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Management of mango diseases anthracnose and blossom blight by ecofriendly methods

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Key words: Ecofriendly management, Anthracnose, Blossom blight, Plant extracts, Fungal isolates.

Abstract

Mango diseases anthracnose and blossom blight has caused intense damage to the mango yield and export so its control is required. Fungicides are being used for their control which effects the environment as well as human health so the introduction of different ecofriendly methods for the control of these diseases is the need of hour. For this purpose the study on the ecofriendly management of mango anthracnose and blossom blight has been carried out. Plant extracts of akk (*Calotropisprocera*), eucalyptus (*Eucalyptus camaldulensis*), neem (*Azadirachtaindica*) and garlic (*Allium sativum*) were prepared. Three different concentrations including 1.25%, 2.5% and 5% of these plants extracts were applied in vitro on fungal isolates of *Choletotrichumgleosporiodes* causal agent of mango anthracnose and blossom blight. Results were assessed for the best concentration application on mango trees. 5% concentration of each extract was proved to be most effective for the inhibition of *Choletotrichumgleosporiodes* and 5% eucalyptus extract displayed maximum percentage growth inhibition of 79%. Consequently 5% concentration of each extract was sprayed on the four replicates of mango trees in field application. Results showed that among all botanical extracts eucalyptus ismore effectivein control of both diseases anthracnose and blossom blight.

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Introduction

Mangobelongs to dicotyledonous family *Anacardiaceae.* Mango is cultivated in tropical and subtropical areas of Pakistan covering area of 170.1 thousand ha (Maqsood *et al.*, 2014). For Pakistan, mango is an important foreign exchange earning commodity. Pakistan is one of the leading mango exporting countries having 4th rank (Maqbool*et al.*, 2007).A large number of mangoes have been destroyed every year due to mango pre and post-harvest diseases.

Among all of the mango diseases, anthracnose is the most common which is caused by *Colletotric-humgloeosporioides* (Nelson, 2008). Symptoms of anthracnose include tan to dark brown spots formation along the margin of leaves. Blossom blight or panicle anthracnose is also caused by fungus *Colletotrichumgloeosporioides* (Kolase *et al.*, 2014). It can affect both the inflorescence stalk and the single flower on which extended dark gray to black lesions or spots appear.

Mostly for the control of mango diseases fungicides and different chemicals are applied. Generally use of different chemicals is effective for preharvest control of mango diseases. Copper fungicides alone and in combination with other fungicides are used worldwide for diseases control (Arauz, 2000). During spring season blossom blight infection is controlled efficiently by two to three sprays of contact or systemic fungicides. These synthetic chemicals have adverse effects on human health and the environment; this led to the intensive worldwide research efforts to develop alternative control strategies that are ecofriendly (Jabbar *et al.*, 2011).Now scientists are started to shift their focus on ecofriendly methods. These ways include use of plant extracts like in a study of Sahi *et al.* (2012) botanical extracts of neem (*Azadirachtaindica), onion (Allium cepa) and safeda (Eucalyptus camaldulensis)* were used against the mycelial growth of *Botryodiplodi-atheobromae* the causal organism of quick decline of mango. Among them safeda and neem extracts were observed to be the most effective. In another study some plants extracts like tobacco leaf *(Nicotiana tabacum)*, keora seed, keora *(Sonneratiaapetala),* mahogoni *(Swieteniamacrophylla)*, gaintindian milky weed *(Calotropis gigantean*), garlic *(Allium sativum)* and ginger *(Zingibeyr officinale)* were used to observe their antifungal effects on the growth and development of *Colletotrichum gloeosporioides,* causal agent of anthracnose of mango. Highest mycelial growth inhibition was observed in case of garlic extracts at 70% concentration (Mukherjee *et al.*, 2011).

In past mostly in vitro application of extracts were done and field application is absent for the control of plant diseases. Significance of the present study is to develop and assess the ecofriendly methods used for the management of mango diseases under in vitro conditions along with their field application. The objectives were the application of different botanical extracts and the assessment of most effective botanical extract for the control of mango diseases anthracnose and blossom blight.

