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Full Length Research Paper

Cymbopogon citrates: A remedy to control selected *Alternaria* species

Sobiya Shafique*, Ruqeyah Abdul Majeed and Shazia Shafique

Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan.

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***Alternaria* species, ubiquitous post harvest pathogens, contribute to the spoilage of 20 to 40% of the agricultural output. *Alternaria* usually attacks aerial parts of its host and symptoms appear as small, circular, dark spots. Control of this pathogen has become an obligatory requirement. In the present study, two species of *Alternaria* (*Alternaria alternata* and *Alternaria tenuissima*) were selected on the basis of their ability to cause economically important plant diseases. These species were treated with aqueous and methanol extracts as well as essential oil of *Cymbopogon citratus* L. (lemongrass) in liquid malt extract medium using different concentrations (1, 2, 3 and 4%). Bioassays revealed that among aqueous and methanol extracts; methanol extract caused maximum inhibition of both the *Alternaria* species at high concentration of 4% after 5 and 10 days of incubation period. *A. alternata* displayed maximum inhibition of 100% at higher concentration of 4% after 5 days and 94% after 10 days of incubation. In contrast to this, *A. tenuissima* exhibited 90% inhibition after 5 days and 92% after 10 days at higher concentration (4%). Essential oil of lemongrass proved the most effective as it induced complete inhibition of both fungal species at all concentrations. This study concludes that aqueous and methanol extracts of *C. citratus* possess the ability for substantial inhibition in growth of *A. alternata* and *A. tenuissima*.**

Key words: Antifungal activity, *Cymbopogon Citratus,* aqueous and methanol extract, essential oils.

INTRODUCTION

Alternaria species induce symptoms on aerial plant parts as small, circular, dark spots. Many *Alternaria* species produce toxins that diffuse into host tissues ahead of the fungus. Dark, sunken lesions are usually the expression of *Alternaria* infections on roots, tubers, stems, and fruits. The fungus may sporulate in these cankers, causing a fine, black, velvety growth of fungus and spores to cover the affected area. Host plants include a variety of crops such as apples, broccoli, cauliflower, carrots, potatoes, cabbage, tomatoes, citrus and many other ornamentals and a number of weeds. *Alternaria* is the commonest species of the tropics and is responsible for diseases of wheat and other economically important plants. Diseases caused by *Alternaria* are very common and occur world wide for example *Alternaria alternata* - causes early blight of tomato, brown spots of tobacco, leaf spot disease in *Withania somnifera* (Pati et al., 2008) and can infest many other plants, *Alternaria tenuissima* causes very serious attack on leaves of apricot.

Due to foregoing mentioned hazards and damages caused to plants and agricultural crops by *Alternaria* species, control of pathogenic fungi has become an obligatory requirement. The control can be done by many methods, chemical control being widely used because of following reasons: Easiest method, time saving, less use of labour, target specific. Mancozeb, maneb, captan, iprodione, chlorothalonil, copper, and triflumizole are known to control *Alternaria* diseases to a varying degree of efficacy. Daconil® Ultrex, Heritage, Medallion®, and Spectro provided the best control under the highest disease pressure (Chase, 1990; Simone and Chase, 1989). Although, chemical control suggests being the best method to control *Alternaria* diseases however in recent years, use of chemicals has increased consumer concern and their use is becoming more restricted due to carcinogenic effects, residual toxicity problems, environmental pollution, occurrence of microbial resistance, high inputs etc. (El-Rokiek et al., 2006).

*Corresponding author. E-mail: drsobi81@gmail.com.

