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Effect of Temperature, Soil Moisture and Elevated CO_2 on Growth of *Rhizoctonia bataticola* and Dry Root Rot Disease of Chickpea in Karnataka State

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Authors Contribution

Gururaj Sunkad & Deepa Dore : Conceptualization, executed the experiment, analysis of data, investigation, writing, methodology, review, editing and revise of this article

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Abstract

Dry root rot caused by *Rhizoctonia bataticola* is becoming an emerging disease as per the new reports and considered as potential threat to chickpea productivity and production under changing climatic scenario. The identity of pathogen was confirmed molecularly using ITS-1 and ITS-4 primers which produced amplified product size of 500-650 bp in all three isolates indicating that all the isolates belonged to species *Rhizoctonia bataticola.* The maximum colony growth of *R. bataticola* and the dry root rot disease severity was recorded at 30-35 ºC which is considered as optimum temperature range for growth of pathogen and development of disease. Highest severity of dry root rot and lesser plant growth parameters such as root length, shoot length and total biomass were observed at 40-60 per cent soil moisture regimes, irrespective of type of soil. Further, elevated CO_2 @ 550 ± 25 ppm with 2 ºC rise in temperature recorded higher dry root rot well as reduced growth parameters of chickpea. With respect to correlation studies, increase in the temperature lead to decreased radial growth of pathogen and dry root rot disease incidence. It is also inferred that increase in the soil moisture led to increase in growth parameters in both black as well as red soils.

Keywords : Dry root rot of chick pea, Climate change, Temperature, Soil moisture, Disease severity

Chickpea is one of the most important food legumes being cultivated in almost all over the world including temperate and sub-tropical regions. The crop faces various problems throughout the growing areas, some related to specific regions and some under wider range of climatic conditions. Chickpea cultivation is often subjected to significant yield losses due to insects and diseases ranging from 5-10 per cent in temperate and 50-100 per cent in tropical regions (Van Emden *et al.,* 1988).

The recent reports indicated that dry root rot is emerging as a potential threat to chickpea productivity and production (Ghosh *et al*., 2013). The disease is more prevalent during hot temperature 30 to 35 °C and low soil moisture conditions (Pande *et al*., 2010). Dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler [Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid] is a soil and seed 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). *Rhizoctonia bataticola* does not produce spores, but are composed of hyphae and sclerotia (hyphal propagules) acing as facultative plant pathogen causing complete loss in grain yield if chickpea crop is infected.

Environmental conditions like temperature, soil moisture and carbon dioxide play an important role in the viability and growth of *R. bataticola* (Khan, 2007). *R. bataticola* is able to produce microsclerotia under relatively low water conditions while viability of microsclerotia is drastically reduced at high water potentials (Olaya and Abawi, 1996). Temperature and soil moisture are the two important weather parameters influencing the dry root rot infection, colonization and development in chickpea. In the changing climatic scenario, studies on impact of climatic factors on pathogen and disease are scanty. Moreover, the crop is largely grown in rainfed environment and change in climatic factors within the rainfed ecologies may lead to varying degrees of growth of pathogen and intensities of dry root rot. Keeping this in view, studies on impact of climatic variables on growth of pathogen and disease incidence is felt necessary under changing climatic scenario. Hence, the effect of three climatic change variables *viz,.* temperature, moisture and carbon dioxide was studied on growth of pathogen and development of disease during present investigation.

Material and Methods

Isolation, Purification and Detection of *R. bataticola* Isolates

Chickpea plants showing typical dry root rot symptoms were collected from three different geographic regions (Region 1, Region 2 and Region 3) of Karnataka and used for isolation of pathogen. Infected roots were cut into pieces of 5-6 mm and were transferred on to sterilized potato dextrose agar (PDA) medium in Petri dishes and incubated at 25 ± 2 °C to obtain mycelial growth. After 48h of incubation, hyphal tips of the growing mycelium were marked on the underside of the petri dish with a glass marker by viewing through a light microscope. The cultures were purified by placing single sclerotial body transferred to PDA slants. Later, Koch postulates were carried out for all three isolates to confirm the pathogenecity of pathogen.

Molecular Detection of Isolates

Total DNA of three isolates of pathogen was extracted using Cetyl-Trimethylammonium Bromide (CTAB) method from young vegetative mycelium using the procedure given by Murray and Thompson (1980). The Internal Transcribed Spacers region was sequenced from three isolates belonging to three different geographic locations to confirm the identity of isolates. PCR (Mastercycler, Hamburg, Germany) amplifications of the ITS of rDNA was performed by using universal primers ITS-1 (52CCTGTGCACCTGTGAGACAG-32) as forward primer and ITS-4 (52 - TGTCCAAGTCAAT GGACTAT-32) as reverse primer. The PCR product was sequenced using forward and reverse primers at Medauxin Biotech. Ltd., Bengaluru. Homology search was done using BLAST algorithm available at the http://www.ncbi.nlm.nih.gov.,

Effect of Climate Change Variables on *R. bataticola* and Dry Root Rot Incidence

The effect of three climatic change variables *viz.,* temperature, moisture and carbon dioxide was studied on growth of pathogen and development of disease during present investigation.

