

Original Research Article

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Evaluation of Plant Extracts against Wilt of Brinjal caused by *Fusarium oxysporium* f.sp. *melongenae* (Schlecht) Mutuo and Ishigami

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ABSTRACT

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Six different plants extracts viz., Neem, Tulsi, Aak, Eucalyptus, Onion and Garlic clove at different concentrations were studied by poisoned food technique against *Fusarium* wilt disease of brinjal to find out their efficacy. Neem leaf extract was found most effective in inhibiting the mycelial growth at 50% concentration of the causal fungus *Fusarium oxysporium* f. sp. *melongenae*. The Aak leaf extract was found second most effective in controlling fungus growth, whereas Tulsi was least effective. Neem leaf extract was observed most effective when used as seed soaking in reducing the incidence of the disease and the Aak leaf extracts was second most effective in controlling wilt in pot condition.

Introduction

Brinjal or eggplant (*Solanum melongena* L.) is one of the most common and principal vegetable crops grown in India and other parts of the world. It can be grown in all seasons and in almost all part of India except those at high altitude. In Rajasthan state, it is grown in all districts where irrigation facilities are

available. It is cultivated over an area of 512,800 hectares with an annual production of 8.450,200 metric tonnes in 2007 (Anonymous, 2006-07). The wilt disease of brinjal is very common in Eastern Rajasthan and U.P. and causes heavy losses. The disease is more severe in soils of pH below 6.4 and above 7. Other conditions which pre dispose the plant to wilt are short day length, low light intensity,

low nitrogen and phosphorous and high potassium nutrition to crop (Singh, 1998). Initial symptoms of disease starts as clearing of the veinlets, with main veins remaining green. This is followed by a unilateral yellowing of the younger leaves, subsequent wilting and death, which begins in the older leaves and progresses up to the main stem and ultimately the whole plant. Xylem vessels of infected plants show brown discoloration. The root system is very much reduced and their color also changes to light black and they becomes spongy. The root cover of affected roots is easily removable. Singh and Shukla (1980) reported that appearance of wilt disease in Kanpur is in the first fortnight of July, which gradually increase up to November and then decline. Crop loss varied from 5-60 percent reported by Mathur and Prasad (1964) an average loss 20 percent in Rajasthan where diseases infection ranged from 70-80 percent in vegetable fields. Management of seed-borne and soil-borne diseases such as wilt caused by *Fusarium* species has always been problematic (Haware and Kannaiyan, 1992 Jatav 2014). Soil solarization/disinfection, crop rotation and mixed cropping are the best ways of eliminating soil borne pathogens (Sullivan, 2004). Seed treatment with synthetic fungicides considerably reduce wilt incidence in brinjal. However, their use is costly as well as environmentally undesirable (Song and Goodman, 2001). The use of resistant varieties is one of the most effective alternative approaches to controlling wilt disease (Singh, 2005) but, due to breakdown of resistance in the face of high pathogenic variability in the pathogen population, the usefulness of many resistant cultivars is restricted to only a few years. In this context, plant extract is an eco-friendly way of managing *Fusarium* wilt in brinjal which offers an alternative to fungicides (Prasad and Rangeshwaran, 2000, Jatav *et al.*, 2013). The persistent and hazardous use of fungicides have posed a serious threat to human health

and to the existing human eco-geographical conditions as some of them have already been proved to be either mutagenic or carcinogenic. Fresh plant extracts from plant parts became valuable to management of pathogens (Grainge and Ahmad, 1988; Jespers and Wards, 1993). Plant extracts have been used as antifungal agents. These extracts can be easily prepared by farmers (Okigbo and Nameka, 2005). Keeping in view the problem of chemical management of plant diseases, the use of locally available plant extracts was evaluated both *in vitro* and *in vivo* to find out their efficacies for eco-friendly in the management of plant diseases is ahead importance

Materials and Methods

The study was conducted in S.K.N. Collage of agriculture Jobner Jaipur Rajasthan during 2001.

