

BIO-RATIONAL MANAGEMENT OF ANTHRACNOSE OF BANANA WITH INDIGENOUS PLANT OILS

MST SAMAYUN AREFIN[†], MST ANANYA KHATUN^{†1}, MD MOBINUL ISLAM,
MD AHSAN HABIB, MUHAMMAD FAZLE RABBE^{2*} AND MD MOHIDUL HASAN*

Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University,
Dinajpur-5200, Bangladesh

Keywords: Plant oils, Anthracnose, Banana quality, Shelf life

Abstract

The present investigation was aimed to screen indigenous plant oils for the management of anthracnose (*Colletotrichum musae*) in banana. Among the plant oils, the highest radial growth inhibition of *C. musae* (77.78%) was observed with eucalyptus oil (4%) followed by neem. This study demonstrated maximum reduction of anthracnose incidence in Sobri by neem and sesame (64.44%) and in Chini Champa by eucalyptus and sesame (~73.73%) compared to control. Maximum reduction of anthracnose severity was achieved in Sobri by neem and black cumin (~60.15%), and in Chini Champa by sesame, linseed, and eucalyptus (~56.31%) in comparison to the control. Compared to fruit inoculated with *C. musae*, neem, black cumin, and eucalyptus increased the fruits' shelf life by two to three days. Neem resulted lowest total soluble solids (~9.07°Brix); eucalyptus gave a maximum reduction of physiological weight loss (~24.93%).

Introduction

Banana (*Musa acuminata*), is one of the most popular tropical fruits cultivated around the globe (Sau *et al.* 2023). The storage and transportation of the fruits are difficult because of their quick ripening tendency and vulnerability to post-harvest diseases (Ahmed and Palta 2016). Anthracnose, a post-harvest disease of banana caused by *Colletotrichum musae* can lead to 30-40% of marketable yield loss (Balendres *et al.* 2020, Ranasinghe *et al.* 2003). Farmers usually apply expensive fungicides to control the disease, which often causes hazardous effects on human health and the environment (Goswami *et al.* 2018, Perdichizzi *et al.* 2014). Global demand for organic fresh fruits is rising as a result of the detrimental effects of agrochemicals in fresh fruits (Şener *et al.* 2020). Therefore, efforts are being for the search for eco-friendly and sustainable approaches alternatives to agrochemicals.

Essential oils derived from medicinal plants function as a reservoir of secondary metabolites (Campos-Requena *et al.* 2017). Plant oils are biodegradable and eco-friendly, which offer a wide range of antifungal activity (Sellamuthu *et al.* 2013). Regarding the use of plant oils to treat banana anthracnose, there has been little research conducted in Bangladesh (Idris *et al.* 2015). Therefore, the present study was taken to evaluate the efficacy of indigenous plant oils for the efficient and eco-friendly management of anthracnose in banana.

Materials and Methods

Banana fruits with prominent symptoms of anthracnose were collected from local market and surface sterilized using 70% ethanol, cut aseptically (4 mm disc), placed (4 pieces/plate) on PDA medium, and incubated at 25±2 °C for 7 days. The fungus grown on PDA was transferred to new PDA plates and identified.

*Author for correspondence: <mhasan@hstu.ac.bd>, <rab-bi.biotech@gmail.com>. ¹Department of Horticulture, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh. ²Department of Biotechnology, Yeungnam University, Gyeongsan 38541, Gyeongbuk, Korea. [†]Authors contributed equally.

Fresh bananas of two local varieties namely Sobri and Chini Champa, were purchased from banana growers based on uniformity in size, appearance, ripeness, and absence of any physical disorders. Five different plant oils namely Neem (*Azadirachta indica*), Sesame (*Sesamum indicum*), Linseed (*Linum usitatissimum*), Eucalyptus (*Eucalyptus globulus*), and Black cumin (*Nigella sativa*) were purchased from the local market in Bangladesh. The antifungal ability of plant oils against *C. musae* was tested by the agar well diffusion method (Valgas *et al.* 2007). In brief, wells (6 mm) were made in PDA by maintaining an equal distance from the center, filled with 100 μ L of plant oils (2, 4, and 6%) and 7-day-old mycelial blocks of *C. musae* (5 mm) were positioned in the middle. The plates were incubated for 7 days at 25 ± 2 °C. The mycelial growth inhibition (MGI) rate was calculated using the formula of Idris *et al.* (2015).

