Journal of Postharvest Technology
2021, **09**(1): 53-63
www.jpht.in

RESEARCHARTICLE

In vitro and *in vivo* activity of essential oils against postharvest pathogen *Colletotrichum musae* of banana

Deepak Kumar[1*], Santosh K. Arya[1], Md. Shamim[2], Deepti Srivastava[3], Manjusha Tyagi[4], K. N. Singh[5]

[1] R&D Division, Shri Ram Solvent Extractions Pvt. Ltd, Jaspur-244712, Uttarakhand, India

[2] Department of Molecular Biology and Genetic Engineering (PBG), Dr Kalam Agricultural College, Kishanganj, Bihar Agricultural University, Sabour, Bhagalpur 813210, Bihar, India

[3] Integral Institute of Agricultural Science and Technology, Integral University, Dasauli, Lucknow 226021, Uttar Pradesh, India

[4] Department of Microbiology, Shri Guru Ram Rai University, Patel Nagar, Dehradun 248001, Uttarakhand, India

[5] Department of Plant Molecular Biology and Genetic Engineering, Acharya Narendra Deva University of Science and Technology, Kumarganj, Ayodhya, 224229, U.P., India

Received: 06.12.2020 Accepted: 20.01.2021

ABSTRACT

Anthracnose caused by *Colletotrichum musae* is a serious postharvest disease of immature and mature fruit of banana. The synthetic fungicides are used to control this disease but in current scenario, fungicides prohibited in many countries that reduce their commercial value. Therefore, presented study was conducted to evaluate the antifungal activity *in vitro* and *in vivo* for find out alternative products to the synthetic fungicides. Two different approaches were used; poisoned food technique was used for *in vitro* studies while pre-inoculated banana fruits with phytopathogen (*C. musae*) and essentials oils' treatments *in vivo* conditions. The results indicated that all essential oils inhibited the mycelial growth of pathogen over untreated PDA plates. *Schleichera oleosa* oil was superior among all treatments *in vitro* against pathogen *C. musae*, it was twofold higher to rest other EOs. Similarly, under *in vivo* conditions, all essential oils showed significant influence on lesion diameter, decay incidence, decay loss, physiological loss in weight and storage life but *Schleichera oleosa* oil was the most effective. The storage life of banana fruits was higher in *Schleichera oleosa* oil (25% v/v) treated fruits (*viz* 4.5 days more) compared to untreated and rest EOs treated banana fruits after 15 days of storage under ambient conditions (22±2^{0}C, 72-75% RH). Thus presented studies revealed that unexploited essential oils have the potential to control anthracnose disease without causing harmful effects on banana fruits, and can be recommended as safe biofungicides for extending storage life and quality attributes.

Keywords: Essential oils, *Colletotrichum musae*. banana quality, disease incidence, storage life.

Citation: Kumar, D., Arya, S. K., Shamim, M., Srivastava, D., Tyagi, M., and Singh, K. N. 2021. Impact of food selection and usage pattern on consumers' attitude towards food label information. *Journal of Postharvest Technology*, **9** (1): 53-63.

INTRODUCTION

Banana is most traded and consumed fruits in the world due to their distinct aroma and taste. It is the economic life line and staple food for many countries because of cheap source of carbohydrate and rich source of potassium, calcium, antioxidants and other micronutrients (Mohapatra et al, 2010). In 2017 alone, 22.7 million tonnes of bananas were traded, representing almost

* *For correspondence*: D. Kumar (Email: deepak_rajora84@rediffmail.com)

ISSN: 2348-4330

20% of global production that year. Current available estimates showed that average global banana production increased from 69 million tonnes in 2000-2002 to 116 million tonnes in 2017-2019, at an approximate value of 31 billion USD (FAO, 2020). However, it is observed that only around 15 percent of the total global banana production is traded in the international market mainly in Europe and United States; the rest is consumed locally in large producing countries such as India, China, and Brazil, and in some African countries where bananas include largely to people's diets (FAO, 2020). The major banana producing states of India are Tamil Nadu, Maharashtra and Gujarat.

In last few years, there is significant loss of the food and quality values of banana due to improper post harvest management practices that causes huge economic loss. In post harvest losses, crown rot and anthracnose are the main diseases that affecting bananas after harvest, mainly caused by *Colletotrichum musae*. For extending the shelf life, banana preservation is used through two phases (i.e. between harvesting and initiation of the ripening process and a second preservation period between initiation of the ripening process and the time of consumption) by fungicidal (imazalil, thiabendazole, pyrimethanil, boscalid, iprdione and fludioxonil) coating along with preservatives like Polyvinylidene chloride copolymer, carboxymethyl cellulose and surfactants (tween 80, tween 20) respectively (Petcavich, 2007; Davie et al., 2007; Stammler et al., 2007; Palou, 2018).

Fungicides like Chloride-based chemicals including chloramines, dichloramines, and trichloromethanes, which are considered respiratory irritants supposed to be carcinogenic (Fallanaj et al., 2013) and moreover On the other hand, caused resistant strains of pathogens resulting in a breakdown of fungicide effectiveness (Hao et al., 2011; Sánchez-Torre et al., 2011). Furthermore, after pandemic COVID 19 disease incidence, consumers are more worried about to health related problems and ecological contamination. Therefore in previous two decades many research have been carried out the impact of non-chemical application, for example, irradiations, natural compounds, essential oils, bio-control agents, hot water and salts applications (Stammler et al., 2007; Gastavsson et al., 2011; Hao et al., 2011; Youssef et al., 2012, 2019, 2020).

