DE GRUYTER OPEN

Acta Bot. Croat. 76 (1), 72–79, 2017
DOI: 10.1515/botcro-2016-0053

CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

Chemical composition and antifungal potential of medicinal plants against seedborne mycoflora of eggplant (*Solanum melongena* L.)

Bushra Ashiq[1], Sobia Chohan[1*], Rashida Perveen[1], Muhammad Abid[1] and Mirza Abid Mehmood[2]

[1] Department of Plant Pathology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan.

[2] Department of Plant Pathology, Mian Nawaz Sharif University of Agriculture, Multan, Pakistan

Abstract – Antifungal activities of medicinal plants were observed against seedborne mycoflora of eggplant (*Solanum melongena*). The effect of ethanolic leaf extracts of *Mangifera indica, Mentha spicata, Citrus limon, Eucalyptus camaldulensis* against four isolated fungal species including *Fusarium oxysporum*, *Aspergillus flavus*, *Rhizopus stolonifer* and *Penicillium digitatum* was evaluated at various concentrations, by using the poisoned food technique. The impact of the extracts on seed germination and growth of eggplant was assessed by seed treatment and growth in a greenhouse experiment. Total flavonoids of *E. camaldulensis* were analyzed through spectrophotometer, using quercetin as a standard. Physico-chemical parameters were also determined. Antifungal activity showed that maximum inhibition percentage of *P. digitatum* (67.78%) and *F. oxysporum* (64.44%) was observed at the highest concentration (80%) of *C. limon* and *E. camaldulensis* extracts, respectively, followed by *M. spicata* extract against *A. flavus* (63.33%) and *R. stolonifer* (52.22%). Least inhibition percentage of *F. oxysporum*, *P. digitatum*, *R. stolonifer* and *A. flavus* was 6.67, 7.78, 14.44 and 16.67%, respectively, at the lowest (20%) concentration of *M. spicata.* The greenhouse experiment showed variations in seedling germination and post-germination growth. *E. camaldulensis* extract showed an increase in percent germination (78.98%) over untreated control (62.83%), root and shoot length and fresh and dry weight of root and shoot with the consequent reduction in disease symptoms. Phytochemical analysis depicted the presence of alkaloids, flavonoids, tannins, saponins in all extracts while steroids and glycosides were absent. A fair amount (10.38 mg QE g^{-1}_{DF}) of flavonoid was present in leaf extract of *E. camaldulensis.* Physico-chemical analysis showed pH of 4.6, ash content of 0.41% and weight loss on drying of 8.14%.

Keywords: medicinal plants, physico-chemical parameters, secondary metabolites, seedborne mycoflora, *Solanum melongena*

Introduction

Eggplant, also commonly known as brinjal or aubergine (*Solanum melongena* L.), is an important Solanaceous vegetable crop of the sub-tropics and tropics and is cultivated for its fleshy fruits (Pandey 2010). It was later domesticated in India and is grown widely in Pakistan, India, Bangladesh, China and the Philippines. In Pakistan it is grown for local consumption with an annual production of 88148 tones over an area of 8673 hectares (GOP 2009). This important summer crop is prone to numerous diseases and pests during various stages of crop growth in all seasons in most of the tropical zones (Ali et al. 2012). The severity in any serious disease depends on the season and the region in which the crop is grown (Dhamdhere et al. 1995). Seedborne diseases are considered important because they bring about a significant reduction in yield. Different microorganisms affect the quality of seed by reducing its vigor, ultimately weakening the plant at its initial growth stage causing seed rot, pre- and post-germination death of seedlings (Ellis et al. 1975). Pathogenic fungi are the largest group of plant pathogens. Seed-borne diseases caused by fungi are relatively difficult to control. A primary inoculum can be avoided by using disease-free seed, choosing pathogen-free seedbed soil, treating infected seed and following a three year rotation. However, some pre-emptive measures in selection of seed such as color and texture can be helpful in avoiding future losses. Healthy seed is always recommended for plantation, to save time and labor. However, if seed is not disease free, it can be treated with pesticides or with hot

* Corresponding author, e-mail:sobia_mustafa2006@hotmail.com

water at 50 °C (Panwar and Chand 1970). Al-Kassimand Monawar (2000) identified more than 30 fungal species belonging to 19 genera on eggplant seeds. The most prevalent seed borne fungi of brinjal were *Aspergillus niger, A. flavus, A. clavatus, Penicilliumdigitatum*, *Pythium* sp., *Rhizoctonia* sp., *Rhizopusarrhizus, R. stolonifer, Alternaria alternate* and *Fusarium oxysporum.*

Large quantities of fungicides have been used all over the world by farmers to control fungal diseases in their fields, thus increasing production costs and contaminating food and the environment with toxic substances (Bowers and Locke 2000). Consequently, indiscriminate use of fungicides poses a serious threat to the environment and health. Use of bio pesticides gives a hope that synthetic pesticides can be replaced.. These pesticides are made up of natural products, and are easily biodegradable. A potentially useful substitute for expensive and possibly toxic fungicides could befound in plant extracts (Joseph et al. 2008).

The plant extracts of many higher plants have for centuries been known for their antimicrobial properties. Medicinal plants are storehouses of secondary phytomedicines. These compounds have additive or synergistic effects on the physiological processes of single or a multiple targets. Ethno-botany surveys all over the world support the medicinal use of these extracts against human and plant pests (Shariff et al. 2006, Gupta et al. 2008). Recently, the antimicrobial activity of some of the higher plant products that are biodegradable and safe for human health has gained the attention of researchers working on plant defenses against microbial ingress (Koirala et al. 2005). Numerous chemical compounds are present in medicinal plants including saponins, tannins, flavonoids, alkaloids, terpenoids, steroids, glycosides and proteins. These substances are potent bioactive compounds and can be used for remedial purposes (Sofowora 1993).

