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EXPLOITATION OF NATURAL PRODUCTS AS AN ALTERNATIVE STRATEGY TO CONTROL STEM END ROT DISEASE OF MANGO FRUIT IN PAKISTAN

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Stem end rot (SER) disease development is the major issue affecting the delivery of quality mangoes to distance markets, especially through sea-shipments. Non availability of registered fungicidal products in Pakistan for the management of SER disease of mango is a major hurdle to overcome this enigma. Investigations were carried out to exploit natural products instead of fungicides for the effective management of SER. Fully mature but unripe mango fruits were collected from major mango growing belt of Punjab Province. The fruits were stored in cold storage (12°C; 4-5 weeks) and ambient storage (25°C; 2 weeks). After ripening, fruit under ambient and cold storage revealed that SER was the most prevalent disease. Associated pathogens appeared during ripening were isolated and identified as *Lasiodiplodia theobromae, Phomopsis mangiferae, Colletotrichum gloeosporioides*, *Alternaria alternata, Botrytis cinerea* and *Cytosphaera mangiferae*. In subsequent research, the efficacy of different plant extracts (*Cichorium intybus, Peganum harmala, Syzygium aromaticum, Moringa oleifera, Coriandrum sativum* and *Cinnamomum zeylanicum*) at different concentrations was evaluated *in vitro* and *in vivo*. Results revealed that *M. oliefera, S. aromaticum* and *C. zeylanicum* showed statistically significant antifungal activity against mycelial growth of tested pathogens and SER development.

Keywords: Mango, postharvest, cold storage, plant extract, *Moringa oleifera, Syzygium aromaticum.*

INTRODUCTION

Mango, being a perishable fruit, is subjected to various diseases caused by different pathogens that results in the loss of huge quantity of produce during post-harvest processes like grading, packing, marketing, storage and shipping to distance markets especially through sea shipment (Jha *et al.,* 2010). Although, Pakistan is the 4th largest producer and exporter of mango but still facing several challenges specifically in term of post-harvest diseases which limits its share in international markets (Ambreen *et al*., 2014; Sharma, 2014).

The post-harvest losses of mango are up to 17-36 % which may rise up to 100 % if proper management strategies are not in place, along with conditions favor the disease development (Johnson, 2008; Haggag, 2010).

Among postharvest diseases of mango fruit, stem end rot caused significant loses that limit the thriving mango industry of Pakistan (Barkai-Golan, 2001; Tarnowski and Ploetz, 2008; Ambreen *et al.* 2014). Several pathogens have been found associated with this disease globally including *Lasiodiplodia theobromae, Neofusicoccum parvum, Fusicoccum aesculi, Nattrassia mangiferae* and *Fusicoccum* spp. in Australia and Brazil (Slippers *et al.* 2005; Costa *et al.* 2010) while *Lasiodiplodia theobromae, Colletotrichum gloeosporioides, Alternaria alternata* and *Botrytis cinerea* in Egypt (Abdalla *et al.,* 2003).

Post-harvest disease management is very crucial issue during mango shipment particularly in distance markets (Amin *et al.,* 2011). A growing number of foreign market places are no more permitting fungicidal application for import of fruits and vegetables because synthetic fungicides are very injurious due to their maximum residue level (MRL) on consumer's health (Athukoralage, 2001; Hussain *et al.,* 2011). Currently, plant extracts has attained consideration because they are non-toxic, safe for humans and environment, have antifungal activity and considered as best alternative of fungicides for post-harvest disease management (Obagwu and Korsten, 2003). Numerous studies have revealed the antifungal activity of plant extracts on a number of phytopathogenic fungi (Bonjar *et al.,* 2005; Panti and kolte, 2006; Bhalodia *et al.,* 2011). The aim of present study was to explore SER associated fungal pathogens, their pathogenicity and *in vitro* as well as *in vivo* screening of medicinal plant extracts as an alternative approach to manage SER disease.

MATERIALS AND METHODS

***Survey and sample collection*:** The survey was conducted during 2013-2014 in the selected orchards of Districts Rahim Yar Khan, Multan and Vehari of Punjab Province. Five mango trees in each selected orchard were randomly tagged. During the course of sampling, general observations were

made *viz*., tree age, the amount of litter beneath the tree, watering regimes and pre-harvest spray programs.