Effect of Temperature on *R. bataticola*

Three isolates of pathogen namely, Rb1, Rb2 and Rb3 were used to study the effect of temperature on pathogen virulence. The isolates were inoculated on PDA medium and kept for incubation at different temperature regimes, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C and 45 °C. Later, the radial growth of mycelium of pathogen was recorded.

Effect of Temperature on Dry Root Rot Disease

Chickpea (Annigeri-1) seedlings were raised in small plastic containers which were filled with pre-autoclaved sand. Chickpea seeds are sown in these containers with 2 cm deep. Moisture was maintained by watering on daily basis, after ten days seedlings were used for the study. To study the effect of temperature on dry root rot, each isolate of pathogen was inoculated to ten days old seedlings separately, grown under *in vitro* conditions using paper towel technique. Inoculated seedlings were placed in folded, moist blotting paper with the shoots left outside and then incubated at different temperature regimes 15, 20, 25, 30, 35, 40, 45 and 50 °C with a 12 h photoperiod. The experiment was conducted with Completely Randomized Block Design (CRBD) with three replications (each replication consisted of 5 plants). Total 15 plants per treatment were scored for disease severity of dry root rot and the disease severity was recorded using 1-9 rating scale (Sharma and Pande, 2013) (Table 1).

Effect of Soil Moisture on Dry Root Rot Disease

Pot experiment was conducted to know the effect of soil moisture on dry root disease in glasshouse conditions. To obtain large amount of inoculum, the fungus was multiplied on sorghum grain medium and sufficient inoculums was applied to pots. The pots were incubated for 4 days to allow the pathogen to multiply in soil. The effect of seven soil moisture regimes, *i.e.* 40, 50, 60, 70, 80, 90 and 100 per cent was studied with black and red soils separately for the development of disease. Each treatment was replicated four times and each replication consisted of three pots (five plants/pot). Deionized water was used for maintaining the soil moisture content (SMC) in each treatment. The SMC was determined using the gravimetric method on an oven-dry basis. The method includes saturation of soil sample followed by removal of available soil moisture by oven drying (100 - 110 °C) until the weight remains constant. After removing from oven, samples were cooled slowly to room temperature and weighed again. The difference in weight was amount of moisture in the soil. The available SMC in the soil was calculated by the following formula.

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

Effect of Carbon Dioxide (CO_2) on Dry Root Rot

The study was conducted in the open top chambers maintained by Centre for Climatic Studies, University of Agricultural Sciences, Raichur. Totally, five sets of treatments were made to study the effect of carbon dioxide on dry root rot of chickpea.

T_1 : Elevated CO_2 @ 550 ± 25 ppm alone

T_2 : Elevated CO_2 @ 550 ± 25 ppm with 2 ºC rise in temperature

T_3 : Ambient CO_2 @ 390 ± 25 ppm with 2 ºC rise in temperature

T_4 : Reference Open Top Chamber

T_5 : Open plot

Under the open top chamber, susceptible chickpea variety (Annigeri-1) was sown in the sick pots. Each sick pot was sown with five seeds and for each treatment five replications were maintained. Observations on growth parameters and disease severity of dry root rot were recorded at 75 DAS.

Statistical Analysis

The data obtained in the laboratory as well as open top chamber experiments through Factorial Completely Randomized Design were analyzed by Statistical Package for Social Sciences (SPSS V.20). Further, correlation analysis was carried out to understand the relationship between the parameters.

RESULTS AND DISCUSSION

Isolation, Purification and Detection of *R. bataticola* Isolates

The results indicated that *R. bataticola* pathogen isolates produced black, brown to grey coloured mycelium that become darker with age (Fig. 1). The young hyphae were thin, hyaline, septate and dichotomously branched and later produce typical black sclerotia. The characteristic features of R. bataticola were right angle branching of the mycelium and constriction of the branch near the point of origin. The sclerotia formed were black, smooth, varying from spherical through oblong to irregular shapes. Later, the pathogenecity was proved successfully by following Koch postulates. In molecular detection, both ITS-1 and ITS-4 primers produced amplified product size of 500-650 bp in all the three isolates indicating that all the isolates are Rhizoctonia bataticola. Further, nucleotide sequencing was done for ITS region of 18S rRNA. The BLAST data results revealed that the R. bataticola species matched with the reference strains of NCBI results and identified as Rhizoctonia bataticola. The sequences are deposited in NCBI GeneBank, Maryland, USA along with location of the isolates and accession number Rb1 (KX270355.1), Rb2 (MG001962.1) and Rb3 (HQ392772.1) were obtained. Similarly, Aghakhani and Dubey (2009) isolated 23 isolates of R. bataticola from chickpea plants showing characteristic dry root rot symptoms from different chickpea growing states of India.

