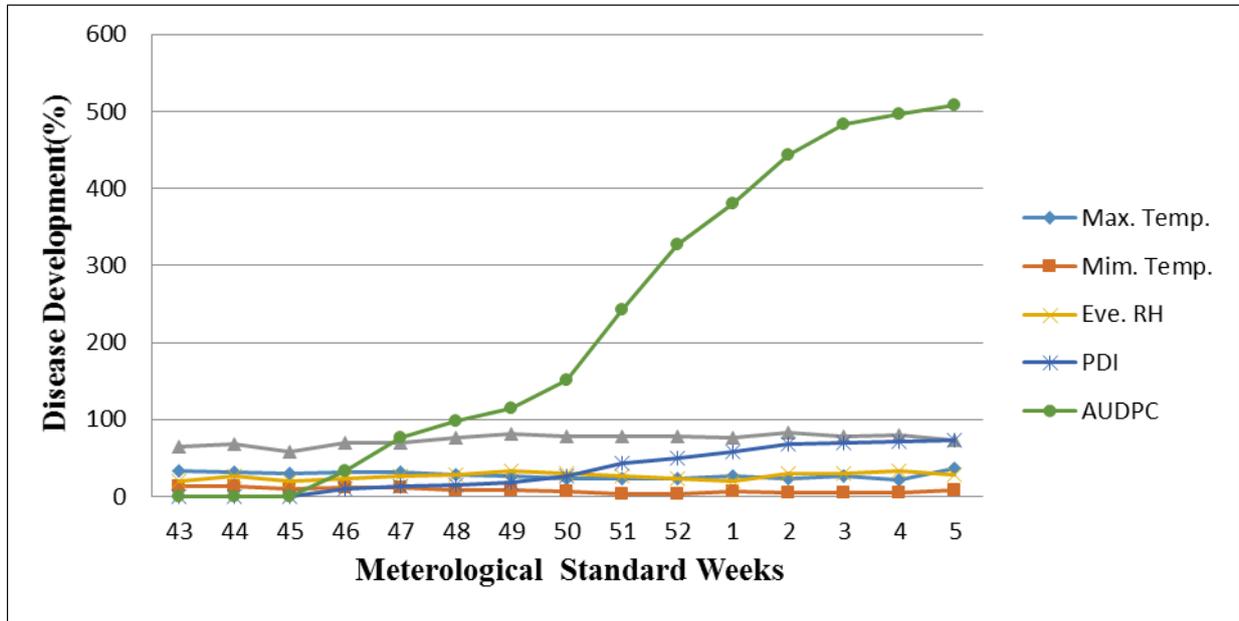


Fig 3: Evaluation of different weather parameter (Temprature and Relative Humidity) on disease development of *Alternaria* leaf spot (artificially inoculated) on Asalio plants in pot culture.

