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Antifungal Activity of Plant Extracts and their Applicability in Extending the Shelf Life of Mango Fruits

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Abstract
The present study was conducted to evaluate the efficacy of aqueous extract of eight plant species against C. gloeosporioides the causal agent of mango anthracnose under natural disease epidemics. A highly significant ($P < 0.001$) difference was observed among treatments in their effect on anthracnose development. None of the extracts were able to completely prevent development of anthracnose. However, most of the extracts significantly reduced disease development over the control. A high level of reduction of anthracnose development was observed when fruits were dipped in aqueous extract of Ruta chalepensis. The extract reduced disease development to below 36% during the experimental period while severity on untreated fruits reached more than 93.4%, and more than 60 % of the fruits were marketable. The treatments also maintained quality of mango; firmness, pH, total soluble solid and titratable acidity of treated mango fruit significantly ($P < 0.001$) differed from those of the control. Present study indicate that postharvest dipping of mango fruits into various plant extracts can suppress the development of anthracnose and improve marketability of mango fruits without pronounced effect on the qualities of the fruit. Moreover, extensive studies are justified towards application of plant extracts as part of integrated disease management to establish effective management tool.
Key words: C. gloeosporioides; Mango anthracnose; Marketability; Plant extract; Ruta chalepensis

1. Introduction

Mango (Mangifera indica L.) is one of the most popular fruits grown throughout the tropics and subtropics worldwide. It is one of the most desirable fruits in the international market because of its delicious taste and high caloric value (Diedhiou et al., 2007). However, mango is prone to many market problems which could limit domestic and international trade of fresh fruits; the major factors involved are the highly perishable nature of the fruit, susceptibility to postharvest diseases and extremes of temperature particularly chilling, and physical injury (Dodd et al., 1997).

Anthracnose caused by Colletotrichum gloeosporioides (Penz.) Penz. and Sacc., is by far the most important field and postharvest disease of mango in all mango producing areas of the world and is often associated with high rainfall and humidity (Arauz, 2000). It affects young leaves and flower panicles, and forms latent infections on the fruit (Dodd et al., 1989). Anthracnose remains quiescent in immature fruits and is more significant in the postharvest period (Spalding and Reeder, 1986).

Synthetic fungicides are currently used as the primary means for the control of postharvest mango anthracnose. However, increasing public concern over the indiscriminate use of pesticides and associated health risks and environmental hazards, as well as occurrence of fungicide resistant pathogen strains, has stimulated research on alternative methods to control postharvest diseases (Yao and Tian, 2005). Recently, plant extracts are emerging as safer alternatives to conventional fungicides for the control of plant diseases (Tripathi and Shula, 2007). Natural product based fungicides have the ability to decompose rapidly, thereby reducing their risk to the environment (Fokialakis et al., 2006). The antifungal activities of different plant species and the importance of plants as possible sources of natural fungicides are well established. They play an important role in the preservation of foodstuffs against fungi and have potential to replace synthetic fungicides (Tripathi and Shukla, 2007). This paper reports on the effect of some plant extract on development of mango anthracnose and their applicability on maintaining the quality of mango fruit.

2. Materials and Methods

2.1. Sample Collection and extraction

Samples of plant materials with potential antifungal activity were collected from different part of Ethiopia. The collected plant materials were washed in distilled water and dried under shade at room temperature. The dried plant parts were chopped and ground to coarse powder. Following the method employed by Ogbebor et al.

(2007) 10, 25 and 50 gram of the pulverized plant specimens were extracted with 100 ml of sterile distilled water by stirring for 24 hours on magnetic stirrer to get a final concentration of 10%, 25% and 50%. The extracts were then filtered through double layer of cheese cloth.

2.2. Fruit sample collection

Physiologically matured mango fruits of single local cultivar "Aba kurfa" were collected directly from trees on which high level of anthracnose had been observed. Such trees were pinpointed in a mango orchard at Bisidimo Leprosy Relief Center, Harar, Ethiopia during the beginning of fruit set. Fruits were grouped in to similar size and color classes, and used for the experiment.

