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Efficacy of Plant Extracts, Bioagents and Fungicides against *Fusarium udum* Causing Pigeonpea Wilt

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A B S T R A C T

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is the most important pulse crop of the India. It is suffered number of diseases like, *Alternaria* leaf spot, *Phytophthora* blight, Sterility mosaic and wilt. Pigeonpea wilt is a most destructive soil borne disease caused by *F. udum.* It is wide spread and caused heavy yeild loss nearly 100%. Plant extracts, bioagents and fungicides were evaluated against Fusarium udum. Out of seventeen treatments tested *in vitro*, propiconazole (0.15%) was found most effective in inhibiting radial growth of the pathogen followed by copper oxychloride, Garlic, Garlic + Tulsi, Tulsi, *T. viride*, Ginger + Mehandi, Neem, Onion, *A. niger*, Ginger, Onion + Beal, Mehandi, Beal, Neem + Marigold, *P. notatum* and Marigold over the check. The highest percent disease control was 97.22 to 94.44 in Garlic and lowest 58.33 to49.44 in Marigold. Among the bio-agents seed treatment with *T. viride* had controlled maximum wilt incidence 47.22 to 88.88% followed by *A. niger* (41.66 to 77.78%) and *P. notatum* (25.00 to 61.11%). Both fungicides were highly effective in reducing the disease incidence. The effectiveness was 100% in propiconazole and 97.22 to 100% in copper oxychloride.

Introduction

Pulses play a major role in nutritional security for the people, particularly those depends on cereal based diet and also play an important role in sustaining intensive agriculture by improving physical, chemical and biological properties of soil and are considered excellent crop for diversification of cereal based cropping system. Pigeonpea [*Cajanus cajan* (L.) Millsp.] is the multipurpose legume crop with diversified uses as food, feed, fodder and fuel. It has been recognized as a valuable source of protein (17.9 to 24.39/100g) particularly in the developing countries where majority of the population depends on the vegetarian foods for meeting its dietary requirements (Ali and Kumar, 2005). It was grown on about 3.53 million ha area producing 2.43 million tonnes with production of 697 kg/ha. In U.P. it is grown in 0.33 million ha area producing 0.30 million tones with productivity of 9.14 q/ha (Anonymous, 2011).

India is the largest producer and consumer of pulses in the world and also key player with 25% share in the global basket from an area

about 33% (Ali, 2007). The yield of pigeonpea is greatly affected by wilt disease caused by fungi Fusarium udum. Pigeonpea wilt is an important disease in India, Kenya, Malawi, Nepal, Tanzania and Uganda (Reddy *et al.,* 1990). In India, it is the most serious problem all over the pigeonpea growing areas especially in U.P, M.P, Bihar and Maharashtra. However, loss in individual plant found nearly 100% when wilt occurred at pre-podding stage, 67% at podding stage and 29.5% at pre-harvest stage (Kannaiyan *et al.,* 1981). *Fusarium udum* is a host specific pathogen of pigeonpea. 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 these chemicals has led to development of fungicide resistance strain (Okigbo, 2004) and more importantly, environmental pollution, posing a potential risk to animal and human health (Lyon *et al.,* 1995). Plant extracts and bioagents are emerging as eco- friendly way to manage pathogen. So, the objective of this investigation was to evaluate the potential of plant extracts, bioagents and fungicides against Fusarium udum.

Materials and Methods

Isolation

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 pieces were transferred in Petri dishes containing potato Dextrose Agar and incubated at 25^0C for 6 days. Pure colonies are isolated from inoculated petriplates separately in aseptic condition.

Efficacy of plant extract against *Fusarium* wilt *in vitro*

In order to find out the efficacy of various plant extract against the *Fusarium* wilt 8 plant extracts viz., leaves of Neem, Mehandi, Tulsi, Beal, Marigold, bulb of Garlic, Onion and Ginger were used. Fresh leaves, bulb and rhizome 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 ^{0}C for 10 minutes in hot water both. 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 5% and 10% concentration were made by adding 90 and 95 ml of sterilized PDA media to obtained 5 and 10% concentration of plant extract.

To study the inhibitory effect of botanicals on mycelial growth of *F. udum*, 5 and 10% concentration were used by applying poison food techniques under *in vitro* condition. Seventeen treatments having four replications were maintained. 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 petriplates. The control petriplates having PDA alone were inoculated in the same manner. These petriplates were incubated at 25±2 ^{0}C. The observations were recorded on radial growth at 48 hrs, 72, 96, 120 and 144 hrs of incubation.