Many researchers have tried to find safe and economical control of plant diseases by using extracts of different plant parts (Bajwa et al., 2004; Bajwa and Iftikhar, 2005; Shafique et al., 2011). The antimicrobial and antitoxin properties of many plants, herbs, and their components have been documented since late 19th century (Reigosa et al., 1999). Natural plants products are biodegradable, exhibit structural diversity and complexity and used traditionally to treat a number of infectious diseases including those caused by bacteria, fungi, protozoa and viruses (Reigosa et al., 1999; Shafique et al., 2005; Javaid and Shah, 2007). These natural plants involve garlic, lemon grass, datura, acacia, ginger, black seed, neem, basil, eucalyptus, alfalfa and many others (Shafique et al., 2006, 2011; Javaid et al., 2010). Recent studies have shown that fungal plant pathogens can be controlled by plant products and there essential oils (Masoko et al., 2007). Lemongrass as well as bay oil has the potential to inhibit the growth of many microorganisms (Hammer et al., 1999). Knowing the high antifungal activity of lemongrass extracts and oil, the aim of present study was to evaluate the *in vitro* antifungal activity of lemongrass aqueous and organic plant extracts and essential oil against A*. alternata* and *A. tenuissima*, the main cause of leaf spot diseases of many plants.

MATERIALS AND METHODS

Pathogenic test fungi

Pure cultures of test fungi, *A. alternata* (Fries) Keissler and *A. tenuissima* (Nees and T. Nees: Fr.) Wiltshire, were obtained from First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences, University of the Punjab, Lahore and maintained on 2% malt extract medium. The inoculum was prepared according to the method of Noomrio and Dahot (1992) containing 6 x 10^5 conidia/ml.

Collection of plant materials

Fresh and healthy leaves of *Cymbopogon citratus* were collected from different nurseries of Lahore. Leaves were washed thoroughly with detergent and surface sterilized with 1% sodium hypochlorite solution. Washed leaves were dried in an electric oven at 40 to 50°C for 96 h and crushed to make powder.

Preparation of extracts

Preparation of aqueous extract

To make 20% stock solution, twenty grams of dried leaf powder of lemongrass was soaked in 100 ml of sterilized distilled water for 24 h. Extract was filtered through a double layered muslin cloth followed by Whatman No. 1 filter paper (Bajwa et al., 2007).

Preparation of methanolic extract

Twenty grams of dried leaf powder was soaked in 100 ml of methanol for 24 h. Extract was filtered as mentioned previously and then volume of organic solvent was reduced to 2 ml by evaporating at 40°C and diluted by adding appropriate quantity of sterilized distilled water to make the final volume 100 ml (Bajwa et al., 2008). For methanolic control 2 ml of methanol was taken and water was added to make the final volume up to 100 ml. The lower concentrations of 1, 2, 3 and 4% were prepared by adding appropriate quantity of distilled water into the stock solution.

Oil extraction

Fresh plant material of lemongrass was cut into small pieces. Oil extraction was made using 50 g fresh plants by steam distillation using Clevenger system, during 10 h. The extracted oil was stored at 15±3°C. Equal amount of oil was dissolved in equal amount of Tween 20 and distilled water was slowly added to this solution to make the stock solution. The lower concentrations of 1, 2, 3 and 4% were prepared by adding appropriate quantity of distilled water into the stock solution.

Aqueous and methanolic extract bioassays

Antifungal bioassays were carried out in liquid 2% malt extract (ME) medium. Conidial suspension (0.2 ml) containing 6 x 10^5 conidia/ml was inoculated aseptically. The flasks were incubated at 25±2°C. For the assessment of fungal biomass yield, two harvests were designed up to 10 days at 5-days interval. The mycelial biomass from triplicate samples for each treatment was collected on pre-weighed filter papers. Their dry weight yield was determined after 24 h oven drying at 60°C (Bajwa et al., 2004).

Oil bioassays

Oil bioassays were also conducted in liquid 2% malt extract (ME) medium in test tubes. To 4 ml of ME, 1 ml of each of 1 to 4% stock solution of lemongrass oil was added. Control received the same quantity of distilled water (Nikos et al., 2007). Then 0.2 ml of conidial suspension was inoculated in each test tube containing 6 x 10^5 conidia/ml aseptically. The test tubes were incubated at 25±2°C. The harvest designed at an interval of 7-days from triplicate samples for each treatment was collected on pre-weighed filter papers. Their dry weight yield was determined after 24 h oven drying at 60°C.

Statistical analysis

All the data were analyzed by applying Duncan's Multiple Range (DMR) Test (Steel and Torrie, 1980) to compare different treatments with one another statistically.