Materials and methods

Four different plant extracts have been used (neem, garlic, eucalyptus and akk) to find out which extract is more effective against mango diseases anthracnose and blossom blight (Table 1).

Table 1. Detail of plants extract used for botanical spray.

Name of plants	Scientific name	Family	Plant parts used
Eucalyptus (Sufeda)	*Eucalyptus camaldulensis*	*Myrtaceae*	Leaves
Akk	*Calotropisprocera*	*Apocynaceae*	Leaves
Garlic	*Allium sativum*	*Liliaceae*	Cloves
Neem	*Azadirachtaindica*	*Meliaceae*	Leaves

For extract formation cloves of garlic and leaves of neem, eucalyptus, akk (1 Kg) were taken, washed and grinded and then put into the pot to boil for 2 hrs. This mixture was left open in air for an hour and then filtered by using muslin cloth. Thus extract is obtained in the form of filtrate.

Before and after spray disease assessment

Experimental farm department of entomology, faculty of agricultural sciences and technology, Bahauddin Zakariya University, Multan is selected as a study area. The study area was surveyed before and after spray application for the sake of before and after spray disease assessment and sample collection was done for fungal isolation. For spray 5% solution of extracts was prepared and sprayed on selected trees. Trees were sprayed three times with the gap of one month and study area was surveyed after each spray application. Total 24 mango trees were carefully chosen and tagged according to their category of applied botanical spray. Four replicates were selected for each extract treatment, positive control (fungicide score) and negative control (without any extract treatment). Disease assessment during survey was based on visual observation of disease symptoms. For the sake of data collection of mango diseases anthracnose and blossom blight a per forma was designed to note down the disease severity. The per forma was filled by rating the severity of each disease according to severity scale of 1-5 by the careful observation from each side of tree i.e. east, west, north, south (Masood *et al*., 2010).

Assessment of severity, incidence and prevalence of mango anthracnose and blossom blight

The performa was evaluated to calculate the incidence, severity and percent disease index of each disease before and after each spray application.

Disease severity

For the calculation of disease severity of mango diseases anthracnose and blossom blight disease severity scale of 1-5 was used (Corikidi *et al*., 2006). (Table 2)

Table 2. Severity scale.

Scale	Percent severity
1	0–1%
2	2-5%
3	6–10%
4	11–49%
5	50–100%

Disease incidence

Disease incidence was assessed by counting the number of apparently diseased plants per site with respect to overall plants in an orchard (Tucho *et al*., 2014).

$$\% \text{ Disease Incidence} = \frac{\text{No. diseased plants}}{\text{Total no. of plants}} \times 100$$

Percent disease index

Percent disease index was measured by using following formula (Prabakar *et al*., 2005).

$$\text{Percent disease index} = \frac{\text{Sum of all individual ratings}}{\text{Total number of trees observed} \times \text{maximum grade}} \times 100$$

Fungal Isolation and purification

For the isolation of causal fungal agent of both diseases sampling of diseases affected parts along with healthy tissue was done. Leaves for anthracnose and inflorescence for blossom blight were cut and packed into brown paper bags and transported from study area to Mycology and Ecotoxicology laboratory of Fatima Jinnah Women University Rawalpindi in cardboard bags. Infected diseased samples along with healthy tissues were washed with tap water and then cut into small pieces by using a cutter. These small pieces were surface sterilized by dipping in hydrogen per oxide solution for two minutes (Gupta, 2003). Then these treated plant pieces were washed three times with sterilized distilled water. Then these pieces were put on the filter paper for drying. These cut pieces were then placed in petri dishes (9 mm diameter) containing solidified media potato dextrose agar. Para-film was wrapped around petri plates after inoculation was done and then plates were placed in an incubator adjusted at 28°C for three days for mycelium formation in petri dish. After three days of

incubation fungal colony in petri plates was observed. From these cultures pure culture of fungal isolates was obtained by Hyphal Tipping method. This technique involved transferring hyphal tip on PDA plate with the help of flame sterilized tip of an inoculation needle from previously prepared culture plates. By this way isolated colonies were sub-cultured two to three times into fresh media plates till the pure cultures were obtained. The plate was incubated at room temperature. Pure cultures were identified by making visual observations.

