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Research Article

BIOCONTROL ACTIVITY OF SOLVENT EXTRACTS OF *DATURA METEL* AND *ACALYPHA INDICA* AGAINST FUNGAL LEAF PATHOGEN OF *ARACHIS HYPOGEA*

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ABSTRACT

Datura metel and *Acalypha indica* are medicinal plants. These plants are rich in a wide variety of secondary metabolites such as tannins, terpenoid, alkaloids and flavonoid. Among the commercial crops, Groundnut (*Arachis hypogea*) is economically important and it is grown in India. The seeds contain 50% of non-drying oil and about 35% protein. Fungi are the most important common plant disease causing pathogen. 18 fungal species were isolated from infected *Arachis hypogea* plants. Bio control activities of the organic solvent plants extracts of *D. metel, A. indica* was carried out on the various test micro organism using agar well diffusion technique. The extracts restricted the growth of pathogen on the media. The maximum inhibition zone was observed in the extracts of *D. metel* and *A. Indica* against *Aspergillus niger* and *Fusarium oxysporum.* The minimum inhibition zone was observed in *Trichoderma harzianum.* Increased concentration of extracts was more effective against fungi. Extracts of *D. metel* have max zone of inhibition compare to that of *A. Indica*. The present study concludes that *Datura metel* was an effective Bio control agent.

Keywords: Biocontrol activity, Fungi, *Datura metel, Acalypha indica, Arachis hypogea*.

INTRODUCTION

India is basically an agro-based country where more than 80% of Indian population depends on agriculture. Insects are known to cause significant damage to crops and affect agricultural productivity. Several pressures have accelerated the search for more environmentally and toxicologically safe and more selective and efficacious pesticides. Most commercially successful pesticides have been discovered by screening compounds synthesized in the laboratory for pesticidal properties. Plants are rich sources of natural substances that can be utilized in the development of environmentally safe methods for insect control (Sadek, 2003). Numerous plant species have been identified as possessing pesticidal properties and have shown potential as alternative to chemical pesticides (Singh, 2000; Sahayaraj *et al*., 2003; Kaushik and Kathuria, 2004).

The plant secondary metabolites that show feeding deterrent or toxic effect to insects in laboratory have been subject of several recent volumes (Dev and Koul, 1997; Koul and Dhaliwal, 2001; Elumalai *et al*., 2008; Pugazhvendan *et al*., 2009). Plant chemicals may produce toxic effects when ingested by insects. Antifeeding activity may determine the extent of insect herbivory. Several papers have been published on the entomotoxic properties of crude extracts from different plant species (Sadek, 1997, 2003; Rodriguez-Saona and Trumble, 1999; Ciccia *et al*., 2000; Tapondiou *et al*., 2005; Ulrichs *et al*., 2008; Baskar *et al*., 2009).

Plant disease is an on-going limiting factor in crop production. Diseases of crops lead to yield losses and are of increasing importance as world population increases. A simple definition of plant disease is any disturbance that interferes with a plants normal structure, function, or economic value (Persley, 1993).

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Plant diseases can be divided conveniently into those caused by parasitic microorganisms or pathogens, and non-parasitic diseases or disorders. The major groups of plant pathogens are fungi, bacteria, viruses, and nematodes. Fungi are the most important common cause of plant disease (Persley, 1993), since they are the most widespread and destructive parasites of plants (Ingold and Hudson, 1993). More than 100,000 species of fungi are known to cause diseases in plants; some 50 species cause diseases in humans and about as many cause diseases in animals, most of them superficial diseases of the skin or appendages (Agrios, 1997).

The effect of fungi on plants can be devastating. Cellular structure can be destroyed, physiological functions of the plant impaired, and rates of metabolism and metabolic pathways can be altered. These biological processes have widespread consequences for infected plants because the impairment of any one function or organ has effects on others (Moore, 1996). Prior to fungal penetration of a plant, many 13 factors influence the sequence of events from germ tube emergence to attachment, adhesion, appresorium development, and growth on the plant surface. These may relate directly to endogenous factors such as influences from the environment, competition from other microbes, or factors relating to the host plant such as leaf age, cultivar type, and physiological condition (Isaac, 1992).

