

IMPACT OF ABIOTIC FACTORS ON THE DISEASE DEVELOPMENT OF ALTERNARIA BLIGHT OF CORIANDER CAUSING *ALTERNARIA POONENSIS* RAGUNATH

Varsha Rani, Mahendra Kumar Meena* and Kusum Mathur

Department of Plant Pathology, Rajasthan College of Agriculture, MPUAT, Udaipur-313001

Email: mahendrameena781@gmail.com

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Abstract: An investigation on Coriander susceptible cultivar Raunak-31 suffered from Alternaria blight caused by *Alternaria poonensis* Raghunath was conducted during Rabi October 2012 to February 2013. Five isolates were recovered from samples collected from Kota (1), Udaipur (3) and Baran (1). Of the three isolates of *A. poonensis* (Ap-01, Ap-02 and Ap-03) evaluated for pathogenic variability on pot -grown plants of susceptible cultivar Raunak-31, the maximum disease (53.2% PDI) was by isolate Ap-01 and minimum (32.4%) by Ap-03, suggesting that considerable variability exists in *A. poonensis*. Disease progress was influenced by different weather factors viz., temperature, relative humidity, sunshine and evaporation. The maximum AUDPC value (area under disease progressive curve) was 322 on plants inoculated on 21st November and the lowest (171.8) was on than inoculated on 20th December in 21- 27 February, standard week followed by maximum AUDPC value 273.7 and minimum 91.0 in 14- 20 February, standard week.

Keywords: Epidemiology, Abiotic, Raunak-31, Temperature, RH, Rainfall, *Alternaria poonensis*

INTRODUCTION

Coriander (*Coriandrum sativum* L.), a major spice crop of the family Umbelliferae (or Apiaceae) is native of the Mediterranean and near Eastern Asian regions. (Saskatchewan Herb & Spice Association, 2007). Alternaria blight is one of the most devastating diseases of coriander occurring in major coriander growing states of India and World.

In India, the total area under coriander cultivation is about 321.6 lac hectares with annual productions of 2.8-3.0 lakh metric tones. In Rajasthan, coriander is cultivated in Kota, Jhalawar, Baran, Bundi, Chittorgarh and Udaipur districts on 2.81 lac hectares with production of 1.21 lac tones. (Rajasthan Agriculture Statistics, 2009-2010). Coriander crop suffers from various diseases caused by fungi and other microorganisms. Important diseases incited by fungi are stem galls/tumours (*Protomyces macrospores*; Unger, 1834), stem rot (*Sclerotinia sclerotiorum*; Korf & Dumont, 1972), wilt (*Fusarium oxysporum* f.sp. *coriandri*; Narula & Joshi, 1963), powdery mildew (*Erysiphe polygoni*; Weiltzein, 1963), root and stem rot (*Rhizoctonia solani* and *Macrophomina phaseolina*; J.G.Kuhn, 1858 & Goid, 1947) and blight (*Alternaria poonensis*; Raghunath, 1962). Alternaria blight of coriander is emerging as a major and wide spread problem in Rajasthan. The pathogen seems to have adaptability to higher temperatures and the disease occurs during February-April, and it is particularly severe at flowering and post flowering stages causing considerable losses to the yield.

With these facts in view, the present study on epidemiology of alternaria blight of Coriander caused by *Alternaria poonensis* was carried out.

MATERIAL AND METHOD

The present investigation was aimed to studying the effect of environmental factors in relation to the disease development conducted at Cage House, Rajasthan College Agriculture, Udaipur, Rajasthan during Rabi, 2012 and 2013.

Coriander susceptible cultivar Raunak-31 was selected for this study from the grown in Cage House and was constantly examined for disease progress/decline.