Efficacy of plant extracts against *Fusarium udum in vivo*

The concentration of plant extracts found effective *in vitro* will be further tested *in vivo*. Soil was autoclaved, filled in 20 cm diameter

earthen pots separately @ 4 kg per pot and inoculation was done by adding *F. udum* grown on wheat grain medium @ 5% of the weight of soil in the pots. Control pots were filled with soil without adding inoculum. Seven days after inoculation of pathogen, 10% concentration of plant extracts @ 100 ml per kg of soil was thoroughly mixed in the pots to determine the effect of plant extract *in vivo*.

The seeds of pigeonpea wilt susceptible variety Bahar were sown in each inoculated pot (15 seed per pot), where finally 12 plants were maintained. The experiment was conducted in CRD with 18 treatments including control.

First appearance of disease, disease incidence and per cent disease control were observed 45, 60, 75, 90, 105 and 120 days after sowing.

Efficacy of bioagents against *Fusarium udum in vitro*

The rhizospheric fungi of pigeonpea namely *Trichoderma viride, Aspergillus niger* and *Penicillium notatum* were isolated from pigeonpea grown in the G.P.B. research farm of N.D.U.A. & T, Kumarganj, Faizabad, U.P. These dominant rhizospheric fungi were isolated by soil plate methods as described by Dhingra and Sinclair (1995) using Martin's agar medium. The rhizospheric fungi were screened for their antagonistic potential against *F. udum* by the method described by Upadhyay and Rai (1987). 5mm agar block of 5 day old culture of pathogen and bio-agents were used for test as dual culture methods. The experiments was performed in four replications for each treatments, for the control set, the same size of agar block of pathogen was put cooled distilled 16 ml of PDA medium. The radial colony growth was measured after 48, 72, 96, 120 and 144 hrs of incubation.

Efficacy of bioagents against *Fusarium udum in vivo*

The mass culture of the antagonists and the pathogen was prepared on wheat grains following the methods of Singh *et al.,* (1996). The sterilized soil was well mixed and separately with 1% (v/w) pure inoculum of the pathogen.

The pathogen infested soil was placed in plastic pots (25x20 cm) and kept at room temperature for 7 days to allow the pathogen to establish well in the soil. The pure inoculum 5% of *T. viride, A. niger* and *P. notatum* were mixed separately with the pathogen infested soil. The pots containing the soil pathogen inocula without the antagonist served as control. Four replications were maintained for each treatment. The 50% soil moisture was maintained by adding water once a day. Disease development was observed regularly. The final record of the incidence of wilting in plants was done at 45, 60, 75, 90, 105 and 120 days after sowing.

Efficacy of fungicides against *Fusarium* wilt *in vitro*

The fungicides viz., Propiconazole (0.15%) and Copper oxychloride (0.30%) were brought from authentic dealer. The required amount of Propiconazole 0.15ml/100 ml of PDA and Copper oxychloride 0.30g/100 ml PDA were mixed in the flask shacked and poured in petriplates replicated. The standard technique was followed for testing of mycelium growth of pathogen.

Efficacy of fungicides against *Fusarium* wilt *in vivo*

Fungicides that showed good inhibitory effect in vitro were selected for seed treatment *in vivo*. Seeds of pigeonpea variety "Bahar" were moist for 12 hrs prior to showing and

then treated with the Propiconazole (0.15%) and Copper oxychloride (0.30%). Twenty (20) treated seeds of susceptible variety "Bahar" were shown in each wilt sick pot and only 12 plants are maintained after germination. Percent wilt incidence was recorded at 45, 60, 75, 90, 105 and120 days after showing.

The percent growth inhibition, percent disease incidence and percent disease control were calculated by using the following formula

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of fungal growth
C = Radial growth of control
T = Radial growth of treated petriplates

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

$$\text{Per cent disease control} = \frac{C - T}{C} \times 100$$

Where,

C = Per cent disease incidence of control plots

T = Per cent disease incidence in treated plots

Results and Discussion

Efficacy of plant extracts, chemicals and bio-agents *in vitro*

During the present investigation of seventeen treatments namely, Neem, Garlic, Onion, Ginger, Marigold, Tulsi, Beal, Mehandi, Neem + Marigold, Garlic + Tulsi, Onion + Beal, Ginger + Mehandi, *T. viride, A. niger, P. notatum,* Propiconazole and Copper oxychloride were evaluated for fungi toxicity against *Fusarium udum* by using poison food technique. The results (Tables 1 and 2) showed that all treatments inhibited the mycelia growth of *F. udum*. The effectiveness of the extracts increased with an increasing concentration. The maximum inhibition was recorded at 10% concentration.

