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Essential Oils to Control *Colletotrichum musae in vitro* and *in vivo* on Banana Fruits

[1]*Fekiya Mohammed Idris,* [2]*Ali Mohammed Ibrahim and* [2]*Sirawdink Fikreyesus Forsido*

[1]Addis Ababa Science and Technology University, Addis Ababa, Ethiopia
[2]Jimma University, College of Agriculture and Veterinary Medicine,
Department of Postharvest Management. P.O.Box 307

Abstract: Owing to increased restriction on use of synthetic fungicides, application of botanical fungicides as alternatives to control postharvest diseases is imperative. Hence, a study was conducted to determine efficacy of essential oils (EOs) in controlling anthracnose of bananas (*C. musae*). First, an *in vitro* experiment was conducted by preparing a pure culture of anthracnose on PDA medium containing EOs of basil (0.10%, 0.15% and 0.20%), cinnamon (0.025%, 0.05% and 0.075%) and rosemary (0.20%, 0.25% and 0.30%). Second, banana fruits (variety Giant Cavendish and Williams) were artificially infected with *C. musae* and sprayed with emulsions of EO. During *in vitro* study, a significant ($P<0.01$) inhibition of mycelial growth was observed in all treatments, except the control 7 days after incubation at 25°C. Similarly, *in vivo* study revealed that all EOs significantly reduced both disease incidence and percent disease index (PDI). However, there was no significant variation for most physicochemical characteristics. Treatment with EO of Basil (0.20%) resulted in the least PDI (26.67%) at 19 days of storage. Findings of this study, both *in vitro* and *in vivo*, confirmed the antifungal effects of basil, cinnamon and rosemary essential oils on anthracnose of banana fruits. Therefore, EOs could be environmentally safe, economical and ideal alternatives to synthetic fungicides.

Key words: Essential oil · Basil · Cinnamon · Rosemary · *C. musae* · Banana quality

INTRODUCTION

Banana is one of the most widely grown tropical fruits, cultivated over 130 countries, along the tropics and subtropics of Capricorn [1]. Its production in the world exceeds 91 million tons [2]. Banana is the second largest produced fruit after citrus, contributing about 16% of the world's total fruit production [3]. In Ethiopia, it is a widely produced crop both in home gardens and commercial farms. In the year 2008, 261,059 tons of bananas had been produced in Ethiopia from 39428 hectares of land [2]. The wide consumption of banana is due to its sensory characteristics [3]; and the caloric contribution of vitamins and minerals, mainly potassium [4].

During storage, banana fruits can develop many postharvest diseases that often affect the quality of the fruit. Anthracnose of banana is caused by the *Colletotrichum* species and is one of the most serious diseases of ripe banana. In the developing world, loses of harvested foods (up to thirty-seven percent) are attributed due to problems in storage and transportation [5].

Particularly during postharvest storage and handling, it is important to encourage the rapid development of alternative approaches to plant disease control. Among the various alternatives, natural plant products, including essential oils that are biodegradable and eco-friendly, are catching the attention of scientists worldwide. Such products from higher plants are bio-efficacious, economical and environmentally safe and can be ideal candidates for use as agrochemicals. Applying postharvest treatment to fruits has advantages such as maintenance of quality (appearance, texture, flavor and nutritive value), assurance of food safety and reduction in harvest and postharvest losses. Storability of fruits can however be improved by postharvest treatments.

Corresponding Author: Ali Mohammed Ibrahim, Jimma University, College of Agriculture and Veterinary Medicine, Department of Postharvest Management. P.O. Box 307,
Tel: +251 917015114. Fax: +251471110934. E-mail: ali.mohammed@ju.edu.et - alimhmd@yahoo.com.

Treatments in the form of essential oil have been developed to control postharvest decay, insect infestation and alleviate storage disorders in a wide range of fresh produce [6].

Essential oils are volatile, natural, complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites. In nature, essential oils play an important role in the protection of the plants through their antibacterial, antiviral, antifungal, insecticidal action and also repellent effect against herbivores by reducing their appetite for such plants [7]. Essential oils are reported to have some fungicidal properties against certain postharvest diseases of tropical fruits and vegetables and they are also safer for the environment than synthetics [6].

The objective of the current study was to investigate the fungicidal effects of the essential oils of basil (*Ocimum basilicum*), cinnamon (*Cinnamomum zeylanicum*) and rosemary (*Rosmarinus officinalis*) against *C. musae* with the aim of developing a cost effective and environmentally friendly treatment system to control postharvest anthracnose of stored banana fruits.

