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Research Paper

EFFECT OF TEMPERATURE, RELATIVE HUMIDITY, PHOTOPERIOD ON ANTHRACNOSE OF HORSE GRAM AND ROLE OF WEATHER PARAMETERS IN DEVELOPMENT OF DISEASE IN HIMACHAL PRADESH

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Abstract

Investigations on anthracnose of horse gram were undertaken to study the pattern of disease development and agrometeorological factors affecting the disease at Palampur, Himachal Pradesh. To study the effect of temperature, moisture chambers of petriplates lined with wet filter paper were made and incubated at different temperatures i.e.15, 20, 25, 30 and 35 °C at 100% RH. To study the effect of light three different photoperiods were created i.e. complete dark (24 hr), complete light (24 hr) and 12/12 hr alternate light/dark period. Different humidity levels ranging from 75 to 100% were created by variable concentration of H_2SO_4 in water. Detached horse gram leaves were then placed in sterilized petriplates and inoculated with the spore suspension of pathogen (*Colletotrichum truncatum*) with the help of sterilized pipette. Maximum lesion size was observed at 25ºC (8.29 mm^2) after 96 hr of inoculation. As the temperature increased and decreased from 25ºC, there was decrease in the lesion size. After 96 hr of inoculation least lesion size was observed at 15ºC (1.81 mm^2). There was progressive increase in lesion size with an increase in relative humidity level. High RH (100%) favoured the lesion size development at 25ºC temperature. Among different RH levels, maximum lesion size of 7.18 mm^2 was found at 100% RH after 96 hr of inoculation and minimum of 2.05 mm^2 after 96 hr of inoculation at 75% RH. Different photoperiods showed significant variation in disease development. Maximum lesion size of 6.23 mm^2 was noticed at 12 hr alternating light and dark conditions after 96 hr of inoculation. Minimum lesion size of 4.51 mm^2 was noticed in complete dark conditions after 96 hr of inoculation. Disease progress as a function of time was recorded at periodical intervals on susceptible cultivar HPK-4. The disease was correlated with meteorological factors such as temperature, relative humidity, bright sunshine hours and rainfall to determine the role of weather variables on the development of disease.

The progress of disease exhibited sigmoidal pattern of the curve during both years. Mean temperature, rainfall and bright sunshine hours showed positive correlation with disease severity during both the years. The correlation coefficient between disease severity and mean relative humidity was negative during first year and positive during second year.
Key words: Horse Gram, Anthracnose, *Colletotrichum truncatum*, temperature, relative humidity, photoperiod, weather parameters.

INTRODUCTION

Pulses are important food crops due to their high protein and essential amino acid content. Like many leguminous crops, pulses play a key role in crop rotation due to their ability to fix nitrogen. Pulses are subjected to the attack of a variety of diseases and insect pests. Horse gram (*Macrotyloma uniflorum*), commonly known as *Kulthi* in north India, is an important edible legume, consumed throughout the country. It is primarily a crop of the dry and upland areas of the peninsular and eastern states of India. Horse gram requires an average temperature of 20–30°C and does not tolerate frost. It is drought-resistant and can be grown with rainfall as low as 380 mm. It is mostly grown in areas with less than 900 mm annual rainfall. In high rainfall areas it is grown on residual moisture in the dry season, e.g. after rice crop. Most horse gram cultivars are short-day plants. During the cropping season, wet and humid environment conditions predispose the crop to the attack of many pathogens. The main diseases of horse gram in India are anthracnose (*Colletotrichum truncatum*), leaf spot (*Cercospora dolichi*, synonym: *Mycosphaerella cruenta*), rust (*Uromyces appendiculatus*), root rot (*Pellicularia filamentosa*, synonym: *Thanatephorus cucumeris*), dry root rot (*Macrophomina phaseolina*) and horse gram yellow mosaic virus (HgYMV). The genus *Colletotrichum* includes more than 900 species responsible for anthracnose disease. In India more than hundred species have been reported [1]. Isolates of *C. truncatum* vary considerably in colony characteristics, size of fruiting structures and pathogenicity.The isolates of *Colletotrichum* from South Africa were collected and their pathogenicity were tested on soybean and it was observed that *Colletotrichum truncatum* was most pathogenic on soybean[2]. Additionally, *C. truncatum* population from different hosts often exhibit different host preferences. Conidia of *C. truncatum* are described as hyaline and one celled, with slightly falcate shape and a size of 17.0-31.5 x 3.0-4.5µm (length x width). Dark brown to black setae are generally produced in abundance [3 ;4]. Anthracnose caused by *C. truncatum* is one of the most important seed-borne fungal

