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EVALUATION OF ANTIFUNGAL EFFECT OF *Croton aromaticus* ON STORAGE LIFE EXTENSION OF BANANA

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ABSTRACT

Effect of *Croton aromaticus* aqueous, hot water and ethanolic leaf extracts alone and in combination with alum against banana crown rot causing fungal pathogens were investigated using 96 well plate bioassay and liquid bioassay *in vitro*. According to the results of 96 well plate assay, *C. aromaticus* aqueous extract successfully inhibited conidial germination of *C. musae* up to 96.9%. Percentage germination inhibition of *F. proliferatum* is less than that of *C. musae*. Hot water extract expressed higher conidial germination inhibition of *F. proliferatum*. Conidial germination of *C. musae* was 100% inhibited at the concentration of 600 (%w/v) of ethanoic extract and at the same concentration *F. proliferatum* was inhibited by 90.4 %. Alum was fungicidal against *C. musae* and *L. theobromae*. *C. aromaticus* leaf extract in combination with alum controlled *F. proliferatum* more effectively compared to leaf extract alone. During liquid bioassay, ethanolic leaf extract totally inhibited the mycelial growth of test pathogens at the concentration of 800 (%w/v). Among three tested extracts, ethanolic extract was most effective in inhibiting both spore germination and mycelial growth of crown rot causing fungal pathogens.

Keywords*:* crown rot, liquid bioassay, 96 well plate bioassay, alum

1. INTRODUCTION

The most common and serious postharvest disease that affect banana is Crown Rot (CR), caused respectively by *Colletotrichum musae, Lasiodiplodia theobromae* and certain other pathogenic species. These pathogens are strict wound pathogens that can infect the fruit in the orchard, in the

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packinghouse or during subsequent handling and storage. Fungal inocula are practically always present on the surface of fruit during the season and after harvest it can build up to high levels unless appropriate packing house sanitation measures are adopted. Contamination and infection by CR pathogenic fungi occur during mechanical injury to the fruits during dehanding process as well as unsanitary handling practices in packhouses (Talibi *et al*., 2014). During CR, infected tissues turn brown and the dry rot advance into the finger stalks and cause the fingers to drop. Crown rot decay is also more prevalent as fruit maturity increases and at favourable temperatures and humidity (Abd-Alla *et al*., 2014).

Crown rot of wheat in Australia and many other countries is a destructive disease caused by species of *Fusarium*. The fungal pathogens *Fusarium graminearum* and *F. pseudograminearum* cause necrosis and dry rot of the crown, basal stem and root tissue in wheat (Chakraborty *et al*., 2010). Besides *Colletotrichum, Lasiodiplodia* and *Fusarium* species, *Verticillium* is also reported as a pathogen responsible for crown rot in banana in cooler climates (Indrakeerthi and Adikaram 2011).

Synthetic fungicides are the primary means of controlling postharvest diseases. Prolonged usage often poses health problems however, modern society is becoming more health-conscious. The excessive use of these chemicals for controlling crown rot fungi especially at postharvest level on fruit has been counterproductive, causing damage to the environment and humans which increase demands to reduce the use of these chemicals that accumulate in fruits. Continuous use of these fungicides has resulted in the appearance of isolates of fungi with multiple fungicide resistance. This further complicates the management of the disease. In addition, these fungicides are not effective against all pathogens which cause crown rot disease complex (Talibi *et al*., 2014; Al-Samarrai *et al*., 2012; Abd-Alla*et al*., 2014). No fungicides are normally applied on Embul banana variety and other varieties in plantations in many developing countries including Sri Lanka. Therefore, fungal pathogens and their reproductive structures such as conidia remain at all times on banana plant surfaces which act as a source of contamination at the point of harvesting, dehanding and preparation for local consumption and export.

Benomyl fungicide has been used on bananas for effective control of post-harvest diseases during preparation for export in several countries, including Sri Lanka (Ranasinghe *et al*., 2005; Lassois *et al*., 2014). However, benomyl is prohibited to be used on perishable commodities due to its potential oncogenic risk (Wilson *et al*., 1992)

Commercially, 1% solution of alum (sodium aluminium sulphate) in a float tank is used to arrest the flow of latex from banana crowns (Wijerathnam, 2002). Preliminary *in vitro* research conducted at the University of Kelaniya, using banana rot pathogens, have indicated that 1% w/v

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solution of alum prevents the mycelial growth of all CR pathogens of banana and stem end rot pathogens of papaya (Abeywickrama *et al*., 2012).

Plant extracts of higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties. Several studies have been focused on screening of plant extracts for new antifungal compounds that can be used to control postharvest diseases (Talibi *et al*., 2014). Pong-pong and Neem have displayed positive effects on the inhibition of postharvest fungi as alternatives to fungicides through cytotoxicity screening using the Brine-shrimp lethality test (BST) and *in vitro* screening on mycelial growth of *Penicillium digitatum* (Al-Samarrai *et al*., 2012). Kuri *et al* (2011) have investigated the potential of certain botanical extracts against important seed-borne pathogens associated with brinjal seeds. Their findings indicate that aqueous leaf extracts of *Azadirachta indica*, *Calotropic procera*, *Clerodendron* spp., *Luffa cylidrica, Croton spasriflorous*, *Moringa oleifera* and seed of *Lantana camara*dis played some potentiality to inhibit the growth of some seed borne fungi such as *Phomopsis vexans*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus nigar*, *Curvularia lunat and Penicillium* spp.

