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Efficacy of phytoalexin against *Fusarium udum* causing wilt of pigeonpea

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ABSTRACT

The investigation was undertaken to test the efficacy of pytoalexin against *Fusarium udum*, Amongst the eleven concentrations of cajanol tested against *F. udum* concentrations causing wilt of pigeonpea *i.e.* 325, 350 and 375 µg/l were found most effective resulting in cent per cent growth inhibition of *F. udum.* The concentrations of cajanol *viz.,* 350 µg/l and 375 µg/l were found to be most effective against conidial germination of *F. udum.* Besides the above, a commercial cajanol formulation was also tested against *F. udum* and the concentrations of 325 µg/l, 350 µg/l and 375 µg/l were againfound to be most effective in inhibiting the conidial growth germination.

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INTRODUCTION

Fusarium wilt is one of the important diseases of pigeonpea. Butler first reported the disease from Bihar during the year 1906. The disease first appears in young seedlings and extend to the entire field late if the pigeonpea is repeatedly cultivated in the same field.

A large number of micro-organisms are known to produce toxic metabolites when cultivated on synthetic media. The pathogen produces fusaric acid that is having phytotoxin properties and its high production has been correlated to virulence of it's pathogenic strains (Xu *et al.*, 1983 and Chakrabarti and Basuchaudhary, 1980).

Fusaric acid (FA) is one of the most important host non-specific toxins produced by several *Fusarium* species. Different parameters like pH, temperature and days of incubation also play important role in fusaric acid production.

Phytoalexins accumulate at the site of infection of pathogen and are thought to restrict ingress of invading pathogen (s). An investigation on the induction and accumulation of phytoalexin in pigeonpea is therefore needed to elucidate the contribution of phytoalexins to resistance. This research will, therefore, provide information on the defensive potential of cultivars by delineating the three probable utility of detected phytoalexin as a chemical marker of resistance in pigeonpea.

MATERIAL AND METHODS

The present investigation was carried out during January 2011 to March 2013 at Department of Plant

Pathology and Agricultural Microbiology and Department of Biochemistry, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri dist. Ahmednagar (M.S.).

An aliquot of 0.00, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000µg of commercial cajanol solution was pipetted out in a series of test tubes in triplicate set. The volume was made 1ml with distilled water in each of these test tubes. The standard curve was prepared by plotting absorbance against the concentrations of commercial cajanol solution and used for further study.

For extraction of phytoalexin, plants were harvested 15 days after inoculation of pathogen into host. The stele tissue was removed from the stem upto 10 cm above the inoculation point, cut into 0.5 cm pieces, weighed and placed overnight in 95 per cent alcohol (5 ml/g tissue). The ethanol extract was then decanted and dried by vaccum evaporation and redissolved in ethyl acetate (0.3 ml/g tissue). Ethyl acetate extract (0.3 ml) was then spotted on thin-layer chromatography (TLC) plates (Merck 20 x 20 cm silica gel 40 F 254) to separate cajanol. The plates were developed in chloroform/methanol solution (50:2) and allowed to dry before being sprayed with a spore suspension of *Fusarium udum* in a nutrient solution, then incubated at 28^{0}C in a moist chamber in order to detect the presence of antifungal bands (Holmans and Fuchs, 1970). All detected spots were cut out and eluted with 3 ml of 80 per cent ethanol (UV range) for 1 hr (Ingham, 1976). After elution, the contents of phytoalexin (cajanol) in each sample were determined spectrophotometrically at absorbance of 254 nm. The quantification of phytoalexin (cajanol) was made according to the calibration curve for standard cajanol (1mg cajanol/10 ml of distilled water). A purified preparation of cajanol was used as a standard.

Phytoalexins were separated on TLC plates from ethyl acetate extracts prepared as described above. The identity of fungitoxic bands was determined by using Gibb's reagent and U V spectral analysis using data available for purified compounds (Ingham, 1976; Smith and Ingham, 1981).