TABLE 1

Disease rating scale of 1-9 for dry root rot of chickpea

Rating	Observation
1	No infection on roots
>1 and <3	Very few small lesions (black discoloration) on roots
>3 and <5	Lesions (black discoloration) on roots clear but less; new roots free from infection
>5 and <7	Lesions (black discoloration) on roots more; many new roots generally free from lesions
>7 and 9	Roots infected and completely discoloured (black)

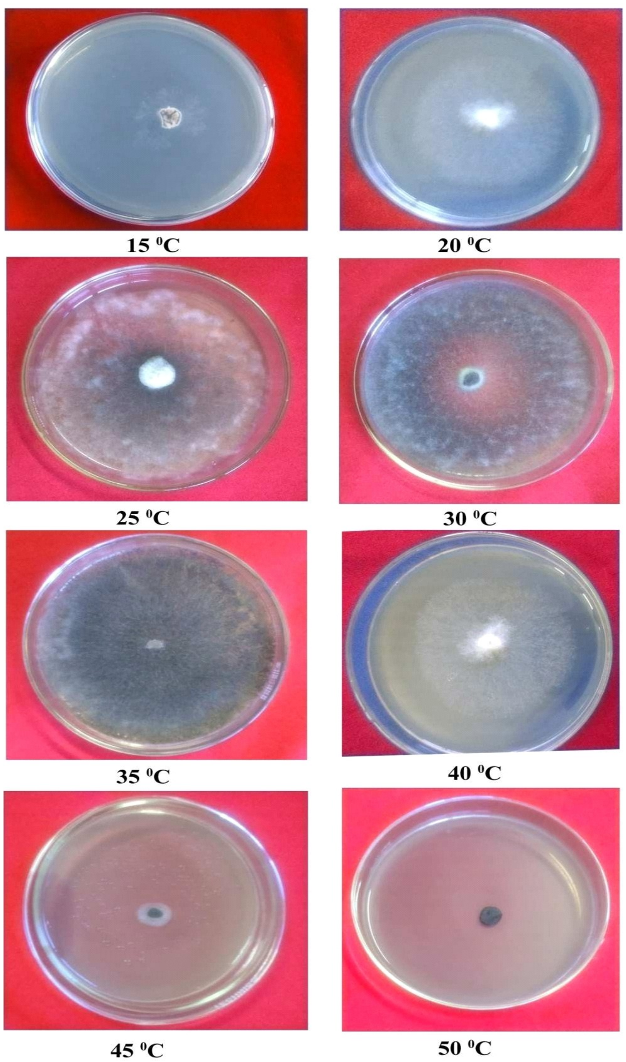

Fig. 1: Effect of temperature on radial growth of *R. bataticola*

Further, Sharma *et al.* (2012) also proved the pathogenicity of 50 isolates of R. bataticola using chickpea cultivar BG 212.

Effect of Climate Change Variables on *R. bataticola* and Dry Root Rot Incidence

The effect of three climatic change variables *viz.,* temperature, moisture and carbon dioxide was studied on growth of pathogen and development of disease during present investigation was studied and results are presented and discussed here under.

Effect of Temperature on Pathogen

Among three isolates of pathogen, the results indicated that the maximum colony growth was observed in Rb3 (4.5, 31, 47, 52, 68 and 15 mm at 15, 20, 25, 30, 35 and 40 ºC, respectively) at 48 h after inoculation. However, the least growth of pathogen was observed in Rb1 (1.40, 29, 32, 40, 50 and 10 mm at 15, 20, 25, 30, 35 and 40 ºC respectively). The results also indicated that the temperature levels such as 45 and 50 ºC recorded lesser growth in all the three isolates (Table 2 and Fig. 2).

At 96 h after inoculation, the maximum colony growth was observed in Rb3 (18.15, 61.00, 75.50, 86.50, 90.00 and 18.00 mm at 15, 20, 25, 30, 35, 40 ºC, respectively) and this is followed by isolate Rb1 (12.55, 50.00, 64.00, 85.00, 90.00 and 17.00 mm) and least growth was observed in Rb2 (10.20, 34.20, 37.00, 47.70, 80.00 and 15.00 mm at 15, 20, 25, 30, 35 and 40 ºC, respectively). All three pathogen isolates did not grow at 45 and 50 ºC as observed at 48 h after inoculation (Table 2).