RESULTS

Effect of aqueous extract of lemongrass on *Alternaria* species after 5 days

The periodic growth assays of pathogenic species *A. alternata,* in terms of dry biomass production against aqueous extract of *C. citratus,* were carried out after 5 days of incubation. The growth assays revealed a rather consistent pattern of growth. All regimes of aqueous extract of *C. citratus* caused considerable inhibition in

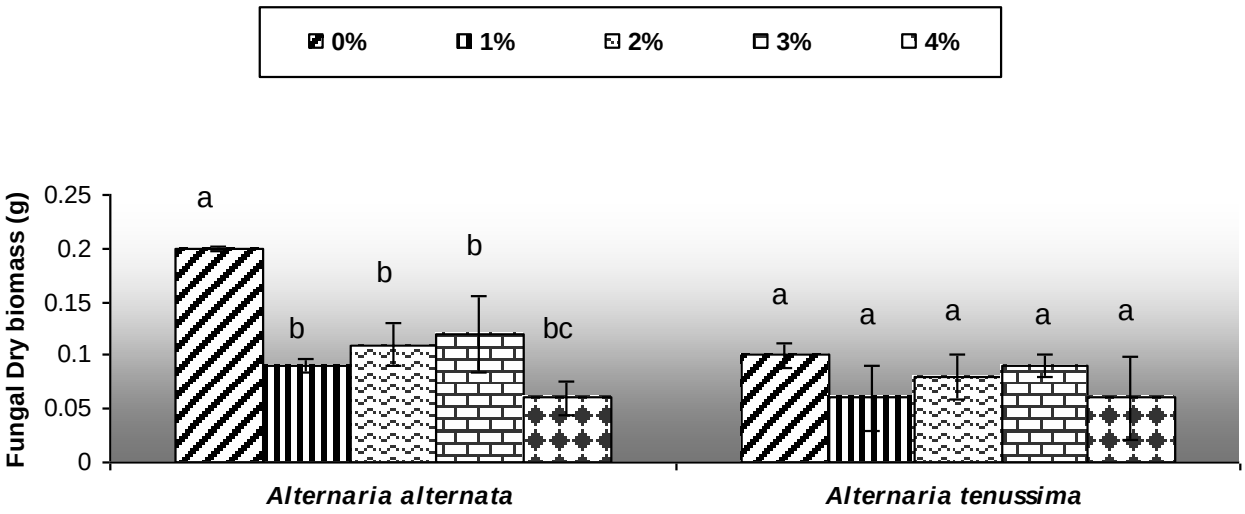

Figure 1. Effect of different concentrations of aqueous extracts of C*ymbopogon citratus* on dry biomass production of A*lternaria alternata* and A*lternaria tenussima* after 5 days of incubation. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P=0.05) as determined by DMR test.

mycelial biomass production of the fungus. However, 4% concentration of aqueous extract of *C. citratus* showed greater reduction causing a decline of about 70%, in dry biomass while 1 to 3% concentrations caused a reduction of only 40 to 55% (Figure 1).

The antifungal bioassays of *A. tenussima* in initial growth period of 5 days depicted a variable pattern of growth. All the concentrations of aqueous extract of *C. citratus* caused substantial inhibition in mycelial biomass production of the fungus. In particular, 1 and 4% concentrations showed maximum inhibition of 40% in dry biomass followed by 2 and 3% concentrations that displayed only 20 and 10% reduction in dry biomass, respectively (Figure 1).

Effect of aqueous extract of lemongrass on *Alternaria* species after 10 days

A. alternata exhibited a quite different pattern of growth after 10 days of incubation in the aqueous extract of *C. citratus*. All the concentrations of aqueous extract of *C. citratus* caused gradual and significant inhibition in mycelial growth of the target fungus. Lower concentrations of 1 and 2% showed significant reduction causing a decline of 51 and 57% in dry biomass, respectively, while 3 and 4% concentrations induced the greatest inhibition of 80% (Figure 2).