In vitro extracts evaluation

For in vitro assessment of plant extracts three concentrations of each extract neem, eucalyptus, akk and garlic were made in a 100 ml conical flask. These three concentrations include 1.25%, 2.5% and 5% (Table 3).

Table 3. Recipe of different concentrations of each extract with PDA.

Concentration (%)	Extract (ml)	PDA (ml)
1.25	1.25	98.7
2.5	2.5	97.5
5	5	95

For control the PDA solution without plant extract was prepared according to protocol. Then this media in conical flasks were sterilized for 20 minutes in an autoclave at temperature of 121°C. After autoclaving sterilized petri plates were poured by 20 ml of the medium in each plate and then kept for 24 hours for solidification. Cork borer (5mm diameter) was used to prepare mycelial discs from 5 days old pure cultures of the fungal isolate. At the center of a petri dish one disc of the fungal isolate was placed. Three replicates for each extract concentration, fungicide score and control were made (Mukherjee *et al.*, 2011).

Collection and analysis of data

Radial growth (mm) of fungus *Colletotrichumg loeosporioides* in each petri plate was recorded after three days of incubation. For this purpose two diameters length (a) and width (b) for each colony were measured. After measuring radial growth for each replicate its average growth was calculated and then percent growth inhibition was measured for each concentration with the help of formula (Vincent, 1947).

$I = C-T/ C \times 100$

Here,

C = Averageradial growth in control

T = Averageradial growth in treatment

Results

Identification and characterization of fungal pathogens of mango

Causal pathogens isolated from disease affected samples were identified by morphological studies based on colony and spore characters of fungi (Table 4). *Colletotrichumgloeosporioides* was identified as causal pathogen of anthracnose and blossom blight.

Table 4. Morphological analysis of *Colletotrichum gloeosporioides*.

Causal FungalAgent	Colony characteristics	Spore Characters
Colletotrichumg-loeosporioides	Dense Whitish mycelium formed with black and orange color fruiting bodies	Smooth, hyaline and sub cylindrical with round end spores.

In vitro disease control assessment

Each extract presented least radial growth at its 5 % concentration as compare to the other concentrations with percentage growth inhibition of 79%, 57%, 56%, and 45% for eucalyptus, akk, neem and garlic respectively (Table 5). Data of growth inhibition after extracts application was statistically analyzed by using analysis of variance (ANOVA) and results revealed that different concentrations and extracts application has significant effect on the fungal growth inhibition at $P< 0.05$ with reference to control.

Table 5. Results of percent growth inhibition of *Colletotrichumgloeosporioides* by each treatment under in vitro conditions.

Treatments	Concen-trations %	Average Growth	Percent growth inhibition
Eucalyptus	1.25	2.02	62
	2.5	1.8	66
	5	1.07	79
Neem	1.25	3.23	39
	2.5	2.58	51
	5	2.32	56
Akk	1.25	3.6	32
	2.5	2.48	53
	5	2.28	57
Garlic	1.25	3.95	25
	2.5	3.4	36
	5	2.9	45
Control		5.33	0

Field assessment of plant extracts against anthracnose and blossom blight

During pre-spray stage all selected 24 mango trees have showed 100% incidence for both diseases while percent disease index was found 79% in case of anthracnose and 66 % in case of blossom blight (Fig. 1, Fig. 2). After each spray diseases incidence was remained same while percent disease index for both diseases was shown a decrease.

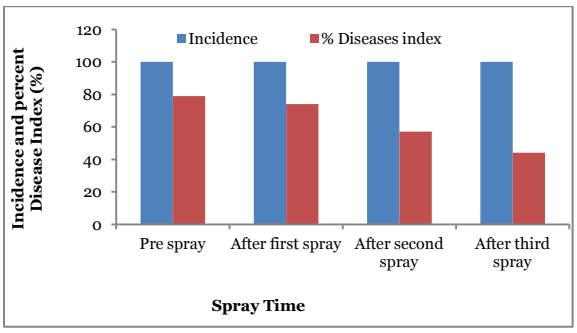

Fig. 1. Incidence and percent disease index of anthracnose on leaves.

