Research Paper

Volume : 4 | Issue : 12 | December 2015 • ISSN No 2277 - 8179

Alteration in total Phenol contents and PPO enzyme activity in wilt infected cluster bean plant parts caused by *Fusarium solani*

Botany

KEYWORDS : Cyamopsis tetragonoloba, Deterioration, Phenol, Polyphenol oxidase(PPO), Wilt.

Vikas Pareek PG Department of Botany, Government College, Kota, Rajasthan-324001

Rashmi Varma PG Department of Botany, Government College, Kota, Rajasthan-324001

ABSTRACT

Field as well as storage fungi cause deterioration of the seeds by affecting planting and edible value. In present paper alteration in phenolic contents (mg/gm) and their related enzymes (units/ sec/mg of fresh weight of tissue) were examined in cluster bean [Cyamopsis tetragonoloba (L.) Taub.] plant parts infected with Fusarium solani caused wilt disease. Initial symptoms of wilt disease appeared as yellow brown patches on cotyledonary leaves and it is characterized by yellowing of lower leaves and stunting or dwarfing of plant growth. Phenolic compounds and related oxidative enzymes are one of the most important disease resistance biochemical parameters. In higher plants they are involved in disease resistance mechanism. Various plant parts viz. leaves, stems and seeds expressed alteration in their phenol contents and related enzyme i.e. polyphenoloxidase. Total phenolic content were measured higher in wilt infected plant parts viz. leaves (0.15 mg/gm), stem (0.16 mg/gm) and seeds (0.53 mg/gm) in comparison to healthy counterparts while polyphenol oxidase (PPO) activities were examined higher in normal leaves (0.021 units/ sec/mg of fresh weight of tissue), stem (0.03 units/ sec/mg of fresh weight of tissue) and seeds (0.026 units/ sec/mg of fresh weight of tissue) as compared to infected counterparts. Increased level of phenols suggested an acceleration of phenols synthesizing following pathogen infection.

INTRODUCTION

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] is one of the oldest known protein rich food legumes grown as green manuring, green fodder and vegetable crop for human consumption since ancient times in India and Pakistan (Patel, Patel and Desai, 2002; Meena, Godara and Gangopadhyay, 2010). The guar seed comprises three parts: the seed coat (14-17%), the endosperm (35-42%), and the germ (43-47%) (Sharma and Gummagolmath, 2012). It is cultivated for its seeds which were among the top three traded agricultural commodity on Indian bourses (Mishra, 2008). Guar seeds are a rich source of proteins and carbohydrates as compared to other bean and legume crops (Deore, Sawant and Ilhe, 2004). The presence of glactomannan gum in endosperm of seeds resulted in to making guar as an important industrial cash crop. Guar meal is the main by-product of guar gum production. It is a mixture of germs and hulls at an approximate ratio of 25 % germ to 75 % hull (Lee et al., 2004). It gained worldwide recognization due to its wide industrial and medicinal uses. A high concentration of flavanoids and other phenolics compounds like kaempherol in guar seeds may expand its neutraceutical and pharmaceutical use (Khare, 2004; Katewa et al, 2004).

Among the different pathogens attacking the cluster bean crop *F. solani* is the most common fungus causing considerable yield losses. The pathogen caused wilt of seedlings/ plant (Pareek and Varma, 2014). Initial symptoms of wilt disease appeared as yellow brown patches on cotyledonary leaves and it is characterized by yellowing of lower leaves and stunting or dwarfing of plant growth. The margins of the cotyledonary leaves curl downward and inwards. Entire seedling appeared dried and collapsed. The stem near the soil line may be slightly thickened and brittle. Seeds covered with white mycelial growth. Brown to black discolouration appears on stem affected with wilt disease.

Present study was carried out to observe physiological changes in various plant parts due to wilt disease caused by *Fusarium solani*. The quantification of alteration in total phenol content and their related enzyme (PPO) activity were estimated.

