Physiology of Resistant Isolates of *Fusarium Udum*, Causal Organism of Wilt of Pigeon Pea

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Abstract:

Effect of various sources of carbon, nitrogen, phosphorus, sulphate, salts, micronutrients, vitamins and amino acids on the growth of Fusarium udum was carried out by incorporating them in Czapek Dox Agar medium. Resistant isolate of Fusarium udum which was determined by taking the sensitivity test of Fusarium udum collected from various localities of Maharashtra and Karnataka were selected for this experiment. Plates without any source served as control.

Key words: *Amino acids, Czapek Dox Agar medium, carbon, Fusarium udum, micronutrients, nitrogen, phosphorus, sulphate, salts, vitamins.*

Introduction:

Pigeon pea (*Cajanus cajan* (L.) Huth. a member belonging to family Fabaceae is one of the most essential leguminous food crop cultivated in tropical and subtropical countries like, Madagascar, India, Myanmar, Philippines, Australia. India, Myanmar, Malawi, Tanzania and Kenya are the top 5 producers of this crop. Amongst them India holds a major contribution of 90% of total world production. India engages an area of 3.85 million hectare with an annual production of 2.68 million tonnes (Anonymous, 2010). The plant helps in re-establishing soil productivity by atmospheric nitrogen fixation (Reddy et al., 1993).

Pigeon pea is a commercially important neutraceutical crop as it contains high level of amino acids like methionine, lysine tryptophan, vitamin B and proteins. The content of protein in seeds is almost similar to Soybean (*Glycine max*) which ranges from 21-28 % (Phatak et al., 1993). Inspite of this, *Cajanus cajan* is affected by various serious diseases and leads to heavy destruction. Pigeon pea is bombarded by numerous bacteria, viruses, fungi but amongst them just a few of them cause a negative impact on the plant. The wilt caused by *Fusarium udum Butler*, is the most destructive disease (Kannaiyan et al., 1985). Genus Fusarium account to the most significant group of ascomycetous fungi, whose members are liable for enormous economic loss due to depletion in yield, quality and quantity of pea (Nelson et al., 1983; Leslie and Summerell, 2006). Many members of Fusarium produce type A and B trichothecene mycotoxins that cause toxicosis in humans and animals (Mali et al., 2015). Several *Fusarium* species cause catastrophic diseases on cereal grains (White, 1980; Parry et al., 1995; Nyvall et al., 1999; Goswami and Kistler, 2004), some are responsible for vascular wilts or root rots on many important vegetable, ornamental and field crops (Kraft et al., 1981; Linderman, 1981) while cankers are produced by others on soft and hardwood trees (Bloomberg, 1981; Dwinell et al., 1981, 2001; Wingfield et al., 2008).

Material and Method:

Fifteen isolates of infected pigeon pea plants were collected from Kolhapur, Sangli districts of Maharashtra and Dharwad, Vijapura (Bijapur) and, Belgavi (Belgaum) districts of Karnataka. The infected plant materials were brought to the laboratory in clean polythene bags, they were cut into small pieces (0.5-1.0cm length) along the symptomatic region of stem, root and leaves, they were subsequently surface sterilized by sequential dipping in 70% ethanol for 30 sec and in 0.1% HgCl2 for 1 min and were later rinsed in sterilized distilled water, and then cultured on Czapek Dox agar (CDA) amended with 25 mg/l of streptomycin.

Plates were incubated at 25± 2°C for 6 days. The plates were observed for fungal outgrowth through the symptomatic parts of plants. After a period of 5-6 days white cottony fungal mass was observed. On the basis of visual morphological and microscopic characters the fungal isolate was identified as

