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In vitro antifungal activity of some plant extracts against *Fusarium oxysporum* f. sp. *lycopersici*

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ABSTRACT

Fusarium oxysporum f. sp lycopersici is an important disease that causes wilt disease in tomato crop world over. Management through chemical fungicides cause serious environmental problems and are toxic to non-target organisms as well. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides. In an approach towards the development of eco-friendly management, in vitro antifungal assay was conducted against Fusarium oxysporum f. sp. lycopersici (FOL), using plant extracts of fifteen plants. Out of fifteen plants, three plants proved to be potential in inhibiting the growth of the FOL viz., Solanum indicum (78.33%), Azadirachta indica (75.00%), Oxalis latifolia (70.33%). Antifungal potency was compared with three chemical fungicides namely via (Mancozeb with 82.66%, Copper oxychloride 79.33% and Copper sulphate 82.33%) in different concentration. The poison food technique was employed for the evaluation of antifungal activity of the extracts at four different concentrations (10%, 20%, 40% and 60%) on mycelial growth of FOL. This study indicates that the botanical extracts could be a good alternative in developing a potent plant based fungicides which can be used in organic farming for the management of Fusarium oxysporum f. sp. lycopersici.

Keywords: Poison food technique, Plant extract, *Fusarium oxysporum* f. sp. *lycopersici*, Tomato, Wilt disease.

INTRODUCTION

Tomato plant (*Solanum lycopersicum* L.) is affected by various fungi and bacterial diseases, which in turn produce a heavy loss to the crop. Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hans is one of the most prevalent and damaging diseases of tomato that causes considerable losses, especially in susceptaible varieties and under favorable weather conditions. The main symptoms of the disease are wilting of seedling and adult plants. The plant infected with this fungus produces wilt in older leaves that droop and afterwards turns yellow. Leaf yellowing can occur on one side of the plant and gradually most leaves from yellow and wilt. In order to prevent the plant diseases and to protect the crop plants against pathogens, chemical control methods were in practice. In view of the high cost of chemical pesticides and their hazardous consequence, use of biodegradable and different material like fresh plant extracts from different parts of the plants gained importance during the last three decades for plant disease control [4].

Although *Fusarium oxysporum* f. sp. *lycopersici* is widely reported as soil–borne pathogens in tomato plants, [12] concluded that this fungus can infest tomato crops via contaminated seeds. Fungal colonizes the xylem of the host plant, and as a result, blockage and breakdown of the xylem lead to wilt disease symptoms such as, leaf wilting, yellowing and eventually the death of the plant [1] [6]. Management of *Fusarium oxysporum* f. sp. *lycopersici* is required, as this pathogen and its many special forms affect a wide variety of crops of economic value.

Chemical fungicides are used to control *Fusarium* wilt of tomato. Unfortunately, these chemical fungicides are not readily biodegradable, tend to persist for years in the environment and few fungi have developed resistance to them [3]. Use of natural products like botanical amendments or botanical extracts for the management of fungal diseases

in plants is considered as a substitute method to synthetic fungicides, due to their less negative impacts on the human and environment health hazard or implications. This may be used for formulating new, safer and ecofriendly fungicides. In the present work, *in vitro* screening for antifungal properties of some plant extracts against *Fusarium oxysporum* f. sp. *lycopersici*, were evaluated.

MATERIALS AND METHODS

Collection of disease samples and calculation of disease severity and percent infection of *Fusarium* wilt of tomato

Assessment of disease severity, collection of disease samples and percentage of infection in tomato crops growing areas in and around Mysore region was carried out. A single plot was divided into 5 micro plots and 100 plants in each plot were considered and evaluated for disease severity and percent infection. Total number of fields surveyed were recorded. Diseased samples like infected stem, leaf and fruits were collected to isolate the causal organism.

Collection of native plants in and around Mysore District

Fifteen native plants were selected in the present study, to evaluate their antifungal activity Table – 1. Native plants were selected from local flora on the basis of criteria such as the presence of antimicrobial properties according to literature or traditional knowledge, easy availability in bulk with very little commercial value. The selected plants were well adapted to the climatic conditions and were well known, among local natives, for their medicinal properties. Different parts of native plants were collected from twenty one different regions, which are in different geographical regions of Mysore district. Mysore district is located between latitude 11°45' to 12°40' N and longitude 75°57' to 77°15' E. The plants were identified by using standard flora [7] and by comparison with herbaria at the Department of Studies in Botany, University of Mysore, Mysore.