MATERIALS AND METHODS

Healthy and wilt infected cluster bean plant parts were collected directly from the field of Sikar (Ac. no. CB70) districts of Rajasthan and their morphological and biochemical studies were undertaken. Estimation of total phenolic content and their related enzyme (Poly Phenol Oxidase) activity in various cluster bean plant parts viz. leaves, stem and seeds were carried out by using standard methods. Phenolic compounds and related oxidative enzymes are one of the important disease resistance biochemical parameters. In higher plants they are involved in disease resistance mechanism. Total phenolic contents in present study were estimated by method of Bray and Thorpe (1954) while the poly phenol oxidase (PPO) enzyme activity was assayed by the method of Shinshi and Noguchi (1975).

Extraction of total phenol contents

500 mg of dried plant tissue sample was homogenized in 10 ml of 80% ethanol. Centrifugation of the homogenate at 1000-3000 rpm for 20 min, collect the supernatant and the residue was re-extracted with 80% ethanol and recentrifuged after supernatant was collected.

Estimation of total phenol content

Estimation of total phenol content by assay method using ethanolic extract (Bray and Thorpe, 1954). Total phenolic contents were estimated by the 1.0 ml Folin Ciocalteau Phenol reagent (diluted with equal volume of distilled water before use) was added to 1 ml of alcohol extract in a test tube followed by 2.0 ml of 20% sodium carbonate solution. Heat the mixture in a boiling water bath for 1 minute and shaken vigorously. Blue colour is obtained which was diluted with 25.0 ml of distilled water and absorbance was recorded at 750nm in UV spectrophotometer (Systronics UV - VIS-118) against a blank. 80% ethanol was used to prepare the blank. Three such replicates were taken for each concentration and the average Optical density was plotted against the respective concentration to compute a regression curve. Standard curve prepared from different concentrations of catechol used to calculate total phenols. Total phenols were expressed as mg/gm fresh weight of tissue.

Extraction of Poly Phenol Oxidase

Crush 1 gm of plant tissue samples in 10 ml of chilled 1 M phosphate buffer PH-6.0. Refrigerated centrifugation of homogenate done at 3000 rpm for 15 minutes at 0°C. The supernatant was collected as enzyme extract and made upto 10 ml with buffer solution.

Estimation of Poly Phenol Oxidase Enzyme

Polyphenol oxidase activity was assayed by the method of Shinshi and Noguchi, 1975. 1.0 ml of enzyme extract and 3.0 ml of buffered catechol (Freshly prepared) [buffered catechol = 0.022 gm/20 ml phosphate buffer pH= 6.0] constitute the reaction mixture. The increase in optical density was recorded at 15 second

intervals at 470 nm after mixing enzyme and substrate. A unit of enzyme activity was chosen as change in absorbance 0.001 per second the reaction mixture without the substrate was used as blank, enzyme activity was expressed in terms of units/sec/mg fresh weight of tissue.

Enzyme reaction

Catechol (Colourless) ⟹ **Quinone** (Yellow coloured)

Catechol oxidized by enzyme polyphenol oxidase into a yellow coloured quinone compounds.

RESULTS AND DISCUSSION

The results revealed slight increase in total phenols in wilt infected plant parts viz. leaves, stem and seeds than the healthy counterparts (Table:-1; Fig. 1A) whereas polyphenol (PPO) enzyme activity was observed higher in healthy plant parts viz. leaves, stem and seeds than the *Fusarium solani* infected counterparts (Table:- 1; Fig. 1B). The total phenol contents was 0.15, 0.16 and 0.53mg/gm in wilt infected leaves, stem and seeds whereas it was 0.06, 0.07 and 0.49 mg/gm in healthy leaves, stem and seeds respectively and the poly phenol oxidase (PPO) enzyme activities was 0.021, 0.03 and 0.026 units/ sec/mg of fresh weight of tissue in healthy leaves, stem and seeds whereas it was 0.012, 0.008 and 0.018 units/ sec/mg of fresh weight of tissue in wilt infected leaves, stem and seeds respectively. The increase in quantity of total phenols might be attributed with defense mechanism (Jain and Yadav, 2003). The resistance to disease caused by fungi was due to presence of high amount of phenols (Parashar and Lodha, 2007). After infection by a pathogen, plant cells synthesize phenol oxidizing enzymes that oxidise phenols into toxic quinines, which play a crucial role in disease resistance (Jiang et al., 2009; Ashry and Mohamed, 2011).

