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Green approach for management of *Fusarium* wilt disease in pigeonpea using botanicals under *in vitro* condition

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ABSTRACT

Pigeonpea (*Cajanus cajan* (L.) Mill sp.) also known as tur or arhar in India is a valuable pulse crop predominantly cultivated in tropical areas and in India. Pigeopea having a significant place among the family fabaceae. The legume crop pigeonpea is kharif season crop and has wider adaptability and requires low input in cropping practice. In India, pigeonpea is the most important pulse crop after chickpea. Among a few variables known to influence pigeonpea development, the most significant is the effect of diseases like Cercospora leaf spot, Fusarium wilt, Phytopthara blight, dry root rot, Alternaria leaf spot, phyllody and sterility mosaic. It just so happens, a couple of them cause monetary misfortunes in India (Kannaiyan *et al*., 1984). Among the illnesses, Fusarium wilt, incited by *Fusarium udum*, is the main soil borne disease and was first revealed from Bihar state in Quite a while (Butler, 1906). For an ecofriendly and sustainable management of *Fusarium wilt*, the effectiveness of botanicals was tested *in vitro* at 5, 10, 15, and 20% concentrations against the pathogen by poisoned food technique. The botanical extract of ashoka leaves, eucalyptus leaves, garlic clove, marigold leaves, Tulsi leaves, neem leaves, dhatura leaves, turmeric rhizome,,ginger rhizome, onion bulb and moringa leaves were found effective in inhibition of *Fusarium udum*.

Key word: *Pigeonpea, Fusarium wilt*

Introduction

Pulse crop grown in a variety of countries worldwide among them pigeonpea [*Cajanus cajan* (L.) Mill sp.] is grown at large scale, (Behera *et al*., 2020 and Saxena *et al*., 2020), covers an area of 6.99 million ha worldwide, producing 5.93 million tonnes with yield of 852 kg/ha. In India this pulse crop is grown in the area of 45 Lakh ha and have an annual production of 42 Lakh tonnes, accounting for nearly 90 percent of global acreage and production

Pigeonpea was grown as a perennial legume in the semiarid tropics and was thought to have originated in India; however the centres of diversity for the genus- Cajanus were in India and Africa, so it is also thought to have originated in Africa. This crop is widely cultivated for a variety of purposes including pulse, animal fodder, legume nitrogen fixation, cover crop, soil conservation, stem as fuel wood, and others. Pigeonpea seeds are rich in protein (21 percent), carbohydrates, minerals, fibre, and essential amino acids as arginine, lysine, methionine, and (Orwa *et al.,* 2009). Pigeonpea seeds have 43% protein, 2% fat, 21% carbohydrate, a calorific value of 343 Cal/100g and minerals (Ca-130, Cu-1.1, P-367, K-1392, Na-17, Zn-2.8, Mg-183, Mn-1.8, Fe-5.2, and Se-8.1) mg/100g. Niacin, Vitamin A, Thiamin, Riboflavin, folate and pantothenic acid are other essential

components present in this crop.

Pigeonpea is widely cultivated in India as a low-input, rain-fed crop that has a direct impact on the financial and economic well-being, as well as the nutritional status of farmers in the country, and yields high profitable returns from each part of the plant (Sharma *et al.,* 2019). The crop represents about more than 70% being produced in India among 5% of total world legume production (Hillocks *et al*.2000). Pigeonpea production stagnation is a significant challenge in increasing pulse production. More than 100 pathogens, including bacteria, viruses, fungi, mycoplasma-like organisms and nematodes, attack the pigeonpea crop (Nene *et al*. 1996). The crop's susceptibility to various diseases (Fusarium wilt, Phytopthora blight, Dry root rot, Sterility mosaic disease, Alternaria leaf spot, Cercospora leaf spot, Powdery mildew and Phyllody) are the major constraints for increasing productivity. Fortunately, only a few of them, including wilt, cause losses conomically in pigeonpea crop (Kannaiyan *et al*., 1984). Fusarium wilt caused by *Fusarium udum* is the most serious disease of pigeonpea. It was first reported in Pusa, Bihar, India (Butler, 1906).