Although use of plant extracts is less laborious, more economical and more eco-friendly than that of fungicides, it is still not popular among stakeholders. The aim of this research was to uncover the potentials of these natural occurring chemicals against existing mycoflora associated with seeds. The present study was undertaken to exploit different plant species that have antifungal properties, primarily under *in vitro* and greenhouse conditions, to control the seedborne diseases of eggplant as an alternative to chemical fungicide.

Materials and methods

Sample collection

Eggplant seeds were obtained from the vegetable section of Ayub Agriculture Research Institute, Faisalabad and preserved in brown paper bags in the laboratory of Plant Pathology at Bahauddin Zakariya University (BZU) Multan, Pakistan, until further investigation.

Detection and isolation of fungi on seeds

Two hundred seeds were taken at random, surface sterilized with 1% (v/v) sodium hypochlorite solution and subjected to the standard blotter paper method for isolations of fungal mycoflora (ISTA1993). Ten seeds were placed on three-layered moistened blotter paper in 9cm diameter petri plates. After one week of incubation, fungal colonies emerging around the seeds were isolated by the single-hyphal isolation technique on potato dextrose agar (PDA) medium and incubated under a 12 h photoperiod at 27 °C for seven days for further identification and purification. The fungi were identified according to the morphological characters and relevant literature (Barnet and Hunter 1972, Ellis et al. 1975, Nelson et al. 1983). Pathogenicity was confirmed according to Koch's postulates.

Preparation of plant extracts

A previously described method (Neycee 2012) was used for extraction procedure. Fresh healthy leaves of indigenous medicinal plants: *Mangifera indica, Citrus limon, Eucalyptus camaldulensis* and *Mentha spicata* were collected in the premises of Bahauddin Zakariya University, Pakistan. Samples were thoroughly washed with tap water, followed by distilled water and finally with 70% ethanol (Merck) to eliminate any traces of contaminants. Blot dried leaves of each sample were then dried in the oven at 50 °C for 2 h, later shade dried and homogenized to fine powder and stored in airtight bottles. 10 g of powdered material was extracted using Soxhlet apparatus (J. P. Selecta-Spain) with 100 mL ethanol for 48 h. The extract solutions were centrifuged at 6000 rpm for 10 min, filtered with filter paper (Whatman No.1) and concentrated over a water bath at 40 °C. After complete solvent evaporation extract residues were sealed in dark bottles at 4 °C for further use.

Fungal growth assay (*in vitro*)

Four out of eight isolated fungal strains including *Fusarium oxysporum, Aspergillus flavus*, *Rhizopus stolonifer* and *Penicillium digitatum* were tested. The comparative toxicity of plant extracts on the growth of fungi was evaluated by the poisoned food technique (Nene and Thapilyal 2000). Prerequisite amounts of different concentrations (20, 30, 40, 60 and 80%) of plant extracts were incorporated aseptically into potato dextrose agar medium for inoculation of test fungi in 9 cm sterilized petri plates. Medium without any plant extract served as a control. A mycelial disk (0.6 cm) of test pathogens was placed at the center of each Petri plate and incubated for 7 days at 27 °C under a 12 h photoperiod. The experiment was carried out in triplicate. Mycelial growth (cm) of fungus was measured after 7 days of incubation. The inhibition percentage (P) was calculated according to the equation of Singhand Tripathi (1999) where dc denotes average increase in mycelial growth in control and dt denotes average increase in mycelial growth in treatment:

$$P=(dc–dt)/dc\times100$$

Greenhouse experiment

Fresh plant extracts were prepared by the method described above with distilled water (50:50 w/v) and filtered

through cheese cloth. Further, these extracts were diluted with distilled water to obtain an 80% concentration. Four hundred eggplant seeds per treatment were immersed in each extract solution for 30 min (ISTA 1996). Then the excess extract was drained off and seeds were blot dried and kept in the open air. The pots were arranged in a randomized complete block design with four replications. The seed rate was 10 seeds/ pot in each replication. Data regarding germination percentage, seedling height, shoot length, root length, fresh shoot weight, fresh root weight, dry root weight, dry shoot weight and average biomass parameters were recorded. Germination percentage was observed at 18 days after sowing (DAS) while other growth characters were recorded at 35 DAS.

Phytochemical analysis

Plant extract were subjected to qualitative phytochemical screening using the methods of Sofowora (1993) and Harborne (1973). For analysis of alkaloids (Wagner's test), a 20 mg of ethanolic extract was warmed with 2% sulphuric acid (H_2SO_4) for 1–2 min, filtered and treated with a few drops of Wagner's reagent. Presence of reddish brown precipitation or turbidity indicated the presence of alkaloids. For tannins (ferric chloride test) a 20 mg plant extract was dissolved in ethanol, a few drops of 0.1% ferric chloride were added and the extract was observed for the formation of blue black coloration. For steroids, a 2 mL of acetic anhydride and concentrated H_2SO_4 were added to 50 mg ethanolic plant extract. A blue green ring indicates the presence of steroids. For terpenoids (Salkowski Test), a 5 mL extract of each sample was mixed in chloroform (2 mL) and H_2SO_4 (3 mL) was added carefully to form a layer. Formation of reddish brown coloration at the interface indicates the presence of terpenoids. For analysis of saponins a 20 mg powdered sample was boiled in 5 mL distilled water and shaken vigorously for a stable persistent froth. Three drops of olive oil were mixed vigorously with the frothing and observed for the formation of emulsion. For flavonoids analysis, a powdered sample (20 mg) was heated with 10 mL of ethyl acetate for 3 min and filtered. The filtrate (4 mL) was mixed with 1 mL of dilute ammonia solution. A yellow coloration that disappears on addition of concentrated HCl indicated the presence of flavonoids.

