

Evaluation of Botanicals Against *Fusarium udum* under *in vitro* Conditions

B. Deepak Reddy^{1*}, Birendra Kumar², Sangita Sahini³, M. Sunitha⁴ and K. Sai Krishna⁵

¹Ph.D. Research Scholar, Department of Plant Pathology,

Dr. Rajendra Prasad Central Agricultural University, Pusa, 848-125, Samastipur, (Bihar), India.

²Professor, Department of Plant Pathology,

Dr. Rajendra Prasad Central Agricultural University, Pusa, 848-125, Samastipur, (Bihar), India.

³Assistant Professor, Department of Plant Pathology,

Dr. Rajendra Prasad Central Agricultural University, Pusa, 848-125, Samastipur, (Bihar), India.

⁴M.Sc. Research Scholar, Department of Plant Pathology,

Dr. Rajendra Prasad Central Agricultural University, Pusa, 848-125, Samastipur, (Bihar), India.

⁵M.Sc. Research Scholar, Department of Basic Sciences and Languages,

Dr. Rajendra Prasad Central Agricultural University, Pusa, 848-125, Samastipur, (Bihar), India.

(Corresponding author: B. Deepak Reddy*)

(Received 16 July 2021, Accepted 21 September, 2021)

(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT. Pigeonpea is one of the staple pulse crop in the world. Yield of pigeonpea is affected by various biotic stresses, among them wilt is the most important disease and it is causing severe yield losses. Use of botanical extracts for the management of fungal diseases in plants is considered as a substitute to synthetic fungicides, due to their less negative effects on the human and environment health hazard or implications. For management of pigeonpea wilt seven botanicals were tested at 10%, 15%, 20% and 30%. Among all the botanicals garlic and turmeric exhibited highest per cent inhibition. At 144 hours the per cent inhibition was 89.63% and turmeric was 87.03%.

Keywords. Pigeonpea wilt, Management, Botanicals.

INTRODUCTION

The pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important pulse crop in India belonging to the family Fabaceae. Globally pigeonpea is cultivated in an area of 4.7 million ha with 3.69 million tonnes annual production (Ravindra *et al.*, 2018). In India, pigeonpea is grown on 45 lakh hectares, with annual production of 42 million tons and yielded 960kg/ha during the year 2017-2018 (Pulses Revolution Bulletin 20119-2020). Though several factors are known to affect pigeonpea cultivation, the most important being the diseases. Some of the important diseases are *Fusarium* wilt, *Phytophthora* blight, *Cercospora* leaf spot, collar rot, dry root rot, *Alternaria* leaf spot, powdery mildew, sterility mosaic and phyllody. Incidentally, only a few of them causes economic losses in India (Kannaiyan *et al.*, 1984). Among the diseases, *Fusarium* wilt caused by *Fusarium udum* is the most important soil borne disease and was first reported from Bihar state in India (Butler, 1906). In India, it is the most serious problem all over the pigeonpea growing states especially in U.P., M.P., Bihar and Maharashtra (Rajvanshi *et al.*, 2018). The yield loss of pigeonpea depends on the stage at which the plants wilt and it can approach 100, 67 and 30 per cent when wilt occurs at pre-pod, maturity and pre-harvest stages, respectively (Kannaiyan and Nene, 1981) and sometimes it causes losses up to 100% loss in grain yield (Okior, 2002). The fungus is primarily a soil

borne facultative parasite and enters the host through fine roots and subsequently colonizes in different plant parts (Khune, 1990).

In discriminate use of the chemicals has led to development of fungicide resistance strain (Lyon *et al.*, 1995; Okigbo, 2004) and these chemical fungicides are not readily biodegradable; tend to persist for years in the environment and few fungi have developed resistance to them and more importantly, environmental pollution, posing a potential risk to animal and human health (Kumar, *et al.*, 2021). Plant extracts are emerging as eco-friendly way to manage pathogen. So, the objective of this investigation was to evaluate the potential of plant extracts, bio agents and fungicides against *Fusarium udum*.