Isolation and identification of the pathogen

Evaluation of infected parts of the tomato plant resulted in isolation, identified and confirmation of *F. oxysporum* f. sp. *lycopersici* based on the examination under different magnifications of a stereomicroscope. The characteristic growth of the fungus on the root and stem samples and the morphological characters of micro conidia and macro conidia and with chlamydospores were observed [5] [14]. Parts of plants with symptoms of *F. oxysporum* infection was surface sterilized with 70% ethanol and immersion in 0.3% sodium hypochlorite for 10 min, rinsed in sterile distilled water, transferred to potato dextrose agar (PDA) medium in petri dishes and incubated in dark at 28 °C for 7 days. The isolated fungal pathogen was tested for pathogenicity on tomato seedlings [12]. Spores were collected in sterile distilled water after 7 days growth in PDA medium, adjusted to 10^5 spores ml^{-1} using a haemocytometer and used as inoculum for further studies. Pure cultures were maintained on PDA slants and Petriplates at 4 °C [15].

Preparation of plant extracts

Plant extracts were prepared by taking plant parts and macerated with mortar and pestle in distilled water. The macerated biomass were kept overnight in culture tubes for the exudation of bio-chemicals at 4 °C. The biomass were filtered using muslin cloth, followed by filtration method by Whatman No. 1 filter paper. The filtrate was subjected to centrifugation at 10, 000 RPM for 5 min and the supernatant was sterilized and stored at 4 °C as stock solutions.

Antifungal activity assay of botanical extracts by using poison food technique

Plant extract at different concentrations 10%, 20%, 40% and 60% from the each stock solution were added in 20 ml of sterilized potato dextrose agar in petri plates. A 5 mm diameter of the actively growing mycelium disc of the pathogen of 6–7 day old culture was placed in the center of the Petri dish. Plates without plant extract served as negative control. Plates were incubated at 27 °C. Triplicates were maintained for each treatment. Radial growth of mycelium was measured after seven days of incubation. The results were compared with negative control. The experiment was repeated thrice and mean of three readings was taken for calculations. The percent inhibition of the fungus in treatments was calculated using following formula;

$$L = [(C – T)/C] \times 100$$

Where, L is the percent inhibition; C is the colony radius in control plate and T is the radial growth of the pathogen in the presence of plant extracts [13].

Evaluation of chemical fungicides by poison food technique on *Fusarium oxysporum* f. sp. *lycopersici.*

Three fungicides, Mancozeb, Copper oxychloride and Copper sulphate, were evaluated for their efficacy against *Fusarium oxysporum* f. sp. *lycopersici in vitro*. Four different concentrations (10ppm, 20ppm, 40ppm and 60ppm)

were used for assessment of their inhibitory activity against the pathogen by poison food technique. The experimental procedure was same as that used with plant extracts.

Data analysis

Statistical analysis of results was performed using IBM SPSS version 20. One way ANOVA (Analysis of Variance) at value $p \leq 0.001$ followed by Tukey's Post Hoc test with $p \leq 0.05$ was used to determine the significant differences between the results obtained in each experiment.

RESULTS AND DISCUSSION

Collection of disease samples and calculation of Disease severity and percent infection of *Fusarium* wilt of tomato

Mysore Taluk, KR Nagar, HD Kote showed more disease severity and percent infection of *Fusarium* wilt of tomato compared to other area [Table- 2, Fig. 1].