Essential oils are natural, volatile and complex compounds known for their antimicrobial, antioxidant and medicinal properties. The volatility, ephemeral nature and biodegradability of essential oil compounds may be especially advantageous for treatment of postharvest diseases because only low levels of residues can be expected (Talibi *et al*., 2014). Essential oils of *Cymbopogon citratus* and *Cinnamomum zeylanicum* have been previously reported as potential fumigants and contact toxicants against *Sitophylus oryzae* which commonly inhabits stored rice (Paranagama *et al*., 2004). Antifungal activity of the essential oils have considered as a potential alternative to the synthetic fungicides for the control of postharvest pathogens in banana. *Cymbopogon nardus* and *Ocimum basilicum* oils have previously displayed fungistatic and fungicidal activity against *C. musae* and *F. proliferatum* isolated from Embul variety banana during an *in vitro* liquid bioassay (Anthony *et al*., 2004).

Croton genus belonging to Euphorbiaceae is widespread in tropical regions. Some species are aromatic due to the possession of volatile oils and rich in bioactive compounds such as diterpenes and alkaloids (Salatino *et al*., 2007). Most isolated compounds in *Croton zambesicus* have reported antimicrobial activity due to presence of diterpenoids, quinines, triterpenoids and flavonoids (Mohamed *et al*., 2009). During a previous research, leaf extracts of *Croton aromaticus* was reported as having antifungal activity against *Cladosporium cladosporioides* (Bandara *etal*., 1987). Recently, ethanolic leaf extract of *C. aromaticus* significantly inhibited the mycelial growth and spore germination of postharvest fungal pathogens of *Colletotrichum musae*, *Alternaria alternata*, *Pestaloptiopsis mangiferae* and *Colletotrichum gleosporioides* isolated from a selected group of tropical fruits (Wijesundara *et al*., 2016).

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The objective of current research is to evaluate the antifungal activity of *C. aromaticus* fresh leaf extract, hot water and ethanolic extracts against CR causing fungal pathogens of banana using 96 well plate bioassay and liquid bioassay *in vitro*. The combined effect of alum (sodium aluminium sulphate) and *C. aromaticus* leaf extract on mycelial growth of crown rot pathogens were also assessed using a liquid bioassay.

2.MATERIALS AND METHODS

In vitro bioassay

2.1 Collection of *Croton aromaticus* leaf samples

Branches containing leaves of *Croton aromaticus* were collected separately from Malabe and Kaduwela areas in Colombo district in Sri Lanka.

2.2 Preparation of leaf extract

2.2.1 Aqueous extract- Fresh leaf samples of *C. aromaticus* were thoroughly washed with tap water and then rinsed with sterile distilled water. Fresh leaves (1 g) were then crushed using a sterilized mortar and pestle with 10 mL of sterile distilled water. Resulting crude extract was filtered through sterilized Whatmann No. 1 filter paper, and the extract was subsequently filter sterilized (Pawar, 2011).

2.2.2 Hot water extract-Fresh leaf samples were crushed as above and sample extract was boiled in a water bath for 6-7 hours at 45 ^{0}C. Extract was filtered after 18 hours and filtrate was taken as the hot water extract.

2.2.3 Solvent extract-Fresh leaf samples were thoroughly washed using tap water, air - dried and powdered using a grinder. The crude ethanolic extract was prepared from dried powdered leaf material (100 g), macerated with absolute alcohol (ethanol) (500 ml)for 72 hours at room temperature (28±2 ^{0}C). Extract was then filtered through Whatmann No.1 filter paper. Filtrate was concentrated and evaporated to dryness under vacuum at 40^{0}C using a rotary evaporator **(**Cole-Parmer Rotary Evaporator System; Diagonal, 230 VAC, U.S.A**).** The crude extract was then stored at 4^{0}C until further use (Britto *et al*., 2012).

2.3 Preparation of concentration series of *Croton aromaticus* leaf extracts

2.3.1 Aqueous extract / Hot water extract- aliquots of 100 µL, 200 µL upto 900 µL of aqueous and hot water crude extract from *Croton aromaticus* were mixed with sterile distilled water to

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obtain 10% to 90% (v/v) concentrations of crude extract. Undiluted aqueous/ hot water extract was also tested.

2.3.2 Solvent extract-Aliquots of 100.0 mg, 200.0 mg upto 700.0 mg of ethanolic crude extract of *Croton aromaticus* were dissolved in 1.0 ml of sterile 95% ethanol to obtain dilutions of 100.0 mg/ml, 200.0 mg/ml up to 700.0 mg/ml concentrations.