Estimation of total flavonoid contents

Total flavonoid contents were determined following the procedure of Chang et al. (2002) with a slight modification. Quercetin was used as a standard to make the calibration curve. Plant extract (0.5 mL) was mixed with 1.5 mL methanol, 10% (w/v) aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL) and distilled water (2.8 mL). It was kept at room temperature for 30 min. Absorbance was measured at 415 nm by spectrophotometer (UV-3000).The calibration curve was prepared using quercetin standard solutions of 25, 50 and 100 mg L^{-1} in ethanol. Total flavonoid values are expressed in terms of mg quercetin equivalent per g of dried fraction (mg QE g^{-1}DF). The experiment was run in triplicate.

Physico-chemical analysis

Total ash contents, pH values and weight loss on drying were determined for ethanolic extract of *E. camaldulensis,* following the methods of Vaghasiya et al. (2008).

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) and treatment means were compared with Fisher's least significance difference (LSD) test at 5% level of significance (Steel and Torrie 1980) by using the statistical software Sigma plot 11.

Results

Eight fungal species belonging to 5 genera were isolated by the standard blotter method from seeds of eggplant. The highest percentage frequency was recorded by *Penicillium digitatum (*49.67%) followed by *Aspergillus flavus, Fusarium oxysporum, Rhizopus stolonifer,Alternaria alternata, Curvularia lunata, Aspergillus niger* and *Fusarium solani* with 47.33, 38.00, 33.00, 15.00, 13.67, 8.33 and 5.67% recovery percentage, respectively (Fig. 1). Out of recovered fungi, *C. lunata, A. alternata, A. niger* and *F. solani* were found in minimum incidence percentages, hence only predominant fungi, viz. *F. oxysporum, A. flavus, P. digitatum* and *R. stolonifer* were purified and subjected to antifungal assay with four plant extracts at five different concentrations (20, 30, 40, 60 and 80%).

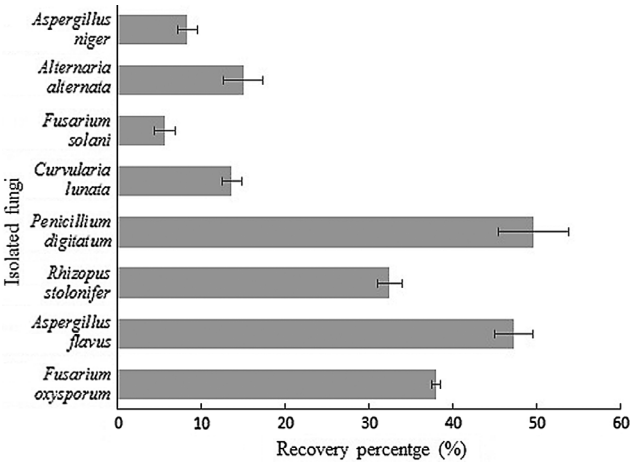

Fig.1. Recovery percentage (%±SD) of fungi isolated from eggplant seeds by using the standard blotter method.

All plant extracts revealed varied inhibitory effects on the mycelial growth of test fungi, by affecting their normal growth. The data regarding mean mycelial growth inhibition of four fungal species and ethanolic leaf extract of four extracts with different concentrations was statistically significant (Fig. 2). Extract of *M. spicata* at 20% concentration was found less effective against *A. flavus, R. stolonifer*, *P. digitatum* and *F. oxysporum* with 7.5, 7.7, 8.3 and 8.4 cm growth, respectively. At 30% concentration, mycelial growth of *P. digitatum* (7.9 cm), *F. oxysporum* (7.7 cm), *A. flavus* and *R. stolonifer* (6.8 cm) was observed. Less myce-

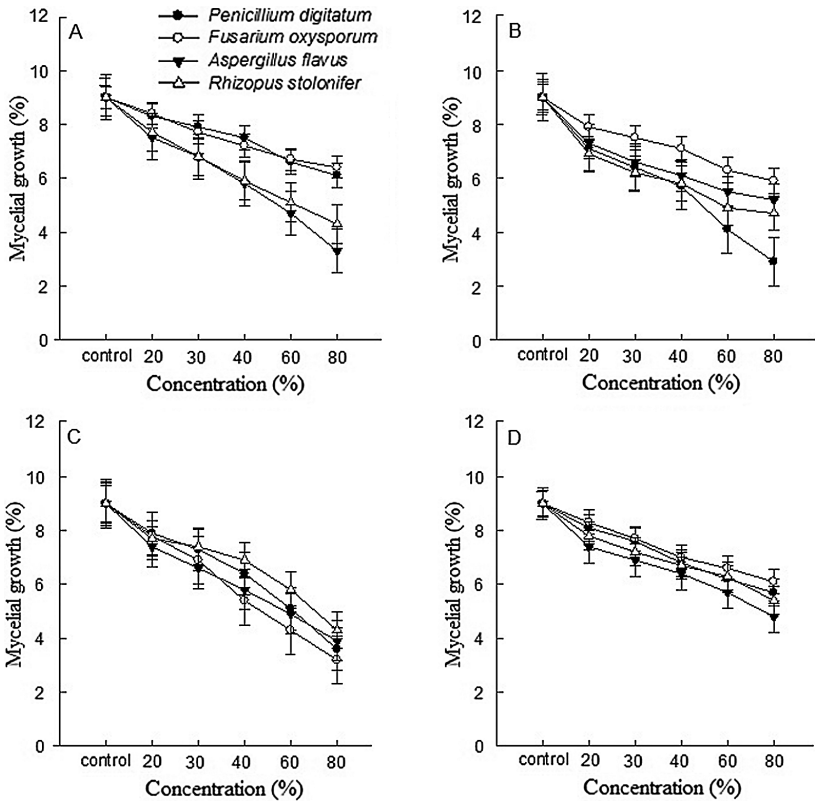

Fig. 2. Mycelial growth (mean±SE) of four tested fungi after using different concentrations of ethanolic leaf extract of four plants: A) *Mentha spicata*, B) *Citrus limon*, C) *Eucalyptus camaldulensis*, D) *Mangifera indica.*