Table 1: List of Plant and part used for screening

Sl No	Botanical Name	Family	Plant Part Used
1	*Tamarindus indicus* L.	Fabaceae	Young twigs with fruits
2	*Eucalyptus globulus* Labill.	Myrtaceae	Young twigs with flowers
3	*Solanum indicum* L.	Solanaceae	Whole plant parts
4	*Oxalis latifolia* Kunth.	Oxalidaceae	Whole plant (aerial)
5	*Agave americana* L.	Asparagaceae	Leaves
6	*Pongamia glabra* Vent.	Fabaceae	Young twigs with flowers
7	*Tridax procumbens* L.	Asteraceae	Whole plant (aerial)
8	*Parthenium hysterophorus* L.	Asteraceae	Whole plant parts
9	*Azadirachta indica* A. Juss.	Meliaceae	Young twigs with fruits
10	*Ficus religiosa* L.	Moraceae	Young twigs with fruits
11	*Ricinus communis* L.	Euphorbiaceae	Young twigs with fruits
12	*Nerium oleander* L.	Apocynaceae	Young twigs with flowers
13	*Cissus quadrangularis* L.	Vitaceae	Whole plant parts
14	*Artocarpus heterophyllus* Lam.	Moraceae	Young twigs
15	*Allium cepa* L.	Lilliaceae	Bulb scales

Table 2: Disease severity and Percent infection of *Fusarium* wilt of tomato

Mysore district	Statistical Analysis
K. R. Nagar	31.00 ± 1.00^{a}
Hunsur	23.85 ± 1.23^{bd}
H.D. Kote	29.00 ± 1.00^{ab}
T. Narasipura	21.00 ± 1.00^{d}
Nanjanagud	23.33 ± 1.53^{d}
Piriyapatna	16.00 ± 4.00^{d}
Mysore	33.00 ± 1.73^{a}

Values sharing the same superscripts are significantly different according Tukey's Post Hoc at $p \leq 0.05$.

Table-3: Antifungal activity of different aqueous plant extracts at different (10%, 20%, 40% and 60%) concentration showing percent inhibition of mycelia growth

Plant Extract	10%	20%	40%	60%
Tamarindus indicus	39.33 ± 0.58^{d}	42.33 ± 0.58^{c}	57.33 ± 0.58^{b}	60.00 ± 0.00^{a}
Eucalyptus globulus	40.33 ± 0.58^{d}	45.33 ± 0.58^{c}	47.66 ± 0.58^{b}	57.33 ± 0.58^{a}
Solanum indicum	68.33 ± 0.58^{c}	72.00 ± 1.00^{b}	72.33 ± 0.58^{b}	78.33 ± 0.58^{a}
Oxalis latifolia	54.33 ± 0.58^{c}	55.33 ± 0.58^{c}	66.33 ± 0.58^{b}	70.33 ± 0.58^{a}
Allium cepa	44.33 ± 1.15^{c}	44.66 ± 0.58^{c}	49.33 ± 0.58^{b}	56.33 ± 0.58^{a}
Agave americana	42.00 ± 1.00^{d}	44.00 ± 1.00^{c}	48.33 ± 0.58^{b}	54.33 ± 0.58^{a}
Pongamia glabra	45.33 ± 0.58^{d}	47.66 ± 0.58^{c}	51.33 ± 0.58^{b}	55.66 ± 0.58^{a}
Tridax procumbens	35.33 ± 0.58^{c}	44.66 ± 0.58^{b}	44.66 ± 0.58^{b}	48.66 ± 0.58^{a}
Parthenium hysterophorus	57.66 ± 0.58^{c}	61.33 ± 0.58^{b}	63.66 ± 0.58^{a}	64.66 ± 0.58^{a}
Azadirachta indica	51.00 ± 0.58^{a}	60.00 ± 1.00^{a}	72.33 ± 0.58^{a}	75.00 ± 0.00^{a}
Ficus religiosa	32.33 ± 0.58^{c}	40.00 ± 1.00^{b}	40.33 ± 0.58^{b}	55.66 ± 0.58^{a}
Ricinus communis	44.00 ± 1.00^{b}	60.00 ± 1.00^{a}	61.00 ± 1.00^{a}	61.66 ± 0.58^{a}
Nerium oleander	24.00 ± 1.00^{d}	54.66 ± 0.58^{c}	57.33 ± 0.58^{b}	60.00 ± 1.00^{a}
Cissus quadrangularis	33.33 ± 0.58^{c}	55.00 ± 1.00^{b}	68.33 ± 0.58^{a}	69.00 ± 1.00^{a}
Artocarpus heterophyllus	32.00 ± 0.58^{a}	53.55 ± 1.00^{a}	55.85 ± 1.00^{a}	57.00 ± 0.58^{a}
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values sharing the same superscripts are significantly different according Tukey's Post Hoc at $p \leq 0.05$ within the column.

