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RESEARCH ARTICLE   Legume Research- An International Journal, Volume Issue : ()

Impact of Soil Moisture and Temperature on the Epidemiology of Collar Rot of Chickpea Caused by *Sclerotium rolfsii* (Sacc.) and Assessment of Yield Loss

Sanju Tamang[1], Poly Saha[2]

10.18805/LR-4855

ABSTRACT

Background: Collar rot (CR) of chickpea instigated by *Sclerotium rolfsii* (Sacc.) is one of the most prevalent soil-borne pathogens and identified as one of the major production constraints in chickpea worldwide. Potential threat exerted by the pathogen is greatly influenced by soil moisture condition (SMC) and soil temperature (ST), which play a substantial role towards the development of host-pathogen dynamics and bring about havoc yield loss. This study was aimed to enumerate the role of soil temperature and soil moisture in the establishment of CR symptoms in chickpea under controlled environment as well as to determine the relationship between disease severity and yield loss.

Methods: All the experiments were carried out at the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, Nadia. One of the most popular chickpea variety Anuradha was used for two consecutive years (2018-19 and 2019-20) of experiment in the present study. This variety showed a moderately susceptible reaction against CR under natural epiphytotic conditions.

Result: The result revealed ST being 25 to 30°C and SM level of 80% is optimal for establishing CR incidence in chickpea. Correlation determined between disease severity and yield loss unveiled loss in yield could be from 0.13 q ha^{-1} with every 1% increase in disease severity.

Key words: Chickpea, Collar rot, *Sclerotium rolfsii*, Soil moisture, Soil temperature, Yield loss.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the world's third most important food legume grown globally over 52 countries and India ranks first in the production of chickpea with 10.12 million tons, which share 46% of the total pulses production in India (FAOSTAT, 2021). The major constraint of chickpea production is due to several biotic stresses aggravated by the interaction of numerous abiotic factors. CR of chickpea has been identified as the most pervasive disease rendering 55-95% mortality in India (Sharma and Ghosh, 2017). In this disease, chickpea is parasitized by the *Sclerotium rolfsii*, at the juvenile stage near the root and collar region and complete crop loss is recorded within 30 days of sowing under conducive condition. Contemporary studies exposed that there is a shift of paradigm in the pathogen's prevalence due to climate change and therefore hamper the yield potential (Rajlaxmi, 2020; Sood *et al*., 2020). Study of yield loss is relevant to comprehend the importance of the disease. Presently, CR becoming more predominant in the areas with erratic rainfall that increases soil moisture for extensive periods along with warm temperatures (Tarafdar *et al*., 2018). Moreover, having highly competitive saprophytic ability and a wide host range makes it fit to survive prolong even in dry climatic regions (Srividya *et al*., 2022). The soil-borne pathogens are greatly influenced by the soil environment *viz*. in chickpea, low soil moisture stress and high temperature significantly increased the incidence of dry root rot caused by *Rhizoctonia bataticola* (Sharma and Pande, 2013) but conversely, it improves the defense response of plants against other pathogens (Sinha *et al*., 2016). Contrary, high soil moisture and low temperature also favour some diseases (Ferraz *et al*., 1999). So, the current study was focused to determine and quantify the influence of differential soil moisture and temperature on the severity of CR infection, colonization and, expression of disease symptoms in chickpea under controlled environment and thereafter, the likelihood of yield loss with each unit increase in disease severity.

[1]Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Nadia-741 252, West Bengal, India.
[2]College of Agriculture, Extended Campus of Bidhan Chandra Krishi Viswavidyalaya, Burdwan-713 101, West Bengal, India.

Corresponding Author: Poly Saha, College of Agriculture, Extended Campus of BCKV, Burdwan-713 101, West Bengal, India.
Email: poly.saha@gmail.com

How to cite this article: Tamang, S. and Saha, P. (2022). Impact of Soil Moisture and Temperature on the Epidemiology of Collar Rot of Chickpea Caused by *Sclerotium rolfsii* (Sacc.) and Assessment of Yield Loss. Legume Research. DOI: 10.18805/LR-4855.

