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In-Vitro Antagonistic Activity of Plant Extract on Fusarium Species

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Abstract

Plant protection is an important area which needs attention since most of the hazardous inputs added into the agricultural system are in the form of plant protection chemicals. Botanicals possess a variety of promising properties which make it a better biocontrol agent. The objectives of the present study were to isolate *Fusarium* sp. from soil and to check the effect of botanicals against this fungal pathogen *in-vitro*. The antagonistic activity of botanicals was studied by co-inoculation with the *Fusarium* sp. isolated from rhizosphere soil. In poison food technique, the botanicals in different concentration, showed decrease in the growth of the fungal pathogen. Maximum inhibition was observed in 10% *Azadiracta* sp. with 64% inhibition followed by 5% *Azadiracta* sp. with 57.8%

Keywords: Plant Protection, Chemical Fungicides, Botanicals, *Azadiracta* sp., *Parthenium* sp., Fungal Pathogens, Poison Food Technique

1. Introduction

Research on a more sustainable and eco-friendly agriculture is the need of the hour as there is a growing concern on the deteriorating

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quality of the environment as a result of chemical intensive agriculture and fast growing population. Despite many achievements of modern agriculture, the indiscriminate use of fertilizers and plant protections chemicals leads to the erosion of physical and chemical qualities of the soil. This leads to an increase in the prevalence of diseases and pest attacks and also in residual toxicity in food leading to various health hazards. The farmers are failing to take proper preventive measures. Plant disease control, therefore has become mainly dependent on fungicides to reduce the wide array of fungal diseases. A landmark study published by the US environment protection agency indicates that in the US alone, 3000-6000 cancer cases are induced annually by pesticide residues on foods and another 50-150 by exposure to pesticides during application [1,2]. For the last two decades, many research results have provided convincing evidence that root health and vigour are directly related to plant productivity. As a consequence, root disease control has become one of the most challenging research areas in the context of improvement of plant productivity [3,4]. Soil-borne plant pathogen *Fusariumoxy sporum* is one of the common diseases causing *Fusarium*wilt in crop of Solanaceae: tomato, potato, eggplant, and chilli. This disease causes serious seedling damping. *Fusarium* also causes plant to grow abnormally, or uses the plant as agent of the pathogen transmission to other host plants [5]. The pathogen infects young root, growing, developing and spreading in root and stem vessel, inhibiting water and nutrient transport [5]. Chilli (*Capsicum annuum* L.) is one of the cash crops of India, grown both for domestic consumption and export purposes. The crop is valued for its pungency which is imparted by an alkaloid, capsaicin and red pigments viz., capsanthin, capsorubin and Capxanthin [6]. Many factors operate in successful cultivation, production, marketing and exporting of quality chilli. Diseases play an important role in this. Amongst the diseases of chilli, Fusarial wilt has become a serious problem in recent years in all chilli growing irrigated tracts of India where the crop is grown especially in black cotton soil leading up to 20% yield loss [6]. The disease is typically soil-borne and the pathogen perpetuates in soil for several years by means of chlamydospores [7]. Chemicals are impressive, quick and convincing even to an illiterate farmer, but there is also an intensified worldwide concern

about environmental pollution due to escalated use of hazardous pesticides. Even otherwise, chemical control of a soil borne plant pathogen is frequently ineffective because of the physical and chemical heterogeneity of the soil which may prevent effective concentration of the chemical from reaching the pathogen. Hence, the best alternative measure is to look for the biological control of the pathogen using botanicals.

Plant protection is an important area which needs attention since most of the hazardous inputs added into the agricultural system are in the form of plant protection chemicals. Previous studies aimed at replacing pesticides with environmentally safer methods. Biological control of plant diseases assumes a greater importance in this context. Various methods for controlling such diseases have been investigated including the use of resistant varieties, cultural practices [8], plant volatile compounds [9] and, plant extracts [10].

The objective of the study was to isolate fungal pathogen from the rhizosphere of diseased plant and to analyse the presence or absence of certain biochemical compounds in the extract and evaluate the antagonistic activity of the aqueous extract of the leaf sample to increase the use of plant extract to control phytopathogens and by extension, the span of organic farming.

2. Methodology

The phytochemical analysis and *in vivo* studies of the leaf extract of *Parthinium* sp. and *Azadiracta* sp. against the plant pathogen *Fusarium* sp., isolated from the diseased plants' rhizosphere was reinoculated to a healthy plant in the laboratory to check the symptoms of the disease. This was done in the microbiology laboratory of MS Swaminathan Research Foundation.

