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Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (*Foc*) is known to produce fusaric acid (FA) which plays an important role during pathogenesis. In the present study, *in vitro* production of fusaric acid in culture filtrate by two races, 8 and 4 was estimated through HPLC and their effect on chickpea in relation to Relative Water Content (RWC) and growth parameters was worked out. The average quantity of FA production by race 4 was approximately 126.88 ± 5.56 mg/ L, whereas race 8 had a production of 820.51 ± 13.37 mg /L FA in the culture filtrate which is significantly higher (> 6 time) than race 4. Treatment of CF containing FA caused a reduction in RWC (10-45 %), shoot length (9-45 %), root length (8-45 %), shoot fresh biomass (38-65 %), root fresh biomass (10-43%), shoot dry biomass (7-50 %), root dry biomass (9-53 %) in chickpea seedlings. Reduction in WRC was recorded higher in JG 62 (60.67, 62.35 and 67.91% at 1, 3, 6 DAI) than WR 315 (39.22, 42.56 and 44.84 % at 1, 3 and 6 DAI) with CF of race 8 with the highest reduction of WRC (67.91%) in JG-62 at 6 DAI. Per cent decrease in root length, root fresh biomass was more than shoot length and root fresh biomass in both WR 315 and JG 62 but reduction in root length, and root fresh biomass was higher in susceptible JG 62. In general, CF of race 8 was found more effective than race 4 in reduction of RWC and growth parameters in both the cultivars, WR 315 and JG 62 because of the higher concentration of FA (205.12 mg/L) in culture filtrate.

Key words: Biomass, *Fusarium oxysporum f.* sp. *ciceris*, Fusaric acid, Relative Water Content, root and shoot growth

INTRODUCTION

Fusarium wilt is one of the major biotic threats to global chickpea production after *Ascochyta* leaf blight. Presently, *Fusarium oxysporum*, an opportunistic soil inhabiting fungus is considered in the top 10 fungal pathogens based on the economic/scientific importance which mainly causes vascular wilt disease in over 100 crop plants (Dean *et al.* 2012). *Fusarium oxysporum*, synthesizes several secondary metabolites that are toxic to plants or animals (Berthriller *et al.* 2013). Fusaric acid (FA) is one such kind of secondary metabolite, which was originally discovered in *Fusarium heterosporium* in Japan. Later, it has been successfully isolated FA from infected host plant. FA was the oldest known non - specific vivotoxin which was reported to produce by several vascular wilt pathogens such as *Fusarium oxysporum f. sp. lycopersici*, *Gibberella fujikuroi*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Fusarium oxysporum* f. sp. *cubense*, *Fusarium oxysporum* f. sp. *udum* (Shinde and Deshmukh, 2014), *Fusarium oxysporum* f. sp. *niveum* (Wu *et al.* 2008) and many more. FA has been proposed to play a functional role in virulence in the pathogen. It is now established that FA production is correlated with virulence of the pathogen. Races/isolates of wilt pathogen are also reported to vary in their capacity to produce FA which are, in turn, found to have positive correlation with lesion size and quantity of FA produced in culture filtrate (Bani *et al*. 2014). Secondary metabolites such as FA have been reported to increase plasma membrane permeability and electrolyte leakage (Bouizgarne *et al*. 2006), reduce relative water content, stunting growth (Puyum *et al*., 2017), destruction of chlorophyll (Khan *et al*. 2004) leading to chlorosis

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and cell death (Singh *et al*. 2017) in various host-pathogen combinations. In low concentration, FA can also trigger the early production of defense related enzymes. But the effect of FA toxin on growth parameters such as root & shoot length, root and shoot fresh and dry biomass in chickpea seedlings are lacking. In the present study, we have examined the production of FA by Race 4 and Race 8 in culture filtrate, and their effect on water retention capacity (WRC) and growth parameters in chickpea.

MATERIALS AND METHODS

Estimation of FA production by race 4 and race 8 of Foc in culture filtrate

Culturing of race 4 and race 8 of *Foc* : Two races, 4 (ITCC 7681) and 8 (ITCC 7682) of Foc which were previously isolated, characterized by the scientists in our Pulse Pathology Laboratory, Division of Plant Pathology, IARI, New Delhi, and deposited in ITCC, were used in the study. Entire study was conducted in the Division of Plant Pathology and partly in the Division of Agriculture Chemicals, Indian Agricultural Research Institute, PUSA, New Delhi during 2020-2021. Each of the two races 4 and 8 was grown in 250 ml of sterile Czapek-Dox broth liquid medium separately in 500 ml conical flasks which were inoculated with 5 mm disc (1 disc per flask) of 7 days old culture of *Foc*. Three replications were maintained for each isolate and incubated for 21 days at 25±2°C.