Ten surface-sterilized bananas were injured using a sterilized needle and *C. musae* (10^8 /mL) was inoculated by spraying (Idris *et al.* 2015). Following inoculation, the fruits were sprayed with each of the plant oil (4%). Negative control was maintained by spraying with *C. musae* only and positive control by spraying with fungicide Mancozeb (0.25%). Anthracnose incidence (%) was counted and severity (%) was calculated (Sivakumar *et al.* 2002).

Total soluble solid (°Brix) [TSS, Digital Refractometer, Model: EMSZ, China], physiological weight loss (%) and pulp to peel ratio were determined. Firmness of the fruits was measured using a penetrometer (kg/cm^2). The shelf life of banana fruits was counted as the days required for complete ripening acceptable for marketing.

Data obtained from different parameters were analyzed using Statistix 10. Multiple means comparison was computed using Tukey's test at 5% level of significance.

Results and Discussion

The colony of *C. musae* was pinkish-white colored, with septate mycelia, and hyaline cylindrical conidia (Fig. 1A, B). The isolated fungi developed characteristic symptoms after inoculation to the healthy banana fruits. In agar-multiwell diffusion culture, the highest radial growth inhibition (77.78%) of *C. musae* occurred owing to eucalyptus oil (4%) followed by neem oil (66.67%), black cumin oil (60%), sesame oil (57.77%) and linseed oil (53.33%) (Table 1, Fig. 1C,D). Similar findings with neem leaf and seed extracts were reported to inhibit the growth of *Colletotrichum* spp. by Okigbo and Emoghene (2003). Not only the extracts, neem, and eucalyptus oil (2-3%) also showed significant inhibition of mycelial growth of *C. musae* (Bhutia *et al.* 2014, De Araújo *et al.* 2018). At 12 days after storage (DAS), neem and eucalyptus result in the lowest incidence of anthracnose in Sobri (40%) and in Chini Champa (23%) (Table 2). Oils derived from neem, eucalyptus, cloves, thyme, basil, cinnamon, rosemary, etc. exhibited notable antifungal activity against *C. musae* (Vilaplana *et al.* 2018, Idris *et al.* 2015, Lundgren *et al.* 2022).

Neem-oil-treated bananas showed the lowest TSS (9.07, 9.10° Brix) in both Sobri and Chini Champa, respectively (Fig. 2A). The apex of firmness was attained in Sobri with sesame oil (1.99) where both sesame and neem oil resulted in higher grades of firmness (2.64, 2.56 kg cm^{-2}) in Chini Champa, respectively (Fig. 2B). The highest pulp-to-peel ratio was found where fruits treated with sesame and black cumin oil (2.01 and 1.90) respectively in Sobri and lowest in black cumin-treated Chini Champa (0.20) (Fig. 2C). Bananas, which did not receive any plant oils, rotted within 9-10 DAS. Only Chini Champa treated with linseed and black cumin oils remained edible even after 14 DAS, whereas, another plant oil kept the banana edible not more than 12 days after storing (DAS) (Fig. 2D). Sobri and Chini Champa treated with eucalyptus oil exhibited minimal weight loss (24.93% and 17.20%, respectively), whereas black cumin oil showed only 4% weight loss in Chini Champa (Fig. 2E). Neem oil also demonstrated its weight-loss reducing effect in both Sobri and Chini Champa (23.07% and 22.97 %, respectively). Anise, ziziphora, and cinnamon

essential oils were found to lower the TSS content of peach fruits (Mohammadi *et al.* 2012) and citronella oil increased firmness (Idris *et al.* 2015). The shelf life and weight loss of post-harvest fruits are indeed influenced by respiration rate and ethylene production. Various plant oils can mitigate these factors, creating a microclimate on the fruit peel that reduces respiration and moisture loss, ultimately extending shelf life (Jhalegar *et al.* 2015).