pathogen of horse gram. The disease causes a significant reduction of seed germination, seed quality thereby limiting its potential yield.

2. MATERIALS AND METHODS

2.1 Effect of different epidemiological parameters on disease development

2.1.1 Effect of temperature

To study the effect of temperature, moisture chambers of Petriplates lined with wet filter paper were made. Three detached horse gram leaves were kept in each Petriplate. The leaves were then inoculated with the spore suspension of pathogen (5-6 drops of suspension per leaf) with the help of sterilized pipette. This was replicated thrice and incubated at different temperatures i.e.15, 20, 25, 30 and 35 °C at 100% RH. Area (L X B mm^2) was calculated by measuring the maximum length (L) and width (B) of the lesion at regular intervals i.e. after 48, 72 and 96 hr of inoculation.

2.1.2 Effect of light

To study the effect of light three different photoperiods were created i.e. complete dark (24 hr), complete light (24hr) and 12/12 hr alternate light/dark period. Three detached horse gram leaves were kept in each Petriplate. Leaves were then inoculated with the spore suspension of pathogen (5-6 drops of suspension per leaf) with the help of sterilized pipette. This was replicated thrice and incubated in three different photoperiodic conditions at 25±1ºC and 100% RH. Data were recorded on lesion development under different photoperiods at regular intervals as mentioned above.

2.1.3 Effect of relative humidity

Different humidity levels ranging from 75 to 100% were created by variable concentration of H_2SO_4 in water as per method described by Dhingra and Sinclair [5]. Detached horse gram leaves were then inoculated with the spore suspension of pathogen (5-6 drops of suspension per leaf) with the help of sterilized pipette and dried in the laminar air flow chamber. Three leaves were then kept in each moisture chambers made up of concentrated H_2SO_4 and water. Data were recorded on lesion development at different humidity levels at regular intervals as mentioned above.

2.2 Role of weather parameters on disease development

Disease progress as a function of time was recorded at periodical intervals on susceptible cultivar HPK4 grown in fields. The disease was correlated with

meteorological factors such as temperature, relative humidity, BSS and rainfall to determine the role of weather variables if any, on the development of disease. All these parameters were recorded from meteorological laboratory set up near by the Department of Agronomy.The role of environmental factors in the development of disease was further established by multiple regression analysis of disease progress with weather parameters. Simple correlation and multiple regression analysis were performed using computer facility, between disease (anthracnose of horse gram) and four independent variables *viz.*, mean temperature (X_1), mean relative humidity (X_2), bright sunshine hours (X_3) and rainfall (X_4). The multiple linear model: $Y = a+b_ix_i+e$ was used to describe the functional relationship, where,

Y = Predicted mean severity

a = Intercept

bi = Regression coefficient for Xi (i = 1.....n)

xi = Independent variables (i =1.....n)

e = Random error

3. RESULTS

3.1 Effect of different epidemiological parameters in disease development

Effect of different ranges of temperature, RH and photoperiod was studied on the appearance of anthracnose of horse gram and the results obtained are presented below:

3.1.1 Effect of temperature

The data presented in Table 1 show the effect of different temperature regimes *viz.,* 15, 20, 25, 30, 35 °C on the anthracnose development of horse gram under laboratory conditions. The lesion size was observed after 48, 72 and 96 hr after inoculation. Maximum lesion size was observed at 25ºC (8.29 mm^2) after 96 hr of inoculation. After 96 hr of inoculation least lesion size was observed at 15ºC (1.81 mm^2). At 20ºC and 30ºC the lesion size was recorded 6.41 mm^2 and 5.57 mm^2 respectively, after 96 hr of inoculation. At 35ºC, however the lesion size after 96 hr was reduced (4.56 mm^2) indicating that 20ºC and 25ºC were the congenial temperatures for anthracnose development.

