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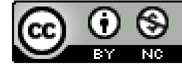

EFFICACY OF PLANT EXTRACTS AND EXTRACTING AGENTS AGAINST *Colletotrichum gloeosporoides* OF PAWPAW FRUITS

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ABSTRACT

Pawpaw (*Carica papaya* L.) is a fruit crop of economic importance in Kenya, where small scale farmers are the major producers. One factor limiting pawpaw production is anthracnose disease caused by *Colletotrichum gloeosporoides,* whose management on farm still remains a major challenge. The objective of this study was to determine the efficacy of plant extracts and extracting agents for controlling anthracnose disease on pawpaw fruits. *In vitro* spore germination and *in-vivo* tests were done using extracts from five plants; *Aloe chiliensis*, *Azadiracta indica*, *Carissa edulis*, *Fuerstia africana* and *Solanum incanum;* and extracting agents methanol, chloroform and ethanol against the fungus. A logistic regression model was used to estimate the botanicals' dose response treatment ranges. The efficacy of the crude extracts was greatest when methanol was used for extraction. The highest inhibition was recorded in pawpaw fruits treated with leaf extracts of *F. africana*. There were significant differences in effects among treatments by methanolic extracts of the five plants on conidial spore germination percentage. *Aloe chilensis* (Aloe) showed a higher spore germination of 35.7%; while *Azadirachta indica* (Neem) resulted in the lowest spore germination of 1.2%. There were significant differences in days to healing of *C. papaya* fruits infected with anthracnose. Pawpaw infected fruits healed fastest (3.5 days) when treated with the methanolic leaf extracts of *F. africana;* while *A. chiliensis* ethanolic leaf extracts resulted in the longest healing time of over 7 days. Although these botanical fungicides present high potentials or controlling anthracnose pathogens of pawpaw fruits, their suitability for application within the socio-economic framework of Kenyan small-scale producers still remains a matter for further investigation.

Key Words: *Aloe chilensis,* botanicals, *Carica papaya*, *Solanum incanum*

RÉSUMÉ

La papaye (*Carica papaya* L.) est une culture fruitière d'importance économique au Kenya, où les petits agriculteurs sont les principaux producteurs. Un facteur limitant de la production de papayes est la maladie de l'anthracnose causée par *Colletotrichum gloeosporoides*, dont la gestion au champ reste encore un défi majeur. L'objectif de cette étude était de déterminer l'efficacité des extraits de

plantes et des agents d'extraction pour contrôler la maladie de l'anthracnose sur les fruits de papaye. La germination des spores *in vitro* et des tests *in vivo* ont été réalisés en utilisant des extraits de cinq plantes; *Aloe chiliensis, Azadiracta indica, Carissa edulis, Fuerstia africana* et *Solanum incanum*; et les agents d'extraction méthanol, chloroforme et éthanol contre le champignon. Un modèle de régression logistique a été utilisé pour estimer les rangées de traitement dose-réponse des plantes. L'efficacité des extraits bruts était la plus élevée lorsque le méthanol était utilisé pour l'extraction. L'inhibition la plus élevée a été enregistrée dans les fruits de papaye traités avec des extraits de feuilles de F. *africana*. Il y avait des différences significatives dans les effets entre les traitements par extraits méthanoliques des cinq plantes sur le pourcentage de germination des spores conidiennes. *Aloe chilensis* (Aloe) a montré une germination des spores plus élevée de 35,7%; tandis qu'*Azadirachta indica* (Neem) a entraîné la plus faible germination des spores de 1,2%. Il y avait des différences significatives dans les jours de guérison des fruits de C. *papaya* infectés par l'anthracnose. Les fruits de papaye infectés guérissaient le plus rapidement (3,5 jours) lorsqu'ils étaient traités avec les extraits de feuilles méthanoliques de F. *africana*; tandis que les extraits de feuilles éthanoliques d'A. *chiliensis* ont donné le temps de guérison le plus long de plus de 7 jours. Bien que ces fongicides botaniques présentent des potentiels élevés ou contrôlent les agents pathogènes de l'anthracnose des fruits de papaye, leur aptitude à être appliquée dans le cadre socio-économique des petits producteurs de Kenya reste encore à étudier.

Mots Clés: *Aloe chilensis*, plantes, *Carica papaya*, *Solanum incanum*

INTRODUCTION

Pawpaw (*Carica papaya* Caricaceae) is predominantly a smallholder farmer crop in the arid and semi-arid areas of Kenya; whose production is widely hampered by anthracnose disease caused by *Colletotrichum gloeosporoides*. Fungicides application has been the most effective method of controlling anthracnose (Ishii *et al*., 2016; Cao *et al*., 2017; Moral *et al*., 2018). However, the practice is associated with ill health, caused by fungicide residues in food value chains. Fungicides have also been reported to affect the fruit ripening process (Domínguez *et al*., 2012) by delaying senescence. Furthermore, their use for extended periods has been reported to lead to the emergence of fungicide-resistant strains (Sanders *et al*., 2000; Vinod *et al*., 2009; Wanyera *et al*., 2009; Phoulivong, 2011).

Botanical fungicides and bactericides are products that are derived from natural plant compounds and have been used to control various bacterial and fungal diseases of plants (Serra *et al*., 2018; Khaliq *et al*., 2019). Among the numerous plant sources of botanicals available are *Azadiracta indica* (Aliero, 2003; Nguta *et al*., 2010; Oyoo-Okoth *et al*., 2011), *Fuerstia africana* (Muthaura *et al*., 2007; Keter and Mutiso, 2012; Okach *et al*., 2013), *Solanum incanum* (Njoroge *et al*., 2004; Keter and Mutiso, 2012), *Carissa edulis* (Koch *et al*., 2005; Tolo *et al*., 2006) and *Aloe chilensis* (Tolo *et al*., 2010). These plants have been widely researched for their mosquitocidal, antiparasitic, larvicidal, bactericidal and viricidal properties; and are currently used for treatment of animal and plant diseases.

Several studies have been conducted on diseases of crops, including avocados (Wasilwa *et al.,* 2005*;* Kimaru *et al*., 2018*)*, snap beans (Wagara and Kimani, 2007; Chemining'wa *et al*., 2011; Wahome *et al*., 2011), sorghum (Ngugi *et al*., 2000, 2001, 2002; Akosambo-Ayoo *et al*., 2011), citrus (Njoroge *et al*., 2004), passion fruits (Amata *et al*., 2009). However, there is negligible literature on their efficacy on anthracnose of *C. papaya*. The objective of this study was to evaluate the antifungal efficacy of selected botanical extracts and extracting agents against *C. gloeosporoides* of pawpaw fruits.

MATERIALS AND METHODS

Study area. This study was conducted in Elgeyo-Marakwet and Baringo Counties in the Rift Valley of Kenya, an area of approximately 8,655 Km^2. Baringo County lies between Latitudes 0°13" South and 1°40" north and Longitudes 35°36" and 36°30" East, with altitude between 1005 and 1280 meters above sea level. Elgeyo-Marakwet County extends from latitude 0°202 to 1°302 North and longitude 35°02 to 35°452 East, with altitude between 1200 and 1400 meters above sea level.

In the two counties, pawpaw is an important fruit crop grown for both local consumption and sale. The area under *C. papaya* ranges between 50,000–90,000 hectares, producing on average 9,000 –70,000 metric tonnes per year (FAOSTAT, 2016). The main pawpaw varieties grown are Kapo Solo, Shillong. Co-1 and Pink fleshed sweet, but only Pink fleshed pawpaw variety was in this study.