One hundred un-ripened but fully mature mango fruits of cv. Samar Bahisht (S.B) Chaunsa were collected from each selected orchards. Mango fruits after harvesting and de-sapping at farm were brought to Post Harvest Research and Training Center, University of Agriculture, Faisalabad in a cool chain van and stored in cold storage (CS) (12ºC; 4-5 weeks) and ambient storage (25ºC; 2 weeks). After removal from storage, these fruits were placed at room temperature (33ºC) for ripening and evaluated for disease prevalence and data was recorded separately.

***Disease assessment study*:** Fruits exhibit SER symptoms after ripening were brought to Plant Disease Diagnostic Laboratory, University of Agriculture, Faisalabad. The isolations were made through isolation technique used by (Ambreen *et al.*, 2014). For this purpose, infected portion from peel of mango fruits along with healthy portion was cut and immersed in 70 % ethanol for 1 minute followed by subsequent washing with sterilized water, dried on sterilized filter papers and finally placed in petri dishes containing Potato Dextrose Agar (PDA) medium under aseptic conditions. The plates were then incubated at 25ºC for 4-5 days and observed for appearance of fungal colonies. Hyphal tip method was used for purification of isolated fungal pathogens. The identification was done on the basis of morphological characteristics like spore color, shape and size (Barnett and Hunter, 1998). Frequency percentage of isolated fungi was calculated by following formula:

$$\text{Frequency \%} = \frac{\text{Number of isolated fungi}}{\text{Total number of isolates}} \times 100$$

***Pathogenicity test*:** Based on the fungal species that were identified after isolation from symptomatic mango tissues, six isolates were selected for this test including *Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Phomopsis mangiferae, Alternaria alternata, Botrytis cinerea* and *Cytosphaera mangiferae*. Spore suspension (1×10^4 spores/ml) was prepared from purified cultures grown on PDA. The mycelial scraped was suspended in 500 ml distilled water and shaking vigorously for 10 minutes on a mechanical shaker. The resulting suspension was passed through two layers of cheese cloth. The concentration of spore suspension was adjusted to (1×10^4 spores/ ml) using haemocytometer (Sivakumar *et al.*, 1997).

Unripe but mature mango fruits were selected for *in vitro* pathogenicity test. Fruits were de-sapped and surface sterilized with 1 % NaOCL solution for 3 minutes then washed with sterilized water and allowed to air dry. Fruits were pricked with a sterilized scalpel to a depth of 4 mm at the stem end portion, then inoculated with 50 µl of the spore suspension using a microliter syringe (Sun *et al.*, 2008). Fruits of control treatment were inoculated with sterilized water only. The experiment was run using three replications for each treatment and each replicate consisted of 15 mangoes. The fruits of each treatment were placed separately into plastic crates followed by incubation at ambient temperature (25°C) for 9 days and in cold storage (12°C) for 21 days, in the Post-Harvest Research and Training Centre, Institute of Horticultural Sciences, University of Agriculture Faisalabad.

***Assessment of post-harvest SER incidence*:** After inoculation, mango fruits were assessed for SER incidence according to scale developed by (Corkidi *et al.*, 2006). The data on percent disease severity was recorded and statistically analyzed.

$$\text{DS\%} = \frac{\text{Sum of all the dis. rating on fruits}}{\text{Number of diseased fruits}} \times \frac{100}{\text{Max. disaese grade}}$$

Where, DS = Disease severity

***Preparation of plant extracts*:** The scientific and common names, families and plant part used are presented in (Table 1). The bark and seeds of the medicinal plants used in present study were purchased from a local market. The plant leaves were collected from Botanical garden, University of Agriculture, Faisalabad. Seeds and other plant parts were taxonomically identified and confirmed from Department of Botany, University of Agriculture, Faisalabad. After collection, all the parts used were washed under tap water and dried under shade and then grounded by using liquid nitrogen and extracted (48 h) with absolute ethanol and methanol in a soxhlet apparatus (Ndukwe *et al.*, 2006). The solvents were removed separately using rotary evaporator (Heidolph, VV2000) under reduced pressure at temperature below 50°C. The resulting crude extracts were stored at 20°C until assayed. Stock solutions and serial dilutions of extracts were prepared in dimethyl sulphoxide (DMSO) (Ambrozin *et al.*, 2004).