The present investigations are in line with Srinivas (2016) who reported that significant difference in the radial growth among the isolates of *R. bataticola* ranging from 17.7 mm to 80.0 mm at 72 h after incubation. Isolate Rb 14, Rb 17, Rb 22, Rb 26, Rb 49 and Rb 54 showed significantly highest colony growth (80 mm). The least colony diameter was observed in the isolate Rb 20 (17.7 mm). The

TABLE 2

Effects of temperature regimes on radial growth of *R. bataticola* at 48 and 96 hours after inoculation

Temperature regimes (^{0}C)	Rb1		Rb2		Rb3		Mean	
	48 HAI	96 HAI	48 HAI	96 HAI	48 HAI	96 HAI	48 HAI	96 HAI
15	2.50	12.55	1.40	10.20	4.50	18.15	2.80	13.66
20	30.00	50.00	29.00	34.20	31.00	61.00	30.00	48.40
25	43.00	64.00	32.00	37.00	47.00	75.50	40.66	58.80
30	50.00	85.00	40.00	47.70	52.00	86.50	47.33	73.06
35	65.00	90.00	50.00	80.00	68.00	90.00	61.00	86.66
40	12.00	17.00	10.00	15.00	15.00	18.00	12.33	16.66
45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	25.31	39.81	20.31	28.01	27.18	43.64	24.26	37.15

*Mean of three replications HAI- Hours after inoculation

Factors	S.Em±	CD @ 1%
Temperature (T)	0.30	0.95
Isolate (I)	0.32	0.98
T x I	0.34	1.11

maximum radial growth (90 mm) of the *R. bataticola* was recorded at 30-35 ^{0}C as observed in present investigation (Veerendra Kumar, 2004)

Effect of Temperature on Dry Root Rot

The maximum disease severity was recorded in Rb3 with 1.7, 3.1, 7.4, 8.2, 9.0 and 3 grade on 1-9 scale followed by Rb1 (1.3, 3.0, 7.2, 8.0, 9.0 and 3.0) at 15, 20, 25, 30, 35 and 40 ºC, respectively. Whereas the least disease severity rating was recorded in Rb2 (1.0, 2.9, 6.7, 7.2, 8.5 and 2.5 at 15, 20, 25, 30, 35 and 40 ºC, respectively (Table 3 and Fig. 2). However, there was dry root rot development at 45 and 50 ºC (Table 3).

The correlation results indicated that the negative correlation coefficients (-0.29 and -0.38 for 48 and 96 hours, respectively) between temperature regimes and radial growth of *R. bataticola* suggest a weak negative relationship between the two variables (Table 4). This means that as the temperature regime increases, the radial growth of *R. bataticola* is likely to decrease. However, the same pattern was observed

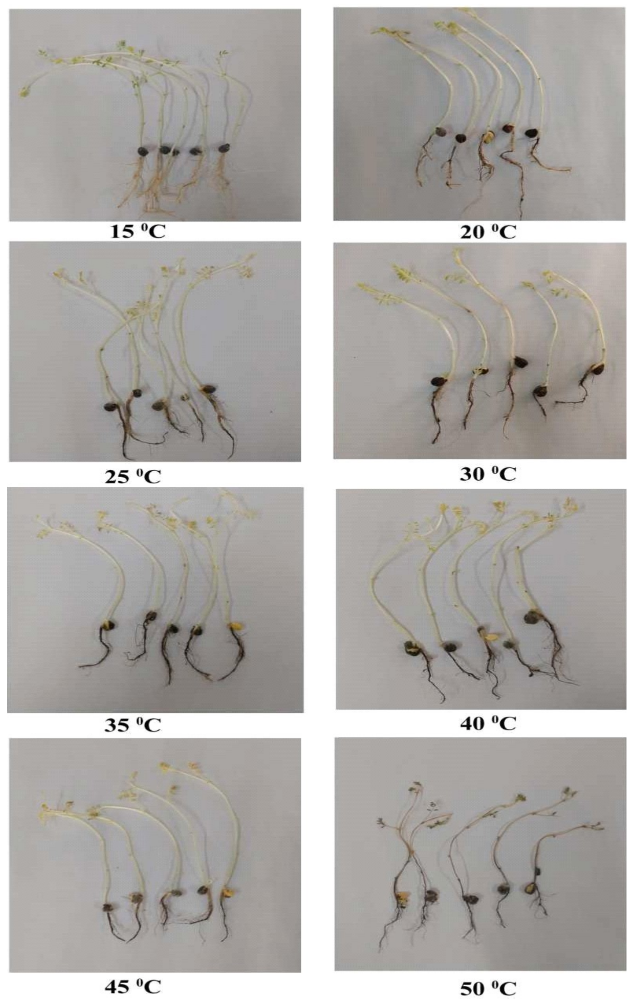

Fig. 2 : Effect of temperature regimes on dry root rot disease in chickpea

TABLE 3

Effects of temperature regimes on dry root rot disease caused by *R. bataticola* isolates on Annigeri-1 variety

Temperature (^{0}C)	*Disease severity (1-9 scale)		
	Rb1	Rb2	Rb3
15	1.3	1.0	1.7
20	3.0	2.9	3.1
25	7.2	6.7	7.4
30	8.0	7.2	8.2
35	9.0	8.5	9.0
40	3.00	2.5	3.0
45	0.00	0.00	0.00
50	0.00	0.00	0.00
Mean	3.93	3.60	4.05

