



E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2019; 7(3): 33-35
Received: 14-03-2019
Accepted: 19-04-2019

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Antimicrobial activity of biocontrol agents against soil born phytopathogens

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Abstract

Soil borne pathogens are one of major concern in agriculture which significantly decreases the plant yield. Among pathogens phytopathogenic fungi is most devastating. Chemical are used to control plant pathogens which cause environmental, ecological and human health hazards, so natural pesticides are used which are known as biopesticides. In this study the effect of plant products and cow urine was observed on certain species of *Alternaria*, *Fusarium* and *Helminthosporium*. The phytopathogens were obtained by different methods were used. For plant extract preparation *Cassia lunata* and *Calotropus procera* were used. The extract antifungal activity of plant and cow urine distillate was observed separately and a combination of both cow urine distillate and plants extract was also used. *Calotropus procera* showed maximum inhibition upto 70% and the inhibition was increased when concentration of extract was increased. Cow urine also showed successful decrease in fungal activity.

Keywords: Phytopathogenic, plant extract, biocontrol agents, antifungal activity, biopesticides

1. Introduction

Agricultural production of the world sustains annual loss of about 20 to 30% on an average due to plant diseases in different crops and in different countries. Plant diseases are one of the major bottlenecks in agricultural production particularly in irrigated crops, in monoculture cultivations and in certain widely grown rainfed crops as well. The micro-organisms responsible for causing the most diseases utilize the plant food available in different parts viz. leaves, flowers, fruits, stems, roots etc. and grow inside or on the surface of these plant parts under favorable conditions and develop different kinds of symptoms on the affected parts. Among the causal agents of infectious diseases of crop plants however, phytopathogenic fungi play a dominant role by causing devastating epidemics. The most promising fungi include *Alternaria*, *Fusarium* and *Helminthosporium* which play an important role in causing various hazardous diseases in food crops. In order to prevent the plant diseases and to protect the crop plants against pathogens chemical control methods are in practice. The use of chemicals has helped increase of yields obtained but, one of the major problems with the constant use of chemicals is that resistance can be induced in target organisms and contaminate the environment with very toxic substances^[1, 2]. So the natural plant products and their analogues are an important source of new agricultural chemicals^[3, 4]. Pesticides of plant origin^[5] plant extracts, Phyto-products; cow urine^[6]. Cow urine possesses antiseptic and mild anti-fungal properties. Cow urine can also be used, mixed with herbal extracts to strengthen the plant, repel pests and reduce fungal problems^[7]. On the basis of efficacy of plant extract and cow urine distillate present investigation is carried out to find out the effective plant products for the management of few species of *Alternaria*, *Fusarium* and *Helminthosporium*.

For this study following objectives are selected:

1. Isolation of microorganism from the infected plant.
2. Characterization of microorganisms.
3. Effects of plant extract on the growth of microorganisms.
4. Effects of cow urine on the growth of microorganisms.

2. Materials and Methods

2.1 Test organism

Four phytopathogens *Alternaria solani*, *Fusarium oxysporum*, *Fusarium graneium* and *Helminthosporium oryzae* were used for the experiments.

2.2 Medium Used

Czapek Dox agar medium was used for the growth of fungus.

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2.3 Isolation of Organism

Fusarium oxysporum were isolated from chickpea rhizosphere by serial dilution method. *Alternaria solani* and *Helminthosporium oryzae* were isolated from brinjal and rice infected leaves respectively. Diseased leaves were thoroughly washed in running tap water. Small piece of the leaves with leaf spot symptoms were cut from the sample, washed with sterile water, and surface sterilized using mercuric chloride solution (1:1000) for 30 seconds and then washing in several changes of sterile water and transferred aseptically on Czapek Dox agar petriplates and incubated at 28°C. Isolates were confirmed by the characters given by Von Arx (1974).

2.4 Preparation of plant extract

Parts of *Cassia lunata* and *Calotropus procera*, were surface sterilized by washing with 70 % ethanol and finally with sterilized water to remove the traces of ethanol. These were then, crushed and extracted in 20 ml of sterilized water.

2.5 Assay of antifungal activity of plant extract

5 ml of prepared aqueous extract of each plant/part was mixed with 10 ml of molten Czapek Dox agar medium in a petriplates separately and agitated thoroughly to mix the extract homogeneously.

Control sets were prepared similarly using 5 ml of sterilized distilled water instead of extract. Fungal disc (5 mm diameter), cut from periphery of 7 days old culture of *Alternaria solani*, *Fusarium oxysporum*, *Fusarium graneium* and *Helminthosporium oryzae* were inoculated aseptically in each assay. Fungi toxicity was calculated and recorded in terms of % inhibition of mycelial growth. Experiments were repeated twice and each set contained three replicates.