Table 1. Medicinal plants and their parts used

Sr	Scientific Name	Common Name	Part Used	Family
1	*Cichorium intybus*	Kasni	Seed	Asteraceae
2	*Peganum harmala*	Hermal	Seed	Nitrariaceae
3	*Syzygium aromaticum*	Clove	Seed	Myrtaceae
4	*Moringa oleifera*	Sohanjna	Leaves	Moringaceae
5	*Coriandrum sativum*	Coriandar	Seed	Apiaceae
6	*Cinnamomum zeylanicum*	Cinnamon	Bark	Lauraceae

***In vitro antifungal assay of medicinal plant extracts*:** Antifungal activity of selected medicinal plants (*Syzygium aromaticum, Cinnamon zelanicum, Moringa oleifera, Cichorium intybus, Coriandrum sativum* and *Peganum harmala*) was assessed against SER associated pathogens using agar well diffusion method (Perez *et al.*, 1990). For this purpose PDA media was prepared and 20 ml media was poured in each 9 cm petri plates. After solidification, four wells of 6 mm diameter were made in each petri plate with the help of a sterilized cork borer. Different concentrations (5, 15, 25 and 50 μg/mL) were prepared in DMSO and applied in each well using a micropipette. Purified fungal colony (5 mm) was picked with the help of a sterilized loop and placed in the

center of each petri plate. The plates were incubated at 28ºC. The experiment was run by using seven treatments with three replications. Control experiment was performed by using DMSO with same concentration used to test the extracts. Extracts were dissolved in DMSO and evaluated for Diameter of Inhibition Zone (DIZ) against the fungal pathogens.

$$\text{Dia. of Inhibition Zone} = \frac{\text{diameter of sample} - \text{diameter of control}}{\text{diameter of control}}$$

(Aznita *et al.*, 2011)

***In vivo antifungal assay of medicinal plant extracts*:** Based on the results of *in vitro* test, two concentrations including 25 and 50 μg/mL of ethanolic and methanolic extracts of *M. oliefera*, *S. aromaticum* and *C. zeylanicum* were selected and used for their effect on SER disease development on harvested fruits. For this purpose, undamaged, mature fruits of comparable size and free from any pesticides were used. Fruits were surface sterilized by dipping in 1 % sodium hypochlorite solution for 3 min and rinsed twice in sterile distilled water. Conidial suspension of *L. theobromae* (most pathogenic fungus) was prepared and adjusted to a concentration (1×10^4 spores/ml) using haemocytometer. Fruit were pricked with a sterilized scalpel to a depth of 4 mm at the stem end portion. Immediately after wounding, these fruits received simultaneously 50 µl of conidial suspension and 50 µl of each plant extract at the above concentrations that showed fungicidal activity during *in vitro* test (Chuku *et al.*, 2010). The control fruits were injected with sterile distilled water only. Nativo (systemic broad spectrum fungicide with protective and curative activity) was used as positive control in this test. The inoculated and treated fruits of each treatment were placed into plastic crates, covered by plastic sheets and stored at ambient temperature (25ºC) till 9 days. The experiment was run using three replications for each treatment and single replicate comprise of 12 fruits. The experiment was laid out in CRD. Three days after storage, the disease severity of inoculated and treated fruits was recorded.

***Statistical analysis*:** Experiments were conducted under completely randomized design. All experiments were performed twice. Analysis showed no significant interaction between the two tests run for any of the treatments. Therefore, results from duplicate tests were combined for the final analysis. The collected data was statistically analyzed using computer software Statistix® 8.1. Analysis of variance techniques were employed to test the overall significance of the data and Tukey's test ($P\leq 0.05$) was used to compare the differences among the treatment mean except pathogenicity data where LSD test was performed (Steel *et al.*, 1997).

RESULTS

***Disease assessment study*:** After isolations, six different fungi including *Alternaria alternata, Lasiodiplodia theobromae, Botrytis cinerea, Colletotrichum gloeosporioides, Phomopsis mangiferae* and *Cytosphaera mangiferae* were found to be associated with the stored mango fruits showing SER symptoms. The data of frequency percentage showed that *L. theobromae*, *C. gloeosporioides*, and *A. alternata* were severely spreading diseases in the evaluated orchards of Rahim Yar Khan, Multan and Vehari. In both storage conditions, maximum disease frequency of *L. theobromae* was recorded followed by *C. gloeosporioides* and *A. alternata*. No association of *C. mangiferae* was found with the fruits collected from Vehari orchards (Fig. 1).

