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BIOCHEMICAL CHANGES IN BENOMYL SENSITIVE AND RESITANT ISOLATES OF *FUSARIUM SOLANI* (MART.) SACC CAUSING ROOT ROT OF CHICKPEA (*CICER ARIETINUM* L.)

[1]Waghmare Vandana U. and [2]*Andoji Yogesh S.

[1]Department of Botany, Willingdon College Sangli.

[2]Department of Botany, Shikshanmaharshee Dr. Bapuji Salunkhe College Miraj Maharashtra, India.

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***Corresponding Author**
Dr. Andoji Yogesh S.
Department of Botany,
Shikshanmaharshee Dr.
Bapuji Salunkhe College
Miraj Maharashtra, India.

ABSTRACT

chickpea (*Cicer arietinum* L.) is an important pulse crop grown for its vegetable, fodder and medicinal value. It was infected by *Fusarium solani* (Mart.) causing root rot disease to chickpea. Benomyl was used for management of the disease. The benomyl sensitive and resistant isolates show biochemical variation when assessed against untreated healthy ones. Biochemical constituents like Carbohydrates, starch, reducing sugar, DNA, RNA as well as Iron. Zinc, Copper, Manganese, and Magnesium contents were seen to be reduced due to infection of *Fusarium solani* in sensitive and resistant isolates as compared to healthy plant, while Calcium, total ash and polyphenol contents were increased in both of the isolates.

KEYWORDS: Root rot chickpea (*Cicer arietinum* L.), *Fusarium solani* (Mart.) Sacc sensitive and resistant, biochemical constituents.

INTRODUCTION

Pulses are an important part of the daily diet for most indians as they contain 2 to 3 times more protein than cereals. Chickpea (*Cicer arietinum* L.) is the most important pulse food crop among major rabi pulses of India and belongs to family Leguminosae. Chickpea is not only important human food but also used in traditional farming systems. According to (Chiranjeevi *et al*., 2002) in the dry land it fixes atmospheric nitrogen in the soil and increases soil fertility. It has very great nutritional value. According to (Cook, 1967) after dehulling chickpea is valued for its nutritive seeds with protein content 25.3 to 28.9 percent.

Raw chickpea seeds contains per 100g: 357 calories, 140-440 mg Ca, 2-4.8 g ash, 190-382 mg P, 4.5-15.69 percent moisture 0.8 to 6.4 percent fat, 9mg Fe, 1.3-2.9 mg niacin, 0.12-0.33 mg riboflavin and 0- 225 µg β carotene. (Chet *et al*., 1981; Kumar and Mukerji, 1996). According to Chiranjeevi *et al*., 2002 chickpea is very richest source of fibre, fat and proteins. Chickpea is used as medicine for cholera, diarrhea, snakebite, warts and blood purification. Chickpea is most hypocholesteremic legume among all food legumes. Seeds are antibilious (Mukhopadhyay, 2005). Chickpea seeds are eaten fresh, roasted and boiled. Seed flour can be used to make soup, tasty food stuffs, bread and served as side dish.

(Mukhopadhyay, 2005) reported that India ranks first in acreage of chickpea (Bengal gram) cultivation (7.49 mha) and annual yield is about 6.33 mtns and produces some fifteen times as much as second largest producer, Australia. The major regions where chickpea is cultivated in India are Maharashtra, Uttarpradesh, Karnatak, Haryana, Westbengal, Gujrat, Bihar, Chhattisgarh.

Black root rot or wilt of Chickpea

At present, chickpea production has either remained static or decreased is mainly due to the diseases and poor management practices.

Nene *et al.* (1996) reported that is about 67 fungi, 3 bacteria, 22 viruses, and 80 nematodes. The major fungal diseases are ascochyta blight, black root rot, rhizoctonia dry root rot, verticillium wilt, rust etc. Among these black root rot of chickpea caused by *Fusarium solani* (Mart.) Sacc. is very serious disease in India. Tewari and Mukhopadhyay (2003) reported that black root rot caused by *Fusarium solani* (Mart.) Sacc. is one of the most serious disease, which causes severe yield loss up to 60 to 80 percent. The disease shows both the symptoms of wilt and root rot. Nene (1985) observed that the disease can appear at any stage of the crop, symptoms in a highly susceptible cultivar can develop any time between 25 days after sowing till as late as podding stage. Chickpea black root rot spread through soil as mycelium grows on roots and enters through soil water, farm equipments and wounds (Agrios, 1984).