MATERIALS AND METHODS

Plant Materials and Extraction of Essential Oils: Fresh banana fruits of two varieties namely Giant Cavendish and Williams were obtained from Jimma Agricultural Research Center, Ethiopia and fruits were selected for uniformity in size, appearance, ripeness and absence of physical defects. Three kindsspices namely cinnamon, basil and rosemary were selected for extraction of essential oils. Those spices were collected from Jimma University College of Agriculture and Veterinary Medicine. After drying the plant materials (basil, rosemary and cinnamon) under shade, essential oils were extracted by hydro-distillation method using a Clevenger-type apparatus. The oils were separated, dried over anhydrous Na_2SO_4andkept in an airtight sealed dark glass at 4°C until used.

Isolation and Purification of Test Pathogen: Banana fingers with clear symptoms of anthracnose were selected and collected from retail market of Jimma town in order to isolate *C. musae*. Disks ($4mm^2$) of diseased banana fruit peel were cut aseptically from anthracnose lesion surfaces. Next the tissues were surface sterilized with freshly prepared NaOCl (3% w/v) for 3 minutes. Then banana tissues received three serial washings in sterile distilled water and were placed (4 pieces per plate) on Potato Dextrose Agar (PDA) and incubated in an incubator (MJX-1508 model) at 25°C. Potential pathogens were transferred to new PDA plates in order to obtain pure cultures [8, 9]. The fungal pathogens were identified by studying their morphological characteristics using slide cultures and comparison with literature [10, 11]. The identity of test pathogens was confirmed by Mycologist from department of biology, Jimma University. The isolated fungi were maintained on PDA medium at 4°C for further studies.

***In vitro* Experiment**

Antifungal Effect of Essential Oils on Mycelial Radial Growth under *in vitro* Conditions: The antifungal activities of essential oils against *C. musae* were tested by poison food medium method [12]. Antifungal activity was studied using a contact assay (*in vitro*) that produced hyphal growth inhibition. Essential oils of basil (100,150 and 200µl), cinnamon (25, 50 and 75µl) and rosemary (200, 250 and 300µl) were aseptically added to 100ml of sterile cooled PDA medium (45°C) to prepare 0.10%, 0.15% and 0.20% (v/v) for basil; 0.025%, 0.05% and 0.075% (v/v) for cinnamon and for rosemary 0.20%, 0.25% and 0.30% (v/v) concentrations with a drop of Tween 80. The resulting media were immediately dispensed (20 ml) into sterilized Petri plates.

Then a 5 mm disc of mycelium was placed on the PDA medium in each dish.

The treated media were incubated at 24°C and mycelial growth was measured daily. The mycelial growth inhibition (MGI) was determined using the formula MGI = [(dc - dt)/dc] × 100, where *dc* is the mycelium diameter in the control Petri dish and *dt* is the mycelium diameter in the essential oil-treated Petri dish.

***In vivo* Experiment**

Effect of Essential Oils on Postharvest Decay and Quality Factors of C. Musaeinoculated Banana Fruits: The pathogenicity and virulence of pure cultures of fungal pathogens isolated from anthracnose lesions were established by inoculating fully matured green but unripe fruits of Cavendish and Williams [13]. Banana fruits were washed thoroughly with tap water, air dried and surface sterilized with 3% NaOCl. Then fruits were injured (pinprick) with sterilized needle and conidia scrapped out from a 7-day old culture of fungus with a glass rod and suspended in sterile distilled water. Then the concentration was adjusted to 105 conidia/ml using a

haemocytometer and this suspension was sprayed over each fruit. Pinpricked fruits inoculated with only pure sterile distilled water were used as control. Subsequently fruits were treated with different concentrations of essential oils basil, cinnamon and rosemary. Finally, fruits were stored in separate but uniform packages and fruits of all treatments were placed under ambient condition (average temperature and relative humidity 22+1°C and 72%, respectively) for 19 days.

Disease Incidence and Percentage Disease Index: The disease incidence (DI) was calculated as number of infected fruits showing symptom of anthracnose out of total number of banana fruits stored.

On the other hand, disease severity was ranked by observing the extent of anthracnose symptom in terms of the percentage infected surface area to the total fruit [14], where no infected surface area was scored as 1, whereas infected surface areas of 0–5%, 5–25%, 25–50%,50–75% and >75%were scored as 2, 3, 4, 5 and 6, respectively. Based on the numerical ratings given above a Percent Disease Index (PDI) for fruit rot was calculated using the formula given below [15]:

$$(PDI) = \frac{sum\ of\ numerical\ ratings}{Number\ of\ fruit\ exa\min ed\ x\ \max imum\ grade} x\,100$$

(Equation 1)

Shelf Life of Fruits: The shelf life of the banana fruits was calculated by counting the days required for the fruits to attain their last stage of ripening, which is still acceptable for marketing [16].

pH, Titratable Acidity and Total Soluble Solids: The pH of juices extracted from sample banana fruits was measured at 20°C using a pH meter (CP-505 Elmetron, Zabrze, Poland). Titratable acidity (TA) was determined by titrating sample juice with 0.1M NaOH to pH 8.1 and the values are reported as equivalent of malic acid (g/100 g fresh weight) [17]. Total soluble solids (TSS) were determined at 20°C using a refractometer (Eclipse 45-02, Bellingham and Stanley, Bellingham, UK) and reported as° Brix. Subsequently, the ratio between TSS and TA was also calculated.