Purification and extraction of FA

Extraction of FA from Foc culture filtrate was carried out following Pritesh *et al.* (2010). The mycelial mat of each isolate was removed by passing the entire broth through cheesecloth; then the filtrate was passed through Whatman filter paper no. 1 and the filtrate was retained. The pH of the filtrate was adjusted to 3.5-4.0 with 2N HCl and re-suspended in an equal amount of ethyl acetate. It was then agitated vigorously in a separator funnel and allowed to stay undisturbed for 30 minutes to separate into two layers. In a conical flask, the upper layer was collected. The extraction was done thrice and the extracts were combined. A rotating evaporator was used to evaporate ethyl acetate under reduced pressure at 70 rpm and 27^{0}C temperature. The residue was collected and suspended in 5 ml ethyl alcohol before being frozen until the test was performed.

HPLC Analysis

Fusaric acid from race 4 and race 8 was estimated using an HPLC (Alliance, Waters Crop., Milford, Mass., USA), equipped with an e2695 quaternary pump, 20 ìL loop auto-injector, 2998 photodiode array detector (PDA). The retrieved data were analyzed using the software program “Empower 2”. The stationary phase, C18 column (Hypersil ODS; 250mm×4.6mm×5ì, Thermo Fischer Scientific, USA) was used for the separation of FA using a gradient solvent system consisting of, solvent A: H_2O acidified with 0.1% FA and solvent B: ACN with 0.1% FA, v/v. Initially, the gradient system started with 90% A for 2 min, then decreased to 20% in 18 min, followed by an increase to 90% in the next 2 min. The flow rate of the gradient phase was maintained at 0.5mL/min. The total run time for the analysis was 20 min. The detector response was recorded at 270 nm. Three replications were maintained for each race. Spectral analysis was conducted to compare the detected peaks within similar retention times in all samples with a spectral pattern of pure FA standard (Sigma Aldrich, USA). FA accumulation was determined by measuring the area under the curve at 270 nm (optimal wavelength) with the diode array detector (DAD).

Treatments preparation

The culture filtrate (CF) of races 4 and 8 were taken to study their effects on growth parameters on seedlings of two chickpea cv. WR 315 and JG 62. The 25% concentration of crude CF of the races 4 and 8 were prepared adding sterile distilled water in the ratio of 1:3. A commercially available FA standard (Sigma Aldrich, USA) was procured, and 25 ppm concentration was used as a positive control. In the negative control, only sterile distilled water was used. Three replications were maintained for each treatment. Therefore, T1 = 25% concentration of CF of race 8; T2 = 25% concentration of CF of race 4; T3 = Positive control with 25 ppm concentration of commercial FA standard; T4 = Negative control with sterile distilled water.

Raising of chickpea seedlings

The seeds of two chickpea cv. WR 315 and JG 62 were procured from the Division of Genetics and

Plant Breeding, IARI, New Delhi, surface sterilized with 1% sodium hypochlorite for 2 min, rinsed in sterile water, and then soaked in distilled water for 24 hrs. Later, seeds were allowed to germinate for two weeks following the paper roll method.

Inoculation of seedlings with CF of race 4 and race 8

Uniformly germinated 7 days old seedlings were carefully transferred into 60 ml sterilized tube (two seedlings/tube) filled with 40 ml Hoagland's solution (Garland, 1992) in each tube and were allowed to grow for a week under proper growth conditions (18-20^{0}C, 70-80% RH) at National Phytotron Facility, IARI, New Delhi. Then, Hoagland's solution in the tube was replaced with different treatments as mentioned above. So, 15 days old chickpea seedlings were given treatments with 25% CF of the races 4, 8 and 25 ppm conc. of commercial FA. Treatments were given to both resistant cv. WR 315 and susceptible cv. JG 62 seedlings with three replications. Samples were collected separately for each treatment from both, WR 315 and JG 62 at 1, 3 and 6 days after inoculation (DAI) for recording growth related parameters.

Effect of CF containing FA on Relative water content (RWC) and growth parameters of chickpea seedlings

Relative water content (RWC) : Relative water content was estimated following the method of Barrs (1968). A fresh biomass of 0.5 g of tissue was collected from the young leaves. After soaking the leaf in distilled water for 6 h, the turgid biomass was determined. Before determining turgid biomass, the leaves were properly wiped dry using tissue paper after soaking. After drying the leaf sample for 48 h at 80°C, the dry biomass was determined. The following equation was used to compute the relative water content.

$$RWC\ \% = \frac{\text{Fresh biomass - Dry biomass}}{\text{Turgid biomass - Dry biomass}} X\ 100$$

RWC percentage was calculated for all the treatments in all the time intervals in both resistant and susceptible cultivars.

