International Journal of Academic Research and Development
ISSN: 2455-4197
Impact Factor: RJIF 5.22
www.academicsjournal.com
Volume 2; Issue 6; November 2017; Page No. 1181-1184

Screening of phytoextracts to control of *Fusarium oxysporum f.sp. vigni* incitant of mungbean (*Vigna radiata*) wilt

Anam Choudhary, Shabbir Ashraf, Nasreen Musheer
Department of Plant Protection, Aligarh Muslim University, Aligarh, Utter Pradesh, India

Abstract
In the present study different phytoextracts were tested for the efficacy against *Fusarium oxysporum f.sp. vigni* causing wilt of mungbean. Plant extracts of eleven different plant species, viz. Garlic, Ginger, Onion, Turmeric, Amla, Castor, Calotropis, Tobacco, Betel, Fennel and Neem were used at three different concentration 10%, 15% and 30%. Inhibitory effect of the different phytoextracts were tested on the mycelial growth of *Fusarium* by using poison food technique *in vitro*. The fungal pathogens were separately inoculated into the media and incubated for seven days. In general, all the test plant extracts checked the mycelial growth to varying degrees. Other plant extracts also exhibited antifungal activity at varying percentage. Radial growth of *Fusarium* was recorded. The growth inhibition increase with the increase of concentration of all the plant extracts. Highest mycelial growth inhibition was recorded with garlic extract at 30% concentration (100%) followed by 91.80% and 80.52% at concentration 10%and 15% respectively. The results of the experiment suggested that the use of garlic extracts are effective for minimizing *Fusarium* wilt incidence of mungbean. Calotropis and castor were found least effective against the fungus. The present study revealed that these plant extracts could be exploited for the possible control of this pathogen accordingly, this is an important proactive measure in preventing the spread of the wilt disease through a more ecofriendly approach.

Keywords: mungbean, *Fusarium oxysporum f.sp. vigni*, phytoextracts

Introduction

Pulses are the cheapest source of protein and are important constituent of daily diet. It is main source of protein for both humans and animals (Sattar *et al*. 1996). Mungbean (*Vigna radiata* (L.) Wilczek) commonly known as green gram is one of the important pulse crops of India. It alone accounts for 65% of its world acreage and 54% of the production. It covers about 3.50 mha area in the country and mainly grown in Rajasthan, Maharashtra, Andhra Pradesh, Karnataka, Orissa and Bihar. A unusual increase in area, production and productivity has been observed since 1964-65. The area and production have increased from 1.99 mha and 0.60 mt in 1964-65 to 3.54 m ha and 1.81 mt in 2010-2011. About sixteen diseases of mungbean have been observed in India (Nene, 1973). Among the various factors, diseases play an important role in low yield of crop. Including various disease causing agents, *Fusarium oxysporum* (causal agent of Fusarium wilt) causing severe losses to various pulse crops is an important soilborne pathogen of Indian Subcontinent. It also causes severe yield losses in the cultivation of mung bean by posing a great menace (Perveen *et al*., 1999; De *et al*., 2000 and Mahapatra and Swain, 2001). *Fusarium oxysporum* has a more specialized host range as compared to other soil borne fungal pathogens (classified as forma speciales). Fusarium is adapted to grow in the vascular system of their host resulting in root diseases of older plants at later stages of crop growth (Chindhelore, 1974). In many states of India seed and seedling rot of mungbean caused by *Fusarium oxysporum* f. sp. *vigni* has gained economic importance (Khare *et al*., 1977). It affects both seedlings and older plants. Affected seedlings wilt and their lower roots rot and develops a basal rot on stems in the affected seedlings. Various symptoms recorded in older plants are vein clearing and leaf epinasty that is often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves which at last results in death of the entire plant (Agrios, 1988). Various management practices including chemical and non chemical are used to manage *Fusarium* wilt of mungbean but due to toxic and residual effect of chemical various other alternatives are taken into consideration to manage the disease. Antifungal properties of several plant species have been reported earlier by many workers (Kubo *et al.,* 1995). Plants contain biologically active molecule that have antimicrobial property and have a great potential to inhibit the fungal growth. According to Isman (2006), these phytochemicals provide a promising and natural source for safer agrochemicals as these are biodegradable, protective, curative and selective in toxicity. Root rot (*Fusarium* spp.) can be managed through phytoextrects in different crops (Mamatha and Ravishankar, 2004; Joseph *et al.,* 2008; Mallesh *et al*., 2009; Patel *et al*., 2010 and Obongoya, 2010).