2.4 Antifungal assay for spore germination inhibition of spore forming crown rot pathogens (microtitre plate technique)

Conidia were harvested from 10- day old pure cultures of spore forming CR fungal pathogens - *Colletotrichum musae* and *Fusarium proliferatum* separately by adding 10 ml of sterile distilled water into the PDA (Oxoid, Basingstoke, UK) petri dishes and gently rubbing the sporulating mycelial mat with a sterile spreader. These conidial suspensions were filtered through a sterilized muslin cloth to remove mycelial fragments. The concentration of each conidial suspension was adjusted to 10^5 conidia/ml by using a hemocytometer. (C.A Hausser and Sons, U.S.A). The influence of different concentrations of aqueous, hot water and ethanolic crude extract of *Croton aromaticus* on the spore germination of postharvest fungal pathogens was determined using microtitre plate method. One hundred µl of each concentration of leaf extract of *C. aromaticus* were placed in separate rows in the same 96 - well plate. Sterile distilled water was used as the control. Subsequently, 200µl of spore suspension of each postharvest fungal pathogen was inoculated into 96-well plates. Content of each well was mixed thoroughly with a micro pipette (Burt and Reinders, 2003). Each 96-well plate was aseptically transferred to a zip-lock bag and incubated at room temperature (28 ± 2^0C) for 48 hours to facilitate germination of conidia. After 48 hours, a drop of cotton blue stain in lactophenol was added to each well to stop conidial germination and to stain conidia. Two drops (0.05 mL each) from a well from each concentration were placed on a clean microscope slide using a micropipette. Four replicate slides from each concentration from different wells were prepared and observed under high power magnification (400×) of binocular compound microscope. One hundred randomly selected conidia were observed and the number of germinated conidia were counted. A conidium was considered as germinated if the length of the germ tube was at least half the length of the conidium (Herath and Abeywickrama, 2008). The average number of germinated conidia were calculated in four replicate slides per concentration and mean number of germinated conidia of each concentration of aqueous, hot water and ethanolic extract were calculated. The percentage inhibition of conidial germination was calculated with respect to control using the formula described by Jayasingha and Wijesundera, (1995). The mean values and standard errors were calculated.

$$\text{Percent inhibition} = \frac{T - C}{100 - C}$$

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Where C is the percent inhibition of germination in control and T is the percent inhibition of germination in treatment.

2.5 Liquid bioassay to screen the antifungal efficacy of leaf extracts of *C. aromaticus* and alum

A semi synthetic liquid medium – SMKY was prepared by dissolving yeast extract (7 g), sucrose (20 g), $MgSO_4.7H_2O$ (0.5 g), KNO_3 (1.5 g) in 1 liter of distilled water. Conical flasks (100 ml) containing 50 ml of semi synthetic liquid medium was autoclaved for 20 minutes at 1.03 $Kgcm^{-2}$ and 121^0C. Concentration series were prepared from aqueous, hot water and ethanolic extracts of *C. aromaticus* as described previously. Five hundred (500) µl of each concentration was added to each conical flask. Each assay flask was then inoculated with a 5- mm fungal disc cut from the periphery of a 7- day-old culture of CR pathogens of banana (*C. musae, F. proliferatum* or *L. theobromae)* using a sterilized cork borer. Contents were mixed thoroughly by placing the flasks on a shaker (Reciprocal water bath shaker, New Brunswick Scientific, Model R76) for 5 minutes and incubated at room temperature (28 ± 2^0C) for 7 days. Control (without leaf extract) was included for comparison purposes. Alum at 2% (w/v) and alum + *C. aromaticus* extracts of aqueous, hot water and ethanolic were similarly tested for their possible fungicidal effect.Five replicates of each treatment and control were arranged according to a completely randomized design (CRD) on a laboratory bench. After 7 days, the appearance of fungal mat was observed and treated samples were compared with controls to determine the Minimum Inhibitory Concentration (MIC) which was needed to inhibit each fungus completely.Where the growth was completely inhibited by plant extracts, fungal discs were transferred to fresh PDA plates without plant extracts to test for the survival of the fungus. After 7 days, the mycelium was recovered on pre- weighed filter paper (Whatmann No. 1: 5.5 cm diameter), washed three times with sterile distilled water and placed in a hot air oven (Memmert, UM 600) at 105^0C overnight. The % inhibition of mycelium was determined (Baratta *et al.,*1998).

$$\% \text{ inhibition} = \frac{(C-I) - (T-I) \times 100}{C-I}$$

Where, *T* = mean weight of mycelium of test flasks, C = mean weight of mycelium of control flasks, I = mean weight of initial inoculum.

After a 7 day incubation period, fungal discs indicating no growth were flooded with a freshly prepared solution of 2,3,5 Triphenyl Tetrazolium Chloride (TTC)(1%) for 30 minutes in order to confirm the death of fungal cells. Minimum Lethal Concentration (MLC) of each type of leaf extract that was needed to kill each fungus completely was noted (Anthony *et al*., 2004).

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3.RESULTS AND DISCUSSION

3.1 Antifungal assay for spore germination inhibition of spore forming Crown rot fungal pathogens (microtitre plate techniques)

Aqueous *C. aromaticus* leaf extract with 10% (v/v) of concentration, resulted in 46.9% of conidial germination inhibition of *C. musae*. Increasing the concentrations of *C. aromaticus* aqueous extracts resulted in elevated levels of germination inhibition. Fifty percent (v/v) extract resulted in 84.6% of conidial germination inhibition, whereas 80% extract successfully inhibited conidial germination up to 96.9% (figure 1, table 1).