2.3. In vivo antifungal assay of plant extracts

Antifungal activity of aqueous extract of selected plant species were tested for their effect on development of anthracnose disease on harvested mango fruit. Within 24 hours after harvest, mango fruits were dipped in to aqueous extracts of the selected plant species separately at three different concentrations (10%, 25% and 50%) prepared as mentioned above. Chemical treatment with carbendazim was used as a bench mark treatment and mango fruits dipped in sterile distilled water served as untreated control. Five fruits were used for each plant extract solutions. After the respective treatments, fruits were allowed to air dry and put on a plastic plate before incubation at 25 $^{\circ}$C. The experiment was set up in completely randomized design (CRD) with three replications.

2.4. Disease assessment

Data on disease severity were scored for evaluation of antifungal activity of botanicals under natural infection conditions at 48 hours interval from the time of symptom appearance to 100% unmarketability of untreated control fruits. Disease severity was recorded as percentage of fruit area covered by anthracnose lesions according to Corkidi et al. (2006). The following scales were used to score disease severity: 1= 0-1%, 2 = 1-5%, 3 = 6-9%, 4 = 10-49% and 5 = 50-100% of the area affected by anthracnose lesion.

2.5. Marketability of mango fruit

Data on the proportion of marketable and unmarketable fruits were collected at the time of disease assessment according to the procedure of Mohammed et al. (1999) based on descriptive quality attributes such as the level of visible lesion, shriveling, smoothness and shininess of the fruit. Percentage of marketable fruits during the experiment was calculated by the following formula:

$$\text{Marketability of mango fruit (\%)} = \frac{\text{Number of marketable fruits}}{\text{Total number of fruits}} \times 100$$

2.6. Determination of quality of mango fruit

Fruit firmness was measured in Newton, using a hand-held penetrometer and measurements were taken near the stem and head in two opposite sides (Abbasi et al., 2009). An aliquot of juice was extracted using a juice extractor (Type 6001x, USA) for determining the TSS, pH and TA content of mango fruit. TSS was measured using a hand healed refractometer with a range of 0 to 32 ^{o}Brix and resolutions of 0.2 ^{o}Brix. The TSS was determined by placing 1 to 2 drops of clear juice on the prism, according to the method described by Wasker et al. (1991). The pH value of the mango juice was measured with a pH meter and the titratable acidity (TA) was measured according to the method described by Maul et al. (2000). The TA, expressed as percent citric acid, was gained by titrating 10 ml of mango juice to pH 8.2 with 0.1 NaOH. The TA was calculated by the following formula:

$$\text{TA (\%)} = \frac{\text{Titer X 0.1N NaOH X 0.67 X 100}}{1000}$$

Where; titre = the amount of NaOH used on the burette; 0.67/100= Acid multiplication factor.

2.7. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using SAS V 9.0 software package. Mean comparisons were made using Least Significant Difference (LSD) test at 5% probability level.

3. Results and Discussion

3.1. Effect of extracts on anthracnose development

The effect of plant extract on the development of anthracnose on naturally infected mango fruit is presented in Figure 1. There was a significant ($P<0.001$) reduction of anthracnose development on mango fruit due to the dipping of the fruits in aqueous extracts of selected plant species. Higher mean disease severity was recorded on the untreated control 4.67 (i.e. nearly 93.4% fruit area affected); while the greatest reduction in severity of anthracnose 1.33 and 1.8 (i.e. nearly 26.7% and 36.0% fruit area affected) was observed from dipping of fruits in

carbendazim and aqueous extract of Ruta chalepensis at 50% concentration, respectively. Extracts from Datura stramonium at 25% and 50%, Eucalyptus globulus at 50% and Vernonia amygdalina at 25% concentration also showed remarkable antifungal activity against the test fungus by reducing the severity within a range of 2.07-2.40.

