Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 2537-2540
Received: 17-11-2017
Accepted: 18-12-2017

SK Arsia
B.M. College of agriculture Khandwa, RVSKVV, Gwalior, Madhya Pradesh, India

SP Mishra
Department of Crop Science, MGCGVV Satna, Madhya Pradesh, India

Moly Saxena
RAK College of Agriculture, Sehore, RVSKVV, Gwalior, Madhya Pradesh, India

Correspondence
SK Arsia
B.M. College of agriculture Khandwa, RVSKVV, Gwalior, Madhya Pradesh, India

In vitro evaluation of bioagents and agrochemicals against *Fusarium udum*

SK Arsia, SP Mishra and Moly Saxena

Abstract
Four antagonists *viz; Trichoderma vridii* (TV-S1) *T. harginum* (TH-S2), *Baccillus subtillis* (BST-S1) and *Pseudomonas fleuroscence* (PSF-S2) were tested against *Fusarium udum*. Among the antagonists PSF-S2 was found most effective with highest mycelial inhibition (77.21%) of the test pathogen followed by BST-S1 with 73.48% and TH-S2 with 66.60% inhibition and the lowest inhibition was found with TV-S1 with mycelial inhibition of 65.95 per cent. Thus, all the fungal and bacterial bioagents tested were found fungistatic against *F. udum* and significantly inhibited its mycelial growth over untreated control. However, fungal and bacterial bioagents isolates found most effective in the order of merit were PSF-S2, BST-S1, TH-S2, and TV-S2.The sporulation was very good in control and PSF-S2 and TH-S2 shows good sporulation where as TV-S1 and BST-S1 shows very low sporulation. Seven agrochemicals were tested against the pigeonpea wilt pathogen under *in vitro*. The evaluation of the best fungicides was done on the basis of the inhibition of the growth of the fungus by the agar plate method after 7 days. Different agrochemicals tested in laboratory, Carboxin was highly inhibited the growth of fungus (97.61%) followed by Copper oxychloride, Hexaconazole, Chlorothalonil and Metalaxyl (84.27, 81.52, 80.80 and 75.98%, respectively). Whereas the least inhibition (62.12%) was observed in Maneb mixed agar medium followed by Benomyle (69.36%). The Carboxin, Copper oxychloride, Metalaxyl, Hexaconazole and Chlorothalonil were recorded low sporulation. The good sporulation was recorded with Maneb and Benomyle.

Keywords: *Fusarium udum*, bioagents, sporulation, fungicides, inhibition

Introduction
The pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important pulse crop in India belonging to the family Fabaceae. It is a profitable and popular crop and fetches good price in the market. It is a hardy plant, when intercropped with cereals, and ensures a measure of income stability. Pigeonpea pods are consumed as green vegetable in many countries. Dry seeds of pigeonpea are consumed as split dhal. Pigeonpea is also used as ration for milch cattle. Globally pigeonpea is cultivated about on 4.7 million ha area with 3.69 million tones annual production. India accounts 78% of the global output with current production of 2.78 million tones from 3.5 million ha. The average yield of pigeonpea in M.P. is 848 kg/ha which is much lesser than the potential yield of crop (1500-2000 kg/ha). Several biotic and abiotic factors are responsible for reducing the yield (Anon. 2015-16). It is widely used as a pulse, green vegetable, fodder and for a variety of other purposes (Nene and Sheila, 1990) [12]. The seed protein content of pigeonpea (21%) compares well with that of other important grain legumes. High sensitivity of the crop to the attack of insect-pests and diseases appears to be the main reason for such low yields. The crop is attacked by more than 100 pathogens (Nene *et al.*, 1996) [11] including fungi, bacteria, viruses, phytoplasma like organisms and nematodes. However, only a few of them cause economic losses (Kannaiyan *et al*., 1984) [5]. Fusarium wilt is the most important disease of pigeonpea in India resulting in yield losses upto 67 per cent at maturity (Kannaiyan *et.al* 1981) [4].

Materials and Methods
Source of seed and other materials
The experiment was conducted at AICRP on Pigeonpea sub centre Sehore and laboratory facility was availed at Department of Plant Pathology, RAK Collage of Agriculture, Sehore (M.P.). Wilted samples were collected from experimental sites (ACRIP on pigeonpea sub centre, Sehore). The pathogen were isolated and grown on potato dextrose agar (PDA). The pathogen was identified according to Rai and Upadhyay (1982) [14]. Colonies of *F. udum* were purified in PDA slants and stored at 4^0C. All the bioagents *viz; Trichoderma vridii, T. harginum, Baccillus subtillis* and *Pseudomonas fleuroscence* are collected from the AICRP on Pigeonpea sub centre Sehore (M.P.).