Physiological Weight Loss (%): In order to determine the physiological weight loss of fruits during storage, the weight of both treated and untreated fruits was taken at the beginning and end of the storage period.

Pulp to Peel Ratio: Pulp to peel ratio was determined by taking the separate weight of the peel and pulp of the sample banana fruits on a sensitive balance (model of TWIII and made in USA). Then the weight of the pulp was divided to that of the peel to arrive at the ratio.

Firmness: Fruits were held firmly with one hand and a penetrometer was tarred to zero and the plunger head was placed against the fruit. Readings were taken from the dual as soon as the fruits showed penetration. The values are expressed as kg/cm^2.

Dry Matter: A 4mm thick slice of banana fruits was taken and weighed on a sensitive balance and then placed in an oven(Cintex, CLC-12, England)at 70°C. The samples were dried and weighed repeatedly until two consecutive measurements were identical. Dry matter percentage (DM%) was calculated by dividing final weight to the initial weight and multiplying by 100 (AOAC, 2000).

$$Dry\ Matter\ (\%) = \frac{Final\ dry\ weight\ (g)}{Initial\ wet\ \ weight\ (g)} x\,100$$

(Equation 2)

Statistical Analysis: The effect of essential oils and varieties on mycelial growth inhibition, physiological weight loss, pulp: peel ratio, firmness, TSS, TA, TSS:TA ratio, dry matter, pH and shelf life was determined using a two-way analysis of variance. For each response variable, the validity of model assumptions, namely normal distribution and constant variance of the error terms assumptions, were verified by examining the residuals [18]. For those responses variables where the effect of essential oils and varieties was significant ($P < 0.05$), multiple means comparison was completed using Tukey's studentized range test at the 5% level of significance and letter groupings were generated. The ANOVA was done using Minitab software version 16.

RESULTS AND DISCUSSION

Table 1 summarizes the ANOVA results for all the response variables and specific discussions are presented below.

Effect of Essential Oils on Radial Growth of C. Musae: Mycelial growth of *C. musae* was significantly ($P < 0.01$) affected by all essential oils (basil, cinnamon and rosemary) as compared to the control during the

Table 1: Means squares for the variance of the effects of essential oils on disease severity, PDI and quality parameters of treated banana fruit

Factor	DF	DI	PDI	Weight Loss (%)	TSS	PH	TSS:TA	TA	Dry matter	Pulp: Peel	Firmness
Var.	1	133.5*	128.2**	1.18^{ns}	1.67^{ns}	0.07^{ns}	0.88^{ns}	0.0008^{ns}	0.54^{ns}	0.00001^{ns}	0.0003^{ns}
EO	9	2239.9**	16377.4**	0.94^{ns}	18.59*	0.04^{ns}	62.57*	0.008*	0.60^{ns}	0.033^{ns}	0.0005^{ns}
EO*Var	9	38.4*	69.6^{ns}	0.93^{ns}	1.78^{ns}	0.04^{ns}	26.54^{ns}	0.0007^{ns}	1.88^{ns}	0.04^{ns}	0.0015^{ns}

*EO: Essential oil, Var: variety. *, ** and ns: Significant at (P< 0.05, P< 0.01) and not significant, respectively*

TSS: Total Soluble solids, TA: Titratable acidity and PDI: percent disease index

Table 2: Mean radial growth and mycelial growth inhibition of*C. musae*on banana fruits as influenced by treatment with essential oils

Essential oils (%v/v)	Radial growth (mm)	Radial growth inhibition (%)
Basil 0.10%	11.33^{d}	87.4^{b}
Basil 0.15%	0.0^{e}	100^{a}
Basil 0.20%	0.0^{e}	100^{a}
Cinnamon 0.025%	24.90^{b}	72.34^{d}
Cinnamon 0.050%	0.0^{e}	100^{a}
Cinnamon 0.075%	0.0^{e}	100^{a}
Rosemary 0.20%	14.67^{c}	83.7^{c}
Rosemary 0.25%	0.0^{e}	100^{a}
Rosemary 0.30%	0.0^{e}	100^{a}
Control (0%)	90^{a}	0^{e}
CV (%)	8.51	0.64
LSD (5%)	2.042	0.92

Within each column, means followed by same letter(s) are not significantly different at the 5% level of significance.