Any chemical with fungicidal properties could be potentially useful to inhibit fungal growth or sporulation. Some chemicals derived from plants that have fungicidal properties may control plant fungal pathogens. Antifungal compounds come from volatile oil producers such as tea tree, Eucalyptus, cinnamon, and others (Carson and Riley 1998; Fluck and Schib 1976; Hay and Waterman 1993; Weiss 1997).

Acalypha indica (Linn.) belongs to the family Euphorbiaceae. It is a common weed in many parts of Asia. It is an annual herb, about 80 cm high and commonly found in waste places or fields. It is locally known as "kucing galak" or "rumput lis-lis", "kuppaimeni" in India and "t‟ie han tsai" in China (Kirtikar and Basu, 1975). It grows in the common farmlands, gardens, roadside waste lands. Parts used are leaves, root, stalk and flowers. The major phytochemical constituents are alkaloids acalypus and aclyphine (Kirtikar and Basu, 1975). This plant is used as diuretic, antihelmintic and for respiratory problems such as bronchitis, asthma and pneumonia (Varier, 1996).

Datura metel (Linn.) is a Nigerian medicinal plant widely used in phytomedicine to cure diseases such as asthma, cough, convulsion and insanity. The leaves and seeds are widely used in herbal medicine as anesthetic, antispasmodic, bronchodilator and as hallucinogenic (Duke and Ayensu, 1985, Dabur *et al*., 2004). A variety of phytochemicals have been found to occur in *D. metel*. These phytoconstituents comprises alkaloids, flavonoids, phenols, tannins, saponins and sterols. The solanaceous alkaloids hyoscyamine and scopolamines have been isolated from *D. metel* (Chopra *et al.*, 1986, Oliver-Bever, 1986). Chemical control has been proved efficient and economical in controlling blight disease. However, increasing public concern on environmental issues desires that alternative management systems be evolved either to reduce pesticide dependant or naturally occurring compounds be explored to constrain the pathogen attack (Singh *et al*., 2003).

In order to search an environmentally safe alternative, scientists considered the pesticides of biological origin (bio-pesticides) in the place of synthetic insecticides. Replacement of synthetic insecticides by bio-rational insecticide is a universally acceptable and practicable approach worldwide. Throughout history, plant products have been successfully exploited as insecticides, insect repellents, and insect antifeedants. Recent plant protection researchers, particularly of the last decade revealed the importance of plant products that disrupt the normal insect growth and development. Plants are rich sources of natural substances that can be utilized in the development of environmentally safe methods for insect control (Sadek, 2003). Plants contain secondary metabolites that are deleterious to insect and other herbivores in diverse ways; through acute toxicity, enzyme inhibition and interference with the consumption and/or utilization of food (Wheeler and Isman, 2001). These botanical pesticides are highly effective, safe and ecologically acceptable.

Among the oil seed crops, Groundnut (*Arachis hypogea*) is economically important and it is grown in India for its seeds which contain 50% of non-drying oil and about 35% protein and are used in oil and feed industries or for confectionary (Mittal and Vyas, 1992). Its cultivation is severely affected by varied insect pests, among which cutworms deserve much attention for control measures. Furthermore, the usefulness of phytochemicals contained in the plants *D. metel* and *A. indica* against groundnut pests is not yet reported. Therefore, the present study was aimed to investigate the biocontrol of these two plant leaf extracts against a common groundnut plants with fungal pathogens.

MATERIALS AND METHODS

Collection and processing of plants sample

For the present study, the healthy plants *A. indica* (Family: Euphorbiaceae) and *D metel* (Family: Solanaceae) were collected in and around of Adirampattinam, Thanjavur District, Tamil Nadu, South India. The plant was identified with the help of flora of presidency, Tamil Nadu and Karnatic flora (Gamble, 1967; Matthew, 1983) and standard references (Kirthkar and Basu, 1935).

