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Research Article

BIO CONTROL POTENTIAL OF *PIMENTA DIOICA* AND *ANACARDIUM OCCIDENTALE* AGAINST *FUSARIUM OXYSPORUM* F. SP. *ZINGIBERI*

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Abstract

Ginger is an important commercial crop attacked by a number of pathogenic fungi and *Fusarium oxysporum* f. sp. *zingiberi* is one among the important fungi. The purpose of the present study was to investigate the *in vitro* antifungal effect of aqueous extracts of leaf and bark of *Pimenta dioica* (Linn.) Merill (family: Myrtaceae) and *Anacardium occidentale* L. (family: Anacardiaceae) against *F. oxysporum* f. sp. *zingiberi* recovered from rhizome rot specimen of ginger. Antifungal potential of leaf and bark extracts was determined by poisoned food technique and the inhibitory effect was observed in terms of reduction in colony diameter of the fungus in plates poisoned with extracts (10 %) when compared with control plates. The leaf and bark extracts were effective in inhibiting the fungus but to varied extent. Leaf extracts were more inhibitory than bark extracts. Leaf extract and bark extract of *P. dioica* showed high and least inhibition of the fungus respectively. The antifungal potential could be related to the presence of inhibitory metabolites present in the extracts. Further studies involving field trials are to be carried out.

Keywords: Rhizome rot of ginger, *Fusarium oxysporum* f. sp. *zingiberi*, *Pimenta dioica*, *Anacardium occidentale*, Poisoned food technique.

INTRODUCTION

Ginger (*Zingiber officinale*, Zingiberaceae) is an important commercial spice crop grown worldwide for its aromatic rhizomes. It is used as spice as well as medicine. India is the largest producer of ginger accounting for about 1/3rd of the total world output. Kerala, Karnataka, West Bengal, Andhra Pradesh, Orissa, Meghalaya, Mizoram, Arunachal Pradesh, Sikkim and Himachal Pradesh, India are the leading ginger growing states in India. The production of ginger is influenced by a number of diseases caused by pathogens such as *Ralstonia solanacearum*, *Pythium* spp., *Fusarium oxysporum* etc. The crop suffers from several diseases and bacterial wilt (*Ralstonia solanacearum*) and rhizome rot (*Pythium* spp., *Fusarium* spp.) are among the important diseases affecting the crop production in India[1,2]. The rhizome rot (soft rot) is one of the most destructive diseases of ginger. The disease affects ginger crop worldwide, with losses of 50–90 % sometimes occurring in major production areas such as the tropical regions of India[2]. The disease is caused by a pathogen complex which includes species of *Pythium* and *Fusarium* as the main causal agents. *Fusarium oxysporum* f. sp. *zingiberi* is one of the important fungal pathogen isolated commonly from the rhizome rot complex of ginger. It is the major and predominant wilt pathogen resulting in yellows of ginger[3-6]. Indiscriminate use of chemical agents to control plant pathogens leads to environmental pollution, adverse effect on human beings and emergence of resistant pathogens. Biological control provides an important avenue to ecofriendly plant protection. It reduces risks associated with the use of synthetic plant protection agents[7]. The present study was conducted to investigate inhibitory potential of aqueous extracts of leaf and bark of *Pimenta dioica* (Linn.) Merill (family: Myrtaceae) and *Anacardium occidentale* L. (family: Anacardiaceae) against *Fusarium oxysporum* f. sp. *zingiberi* isolated previously from rhizome rot complex of ginger.

MATERIALS AND METHODS

Collection and identification of plant materials

The leaves and barks of *P. dioica* and *A. occidentale* were collected at a place called Maragalale, Thirthahalli (taluk), Shivamogga (district), Karnataka, India during July 2013. The plant materials were washed thoroughly, shade dried and powdered. The powdered leaf and bark materials were stored in air-tight containers until extraction.

Extraction

10 g of leaf and bark of *P. dioica* and *A. occidentale* were added separately to 100 ml of distilled water and boiled for about 10 minutes. After cooling, the contents were filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrates were used to poison the medium[8].

Antifungal activity of leaf and bark extracts

The antifungal efficacy of leaf and bark of *P. dioica* and *A. occidentale* was determined by Poisoned food technique[9]. Here, Potato dextrose agar (PDA) was amended with 10 % leaf extracts (LE) and bark extracts (BE) and sterilized by autoclaving. The poisoned medium was added to sterile petriplates and allowed to solidify. Later, fungal discs of 5mm diameter were cut from periphery of 5 days old culture of the test fungus, transferred aseptically on poisoned PDA

plates and incubated for 5 days at 28°C. Colony diameters in mutual perpendicular directions were measured on 5th day. The experiment was carried out in triplicate and average colony diameter was recorded. Antifungal activity was recorded in terms of inhibition of mycelial growth (%) and calculated using the formula:

Inhibition of mycelia growth (%) = ([C-T]/C) x 100,
Where C is average diameter of fungal colony in control plates and T is average diameter of fungal colony in poisoned plates.

