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Efficacy of botanicals, bio-agents and fungicides against *Fusariumudum* causing wilt disease of pigeonpea *In Vitro*

Neeraj Kumar Rajvanshi, Subhash Chandra and Ajay Kumar

Abstract
Pigeon pea is an important pulse crop of India and its production is greatly affected by wilt disease. Pigeon pea wilt is a very destructive soil borne disease caused by *Fusariumudam*. In present research work plant extracts (Garlic, Neem, Tulsi), bio-agents (*Trichodrema viride* and *Pseudomonas fluorescens*) and fungicides (Vitavax, Bavistin and Thiram + Carbendazim) were tested against *Fusarium udum*. Fungicides namely Vitavax Power (0.2%), Bavistin 25SD (0.1%), Thiram + Carbendazim (0.3%) were tested by poisoned food technique *in vitro* and resulted cent per cent restriction of radial growth of *F. udum* up to 96 hrs. The results indicate that the antagonist effect on radial growth of *F. udum* was highest in *Pseudomonas fluorescens* (4.82 to 5.95mm) followed by *T. viride* (5.15 to 6.66mm) in comparison to check (81.00 to 90.00mm). The maximum inhibition was recorded at 10% concentration. The radial growth in plant extracts ranged from 2.32mm (Garlic bulb) to 6.15mm (Neem leaves) at 48 hrs of incubation period. The similar trends were also observed in 5% concentration of all incubation period.

Keywords: Pigeon pea, *Fusariumudum*, bio-agent, fungicide

Introduction
Pigeon pea (*Cajanus cajan* L. Millsp.) is one of the major pulse crops grown in the tropics and subtropics belonging to family Leguminaceae. It has wide adaptability and low input requirements mostly grown in *kharif* season. In India, the crop is mainly grown in Andhra Pradesh, Bihar, Uttar Pradesh, Karnataka, Gujarat, Madhya Pradesh, Maharashtra, Orissa and Tamil Nadu. In Uttar Pradesh, it is grown 311.0 thousand ha area producing 325.0 thousand tons, with an average yield of 1040 kg/ha (Anonymous, 2014) [1]. The poor yield of pigeon pea is mainly due to biotic stress like diseases and insect pests but only few of them like wilt, sterility mosaic, *Phytophthora* blight and *Alternaria* blight are major and destructive ones. Among fungal pathogens, wilt disease caused by *Fusarium udum* Butler is an important disease in India, Kenya, Malawi, Nepal, Tanzania and Uganda (Reddy *et al.*, 1990) [7]. In India, it is the most serious problem all over the pigeon pea growing states especially in U.P, M.P, Bihar and Maharashtra. In Bihar and Uttar Pradesh, 5-10 per cent losses in standing crop are common feature every year (Singh, 2006) [9]. Management of pigeon pea wilt through fungicides applied as soil treatment such as Thiram, Ferbam, Carbendazim and Copper oxychloride have been reported by different workers. In discriminate use of these chemicals has led to development of fungicide resistance strain and more importantly, environmental pollution, posing a potential risk to animal and human health. Hence, for minimizing the losses caused by wilt need inexpensive and environmentally safe management practices. It was therefore considered desirable to evaluate botanicals, bio-agents and fungicides against wilt pathogen for effective management.

Material and Methods
Pigeonpea plants showing characteristic symptoms of *Fusarium* wilt were collected from pigeonpea experimental field, Department of Genetics and Plant Breeding, NDUA&T, Kumarganj, Faizabad for isolation and identification. The infected plant parts were cut in to small pieces and surface sterilized with 0.1 per cent mercuric chloride solution and washed

thoroughly 3 to 4 times with sterilized water to remove the traces of Mercuric Chloride. The spieces were transferred in Petri dishes containing potato dextrose agar and incubated at 25 ^{0}C for 6 days.