Submitted: 17-12-2021 **Accepted:** 22-04-2022 **Online:** 31-05-2022

MATERIALS AND METHODS

All the experiments were done at the Department of Plant Pathology, BCKV, WB in the year 2018-19 and 2019-20. The location of farm is 22°93'N latitude to 88°33'E longitude at an elevation of 9.75 meters above MSL. Chickpea variety Anuradha was used for two consecutive experimental years and this variety showed moderately susceptible reaction against CR under natural epiphytotic conditions. Diseased chickpea plants exhibiting typical symptoms of CR were collected from the research field and pathogen was isolated following the standard procedure and pure culture was maintained in the PDA medium throughout the experiment for artificial inoculation in subsequent studies. For mass multiplication of *Sclerotium rolfsii* wheat grain was used. Grains were soaked in water for 6 hrs then boiled little, excess water drained, air-dried and complemented with 50 g calcium carbonate in 1 kg wheat grains. Two hundred grams of wheat grains were packed in each of 6 × 11 inches polypropylene bags and plugged with non-absorbent cotton with the support of a one-inch diameter PVC ring. These bags were sterilized and after sterilization the bags were inoculated with 2-3 mycelial discs (5 mm) taken from the periphery of the 5-day old culture of *S. rolfsii* grown on PDA earlier. The inoculated bags were kept at 25±2°C for 15 days. This multiplied culture was inoculated in the soil 2 days before sowing.

To study the effect of temperature on *S. rolfsii* four temperature regimes 20°C, 25°C, 30°C and 35°C were chosen. Under *in vitro* conditions paper towel technique was used as given by Pandey *et al*. (2006). Seeds of Anuradha were grown in sterilized media and 7 days old seedlings were uprooted inoculated at the collar region by the pin-pricking method. An active growing culture of *S. rolfsii* grown in PDA petri plates was cut into a 5 mm disc and wrapped lightly with tape around the pricked region. Now, inoculated seedlings were placed inside the folded, moist blotting paper leaving the shoots outside and then incubated at different temperature regimes inside BOD allowing 14 h of light.

The experimental design followed was a completely randomized block design (CRBD) and in each case, three replications allowed consisted of 10 plants. Total 30 plants per treatment were scored for disease severity. Seedlings inoculated with only sterile distilled water served as control and disease severity was recorded seven days after inoculation using 1-5 rating scale. Experiments performed batch-wise and moisture maintained not less than 50%.

The influence of four soil moisture regimes, *i.e*. 40%, 60%, 80% and 100% were studied on the development of CR in chickpea. The gravimetric method was used to determine the soil moisture given by Sharma and Pandey, **(**2013) under laboratory conditions. The following formula was used to calculate the SMC:

$$\text{SMC \%} = \frac{\text{Saturated soil weight-oven-dry soil weight}}{\text{Oven dry soil weight}} \times 100$$

The seeds were surface sterilized and sown in pre-weighted pots (5 seeds per pot). The experiment was set up in four different sets with four replications and four different soil moisture conditions were maintained in the pot *i.e*., maximum soil moisture 100%, 80%, 60% and 40% conditions. For pots filling, sterilized soil was mixed with *Sclerotium rolfsii* mass multiplied in wheat seed medium @50 g/2kg soil (Gupta, 2001) and this pathogen infested soil was filled in the 6inch pots (2.0 kg/pot). Control pots were maintained without inoculations. Sowing was done 2 days after inoculation. The seedlings were allowed to grow under normal conditions for 7 days. After 7 days different levels of SMC were created. The experiment was repeated twice with an equal number of treatments and replications. The four levels of SMC (40%, 60%, 80% and 100%) were adjusted by maintaining the constant weight by regular weighing and replacing the moisture deficit in each pot by watering (Suriachandraselvan and Seetharaman, 2003).