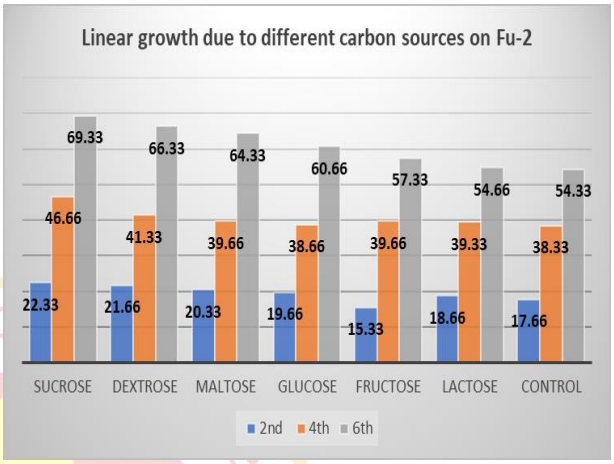
Fusarium udum (Butler). *Fusarium udum* was consistently isolated from infected tissues which were purified by single-spore culture method. The sensitivity of *Fusarium udum* was carried out by using Food Poisoning Technique (Dekker and Gielink, 1979) by deploying various concentrations of benomyl a systemic benzimidazole fungicide. The treatment was carried out by preparing benomyl dilutions from 1000 µg/ml stock solution by dissolving it in sterilized distilled water and then mixed in autoclaved Czapek Dox Agar (CDA). The mixture was prepared in proportion of 1:1 and final volume was made up to 30 ml. The media containing Benomyl solution of various concentrations was poured into Petri plates until solidification of media. Pure actively growing fungal mycelium was transferred on the solidified culture media plates by cutting 8 mm diameter discs. These plates were then incubated at 28-30°C in dark and then continuous growth was measured after various time intervals. A plate without benomyl was served as control. For in-vitro experiment, the work was carried out in triplicates. After determining Minimum Inhibitory Concentration (MIC) of benomyl effects of different sources on the development of benomyl resistance was studied in continuous, alternate and mixed pattern along with different fungicide for in vitro experiments.

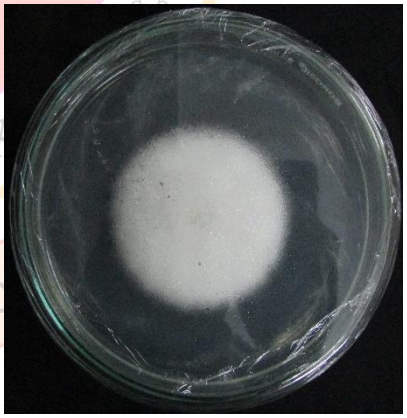
Result and Discussion:

Carbohydrate nutrition

Different carbohydrate sources like sucrose, fructose, dextrose, maltose, lactose and glucose were amended in Czapek Dox agar at 3% and the linear mycelial growth of the resistant isolate Fu- 2 was recorded. Observations showed that sugars are very much necessary for the growth of both sensitive and resistant isolates. There was maximum increase in the growth of both the isolates over the control. It was found that the resistant isolate's growth rate was higher in comparison with the sensitive isolate. The sensitive and resistant isolate showed a very good rate of growth on sucrose then followed by dextrose, maltose, glucose, fructose and lactose.

Graph 1. Effect of Different carbon sources on the linear growth (mm) of *Fusarium udum* resistant isolate Fu-2 on Czapek Dox agar.





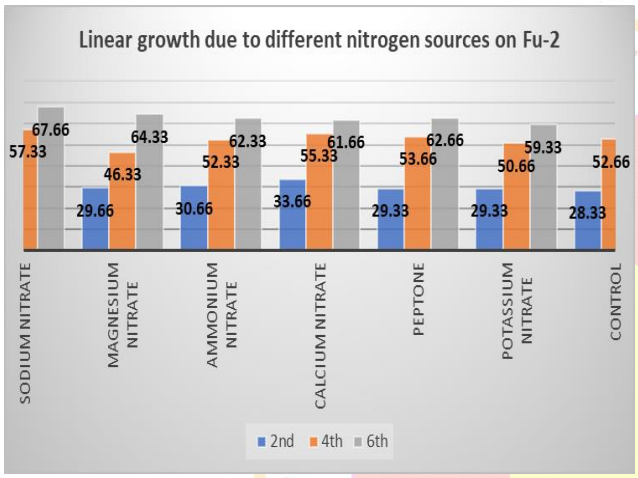
Nitrogen nutrition

Various nitrogen sources were utilised to check the effect of nitrogen on the growth of resistant isolate Fu – 2 of *Fusarium udum*. Different nitrogen sources like ammonium, potassium, sodium, magnesium, calcium nitrates and peptone were utilised at 0.2%.