Preparation of powder

The leaves of *D. metel* and *A. indica* was collected washed, cut into small pieces and dried at room temperature (28ºC±1) for two weeks and made into powder by using mixture for further analysis.

Preparation of plant extract

Extraction is a process to separate or isolate the secondary metabolites from plant material. It is basically two types i.e. heat and cold extraction. Heat extraction has some advantage over cold extraction like time consistency and also no contamination by microbes. An apparatus called soxhlet did heat extraction. 100g of the plant leaf powder were packed into the thimble of a soxhlet apparatus. The ratio of the plant powder and solvents were maintained at 1: 4.

Methanol extract

The methanol solvent was used for the extraction of plant powder through the soxhlet apparatus to test the presence or absence of alkaloids, flavanoids, steroids, etc. The predigested dried explants were also extracted by the above procedure. The methods of preliminary phytochemical analysis have been presented. The mark root after the Methanol extraction was dried and extracted with 100 ml of Methanol (77.5-78.5°C) by continuous hot percolation, until the extraction was completed. After the completion of extraction, the extract was filtered and the solvent was removed by distillation under reduced pressure. A dark green coloured residue was obtained.

Isolation of Fungal pathogens

Pieces of fresh leaves of *Arachis hypogea* plants from were cut from diseased and healthy parts of the leaves of plant (Agrios, 1997). These leaf pieces or whole pepper berries were dipped momentarily in 70% ethanol and then soaked in a solution of 1% sodium hypochlorite for five or ten minutes. The sterilized tissues then were washed once in sterile distilled water, placed in Petri dishes containing PD agar, and maintained in an incubator at 28°C.

Leaf microflora

Leaflets (i.e. pieces of *Arachis hypogea* plant leaves up to 10 x 10 mm in area) were prepared for assessment of their active fungal populations by serial washing with distilled water (Shipton *et al*., 1981). Washing schedules were 2, 4, 7, and 10 times (two replicates each). Twelve pieces of 100 mm 2 leaflets (four pieces from one leaf) were placed in Universal bottles for each washing schedule (two replications) and washed by shaking for 2 minutes first with 1 change of 10 ml sterile surfactant Tween 80 (2 ml /litre water) using a Griffin Flask Shaker. This was followed by additional changes of sterile distilled water (10 ml).

Spread plate assessment of fungal fragments and spores recovered followed the method of Black (1999) by plating out 0.25 ml aliquots of selected washings on PDA agar in three replications. Finally, 4 pieces of washed leaflets from each washing schedule were plated (leaf plate method; Black, 1999) onto PDA agar in three replications. After incubation for 5 days at 280C, the plates were examined and the species of each fungus present were counted under a bacterial colony counter (Black, 1999) and identified.

Identification of fungi

The fungal colonies growing on the culture plates were identified morphologically on the basis of their colour, type

of spores, colony texture and other growth characteristics of the fungi (Dhingra and Sinclair, 1985). Fungal species from *A. hypogaea* plants were identified by using information from Arx (1981), Bailey and Jeger (1992), Barnett and Hunter (1972), Barron (1972), Ellis (1971), Ellis (1976), Kirk *et al*. (2001), and Rayner (1970).

Preparation of plant extracts for antifungal activity

For the preparation of extracts from leaves of *Acalypha indica* and *Datura metel* they were shade dried at room temperature and powdered by electric blender. 50g of each of the dried and powdered materials were macerated separately with 200 ml of ethanol for 48h. Extracts were concentrated under reduced pressure. The condensed products were weighed and kept at 4ºC prior to test. The unidentified fractions were thus separated and tested for antifungal activities.

Test Microorganisms

Eight fungal species were employed as fungal test organism. These include *Cladosporium sp, Aspergillus flavus, Colletotrichum gloeosporioides, Curvularia inaequalis, Fusarium verticillioides, Helminthosporium sp, Rhizopus sp,* and *Trichoderma sp* which were isolated from infected plants leaves and pure cultures were isolated and identified from the PRILS Pathological Lab, Thanjavur.