Extract from Ruta chalepensis showed potent antifungal activity in reducing the level of anthracnose development in naturally infected mango fruit. The extract from Datura stramonium, Eucalyptus globulus and Vernonia amygdalina also showed good antifungal activity at their higher concentration. The antifungal effect of Eucalyptus globulus against various pathogens is well documented (Takahashi et al., 2004; Alabi et al., 2005). Similarly, it was reported that the aqueous extract of Vernonia amygdalina leaves can inhibit the growth and conidial germination of C. gloeosporioides (Ogbebor et al., 2007). The results showed that extracts of the different plant species are substantially varied in their antifungal potentials. These differences are to be expected since plants vary in their chemical constituents, habitats and stages at which they were collected. Differences in the nature and concentration of inhibitory material even between different plants parts have been reported elsewhere (Ogbebor and Adekunle, 2008).
In the present study, extracts from selected plant species showed a potential on reduction of anthracnose development in naturally infected mango fruit, indicating that biologically active plant derived product could play significant role in crop protection strategies. Plant extracts are considered non-phytotoxic compounds and are potentially effective as natural pesticides for crop protection (Isman, 2000). Antifungal activity exhibited by these plants may attribute to the presence of secondary metabolites. Similarly, Sunayana et al. (2003) reported that plants contain thousands of constituents which are valuable sources of new and biologically active molecule. These compounds can combat with pathogens by different mode of action. In the present study the biologically active compounds of plant extracts were not identified.

3.2. Marketability of mango fruit

Dipping mango fruits in aqueous extracts of selected plant species showed a significant ($p<0.001$) difference on their potential to maintain fruit marketability in naturally infested mango fruit (Figure 2). Fruits in untreated control were 100% unmarketable while the highest marketability of fruit was obtained from fruit treated with commercial fungicide carbendazim, accounting 95.2% marketable fruits. Aqueous extract from Datura stramonium, Eucalyptus globulus and Ruta chalepensis at 50% concentration showed a potential to maintain the marketability of mango fruits in a range of 57 to 62% with no significant difference among them. Aqueous extract of Vernonia amygdalina also showed a potential in prolonging the shelf life via increasing the marketability of mango fruits above 42%. Extracts from Adatoda schimperiana and Lantana camara showed the least potential in maintaining the market value of mango fruit. The use of plant products for the management of postharvest plant diseases, besides maintaining the quality of the product, has a special significance in the context of environmental pollution, accumulation of toxic substances in the produce and development of resistance by plant pathogens (Anand and Bhaskaran, 2009). In the present study too, it was observed that natural plant products from different plant species played role in maintaining the marketability of mango fruits.

Desiccation and decay are the two major causes of the termination of commercial life span of fruits, which can be the result of various postharvest disease and other physiological disorders. The present study showed that fruit treated with various plant extracts had better marketability. This may be due to the fact that plant extract check the growth of microbes that are responsible for rotting and reduce metabolic rate of the fruits, which cause loss in weight through respiration (Bautista-Banos et al., 2002; Bhardwaj et al., 2010). It was also reported that the use of various plants extract acts as anti-senescent and arrest the metabolic break down deterioration caused by various bio-chemical activities in fruits (Bhardwaj et al., 2010). Effectiveness of plant leaf extracts in reducing the physiological loss and prolonging the shelf life of fruits in comparison to control, as found in present study, can be corroborated by previous findings in various fruits (Bautista-Banos et al., 2002; Win et al., 2007).

4.4. Postharvest Quality of Mango Fruit

4.4.1. Firmness of mango fruit

Firmness is widely used as a ripeness test for many fruits and the values of firmness are effective for evaluating fruit maturity as the fruit ripens (Olmo et al., 2000). In the present study, a significant ($P < 0.001$) difference was recorded among treatments with respect to fruit firmness. The highest firmness was recorded in fruit dipped in extracts of Ruta chalepensis and carbendazim (8.93 N and 9 N), respectively. This was followed by Eucalyptus globulus and Datura stramonium at 50% and extract from Vernonia amygdalina at 25% concentration with values of 8.20 N, 8.13 N and 7.93 N, respectively, and no significant difference among them and the former treatments. On the other hand, the lowest fruit firmness was exhibited in untreated control,

Adatoda schimperiana, Ocimum basillicum and Rosmarinus officinalis at 10% concentration ranged from 4.93 N to 5.47 N. The lowest firmness appears to be due to the high level of anthracnose infection which could have enhanced respiration rate of the fruit and thus resulted in loss of structural integrity of cell wall in the fruit (Abbasi et al., 2009). The highest firmness indicates low level of anthracnose infestation, as fruits firmness and disease severity are negatively correlated with each other.

