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Antagonistic activities of some plant extract against *Trichoderma harzianuum* and *Pseudomonas agarici* causing disease in mushroom

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ABSTRACT

In this study, antimicrobial activities of different plant samples were tested against *Trichoderma harzianum* and *Pseudomonas agarici*, which causes disease green mold and yellow blotch in mushroom respectively. For this more than 100 plant leaf samples were collected from different regions of eastern Uttar-Pradesh like Varanasi, Mirzapur, Chandauli, Chunar, Jaunpur and Gorakhpur. Some of these plants are used for the treatment of various ailments by the indigenous population. The antimicrobial activities were evaluated according to the poisoned food technique using aqueous and alcoholic extract of the leaf samples against both, the test fungus and bacteria. In the end of the experimental studies the plants such as; *Psidium guajava, Polyalthia longifolia, Andrographis paniculata, Ocimum sanctum, Jatropha sp, Parthenium hysteroporus, Ficus religiosa* etc. leaf samples were found to be effective against the fungus *T. harzianum.* On the other hand the plant extracts of *Ricinus communis, Parthenium hysteroporus, Syzygium cumuni, Emblica officinalis, Woodfordia floribenda, Polyalthia longifolia, Andrographis paniculata, Euphorbia hirita and Nerium indicum* were found to be effective against the bacteria *P. agrici*. We are reporting about antifungal as well as antibacterial property of some plant extract against *Trichoderma harzianum* and *Pseudomonas agarici*.

Keywords: Alcoholic Extract, Antimicrobial, Aqueous extract, Mushroom, *Pseudomonas agarici*, *Richoderma harzianum*

Introduction

Mushroom is a fungus belonging to *Basidiomycota*. The part of the organism that we see and call a mushroom is just the fruiting body. It is taken as a good source of edible food in whole world. But disease like Green mold caused by *Trichoderma harzianum* and Yellow blotch caused by *Pseudomonas agarici* are the major obstacles to the mushroom industry. They can reduce the mushroom production up to a great extent. It has been tradition in India that since ancient times plants and its extracts have been used as a good source of medicine for the treatment of human diseases. According to world Health Organisation, plant extracts are used by more than 70% of world population for traditional treatment (Anonynous, 1993). Over fifty percent of modern drugs are obtained from plant origin (Baker *et al*., 1995).The extracts of various plants possess antimicrobial activities. These plants may be herbs, shrubs and trees belonging to different families and distributed all over the world. The extracts are obtained from various plant parts such as leaves, stems, roots, flowers, fruits and oils which inhibit the growth of the pathogen. By applying these extracts on crops the disease is checked without disturbing the ecosystem. Thus the plant extracts are eco friendly. The antagonistic activities of plant extracts against

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micro-organisms have been studied by a large number of researchers in all over the world (Triach *et al*., 1998, Hammer *et al*., 1999, Jiapiyasakul *et al*., 2001, Sinha & Verma 2002, Russo *et al.*, 2003, Mahansen, 1996, Ates *et al.*, 2003, Prabhakar *et al*., 2005, Bobbarala *et al.* 2009, Pundir & Jain, 2010).The objective of the present study was to evaluate the antimicrobial activities of the collected plant samples from different regions of eastern Uttar Pradesh.

Materials and Methods

Plant materials

Leaf samples were collected from different regions and districts of eastern UP like Mirzapur, Varanasi, Chandauli, Jaunpur, Chunar and Gorakhpur. More than 100 plant samples were collected.

Extract preparation

Two types of extracts (aqueous and alcoholic) were prepared by the method of Saha *et al*., 2004. In aqueous extract preparation 2 gm of fresh leaf samples were crushed in a mortar and pestle with four ml sterilized distilled water. The solution was filtered through muslin cloth and centrifuged at 10,000 rpm for 10 minutes. Supernatant were taken as the aqueous extract. In alcoholic extract preparation leaf samples were dried in shade for 3-4 days. It was grounded to make powder and 1gm of the powder was taken with 4 ml of ethanol. After 4-5 days it was filtered through what man filter paper and used as alcoholic extract.