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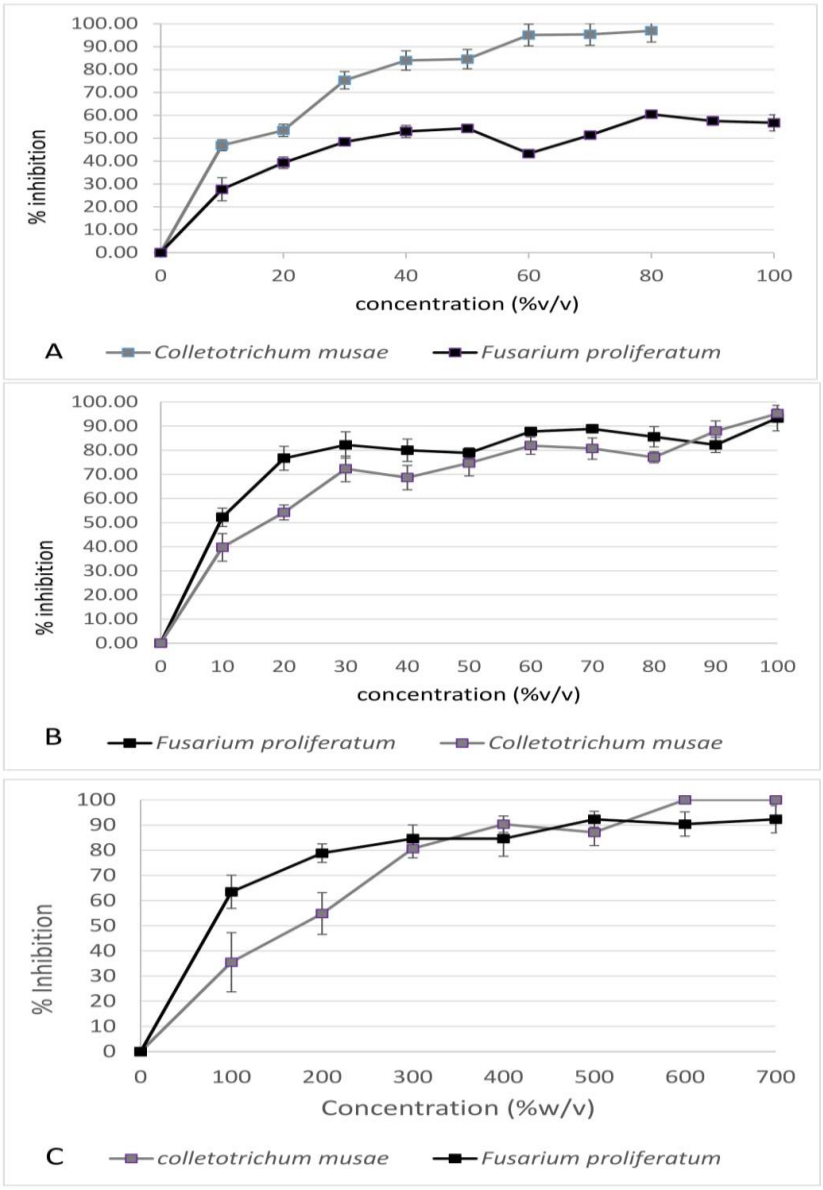

Figure 01: The inhibitory effect of *Croton aromaticus* aqueous (A), hot water (B)and ethanolic (C)leaf extract on conidial germination of *Colletotrichum musae* and *Fusarium proliferatum*. Each data point represents the mean of 4 replicates ± standard error.

When percentage conidial germination inhibition of *F. proliferatum* was assessed using aqueous leaf extract, a fairly linear increase in response was observed up to 50% (v/v). The highest percentage germination inhibition of conidia of *F. proliferatum* (i.e. 60.5±1.48) was observed with 80% (v/v) concentration of extract. Conidial germination inhibition data of both *C. musae*

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and *F. proliferatum* were significantly different at different concentrations of treatment ($P< 0.05$) (table 1).

The effect of different concentrations of *C. aromaticus* hot water extract on conidial germination inhibition of *C. musae*is shown in table 1. Percentage germination inhibition increased with the increasing concentration of hot water extract except at 40% (v/v). A slight decrease of conidial germination inhibition was observed at 40% (v/v). Highest conidial germination inhibition (i.e. 95.2± 1.97) was achieved at the concentration of 100% (v/v) of hot water extract.