4.4.2. pH of mango fruit

A highly significance difference was exhibited among treatment with respect to pH of the juice of fruits that were left to natural infection (Table 1). The highest pH was recorded in untreated control (5.14) and fruit treated with extract of Lantana camara at all concentration, Adatoda schimperiana, Datura stramonium and Vernonia amygdalina at 10% and Ocimum basillicum at 25% ranges from 4.86 to 5.10 with no significant difference among them. On the other hand, the lowest pH was recorded in fruits treated with the aqueous extract of Adatoda schimperiana, Datura stramonium, Eucalyptus globulus, Rosmarinus officinalis, Ruta chalepensis and Vernonia amygdalina at 50% and the synthetic fungicide all of which did not vary significantly. Jitareerat et al. (2007) suggested that the change in pH is associated with the effect of treatment on the respiration and metabolic activity of the fruits. In this study, it seems that fruits with high rate of disease incidence and severity had higher rates of respiration which would raise pH of the fruit juice as ripening advances (Abbasi et al., 2009; Tehrani et al., 2011).

4.4.3. TSS of mango fruit

There was a significant difference in the TSS of mango fruit as a result of treatment of the fruits with plant extracts (Table 10), the maximum TSS value (17.2, 17.47 and 17.93 ^{o}Brix.) was exhibited in fruits treated with extracts of lantana camara of 10 and 25% and Vernonia amygdalina at 10% concentration, respectively. A high level of TSS was also recorded in the untreated control and plant extracts at lower concentration. Extract from Ocimum basillicum at 25%, Datura stramonium, Ruta chalepensis and Vernonia amygdalina at 50% were effective in lowering the TSS of the fruit by delaying early senescence of the fruit. The high TSS content of mango fruits could be due to the high level of anthracnose severity, which could have accelerated ripening, thus, resulting in increment of sugar level of the fruits before the other treatments. The low TSS may be the attribute of the low ripening process as a result of low level of anthracnose infection on the fruits. This could also be justified by the positive and highly significant correlation between TSS and disease infection. The change in TSS could be due to hydrolytic changes in starch, and conversion of starch to sugar which is an important index of ripening process in mango and other climacteric fruit (Kittur et al., 2001).

4.4.4. Titrable acidity of mango fruit

A highly significant ($P < 0.001$) difference was recorded among treatment with respect to titratable acidity (TA) of the juice of fruits. The lowest TA was recorded in 10% Vernonia amygdalina and 25% Ocimum basillicum with a value of 0.49. While fruit treated with the aqueous extract of Datura stramonium and Vernonia amygdalina at 50%, Eucalyptus globulus, Rosmarinus officinalis and Ruta chalepensis at both 25% and 50% concentration relatively exhibited significantly higher TA contents, which was not statistically significant with that of carbendazim. The reduced TA may be the attribute of anthracnose disease, which enhance ripening and senescence of fruit (Jabbar et al., 2011); and thus reduced TA possibly through the use substrate for respiration. Earlier, Abbasi et al. (2009) demonstrated that the ascorbic acid and TA of mango fruit first increases then decrease, while pH and the TSS values increase during senescence.

4. Conclusion

Postharvest dipping of mango fruits into various plant extracts can suppress the development of anthracnose and improve marketability of mango fruits without pronounced effect on the qualities of the fruits. Aqueous extract of Ruta chalepensis at its high concentration showed superior performance in the experiment which was comparable with the bench mark treatment; indicating that, the exploitation of plant extracts for the control of anthracnose disease on mango can be used as a potential source of sustainable environmentally-friendly botanical fungicides. Moreover, further studies are required to isolate and characterize the active components of the plant extracts that responsible for the antifungal property and the potentially use for fumigation in cold storage or for active packing.