lial growth, of 5.8 and 5.9 cm, was found for *A. flavus* and *R. stolonifer,* while more growth, of 7.2 and 7.5 cm, was observed for *F. oxysporum* and *P. digitatum* respectively at 40% concentration of *M. spicata* extract. Maximum mycelial growth at 60% concentration of *M. spicata* extract was of *F. oxysporum* (6.7 cm) and *P. digitatum* (6.6 cm) and minimum growth was of *R. stolonifer* (5.1 cm) and *A. flavus* (4.7 cm). At 80% concentration of *M. spicata* extract, the mycelial growth of *A. flavus* was best inhibited with only 3.3 cm growth followed by *R. stolonifer* (4.3 cm), *P. digitatum* (6.1 cm) and *F. oxysporum* (6.4 cm) compared to control fungi with 9.0 cm growth (Fig. 2A).

At 20% concentration of *C. limon*, the diagonal growth of *F. oxysporum* (7.9 cm), *A. flavus* (7.3 cm), *P. digitatum* (7.1 cm) and *R. stolonifer* (6.9 cm) was observed. *R. stolonifer*, *P.digitatum*, *A. flavus* and *F. oxysporum* showed 6.2, 6.4, 6.6 and 7.5 cm mycelial growth respectively at 30% concentration of *C. limon* extract. The minimum growth (5.7 cm) was of *P. digitatum* after *R. stolonifer* (5.8 cm), *A. flavus* (6.1 cm) and *F. oxysporum* (7.1 cm) at 40% concentration of *C. limon* extract. Maximum mycelial growth (6.3 cm) was of *F. oxysporum* followed by *A. flavus* (5.5 cm), *R. stolonifer* (4.9 cm) and *P. digitatum* (4.1 cm) at 60% concentration of *C. limon* extract. On the other hand, 80% concentration of *C. limon* extract was found to be the most effective and illustrated maximum growth inhibition of *P. digitatum* (2.9 cm) and minimum inhibition in *R. stolonifer*, *A. flavus, F. oxysporum,* with 4.7, 5.2 and 5.9 cm growth respectively compared to control fungi with 9 cm growth (Fig. 2B).

The mycelial growth of *P. digitatum*, *F. oxysporum, R. stolonifer* and *A. flavus* on agar plate containing different concentrations of ethanolic leaf extract of *E. camaldulensis* was highest with 7.9, 7.8, 7.7 and 7.4 cm at 20% concentration. At 30% concentration, the growth of *P. digitatum* and *R. stolonifer* was 7.3 and 7.4, whereas in *F. oxysporum* and *A. flavus* it was 6.9 and 6.6 cm respectively. Maximum mycelial growth (6.9 cm) was of *R. stolonifer* followed by *P. digitatum* (6.4 cm), *A. flavus* (5.8 cm) and *F. oxysporum* (5.4 cm) at 40% concentration of *E. camaldulensis* extract. Mycelial growth of *F. oxysporum* (4.3 cm), *A. flavus* (4.9 cm), *P. digitatum* (5.1 cm) and *R. stolonifer* (5.8 cm) decreased at 60% concentration *E. camaldulensis* extract. Minimum mycelial growth of 3.2, 3.6, 3.9 and 4.3 cm was observed in *F. oxysporum*, *P. digitatum*, *A. flavus* and *R. stolonifer* respectively, at 80% concentration of *E. camaldulensis* extract compared to control fungi with 9 cm growth (Fig. 2C).

At 20% concentration of ethanolic leaf extract of *M. indica,* mycelial growth of *F. oxysporum* (8.3 cm) was greater than in *P. digitatum* (8.1 cm), *R. stolonifer* (7.8 cm) and *A. flavus* (7.4 cm). At a 30% concentration, the mycelial growths of *A. flavus* (6.9 cm), *R. stolonifer* (7.2 cm), *P. digitatum* (7.6 cm) and *F. oxysporum* (7.7 cm) were observed. Growth of mycelia was 6.4, 6.7, 6.8 and 7 cm in *A. flavus*, *R. stolonifer*, *P. digitatum* and *F. oxysporum* respectively at a 40% concentration of *M. indica* extract. At a 60% concentration, maximum growth (6.6 cm) was seen for *F. oxysporum* followed by *R. stolonifer* (6.3 cm), *P. digitatum* (6.2 cm) and *A. flavus* (5.7 cm). Minimum mycelial growth (4.8

cm) was observed in *A. flavus* after *R. stolonifer* (5.4 cm), *P. digitatum* (5.7 cm) and *F. oxysporum* (6.1 cm) at 80% concentration of *M. indica* extract, compared to control fungi with 9 cm growth (Fig. 2D).