Out of all alternative methods, essential oils (EOs) have been used in many studies to control postharvest decay, insect infestation and alternative disorders in wide range of fresh horticultural produce (Idris et al., 2015; Jhalegar et al., 2015; Wisniewski et al., 2016; Sanzani et al., 2019). Essential oils are natural and complex compounds containing secondary metabolites, which play an important role as antibacterial, antifungal; antiviral and insecticidal against pathogens and pests (Asghari Marjanlo et al., 2009; Maqbool et al., 2010). Keeping the benefits of essential oils as biodegradable and ecofriendly bio-fungicide, this study was taken to investigate the fungicidal effects of the essential oils of neem *Azadirachta indica*), karanj (*Pongamia pinnata*), kusum (*Schleichera oleosa*) and jatropha (*Jatropha curcas*) against *Colletotrichum musae.* This research had main aim of developing a cost effective and sustainable treatment system to control postharvest anthracnose of stored banana fruits.

MATERILAS AND METHODS

Experimental site and fruit material

The study was conducted in the R&D Division, Shri Ram Solvent Extractions Pvt. Ltd., Jaspur, Uttarakhand, India during 2018-19 (October-November). Fresh banana fruits of variety Dwarf Cavendish were obtained from banana ripening facility of Shri Ram Solvent Extractions Pvt Ltd, Jaspur, Uttarakhand, India and fruits were manually selected for uniformity in size, appearance, ripeness and absence of physical defects.

Isolation, purification and identification of test pathogen

Banana fruits with clear symptoms of anthracnose were selected and collected from retails market of Jaspur town, Uttarakhand. Anthracnose infected banana fruit peel's surface lesions disks (4 mm^2) were cut aseptically to isolate *Colletotrichum musae*. The

tissues were surface sterilized with freshly prepared NaOCl (3% w/v) for three minutes, then three serial washings with sterilized distilled water and placed (3 pieces per plate) on potato dextrose agar (PDA). For proper growth of test pathogen, inoculated plates were incubated in incubator (SIS-1302 model) at 25±2^{0}C for one week (Idris et al., 2015). The potential pathogens were sub-cultured to new PDA containing plates in order to achieve pure cultures (Shivas and Beasley, 2005). The identification of the fungal isolates were carried out by microscopic observation (Olympus CH 20i, New York, USA) and studying their morphological characteristics using slide cultures and comparison with taxonomic key description and literatures (Lim et al., 2002; Abd-Elsalam, 2010).

Preparation of essential oil solution

For *in vitro* and *in vivo* studies, four different essential oils namely Neem (*Azadirachta indica*), Karanj (*Pongamia pinnata*), Kusum (*Schleichera oleosa*) and Jatropha (*Jatropha curcas*) were used and collected from Suyash Ayurveda, Surat Gujarat, R.S. Oil industries, Raipur, Chhattisgarh and Shri Ram Solvent Extractions Pvt. Ltd., Uttarakhand, India respectively with detailed technical specifications (Table 1). For optimization of all essential oils' doses, different concentrations of EOs (0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0, 25.0% v/v) were prepared using Tween 20 (0.1%) as surfactant and amended in PDA. The pH of media was adjusted 5.6 by adding 1N NaOH, using digital pH meter (Systronics-M362 model) and autoclaved for 15 min at 121^{0}C (Jhalegar et al., 2015; Idris et al., 2015).

Table 1: Technical specifications of all essential oils used in presented studies

S. No.	Technical Parameters	Essential oils			
		Neem (*Azadirachta indica*)	**Karanj (*Pongamia pinnata*)**	**Kusum (*Schleichera oleosa*)**	**Jatropha (*Jatropha curcas*)**
1.	Colour	Yellowish brown	Yellowish	Yellowish brown	Yellowish
2.	pH	6.8	6.7	5.6	6.6
3.	Specific gravity @30^{0}C (g/cm^3)	0.935	0.925	0.869	0.910
4.	Moisture (%)	0.28	0.11	0.25	0.30
5.	Refractive index @40^{0}C	1.4670	1.4752	1.4602	1.4625
6.	Acid Value (mg/KOH/g)	7.50	7.07	10.52	5.02
7.	Iodine Value (ml/g) wijs	75.12	85.61	55.24	96.30
8.	Viscosity (mm^2/sec)	45	40	52	37
9.	Saponification Value (ml/g)	187	189	220	122
10.	Unsaponifiable matter	1.86	2.58	3.00	0.10
11.	Active metabolites (ppm)	Azadirachtin (350 ppm), Nimbin (2500 ppm)	Karanjin (21000 ppm)	Hydrocyanic acid (420 ppm)	Curcin (25 ppm)

***In vitro* antifungal assay of essential oils (EOs)**

The antifungal assay of essential oils (EOs) against phytopathogen *C. musae* was carried out by poison food medium method (Mishra and Dubey, 1994). An PDA disk (5 mm diameter) from pure culture of *C. musae* was transferred in the centre of a EOs (0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0, 25.0% v/v) containing PDA plates. In control plates, PDA was used for referral comparison with EOs treated plates. The inoculated PDA Petri-plates were incubated at 25±1^{0}C for 7 days. The radial mycelial growth was measured in all treatments daily until the fungus reached the edge of the control plate. The mycelial growth inhibition (MGI) of pathogen *C. musae* was calculated using the formula MGI = [(dc-dt)/dc] x 100, where *dc* is the fungal mycelial growth (mm) in the control Petri-dish and *dt* is the mycelial diameter in Petri-dish treated with essential oils (Idris, 2015).