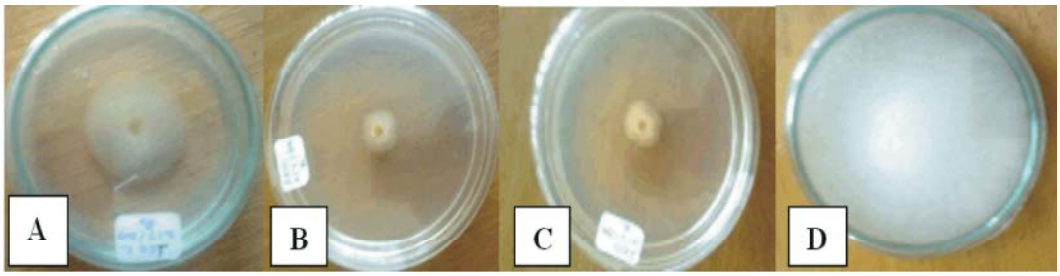

Fig. 1: Radial growth of *C. musae* after 7 days of incubation following treatment banana fruits with different essential oils:(A) 0.1% basil oil, (B) 0.2% rosemary, (C) 0.25% cinnamon and (D) control

incubation period of 7 days at 25°C. The myceilal growth inhibition was directly related to the concentrations and type of essential oil (Table 2). The findings of the present study are in agreement with Thangavelu *et al.* [19]who revealed that extracts of *Solanum torvum, Jatropha glandulifera* and *Emblica officinalis* were highly inhibitory to mycelial growth of *C. musae* and the inhibitory effect was directly related to the quantity of extract added to the medium. *O. basilicum* oil completely (100%) inhibited the growth of the test pathogen, *C. musae*, at a concentration of 0.15-0.2% (v/v) (Figure 1). Basil oil was reported to be fungicidal to *Aspergillus flavus* and *A. parasiticus* at a concentration of 0.6% (v/v) [20]. According to Marandi *et al.* [21], based on results of an *in vitro* assay on pear fruit stated that treatment with essential oil of *O. basilicum* resulted in high antifungal effect on mycelial growth of *Penicillium expansum*.

A study conducted to identify the efficacy of nine essential oils on *C. gloeosporiodes* isolated from papaya [22] indicated that *C. zeylanicum* and *Syzygium aromaticum* oils had better antifungal effects in which the extent of mycelia growth inhibition was dose dependent. Similarly, Zaika[23] reported that essential oil of cinnamon had strong antimicrobial effect. Cinnamon leaf oil was fungicidal against *C. musae, Lasiodiplodia theobromae* and *Fusarium proliferatum*; *in vitro* test of this essential oil was effective at low concentration against the pathogenic organisms isolated from banana fruits and the main constituent of cinnamon leaf oil was eugenol [24]. Cinnamaldehyde, linalool, eugenol and 1,8-cineol have been reported as active components in inhibiting the growth of several fungi [25].

A study pertaining to the antifungal impacts of the essential oil of rosemary against *Aspergillus flavus* under scores the high controlling and antifungal power of

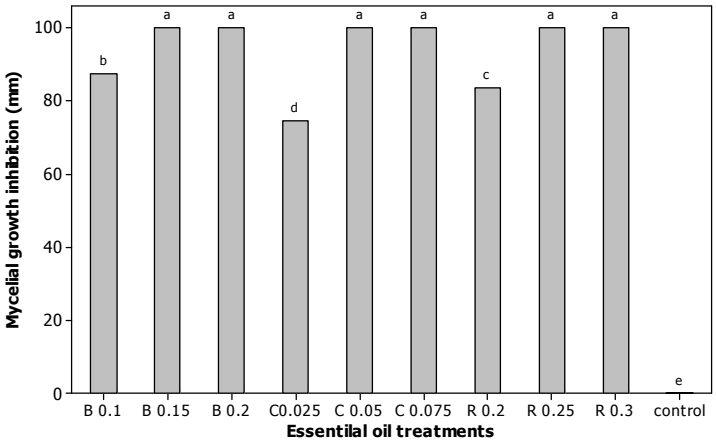

Fig. 2: Mean mycelia growth inhibition of *C. Musae* after 7 days of incubation of banana fruits treated with different essential oils (*B=Basil, C=Cinnamon, R=Rosemary)*

Table 3: Mean disease incidence of anthracnose on banana fruitsas affected by treatment with essential oils