M. indica at 20% concentration showed minimum mycelial growth inhibition (13.33%) while maximum inhibition (52.22%) was observed for *M. spicata* and *E. camaldulensis* extracts at 80% concentration against *R. stolonifer.* Growth of *A. flavus* was inhibited by 16.67% and 63.33% at 20% and 80% concentration of *M. spicata* extract, respectively. Mean mycelial growth inhibition of *F. oxysporum* was lower (6.67%) at 20% concentration but maximum inhibition of 64.44% was observed at 80% concentration of *E. camaldulensis.* A maximum inhibition percentage (67.78%) of *P. digitatum* was observed at 80% concentration of *C. limon* with, while a minimum inhibition (7.78%) was observed at a 20% concentration of *M. spicata* extract (Fig 3A–D).

Results of variance analysis for the germination experiment showed that germination percentage and growth characters of eggplant were statistically significant except for dry root weight. Maximum germination was observed after treatment with *E. camaldulensis* extract (78.98%) followed by extracts of *C. limon* (73.05%), *M. spicata* (71.00%) and *M. indica* (64.10%), and control untreated plants (62.83%), respectively. Maximum seedling height was observed in seeds treated with E. *camaldulensis* (18.57 cm) and *C. limon* extracts (18.27 cm) and a minimum in control plants (12.87 cm). Shoot length was greater (15.25 cm) after treatment with *E. camaldulensis* extract than in control plants

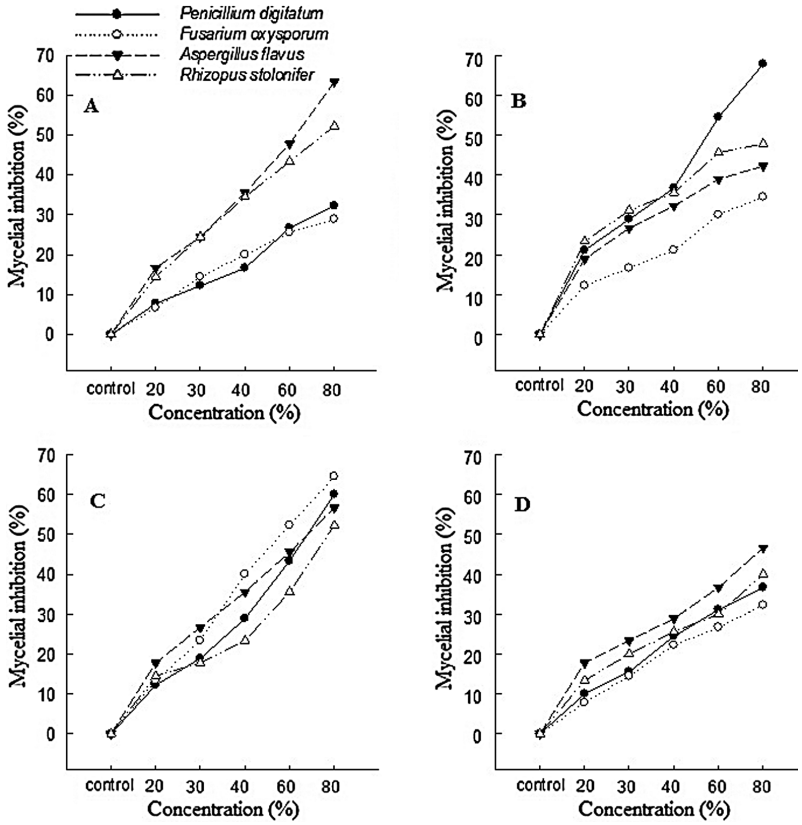

Fig. 3. Mycelial inhibition (mean±SE) by four different plant extracts at different concentrations: A) *Mentha spicata*, B) *Citrus limon*, C) *Eucalyptus camaldulensis*, D) *Mangifera indica.*

Table 1. Effect of different plant extracts on eggplant seed germination and growth parameters. Means sharing different letters are statistically different at 5% level of significance. Values are means of four replications. T1 – *Eucalyptus camaldulensis*, T2 – *Mangifera indica*, T3 – *Citrus limon*, T4 – *Mentha spicata*, T5 – negative control; LSD – least significant difference.

Treatments	Germination (%)	Seedling height (cm)	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Dry root weight (g)	Biomass (g)
T1	78.98 a	18.575 a	15.25 a	4.05 a	0.825 a	0.0723 a	0.0805 a	0.0080 a	1.015 a
T2	64.10 c	14.175 c	11.95 bc	2.73 c	0.550 c	0.0463 d	0.0588 d	0.0053 a	0.685 cd
T3	73.05 b	18.275 a	14.85 a	3.85 ab	0.900 ab	0.0660 b	0.0730 b	0.0078 a	0.898 b
T4	71.00 b	16.125 b	12.90 b	3.40 b	0.750 abc	0.0593 c	0.0660 c	0.0065 a	0.738 c
T5	62.83 c	12.875 d	11.53 c	2.80 c	0.625 bc	0.0478 d	0.0565 d	0.0070 a	0.648 d
LSD P=0.05	2.129	1.113	1.323	0.514	0.205	0.005	0.006	0.0028	0.075

(11.53 cm). Minimum root length was observed in seeds treated with *M. indica* extract (2.73 cm) and maximum in seeds treated with *E. camaldulensis* extract (4.05 cm). Fresh shoot weight was greater after seed treatment with *C. limon* extract (0.90 g) than after treatment with *M. indica* extract (0.55 g). Highest fresh root weight was after seed treatment with *E. camaldulensis* extract (0.072 g) and the lowest after treatment with *M. indica* extract (0.046 g). Greatest dry shoot weight and biomass was observed in seeds treated with *E. camaldulensis* (0.08 g and 1.015 g) and lowest in control (0.05 g and 0.64 g), respectively (Tab. 1).

