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Biochemical and Physiological changes in groundnut (*Arachis hypogaea* L.) in response to stem rot (*Sclerotium rolfsii*) infection

HR Pipaliya, Dr. GV Marviya, PK Ukani, KR Khunt, HM Movaliya and Dr. SK Bhuva

Abstract
Studies on biochemical aspects of groundnut and *Sclerotium rolfsii* interaction are sparse, probably due to complex biology and nature of the biotropic parasitim. The present investigation was carried out on stem rot (*Sclerotium rolfsii*) of groundnut with two varieties (GG-20 and GJG-22) and sampling of leaf tissues were done at 2 days after inoculation (DAI), 7 DAI, and 14 DAI from inoculated soil (T_2) and sampling time was kept for normal soil (T_1) to observe the changes in physiological and biochemical parameters as well as altered enzyme activities due to stem rot infection in groundnut. Physiological parameters in leaf tissues of groundnut collected from inoculated soil showed reduction in their values by 13.24%, 17.21% and 20.08% in chlorophyll a, chlorophyll b and total chlorophyll respectively compared to normal soil. Significant interaction effect was observed for treatment and stages of groundnut in respect to all physiological parameters. Biochemical parameters like total phenol and ascorbic acid in leaves and oxalic acid in stem were increased due to infection. Leaf samples from inoculated soil showed rise in the biochemical parameters like phenol, ascorbic acid and oxalic acid by 77.08%, 50.94%, and 95.68 %, respectively. Same trend of increase or decrease was observed with the stage wise progress of disease from S_1 to S_3 stage as well as same result were found in the interaction of treatment and stages from T_2S_1 to T_2S_3 treatments.

Keywords: groundnut, *Sclerotium rolfsii*, chlorophyll, total phenol and ascorbic acid.

Introduction
Groundnut (*Arachis hypogaea* L.) is a member of *Fabaceae* family which comprises important edible oil seed crops in the wodrld. India, China, Nigeria, Senegal, Sudan, Burma and the USA are the major groundnut producing countries of the world. These countries together accounts for a total area of 18.9 million hectares and a production of 17.8 million tones i.e. 69% of the area and 70% of the production. In terms of cultivated area and production in the world, India is one of the largest producers of oilseeds occupying an important position in the Indian agricultural economy. It is estimated that nine oilseeds namely groundnut, rapeseed-mustard, soybean, sunflower, safflower, sesame, niger, castor and linseed, accounted for an area of 26.71 million ha with the production of 32.88 million tons (Anon., 2014) [2]. Groundnut is called as the 'King' of oilseeds. It is one of the most important food and cash crops of our country.

The pathogen *S. rolfsii*, is a soil borne pathogen that commonly occurs in the tropics, sub-tropics and other warm temperate regions of the world. In India, it is wide spread in almost all the states especially in Gujarat, Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh (Kumar *et al.*, 2013) [7]. Groundnut crop grown in the post rainy and summer seasons in India are often infected by the pathogen. It is predominant especially in vertisols and relatively less severe in sandy loam soils. Yield losses due to stem rot usually range from 10 to 25 %, but may reach up to 80% in severely infested fields in India (Mayee and Datar, 1988) [9]. It was also observed that about 20-60% of pod yield loss occur due to the disease in Karnataka and Andhra Pradesh (Anon., 1992) [1].

Materials and Methods
(A) Physiological Parameters
Chlorophyll content

The fresh groundnut leaf weighed to 0.1 gm and were cut into small pieces and crushed into chilled 80% acetone. The whole paste was filtered with whatman No. 1 filter paper. Filtrate collected and volume made to 10 ml with chilled 80% acetone. Absorbance was measured in spectrophotometer at 645nm and 663nm for determination of total chlorophyll a, b and total using following formula (Arnon, 1949) [3].

$$\text{Chlorophyll a (mg g}^{-1}\text{ fresh tissue)} = \frac{12.7\ (A_{663}) - 2.69\ (A_{645})}{a \times wt \times 1000} \times V$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ fresh tissue)} = \frac{22.9\ (A_{645}) - 4.68(A_{663})}{a \times wt \times 1000} \times V$$

$$\text{Chlorophyll total (mg g}^{-1}\text{ fresh tissue)} = \frac{20.2\ (A_{645}) + 8.02\ (A_{663})}{a \times wt \times 1000} \times V$$

Where, A_{663} = Absorbance at 663nm
A_{645} = Absorbance at 645nm
a = Length of light path in cell (Usually 10 cm)
V= Total volume of chlorophyll the extract in 80% acetone.
wt. = Fresh weight of tissue extracted.

(B) Biochemical Analysis

(1) Total phenol

Suitable aliquot (0.1 ml) of sample extract was taken from methanol extract prepared for total free amino acids analysis and evaporated to dryness in water bath. One ml of distilled water in each test tube and 0.5 ml of Folin Ciocalteu's phenol reagent (1:1 with water) was added and kept for 3 min. After this 2 ml of 20% Sodium carbonate was added and mixed thoroughly. The tubes were placed in boiling water for exactly one minute and cooled in ice water. The absorbance was read at 650 nm against a reagent blank (Bray and Thorpe, 1954). A standard graph was prepared using catachol ranging between 10-50µg concentrations. The amount of phenols present in the sample was calculated as %.