***In vitro* antifungal assay of essential oils (EOs)**

Effect of essential oils on postharvest factors (lesion diameter, decay incidence, decay area, weight lose, and storage time) of *C. musae* inoculated banana fruits

The pathogenicity of pure culture of *C. musae* was established by inoculating fully matured green but unripe banana fruits of Dwarf Cavendish. Banana fingers were washed thoroughly with tap running water, air dried at ambient temperature (25-28^{0}C) and surface sterilized with 3% sodium hypochlorite. The spore suspension containing 10^6 conidia ml^{-1} was prepared from 7 days old culture of fungus and inoculated on banana fruits and held at room temperature (25±2^{0}C) for 2 hours. Inoculation was carried out with sterilized needle (1 mm wide and 2 mm long) and making a punctures for injuries in each banana fruit (Eckert and Brown, 1986; Idris et al., 2015). The treatments were made by suspension solution spraying over each fruit for 60 seconds at 25±2^{0}C. Then air dried banana fruits were dipped in known concentration (25% v/v) of all essential oils (*Azadirachta indica*, *Pongamia pinnata*, *Schleichera oleosa* and *Jatropha curcas*) and again kept them at ambient conditions for drying. After treatments, fruits were packed in separate and uniform packages and stored under ambient condition (average temperature 22±2^{0}C and 72±5 relative humidity) for 15 days.

The effect of all EOs on various physiological and pathological parameters was studied at regular time intervals for 15 days during storage. The disease incidence (DI) was expressed in percentage (%) and calculated as number of infected fruits showing positive symptoms of anthracnose out of the total number of banana fruits stored in each treatment. Lesion diameter (mm) and decay area were expressed accordingly same as disease incidence accordingly (Sivakumar et al., 2002). Physiological weight loss was measured by subtracting the initial banana fruit from final weight after storage time and expressed as a percentage (%). Similarly fruit decay was calculated by counting the number of rotten banana fruits, divided by the total number of fruit, and expressed as percentage (%) like weight loss. The number of fruits for observation was taken 6 in each treatment.

The shelf life of banana fruits was calculated through counting the days required for fruits to attain their last stage of ripening which is suitable and acceptable for marketing (Thangavelu et al., 2004).

Statistical design and analysis of data

The experiments were carried out in factorial CRD design with each treatment consisting of six fruits with three replications. The obtained data of experiments were analysed as per design and results were compared with ANOVA through calculating CD (Panse and Sukhatme, 1984).

RESULTS AND DISCUSSION

***In vitro* studies**

Effect of essential oils (EOs) on radial growth of *Colletotrichum musae*

The presented studies have indicated that mycelial growth of *C. musae* was inhibited by all the essential oils (*Azadirachta indica*, *Pongamia pinnata, Schleichera oleosa,* and *Jatropha curcas*) as compared to the control during the incubation period of 7 days at 25±1^{0}C. The radial mycelium growth was directly related to different concentrations and types of essential oils (Table 2 and Fig. 1). Among different EOs, maximum radial mycelial growth of *C. musae* was inhibited by Kusum (*Schleichera oleosa*) oil (0.09 mm, 98.68%) compared to rest others EOs at 25% (v/v) concentration incorporated into potato dextrose agar (PDA).