*Mean of three replications

Factors	S.Em±	CD @ 1%
Temperature (T)	0.20	0.65
Isolate (I)	0.22	0.72
T x I	0.16	0.69

with disease severity in *R. bataticola* isolates that is as the temperature regime increases, the disease severity by *R. bataticola* isolates is likely to decrease (Table 4). Sharma and Pande (2013) reported that the disease incidence of dry root rot was significantly affected by high temperature. Out of five temperature levels *viz.*, 15, 20, 25, 30 and 35 °C tested, chickpea predisposed to dry root rot early and severity was more at 35 °C. Savary *et al*. (2011) observed the effect

TABLE 4

Correlation Analysis of temperature regimes on radial growth of *R. bataticola* and Disease severity *R. bataticola* isolates on Annigeri-1 variety

Temperature Levels	Radial growth	Correlation Coefficent
Temperature	48 HAI(mm)	-0.29*
	96 HAI(mm)	-0.38*
	Disease severity	
	Rb1	-0.28*
	Rb2	-0.28*
	Rb3	-0.31*

** Significant at 5% LOS

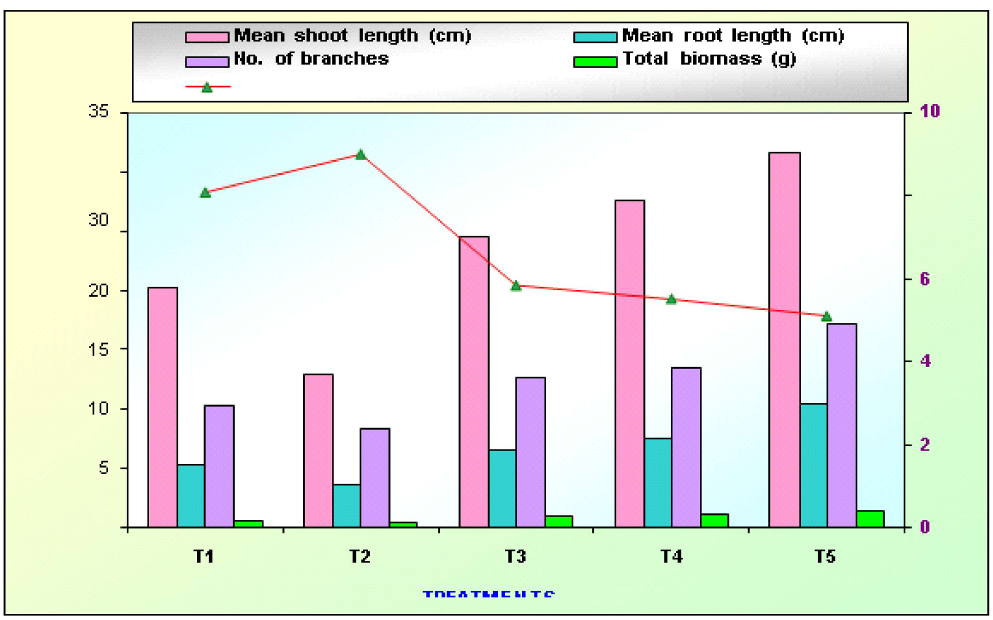

Fig. 3: Effect of carbon and temperature on dry root rot disease

Treatment details:

T_1 - Elevated CO_2 @ 550 ± 25 ppm alone; T_2 - Elevated CO_2 @ 550 ± 25 ppm with 2 ºC rise in temperature
T_3 - Ambient CO_2 @ 390 ± 25 ppm with 2 ºC rise in temperature T_4 - Ambient CO_2 @ 390 ± 25 ppm; T_5 - Open plot

TABLE 5

Effect of soil moisture levels on growth parameters of chickpea in black soil inoculated with *R. bataticola*

Moisture (%)	Growth parameters											
	Root length (cm)				Shoot length (cm)				Total biomass (g)			
	Rb1	Rb2	Rb3	Mean	Rb1	Rb2	Rb3	Mean	Rb1	Rb2	Rb3	Mean
40	2.05	2.10	2.00	2.14	8.20	8.67	8.50	8.45	0.37	0.40	0.38	0.38
50	2.76	2.80	2.70	2.75	10.00	11.00	10.07	10.35	0.45	0.55	0.37	0.45
60	3.10	3.45	3.00	3.18	11.23	11.50	11.00	11.24	0.48	0.65	0.35	0.49
70	3.57	3.55	3.38	3.50	20.10	23.00	19.19	20.76	0.67	0.70	0.61	0.66
80	4.56	4.80	4.00	4.16	25.04	26.00	24.59	25.21	1.10	1.54	1.33	1.32
90	4.16	4.20	4.15	4.45	29.54	29.70	29.20	29.48	3.40	3. 27	3.51	3.45
100	4.88	5.23	4.80	4.97	29.81	29.88	29.00	29.56	3.77	3. 90	3.80	3.82
Mean	3.59	3.74	3.43	3.60	19.13	19.96	18.79	19.29	1.36	1.89	1.35	1.42

* Mean of three replications

Factors	S.Em±	CD @ 1%
Temperature (T)	0.29	0.93
Isolate (I)	0.32	1.01
T x I	0.41	1.31

of temperature on increasing incidence of dry root rot at various locations over the years which suggests a strong influence of rising temperature (above 30 ºC) on *R. bataticola*.