(2) Oxalic acid

Groundnut stem and leaf (500 mg) of all treatments were extracted with 1.5 ml of 4N H_2SO_4 using mortar and pestle. To that, 5ml diethyl ether was added, and tubes were crocked appropriately, and then put it on heating block for 6 h at 60^0C. After that, 5ml of 1N NaOH and 7 ml of distilled water were added to the tubes and shaken well. Ether layer was evaporated by keeping the tubes open. Then, the water phase was transferred to centrifuge tubes and added 4 ml of calcium acetate buffer (100mM, pH 6.8) to each tube and mixed thoroughly.

The mixture was allowed to stand overnight and then centrifuged for 10 minutes at 3000 rpm. The supernatant was discarded, and the pellet was washed with 5 ml of 5 % acetic acid solution saturated with calcium oxalate and centrifuged again. The supernatant was discarded and the residue was dissolved in 5 ml of 4N H_2SO_4. The content of the tube was transferred to a conical flask and heated to 90ºC on water bath. It was immediately titrated while still hot to a faint pink colored end point with 0.02N $KMNO_4$. The amount of oxalic acid present was calculated on the basis that one ml of 0.02N $KMNO_4$ was reacting with 1.78 mg oxalic acid (Mahadevan and Sridhar, 1986) [8].

(3) Ascorbic acid

Ascorbic acid was estimated by Dinitrophenyl hydrazine (DNPH) method (Sadasivam and Manickam, 1992) [13] Groundnut leaf (500 mg) of all treatments were extracted with 5 ml of 6% TCA (Trichloro acetic acid) using mortar and pestle. Homogenized material was centrifuged or filtered and the supernatant was collected. Known aliquot (0.1 ml) was taken and volume made up to 1 ml by adding distilled water. Two ml DNPH reagent (2% DNPH in 9N H_2SO_4) was added in each tube. Then added 2 drop of 10% thiourea. Ten kept in boiling water bath at 80 ºC for 15 min. Finally 5 ml 80% H_2SO_4 was added and cooled the intensity of colour was read at 540 nm on spectrophotometer

(B) Biochemical Analysis

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Suitable aliquot (0.1 ml) of sample extract was taken from methanol extract prepared for total free amino acids analysis and evaporated to dryness in water bath. One ml of distilled water in each test tube and 0.5 ml of Folin Ciocalteu's phenol reagent (1:1 with water) was added and kept for 3 min. After this 2 ml of 20% Sodium carbonate was added and mixed thoroughly. The tubes were placed in boiling water for exactly one minute and cooled in ice water. The absorbance was read at 650 nm against a reagent blank (Bray and Thorpe, 1954). A standard graph was prepared using catachol ranging between 10-50µg concentrations. The amount of phenols present in the sample was calculated as %.

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Results and Discussion

(A) Physiological Parameters

Chlorophyll

(a) Chlorophyll a

Table 1: Changes in chlorophyll a (mg.gm^{-1}fr.wt.) in healthy and stem rot infected leaves tissue of groundnut.

Treatment (T)	Variety (V)	Stages (S)			Mean V	Mean T
		Pre infectional (S_1)	Infectional (S_2)	Post infectional (S_3)		
Normal soil (T_1)	GG-20 (V_1)	0.0620	0.0642	0.0657	0.0640	0.0642
	GJG-22 (V_2)	0.0624	0.0647	0.0661	0.0644	
	Mean T X S	0.0622	0.0644	0.0659		
Inoculated soil (T_2)	GG-20 (V_1)	0.0636	0.0586	0.0450	0.0557	0.0557
	GJG-22 (V_2)	0.0628	0.0589	0.0453	0.0557	
	Mean T X S	0.0632	0.0588	0.0452		
	Mean S	0.0627	0.0616	0.0555		
	C.V. % = 2.17					

	S.Em. ±	C.D. at 5%
T	0.0003	0.0008
V	0.0003	NS
T X V	0.0004	NS
S	0.0003	0.0010
T X S	0.0005	0.0014
V X S	0.0005	NS
T X V X S	0.0007	NS

Chlorophyll a content in normal soil (T_1) recorded significantly higher value (0.0642mg.gm^{-1}fr.wt.) compared to inoculated soil (T_2) (0.0557mg.gm^{-1}fr.wt.). In the inoculated soil (T_2) chlorophyll a reduced by 13.24 %.

Varieties did not differ significant for chlorophyll a content.

The value for chlorophyll a for the pre infectional stage (S_1) was significantly the highest (0.0627 mg.gm^{-1}fr.wt.) followed by infectional stage (S_2) (0.0616 mg.gm^{-1}fr.wt.). Post infectional stage (S_3) (0.0555 mg.gm^{-1}fr.wt.) significantly the lowest chlorophyll a content was found compared to the other stages. Reduction in chlorophyll a content progressively was observed from S_1 to S_3 stages. The total reduction in chlorophyll a content was about 11.48% in S_3 stage compared to S_1 stage.