3.1.2 Effect of relative humidity

Data on the effect of relative humidity (RH) levels on the anthracnose development of horse gram are presented in Table 2. There was progressive increase in lesion size with an increase in relative humidity level. High RH (100%) favoured the lesion size development at 25 ºC temperature. Among different RH levels, maximum lesion size of 7.18 mm^2 was found at 100% RH after 96 hr of inoculation and minimum of 2.05 mm^2 after 96 hr of inoculation at 75% RH.

Table 2: Effect of relative humidity on the anthracnose development of horse gram

	Lesion size (mm^2)*		
Relative humidity (%)	48hr	72hr	96hr
75	1.13	1.55	2.05
80	2.48	1.87	2.57
85	1.77	2.39	4.78
90	2.41	4.29	5.23
95	3.61	5.29	5.90
100	4.74	6.32	7.18
CD (P=0.05)	0.31	0.25	0.19

*Mean of three replications

Each replication consists of observations on 3 leaves

3.1.3 Effect of photoperiod

Data on the effect of photoperiod on the anthracnose development of horse gram are presented in Table 3. Different photoperiods showed significant variation in disease development. Maximum lesion size of 6.23 mm^2 was noticed at 12 hr alternating light and dark conditions after 96 hr of inoculation. Minimum lesion size of 4.51 mm^2 was noticed in complete dark conditions after 96 hr of inoculation.

Table 3: Effect of photoperiod on the anthracnose development of horse gram

	Lesion size (mm^2)*		
Photoperiod (hr)	48hr	72hr	96hr
12/12 alternate	2.25	3.44	6.23
Light (24)	2.04	3.35	5.39
Dark (24)	1.75	2.42	4.51
CD(P=0.05)	0.13	0.34	0.43

*Mean of three replications

Each replication consists of observations on 3 leaves

3.2 Role of weather variables on disease development

An experiment was conducted on anthracnose development on susceptible horse gram cultivar HPK4 for consecutive two years. The anthracnose progress was recorded periodically and was plotted against time during both the years (Figures 1 and 2). The pattern of disease progress during both the years was similar. The progress of disease started picking up from the first week of August and maximum progress was observed in mid August, when the weather variables were most favourable.

Fig 1: Role of meteorological factors on anthracnose development of horse gram in 1st year