Effect of Soil Moisture on Dry Root Rot

The effect of dry root rot on different growth parameters such as root length, shoot length and total biomass was studied at different moisture levels and results are presented in Table 5 and 6.

Root Length

The highest mean root length of three isolates (Rb1, Rb2 and Rb3) was observed in red soil (3.67 cm) compared to the black soil (3.60 cm), but there was no significant difference (Table 5 and 6). Among the three isolates, maximum mean root length of 3.74 cm and 3.90 cm was recorded in both the soils by Rb2 followed by Rb1 (3.59 cm and 3.60 cm). Whereas Rb3 recorded the least root length in two types of soils (3.43 cm and 3.54 cm).

Among the seven soil moisture regimes tested against the dry root rot and its effect on plant root length, the maximum mean root length was recorded in both black and red soil at 100 per cent moisture level (4.97 cm and 5.29 cm) followed by 90 per cent (4.45 cm and

TABLE 6

Effect of soil moisture levels on growth parameters of chickpea in red soil inoculated with *R. bataticola*

Moisture (%)	Growth parameters											
	Root length (cm)				Shoot length (cm)				Total biomass (g)			
	Rb1	Rb2	Rb3	Mean	Rb1	Rb2	Rb3	Mean	Rb1	Rb2	Rb3	Mean
40	1.84	2.00	2.1	1.98	7.50	7.65	7.00	7.30	0.35	0.50	0.25	0.36
50	2.64	2.67	2.63	2.60	9.53	10.89	9.55	10.00	0.66	0.85	0.70	0.73
60	2.60	3.40	3.00	3.00	11.46	11.66	11.38	11.54	0.60	0.64	0.57	0.60
70	3.33	3.60	3.30	3.46	20.76	26.23	18.00	21.66	0.98	1.00	0.83	0.93
80	4.50	4.75	4.56	4.60	27.20	27.24	27.00	27.14	1.39	1.43	1.35	1.39
90	5.00	5.06	4.35	4.80	29.07	29.00	28.50	28.85	3.54	3.45	3.10	3.35
100	5.24	5.85	4.8	5.29	29.33	29.20	29.00	29.13	3.88	3.90	3.90	3.81
Mean	3.60	3.90	3.54	3.67	19.26	20.26	18.63	19.38	1.61	1.68	1.52	1.59

Factors	S.Em±	CD @ 1%
Moisture (M)	0.21	0.70
Isolates (I)	0.26	0.82
M x I	0.32	1.06

4.80 cm) and 80 per cent (4.16 cm and 4.60 cm). The least root length was observed at 40 per cent (2.14 cm and 1.98 cm) soil moisture regime (Table 6).

Shoot Length

The highest mean shoot length of three isolates (Rb1, Rb2 and Rb3) was observed in red soil (19.38 cm) when compared to the black soil (19.29 cm). Among the three isolates, maximum mean shoot length was recorded Rb2 (19.96 cm and 20.26 cm in black and red soil, respectively) followed by Rb1 (19.13 cm and 19.26 cm) and least was observed in Rb3 (18.79 cm and 18.63 cm) (Table 5 and 6).

With respect to seven soil moisture regimes tested against the dry root rot,the maximum mean shoot length was recorded in both black and red soil at 100 per cent (29.56 cm and 29.13 cm, respectively) followed by 90 per cent (29.48 cm and 28.85 cm) and 80 per cent (25.21 cm and 27.14 cm) when compared to least shoot length of 8.45 cm and 7.30 cm at 40 per cent in both soils (Table 5 and 6).

Total Biomass

The highest mean total biomass of three isolates (Rb1, Rb2 and Rb3) was observed in red soil (1.59 g) compared to the black soil (1.42 g). Among the three isolates, maximum mean total biomass was in Rb2 (1.89 g and 1.68 g in black and red soils, respectively) followed by Rb1 (1.36 g and 1.61 g) and Rb3 (1.35 g and 1.52 g) (Table 5 and 6).

Among the seven soil moisture regimes, the maximum mean total biomass was recorded at 100 per cent soil moisture (3.82 g and 3.81 g in black and red soils, respectively) followed by 90 per cent (3.45 g and 3.35 g) and 80 per cent (1.32 g and 1.39 g) in comparison to least moisture level of 40 per cent (0.38 g and 0.36 g) (Table 5 and 6).