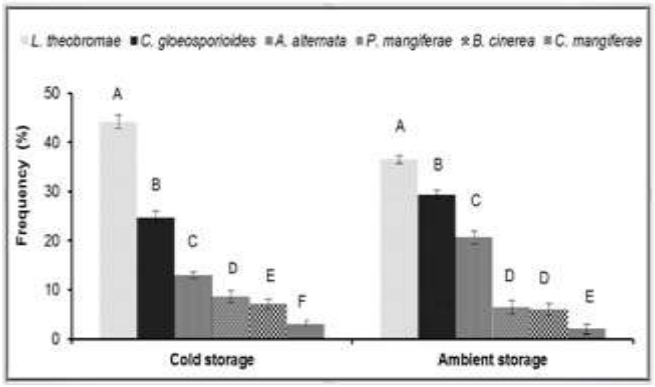

Figure 1. Frequency of fungal pathogens of stem end rot (SER) isolated from symptomatic mango fruit (cv. Samar Bahisht Chaunsa) that were exposed to cold storage (12ºC) or ambient storage (25ºC). Means within a cluster of bars (± S.E) that are followed by the same letter do not differ significantly according to the Tukey's test ($P\leq 0.05$). n=100

Morphological characteristics of isolated fungal pathogens

***Alternaria alternata*:** Mature conidia were beakless or short conical beak, elongated on branching chains, individual chains of 5-15 conidia. Discrete conidiophores rise freely from substrate developing bushy heads containing 4-8 large conidia chains, secondary conidiophores are generally 1-celled and short (Simmons, 2007).

***Lasiodiplodia theobromae*:** Paraphysis was hyaline, cylindrical, septate, occasionally branched ends rounded. Conidiogenous cells were thin walled, smooth, hyaline, cylindrical and holoblastic. Conidia were broadly rounded, thick-walled, initially aseptate and hyaline, finally becoming one-septate and dark brown but only when release from the pycnidia (Alves *et al.*, 2008).

***Botrytis cinerea*:** Conidiophores arise directly. Conidiophores and conidium were grape shape. The conidia observed were ellipsoidal or sometimes globose, often smooth with a slightly protuberant hilum and unicellular (Vasilica *et al.*, 2012).

***Colletotrichum gloeosporioides*:** Colonies on PDA were pale oliveaceous, grey to oliveaceous grey merging to grey oliveaceous in the centre, reverse iron grey to oliveaceous

black, with copious floccose to woolly aerial mycelium. Conidiomata with masses of orange conidia, forming irregular masses of dark brown angular cells from which setae and conidiophores are produced. Conidiophores were irregularly branched, conidia were hyaline, smooth, sub-cylindrical with rounded ends and slightly flattened base (Cannon *et al.,* 2008).

***Phomopsis mangiferae*:** Colonies on potato dextrose agar appear as white-grey mycelium, with scattered pycnidia in small clumps, with conidia extruding in droplets. Conidiophores hyaline, bearing two kinds of conidia, α conidia hyaline, fusiform to ellipsoidal and biguttulate while ß conidia hyaline, unicellular and filiform (Johnson *et al.,* 1993).

Cytosphaera mangiferae: Conidiomata produced in concentric bands, superficial and pulvinate. Conidiogenous cells simple, cylindrical, aseptate, hyaline, smooth and formed from cells of the locule wall. Conidia hyaline, oblong to ovate, unicellular, basally truncate and very thick-walled (Johnson and Hyde, 1992).

***Pathogenicity test*:** All the inoculated fungal pathogens produced irregular shape, black-brown lesions on stem end of fruits when examined after inoculation. The pathogens were re-isolated from infected fruits to fulfill Koch's postulates and similar pathogens were obtained that were used in inoculations. The disease severity was increased with increased storage period in both storage conditions. Maximum disease severity in ambient storage fruits was recorded when spore suspension of *L. theobromae* (79.8 %) was inoculated in mature mango fruits followed by *A. alternata* (67.6 %) and *C. gloeosporioides* (64.4 %) while *L. theobromae* showed the maximum disease severity (70.2 %) when its spore suspension was inoculated in cold storage fruits followed by *C. gloeosporioides* (62.7 %) and *P. mangiferae* (50.0 %). *B. cinerea* exhibited least disease severity in both the storage conditions (Table 2).