Collection, isolation and purification of the pathogen

To study the populations of *Alternaria poonensis* prevalent in different places of Rajasthan, infected disease leaf samples of coriander were collected from the diseased coriander plants from the farmers fields of Kota, Lakadwas, Udaipur and Baran, RCA, Udaipur and Bhuwana, Udaipur. Cultures of coriander blight pathogen were isolated from the infected leaves. Small pieces of infected portion from diseased leaves were taken from the margin of the spots and were surface sterilized with 0.1 per cent mercuric chloride for 1 to 2 min. and were then washed in two changes of sterile distilled water for removing the disinfectant and aseptically transferred to potato dextrose agar (PDA) in Petri plates. The plates were incubated at 25 ± 2°C for growth. Sub-culture was made by removing 5 mm bit of the culture from the periphery of the mycelial growth of 4-5 days old colonies on (PDA) slants. The cultures were incubated at 25 ± 2°C for growth and sporulation. The microscopic examination of cultures indicated that the fungus belonged to the genus *Alternaria*.

*Corresponding Author

Pure culture of the pathogen was prepared by demarcating single hyphal tips on 2 per cent water agar with dummy objective which were then

transferred to PDA slants and were allowed to grow. The culture was maintained at 4°C and also by periodical transfers on PDA slants for further use.

Table 1. Isolates of *A. poonensis* recovered from samples collected from fields in coriander growing areas of Rajasthan

S.No.	Place of Collection	Cultivar	Isolated designation
1.	Kota	Local landrace	Ap-01
2.	Lakadwas, Udaipur	Local landrace	Ap-02
3.	Baran	Local landrace	Ap-03
4.	RCA, Udaipur	Local landrace	Ap-04
5.	Bhuvana, Udaipur	Local landrace	Ap-05

Pathogenicity test

Pathogenicity of the 5 isolates of *A. poonensis*, was tested by spray inoculating 21-days-old plants on pot grown plants of coriander (Raunak- 31 and Pratap raj dhaniya). The plants were raised in soil: FYM(3:1) mixture and surface sterilized seeds (0.1% HgCl₂) for two minutes) were sown @ 5 per pot. For preparation of the inoculum the pure cultures of different isolates were grown on PDA for 10 days on 28±1°C in Petri plates so as to allow profuse sporulation. The spores were harvested by flooding the plates with sterile distilled water and gently scrapping the colony with the help of a sterilized plastic loop and the conidial suspension was strained through muslin cloth. Final concentration of the spores was maintained 1×10³ conidia ml⁻¹. 21-days-old plants were spray inoculated with the suspension using a hand held atomizer. The inoculated plants were kept in humid chamber for 24 hours and then transferred to cage house and high humidity was maintained throughout the disease development period by frequent irrigations.

Infection started as minute necrotic areas on all the above ground plant parts which turned purple at advanced stages and later turned brown to black during 36-71 hours and the affected parts of the plant got blighted. These typical leaf blight symptoms appeared in 7-10 days after inoculation. Re-isolation was done from infected plant parts collected 10 days after inoculation. The resultant cultures were compared with the original ones to confirm the pathogenicity.

Identification of the culture

Cultural characters of all isolates of *Alternariaspp.* were studied by growing them on PDA medium for the identification of the species and other characteristic of the fungus at 25±2°C for 10 days. Temporary mount on the slides in lactophenol and cotton blue were prepared from 10-days-old culture. These slides were examined thoroughly under the microscope for observing the characters of hyphal, colour, shape, size and septation of conidia and various cultural characters on PDA. The cultures were identified by following the standard references of *A. poonensis* (Raghunath, 1962 and Rao, 1963)

Effect of weather condition on development of alternaria blight

Staggered sowing was done from 1st Nov. and dates were as followed 7th Nov., 15th Nov., 22nd Nov. and 29th Nov.12). After germination 15-days-old plants were inoculated on 21st Nov.12, 28th Nov.12, 5th Dec.12, 12th Dec.12 and 19th Dec.2012 with a spore suspension of 1×10³ conidia ml⁻¹. Disease severity from initiation and at interval of seven days was recorded following the 0-5 scale. Weather variables viz., temperature, RH, sunshine hours, rainfall and evaporation etc. were also recorded for crop season and correlation worked out.