The Fungus- *Fusarium udum* have ability to survive in the soil saprophytically for up to eight years, causing disease with various symptoms such as inter-veinal chlorosis ,loss of turgidity, brown discoloration of xylem tissue, and a purple band on the stem increasing upwards from the base (Swamy, 2011). In one season, about 3 metres the fungus spreads through the soil, along roots. The yield losses to crop is determined by the stage at which it infects the plants, at pre-pod up to 100% losses, 67% at pre-harvest, and 30% at maturity (Kannaiyan and Nene, 1981), and under severe conditions can cause yield losses of up to 100% (Pande *et al .,* 2011; Okiror, 2002). Fusarium wilt is causing significant losses in pigeon pea production, with yield losses of up to 90% recorded (Datta *et al.,* 2013), thus adversely affecting on pigeonpea production.

Materials and Method

A field trial experiment was conducted at Research Farm, Tirhut College of Agriculture Dholi (Bihar) during the *kharif* season of 2021-22. The research farm is located above mean sea level at an elevation of 160 metres, at latitude 25.98^{o}N and longitude 85.6^{o}E. The area had a reasonably consistent topography and the soil texture and pH were silty loam and 8.5, respectively. The centre is having the 50 years old sick plot for wilt even though every year, chopped stem pieces of wilted plants of pigeonpea utilized for artificial inoculation of research plot at the time of final land preparation to maintain in the sickness of the plot. This method called wilt sick plot technique.

Observation of symptoms of wilt affected pigeonpea plants in fields

To investigate the symptoms presented in the field, naturally wilted infected plants were attentively monitored in the field. The wilting plant was examined for anomalies such as uprooted roots and broken roots and stems. To determine the presence of pathogens, a microscopic examination of aberrant parts and discolored splited root and stem was performed. The symptoms on wilted plants were documented after verifying the presence of the pathogen, *Fusarium sp*.

Pathogen isolation and purification

Plants exhibiting Fusarium wilt signs were pulled from the experimental field. Partly wilted plant roots were washed gently and thoroughly in running tap water. Affected portions of root were cut into 2 mm consisting of both diseased and healthy tissue. Selected cut parts splits open so that inner browning is seen. The water cleaned pieces were surface sterilized with mercuric chloride (0.1 percent) solution for 30 seconds followed by rinsed twice in sterilized water so that no chemical remains with sterilized pieces of plant part, further sterilized plant part were transferred to its selective media, i.e. Potato Dextrose Agar (PDA) medium. Incubation of this plate at 28^{0}C in a B.O.D. incubator was done for five days to grow desired micro-organism. The fungus purification was done by transferring the developing tip of the fungus on other plate poured with PDA medium in LAF and then isolated single spore for purpose of identification of pathogen. On the basis of colony characteristics and physical characteristics given by Booth, the desired fungus was isolated and recognised as *Fusarium udum* (Butler, 1971). The obtained culture of *Fusarium udum* was transferred to PDA slants for purpose of further study.

Pathogen identification

On Czapek's Dox agar medium, the pathogen's

morphological characteristics such as colour growthof mycelium, colony characteristics are visible characteristics with naked eye while micro- and macro-conidia, and chlamydospore production observed under compound microscope were analysed and compared to Booth's (1971) description of *F. udum* Butler.

Morphological studies

Fresh cultures of desired pathogen grown on PDA plates were utilized to know more details about the morphological characteristics of the pathogen *Fusarium udum* Butler, and slides for these cultures were made so that they could be examined under a compound microscope. Size, form, and septation of morphological characteristics were noted in a culture that had been cultured on PDA for seven days at a temperature of 27 ± 2°C (30 days for chlamydospores). The Rilax micrometre was used to measure the conidial and chlamydospres levels.

Evaluation of botanicals against *Fusarium udum* Butler under *in vitro* condition

Efficacy of various phytoextracts were experimentally tested on PDA for finding its effectiveness against *Fusarium udum* Butler using poisoned food technique given by Grover and Moore in 1961.