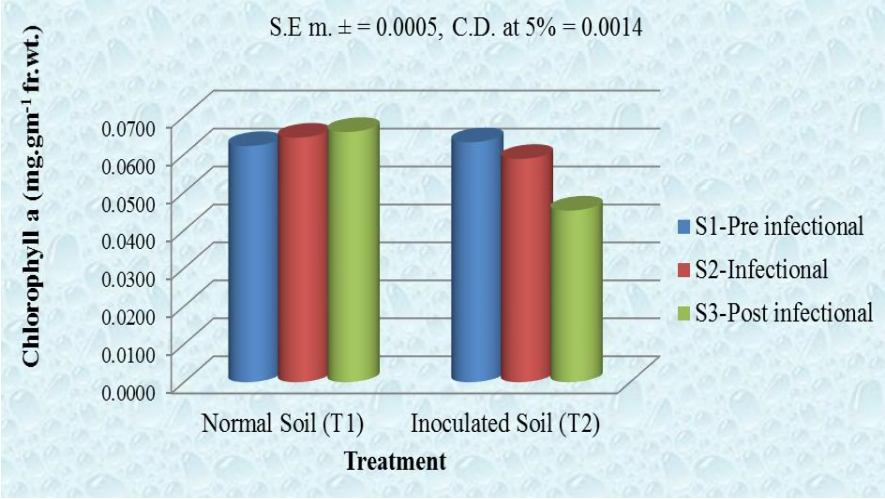

Fig 1: Interaction effect of Normal soil and Inoculated soil in response to stem rot disease on chlorophyll a in leaves of groundnut T X S.

Interaction effect for treatments and stages (T X S) was found significant

(Fig. 1). In normal soil, the highest chlorophyll a was observed with the T_1S_3 (0.0659 mg.gm^{-1}fr.wt.) treatment while lowest was with T_1S_1 (0.0622 mg.gm^{-1}fr.wt.) treatment. Opposite to inoculated soil the chlorophyll a was minimum with T_2S_3 (0.0452 mg.gm^{-1}fr.wt.) treatment and maximum with the T_2S_1 (0.0632 mg.gm^{-1}fr.wt.) treatments in the inoculated soil (T_2). Stage wise increase was observed in normal soil (T_1) while it was decreased from S_1 to S_3 stage in inoculated soil (T_2).

Rathod and Chatrabhuji (2010) [11] carried out an experiment on the changes in total chlorophyll in five different cv. of mustard *viz*, Skm-9801, skm-9804, GM-1, Varuna and Skm-9818 in response to powdery mildew at different stages of disease infection. In fungicide treated plant i.e. control plants significantly higher value for total chlorophyll content was recorded in all cultivars. Among the different cultivars no similar trend was observed in diseased infectional stages.

(b) Chlorophyll b

Table 2: Changes in chlorophyll b (mg.gm^{-1}fr.wt.) in healthy and stem rot infected leaves tissue of groundnut.

Treatment (T)	Variety (V)	Stages (S)			Mean V	Mean T
		Pre infectional (S_1)	Infectional (S_2)	Post infectional (S_3)		
Normal soil (T_1)	GG-20 (V_1)	0.0353	0.0348	0.0306	0.0336	0.0337
	GJG-22 (V_2)	0.0360	0.0346	0.0310	0.0339	
	Mean T X S	0.0357	0.0347	0.0308		
Inoculated soil (T_2)	GG-20 (V_1)	0.0351	0.0241	0.0236	0.0276	0.0279
	GJG-22 (V_2)	0.0370	0.0239	0.0236	0.0282	
	Mean T X S	0.0361	0.0240	0.0236		
	Mean S	0.0359	0.0294	0.0272		
	C.V. % = 3.16					
	S.Em. ±		**C.D. at 5%**			
T	0.0002		0.0006			
V	0.0002		NS			
T X V	0.0003		NS			
S	0.0002		0.0007			
T X S	0.0003		0.0010			
V X S	0.0003		NS			
T X V X S	0.0005		NS			

In case of chlorophyll b normal soil (T_1) showed significantly higher value (0.0337 mg.gm^{-1}fr.wt.) compared to inoculated soil (T_2) (0.0279 mg.gm^{-1}fr.wt.). The reduction in chlorophyll b content was about 17.21% under inoculated soil.

Varieties exhibited non-significant difference for chlorophyll b content.

A decrease was observed in chlorophyll b content with the progress stem rot disease form S_1 to S_3 stages. The value for chlorophyll b for the pre infectional stage (S_1) was significantly the highest (0.0359 mg.gm^{-1}fr.wt.) followed by infectional stage (S_2) (0.0294 mg.gm^{-1}fr.wt.) and post infectional stage (S_3) (0.0272 mg.gm^{-1}fr.wt.). The total reduction in chlorophyll b content was about 24.23% in S_3 stage compared to S_1 stage.