In vitro antifungal assay of medicinal plant extracts

***Ethanolic extracts*:** The results revealed that all the tested plant extracts at each concentration showed more or less antifungal activity against evaluated pathogens. The extracts of *M. oleiferae*, *S. aromaticum* and *C. zeylanicum* were found to be prominently active against all the test fungal pathogens. The maximum DIZ value recorded was from *M. oleiferae* and *S. aromaticum* (2.82 and 2.13 mm) at 50*µ*g/mL when evaluated against *C. gloeosporioides*. At higher concentrations, *M. oleiferae* and *S. aromaticum* with 3.38 and 3.2 mm DIZ values showed significant antifungal activity against *L. theobromae*. *M. oleiferae* and *C. zeylanicum* at 25 and 15*µ*g/mL were effective against *P. mangiferae* with 3.53 and 3.13 mm DIZ values respectively. In case of *A. alternata*, *M. oleiferae* and *C. zeylanicum* were effective at 25 and 50 *µ*g/mL with DIZ values of 3.42 and 3.10 mm, respectively. *S. aromaticum* and *M. oleiferae* at 15 *µ*g/mL showed maximum DIZ values with 3.12 and 3.16 mm against *B. cinerea*. Against *C. mangiferae*, the highest DIZ values (3.31 and 3.06 mm) were recorded by the application of *C. zelanicum* and *M. oleiferae* at 25 and 50 *µ*g/mL respectively (Fig. 2).

***Methanolic extracts*:** Methanolic extracts also showed significant activity against all the tested pathogens. The results were more or less similar with ethanloic extracts. *M. oleiferae* and *C. zelanicum* at 50 *µ*g/mL showed effective results with 3.4 and 3.23 mm DIZ values against *C. gloeosporioides*. *S. aromaticum* and *M. oleiferae* at 50 and 15 *µ*g/mL showed significant results against *L. theobromae* with 3.35 and 3.0 mm DIZ values. *M. oleiferae*, *S. aromaticum* and *C. sativum* at 25, 50 and 50 *µ*g/mL respectively were statistically significant against *P. mangiferae* and *A. alternata. S. aromaticum* and *C. zelanicum* at 25 and 50 *µ*g/mL was effective with 3.32 and 3.15 mm DIZ values

Table 2. Disease severity in mango fruits after artificial inoculation with stem end rot (SER) associated fungal pathogens.

Treatments	Disease severity (%)					
	Ambient storage			Cold storage		
	3 days	6 days	9 days	7 days	14 days	21 days
L. theobromae	32.6±1.70I	49.6±1.2E	79.8±1.3A	26.6±1.4H	53.3±0.7C	70.3±1.3A
C. gloeosporioides	28.6±1.20JK	40.5±1.1G	64.4±1.3C	24.4±1.3I	42.8±1.0F	62.7±1.5B
P. mangiferae	24.2±1.27L	36.9±0.8H	59.9±1.1D	20.0±1.1J	34.6±1.3G	50.3±1.3D
A. alternata	27.4±0.23K	42.2±1.2F	67.6±1.3B	17.7±0.7K	33.7±1.4G	46.5±1.4E
C. mangiferae	20.0±1.70M	29.7±0.4J	42.3±1.0F	13.3±0.7L	26.6±1.5H	34.0±1.1G
B. cinerea	14.0±1.10N	23.8±1.2L	35.5±0.8H	8.5±0.2M	17.7±1.3K	26.3±1.3H
Control	1.8±0.00Q	4.7±0.3P	8.9±0.9O	0.00±0.0O	0.0±0.0O	2.4±0.2N
	Treatment p≤0.00; Duration p≤0.001			Treatment p≤0.00; Duration p≤0.001		
	Treatment × Duration p≤0.001			Treatment × Duration p≤0.001		

Values in rows and columns (Treatment × Duration p≤0.001) followed by different letters, differ significantly according to the LSD test ($P \leq 0.05$). Fruits were stored under ambient (25ºC; 9 days) or cold (12ºC; 21 days) conditions and 70 to 80% RH. n=45