Initially infected crop do not show external rotting, but their roots spread vertically and shows blackish rotten patches on the internal tissues. The main symptoms of diseased plant are get stunted growth, yellowing and drying of leaves and browning of vascular bundle.Mycelium enters in to xylem vessels and acquire whole vascular system of host results in to wilting and yellowing of plant. According to Haware (1993) in the absence of

host *Fusarium solani* can survive in the soil up to six years. The disease has assumed great importance in Maharashtra state during the past few years due to severe yield loss.

The sensitivity of pathogen was calculated in both sensitive (Fs 3) and resistant isolates (Fs 13) of *Fusarium solani*. The root rot causes infection to plant and reduces the quality of plant. The plant is rich in carbohydrate, starch and other elements which make the plant valuable for use for various purposes. The biochemical analysis of the plant in healthy and infected plant shows lot of variations during biochemical analysis. The aim of present investigation is to analyse the biochemical constituents in healthy and infected chickpea.

2. MATERIAL AND METHODS

2.1. Material

Fenugreek (*Trigonella foenum* graecum L.), healthy and infected by *Alternaria alternata*. This was studied by inoculating the *Trigonella foenum* graecum (L.) plants with spore suspension of resistant and sensitive isolates of *Alternaria alternata*. After 10 days of inoculation plants were dried at 40°C in hot air oven and powder was obtained in grinder. Healthy and inoculated samples were extracted in ethanol and were analyzed for all the biochemical estimations.

2.2. Preparation of Reagents and Solutions

2.2.1. Alkaline Copper tartarate

Solution A

Anhydrous sodium carbonate 2.5 gm, Potassium sodium tartarate 2.5 gm, sodium sulphate (anhydrous) 20 gm were dissolved in distilled water and final volume was made up to 100 ml with distilled water, filtered and stored at room temperature.

Solution B

Copper sulphate 15 gm were dissolved in a small volume of distilled water and one drop of sulphuric acid was added and final volume was made up to 100 ml with distilled water. 4 ml of solution B and 96 ml of solution A were mixed before use.

2.2.2. Arsenomolybdate Reagent

2.5 gm ammonium molybdate was dissolved in 45 ml distilled water. To this 2.5 ml sulphuric acid was mixed well. Then 0.3 gm disodium hydrogen arsenate dissolved in 25 ml distilled water was added and incubated at 37ºC for 24 to 28 hours.

2.2.3. Standard Glucose Solution

Stock

100 mg of Glucose was dissolved in 100 ml distilled water.

Working Standard

10 l of stock diluted to 100 ml with distilled water. (0.1mg/ml).

2.2.4. Folin –Ciacolteu reagent-

Commercially prepared reagent (2N) was mixed with equal quantity of distilled water and stored in amber color bottle at 2 °C.

2.2.5. DNA Standard solution

30 mg deoxyribonucleic acid (DNA) salt from Herring sperm free acid. Hi Media Laboratory, Pvt. Ltd. Mumbai was dissolved in 100 ml 0.3 N KOH solution to give a solution containing 30 mg DNA per ml of KOH.

2.2.6. Diphenylamine Reagent

1 gm dipheylamine was dissolved in 96 ml glacial acetic acid to which 4 ml concentrated H2SO4 added and stored at 20ºC.

2.2.7. RNA Standard solution

50 mg of RNA from yeast, pure (BDH) chemicals Ltd. Poole (England) was dissolved in 100 ml 0.3 N KOH and stored at 20C. Each ml of this solution contained 500 mg RNA. This was diluted with KOH to obtain various concentrations of RNA.

2.2.8. Orcinol Reagent

2 ml of 10% solution of ferric chloride 6H2O was added to 400 ml of concentrated HCl.