It was observed that there was variation in the growth of both sensitive as well as resistant isolates of various nitrogen sources and in between different incubation periods. The radial mycelial growth of

resistant isolate Fu – 2 was found to be good. Amongst the nitrogen sources provided source of sodium nitrate showed maximum growth followed by calcium nitrate, ammonium nitrate, potassium nitrate, magnesium nitrate and peptone on the resistant isolate Fu – 2

Graph 2. Effect of Different Nitrogen sources on the linear growth (mm) of *Fusarium udum* Fu-2 resistant isolate on Czapek Dox agar with benomyl.

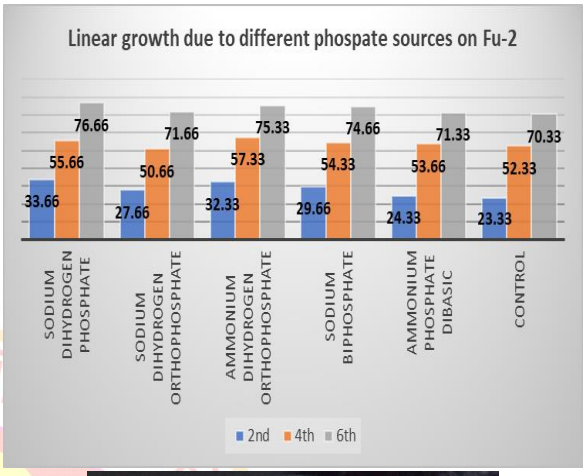


Phosphate nutrition

Various phosphate sources were utilised to check the effect on the development of resistant isolate Fu – 2 of *Fusarium udum*. Different phosphate sources like ammonium phosphate dibasic, sodium dihydrogen phosphate, ammonium dihydrogen orthophosphate, sodium dihydrogen orthophosphate and sodium biphosphate were utilised in the study at 0.1mg. The radial mycelial growth of resistant isolate Fu – 2 was found to be good in.

Significant variation was observed on the growth of *Fusarium udum* isolate at different periods of incubation and between various phosphate sources. A good response of growth was achieved on sodium dihydrogen phosphate followed by sodium dihydrogen orthophosphate, sodium biphosphate, ammonium dihydrogen orthophosphate and ammonium phosphate dibasic.

Graph 3. Effect of Different Phosphate sources on the linear growth (mm) of *Fusarium udum* resistant isolate on Czapek Dox agar with benomyl.

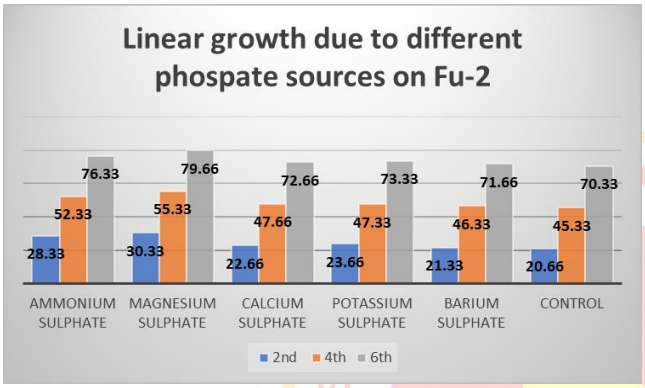


Sulphate nutrition

The effect of how, various sulphate nutrition affects the growth of resistant isolate Fu -2 studied by amending different sulphate sources in Czapek Dox agar at 0.05 mg. Various sulphate sources like calcium sulphate, magnesium sulphate, iron sulphate, ammonium sulphate, barium sulphate, potassium sulphate and zinc sulphate were utilised. It was seen that there was a significant difference in the development resistant isolate Fu- 2 of *Fusarium udum*. The growth of resistant isolate Fu - 2 was found to be more. It was seen that magnesium

sulphate helped in good development of *Fusarium udum* followed by ammonium sulphate, calcium sulphate, magnesium sulphate, potassium sulphate and barium sulphate.