Efficacy of plant extracts against *Fusariumudum*

In order of find out the efficacy of various plant extracts against the *Fusarium* wilt three plant extracts *viz*., bulb of Garlic, leaves of Neem and Tulsi were used. Fresh leaves and bulb were collected and washed thoroughly in clean water. Hundred gram of each washed plant material was grinded in Pestle and Mortar by adding equal amount (100 ml) of sterilized water (1: 1 w/v) and heated at 80^0 C for 10 minutes. The materials was filtered through double layered muslin cloth followed by filtering through sterilized Whatman No. 1 filter paper and treated as standard plant extract (100%). The 5.0 and 10.0 per cent concentration were made by adding in requisite amount of sterilized PDA medium. To study the inhibitory effect on mycelial growth of *Fusarium udum*, 5.0 and 10.0 per cent concentration of botanicals were evaluated through poison food techniques under *in vitro* condition by adding Five and ten ml plant extract of stock solution to the 95.0 ml and 90.0 ml of sterilized cooled PDA medium. Twenty ml medium was poured into each Petri plate. Three treatments having three replications were maintained. Control treatment was maintained by pouring PDA medium without plant extract. Five mm discs of 7 days old culture of *Fusarium udum* were placed in the centre of plant extract amended Petri plates. The Petri plate having PDA alone were inoculated in the same manner. These Petri plates were incubated at 28 ± 2 °C. The observations were recorded on radial growth at 48 hrs, 72 hrs, and 96 hrs of incubation in plant extracts amended Petri plate as well as in control.

Efficacy of bio-agents against *Fusariumudum* using dual culture method

Two bioagents (*Trichoderma viride* and *Pseudomonas fluorescens*) were used against *F. udum* to test the antagonistic effect. Culture of *Trichoderma viride* was obtained from the Department of Plant Pathology, NDUA&T, Kumarganj, Faizabad (UP). For *Pseudomonas fluorescens*, soil sample were collected around the rhizosphere of wilt affected plant from Student Instructional Farm, NDUA&T, Kumarganj Faizabad (UP). Isolates of *Pseudomonas fluorescens* were isolated and identified using Bergey's Manual of Determinative Bacteriology. Bacterial colonies identified as *Pseudomonas fluorescens* were picked and pure culture of isolates was maintained. The potential of cultured *Trichoderma viride* and *Pseudomonas fluorescens* was evaluated *in vitro* using dual culture technique by 5 mm disc cut from the actively growing margins of 72h old culture was placed at the margin of the 90mm Petri plates containing 20 ml potato dextrose agar (PDA). Disc of 5 mm size of 72h old culture of *Fusarium udum* was placed opposite to the antagonist under aseptic conditions. The plates were incubated at 28 ± 2^o C. Each treatment was replicated thrice. A Petri plate inoculated with pathogen alone served as the control. Mycelial growth of the pathogen was observed regularly from 48 hrs to 96 hrs after the inoculation of pathogen.

Efficacy of fungicides against *Fusarium udum* using poison food technique

Three fungicides (Vitavax Power, Bavistin 25SD, Thiram + Carbendazim) were used in three concentrations of each (0.2, 0.1 and 0.3 per cent). Three concentrations of fungicides were bio-assayed against the test pathogen under laboratory condition to find out their relative efficacy for inhibiting the mycelial growth of the pathogen by poison food technique. The efficacy of fungicides was observed by measuring the radial growth of the fungal colony at 48 hrs, 72 hrs, and 96 hrs after incubation.

Result and Discussion

During the present investigation of the treatments namely, Garlic bulb extract, Neem leaves extract, Tulsi leaves extract, *T. viride*, *Pseudomonas fluorescens* and Vitavax Power, Bavistin 25SD, Thiram + Carbendazim were evaluated for fungi toxicity against *Fusarium udum* by using poison food technique. The results showed that all treatments inhibited the mycelia growth of *F. udum*. The maximum inhibition was recorded at 10% concentration. The radial growth in plant extracts ranged from 2.32mm (Garlic bulb) to 6.15mm (Neem leaves) at 48 hrs of incubation period. The similar trends were also observed in 5% concentration of all incubation period. Devi and Charley (2012) reported the effect of different plant extracts against the mycelial growth of *F. udum*. Among them extract of *A. sativum* showed complete inhibition of radial growth of *F. udum* followed by *A. indica* (74.4%), *Spilanthes acemella* (68.8%) and *Aloe very* (55.9%). Mehta *et al.* (2010) found the Garlic bulb extract was significantly superior to inhibit the growth of *F. udum*. Same results were found by Dwivedi and Shukla (2000).