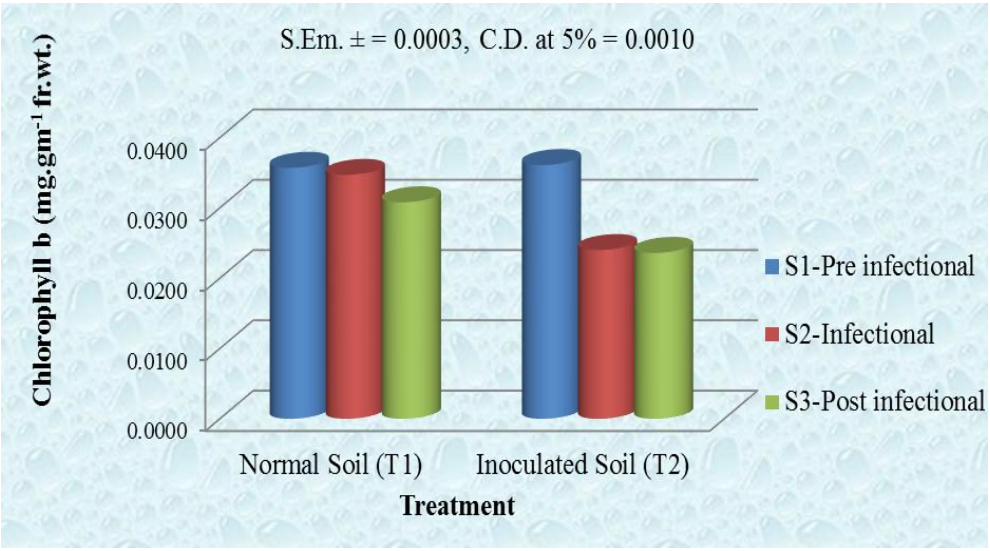

Fig 2: Interaction effect of Normal soil and Inoculated soil in response to stem rot disease on chlorophyll bin leaves of groundnut T X S.

Interaction effect for treatments and stages (T X S) was found significant

(Fig. 2). Both the treatments showed reduction in chlorophyll b content from S_1 to S_3 stage but reduction was more in inoculated soil (T_2) compared to normal soil (T_1). In normal soil, T_1S_1 reduced maximum value (0.0357 mg.gm^{-1}fr.wt.) for chlorophyll b while minimum was with T_1S_3 (0.0308 mg.gm^{-1}fr.wt.) treatment. Same tread was observed inoculated soil (T_2) in which treatment T_2S_1 progressed maximum value (0.0361 mg.gm^{-1}fr.wt.) for chlorophyll b and minimum was (0.0236 mg.gm^{-1}fr.wt.) with T_2S_3 value. The reduction in the chlorophyll b content was about 34.63% in T_2S_3 as compared to T_2S_1.

Arya and Buch (2013) studied the effect of *Arbuscular mycorrhizal* fungi on chlorophyll content in three different varieties of cotton and observed changes in chlorophyll a. The maximum total chlorophyll content was noticed at 90 days old sampling leaves as compared to the inoculated plants.

(c) Total Chlorophyll

Table 3: Total chlorophyll (mg.gm^{-1}fr.wt.) in healthy and stem rot infected leaves tissue of groundnut.

Treatment (T)	Variety (V)	Stages (S)			Mean V	Mean T
		Pre infectional (S_1)	Infectional (S_2)	Post infectional (S_3)		
Normal soil (T_1)	GG-20 (V_1)	0.0972	0.1100	0.1052	0.1041	0.1046
	GJG-22 (V_2)	0.0984	0.1099	0.1071	0.1051	

	Mean T X S	0.0978	0.1100	0.1062		
Inoculated soil (T_2)	GG-20 (V_1)	0.0986	0.0827	0.0686	0.0833	0.0836
	GJG-22 (V_2)	0.0998	0.0828	0.0689	0.0838	
	Mean T X S	0.0992	0.0828	0.0688		
	Mean S	0.0985	0.0964	0.0875		
	C.V. % = 1.09					

	S.Em. ±	C.D. at 5%
T	0.0002	0.0006
V	0.0002	0.0006
T X V	0.0003	NS
S	0.0003	0.0007
T X S	0.0004	0.0010
V X S	0.0004	NS
T X V X S	0.0005	NS

Significantly the highest value (0.1046 mg.gm^{-1}fr.wt.) was recorded in case of normal soil (T_1) as compared to inoculated soil (T_2) (0.0836 mg.gm^{-1}fr.wt.). The reduction in total chlorophyll content was about 17.21% under inoculated soil. Varieties also exhibited significant difference for total chlorophyll content and mean value was higher in GJG-22 compared GG-20.

The value for total chlorophyll for the pre infectional stage (S_1) was significantly the highest (0.0985 mg.gm^{-1}fr.wt.) followed by infectional stage (S_2) (0.0964 mg.gm^{-1}fr.wt.). Post infectional stage (S_3) (0.0875 mg.gm^{-1}fr.wt.) recorded significantly the lowest total chlorophyll content compared to the other stages. The reduction of total chlorophyll content was about 11.17% in S_3 stage compared to S_1 stage.