MATERIALS AND METHODS

Isolation of pathogen: Pigeonpea plants exhibiting typical symptoms of *Fusarium wilt* were collected from AICRP pigeonpea wilt sick plot Dholi. Tissue segment technique were followed for isolation pathogen of the diseased samples. Diseased plants collar portion were split longitudinally with sterile knife and brown discoloured vascular tissues of plants were into cut into small bits. Surface sterilization were done by dropping diseased plant pits in sodium hypochlorite solution (1%) for one minute, cleaned with 3 changes of sterile distilled water, dried on blotting paper and then moved aseptically on to PDA medium @ 4 bits/Petri plate and

incubated in an incubator at $25 \pm 2^{\circ}\text{C}$. Single spore isolation technique was used for obtaining pure and homogenous cultures. Spore suspensions of *Fusarium udum* were prepared in test tubes with sterile distilled water and concentration were adjusted to 4-5 spores for per field of microscope. In sterilized Petri plates one ml of spore suspension was added, into which 2% water agar medium was poured. For getting uniform spread of spores in the medium, plates were rotated gently. Isolated single spores were located after twenty four hours and marked by observing the plates through microscope. Single spores were picked along with medium, transferred to PDA slants under aseptic conditions and incubate at $25 \pm 2^{\circ}\text{C}$ in an incubator.

Efficacy of plant extract against Fusarium wilt in vitro: In order to find out the efficacy of various plant extract against the *Fusarium udum* plant extracts viz., Turmeric (Rhizome), Black pepper (Pepper), Garlic (Bulb), Bitter guard (Leaf), Neem (Leaf), Papaya (Leaf) and Aloe Vera, were collected and washed thoroughly in clean water. 100 g of each washed plant material was grinded in Pestle and Mortar by adding equal amount (100 ml) of sterilized water (1:1 V/W) and heated at 80°C for 10 minutes in hot water bath. The materials was filtered through double layered muslin cloth followed by filtering through sterilized What man No. 1 filter paper and treated as standard plant extract (100%). The stock solution 10%, 15%, 20% and 30% concentration were made by adding 90, 85, 80 and 70 ml of sterilized PDA media. To study the inhibitory effect of botanicals on mycelial growth of *F. udum*, 10%, 15%, 20% and 30% concentration were used by applying poison food techniques under in vitro condition. Five mm discs of 7 days old culture of *F. udum* were cut with sterilized cork borer and placed in the centre of plant extract amended Petri plates. The control Petri plates having PDA alone were inoculated in the same manner. These Petri plates were incubated at $25 \pm 2^{\circ}\text{C}$. The observations were recorded on radial growth were recorded.

Per cent inhibition of mycelial growth will be estimated following the formula given by Vincent, (1927).

$$I = [(C - T)/C] \times 100$$

Where, I is the percent inhibition; C is the colony radius in control plate and T is the radial growth of the pathogen in the presence of plant extracts.

RESULTS

The efficacy of eight botanicals were tested at 10%, 15%, 20% and 30% concentrations and growth rate was recorded at twenty four hours interval from 72 hours to 144 hours. At seventy two hours the percent inhibition in garlic was 66.67%, 73.33%, 73.55% and 79.9%, in turmeric 77.77%, 79.99%, 83.33% and 83.33, neem, 25.5%, 25.5%, 33.3% and 33.3%, black pepper 18.89%, 25.55%, 25.55% and 25.55%, papaya 16.6%, 16.6%, 16.6% and 16.6% , bitter guard 16.6%, 16.6%, 16.6% and 16.6 alove vera was 33.3%, 33.3%, 33.3% and 66.67% at 10%, 15%, 20% and 30% respectively. Similarly at ninety six hours the percent inhibition in garlic were 72.66%, 80.00%, 81.33 and 84.00, while in turmeric was 80.00%, 80.00%, 80.00%, and 80.00% neem 34.66%, 36.6%, 40.00% and 53.33, black pepper 33.3%, 36.6%, 40.00% and 40.00%, papaya 16.6%, 16.6%, 16.6% and 16.6%, bitter guard 10.0%, 13.3%, 16.6% and 20.0% and alove vera 20.0%, 30.0%, 33.3% and 60% at all 10%, 15%, 20% and 30%, respectively. At one twenty hours the percent inhibition in garlic was 74.28, 83.33, 85.7 and 87.61, in turmeric 78.57, 83.33, 85.71 and 85.71, neem 36.66%, 45.2%, 47.6% and 61.43%, black pepper 42.86%, 47.62%, 50.00% and 52.38%, papaya 20.95%, 23.81%, 28.57 and 28.57, bitter guard 23.81%, 26.19%, 26.19% and 28.17, alove vera 26.19%, 40.47%, 42.86% and 71.43 at 10%, 15%, 20% and 30% concentrations respectively. While at one forty hours the percent inhibition in garlic were 77.40%, 83.88%, 88.89% and 89.69%, in turmeric 85.18%, 85.18%, 85.18%, and 87.03%, neem 46.29%, 46.29%, 53.70% and 64.81, black pepper 51.85%, 53.33%, 55.56% and 64.81, papaya 33.33%, 34.44%, 34.8% and 35.8%, bitter guard 31.48%, 35.18%, 42.59% and 44.44% and alove vera 35.92%, 46.29%, 48.14% and 70.37% at 10%, 15%, 20% and 30% concentrations respectively.