Laboratory bioassay of fungicides evaluation of bioagents against *Fusarium udum*

Five fungicides given in table-2 were evaluated against the pathogen *F. oxysporum* f. sp. *udum* by poison food techniques (Nene and Thapliyal, 1979) [4]. The details of the fungicides used in the present investigation are summarized in table-1. The different fungicides were screened for their efficacy against the pathogen by "Food Poison Techniques" described by *Schmitz* (1930) in which required quantity of each fungicides was thoroughly mixed with 60 ml well sterilized potato dextrose agar medium contained in 100 ml flasks. Concentration of all the fungicides was maintained 500 ppm. Now 20 ml of this medium mixed with fungicides was poured in Petri-plates and allowed to solidify. Inhibition were calculated with each replication and the data used for the analysis.

The efficacy of biocontrol agents was evaluated *in vitro* against *F. udum* by dual culture method (Dhingra and Sinclair, 1985) and the seven days old culture grown on PDA media was used. The inoculum disc of 5 mm bio-agent and 5 mm pathogen was slotted with cork borer were picked up with sterile needle. The autoclaved and cooled PDA medium was poured in sterilized glass petri plates (90 mm dia.) allowed to solidify and 5 mm disc one each of the bioagent and the test pathogen were picked up with sterile needle. They were incubated for 3 days at temperature 28 + 2ºC and the observation on colony diameter of the antagonist and target pathogen were noted vertically and horizontally and their mean was noted as average colony diameter. The four biocontrol agents *viz*; *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluroscence* and *Baccilus subtilis* with control were evaluated and the experiment in CRD was planned in three replications and five treatments for dual culture testing. The percentage inhibition over control was calculated by following formula (Vincent, 1947) [20].

$$\text{Inhibition (\%) I} = \frac{C - T}{C} \times 100$$

Where, I = inhibition (%) C = Colony growth of the target pathogen in control plate. T = Colony growth of the target pathogen in intersecting plate

Table 1: biocontrol agents used and their symbols

S. No.	Bio control Agents	Symbol
1.	*Trichoderma viride*, and	TV- S1
2.	*Trichoderma harzianum*,	TH- S2
3.	*Pseudomonas fluroscence*	BST-S1
4.	*Baccilus subtilis*	PSF-S2
5.	-	Control

Table 2: Fungicide with their coined, trade and chemical name

Fungicide	Trade name	Formulation	Chemical name
Carboxin	Vitavax	37.5%WP	5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilido
Copper oxychoride	Blue Copper	50%WP	Dicopper Chloride trioxide
Benomyle	Benlate	50% WP	Methyl 1-(butylcarbamoyl) benzimidazol-ylcarbamate
Metalaxyl	Ridomil	35% WS	methyl N-(methoxyacetyl)-N-(2,6 xylyl)-DL-alaninate
Maneb	Dithane M-45	37%WP	Manganese ethylenebis (dithiocarbamate)
Hexaconazole	Trigger	5%WP	2-(2,4-dichlorophenyl)-1-(1H-1,2,4-trizol-1-yl)hexan-2-ol
Cholorthelonil	Bumper	25%WP	1-2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole

Each treatment was replicated three times. One set of control was also kept in which the medium was not mixed with fungicides. Equal pieces of the fungal growth, cut by the cark borer were inoculated in each Petri-plate at the center. These inoculated Petri-dishes were incubated at 28±1^{0}C for 7days and after 7 days of the incubation, the fungal growth was recorded in each Petri- dishes.

Statistical analysis

The data were subjected to statistical analysis after mean of three replications was testing by computing critical difference at 5% probability level.

1) Standard error for treatment mean

$$\text{S. Em.} \pm = \sqrt{\frac{Em.s}{r}}$$

2) Critical difference

$$\text{C.D.} = \text{S. Em.} \times \sqrt{2} \times t$$

Where,

Ems = Error means sum of square
r = Number of replication
t = 't' value at 5% probability levels

Results

In vitro* evaluation of bioagents against *Fusarium udum

The results obtained on mycelial growth and inhibition of *Fusarium udum* with two fungal and two bacterial antagonists are presented in Table-3. Results revealed that all the bioagents significantly inhibited the growth over untreated control against *Fusarium udum.*

Among the antagonists tested against *F. udum* all the tested bioagents were found significantly inhibited the mycelia growth of test pathogen as compared to control. The table-3 revealed that the maximum percent of inhibition was recorded by PSF-S2 (77.21%) followed by BST-S1 (73.48%) and TH-S2 (66.60%). The minimum inhibition was found by TV- S1 (65.95) as compared to control. The sporulation was maximum recorded in control and PSF-S2 and TH-S2 shows good sporulation where as TV-S1 and BST-S1shows low sporulation.