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%).

Two fungicides namely propiconazole (0.15%) and copper oxychloride (0.30%) were tested by poisoned food technique in vitro and resulted cent percent restriction of radial growth of *F. udum* up to 144 hrs. Mehta *et al.,* (2010) reported laboratory test that the propiconazole (1000 & 1500 ppm) and copper oxychloride (3000 ppm) had inhibited cent percent growth of the *F. udum* upto 7 days.

It is found from the experiment that Garlic extract inhibit the growth of *F. udum* up to 76.87 to 83.22% at 5% concentration and 85.41 to 90.37% at 10% concentration followed by Garlic + Tulsi (80.20 to 88.14%) up to 144 hrs incubation. Mehta *et al.,* (2010) found the Garlic bulb extract was significantly superior to inhibit the growth of *F. udum*.

The growth of *F. udum* reduced from 72.96% to 81.27% at 5% concentration and 73.95% to 85.92% at 10% concentration up to 144 hrs incubation followed by Neem (54.21-74.41%, 61.00-79.25%), Onion (49.21-70.77%, 55.20-74.81%), Ginger (41.87-68.55%, 52.00-72.96%), Mehandi (38.90-66.11%, 30.55-66.66%), Beal (31.09-64.11%, 33.33-69.44%) and Marigold (26.87-62.27%, 41.66-77.78%). The mycelial growth inhibition rate was increased with increasing concentration of

plant extracts. Baby Joseph *et al.*, (2008) tested different concentration i.e, 5%, 10%, 15% and 20% extracts of different plant viz., *Artemisia annua, Eucalyptus globules, O. sanctum* and *Rheum emodi* and found significant reduction in growth of *F. solani*. Plant extracts were found most effective at 20% concentration. The mixture extract of Ginger + Mehandi inhibited the germination of *F. udum* from 66.37 to 80.37% followed by Onion + Beal (48.95-70.37%) and neem + marigold (36.45-65.18%) from 48 hrs to 144 hrs. incubation period.

Table.1 Effect of plant extracts (5% conc. each) on the per cent mycelial Growth inhibition of *F. udum*

Treatments	**Per cent inhibition**				
	48 hrs	**72 hrs**	**96 hrs**	**120 hrs**	**144 hrs**
Neem	54.21 (47.14)	59.88 (50.73)	66.33 (54.57)	72.56 (58.44)	74.61 (59.77)
Garlic	76.87 (61.28)	78.33 (62.28)	80.17 (63.58)	82.76 (65.48)	83.22 (65.82)
Onion	49.21 (44.55)	56.07 (48.48)	63.48 (52.82)	68.02 (55.56)	70.77 (57.27)
Ginger	41.87 (40.31)	51.66 (45.95)	60.17 (50.87)	65.39 (53.97)	68.55 (55.40)
Marigold	26.87 (31.16)	40.59 (39.54)	48.30 (44.02)	59.27 (50.34)	62.22 (52.52)
Tulsi	72.96 (58.69)	74.16 (59.46)	77.05 (61.38)	80.46 (63.77)	81.27 (64.36)
Beal	31.09 (33.86)	42.26 (40.54)	53.12 (46.79)	60.13 (50.84)	64.11 (53.20)
Mehandi	38.90 (38.57)	47.97 (4383)	53.57 (47.05)	62.50 (52.24)	66.11 (54.40)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD at 5%	6.01	5.10	3.30	2.55	2.15

Figure given in parenthesis are angular transformed value

Table.2 Effect of plant extracts (10% conc. each), chemicals and bio-agents on the per cent Mycelial growth inhibition of *F. udum*

