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Efficacy of Certain Plant Extracts as Safe Fungicides Against Phytopathogenic and Mycotoxigenic Fungi

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Abstract

The efficiency of different natural plant extracts and of the chemical fungicide Micronite was carried out to determine their effects on the soil fungi particularly phytopathogenic and mycotoxigenic fungi *Fusarium oxysporum*, *Alternaria alternata* and *Aspergillus flavus*. *In vitro* studies were carried out to test the antifungal activity of 4 plant extracts; performed with either cold distilled water. The results revealed that plants extracts had a strong antifungal activity with significant inhibition on the growth of the all tested fungi. Extracts of *Azadirachta indica* and *Jatropha curca*s were the most effective to inhibit the growth of the tested fungi. On the other hand, the chemical fungicide was more efficient than the natural compounds. Different concentrations of plant extract of *A. indica* and of chemical fungicide were studied on the growth of *Aspergillus flavus* and *Alternaria alternate*. Findings from this study confirmed that plant extracts can be used as natural fungicides to control pathogenic fungi, thus reducing the dependence on the synthetic fungicides. *Azadirachta indica* extract, which was found to be the most efficient extract, might be a promising agent for controlling these fungi.

Keywords

Fungi, Bioevaluating, Fungicide, Fungi, Mycotoxigenic, Phytopathogenic

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1. Introduction

To avoid the hazardous effects of chemicals, natural products of some plants have been used to control plant disease (Rahber-Bhatti, 1986; Bowers and Locke, 2000; Momin *et al.,* 2001). Development of safer anti-fungal agents such as plant extracts to control phytopathogens in agriculture was reported in recent years (Imtiaj et al., 2005; Tumen et al., 2013).The essential oils and their constituents have been found effective as antifungal agent (Daferera *et al.,* 2000; Sridhar *et al.,* 2003). Several reports have been made on the fungicidal properties of neem oil (Kazmi *et al.,* 1995). Locke (1995) reported that in field *Alternaria alternata, Aspergillus niger and Fusarium oxysporum* has been completely controlled by using 2-10% neem oil. It is observed that mustard seed oil also showed antifungal activity (Dhingra *et al.,* 2004). Houghton *et al*., (2006) reported antifungal activity of asafoetida against *Microsporeum gypseum* and *Trichophyton interdigitale*. Thyagaraia & Hosono (1996) also studied the inhibition effect of asafoetida on *Rhizopus sporus, Mucor dimorphosphorous, Penicillium commune* and *Fusarium solani.* Extracts from plants such as garlic (*Allium sativum*) (Obagwu and Korsten, 2003), *Azadirachta indica*, *Moringa oleifera* (Adandonon *et al*., 2006), *Ferula communis* and *Dittrichia viscose*, *Juniperus communis* (Menghani and Sharma 2012) have been tested on many other soil borne fungi. Alkhail (2005) showed that extracts of *Allium sativum, Azadirachta indica* and *Eugenia caryophyllus* presented remarkable biological activity when tested against fungi viz., *F. oxysporum, Botrytis cinerea*.

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The antimicrobial and antitoxin properties of some plants, herbs, and their components have been documented since the late 19th century (Saadabi, 2006, Fawzi *et al.*2009, Zaker and Mosallanejad 2010, Abdulghaffar *et al*. 2010; Abdel Ghany and Hakamy 2014). These natural plants involve garlic, lemon grass, datura, acacia, a triplex, ginger, black seed, neem, basil, eucalyptus, *Juniperus procera*, alfalfa and basil (Omar and Abd-El-Halim, 1992; Aly *et al.,* 2000; Aly and Bafiel, 2008; Abdel Ghany 2014). They are safer to human and the ecosystem than the chemical antifungal compounds, and can easily be used by the public who used them for thousands of years to enhance flavor and aroma of foods as well as its economic value (Shelef, 1983). An emerging alternative to random chemical synthesis is the study and exploitation of naturally occurring products with fungicidal properties. Plants produce an enormous array of secondary metabolites, and it is commonly reasoned that a significant part of this chemical diversity serves to protect plants against plant pathogens. A problem with plant-produced compounds as potential fungicides is that in the natural state, they are generally only weakly active compared to synthetic fungicides. The efficiency of different natural plant extracts and chemical fungicide (Micronite) was carried out to determine their effects on the soil fungi particularly phytopathogenic and mycotoxigenic fungi *Fusarium oxysporum* and *Alternaria alternata* and *Aspergillus flavus* . *In vitro* studies were carried out to test the antifungal activity of 4 plant extracts; performed with either cold distilled water. Therefore, this work aimed to study the evaluating the antifungal activity of natural compounds of plant extracts with comparing of chemical fungicide (Micronite).