Ethanol leaf extract of *E. camaldulensis* was found rich in flavonoids, alkaloids, tannins and saponins while steroids and glycosides were absent. Quantitative analysis of ethanol extract resulted in 10.38 mg QE g^{-1} flavonoid contents in *Eucalyptus* leaves. The results of physico-chemical analysis revealed pH value of 4.6, ash content of 0.41% and weight loss on drying of 8.14%.

Discussion

Seedborne microorganisms play an important role in affecting the quality of seed, causing significant crop losses. Predominant fungi associated with seed samples of eggplant were identified as *Fusarium oxysporum, Aspergillus flavus, Penicillium digitatum* and *Rhizopus stolonifer,* causing quantitative and qualitative losses to seeds during storage (Neergard 1977). Opportunistic pathogens such as *Aspergillus sp.* and *Penicillum sp.* were found associated with seed infection and caused severe damage to both quality and quantity of seed production (Kumar et al. 2005). Traditionally, seeds have been treated with fungicides to kill microorganisms. However, the World Health Organization (WHO) banned many agriculturally important pesticides due to their toxicity against non-target organisms including humans, and because they are known to cause pollution problem (Barnard et al. 1977). Many plant extracts and oils, such as tea tree and clove had been used as contemporary antiseptics, or had been reported to have antimicrobial properties (Hoffman 1987, Rice 2012). Extracts from different plants have been reported to have antifungal, antibacterial and antioxidant activities. The antifungal activity of diverse plants against different fungi has been reported (Farrag and Moharam 2012, Vidua-Martos et al. 2008).

Results in the present study showed that four fungal species were isolated from eggplant seeds. Their growth was best during February, March and confirms the results of Pandey (2010) who detected five fungi on brinjal viz. *F. solani, Helminthosporium spiciferum, Phomopsis vexan, C. lunata, Trichothecium roseum.* The growth of these fungi was particularly luxurious from October to November, and February to April. Different concentrations of different plant extracts exhibited different activities. In this study five extract concentrations (20, 30, 40, 60 and 80%) were used against test fungi and illustrated varying results. Some concentrations were weak while few were effective in controlling mycelial growth of test fungi. Similarly, Joseph et al. (2008) used different concentrations, i.e., 5, 10, 15 and 20% of plant extracts. Among the different extracts 20% of *Azardiachta indica* was found most effective followed by *Rheum emodi, Eucalyptus globulus, Artemessia annua* and *Ocimum sanctum*. In our study, in the case of *R. stolonifer* and *A. flavus* the best inhibition was exhibited by *E. camaldulensis* and *M. spicata* extracts, respectively, at 80% concentration. Similar results were also reported by different scientists at different times (Satish et al. 2007). On the other hand, Tzortzakis and Economakis (2007) found lemongrass (*Cympopogon citratus*) extract to be effective against *R. stolonifer* and *A. niger.* In our study, *F. oxysporum* was best inhibited by *E. camaldulensis* extract at 80% concentration. The results correlated with previous findings that aqueous leaf extracts of *E. citriodora*, *M. indica*, *Accacia nilotica*, *A. indica* and *Syzygium cumini* significantly reduced the incidence of the two most frequent seed-borne fungi, viz., *F. solani*, and *A. alternata* (Shafique et al. 2007, Marzoug et al. 2011). *P. digitatum* was best inhibited at higher concentration by *C. limon* extract in our study. These findings are in line with the results of Kanan et al. (2008), who evaluated the effect of different plant extracts and liquid fractions against citrus post-harvest disease agent *P. digitatum.* Similarly, Ragab et al. (2012) found that mint, thyme, peppermint and clove had significant inhibitory effects on fungal mycelia. Fungal mycelial growth decreased significantly as the concentrations of extracts increased. Mango leaf extract moderately reduce the growth of pathogenic fungi. In the same way Suvarna and Patil (2009) showed that extract of *M. indica* had moderate activity against the human pathogenic fungi *Candida albicans*.

Seed germination and other growth parameters of eggplant were greater in seeds treated with *E. camaldulensis* extract as compared to control treatment. Conversely *E. camaldulensis* significantly reduced seed germination of sorghum (Mohamadi and Rajaie 2009). *M. indica* extract was found least effective among all the extracts used. Present results are in harmony with the findings of Kumar et al. (2005) and Alberts et al. (2006). They reported that pre- and post-harvest bio-deterioration of crop seeds are mainly due to seed infestation by microorganisms and insects, causing up to 100% losses. Contrary to our results, leaf extract of *Moringa oleifera* increased seed germination of eggplant up to 92% over control treatment (Kuri et al. 2011).

E. camaldulensis extract was found most effective in reducing the fungal growth of seedborne mycoflora, eventually increasing the germination of eggplant seed. Similarly *E. camaldulensis* exhibited pronounced antifungal activity, among different species of eucalyptus (May and Ash 1990, Rukhsana. 2005, Babayi et al. 2004, Ghalem and Mohamed 2008) and this might be due to the presence of secondary compounds. Thus the phytochemical and physico-chemical parameters of *E. camaldulensis* were studied. Phytochemical screening of leaf extract of *E. camaldulensis* extract revealed the presence of tannins, flavonoid, alkaloids and saponins, while steroids and glycosides were absent, and these results are similar to earlier findings (Vaghasiya et al. 2008).