Treatments	Per cent inhibition				
	48 hrs	**72 hrs**	**96 hrs**	**120 hrs**	**144 hrs**
Neem	61.45 (51.60)	65.07 (53.75)	72.01 (58.04)	78.06 (62.05)	79.25 (62.87)
Garlic	85.41 (67.53)	86.25 (68.22)	88.09 (69.67)	89.90 (71.45)	90.37 (71.89)
Onion	55.20 (47.96)	60.31 (50.93)	67.85 (55.44)	71.92 (57.98)	74.81 (59.85)
Ginger	52.08 (46.17)	58.72 (50.00)	64.87 (53.63)	67.97 (55.51)	72.96 (58.64)
Marigold	30.20 (33.32)	36.25 (37.00)	47.61 (43.61)	58.76 (50.02)	61.96 (52.49)
Tulsi	73.95 (59.29)	77.77 (61.85)	80.94 (64.09)	84.64 (66.90)	85.92 (67.93)
Beal	40.62 (39.57)	46.81 (43.15)	55.35 (48.05)	62.71 (52.34)	66.29 (54.48)
Mehandi	47.91 (43.78)	51.58 (45.89)	57.14 (49.08)	64.03 (53.12)	67.40 (55.16)
Neem + Marigold	36.45 (37.12)	44.43 (41.78)	52.37 (46.34)	61.40 (51.56)	65.18 (53.81)
Garlic + Tulsi	80.20 (63.56)	82.53 (65.27)	85.11 (67.28)	87.27 (69.07)	88.14 (69.83)
Onion + Beal	48.95 (44.38)	55.15 (47.93)	60.71 (51.16)	65.80 (58.54)	70.37 (56.99)
Ginger + Mehandi	66.33 (54.51)	69.83 (56.66)	73.80 (59.19)	78.94 (62.65)	80.37 (63.67)
T. viride	67.70 (55.35)	71.42 (57.66)	76.18 (60.76)	79.38 (62.97)	81.48 (64.48)
A. niger	53.12 (46.77)	59.52 (50.47)	66.66 (54.71)	70.61 (57.14)	72.59 (58.40)
P. notatum	32.29 (34.61)	39.67 (39.02)	50.59 (45.32)	60.08 (50.79)	63.70 (52.93)
Propiconazole (0.15%)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Copper oxychloride (0.30%)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Check	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD at 5%	1.79	1.12	1.26	1.05	0.85

Table.3 Effect of plant extracts (10% conc. each), chemicals and bio-agents on the per cent Disease control *Fusarium* wilt of pigeon pea

Treatments	Percent disease Control					
	45 day	**60 day**	**75 day**	**90 day**	**105 day**	**120 day**
Neem	83.33 (65.87)	75.00 (59.97)	66.66 (54.71)	58.33 (48.77)	50.00 (44.98)	44.44 (42.98)
Garlic	97.22 (83.38)	94.44 (78.17)	91.66 (73.18)	83.33 (65.87)	75.00 (59.97)	66.66 (53.47)
Onion	80.55 (63.88)	72.22 (58.20)	63.88 (53.05)	55.55 (48.17)	47.22 (43.38)	41.66 (38.94)
Ginger	75.00 (59.97)	63.89 (53.17)	58.33 (49.77)	50.00 (44.98)	44.44 (42.98)	38.88 (39.74)
Marigold	58.33 (49.77)	49.44 (56.45)	41.66 (40.18)	33.33 (35.24)	33.33 (35.24)	22.22 (25.11)
Tulsi	91.66 (73.18)	83.33 (65.87)	75.00 (59.97)	66.66 (54.71)	58.33 (48.57)	50.00 (44.98)
Beal	66.66 (54.79)	55.55 (48.17)	50.00 (44.98)	41.66 (40.18)	38.89 (38.51)	30.55 (33.50)
Mehandi	69.44 (56.53)	58.33 (49.81)	52.77 (46.57)	44.44 (42.98)	41.66 (40.18)	33.33 (35.24)
Neem + Marigold	63.88 (53.05)	52.78 (46.57)	47.22 (43.38)	38.89 (38.51)	38.89 (38.51)	27.78 (31.75)
Garlic + Tulsi	94.44 (78.17)	91.66 (73.18)	83.33 (65.87)	75.00 (59.97)	66.66 (54.71)	58.33 (48.57)
Onion + Beal	72.22 (58.44)	61.11 (51.45)	55.55 (48.20)	47.22 (43.38)	44.44 (41.75)	33.33 (35.24)
Ginger + Mehandi	86.11 (68.25)	77.78 (61.92)	69.44 (56.45)	61.11 (51.45)	52.78 (46.57)	44.44 (42.98)
T. viride(4g/kg seed)	88.88 (70.68)	80.55 (63.88)	72.22 (58.20)	63.88 (53.05)	55.55 (48.17)	47.22 (40.95)
A. niger (4g/kg seed)	77.78 (61.92)	66.66 (54.71)	61.11 (51.41)	52.78 (46.57)	46.66 (40.18)	41.66 (38.94)
P. notatum (4g/kg seed)	61.11 (51.41)	50.00 (44.98)	44.44 (41.78)	36.11 (36.78)	36.11 (36.78)	25.00 (49.98)
Propiconazole (0.15%)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Copper oxychloride (0.30%)	100.00 (90.00)	100.00 (90.00)	97.22 (8338)	97.22 (83.38)	97.22 (83.38)	97.22 (83.38)
Check	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD at 5%	1.99	2.10	2.20	2.59	2.53	2.92