Anthracnose of onion, banana, pawpaw, mango, lettuce and all other fruit trees occur as small angular, brown to black spots that coalesce to form large extensive lesions on the leaf; while on the fruits it occurs as tear strain of linear necrotic regions associated with superficial cracking of the fruit epidermis (Sadhu and Chattopadhyay, 2011). The average loss of pawpaw fruits due to fungal infection (including Anthracnose) and bad weather at the farm is reported to be 28 percent, with some farmers reporting up to 50 percent (FAOSTAT, 2016).

Control of pawpaw anthracnose in the area includes siting of pawpaw orchards in drier areas, use of resistant cultivars, farm sanitation and the application of fungicides (Diehl, 2008). Washing off of fungicides during heavy rainy spells and increased resistance to diseases, weeds and insect pests alike due to increased use of synthetic chemicals are some of the challenges in anthracnose disease control (Forcelini and Peres, 2018).

Study plant materials. Selection of the plants for biofungicidal extraction (Table 1) was based on available ethnobotanical information from traditional health practitioners and literature (Omwenga *et al*., 2009; Gakuya *et al*., 2013). The plants were identified using morphological characteristics and taxonomic keys (Rainer, 2014). The leaves, fruits and roots of *Aloe chiliensis* (Linn.), *Azadirachta indica* (A. Juss), *Carissa edulis* (Forssk.), *Fuerstia africana* (T.C.E. Frie) and *Solanum incanum* (Linn.) were collected from several areas in Baringo and Marakwet Counties. Samples of the plant parts were separately packaged in clean paper bags and identified and extracted at the Department of Biological Sciences at the University of Eldoret in Kenya. Voucher specimens were deposited at the University of Eldoret herbarium.

Extracting reagents. The reagents used in the extraction of plant parts were ethanol (95%), methanol (70%), chloroform (5-10% DMSO). Methanol being a polar solvent, is generally used as a first solvent for extraction purpose to look for bioactives in medicinal plants (Tasiwal *et al*., 2009). Ethanol has been known as a good solvent, and is used for

TABLE 1. Plant parts collected from Baringo and Marakwet Counties in Kenya used in the study

Plant species	Family	Parts	Collection area
Aloe chilensis	Asphodelaceae	Leaves, fruits and roots	Baringo - Kapsergong
Azadirachta indica	Meliaceae	Leaves, fruits and roots	Baringo – Ossen
Carissa edulis	Apocynaceae	Leaves, fruits and roots	E/Marakwet - Kimwarer
Fuerstia africana	Labiatae	Leaves, fruits and roots	E/Marakwet - Tambach
Solanum incanum	Solanaceae	Leaves, fruits and roots	Baringo - Sesia

polyphenol extraction and is safe for human consumption (Vinod *et al*., 2009).

Plant crude extracts. The plant parts used for plant crude extraction were collected, separated and rinsed with tap water; and sterilised using 2% Sodium hypochlorite for two minutes. They were then rinsed three times with sterile distilled water; dried separately on blotting paper at room temperature (26 ^{0}C) for two weeks before being ground into fine powder using an electric grinder (GR8TR V2 GRINDER).

Crude extracts of the plants were prepared using the method described by Cakir *et al.* (2004). The dried preparations of leaves, fruits, and roots were extracted separately with methanol (70%), chloroform (5-10% DMSO) and ethanol (95%) at room temperature. Extraction with each solvent was done three times per sample. After the extraction of each plant part, the extract was filtered through filter paper (Wattman No. 1). The extracts were then concentrated under low pressure at 40 ^{o}C, using a rotary disk. The extracts were then reconstituted into a concentration of 80 mg ml^{-1} using the different solvents. The negative control was a 6 mm disk treated with distilled water and inoculated into the petri plate containing PDA in the bioassay; while the positive control was carbendazim (1 x 10^{-6} ppm).

Sources of anthracnose inocula. Anthracnose-infected pawpaw plants, obtained from 32 farms in Baringo and Elgeyo-Marakwet Counties where the disease is particularly severe (unpublished reports), based on typical anthracnose symptoms, were used as sources of *Colletotrichum* spores. The *C. gloeosporioides* isolates were transferred into PDA medium, and incubated for 3 to 4 days at 25 + 1 ^{o}C in PDA. The cultures were then sub - cultured onto fresh plates containing the PDA medium. In determining the conidial morphology, the lengths and widths were measured for 50 conidia, and conidial shape (acute and obtuse apices) recorded at X600 or X1250 magnification.

Isolation of *C. gloeosporoides.* The diseased parts of pawpaw fruit were excised using sterile blades, packaged in zip-lock bags and placed in cool (26 ^{o}C) boxes; then transported to the laboratory. The diseased plant samples were then rinsed with tap water and subsequently sterilised using 2% Sodium hypochlorite for two minutes; before being rinsed three times with sterile distilled water and then dried on blotting paper. Diseased sections were excised using sterile scalpels, and placed on chloramphenicol amended-potato dextrose agar (PDA) for seven days at 25 ^{o}C in a non-illuminated incubator.

Identification of *C. gloeosporoides.* Single spore isolates were sub-cultured onto PDA at room temperature, and identified using cultural and mycelial morphological characteristics. The colour of the aerial mycelium was determined using a Morphological Colour Chart. Synoptic keys and Riddell slide cultures were used to classify the *Colletotrichum* species (Riddel, 2019). Infected pawpaw of anthracnose that were collected from the samples were stained and observed at the mechanical stage of a light microscope at a magnification of × 400 for the identification of *C. gloeosporoides*.

Preparation of anthracnose inoculum. Sterile distilled water (5 ml) was added to each PDA plate and stirred up gently using the edge of a sterile glass slide to obtain a spore suspension. The suspension was then sieved through four layers of cheesecloth to remove mycelia fragments.

***In vitro* bioassay of plant extracts.** Antifungal activity using methanol, chloroform and ethanol crude extracts of *F. africana*, *S. incanum, C. edulis*, *A. indica* and *A. chilensis* were evaluated, separately by the serial dilution/ paper disk-agar diffusion method to achieve

six different concentrations of the form 10^n. The six concentrations (10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 10°) of the solvent plant extracts were prepared on and laid onto the PDA medium using 6 mm – paper disks. Test plates (diameter = 6 cm) were prepared with PDA medium and inoculated on surface with a pore suspension in sterile dissolution of 0.9% saline. Sterile distilled water was used as the control. The entire set-up was used to evaluate antifungal activity from the zones of inhibition that formed after incubation for 48 hours at 25 ± 1 ^{o}C.

Minimum inhibitory concentration. Serial dilutions (25 - 150 mg ml^{-1}) of the plants extracts were prepared and 60 µl of the reconstituted samples impregnated on paper discs (diameter 6 mm). Inoculation with *C. gloeosporoides* was done where the test fungus was transferred *in-vitro* to the PDA medium, followed by the aseptic transfer of the discs into the same medium. The minimum inhibitory concentration (MIC) was regarded as the lowest concentration that produced a visible zone of inhibition (Kariba *et al*., 2001). The plates were incubated at 27 ^{o}C for 4–6 days.

Experimental design. The experimental design employed a 5 x 3 x 1 three factorial design (5 plant species, 3 plant parts and one fungus), arranged in a completely randomised design (CRD) and replicated three times. Papaya belonging to pink fleshed sweet variety, with no visible cracks, rots or no visible deformities used in this study. Fruits were disinfected by immersing them in 1% NaOCl solution for 1 minute, washed twice with sterile distilled water and dried at room temperature (26 ^{o}C).

Conidial germination test. In conidial germination tests, a spore concentration of *C. gloeosporioides* (10^5 conidia ml^{-1}) was adjusted using a haemocytometer. A 10 ml sample of plant extract and a 90 ml sample of the spore suspension were mixed and the mixture placed of a sterile slide. The slide was then supported using two glass rods in a Petri dish, under moistened conditions and incubated at 25 ^{0}C, for 24 hr. The control conidia treatment received an equivalent amount of the solvent and sterile water, instead of plant extracts.

