Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(1): 2123-2126
Received: 26-11-2018
Accepted: 30-12-2018

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In vitro evaluation of biocontrol agents and botanicals against onion basal rot caused by *Fusarium oxysporum* f. sp. *cepae*

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Abstract
Efficacy of biocontrol agents and botanicals was evaluated for their potential to manage the basal rot of onion caused by *Fusarium oxysporum* f. sp. *cepae*. Six biocontrol agents such as, *Trichoderma harzianum*, *Trichoderma viride* Persex. Fr., *Trichoderma koningii* Oudern, *Trichoderma virens* Miller, *Pseudomonas fluorescens* Migula and *Bacillus subtilis* were tested for their antagonistic activity against the pathogen by dual culture technique. Among the tested isolates *T. harzianum* recorded the maximum (75.92%) inhibition. Among the tested botanicals NSKE (43.95%) was found effective in inhibiting mycelial growth which was followed by prickly chaff flower (34.81%).

Keywords: *Allium cepae, Fusarium oxysporum f.* sp *cepae*, basal rot, biocontrol agents

Introduction
Onion (*Allium cepa*) is one of the major bulb crop of the world, which has been considered as rich source of carbohydrates and minerals like phosphorus, calcium. It also contains protein and vitamin C. Onion is a seasonal crop and has comparatively low storage ability and bulbs are usually stored until the harvest of next season crop or for longer period due to seasonal glut in the market. The major constraints in onion production are lack of varieties capable of producing high yields of uniform sized bulbs, imbalanced use of fertilizer, poor management of disease and pest. Defects which are commonly seen are premature bolting, doubling or splitting of bulb at initial stages of crop growth as well as after bulb development and also after harvesting. As with other vegetables crops onions are susptable to numerous foliar bulb, root fungal pathogens that reduces yield and quantity one of those disease fusarium basal rot is more prevalent where onion is grown under high temperature condition. It was first reported in United States in 1910. Basal rot of onion caused by *Fusarium oxysporum* f.sp *cepae* is an economically important, causing rot of basal plate of the bulb, further infection of bulb scales occurs and most severe loss are found in storage period. *F. oxysporum* f. sp. *cepae* causing fusarium basal rot in a number of *Allium* species in addition to onion, such as chive, garlic and shallot (Schwartz *et al*., 1995) [10]. *F. oxysporum* f. sp. *cepae* produces mycelium as well as three types of asexual spores: microconidia, macroconidia and chlamydospores. Chlamydospores are produced in or on older mycelium, have one or two round cells and have thick cell walls, which defend the cells against degradation and antagonists (Vincent, 1947) [12].

Materials and Method
The standard tissue isolation procedure was followed to isolate the pathogen. The infected parts were surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) solution for 60 seconds and washed separately in sterilized distilled water to remove the traces of mercury if any and then transferred to sterilized petriplates containing potato dextrose agar (PDA). The petriplates were incubated at room temperature (27±1 °C) and observed periodically for the growth of pure colonies. The pure colonies which developed from the bits were transferred to PDA slants and incubated at 27±1 °C for 15 days. Then such slants were used.
Six biocontrol agents such as, *Trichoderma harzianum*, *Trichoderma viride* Persex. Fr., *Trichoderma koningii* Oudern, *Trichoderma virens* Miller, *Pseudomonas fluorescens* Migula and *Bacillus subtilis* Cohn Emend Pras were tested against *Fusarium oxysporum* f. sp. *cepae*. The biocontrol agents and test fungus were cultured on potato dextrose agar by using dual culture technique.
Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile petriplates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelial discs of test fungus

was inoculated at one end of the petriplate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist the bacterium was streaked at ends of the petriplates and mycelial discs of the fungus was placed at the centre. The plates were incubated at 27±1 °C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The percent inhibition of the growth of the pathogen was calculated by using the formula (Cramer, 2000) [2]

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

Where,
I = Per cent inhibition.
C = Radial growth in control.
T = Radial growth in treatment.