The experiment was designed in a completely randomized design (CRD) and the disease severity was recorded at regular time intervals up to 7 days starting from inoculation and continued till the mortality. Disease severity was scored using 1-5 rating scale as recommended by Indian Institute of Pulses Research (IIPR) rating scale for scoring CR of chickpea. Five distinct groups denoted as 1= highly resistant (less than 10% mortality); 2= moderately resistant (11-20 % mortality); 3= moderately susceptible (21-30% mortality); 4= susceptible (31-40% mortality); and 5 = Highly susceptible (greater than 40% mortality), as per the standard procedure (Chaudhary, 2009)**.** The per cent of incidence measured using the following formula:

$$\text{Percent incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of plants}} \times 100$$

The correlation between disease severity with SMC and ST were counted separately and a regression model was developed for future projection of the diseases under the given soil moisture and temperature situation. The yield loss experiment was set up in two situations. Locally popular variety ‘Anuradha’ was grown in both natural and controlled condition, where no growth of *Sclerotium rolfsii* was allowed. Disease incidence scored from natural epiphytotic state and yield data collected from both the situations and compared. The linear regression equation was used to draw the correlation between disease severity and yield loss and a prediction equation was developed. The data were analyzed using MS Excel. Percent disease severity was subjected to arcsine transformation (Gomez and Gomez, 1984). Transformed data were analysed through MS Excel and ANOVA developed. The level of significance and interaction effects were evaluated. Linear regression and correlation between ST, SMC and CR severity were also determined.

RESULTS AND DISCUSSION

Appearances of the initial symptom after artificial inoculation were recorded starting from 24 hours post-inoculation (hpi) and continued up to 7 days. At 20°C the first symptoms appear 48 hpi with minute speck at the point of infection. At 25°C and 30°C the symptoms appear 30 hpi and at 35°C the symptoms appear nearly 36 hpi. After 72 hpi the extend of the infection was found to be similar at 25°C, 30°C and

35°C and it remains the same up to 96 hpi (4 days). The rate of infection was maximum at 30°C as observed followed by 25°C and the ultimate score on disease severity was computed at 7 days after inoculation presented in (Table 1). The rate of progression of the disease was much lower at 20°C and the ultimate disease establishment was also recorded at rated. At 35°C disease initiation was delayed by 6 hr. than 30°C but the rate of progression was found at par with 30°C up to 4 days but then the growth was checked and final disease establishment went below 30°C (Table 1). In control pots, healthy crop appearance noticed.

The test result showed that temperature changes counted a significant effect on the disease development of CR of chickpea though raise in temperature from 25°C to 30°C was found non-significant. All the other treatment showed significant differences except temperature 30°C and 35°C. Scattered plot technique was used to depict the disease severity recorded on 0-5 scale along with temperature ranges showed maximum disease severity at 30°C almost at par with 25°C (Fig 1).

The correlation derived between the disease severity and different temperature was found to be positively correlated and statistically significant with a high correlation coefficient value (r = 0.863), which means variation in the disease severity can be explained up to 86.3 per cent with the change in a temperature gradient and data revealed optimum temperature is 25 to 30°C beyond which the strength of correlation declined. The similar result was earlier reported by Ayed *et al*., 2018 who reported radial growth and dry mycelium production of *Sclerotium rolfsii* were highest at 30°C.

The four-level of SMC *viz*., 40%, 60%, 80% and 100% was considered for study and temperature maintained between 25 to 30°C. Table 2 contain the detail observation on the behaviour of the pathogen upon artificial inoculation during the establishment of the disease in the host when imposing different soil moisture condition.