The setup was laid out in a Completely Randomised Design, with three replications and approximately 200 conidia counted for each slide (Mwamburi *et al*., 2015). Conidia were observed at 400x magnification and germination was recorded when a germ tube was visible. After incubation, slides were fixed with a drop of lactophenol cotton blue and observed microscopically, for spore germination (Mwamburi *et al*., 2015). A conidium was considered germinated when the length of the germ tube exceeded its diameter. The number of conidia germinated was scored to calculate the percentage inhibition of conidial germination. The number of conidia germinated was expressed as percentage of germination.

***In vivo* efficacy tests.** Plant extracts that showed antifungal activity were further tested for their effects against papaya anthracnose, on harvested pawpaw fruits. Papaya fruits (pink fleshed sweet variety) were obtained from Marigat farm in Baringo County. The undamaged, matured fruits of comparable size and colour were visually selected and used. Methanol, chloroform and ethanol Aqueous extracts of *F. africana*, *S. incanum*, *A. indica*, *A. chiliensis* and *C. edulis* were evaluated at concentrations of 10 and 25% (w/v). Conidial suspensions of *C. gloeosporioides* prepared from 10-day old cultures and adjusted to 10^8 conidia per ml using hemacytometer were used.

Papaya fruits were surface sterilised by dipping them in 1% Sodium hypochlorite solution for 10 minutes then rinsed in sterile distilled water; and inoculated by dipping into spore suspensions of *C. gloeosporioides*. The fruits were covered using a plastic sheet and

incubated for 15 hr until conidia germinated. The fruits were dipped into aqueous extracts of the test plants extracts; while the control fruits were dipped into sterile water. Carbendazim (1 x 10^{-6}) was used as positive control. Three fruits of pink-fleshed sweet variety were used for each of the treatments.

The experiment was laid out in a completely randomised design and treatments were replicated three times. Disease severity was assessed using systemic symptoms such as lesions based on visual rating. It was recorded using a scale developed by Gurjar *et al.* (2012), where 1=0% of fruit area affected, 2=1–25%, 3=26 – 50%, 4=51-75%, and 5 =76-100% fruit area affected.

Statistical analysis. All data obtained were subjected to One-Way Analysis of Variance (ANOVA) of the SAS v. 9.0. Least Significant Difference (LSD) at 5% probability level was used for mean comparison. Disease severity ratings were square root transformed; while percentage spore germination was arcsine (%/ 100) transformed for normal distribution before statistical analysis.

RESULTS

***In vitro* efficacy of plant extracts.** The results for the efficacy of leaf crude extracts of five test plants against *Colletotrichum gloeosporioides* for methanolic, chloroform and ethanolic extracts of different plant parts and the response of *C. gloeosporoides* to the leaf, fruit and root extracts of five plant species are presented in Figure 1. The maximum zone of inhibition of *C. gloeosporoides* (12.4mm) was recorded in *Fuerstia africana* chloroform (10° ppm) leaf extracts; followed by that of 11.0mm by *Azadirachta indica* methanolic (10° ppm) fruit extracts and *Carisa edulis* methanolic (10° ppm) root extracts; while the lowest maximum zone of inhibition was achieved in *Solanum incanum*-ethanolic (10° ppm) fruit extracts and *Aloe chiliensis*-chloroform (10^{0} ppm) root extracts (2.1 mm and 3.0 mm, respectively).

The response of *C. gloeosporioides* to the methanolic leaf ($R^2 > 0.96$) (Fig. 1A), chloroform fruit ($R^2 > 0.94$) (Fig. 1B) and chloroform root ($R^2 > 0.95$) (Fig. 1C), extracts of the five species were significantly different ($P < 0.05$) with extracts from *F. africana*, *A. indica* and *C. edulis,* displaying dose-response relationships. The trend of the estimated minimum zones of inhibition in the plates after treatment with solvent plant parts extracts of the five species was *F. africana* > *A. indica* > *C. edulis* > (*A. chiliensis* = *S. incanum*).

Conidial spore germination test. The *in vitro* screening tests against *C. gloeosporoides* revealed that there were high differences ($P<0.05$) in efficacy among methanolic (Table 2a), chloroform (Table 2b) and ethanolic (Table 2c) extracts of different plant parts on spore germination (Table 2). The effect of the extracts ranged from weak to strong on a 0-4 scale. Strong antifungal activity was exhibited by the extracts of *Fuerstia africana* chloroform leaf extracts, with a diameter of inhibition of 4.5 mm and a growth inhibition score of 4; indicating the complete inhibition of growth and sporulation of *C. gloeosporoides*. *F. africana* methanolic leaf extracts followed with a diameter of inhibition of 4.4 mm, and a growth inhibition score of 3. *Aloe chiliensis*-ethanolic extracts diameter of inhibition of 1.3 mm and a growth inhibition score of 3 had the weakest antifungal activity.

Similar to growth inhibition score and inhibition zone diameter, there were significant differences among treatments by the solvent extracts of the five plant parts on germination of spores (Table 2). *Aloe chilensis* chloroform fruit extracts (Table 2b) showed spore germination of 35.7%, followed by *F. africana* methanolic leaf extracts (Table 2a) (36.6%) and *F. africana* chloroform leaf extracts (35.5%). *Aloe chiliensis* ethanolic root extracts (Table 2c) had the lowest spore germination of 0.7%.