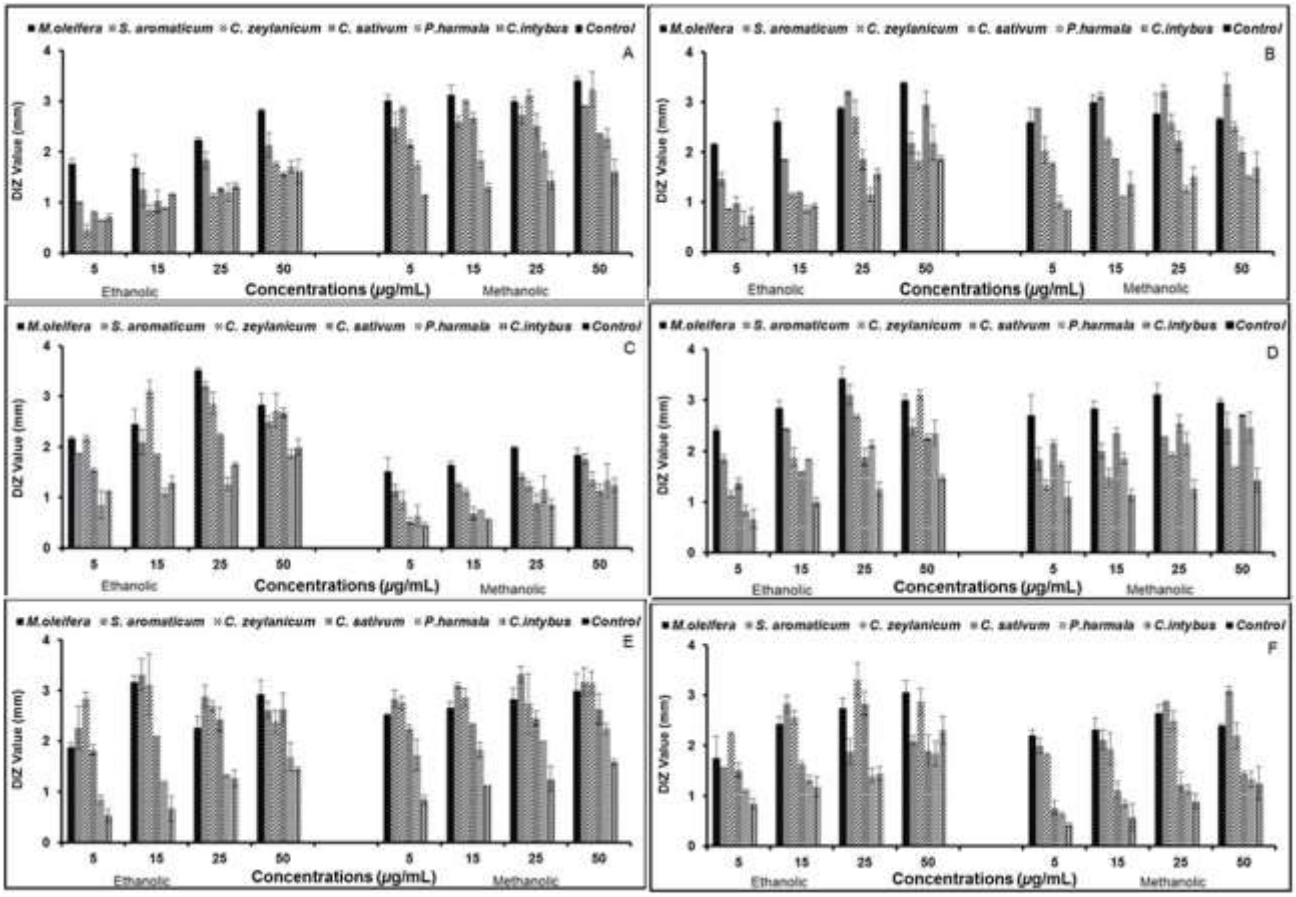

Figure 2. Effect of various concentrations of ethanolic and methanolic extracts of medicinal plants against A) *C. gloeosporioides*; B) *L. theobromae*; C) *P.mangiferae*; D) *A. alternata*; E) *B. cinerea*; F) *C. mangiferae.* Vertical bars represent ± S.E. of means and are invisible when the values are smaller than the symbol. n=3

against *B. cinerea*. Against *C. mangiferae*, the highest DIZ values (3.10 and 2.65 mm) were recorded by the application of *S. aromaticum* and *M. oleiferae* at 25 µg/mL (Fig. 2).

In vivo antifungal assay of medicinal plant extracts: All the three ethanolic and methanolic plants extracts evaluated, significantly reduced the SER severity on mango fruits that were artificially inoculated with *L. theobromae*. Increased in the severity of the disease resulted in softening and rotting of fruit tissues. From ethanolic extracts, the maximum reduction (77.7 %) in disease severity was obtained by the application of *M. oleiferae* extract at 50 µg/mL concentration followed by the extract of *S. aromaticum* at same concentration with 64.4 % reduction in disease severity. From methanolic extracts, the greatest reduction (71.1 %) in disease severity was achieved by extract of *M. oleiferae* at 50 µg/mL concentration followed by extract of *S. aromaticum* at same concentration with 60.0 % reduction in disease severity. The extract of *C. zeylanicum* in both ethanolic and methanolic, showed least reduction in SER disease severity. The effect of positive control (Nativo) was at par with evaluated plant extracts in reducing SER disease severity with the value of 77.5 % (Table 3).