Table 1: Per cent inhibition of botanicals at 72 hours.

Treatments	Radial growth of pathogen at 72 hours				Percent inhibition at 72 hours			
	10	15	20	30	10	15	20	30
Garlic	1.00	0.80	0.79	0.60	66.67	73.3	73.55	79.9
Neem	2.23	2.16	2.00	2.00	25.55	27.7	33.33	33.33
Black pepper	2.33	2.00	2.00	2.00	18.89	25.55	33.33	33.33
Papaya	3.00	3.00	3.00	3.00	16.67	16.6	16.67	16.67
Turmeric	0.83	0.76	0.66	0.50	77.77	79.9	83.33	83.33
Bitter Gaurd	3.00	3.00	3.00	3.00	16.67	16.67	16.67	16.67
Alove vera	2.00	2.00	2.00	1.00	33.3	33.33	33.3	66.67
Control	3.00	3.00	3.00	3.00	0	0	0	0
Factors	C.d @5%							
Factor(A)					2.68			
Factor(B)					1.89			
Factor(A X B)					5.36			

Table 2. Per cent inhibition of botanicals at 96 hours.

Treatments	Radial growth of pathogen at 96 hours				Percent inhibition at 96 hours			
	10	15	20	30	10	15	20	30
Garlic	1.367	1.000	0.933	0.800	72.66	80.00	81.33	84.00
Neem	3.267	3.167	3.000	2.333	34.66	36.6	40.00	53.33
Black pepper	3.333	3.167	3.000	3.000	33.3	36.66	40.00	40.00
Papaya	4.400	4.500	4.300	4.000	16.00	16.0	16.00	16.00
Turmeric	1.000	1.000	1.000	1.000	80.0	80.00	80.00	80.00
Bitter Gaurd	4.500	4.333	4.167	4.000	10.00	13.33	16.6	20.0
Alove vera	4.000	3.500	3.333	2.000	20.00	30.00	33.3	60.00
Control	5.000	5.000	5.000	5.000	0.0	0.0	0.0	0.0
Factors	C.d @5%							
Factor (A)	0.13				2.65			
Factor (B)	0.09				1.87			
Factor(A × B)	0.26				5.30			

Table 3: Per cent inhibition of botanicals at 120 hours.

Treatments	Radial growth of pathogen at 120 hours				Percent inhibition at 120 hours			
	10	15	20	30	10	15	20	30
Garlic	1.800	1.167	1.000	0.867	74.28	83.33	85.7	87.61
Neem	4.433	3.833	3.667	2.700	36.66	45.2	47.6	61.43
Black pepper	4.000	3.667	3.500	3.333	42.86	47.62	50.00	52.38
Papaya	5.533	5.333	5.000	5.000	20.95	23.81	28.57	28.57
Turmeric	1.500	1.167	1.000	1.000	78.57	83.33	85.71	85.71
Bitter Gaurd	5.333	5.167	5.167	5.000	23.81	26.19	26.19	28.57
Alove vera	5.167	4.167	4.000	2.000	26.19	40.47	42.86	71.43
Control	7.000	7.000	7.000	7.000	0.00	0.00	0.00	0.00
Factors	C.d @5%							
Factor (A)	0.19				2.754			
Factor (B)	0.13				1.94			
Factor(A × B)	0.38				5.50			

Table 4: Per cent inhibition of botanicals at 144 hours.

