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Effect of temperature on *Rhizoctonia bataticola* and dry root rot in chick pea

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A B S T R A C T

Chickpea dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler is a soil borne fungal pathogen causing significant yield losses due to change in environmental conditions. Influence of seven temperatures regimes (15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 45°C) were tested on growth of *R. bataticola* isolates representing Madhya Pradesh, Karnataka, Maharashtra, Andhra Pradesh and Telangana states of India. The maximum mycelial growth was observed at 35ºC followed by 30 and 25ºC in all the isolates. The optimum temperature for dry root rot severity rating was at 35°C (8.5) followed by 30°C (7.9) followed by 25°C (7.0). Among the isolates, Telangana isolate was virulent and caused maximum disease severity.

Introduction

Chickpea (*Cicer arietinum* L.) is the premier pulse crop grown in more than 50 countries originated in south west Asia and is cultivated from ancient times both in Asia and European countries. It is the world's second most important food legume next to common bean. In India, chickpea is grown in an area of 10.22million hectares with a production of 9.53 million tonnes and productivity of 967kgha^{-1} (Ministry of Agriculture, Govt. of India, 2013-14). Chickpea usually grown after rainy season on conserved soil moisture during winter in the tropics; in spring in the temperate and Mediterranean regions. Chickpea cultivation is often subjected to significant yield losses due to insects and diseases ranging from 5-10% in temperate and 50-100% in tropical regions (Van Emden *et al.*, 1988). Among the diseases of chickpea, dry root rot is emerging as the most destructive constraint to chickpea productivity and production. Dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler [Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid] is a soil borne necrotrophic fungal pathogen that has a global distribution, which can infect more than 284 plant species throughout the world including monocot and dicots (Farr *et al.*, 1995). The disease is more prevalent during high temperature and low soil moisture conditions (Pande and Sharma, 2010). The most favorable temperatures for development of the *Rhizoctonia bataticola* ranged between 25 and 35ºC.

In chickpea field, the onset of the disease appears as scattered drying of the plants. Affected plants are usually straw coloured,

but in some cases the lower leaves and stems show brown discolouration. The tap root appears black, rotten and devoid of most of the lateral and fine roots. The dead root become quite brittle and shows shredding of bark. Dark minute sclerotial bodies can be seen on the roots exposed or inside the wood. When the dry stem of the collar region is split vertically, sparse mycelium or minute sclerotia can be seen in the pith (Nene *et al.*, 1991). The effect of temperature on the growth of *Rhizoctonia bataticola* has been investigated by several authors, but up to now no satisfactory answer has been given on the optimal temperature conditions required for this necrotrophic pathogen on chickpea and its isolates from different locations.

In this paper, we report the response of isolates of *Rhizoctonia bataticola* at different temperatures on the growth and the disease severity on susceptible cultivar.

Materials and Methods

A roving survey was conducted to collect dry root rot effected chickpea plants in central and southern parts of India *viz*. Madhya Pradesh, Karnataka, Maharashtra, Andhra Pradesh and Telangana. The Isolates collected from these locations were studied for pathogenicity on chickpea. The isolates representing five states which are showing disease severity more than seven rating were selected for the temperature studies. The five isolates selected were Rb 1 (Madhya Pradesh), Rb 2 (Karnataka), Rb 3 (Maharastra), Rb 4 (Andhra Pradesh) and Rb 5 (Telangana).

Effect of temperature on growth of *Rhizoctonia bataticola*

Effect of seven different temperatures *viz.* 15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 45°C were studied on growth of *Rhizoctonia bataticola* on potato dextrose agar. Mycelial discs of 5mm diameter were cut from the edge of 3 days old culture of five isolates grown in 25ºC were transferred to the center of 90mm Petri dish and incubated at different temperatures with 12h photoperiod. Each treatment was replicated three times in a completely randomized design. The average diameter of the fungal colony was recorded at 48h, 72h and 96h after incubation.