Collection of root samples

Roots samples were collected from fields in vicinity of Jobner and from collage farm and department of Plant Pathology, cage house. Collected samples were kept in polythene bags and stored at 10 c° for some further studies.

Isolation

Roots samples from wilted were used for isolation of pathogen employing standard method on PDA (Potato Dextrose Agar), maintaining aseptic condition.

Isolation method

Bits of roots samples were taken for isolation of *F. oxysporium*. Roots pieces were surface sterilized by dipping in 0.1% Hgcl₂ solution for 2 min., followed by three washing in sterilized distilled water. Sterilized petridishes each containing, about 20ml PDA(1000ml

water, 20g dextrose + 250g potato extract + 20g agar – agar). Medium was autoclaved at 1.045kg/cm² for 15 min. before pouring in plates. The medium sufficiently cooled before pouring into petriplates. Ten roots bits were placed in petridishes equispaced aseptically and incubated at 25 c with 12hr. of light alternating with 12hr. of dark period. The fungal colonies emanating from root bits were examined from 3rd to 7th day of incubation and used for purification.

Purification and Identification of wilt pathogen

Isolated root wilt pathogen was purified by two methods (i) single spore method (ii) Hyphal tip method.

Single spore method

In case of single spore technique, the serial dilution of the spore suspension from 7th day old culture were made in sterilized distilled water until a solution containing 10-15 spore/ml was achieved. One ml of this diluted suspension was poured in petridish containing cooled 2% plain agar (20g agar in 1000ml distilled water autoclaved at 1.045kg/cm² for 15 min.) under aseptic condition. Spore suspension was evenly distributed by tilting the Petridis in various directions. After few min. excess suspension was poured off from the petridish. Incubated Petridis were incubated at 25c for 24hr. Germinating single macro conidia (spore) were located under the microscope and marked with help of dummy objective and transferred to 2% PDA slants aseptically. Inoculated slants were subsequently allowed to grow and sporulate.

Hyphal tip method

The method is same as described earlier except instead single spore, hyphal tip method was marked and transferred on PDA. The

fungi isolated and purified were maintained by periodical transfer on PDA. Root pathogen was identified on the basis of morphological characteristics

The effect of six plant extracts namely neem (*Azadirachita indica*), onion (*Allium cepa*), garlic (*A. sativum*) Aak (*Calotropis procera*), eucalyptus (*Eucalyptus citridoria*) and tulsi (*Ocimum sanctum*) were tested at two different concentrations 25 and 50% following the method of Singh and Majumdar (2001) with certain modifications. The required plant parts were thoroughly washed with sterile water and were ground separately in electric grinder using equal amount of sterile distilled water. The mixture was squeezed with double layered sterilized Cheese cloth. The plant extracts were sterilized by passing through 0.22 µm filter (Millipore). Required amount of stock solution was added to potato dextrose agar (PDA) to get desired concentrations. The antifungal activity of these various plant sources, under study was tested *in vitro*. Test against growth of *Fusarium oxysporium* fungi by poisoned food technique (Schimitz, 1930) at different concentration of the stock solution. PDA medium was supplemented aseptically with the above two concentration of extract and sterilized at (1.045kg/cm² pressure for 20 minute.) 20 ml of sterilized PDA was poured in each Petri dish. The Petri dish were seeded with 2 mm disc taken from the periphery of 7 day old *Fusarium oxysporium* culture, and incubated at 25±1°C. Experiment was replicated thrice. Distilled water was used as a negative control. The radial growth of the fungi was measured in mm, after 7 days incubation at 25±1°C. Observations were recorded on radial growth along the two diagonals passing through the center of the colony. Colony and culture characteristics were recorded. The measuring of radial growth of the fungus was recorded. Percent growth inhibition was calculated using following formula called Vincent formula

(Vincent, 1947). The experiment was set up using CRD design.