2.6 Assay of antifungal activity of cow urine distillate

The cow urine distillate was prepared and was mixed in 12 ml of molten Czapek Dox agar medium in petriplates. Control sets were prepared similarly using 1 ml of sterilized distilled water instead of urine. Fungal disc (5 mm diameter), cut from 7 days old culture of *Alternaria solani*, *Fusarium oxysporum*, *Fusarium graneium* and *Helminthosporium oryzae* were inoculated aseptically in each assay. The plates were incubated at 28°C for 5 days. Fungi toxicity was calculated and recorded in terms of % inhibition of mycelial growth.

2.7 Determination of minimum inhibitory concentration (Mic) level

Minimum inhibitory concentration (MIC) was determined using different concentration (0.5 to 5 ml) of extracts. Assay method is same as above.

2.8 Combined effect of cow urine distillate and plant extract against Phyto pathogen

1 ml cow urine distillate and 1 ml plant extract mixed in 15 ml of molten Czapek Dox agar in petriplates. Control sets were prepared similarly using 2 ml of sterilized distilled water instead of urine distillate and plant extract. Assay method is same as above.

3. Results and Discussion

Extract of *Cassia lunata* and *Calotropus procera* used in this study were tested form *Fusarium oxysporum*, *Helminthosporium oryzae*, *Alternaria solani* and *Fusarium graneium* Phyto fungi to determine their antifungal activity.

Different concentration of each plant extract (0.5ml 1ml 2ml 3ml 4ml 5ml) was tested against fungi. The microbial culture was grown in Czapek Dox medium with different concentration of plant extract at 28^o C. After 7 days of incubation the % of growth inhibition were measured.

The inhibition effect of plant extract used against plant pathogenic fungi were represented in tables 1, 3, 5. The highest inhibition effect of extract of *Calotropus procera* leaves showed 70.3% inhibition on *Fusarium oxysporum*, 37.5% inhibition on *Alternaria solani*, 32.5% inhibition on *Fusarium graneium* and 22.2% inhibition on *Helmeinthosporium oryzae*.

Fresh flower of *Calotropus procera* showed maximum inhibition activity against *Fusarium oxysporum* and *Helminthosporium* respectively (Table-3). The lowest 57.1% inhibition of *Fusarium graneium* record in 5ml concentration after 7 days of incubation (Table-3). It has showed the rate of inhibition increase with the increase of concentration of plant extract.) Same finding were observed with extract of *Calotropus procera* showed high inhibitory effect against *Fusarium oxysporum* [8]. But the aqueous extract of *Calotropus procera* leaves showed maximum growth inhibition 87.5% against *Fusarium graneium* and 78.1% *Alternaria solani*. *Fusarium oxysporum* showed less than 50% inhibition (Table-5). It was also reported in other studies that only 24% inhibition of *Fusarium oxysporum* with different plant extract [8]. Few other studies supported that *Calotropus procera* completely inhibit the growth of *Fusarium solani* [9].

Table 2, 4 and 6 showed that the effect of cow urine distillate (1ml) on the growth of *Fusarium graneium*, *Fusarium oxysporum*, *Helminthosporium oryzae* and *Alternaria solani* Phyto pathogen. The result were comparable to the studies of Basale and Lee, According to them fresh cow urine distillate has positive response in suppression of mycelial growth of *Fusarium solani*, *Fusarium oxysporum* and *S. sclerotiorum* and 72.1% and 61% inhibition with 1ml *Calotropus procera* leaf extract [10].

Alternaria solani showed 60% inhibition but other three test organism inhibitions were less than 50% (36.6%, 47.3%, 42.1%) but cow urine distillate showed maximum inhibition 90% of *Alternaria solani* and 80% of *Fusarium graneium* with 1ml *Calotropus* flower (Table-3). In this study concentration (1ml) of *Calotropus procera* extract in combination with cow urine showed the highest inhibition of mycelial growth. Jabin (2003) reported that cow urine has inhibitory activity against *Fusarium semitectum* and cow urine distillate mixed with leaf extract of *Calotropus procera* and *Calotropus alata* completely (100%) inhibited the mycelial growth of the pathogen [11]. Cow urine has been proved to be inhibiting to the mycelial growth of *Fusarium oxysporum*, *Fusarium solani* that cause wilt in different plants [12].

3.1 Tables and Figures

Table 1: % Inhibition of mycelial growth by *Cassia lunata* leaf.