Effect on root and shoot growth

Plant samples were collected in all the intervals from all the treatments and lengths of shoots and roots of all the plants were measured separately. The trend in the increase/ decrease in the shoot length and shoot length of all the samples of resistant and susceptible cultivars at a particular concentration at a particular interval was noted and correlated

Effect on root and shoot biomass

Samples of treated chickpea plant were collected from all the treatments for all the three intervals and the fresh biomass of shoots and roots of all the plants were measured separately. All the shoot and root samples of all the treatments were placed in paper envelopes for drying in a hot air oven for 48 hrs at 80^{0}C. The dry biomass of shoot and root samples of respective treatments in the respective intervals was measured and recorded. The trend in the increase/ decrease in the fresh and dry biomass of the shoot and root samples of resistant and susceptible cultivars at a particular concentration at a particular interval was noted and correlated.

Statistical calculation

Standard statistical calculations like Duncan multiple tests etc. were done wherever required following SPSS Software 16.0 version.

RESULTS AND DISCUSSION

Estimation of FA production by race 4 and race 8 of Foc in culture filtrate

Analysis of HPLC data indicated that the average quantity of FA production by race 4 was approximately 126.88 ± 5.56 mg L^{-1} (Fig.1), whereas race 8 had a production of 820.51 ± 13.37 mg L^{-1} (Fig.2) FA in the culture filtrate which is significantly higher (> 6 time) than race 4. Bani *et al.* (2014) reported that different strains within the *Fusarium oxysporum* f. sp. *pisi* race 2 produced different quantities of toxin Fusaric acid and 9, 10 dehydrofusaric acids. Selim and El-Gammal (2015) determined the relative capacity of Fusaric acid production by 12 Egyptian isolates of *Fusarium oxysporum* f. sp. *lycopersici* infecting tomatoes and showed that different isolates produced different quantities of Fusaric acid in *in vitro* culture filtrate. Concentration of Fusaric acid produced is highly correlated with the time taken to reproduce wilt symptoms.