Preparation of Test Microorganisms

Test microorganisms used were *Trichoderma harzianum and Pseudomonas agarici* (which causesgreen mold and yellow blotch disease in mushroom, respectively. These two test microorganisms were obtained from the Mushroom laboratory, Department of Mycology and Pathology, Institute of Agricultural Science, BHU, Varanasi. Spore suspension of *Trichoderma* was prepared in 10 ml sterilized distilled water. Bacterial culture was prepared in a 10 ml nutrient broth and incubated for 24 hrs. at 28±1ºC**.** Antifungal activity estimated and it was studied by poisoned food technique (Nene & Thapliyal 1982).

Food poisoned technique for test fungus

For food poisoned technique about 200 µl of spore suspension was poured in a sterilized petriplate and after this approx 20 ml sterilized, cooled PDA media was poured in the plate. After solidification one 8 mm well was made in the middle of the plate by cork-borer. 200 µl of plant extract (aqueous and alcoholic separately) was again poured into the well. Different solutions were made for each plant extracts. Plate without any botanical extract was treated as control. All the plates were kept at 28±1ºC for 3 days in a BOD incubator. Percentage of inhibition was measured by the formula given by Vincent (1947).

Formula for % inhibition 'I' = $\frac{(C-T)}{C} \times 100$ (given by Vincent 1947)

Where, **I** = Per cent inhibition

C = Radial growth in control

T = Radial growth in treatment

Food poisoned technique for Test bacteria

For evaluation of anti-bacterial activity nutrient agar plates were prepared. Four wells of 8 mm diameter were made by using a sterile cork-borer. One well in the middle and rest three in a triangular form). 50µl of bacterial culture was poured into the mid well and 100 µl of botanical extract (aqueous and alcoholic separately) poured in each of the three wells. Plates without botanical extract were treated as control. All the plates were incubated at 28±1ºC for 24hrs. Percentage of inhibition was calculated by the formula given by Vincent, 1947.

Results and Discussion

In the present investigation all the plant extracts were evaluated under *in vitro* condition against both

Table 1 Result of Food poisoning technique against *Trichoderma harzianum.*

Plant samples	Colony growth in control (cm)	Zone of inhibition for aqueous extract treatment (cm)	% inhibition	Zone of inhibition for alcoholic extract treatment (cm)	% inhibition
Jatropha gossypiflia	9	3.4	37.78	1.4	15.56
Plumbago zeylanicia	9	1.2	13.33	1.3	14.44
Parthenium hysteroporus	9	1.3	14.44	2.1	23.33
Psidium guajava	9	1.4	15.56	3.8	42.22
Ficus religiosa	9	1.5	16.67	1.4	15.56
Ocimum sanctum	9	1.9	21.11	1.2	13.33
Andrographis paniculata	9	1.1	12.22	1.3	14.44
Annona squamosa	9	1.3	14.44	1.2	13.33
Lycopersicum esculentum	9	1.4	15.56	1.5	16.67
Polyalthia longifolia	9	1.4	15.56	1.7	18.89
Musa paradisiaca	9	1.3	14.44	1.1	12.22
Trichodesma indicum	9	1.2	13.33	1.3	14.44