The comparative histogram shows that in present study the total phenol activity was highest in infected seeds and lowest in healthy leaves whereas polyphenol oxidase (PPO) enzyme activity was highest in healthy stem and lowest in infected stem (Fig. 1C). Increased level of phenols suggested an acceleration of phenols synthesizing following pathogen infection.

Several workers (Cruickshank and Perrin, 1964; Bhatia, Uppal and Bajaj, 1972; Chopra, Jhooty and Bajaj, 1974; Nemec, 1976) have emphasized a definite correlation between degree of resistance and level of phenol while Singh, Nagra and Mehrotra (1982) observed that phenol have no relation with resistance.

Kamble and Gangawane (1987) reported increase in total phenol content in ground nut seeds due to infection of *Curvularia lunata*, *Aspergillus flavus*, *Penicillium fumiculosum*, *P. varians* and *Fusarium oxysporum*. Singh and Shrivastav (1988) have showed increase in total phenol content in seedlings of moth bean infected with *Macrophomina phaseolina*. Agarwal (1989) observed that total phenol content in pigeon pea increased after infection of *Phytophthora drechsleri* in resistant variety whereas it was reduced in leaves of susceptible variety. Jain and Yadav (2003) reported the increase in quantity of total phenols quantity of total phenol in infected finger millet plant parts. Similar increase in phenol content in maize crop infected with leaf and sheath blight (BLSB) disease caused by *Rhizoctonia solani*.

Rao and Panwar (2001) revealed decrease poly phenol activities in *Sesamum indicum* infected with *Curvularia phaseoli*. Saharan et al. (2001) observed phenolic compound and oxidative enzymes in cluster bean leaves infected with *Alternaria* blight. They were reported that the amount of poly phenol oxidase (PPO) increased with the increase in intensity of *Alternaria* blight up to 50% in highly susceptible varieties as compared to their respective healthy leaves. Similar observations were reported in cluster bean by Joshi, Gupta and Singh (2003) during study on root and leaves infected with *Macrophomina phaseolina* and by Joshi, Gupta and Kumar (2004) during study on *Alternaria* blight.

Varma (2003) reported that both asymptomatic seeds and symptomatic soybean seeds infected with Rhizoctonia bataticola showed initial increase in phenol content but the amount was always low in symptomatic seeds than the asymptomatic during germination whereas peroxidase activity was high in asymptomatic soybean seeds than the symptomatic seeds infected with Rhizoctonia bataticola. Singh (2004) reported increase in the phenolics in relation to resistance in *Brassica* (Ghosal et al., 2004).

Marmit et al. (2008) reported the role of phenolic compounds in the defence mechanism of the plants and subsequently their accumulation in the cells followed by insect induced galls. Accumulation of total phenol caused the hyperphenolicity in infected resistant host tisuue in pearl millet infected with *Sclerospora graminicola* whereas polyphenol oxidase activity increased (Arun, Mali and Manga, 2012).

Singh and Varma (2010) estimated total phenol and related polyphenol oxidase enzyme activity in *Lens culinaris Medic* infected with *Fusarium oxysporum* and reported higher phenol contents in infected plant parts than the healthy plant parts whereas poly phenol oxidase activity was higher in normal plant parts viz. leaf, stem, fruit and seeds compared to infected counterparts. Anjum, Fatima and Amjad (2012) determined the dynamics of total phenolic content and polyphenol oxidase activities in wheat infected with *Ustilago tritici*. Sadda (2012) revealed high phenolic contents and low polyphenol oxidase activity in *Rhizoctonia solani* and *Colletotrichum orbiculare* infected *Luffa cylindrica* plant parts viz. leaf, stem and seed as compared to healthy counterparts. Dahima et al. (2014) reported maximum total phenol contents in *Rhizoctonia solani f. sp. sasakii* (banded leaf and sheath blight) infected maize plant parts. The alteration in the oxidative enzyme level in maize infected with *R. solani* may be due to injury of the host tissues by fungal hyphae leading to the oxidation of phenolic compounds, however, the amount of oxidation products is insufficient to stop the invasion of mycelium in the leaf tissues (Liu et al., 2012).