Table 1: Mean % inhibition of conidial germination of *Colletotrichum musae and Fusarium proliferatumin different concentration Croton aromaticus* aqueous leaf extract and hot water extract

Concentration (%v/v)	mean % inhibition			
	Aqueous extract		Hot water extract	
	C. musae	*F. proliferatum*	*C. musae*	*F. proliferatum*
0	0.0± 0.00^{a}	0.0±0.00^{a}	0.0± 0.00^{a}	0.0± 0.00^{a}
10	46.9±1.13^{b}	27.7±5.01^{b}	39.8± 5.74^{b}	52.2± 3.80^{b}
20	53.4±3.47^{b}	39.3±2.50^{b,c}	54.2± 3.11^{b,c}	76.7± 4.93^{c}
30	75.3±2.08^{c}	48.4±1.58^{c,d,e}	72.3± 5.34^{c,d}	82.2± 5.44^{c}
40	83.9±1.33^{d}	53.0±2.61^{d,e}	68.7± 5.02^{c,d}	80.0± 4.63^{c}
50	84.6±0.36^{d}	54.3±1.55^{d,e}	74.7± 5.34^{c,d,e}	78.9± 2.13^{c}
60	95.1±1.33^{e}	43.3±1.78^{c,d}	81.9± 3.61^{d,e}	87.8±1.11^{c}
70	95.4±0.59^{e}	51.3±1.54^{c,d,e}	80.7± 4.40^{d,e}	88.9± 1.28^{c}
80	96.9±0.36^{e}	60.5±1.48^{e}	77.1± 2.31^{d,e}	85.6± 4.21^{c}
90	—	57.5±1.89^{e}	88.0± 4.17^{d,e}	82.2± 3.14^{c}
100	—	56.7±3.52^{e}	95.2± 1.97^{e}	93.3± 5.29^{c}

Each data value represents the mean of 4 replicate countings ± standard error. Mean values sharing common letters in each column are not significantly different ($P> 0.05$) according to Tukey's multiple comparison test.

A significant increase of percentage conidial germination inhibition of *F. proliferatum* was observed with the hot water extract of *C. aromaticus* when compared to aqueous extract. Roughly fifty percentconidial germination inhibition was seen for the concentration of 10% (v/v) and it increased with increasing concentration of hot water extract upto 30% (v/v). Hot water extract at 100% v/v achieved 93.33% conidial germination inhibition. None of the hot water extracts tested were successful in complete inhibition (100% inhibition) of conidial germination of *C. musae* and *F. proliferatum* (One way ANOVA $P<0.05$).

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Conidial germination inhibition increased with increasing concentration of ethanolic leaf extracts of *C. aromaticus* when tested against *C. musae* and *F. proliferatum*. Concentration of ethanolic extract at 700 mg ml^{-1} indicated the highest percentage inhibition of conidia of *F. proliferatum* (i.e. 92.3±5.44). Conidial germination of *C. musae was completely inhibited at* 600 mg ml^{-1} and 700 mg ml^{-1}**.** Concentrations of ethanolic extract (table 2). None of the ethanolic extracts tested were successful in inhibiting conidial germination of *F. proliferatum* completely (One way ANOVA $P<0.05$)

Table 2: Mean % inhibition of conidial germination of *C. musae and F. proliferatumat different concentrations of Croton aromaticus* ethanolic leaf extract.

concentration (%w/v)	mean % inhibition	
	C. musae	*F. proliferatum*
0	0.0±0.00	0.0±0.00
100	35.5±11.78	63.5±6.57
200	54.8±8.33	78.8±3.68
300	80.6±3.72	84.6±5.44
400	90.3±3.23	84.6±7.02
500	87.1±5.27	92.3±3.14
600	100.0±0.00	90.4±4.84
700	100.0±0.00	92.3±5.44

Each data value represents the mean of 4 replicates ± standard error.

3.2 Liquid bioassay

The inhibitory effect of *Croton aromaticus* leaf extracts on pathogens increased with the increase in concentrations. Both aqueous and hot water leaf extracts were not successful in inhibiting the mycelial growth of *Fusarium proliferatum, Colletotrichum musae* and *Lasiodiplodia theobromae* completely.

Alum (1 % w/v) was fungicidal against only two of the test pathogens i.e. *C. musae* & *L. theobromae*. No mycelial growth was observed in the liquid medium which was treated with 1% alum. When the fungal discs were transferred to freshly prepared PDA plates and incubated at room temperature (28 ± 2 ^{0}C) for 7 days, mycelia did not revive. However, alum at 1 % w/v was

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not fungicidal against *F. proliferatum* .*F. proliferatum* grew in the form of a mat on surface of the liquid medium as in the control.

During the liquid bioassay, a dry weight of 0.21± 0.04 g of mycelia was obtained for the control treatment of *C. musae*. Negligible change in fungal growth was observed due to ethanol (0.20 ± 0.01 g) indicating ethanol alone does not have a major effect on the growth of test fungi. Ethanolic leaf extract of *C. aromaticus* completely inhibited the growth of *C. musae* at a concentration of 400 mg ml^{-1}(Table 03). When the fungal discs of *C. musae* were introduced into PDA plates, no mycelial growth was observed from 600 mg ml^{-1} and above concentrations**.** The Minimum Inhibitory Concentration value of *C. aromaticus* ethanolic extract against *F. proliferatum* was 800 mg ml^{-1} and this concentration was not strong enough to kill *F. proliferatum* completely. *C. aromaticus* ethanolic extract in combination with alum showed higher percentage of mycelial inhibition of *F. proliferatum* when compared to ethanolic extract alone.