Material and method

Collection of the sample

Diseased roots of mungbean showing typical wilt symptoms of *Fusarium oxysporum* infection was collected from the field of Department of Plant Protection, A.M.U., Aligarh.

Isolation of the fungal pathogen

Using taxonomic and morphological references, the identified pathogen was *Fusarium oxysporum f.sp. vigni.* The fungus was isolated from the infected mango fruit following standard procedures (Dasgupta, 1981; Agostini and Timmer, 1992). The infected diseased samples along with healthy tissues were cut into small pieces and were surface sterilized by dipping in 0.1% sodium hypochloride (NaOCl) solution for two minutes. The treated plant tissues were washed three times with sterilized distilled water. Excess water was decanted by soaking with sterilized blotting paper. The cut pieces were then placed onto sterilized potato dextrose agar (PDA) in glass Petridish (20ml/petridish) and incubated at 28±1°C for three days for mycelium formation in petridish.

Purification and preservation

To obtain pure culture of hyphal tip was transferred aseptically onto PDA plate by using the flame sterilized tip of an inoculation needle. The plate was incubated at room temperature for three days. Mature hyphae were collected and transferred into the test-tube slants containing PDA and incubated at room temperature for seven days. After incubation, the slants were carefully checked for contamination and then preserved at 4 °C in a refrigerator for further use.

Identification of fungus isolates upto species

The fungus was identification on the basis of morphological characteristics suggested by Ellis (2009) and Agron (2009).

Collection of plant parts for phytoextraction

Plant products were prepared from different plant species (Table 1). Fresh leaves were collected for preparing their extract whereas bulbs, rhizomes were used in case of Garlic, Onion and Ginger, respectively.

Table 1: The parts of the plants used in the experiment

Name of plants	Scientific name	Family	Plant parts used
Onion	*Allium cepa*	Amaryllidaceae	Bulb
Ginger	*Zingibeyr officinale*	Zingiberaceae	Rhizomes
Tobacco leaf	*Nicotiana tabacum*	Solanaceae	Leaves
Giant indian milky weed	*Calotropis gigantean*	Asclepiadaceae	Leaves
Garlic	*Allium sativum*	Liliaceae	Cloves
Turmeric	*Curcuma longa*	Zigerberaceae	Rhizome
Indian gooseberry	*Phyllanthus emblica*	Phyllanthaceae	Fruit
Tobacco	*Nicotiana tobacum*	Solanaceae	Leaves
Fennel	*Foeniculum vulgare*	Apiaceae	Leaves
Neem	*Azadiractica indica*	Meliaceae	Leaves
Castor	*Ricinus communis*	Euphorbiaceae	Leaves
Betel	*Piper betle*	Piperacea	Leaves

Preparation of phytoextract

Aqueous extracts of plant parts such as leaves, rhizomes were obtained by using standard protocols as mentioned (Ezhilan *et al*., 1994). Healthy plant parts such as fresh leaves and rhizome (50 gm.) of ten different plant species were collected. These were surface sterilized with 0.02 per cent mercury chloride and thoroughly washed with distilled water. The sterilized leaves were crushed by adding equal amount (50 ml) of sterilized distilled water (1: 1, w/v). with the help of mortar and pestle. The pulverized mass was first passed through four layers of muslin cloth followed by filtering through Whatman's filter paper No.41. The filtered extracts were poured into 150 ml conical flasks and plugged with non-absorbent cotton. The flasks were tightly wrapped with aluminum foil and autoclaved at 121°C at 15psi pressure for 20 minutes. The crude solution was considered as standard plant extract having a concentration of 100%. Three different dilutions of extract (10, 30, and 50 %) were made from initial concentration.

***In vitro* Antifungal assay**

The effect of plant extracts on mycelial growth was studied by 'Poisoned Food Technique' (Nene and Thapliyal, 1993). Antifungal activity of plants extracts were determined by food-poisoned technique (Schmitz, 1930). Autoclaved extract were individually added into previously sterilized Potato Dextrose Agar (PDA) plates @ 10, 30 and 50 % and mixed thoroughly at the time of pouring in the previously sterilized Petri plates. The seven day old fungal culture disk of 5 mm diameter was inoculated to the center of Petri plates containing plantextracts aseptically after solidification. Without plant extracts PDA medium served as control. All plates were incubated at 28 ±2˚C and radial growth of colony was measured after three consecutive days viz. after third , fifth and seven day of incubation. Three replications were maintained for each treatment. All the inoculated Petri dishes were incubated at 25±1°C.