***In vitro* evaluation of botanicals**

Plant based pesticides which are relatively economical, safe and non-hazardous can be used successfully against the plant pathogenic fungi. The present investigation was aimed to study the antifungal activity of some plant extracts. The following plant leaf extracts were selected. Preparation of cold aqueous extract

Fresh plant leaf materials and seeds were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Finally filtrate thus obtained was used as stock solution.

To study the antifungal mechanism of plant extracts, the poisoned food technique was used (Nene, 1982) [6]. Five, seven point five and ten ml of stock solution was mixed with 95, 92.5 and 90 ml of sterilized molten PDA media, respectively so as to get 5, 7.5 and 10 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract. Twenty ml of medium was poured into sterile petriplates, mycelium of five mm size discs form periphery of actively growing culture were cut out by sterile cork borer and one such disc was placed on the center of each agar plate. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 27±1 °C temperature for ten days and radial growth was taken when maximum growth occurred in the control plates. The efficacy of plant products or botanicals was expressed as per cent of radial growth over the control which was calculated by using the formula suggested by Cramer, 2000 [2].

Results

***In vitro* evaluation of bio control agents.**

Efficacy of bacterial and fungal bioagents *viz*., *Bacillus subtilis, Pseudomonas fluorescens*, *Trichoderma koningii, T. harzianum*, *T. viride*, and *T. virens* were evaluated against *F. oxysporum* f. sp. *cepae* under *in vitro* condition by following dual culture method as described in "Material and Methods" the results are presented in Table (1)

The results revealed that, the antagonists significantly reduced the growth of *F. oxysporum* f. sp. *cepae* either by over growing or by exhibiting inhibition zones. Maximum reduction in colony growth was observed in *T. harzianum* (75.92%) which was significantly superior over all other bioagents tested. Next best was T. *virens* (74.81%) followed by *T. koningii* (73.7%) and *T. viride* (72.59%). However, *B. subtilis* (57.4%) and *Pseudomonas fluorescens* (52.96%) were least effective in inhibiting mycelia growth of the pathogen.

***In vitro* evaluation of botanicals**

The antifungal activity of ten leaf extracts was assayed, at three concentrations in the laboratory for their efficacy against the *F. oxysporum* f. sp. *cepae* using poisoned food technique as described in Material and Methods. The data are presented in Table 2.

The effect of plant extracts on the per cent inhibition of mycelial growth of *F. oxysporum* f. sp. *cepae* at three concentrations differed significantly. The results revealed that, among the eight plant extracts, Neem seed kernel extract (43.95%) was found effective in inhibiting mycelial growth which was followed by prickly chaff flower (34.81%), turmeric (32.53%) and datura (31.95%), which were on par with lantana (31.85), tulsi (25.92%). The least inhibition was observed in garlic bulb extract (17.90%) followed by onion bulb extract (19.50%).

The plant extract at 10 per cent was significantly superior over 7.5 and 5 per cent. Neem seed kernel extract (54.1%) at 10 per cent was the best and significantly superior over all other plant extracts. Next best was prickly chaff flower (41.9%) and lantana (39.03%). Least inhibition was observed in onion bulb extract (23.0%) and garlic bulb extract (23.3%). Similar trend was observed at 5 and 7.5 percent concentrations.

Discussion

***In vitro* evaluation of bioagents**

Management of the disease through chemicals and use of resistant varieties is possible to some extent. But the hazardous impact of agrochemicals on the environment, development of resistant mutants, escalating cost of pesticides and frequent breakdown of resistance strongly demand a sustainable and an alternative management approach to disease. Biological control assumes special significance as it is ecology conscious and cost-effective alternative strategy for disease management.

Chemical measures in management of soil borne pathogens, especially those infecting onion in late vegetative phase, are of limited. Therefore the presence of root colonizing antagonist seems acceptable for many reasons, including self-propagation, longer persistence in soil environment, and ecological and toxicological benefit for workers and consumers. Hence, the present investigation was taken up to screen the bioagents for effective management of basal rot of onion.