The growth assays of *A. tenussima* in 10 days incubation period also presented a significant growth pattern. A considerable inhibition in mycelial biomass production of *A. tenussima* was observed by all the concentrations of aqueous extract of *C. citratus* with respect to control however this suppression was invariable among the treatments. The highest concentration of aqueous extract of *C. citratus* (4%) caused the greatest reduction (up to 65%) while lower concentrations of 1 to 3% showed comparatively lesser decrease causing a decline of 45 to 55% in dry biomass production of the target fungus than 4% concentration (Figure 2).

Effect of methanol extract of lemongrass on *Alternaria* species after 5 days

The periodic growth assays of *A. alternata* in terms of dry biomass production against exposure to methanolic extract of *C. citratus* after 5 days of incubation period revealed a rather different and drastic pattern of growth in comparison to growth of *A. alternata* in aqueous extract. All regimes of methanolic extract of *C. citratus* caused considerable inhibition in dry biomass production of the fungus with insignificant difference among each other. Lower concentrations of 1 to 3% demonstrated remarkably suppressive effect in dry biomass by reducing mycelial growth up to 61 to 65%. However, 4% concentration displayed complete reduction causing a decline of 100% as compared to the lower concentrations (1 to 3%) (Figure 3).

The dry biomass assays of *A. tenussima* in the initial period of 5 days demonstrated again an inconsistent and statistically slightly significant pattern of growth. All regimes of methanol extract of *C. citratus* caused considerable inhibition in mycelial biomass production of *A. tenussima*. Lower methanolic concentration of 2% depicted minimum reduction of 37% while 1 and 3% concentrations showed considerable inhibition of 50 and 53% of *A. tenussima,* respectively. Higher concentration of 4% showed significantly greater depression in dry

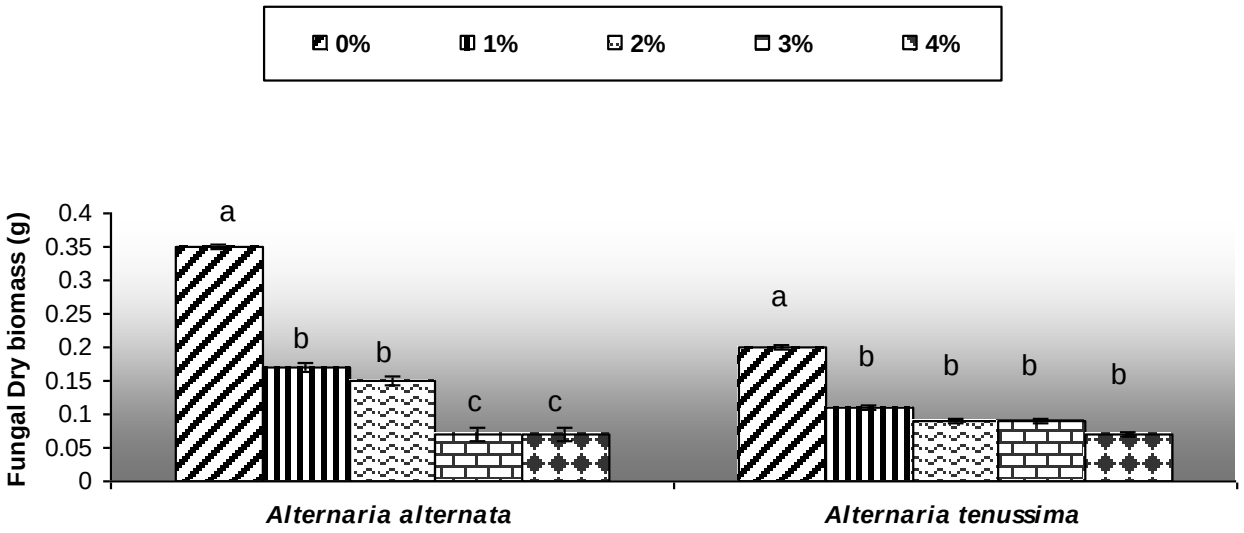

Figure 2. Effect of different concentrations of aqueous extracts of C*ymbopogon citratus* on dry biomass production of A*lternaria alternata* and A*lternaria tenussima* after 10 days of incubation. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P=0.05) as determined by DMR test.