2.3 Biochemical Estimation

2.3.1. Estimation of carbohydrates

Carbohydrates were estimated according to the method described by Nelson (1944).0.5 gm oven dried plant material was homogenized in mortar and pestle and extracted with 80% alcohol. It was filtered through Buckner's funnel using whatman no. 1 filter paper. The residue on filter paper was washed with 80% alcohol repeatedly. All the washing and filtrate were mixed together. The residue was used for starch estimation.

2.3.2. Estimation of Reducing Sugar

The Filtrate was condensed on the water bath to about 2-3 ml and to it added lead acetate and potassium oxalate (1:1) to decolorize the extract. It was mixed together with the help of glass rod with addition of some distilled water. It was again filtered and washed with distilled water, collecting the washings in the same filtrate. The final volume of filtrate was made to 50 ml with distilled water. The filtrate was used for estimation of reducing sugars.

2.3.3. Estimation of Starch

The residue on the filter paper used for estimation of starch was transferred to a conical flask with 50 ml of distilled water and 2-3 ml of concentrated HCL, autoclaved at 15 lbs pressure for 30 minutes. The autoclaved filtrate was cooled and neutralized with Na2Co3 and filtrated. This filtrate contained reducing sugars produced as a result of hydrolysis of starch. Aliquots of 2ml (from reducing sugar and starch) were taken separately in 10ml marked test tubes. 0.5, 1.0, 1.5, 2.0, 2.5 ml of working standard glucose solution were taken in a series of test tubes. The Alkaline copper tartarate reagents 1 ml was added to each test tube. Test tubes were then kept in boiling water bath for 10 minutes. After cooling the tubes, 1 ml Arsenomolybdate reagent was added to all the tubes. The final volume was made 10 ml with distilled water. A blank was prepared as stated above with working standard glucose solution. After adjusting the spectrophotometer with blank after 10 minutes, the absorbance was read at 560 nm on double beam spectrophotometer (Shimadzu, UVVIS-190). A standard curve of glucose (0.1 mg/ml) was prepared and the sugar content was calculated.

2.3.4. Estimation of Polyphenol

The polyphenols were estimated following the method by Folin and Denis (1951). 1 gm oven dried root infected powder of *Trigonella foenum graecum* was homogenized in mortar and pestle and extracted with 80% acetone. It was filtered through Buckner' funnel, using Whatman No.1, filter paper. The total volume was made 25ml with 80% acetone. This filtrate was used for estimation of polyphenols. From the acetone extract, 2ml were taken separately in 10 ml marked test tubes. 1.00, 2.00, 3.00, 4.00 ml of the standard tannic acid solutions were taken in a series of test tubes. To this mixture 10 ml 20% Na2CO3 was added in each test tube. In the above mixture 2 ml of Folin- Denis reagent was added mixed well and diluted to 50 ml with distilled water. The standard tannic acid solution (0.1 mg/ml) was used for the preparation of standard polyphenol curve. A blank was prepared without standard

tannic acid. After development of colour the absorbance was read at 660 nm on Shimadzu, UVVIS, and 190 double beam, spectrophotometer.

2.3.5. Estimation of nucleic acids

The nucleic acids were separated from the roots of fenugreek by using method of Cherry (1962). Oven dried root infected powder (200 mg) of *Trigonella foenum graecum* roots were homogenized in 2 ml cold methanol. Insoluble pellet was washed with 2 ml methanol and centrifuged at 500 rpm for 15 minutes. Aqueous state discarded. Pellet was again washed with 4 ml cold 0.2 N perchloric acid and centrifuged at 5000 rpm for 15 minutes at 40°C aqueous state discarded. Pellet was suspended in 5 ml ethanol. (other mixture (2:1) and kept at 50°C for 15 minutes, centrifuged in cooling centrifuge at 5000 rpm for 15 minutes. Pellet was discarded. Supernatant was used for estimation of DNA with a Diphenylamine and RNA with Orcinol.

2.3.6. Estimation of DNA

1 ml of supernatant was added to 2.5 ml freshly prepared diphenyl amine reagent. The contents were mixed and heated for 5 minutes in boiling water bath. These samples were cooled and absorbance read at 540 nm in a spectrophotometer (Schimadzu-UV-VIS-190). A similarly treated blank was used for zero setting. The quantity of DNA per test was determined from the standard curve by using standard DNA solution.