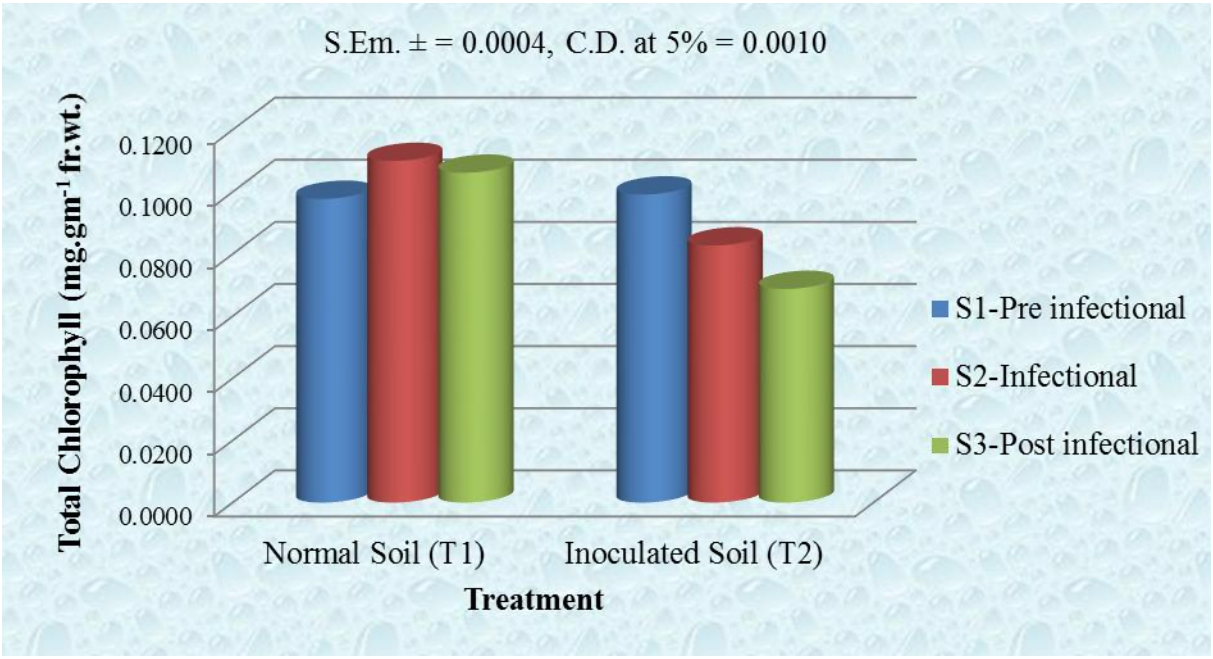

Fig 3: Interaction effect of Normal soil and Inoculated soil in response to stem rot disease on total chlorophyll in leaves of groundnut T X S

Interaction effects for treatments and stages (T X S) were found significant (Fig. 3). In normal soil, the highest total chlorophyll was observed with the T_1S_2 (0.1100 mg.gm^{-1}fr.wt.) treatment while lowest was with T_1S_1 (0.0978 mg.gm^{-1}fr.wt.) treatment. Opposite to inoculated soil the total chlorophyll was minimum with T_2S_3 (0.0688 mg.gm^{-1}fr.wt.) treatment and maximum with the T_2S_1 (0.0992 mg.gm^{-1}fr.wt.) treatments in the inoculated soil (T_2). Stage wise increase was observed in normal soil (T_1) while it was decreased from S_1 to S_3 stage in inoculated soil (T_2).

Arya and Buch (2013) studied the effect of *Arbuscular mycorrhizal* fungi on chlorophyll content in three different varieties of cotton and observed changes in chlorophyll b. The maximum total chlorophyll content was noticed at 90 days old sampling leaves as compared to the inoculated plants.

(B) Biochemical Analysis

(1) Total Phenol

Table 4: Changes in Total phenol (%) in healthy and stem rot infected leaves tissue of groundnut.

Treatment (T)	Variety (V)	Stages (S)			Mean V	Mean T
		Pre infectional (S_1)	Infectional (S_2)	Post infectional (S_3)		
Normal soil (T_1)	GG-20 (V_1)	0.170	0.193	0.213	0.192	0.192
	GJG-22 (V_2)	0.170	0.196	0.212	0.193	
	Mean T X S	0.170	0.195	0.213		
Inoculated soil (T_2)	GG-20 (V_1)	0.169	0.314	0.533	0.339	0.340
	GJG-22 (V_2)	0.169	0.314	0.540	0.341	

	Mean T X S	0.169	0.314	0.537		
	Mean S	0.170	0.254	0.375		
	C.V. % = 1.45					
	S.Em. ±		C.D. at 5%			
T	0.0008		0.0023			
V	0.0008		NS			
T X V	0.0011		NS			
S	0.0010		0.0028			
T X S	0.0014		0.0039			
V X S	0.0014		NS			
T X V X S	0.0019		NS			

Imposition to different treatments resulted in significant changes in total phenol contents. The leaf tissues obtained from inoculated soil (T_2) revealed higher amount of total phenol (0.340 %) as compared to normal soil (T_1) (0.192 %) and the increase was about 77.08%.