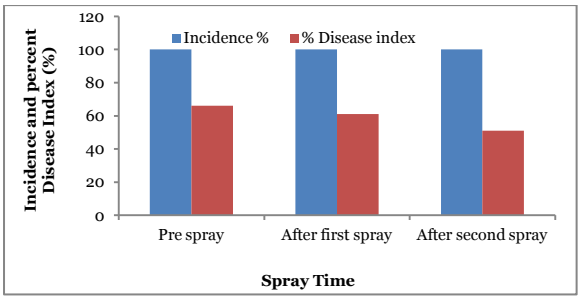

Fig. 2. Incidence and percent disease index of blossom blight.

Evaluation of effectiveness of each botanical extracts against anthracnose

Different botanical extracts showed different effectiveness for the inhibition of anthracnose. In tree replicates of garlic, eucalyptus, neem and akk average disease severity of anthracnose was found to be 3.25, 4, 3.75 and 4.25 however after respective spray applications it was decreased showing disease severity of 1.75, 1.75, 2.25 and 2.75 respectively (Fig. 3, Fig. 4, Fig. 5, Fig. 6). Eucalyptus extract has showed significant disease severity reduction so it is proved to be the best for suppressing the anthracnose. After eucalyptus akkwas found to be effective against anthracnose whereas neemandgarlic extract has presented similar efficiency in suppressing the anthracnose disease. ANOVA was used to statisticaly analyzed the data and it showed that results of extracts application were highly signifiant at $P<0.05$. So these extracts has indicated a significant effect for the control of anthracnose.

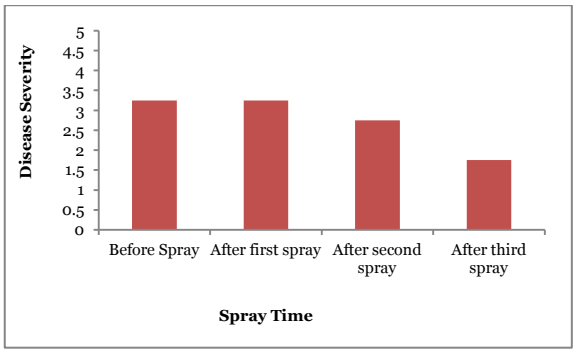

Fig. 3. Effect of Garlic extract on anthracnose.

* Severity Scale: 1 = 0–1%, 2 = 2-5%, 3 = 6–10%, 4 = 11–49%, 5 = 50–100%

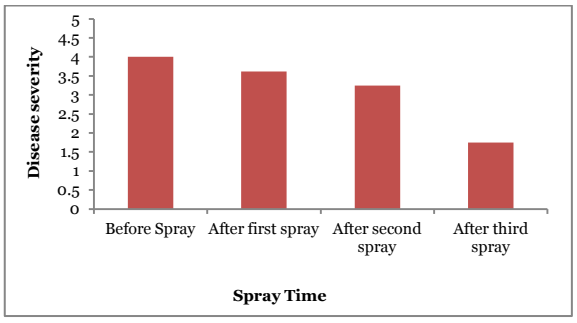

Fig. 4. Effect of Eucalyptus extract on anthracnose.

* Severity Scale: 1 = 0–1%, 2 = 2-5%, 3 = 6–10%, 4 = 11–49%, 5 = 50–100%

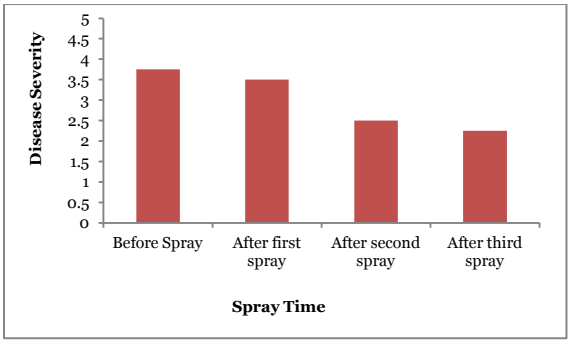

Fig. 5. Effect of Neem extract on anthracnose.