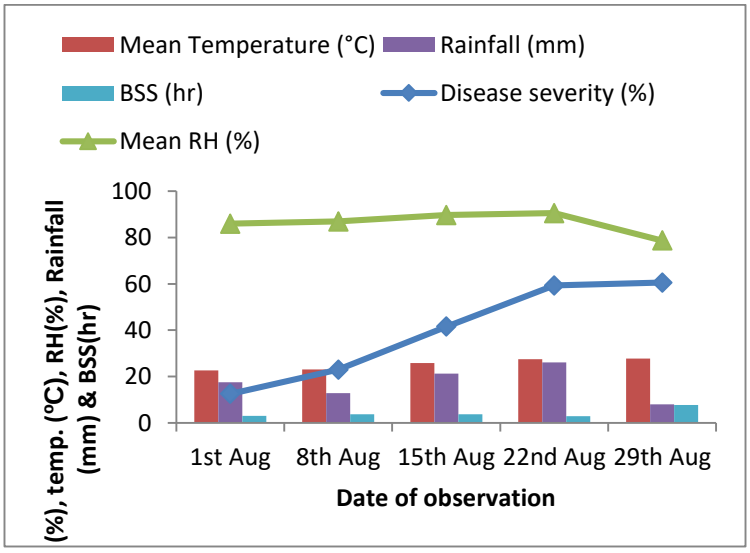
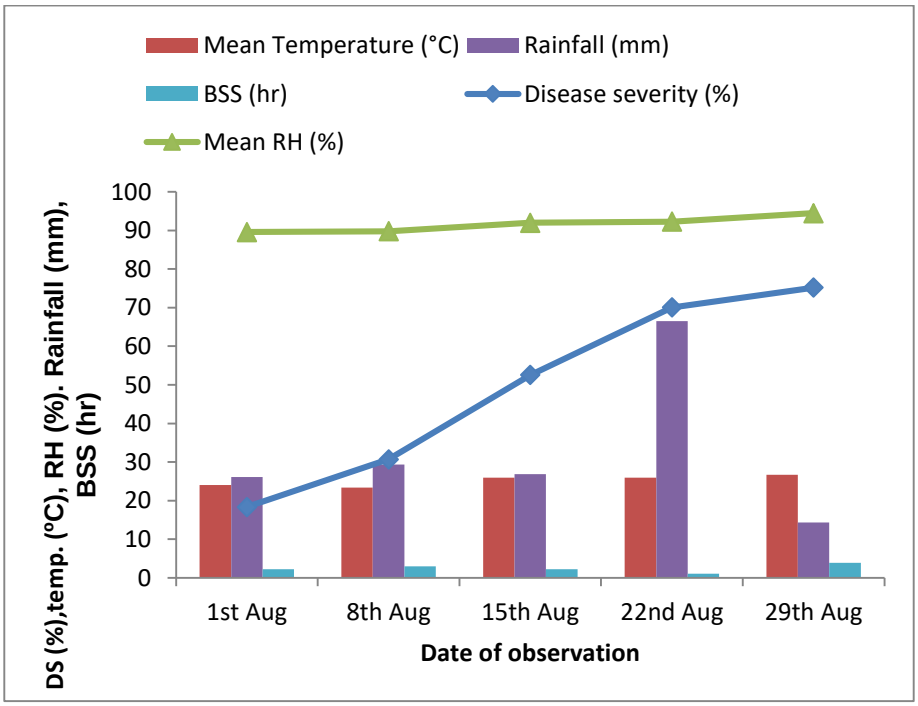


Fig 2: Role of weather variables on anthracnose development of horse gram in 2nd year



The disease progress was correlated with weather variables *viz.,* mean temperature (ºC), mean relative humidity (%), bright sunshine (hr) and rainfall (mm) (Table 4). There was a significant positive correlation between disease severity and mean temperature during both the years. Correlation coefficient (r) 0.95 and 0.88 were highly significant during 1st year and 2nd year, respectively. The correlation coefficients between disease severity and mean relative humidity were negative (r=-0.24) during 1st year and significantly positive (r=0.90) during 2nd year. There was significant positive correlation between disease severity and bright sunshine hours (r=0.56) during 1st year but was non-significant during 2nd year. Rainfall though showed positive correlation (0.02 and 0.23) with disease severity during both years, but were non-significant. The correlation of disease severity with rainfall was non- significant during both the years. In pooled analysis, all factors are positively correlated with disease severity but only correlation with mean temperature was significant.

Table 4: Simple correlation coefficients between disease severity and weather variables

Weather variable	Simple correlation coefficient (r)		
	1st year	2ndyear	Pooled
Mean temperature	0.95*	0.88*	0.85*
Mean relative humidity	-0.24	0.90*	0.21
Bright sunshine hours	0.56*	0.05	0.19
Rainfall	0.02	0.23	0.24

* Significant at 5% level of significance

Multiple correlation coefficients between disease severity and group of weather variables (mean temperature, mean relative humidity, bright sunshine hours and rainfall) were highly significant during both the years (Table 5).

During the year 1st year best fit regression equation for anthracnose of horse gram was found to be $Y = - 252.8890 + 13.2972X_1 + 0.2385X_2 - 6.844X_3 + 2.0686 X_4$ with coefficient of determination (R^2) 0.92. Coefficient of determination revealed that all the weather variables contributed up to 92 per cent towards disease severity. Similarly during the year 2nd year best fit regression equation for anthracnose of horse gram was found to be $Y= 4831.3744 + 132.9740X_1 - 93.8528X_2 + 124.3477X_3 + 4.8167X_4$ with coefficient of determination (R^2) 0.93. Coefficient of determination revealed that all the weather variables contributed up to 93 per cent towards disease severity.