Disease scored 7 days after exposing the chickpea at different SMC levels revealed maximum disease incidence at SMC 80% followed by SMC 100% but their differences were statistically at par (Table 3). Other two treatments showed lowest disease incidence though a positive significant relationship had been observed between the CR incidence and SMC that was proved by a high correlation coefficient value (r= 0.872) (Table 3), which means variation in the disease severity can be explained up to 87.2 percent with the change in moisture gradient. On the basis of result it can be concluded that 80% soil moisture condition is optimum for the establishment of CR in chickpea grown under West Bengal conditions. The regression equation developed for each soil moisture level showed a positive significant relationship among the two variables and their goodness of fit was determined by the R^2 value. High Co-efficient of determination value (R^2) explained variation in disease severity could be expected from 85.6% to 97.0% with the change in SMC level proved that SMC has a pronounced and significant effect on CR disease severity (Fig 2). The similar result was earlier reported by Tarafdar *et al*. (2018), on CR of chickpea where the highest severity was detected at 80% SMC, followed by 100 and 60% SMC; Prasad and Saifulla (2012), on the population of *F. udum* causing pigeonpea wilt revealed a decrease in growth of the pathogen at SMC 25 to 50% while 75% SMC was favorable for its establishment; Sharma and Pande (2013) carried out a similar type of experiment on dry root rot of chickpea (*Rhizoctonia bataticola*) where pathogen preference was observed just reverse to *S. rolfsii i.e*. high temperature (35°C) coupled with SMC 60% found to be the main predisposing factors for chickpea to the disease. The experiment proved temperature and soil moisture are the two most vital variables having a positive significant effect on the colonization and development of *Sclerotium rolfsii* in chickpea.

Correlation evaluated between different ST and SMC levels to find out the optimum combination for the growth and development of CR in chickpea. Correlation coefficient values (r) were determined in various combinations and plotted in the graph (Fig 3). In the graph, Y-axis represents the correlation coefficient value (r) that ranges between -1 to +1. The X-axis represents the ST ranges 20°C, 25°C, 30°C and 35°C. r values calculated from different combinations of ST and SM level *i.e*. SMC 40%, SMC 60%, SMC 80% and SMC 100% are plotted showed a negative significant correlation between ST 20°C with all the combination of SMC percentage tested but at the temperature between 25-30°C the trend line found to touch the X-axis (r = 0) means from this temperature disease incidence initiate and get established along with the SMC

Table 1: Disease severity of collar rot in chickpea recorded at different temperature levels and different hours post-inoculation.

Temperature °C	Hours post-inoculation (hpi)							Mean	Tukey t grouping	Correlation co-efficient value (r)
	24 hpi	48 hpi	72 hpi	96 hpi (4 day)	120 hpi (5 day)	144 hpi (6 day)	168 hpi (7 day)			
20	-	0.463	1.240	2.040	3.477	4.763	5.967	2.992	a	0.863
25	-	1.217	2.743	9.667	13.600	18.813	20.717	11.126	b	
30	-	1.737	2.930	11.073	15.067	22.250	29.643	13.783	bc	
35	-	1.113	2.800	10.907	14.143	21.147	22.023	12.022	bc	

hpi= Hour post inoculation. Grouping done by Tukey t-test at 5% probability level. Means followed by the same uppercase do not differ from each other.

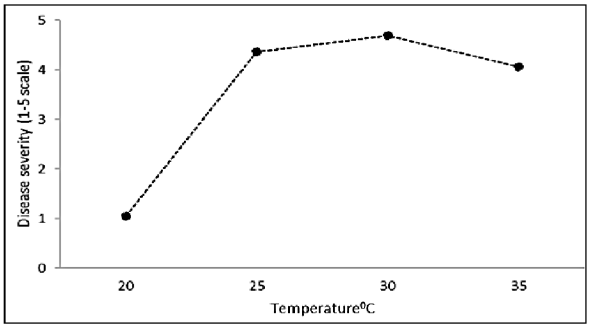

Fig 1: Effect of temperature on collar rot severity (1 - 5 scale) under *in vitro* condition.