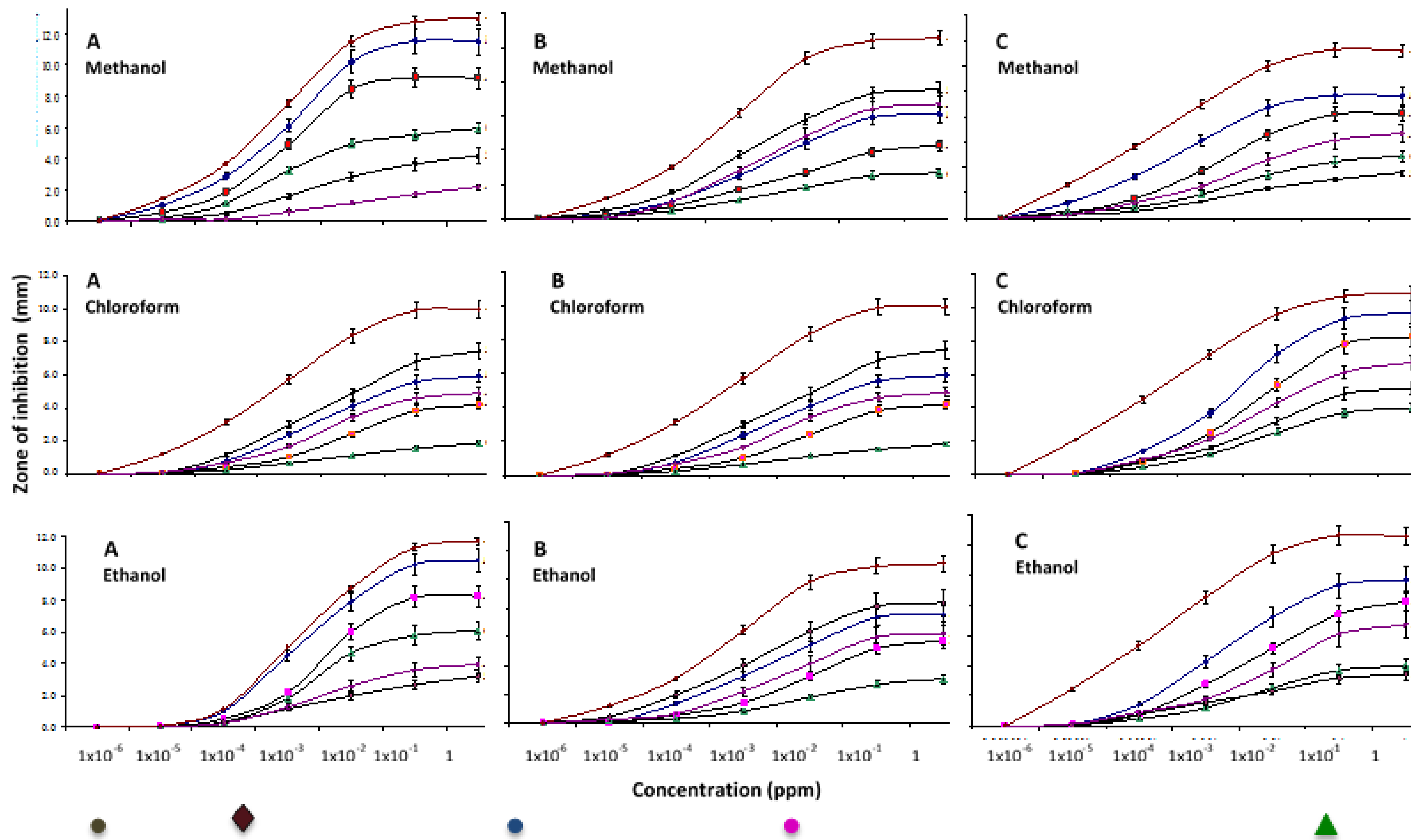

Figure 1. Efficacy of methanol, chloroform and ethanol of A. leaves, B. fruits and C. roots crude extracts of five plants against *C. gleosporoides* Control, *Solanum incarnum*, *Fuerstia africana*, *Azadirachta indica*, x *Aloe chilliensis, Carisa edulis.*

TABLE 2 (a). Antifungal activity of methanolic (1 x 10^{-3}) plant part extracts of some plant species from Kerio Valley region in Kenya against *C. gloeosporoides*

Plant species	Plant parts	DI (mm)[a]	IE[b]	Spore germination (%)[c]
F. africana	Leaves	4.4	3	12.9
	Fruits	4.2	3	12.3
	Roots	4.1	3	12.5
S. incanum	Leaves	13.5	4	3.0
	Fruits	13.2	4	2.6
	Roots	13.1	4	3.1
C.eduis	Leaves	2.7	1	20.2
	Fruits	2.5	1	20.1
	Roots	2.0	1	20.0
A.indica	Leaves	1.9	3	1.2
	Fruits	1.6	3	1.2
	Roots	1.6	3	1.1
A.chilensis	Leaves	2.3	1	35.6
	Fruits	2.3	1	35.5
	Roots	3.5	1	28.9
Control		0.0	4	9.3

[a]diameter of inhibition zone in mm measured after 4 days of incubation. [b]inhibition effect on a 0-4 scale, where 0 = none and 4 = strong. [c]spore germination 24 h after treatment

TABLE 2(b). Antifungal activity of chloroform (1 x 10^{-3}) plant part extracts of some plant species from Kerio Valley region in Kenya against *C. gloeosporoides*

Plant species	Plant parts	DI (mm)[a]	IE[b]	Spore germination (%)[c]
F. africana	Leaves	4.5	3	10.3
	Fruits	3.9	3	9.7
	Roots	3.6	3	9.9
S. incanum	Leaves	12.6	4	2.1
	Fruits	11.9	4	2.0
	Roots	11.2	4	1.4
C.eduis	Leaves	3.7	1	17.3
	Fruits	2.1	1	18.4
	Roots	1.8	1	15.1
A.indica	Leaves	3.9	3	1.3
	Fruits	3.6	3	1.2
	Roots	3.8	3	2.0
A.chilensis	Leaves	2.5	1	35.6
	Fruits	2.1	1	35.7
	Roots	2.2	1	32.8
Control		0.0	4	9.0

[a]diameter of inhibition zone in mm measured after 4 days of incubation. [b]inhibition effect on a 0-4 scale, where 0 = none and 4 = strong.. [c]spore germination 24 h after treatment

TABLE 2 (c). Antifungal activity of ethanolic ((1 x 10^{-3}) plant part extracts of some plant species from Kerio Valley region in Kenya against *C. gloeosporoides*

Plant species	Plant parts	DI(mm)[a]	IE[b]	Spore germination (%)[c]
F. africana	Leaves	2.8	3	9.3
	Fruits	3.2	3	1.6
	Roots	2.9	3	9.0
S. incanum	Leaves	11.2	3	1.4
	Fruits	12.5	4	1.6
	Roots	11.5	4	1.5
C.edulis	Leaves	1.8	1	15.1
	Fruits	2.1	1	16.4
	Roots	1.9	1	14.5
A.indica	Leaves	3.3	3	0.9
	Fruits	3.0	3	1.0
	Roots	3.0	3	0.7
A.chilensis	Leaves	3.0	1	29.3
	Fruits	2.0	1	32.3
	Roots	2.0	1	24.9
Control		1.3	4	9.5

[a]diameter of inhibition zone in mm measured after 4 days of incubation. [b]inhibition effect on a 0-4 scale, where 0 = none and 4 = strong. [c]spore germination 24 h after treatment

***In vivo* efficacy of plant extracts.** The results of infections (%), severity (on a 1 - 5 scale) of infection, soluble solid content and mass loss (percent) after papaya (pink fleshed sweet) fruit were treated with solvent plant parts extracts of five plants are presented in Table 3a-c. The ranges of infection, soluble solid content and mass loss were (19 - 52%), (4.1 - 10.1) and (4.0 - 9.6)%, respectively. The highest infection of 52% was recorded in *F. africana* ethanolic fruit extracts (Table 3a), with a severity of infection of 2, soluble solid content of 6.5 and mass loss of 6.6%; followed by *F. africana* chloroform root extracts (Table 3b) with 51% with severity of infection of 2, soluble solid content of 6.4 and mass loss of 6.4%. The lowest infection of 19% was achieved in *A. chiliensis* ethanolic root extracts (Table 3c) with severity of 3, soluble solid content of 4.1 and mass loss of 4.0%.

Significant differences were observed between the response of the fruits to the treatments in terms of the number of days to healing after treatment of the *C. papaya* infected fruits with the solvent leaf extracts of different plant parts of herbal plants (χ^2 = 7.2442, P = 0.0007) (Fig. 2). Healing of the infected fruits occurred in the shortest time in *C. papaya* treated with *F. africana* ethanolic leaf (Fig. 2A) extracts (1.85 days); followed by *F. africana* methanolic leaf extracts (3.37 days) and *Azadirachta indica* (3.45 days); while healing was delayed in *C. papaya* treated with *A. chiliensis* methanolic leaf extracts and *Solanum incanum* ethanolic leaf extracts (8.04 and 8.35 days, respectively).