Treatments	Radial growth of pathogen at 144 hours				Percent inhibition at 144 hours			
	10	15	20	30	10	15	20	30
Garlic	2.033	1.450	1.000	0.933	77.40	83.88	88.89	89.63
Neem	4.833	4.833	4.167	3.167	46.29	46.29	53.70	64.81
Black pepper	4.333	4.200	4.000	3.767	51.85	53.33	55.56	58.15
Papaya	6.000	5.900	5.867	5.833	33.33	34.44	34.8	35.18
Turmeric	1.667	1.333	1.333	1.167	81.48	85.18	85.18	87.03
Bitter Gaurd	6.167	5.833	5.167	5.000	31.48	35.18	42.59	44.44
Alove vera	5.767	4.833	4.667	2.667	35.92	46.29	48.14	70.37
Control	9.000	9.000	9.000	9.000	0.00	0.00	0.00	0.00
Factors	C.d @5%							
Factor (A)	0.20				2.23			
Factor (B)	0.14				1.58			
Factor(A × B)	0.40				4.47			

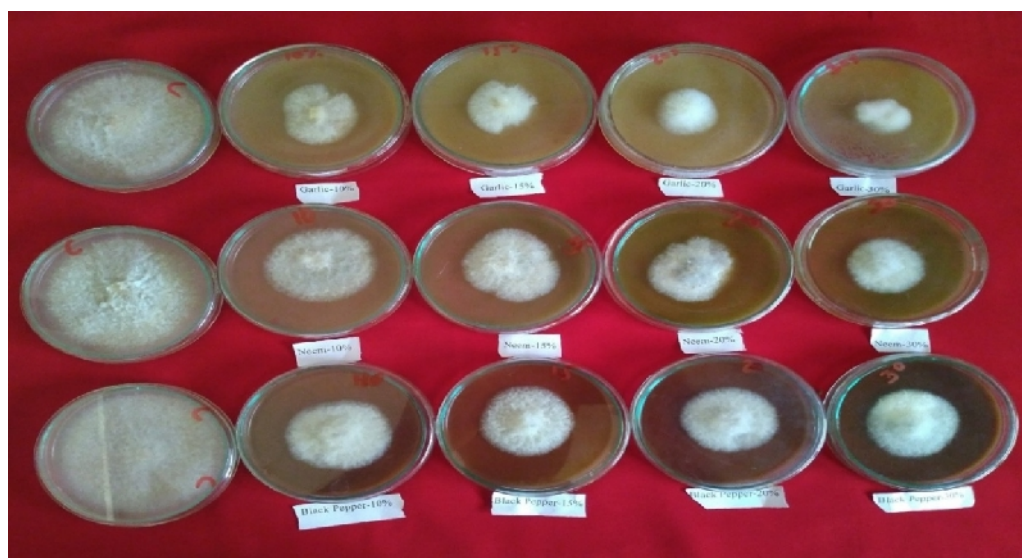


Plate 1: In vitro evaluation of botanicals against *Fusarium udum*.

DISCUSSION

Among all the botanicals garlic and turmeric exhibited highest per cent inhibition. At 144 hours the per cent inhibition was 89.63% and turmeric was 87.03%. The present results on evaluation of botanical against *Fusarium udum* were in conformity with the findings of several researchers. Kumar *et al.*, (2018) evaluated eight botanicals i.e., Neem, Garlic, Onion, Ginger, Marigold, Tulsi, Beal, Mehandi, Neem + Marigold, Garlic + Tulsi, Onion + Beal, Ginger + Mehandi against *Fusarium udum* and reported that inhibition per cent age of garlic were 76.87 to 83.22% at 5% concentration and 85.41 to 90.37% at 10% concentration. Ghante *et al.*, (2019) evaluated 12 phyto extracts against *Fusarium udum* under in vitro conditions. Among them garlic at 20% exhibited inhibition per cent of 84.44%. Similarly Chaudhary *et al.*, (2019) also tested seven botanicals at 5%, 10% and 15% concentrations. Among them garlic at 15% concentration exhibited 62.8% inhibition. Rao *et al.* (2020) reported that inhibition per cent of garlic at 10% were 73.75% and turmeric was 34.39% against *Fusarium oxysporum* causing wilt in tomato.