Table 3: The minimum inhibitory concentrations (MIC) and minimum lethal concentration (MLC) of *C. aromaticus* ethanolic leaf extract on banana crown rot causing pathogens in liquid bioassay.

Test pathogen	MIC [a] mg ml^{-1}	MLC [b] mg ml^{-1}
C. musae	400	800
F. proliferatum	800	-
L. theobromae	800	-

[a]Zero weight of mycelia at **M**inimum **I**nhibitory **C**oncentration (MIC**)**

[b] Zero revival of fungi at **M**inimum **L**ethal **C**oncentration (MLC)

According to the results, aqueous leaf extract of *C. aromaticus* inhibited the mycelial growth of *F. proliferatum* to about 50% at the concentration of 100% and at the same concentration it achieved 70% of mycelial inhibition against *L. theobromae*. Aqueous extract in combination with alum could control the mycelial growth of *F. proliferatum* to a higher extent except at the concentration of 70% (v/v).

Results revealed that mycelial growth of *C. musae, F. proliferatum* and *L. theobromae* were inhibited by hot water extract at higher levels when compared to aqueous extract. Hot water

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extract in combination with alum expressed better inhibition of mycelial growth than hot water extract alone except at the concentration of 60% (v/v) (figure 3).

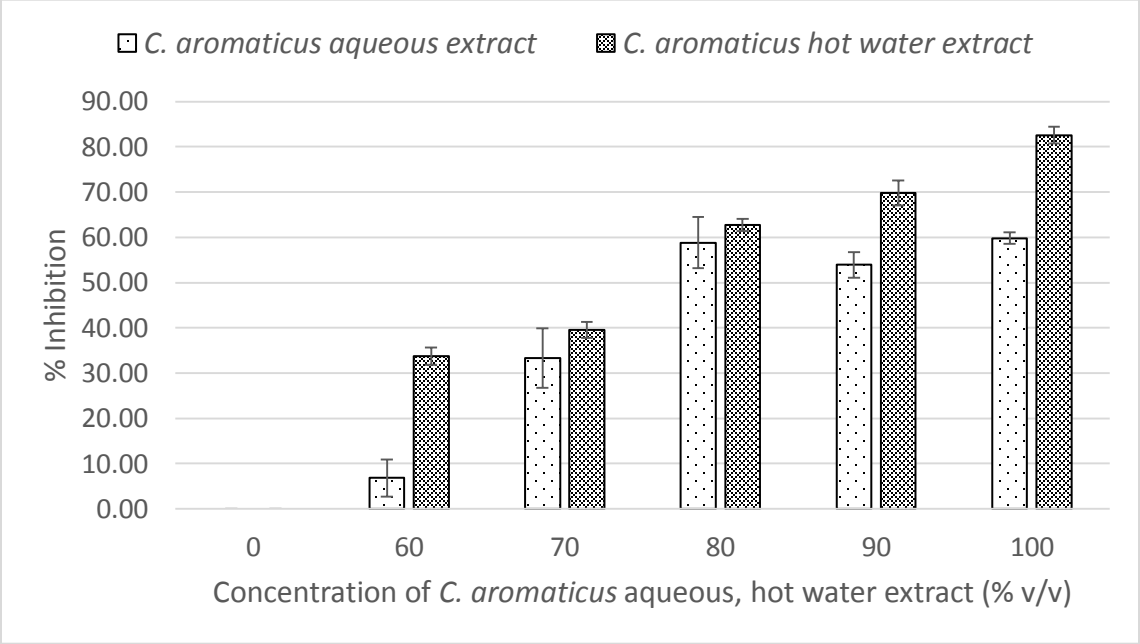

Figure 03: The inhibitory effect of *C. aromaticus* aqueous and hot water extract on the growth of *C. musae* in liquid bioassay. Each data point represents the mean of 5 replicates ± standard error.

Many plants have the ability to synthesize secondary metabolites, like phenols, terpinoids, glycosides, steroids, quinones, flavonoids, tannins and coumarins, which have toxic effects on microorganisms causing plant diseases (Enyiukwu *et al.,* 2013). Wijesundara *et al*., *(*2016*)* reported the presence of alkaloids, terpinoids, quinones, steroids and flavonoids, but not phenol, tannin and saponins in the ethanolic extracts of *C. aromaticus* leaves. According to Bandara *et al*., (1990) the roots of *C. aromaticus* yield β amyrin and **(-)-** hardwickiic acid and sesquiterpinoids as major secondary metabolites.