		Days After Storage		
Variety	Essential Oil (%)	15th	17th	19th
Giant Cavendish	B 0.10	(30.79)26.20^{de}	(43.07)46.66 ^{de}	(88.72)100^{a}
	B 0.15	(1.28)0^{f}	(35)33.33^{efg}	(71.86)90.31^{bc}
	B 0.20	(1.28)0^{f}	(1.28)0^{h}	(42.7)46^{e}
	C0.025	(35)33.33^{cd}	(55.37)67.7^{bc}	(88.72)100^{a}
	C 0.05	(26.56)20^{e}	(46.92)53.33^{cd}	(88.72)100^{a}
	C 0.075	(1.28)0^{f}	(35)33.33^{efg}	(54.99) 67.01^{d}
	R 0.20	(30.79)26.20^{de}	(43.07)46.66^{de}	(88.72)100^{a}
	R 0.25	(26.56)20^{e}	(43.07)46.66^{de}	(88.72)100^{a}
	R 0.30	(1.28)0^{f}	(30.78)26.67^{fg}	(54.99)67.01^{d}
	Control	(88.72)100^{a}	(88.72)100^{a}	(88.72)100^{a}
Williams	B 0.1	(30.79)26.20^{de}	(59.21)73.8^{b}	(88.72) 100^{a}
	B 0.15	(1.28)0^{f}	(46.92)53.33^{cd}	(80.29)97.02^{ab}
	B 0.2	(1.28)0^{f}	(26.56)20^{g}	(54.99)67.01^{d}
	C0.025	(39.23)40^{e}	(59.21)73.8^{b}	(88.72)100^{a}
	C 0.05	(26.56)20^{e}	(46.92)53.33^{cd}	(88.72) 100^{a}
	C 0.075	(1.28)0^{f}	(39.23)40^{def}	(59.21)73.8^{d}
	R 0.2	(35)33.33^{cd}	(59.21)73.8^{b}	(80.29) 93.33^{ab}
	R 0.25	(30.79)26.20^{de}	(43.07) 46.66^{de}	(63.44) 80^{cd}
	R 0.3	(1.28)0^{f}	(35)33.33^{efg}	(59.21) 73.8^{d}
	Control	(88.72)100^{a}	(88.72) 100 ^{a}	(88.72)100^{a}
	LSD (5%)	6.616	11.89	12.24
	CV (%)	19.46	15.55	9.82

Data in the brackets are transformed mean. Within each column, means followed bysame letter(s) are not significantly different at the 5% level of significance.B=Basil, C=Cinnamon, R=Rosemary

rosemary essential oil [26]. The extent of inhibition of fungal growth varied depending on the levels of essential oil used in the experiment [27]. The antimicrobial impact of the essential oils of rosemary is due to the active components like α-pinene, camphor, verbenone and 1, 8-cineole [28]. In the poisoned food bioassay, there was zero percent inhibition of mycelia growth *C. musae* (Fig. 1 and 2) in the control plates. The different essential oils tested in the present study exhibited different minimum inhibitory concentration (MIC) and this result is in agreement with reports of Maqbool *et al*. [29, 24, 30].

Effect of Essential Oils on Disease Incidence and Percentage Disease Index

Disease Incidence: The disease incidence on banana fruits increased with time and almost all treated banana fruits showed the disease symptom after nineteen days of storage while all the fruits in the control treatment developed the typical symptoms of anthracnose after seven days of storage. The different essential oils (basil, cinnamon and rosemary) delayed the occurrence of anthracnose disease development and later on manifested few symptoms. Table 3 shows that there was a significant

Table 4: Mean percent disease index of anthracnose as influenced by treatment of banana fruits with essential oils

	Days After Storage		
Essential oils	15th	17th	19th
B 0.10	21.67^{bc}	29.44^{bc}	37.78^{cd}
B 0.15	18.33^{cd}	23.33^{ef}	36.66^{cd}
B 0.20	16.67^{d}	21.11^{f}	26.67^{f}
C0.025	23.82^{b}	31.67^{b}	46.11^{b}
C 0.05	19.93^{bcd}	26.68^{cd}	34.44^{d}
C 0.075	16.67^{d}	23.8^{def}	27.78^{ef}
R 0.20	23.33^{b}	30.55^{b}	42.77^{bc}
R 0.25	20.55^{bcd}	25.56^{de}	36.11^{d}
R 0.30	16.67^{d}	25.002^{de}	33.8^{de}
Control	63.89^{a}	77.22^{a}	100^{a}
CV (%)	14.04	9.61	13.24
LSD (5%)	3.9	3.29	6.5

Within each column, means followed by same letter(s) are not significantly different at the 5% level of significance.B=Basil, C=Cinnamon, R=Rosemary

difference among treatments for both cultivars of banana examined. The maximum average disease incidence (100%) was observed in the control treatment on all days of assessment with the entire samples of fruits showing symptom for anthracnose. On the other hand, the minimum disease incidence (0%) was observed on the 15th day of storage on banana fruits which were treated with 0.15% and 0.20% basil, 0.075% cinnamon and 0.30% rosemary essential oil treated. Even after 17 days of storage, treatment with 0.20% basil resulted in the smallest anthracnose incidence compared with other treatments. These results are in agreement with that of Maqbool *et al.* [6, 29], Anthony *et al.* [31], Ranasinghe *et al.* [32] and Marandi *et al.* [21].