In the present investigation four fungal and two bacterial biocontrol agents are tested against *F. oxysporum* f. sp. *cepae*. The results of dual culture technique on *F. oxysporum* f. sp. *cepae* revealed that all the four fungal antagonists significantly reduced the growth of *F. oxysporum* f. sp. *cepae* either by over growing or by exhibiting inhibition zones, except bacterial bioagents. Most of the antagonists inhibited colony growth of *F. oxysporum* f. sp. *cepae* by their fast and over growing nature as observed in antagonists. It was noticed that maximum reduction in colony growth was observed in *T. harzianum* (75.92%) which was significantly superior over all the bioagents tested. Next best was *T. virens* (74.81%) and *T. koningii* (73.7%).

Present studies recorded significant my coparasitism of *Trichoderma harzianum* and *Trichoderma viride* on basal rot

fungus that caused lysis of the hyphae and the spores *in vitro*. The results of the present study are in line with results of conditions (Bhatnagar and his coworkers, 2004) [1] who reported that fungal antagonists *T. viride, T. harzianum, T. hamatum, T. koningii, T. pseudokoningii* were effective against *Fusarium oxysporum* f. sp. *cepae* infecting onion under *in vitro*. Flori and Roberti., 1993 [3] reported that *T. harzianum* and *T. viride* reduced onion basal rot caused by *Fusarium oxysporum* f. sp. *cepae* to an extent of 88.7 and 77.3 per cent respectively and (Ozer *et al*., 2004) [8] reported that seed treatment with *T. harzianum*, gave significant reduction in basal rot incidence on onion under pot and field conditions and (K. Sumana and N. S Devaki 2012) [4] reported that *T. harzianum* has shown significant reduction in growth of *F. Oxysporum f. sp* nicotianae. Similar results were also found in S. Pavan Kumar *et al.*, reported that *T. harzianum* were found effective in controlling the pathogen *F. Oxysporum.*

In vitro evaluation of botanicals

In the present investigation, eight plant extracts were evaluated under *in vitro* condition against *F. oxysporum* f. sp. *cepae* to know the fungitoxic nature. Though complete inhibition of the pathogen was not observed in any of the leaf extract tested but considerable amount of inhibition was noticed in some of them.

Among eigtht plant extracts tested against *F. oxysporum* f. sp. *cepae*, Neem seed kernel extract at 10 per cent (54.1%) was significantly superior over all other plant extracts. Next best was prickly chaff flower leaf extract at 10 per cent (41.90%) followed by lantana leaf extract at 10 per cent (39.6%), which is on par with turmeric (39.3) and datura leaf extract (38.10%) and followed by tulsi at 10 per cent (39.1%). The least inhibition was noticed in garlic bulb extract (23.00%).In the present investigation, the mycelial growth of fungus was inhibited to a greater extent by neem seed kernel extract, which is said to have insecticidal property also. The fungicidal spectrum of *Azadirachta indica* has been attributed to azadirachtin which belongs to C_{25} terpenoides (Subramaniam, 1993) [11]. Similar results were observed by Kulkarni., 2004 who reported that among the ten botanicals tested *in vitro* against *F. oxysporum* f. sp. *gladioli*, neem seed kernal extract at 10 per cent (54.49%) was found superior.one of the report shown that that the highest percentage of inhibition for *Fusarium oxysporum* f. sp *psidi* was achieved by extacts from *Achyranthes rosea*, and *Curcuma longa* L. nd similar results were also found in Omer Jan. *et al.*,2015 [7] Who reported that about 4 botanicals were tested near seed karnal extract proved significantly superior.

Table 1: Evaluation of biocontrol agents against F. oxysporum f. sp. cepae a causal agent of basal rot

Biocontrol agents	Per cent inhibition of mycelial growth
Bacillus subtilis	57.40 (49.28)*
Pseudomonas fluorescens	52.96 (46.72)
Trichoderma harzianum	75.92 (60.64)
Trichoderma koningii	73.70 (59.18)
Trichoderma virens	74.81 (59.90)
Trichoderma viridae	72.59 (58.46)
S.Em±	0.37
CD at 1%	1.59