Cajanol was separated from ethyl acetate by TLC plates and eluted from plates for quantitative determination by U V spectral analysis and fungotoxicity tests. To test the effect of cajanol on germination of *F. udum* conidia, various concentrations of cajanol was placed in Ependorf tubes and allowed to evaporate to dryness in the dark at root temperature. The residue was then redissolved in 0.03 ml of absolute alcohol to which was added 1ml of suspension of 10^6 conidia/ml made up in sterile Armstrong's *Fusarium* medium. Before adding them to nutrient solution, the conidia were washed several times in sterile water by centrifugation to remove any auto-inhibitors that may have accumulated in the growth medium. The Eppendorf tubes were placed in the incubator at 30^{0}C and conidial germination was assessed after 24 h, by counting out number of spores those that had germtubes longer than the spores. In this way different concentrations *viz.,* 100, 150, 200, 225, 250, 275, 300, 325, 350 and 375 µg of cajanol were prepared and their fungitoxicity tested.

In vitro poisoned food technique was adopted for evaluation of cajanol against *F. udum.* For this experiment, Czapeck's dox agar medium was used as a basal medium and various concentrations of cajanol were incorporated.

Czapeck's dox agar medium was prepared and distributed @ 300 ml in 500 ml conical flasks, autoclaved at 1.05 kg/cm^2 for 20 min. Before solidification of media, different cajanol concentrations *viz.,* 50,100,150, 200, 225, 250, 275, 300, 325, 350 and 375 µg/l were incorporated aseptically in different flasks. These flasks were shaken thoroughly and poured in plates @ 20 ml/plate. Likewise, three plates for each treatment were poured. Plates without cajanol served as control. After solidification of medium, plates were inoculated with seven days old culture of *F. udum*. Five mm mycelial discs from peripheral growth of fungus were cut with the help of sterilized cooled cork borer. The plates were inoculated by keeping one disc per plate in the centre, so as to make the mycelial growth touch to the surface of the medium. The inoculated plates in inverted position were incubated at room temperature for seven days. Each treatment was replicated thrice. The colony diameter of the fungal pathogen was recorded and per cent inhibition was calculated by using the formula:

$$\text{Per cent growth inhibition} = \frac{C - T}{C} \times 100$$

C = Mycelial growth in control (mm)

T = Mycelial growth in treatment (mm)

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dryness in the dark at root temperature. The residue was then redissolved in 0.03 ml of absolute alcohol to which was added 1ml of suspension of 10^6 conidia/ml made up in sterile Armstrong's *Fusarium* medium. Before adding them to nutrient solution, the conidia were washed several times in sterile water by centrifugation to remove any auto-inhibitors that might have accumulated in the growth medium. The Eppendorf tubes were placed in the incubator at 30^0C and conidial germination was assessed after 24 h, by counting out number of spores where germ tubes had allowed more than one fourth size of the spores. In this way different concentrations *viz.,* 100, 150, 200, 225, 250, 275, 300, 325, 350 and 375 µg of cajanol were prepared and their fungitoxicity tested.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Fungitoxicity of cajanol on conidial germination of *F. udum*

The data (Table 1) revealed that amongst the fifteen concentrations of cajanol tested against conidial germination of *F. udum* the concentrations of 350 µg/l and 375 µg/l were found to be most effective resulting in cent per cent inhibition of conidial germination followed by 325 µg/l (90.38 % inhibition), 300 µg/l (82.00 % inhibition), 275 µg/l (76.59% inhibition). The least inhibition of conidial germination was shown by 100 µg/l (5.26% inhibition), 125 µg/l (10.00 % inhibition) and 150 µg/l (14.51 % inhibition). No inhibition of conidial germination was recorded at the concentrations of 25 µg/l, 50 µg/l and 75 µg/l of cajanol.