CONCLUSION

A total of seven botanicals were evaluated against *Fusarium udum* under in vitro conditions at 10%, 15%, 20% and 30%. In all the test concentrations garlic and neem exhibited highest per cent inhibition. Field experiments were needed for further confirmation.

Acknowledgement. Authors were thankful to Department of Plant Pathology & DR. RPCAU.

Conflict of Interest. None.

REFERENCES

Butler, E. J. (1906). The wilt disease of Pigeonpea and Pepper. *Agriculture Journal of India*, 1: 25-26.

Chaudhary, B., Kumar, S., & Kushawaha, S. K. (2019). Evaluation of plant extracts and fungicides against *Fusarium udum* causing pigeonpea wilt. *Chem. Sci. Rev. Lett.*, 8(32): 340-344.

FAOSTAT (2020). Food and agriculture organization of United Nations. Retrieved from <http://faostat.fao.org>

Ghante, P. H., Kanase, K. M., Markad, H. N., Suryawanshi, A. P., & Chavan (2019). In vitro efficacy of phyto- extracts against *Fusarium oxysporum f. sp. udum* causing wilt disease of pigeonpea. *Journal of Pharmacognosy and Phytochemistry*, 8(2): 19-21.

Kannaiyan, J., & Nene, Y. L. (1981). Influence of wilt at different growth stages on yield in pigeonpea. *Tropical pest management*, 27: 141.

Kannaiyan, J., Nene, Y. L., Reddy, M. V., Ryan, J. G., & Raju, T. N. (1984). Prevalence of pigeonpea diseases and associated crop losses in Asia, Africa and the Americas. *International Journal of Pest Management*. 30(1): 62-72.

Khune, R. (1990). Biological control of soil borne plant pathogens. *Indian J. of Mycol. Plant Pathology*, 17: 1-9.

Kumar, S., Singh, D., Yadav, K. J., Kumar, S., & Verma, K.S. (2017). Efficacy of Plant Extracts, Bio agents and Fungicides against *Fusarium udum* causing Pigeonpea wilt. *International Journal of Current Microbiology and Applied Sciences*, 6(9): 2652-2660.

Kumar, S., Meena N. L., Meena, N. K., Rohith M., & Deora, A. (2021). Management of *Fusarium oxysporum f. sp. Radices cucumerinum* causing root and stem rot of cucumber by In vitro evaluation of bio-efficacy of botanicals: A review. *The Pharma Innovation Journal*, 10(9): 1072-1075.

Lyon, G. D. T., Beglinski., & A.C. Newton. (1995). Novel disease control compound: the potential to immunis plants against infection. *Plant Pathology*, 44, 407-427.

Okigbo, R. N. (2004). A review of botanicals control methods for post-harvest yams (*Dioscorea spp.*) in storage in South Eastern Nigeria, *KMITL Sci. J.*, 4(1): 207- 215

Okior, M. A. (2002). Genetics of wilt resistance in pigeonpea. *Indian Journal of Genetics and Plant Breeding*, 62: 218-220.

Rajvanshi, N. K., Chandra, S., & Kumar, A. (2018). Evaluation of pigeonpea genotypes against *Fusarium udum* butler under artificial epiphytotic condition. *Journal of Pharmacognosy and Phytochemistry*, 4: 195-196.

Ravindra, M., Arsia, S. K., Jain, Y. K., & Mukesh, D. (2018). Compatibility of fungicides with *Trichoderma viridae* against *Fusarium* wilt caused by *Fusarium udum*. *International Journal of Agriculture Sciences*, 10(5): 5268-5271.

Rao, Y. H., Devi, S., Vemavarapu, V. V., & Chowdary, K. R. (2020). Effect of *Trichoderma spp.*, botanicals and fungicides against *Fusarium oxysporum*. *IJCS*, 8(5): 2115-2119.

How to cite this article: Reddy, B. D., Kumar, B., Sahini, S., Sunitha, M. and Krishna, K.S. (2021). Evaluation of Botanicals Against *Fusarium udum* under in vitro Conditions. *Biological Forum – An International Journal*, 13(3a): 495-498.