Per cent disease intensity (PDI) was calculated based on each reading till maturity of crop. Weekly meteorological data on maximum and minimum temperature morning and evening relative humidity, rainfall and duration of sunshine hours and evaporation were obtained from agromet observatory, Agronomy farm RCA, Udaipur for the period between disease recordings to establish their correlation with disease development. Area under disease progressive curve (AUDPC) values were calculated for different recording by the formula given by Campbell and Madden (1990). Sequential apparent infection rate was calculated between each two subsequent observations. AUDPC and r_c was calculated by the method described by Vander Plank (1963), Johnson and Wilcoxson (1982) and later described by Campbell and Madden (1990) as follows:

$$AUDPC = \left[\left(\frac{X_{i+1} + X_i}{2} \right) \times (t_{i+1} - t_i) \right]$$

Where

X_i = the cumulative disease index expressed as a proportion at the ith observation

t_i = Time (days after planting) at the ith observations.

n = Total number of observations

$$r_c = \frac{1}{t_2 - t_1} \log_e \frac{n_2(1-n_1)}{n_1(1-n_2)}$$

Where t₁ = Time (days) during the 1st observations

t₂ = Time during the 2nd observation.

t₂ - t₁ = Time interval between two observations and subsequently so on.

n_1 = Per cent disease index value in decimal at corresponding t1 time.

n_2 = Per cent disease index value at t2 time.

The disease severity was recorded on standard 1 to 5 disease rating scale. Details of the disease rating scale are given below:

Per cent all above ground parts infected	Score
Free from disease	0
1 to 10% area of leaf and umbel blighted	1
11 to 20% area of leaf, stem and umbel blighted	2
21 to 35% area of leaf, stem and umbel blighted	3
36 to 60% area of leaf, stem and umbel blighted	4
More than 61% area of leaf, stem and umbel blighted	5

The per cent infection index (Mckinney, 1923; Chester, 1959 and Wheeler, 1969) was calculated as

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all individual disease rating}}{\text{Total No. of plants ass.} \times \text{maximum rating}} \times 100$$

RESULT AND DISCUSSION

The experiment was conducted to find out the effect of environmental factors on the development of Alternaria blight on the susceptible coriander cultivar Raunak-31 of coriander in Rabi 2012-13. Weekly sowing was done start from 1st Nov. and the following dates were 7th Nov., 15th Nov., 22nd Nov. and 29th Nov.). After germination 15-days-old plants were inoculated with virulent isolate Ap-01 with $1 \times 10^{-3} \text{ ml}^{-1}$ spore suspension, to know the

relationship between the disease severity (dependent variable) and the weather factors (maximum temperature, minimum temperature, maximum per cent relative humidity and minimum per cent relative humidity) multiple linear regression analysis and for (sunshine, rainfall and evaporation), simple regression analysis was done with 1st Nov. sown plants. By fitting this equation, the contribution of weather factors in the development of Alternaria leaf spot was observed.

Table 2.

S.No.	Disease severity PDI on 1 st sown	Independent value				
		Weather factors	R	R ²	A	B
1.	4.4	T max.	-0.225	0.051	64.105	-1.733
2.	9.3	T min.	-0.017	0.00	19.999	-0.092
3.	25.4	RH max.	0.110	0.012	-20.813	0.506
4.	36.6	RH min.	0.246	0.060	-5.174	0.909
5.	41.1	SSH	0.425	0.181	-60.906	9.736
6.	50.4	Evap.	0.287	0.083	4.399	4.902

$$Y = a + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_4$$

$R^2 = 0.8972$
 $a = -321.714$
 $b_1 = 3.602$
 $b_2 = -2.414$
 $b_3 = 2.0142$
 $b_4 = -0.6834$

Thus, the equation comes to:

$$Y = -321.714 - 3.609 X_1 - 2.414 X_2 + 2.0142 X_3 - 0.6834 X_4$$

$b_1 = 3.602$: It means that holding X_2 (minimum temperature), X_3 (maximum relative humidity) and X_4 (minimum relative humidity), constant a 1 per cent increase in X_1 (maximum temperature), led on average to about 1.694 per cent decrease in Y (per cent disease index)

$b_2 = -2.414$: It means that holding $X_1 = X_3$ and X_4 constant a 1 per cent increase in X_2 lead on average to about -7.715 per cent increase in Y.