Wijesundara *et al*., *(*2016*)* reported the antifungal activity of ethanolic extract of *C. aromaticus* leaves, against mycelial growth and spore germination of postharvest fungal pathogens isolated from banana *(Colletotrichum musae, Lasiodiplodia theobromae)* papaya *(Colletotrichum gleosporioides, L.theobromae)* and mango *(Alternaria alternata, Pestalotiopsis mangiferae, L. theobromae)* using well diffusion assay. Significant inhibitory effects *(*$P < 0.05$*)* were exhibited by the extracts against *C. musae, P. mangiferae, A. alternata* and *C. gleosporioides* except *L. theobromae*. According to results of present study, no mycelial growth of *L. theobromae* was

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observed at 800 mg ml^{-1} concentration of ethanolic leaf extract of *C. aromaticus* however, none of tested concentrations of *C. aromaticus* ethanolic leaf extract were leathal for *L. theobromae*. Further, significantly higher inhibition of both mycelial growth and conidia germination of *C. musae* was observed in the presence of *C. aromaticus* leaf extract when compared to *F. proliferatum*.Findings of the present study clearly indicates the possibility of suppressing the growth of crown rot fungal pathogens, *C. musae, F. proliferatum* and *L. theobromae* using ethanolic leaf extract of *C. aromaticus* using liquid bioassay. Further, the results obtained from the present study highlights the possibility of reducing the growth of postharvest fungal pathogens to some extent using aqueous and hot water extracts of *C. aromaticus*. Hot water extract was more effective in inhibiting mycelial growth of test fungal pathogens when compared to aqueous extract. According to Ansari *et al*., (2010) an alcoholic solution could extract more melatonin from flowers of certain herbs than hot water. Mohammad., (2014) reported that the hot water extract of parsley leaves was more efficient on five genera of bacteria belong toenterobacteriaceae family compared to cold water extract. During the current research, ethanolic extract showed higher inhibition of both mycelia and spores of test pathogens than hot water extract of *C. aromaticus*. Inhibitory ability of aqueous extract was minimum when compared to ethanolic and hot water extracts.

CONCLUSION

Ethanolic leaf extract of *C. aromaticus* significantly inhibited the mycelial growth and spore germination of crown rot fungal pathogens *C. musae, F. proliferatum* and *L. theobromae*. *C. aromaticus*aqueous and hot water leaf extracts have the potential to control crown rot disease of banana by inhibiting spore germination and mycelial growth to some extent. Research data support the idea that *C. aromaticus* plant extracts could be a promising source of potential antifungal agent to control crown rot disease in banana.

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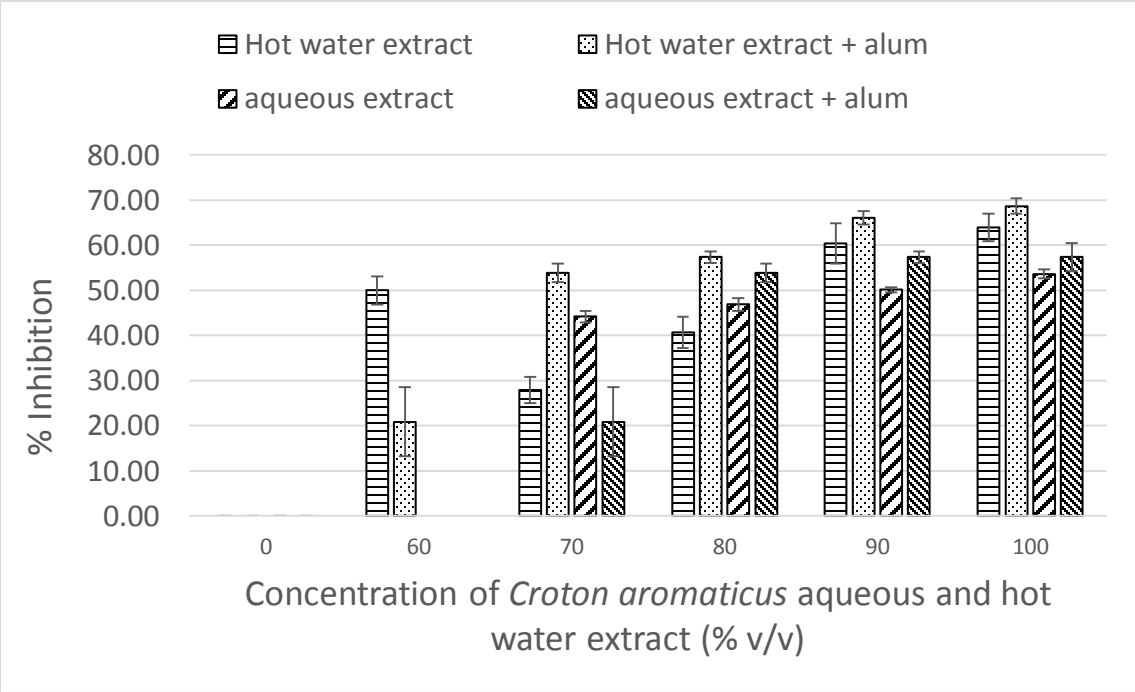

Figure 02: The inhibitory effect of *C. aromaticus* aqueous and hot water leaf extract on the growth of *Fusarium proliferatum* in liquid bioassay. Each data point represents the mean of 5 replicates ± standard error.