It is evident from the data (Table 2) that the amount of commercial cajanol was proportionately responsible for inhibition of conidial germination. Among the eleven concentrations of commercial cajanol tested against conidial germination of *F. udum,* the concentrations of 325 µg/l, 350 µg/l and 375 µg/l were found to be most effective (100 % inhibition of conidial germination) followed by 300 µg/l (92.59 % inhibition), 275 µg/l (83.33 % inhibition), 250 µg/l (70.00 % inhibition), respectively. The least conidial inhibition (4.87 %) was recorded at the concentration of 50 µg/l.

The above findings correlate with the reports of Marley and Hillocks (1993) who reported similar level of inhibition with extracts from susceptible plants after 15 days of inoculation.

Stevenson *et al.* (1997) reported that the conidial germination of race 1 of *Fusarium oxysporum* f. sp. *ciceri* was significantly inhibited by the root exudates of susceptible cultivars. The hyphal growth of germinated conidia was also strongly inhibited by the concentrated

Table 1 : Fungitoxicity study of cajanol on conidial germination of *Usarium. udum*

Sr. No.	Cajanol conc.(µg/l)	conidia tested	Conidia germinated	Inhibition of conidial germination
1.	25	47	47	0.00
2.	50	47	47	0.00
3.	75	43	43	0.00
4.	100	57	54	5.26
5.	125	60	54	10.00
6.	150	62	53	14.51
7.	175	55	41	25.45
8.	200	51	32	37.25
9.	225	51	26	49.01
10.	250	45	16	64.44
11.	275	47	11	76.59
12.	300	50	09	82.00
13.	325	52	05	90.38
14.	350	46	00	100.00
15.	375	54	00	100.00
16.	Control	47	47	0.00

exudates of CPS1 and WR 315 while diluted exudates were less potent. The present findings are collaborate with those findings. Sundaresan *et al.* (1993) reported that the conidial germination of *F. oxysporum* was strongly inhibited by a compound III than other 2 compounds. It has suggested that the production of phytoalexin compounds in mycorrhizal plants could be one of the mechanisms imparting tolerance of the plants to wilt disease. The present findings are also in agreement with Brinker and Seigler (1991) who isolated 'piceatannol' phytoalexin from sugarcane (*Saccharum* sp.) infected with *Colletotrichum falcatum*. The compound inhibited the conidial germination and germ tube growth of *C. falcatum*.

The data (Table 3) revealed that amongst the eleven concentrations of cajanol tested against *F. udum,* the concentration of 325, 350 and 375 µg/l were found to be most effective (100 % growth inhibition of *F. udum*) followed by 300 µg/l (90.30 % growth inhibition) and 275 µg/l (75.55 % growth inhibition). There was no growth inhibition of a pathogen with 50 µg/l concentration of commercial cajanol. The least inhibition of pathogen was shown by 100 µg/l (8.82 % inhibition). There was more than 50 per cent growth inhibition (50.07 %) with

Table 2 : Effect of different concentrations of commercial cajanol formulation on conidial germination of *Fusarium udum*

Sr. No.	Cajanol concentration (µg/l)	No. of conidia in tested	No. of conidia germinated	Inhibition of conidial germination (%)
1.	50	41	39	4.87
2.	100	53	47	11.32
3.	150	45	35	22.22
4.	200	47	29	38.29
5.	225	48	23	52.08
6.	250	50	15	70.00
7.	275	48	08	83.33
8.	300	54	04	92.59
9.	325	58	00	100.00
10.	350	60	00	100.00
11.	375	58	00	100.00
12.	Control	58	58	0.00

Table 3 : Efficacy of different concentrations of commercial cajanol solution against *F. udum*