(1) Isolation and identification of the fungal pathogen *Fusarium*sp.

The plant pathogens were isolated from the rhizosphere of the diseased chilli plant during the monsoon season. The soil was kept in sterile bottles labeled by the collection date, site and name of the person and brought to the laboratory immediately

after the collection. Serial dilution technique was done up to the dilution 10^{-4} by using potato dextrose agar (PDA). Based on morphological characters and keys and by slide culture, the pathogen was identified as *Fusarium* sp.

(2) Preparation of dried leaves powder

Parthenium sp. and *Azadiracta* sp. leaves were carefully washed using tap water to remove the dust and then dried in an oven at 60°C for 8 h. The dried leaves were ground separately in an electric blender. The powdered leaves of these plants were transferred to sterile bottles and placed in the refrigerator before the extraction process [11].

(3) Extraction of plant material

The aqueous extract of dried plant leaves was made in the distilled water. About 5 grams of each plant leaves powder (*Parthenium* sp., and *Azadiracta* sp.,) were taken and mixed in 50 ml of distilled water. The mixture was taken into 250 ml sterile conical flasks, plugged with sterile cotton and kept in rotary Shaker for 24 h. The solution was filtered through muslin cloth. This process was repeated three times after which a clear aqueous extract of the plant was taken [11]. Preliminary phytochemical study was screened for presence of alkaloid, anthraquinon, saponnins, tannins, flavonoid, glycosides, reducing sugar and terpinoides [12].

(4) Determination of mycelial inhibition by Poisoned food technique

The aqueous extract of *Parthenium* sp. and *Azadiracta* sp. were subjected to antifungal activity assay. Potato Dextrose Medium with 10 %, 5% and 2% aqueous extract of the test plants were prepared and sterilized at 121°C, 15 lbs pressure for 15 minutes. 15 ml of each media was separately poured into petriplates, allowed to cool and solidify. After

complete solidification of the medium, 9 mm disc of seven day old culture of the *Fusarium* sp. was inoculated in to the centre of respective Petri plates. The plates were incubated at 28 ± 1 ^{0}C for seven days. The Petri dishes containing media devoid of the extract served as control. After incubation, the colony diameter was measured in mm. For each, treatment was repeated three times. The fungi toxicity of the extract in terms of percentage inhibition of mycelial growth was calculated using the formula:-I $= \frac{C-T\times 100}{C}$

Where: I = Percentage of inhibition, C= Growth of mycelium in control, T=Growth of mycelium in treatment [13,14]

3. Result and Discussion

Identification of the plant: The plant species was identified by the taxonomist at MS Swaminathan Research Foundation Kalpetta Wayanad and the herbarium was maintained in the MSSRF herbarium center with authentication number MSSH Wayanad 0463 and MSSH Wayanad 0464 respectively for *Azadiracta* species and *Parthenium* species respectively.

Identification of fungal pathogen: The fungal pathogen responsible for the fusarial wilt was isolated from diseased plant parts were identified based on the morphological characters and microscopic observations. The culture was stained with lactophenol cotton blue and observed under high power microscope. *Fusarium* can be identified based on microconidia and macroconida [15,16,17,18]. The Fungal isolates obtained were cultured on potato dextrose agar which has a capacity to grow a wide range of fungi [19]. Based on the cultural characters and microscopic observations, the fungus was identified as *Fusarium* sp.

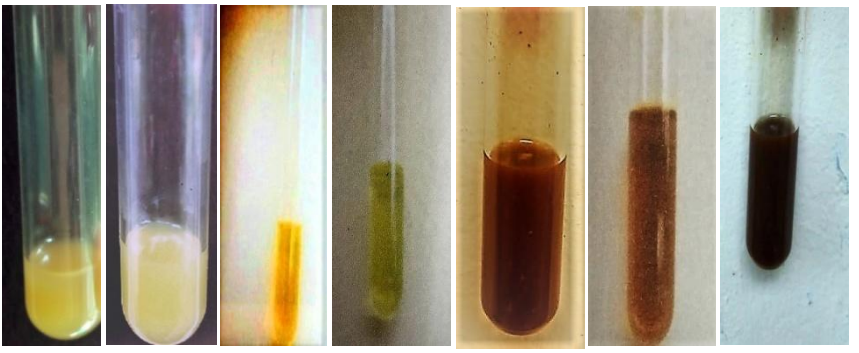
The preliminary phytochemical studies were done to detect the presence or absence of Alkaloides, Saponnins, Tannins, Glycosides, Flavanoides, Reducing sugars, Anthraquinone and terpinoides. Both the samples showed the presence of Alkaloides, Glycosides and flavanoides. Azadiracta extract showed positive for the

presence of glycosides and reducing sugar. All other tested phytochemicals are absent in both the plant extracts (Plate 1). The study results were shown in table 1