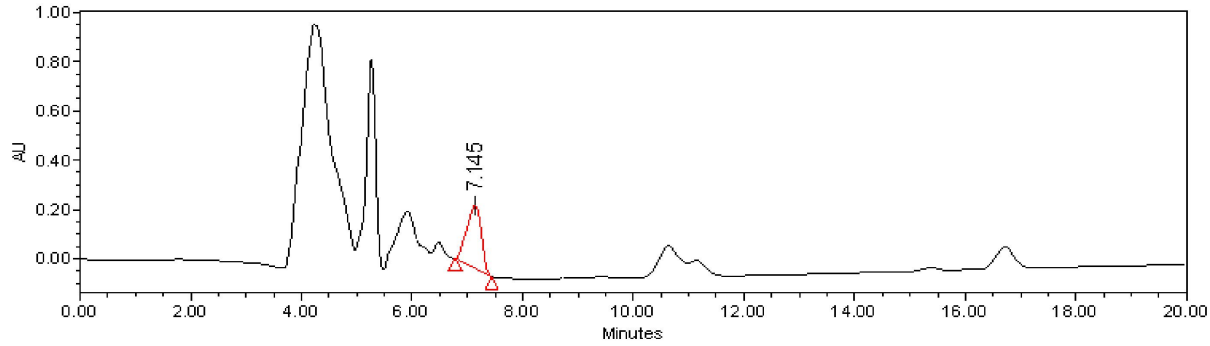

Fig.1 : HPLC Chromatogram for Fusaric acid in culture filtrate of race 4

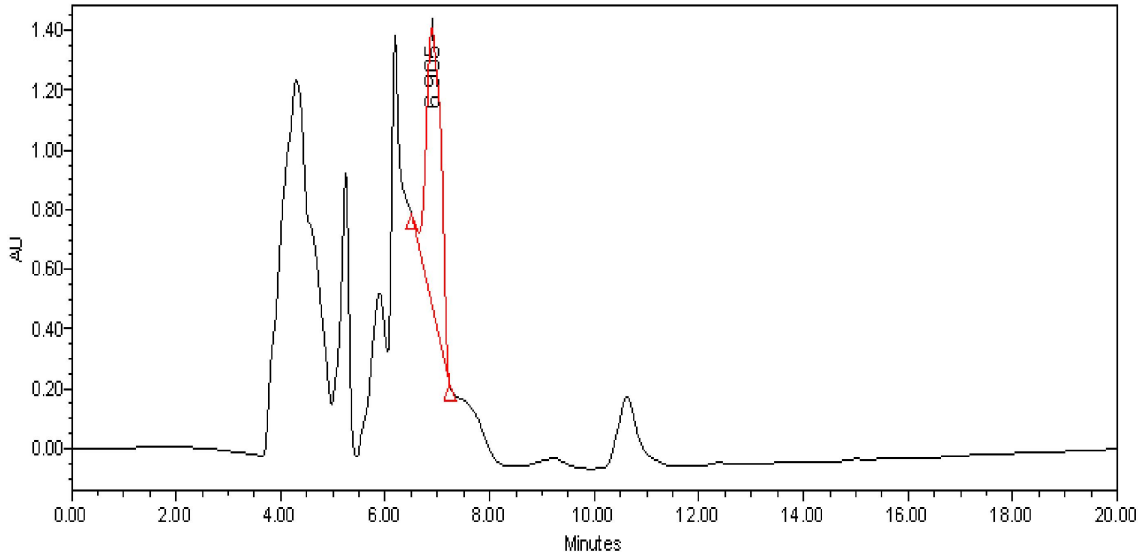

Fig.2: HPLC Chromatogram for Fusaric acid in culture filtrate of race 8

Effect of FA contained in cf on Relative Water Content (RWC) in chickpea seedlings

From the experimental data (Table 1) it was evident that RWC was higher in resistant *cv.* RW 315 than susceptible *cv.* JG 62 of chickpea in all three intervals. But reduction of RWC was more in susceptible *cv.* JG 62 than in resistant *cv.* RW 315 in all three intervals of 1, 3 and 6 DAI with CF of race 8, 4 and pure FA. Higher reduction of RWC was noticed with the increase of time of exposure of chickpea roots to CFs and pure FA.

Maximum reduction of RWC was 44.84 % in WR 315 and 67.91% in JG 62 at 6 DAI with CF of race 8. Similarly reduction of RWC was 35.81 % in WR 315 and 57.52% in JG 62 at 6 DAI with CF of race 4. It was found that RWC reduction was more by 3.34 and 5.62% in WR 315 and 1.68 and 7.24% in JG 62 at 3 and 6 DAI respectively with CF of race 8 indicating loss of water was more with the increase in time of exposure to culture filtrate containing FA. The trends were recorded similarly with respect to the treatments of culture filtrate of *Foc* race 4 and pure FA standard.

Relative Water Content (RWC) is usually measured to understand the relative amounts of water retained within the plant tissues. As culture filtrate containing fusaric acid is phytotoxic to plants, exposure of plants to this toxin cause cell damage/ injury leading to loss of permeability of plasma membrane and thereby enhances loss of electrolytes and water (Wang *et al.* 2014). At the same time RWC will be less in the damaged tissues. In the present study, CF of race 8 and 4 contained fusaric acid @ ~205.12 mg/L and @ ~31.72 mg/L respectively in 25% diluted filtrate as per the chromatogram analysis of HPLC data. These culture filtrates damage the chickpea plant tissue and enhance loss of water. Damage of plant tissues increased with higher concentration of culture filtrate and longer time of exposure. Thus loss of water in chickpea seedlings was more with

Table 1 : Effect of CF containing fusaric acid on Relative Water Content in resistant *cv*. WR 315 and susceptible *cv*. JG 62

Treatments	WR 315		JG 62	
	(Mean ± SE) (%)	Decreased over control (%)	(Mean ± SE) (%)	Decreased Over control (%)
1 DAI				
T1=CF of race 8	60.15±2.23^{ab}	39.22	37.20±2.42^{ab}	60.67
T2=CF of race 4	68.35±0.42^{c}	30.94	47.36±1.12^{c}	49.93
T3= Pure FA (+ve control)	88.31±4.83^{d}	10.77	83.95±0.59^{d}	11.25
T4= Control (-ve control)	98.97±0.15^{e}		94.59±0.03^{e}	
3 DAI				
T1=CF of race 8	57.19±2.42^{a}	42.56	35.71±1.34^{ab}	62.35
T2=CF of race 4	66.57±1.41^{c}	33.14	42.10±5.44^{bc}	55.61
T3= Pure FA (+ve control)	87.51±2.35^{d}	12.11	81.60±1.44^{d}	13.97
T4= Control (-ve control)	99.57±0.10^{e}		94.85±0.66^{e}	
6 DAI				
T1=CF of race 8	55.01±0.29^{a}	44.84	30.60±0.56^{a}	67.91
T2=CF of race 4	64.01±1.41^{bc}	35.81	40.50±4.38^{b}	57.52
T3= Pure FA (+ve control)	84.52±2.35^{d}	15.24	81.31±1.78^{d}	14.72
T4= Control (-ve control)	99.72±0.15^{e}		95.35±1.13^{e}	