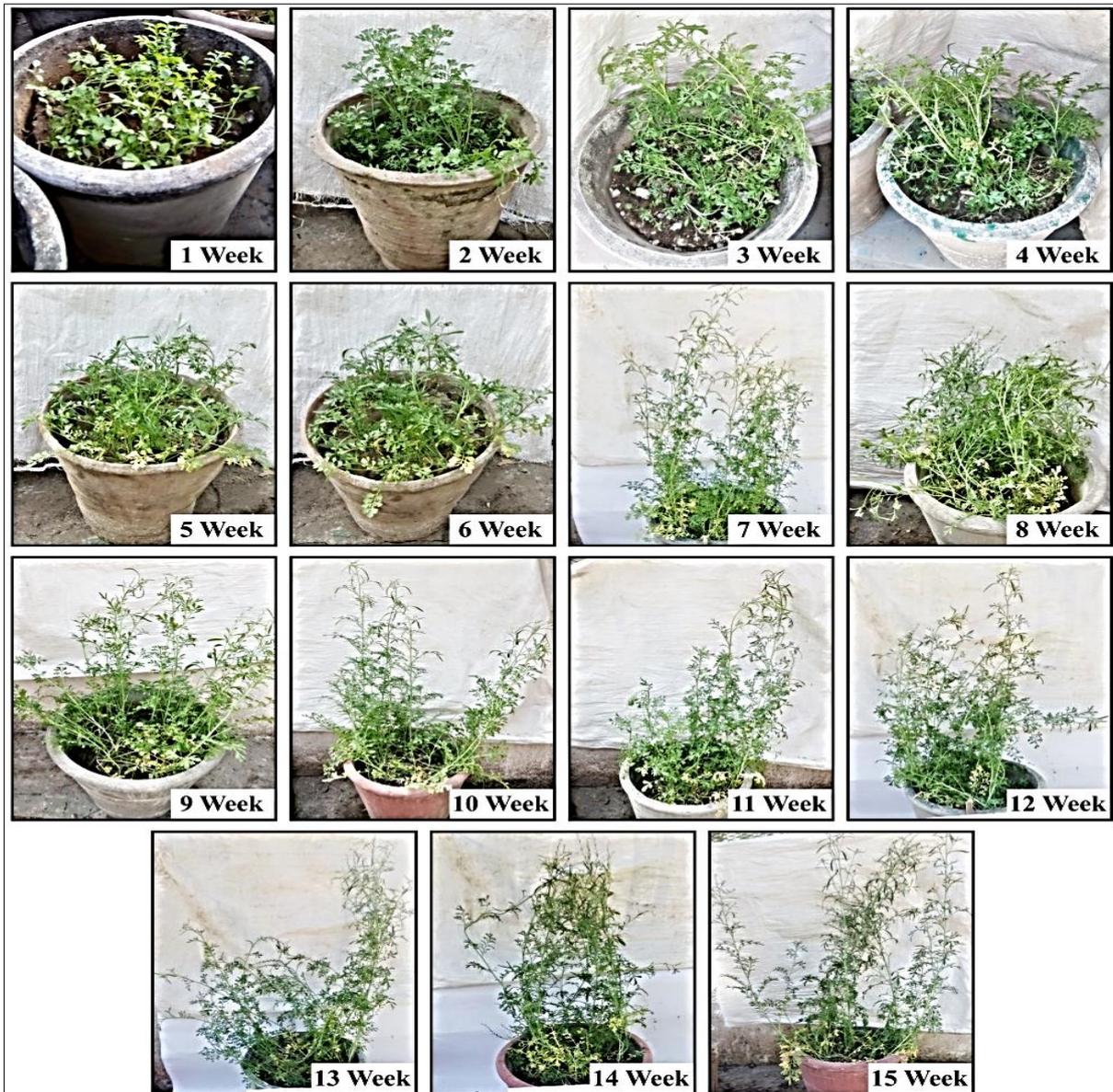


Plate 4: Epidemiological evaluation of different weather parameters with disease development of *Alternaria* leaf spot on pot grown Asalio plants

Discussion

The area under disease progress curve (AUDPC) is a quantitative measure of disease intensity with time. It is used in plant pathology to observe effect of weather factors on disease development of *Alternaria* leaf spot of Asalio. It is preferred by using a formula devised by Campbell and Madden (1990)^[1]. Lower AUDPC represented slower disease progression and the high AUDPC represents faster disease progression. In the present study *Alternaria* leaf spot of Asalio, AUDPC values were low during 12 to 25 November. When temperature ranges in 31.1 (46 SNW)- 30.9 °C (47 SNW) and relative humidity 69.9 - 69.7%. The AUDPC values were moderate during 26 Nov. to 16 Dec. 2018. While decrease in temperature (24.0-4.1 °C - 26.9-5.6 °C) and increase in RH (77.3- 26.3% - 78.7- 29.7%) during 17 Dec. to 21 Jan. 2019, the disease progression was much faster and high AUDPC values were obtained. It appeared that moderate temperature (24.0- 26.9 °C) and high humidity (77.3- 78.7%) most favorable to caused *Alternaria* leaf spot disease of Asalio. These observations are useful for timely application of fungicides and botanicals for checking further spread of the disease. This seems to be crucial stage when prophylactic control measures may be applied to suppress the disease. The present observations are tune with the onward said reported reviews. Pleysier *et al.* (2006)^[18] also reported that temperature had a significant effect on infection, with lesion development and expansion observed to increase from 15-25 °C, declining between 30-37 °C. Relative humidity (RH) also influenced infection with limited lesion development observed at <=92% RH whereas incubation at 98 and 100% RH resulted in large lesions.

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