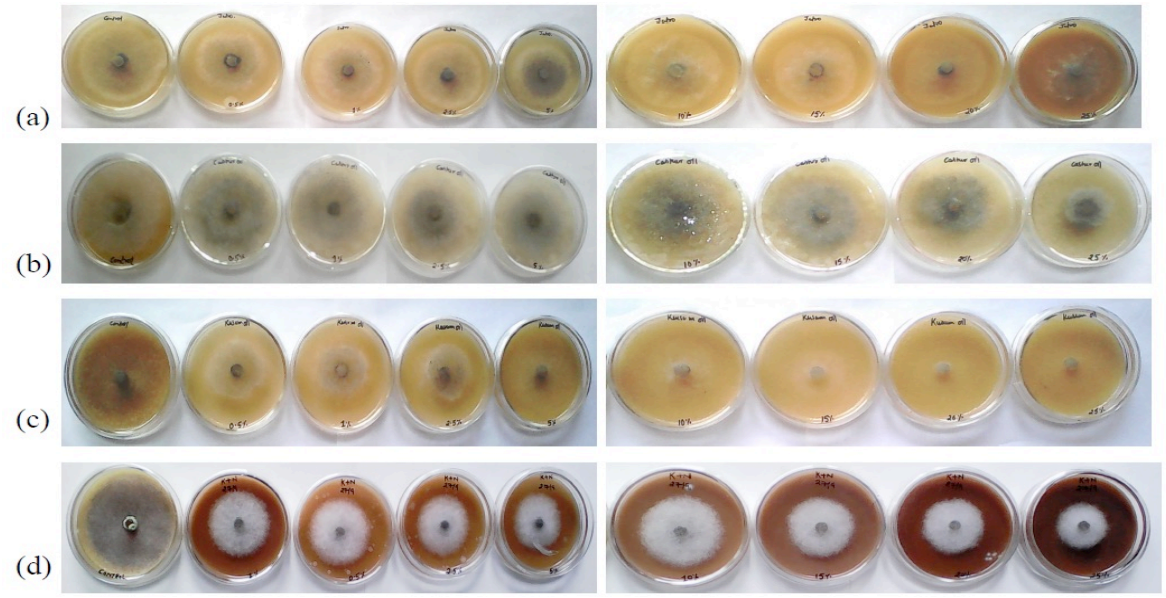

Fig 1: *In vitro* efficacy of different concentrations of essential oils (0.0, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0, 25.0% v/v) (a) neem (*Azadirachta indica*), (b.) Karanj (*Pongamia pinnata*),(c.) Kusum (*Schleichera oleosa*),(d.) Jatropha (*Jatropha curcas*) incorporated into PDA on mycelial radial growth of pathogen *C. musae* after 7 days of incubation.

Table 2: *In vitro* efficacy of essential oils (%v/v) incorporated into PDA on mycelium radial growth (mm) and growth inhibition (%) of pathogen *Colletotrichum musae* after 7 days of incubation at 25^{0}C

S.No.	Conc. of EOs (%v/v)	Radial growth of *Colletotrichum musae* (mm) into essential oils (%v/v) incorporated into PDA				Radial growth inhibition (%) of *Colletotrichum musae* into essential oils (%v/v) incorporated into PDA			
		Neem oil	Karanj oil	Kusum oil	Jatropha oil	Neem oil	Karanj oil	Kusum oil	Jatropha oil
1.	0.00	7.25	6.96	7.13	6.90	0.00	0.00	0.00	0.00
2.	0.50	6.11	6.75	3.87	4.04	15.67	3.10	45.64	41.47
3.	1.00	5.29	5.56	3.64	3.91	27.05	20.14	48.96	43.31
4.	2.50	5.12	5.34	3.24	3.74	29.37	23.25	55.43	45.82
5.	5.00	4.46	5.12	2.53	3.64	38.43	26.46	64.44	47.26
6.	10.00	4.38	4.46	2.28	3.30	39.54	35.99	67.99	52.19
7.	15.00	4.09	3.90	2.09	3.03	43.64	43.93	70.62	56.06
8.	20.00	3.77	3.61	1.21	2.83	48.01	48.20	82.97	58.95
9.	25.00	2.96	3.21	0.09	1.76	59.13	53.85	98.68	74.50
LSD (P=5%)		0.22	0.16	0.10	0.15	2.95	2.49	1.68	2.11
CV (%)		2.43	1.69	1.87	2.14	4.69	4.67	1.49	2.40

*Mean of three replicates

The results of the present study are in the findings of Idris et al. (2015) who revealed that essential oils of Basil, Cinnamon and Rosemary were highly inhibitory effect to mycelial growth of *C. Musae* and this inhibitory effect was directly related to the concentrations of EOs incorporated to the medium. Wang et al. (2019) was also used seven essential oils in suppressing *Collelotrichum gloeosporioides* at different concentrations *in vitro* and identified clove oil to be the most promising inhibitor.

Similarly, four essential oils of tea tree (*Melaleuca alternifolia*), clove (*Eugenia caryophyllus*), ginger (*Zingiber officinale*) and thyme (*Thymus vulgaris*) species were used at different concentrations to control and inhibiting mycelial growth, germination and sporulation of *C. Musae* 'Prata Anã' banana by Rodrigues et al. (2018).

The inhibitory effects of all the EOs shows positive activities against fungi due to their volatile and non-volatile chemical constituents or active ingredients that are produced during different stages of developments. The essential oils (EOs) used in this research, have many active components which showed antifungal activities. Tetranortriterpenoid azadiradione isolated from neem (*Azadirachta indica*) seed oil, has evaluated and found a reduction of 76 % in the rust pustule count of *P. arachidis* by Govindachari et al. (2000). Similarly, Ospina Salazar et al. (2015) used neem oil extract (Azadirachtin) against 14 isolates of the dermatophytes *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis* and *Epidermophyton floccosum* and found effective. Karanj (*Pongamia pinnata*) showed inhibitory effect against *C. Musae* (53.85%, 59.13%) parallel to Neem **(***Azadirachta indica*). Wagh et al. (2007) studied the antifungal and antibacterial activity of *Pongamia pinnata* oil with different concentrations against *Aspergillus fumigatus*, *A. niger*, *Pseudomonas aerugina. Pongamia pinnata* showed high efficacy and antifungal activity because of presence of flavonoids, triterpenoids (Karanjin) and thermostatic nature (Tim and Lamb, 2005; Akram et al., 2016). The essential oil of Kusum *Schleichera oleosa* extracted from seeds was reported for antifungal activity having terpenoids, flavonoids and cyanogenic compounds that proved to be a significant member to inhibit *Candida albicans* isolated from eye-patients (Ghosh et al., 2011; Goswami and Singh, 2017). Jatropha (*Jatropha curcas*) oil was second most promising antifungal activity (1.76mm, 74.50%) on *C. Musae* after *Schleichera oleosa* among all the essential oils. The similar findings by Srivastava et al. (2012) supported the presented results, in which *Jatropha curcas* oil was tested against six fungi and found very effective *Penicillium gkabrum* and *Aspergillus niger* respectively.