2.3.7 Estimation of RNA

1ml distilled water and 9 ml freshly prepared Orcinol reagent was added to 0.2 ml of extract. The contents were mixed and heated for 20 minutes in a boiling water bath. The solution was cooled and absorbance read at 260 nm (Schimadzu-UVVIS-190). A blank was prepared by using distilled water for zero setting. Standard curve for RNA was prepared by using standard RNA solution.

2.3.8 Estimation of Total ash

The residue after incineration of the sample at 550-660 ºC was known as ash. For this purpose the sample was subjected to a high temperature up to 600 ºC and then ash content was determined. During ignition to such a high temperature all organic compounds decomposed and pass off in the form of gases, while the mineral elements remained in the form of ash.

2.4 Procedure

1 gm oven dried powder of sample was taken in a previously weighed vitrosil silica crucible. It was heated on a hot plate for about 30 minutes, till the sample was sufficiently charred and turns black. The lid of crucible was replaced and kept it in a muffle furnace. The temperature was allowed to raise up to 550 ºC and kept constant for 4 hrs. The crucible was removed on cooling and transferred directly to desiccators and weighed immediately. The weight of ash obtained was determined per 1 gm of sample and calculated the ash content as percent dry matter.

3. RESULT AND DISCUSSION

In the experiment it was investigated that by inoculating the chickpea (*Cicer arietinum* L.) plants with spore suspension of resistant isolate and sensitive isolate of *Fusarium solani* and compared with healthy plant, (Table 1) indicate that, carbohydrates, starch, reducing sugar, DNA, RNA as well as Iron. Zinc, Copper, Manganese, and Magnesium content reduced due to infection of sensitive and resistant isolates of *Fusarium solani* as compared to healthy plant, while Calcium, total ash and polyphenol content was increased due to the infection of both the isolates of *Fusarium solani.* The biochemical comparison among sensitive and resistant isolates show highest reduction in biochemical constituents like Carbohydrates, starch, reducing sugar, DNA, RNA, Iron, Zinc, Copper, Manganese, and Magnesium contents, while Calcium, total ash and polyphenol contents were highly increased. Rana and Sengupta (1976), reported more production of amino acid in benomyl resistant strain of *Macrophomina phaseolina* than the sensitive one. Jagtap *et al*., (2011), studied biochemical changes in turmeric rhizome infected with *Sclerotium rolfsii*. There are similar results observed by other workers and the results are in aggregation of earlier workers who have studied biochemical characteristics of fungal pathogens Nagaraja, T.G. (2007), Sindhan and Parshar (1996), Ushamalini, *et al* (1998) and Choudhari and Kareppa **(**2013).

Table 1: Biochemical constituents of benomyl sensitive, resistant and healthy (untreated) Chickpea.

Sr. No.	Estimation	Healthy chickpea roots	Chickpea roots infected with sensitive isolate (Fs3)	Chickpea roots infected with resistant isolate (Fs13)
1	Carbohydrate gm/100gm	9.5	8.9	8.3
2	Reducing sugar	1.90	1.10	1.0

	gm/100gm			
3	Starch gm/100gm	6.20	4.80	4.10
4	Polyphenols mg/100g	910	930	970
5	DNA mg/g	1.80	0.625	0.775
6	RNA mg/g	2.50	2.20	1.40
Mineral analysis (mg/100g)				
a	Iron	460	430	410
b	Zinc	15.90	4.60	2.30
c	Copper	11.15	7.50	5.20
d	Calcium	1.34	2.30	2.58
e	Manganese	0.83	0.20	0.5
f	Magnesium	13.86	9.10	8.5

4. CONCLUSION

The sensitive and resistant isolates after biochemical comparison with healthy one show reduction in biochemical constituents like Carbohydrates, starch, reducing sugar, DNA, RNA as well as Iron, Zinc, Copper, Manganese, and Magnesium contents, while Calcium, total ash and polyphenol contents were increased in both of the isolates. The resistant isolate show highest reduction in biochemical constituents like Carbohydrates, starch, reducing sugar, DNA, RNA, Iron, Zinc, Copper, Manganese, and Magnesium contents, while Calcium, total ash and polyphenol contents were highly increased.

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