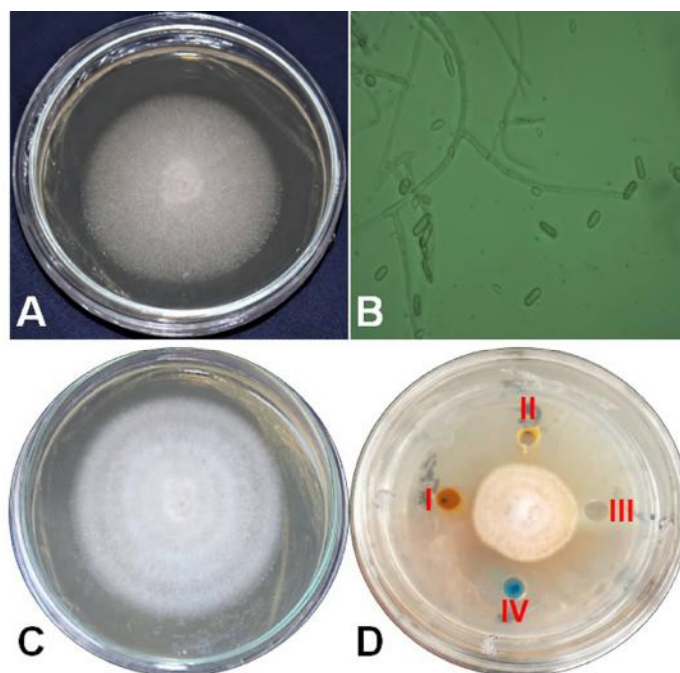


Fig. 1. Characteristics of *Colletotrichum musae*. A: Pure culture, B: Conidia, C: *C. musae* without oils, D: *C. musae* with oils; I. Neem, II. Eucalyptus, III. Sesame and IV. Mancozeb

Table 1. Inhibition of radial growth of *Colletotrichum musae* owing to various plant oils at different concentration.

Plant oils	Concentration (%)	<i>Colletotrichum musae</i>	
		Radial growth (mm)	% inhibition
Neem	2	20 d	55.56
	4	15 d	66.67
	6	21 c	53.33
Sesame	2	21 c	53.33
	4	19 d	57.77
	6	24 c	46.67
Linseed	2	27 b	40.00
	4	22 c	51.11
	6	29 b	35.56
Eucalyptus	2	11 e	75.56
	4	10 e	77.78
	6	20 d	55.56
Black Cumin	2	19 d	57.77
	4	18 d	60.00
	6	21 c	53.33
Mancozeb	0.25	20 d	55.56
Control		45 a	0

Table 2. Efficacy of plant oils on the incidence and severity of anthracnose of banana at different intervals.

Variety	Plant oils	% incidence (DAS)			% severity (DAS)			Avg.	12	6	3	Avg.	12	6	3	Avg.
		3	6	12	3	6	12									
Sobri	Neem	30.00±0.00 cdef	36.67±5.77 defg	40.00±0.00 def	35.55	22.77±0.95 ef	25.56±1.93 ef	31.11±0.96 de	26.48							
	Sesame	43.33±5.77 c	46.67±5.77 d	46.67±5.77 cd	45.55	21.11±0.96 ef	31.11±1.92 d	33.89±1.92 d	28.70333							
	Linseed	26.67±5.77 def	26.67±5.77 fg	53.33±5.77 c	35.55	23.89±0.96 de	26.11±0.96 e	31.11±0.96 de	27.03667							
	Eucalyptus	36.67±5.77 cde	36.67±5.77 defg	40.00±0.00 def	37.78	24.44±0.96 de	28.89±1.92 de	31.11±0.96 de	28.14667							
	Black Cumin	36.67±5.77 cde	46.67±5.77 d	46.67±5.77 cd	43.33	21.11±0.96 ef	27.67±1.73 de	31.11±0.96 de	26.63							
	Mancozeb	20.00±0.00 f	43.33±5.77 de	43.33±5.77 cde	35.55	23.89±0.96 de	26.11±0.96 e	28.89±1.92 ef	26.29667							
	Only fruit	63.33±5.77 b	73.33±5.77 c	86.67±5.77 b	74.44	27.22±0.95 d	42.78±0.95 c	65.56±0.96 b	45.18667							
	Only <i>C. musae</i>	96.67±5.77 a	100.00±0.00 a	100.00±0.00 a	98.89	57.22±0.95 a	66.03±0.57 a	76.11±0.96 a	66.45333							
	Neem	40.00±0.00 cd	40.00±0.00 def	43.33±5.77 cde	41.11	24.44±1.92 de	26.11±0.96 e	29.44±0.96 e	26.66333							
	Sesame	26.67±5.77 def	36.67±5.77 defg	36.67±5.77 efg	33.33	21.11±0.96 de	21.11±1.92 g	22.78±1.92 g	21.66667							
Chini Champa	Linseed	26.67±5.77 def	30.00±0.00 efg	33.33±5.77 efg	30	22.78±0.95 ef	25.56±1.93 ef	28.89±1.92 ef	25.74333							
	Eucalyptus	23.33±5.77 ef	23.33±5.77 g	23.33±5.77 h	23.33	21.11±0.96 ef	22.22±1.92 fg	25.44±0.76 fg	22.92333							
	Black Cumin	16.67±5.77 f	26.67±5.77 fg	30.00±0.00 fgh	24.44	21.11±0.96 ef	22.22±0.95 fg	23.89±0.96 g	22.40667							
	Mancozeb	16.67±5.77 f	23.33±5.77 g	26.67±5.77 gh	22.22	19.44±0.96 f	20.56±1.93 g	22.22±0.95 g	20.74							
	Only fruit	66.67±5.77 b	76.67±5.77 bc	90.00±0.00 ab	77.78	32.78±1.92 c	43.89±0.96 bc	54.44±1.93 c	43.70333							
	Only <i>C. musae</i>	83.33±5.77 a	90.00±0.00 ab	93.33±5.77 ab	88.88	38.89±0.96 b	47.22±1.92 b	67.78±1.92 b	51.29667							