* Severity Scale: 1 = 0–1%, 2 = 2-5%, 3 = 6–10%, 4 = 11–49%, 5 = 50–100%

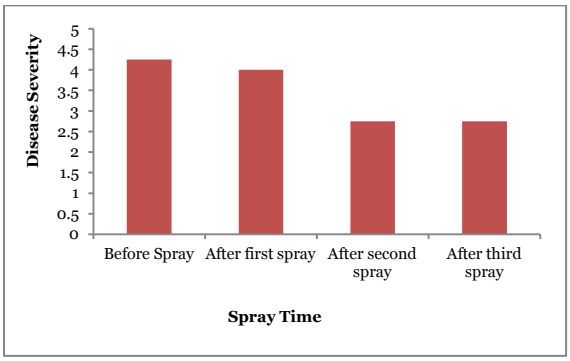

Fig. 6. Effect of Akk extract on anthracnose.

* Severity Scale: 1 = 0–1%, 2 = 2-5%, 3 = 6–10%, 4 = 11–49%, 5 = 50–100%

Evaluation of effectiveness of each botanical extracts against blossom blight

Each botanical extract exhibited different trend of effectiveness for the inhibition of blossom blight. In tree replicates of garlic, eucalyptus, neem and akk average disease severity of blossom blight was found to be 3, 3, 3.5 and 3.75 however after respective spray applications it was decreased showing disease severity of 2.75, 1.75, 2.75 and 3 respectively (Fig. 7, Fig. 8, Fig. 9, Fig. 10, Fig. 11, Fig. 12 Fig. 13 Fig. 14). Among all botanical extracts, eucalyptus extract proved to be the best for suppressing the blossom blight for its all tree replicates as it showed substantial disease severity reduction. After eucalyptus extract neem and akk extract were most effective plant extract. Garlic extract presented least efficiency in suppressing the blossom blight disease. Data was statisticaly analyzed by using ANOVA it showed that after the application of extracts results are highly signifiant at $P<0.05$. So the extracts has showed a significant effect for the control of blossom blight.

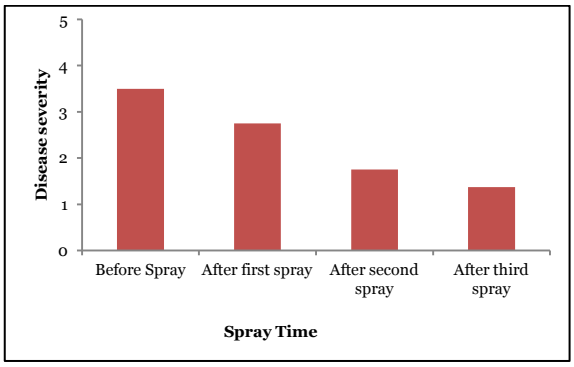

Fig. 7. Effect of Score on anthracnose.

* Severity Scale: 1 = 0–1%, 2 = 2-5%, 3 = 6–10%, 4 = 11–49%, 5 = 50–100%

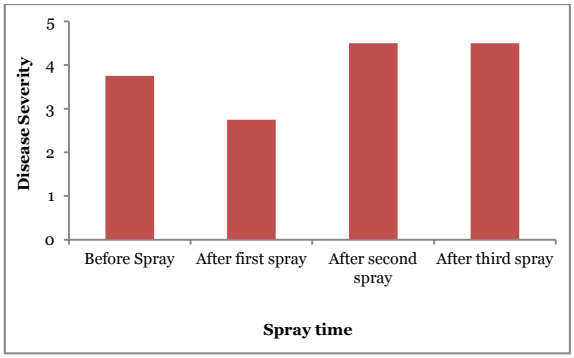

Fig. 8. Results of control replicates for anthracnose.