Table 1: Phyto chemical analysis of the plant extract

Chemical compound	Aqueous extract	
	***Azadiracta* sp.**	***Parthenium* sp.**
Alkaloides	+	+
Saponnins	-	-
Tannins	-	-
Glycosides	+	+
Flavanoides	+	+
Reducing sugar	+	_
Anthraquinone	-	-
Terpinoides	-	-

+ present , _ absent



1 2 3 4 5 6 7

Plate 1: Phytochemical analysis of the aqueous extract of *Azadiracta* and *Parthenium* sp

The authors performed the poisoned food technique to study the effect of commonly available plant extract on the growth of *Fusarium* sp.

The results were presented in Table 2, Plate 2 and Figures 3 and 4.

Table 2: Results of poison food technique against *Fusarium* sp.

Particulars	*Azadiracta* sp.			*Parthenium* sp.		
Concentration of the plant extract (%)	2	5	10	2	5	10
Mycelial growth (mm)	42	38	32	74	65	60
Percentage of inhibition	53.3	57.8	64.4	17.8	27.8	33.33

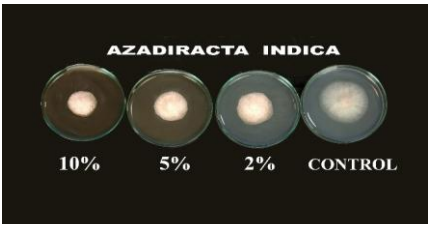


Plate 2: Mycelial inhibition of Azadiracta and Parthenium

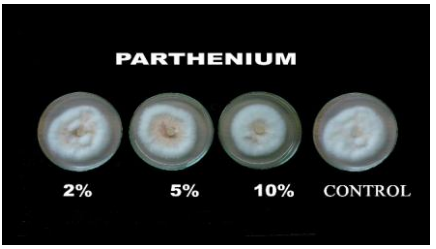


Fig 3:

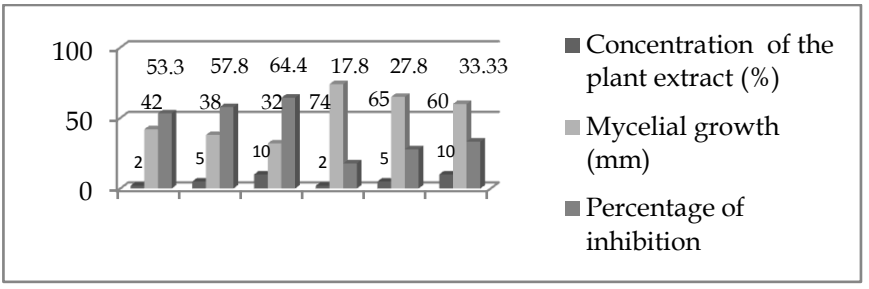


Figure 4: Mycelial inhibition of plant extract in growth of *Fusarium* sp.

The study revealed that Azadiracta at 10% is very effective to control *Fusarium* sp. followed by 5% and 2% of Azadiracta respectively. Azadiracta at 10% showed 64.4% of mycelial inhibition followed by 57.8 and 53.3 % for 5 and 2% of the Azadiracta extract respectively. In case of the *Parthenium* sp. extract at 10% concentration, it showed only 33.33%. From this study it was clear that the plant extract Azadiracta was very

effective to control *Fusarium* sp when compared to *Parthenium* sp. The study highlights the need to explore the potential or using the plant extract for developing plant based natural fungicide to control seed.

The popularisation and much desired mass adoption of botanicals by the farmers cannot be achieved only through workshops, farmers meet, exhibitions and policy decisions. The real imputer to this shall come only through coordinated and interactive joint field work of people involved with extension activities, industry staff and farmers in field situation and by adoption of botanicals. Non acceptability of many of the antagonistic plants by farmers, on account of farm economics is one of the major constraints in their utilisation. An ideal antagonistic plant extract is required to be used for the control of plant pathogen. The plant based formulation must be compactable with the crop and should be antagonistic to far set pathogens but should not have adverse effect on beneficial flora and fauna, particularly, natural enemies of pathogen and pests. It should keep the pest and pathogen in its economic threshold level and not be highly deleterious to non target organism. In addition to the antagonistic use, it is better to have the plant having other economic uses to contribute the revenue of the farmer.

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