Preparation of water extracts

Collection of fresh, healthy looking and disease free plant parts were was done based on appearance, washed thoroughly in running tap water to get rid of dust and other unwanted particles followed by rinsing with sterilized water and then crushed in 1: 1 ratio in a electric grinder *i.e.,* 100 g of plant parts were homogenized in 100 ml of sterilized water. The botanical extract prepared was strained through two folds of muslin cloth twice so that fine homogenous extract can be obtained. Then the crude extract was filtered by using Whatman filter paper No. 44. The filtrates obtained were regarded as 100% concentration for experiment to be performed.

Evaluation of aqueous extracts of botanicals against *Fusarium udum* Butler

Aqueous extracts of botanicals were evaluated at different concentrations of botanical extracts *viz.*5%, 10%, 15% and 20% concentrations. To maintain the above mentioned concentrations, 5 ml, 10 ml, 15 ml and 20 ml crude botanical extracts were poured in 95 ml, 90 ml, 85 ml and 80 ml of unsterilized Potato Dextrose Agar medium respectively to maintain required concentration for testing. Mixture prepared is autoclaved for 15 minutes at pressure 10 lb p.s.i. Following sterilization, the melted medium was aseptically poured at a rate of 20 ml/plate into sterilized petri plates under LAF. After solidification of media, a 10 mm diameter of fungal disc was cut from the perimeter of a pure culture of 8 days old culture of pathogen *Fusarium udum* Butler, it was kept in concern that disc to be transferred centrally in poured plate media under LAF and incubated in B.O.D. at 28 ^{0}C. PDA without plant extract were tested by utilizing pure water and considered as control. Each treatment was replicated three times. Percent inhibition was recorded by using the following formula:

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

Where,

C = Test fungus mycelialgrowth in untreated control plates.

T = Test fungus mycelial growth in treated plates.

Statistical Investigation

The data was statistically analysed using the statistical method proposed by Panse and Sukhatme (1978). The 'F' test of the treatment effects that were significant were used to determine whether the observed treatment effects were real or not. At a 1% level of probability, the standard error (SE) and critical difference (CD) were calculated. The results were interpreted using critical difference values. The data has been graphically illustrated at appropriate points in the text.

Results and Discussion

For quick detection of virulent isolates, pathogenicity test was conducted by water culture technique. The initial symptoms were appeared 3rd day after placing the seedlings in spore suspension, and on 5th day the entire seedlings developed loss of turgidity followed by withering and wilting.

Pathogenicity of 50 Fusarium isolates was tested on susceptible cultivar ICP2376. All the isolates exhibited characteristic wilt symptoms and the wilt incidence in different isolates ranged between 70.81% (Fsp-45) to 100% (Fsp-11).

Seven botanicals were tested for their antagonistic against *Fusarium udum* under in vitro conditions.

Table 1. *In vitro* evaluation of aqueous extracts of botanicals against *Fusarium udum* under *in vitro* condition

Sl. No.	Treatments	Mycelial growth (in mm) (after 120 hours)				Percent inhibition over check			
		5%	10%	15%	20%	5%	10%	15%	20%
1	Ashoka	28.77	28.77	27.80	28.29	30	30	32	37
2	Datura	32.8	32.47	32.47	28.7	20	20.8	20.8	30
3	Eucalyptus	20	20	15	8.2	51.21	51.21	63.41	80
4	Garlic	18	18	14	12.3	56.09	56.09	65.85	70
5	Ginger	22	9.6	9.8	9.8	46.34	76.55	76.55	76.09
6	Marigold	26	24	24	24	36.58	41.46	41.46	41.46
7	Moringa	23.50	10.4	11.2	7	42.68	74.63	72.68	82.92
8	Neem	24	22.2	22.4	18	41.16	45.85	45.36	56.09
9	Onion	9.8	9.8	8	8.45	76.4	76.09	80.48	79.39
10	Tulsi	15	15	15	10	63.41	63.41	63.41	75.60
11	Turmeric	12.3	12.1	11.1	10	70	70.48	72.92	75.60
	Control	41	41	41	41	-	-	-	-