Table 4. Evaluation of Chemical Fungicides of poison food technique

Chemical Fungicides	10%	20%	40%	60%
Mancozeb	74.11±0.85[a]	80.66±1.15[a]	81.53±0.58[a]	82.66±0.58[a]
C. Sulphate	74.33±0.58[a]	77.33±0.58[b]	78.33±0.58[b]	79.33±0.58[a]
C. oxychloride	72.00±1.00[b]	74.85±0.78[c]	76.33±0.58[c]	82.33±2.51[a]
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values sharing the same superscripts are significantly different according Tukey's Post Hoc at p≤0. 05 within the column.

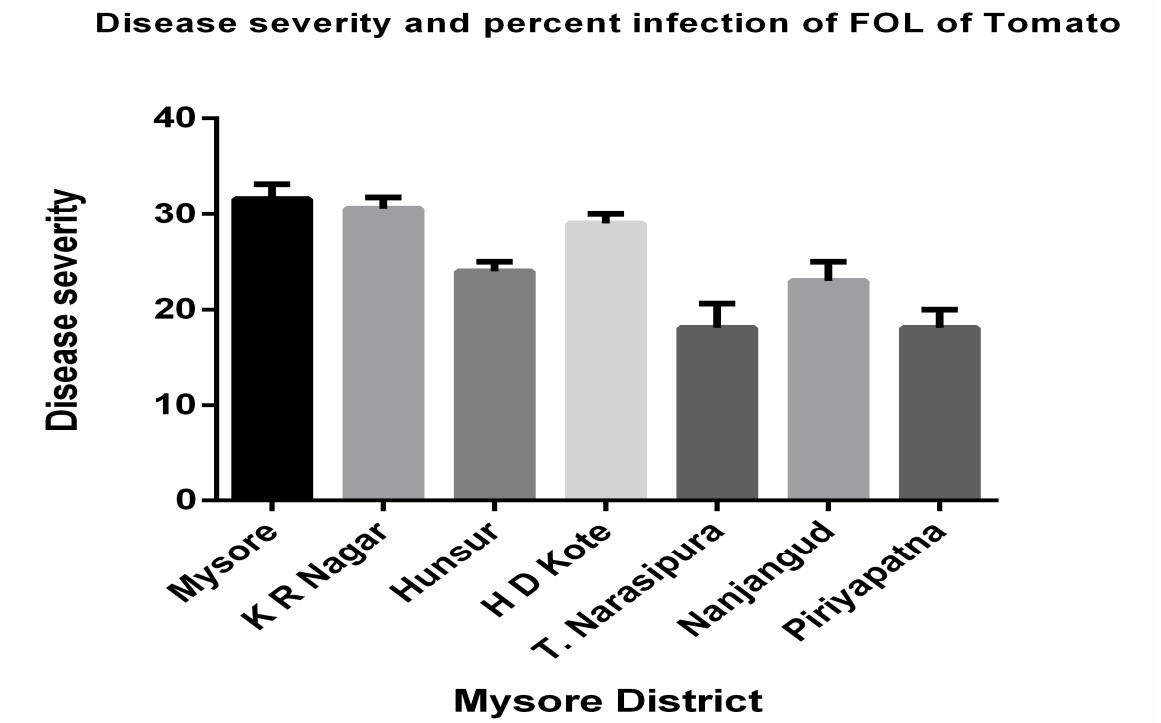

Figure-1: Disease severity and Percent infection of *Fusarium* wilt of tomato

Antifungal activity assay of plant extracts by using poison food technique

The effects of different extracts of selected plants were observed for the percentage of mycelial growth inhibition of *Fusarium oxysporum f. sp. lycopersici* (Fig. 2). A total of fifteen extracts were screened for their antifungal properties at four different concentrations (10%, 20%, 40% and 60%). Three plant extracts were found to be significantly inhibited growth of FOL pathogen, at the four tested concentrations viz., *Solanum indicum* (78.33%), *Oxalis latifolia* (70.33%), *Azadirachta indica* (75.00%) [Fig. 2, Table-3]. The rest of the plant extracts exhibited lesser percentage of inhibition at the tested concentrations [Table-3].