Varieties did not show significant difference for total phenol.

The value for total phenol for the post infectional stage (S_3) was significantly the highest (0.375 %) followed by infectional stage (S_2) (0.254 %). Pre infectional stage (S_1) (0.170 %) found significantly lowest total phenol content compared to the other stages. The increased of total phenol was about 2.2 fold in S_3 stage compared to S_1 stage.

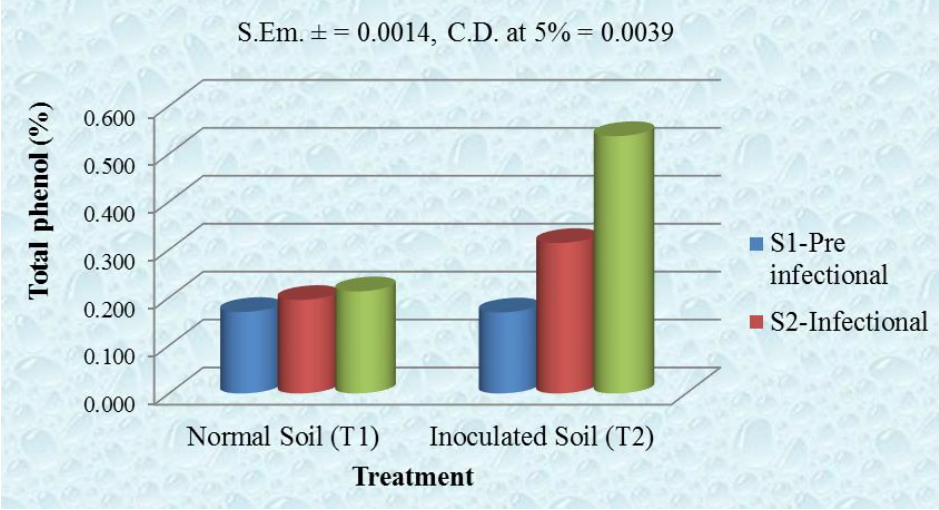

Fig 4: Interaction effect of Normal soil and Inoculated soil in response to stem rot disease on total phenol in leaves of groundnut T X S

Interaction effect for treatments and stages (T X S) was found significant

(Fig. 4). In normal soil, the highest total phenol was observed with the T_1S_3 (0.213 %) treatment while lowest was T_1S_1 (0.170 %) with the treatment. Treatments T_1S_3 were statistically at par with treatment T_1S_2. In inoculated soil the total phenol was minimum with T_2S_1 (0.169 %) treatment and maximum with the T_2S_3 (0.537%) treatments in the inoculated soil (T_2). Both the treatments stage wise increase in total phenol was observed in normal soil (T_1) and inoculated soil (T_2) from S_1 to S_3 stage.

The results of present study are in good agreement with the findings of Rathod and Vakharia (2011) study days after infection conducted to the changes in total phenol content atdifferent stages of infection of wilt disease in chickpea (*Cicer arietinum* L) roots tissues. The results indicated that total phenol content was significantly higher in root of all the cultivars obtained from sick plot. The level of phenol declined from pre infection (S_1) to post infection stage (S_2) and further it increased in all the cultivars among six cultivars tested, JG-62 and GG-1 had lower concentration of total phenol than others.

(2) Ascorbic acid

Table 5: Changes in Ascorbic acid (mg.g.$^{-1}$) in healthy and stem rot infected leaves tissue of groundnut

Treatment (T)	Variety (V)	Stages (S)			Mean V	Mean T
		Pre infectional (S_1)	Infectional (S_2)	Post infectional (S_3)		
Normal soil (T_1)	GG-20 (V_1)	0.199	0.247	0.357	0.268	0.267
	GJG-22 (V_2)	0.194	0.249	0.354	0.266	
	Mean T X S	0.197	0.248	0.356		
Inoculated soil (T_2)	GG-20 (V_1)	0.182	0.369	0.679	0.410	0.403
	GJG-22 (V_2)	0.196	0.333	0.660	0.396	
	Mean T X S	0.189	0.351	0.670		
	Mean S	0.193	0.300	0.513		
	C.V. % = 1.73					
	S.Em. ±		C.D. at 5%			
T	0.0025		0.0073			
V	0.0025		0.0073			

T X V	0.0036	NS
S	0.0031	0.0090
T X S	0.0044	0.0127
V X S	0.0044	NS
T X V X S	0.0062	0.0179

Leaf tissue from inoculated soil (T_2) found highest ascorbic acid content compared to normal soil (T_1). However, T_1 showed minimum differences in the ascorbic acid content during disease developmental stages. Seeds sown in inoculated soil (T_2) had recorded maximum (0.403 mg.g^{-1}) followed by normal soil (T_1) (0.267 mg.g^{-1}). Ascorbic acid content was increased by 50.94 % in inoculated soil (T_2) as compared to normal soil (T_1).

Varieties also differed significantly for ascorbic acid content. Variety GG-20 (V_1) possessed increased ascorbic acid (0.339 mg.g^{-1}) as compared to GJG-22 (V_2).