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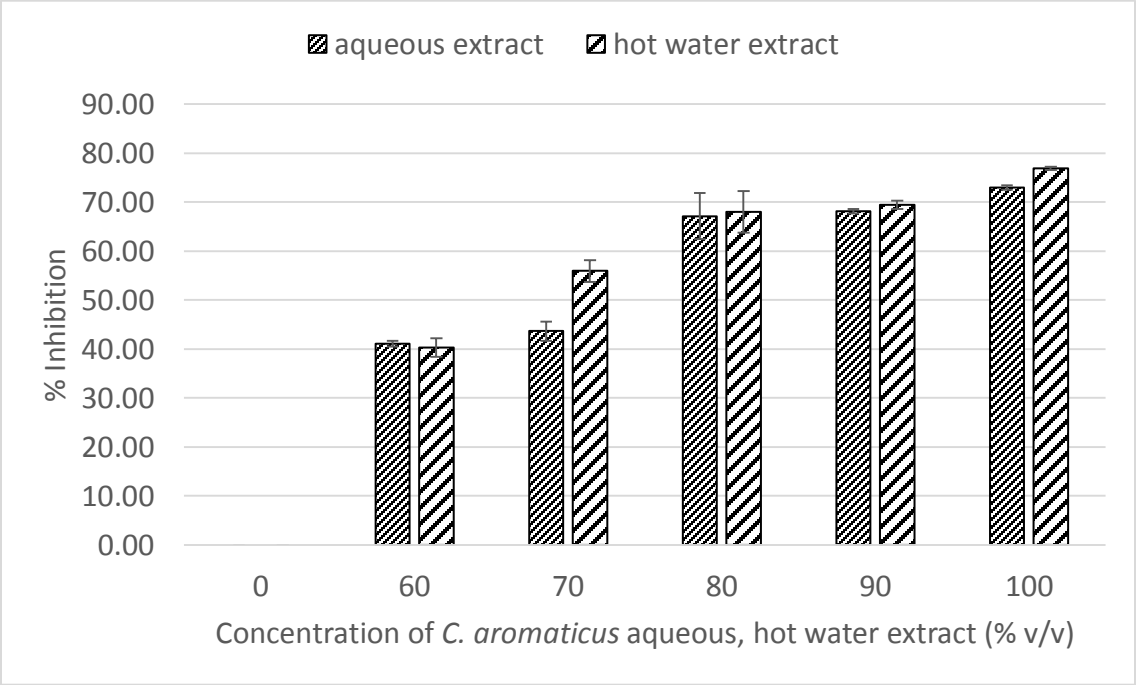

Figure 04: The inhibitory effect of *C. aromaticus* aqueous and hot water extract on the growth of *L. theobromae* in liquid bioassay. Each data point represents the mean of 5 replicates ± standard error.

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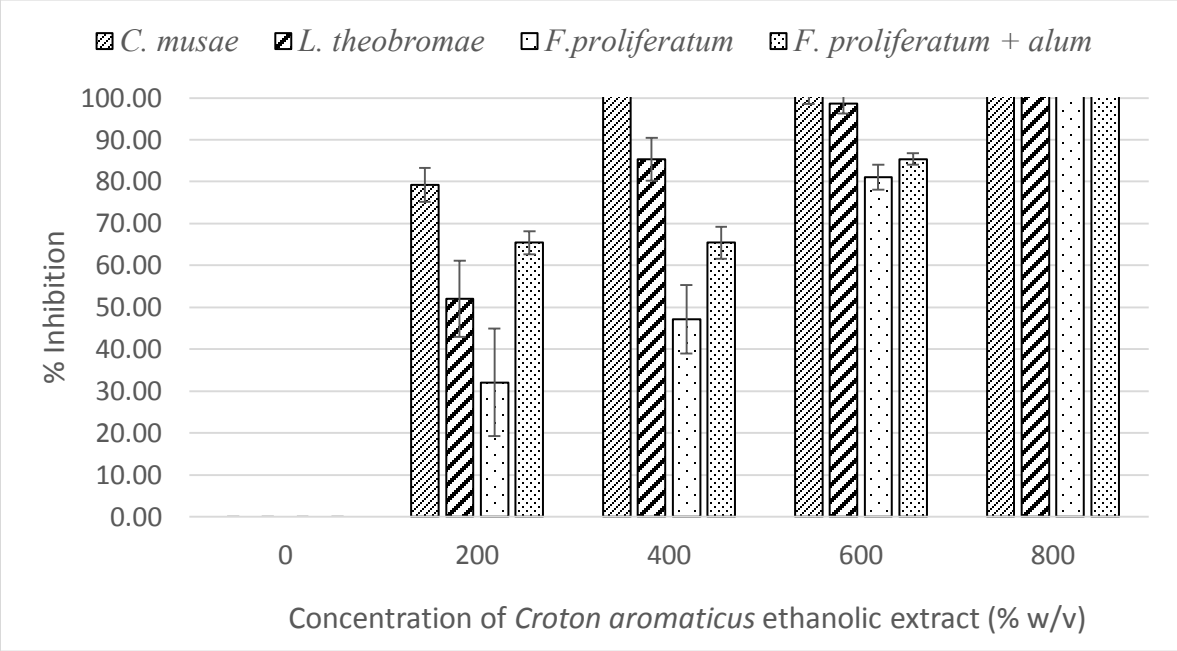

Figure 05: The inhibitory effect of *C. aromaticus* ethanolic extract on the growth of *F. proliferatum, C. musae* and*L. theobromae* in liquid bioassay. Each data point represents the mean of 5 replicates ± standard error.