The growth inhibition of *F. udum* was found highest in *T. viride* (67.70-81.48%) followed by *A. niger* (53.12-72.54%) and *P. notatum* (32.29-63.70%) at 48 hrs to 144 hrs incubation. Singh *et al.,* (2010) observed maximum inhibition of radial growth of *F. udum* with *T. viride* (83.48%) followed by *A. niger* (52.3%) and *T. harzianum* (35.8%).The growth inhibition of *F. udum* by culture filtrates of the test fungi is possibly attributed

to the secretion of antibiotics by the fungi. Upadhyay and Rai (1987) on the inhibitory substances produced by the antagonists such as geodin, terricin, terric acid aspergillic acid dermadin etc.

Fungicides propiconazole (0.15%) and copper oxychloride (0.30%) evaluated against *F. udum* were highly effective for inhibiting the growth (100%). Mahesh and Saifulla (2006) evaluated the contact fungicides *in vitro* and found copper oxychloride was the most effective, which inhibited mycelia growth 97.66% at 2000ppm.

Out of seventeen treatments tested *in vitro*, propiconazole (0.15%) was found most effective in inhibiting radial growth of the pathogen followed by copper oxychloride, Garlic, Garlic + Tulsi, Tulsi, *T. viride*, Ginger + Mehandi, Neem, Onion, *A. niger*, Ginger, Onion + Beal, Mehandi, Beal, Neem + Marigold, *P. notatum* and Marigold over the check.

Efficacy of plant extracts, chemicals and bio-agents *in vivo*

Plant extracts (10% concentration), *T. viride, A. niger, P. notatum* (each 4g/kg seed). Propiconazole (0.15%) and Copper oxychloride (0.30%) were used for the test *in vivo* to find out the effectiveness against pigeonpea wilt (Pot experiment) at 45, 60, 75, 90, 105 and 120 days after sowing. On the basis of screening results *in vitro*. 10% concentration of plant extracts were amended in sterilized soil (w/v). The disease was controlled significantly in all plant extracts (Table 3). The effectiveness of more than 50% was recorded in all treatment at 45 and 60 days after sowing.

The highest percent disease control was 97.22 to 94.44 in Garlic and lowest 58.33 to 49.44 in Marigold. At 75 days after sowing, 50 % disease control was recorded in Garlic, Garlic + Tulsi, Tulsi, Ginger + Mehandi, Neem, Onion and Ginger, but only Garlic (66.66%), Garlic + Tulsi (58.37%) and Tulsi (50.00%) gave more than 50% disease control at 120 days after sowing.

Effectiveness of Garlic and Neem as bio-fungicides has already been reported by many works against different fungi. Chandra and Singh (2005) described that plant extracts of *A. sativum, A. indica* and *Calotropis procera* significantly reduced the wilt incidence in *Cicer arietinum.*

Among the bio-agents seed treatment with *T. viride* had controlled maximum wilt incidence 47.22 to 88.88% followed by *A. niger* (41.66 to 77.78%) and *P. notatum* (25.00 to 61.11%). It was significantly higher than the check up to 120 days after sowing. Adoms (1990) has been reviewed the fungal bio-agent like spp. of *T. viride*, *Penicillium* and *Aspergillus* against the successful control of *Fusarium* wilt.

Propiconazole (0.15%) and Copper oxychloride (0.30%) applied in the sterilized soil before the sowing of seed. Both fungicides were highly effective in reducing the disease incidence.

The effectiveness was 100% in propiconazole and 97.22 to 100% in copper oxychloride. Mehta *et al.*, (2010) have also reported the high concentration of propiconazole (1000 &1500ppm) and copper oxychloride inhibited cent percent mycelium growth of *F. udum*. The percent inhibition was decrease in copper oxychloride with increase in period of fungicides application.

Among the seventeen treatments the seed germination in fungicides were found significantly superior than the bioagents and plant extracts. The bioagents were also superior over the plants extracts.

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