Antifungal Activity

Antifungal activity of the extracts and fractions were tested using the agar diffusion method described by Collin and Lyne (1970). The extract was tested with different concentration (10, 20 and 30µl) for the antifungal activity against the fungi such as such as *Cladosporium sp, Aspergillus flavus, Colletotrichum gloeosporioides, Curvularia inaequalis, Fusarium verticillioides, Helminthosporium sp, Rhizopus sp,* and *Trichoderma sp*. Varying concentrations of the extracts and fractions were prepared and incorporated into Potato dextrose agar. The plates were incubated at 28°C for 48 hours and inhibition of growth was noted.

RESULTS

The use of herbal preparations in the treatment of plant diseases is very common in the rural communities of world. *D. metel* and *A. indica* is frequently used for the treatment of infections, pathologies of the leaf spot disease and others diseases. The importance of this plant in folk medicine as well as its promising phytochemical properties was verified in our laboratories. The leaves powder of *D. metel* and *A.*

indica was taken in an apparatus and refluxed serially using methanol solvent system depending upon the polarity. The extracts of methanol solvent system were transferred separately in previous weighted beaker; the weight of the sample was calculated. Weight and character of the sample was found to be more in methanol leaching out of the compounds.

Isolating the fungal species from plant leaves

Fungi isolated from *A. hypogaea* leaves. A total of 18 fungal species were isolated from *A. hypogaea* plants. They are *Aspergillus flavus, A. caelatus, A. niger, Fusarium oxysporum, Verticillium lecanii, Rhizoctonia bataticola, R. solani, Trichoderma viride, T. harzianum, T. virens, T. hamantum, T. longibrachiatum, T. reesi, T. Konongi, T. pseudokoningi, Cercospora arahidicola, Puccinia arachidis* and *Sclerotium rolfsii*. The fungal genera most frequently isolated from the plant samples were *A. flavus, A. niger, Fusarium oxysporum, Verticillium lecanii, Rhizoctonia solani, Trichoderma viride, T. harzianum, Puccinia arachidis* and *Sclerotium rolfsii*.

Antifungal activity

In this study, medicinal herbs, i.e., *D. metel* and *A. indica* were extracted with 95% methanol. The extracts were used to study antifungal effects by the agar disc diffusion technique. The fungal strains such as *A flavus, A. caelatus, A. niger, F. oxysporum, V. lecanii, R bataticola, R. solani, T. viride, T. harzianum, T. virens, T. hamantum, T. longibrachiatum, T. reesi, T. Konongi, T. pseudokoningi, C. arahidicola, P. arachidis and S. rolfsii* were used.

The ethnobotanical screening tests of hydroalcoholic extract of *D. metel* and *A. indica* against fungi by using the micro dilution technique were given in the Table 1). The inhibition zones of extracts against the specific test organisms were measured. *D. metel* and *A. indica* were screened against nine pathogenic fungal strains for antimicrobial activities. The inhibition zones of extracts against the specific test organisms were measured. The extract restricted the growth of pathogen on the media around the well. The extracts showed the inhibitory effect on the all test organisms. In general the increased concentration of the plants extract was more effective against the fungal growth than the minimum concentration. Significantly, the extracts of *D. metel* showed the maximum zone of inhibition when compared *A. indica*.

This is interesting in that the traditional method of treating a microbial infection was by administering a decoction of the plant, whereas according to our results a high concentration was better; hence this may be more beneficial. Amongst 9 fungal strains investigated *A. niger, F. oxysporum* were the most sensitive and *Trichoderma harzianum* was resistant.