The mean value for ascorbic acid for the post infectional stage (S_3) was significantly the highest (0.513mg.g^{-1}) as compared to infectional stage (S_2) (0.300 mg.g^{-1}) and pre infection stage (S_1) (0.193 mg.g^{-1}). There was 55.44 % increase in S_2 stage as compared to S_1 while in S_3 it was 2.66 fold increased

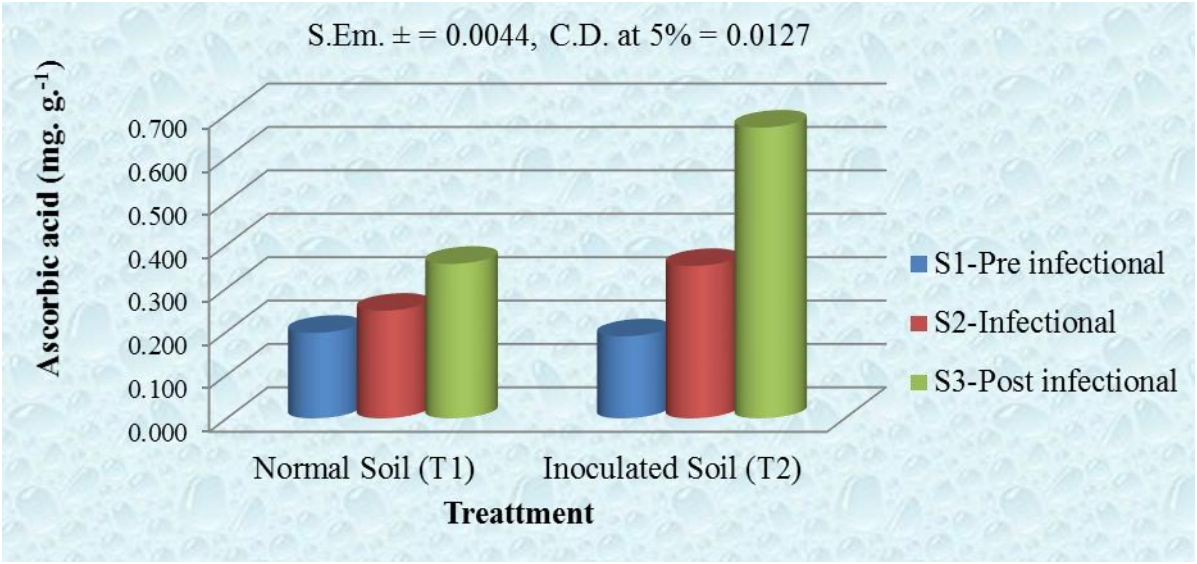

Fig 5: Interaction effect of Normal soil and Inoculated soil in response to stem rot disease on ascorbic acid in leaves of groundnut T X S.

Interaction effect for treatments and stages (T X S) was found significant (Fig. 5). In normal soil, the highest ascorbic acid was observed with the T_1S_3 (0.356 mg.g^{-1}) treatment while lowest was T_1S_1 (0.197 mg.g^{-1}) with the treatment. In inoculated soil the ascorbic acid was minimum with T_2S_1 (0.189 mg.g^{-1}) treatment and maximum with the T_2S_3 (0.670mg.g^{-1}) treatments in the inoculated soil (T_2). Both the treatments stage wise increase was ascorbic acid observed in normal soil (T_1) and inoculated soil (T_2) from S_1 to S_3 stage.

Similar results were observed by Chhabra *et al*. (2000) from analyzed leaf sample of tomato infected with (*Alternaria solani*), and found that at pre infection stage; there was no significant difference in the ascorbic acid content of the leaves of different cultivars. After infection, drastic reductions in ascorbic acid content were observed, being highest (63.30 %) in the highly susceptible cultivar and lowest (24.55 %) in the resistant cultivar.

(3) Oxalic acid

Table 6: Changes in Oxalic acid (mg.g^{-1}) in healthy and stem rot infected stem tissue of groundnut.

Treatment (T)	Variety (V)	Stages (S)			Mean V	Mean T
		Pre infectional (S_1)	Infectional (S_2)	Post infectional (S_3)		
Normal soil (T_1)	GG-20 (V_1)	6.141	6.186	6.275	6.201	6.201
	GJG-22 (V_2)	6.186	6.141	6.275	6.201	
	Mean T X S	6.164	6.164	6.275		
Inoculated soil (T_2)	GG-20 (V_1)	6.230	9.879	20.248	12.119	12.134
	GJG-22 (V_2)	6.275	9.523	20.648	12.149	
	Mean T X S	6.253	9.701	20.448		
	Mean S	6.208	7.932	13.362		
	C.V. % = 5.84					
	S.Em. ±		C.D. at 5%			
T	0.1094		0.3141			
V	0.1094		NS			
T X V	0.1547		NS			
S	0.1340		0.3847			
T X S	0.1895		0.5440			
V X S	0.1895		NS			
T X V X S	0.2680		NS			

Treatments were found to be significant for oxalic acids content**.** Inoculated soil treatment (T_2) showed the increase in oxalic acid content (12.13 mg.g^{-1}) and the content was about double than normal soil (T_1) (6.20 mg.g^{-1}).
Varieties did not differ significantly in oxalic acid content.