Means followed by the same letter (s) are not significantly different at 5% level.

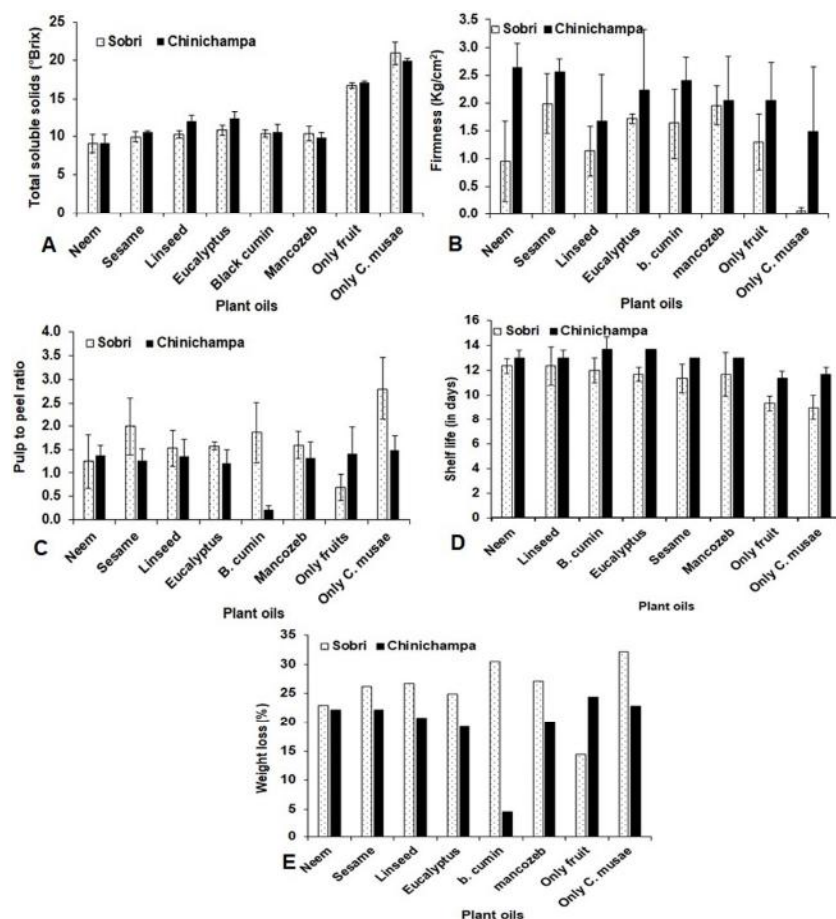


Fig. 2. Effects of plant oils in Sobri and Chini Champa banana fruit. A: Total soluble solid, B: Firmness, C: Pulp to peel ratio, D: Shelf life and E: Physiological weight loss.

The agar well diffusion method revealed the potentiality of the selected plant oils, especially eucalyptus in the inhibition of the mycelial growth of *C. musae*. In addition, all the plant oils extended the shelf life of bananas, and reduced the weight loss by mitigating the incidence and severity of anthracnose.

Acknowledgement

The authors are grateful to the Ministry of Science and Technology, Government of Bangladesh for providing the fund to accomplish the research work.

References

- Ahmed ZFR and Palta JP 2016. Postharvest dip treatment with natural lysophospholipid plus soy lecithin extended the shelf life of banana fruit. *Postharvest Biol. Tech.* **113**: 58-65.
- Balendres MA, Mendoza J and Dela Cueva F 2020. Characteristics of *Colletotrichum musae* PHBN0002 and the susceptibility of popular banana cultivars to postharvest anthracnose. *Indian Phytopathol.* **73**(1): 57-64.