DISCUSSION

Mango SER is characterized by appearance of dark discolored area towards stem end that turns dark brown to purplish black with water soaked lesions, infected fruit rots within three days (Johnson *et al.*, 2012). Therefore, once infected, mango fruits completely lose their marketable value. As revealed by its high incidence, SER has become an imperative disease of mango in Pakistan which bring substantial losses in the form of reduced quantity and quality and have a considerable role in declining its export potential (Ambreen *et al.*, 2014). In present research, on the basis of morphology of fungal colonies and conidia, we have identified the fungal flora responsible for SER infection under agro-ecological conditions of Punjab Province of Pakistan as *L. theobromae*, *P. mangiferae*, *C. gloeosporioides*, *A. alternata*, *B. cinerea* and *C. mangiferae*. In fact, all these pathogens have been

Table 3. Effect of ethanolic and methanolic extracts of plants on severity of SER disease development after 9 days of incubation at 25ºC

Treatments	Concentrations	Ethanolic extracts		Methanolic extracts	
	(*µ*g/mL)\	Disease Severity*	Disease Reduction (%)	Disease Severity*	Disease Reduction (%)
M. oleiferae	25	1.7±0.9CD	62.2	2.4±0.4C	46.6
	50	1.0±0.4F	77.7	1.3±0.7E	71.0
S. aromaticum	25	2.2±0.6BC	51.1	2.6±1.0C	42.2
	50	1.6±1.0DE	64.4	1.8±0.6D	60.0
C. zeylanicum	25	2.5±0.5B	44.4	3.2±0.7B	28.8
	50	1.9±0.2CD	57.7	2.5±0.2C	44.4
Control		4.5±1.2A	0.0	4.5±1.0A	0.0
Nativo		1.0±0.1EF	77.7	1.0±0.3EF	77.7

***=** Square root transformed disease severity (mean of three replications) was measured on a 1-5 scale. Values are means ± S.E. Different letters indicate significant differences among treatments according to the Tukey's test (P =0.05).

reported to be associated individually with SER disease in various research reports globally (Slippers *et al.*, 2005; Amin *et al.*, 2011; Johnson *et al.*, 2012; Ni *et al.*, 2012).

In present research, *B. cinerea* reported to be associated with SER disease of mango first time in Pakistan (Alam *et al.*, 2016). The pathogen was isolated from symptomatic fruit samples and found pathogenic through pathogenicity test. But to delineate either the fungus is the dominant pathogen for causing mango SER or not, can be problematical, because factors such as existence of fungus in the field, source of inoculum, density of inoculum, climate, and agronomic practices all could affect the dynamics of pathogens as well as the frequency of disease prevalence in the field (Ni *et al.*, 2012).

In present study, it was verified through pathogenicity test that *Lasiodiplodia theobromae* was the most aggressive pathogen, with the maximum disease severity on artificially inoculated fruit as compared to other inoculated pathogens. *L. theobromae* also known to cause fruit rot and stem canker of guava, and stem end rot of papaya (Wang *et al*., 2005). Our results strengthened by the findings of different researchers, they inoculated *L. theobromae* along with other SER pathogens and observed that *L. theobromae* is most virulent pathogen of SER of mango as compared to other pathogens (Sangchote, 1991; Rawal, 1997; Diedhiou *et al.*, 2007; Costa *et al.*, 2010; Ni *et al.*, 2012).