***In vivo* Studies**

Effect of essential oils (EOs) on lesion diameter, decay area, decay incidence and physiological weight loss

Lesion diameter: Essential oils treated banana fruits showed the lesser mean lesion diameter compared to untreated fruits, when they were pre-inoculated with post harvest pathogen *C. musae*, known to cause deadly post harvest losses in banana industry. Among all treatments (25% v/v), fruits treated with Kusum (*Schleichera oleosa*) oil had the least lesion diameter, followed by Jatropha (*Jatropha curcas*) oil, Karanj (*Pongamia pinnata*) oil and Neem (*Azadirachta indica*) oil with pre-inoculated *C. musae* at different time intervals of storage (Table 3 and Fig. 2). In presented results, the lesion diameter exhibited an increasing trend with the number of days increase (storage period) from 5th day to 15th day of storage. Lesser lesion diameter among all EOs treated banana fruits compared to untreated could be attributed to the reason that the EOs have many flavonoids, triterpenoids, and active bio-molecules (azadiradione, azadirachtin, nimbin, salanin, karanjin, cyanogenic compounds) which pass through the cell wall, cytoplasmic membrane, different layers of polysaccharides and phospholipids and permeabilize them and protect from pathogen infestation. The our results showed agreement with the results of Idris et al. (2015), who reported the different essential oils as pre-harvest application to control banana anthracnose decay and have the potential for commercialization in banana industry. Moreover, the findings of Jhalegar et al. (2015) who used different EOs for controlling of postharvest losses in citrus fruits, have also support the presented results.

Decay area: During different time intervals of storage, EOs treated banana fruits showed lesser mean decay area than untreated fruits, when they were simultaneously pre-inoculated with pathogen *C. musae*. Among all EOs treated treatments, fruits treated with Kusum oil (*Schleichera oleosa*) oil showed least decay area caused by *C. musae,* significantly followed by Jatropha (*Jatropha curcas*) oil, Karanj (*Pongamia pinnata*) oil and Neem (*Azadirachta indica*) oil and maximum in untreated fruits (Table 3 and Fig. 2). The trends of decay area in all EOs treated banana fruits were in a increasing order according to storage time but much higher in untreated fruits. This could be attributed to the effect of EOs on biological membranes of pathogens, as several

investigations support the antimicrobial and antifungal action of Eos (Bakkali et al., 2008; Idris et al., 2015). At the end of storage period (15th days), decay area caused by *C. musae* was nearly two times higher than those treated with Kusum (*Schleichera oleosa*) oil (Table 3 and Fig. 2). The effects of other EOs (*Azadirachta indica, Pongamia pinnata, Jatropha curcas)* on decay area were also significant over untreated (control) but were less effective than *Schleichera oleosa* oil. Thus, the better mycelium growth inhibition of *C. musae* by *Schleichera oleosa* oil may be due to high content of terpenoids, flavonoids and cyanogenic compounds (Goswami and Singh, 2017). Although, no direct scientific evidence available to prove this fact but the finding of Ghosh et al. (2011) support the presented data who used *Schleichera oleosa* oil to control *Colletotrichum camelliae* and found positive results.