- Bhutia D, Zhimo VY, Kole R and Saha J 2016. Antifungal activity of plant extracts against *Colletotrichum musae*, the post-harvest anthracnose pathogen of banana cv. Martaman. *Food Sci. Nutr.* **46**: 2-15.
- Campos-Requena VH, Rivas BL, Pérez MA, Figueroa CR, Figueroa NE and Sanfuentes EA 2017. Thermoplastic starch/clay nanocomposites loaded with essential oil constituents as packaging for strawberries - *In vivo* antimicrobial synergy over *Botrytis cinerea*. *Postharvest Biol. Technol.* **129**: 29-36.
- De Araújo AC, Toledo ED and de Oliveira Soares WR 2018. Produtos alternativos no controle de *Colletotrichum* spp. isolados de manga e banana. *Científic@-Multidiscip. J.* **5**: 104-112.
- Goswami SK, Singh V, Chakdar H and Choudhary P 2018. Harmful effects of fungicides-Current status. *Int. J. Agric. Environ. Biotech.* **11**: 1011-1019.
- Idris FM, Ibrahim AM and Forsido SF 2015. Essential oils to control *Colletotrichum musae* *in vitro* and *in vivo* on banana fruits. *Am. Eurasian. J. Agric. Environ. Sci.* **15**(3): 291-302.
- Jhalegar MD, Sharma RR and Singh D 2015. *In vitro* and *in vivo* activity of essential oils against major post-harvest pathogens of Kinnow (*Citrus nobilis* × *C. deliciosa*) mandarin. *J. Food Sci. Technol.* **52**(4): 2229-2237.
- Lundgren GA, Braga SDP, de Albuquerque TMR, de Oliveira KÁR, Tavares JF, Vieira WADS, Câmara MPS and de Souza EL 2022. Antifungal effects of *Conyza bonariensis* (L.) Cronquist essential oil against pathogenic *Colletotrichum musae* and its incorporation in gum arabic coating to reduce anthracnose development in banana during storage. *J. Appl. Microbiol.* **132**(1): 547-561.
- Mahmuda S, Khan S, Tabassum P and Sultana M 2018. Effects of neem leaf extract and hot water treatments on shelf life and quality of banana. *J. Bangladesh Agril. Univ.* **16**: 351-356.
- Mohammadi S and Aminifard MH 2012. Effect of essential oils on post-harvest decay and some quality factors of peach (*Prunus persica* var. Redhaven). *J. Biol. Environ. Sci.* **6**: 147-153.
- Okigbo RN and Emoghene AO 2003. Effects of leaf extracts of three plant species on *Mycosphaerella fijiensis* Morelet the causal organism of black sigatoka disease of banana (*Musa acuminata*). *Nigerian J. Plant Protection* **20**: 101-110.
- Perdichizzi S, Mascolo MG, Silingardi P, Morandi E, Rotondo F, Guerrini A, Prete L, Vaccari M and Colacci A 2014. Cancer-related genes transcriptionally induced by the fungicide penconazole. *Toxicol. In Vitro* **28**(1): 125-130.
- Ranasinghe LS, Jayawardena B and Abeywickrama K 2003. Use of waste generated from cinnamon bark oil (*Cinnamomum zeylanicum* Blume) extraction as a post-harvest treatment for Embul banana. *J. Food Agric. Environ.* **1**(2): 340-344.
- Sau S, Bhattacharjee P, Kundu P and Mandal D 2023. Banana In Tropical and Subtropical Fruit Crops, Apple Academic Press, pp. 1-62.
- Sellamuthu PS, Sivakumar D, Soundy P and Korsten L 2013. Essential oil vapors suppress the development of anthracnose and enhance defense related and antioxidant enzyme activities in avocado fruit. *Postharvest Biol. Technol.* **81**: 66-72.
- Şener S, Türemiş NF and Tanır F 2020. Agrochemical usage for sustainable fruit production and human health. In: *Agrochemicals Detection, Treatment and Remediation*, Butterworth-Heinemann, pp. 291-305.
- Sivakumar D, Hewarathgamagae NK, Wijeratnam RSW, Wijesundera RLC and Abeysekere M 2002. Combined effect of generally regarded as safe (GRAS) compounds and *Trichoderma harzianum* on the control of postharvest diseases of rambutan. *Phytoparasitica* **30**: 43-51.
- Valgas C, Souza SMD, Smânia EF and Smânia Jr A 2007. Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.* **38**(2): 369-380.
- Vilaplana R, Pazmino L and Valencia-Chamorro S 2018. Control of anthracnose, caused by *Colletotrichum musae*, on postharvest organic banana by thyme oil. *Postharvest Biol. Technol.* **138**: 56-63.