The results (Table-1&2) indicate that the antagonist effect on radial growth of *F. udum* was highest in *Pseudomonas fluorescens* (4.82 to 5.95mm) followed by *T. viride* (5.15 to 6.66mm) in comparison to check (81.00 to 90.00mm). This was due to inoculation of the antagonists, that produces fungistatic activity of the treated seed and might have increased certain other substances of antibiotic/ toxic nature in soil. Singh *et al.*, (2015) [8] found that the *Pseudomonas fluorescens* was significantly superior to inhibit the growth of *Fusarium udum*. These findings are in accordance of Khune (1990).

Three fungicides namely Vitavax Power (0.2%), Bavistin 25SD (0.1%), Thiram + Carbendazim (0.3%) were tested by poisoned food technique *in vitro* and resulted cent per cent restriction of radial growth of *F. udum* up to 96 hrs. Karande (2007) reported that Bavistin 25SD (0.1%) and Thiram + Carbendazim (0.3%) at higher concentration gave 100% inhibition of mycelial growth of *Fusarium udum* on PDA medium.

Tables and Figures

Table 1: Effect of plants extracts at 5% conc., on mycelial growth of *F. udum.*

Treatment	Mycelial growth (mm)		
	48 hrs.	72 hrs.	96 hrs.
Garlic bulb extract	3.71	4.56	5.56
Neem leaves extract	7.33	8.43	9.43
Tulsi leaves extract	4.33	5.53	6.43
Check	80.00	84.00	90.00
C.D. at 5%	3.12	3.45	4.10

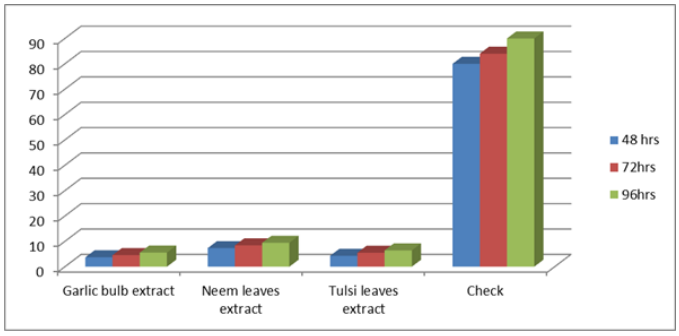

Fig 1: Effect of plant extracts (5% conc.) on mycelial growth of *F. udum.*

Table 2: Effect of plants extracts at 10% conc., chemicals and bio-agents on mycelial growth of *F. udum*.

Treatment	Mycelial growth (mm)		
	48 hrs.	**72 hrs.**	**96 hrs.**
Garlic bulb extract	2.32	2.82	3.32
Neem leaves extract	6.15	7.21	7.82
Tulsi leaves extract	4.15	4.65	5.32
T. viride	5.15	6.01	6.66
P.fluorescens	4.82	4.95	5.95
Vitavax.Power (0.2%)	0.00	0.00	0.00
Bavistin 25 SD (0.1%)	0.00	0.00	0.00
Thiram + Carbendazim (0.3%)	0.00	0.00	0.00
Check	81.00	85.00	90.00
C.D. at 5%	1.65	2.10	2.85

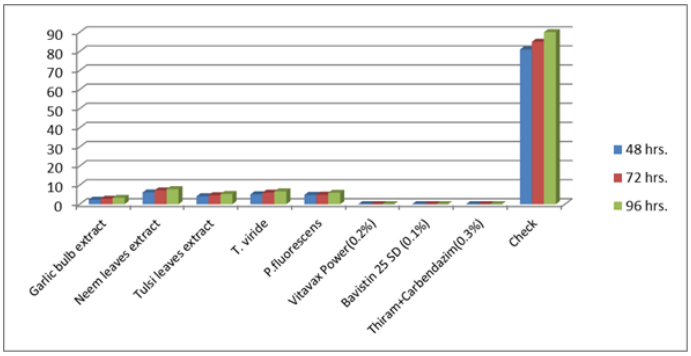

Fig 2: Effect of plants extracts (10% conc.), chemicals and bio-agents on mycelial growth

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