60% and SMC 80%. At SMC 100% and ST 20°C again line moved to a downward direction representing a negative significant correlation. In all the other cases, soil temperature and moisture were noticed to share a positive significant correlation ranging from (r= 0.372 to 0.89) but among them, SMC 80% and temperature 30°C showed the highest value of correlation coefficient r= 0.89 followed by SMC 80% and temperature 25°C r= 0.82. The temperature ranges between 25-30°C and 80% soil moisture are most congenial for the development of CR in chickpea.

The yield loss experiment revealed a negative significant correlation between the two variables *i.e* disease severity and yield and it was confirmed by a high coefficient

Table 2: Observations were recorded on the physical appearance of chickpea upon infection with collar rot at different moisture levels.

Soil moisture level	Initial Establishment of the infection after hr. of inoculation		Ultimate disease scored upto 7 days of inoculation
	Inoculated	Non-inoculated	
40%	i. Minute speck observed at the days point of inoculation 24 hpi. ii. Very low pathogen growth result after 48 hpi. iii. Physiological symptoms started aggravating gradually.	Water stress symptoms appear	No disease symptoms were observed in chickpea even after 7 of inoculation. Physiological wilting was noticed in both inoculated and un-inoculated plants but without any fungal growth inoculated that prolonged exposure to insufficient SMC may from poor growth of the plant and also checked the activity of the pathogen.
60%	i. Initial response was the same as previous and moderate disease incidence at 48 hpi.	Combine effect of biotic and abiotic stress observed	After 48 hpi blackening, tissue maceration, conspicuous hyphal growth and rotting started.
80%	i. Unlike the other two treatments, here initiation of infection started at 16 hpi ii. Rate of progression of disease was found faster than the other treatments. iii. Plants showed symptoms other than wilting like blackening of the collar region that spread both ways up and down and girdles the stem.	Healthy growth of the plant recorded	Here also disease symptoms started to appear but with more fungal mantle covering around the infected region.
100%	i. Establishment of the pathogen occurred 24 hpi but after the initial the colonization spread of the pathogen was found same at 48 hpi as recorded in 80% SMC. ii. Nature of spread of the pathogen and plant growth found at par with 80% SMC	Healthy growth of plant recorded	Similar to 80% SMC up to 4 days after inoculation but ultimate disease score was lower than 80% SMC.

*hpi= hour post inoculation. SMC = Soil moisture condition.

Table 3: Disease severity of collar rot in chickpea recorded at different soil moisture condition levels and different hours post-inoculation.

SMC %	Hours post-inoculation (hpi)							Mean	Tukey t grouping	Correlation coefficient (r)
	24 hpi	48 hpi	72 hpi	96 hpi (4 day)	120 hpi (5 day)	144 hpi (6 day)	168 hpi (7day)			
40	0.323	0.600	0.767	0.747	1.027	1.237	2.050	0.964	a	0.872
60	0.343	2.133	9.870	21.273	26.183	35.673	39.890	19.338	a	
80	1.297	2.757	11.687	21.340	33.770	48.397	56.840	25.155	b	
100	0.707	2.820	12.377	21.627	34.417	44.287	45.503	23.105	b	

SMC= Soil moisture condition, hpi= Hour post inoculation. Grouping done by Tukey t test at 5% probability level. Means followed by the same uppercase do not differ from each other.

of determination (R^2) value close to 1. Fig 4 represented two years pooled (2018-19 and 2019-20) mean that confirmed with the increase in disease severity there was a significant decrease in the seed yield of chickpea. Equation established was Y= 11.34-0.13X, R^2 =0.907 *i.e*. attainable yield could be 11.34 q ha^{-1} and loss due to the CR predicted 0.13 q ha^{-1} for every 1% increase in disease severity. Here, also high co-efficient of determination value R^2 =0.907 established the feasibility of the result obtained. Similar results on disease severity and yield loss were recorded earlier by Das *et al*., (1995) in Cercospora-groundnut pathosystem and Saha and Das (2012) in early blight tomato pathosystem.