Conclusion:-

Present investigation clearly indicates the role of phenolic contents in resistant mechanism of plant. Highest phenolic contents were observed in infected plant parts than the healthy counterparts whereas related PPO enzyme activity was found higher in healthy counterparts than the infected counterparts. Phenolic compounds present in plant parts oxidized in quinone in the presence of PPO enzyme and this binds to protein present in that plant parts and inhibit the action of enzymes like lignin peroxidase & laccase. Due to wide application of guar in traditional medicine, pharmaceutical, cosmetics, paper industries, textiles, backery and oil field present study has significance to understand the alteration in biochemical contents and related enzymes caused by pathogen like Fusarium solani.

Acknowledgement

The author Pareek V and Varma R wishes to thank Department of Science and Technology, Jaipur for their financial assistance.

TABLE 1: Quantification of total Phenol contents and their related enzyme (polyphenol oxidase) activity in Cyamopsis tetragonoloba (L.) Taub.

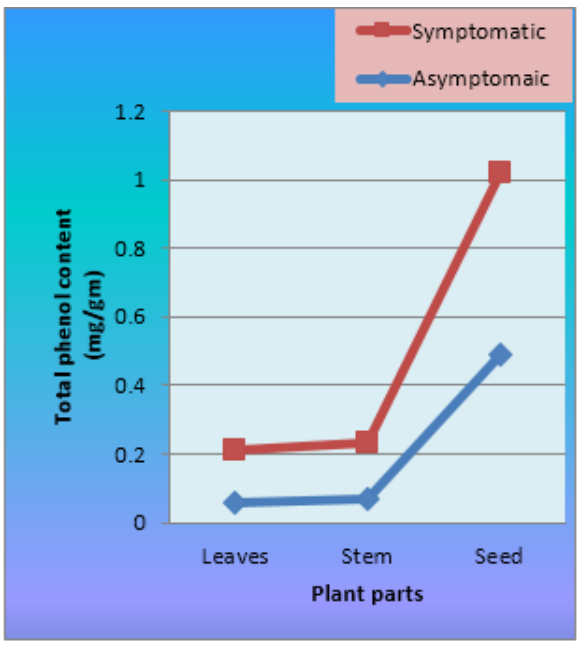
Concentration (mg/g)	NSd	ISd	NS	IS	NL	IL
Total Phenol content (mg/g)	0.49±	0.53±	0.07±	0.16±	0.06±	0.15±
Polyphenol Oxidase activity (units/sec/mg. weight of fresh tissue)	0.026±	0.018±	0.03±	0.008±	0.021±	0.012±

Volume : 4 | Issue : 12 | December 2015 • ISSN No 2277 - 8179

NSd = Non- infected seeds **ISd = Infected seeds**
NS = Non- infected stem **IS = Infected stem**
NL = Non- infected leaves **IL = Infected leaves**

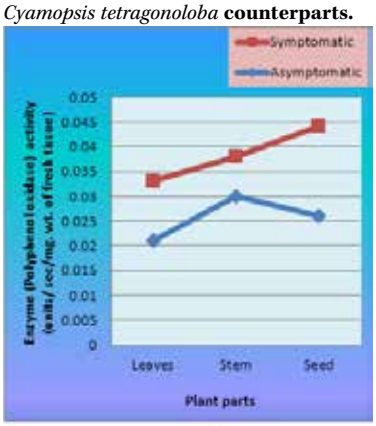
Figure 1

A. Comparison of total phenol in healthy (Asymptomatic) and wilt infected (Symptomatic) *Cyamopsis tetragonoloba* **counterparts.**