Mean, ± SE values for Relative Water Content

$^{a-e}$ significant differences ($p<0.05$)

treatment of CF of race 8 (with ~205.12 mg/L) than race 4 (with ~31.72 mg/L) in this study. Further, loss of water was more after 6 days of exposure than 1 or 3 days of exposure treatment. Reduction of RWC was more in susceptible *cv.* JG 62 than resistant WR 315. This may be due to the resistant cv. WR 315 have more tolerance to fusaric acid damage than susceptible *cv*. JG 62. Bhar *et al*. (2018) reported that loss of water is more in susceptible *cv*. JG 62 with the progress of wilt disease caused by *Fusarium oxysporum f. sp*. *ciceris* race 1 than resistant *cv*. 315. The WRC has also been found to reduce in WR 315 but rate of reduction of WRC in WR 315 was not as per the rate of JG 62. Result of this study is also substantially supported by the work of Dong *et al*. (2012) who demonstrated that the relative water content of leaf was significantly lower in *F. oxysporum* f. sp. *cubense* infected banana seedlings as compared with non-infected control seedlings. As a mark of the positive role of fusaric acid in banana wilt they also recovered fusaric acid from infected banana seedlings by HPLC but failed to detect fusaric acid in healthy banana seedlings. Yan *et al.* (2018) also reported that relative water content reduced remarkably by 57.7% in *Fusarium solani* infected apple seedlings after 18 days.

Effect of CF containing fusaric acid on root and shoot length

Under the stress condition of secondary metabolites such as fusaric acid toxin, growth of plants gets affected. In the present study it was attempted to visualize the influence of CF containing fusaric acid on root and shoot length in chickpea seedlings at different intervals. From the results (Table 2), it was observed that under all treatment conditions, root and shoot were continued to grow both in resistant *cv*. WR 315 and susceptible *cv*. JG 62 but the rate of growth, both in root and shoot, was slowed down under CFs and pure fusaric acid treated plants as compared to the negative control where there was no stress of fusaric acid. Reductions were recorded 44.61 and 35.33% in root length; and 13.71 and 11.37 % in shoot length in WR 315 treated with CF of race 8 and race 4 respectively at 1 DAI, however

Table. 2: Effect of CF containing fusaric acid on root and shoot length in resistant *cv.* WR 315 and susceptible *cv*. JG 62

Treatments	Wr 315 (Mean ± SE)		Jg 62 (Mean ± SE)	
	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)
1 DAI				
T1=cf of race 8	11.05± 0.14[a] (44.61)	23.90±0.17[a] (13.71)	7.85 ± 0.20[a] (44.12)	15.25±0.25[a] (39.00)
T2=cf of race 4	12.90±0.17[b] (35.33)	24.55±0.31[b] (11.37)	10.05±0.08[b] (28.46)	18.05±0.08[b] (27.80)
T3= Pure FA (+ve control)	18.20±0.17[d] (8.77)	26.60±0.05[d] (3.97)	12.75±0.08[d] (9.25)	22.30±0.23[d] (10.80)
T4= Control (-ve control)	19.95±0.02[e]	27.70±0.11[e]	14.05±0.08[e]	25.00±0.11[e]
3 DAI				
T1=CF of race 8	13.65±0.08[b] (41.79)	25.70±0.11[c] (19.68)	9.95 ± 0.08[b] (41.64)	17.05±0.08[c] (40.90)
T2=CF of race 4	15.65±0.14[cd] (33.26)	25.90±0.11[c] (19.06)	12.25±0.14[cd] (28.15)	19.05±0.08[c] (33.96)
T3= Pure FA (+ve control)	21.85±0.08[e] (6.82)	29.00±0.05[f] (9.37)	14.35±0.08[e] (15.83)	24.45±0.20[f] (15.25)
T4= Control (-ve control)	23.45±0.20[f]	32.00±0.28[h]	17.05±0.35[f]	28.85±0.20[h]
6 DAI				
T1 = CF of race 8	13.85±0.14[c] (43.70)	27.60±0.11[e] (21.14)	12.00±0.14[c] (44.82)	20.05±0.14[e] (34.04)
T2=CFof race 4	17.35±0.14[e] (29.47)	27.55±0.14[e] (21.28)	14.40±0.14[e] (33.79)	22.85±0.25[e] (24.83)
T3= Pure FA (+ve control)	22.45±0.08[f] (8.73)	30.75±0.14[g] (12.14)	17.35±0.08[f] (20.22)	26.80±0.17[g] (11.84)
T4= Control (-ve control)	24.60±0.237[g]	35.00±0.11[i]	21.75±0.23[g]	30.40±0.05[i]

Mean ±SE values for root length and shoot length
[a-g] significant differences ($p<0.05$)
Data in parentheses are % reduction with respect to the negative control (T4).