Table 1: Colony diameter of *F. oxysporum* in control and poisoned plates

Treatment	Colony diameter (cm)
Control	2.9
Pd-LE	1.6
Pd-BE	2.5
Ao-LE	2.1
Ao-BE	2.3

Pd- *Pimenta dioica*, Ao- *Anacardium occidentale*

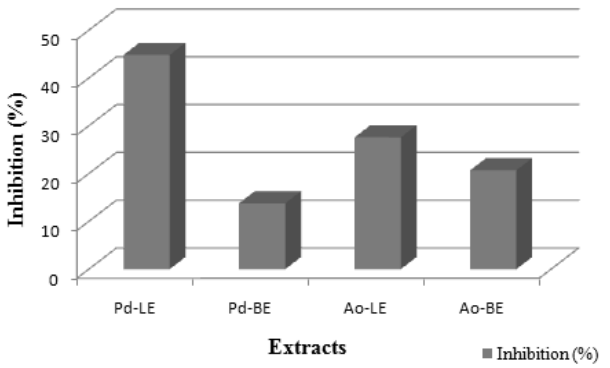

Figure 1: Inhibition (%) of *F. oxysporum* by LE and BE

RESULTS

Table 1 and Figure 1 show the reduction in the colony diameter of test fungus in poisoned plates when compared to control plates. LE of both *P. dioica* and *A. occidentale* showed high inhibition of test fungus when compared to BE of both the plants. Among LE, LE of *P. dioica* caused marked reduction in the colony diameter of test fungus when compared to LE of *A. occidentale*. In case of BE, BE of *A. occidentale* showed high inhibition of test fungus when compared with BE of *P. dioica*. LE of *P. dioica* and BE of *A. occidentale* displayed 44.83 % and 20.69 % inhibition of test fungus respectively.

DISCUSSION

Fusarium oxysporum Schlecht is found in soils worldwide and most strains are saprophytic. Some strains of *F. oxysporum* are the most important plant pathogenic fungi and causes wilt diseases in >100 species of vascular plants. The pathogenic strains are highly host specific and are identified to *Formae speciales* and race. The fungus colonizes the water-conducting vessels (xylem) of the plant and blocks or breakdown xylem. The symptoms appear as leaf wilting, yellowing and eventually the plant death take place. A plenty of measures have been taken to combat the diseases caused by *F. oxysporum* and a number of chemical fungicides have been tried. However, the use of these fungicides of chemical origin resulted in adverse effect on the environment. Bio control seems to be an alternate for the disease control and in this context several scientists screened the efficacy of natural products such as cow urine, cow urine extract of plants, plant extracts etc., and antagonistic microbes against the fungal pathogen[1,8-14]. *F. oxysporum* f. sp. *zingiberi* is the major and predominant wilt pathogen causing yellows of ginger and is isolated commonly from the rhizome rot complex of ginger[5-6]. In the present study, we have assessed the antifungal potential of aqueous extracts of leaf and bark of *P. dioica* and *A. occidentale* by poisoned food technique. Poisoned food technique is routinely employed to screen the antifungal effect of plants and their compounds. The antifungal activity is determined in terms of reduction in the mycelial growth of fungi in poisoned plates when compared to control plates. It has been employed by several researchers to evaluate antifungal activity of plants[8,9,15,16]. It was observed that the fungus was found to be susceptible to extracts of both plants but to a varied extent. Leaf extracts were more effective against the fungus than bark extracts. Yield loss due to fungal pathogens is a significant problem for production of ginger all over the world. The management of rhizome rot disease of ginger involves cultural, biological and chemical approaches for pathogen suppression. The most common method to get rid of fungal diseases of plants is to use chemical fungicides. However, environmental concerns, costs, development of resistance in pathogens stimulated search for alternatives to traditional synthetic chemical fungicides[17,18]. Plants and their products have shown to be promising as potent antifungal agents against rhizome rot pathogens. Sagar *et al*.[19] showed the efficacy of some plant extracts against rhizome rot pathogens *Pythium aphanidermatum* and *F. solani*. In a previous study, Dileep *et al*.[8] showed inhibition of *P. aphanidermatum* and *F. oxysporum* f. sp. *zingiberi* by leaf and pericarp of *Polyalthia longifolia*. In another study, Rakesh *et al*.[14] found inhibitory potential of cow urine extracts of nine plants against *P. aphanidermatum* and *F. oxysporum* f. sp. *zingiberi*.

CONCLUSION

The present study was successful in determining the potential of leaf and bark extracts of *P. dioica* and *A. occidentale* against *F. oxysporum* f. sp. *zingiberi* isolated from rhizome rot specimen of ginger. The inhibitory effect could be attributed to the presence of antifungal principles in the extracts. These plants, especially leaf of *P. dioica*, can be the potential candidates for the development of antifungal agents which can be used against the rhizome rot of ginger. Further, *in vivo* experiments are to be conducted.

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