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Short Communication

Evaluation of *Eucalyptus camaldulensis* against *Fusarium solani*

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ABSTRACT

The use of allelopathic plant extract against pathogenic fungi is gaining attention of the scientists for the last many years. Allelopathic compounds being non hazardous and environment friendly are also being tested as natural substituent of fungicides. Keeping in view this aspect of plants, the present study was therefore designed to evaluate *Eucalyptus camaldulensis* for its antifungal properties against *F. solani*, the causal organism of root rot disease. For this purpose the test fungal species was grown in 100 mL ME broth medium in various concentrations (0, 5, 10 & 15% w/v) of aqueous and organic solvent extract for 10 days. The results of this study showed strong allelopathic effects of test plant parts however, eucalyptus leaf extracts proved more effective than stem and bark for controlling the growth of target fungus. © 2012 Friends Science Publishers

Key Words: *Eucalyptus camaldulensis*; Allelopathy; Aqueous and organic solvent extracts; *Fusarium solani*

INTRODUCTION

Eucacalyptus is belongs to family Myrtaceae is a native of Australia. It is widely planted evergreen genra due to its wide adaptability. Mainly it is cultivated for its timber, fuel and paper pulp, but it has also wide range of medicinal properties.it is a source of essential oil used in medicines and perfumes. Its oil has pesticidal, nemticidal and insecticidal activity. It has been reported that 22 tested bacterial strains and 11 fungal strains were instantly killed by using eucalyptus oil (Pattanaik *et al*., 2002). Extensive research has shown that its oil have marked antiseptic action against a wide variety of infectious bacteria, viruses and fungi (Inouye *et al*., 2001).

Plant pathogens are of serious concern, as they cause huge damage to economic crops. *Fusarium* spp., are well known for their pathogenicity and cause, seed abortion, seed, root, stem and seedling rots, vascular wilt, damping off, die back, stunting and reduction in growth in a variety of host plants (Ahmad *et al*., 1994; Sharfun-Nahar *et al*., 2005).

Fusarium solani is, often an invader on lesions caused by other fungi such as anthracnose. A plant that is infected early in the production season has lesions near the base of the stem, while those that are infected later in the season are more likely to have lesions at upper nodes. The number of infected plants and the severity of disease symptoms increase with time during the growing season. The fungus is also known to cause a rot of young plants like cucurbits, maize, capsicum, soybean and many other plants during wet weather. The fungus enters the seed cavity through the blossom end, where it quickly spreads within the fruit and causes the fruit to abort and fall from the tree (Hunter & Buddenhagen, 1996).

Plant diseases caused by plant pathogenic fungi are among the most important factors that reduce yield losses; farmers apply large quantities of fungicides every year. The continuous application of chemicals will lead to destruction of the ecosystem and result in outbreaks of new strains of fungi that are difficult to control. To minimize the side effects of chemical application, many efforts have been carried out to utilize the antimicrobial activity of plant extracts. Several studies showed the importance of natural chemicals from medicinal plant extracts as a possible source of non-phototoxic, systemic and easily biodegradable alternatives (Amadioha, 1998 & 2002).

The present study was carried out to investigate the antifungal potential of aqueous and organic solvent extract of *Eucalyptus camaldulensis* against wilt and rot causing fungi *F. solani.*

MATERIALS AND METHODS

Selection of test plant: Being potential allelopathic plant *Eucalyptus camaldulensis* was selected to investigate its antifungal activity against the rot causing target fungus *Fusarium solani*. Leaf, Stem and bark material of the selected plant were collected from Quide-e-Azam campus, University of The Punjab, Lahore, Pakistan.

After thorough washing with tap water, the materials were surface sterilized with 1% sodium hypochlorite solution followed by washing with sterilized water. These plant materials were dried in sunlight, crushed and stored in polythene bags. These plant materials were used according to the need of experimental work to make different extract concentrations with water, methanol and n-hexane solutions.
Isolation of target fungal species: *F. solani* was isolated from capsicum plants infected with stem rot disease.

The tissue was cut into 5 mm long and 2-3 mm thick pieces. The pieces were surface sterilized with 1% NaOCl solution for about 2 minutes followed by thorough washing with sterilized water. These surface sterilized pieces were transferred to malt extract agar (MEA) medium in 9 cm diameter petri plates. The plates were incubated at 30±2°C for five days. Morphological studies were done for the identification of fungus. The pure culture was maintained in refrigerator at 4°C.