Collection of data

The percent growth inhibition (PGI) of the pathogen was recorded using formula given by Vincent (1947) The radial growth of the test fungus was measured in all the treatments after three days and compared with the control. The per cent inhibition of fungal growth was estimated by using following formula (Vincent, 1927):

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

Where,

I = per cent inhibition

C = Colony diameter in control

T = Colony diameter in treatment

Statistically analysis of data

Three replicates were taken for each treatment and colony growth were taken for each Petri dish. Mean colony diameters (mm) of *F. oxysporum* were used to calculate percent decrease over the control for each treatment. Mean colony growth in different phytoextract treatments and percent growth inhibition in each treatment over control were analyzed by analysis of variance at three concentration and days. Means were compared at $P= 0.05$ by Least significant difference (LSD) test. The calculated value of F was compared with the tabulated values at 5% level of significance for an appropriate degree of freedom.

It was observed that all the aqueous plant extracts tested showed antifungal activity against the growth of *F oxysporum in vitro*.

Table 2: Antifungal activity of phytoextracts

Treatment	Concentration/Percent of Mycelial growth Inhibition					
	10%		15%		30%	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Garlic	8.70 ±0.78	80.52	3.66±0.64	91.8	0	100
Onion	40.33±0.21	9.7	32.66±0.12	26.87	22±0.20	50.74
Betel	37±0.10	17.15	35±0.20	21.63	28±0.10	37.3
Turmeric	31.33±0.06	29.85	28±0.10	37.3	21.33±0.06	52.24
Amla	28.33±0.38	36.57	22.33±0.21	50	18±0.30	59.7
Castor	40.33±0.06	9.7	39.33±0.15	11.93	38±0.15	14.91
Calotropis	40±0.20	10.43	36±0.10	19.39	33±0.20	26.11
Neem	26.66±0.15	40.3	20.06±0.06	55.08	14.33±0.15	67.91
Fennel	33.33±0.21	25.37	30±0.10	32.83	24±0.15	46.26
Tobacco	36.66±0.15	17.91	32.66±0.25	26.87	26.66±0.21	40.3
Ginger	29±0.20	35.06	24.66±0.15	44.78	21.33±0.15	52.24
Control	44.66±0.06	0	44.66±0.06	0	44.66±0.06	0
S.E.D±	0.23		0.19		0.12	
F value	33.23		58.4		185.3	
LSD(P=0.05)	0.48		0.40		0.25	

*Mean of three replicates

Result and Discussion

In this study phytoextarcts were evaluated for their effect on the mycelia growth of Fusarium oxysporum to identify best effective botanicals. Eleven different botanicals were used to evaluate the efficacy and its effective in reducing the mycelial growth of fungus. They showed variable response in inhibiting the colony growth of fungus according to antimicrobial property present. The result shown in table 2 revealed that maximum mean inhibition was shown in garlic (100%) at 30% concentration followed by neem (67.91%) and amla (59.7%) other phytoextracts showed lower growth inhibition ranging from 52.24% to 14.91%. Garlic showed highest growth inhibition 91.8% and 80.52% at 15% and 10% concentration also. The findings of present investigation were in the favour of the work done by Hossain *et al*. (1993). They reported that garlic extract was found successful in controlling seedborne pathogenic fungi such as *Fusarium* spp. in wheat. Mallesh *et al*. (2009) and Joseph *et al.* (2008) also reported that garlic clove extract and neem leaf extract were most effective for the growth inhibition of *F. solani* Nwachukwu and Umechuruba (2001) recorded that plant extracts have played a significant role in the inhibition of seedborne pathogens such as *Fusarium oxysporum*. Higher concentration of all the phytoextracts gave significantly higher inhibition as compared to their lower level. Among the different phytoextracts, garlic is the most efficient phytoextract for wilt of mungbean as compared to other phytoextracts. There are many botanical products commercialized and reported as antifungal compounds that are present among higher plants, and are well-known entities for disease resistance.

Conclusion

Fusarium oxysporum is a soil borne pathogen and difficult to manage. Use of chemical fungicides leads to pollution of soil and ground water. Use of organic amendments and bioagents is a good option to manage the diseases without polluting the environment to the possible extent. Overall suppressive effect was displayed by all the phytoextarcts and the colony diameter decreased with increase in concentration. Biological control of fungal diseases using plant extracts has been anticipated as a promising alternative control strategies to reduce reliance on synthetic fungicides. Therefore, plant extracts stands as an alternative source for controlling plant diseases. It can be concluded that plant extracts managed Fusarium wilt under in vitro conditions.

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