Effect of temperature on disease development

Effect of different temperatures *viz.* 15˚C, 20˚C, 25˚C, 30˚C, 35˚C, 40˚C and 45˚C on severity of dry root rot on susceptible genotype BG 212 with same five representative isolates was studied by adopting paper towel technique. Inoculum was prepared from the seven days old culture of *Rhizoctonia bataticola* isolates grown on 100ml potato dextrose broth medium (PDB). The culture of each isolate was grinded in a blender by adding 50ml of sterile distilled water to each fungal mat. Seven day old seedlings of BG 212 grown in sterilized sand were uprooted, washed under running water and were inoculated by dipping in the inoculum of *Rhizoctonia bataticola* isolates for 2 min. Inoculation was done through root dip inoculation technique.

Seedlings inoculated with sterile deionized water served as control. Seven to ten inoculated seedlings were placed in paper towel with the shoot left outside, folded, moistened and placed in trays. Trays were transferred to incubators maintained at seven different temperatures with 12h photoperiod and regularly moistened with sterile deionized water for seven days. At seven days after inoculation, the data on disease severity was recorded using 1 to 9 disease severity rating scale (Table 1) developed by Nene *et al.*, (1991).

Results and Discussion

Temperature was known to have profound effect on the growth of fungal organism. Present studies were taken up to know the optimum, minimum and maximum temperature requirements for the growth of pathogen. Five isolates (Rb 1, Rb 2, Rb 3, Rb 4 and Rb 5) representing 5 different states were selected to conduct the study. All the isolates were grown at seven different temperatures *viz.*, 15ºC, 20ºC, 25ºC, 30ºC, 35ºC, 40ºC and 45ºC.

At 48h after inoculation, among the seven different temperatures tested, the maximum colony growth was observed in Rb 3 (13.0mm) and no growth was observed in Rb 5 at 15ºC whereas the maximum colony growth was observed in Rb 5 (38.3mm) and the least in Rb 1 (27.7 mm) at 20ºC. Maximum colony growth of 54.0, 77.0 and 15.0 mm in Rb 3 and the least of 44.0, 62.3 and 3.7 mm in Rb 1 was recorded at 25, 30 and 40ºC respectively. In contrast to this, maximum colony growth was observed in Rb 4 (85.3mm) and the least in Rb 1 (71.3 mm) at 35ºC. The difference between isolates at different temperatures was significant except in Rb 2 and Rb 5 at 30 ºC.

At 72h after inoculation, the maximum colony growth of 25.3 and 67.0 mm in Rb 3 and the least growth of 17.3 and 60.3 mm was observed in Rb 2 at 15 and 20ºC respectively while at 25ºC, maximum colony growth was observed in Rb 2 (84.7mm) and the least in Rb 1 (67.0 mm). At 30ºC, maximum colony growth was observed in Rb 2, Rb 4, Rb 3 (90 mm) whose difference is at par with each other and the least in Rb 1 (82.0mm). At 35ºC, colony growth reached its maximum (90mm) in all the isolates which were at par with each other. At 40ºC, maximum colony growth was observed in Rb 3 (20.7mm) and the least in Rb 1 (8.3mm). There was significant difference between isolates at 15, 20, 25 and 40ºC except in Rb 2 and Rb 4.

At 96h after inoculation, maximum colony growth of 35.3 and 85.3 mm was recorded in Rb 3 while the least was observed in Rb 4 (20.3mm) and Rb 2 (74.7mm) at 15 and 20ºC respectively. At 25ºC, 30ºC and 35ºC, all the isolates had covered the entire 90 mm Petri plate. The difference between the isolates at 25, 30 and 35ºC was at par with each other. Maximum colony growth was observed in Rb 3 (24.0mm) and the least in Rb 1 (10.7mm) at 40ºC. No growth was observed till 96h after inoculation in any isolate and medium also became dry at 45ºC.