Bioassay with aqueous extract: Twenty grams of dried powder of plant parts (e.g. leaf, stem & bark) was soaked in 100 mL of water for 24 h to prepare 20%w/v plant extract. Materials were filtered through muslin cloth followed by Whatman No 1 filter paper. Malt extract broth was prepared by autoclaving and was cooled to 40°C. In 250 mL flask 80 mL of malt extract broth was prepared and appropriate amount of plant extract and distilled water was added to it. Appropriate concentration i.e., 5, 10 and 15% were made by soaking the plant material.

The control set received 80 mL of broth medium with 20 mL of distilled water in it instead of extract. Actively growing disc of *F. solani* were prepared by using a cork borer of 5 mm diameter and transferred to the flask in aseptic condition. Each treatment was replicated thrice and flasks were incubated at 30±2°C for 10 days in incubator After 10 days, fungal biomass in each flask was filtered, fresh and dried weight was taken to conclude the results.

Bioassay with organic solvent extract: Two organic solvents e.g., n-hexane and Methanol were selected for organic bioassay. Twenty grams of dried powder of plant parts (e.g., leaf stem & bark) was soaked in 100 mL of N-hexane and methanol separately for 24 h to prepare 20% (w/v) extract. The organic compounds were evaporated at room temperature and then its volume was raised up to 100 mL by adding sterilized water in it. In next step, materials were filtered through muslin cloth followed by Whatman No. 1 filter paper. Further same procedure was done as described for aqueous extracts bioassays.

Statistical analysis: Experimental was laid out in completely randomized block design and each treatment was in triplicate. Data was subjected to analysis of variance (ANOVA) followed by Duncan's multiple range (DMR) tests using computer software COSTAT.

RESULTS

Growth response of *F. solani* to plant extracts: Leaf extracts in water, methanol and n-hexane proved more inhibitory against *Fusarium solani* than other parts. However, methanol leaf extract gave 66% biomass reduction under 10% concentration followed by 57% in 15% aqueous extract and 45% reduction in n-hexane leaf extract.

Effect of leaf extracts: In case of aqueous extract of leaf, the highest concentration showed a clear decrease in biomass in 15% concentration, which was 57% and statistically significant. The reduction of 50-54% appeared in the biomass was observed in 5% aqueous extract concentration as compared to control (Fig. 1a).

As far as the methanol extract of stem was concerned the result showed same results as is aqueous extract in methanol extract concentration 66% biomass reduction was observed in 10% leaf extract concentration. The least reduction was observed in highest concentration of methanol that was 55%, which was followed by 56% in 5% concentration gradual decrease in fungal biomass was present with the increase in concentration (Fig. 1a).

In the n-hexane extract seem a little different from aqueous and methanol extract concentration the best growth in leaf extract was observed in 15% n-hexane extract concentration, which is 49% as compared to control. When 15% was compared with 5 and 10% of n-hexane extract, 10% concentration proved to be better than 5% (Fig. 1a). In contrast to aqueous, methanol extract 66% reduction found in leaf 10% extract concentration because it showed 44% reduction in biomass the least decline in fungal biomass was in 5%, which was 13% and all the data was statistically insignificant.

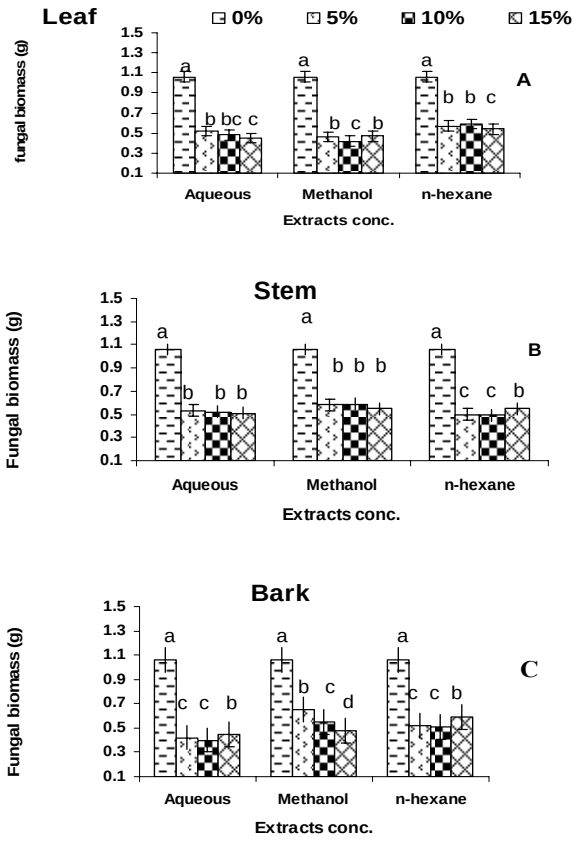
Effect of stem extracts: The finding of stem extract on fungal biomass weight remained significant under different applied treatment. All three concentrations of aqueous extracts showed decrease from 50-52% as compared to control, which is statistically significant reduction in biomass (Fig. 1b).