Antifungal activity of chemical fungicides on *F. oxysporum* f. sp. *lycopersici*

Three chemical fungicides were treated with different concentrations in ppm levels (10ppm, 20ppm, 40ppm and 60ppm). All four concentrations showed percentage inhibition of mycelial growth compared to control [Fig- 3]. All three fungicides significantly reduced the mycelial growth of pathogen in culture. The results showed that Mancozeb proved to be the most effective in inhibiting mycelial radial growth of the pathogen, followed by Copper sulphate and Copper oxychloride [Table- 4].

The management of *Fusarium* wilt disease of tomato is mainly based on the application of chemical fungicides, crop rotation and the use of pathogen-resistant varieties. Application of chemical fungicides was a conventional method to control diseases caused by fungal pathogens, but tremendous health hazards have been reported from time to time during the application of chemical fungicides, in the field conditions [2]. However, fungicide application has resulted in the accumulation of residual toxicity in soil and vegetables, increase environmental pollution and alter the biological balance in the soil by decimating non - target and beneficial microorganisms [11]. Adverse effects of chemical fungicides on the environment and human health are burning issues and there is a need to search for new fungicides which are eco-friendly in nature. The results of present investigation showed that botanical extracts of *Solanum indicum*, *Azadirachta indica*, *Oxalis latifolia* were the most effective to inhibit the growth of the tested pathogen followed by *Cissus quadrangularis* and *P. hysterophorus*.

In vitro efficacy of different plant extracts viz., *Azardiachta indica*, *Artemessia Annua*, *Eucalyptus globules*, *Ocimum sanctum* and *Rheum emodi* were tested to control Brinjal wilt pathogen *Fusarium solani* at different concentrations and showed a significant reduction in the growth of pathogen [9]. *A. indica* was also found to be effective against *A. solani* [10]. Fifteen seed extracts were evaluated 10% concentration by using poison food technique. *Solanum indicum* exerted the maximum mycelial growth inhibition against *Helminthosporium oryzae* [8]. It is noteworthy to mention that plant extract of *Solanum indicum*, *Azadirachta indica* and *Oxalis latifolia*, we're able to inhibit the growth of FOL in the present study as well.

Figure-2: Evaluation of aqueous plant extracts by poison food technique (reference table 2) (A) *Tamarindus indicus* (B) *Eucalyptus globules* (C) *Solanum indicum* (D) *Oxalis latifollia* (E) *Allium cepa* (F) *Agave amaricana* (G) *Pongamia glabra* (H) *Tridax procumbens* (I) *Parthenium hysterophorus* (J) *Azadirachta indica* (K) *Ficus religiosa* (L) *Ricinus communis* (M) *Nerium oleander* (N) *Cissus quadrangularis* (O) *Artocarpus heterophyllus* with control

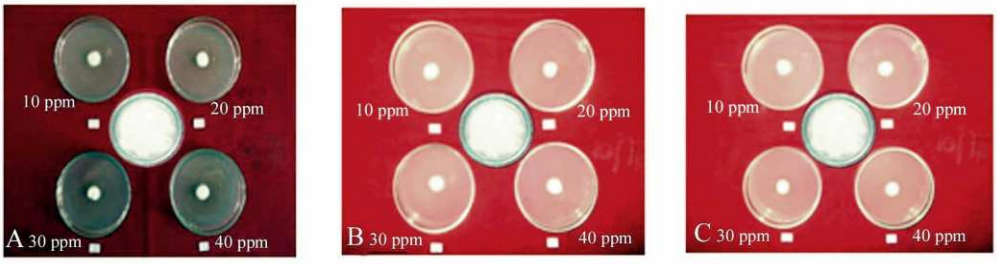

Figure-3: Antifungal activity of chemical fungicide A. Mancozeb, B. Copper sulphate and C. Copper oxychloride at (10ppm, 20ppm, 40ppm and 60ppm). Control at the center

CONCLUSION

Knowledge of compatibility of bio-control agents with other components of the production system is needed to develop feasible management strategies. *F. oxysporum* f. sp. *lycopersici* disease can cause severe losses in tomato. It has been concluded from present research that certain botanical extracts are a source of cost effective and non-

hazardous fungicide against FOL, also they don't have human and environment, health hazard or implications, so same plant extracts such as *Solanum indicum*, *Azadirachta indica*, *Oxalis latifolia* could be a good antifungal efficacy, which may be used for formulating new, safer and ecofriendly fungicides.

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