Sr. No.	Cajanol concentration (µg/l)	Mean colony diameter (mm)				Growth inhibition (%)
		I	II	III	Mean	
1.	50	90.00	90.00	90.00	90.00	0.00
2.	100	80.20	83.20	82.80	82.06	8.82
3.	150	69.10	69.80	69.10	69.33	22.96
4.	200	57.60	57.40	57.30	57.43	36.18
5.	225	45.60	44.10	45.10	44.93	50.07
6.	250	32.50	32.50	30.80	31.93	65.63
7.	275	20.80	18.10	21.70	20.20	77.55
8.	300	7.00	9.40	9.80	8.73	90.30
9.	325	0.00	0.00	0.00	0.00	100.00
10.	350	0.00	0.00	0.00	0.00	100.00
11.	375	0.00	0.00	0.00	0.00	100.00
12.	Control	90.00	90.00	90.00	90.00	0.00
	S.E.±				0.67	
	C.D. (P=0.05)				2.02	
	CV (%)				2.30	

the concentration of 225 µg/l.

Sharon *et al*. (1992) isolated and purified phytoalexin from 12 to 14 days old leaves of *Cassiae obtusifolia* L. inoculated with *Alternaria cassiae* and observed 50 per cent inhibition of radial growth of *A. cassia* by the compound at 225 µg/ml. Fumiya and Arasuke (1983) isolated phytoalxin '6- methoxymellein' from carrot roots and tested against fungi, bacteria and yeast. They observed that, the compound has a broad antimicrobial activity that inhibited the growth of several fungi, bacteria and yeasts. These findings are also in agreement with those obtained from the present findings.

REFERENCES

Brinker, A.M. and Seigler, D.S. (1991). Isolation and Identification of piceatannol as a phytoalexin from sugarcane. *Phytochemistry*, **30** (10) : 3229-3232.

Chakrabarti, D.K. and Basuchaudhary, K.C. (1980). Correlation between virulence and fusaric acid production in *F. oxysporum* f. sp. *carthami*. *Phytopathologia*, **99**: 43-46.

Fumiya and Arasuke (1983). Isolation and antimicrobial activity of the phytoalexin 6-methoxymellein from cultured carrot cells. *Phytochemistry,* **22** (3): 669-672.

Hillocks, R.J. (1986). Resistance to *Fusarium* wilt in cotton and effects of interaction with root-knot nematode. Ph.D. Thesis, University of Reading, U.K. pp. 419.

Holmans, A.L. and Fuchs, A. (1970). Direct autobiography on thin-layer chromatograms as a method for detecting fungitoxic substances. *J. Chromatography,* **5** : 327-329.

Ingham, J.L. (1976). Induced isoflavonoids from fungus infected stems of pigeonpea. *Zeitschrift fur nature ferschung*. **31**: 504-508.

Marley, P.S. and Hillocks, R.J. (1993). The role of phytoalexins in resistance to fusarium wilt in pigeonpea (*Cajanus cajan*). *Plant Pathol*., **42**: 212-218.

Sharon, A., Ghirlando, R. and Gressel, J. (1992). Isolation, purification and identification of 2-(p-Hydroxyphenoxy)-5, 7-Dihydroxychromone : A fungal induced phytoalexin from cassia obtusifolia. *Plant Physiol*., **98**: 303-308.

Smith, D.A. and Ingham, J.L. (1981). Phytoalexins and plant disease resistance. *Biologist*., **28**(2): 69-74.

Stevenson, P.C., Turner, H.C. and Haware, M.P. (1997). Phytoalexin accumulation in the roots of chickpea (*Cicer arietinum* L.) seedling associated with resistance to *Fusarium* wilt (*Fusarium oxysporum* f. sp. *ciceri*). *Physiological & Molecular Plant Pathol*., **50**: 167-178.

Sundaresan, P., Ubalthoose, N. and Gunasekarn, P. (1993). Induction and accumulation of phytoalexins in cow pea roots infected with a mycorrhizal fungus *Glomus fasciculatum* and their resistance to *Fusarium* wilt disease. *J. Biosci.,* **18**(2): 291-301.

Xu, X.H., Gu, G.F. and Shen, F.D. (1983). Studies on the relationship between fusaric acid and cotton fusarial wilt disease. *Alta. Agric. Univen Pekinensis*, **9**: 29-35.

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