There were significant differences in the number of days among the solvent fruit (Fig. 2B) extracts of dirrerent plant parts of five species and the number of days to heal anthracnose infected *C. papaya* fruits (P = 0.0002) with a maximum of 10 days (Fig. 2). The fruits of *C. papaya* infected with anthracnose healed fastest when treated with the *A. indica* ethanolic fruit extracts and F. *africana* ethanolic fruit extracts (2.97 and 2.25,

TABLE 3(a). Effect of methanolic plant part extracts on infection percentage and severity, soluble solid content and fruit mass loss of papaya (pink-fleshed sweet) inoculated with *C. gloeosporioides* and stored for 8 days at 27 ^{0}C

Plant specimen	Plant parts	Papaya fruit (pink fleshed sweet)			
		Infection (%) +/- 0.780	Severity of infection +/- 0.342	Soluble solid content +/- 0.415	Mass loss (%) +/- 0.431
Control		80	4	7.5	6.7
F. africana	Leaves	49	2	5.3	5.3
	Fruits	52	2	6.5	6.6
	Roots	49	2	6.2	4.5
S. incanum	Leaves	41	2	5.3	5.3
	Fruits	44	2	6.1	6.2
	Roots	36	2	5.7	4.4
C. edulis	Leaves	34	3	6.1	6.2
	Fruits	33	3	6.8	6.0
	Roots	29	3	5.3	4.9
A. indica	Leaves	25	3	6.0	6.2
	Fruits	37	3	6.7	5.8
	Roots	23	3	6.2	3.3
A. chiliensis	Leaves	30	3	5.4	5.9
	Fruits	35	2	5.8	5.9
	Roots	19	3	4.1	4.0

TABLE 3(b). Effect of chloroform plant parts extracts on infection percentage and severity, soluble solid content and fruit mass loss of papaya (pink-fleshed sweet) inoculated with *C. gloeosporioides* and stored for 8 days at 27 ^{0}C

Plant specimen	Plant parts	Papaya fruit (pink fleshed sweet)			
		Infection (%) +/- 0.780	Severity of infection +/- 0.342	Soluble solid content +/- 0.415	Mass loss (%) +/- 0.431
Control		80	4	9.3	7.5
F. Africana	Leaves	42	2	7.0	6.3
	Fruits	37	2	6.7	6.0
	Roots	51	2	6.4	6.4
S. lncanum	Leaves	37	2	7.2	6.1
	Fruits	38	2	6.0	6.2
	Roots	43	2	5.9	6.2
C. edulis	Leaves	29	3	7.1	5.6
	Fruits	30	3	7.2	5.9
	Roots	31	3	5.6	5.9
A. indica	Leaves	35	2	6.9	6.5
	Fruits	29	3	6.8	5.7
	Roots	25	3	6.7	6.0
A. chiliensis	Leaves	20	3	6.8	6.0
	Fruits	32	3	6.5	5.2
	Roots	36	2	5.8	6.1

TABLE 3(c). Effect of ethanolic plant parts extracts on infection percentage and severity, soluble solid content and fruit mass loss of papaya (pink-fleshed sweet) inoculated with *C. gloeosporioides* and stored for 8 days at 27 ^{0}C.

Plant specimen	Plant parts	Papaya fruit (pink fleshed sweet)			
		Infection (%) +/- 0.780	Severity of infection +/- 0.342	Soluble solid content +/- 0.415	Mass loss (%) +/- 0.431
Control		80	4	7.5	6.7
F. africana	Leaves	49	2	5.3	5.3
	Fruits	52	2	6.5	6.6
	Roots	49	2	6.2	4.5
S. incanum	Leaves	41	2	5.3	5.3
	Fruits	44	2	6.1	6.2
	Roots	36	2	5.7	4.4
C. edulis	Leaves	34	3	6.1	6.2
	Fruits	33	3	6.8	6.0
	Roots	29	3	5.3	4.9
A. indica	Leaves	25	3	6.0	6.2
	Fruits	37	3	6.7	5.8
	Roots	23	3	6.2	3.3
A. chiliensis	Leaves	30	3	5.4	5.9
	Fruits	35	2	5.8	5.9
	Roots	19	3	4.1	4.0

respectively). This was followed by *F. africana* methanolic extracts (2.5 days). *Carisa edulis* ethanolic fruit extracts took the longest time of over 10.66 days.

There were significant differences in the time taken by the solvent root (Fig. 2C) extracts of test plants in healing *C. papaya* infected fruits (χ^2 = 4.6674, P = 0.0012). Healing of infected fruits occurred in the shortest time in *C. papaya* treated with solvent extracts of *F. africana* ethanolic root extracts (3.95 days) and *F. africana* methanolic root extracts (4.0 days). This followed by fruit healing after treatment with *A. chiliensis* chloroform root extracts (4.38 days); while methanolic root extracts from *A. chilensis* and ethanolic root extracts from *C. edulis* (8.55 and 10.66 days, respectively) delayed healing of infected *C. papaya*.

Overall, there were significant differences in the time taken by the solvent plant extracts of the different plant parts of the five species. Healing of infected fruits occurred in the shortest time in *C. papaya* treated with *F. africana* ethanolic leaf extracts (1.85 days) and *F. africana* ethanolic fruit extracts (2.25 days), *C. edulis* ethanolic fruit extracts and *C. edulis* ethanolic root extracts took the longest time of over 10.27 and 10.66 respectively..

DISCUSSION

***In vitro* efficacy of plant extracts.** The result demonstrated that botanical solvent extracts vary in their efficacy in inhibiting *Colletotrichum gloeosporoides* growth, which is likely to be due to the extraction solvents and the plant parts used. The findings of this study are in agreement with previous reports on the antifungal activity of *Hypericum hyssopifolium* and *Hypericum heterophyllum* (Cakir *et al*., 2004).

The fact that the plant methanolic leaf extracts of *F. africana*, chloroform fruit extracts of *A. indica* and chloroform root