The maximum colony growth was observed 72h after inoculation in all five isolates at 35ºC. After 96h of incubation, all the isolates had covered Petri plates at 25ºC and 30ºC. The sclerotial initiation was started after 48 hours at 30ºC and 35ºC. The sclerotial initiation started 72h after inoculation at 25ºC and it was observed at 96h after incubation in 20ºC. At 15ºC, it was observed that the growth was very slow and sclerotial initiation was observed after 144 hours after inoculation.

The above results were supported by Khan *et al.*, (2012) as they also observed pathogen growth over a wide range of temperature from 10^0C to 45^0C, but the optimum temperature for its growth was found to be 30^0C. The next best temperature for its growth was recorded 35^0C. Statistically the growth of the pathogen gradually decreased both at below 30^0C and above 35^0C. Patel and Patel (1990) also reported 35°C to be optimum temperature for growth and sclerotial formation of *M. phaseolina* in sesame. The results of Cesondes *et al.*, (2012) also showed that the cultures were well grown at 20ºC, their colony size on the 3rd day were 14 times larger than at 10 and 15ºC.

Table.2 Colony diameter of *Rhizoctonia bataticola* isolates at different temperatures under *in vitro* condition

48 HAI (mm)*						
Temperature (˚C)	**Rb 1**	**Rb 2**	**Rb 3**	**Rb 4**	**Rb 5**	**Mean**
15	4.0	3.3	13.0	6.7	0.0	5.4
20	27.7	32.7	37.0	31.7	38.3	33.5
25	44.0	53.7	54.0	51.3	50.0	50.6
30	62.3	71.3	77.0	75.0	71.0	71.3
35	71.3	82.0	80.0	85.3	77.7	79.3
40	3.7	9.3	15.0	13.7	10.3	10.4
45	0.0	0.0	0.0	0.0	0.0	0.0
Mean	30.4	37.7	39.4	36.5	35.3	
72 HAI (mm)						
Temperature (˚C)	**Rb 1**	**Rb 2**	**Rb 3**	**Rb 4**	**Rb 5**	**Mean**
15	20.0	17.3	25.3	17.7	18.3	19.7
20	64.0	60.3	67.0	61.0	61.7	62.8
25	67.0	84.7	82.0	80.0	71.7	77.1
30	82.0	90.0	90.0	90.0	88.3	88.1
35	90.0	90.0	90.0	90.0	90.0	90.0
40	8.3	14.7	20.7	18.3	13.0	15.0
45	0.0	0.0	0.0	0.0	0.0	0.0
Mean	47.3	52.3	53.6	50.0	49.0	
96 HAI (mm)						
Temperature (˚C)	**Rb 1**	**Rb 2**	**Rb 3**	**Rb 4**	**Rb 5**	**Mean**
15	25.3	21.3	35.3	20.3	24.3	25.3
20	80.3	74.7	85.3	78.3	75.0	78.7
25	90.0	90.0	90.0	90.0	90.0	90.0
30	90.0	90.0	90.0	90.0	90.0	90.0
35	90.0	90.0	90.0	90.0	90.0	90.0
40	10.7	17.3	24.0	20.0	15.3	17.5
45	0.0	0.0	0.0	0.0	0.0	0.0
Mean	55.2	57.0	59.2	55.2	54.9	

Factors	**CD**		
	48 HAI	**72 HAI**	**96 HAI**
Temperature	0.64	0.54	0.55
Isolate	0.54	0.46	0.47
Temperature x Isolate	1.42	1.21	1.23

HAI – Hours after inoculation * mean of three replications

Table.3 Disease severity (1-9 rating) of *Rhizoctonia bataticola* isolates on BG 212 at different temperatures

Isolates	Temperatures (˚C)							
	15	20	25	30	35	40	45	Mean
Rb 1	1.7	3.1	6.7	7.4	8.3	9.0#	9.0#	6.5
Rb 2	1.3	3.0	7.2	8.2	8.7	9.0#	9.0#	6.6
Rb 3	1.3	2.9	7.0	7.9	8.3	9.0#	9.0#	6.5
Rb 4	1.7	3.2	6.5	8.0	8.1	9.0#	9.0#	6.5
Rb 5	1.0	3.4	7.4	8.2	9.0	9.0#	9.0#	6.7
control	1.0	1.0	1.0	1.0	1.0	1.0##	1.0##	1.0
Mean	1.4	3.1	7.0	7.9	8.5	9.0	9.0	