$$\text{Percent inhibition} = \frac{C-T}{T} \times 100$$

C= Diameter of colony in the control

T= Diameter of colony in the treatment

Effect of plant extract against pathogen under pot condition

The experiment was conducted under pot conditions in *rabi* 2000-01. With a view to study the efficacy of various plants extract against inducing brinjal wilt pathogen. Seeds of brinjal var. Pusa purple long were soaked for 1hr in various leaf extracts separately at 25% and 50% concentration. Soaked seeds were air dried and sown at 10 seeds/ pot in the pre inoculated with pathogen. A control was also maintained where seeds were soaked in sterilized distilled water. Observation was recorded on seed germination, pre and post emergence. Plant mortality was observed up to 30 day of planting. Disease incidence and percentage disease control was calculated. Pre and post emergence mortality and percent disease control was calculated using following formula.

$$\text{Percent disease control} = \frac{\text{Disease in control} - \text{Disease in treatment}}{\text{Disease in control}} \times 100$$

Results and Discussion

Isolation of pathogen

Fungus was isolated on PDA from roots of brinjal plants under aseptic condition. The fungus emerging from roots bits placed on

PDA was purified by single spore and single mycelium bit transfer method and transferred to slants in tubes. Majority of roots bits yielded similar fungus. Hacer and Altinok (2013), Montaser *et al.*, (2014) and Hacer *et al.*, (2014) isolated the *Fusarium oxysporium* f.sp *melongenae* from different part of wilted egg plant and identified the pathogen.

Identification of the fungus

The pathogen was observed to have white cottony mycelia growth. In subsequent culturing the fungus produce white aerial mycelium on PDA. The whitish fungal growth covered the entire surface of the medium in Petridis within 7 days of old mycelium turn to whitish peach in color but usually with purple tinge. Sparse to abundant growth than becomes felted and sometimes wrinkled in older cultures. Abundant production of macro and micro conidia were observed in this culture of *Fusarium*. Micro conidia borne on simple phialides arising literally on the hyphae or from short sparsely branched conidiophores are generally abundant, variable in shape oval to ellipsoidal somewhat cylindrical, straight to curved 5-12×2.2-3.6µ. Macro conidia are thin walled generally 3-5 septate fusoid and pointed at the both ends measuring 37×3.4µ to 45×3.4µ. The pathogen was identified as *Fusarium oxysporium* f.sp. *melongenae* on the basis of morphological characters.

Efficacy of plant extracts on growth of *Fusarium oxysporium* in vitro

Six plant extracts were tested for inhibition of growth of *Fusarium oxysporium* in vitro condition by poisoned food technique. All plant extracts tested in the experiment at 25% and 50% concentration inhibited the fungal growth significantly. The data presented in Table-1 revealed that all six plant extracts were effective in inhibiting the mycelial growth of fungus over control. Irrespective of

the concentrations maximum inhibition of fungal growth was observed in Neem extract which reduced growth by 65% followed by aak, garlic, onion, eucalyptus and tulsi extract. The mycelial growth inhibition rate increased with increasing plant extract concentration.

Other workers have also reported fungal growth inhibition by plant extracts. Kumar (1979) tested aqueous extracts of different parts of many plant *in vitro* against *Fusarium oxysporium*. The spore germination was completely inhibited by Onion, Garlic, Kalanchae, Congress grass, Cotton and *Phaseolus atropurpureus* extract. Vimla and Sheetharaman (1993) Najar *et al.*, (2011) Chakraborty *et al.*, (2009) and Mandall *et al.*, (2009) also reported effectiveness of plant extract in inhibiting the growth of *Fusarium oxysporium*. Maximum inhibition was

observed by extract of *Parthenium bystorophus* followed by *Carthentum roseus* and *Prosopis juliflora*, *Leuceena leucocephala* and *Eucalyptus* spp.