Methanol extract of Stem also showed variation in results higher the concentration high is the biomass reduction as in 15% concentration of methanol showed the maximum reduction of 54% (Fig. 1b). The same result as with leaf extracts. While minimum reduction of 45% was found in 5% methanol extract concentration as compared to control. Methanol extract of stem in 10% concentration showed 52% reduction in biomass (Fig. 1b). In case of n-hexane stem extract the least reduction of 47% was observed in 15% as compared to control. While in to 5 and 10% concentration it remained as much as 52-53% respectively (Fig. 1b).

Effect of bark extracts: In case of Bark extract fungal dry biomass weight showed significant decrease of 66% in 5% aqueous extract concentration and 52% decline was observed in 10% aqueous extract concentration as compared to control (Fig. 1c). It was evident from the results that 57% decrease in biomass was in 15% extract concentration (Fig. 1c). The findings shows that highest reduction in fungal biomass was observed in 10% and 15% methanol extract, which was 54% in both concentrations the least reduction in biomass was in 5% Methanol extract 30% (Fig. 1c).

The data regarding to bark extract in n-hexane is similar like the stem. It showed maximum reduction in 10% extract concentration which reduced the fungal biomass up to 51% followed by 50% decrease in 5% extract concentration as compared to control (Fig. 1c). However, the least reduction in biomass was found in the highest

Fig. 1: Effect if different concentrations of aqueous, methanol and n-hexane extracts of leaf (a), stem (b) and bark (c) of *E. camaldulensis* on dry biomass of *F. solani.* Verticals bars show standard errors of means of three replicates. Bars with different letters show significant difference (p≤ 0.05) as determined by Duncan multiple range (DMR) tests



concentration of 15%, which gave 44% reduction in biomass (Fig. 1c).

DISCUSSION

Synthetic fungicides have caused many serious economical and environmental problems due to their broad spectrum toxicity. Scientists are continuously trying to search for ecofriendly and non-hazardous means for controlling plant pathogenic fungi.in last few years many research workers have successfully used plant derived chemicals from many allelopathic plants for controlling plant pathogenic fungi.

In present study antifungal activity of aqueous and organic solvent extracts of *E. camaldulensis* was evaluated against pathogenic fungi *F. solani* in all types of extracts leaf extract drastically reduced the growth of target fungal species. However, effect was found variable depending upon the concentrations i.e., high concentration caused more inhibition as compared to lower concentrations. Similar inhibitory effect due to allelopathic plant extract was reported by Bajwa and Ifftikhar (2005). They checked antifungal activity of two eucalyptus species, according to their findings *E. camaldulensis* was more effective in causing growth inhibition in target fungal specie *D. tetramera*. Mughal *et al*. (1996) have reported the fungicidal effects of *Allium cepa* and *A. sativum* against many fungal species. Such growth inhibition may be due to reduced rate of cell division and enhanced cellular respiration in the presence of allelochemicals (Blake, 1985). Shafique *et al.* (2005) found that extracts of sorghum, sunflower and *M. azerdarch* showed fungicidal properties against many plant pathogenic fungi. Furthermore the antifungal compounds present in allelopathic plants can be used for the preparation of natural fungicides to replace chemical compounds for the protection of the environment.

It is concluded that aqueous, methanol and n-hexane extract may be used as natural fungicides because it contain antifungal constituents. Among the different extract concentrations 0, 5, 10 and 15% higher concentration (15%) is more effective for reducing the growth of *F. solani*. Among various aerial parts, leaf extract was more effective as compare to others. The test plant needs to be used as biological control against some other pathogenic fungi by isolating and identifying the antifungal constituents for their use in managing the pathogenic fungi and as agrochemicals.

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