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Table 1: Effect of botanicals on some quality parameters of naturally infected mango fruits

Plant species	Conc. (%)	Quality parameter of mango			
		Firmness	pH	TSS (^{o}Brix)	TA (%)
Adatoda schimperiana	10	5.47^{hij}	4.89^{abc}	17.40^{bcde}	0.52^{fg}
	25	7.70^{bcd}	4.64^{bcd}	16.93^{cdefgh}	0.53^{fg}
	50	7.47^{bcde}	4.30^{efghi}	17.13^{bcdefg}	0.65^{bcde}
Datura stramonium	10	7.40^{bcdef}	5.05^{a}	16.47^{fghi}	0.53^{fg}
	25	8.13^{ab}	4.27^{fghi}	16.60^{efghi}	0.65^{bcde}
	50	7.50^{bcde}	4.21^{hi}	16.20^{hij}	0.70^{ab}
Eucalyptus globulus	10	6.73^{defg}	4.60^{cdef}	17.20^{bcdef}	0.58^{defg}
	25	6.13^{ghi}	4.38^{defghi}	17.47^{bcd}	0.67^{abcd}
	50	8.20^{ab}	4.22^{hi}	16.80^{defghi}	0.71^{ab}
Lantana camara	10	6.53^{efgh}	5.04^{a}	18.53^{a}	0.51^{fg}
	25	6.30^{fgh}	5.20^{a}	17.73^{abc}	0.56^{efg}
	50	6.60^{defg}	4.97^{ab}	17.47^{bcd}	0.52^{fg}
Ocimum basillicum	10	5.13^{ij}	4.49^{defgh}	17.53^{bcd}	0.59d^{efg}
	25	6.90^{cdefg}	5.09^{a}	15.60^{j}	0.49^{g}
	50	6.87^{cdefg}	4.49^{defgh}	16.47^{fghi}	0.53^{fg}
Rosmarinus officinalis	10	4.93^{j}	4.61^{cde}	17.13^{bcdefg}	0.59^{defg}
	25	6.40^{efgh}	4.35^{defghi}	17.33^{bcde}	0.68^{abcd}
	50	7.67^{bcd}	4.09^{i}	17.27^{bcdef}	0.77^{a}
Ruta chalepensis	10	6.93^{cdefg}	4.57^{cdefg}	16.93^{cdefgh}	0.65^{bcde}
	25	7.47^{bcde}	4.38^{defghi}	17.40^{bcde}	0.69^{abc}
	50	8.93^{a}	4.24^{ghi}	16.07^{ij}	0.71^{ab}
Vernonia amygdalina	10	6.40^{efgh}	5.09^{a}	17.93^{ab}	0.49^{g}
	25	7.93^{abc}	4.63^{bcde}	17.60^{bcd}	0.60^{cdef}
	50	7.13^{bcdefg}	4.42^{defghi}	16.33^{ghij}	0.68^{abcd}
Carbendazim		9.00^{a}	4.13^{i}	16.47^{fghi}	0.76^{a}
Untreated Control		5.00^{j}	5.15^{a}	17.67^{bc}	0.53^{fg}
CV (%)		9.93	4.46	2.94	9.82
LSD (0.05)		1.13	0.33	0.82	0.098

Values are means of three replications
Within parameter, means followed by the same letter do not differ significantly at P <0.001