S. N.	Organisms	Different conc. of Plant extract					
		0.5 ml	1.0 ml	2.0 ml	3.0 ml	4.0 ml	5.0 ml
1	<i>Alternaria solani</i>	27.2	33.3	39.3	44.4	45.4	57.5
2	<i>Fusarium oxysporum</i>	37.0	40.7	51.8	55.5	62.9	70.3
3	<i>Fusarium graneium</i>	20.0	25.0	27.5	30.0	30.0	32.5
4	<i>Helminthosporium oryzae</i>	13.8	13.8	16.6	16.6	19.4	22.2

Table 2: % Inhibition of mycelial growth by Cow urine distillate & *Cassia lunata* leaf

S. N.	Organisms	Cow Urine distillate & <i>Cassia lunata</i> (1 ml each)
1	<i>Alternaria solani</i>	60
2	<i>Fusarium oxysporum</i>	36.6
3	<i>Fusarium graneium</i>	47.3
4	<i>Helminthosporium oryzae</i>	42.1

Table 3: % Inhibition of mycelial growth by *Calotropus procera* flower

S. N.	Organisms	Different conc. of Plant extract					
		0.5 ml	1.0 ml	2.0 ml	3.0 ml	4.0 ml	5.0 ml
1	<i>Alternaria solani</i>	27.7	36.1	41.6	47.2	52.7	61.1
2	<i>Fusarium oxysporum</i>	10.0	20.0	3.33	43.3	53.3	76.6
3	<i>Fusarium graneium</i>	28.5	39.2	42.8	50.0	50.0	57.0
4	<i>Helminthosporium oryzae</i>	29.7	32.4	37.8	59.4	62.1	64.8

Table 4: % Inhibition of mycelial growth by Cow urine distillate & *Calotropus procera* flower

S. N.	Organisms	Cow Urine distillate & <i>Calotropus procera</i> flower (1 ml each)
1	<i>Alternaria solani</i>	80
2	<i>Fusarium oxysporum</i>	63.3
3	<i>Fusarium graneium</i>	90.0
4	<i>Helminthosporium oryzae</i>	52.6

Table 5: % Inhibition of mycelial growth by *Calotropus procera* leaf

S. N.	Organisms	Different conc. of Plant extract					
		0.5 ml	1.0 ml	2.0 ml	3.0 ml	4.0 ml	5.0 ml
1	<i>Alternaria solani</i>	12.5	43.0	62.5	65.6	71.8	78.0
2	<i>Fusarium oxysporum</i>	10.0	20.0	26.6	33.5	36.6	43.0
3	<i>Fusarium graneium</i>	55.0	60.0	70.0	75.0	77.5	87.5
4	<i>Helminthosporium oryzae</i>	54.5	61.3	63.6	65.9	70.4	75.0

Table 6: % Inhibition of mycelial growth by Cow urine distillate & *Calotropus procera* leaf

S. N.	Organisms	Cow Urine distillate & <i>Calotropus procera</i> leaf (1 ml each)
1	<i>Alternaria solani</i>	77.1
2	<i>Fusarium oxysporum</i>	33.3
4	<i>Helminthosporium oryzae</i>	44.7

4. Conclusions

The effects of different herbal extract were observed on *Helminthosporium oryzae*, *Fusarium graneium*, *Fusarium oxysporum* and *Alternaria solani*. We have prepared these herbal extracts by grinding plant parts in water. These herbal extract have a toxic effect on organism and showed by zone of inhibition. Dry leaf extracts having shaking time of 24 hrs. Of *Calotropus procera* showed maximum zone of inhibition of against. Dried flower of *Calotropus procera* having 24 hrs shaking time showed maximum zone of inhibition of with water against. Fresh leaves of *Cassia lunata* having shaking time of 30 minutes showed maximum activity of inhibition of diameters with water extract against. The highest inhibition effect of extract of *Calotropus procera* leaves was 5ml and it showed 70.3% inhibition on *Fusarium oxysporum*, 37.5% inhibition on *Alternaria solani*, 32.5% inhibition on *Fusarium graneium* and 22.2% inhibition on *Helminthosporium oryzae*. Fresh flower of *Calotropus procera* showed maximum inhibition activity against *Fusarium oxysporum* and

Helminthosporium respectively. The lowest 57.1% inhibition of *Fusarium graneium* record in 5ml concentration after 7 days of incubation. It has showed the rate of inhibition increase with the increase of concentration of plant extract. Fresh cow urine distillate has positive response in suppression of mycelial growth of *Fusarium solani*, *Fusarium oxysporum* and *Sclerotinia sclerotiorum* and 72.1% and 61% inhibition with 1ml *Calotropus procera* leaf extract. *Alternaria solani* showed 60% inhibition but other three test organism inhibitions were remain less than 50% (36.6%, 47.3%, 42.1%), but cow urine distillate showed maximum inhibition 90% of *Alternaria solani* and 80% of *Fusarium graneium* with 1ml *Calotropus* flower.

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