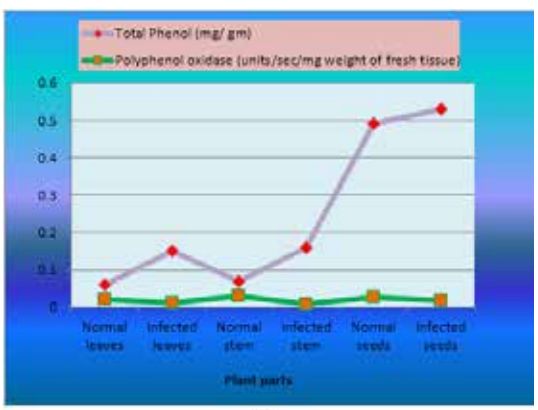
A

B.Comparison of enzyme (Polyphenol oxidase) activity in healthy (Asymptomatic) and wilt infected (Symptomatic) *Cyamopsis tetragonoloba* **counterparts.**



B

C.Comparative line histogram for total phenol and Polyphenol oxidase (PPO) activity.



C

REFERENCE

Agarwal S.C. (1989). Effects of stem inoculation of Phytopthora stem blight on phenol contents in pigeonpea. Indian Journal of Pulse Res. 2: 39-41 Anjum T., Fatima S. and Amjad S. (2012). Physiological changes in wheat during development of loose smut. Trop. Plant pathol. 37(2): Barsilia Mar./ Apr. 2012 Arun K., Mali P.C. and Manga V.K. (2012). Changes of some phenolic compounds and enzyme activities on infected pearl millet caused by Sclerospora graminicola. International Journal of Plant physiology and biochemistry. 2(1): 6-10 Ashry N.A. and Mohamed H.I. (2011). Impact of secondary metabolites and related enzymes in flax resistance and or susceptibility to powdery mildew. World J. Agri. Sci. 7: 78-85 Bhatia I.S., Uppal D.S. and Bajaj K.L. (1972). Studies on phenolic contents and resistant and susceptible varients of tomato (Lycopersicon esculentum) in relation to early blight disease. Indian Phytopathol. 25: 231-235 Bray H.G. and Thorpe W.V. (1954). Analysis of phenolic compounds of interest in metabolism. Methods in Biochemistry Analysis. 1: 27-52 Chopra B.L., Jhooty J.S. and Bajaj K.L. (1974). Biochemical differences between two varieties of watermelon resistant and susceptible to Alternaria cucumerina. Phytopath. Z. 79: 47-52 Cruickshank I.A.M. and Perrin D.R. (1964). In biochemistry of phenolic compounds. Ed. J. Harborne. Academic Press, New York and London. 511-514 Dahima V., Sharma S.S., Khokhar M.K. and Hooda K.S.(2014). Post-infectional biochemical changes in maize leaves affected by banded leaf and sheath blight disease. Indian Phytopath. 67(4): 370-373 Deore P.B., Sawant D.M. and Ilhe B.M. (2004). Comparative efficacy of Trichoderma spp. for the control of powdery mildew of cluster bean. Indian Journal Agric. Res. 38(3): 212-216 Ghosal T.K., Dutta S., Senapati S.K. and Deb D.C. (2004). Role of phenol contents in legume seeds and its effect on the biology of Collosbrchus chinensis. Ann Pl Protect Sci. 12: 442-444 Jain A.K. and Yadav H.S. (2003). Biochemical constituents of finger millet genotypes associated with resistant to blast caused Pyricularis grisea. Ann. Pl. Protec. Sci. 11: 70-74 Jiang Y.F., Cheng W.D., Qin L.Q., Tan X.J., Zhou J.G., Xie H.X., Chen L. (2009). Physiological mechanism of exogenous salicylic acid induced resistance to sheath blight of maize (Zea mays L.). Guangxi Agri. Sci. 40: 242-245 Joshi U.N., Gupta P.P., Gupta V. and Kumar S. (2004). Biochemical factors in cluster bean that impart Alternaria blight resistance. J. Mycol. Pl. Pathol. 34(2): 581-583 Joshi U.N., Gupta P.P. and Singh J.V. (2003). Biochemical parameters involved in resistance against root rot of cluster bean. Advances in Arid Legumes Research. Pp. 405-411 Kamble B.R. and Gangawane L.V. (1987). Biochemical changes in groundnut at influenced by fungi. Seed research. 15: 106-108 Katewa S.S., Caudhary B.L. and Jain A. (2004). Folk herbal medicine from tribal areas of Rajasthan, India. J. Ethano Pharmacol. 