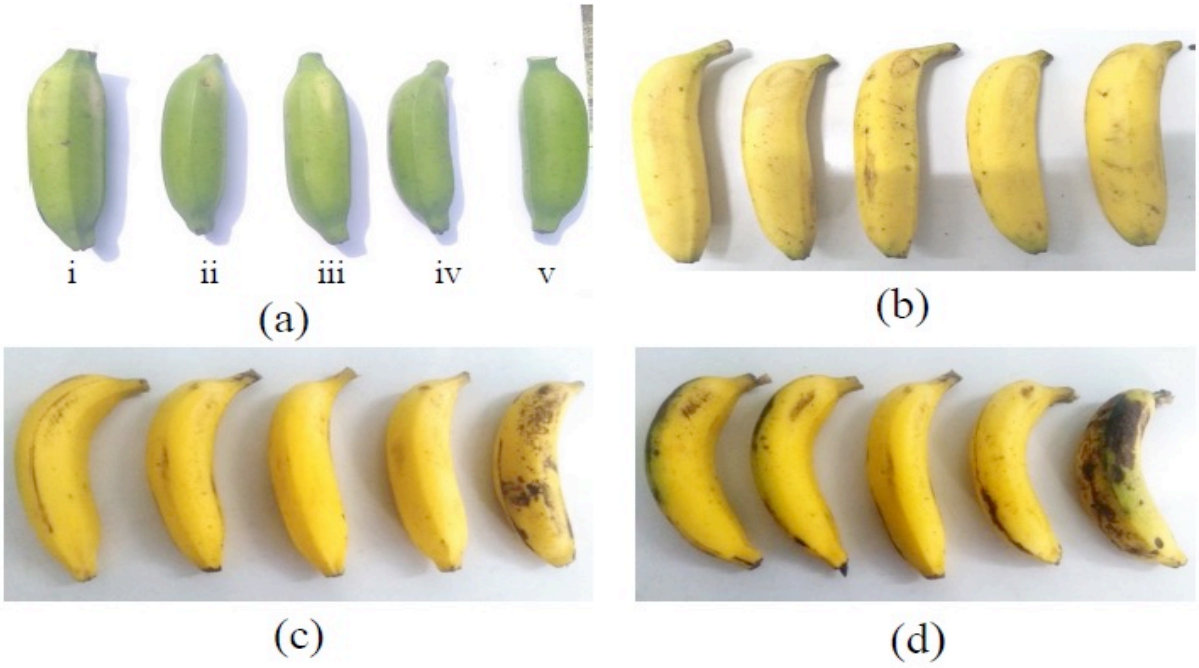

Fig 2: *In vivo* efficacy of different essential oils (25%v/v) (i) neem (*Azadirachta indica*), (ii) Karanj (*Pongamia pinnata*),(iii.) Kusum (*Schleichera oleosa*),(iv) Jatropha (*Jatropha curcas*), (v) Control (untreated) on banana against pathogen *C. musae* at different time intervals (a) 0 days (b) 5 days (c) 10 days and (d) 15 days under ambient conditions (72±5 RH, average temp. 22±2^{0}C) under storage.

Decay incidence (%): The decay incidence on banana fruits increased with time and all EOs treated banana fruits showed the decay incidence after 15th days of storage while all the fruits in untreated treatment developed symptoms of anthracnose after 5th days of storage. Simultaneously pre-inoculated with *C. musae*, EOs treated banana fruits showed lesser decay incidence than untreated fruits. Among all treatments, banana fruits treated with *Schleichera oleosa* oil had least decay incidence followed by Jatropha (*Jatropha curcas*) oil, Karanj (*Pongamia pinnata*) oil and Neem (*Azadirachta indica*) oil respectively (Table 3, Fig 2). After 15 days of storage, untreated banana fruits completely decayed by *C. musae* (100%) and least in *Schleichera oleosa* (61.11%) which was promising result for extending shelf life of banana fruits during storage. Various researchers studied and reported that essential oils have been showed successful in controlling decay of fruits. For instances, Idris et al. (2015) reported the effect of EOs (Basil, Cinnamon, Rosemary) against *C. musae* and showed that all EOs had both *in vitro* and *in vivo* antifungal influence on anthracnose of banana fruits. Moreover, Jhalegar et al. (2015) also evaluated different EOs (Lemon grass, Clove, Eucalyptus, and Neem) against post harvest pathogens *Penicillium digitatum* and *P. italicum* on Kinnow (citrus) fruits and found that EOs significantly increase storage life by controlling them.

Physiological weight loss (%): It is an important parameter which directly linked to the shelf life of any horticultural crop produce. Presented studies revealed that a steady increase in physiological loss in weight as with the increase in storage period from 5th day to 15th day of storage (Table 3 and Fig. 2). The trends of weight loss during storage is similar to other parameters, means *Schleichera oleosa* treated banana fruits showed lesser physiological weight loss than other EOs treated and untreated banana fruits at different time intervals (5,10 and 15 days) of storage. The positive effects of essential oils on decreasing the weight loss could be attributed with hydrophobic coatings of EOs, lesser respiration rate and ethylene production rate, which might have control the water loss from banana fruits (Jhalegar et al., 2015). Essential oils form a thin layer over the fruit peel and create a modification of microclimate on fruits, and induce hindrance in respiration rate resulted in lesser moisture loss from fruits and significantly increased their shelf life (Samra et al., 2006).

Table 3: Efficacy of essential oils (25% v/v) on (a) lesion diameter (mm), (b) decay area (%), (c) decay incidence (%), and physiological weight loss on banana fruits pre-inoculated with *Colletotrichum musae* at different time intervals during storage under ambient conditions (72±5 RH, average temp. 22±2^{0}C) for 15 days.

S.No.	Essential Oils	Lesion diameter (mm)			Decay area (%)			Decay incidence (%)			Physiological weight loss (%)		
	(25% v/v)	5 days	10 days	15 days	5 days	10 days	15 days	5 days	10 days	15 days	5 days	10 days	15 days
1.	Neem oil	13.17	51.50	92.33	23.33	46.83	73.67	30.55	46.83	86.11	0.33	0.67	1.60
2.	Karanj oil	11.33	40.33	90.83	18.00	39.33	69.33	27.78	39.33	83.33	0.33	0.65	1.56
3.	Kusum oil	2.83	23.00	58.00	4.83	23.50	49.33	13.88	23.50	61.11	0.22	0.47	0.88
4.	Jatropha oil	6.67	35.33	72.17	12.17	34.17	58.33	25.00	34.17	72.28	0.24	0.79	1.05
5.	Control	56.00	91.00	137.33	46.17	82.17	100.00	53.56	82.17	100.00	0.65	1.42	2.39
LSD (P=5%)		8.49	11.18	18.52	6.62	7.24	6.20	17.49	7.24	13.48	0.11	0.19	0.32
CV (%)		20.79	10.22	9.065	14.09	7.06	3.89	25.58	7.061	7.37	14.11	10.59	9.34

*Mean of 6 fruits across six replications.