In the present study all banana fruits were infected at the end of the storage period; 19 days after storage. This result fits to the results of Abdolahi *et al*. [33], who reported that treatment with ajowan, fennel and caraway essential oils had no effect on the control of fungal infection in inoculated tomatoes after 20 days of storage. The ineffectiveness of essential oils in providing full control against anthracnose pathogen at ambient temperature could be due to their high volatility [34].

Percent Disease Index (PDI): Fruits treated essential oils were better maintained and had lower severity scores, whereas non-treated fruits suffered increased deterioration. The symptoms of anthracnose appeared on the control fruits seven days after storage while most of the bananas were spoiled 19 days after storage due to severe disease infection. However, there was no significant difference between the two varieties of banana used for the study, namely Giant Cavendish and Williams, in respect of PDI (Table 4). The PDI on bananas reached up to 100% (score 6) 19 days after storage in the control fruits.

The highest fungicidal effect was observed from the use of 0.2% basil oil, in which the average percent disease index was 22.5% for Giant Cavendish and 27% for Williams' banana variety with fruit surface infection close to 1.3 and 1.6, respectively. Banana fingers treated with *O. basilicum* oil had almost no symptom of anthracnose (AS<2) even after 15 days of storage unlike cinnamon and rosemary treated fingers, both of which had developed some anthracnose symptoms (AS >2) and the control treatments (AS > 4, after15 days of storage). The result is in agreement with that of Anthony *et al*. [34] who reported that *O. basilicum* was the most efficacious essential oil. It controlled fungal pathogens of Embul banana to the same extent as Benomyl and has potential as an alternative to synthetic fungicides in the horticultural industry.

Effect of Essential Oils on Postharvest Quality Factors of Banana Fruits

Storage Life: In the present study, it was noticed that most banana fruits were spoiled on the 19thday of storage. Between days 7 and 15, all of the untreated banana fruits deteriorated and on day 17 cinnamon, basil (0.1% and 0.15%) and rosemary treated banana fruits became unmarketable. However, only banana fruits treated with 0.2% basil essential oil kept well till the 19thday of storage. This result is in alignment with that of Abdolahi *et al*. [35], who reported that treatment with essential oils of ajowan, fennel and caraway had no effect on the number of infected tomato fruits until the end of storage period (20 days) when all the fruits were found to be infected.

Total Soluble Solids: Essential oil treatments significantly ($P<0.05$) affected the TSS content of all treated fruits throughout storage period. This was possibly due to increased fungal infection (Fig. 3). TSS increased as ripening progressed. Basil treated fruits had the lowest value of TSS (8.93 and 8.99°Brix in variety Cavendish and Williams, respectively). However, there was no significant difference in terms of TSS content of the two varieties during the storage period.

Similar findings were reported by Abdolahi *et al*. [33], who stated that TSS level was lower in essential oil treated grapes than controls. Moreover, in coherence with the result of the present study, the effects of ammi, anise, ziziphora and cinnamon essential oils on TSS content of peach fruits were found to be significant [36].

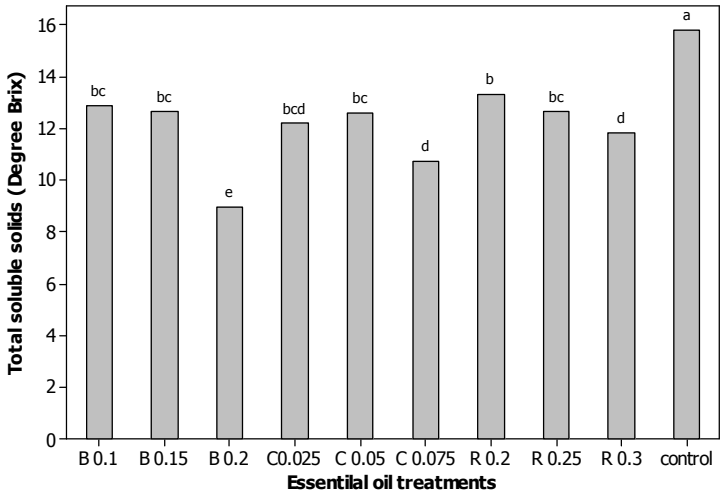

Fig. 3: Mean TSS of banana fruits treated with different essential oils(*B=Basil, C=Cinnamon, R=Rosemary)*