Graph 4. Effect of Different Sulphate sources on the linear growth (mm) of *Fusarium udum* resistant isolate on Czapek Dox agar with benomyl.



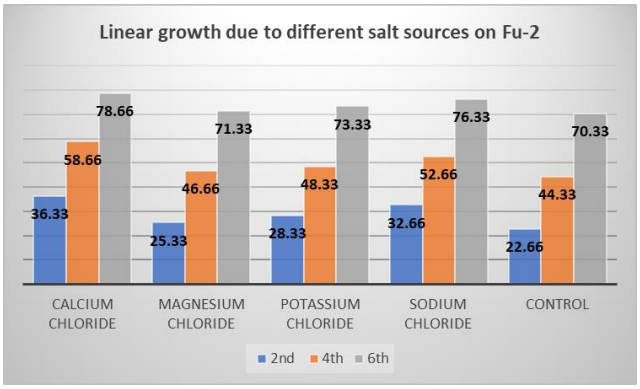
Effect of salts.

In total 4 different salts were selected to see the effect on resistant and sensitive isolates of *Fusarium udum*. For the study sodium chloride, calcium chloride, potassium chloride and magnesium chloride were used. They were incorporated at 0.05 mg in Czapek Dox agar medium. Magnesium chloride was found to inhibit the growth of both the isolates.

Growth of resistant isolate Fu- 2 was found to be more luxuriant. It was found that mixture of benomyl along with calcium chloride proved to provide good growth in the resistant and sensitive isolate of *Fusarium udum* followed by sodium chloride, potassium chloride and magnesium chloride.

Graph 5. Effect of different salts sources on the linear growth (mm) of *Fusarium udum* resistant isolate on Czapek Dox agar with benomyl.

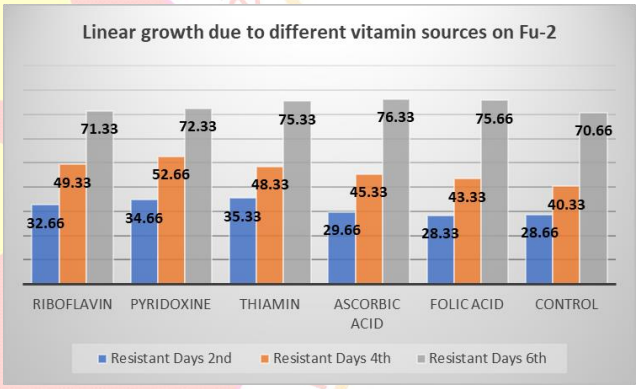
Vitamin nutrition



Effect of vitamins was tested on the growth of the resistant isolate Fu- 2. It was mixed in Czapek Dox agar medium at 0.01 mg. It was observed that there was a significant difference on the growth of resistant in the incubation period. Growth of resistant isolate was found to be higher. Plate without any source of vitamin was served as control.

Various vitamins used during the study were riboflavin, ascorbic acid, thiamin, pyridoxine and folic acid. Among all vitamin sources used, ascorbic acid showed a good growth for the resistant isolate.