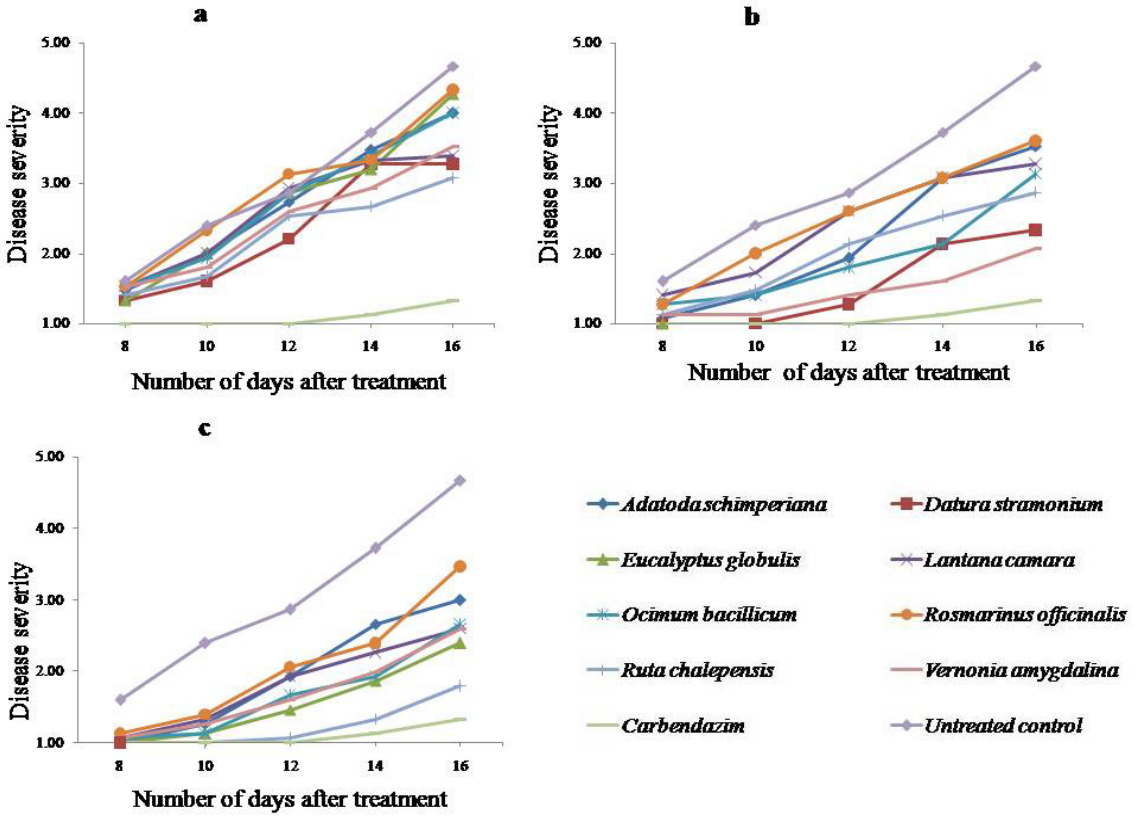

Figure 1: Effect of botanicals on anthracnose development on naturally infected mango fruit. Harvested fruit were dipped in aqueous extracts of selected plant species at (a) 10% concentration (b) 25% concentration and (c) 50% concentration.

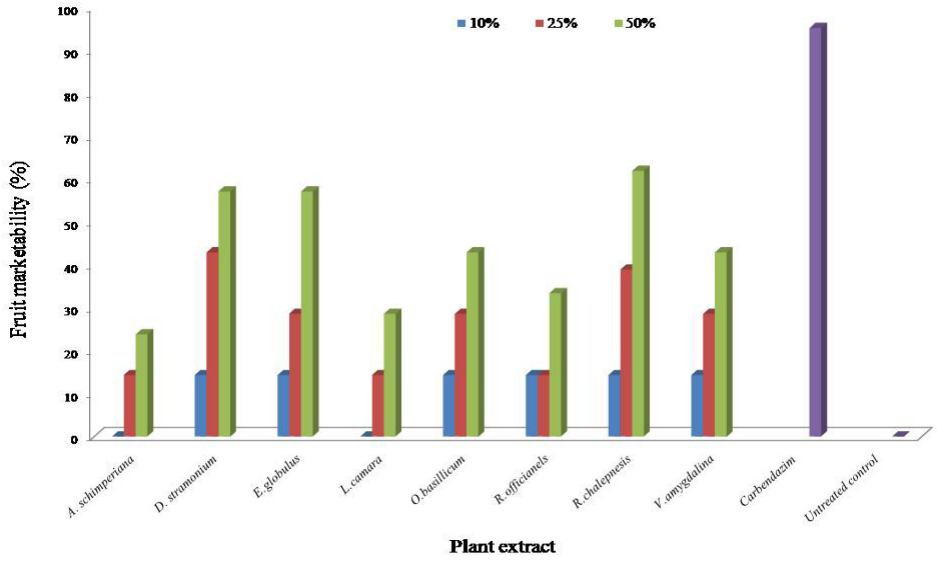

Figure 2. Effect of plant extracts on marketability of mango fruits

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