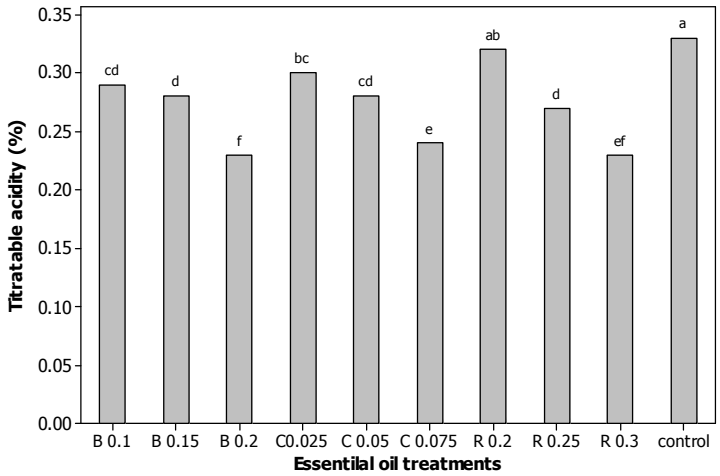

Fig. 4: Mean TA of banana fruits treated with different essential oils(*B=Basil, C=Cinnamon, R=Rosemary)*

Titratable Acidity: Considering the TA of treated fruits, it was significantly (P<0.05) affected by essential oil treatment in all the treated fruits throughout storage period (Fig. 4). There was a significant difference between the TSS values of fruits in the control and the rest of treatments except those fruits treated with 0.20% rosemary. The minimum TA value (0.23%) was recorded from banana fruits which were treated with 0.20% basil and 0.30% rosemary oils, while the maximum TA (0.33%) was registered in the control treatment.

The result of the present study is in accordance with the of findings of Asghari *et al.* (2009), who reported that treatment of strawberry fruits with cumin essential oil had significant influence on the TA of fruits on the 3rd, 12th and 15th days of storage (P<0.05). Similarly, increased TA levelswerereported in essential oil-treated clusters of grapes compared with the control [33]. On the contrary, Marandi *et al.* (2010) and Maqbool *et al.* [37] reported that *Thymus kotschyanus* and *Carum copticum* essential oil treatment had no significant effect on TA table grapes and bananas, respectively.

TSS to TA Ratio: There was a significant (P< 0.05) effect of essential oil on TSS to TA ratio of banana fruits. The control treatment was only significantly different with the rest of the treatments i.e. 0.2% basil, 0.025% cinnamon and 0.3% rosemary essential oil treated banana fruits. But there was no significant difference between the control and other treatments. Investigation done by Samane and Aminifard [36] on effect of ammi, anise, ziziphora and cinnamon essential oils on post harvest decay and some quality factors of peach fruit indicated that there was no significant difference in respect of TSS to TA ratio content of fruits among treatments.

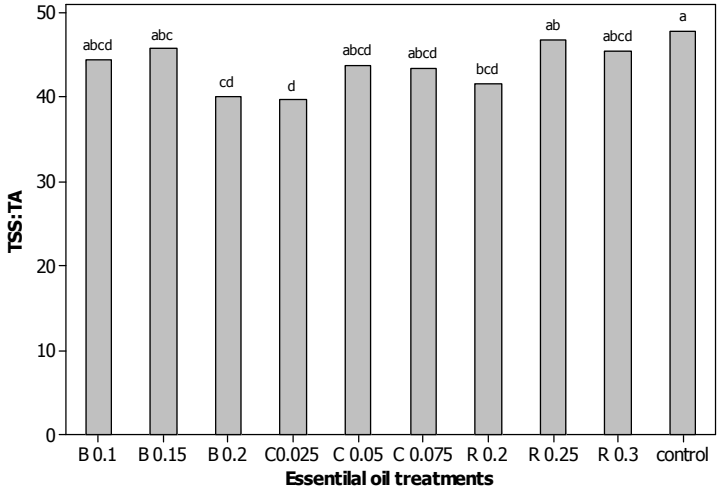

Fig. 5: Mean TSS to TA ratio of banana fruits treated with different essential oils(*B=Basil, C=Cinnamon, R=Rosemary)*

pH: The effects of both essential oil and variety on pH of the banana fruits were not significant throughout the storage time. In a similar study, basil essential oil spray emulsion (0.16% v/v) treatment on banana to control crown rot disease did not have any significant effect on pH after induced ripening [31]. Treatment with cinnamon (bark and leaf) essential oil had no effect on pH of bananas after 14 days of storage at 28°C or 21 days at 14±1°C [32]. Moreover, treatment with cinnamon and eucalyptus vapor had no significant effect on pH of tomato fruits [38].