* Figures in parenthesis indicate arc sin transformed values

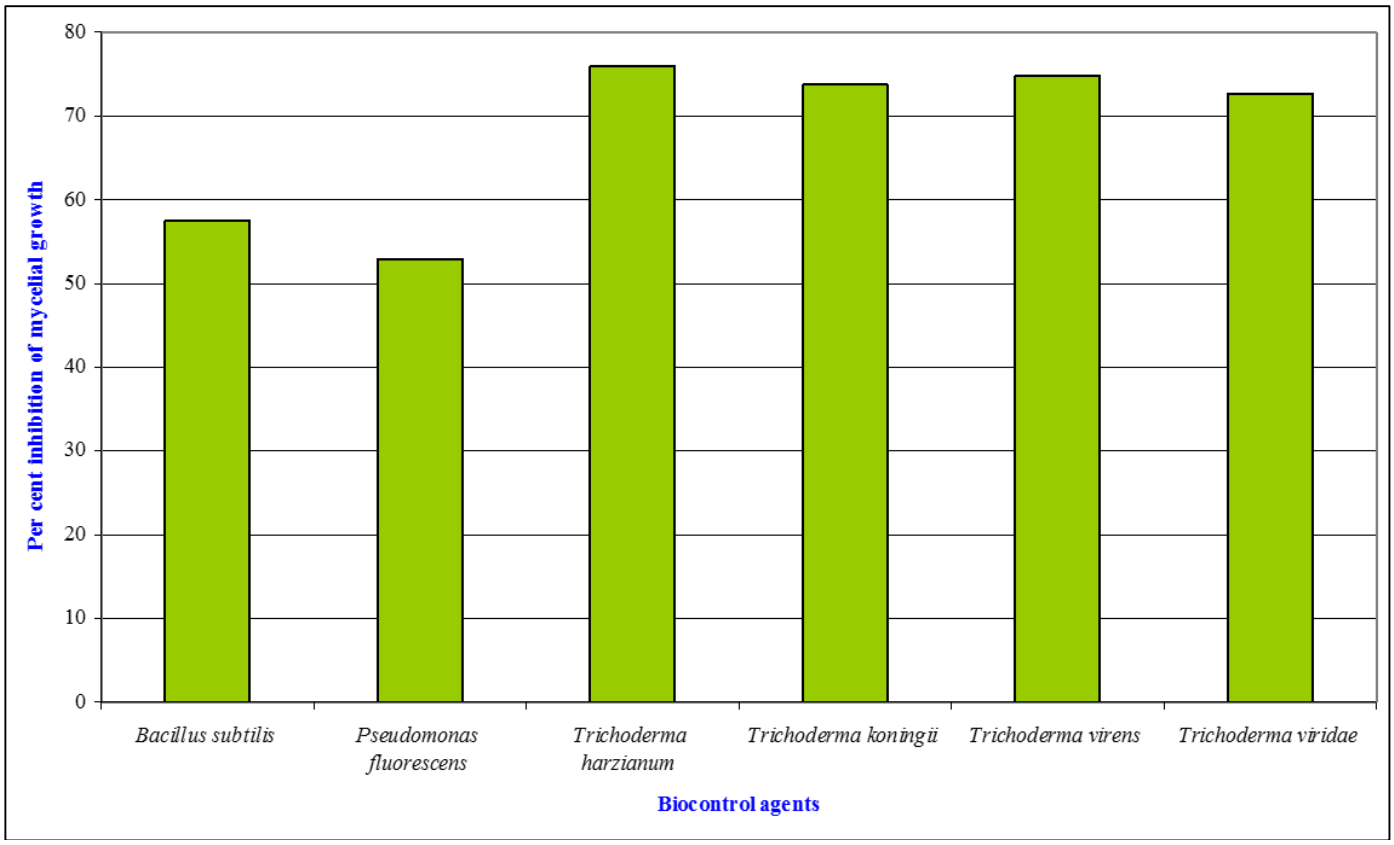

Fig 1: Evaluation of bio control agents F. oxysporum f. sp. cepae

Table 2: *In vitro* evaluation of plant extracts against *Fusarium oxysporum* f. sp. *cepae*