During the past decade, pesticide remnants are prevailing in all compartments of agro-ecosystem, decreasing flora and fauna biodiversity (Koul *et al.*, 2008). Its consumption in food signifies a genuine threat to the health of humans (Price, 2008). That is why the use of different chemicals for post-harvest disease management in fruits production is becoming increasingly restricted by various markets who desired technologies which are nontoxic for humans and environment (Ganmor *et al.*, 2011). Therefore, in present study, we identified extracts from various plant species that are ecofriendly and showed adequate antifungal activity against mango post-harvest fungal pathogens *in vitro* and *in vivo*. The results of antifungal assay revealed that *S. aromaticum*, *M. oleifera* and *C. zeylanicum* were very effective as compared to *C. intybus*, *P. harmala* and *C. sativum* against SER associated pathogens due to their antifungal activities. *S. aromaticum* is known to have antibacterial and anti-inflammatory activity as it contains eugenol (Shafi *et al.*, 2002) and it is among the strong inhibitors of fungal enzymes and responsible for disruption of fungal, bacterial membranes (Pepeljnjak *et al.*, 2003). Antimicrobial activity of eugenol might be due to the existence of a phenolic OH group and an aromatic nucleus that are well-known to be reactive and formed hydrogen bonds with –SH groups in the dynamic sites of targeted enzymes, resulting in de-activation of enzymatic system of pathogenic fungi (Velluti *et al.*,2003; Alma *et al.*, 2007). *M.oleifera* is rich in glucosinolates and reported to have antimicrobial potential (Kumar and Pari, 2003). *M. oleifera* had numerous biological activities as it contains anthocyanins, flavonoids, alkaloids, cinnamates and proanthocyanidins which are being used as an anti-herpes simplex virus type I (Lipipun *et al.*, 2003). *C. zeylanicum* has the active compound cinnamaldehyde which has anti-microbial potential. It has strong anti-fungal activities against wide range of wood rotting fungi and is consider as excellent wood preservatives and occurs naturally in the bark and leaves the genus *Cinnamomum* (Wang *et al.*, 2005; Cheng *et al.*, 2006).

The *in vitro* screening of *S. aromaticum*, *M. oleifera* and *C. zeylanicum* showed effective results at higher concentrations against all tested pathogens. The significant fungicidal activity of these plant extracts may be due to the presence of anthocyanins, flavonoids, alkaloids, cinnamates, proanthocyanidins, glucosinolates, eugenol and cinnamaldehyde. Our results are in close agreement with previous research reports. *M. oleifera* and *S. aromaticum* showed highest fungicidal activity at the concentration of 100*µ*g/ml against *L theobromae* and *C. gloeosporioides* isolated from mango fruits (Khewkhom and Shangchote, 2009). Conidial germination and mycelial growth of *C. musae*

and *C. gloeosporioides* isolated from papaya and mango fruits were suppressed by ethanolic extracts of *S. aromaticum* and *C. zeylanicum* at higher concentrations (Maqbool *et al.,* 2010; Necha *et al.,* 2008). *C. zelanicum* and *S. aromaticum* inhibited the mycelal growth of *L. theobromae, C. gloeosporioides, P. veticola* and *R. stolonifera*, responsible for postharvest decay of grapes (Sukatta *et al.,* 2005; Sukatta *et al.,* 2008). Excellent activity of *S. aromaticum* has been observed against *B. cinerea* at relatively higher concentration (Magdy *et al.,* 2007). *In vivo* efficacy of plant extracts proved that ethanolic and methanolic extracts of *M. oliefera, S. aromaticum* and *C. zeylanicum* not only suppress mycelial growth *in vitro* but also control SER disease development on mango fruits *in vivo*. Comparable effects of these plant extracts were observed with that of Nativo against SER disease development. Similar results have been obtained when ethanolic extract of *M. oliefera* was investigated against *L. theobromae* and *Fusarium moniliforme* (Edward *et al.,* 2015). Post-harvest antifungal activity of extracts from *C. zeylanicum*, and *S. aromaticum* was evaluated against *L. theobromae* and *C. gloeosporioides* and found that both extracts were excellent in reducing disease development on mango fruits (Khewkhom and Shangchote, 2009). Different concentrations of *C. zeylanicum* did not only delay the onset of SER and anthracnose disease, but also maintained the freshness of fruits during storage (Maqbool *et al.,*2010). Moreover, extracts of *C. zeylanicum* and *S. aromaticum* have been successfully tested to minimize the post-harvest disease development on mandarin, Kiwi and Rambutan fruits (Arras, 1988; Sivikumar *et al.,* 2002).

Conclusion: This work has revealed exploitable potential of extracts derived from *M. oliefera, S. aromaticum* and *C. zeylanicum* as alternative of synthetic fungicides against most prominent and destructive post-harvest fungal pathogens. Current approach of post-harvest disease management can contribute to minimize the risks and hazards of toxic fungicides on environment and consumer's health, being the main reason for their suitability and natural origin.

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