Mayek *et al.* (2002) reported that the stress prone and infected plants had poor growth compared to healthy and irrigated plants. Drought stress showed higher negative effects coupled with *R. bataticola* which attack in vegetative growth of the plant which decreased leaf area and dry weight of all vegetative structures significantly. Srinivas (2016) studied different soil moisture levels on growth of chickpea and reported that 100 and 90 per cent soil moisture levels recorded the highest root length, shoot length and total dry weight of chickpea plants in as observed in present investigation.

Effect of Soil Moisture on Disease Incidence

The results indicated that there was significant difference between moisture levels but not with respect to soil types. In black soil the highest disease severity rating was 9.0 grade, 9.0, 8.91, 8.03, 5.62, 4.86 and 3.30 while in red soil, it was 9.0, 9.0, 8.95, 8.33, 5.73, 5.40 and 3.56 at 40, 50, 60, 70, 80, 90 and 100 per cent soil moisture, respectively. The disease severity decreased as the soil moisture increased in both the types of soils (Table 7).

Among the seven levels of moisture content, the disease severity decreased slowly with respect to the increase in soil moisture. At 40 and 50 per cent soil moisture, there was early development of disease symptoms. Yellowing of leaves was started at ten days after sowing and at twenty days after sowing the plants completely dried and root system was completely black. The plants recorded the highest disease severity grade of 9.0 in both the soils at 40 and 50 per cent soil moisture. While in 60 per cent (8.91 and 8.95) and 70 per cent (8.03 and 8.33) soil moisture, the dry root symptoms such as yellowing and upward turning of leaflets was started after 20 days after sowing and recorded the moderate disease severity in both black and red soils. The lesser disease severities were recorded at 100 per cent (3.30 and 3.56) followed by 90 per cent (4.86 and 5.40) and 80 per cent (5.62 and 5.73) soil moisture levels in black soil and red soil, respectively (Table 7).

TABLE 7

Effect of soil moisture levels on disease severity of dry root rot caused by *R. bataticola* isolates in black soil and red soil

Moisture (%)	Disease severity (1-9 scale)							
	Black soil				Red soil			
	Rb1	Rb2	Rb3	Highest scale	Rb1	Rb2	Rb3	Highest scale
40	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
50	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
60	8.90	8.35	8.91	8.91	9.0	8.45	8.95	8.95
70	7.80	6.60	8.03	8.03	8.00	7.82	8.33	8.33
80	5.00	4.87	5.62	5.62	5.25	5.22	5.73	5.73
90	4. 55	4.32	4. 86	4.86	5.08	4.25	5.40	5.40
100	3.10	2.80	3.30	3.30	3. 16	3.00	3. 56	3.56

*Mean of three replications

Factors	S.Em±	CD @ 1%
Soil type (S)	0.20	0.81
Isolate (I)	0.23	0.93
Moisture (M)	0.20	0.42
S x I x M	0.35	1.17

The results (Table 8) on correlation analysis of moisture levels percentage and growth parameters indicated that, the high correlation coefficients of 0.98, 0.95, and 0.88 in black soil and 0.98, 0.98, and 0.90 in red soil suggest a strong positive relationship between the two variables. This means that as the moisture level increases, the growth parameters such as root length, shoot length and total biomass are also likely to increase. The high negative correlation is observed with -0.94 and -0.93 coefficients values for black and red soil between moisture levels percentage and disease severity. This means that as the moisture level increases, the severity of disease in plants is likely to decrease.

The present investigations are in line with Srinivas (2016) who also observed difference in the dry root rot incidence with change in soil moisture content. The plants grown in 50%, 60% soil moisture recorded highest disease severity grade compared to the 80%, 90% and 100% soil moisture. Further, Sharma and Pande (2013) reported that plants exposed to 40% and 60% soil moisture, dry root rot severity was maximum,

TABLE 8

Correlation analysis of soil moisture levels on growth parameters of chickpea in black and red soil inoculated with *R. bataticola* and disease severity

Moisture Levels	Type of Soil	Growth Parameters	Correlation Coeffient	Disease severity
Moisture (%)	Black	Root length (cm)	0.97**	-0.94**
		Shoot length (cm)	0.96**	
		Total biomass (g)	0.88**	
	Red	Root length (cm)	0.98**	-0.93**
		Shoot length (cm)	0.95**	
		Total biomass (g)	0.90**	

** Significant at 1% LOS

showed higher mortality as compared to 80 and 100 per cent.

Effect of Carbon Dioxide Combined with Temperature on Growth Parameters

The study was conducted in the open top chambers with five sets of treatments (carbon dioxide levels) on growth parameters and dry root rot of chickpea and results are presented in Fig 3.