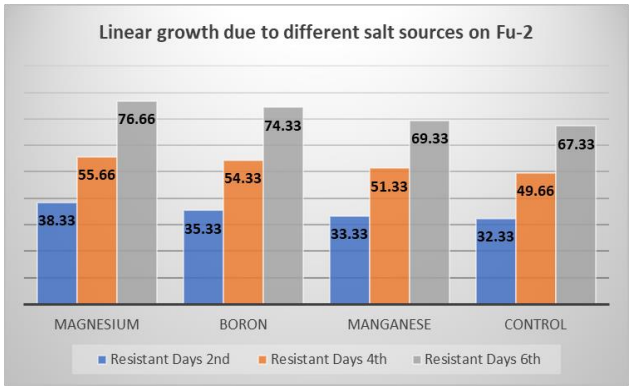
Graph 6. Effect of different vitamins on the linear growth (mm) of *Fusarium udum* resistant isolate on Czapek Dox agar with benomyl.



Effect of Micronutrients

Effect of different micronutrients was tested on the growth of resistant isolate Fu- 2. It was mixed in Czapek Dox agar medium at 0.01 mg. Magnesium, boron and manganese were used to study the effect when amended with Czapek Dox agar medium. Growth of resistant isolate was found to be higher. Plate without any source of micronutrient was served as control. Magnesium source proved to be good for growth of the isolate. Manganese and boron inhibited the growth of the resistant fungal isolate.

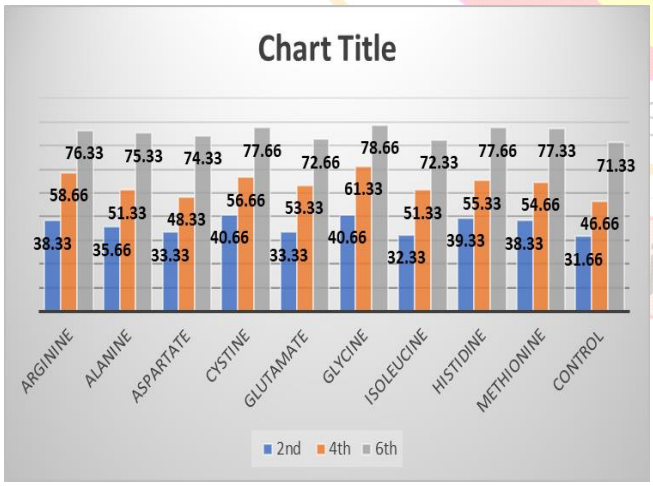
Graph 7. Effect of different micronutrients on the linear growth (mm) of *Fusarium udum* resistant isolate on Czapek Dox agar with benomyl.



Amino acid nutrition

Various amino acid nutrition were utilised for the study viz. Arginine, Alanine, Aspartate, Cystine, Glutamate, Glycine, Isoleucine, Histidine and Methionine. A significant variation in the growth was observed in resistant isolate Fu -2 . It was mixed in Czapek Dox agar medium at 0.02 mg. Growth of resistant isolate was found to be higher. Plate without any source of amino acid nutrition was served as control. It was interesting to note that almost all the amino acid nutrition showed a good growth on the isolate only isoleucine showed certain amount of inhibition.

Graph 8. Effect of different amino acids on the linear growth (mm) of *Fusarium udum* resistant isolate on Czapek Dox agar with benomyl.



Conclusion:

Various agrochemicals which are being used by farmers were implied to study their effect to control wilt such as, various fungicides, herbicides, insecticides, antibiotics, micronutrients, salts, fertilizers etc. There are chances that these agro chemicals may influence the development of Benomyl resistance in fungal pathogen hence, both *in vitro* and *in vivo* experiments were conducted.

The foresaid sources show a varying result while treating the resistant isolate of *Fusarium udum* i. e, F-2 in this case. These sources directly or indirectly increase the resistance in the pathogen.