Factors	C.D.
Temperature (T)	0.2
Isolates (I)	0.17
T x I	0.45

#Mortality of plants due to combined effect of physiological wilting and Pathogen
##Plants showed physiological wilting
* mean of three replications

Table.1 Rating scale used to record disease severity of dry root rot in chickpea

Rating	Observation
1	No infection on roots
>1 - ≤3	Very few small lesions on roots
>3 - ≤5	Lesions on roots clear but small, new roots free from infection
>5 - ≤7	Lesions on roots many, new roots generally free from lesions
>7 - 9	Roots infected and completely discoloured

Effect of temperature on development of disease

There was very significant relation between the temperature and the development of the disease on the chickpea. The disease severity on the BG 212 cultivar was observed after seven days of incubation at different temperatures. The average disease severity at different temperatures was given in the Table 3. Maximum disease severity rating was recorded in Rb 1 and Rb 4 (1.7) and no symptom was observed in Rb 5 (1.0) inoculated plants at 15ºC. In contrast, maximum disease severity rating was recorded in Rb 5 (3.4) and the least in Rb 3 (2.9) inoculated plants at 20ºC. Maximum disease severity rating of 7.4 in Rb 5 inoculated plants and the least in Rb 4 (6.5) and Rb 1 (6.7) which was at par with each other in inoculated plants at 25ºC. At 30ºC, maximum disease severity was observed in Rb 5 and Rb 2 (8.2) and the least in Rb 1 (7.4). At 35ºC, maximum disease severity was observed in Rb 5 (9.0) and least was observed in Rb 4 (8.1) inoculated plants. At 40 and 45ºC, mortality of plants was due to combined effect of physiological wilting and *Rhizoctonia bataticola* isolates. In control, plants were shriveled at 40ºC whereas at 45ºC

there was complete physiological wilting. It was also supported by the scarce and absence of mycelial growth in Petriplates at 40 and 45ºC respectively in all the isolates.

The optimum temperature for dry root rot development was 35°C as maximum disease severity rating of 8.5 was observed irrespective of the isolate. This was followed by 30°C (7.9) followed by 25°C (7.0). It was observed that 20°C and 15°C had helped in the development of the lesions but could not develop further. The disease severity was very low 1.4 rating at 15°C while it was 3.1 at 20°C.

Among Rb 1, Rb 2, Rb 3 and Rb 4 isolates, there was no significant difference while Rb 5 was virulent isolate compared to others. The plants had showed symptoms on the tap root leaving the lateral roots unaffected at 25°C. At 30, 35, 40, 45°C, there was complete blackening of the roots and the reisolation from the roots showed the presence of *Rhizoctonia batatiocla*. The uninoculated plants did not show any symptoms except at 40 and 45°C which showed physiological wilting and death of plants by complete drying respectively.

Similar observations were recorded by Sharma and Pande (2013) as disease incidence of dry root rot was significantly affected by high temperature. Out of five temperature levels *viz.*, 15°C, 20°C, 25°C, 30°C and 35°C tested, chickpea predisposed to dry root rot early and severity was more at 35°C. Singh and Mehrotra (1982) observed increased levels of seed exudates when incubated at 35ºC than at 15 and 25ºC which contributed to increased pre-emergence damping off in gram seedling by *R. bataticola*. This study clearly demonstrated high temperature (35ºC) was predisposing chickpea to *R. bataticola* infection, colonization and development of disease.

From the results we can say there is variation among the isolates of *Rhizoctonia bataticola* and temperature had profound effect on the growth and disease severity on chickpea. The optimum growth of the fungal isolates was found in 35ºC. The next best temperature was 30ºC followed by 25ºC, 20ºC and 15ºC. There was very meager growth in 40ºC and no growth was observed at 45ºC.

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