Shoot length

Elevated carbon dioxide combined with temperature on phenology of chickpea crop revealed that the highest shoot length of 31.66 cm was observed in open plot followed by 27.66 cm in ambient CO_2 @ 390 ± 25 ppm. These two treatments significantly differed with respect of shoot length in elevated CO_2 @ 550 ± 25 ppm with 2 ºC rise in temperature (12.91 cm) followed by elevated CO_2 @ 550 ± 25 ppm alone (20.33 cm) and 24.58 cm in ambient CO_2 @ 390 ± 25 ppm with 2 ºC rise in temperature. The results indicated that the carbon dioxide alone and coupled with increased temperature has detrimental impact on shoot length of chickpea plants.

Root Length

There was a significant difference between root length of plants in the different carbon dioxide levels tested. Highest root length of 10.41 cm was observed in open plot followed by 7.45 cm in ambient CO_2 @ 390 ± 25 ppm while the least growth of 3.66 cm was observed in elevated CO_2 @ 550 ± 25 ppm with 2 ºC rise in temperature. Further, the treatment that is elevated CO_2 @ 550 ± 25 ppm alone recorded root length of 5.30 cm and it was 6.61cm in ambient CO_2 @ 390 ± 25 ppm with 2 ºC rise in temperature. Finally, it is inferred that the elevated carbon dioxide combined with increased temperature of 2 ºC resulted in increased the severity of disease and reduced the root length of plants (Fig. 3).

Number of Branches

The results indicated that there was a significant difference between number of branches of plants in the different sets of treatments. The maximum number of branches (17.26) was observed in open plot followed by in ambient CO_2 @ 390 ± 25 ppm (13.33) and ambient CO_2 @ 390 ± 25 ppm with 2 ºC rise in temperature (12.55). In contrast to this, there was a reduction in the number of branches in elevated CO_2 @ 550 ± 25 ppm with 2 ºC rise in temperature (8.30) followed by elevated CO_2 @ 550 ± 25 ppm alone (0.29) (Fig. 3).

Total Biomass

The maximum total biomass (1.43 g) was recorded in open plot followed by 1.07 g in ambient CO_2 @ 390 ± 25 ppm and 0.85 in ambient CO_2 @ 390 ± 25 ppm with 2 ºC rise in temperature (Fig. 3). However, the reduced biomass of chickpea was recorded in elevated CO_2 @ 550 ± 25 ppm with 2 ºC rise in temperature (0.36 g) followed by elevated CO_2 @ 550 ± 25 ppm alone (0.54 g).

The present investigations on effect of carbon dioxide with temperature levels are supported by Jagadish *et al*. (2007) who reported that reduction in the weight of grains of pigeon pea under increased temperature with high levels of CO_2. Further, Baker (2004) also studied the effect of elevated CO_2 (700 ppm) under different temperature regimes (24, 28, 32, 36 and 40 °C) and found that there was no increase in grains weight of chickpea under enriched CO_2 combined with high temperature.

Severity of Dry Root Rot

The results revealed that at higher concentration of carbon dioxide alone and in combination with increased temperature of 2 ºC has aggravated the disease. Among the five treatments, elevated CO_2 @ 550 ± 25 ppm with 2 ºC rise in temperature showed higher disease severity (9.00 grade) with early infection showing drying of leaves and wilting of entire plant and roots were completely rotten. This treatment was followed by elevated CO_2 @ 550 ± 25 ppm alone with disease rating of 8.10, here also similar kind of symptoms but expression of symptoms was slow compared to elevated CO_2 @ 550 ± 25 ppm with 2 ºC rise in temperature. The least disease severity was observed in open plot

(5.10 grade). Apart from this, the treatment that is Ambient CO_2 @ 390 ± 25 ppm showed moderate disease severity of 5.50 grade and ambient CO_2 @ 390 ± 25 ppm with 2 ºC rise in temperature recorded 5.83grade (Fig. 3).

Similarly, Jagadish *et al*. (2007) studied the effect of carbon dioxide on root rot of pigeon pea grown in elevated CO_2 condition of 550 ± 25 ppm with raised temperature which increased disease incidence in pigeon pea. Further, Chakraborty and Datta (2003) studied the effects of increasing atmospheric CO_2 (increasing CO_2 from 365 ppm to 550 ppm) on plant-pathogen interactions and reported that there was an increased incidence of root rot and reduced yields of pulses in controlled conditions.

It is concluded from the present study that the there was a significant of impact of temperature, soil moisture and elevated CO_2 on growth of pathogen as well as dry root rot Disease of chickpea grown in different parts of Karnataka State. The temperature of 30-35 ºC was optimum for growth of pathogen. The maximum colony growth of *R. bataticola* and the dry root rot disease severity was recorded at 30-35 ºC which is considered as optimum temperature range for growth of pathogen and development of disease. Highest severity of dry root rot and lesser plant growth parameters such as root length, shoot length and total biomass were observed at 40-60 per cent soil moisture regimes, irrespective of type of soil. Further, elevated CO_2 @ 550 ± 25 ppm with 2 ºC rise in temperature showed higher dry rot severity and reduced growth parameters of chickpea.

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