Table. 1. Antimicrobial activity of *Acalypha indica* and *Datura metel.*

S.No.	Name of organism	Zone of inhibition (in mm)					
		Datura metel			*Acalypha indica*		
		10 µl	20 µl	30 µl	10 µl	20 µl	30 µl
1	*Aspergillus flavus*	10	12	14	09	10	12
2	*A. niger*	11	13	15	11	12	14
3	*Fusarium oxysporum*	12	13	15	09	10	13
4	*Verticillium lecanii*	08	09	11	07	09	10
5	*Rhizoctonia solani*	09	11	12	10	11	13
6	*Trichoderma viride*	09	11	13	08	09	12
7	*T. harzianum*	06	08	10	05	06	08
8	*Puccinia arachidis*	08	10	12	08	10	11
9	*Sclerotium rolfsii*	08	09	11	07	08	10

DISCUSSION

Much attention on the discovery of plant origin insecticides was paid after coming across the hazardous consequences of the use of chemical pesticides and the resistance of insects towards them. Several attempts have been made to extract plants for new insecticides which may have been hazardous and more economical due to easy availability of plants.

Isolating the microorganisms from plant leaves

A variety of different organisms, especially bacteria and fungi, were isolated from *A. hypogaea* leaves. Several pigmented fungi dominated the bacterial population. There was a strong trend in the number of isolated fungal colonies to increase from lower to higher washing cycles in all of the plant samples. In general, the data show that microorganisms can be washed from a leaf surface, but with some apparent difficulty. The number of colony-forming units of fungi recovered increased with the number of washes. While washing presumably releases more microflora from the leaf surface, part of this increase may have been due to the fragmentation of leaves and fungal hyphae. Washing 7 and 10 times did not significantly increase the colonies of microflora recovered from *A. hypogaea.* All of the colonies recovered after 10 washes were significantly greater ($P<0.05$) than those found after

seven. When extended beyond 10 washing cycles, there was no further increase in fungal recovery.

There was a strong trend in the number of isolated fungal colonies to increase from lower to higher washing cycles in all of the plant samples.

Fungi isolated from *A. hypogaea* leaves. A total of 18 fungal species were isolated from *A. hypogaea* plants. The macroscopic and microscopic characteristics of all the isolated fungi and identifications are included where possible. Eighteen fungal species were isolated from fungal genera could not be identified because they failed to sporulate in pure cultures. The identified species of fungi belonged to the *A. flavus, A. caelatus, A. niger, F. oxysporum, V. lecanii, R. bataticola, R. solani, T. viride, T. harzianum, T. virens, T. hamantum, T. longibrachiatum, T. reesi, T. Konongi, T. pseudokoningi, C. arahidicola, P. arachidis* and *S. rolfsii*.

The fungal genera most frequently isolated from the plant samples were *A. flavus, A. niger, F. oxysporum, V. lecanii, R. solani, T. viride, T. harzianum, P. arachidis and S. rolfsii*. These fungal genera, with the exception of Trichoderma, have been previously found to be the most common resident fungi isolated from medicinal plants under field conditions (Aziz *et al*. 1998). Members of the genera *C., Pestalotiopsis*, and *Phoma* are commonly pathogenic (Agrios 1997; Barnett and Hunter 1972). Trichoderma is recognized as a successful saprophytic fungus, besides being reported as a parasite on other fungi (Agrios 1997; Barnett and Hunter 1972). *Aspergillus* and *Rhizopus* are recognized as the most common contaminant fungi of stored plant materials (Aziz *et al*. 1998).

The fungi *C. gloeosporioides, Pestalotiopsis sp*., and *Phoma s*p. are presumed as a major cause of the disease on *A. hypogaea* on account of their frequent occurrence and their known behavior on other host plants. Several fungal species were not recovered from the higher washing cycles. These were fast-growing species in which the colonies grow quickly without producing spores or conidia. They were assumed to be either weak pathogens or saprophytic fungi on account of their fast growth. Fast growing fungi are commonly isolated as saprophytic contaminants (Barnett and Hunter, 1972).

In order to avoid excessive work in maintaining fungal cultures and more importantly, to maintain cultures with a minimum of genetic change (Cochrane, 1963), the spores of all of the isolated fungi were preserved in micro beads that were frozen at –73°C (Madigan *et al*., 2000). Through this preservation technique, the fungi retain their abilities to grow, germinate, and sporulate well when they are regrown.