2. Materials and Methods

2.1. Plant samples and Fungal Culture Used

Leaves of *Azadirachta indica*), *Jatropha curcas*, *Ricinus communi and Allium sativum* were dried and ground into a fine powder in an electric grinder and extracted with distilled water. The extract was added for growth medium for testing the antifungal properties against fungal species *Aspergillus flavus, Alternaria alternate, Fusarium oxisporium, Rhizopus stolinifier* and *Cladosporium herbaru*.

2.2. Poisoned Food Technique

Potato dextrose agar medium (PDA) with different concentration plant extracts of the test plantswere prepared. About 25 ml of the growth medium was poured into each petri-dish and allowed to solidify. Five mm disc of 5-day-old culture of the test fungi was placed at the center of the petridish and incubated at 27°C for 7 days, the growth was measured in millimeter. For each treatment three replicates were maintained. PDA medium with micronite (chemical fungicide was used as a positive control. PDA medium without the methanolic extract served as control. The fungitoxicity on the extracts in terms of percentage inhibition of mycelia growth was calculated by using the formula: % inhibition =dc–dt/dc.100; Where, dc = average increase in mycelial growth in control, dt = Average increase at each treatment (Singh and Tripathi, 1999).

2.3. Chemical Fungicide

Micronite: Micronite containing active ingredient 80% sulfur (Fig. 1) produced by National company for agrochemical production used as fungicide 25g / 10 liter water.

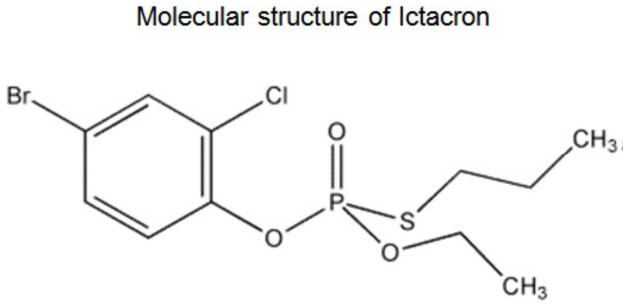

Fig. 1. Chemical structure of Micronite.

3. Result and Discussion

The results revealed that plants extracts had a strong antifungal activity with significant inhibition on the growth of the all tested fungi. Extracts of *Azadirachta indica* and *Jatropha curcas* were the most effective to inhibit the growth of the tested fungi. On the other hand, the chemical fungicide was more efficient than natural compounds. Garlic plant extract in this study showed antifungal activity (Table 1) The results of this study corresponds with work done by William (2008) who reported that sprays made from aqueous garlic extracts have antibiotic and antifungal properties and will suppress a number of plant diseases, including powdery mildew on cucumbers and to some extent black spot on roses. Similar results were reported by Slusarenko *et al.* (2008) who tested the effectiveness of garlic juice against a range of plant pathogenic bacteria, fungi and oomycetes *in vitro*. The effects of the antifungal compounds may be on spore germination leading to its inhibition or may be due to effect of these compounds on the cell wall altering its permeability (William, 2008). Our results showed the antifungal activity of *Azadirachta indica* extract. Mycelial growth of various species of *Fusarium* was inhibited by the plant extracts of *Allium cepa* (Patel, 1989), *Cassia nodosa* (Reddy and Reddy, 1987); *Azadirachta indica* (Eswaramoorthy *et al*, 1989);, *Allium sativum* and *Sapindus trifoliata* (Gohil and Vala, 1996); Neem seed extract (Gour and Sharmaik, 1998),