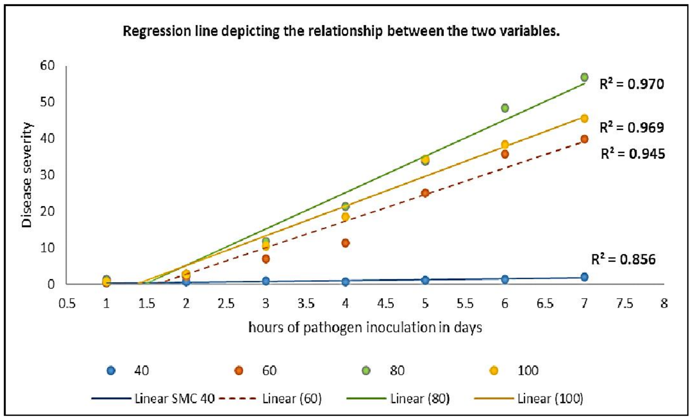

Fig 2: Co-efficient of determination value (R^2) at different soil moisture condition (SMC) level.

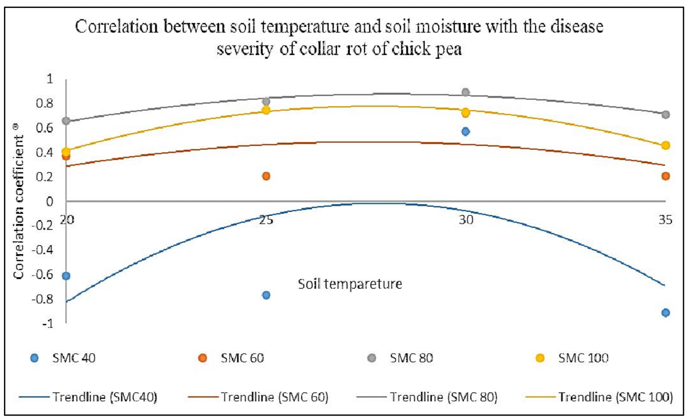

Fig 3: Correlation between temperature and soil moisture with the disease severity of collar rot in chickpea.

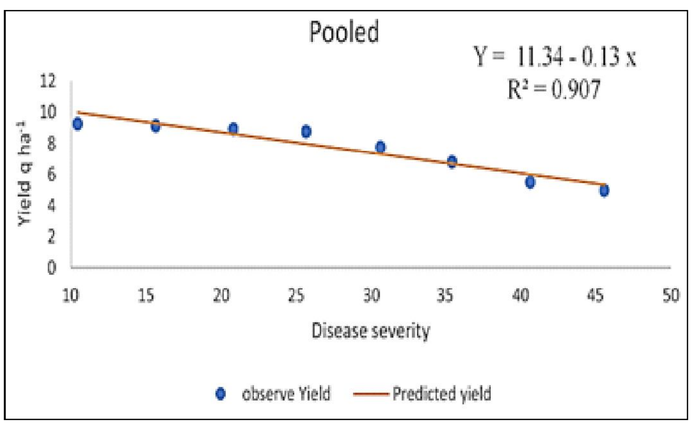

Fig 4: Pooled data of linear regression equation and corresponding regression line for collar rot disease severity in chickpea and seed yield.

Conclusion

This research results interpreted, higher incidence of CR ensues at the temperature 25-30°C along with SMC 80%. This result was validated through different combinations of experiments upon temperature and soil moisture. Correlation coefficient (r) and coefficient of determination (R^2) value determined a positive significant relationship between the dependent variable (disease severity) and the independent variables (ST and SMC). The findings showed a good correspondence between the two factors *i.e* ST and SMC r = 0.863 and 0.872 respectively.

The yield loss could be expected of 0.13 q ha^{-1} for every 1% increase in disease severity. These findings would give an insight to future researchers to forecast the estimation of yield loss in correspondence to the amount of disease prevalence that could steer up the development of a computer-simulated disease forecast system as well as to develop an effective disease management strategy.

Conflict of interest: None.

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