	Botanicals	**Concentration**	**Interaction (BXC)**
SEm(+)	0.26	0.15	0.52
C.D.	0.74	0.48	1.42
C.V.		4.84	

The maximum per cent inhibition were observed in Garlic (74.16%, 75%, 75%, and 75%) and turmeric (74.00%, 75.00%, 75.00%. and 75.00%) at 10%, 15%, 20% and 30% respectively, while per cent inhibition of neem, black pepper were (50%) and bitter guard were (25%) at all 10%, 15%, 20% and 30% concentrations. Several researchers screened various botanicals for management of pigeonpea wilt. Ghante *et al.* (2019b) tested various botanicals and reported that inhibition per cent of garlic against *Fusarium udum* were 84.44% at 20% concentration. Kumar *et al.* (2017) evaluated eight botanicals *Fusarium udum* and reported that inhibition per cent of garlic were 76.87 to 83.22% at 5% concentration and 85.41 to 90.37% at 10% concentration. The inhibitory effect of garlic under *in vitro* conditions also reported by Nandeesha *et al.*, (2020); Nayak *et al.*, (2020); Rao *et.al.*(2020).

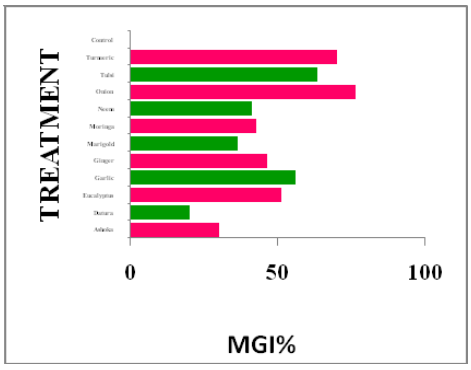

Fig. 1. Mycelial growth inhibition of *Fusarium udum* at 5% concentration of botanical under *in vitro* condition

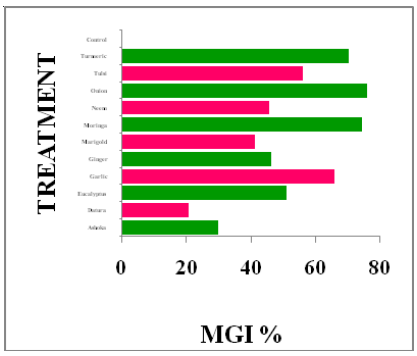

Fig. 2. Mycelial growth inhibition of *Fusarium udum* at 10% concentration of botanical under in vitro condition

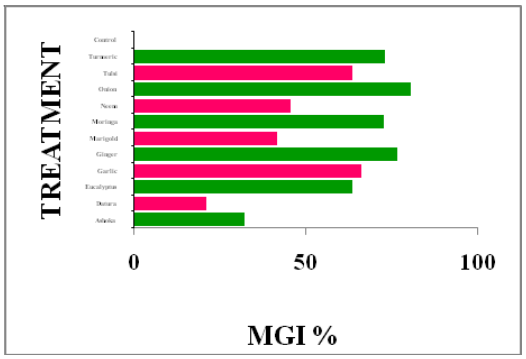

Fig. 3. Mycelial growth inhibition of *Fusarium udum* at 15% concentration of botanical under *in vitro* condition

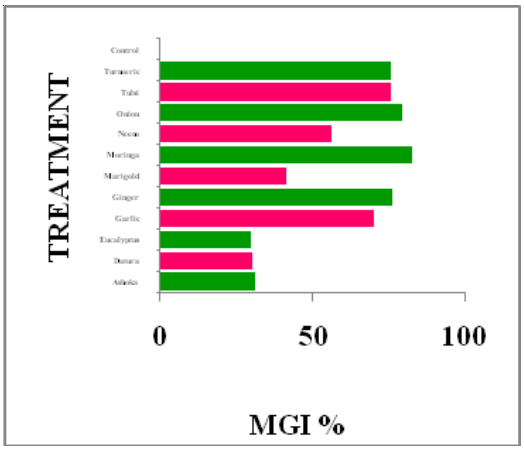

Fig. 4. Mycelial growth inhibition of *Fusarium udum* at 20% concentration of botanical under *in vitro* condition

Conclusion

In the current study, coordinated efforts have been made to assess various phytoextracts under *in vitro* conditions for the control of pigeonpea Fusarium wilt in order to develop an environmentally friendly and commercially viable disease management module.