Efficacy of plant extract against pre emergence mortality and wilt of brinjal under pot condition

All the six plant extracts were tested against *Fusarium oxysporium* in pot condition. The data presented in Table-2 revealed that all plant extracts were significantly superior over control in reducing the disease incidence (seedling mortality). Irrespective of the concentrations Neem extract was found most effective in reducing pre emergence to 60% to 79% post emergence mortality followed by aak, garlic and onion. The extract of *Eucalyptus* and Tulsi was less effective.

Table.1 Growth inhibition of *Fusarium oxysporium* by different plant extracts

Growth inhibition		
Plant extract	Concentration of plant extract (%)	
	25	50
Aak	59.96 (50.73)	75.47 (60.08)
Eucalyptus	52.00 (46.14)	64.89 (53.66)
Neem	65.99 (54.30)	83.56 (61.28)
Tulsi	52.00 (46.14)	64.89 (53.66)
Garlic	56.58 (48.75)	70.17 (56.89)
Onion	54.54 (47.60)	67.12 (55.01)
Control	0.00	0.00
CD at 5%	0.78	0.81
SEm±	0.260	0.270
C.V.	1.075	0.950

*Average of the three replications.

**Figures in parenthesis are angular values

Table.2 Effect of different plant extracts used as seed treatment on the incidence of wilt of brinjal

Plant extract	Germination (%)	Pre emergence mortality (%)	Disease control (%)	Post emergence mortality (%)	Disease control (%)
Aak	76.00 (60.67)	24.00 (29.32)	52.00	16.66 (24.08)	72.23
Eucaluptus	68.00 (55.32)	32.00 (34.45)	36.00	20.95 (27.21)	65.08
Neem	80.00 (63.44)	20.00 (26.56)	60.00	12.50 (20.68)	79.16
Tulsi	68.00 (55.32)	32.00 (34.45)	36.00	20.95 (27.21)	65.08
Garlic	73.33 (58.89)	26.67 (31.08)	46.66	18.00 (25.09)	70.00
Onion	70.00 (56.77)	30.00 (33.39)	40.00	19.57 (26.24)	67.38
Control	50.00 (45.00)	50.00 (45.00)	-	60.00 (50.77)	-
CD at 5%	0.83	0.82	-	0.62	-
SEm±	0.275	0.273	-	0.205	-
C.V.	0.931	1.416	-	1.230	-

*Average of the three replications.

**Figures in parenthesis are angular values

The antifungal properties of the plant extract may be due to presence of some antimicrobial substances in the extract. Chaumont (1979) tested aqueous extract of flowering plant on *Fusarium oxysporium* and reported that extracts of *Chrysonthium alpinum*, *Digitalis grandiflora* are more effective. The effect of these extracts was similar on 5 isolates of *Fusarium oxysporium* isolated from different plants. Raja (1999) studied the effect of extract of garlic bulbs, rhizomes of ginger and turmeric and leaves of *Callistemon lanceolatis* and *Euphorbia hirta* for their efficacy in controlling *Fusarium oxysporium*. His results indicated that garlic extract was superior to all treatment in reducing the growth of *Fusarium oxysporium* with no phytotoxic effects on cotton and tomato. Padmodaya and Reddy (1999) Suprakash *et al.*, (2012), Bhatnagaret *al.*, (2013) Dabbas *et al.*, (2012), Chakraborty *et al.*, (2009) and Dwivedi *et al.*, (2012) studied the effect of six organic amendments including Neem cake and eucalyptus dry leaves for their efficacy against

seedling disease in tomato caused by *Fusarium oxysporium* under pot condition. They reported that all amendments increased healthy seedling stand.

It is concluded from above results that the relative efficacy of plant extracts *in vitro* and pot condition showed Neem and Aak to be most effective followed by Garlic and Neem and Aak to be most effective in lowest incidence was recorded wilt and maximum percent germination of brinjal 80 % and 76 %. From present study it is postulated that plant extract can be used as a viable option for natural pesticides.

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