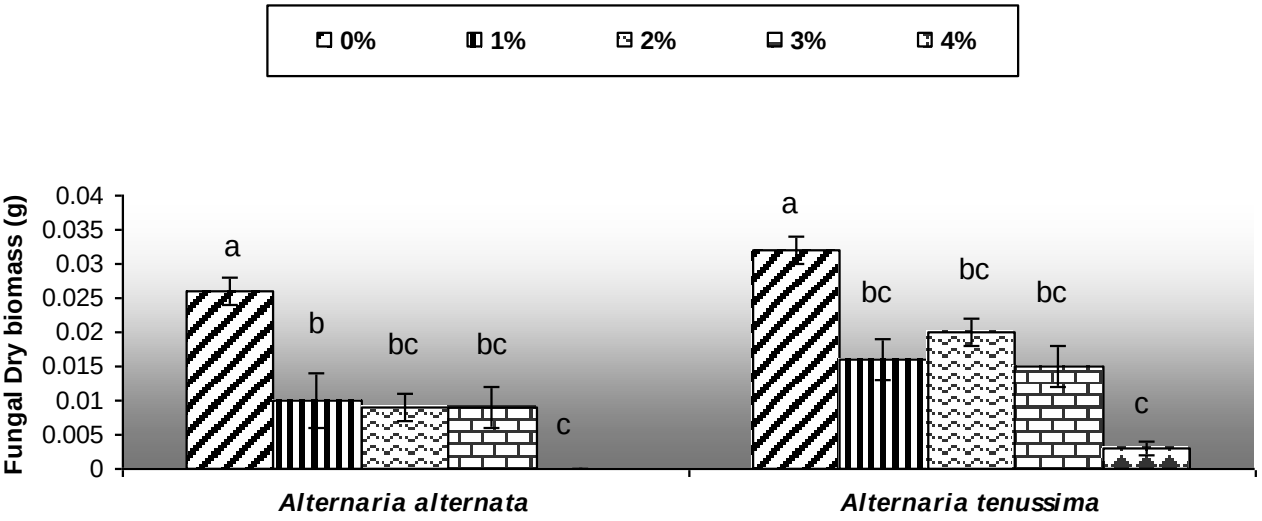

Figure 3. Effect of different concentrations of methanolic extracts of C*ymbopogon citratus* on dry biomass production of A*lternaria alternata* and A*lternaria tenussima* after 5 days of incubation. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P=0.05) as determined by DMR test.

biomass of 91% (Figure 3).

Effect of methanol extract of lemongrass on *Alternaria* species after 10 days

The dry biomass production of *A. alternata* in final phase of 10 days of incubation exhibited that all the concentrations of methanolic extract of *C. citratus* provoked significant decline in mycelial biomass production of the target fungus. Lower concentrations of 1 to 3% displayed significant inhibition of 59 to 62% whereas 4% concentration of methanol extract caused a maximum reduction of 94% (Figure 4).

In this phase of incubation period of 10 days, the dry biomass production of *A. tenussima* showed a precipitous inhibition pattern of growth with varying concentrations. All extract regimes caused significant inhibition in mycelial biomass production of the fungus with maximum arrest of about 92% in dry biomass production (Figure 4).