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References

1. **Anonymous.** (2010). Agriculture statistics at a glance, Department of Agriculture and Cooperation, Ministry of Agriculture, Govt. of India, New Delhi, 108-109.
2. **Reddy, M. V. Nene, Y. L, Kannaiyan, J. Raju, T. N. Saka, V. N. Davor, A. T. Songa, W. P** and**. Omanga, P.** (1993). "Pigeonpea lines resistant to wilt in Kenya and Malawi", *International Pigeonpea News letter*, Vol 6, 1990, p. 34.
3. **Phatak, S. C., Nadimpalli, R. G., Tiwari S. C.** and**. Bharadwaj**. **H. L** (1993). Pigeon peas: potential new crop for the southeastern United States. In: Janick J. and Simon J. E, editors, *New Crops*. Wiley, Newyork. p. 597-599. http://hort.purdue.edu/newcrop/proceedings1993/v2-597.html (accessed 24 July 2012).
4. **Kannaiyan, J., Nene. Y. L** and **Raju, T. N.** (1985). Host Specificity of pigeonpea wilt pathogen *Fusarium udum*. *Indian Phytopath*. 38: 553.
5. **Nelson PE, Toussoun TA, and Marasas WFO** (1983) Fusarium species: an illustrated manual for identification. The Pennsylvania State University Press, University Park
6. **J. F. Leslie, B.A. Summerell (2006)**. Fusarium laboratory workshops- A recent history Mycotoxin Research Vol. 22, No. 2, 73-74.
7. **Mali A M, Patil V B, Ade A B, Chavan N S And Kamble S S** (2015) First Report Of Fusarium Sp. FIESC_17 On *Cucumis*

trigonus In India. Plant Disease. 10.1094/PDIS-09-14-0881-PDN. http://dx.doi.org/10.1094/PDIS-09-14-0881-PDN.

8. **White D. G**. (1980). Compendium of corn diseases. APS, St. Paul, Minnesota.
9. **Parry, D., Jenkinson, P., McLeod, L.** (1995) Fusarium ear blight (scab) in small grain cereals—a review. Plant pathology 44:207-238.
10. **R. F. Nyvall, J. A. Percich and C. J. Mirocha.** (1999).Fusarium head blight of cultivated and natural wild rice (*Zizania palustris*) in Minnesota caused by *Fusarium graminearum* and associated Fusarium spp. Plant Disease 83: 159-164.
11. R. S. GOSWAMI, H. C KISTLER (1994): Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Path Vol 5, 2004, 515-525.
12. J. M. Kraft, D. W. Burke and W. A. Haglund. (1981). Fusarium diseases of beans, peas and lentils. In: Fusarium: Diseases, Biology and Taxonomy (eds. Nelson PE, Toussoun TA and Cook RJ). The Pennsylvania State University Press, University Park: 142-156
13. R. G. Linderman. (1981). Fusarium diseases of flowering bulb crops. In Fusarium: Diseases, Biology and Taxonomy (eds. Nelson PE, Toussoun TA and Cook R J). The Pennsylvania State University Press,University Park: 129-141.
14. W.J. Bloomberg. (1981). Diseases caused by Fusarium in forest nurseries. In: Fusarium: Diseases, Biology and Taxonomy (eds. Nelson PE, Toussoun TA and Cook R J). The Pennsylvania State University Press, University Park: 178-187.
15. L. D. Dwinell, E.G. Kuhlman and G.M. Blakeslee (1981). Pitch cancer of southern pines. In: Fusarium: Diseases, Biology and Taxonomy (eds. Nelson PE,Toussoun TA and Cook R J). The Pennsylvania State University Press, University Park: 188-194
16. L. D. Dwinell, S.W. Fraedrich and D. Adams. (2001).Diseases of pines caused by the pitch canker fungus. In: Fusarium: Paul E. Nelson Memorial Symposium (eds. B.A. Summerell, J.F. Leslie, D. Backhouse, W.L. Bryden and L.W. Burgess). APS Press, St.Paul, Minnesota: 225-232.
17. M. J. Wingfield, A. Hammerbacher, R. J. Ganley, E. T. Steenkamp, T. R. Gordon, B. D. Wingfield and T. A.Coutinho (2008). Pitch canker caused by Fusarium circinatum - a growing threat to pine plantations and forests worldwide. Australasian.
18. Dekker, J. and Gielink, A. J. (1979). Acquired resistance to pimaricin in Cladosprium f. sp. Narcissi associated with decreased virulence. Neth. J. Pl. Pathol. 85: 67-73