A good amount of flavonoids were also detected in leaves of *E. camaldulensis,* confirming their role in plant growth and defense against infections (Abd-Alla et al. 1980, Larson 1998, Pourmorad et al. 2006). The present results are in harmony with previous researchers who also extracted higher contents of flavonoids from leaves of *E. camaldulensis* in ethanol solvent (El-Ghorab et al. 2003, Gharekhani et al. 2012). According to Takahashi et al. (2004) three flavonoids (2',6'-dihydroxy-3'-methyl-4'-methoxy-dihydrochalcone,8-desmethyl-eucalyptin and eucalyptin) isolated from *Eucalyptus maculata* leaf extracts commonly exhibited potent antimicrobial activities against seven micro-organisms causing food poisoning. The physico-chemical analysis showed that the compounds present in *E. camaldulensis* were acidic in nature. These results were correlated with previous results obtained by Vaghasiya et al. (2008) when analyzing pH, ash values and loss on drying.

Conclusion

It was presumed that different plant extracts performed differently at different concentrations and exhibited significant antifungal activity against different pathogenic fungi. The antifungal effect exhibited by the selected plants might be credited to the existence of either single or synergistic consequences of more than one compound. The results of the present study can be further applied to the formulation of an integrated disease management program for eggplant seedborne mycoflora. More investigations are needed to examine the separation and depiction of antifungal moieties and acclamation in field applications.

Acknowledgments

We are very thankful to Seed Certification Department, Islamabad for providing seeds of eggplant.

References

Abd-Alla, M. F., El-Negoumy, S. I., El-Lakany, M. H., Saleh, N. A. M., 1980: Flavonoid glycosides and the chemosystematics of *Eucalyptus camaldulensis*. Phytochemistry 19, 2629–2632.

Alberts, J. F., Engelbrecht, Y., Steyn, P. S., Holzapfel, W. H., Vanzyl, W. H., 2006: Biological degradation of aflatoxin B1 by *Rhodococcus erythropolis* cultures. International Journal of Food Microbiology 109, 121–126.

Ali, M., Ashfaq, M., Akram, W., Sahi, S. T., Ali, A., 2012: The physio- morphic characters of the brinjal (*Solanum melongena* L.) plant and their relationship with the jassid (*Amrasca biguttulla*) population fluctuation. Pakistan Journal of Agricultural Science 49, 67–71.

Al-Kassim, M. Y., Monawar, M. N., 2000: Seed-borne fungi of some vegetable seeds in Gazan province and their chemical control. Saudi Journal of Biological Sciences7, 179–185.

Babayi, H., Kolo, I., Okogun, J. I., Ijah, U. J. J., 2004: The antimicrobial activities of methanolicextracts of *Eucalyptus camaldulensis* and some pathogenic microorganisms. Biokemistri 16, 106–111.

Barnard, C., Padgitt, M., Uri, N. D., 1997: Pesticide use and its measurement. International Pest Control 39, 161–164.

Barnett, H. L., Hunter, B. B., 1972: Illustrated genera of imperfect fungi, 3rd edition. Burgess Publishing Co. Minnesota.

Bowers, J. H., Locke, J. C., 2000: Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil and control of *Fusarium* wilt in the greenhouse. Plant Disease 84, 300–305.

Chang, C. C., Yang, M. H., Wen, H. M., Chern, J. C., 2002: Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10, 178–182.

Dhamdhere, S., Dhamdhere, S.V., Mathur, R., 1995: Occurrence and succession of pests of brinjal, *Solanum melongena L.* at Gwalior (M.P.). Indian Journal of Entomological Research 19, 71–77.

El-Ghorab, A. H., El-Massry, K. F., Marx, F., Fadel, H. M., 2003: Antioxidant activity of *Eucalyptus camaldulensis* var. *brevirotsris* leaf extracts. Food/Nahrung 47, 41–45.

Ellis, M. A., Ilyas, M. B., Sincair, J. B., 1975: Effect of three fungicides on internally seedbornefungi and germination of soybean seeds. Phytopathology 65, 553–556.

Farrag, E. S., Moharam, M. H., 2012: Pathogenic fungi transmitted through cucumber seeds and safely elimination by application of peppermint extract and oil. Notulae Scientia Biologicae 4, 83–91.

Ghalem, B. R., Mohamed, B., 2008: Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*. African Journal of Pharmacy and Pharmacology 2, 211–215.

Gharekhani, M., Ghorbani, M., Rasoulnejad, N., 2012: Microwave-assisted extraction of phenolic and flavonoid compounds from *Eucalyptus camaldulensis* Dehn leaves as compared with ultrasound-assisted extraction. Latin American Applied Research 42, 305–310.

Government of Pakistan, GOP, 2009: Fruits vegetables and condiments statistics of Pakistan. Ministry of Food and Agriculture (Economic wing) Islamabad. pp. 11–12.

Gupta, C., Garg, A.P., Uniyal, R. C., Kumari, A., 2008: Antimicrobial activity of some herbal soils against common food borne pathogens. African Journal of Microbiology Research 2, 254–261.

Harborne, J. B., 1973: Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall Ltd, London.

Hoffman, D. L., 1987: The herb user's guide. Thorsons Publishing Group, Wellingborough, UK.

ISTA, 1993: International rules for seed treating. Seed Science and Technology 21, 160–186.

ISTA, 1996: International rules for seed testing. International Seed Testing Association, Switzerland.

Joseph, B., Muzafar, A., Vinod, K., 2008: Bioefficacy of plant extracts to control *Fusarium solani* f. sp. melongenae incitant of brinjal wilt. Global Journal of Biotechnology and Biochemistry 3, 56–59.

Kanan, G. J., Rasha, A., Al-Najar., 2008: *In vitro* antifungal activities of various plant crude extracts and fractions against citrus post-harvest disease agent *Penicillium digitatum*. Jordan Journal of Biological Science 22, 89–99.