Regression equation was also developed from the pooled data of disease severity and weather variables of 1st year and 2nd year. The forecasting model for anthracnose of horse gram developed from pooled data was found to be, $Y = - 475.3750 + 6.5475X_1 + 3.3194X_2 + 11.6253X_3 + 0.8087X_4$ with R and R^2, 0.93 and 0.87, respectively. Multiple correlation coefficients between disease severity and weather variables were significant. Coefficient of determination revealed that all the weather variables contributed up to 87 per cent towards disease severity.

Table 5: Multiple correlation coefficients between epidemiological parameters of anthracnose of horse gram during 1st year and 2nd year

Year	Regression equation	R	R^2
1st year	$Y = - 252.8890 + 13.2972X_1 + 0.2385X_2 - 6.844X_3 + 2.0686 X_4$	0.96*	0.92
2nd year	$Y = 4831.3744 + 132.9740X_1 - 93.8528X_2 + 124.3477X_3 + 4.8167X_4$	0.97*	0.93
Pooled	$Y = - 475.3750 + 6.5475X_1 + 3.3194X_2 + 11.6253X_3 + 0.8087X_4$	0.93*	0.87

* Significant at 5% level of significance

Y= Disease severity, X_1 = Mean temperature, X_2 = Mean RH, X_3 = Bright sunshine hours, X_4 = Rainfall, R= Multiple correlation coefficient, R^2 = Coefficient of multiple determination

4. DISCUSSION AND CONCLUSION

4.1 Effect of different epidemiological parameters in disease development

4.1.1 Effect of temperature

Temperature was found to play crucial role in disease development. The results show that under optimum conditions of temperature (25ºC) disease severity is high and with increase in the period after inoculation, there is a corresponding increase in disease severity. Mishra and Gupta [6] reported minimum increase in lesion size at 35ºC temperature and maximum (3.81 mm^2) was noticed at 25 ºC temperature. Thakur and Khare [7] reported maximum increase in lesion size of mungbean at 25 ºC temperature. These findings indicate that *Colletotrichum* leaf spot does not appear in plain areas where temperature is ≥ 35ºC in July- August.

4.1.2 Effect of relative humidity

Hundred per cent RH was best for disease development. Disease severity was significantly higher at more than 90 per cent RH as compared to low levels of humidity. Thakur and Khare [7] reported maximum increase in lesion size at 100 per cent RH. When RH was decreased to 50 and 25 per cent, no increase in lesion size was recorded. It is inferred from these findings that the disease does not appear in areas with low or scanty rainfall i.e. in dry temperate zone of Himachal Pradesh.

4.1.3 Effect of photoperiod

Faster disease development occurred at 12/12 hr alternate photoperiod where as complete dark or light induced slower disease development. Mishra and Gupta [6] reported increase in lesion size in alternate light and dark conditions as compared to continuous light and dark. Wong [7] reported 12 hr alternate light and dark best for disease development and sporulation of the pathogen in soybean.

4.2 Role of weather variables on disease development

Under field conditions large number of biotic and abiotic factors influences the anthracnose of horse gram. During the present study the overall disease severity was moderate to high during both the years. Disease progressed readily when environmental conditions were favourable.

Mean temperature and relative humidity were found to be positively correlated, therefore it is evident that disease severity progressed significantly with rise in mean temperature and mean relative humidity. Rainfall and bright sunshine hours were found to influence disease insignificantly. This could be due to desiccation and washing of spores by bright sunshine and heavy rainfall. However, more information on other weather variables needs to be studied.

Multiple regression analysis highlighted significant effect weather parameters on disease development. The effect of weather variables on anthracnose development has been studied by many workers. Mittal [9] reported that daily average maximum temperature of 26-28ºC and minimum 18-20ºC, RH of about 80 per cent and moderate, steady rainfall (40-120 mm in 4-5 rainy days/week) favoured the progress of anthracnose of black gram. Rana and Kaushal [10] reported that temperature near 23ºC, higher relative humidity combined with more number of rainy days and 2.62 sunshine hours were favourable for Colletotrichum leaf spot development in urdbean.

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