92: 41-46 Khare C.P. (2004). Indian Herbal Remedies. Springer, New York. 171-172 Lee J.T., Connor-Appleton S., Haq A.U., Bailey C.A. and Cartwright A.L. (2004). Quantitative measurement of negligible trypsin inhibitor activity and nutrient analysis of guar meal fractions. J. Agric. Food Chem. 52: 6492-6495 Liu Y., Wang R., Hua L.M. and Xie Z.J. (2012). Effects of N applied rate on leaf senescence and protective enzyme system at later growth stage of maize. J. Maize Sci. 20:124-127 Marmit S.K., Meena V.P. and Sharma S.L. (2008). Quantitative estimation of phenolics and related enzymes in insect induced leaf galls of Mangifera indica. Annals of Plant Protection Sciences. 16(2): 306-308 Meena A.K., Godara S.L. and Gangopadhyay S. (2010). Efficacy of fungicides and plant extracts against Alternaria blight of cluster bean. J. Mycol. Pl. Pathol. 40(2): 272-275 Mishra S. (2008). Indian guar gum exports up on industry demand. Reuters, April 28, 2008 Nemec S. (1978). Symptomatology and histopathology of fibrous roots of rough lemon (Citrus limon) infected with Fusarium solani. Mycopathologia. 63(1): 35-40 Parashar A. and Lodha P. (2007). Phenolics estimation in Foeniculum vulgare infected with Ramularia blight. Ann. Plant Prot. Sci. 15: 396-398 Pareek V. and Varma R. (2014). Phytopathological effects and disease transmission inCluster bean seeds grown in Rajasthan. Indian Journal of Plant Sciences. 3(2): 26-30 Patel D.S., Patel S.I. and Desai A.G. (2002). Cluster bean varietal reaction to wilt disease caused by Neocosmospora vasinfecta E.F. Smith. J. Mycol. Pl. Pathol. 32(1): 120-121 Rao C.N. and Panwar V.P.S. (2001). Biochemical plant factors affecting resistance to Atherigona spp. in maize. Ann. Pl. Protec. Sci. 9: 37-41 Sadda N. (2012). Studies on anthracnose and root rot disease of smooth gourd (Luffa cylindrica) and rough gourd (Luffa acutangula) grown in Kota district of Rajasthan. Ph.D. Thesis, University of Kota, Kota, Rajasthan. Saharan M.S., Saharan G.S., Gupta P.P. and Joshi U.N. (2001). Phenolic compounds and oxidative enzymes in cluster bean leaves in relation to Alternaria blight severity. Acta Phytopathologica et Entomologica Hungarica. 36(3/4): 237-242 Sharma P. and Gummagolmath K.C. (2012). Reforming guar industry in India: Issues and strategies. Agricultural Economics Research Review. 25(1): 37-38 Shinshi H. and Noguchi M. (1975). Relationship between peroxidase, IAA-oxidase and polyphenol oxidase. Phytochemistry. 14: 1255-1258 Singh A. and Varma R. (2010). Quantification of total phenolics and related enzymes in Fusarium wilt infected Lens culinaris Medic. Indian J. Appl. and Pure Biology. 25(2): 269-272 Singh H.V. (2004). Biochemical transformation in Brassica spp. due to peronospora parasitica infection. Ann. Pl. Protec. Sci. 12: 301-304 Singh P.J., Nagra P. and Mehrotra R.S. (1982). Relation between Phenol and Rhizoctonia bataticola infection in grain. Indian Journal of Mycology and Plant Pathology. 12: 46-48 Singh S.K. and Srivastava H.P. (1988). Phenolic changes in moth bean [Vigna aconintifolia (Jacq.)Marechal] during Macrophomina phaseolina infection. Indian J. Mycol. Pl. Pathol. 18: 314-316 Varma R. (2003). Biochemical analysis of soybean (Glycine max) seeds and seedlings infected with Rhizoctonia bataticola. Indian J. of Applied and Pure Biol. 18(1): 67-71