The oxalic acid content increased with the progress of disease developmental stages. Value for oxalic acid in post infectional stage (S_3) was significantly the highest (13.362 mg.g^{-1}) compared to infectional stage (S_2) (7.932 mg.g^{-1}) and pre infectional stage (S_1) (6.208 mg.g^{-1}) stages. The total increase was about 3.27 fold in S_3 stage compared to S_1 stage.

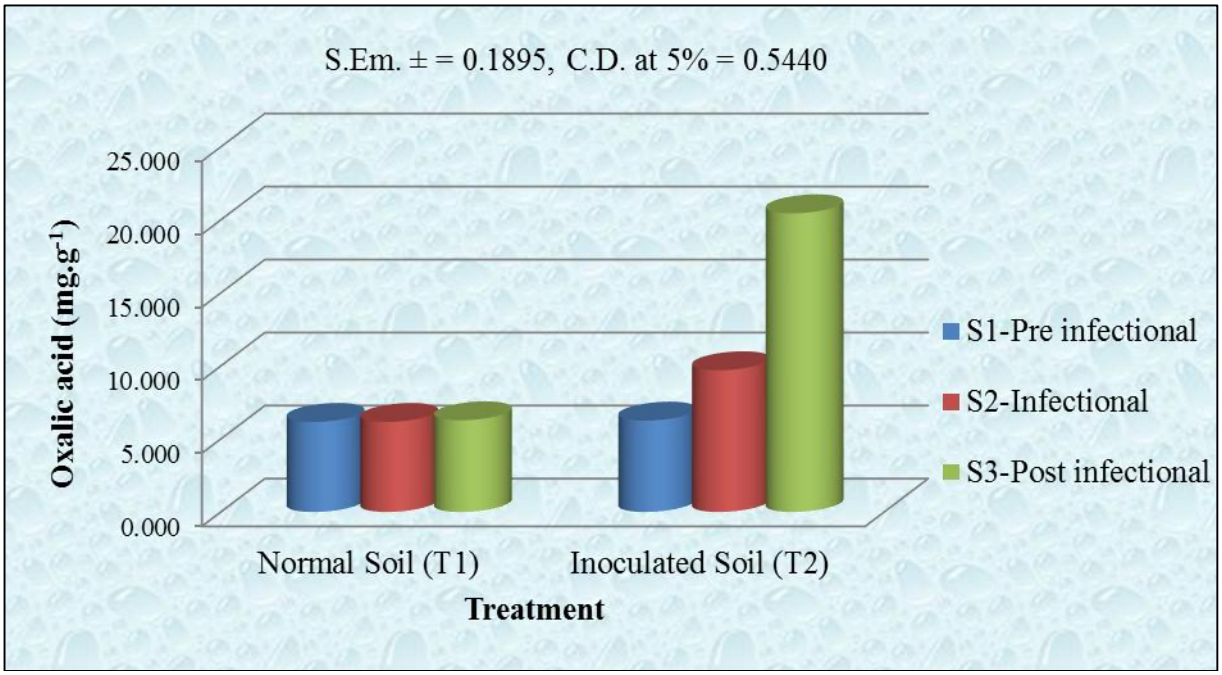

Fig 6: Interaction effect of Normal soil and Inoculated soil in response to stem rot disease on oxalic acid in stem of groundnut T X S.

Interaction effect for treatments and stages (T X S) was found significant (Fig. 6). The highest oxalic acid was recorded with the T_2S_3 (20.448 mg.g^{-1}) treatment and the lowest was T_1S_1 (6.164 mg.g^{-1}) treatment and it was at par with the T_1S_2 and T_1S_1 treatments. The T_2S_2 treatment had (9.701 mg.g^{-1}) oxalic acid content. The T_2S_1 treatment had (6.253 mg.g^{-1}) oxalic acids which reached to (20.448 mg.g^{-1}) oxalic acid in T_2S_3. The increase was about 3.27 fold.
Results of present studies are in agreement with Nagarajkumar *et al*. (2004) [10] who observed that the virulent fungal isolates produced more oxalic acid. They have also reported that effective reduction of oxalic acid by *Pseudomonas fluorescens*strain pfMDU2 in a culture medium proves ability of *Pseudomonas fluorescens*as detoxification agent of oxalic acid produced by fungal pathogen in disease conditions.

Conclusions

The physiological parameters chlorophyll a, chlorophyll b and total chlorophyll was declined due to disease infection. Leaf sample collected from inoculated soil observed an increase in phenol, ascorbic acid and oxalic acid content.
The changes in various parameters were distinguished clearly in inoculated soil compared to normal soil as well as trend of either increase or decrease observed more prominent with the stage wise progress of stem rot disease in inoculated soil. Treatments, stages and interactions of both were found significant while variety, treatment and variety, variety and stage as well as overall interactions were found non-significant. Varieties varied significantly only for total chlorophyll and ascorbic acid content while, they were non-significant for other parameters.

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