Koirala, P., Kumar, S., Yadar, B. K., Premarajan, K. C., 2005: Occurrence of aflatoxin in some of the food and feed in Nepal. Indian Journal of Medical Sciences 59, 331–336.

Kumar, R. S., Sivakumar, T., Sunderam, R. S., 2005: Antioxidant and antimicrobial activity of *Bauhinia racemosa* L. stem bark. Brazilian Journal of Medical and Biological Research 38, 1015–1024.

Kuri, S. K., Islam, M. R., Mondal, U., 2011: Antifungal potentiality of some botanical extracts against important seedborne fungal pathogen associated with brinjal seeds, *Solanum melongena* L. Journal of Agricultural Technology 7, 1139–1153.

Larson, R. A., 1988: The antioxidants of higher plants. Phytochemistry 27, 969–978.

Marzoug, H. N. B., Romdhane, M., Lebrihi, A., Mathieu, F., Couderc, F., Abderraba, M., Khouja, M.L., Bouajila, J., 2011: *Eucalyptus oleosa* essential oils: chemical composition and antimicrobial and antioxidant activities of the oils from different plant parts (stems, leaves, flowers and fruits). Molecules 16, 1695–1709.

May, F. E., Ash, J. E., 1990: An assessment of the allelopathic potential of eucalyptus. Australian Journal of Botany 38, 245–254.

Mohamadi, N., Rajaie, P., 2009: Effect of aqueous eucalyptus (*E. camaldulensis* Labill) extracts on seed germination, seedling growth and physiological responses of *Phaseolus vulgaris* and *Sorghum bicolor*. Research Journal of Biological Sciences 4, 1291–1296.

Neergard, P., 1977: Seed Pathology**.** New York: John Wiley.

Nelson, P. E., Toussoun, T. A., Marasas, W. F. O., 1983: *Fusarium* species – an illustrated manual for *Fusarium* research. The Pennsylvania State University Press, University Park and London.

Nene, Y., Thapilyal, L., 2000: Poisoned food technique of fungicides in plant disease control. 3rd Edn, Oxford and IBH Publishing Company, New Delhi.

Neycee, M. A., Nematzadeh, G. H. A., Dehestani, A., Alavi, M., 2012: Assessment of antifungal effects of shoot extracts in chinaberry (*Melia azedarach*) against 5 phytopathogenic fungi. International Journal of Agriculture and Crop Science 4, 474–477.

Pandey, A., 2010: Studies on fungal diseases of eggplant in relation to statistical analysis and making of a disease calendar. Recent Research in Science and Technology 2, 1–3.

Panwar, N. S., Chand, J. N.,1970: Phomopsis fruit rot of brinjal (*Solanum melongena*) in the Punjab. Viability of fungus and role of seeds in disease development. Journal of Research-Punjab Agricultural University 7, 641–643.

Pourmorad, F., Hosseinmehr, S. J., Shanabimajd, N., 2006: Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology 5, 1142–1145.

Ragab, M. M., Ashour, A. M. A., Abdel-Kader, M. M., El-Mohamady, R., 2012: In vitro evaluation of some fungicides alternatives against *Fusarium oxysporum* the causal of wilt disease of pepper (*Capsicum annum* L.). International Journal of Agriculture Forestry 2, 70–77.

Rice, E. L., 2012: Allelopathy. Academic press.

Rukhsana, B., 2005: Antifungal activity of allelopathic plant extracts VI: in vitro control of fungal pathogen by aqueous leaf extracts of eucalyptus. Mycopath 3, 7–12.

Satish, S., Mohana, D.C., Ranhavendra, M.P., Raveesha, K.A., 2007: Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. Journal of Agricultural Technology 3, 109–119.

Shafique, S., Javaid, A., Bajwa, R., 2007: Effect of aqueous leaf extracts of allelopathic trees on germination and seed borne mycoflora of wheat. Pakistan Journal of Botany 39, 2619–2624.

Shariff, N., Sudarshana, M. S., Umesha, S., Hariprasad, P., 2006: Antimicrobial activity of *Rauvolfia etraphylla* and *Physalis minima* leaf and callus extracts. African Journal of Biotechnology 5, 946–950.

Singh, J., Tripathi, N. N., 1999: Inhibition of storage fungi of blackgram (*Vigna mungo*) by some essential oils. Flavor and Fragrance Journal 14, 1–4.

Sofowora, A., 1993: Medicinal plants and traditional medicine in Africa. Chichester John, Willey and Sons, New York, p. 256.

Steel, R. G. D., Torrie, J. H., 1980: Principles and procedure of statistics approach. 2nd edn, McGraw Hill Company, New York.

Suvarna, V., Patil, S., 2009: Antifungal activity of selected plant extracts against human fungal pathogens. Journal of Herbal Medicine and Toxicology 3, 151–153.

Takahashi, T., Kokubo, R., Sakaino, M., 2004: Antimicrobial activities of eucalyptus leaf extracts and flavonoids from *Eucalyptus maculata*. Letters in Applied Microbiology 39, 60–64.

Tzortzakis, N. G., Economakis, C. D., 2007: Antifungal activity of lemongrass (*Cymbopogon citratus* L.) essential oil against key postharvest pathogens. Innovative Food Science and Emerging Technologies 8, 253–258.

Vaghasiya, Y., Nair, R., Chanda, S., 2008: Antibacterial and preliminary phytochemical and physico-chemical analysis of *Eucalyptus citriodora* Hk leaf. Natural Product Research 22, 754–762.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernandez-Lopez, J., Perez-Álvarez, J., 2008: Antifungal activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. Food control 19, 1130–1138.