decreases were 44.12 and 28.46% in root length; 39.00 and 27.80% in shoot length in JG 62 treated with CF of race 8 and race 4 respectively after the same corresponding period. Reductions in root length were more than shoot length in WR 315 treated with CF of race 8 and race 4; however, both root and shoot length were substantially reduced in JG 62 by CF of race 8 at 1, 3 and 6 DAI; although root and shoot length were marginally reduced by race 4 at 1, 3 and 6 DAI. Reductions in root and shoot length were at par (3.97 to 10.80%) in both resistant and susceptible cultivars treated with pure fusaric acid @ 25ppm. Trends in reduction of root and shoot length were found similar after 3 and 6 DAI in both WR 315 and JG 62. Reductions in root and shoot length were more in both WR 315 and JG 62 treated by CF of race 8 and race 4 than pure Fusaric acid (25 mg L^{-1}) treatment because of the presence of higher amount of FA per litre in cf of race 8 (~205.12 mgL^{-1}) and race 4 (~31.72 mgL^{-1}). Our present results are substantially supported by various past findings

Table 3:.Effect of cf containing FA on root and shoot fresh biomass in resistant *cv.*WR315 and susceptible *cv*. JG 62

Treatments	WR315(Mean ± SE)		JG62 (Mean ± SE)	
	Root fresh biomass (g)	Shoot fresh biomass (g)	Root fresh biomass(g)	Shoot fresh Biomass (g)
1 **DAI**				
T1 = CFof race 8	0.18± 0.005^{b} (40.00)	0.41± 0.011^{a} (42.25)	0.09± 0.133^{a} (59.09)	0.18± 0.005^{a} (58.14)
T2 = CF of race 4	0.17± 0.011^{ab} (43.33)	0.51± 0.011^{cd} (28.17)	0.07± 0.166^{a} (68.18)	0.26± 0.017^{bc} (39.53)
T3 = Pure FA(+ve control)	0.28± 0.005^{ef} (06.67)	0.56± 0.002^{e} (21.13)	0.17± 0.164^{cd} (22.73)	0.31± 0.011^{d} (27.91)
T4 = Control (-ve control)	0.30± 0.008^{f}	0.71± 0.031^{fg}	0.22± 0.167^{f}	0.43± 0.008^{f}
3 **DAI**				
T1 = CF of race 8	0.15± 0.023^{a} (58.33)	0.43± 0.005^{ab} (42.67)	0.11±0.0002^{b} (63.33)	0.19± 0.008^{a} (56.82)
T2 = CFof race 4	0.17± 0.003^{ab} (52.78)	0.53± 0.002^{de} (29.33)	0.15± 0.0008^{c} (50.00)	0.24± 0.005^{b} (45.45)
T3 = Pure FA (+ve control)	0.21± 0.001^{c} (41.67)	0.66± 0.014^{f} (12.00)	0.19± 0.0017^{de} (36.67)	0.37± 0.005^{e} (15.91)
T4 = Control (-ve control)	0.36± 0.006^{f}	0.75± 0.021g^{h}	0.30± 0.0115^{g}	0.44± 0.025^{f}
6 **DAI**				
T1 = CF of race 8	0.23± 0.003^{cd} (48.89)	0.47± 0.012^{bc} (41.25)	0.15± 0.001^{c} (61.54)	0.24± 0.008^{b} (53.85)
T2 = CF of race 4	0.25± 0.003^{de} (44.44)	0.56± 0.034^{e} (30.00)	0.21± 0.009^{ef} (46.15)	0.28± 0.005^{cd} (64.86)
T3 = Pure FA(+ve control)	0.28± 0.007^{f} (37.78)	0.70± 0.008^{fg} (12.50)	0.25± 0.008^{f} (35.90)	0.37± 0.002^{e} (28.85)
T4 = Control(-ve control)	0.45± 0.008^{g}	0.80± 0.005^{h}	0.39± 0.021^{g}	0.52± 0.005^{g}

Mean ± SE values for root and shoot fr esh biomass
$^{a-g}$ significant differences ($p<0.05$)
Data in parentheses are % reduction with respect to negative control (T4)

from other host pathogen combinations. Reduction in root growth by 74 and 42% was reported in susceptible cv. Aug- 424 and resistant cv. CM-97 respectively in chickpea treated with culture filtrate of Foc (Khan *et al.* 2004). Wu *et al*. (2008) have shown that Fusaric acid (@ 400 mg/L) extracted from *Fusarium oxysporum* f. sp*. niveum* decreased root and shoot length by 23.1 and 23.0% in water melon seedling. Further, different degrees of stunting of shoot in rice seedling have also been reported by various isolates of *Fusarium moniliforme* causing bakanae disease of rice (Puyam *et al.* 2017*).*

Effect of CF containing FA on root and shoot fresh biomass in cvs. WR 315 and JG 62