Eucalyptus amygdalina, (Bansal and Gupta, 2000). In accordance with the above reports, in the present study, 87.76 % inhibition of mycelial growth of *Aspergillus flavus* of leaf extracts of *Azadirachta indica* and 79.09 % inhibition of *Alternaria alternata* of mycelial growth (Table 1). Different concentrations of plant extract of *Azadirachta indica* and chemical fungicide were studied on the growth of *Aspergillus flavus*, *Fusarium oxysporum* and *Alternaria alternate* (Fig.2). As well as increasing in the concentration of *Azadirachta indica* extract and chemical fungicide inhibition of *A. flavus* and *Alternaria alternata* increased (Table 2 and 3). The plant extract of *Azadirachta indica* exhibited effect on *Alternaria alternata* spores causing decreasing in the size and rate of sporogenesis. This result is agree with result obtained by Mondall *et al*. (2009) where the crude aqueous and alcoholic leaf extracts of *Azadirachta indica* was more effective in inhibitions of growth of the fungi *Aspergillus* in comparison to inhibitory effects on *Rhizopus* growth in the artificial culture medium. Alam *et al.* (2004) tested five plant extracts against conidial germination of *Fusarium oxysporum* and reported that the extract of *Calotropis procera* showed high inhibitory effect. Effect of plant extracts on conidial germination, mycelial growth and sporulation of *Aspergillus flavus, A. niger* and *A. fumigatus* were examined (Locke, 1995; Bansal and Gupta, 2000; William, 2008) where, *Lowsonia inermis* inhibited conidial germination of *A. flavus* and *A. fumigatus*, while *A. niger* was mostly inhibited by *A. indica*. On the other hand chemical fungicide exhibited more effect on spores causing very reductin in the size and number of cells per conidiospores. Findings from this study confirmed that plant extracts can be used as natural fungicides to control pathogenic fungi, thus reducing the dependence on the synthetic fungicides. *Azadirachta indica* extract, which was found to be the most efficient extract, might be a promising material for controlling these fungi. Mixture of chemical fungicide with *A. indica* extract (Table 4) increased the antifungal activity against tested fungi.

4. Conclusion

The results of the present study revealed that *J. curcas*, *A.indica*, *A. sativum* and *R. communis* extracts have been emerged as safe alternatives to replace chemical fungicides and can be used as eco-friendly fungicides. Further work is required to increase the efficacy of these plant extracts in the field and also to determine the biologically active ingredient present in extracts as well as its mode of action.

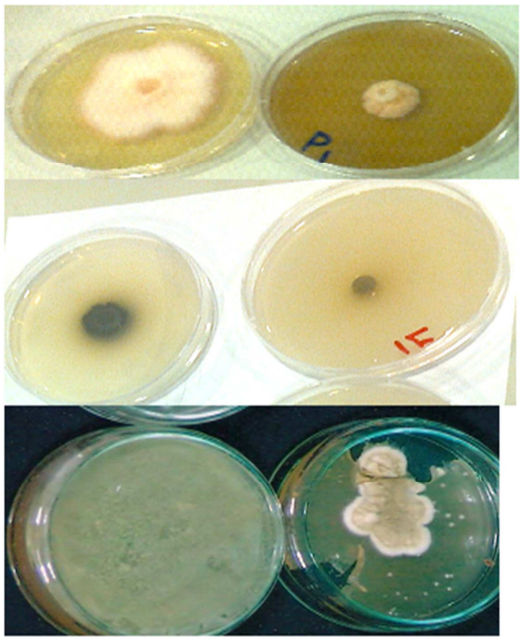

Fig. 2. Effect of *Azadirachta indica* extract on *Fusarium oxysporum*, *Alternaria alternata* and *Aspergillus flavus* From higher to lower respectefly. Left is Control without treatment; Right is treated with Azadirachta indica extract.

Table (1). Inhibitory effect of natural plant extracts and chemical fungicide. Data are expressed as % of fungal growth inhibition.

Chemical fungicide	Control	*Jatropha curcas*	*Azadirachta indica*	*Allium sativum*	*Ricinus communis*	Test fungi
94.98	0.00	77.08	87.76	66.8	45.9	*A. flavus*
80.76	0.00	56.80	78.76	54.87	78.77	*P. cyclopium*
88.67	0.00	67.54	49.65	43.98	48.98	*F. oxysporum*
95.00	0.00	78.00	79.09	45.89	69.32	*A. alternata*
90.45	0.00	70.60	77.08	48.78	70.45	*R. nigricans*
94.50	0.00	89.50	84.56	76.76	48.34	*C. herbarum*

Table (2). Effect of different concentrations of chemical fungicide.

Colony radius (cm)		Concentration mg %
Alternaria alternata	*Aspergillus flavus*	
6.3	6.5	Control
5.5	4.0	1
2.0	2.5	2
1.3	1.6	4
0.0	0.0	6

Table (3). Effect of different concentrations of *Azadirachta indica* extract.

Colony radius(cm)		Concentration mg %
Alternaria alternata	*Aspergillus flavus*	
6.3	6.5	Control
6.3	6.0	1
4.0	5.5	2
3.4	3.7	4
2.5	3.3	6

Table (4). Effect of different concentrations of mixture in equal amount of *Azadirachta indica* extract and Chemical fungicide.

Colony radius (cm)		Concentration mg %
Alternaria alternata	*Aspergillus flavus*	
6.3	6.5	Control
5.1	4.0	1
1.5	1.5	2
1.2	1.2	4
0.0	0.0	6

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