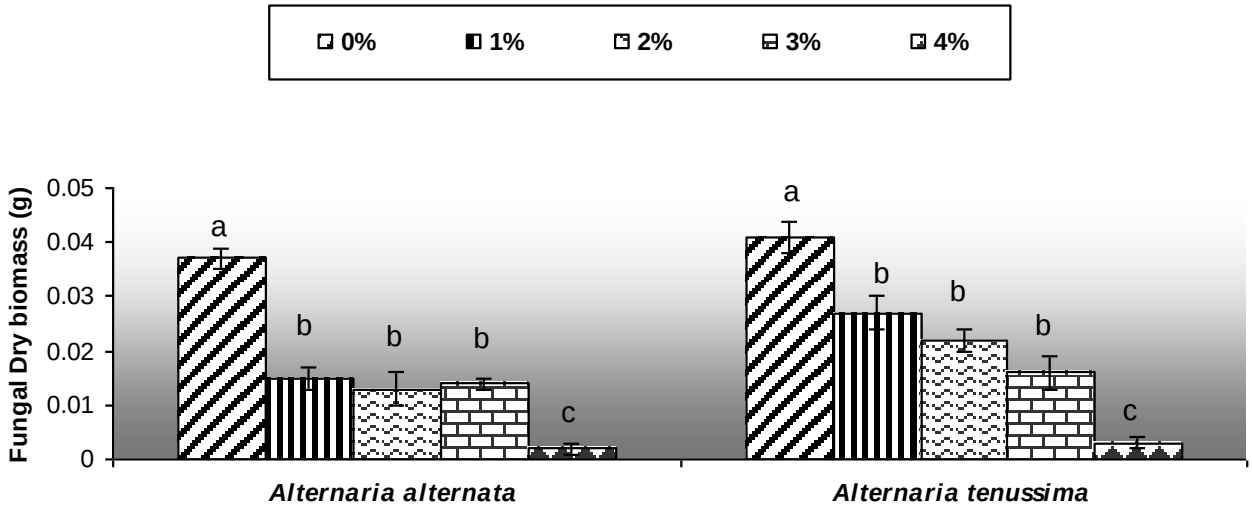

Figure 4. Effect of different concentrations of methanolic extracts of C*ymbopogon citratus* on dry biomass production of A*lternaria alternata* and A*lternaria tenussima* after 10 days of incubation. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P=0.05) as determined by DMR test.

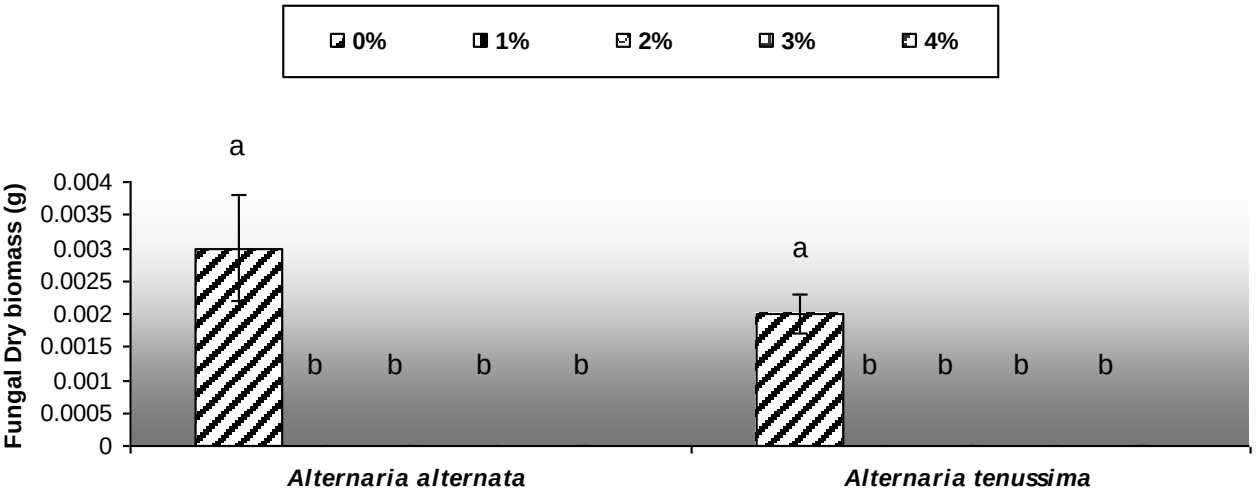

Figure 5. Effect of different concentrations of essential oil of C*ymbopogon citratus* on dry biomass production of A*lternaria alternata* and A*lternaria tenussima*. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P=0.05) as determined by DMR test.