Culture filtrate of *Fusarium oxysporum f. sp.ciceris* contains Fusaric acid which is known to be phytotoxic to chickpea plants at higher concentrations. In the present study, results (Table.3) indicated both resistant *cv.* WR 315 and susceptible *cv*. JG 62 had reduced root and shoot fresh biomass with CF of race 8, race 4 and pure FA standard with respect to control in all three intervals. Reductions of root biomass by 40.00 and shoot biomass by 42.25% in resistant cv. WR 315; and root biomass by 59.09 and shoot biomass by 58.14% in susceptible cv. JG 62 were observed 1

Table 4: Effect of culture filtrate (containing Fusaric acid) on root and shoot dry biomass in resistant *cv.* WR 315 and susceptible *cv.* JG 62

Treatments	WR 315 (Mean + SE)		JG 62 (Mean + SE)	
	Root dry biomass (g)	Shoot dry biomass (g)	Root dry biomass (g)	Shoot dry biomass (g)
1 DAI				
T1 = CF of race 8	0.027 ± 0.0008^{a} (34.15)	0.033 ± 0.001^{a} (28.26)	0.013 ± 0.0002^{a} (53.57)	0.014 ± 0.0001^{a} (54.84)
T2 = CF of race 4	0.032 ± 0.0008^{c} (21.95)	0.044 ± 0.002^{abc} (04.35)	0.013 ± 0.0005^{a} (53.57)	0.016 ± 0.0002^{a} (48.39)
T3 = Pure FA (+ ve control)	0.038 ± 0.0001^{e} (07.32)	0.047 ± 0.001^{bcd} (-2.17)	0.022 ± 0.0008^{d} (21.43)	0.028 ± 0.0005 ^{bcd} (09.68)
T4 = Control (- ve control)	0.041 ± 0.0005^{f}	0.046 ± 0.003^{de}	0.028 ± 0.0008^{e}	0.031 ± 0.0002 ^{cde}
3 DAI				
T1 = CF of race 8	0.031 ± 0.0005^{b} (48.33)	0.040 ± 0.002^{ab} (41.18)	0.016 ± 0.0002^{b} (50.00)	0.021 ± 0.0005^{ab} (52.27)
T2 = CF of race 4	0.038 ± 0.0002^{ef} (36.67)	0.047 ± 0.002^{bcd} (37.50)	0.017 ± 0.0005^{b} (46.88)	0.017 ± 0.0011^{a} (61.36)
T3 = Pure FA (+ ve control)	0.051 ± 0.0002^{g} (15.00)	0.056 ± 0.004^{ef} (17.65)	0.029 ± 0.0008^{e} (09.38)	0.028 ± 0.0002^{bcde} (36.36)
T4 = Control (- ve control)	0.060 ± 0.0008^{h}	0.068 ± 0.007^{g}	0.032 ± 0.0005^{f}	0.044 ± 00005^{f}
6 DAI				
T1 = CF of race 8	0.034 ± 0.0002^{d} (50.00)	0.044 ± 0.002^{abc} (48.24)	0.021 ± 0.0005^{c} (52.27)	0.022 ± 0.0002^{abc} (42.11)
T2 = CF of race 4	0.039 ± 0.0001^{ef} (42.65)	0.056 ± 0.003^{cde} (34.12)	0.022 ± 0.0005^{cd} (50.00)	0.027 ± 0.0002^{bcd} (28.95)
T3 = Pure FA (+ ve control)	0.056 ± 0.0005^{g} (17.65)	0.075 ± 0.003^{fg} (11.76)	0.034 ± 0.0011^{f} (22.73)	0.034 ± 0.0011^{de} (10.53)
T4 = Control (- ve control)	0.068 ± 0.0004^{h}	0.085 ± 0.001^{h}	0.044 ± 0.0011^{g}	0.038 ± 0.0103^{ef}

Mean ± SE values for root and shoot dry biomass
$^{a-h}$ significant differences ($p<0.05$)
Data in parentheses are % reduction with respect to negative control (T4)

DAI of CF of race 8. However, reduction of root fresh biomass by 43.33% and shoot fresh biomass by 28.17% in resistant cv. WR 315; and root biomass by 68.18% and shoot biomass by 39.53% in susceptible *cv*. JG 62 were recorded with the treatment of cf of race 4 for the same period. Similar trends of decrease root and shoot fresh biomass were also recorded in both WR 315 and JG 62 after 3 and 6 DAI with CF of race 8, race 4 and pure FA standard. However, reductions in root and shoot fresh biomass were more in susceptible *cv*. JG 62 as compared to the resistant *cv.* WR 315. Further, reductions of root and shoot fresh biomass were comparatively less in both resistant and susceptible cultivars treated with pure fusaric acid standard @ 25 ppm than FA present in CF of race 8 and 4. However, reductions of root and shoot fresh biomass by pure FA standard @ 25 ppm were also clearly visible in both JG 62 and WR 315. Culture filtrate of *Fusarium oxysporum* f. sp. *ciceris* contains fusaric acid which is known to be phytotoxic to chickpea plants at higher concentration. General observations that infected plants show reduced growth and over all biomass. In the present experiment, results (Table 3) indicated both resistant *cv*. WR 315 and susceptible *cv.* JG 62 showed continuous increase in fresh biomass of root and shoot with the increase in time from 1, 3 and 6 days. However, increase in root and shoot fresh biomass was less with respect to