Botanicals	Per cent inhibition of mycelial growth			Mean
	Concentration (%)			
	5	7.5	10	
Garlic bulb extract	12.20 (20.47)*	18.50 (25.50)	23.00 (28.65)	17.90
Onion bulb extract	15.2 (22.94)	20.00 (26.58)	23.30 (28.90)	19.50
NSKE	34.80 (36.18)	43.00 (40.97)	54.10 (47.36)	43.95
Datura leaf extract	25.60 (30.38)	32.20 (34.60)	38.10 (38.16)	31.97
Lantana leaf extract	22.60 (28.39)	33.30 (35.28)	39.60 (39.03)	31.85
Tulsi leaf extract	20.40 (26.84)	25.90 (30.62)	31.50 (34.15)	25.92
Prickly chaff flower leaf extract	27.40 (31.58)	35.20 (36.40)	41.90 (40.33)	34.81
Turmeric rhizome extract	26.80 (31.22)	31.50 (34.15)	39.30 (38.81)	32.53
	Botanicals (B)	**Concentration (C)**	**B × C**	
S.Em±	0.22	0.13	0.39	
CD at 1%	0.84	0.53	1.50	

* Figures in parenthesis indicate arc sin transformed values

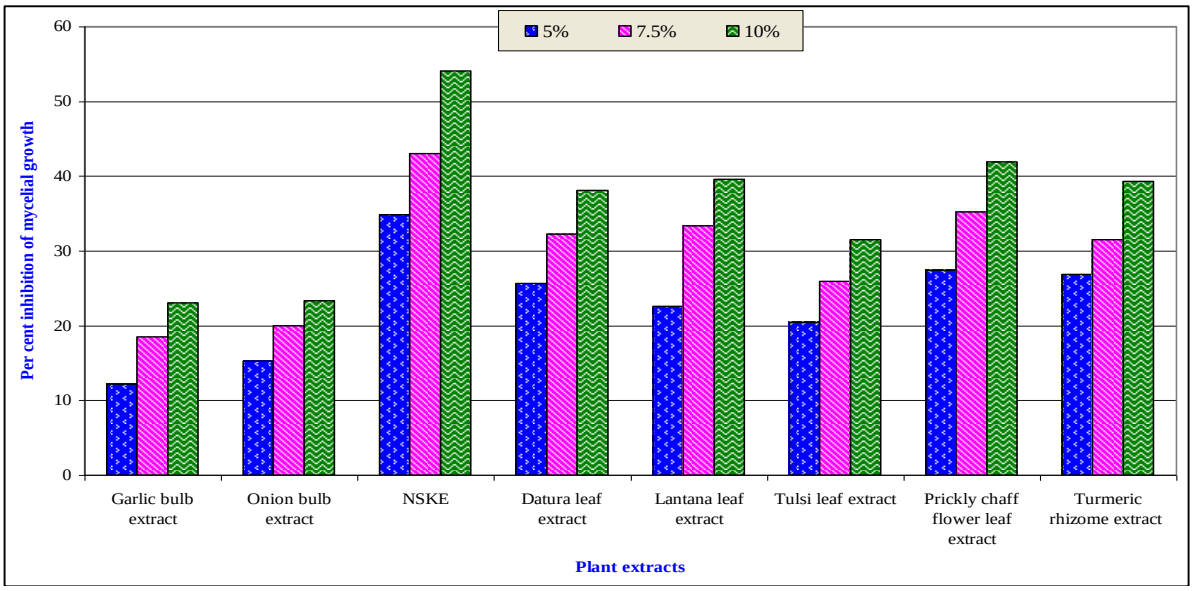

Fig 2: *In vitro* evaluation of plant extracts against fusarium oxysporum f. sp cepae

Conclusions

Among all tested bioagents and botanicals, *T. harzianum*, and NSKE 10 *per cent* (54.1%) were found significantly effective against control of *F. oxysporum* f. sp. *cepae* causing basal rot of onion. These are non-hazardous and ecofriendly management of basal rot of onion as compared to hazardous fungicides. Hence, we can adopt these bioagents in integrated disease management for effective control of onion basal rot.

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