$R^2 = 89$ per cent, it means variation at 89 per cent explained by dependent variable (disease severity) and remaining 11 per cent variable is unexplained.

$r = 0.370$ (Sunshine):

The correlation coefficient between average per cent disease index and sunshine hrs was 0.370, it indicates is a positive correlation, between the in per cent disease index, indicating higher disease severity with the increase in sunshine.

$r = -0.246$ (Evaporation):

There is negative correlation between average per cent disease index and evaporation (-0.246), it indicate that increase in evaporation resulted in decrease in per cent disease index.

Table 3. Comparative pathogenic potential of different isolates of *A. poonensis* on pot grown plants of coriander cultivar Raunak-31

S.No.	Isolate	Latent period (in hrs)	Percent disease index
1.	Ap-01	38	53.2 (46.84)
2.	Ap-02	52	49.8 (44.89)
3.	Ap-03	73	46.3 (42.88)
SEm±			0.92
CD at 5%			2.80
CD at 1%			3.88

Table 4. Progression of Alternaria blight on coriander in relation to weather parameters with different date of date of sowing during *Rabi* 2012-13

Standard week	Metrological weeks	Temperature (°C)		Relative humidity (%)		Sunshine (hrs)	AUDPC*				
		Max.	Min.	Max.	Min.		Inoculated on				
							21 st Nov.	27 th Nov.	5 th Dec.	13 th Dec.	20 th Dec.
47	21Nov.-27Nov.12	30.1	12.6	80	45.7	8.5	15.4	0	0	0	0
48	28Nov.-4Dec.	27.2	12.1	84.9	41.3	6.8	35.7	9.8	0	0	0
49	5Dec.-12Dec.	30.4	13.5	81.4	43.6	8.9	45.1	22.4	7.0	0	0
50	13Dec.-19Dec.	25.4	6.4	76.3	33	8.6	53.9	25.9	14.3	5.6	0
51	20Dec.-26Dec.	26.5	7.6	87	41.1	7.7	60.9	27.3	16.4	11.5	5.6
52	27Dec.-2Jan.13	24.2	7.4	95.5	41	7.2	64.4	28.7	18.5	11.9	11.2
1	3Jan.-9Jan.	23.0	4.4	85.6	30.7	7.8	65.4	30.4	18.9	13.3	12.2
2	10Jan.-16Jan.	23.1	6.4	75.3	21.1	8.5	71.7	38.1	26.6	22.0	16.1
3	17Jan.-23Jan.	23.9	7.5	80.3	28.1	7.6	97.6	68.2	51.4	44.8	25.5
4	23Jan.-30Jan.	22.7	4.0	74.9	19.6	9.0	147.7	124.9	76.3	64.4	35.7
5	31Feb.-6Feb.	25.9	10.0	76.3	26.9	5.7	209.3	189.7	105.0	77.7	39.9
6	7Feb.-13Feb.	24.3	7.6	78	28.2	8.3	248.5	216.1	130.2	100.8	52.5
7	14Feb.-20Feb.	26.3	11.7	83.3	34.3	8.3	273.7	235.7	175.0	159.6	91.0
8	21Feb.-27Feb.	26.8	10.6	82	31	8.7	322	288.4	255.1	250.6	171.8

*Mean of five replications*Observation started 7 days after inoculation and at weekly intervals (please see Materials & Methods)

1st sown-1st Nov., 2nd -7th Nov., 3rd -15th Nov., 4th -22nd Nov. and 5th -29th Nov.12

Considerable variations were observed in AUDPC in five different dates of sowing and correlation with weather factors on disease development. In the first planting on 21st Nov. the AUDPC ranged 15.4, in the next week it was 35.4 and in the third week (42 days old plant) it was 45.1. The AUDPC in the following 7 weeks ranged for 60.9-147.7. In the 8th week the AUDPC given high (209.3-322) in the plants sown on 28th Nov.2012.