the non-treated control. In general reduction in root and shoot fresh biomass were more in susceptible *cv.* JG 62 than resistant *cv.* WR 315 across the treatment in all intervals as compared to the non-treated control. Further, reduction in fresh root biomass was higher than shoot fresh biomass in JG 62. The highest reduction in fresh biomass (64.4%) was observed in JG 62 after 6 days of treatment. Reduction of fresh root and shoot biomass were comparatively higher in both WR 315 and JG 62 across the treatment and interval with the culture filtrates of race 8, 4 as compared to the non-treated control. Effect of pure FA standard was also significantly visible in WR 315 and JG 62 in all three intervals as compared to the non-treated control.

With the increase in time of exposure of chickpea seedling with higher concentration of FA, there would be considerable damage /injury to the root tissues and destruction of chlorophyll affecting overall photosynthesis and biomass accumulation in root and shoot. Here in this study cfs of race 8, 4 contained fusaric acid @ ~205.12 mg/L and @ ~31.72 mg/L respectively in 25% diluted filtrate which have been shown to reduce chlorophyll content also (data not shown) might be affecting photosynthesis leading to higher reduction in fresh root and shoot biomass as compared to the non-treated control. Reduction of total biomass (fresh biomass) by 23.1%was reported in water melon seedlings treated with fusaric acid extracted from *F. oxysporum* f.sp. *niveum* (Wu *et al.*2008) which corroborate the present result in this study.

Effect of CF containing FA on root and shoot dry biomass in resistant cv. WR 315 and susceptible cv. JG 62

The data (Table 4) obtained in the present study indicated that there was continuous decrease of dry biomass in root and shoot of both resistant and susceptible cultivar across the treatments in all three intervals of 1, 3, 6 days with respect to the non-treated control. Reductions of root and shoot dry biomass were comparatively higher in treatments with CF of race 8 (34.15, 48.33 and 50.00% reduction in root biomass; 28.26, 41.18 and 48.24% reduction in shoot biomass) and race 4 (21.95, 36,67 and 42.65% reduction in root ; 04.35, 37.50 and 48.24 % reduction in shoot biomass) in resistant *cv*. WR 315 with the increase in exposure time from 1, 3 and 6 days respectively, whereas reductions of root and shoot dry biomass gradually slowed down or variable with the increase in time of exposure in susceptible *cv*. JG 62 with the treatment of CF of race 8 (53.57, 50.00 and 52.27 % reduction in root biomass; 54.84, 52.27 and 42.11 % reduction in shoot biomass) and race 4 (53.57, 46.88, 50.00 % reduction in root biomass; 48.39, 61.36 and 28.95% reduction in shoot biomass) as compared to pure FA and control. Higher reduction of root and shoot dry biomass may be due to the higher concentration of Fusaric acid in cf of race 8 (@ ~205.12 mg/L) and race 4(@ ~31.72 mg/L) in 25% diluted filtrates. Higher concentration of Fusaric acid possibly caused early and severe damage/injury in cell membrane and destruction of chlorophyll. Therefore, photosynthesis might be affected leading to less accumulation of biomass in root and shoot which would possibly be the reason for reduction of root and shoot dry biomass in the treatment of CF of race 8 and 4. Reductions in root biomass were more as compared to the shoot biomass. This may be due to the direct contact of roots with higher concentration of FA contained in CF of race 8 and 4. Further, reductions of root shoot dry biomass were comparatively less in resistant *cv*. WR 315 than susceptible *cv*. JG 62. This may possibly be the resistant *cv*. WR 315 has some higher tolerance to fusaric acid. Reductions in root and shoot dry biomass were minimal in both WR 315 and JG 62 treated by pure FA standard in all three intervals of 1, 3 and 6 days which might be due to the low concentration of fusaric acid (@ 25ppm). Wu *et al.* (2008) tested the effect of different concentrations of fusaric acid (extracted from *F. oxysporum* f. sp. *niveum*) on water melon seedlings and found that there was gradual decrease in biomass with the increase in concentration of fusaric acid and time of exposure.

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