ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

In Vitro Evaluation of Effect of Different Essential Oils on the Management of Postharvest Fruit Rot of Banana (*Musa Paradisiaca*) caused by *Colletotrichum* spp.

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Article Information

Received: 27 July 2021
Revised version received: 18 September 2021
Accepted: 21 September 2021
Published: 29 September 2021

Cite this article as:
P. Gairhe et al. (2021) Int. J. Appl. Sci. Biotechnol. Vol 9(3): 187-192. DOI: 10.3126/ijasbt.v9i3.38614

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Abstract

An experiment was conducted to evaluate the in vitro efficacy of different essential oils on the management of postharvest fruit rot of banana caused by *Colletotrichum* spp. It was carried out in completely randomized design (CRD) with three replications and six treatments at Nepal Plant Diseases and Agro Associates (NPDA). Each of the treatments: cinnamon oil (*Cinnamomum verum*), mustard oil (*Brassica oleracea*), castor oil (*Ricinus communis*), neem oil (*Azadirachta indica*), coconut oil (*Cocos nucifera*) and control were used at concentrations 200 ppm, 500 ppm and 1000 ppm. The radial growth of mycelium (mm) and percent growth inhibition (%) of *Colletotrichum* spp. was recorded. The decrease in the radial growth and increase in the percent growth inhibition was found in all the treatments except control as their concentration increased, in which the lowest radial growth and the highest percent growth inhibition was found at 1000 ppm concentration. At 1000 ppm concentration, cinnamon oil shows the lowest radial growth and the highest percent growth inhibition (1.67mm and 98.15%) followed by mustard oil (54.00mm and 40.00%), neem oil (55.17mm and 38.70%), castor oil (55.83mm and 37.96%), coconut oil (61.17mm and 32.04%) and control (90mm and 0.00%) respectively. Thus, cinnamon oil is considered as a good essential oil in regards to the management of postharvest disease of banana.

Keywords: castor oil; cinnamon oil; coconut oil; *Colletotrichum* spp.; mustard oil; neem oil.

Introduction

Banana is a popular fruit crop grown widely in tropical countries and has a high consumer demand worldwide due to its flavor, texture, nutritional value and eating convenience. The wide consumption of banana is due to its sensory characteristics and the caloric contribution of vitamins and minerals, mainly potassium (Idris *et al*., 2015). India, China, Indonesia, Brazil, Ecuador, Philippines, Guatemala, Angola, Tanzania and Colombia are major banana producing countries in the world. It is cultivated on an area of 51, 58, 582 ha with an average production of 11, 67, 81, 658 tonnes in the world (FAOSTAT, 2019). Postharvest losses are often more severe due to inadequate storage and transportation facilities in developing countries (Rashad *et al*., 2011). Major economic part of the banana

plant is the fruit, suffers from many postharvest diseases. This disease has considerable influence on different aspects of cultivation, nutritive value, harvesting, transit and transhipment, storage of fruits. During postharvest handling it is estimated that 20 to 25 per cent of harvested fruits are decayed by pathogens even in developed countries (Zhu & Ma, 2007). The microorganisms associated with postharvest spoilage of fruits have engaged the attention of mycologists for many years (Okigbo, 2001). Anthracnose in banana fruit is caused by *Colletotrichum musae* (Berk and Curt) and is confined to mature fruits (Waller, 1992). The phytopathogenic fungus *Colletotrichum musae* is responsible for highly destructive anthracnose fruit rot in many Sri Lankan cultivars of banana that cause high postharvest losses (Perera *et al*., 1999). Infection on banana usually starts during the month after flowering when conidia contaminate the banana fruits (Chillet *et al*., 2000). It is a widely distributed and causes significant damage to crops in tropical, subtropical and temperate regions.

In the morphological characters of *C. gloeosporioidesas*, the acervuli were usually setose or glabrous, round to elongate or irregular in shape. The microscopic view of *Colletotrichum* spp. with setae is shown in Fig. 1. Numerous acervuli having hyaline, one celled oblong to cylindrical conidia measured 13-17 × 5-7 µm on rotted fruits of pomegranate by *C. gloeosporioides* (Singh & Chohan, 1972).

The use of different kinds of inorganic chemicals are increasing in the present situation due to their easy availability, cheapness and instant result in the management of postharvest disease and pests. But these types of chemicals have negative impact in the environment and health of the living organism. The use of the different botanical extracts, their oil, pastes, etc. can be used in the substitution of the inorganic chemical in order to control the various postharvest diseases and pests. The use botanical extracts and essential oils are eco-friendly and safe to the health of living organism.