Conflict of Interest -None

References

Behera, S.K., Shukla A.K., Tiwari P.K., Tripathi, A., Singh, P., Trivedi, V., Patra, A.K. and Das, S. 2020. Classification of Pigeonpea (*Cajanus cajan*(L.) Millsp.) Genotypes for Zinc Efficiency. *Plants.* 9(8): 952.

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

Butler, E.J. 1910. The wilt disease of Pigeonpea and parasitism of *Neocosmospora vasinifectum*. *Department Agricultural Bulletin of India.* 2: 1-64.

Datta, J. and Lal, N. 2013. Genetic diversity of Fusarium wilt races of pigeonpea in major region of India. *African Crop Science Journal.* 21(3): 201-211.

Hillocks, R.J., Minja, E.M., Nahdy, S. andSubrahmanyam, P. 2000. Diseases and.pests of pigeonpea in eastern Africa.*International Journal of Pest Management*. 46: 7–18.

Kannaiyan, J. and 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. and Raju, T.N. 1984. Prevalence of pigeonpea diseases and associated crop losses in Asia, *Africa* and the Americas. *Tropical Pest Managemen.* 30(1): 62-71.

Nandeesha, K.L., Huilgol, S.N. and Goudar, G.D. 2020. Eco Friendly Management of Fusarium Wilt of Chickpea with Botanicals and Bio Agents in In-vitro. *International Research Journal of Pure and Applied Chemistry.* 21(24): 192-196.

Nayak, D., Mishra, M.K., Sharma, K.K. and Pradhan, B. 2020. *In vitro* evaluation of different Phyto-extracts against *Fusarium oxysporum* f. sp. *cubense* causing Panama wilt disease in Banana.*Journal of Pharmacognosy and Phytochemistry.* 9(3): 747-750.

Nene, Y.L., Sheila, V.K., Sharma, S.B. 1996. *A World List Of Chickpea And Pigeonpea Pathogens* (5thEds). ICRISAT, Patancheru, A.P. India.

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

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Simonas, R. 2009. *Agroforesttree Database A Tree Reference And Selection Guide Version 4.0.* Oxford and IBHI Publishing Co Pvt Ltd New Delhi 457-463.

Pande, S., Sharma, M., Mangla, U. N., Ghosh, R. and Sundaresan, G. 2011. Phytophthora blight of Pigeonpea [*Cajanus cajan* (L.) Mill sp.]: an updating review of biology, pathogenicity and disease management. *Crop Protection.* 30(8): 951-957.

Panse, V. G. and Sukhatme, P.V. 1978. *Statistical Methods for Agricultural Workers*, Indian Council of Agricultural Research, p. 357 -358.

Rao, Y.H., Devi, P.S., Vemavarapu, V.V. and Chowdary, K.R. 2020. *In vitro* evaluation of antagonistic potential of native *Trichoderma* spp., botanicals and fungicides against *Curvularia spicifera* causing Curvularia Leaf Spot of Tomato in Manipur. *International Journal of Current Microbiology and Applied Sciences.* 9(10): 1815-1823.

Saxena, R.K, Saxena, K.B., Kumar, R.V., Hoisington, D.A. and Varshney, R.K. 2010. Simple sequence repeat-based diversity in elite pigeonpea genotypes for developing mapping populations to map resistance Fusarium wilt and sterility mosaic disease. *Journal of Plant Breeding*. 129: 135-141.

Sharma, R.L., Mishra, T., Bhagat, R. and Swarnkar, V. 2019. Integrated Disease Management for Pigeonpea Wilt Caused by *Fusarium udum*. *Agricultural Science Digest-A Research Journal.* 39(2): 119-123.

Swamy, R.E. 2011. *Studies on variability in Fusarium udum the incitant of wilt in pigeonpea* (Doctoral dissertation, Acharya NG Ranga Agricultural University).