Physiological Weight Loss (WL) (%): Treatment of banana fruits with essential oils of basil, cinnamon and rosemary had no substantial effect on weight loss of banana fruits throughout storage period. Physiological weight losses recorded from fruits in all treatments increased with the number of days of storage. However, there were no significant ($P < 0.05$) treatment effects on weight loss during subsequent sampling dates during the storage period. Among samples, the highest value for weight loss (11%) was obtained from Cavendish fruits which were treated with 0.025% (v/v) cinnamon essential oil as well as in the control. Whereas, the lowest weight loss (9.03%) was recorded from William banana fruits treated with rosemary oil 0.30% (v/v) and the grand mean for weight loss was 9.55%. According to the results of the present experiment, essential oil treatment had no vivid and significant effect on weight loss of banana fruits.

This result is in conformity with earlier reports of different researchers. Treatment with cinnamon essential oil had no effects on weight loss [37]. Win *et al*. [38] also reported that treatment with cinnamon essential oil had no effect on weight loss throughout the storage period of banana fruit. Similarly, Anthony *et al*. [31] reported that weight losses were not significantly different ($P<0.05$) among all treatments of oils from *O. basilicum, Cymbopogon nardus* and *Cymbopogon fexuosus* when banana fruits were incubated at ambient temperatures. Moreover,[42 indicated that *Thymus kotschyanus* and *Carum copticum* essential oil treatments on table grape had no significant effect on weight loss. Strawberries treated with essential oils of *Eucalyptus globulus* and *C. zeylanicum* did not differ in respect of weight loss, organic acid content and sweetness compared with untreated fruits [38]. In addition, these results are in accordance with that of Ranasinghe *et al*. [40] who stressed that *C. zeylanicum* oil had no effect on weight loss of banana fruit. On the other hand, results of the present investigation contradict with opinion of Abdolahi *et al.* [35] who reported that *T. vulgaris, S. hortensis, F. vulgare and O. basilicum* essential oils reduced weight loss (%) of table grapes. The positive effect of eugenol, thymol and menthol in reducing of weight loss in sweet cherries treated with essential oils was reported [41].

Dry Matter: No significant effect of essential oils was observed on dry matter content of treated fruits compared to the controls for all sampling dates. There was no significant effect attributable to banana varieties and interaction effect of essential oil with variety. Study on the effect of postharvest treatment of Maghrarbi banana fruits with mandarin essential oil on quality indicated that there was no significant effect as compared with the untreated banana fruits [11].

Firmness: Firmness of the banana fruits showed a significant ($P<0.05$) difference between the treatments on day three of storage but there was no significant difference between the varieties. Moreover, no significant difference was observed with regard to the fruit firmness of treated fruits compared to the controls at all other determination times. These results are in accordance with the findings of Ranasinghe *et al*. [40] who stressed that *C. zeylanicum* oil had no significant effect on the fruit firmness of banana fruits. Likewise, Ranasinghe Lakshmie *et al.* [32] stated that cinnamon leaf essential oil treatments had no effect on flesh firmness of bananas fruits after 14 days of storage at 28°C or 21 days at 14 ± 1°C. Moreover, cinnamon essential oils were reported to have no effect on fruit firmness of bananas fruits after 28 days storage at 13±1°C [6]. An investigation on effect of basil, lemon grass and Ceylon citronella oil on postharvest diseases and storage life of Embul banana showed that fruit firmness fell over time in all treatments regardless of whether fruit ripening was natural or induced [31]. However, only fruits treated with citronella oil were significantly firmer ($P<0.05$) than fruits from all other treatments.

Pulp to Peel Ratio: Following the treatments with essential oils and storage, treatments couldn't impart a significant difference in respect of pulp to peel ratio of banana fruits throughout the storage period. In addition, no significant difference was apparent in terms of the pulp to peel ratio of fruits between the two varieties; Giant Cavendish and Williams.

CONCLUSION

Results of the present study showed that treatments of banana fruits with essential oils of basil, cinnamon and rosemary reduced mycelial growth of *C. musae* in vitro. Incidence of disease was reduced on essential oil-treated banana fruits and their storage life was increased considerably. Previous studies have also indicated that constituents of these essential oils are generally regarded as safe compounds, since their toxicity to the human being is very low. Based on these evidences we can conclude that basil, cinnamon and rosemary essential oils could be used as possible bio-fungicides, as an alternative to synthetic fungicides, against phytopathogenic fungi on banana fruits. However, similar experiments need to be conducted before making recommendations with regard to the efficacy of these essential oils on other horticultural crops as antifungal agents to increase the postharvest storage life of other horticultural crops.

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