Table 3: *In vitro* bioefficacy of bioagents on mycelial inhibition and sporulation of *Fusarium udum*

S. No	Bioagents	Inhibition (%)	Sporulation
1	TV- S1	65.95	+
2	TH- S2	66.60	++
3	BST-S1	73.48	+
4	PSF-S2	77.21	++
5	Control	0.00	++++
SEm±		0.98	
CD at 5%		2.13	

+ low, ++ Good and +++ very Good

Effect of new agrochemical on the growth and sporulation of wilt pathogen

Seven agrochemicals were tested against the pigeonpea wilt pathogen under *in vitro*. The evaluation of the best fungicides was done on the basis of the inhibition of the growth of the fungus by the agar plate method after 7 days. It is evident from the results of Table-4 showed that out of seven different agrochemicals tested in laboratory, Carboxin was highly inhibited the growth of fungus (97.61%), followed by Copper oxychloride, Hexaconazole, Chlorothalonil and Metalaxyl (84.27, 81.52, 80.80 and 75.98%, respectively). Whereas the least inhibition (62.12%) was observed in Maneb mixed agar medium followed by Benomyle (69.36%). All the tested chemical fungicides were found significantly inhibited the mycelia growth of test pathogen as compared to control. The Carboxin, Copper oxychloride, Metalaxyl, Hexaconazole and Chlorothalonil were recorded low sporulation. The good sporulation was recorded with Maneb and Benomyle.

Table 4: *In vitro* evaluation of agrochemicals against *Fusarium udum*

S. No	Agrochemicals	Inhibition (%)	Sporulation
1	Carboxin	97.61	+
2	Copper oxychoride	84.27	+
3	Benomyle	69.36	++
4	Metalaxyl	75.98	+
5	Maneb	62.12	++
6	Hexaconazole	81.52	+
7	Chlorothalonil	80.80	+
8	Control	0.00	++++
SEm±		0.86	
CD at 5%		1.78	

+ low, ++ Good and +++ very Good

Discussion

In the present investigation results of antagonist effect revels that *Trichoderma harzianum inhibited the growth more than T. viride where as* the maximum percent of inhibition was recorded by PSF-S2 (77.21%) followed by BST-S1 (73.48%) and TH- S2 (66.60%). The minimum inhibition was found by TV- S1 (65.95) as compared to control. Ramezani (2009) [15] studied on two species of Glomus and Trichoderma for control in wilt disease under *In vitro* condition. Among the bioagents, *T. harzianum* produced the maximum inhibition zone of 18.20 percent as compared to *T.viride*. Sundaramoorthy and Balabaskar (2013) [19] evaluate the Biocontrol efficacy of Trichoderma spp. against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* Under in vitro conditions, the results revealed that *Trichoderma harzianum* (ANR-1) isolate was found to effectively inhibit the radial mycelial growth of the pathogen (by 53%) when compared to all other isolates. Under greenhouse conditions, the application of *Trichoderma harzianum* (ANR-1) exhibited the least disease incidence (15.33%). Kumar *et al*. (2010) [6] reported that *Pseudomonas fluorescens* strongly inhibited the growth of *Fusarium udum*. It also caused degradation and digestion of cell wall components, resulting in hyphal perforations, empty cell (halo) formation, shrinking and lysis of fungal mycelia along with significant degeneration of conidia and suggested it has potential biocontrol efficacy against Fusarium wilt in *C. cajan.* Naik, *et.al.* (2010) [9] evaluate *in vitro* the ability of biocontrol agents in suppressing the growth of *Fusarium oxysporum* f. sp. *vanillae* causing stem rot in Vanilla. Among the tested biocontrol agents *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtili*s was found on betted to the growth of pathogen. Mezeal (2014) [8] evaluated four strains of *Bacillus subtilis* and five strain of *Pseudomonas fluorescens* against tomato disease caused by *Rhioctonia solani* and *Fusarium oxysporum* by dual culture technique. Isolates of *P. fluorescens* showed highest growth inhibition against *R. solani* and *F. oxysporum* while *B. Subtilis* 77.4% and 73.2 of growth inhibition against test pathogens respectively.

The basic approach before recommending chemical control against a particular disease is to evaluate the fungicides against pathogen under laboratory conditions by poison food technique. Among the fungicides carboxin were highly inhibited the growth of *F. udum* followed by Copper oxychoride, hexaconazol, chlorothalonil, manab and benomyl as compared to control in the present investigation. Chennakesavulu *et.al.* (2013) [2] evaluated the fungicides and stated that the propeconazol and hexaconazol significantly inhibited the *Fusarium udum in vitro* conditions. In support of present investigation, Sumitha and Gaikwad (1995) [18] also reported that *F. udum* was inhibited by captan (0.15%) and dithane-M-45 (0.37) in *in vitro*. Shah *et al*. (2006) [17] reported that mancozeb inhibited the growth of *F. udum* as compared to control. Mamzaa *et al.* (2012) [7] reported reduction in sporulation of fusarium by benomyl and Trycyclozol at 500ppm concentration as compare to control. Patil *et.al* (2015) [13] among all the fungicides Carbendazim and Benomyl Very scarce sporulation was observed and acted as antisporulant.