Table 4: Efficacy of essential oils (25%v/v) to measure the number of days increased after 15 days of storage under ambient conditions (72±5 RH, average temp. 22±2^{0}C)

S.No.	Essential Oils (25% v/v)	Number of days increases
1.	Neem (***Azadirachta indica***)	1.83
2.	Karanj (***Pongamia pinnata***)	2.17
3.	Kusum (***Schleichera oleosa***)	4.50
4.	Jatropha	3.50
5.	Control	0.00
LSD (P=5%)		0.83
CV (%)		15.21

*Mean of 6 fruits across six replications

Effect of essential oils on storage life

In the presented study, it was observed that untreated banana fruits were spoiled on the 15th day of storage than EOs treated fruits. Between days of 10th and 15th, maximum incidence of partially deterioration was noticed in all EOs (*Jatropha curcas*,

Pongamia pinnata, *Azadirachta indica*) treated fruits than *Schleichera oleosa* oil treated banana fruits (Table 4 and Fig. 2). The increasing time was two times more (4.5 days) to rest other EOs treated banana fruits after 15 days of storage. The result is in line of agreement with Idris et al. (2015) who found antifungal effect on postharvest anthracnose of banana fruits and reported positive effect on banana fruits storage life by EOs of basil, cinnamon, and rosemary respectively.

CONCLUSION

The presented study showed that treatments of EOs have the potential to control the *C.musae* which causes anthracnose of banana fruits. Mycelial growth of *C. musae* was clearly affected by increasing the concentration and type of essential oils at various stages of the development of phytopathogen. Among all EOs, *Schleichera oleosa* oil was found superior to rest others in controlling the incidence of *C. musae* and increased the storage life considerably. Moreover, previous studies revealed that EOs are generally regarded as safe compounds, and very less toxic to the human. Based on the these results, it can be concluded that *Schleichera oleosa, Jatropha curcas*, *Pongamia pinnata*, and *Azadirachta indica* oils could be used as bio-fungicides, alternative to synthetic fungicides against pathogenic fungi on banana fruits. However, further experiments need to be conducted before making any mutual recommendations for all stakeholders with regard to the efficacy of these essential oils as antifungal agents on other horticultural crops.

ACKNOWLEDGEMENTS

The first author is greatly thankful to the Director, Shri Ram Solvent Extractions Pvt. Ltd, Jaspur for providing financial support to the presented research work.

REFERENCES

Abd-Elsalam K.A., Roshdy S., Amin O.E. and Rabani M. 2010. First morphogenetic identification of the fungal pathogen Colletotrichum musae (Phyllachoraceae) from imported bananas in Saudi Arabia. Genetics and Molecular Research. 9(4), 2335-2342.

Akram A., Shaheen I., Akhund S., Nayyar B.G. and Seerat W. 2016. In vitro antifungal activity of Pongamia pinnata against collar rots pathogen (*Sclerotium rolfsii*) of chickpea of chickpea. Pure and Applied Biology. 5(3), 520-528.

Asghari Marjanlo A., Mostofi Y., Shoeibi S. and Fattahi M. 2009. Effect of cumin essential oil on postharvest decay and some quality factors of strawberry. Journal of Medicinal Plants. 8, 25-43.

Bakkali F., Averbeck S., Averbeck D. and Idaomar M. 2008. Biological ffects of essential oils-a review. Food Chemistry and Toxicology. 46, 446–475.

Davie I. and Kinasz R. 2007. Method for Suppressing Post-harvest Biological Infestation and Disease in Fruit. United States Patent Application 20070166440.

Eckert J.W. and Brown G.E. 1986. Post harvest citrus disease and their control. In: Fresh citrus fruits. AVI publishing Co., Westport, pp 315–360

Fallanaj F., Sanzani S.M., Zavanella C. and Ippolito A. 2013. Salt addition improves the control of citrus post-harvest diseases using electrolysis with conductive diamond electrodes. Journal of Plant Pathology. 95, 373–383.

FAO 2020. http://www.fao.org/economic/est/est-commodities/bananas/en/

Gastavsson J., Cederberg C. and Sonesson U. 2011. Global Food Losses and Food Waste; Food and Agriculture Organization (FAO) of the United Nations: Rome, Italy

Ghosh P., Chakraborty P., Mandal A., Rasul M.G., Chakraborty M. and Saha A. 2011. Triterpenoids from *Schleichera oleosa* of Darjeeling foothills and their antimicrobial activity. Indian Journal of Pharmaceutical Science. 73(2), 231–233.

Goswami S. and Singh R.P. 2017. Ayurvedic, phytochemical and pharmacological review of Scleichera olerosa (Lour) Oken: A traditional plant with enormous biological activity. World Journal of Pharma Research. 6(10), 295-309.

Govindachari T.R., Suresh G., Gopalakrishnan G., Masilamani S. and Banumathi B. 2000. Antifungal Activity of Some Tetranortriterpenoids. Fitoterapia.71, 317-320.

Hao W., Li H., Hu M., Yang L. and Rizwan-ul-Haq M. 2011, Integrated control of citrus green and blue mold and sour rot by Bacillus amyloliquefaciens in combination with tea saponin. *Post-Harvest Biology and Technology*. 59, 316–323.

Idris F.M., Ibrahim A.M. and Forsido S.W. 2015. Essential oils to control *Colletotrichium musae in vitro* and *in vivo* on banana. American-Eurasian Journal of Agriculture and Environment Science. 15(3), 291-302.

Jhalegar J.M., Sharma R.R. and Singh D. 2015. *In vitro* and *in vivo* activity of essential oils against major postharvest pathogens of Kinnow (*Citrus nobilis x C. deliciosa*) mandarin. *Journal of Food Science and Technol*ogy. 52 (4), 2229-2237.