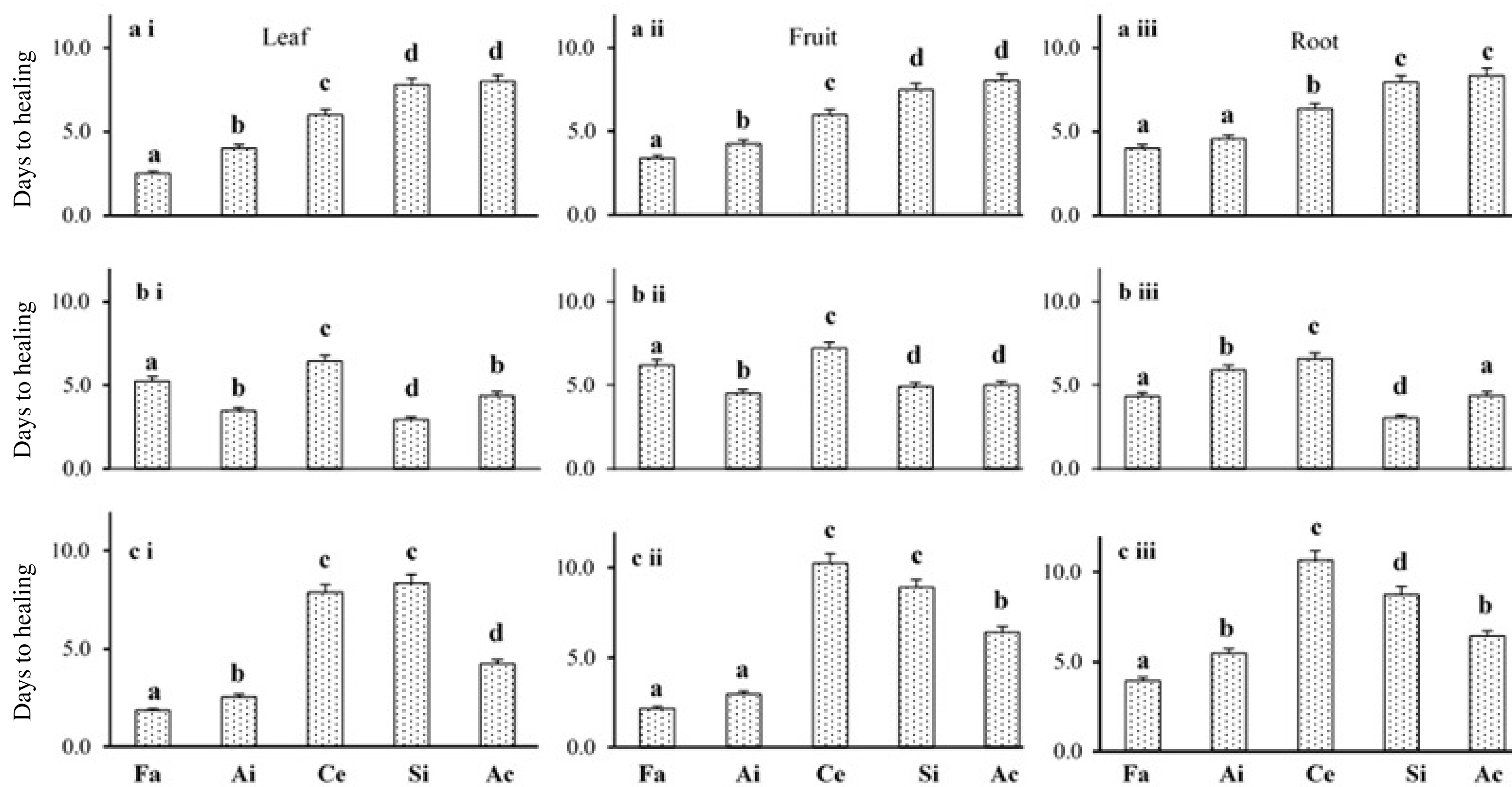

Figure 2. Number of days to healing of infected *C. papaya* after being treated with (a.). methanolic (b) chloroform and (c) ethanolic extracts of (i) leaves, (ii) fruits and (iii) roots of five plant species from Kerio Valley region. Fa = *Fuerstia africana,* Ai = *Azadirachta indica,* Ce = *Carisa edulis,* Si = *Solanum incanum* , Ac = *Aloe chiliensis.*

extracts of *C. edulis* exhibited significant inhibition against in *C. gloeosporioides,* suggests that these plants contain metabolites with antifungal efficacies (Alves *et al*., 2000; Ajila *et al*., 2007; Sasidharan *et al*., 2011). The most probable compounds responsible for antifungal effects in *F. africana*, and *A. indica* have been found to be lignans, pipenoiols, steroids, neolignans, alkaloids, propenylphenols, terpenes, piperolides, chalcones, flavanones, flavones and amides (Navickiene *et al*., 2000; Villaseñor *et al*., 2002; Johann *et al*., 2010). The same studies reported that pyrrolidine, dihydropyridine, and piperidine had antifungal effects in *A. indica*. Differences observed in antifungal activity of the different plant parts may be as a result of different factors. For instance, whereas the methanolic leaf extracts of *A. indica* displayed the highest antifungal activity against *Colletotrichum*, less activity resulted from the fruits and roots. Hence, the active compounds may be more concentrated in the leaves of *A. indica*. Our study showed that the ethanolic root extracts of *F. africana* displayed the lowest antifungal activity after recording the lowest maximum zone of inhibition, suggesting that they have a low concentration of active compounds. Similarly, *S. incanum* chloroform fruit extracts exhibited higher antifungal activity, whereas the chloroform leaf extracts displayed minimal activity. Another factor that had been found to influence the efficacy of the plants includes extraction solvent, (Okigbo, 2005). In the current study, the maximum zones of inhibition were overally recorded in methanolic plant extracts, followed by those of chloroform. The lowest maximum zones of inhibition were achieved in the plant extracts of ethanol.

Solvent crude extracts of plant parts of the five species had significant effects on the growth of *C. gloeosporoides* ($P < 0.05$), and were found to be more effective when methanol was used for extraction (Fig. 1). Methanolic and chloroform extracts exhibited higher efficacy than ethanolic extracts, possibly because they are very suitable for the extraction of polar and non-polar metabolites (Ajila *et al*., 2007). The highest maximum inhibition was recorded in methanolic leaf extracts of *F. africana,* followed by chloroform fruit extracts of *A. indica* and chloroform root extracts of *C. edulis;* while the lowest maximum zone of inhibition occurred in extracts obtained from *S. incanum* and *A. chiliensis*.

Crude extracts of *F. africana* exhibited antifungal activity and the groups of compounds present that were likely to be responsible for the fungitoxicity were identified by Gurjar *et al*. (2012) to be saponins, flavonoids and alkaloids. Methanolic, chloroform and ethanolic extracts of *F. africana*, *Solanum incanum*, *Azadirachta Indica*, *Carisa edulis* and *Aloe chiliensis* that suppressed the growth of *C. gloeosporoides* in the PDA and other media should be used for antifungal treatments of anthracnose in Baringo and Elgeyo-Marakwet in Kenya to encourage herbal plant controls in management of the fungus. The preservative nature of some plant part extracts have been known for centuries, and there has been a renewed interest in the antimicrobial properties of extracts from aromatic plants (Moral *et al*., 2018). The active compounds of the respective botanicals used in this study should be isolated, tested and formulated into affordable and eco-friendly antifungal agents for pawpaw growing farmers in Kenya.

Conidial germination test. The plant parts extracts of respective solvents of tested plants showed a higher inhibition on spore germination percentage compared to the untreated control (Table 2 a-c). This agrees with the report of Vinod *et al*. (2009), which reported the *In vitro* evaluation of botanicals and bioagents on spore germination, and fungicides against anthracnose of papaya caused by *Colletotrichum gloeosporioides*. This study has demonstrated the possibility of using plant extracts to impair conidial germination of *C. gloeosporoides;* and thus may be effective in controlling Anthracnose

in pawpaw fruits. *Fuerstia africana* chloroform leaf extracts exhibited a strong antifungal activity, followed by *F. africana* chloroform leaf extracts. Chloroform leaf extracts of *Aloe chilensis* ethanolic root extracts were the least effective in inhibiting conidial germination of *C. gloeosporoides*, followed by the *Carisa edulis* ethanolic leaf extracts of *S. incanum*. The inhibitory properties of genus *Fuerstia* to spore germination may be attributed to its glycoalkaloid effect of its toxic fruits at 0.1-0.3% wet mass and its relatively strong antifungal activity (Gurjar *et al*., 2012). The fungicidal spectrum of *Azadirachta indica,* which had above average inhibition in this study, has been attributed to Azadiractractrachin, which belongs to C25 terpeniodes (Moral *et al*., 2018). The preservative nature of some plant extracts has been known for centuries, and there has been renewed interest in the antifungal properties of extracts from aromatic plants (Alves *et al*., 2000).

Similar to growth inhibition, there were significant differences among treatments by the solvent extracts of the five plant parts on germination of spores. Higher spore germination was recorded in *Aloe chilensis* chloroform fruit extracts; followed by *F. africana* methanolic leaf extracts. The lowest spore germination was exhibited by *A. chilensis* ethanolic root extracts.

The failure of some spores of *C. gloeosporoides* to germinate after 24 hours exposure to crude plant extracts indicates that the metabolites produced by *F. africana*, *A. indica* and *C. edulis* were not only fungistatic, but also fungicidal to the spore of the test fungus (Sasidharan *et al*., 2011). It is noteworthy that the inhibition of spore germination by the extracts is desirable towards the management of papaya (Kimaru *et al*., 2018).

***In vivo* efficacy of plant extracts.** In this part of the study, there was a highly (P<0.05) significant difference in marketability of the fruits treated with botanical extracts of various solvents (Table 3 a - c). Postharvest diseases like *C. gleospoiroides* greatly reduce the storage life of pawpaw fruits. Hence, dipping the fruit in botanical extracts of various solvents inhibited rot development during storage (Tasiwa *et al*., 2009). The plant part extracts of the five species and their extraction solvents considered in this study had significant effects on the growth of *C. gloeosporoides* (P < 0.05); but were more effective when methanol and ethanol used for extraction (Fig. 2). Healing was most improved when the pawpaw fruits were treated with *F. africana* ethanolic leaf extracts, followed by *F. africana* ethanolic leaf extracts, and by *F. africana* ethanolic fruit extracts. Treatment with *Carica edulis* ethanolic fruit extracts and *C. edulis* ethanolic root extracts resulted in the longest healing time of over ten days. The report by Johann *et al.* (2010) also showed that solvent extracts were effective in controlling postharvest diseases, while maintaining the fruit quality. Plants are known to contain a number of secondary substances, like phenols, flavonoids, quinines, essential oils, saponins alkaloids and steroids which serve as chemical defenses to ward off pathogens (Ajila *et al*., 2007).