References

1. Anonymous. Annual Report of AICRP on pigeonpea. 2015-16, 192-193.
2. Chennakesavulu M, Reddikumar M, Eswara reddy NP. Evaluation of different fungicides and their compatibility with *Pseudomonas fluorescence* in the control of redgram wilt incited by *Fusarium udum*. J Biol. Control. 2013; 27(4):354-361.
3. Dhingra OD, Sinclair JB. Basic Plant Pathology Methods. CRC Press, Florida. Vincent, J. M. 1947. Distortion of fungal hyphae in presence of certain inhibitors. Nature. 1985; 159:325-850.
4. Kannaiyan J, Nene YL, Reddy MV, Ryan JG, Raju TN. Prevalence of pigeonpea diseases and associated crop losses in Asia, Africa and The Americans. Tropical Pest Management. 1984; 30:62-71.
5. Kannaiyan J, Reddy MV, Nene YL, Ryan JG, Raju TN. Prevalence of pigeonpea wilt and sterility mosaic in India: 1975-1980. International Pigeonpea Newsletter. 1981; 1:24-26.
6. Kumar H, Bajpai VK, Dubey RC, Maheshwari DK. Kang SC. Wilt disease management and enhancement of growth and yield of *Cajanus cajan* (L.) by bacterial combinations amended with chemical fertilizer. Crop Protec. 2010; 29:591-598.
7. Mamzaa WS, Zarafib AB, Alabib O. Effect of six fungicides on sporulation of *Fusarium pallidoroseum* isolated from castor (Ricinus communis) in Samaru, Nigeria, IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS). 2012; 1(5):40-42.
8. Mezeal IA. Study biocontrol efficacy of *Pseudomonas fluorescens* and *Bacillus subtilis* against *Rhizoctonia solani* and *Fusarium oxysporum* causing disease in tomato (*Lycopersicon esculentum* L.). Indian J of Funda. and App. Life Sciences. 2014; 4(4):175-183.

9. Naik B, Nagaraja R, Basavaraja MK. Biological control of *Fusarium oxysporum* f. sp. *vanillae*, the casual agent of stem rot of vanilla *in vitro.* I.J.S.N. 2010; 1(2):259-261.
10. Nene YL, Sheila VK. Pigeonpea geography and importance. *In : Nenc Y L, Hall D and Sheila V K. The Pigeonpea,* CABI, ICRISAT, Patancheru, A.P. 1990, 1-14.
11. Nene YL, Thapliyal PN. Fungicides in Plant Disease Control, Oxford and IBH Publishing House, New Delhi. 1979, 163.
12. Nene YL, Sheila VK, Sharma SB. A world list of chickpea and pigeonpea pathogens, fifth edition, ICRISAT, Patancheru, India. 1996, 27.
13. Patil VB, Gawade DB, Surywanshi AP, Zagade SN. Biological and fungicidal management of chickpea wilt caused by *fusarium oxysporum f. Sp.* Ciceri, The Bioscan. 2015; 10(2):685-690.
14. Rai B, Upadhyay RS. *Gibberella indica*, the perfect state of *Fusarium udum.* Mycologia. 1982; 74:343.
15. Ramezani H. Efficacy of some fungal and bacterial bioagents against *Fusarium oxysporum* f. sp. *ciceri on chickpea.* Plant Protection Journal. 2009; 1(1):108-113.
16. Schmitz H. Poison food technique, industrial and Engineering Chemistry. Analyst. 1930; 2:361.
17. Shah MI, Phalisteen Sultan, Nasier A, William SP, Arif Jan, Sajad M, Suriya Rehman, Shawi AS. *In vitro* study of effect of some fungicides *viz*., carbendazim, mancozeb, conjoint carbendazim, mancozeb and sulphur against *Fusarium oxysporum*. Res. J Microbiol. 2006; 1(4):360-365.
18. Sumitha R, Gaikwad ST. Efficacy of some fungicides against *Fusarium udum* in pigeonpea. J Soils Crops. 1995; 5(2):137-140.
19. Sundaramoorthy S, Balabaskar P. Biocontrol efficacy of Trichoderma spp. against wilt of tomato caused by *Fusarium oxysporum* f .sp. *lycopersici.* J App. Bio. & Biotechnology. 2013; 1(3):36-40.
20. Vincent JM. Distoration of fungal hyphae in the presence of certain inhibitors. Nature. 1947; 150:850.