Table 2 Result of Poisoned food technique against *Pseudomonas agarici.*

Plant samples	Colony growth in control (cm)	Zone of inhibition for aqueous extract treatment (cm)				% Inhibition	Zone of inhibition for alcoholic extract treatment (cm)				% Inhibition
		W_1	W_2	W_3	Avg.		W_1	W_2	W_3	Avg.	
Pongamia pinnata	9	2.5	2.5	2.0	2.33	25.89	2.3	2.0	2.2	2.16	24.07
Ricinus communis	9	3.5	3.5	4.0	3.67	40.74	2.5	2.5	2.0	2.33	25.92
Emblica officinalis	9	4.5	3.5	4.0	4.00	44.44	3.5	2.5	2.0	2.67	29.62
Woodfordia floribenda	9	3.0	2.5	3.0	2.83	31.48	3.3	3.3	3.0	3.20	35.56
Euphorbia hirita	9	3.0	3.5	3.0	3.16	35.19	2.7	3.2	3.0	2.97	32.96
Syzygium cumuni	9	2.5	3.5	3.0	3.00	33.33	2.3	3.0	2.8	2.70	30.00
Barleria prionites	9	4.0	3.0	3.0	3.33	37.03	2.8	2.8	2.8	2.80	31.11
Lycopersicum esculentum	9	2.3	2.4	2.0	2.33	24.81	3.0	2.3	2.5	2.60	28.89
Polyalthia longifolia	9	2.5	2.5	2.5	2.50	27.78	3.8	4.2	3.7	3.90	43.33
Rosa indica	9	2.5	2.7	2.3	2.50	27.78	3.0	3.0	2.3	2.76	30.74
Nerium indicum	9	3.2	2.7	2.4	2.77	30.74	2.8	3.0	1.5	2.43	27.03
Andrographis paniculata	9	2.2	2.4	2.0	2.20	24.44	4.0	3.0	4.0	3.67	40.74
Parthenium hysteroporus	9	1.6	2.4	2.4	2.13	23.70	2.3	2.0	3.0	2.43	27.03
Magnifera indica	9	2.4	1.6	2.1	2.00	22.22	3.0	2.8	2.0	2.60	28.89
Carissa carandas	9	2.0	1.6	2.4	2.00	22.22	3.0	2.8	2.5	2.76	30.74

Trichoderma harzianum and *Pseudomonas agarici* to know the antagonistic nature of these extracts. Though complete inhibition (100% inhibition) of the pathogen was not observed but significant amount of inhibition was noticed in some of them. In the study of poisoned food technique against *T. harzianum* alcoholic extract of *Psidium gujava* showed the best result. It reduced the mycelial growth upto 42.22% followed by aqueous extract of *Jatropha gossypiflia*, which reduced the mycelial growth upto 37.78%. Alcoholic extract of *Parthenium hysteroporus* and aqueous extract of *Ocimum sanctum* also showed the good result.

In case of *Pseudomonas agarici* alcoholic extract of *Polyalthia longifolia* showed the best result which reduced the bacterial growth upto 43.33% followed by

Andrographis paniculata (40.74% inhibition). In aqueous extract treatment, aqueous extract of *Emblica officinalis* showed the best result 44.44% inhibition followed by *Ricinus communis* and *Barleria prionites* which reduced the mycelial growth upto 40.74% and 37.03%, respectively. The extract of *Parthenium hysteroporus*, *Lycopersicum esculentum*, *Andrographis paniculata* and *Polyalthia longifolia* showed the antifungal as well as antibacterial activity. There are many more scientists who have worked on antimicrobial activity of plant extracts. Sinha and Verma (2002) took the extract of different plant species like *Azadirachta indica, Datura stramonium, Cassia tora, Side aquta, Ocimum sanctum* and *Cuscuta reflexa*. By poisoned food technique, they showed that all the plant extract influence the growth of fungus. By poisoned food technique Satish and Raghwendra, (2002), found that aqueous extract of *Accacia nilotia, Datura stomonium, Eucalyptus globus* and *Lawsonia inermis* having antifungal activity against *Fusarium.*

Bhaskarwar *et al.,* (2008) worked on antibacterial activity of *Jatropa podagrica* against 10 clinical isolates of *S. aureus*, *E. coli* and *Candida ablicans.* They found that stem bark extract showed the remarkable antibacterial activity as compared to stem extract. Dambhla and Joshi (2001) recorded that the turmeric powder and extract of black Tulsi leaves showed the best result on fungal growth inhibition against *Botryodiploidia theobromal.*

The present study indicates that many local plants and their extract do have anti fungal and anti bacterial activities and potential to inhibit diseases caused by *Trichoderma harzianum* and *Pseudomonas agarici.* These extracts require further study and extraction before they are recommended for commercial use.

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