Hamidson *et al*. (2018) on use *Piper betle* to control anthracnose showed that a concentration of 10 ìg ml^{-1} inhibited the growth of *C. gleospoiroides*, where crude extracts of methanol chloroform and ethanol of *F. africana* fruits*,* followed by *A. indica* leaves at the same concentration were found to prevent *C. capsici* spore germination at 80.93, 74.09 and 72.91%, respectively. Furthermore, the chloroform leaf extracts of *S. incanum*, *O. bacillicum* and *Allium sativum* exhibited 100% inhibition of *C. gloeosporioides* (responsible for anthracnose in para rubber) mycelial growth when applying at 50 and 100% w/v, respectively (Ogbebor *et al*., 2007).

The strength of the botanical extracts and the extracting agents during healing the papaya

infected with anthracnose followed the trend *viz F. africana* ethanolic leaf extracts > *F. africana* ethanolic fruit extracts > *Azadirachta indica* ethanolic fruit extracts > *F. africana* methanolic leaf extracts > *A. indica* ethanolic fruit extracts.

CONCLUSION

The efficacious effects of the plant parts of the five species and the extracting agents considered in the present study, on the growth of *C. gloeosporoides* ($P < 0.05$), has been ascertained in the present study. Their effectiveness is even more enhanced by methanol and chloroform as extracts compared to ethanolic and water as solvents. The highest maximum inhibition was recorded in *Fuerstia africana* chloroform (10° ppm) leaf extracts followed by *Azadirachta indica* methanol (10^{0} ppm) fruit extracts and *Carisa edulis* methanolic (10° ppm) root extracts while the lowest zone of inhibition occurred in *Solanum incanum* ethanolic (10^{0} ppm) fruit extracts. Among treatments by methanolic, chloroform and ethanolic plant part extracts of the five plants on spore germination, *Aloe chilensis* chloroform fruit extracts showed a higher spore germination followed by *A. chilensis* methanolic leaf extracts and *A. chilensis* chloroform leaf extracts. *Azadirachta indica* ethanolic root extracts had the lowest spore germination overall. There were differences in the number of days to healing of the *C. papaya* (pink fleshed sweet) infected with anthracnose; with *F. africana ethanolic* leaf extracts having the most effective healing effect on *C. papaya* fruits; while *C. edulis* ethanolic root extracts was the least effective. Th*e* overall strength of the botanicals during healing the papaya infected of anthracnose followed the trend: *F. africana* ethanolic leaf extracts > *F. africana* ethanolic fruit extracts > *F. africana* methanolic leaf extracts > *A. indica* ethanolic leaf extracts > *A. indica* ethanolic fruit extracts. Further study should be conducted to determine the active compounds contained in the botanicals which have fungicidal activity against *C. gloeosporoides* due to emergence of drug resistant factors.

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REFERENCES

Ajila, C., Naidu, K., Bhat, S. and Rao, U.P. 2007. Bioactive compounds and antioxidant potential of mango peel extract. *Food Chemistry* 105:982-988.

Akosambo-Ayoo, L., Bader, M., Loerz, H. and Becker, D. 2011. Transgenic sorghum (*Sorghum bicolor* L. Moench) developed by transformation with chitinase and chitosanase genes from *Trichoderma harzianum* expresses tolerance to anthracnose. *African Journal of Biotechnology* 10:3659-3670.

Aliero, B. 2003. Larvicidal effects of aqueous extracts of *Azadirachta indica* (neem) on the larvae of Anopheles mosquito. *African Journal of Biotechnology* 2:325-327.

Alves, T.M., de A., Silva, A.F., Brandão, M., Grandi, T.S.M., Smânia, E. de F.A., Smânia Júnior, A. and Zani, C.L. 2000. Biological screening of Brazilian medicinal plants. *Memórias do Instituto Oswaldo Cruz* 95:367-373.

Amata, R., Otipa, M., Waiganjo, M., Wasilwa, L., Kinoti, J., Kyamanywa, S. and Erbaugh, M. 2009. Management strategies for fungal diseases in passion fruit production systems in Kenya. *All Africa Horticultural Congress* 911: 207-213.

Cakir, A., Kordali, S., Zengin, H., Izumi, S. and Hirata, T. 2004. Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifolium* and

Hypericum heterophyllum. *Flavour and Fragrance Journal* 19:62-68.

Cao, X., Xu, X., Che, H., West, J.S. and Luo, D. 2017. Distribution and fungicide sensitivity of *Colletotrichum* species complexes from rubber tree in Hainan, China. *Plant Disease* 101:1774-1780.

Chemining'wa, G., Kimani, P. and Nderitu, J. 2011. Snap bean breeding activities in Kenya. Presented at the Regional Stakeholders' Workshop on Snap Beans Commodity Value Chain. 71pp.

Diehl, K.B. 2008. Topical antifungal agents: an update. *American Family Physician* 54: 1687-1692.

Domínguez, I., Ferreres, F., Riquelme, F.P., Font, R. and Gil, M.I. 2012. Influence of pre-harvest application of fungicides on the postharvest quality of tomato (*Solanum lycopersicum* L.). *Postharvest Biology and Technology* 72:1-10.

FAOSTAT. 2016. Crop Production. http://faostat.fao.org/site/ 567/default.aspx# ancor.

Forcelini, B.B. and Peres, N.A. 2018. Widespread Resistance to QoI Fungicides of *Colletotrichum acutatum* from strawberry nurseries and production fields. *Plant Health Progress* 19:338-341.

Gakuya, D.W., Itonga, S.M., Mbaria, J.M., Muthee, J.K. and Musau, J.K. 2013. Ethnobotanical survey of biopesticides and other medicinal plants traditionally used in Meru central district of Kenya. *Journal of Ethnopharmacology* 145:547-553.

Gurjar, M.S., Ali, S., Akhtar, M. and Singh, K.S. 2012. Efficacy of plant extracts in plant disease management. *Agricultural Sciences* 3:425-433.

Hamidson, H., Damiri, N. and Angraini, E. 2018. Effect of medicinal plants extracts on the incidence of mosaic disease caused by cucumber mosaic virus and growth of chili. Presented at the IOP Conference Series: *Earth and Environmental Science, IOP Publishing, p*. 012062. pp 1 - 7.

Ishii, H., Zhen, F., Hu, M., Li, X. and Schnabel, G. 2016. Efficacy of SDHI fungicides, including benzovindiflupyr, against *Colletotrichum* species. *Pest Management Science* 72:1844-1853.

Johann, S., Cisalpino, P.S., Watanabe, G.A., Cota, B.B., de Siqueira, E.P., Pizzolatti, M.G., Zani, C.L. and de Resende, M.A. 2010. Antifungal activity of extracts of some plants used in Brazilian traditional medicine against the pathogenic fungus *Paracoccidioides brasiliensis*. *Pharmaceutical Biology* 48:388-396.

Kariba, R.M., Siboe, G.M. and Dossaji, S.F. 2001. *In vitro* antifungal activity of *Schizozygia coffaeoides* Bail. (Apocynaceae) extracts. *Journal of Ethnopharmacology* 74:41-44.

Keter, L.K. and Mutiso, P.C. 2012. Ethnobotanical studies of medicinal plants used by traditional health practitioners in the management of diabetes in lower Eastern Province, Kenya. *Journal of Ethnopharmacology* 139:74-80.