In the plants sown on 7th Nov. and inoculated on 28th Nov. the AUDPC in the first week after inoculation (28th Nov.- 4th Dec.) was 9.8. It progressed slowly and up to next six weeks (up to 9th Jan.) AUDPC ranged between 22.4- 30.4. In the following weeks it increased slightly and was 38.1, but after that there

was a sudden increase in AUDPC to almost double, then it was 124.9, 189.7, 216.1, 235.7 and 288.4.

In the plants sown on 15th Nov. and inoculated on 5th Dec., the AUDPC was at first lower (7.0) than the first two dates of sowings (15.4 & 9.8 respectively) and from 2nd week to 5th week (16th Jan. 2013), it remained in the range of 14.3-26.6. But in the next week there was sudden increase and AUDPC reached to 51.4, 76.3, 105.0, 130.2, 175 and 255.1. It was lower than that observed these stages in the first two sowings. Similarly, in the plants sown on 22nd Nov. and inoculated on 5th Dec. and also than sown on 29th Nov. and inoculated on 19th Dec. the AUDPC was 5.6. It increased to 11.5 & 11.2, respectively on the next week and remained in the range of 11.9-22.0 during next four weeks. But there were difference in

the two dates of sowing after 4th weeks. Higher AUDPC was recorded in the 5th week in plants sown on 22nd Nov. (44.6) as compared to 25.5 in those sown on 29th Nov. In the former set, the AUDPC in the following weeks was 64.4, 77.7, 100.8, 159.6 & 250.6, while on the later sown; the AUDPC on the 5th week much was lower and was 35.7, 39.9, 52.5, 91.0 and 171.8 only.

It was observed that the disease progressed faster when the maximum temperature ranged from 24.3 to 26.8^oC and minimum temperature was from 7.6 to 11.7^oC as compared to 22. To 24.2^oC maximum and 4.0 to 10.0^oC minimum during 27th Dec.2012 to 3 Jan.2013. The age of the plant also seemed to be important as disease progress was higher on 12-15 week old plants.

Studies on the effect of weather factors on disease development revealed that 21 Nov. to 10 Jan. 2013 due to 30.7-95.5 per cent relative humidity and 13.5-30.4^oC temperature range AUDPC was 71.1 on 21st Nov. sown plants followed by 38.1, 26.6, 22.0 and 16.1 on 27th Nov., 5th Dec., 13 Nov. and 20th Nov. sown plants. During 11 Jan. to 24 Jan.2013 was moderate period for *Alternaria* blight development as during this period the minimum and maximum relative humidity ranged between 21-1-30.7 and per cent coupled with minimum and maximum temperature i.e., 4.4-7-5^oC and 23-25.1^oC and AUDPC was 147.7 on 21st sown plants followed by 124.9, 76.3, 64.4 and 35.7 on 27th Nov., 5th Nov., 13 Nov. and 20th Nov. sown plants.

The period between 25 Jan. to 27 Feb. 2013 was much favourable for disease development and more severity during this period was observed in comparison to progressive phase. The minimum and maximum temperature range was 4-11.7^oC and 22.7-26.8^oC accompanied with minimum and maximum relative humidity i.e., between 19.6-31 and 74.9-83.3 per cent respectively in progress phase AUDPC value ranged from 250-322 per cent while disease was found almost stable. The area under disease progressive curve (AUDPC) is a quantitative measure of disease intensity with time. It is used in

plant pathology to indicate and compose levels of resistance to disease among different varieties of crops, effect of weather factors and various disease suppression treatments. It is preferred by using a formula devised by Campbell and Modden (1990). Lower AUDPC represented slower disease progression and the high AUDPC represents faster disease progression. In the present study, AUDPC values were lower during 21st Nov. to 27th Dec. when temperature ranges in 7.6-30.4^oC and relative humidity 33-87 per cent. The AUDPC value was moderate (ranging from) during 28th Dec.12 to 7th Feb.13. With increase in Temperature (7.6-26.8^oC) during 8th Feb. to 27th Feb.13 period, the disease progression was much faster and high AUDPC values were obtained. It appeared that despite different ages of the coriander, the temperature of 7.6 to 26.8^oC were the most confined during 8th Feb. to 27th Feb.13.

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