Materials and Methodology

Experimental site and design

The experiment was carried out in completely randomized design (CRD) with three replications and six treatments at Nepal Plant Diseases and Agro Associates (NPDA), Balaju, Kathmandu, Nepal. The name of treatments used are listed in Table 1.

Isolation and identification of pathogen

Protocol of Tuite (1969) was adopted for identification of pathogens. Rotted diseased fruits of banana collected from various locations were subjected to tissue isolation. For the purpose, the diseased specimens were washed gently in running tap water; blot dried and cut with sharp sterilized blade into small bits (5 mm), keeping half healthy and half diseased portion intact. Such leaf and fruit bits were surface sterilized with 0.2% aqueous solution of sodium hypochlorite in glass Petriplates for two minutes and washed by giving three sequential changes with sterile distilled water to remove traces of sodium hypochlorite. Those sample pieces were then blot dried and inoculated aseptically on autoclaved and cooled potato dextrose agar (PDA) medium in Petriplates under laminar-air-flow cabinet and incubated in incubator at 25±2°C temperature. Within a week of incubation, profuse fungal mycelial growth was obtained. Applying hyphal-tip technique, the test isolates of the test pathogen was aseptically sub-cultured, purified and maintained the pure cultures separately on agar slant tubes in refrigerator for further studies. On the basis of symptomatology, cultural, morphological and microscopic characters, the pathogen were identified as *Colletotrichum* spp.

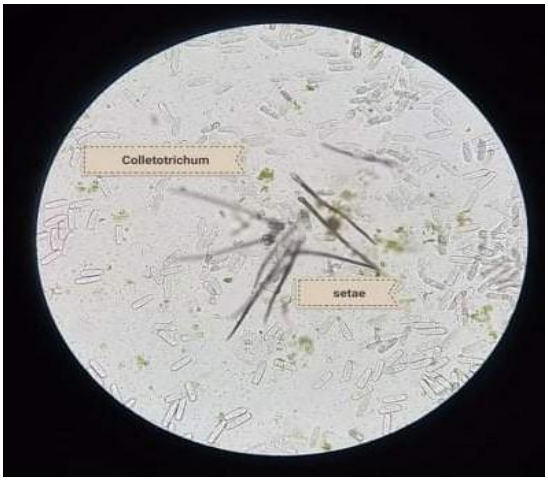

Figure 1: Microscopic view of *Colletotrichum* spp. with setae.

Table 1: List of treatments

Treatment number	Treatments	Extracted From
T1	Cinnamon oil (essential)	*Cinnamomum verum*
T2	Mustard oil	*Brassica oleracea*
T3	Castor oil	*Ricinus communis*
T4	Neem oil	*Azadirachta indica*
T5	Coconut oil	*Cocos nucifera*
T6	Control	-

Note: Above oil and essential oils reported to possess antimicrobial and therapeutic properties and they were collected from local market and Herbs Products and Processing Co. Ltd, Koteshwor, Kathmandu.

In vitro evaluation of essential oils

Essential oils were evaluated (each @ 200 ppm, 500 ppm and 1000 ppm) in vitro against the test pathogen applying poisoned food technique using PDA as a base medium. Twenty ml of PDA was poured into sterilized petriplate of 9 cm diameter (inner) and measured amount of essential oil after sterilizing in water bath for 20 minutes, was added in each treatment, allowed them to mix homogeneously and to be solidified. Fungal disks of 5 mm in diameter from 7 days old pure culture was placed in the center of the petriplate containing medium under aseptic condition, incubated at 25◦C ± 2◦C for 7 days.

Observation

Observations on radial mycelial growth were recorded in all the replicated treatments. Percent inhibition of the growth was calculated by using the formula comparing with the control. The data obtained was averaged and analysed statistically (Vincent, 1947).

Percent inhibition (I) = $\frac{C-T}{C} X100$

Where,

C = Diametric growth in control (mm)

T = Diametric growth in treatment (mm)

Data collection and analysis

The recorded data were entered into Microsoft Excel and analysis of data was carried out using R Studio software. The mean comparison between treatments during analysis was carried out by Duncan's multiple range test (DMRT).

Result and Discussion

The significant difference was found among the treatments at 200 ppm, 500 ppm and 1000 ppm. The decrease in the radial growth and increase in the percent growth inhibition of test isolate of *Colletotrichum* spp. was found in all the treatments except in control (T6), with increasing in their concentration. On 7th day after inoculation, the lowest radial growth and the highest percent growth inhibition were found at 1000 ppm concentration. At 1000 ppm concentration, cinnamon oil shows the lowest radial growth and the highest percent growth inhibition (1.67mm and 98.15%) followed by mustard oil (54.00mm and 40.00%), neem oil (55.17 mm and 38.70%), castor oil (55.83mm and 37.96%), coconut oil (61.17mm and 32.04%) and control (90mm and 0.00%) respectively (Table 2).