Effect of essential oil of lemongrass on *Alternaria* species

The data regarding the growth assays of pathogenic fungal species, *A. alternata* and *A. tenussima,* to essential oil of *C. citratus* after 7 days of incubation is presented in Figure 5. The essential oil of *C. citratus* was proved to be the most effective among all the extract types. The dry biomass assays of both the target species revealed a significant inhibition pattern of growth. All regimes of essential oil of *C. citratus* caused complete inhibition in mycelia biomass production of the fungus causing 100% decline in dry biomass by essential oil of *C. citratus* (Figure 5).

DISCUSSION

Biocontrol is the safest and economical method of controlling plant pathogens by using extracts of different plant parts (Bajwa and Iftikhar, 2005; Shafique et al., 2011). Presently, the study was conducted to assess the *in vitro* efficacy of aqueous and methanol extracts of *C. citratus* against two pathogenic test species of *Alternaria.*

Different concentrations of aqueous and organic extracts were employed which produced variable results. The effect of aqueous and organic extract varied with the test species used, concentrations of aqueous and organic extracts and the incubation period (Bajwa et al., 2008). The variation in antifungal activity of extracts in different solvents may be attributed to the different chemical nature of the solvents. It is likely that different types of chemicals were dissolved in different solvents that resulted in variable activity of the extracts of same plant in different solvents. There are many examples in literature which support these findings. Similarly, Bajwa et al. (2007) carried out the study on antifungal activity of aqueous and n-hexane shoot extracts of *Aloe vera* against *A. alternata*, *Alternaria citri* and *A. tenuissima*. They reported that inhibitory effect was variable with applied concentrations and caused a significant inhibition in biomass production of the three test fungi.

It is clearly shown by the present study that average mycelial growth rate per day varied with the concentrations and also with the incubation period. At 10 days incubation, *A. alternata* showed greatest inhibition at higher concentrations while displayed minimum inhibition at lowest concentration of 1% aqueous extract. This work is supported by the findings of previous study in which aqueous extract of *Parthenium hysterophorus* showed maximum inhibition at lowest concentration at 5 days of incubation while gives minimum inhibition at same concentration at 10 days of inhibition against *A. alternata* (Bajwa et al., 2004). Presently, *A. tenussima* showed minimum inhibition at 3% concentration while maximum inhibition at 1 and 4% concentration of aqueous extract. With an increase in incubation period, minimum inhibition was noticed by lower concentration (1%) and maximum inhibition at higher concentration (4%). Another study by Hassan et al. (1992) evidenced similar effect of leaf extracts of *Datura stramonium* on decline of rust pustules on leaves of wheat.

A. alternata and *A. tenussima* both showed significant inhibition in 4% concentration at both incubation periods of 5 and 10 days. At lower concentration of methanol extract both strains exhibited minimum inhibition at both time periods. This demonstrated that methanol extract of lemongrass at high concentration may possess different and potential antifungal components. These findings are in line with the work of Bajwa et al. (2008b) who have reported maximum antifungal activity of methanol extract of rice varieties against *Macrophomia phaseolina* and *Ascochyta rabiei*. Earlier Daoud et al. (1990) have reported good antifungal activity of *Malia azedarach* against *Alternaria, Aspergillus* and *Penicillium spp*. Similarly, Iqbal et al. (2002) have accounted that extract of *M. azedarach* was effective against *Fusarium chlamydosporum, Aspergillus niger* and *Hyloflora ramosa.*

Earlier, there has been a considerable interest in extracts and essential oils from aromatic plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms in foods (Soliman and Badeaa, 2002). Presently, all the concentrations of essential oil of *C. citratus* caused complete inhibition in mycelial biomass production of both species *viz., A. alternata* and *A. tenussima*. In a study, Lemongrass oil decreased *Fusarium verticillioides* growth in PDA by 90 and 100% at 500 and 1000 ppm, respectively, being in accordance with the present study (Mishra and Dubey, 1994). In the same way, Baratta et al. (1998) reported 91% inhibition of the growth of *A. niger* in liquid culture media when treated with 1000 ppm lemongrass oil.

This study concludes that aqueous and methanolic extracts contain potential antifungal compounds, especially; essential oils possess strong antifungal activity and can be exploited as an ideal treatment for future plant disease management programs eliminating fungal spread.

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