Antimicrobial activity

In this study, medicinal herbs, i.e., *D. metel* and *Acalypha indica* were extracted with 95% methanol. The extracts were used to study antifungal effects by the agar disc diffusion technique. Fungal strains such as *A. flavus, A. niger, F. oxysporum, V. lecanii, R. solani, T. viride, P. arachidis and S. rolfsii* were used.

In the present study that methanol extract was found to inhibit the growth of twelve organisms tested. Similarly, *D. metel* and *A. indica* has been reported to contain anthraquinone, the principal laxative constituent of many plants used as purgatives. Thus, from literature search, there is no evidence that flavonoid glycoside; a main constituent of the leaf extract is responsible for antifungal activity. Meanwhile, the ethanolic extracts of leaves, flowers, stem and root of *C. alata*

The dichloromethane fraction of the flower extract was found to be the most effective (Khan *et al*., 2001). In a recent review, the methanolic fraction of the leave has been shown to be active against *Trichophyton mentagrophytes* at a concentration of 50mg/ml but has no activity against moulds and *Candida albicans* (Villasenor *et al*., 2002). Much earlier, the antimicrobial activity of *Cassia alata* leaf extract has been reported (Palanichamy and Nagarajan, 1990).

The ethnobotanical screening tests of hydroalcoholic extract of *D. metel* and *A. indica* against fungi by using the micro dilution technique were given in (Table 2). The inhibition zones of extracts against the specific test organisms were measured. *D. metel* and *A. indica* were screened against nine pathogenic fungal strains for antimicrobial activities. The inhibition zones of extracts against the specific test organisms were measured. The extract restricted the growth of pathogen on the media around the well. The extracts showed the inhibitory effect on the all test organisms. In general the increased concentration of the plants extract was more effective against the bacterial and fungal growth than the minimum concentration. Significantly, the extracts of *D. metel* showed the maximum zone of inhibition when compared *A. indica*.

Boominathan and Ramamurthy (2009) reported that the ethanolic extracts were tested against bacteria and fungi. Among the extracts, the leaf extract of *Heliotrpium indicum* were effective against bacteria and fungi. The other three extracts have less inhibitory effect been noted in bacteria and fungi.

The methanol extracts of *Castanopsis acuminatissima* leaves, stem and root barks were partitioned (petrol, dichloromethane, ethyl acetate). Though all of the crude methanol extracts and obtained fractions from them, showed a broad spectrum of antibacterial activity, in most cases the activity was decreased on fractionation. None was active against tested moulds (Khan *et al*., 2001). In this study alcohol and aqueous extract of *D. metel* and *A. indica* showed that a broad spectrum of antifungal activity.

In the present study the ethanol extracts were found to inhibit the growth of six organisms tested. Similarly, *D. metel* and *A. indica* has been reported to contain anthraquinone, the principal laxative constituent of many plants used as purgatives (Ogunti and Elujoba, 1993). Thus, from literature search, there is no evidence that flavonoid

glycoside; a main constituent of the leaf extract of *Aegle marmelos* was responsible for antifungal activity. Meanwhile, the ethanol extracts of leaves, flowers, stem and root of *A. marmelos* had been shown to have a broad spectrum of antimicrobial activity after fractionating with petroleum spirit, dichloromethane and ethyl acetate. The dichloromethane fraction of the flower extract was found to be the most effective (Khan *et al.,* 2001). In a recent review, the methanolic fraction of the leaves has been shown to be active against *Candida albicans* at a concentration of 50mg/ml but has no activity against *Trichophyton mentagrophytes* (Villasenor *et al.,* 2002). Much earlier, the antifmicrobial activity of *Cassia alata* leaf extract has been reported (Palanichamy and Nagarajan, 1990).

CONCLUSION

The search for new antimicrobial agents is a field of utmost importance. The prevalence of antimicrobial resistance among key microbial pathogens is increasing at an alarming rate worldwide. Current solutions involve development of a more rational approach to antibiotic use and discover of new antimicrobials, but the problem of antibiotic resistance is increasing globally and may render the current antimicrobial agents insufficient to control at least some bacterial infections.

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