Thus, these results clearly indicated that, among the five essential oils tested, the most effective was cinnamon essential oil followed by mustard oil, neem oil, castor oil and coconut oil against the test isolate of *Colletotrichum* spp. (Figure 2)

Table 2: In vitro evaluation of effect of different oils at their various concentrations against *Colletotrichum* spp. in radial growth and percent growth inhibition at 7th Day After Inoculation (DAI).

Treatment	Radial growth (mm) and Percent growth inhibition (%)		
	200 ppm	**500 ppm**	**1000 ppm**
Cinnamon oil(T1)	23.17^{d} (74.26%)	13.00^{e} (85.56%)	1.67^{d} (98.15%)
Mustard oil (T2)	58.83^{c} (34.63%)	56.50^{d} (37.22%)	54.00^{c} (40.00%)
Castor oil (T3)	67.50^{b} (25.19%)	64.50^{b} (28.33%)	55.83^{c} (37.96%)
Neem oil (T4)	66.00^{b} (26.67%)	61.50^{c} (31.67%)	55.17^{c} (38.70%)
Coconut oil (T5)	66.17^{b} (26.48%)	63.50^{bc} (29.44%)	61.17^{b} (32.04%)
Control (T6)	90.00^{a} (0.00%)	90.00^{a} (0.00%)	90.00^{a} (00.00%)
LSD (0.05)	1.62	2.18	2.87
GM	61.9	58.2	53.0
SEM	4.82	5.55	6.34
CV (%)	1.47	2.11	3.04

Note: LSD: Least Significant Difference, GM: Grand Mean, SEM= Standard Error of Mean, CV: Coefficient of Variation; Means followed by the same letter in a column are not significantly different by DMRT at 5% level of significance.