Lim J., Lim T.H. and Cha B. 2002. Isolation and Identification of Colletotrichum musae from imported bananas. Plant Pathology Journal. 18(3), 161-164.

Maqbool M., Ali A. and Alderson P.G. 2010. Effect of cinnamon oil on incidence of anthracnose disease and postharvest quality of bananas during storage. International Journal of Agriculture and Biology. 12, 516-520.

Mishra A.K. and Dudey N.K. 1994. Evaluation of some essential oil for their toxicity against fungi causing deterioration of stored food commodities. Applied and Environmental Microbiology. 60, 1101-1105.

Mohapatra D., Mishra S. and Suthar N. 2010. Banana post harvest practices: Current status and future prospects-A review: Agricultural Reviews. 31(1), 56-62.

Ospina Salazar D.I., Hoyos Sánchez R.A., Orozco Sánchez F., Arango Arteaga M. and Gómez Londoño L.F. 2015. Antifungal activity of neem (*Azadirachta indica*: Meliaceae) extracts against dermatophytes. Acta Biologica Colombiana. 20(3), 201-207.

Palou L. 2018. Post-harvest treatments with GRAS salts to control fresh fruit decay. Horticulturae. 4, 46.

Panse V.G. and Sukhatme P.V. 1984. Statistical methods for agricultural workers, ICAR, New Delhi, pp. 228-290.

Petcavich R.J. 2007. Process and Coating Composition for Extending the Shelf Life of Post Harvest Produce. United States Patent Application 20070055003

Rodrigues M.L.M., Mizobutsi E.H., Naearath I.R.F.F., Fernandes M.B., Mizobutsi G.P., Ribeiro R.C.F., Dos Reis S.T., Pinheiro J.M.S., Prates P.J.L. and Lage G.G.A. 2018. Essential oils in the control of anthracnose on 'Prata Ana' Banana. Journal of Agricultural Science. 10(9), 116-124.

Samra N.R., Mansour A.M., Tourky M.N. and Tarbih M.E. 2006. Pre and post harvest treatments on peach fruit grown under desert conditions. Journal of Agricultural Science Mansoura University. 31, 7835–7846.

Sánchez-Torres P. and Tuset J.J. 2011. Molecular insights into fungicide resistance in sensitive and resistant Penicillium digitatum strains infecting citrus. Post-Harvest Biology and Technology. 59, 159–165.

Sanzani S.M. and Ippolito A. 2019. New techniques for managing post-harvest diseases of fruit. In Integrated Management of Diseases and Insect Pests of Tree Fruit; Xu, X.M., Fountain, M., Eds.; Burleigh Dodds Science Publishing: Cambridge, UK,; pp. 748.

Shivas R. and Beasley D. 2005. Management of Plant Pathogen Collections manual. Australian Government Department of Agriculture, Fisheries and Forestry. Available online at: http:// www.daff.gov.au/planthealth[Accessed 11/5/2011]

Sivakumar D., Hewarathgamagae N.K., Wijeratnam R.S.W. and Wijesundera R.L.C. 2002. Effect of ammonium carbonate and sodiumbicarbonate on anthracnose of papaya. Phytoparasitica. 30, 1–7.

Srivastava S., Kumar R. and Sinha A. 2012. Antifungal Activity of *Jatropha curcas* Oil Against Some Seed-borne Fungi. Plant Pathology Journal. 11, 120-123.

Stammler G., Brix H.D., Nave B., Gold R. and Schoefl U. 2007. Studies on the biological performance of boscalid and its mode of action. In Proceedings of the Modern Fungicides and Antifungal Compounds V, Friedrichroda, Germany, 6–10 May, 2007.

Thangavelu R., Sundararaju P. and Sathiamoorthy S. 2004. Management of anthracnose disease of banana caused by Colletotrichummusae using plant extracts. The Journal of Horticultural Science and Biotechnology. 79, 664-668.

Tim T.P. and Lamb A.J. 2005. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 26, 343-356.

Wagh P., Rai M., Deshmukh S.K. and Durate M.C.T. 2007. Bio-activity of oils of *Trigonella foenum-graecum* and *Pongamia pinnata*. African Journal of Biotechnology. 6, 1592-1596

Wang D, Zhang J; Jia J, Xin L, Zhai H. (2019). Antifungal effects and potential mechanism of essential oils on Collelotrichum gloeosporioides *in vitro* and *in vivo*. *Molecules:* 24: 3386. doi:10.3390/molecules24183386

Wisniewski M., Droby S., Norelli J., Liu J. and Schena L. 2016. Alternative management technologies for post-harvest disease control: The journey from simplicity to complexity. Post-Harvest Biology and Technology. 122, 3–10.

Youssef K. and Hussien A. 2020. Electrolysed water and salt solutions can reduce green and blue molds while maintain the quality properties of 'Valencia' late oranges. Post-Harvest Biology and Technology. 159, 111025.

Youssef K., Ligorio A., Sanzani S.M., Nigro F. and Ippolito A. 2012. Control of storage diseases of citrus by pre- and post-harvest application of salts. Post-Harvest Biology and Technology. 72, 57–63.

Youssef K., Roberto S.R., Colombo R.C., Canteri M.G. and Abd-Elsalam K.A. 2019. Acibenzolar-S-methyl against Botrytis mold on table grapes in vitro and in vivo. Agronomy Science and Biotechnology. 5:52–61.