* Severity Scale: 1 = 0–1%, 2 = 2-5%, 3 = 6–10%, 4 = 11–49%, 5 = 50–100%

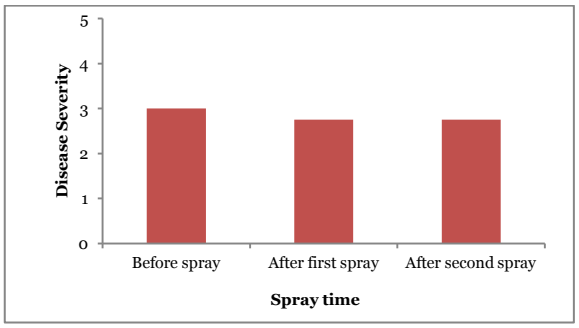

Fig. 9. Effect of Garlic extract on blossom blight.

* Severity Scale: 1 = 0–1%, 2 = 2-5%, 3 = 6–10%, 4 = 11–49%, 5 = 50–100%

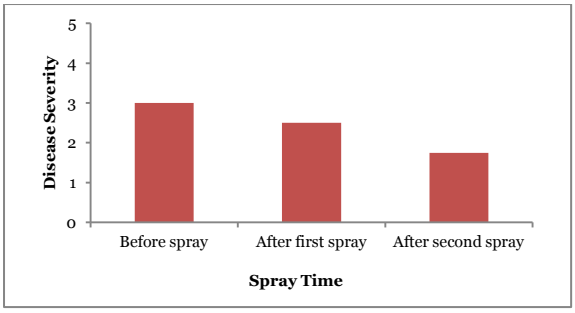

Fig. 10. Effect of Eucalyptus extract on blossom blight.

* Severity Scale: 1 = 0–1%, 2 = 2-5%, 3 = 6–10%, 4 = 11–49%, 5 = 50–100%

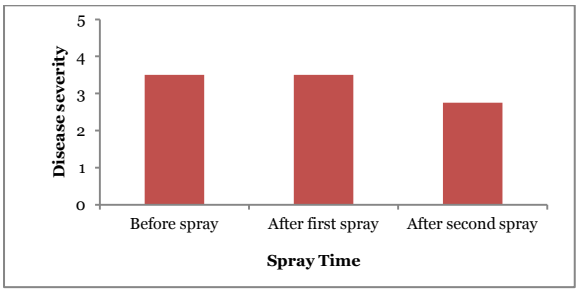

Fig. 11. Effect of Neem extract on blossom blight.

* Severity Scale: 1 = 0–1%, 2 = 2-5%, 3 = 6–10%, 4 = 11–49%, 5 = 50–100%

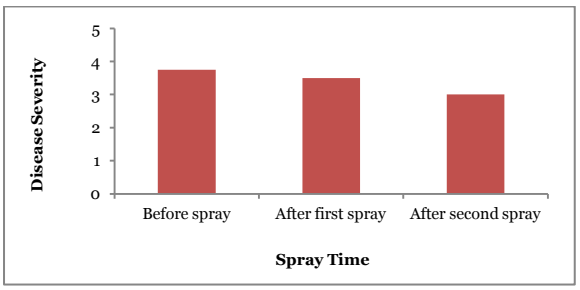

Fig. 12. Effect of Akk extract on blossom blight.

* Severity Scale: 1 = 0–1%, 2 = 2-5%, 3 = 6–10%, 4 = 11–49%, 5 = 50–100%

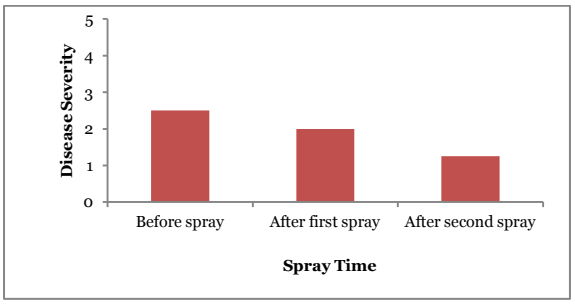

Fig. 13. Effect of Score on blossom blight.