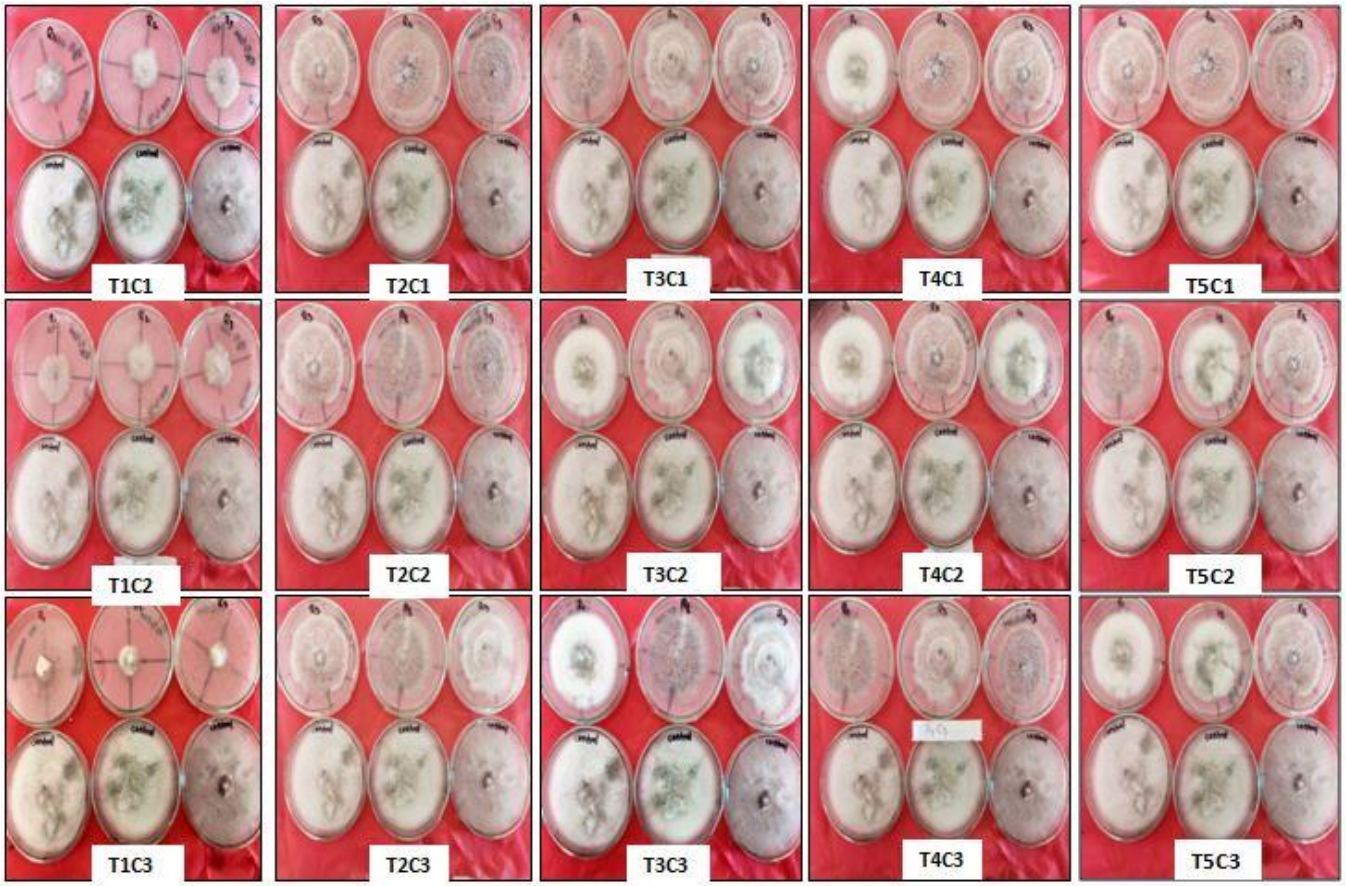

Figure 2: In vitro evaluation of effect of different essential oils against *Colletotrichum* spp.

The cinnamon oil was reported to cause maximum mycelial growth inhibition in many *Colletotrichum* spp. The present results observed are in conformity to the findings of several earlier workers Ranasinghe *et al*. (2002), Singh and Tripathi (2015) and Sefu *et al*. (2015). Maqbool *et al*.(2010) reported the suppressing of mycelial growth and conidial germination inhibition (83.3%) when cinnamon oil is applied at concentration 0.4%. Kowalska *et al.* (2020) also reported the antifungal property of cinnamon water filtrates against *Botrytis cinerea* and inhibit the mycelium growth (81.4%) at 1% concentration. The cinnamon oil results 100% antifungal activities against different postharvest pathogens *Aspergillus niger*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae* and *Phomopsis viticola* (Sukatta *et al.*, 2008). The important constituents present in cinnamon essential oil is Cinnamaldehyde, which acts as antifungal agent and is effective against the different fungi. (Copping, 2004; Wang & Chen, 2005). Cheng *et al.* (2008) reported the antifungal activity of cinnamaldehyde and eugenol congeners against wood-rot fungi.

Similarly, mustard oil, neem oil, castor oil and coconut oil also shows the inhibition of mycelium growth of *Colletotrichum* spp. respectively, which was similar to the result reported by Burgute *et al.*, (2019) *Colletotrichum gloeosporioides*. The seeds of mustard contain allyl isothiocyanate which acts as antifungal agent and inhibits the growth of fungus (Chung *et al.*, 2002).The effect of neem oil against the growth of *Colletotrichum* spp. was also reported by Musakhan *et al.*(2017) and Vi & Ao (2017). Propyl disulfide that was present in Neem has ability to inhibit the growth of *Colletotrichum* spp.(Khan *et al.* 2021).

Conclusion

Colletotrichum spp. is the most predominant fungus of banana responsible for postharvest infection that causes considerable loss in storage. From the in vitro experiment, it is concluded that different essential oils can be used on banana fruits as alternatives to synthetic fungicides for reducing postharvest loss caused by *Colletotrichum* spp. and enhancing storage life considerably. However, for the most effective method of control, cinnamon oil is preferred.

Acknowledgement

Authors heartfelt regards to Nepal Plant Disease and Agro Associates (NPDA) executive and former senior scientist of Nepal Agricultural Research Council (NARC), Ram Devi Timila, Ph.D. and Prof. Sundar Man Shrestha, Ph.D., of Department of Plant Pathology, Agriculture and Forestry University (AFU) for their constant guidance and valuable suggestion as well as to entire Nepal Plant Disease and Agro Associates (NPDA), Kathmandu for facilitating technical, laboratory and partial fund support in research.

Conflict of Interest

There is no any conflict of interest among the authors for the present study.

Authors' Contribution

Pramod Gairhe: Experimental design, conduction, data analysis, interpretation and paper writing.
Sandesh Bhandari: Data recording, data analysis and interpretation.
Hom Prasad Sitaula: Data recording, data analysis and interpretation.
Beautina Karki: Data recording, data analysis and interpretation.
Hira Kaji Manandhar: Supervision, suggestion and recommendation during experiment.

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