Khaliq, G., Ramzan, M. and Baloch, A.H. 2019. Effect of *Aloe vera* gel coating enriched with *Fagonia indica* plant extract on physicochemical and antioxidant activity of sapodilla fruit during postharvest storage. *Food Chemistry* 286:346-353.

Kimaru, S., Monda, E., Cheruiyot, R., Mbaka, J. and Alakonya, A. 2018. Morphological and molecular identification of the causal agent of anthracnose disease of avocado in Kenya. *International Journal of Microbiology* 4:1-10.

Koch, A., Tamez, P., Pezzuto, J. and Soejarto, D. 2005. Evaluation of plants used for antimalarial treatment by the Maasai of Kenya. *Journal of Ethnopharmacology* 101:95-99.

Moral, J., Agustí-Brisach, C., Agalliu, G., de Oliveira, R., Pérez-Rodríguez, M., Roca, L.F., Romero, J. and Trapero, A. 2018. Preliminary selection and evaluation of fungicides and natural compounds to control olive anthracnose caused by

Colletotrichum species. *Crop Protection* 114:167-176.

Muthaura, C., Rukunga, G., Chhabra, S., Omar, S., Guantai, A., Gathirwa, J., Tolo, F., Mwitari, P., Keter, L. and Kirira, P. 2007. Antimalarial activity of some plants traditionally used in treatment of malaria in Kwale district of Kenya. *Journal of Ethnopharmacology* 112:545-551.

Mwamburi, L.A., Laing, M.D. and Miller, R.M. 2015. Effect of surfactants and temperature on germination and vegetative growth of *Beauveria bassiana*. *Brazilian Journal of Microbiology* 46:67-74.

Navickiene, H.M.D., Alécio, A.C., Kato, M.J., Bolzani, V. da S., Young, M.C.M., Cavalheiro, A.J. and Furlan, M. 2000. Antifungal amides from *Piper hispidum* and *Piper tuberculatum*. *Phytochemistry* 55:621-626.

Ngugi, H., Julian, A., King, S. and Peacocke, B. 2000. Epidemiology of sorghum anthracnose (*Colletotrichum sublineolum*) and leaf blight *(Exserohilum turcicum*) in Kenya. *Plant Pathology* 49:129-140.

Ngugi, H., King, S., Abayo, G. and Reddy, Y. 2002. Prevalence, incidence, and severity of sorghum diseases in western Kenya. *Plant Disease* 86:65-70.

Ngugi, H., King, S., Holt, J. and Julian, A. 2001. Simultaneous temporal progress of sorghum anthracnose and leaf blight in crop mixtures with disparate patterns. *Phytopathology* 91:720-729.

Nguta, J., Mbaria, J., Gakuya, D., Gathumbi, P. and Kiama, S. 2010. Antimalarial herbal remedies of Msambweni, Kenya. *Journal of Ethnopharmacology* 128:424-432.

Njoroge, G., Bussmann, R., Gemmill, B., Newton, L.E. and Ngumi, V. 2004. Utilisation of weed species as sources of traditional medicines in central Kenya. *Lyonia* 7:71-87.

Ogbebor, N., Adekunle, A. and Enobakhare, D. 2007. Inhibition of *Colletotrichum gloeosporioides* (Penz) Sac. causal organism of rubber (*Hevea brasiliensis Muell*. Arg.) Leaf spot using plant extracts. *African Journal of Biotechnology* 6:213-218

Okach, D., Nyunja, A. and Opande, G. 2013. Phytochemical screening of some wild plants from *Lamiaceae* and their role in traditional medicine in Uriri District-Kenya. International *Journal of Herbal Medicine* 1:135-143.

Okigbo, R. 2005. Biological control of postharvest fungal rot of yam (*Dioscorea* spp.) with *Bacillus subtilis*. *Mycopathologia* 159:307-314.

Omwenga, E., Okemo, P.O., Mbugua, P.K. and Ogol, C. 2009. Ethnobotanical survey and antimicrobial evaluation of medicinal plants used by the Samburu community (Kenya) for treatment of diarrhorea. *Pharmacognosy Magazine* 4:165-176.

Oyoo-Okoth, E., Ngugi, C.C. and Chepkirui-Boit, V. 2011. Physiological and biochemical responses of Nile tilapia (*Oreochromis niloticus)* exposed to aqueous extracts of Neem (*Azadirachta indica*). *Journal of Applied Aquaculture* 23:177-186.

Phoulivong, S. 2011. *Colletotrichum,* naming, control, resistance, biocontrol of weeds and current challenges. *Current Research in Environmental & Applied Mycology* 1: 53-73.

Rayney, IOL. 2014. Manufacture of surgical instruments. Brighton and Hove East Sussex. St. Thomas Hospital, London.

Riddel John. 2019. Medical and colour dictionary. Spanish – English translation. Webstar.

Sadhu M K and Chattopadhyay P K. 2001. Introductory Fruit Crops, Naya Prakash Publishers, Kolkata.

Sanders, G.M., Korsten, L. and Wehner, F. 2000. Survey of fungicide sensitivity in *Colletotrichum gloeosporioides* from different avocado and mango production areas in South Africa. *European Journal of Plant Pathology* 106: 745-752.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. and Latha, L.Y. 2011.

Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines* 8:1-10.

Serra, E., Hidalgo-Bastida, L.A., Verran, J., Williams, D. and Malic, S. 2018. Antifungal activity of commercial essential oils and biocides against *Candida albicans*. *Pathogens* 7:15.

Tasiwal, V., Benagi, V.I., Hegde, Y.R., Kamanna, B.C. and Naik, K.R. 2009. *In vitro* evaluation of botanicals, bioagents and fungicides against anthracnose of papaya caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. *Karnataka Journal of Agricultural Science* 22:803-806.

Tolo, F., Rukunga, G., Muli, F., Ochora, J., Irungu, B., Muthaura, C., Wanjiku, C., Mungai, G., Ngoc, Q. and Hashimoto, K. 2010. The antiviral activity of compounds isolated from Kenyan Carissa edulis (Forssk.) Vahl. *Journal of Medicinal Plants Research* 4:1517-22.

Tolo, F.M., Rukunga, G.M., Muli, F.W., Njagi, E.N., Njue, W., Kumon, K., Mungai, G.M., Muthaura, C.N., Muli, J.M. and Keter, L.K. 2006. Anti-viral activity of the extracts of a Kenyan medicinal plant *Carissa edulis* against herpes simplex virus. *Journal of Ethnopharmacology* 104:92-99.

Villaseñor, I.M., Angelada, J., Canlas, A.P. and Echegoyen, D. 2002. Bioactivity studies on â-sitosterol and its glucoside. *Phytotherapy Research* 16:417-421.

Vinod, T., Benagi, V., Hegde, Y.R., Kamanna, B. and Bank, K.R. 2009. In vitro evaluation of botanicals, bioagents and fungicides against anthracnose of papaya caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. *Karnataka Journal of Agricultural Sciences* 22:803-806.

Wagara, I. and Kimani, P. 2007. Resistance of nutrient-rich bean varieties to major biotic constraints in Kenya. *Presented at the African Crop Science Conference Proceedings*, pp. 2087-2090.

Wahome, S., Kimani, P., Muthomi, J., Narla, R. and Buruchara, R. 2011. Multiple disease resistance in snap bean genotypes in Kenya. *African Crop Science Journal* 19: 289–302.

Wanyera, R., Macharia, J., Kilonzo, S. and Kamundia, J. 2009. Foliar fungicides to control wheat stem rust, race TTKS (Ug99), in Kenya. *Plant disease* 93:929-932.

Wasilwa, L.A., Njuguna, J.K., Kirigua, V., Waturu, C.N., Stafne, R.A. and Morelock, T.E. 2005. Impact of Anthracnose on Avocado Production in Kenya. *HortScience* 40:1103D - 1104.