* Severity Scale: 1 = 0–1%, 2 = 2-5%, 3 = 6–10%, 4 = 11–49%, 5 = 50–100%

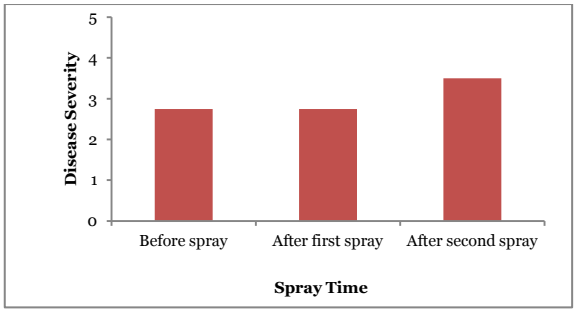

Fig. 14. Results of control replicates for blossom blight.

* Severity Scale: 1 = 0–1%, 2 = 2-5%, 3 = 6–10%, 4 = 11–49%, 5 = 50–100%

Discussion

In the present study neem, garlic, eucalyptus and akk were used to check their anti-fungal activity against fungus *Colletotrichumgloeosporioides* causal agent of mango anthracnose and blossom blight. Both in vitro and field application showed the same results of inhibition of diseases. Eucalyptus has showed the highest antifungal activity for the control of *Colletotrichumgloeosporioides* among the selected extracts. A study has showed somewhat similar trend of antifungal activity of Eucalyptus, Neem and Garlic (Sahi *et al.,* 2012). In this study Eucalyptus extract proved to be the best plant extract inhibiting the growth of fungus *Botryodiplodiatheobromae;* the causal agent of quick decline of mango. *Eucalyptuscamaldulensis* can be used as an alternative to chemical fungicides exhibited highly prominent antifungal potential against pathogenic fungi (Bajwa, 2005) which is similar with the present study.In a study of (Rukhsana *et al.,* 2005) *Eucalyptus camaldulensis* displayed highly pronounced antifungal potential. In another study antifungal activities of twenty plant species were evaluated against fungus *Colletotrichum gloeosporioides.* Among the twenty botanicals *Eucalyptus* showed the highest anti-fungal activity (Alemu *et al.,* 2013).

Neem extract used to be a prominent agent for the control of fungus growth. In present study it was found effective against *Colletotrichumgloeo sporioides.* Neem was also found to be effective under in vitro condition at 5% concentration to inhibit the

growth of *Colletotrichumgloeosporioides*in other study (Kolase *et al.*, 2014). Akk has antifungal activity due to which it is also being used in different studies for the fungal growth inhibition. Five plant extracts were tested by (Alam *et al.,* 2014) against conidial germination of *Fusariumoxysporum* and reported that the extract of Akk showed high inhibitory effect. In a study of (Bazie *et al.,* 2014) extract of Akk was observed to be least effective in reducing conidial germination of *Colletotrichummusae* the Causal agent of postharvest anthracnose of banana. Garlic extract showed different trend in different studies. In present study it is found to be moderate to least affective for the fungal disease control. This is contradicted to the results of the study of (Mukherjee *et al.,* 2011). In this study garlic presented highest percentage inhibition (60.46%) against mycelial growth of *Colletotrichumgloeosporioides*. According to the study of (Sehajpal *et al.,* 2009) plant extract of *Allium sativum* showed significant antifungal activity against fungal pathogen *Rhizoctoniasolani*.

Conclusion

This study concluded that mango diseases anthracnose and blossom blight can be managed by using ecofriendly methods. Botanical extracts of eucalyptus, neem, akk and garlic has showed the antifungal activity against *Colletotrichumglo eosporioides* (causal agent of mango anthracnose and blossom blight).Each botanical extract were successfully control the fungus *Colletotrichumgloe osporioide s*under in vitro condition. Field application has also showed the effectiveness of each extract for the ecofriendly management of mango diseases anthracnose and blossom blight. Among all botanical extracts eucalyptus extract showed the highest antifungal activity in suppressing the fungal growth under in vitro and field application. So it can be concluded that botanical extracts specifically eucalyptus can be used as an ecofriendly method for the control of mango diseases anthracnose and blossom blight.

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