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The Antifungal Activity of Some Medicinal Plants Against Coffee Berry Disease Caused by *Colletotrichum kahawae*

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ABSTRACT

This study was carried out with the objective of evaluating the antifungal potential of aqueous and ethanol extracts of eight different plant species *in vitro* and *in vivo* against *Colletotrichum kahawae* in completely randomized design with three replications. The extracts were from *Hagenia abyssinica, Allium sativum, Phytolacca dodcandera, Croton macrostachyus, Maesa lanceolata, Eucalyptus globules, Eucalyptus citriodera* and *Lippia adoensis*. Subsequently, two most effective plant extracts were tested *in vivo* against the disease on detached green coffee berries and seedling applying the extracts at 3 different times of application (at the time of inoculation and 48 h before and after inoculation) on the pathogen. The study indicated that the inhibitory effect of the extracts depended on the type of plant species used, method of extraction and time of application of the extracts. Generally, *A. sativum* and *C. macrostachyus* aqueous and ethanol extracts were the most effective plants that significantly reduced radial growth of the pathogen compared to the control. *A. sativum* reduced radial growth of the pathogen in ethanol and aqueous extracts by 83 and 100%, respectively and *C. macrostchyus* by 68 and 88%, respectively. Furthermore, *A. sativum* extracts consistently reduced disease severity on detached green berries and seedling in greenhouse at all times of application. Nevertheless, the efficacy of *C. macrostachyus* on detached green berries and seedlings was inconsistent and variable based on method of extraction and time of application of the extracts. The study indicated the possible use of extracts of *A. sativum* as an alternative means of CBD (coffee berry disease) management but further study at field conditions should be carried out to verify the result.

Key words: *Allium sativum*, anthracnose, *Croton macrostachyus*, inhibition, *Coffea arabica*

INTRODUCTION

Arabica coffee (*Coffea arabica* L.) is the single most important cash crop that has been contributing a lion's share to the Ethiopian economy (Girma *et al.*, 2007). Although, it plays significant role in the economy of the country, the crop suffers from many production constraints.

Coffee Berry Disease (CBD) is the major threat to Arabica coffee production since its outbreak in 1971 (Arega, 2006). It is an anthracnose of green coffee berry, caused by the infection of a fungal pathogen *Colletrichum kahawae*. Arega *et al.* (2008) pointed out the concomitant spread of CBD to Southwest and Southeast of Ethiopia in the early 1970s. Furthermore, the disease was

extended to Shewa and Gamugofa and Harraghe areas of the country in the year 1971 and 1978, respectively (Hindorf and Arega, 2006). Latter on it was also disseminated to other coffee growing areas of the country.

CBD causes significant yield losses. The average national yield losses were about 28% between 1974 and 1978 (Arega, 2006). Similarly in Harraghe, the losses were estimated to be as high as 100% (Eshetu *et al.,* 2000). The severity of CBD and the losses caused are often under estimated annually since young coffee berries drop off the tree at an early stage of the disease. Largely, CBD causes 30% national average crop losses to total harvestable coffee yield every year in Ethiopia (Eshetu *et al.,* 2000). At present, CBD has rapidly spread to all coffee growing areas of Ethiopia and still inflicting significant crop loss (up to 100% on susceptible land races) although the magnitudes vary from place to place and from time to time (Arega, 2006).

Fungicides such as Daconil and Delan were considered as promising chemicals against *C. kahawae*. However, later these products including Dyrene and Octave were banned for a number of side effects (Eshetu *et al.,* 2000). The use of fungicides against CBD has been shown to induce negative effects on beneficial microorganisms that can antagonize the CBD pathogen (Masaba, 1991). The high cost of pesticides, the appearance of fungicide resistant pathogen biotype and other social and health related problems of the conventional agriculture on the environment have increased interest in sustainable agriculture and biodiversity conservation. Additionally, millions of coffee farmers are facing problem not only with low coffee prices but also a growing interest in organically grown coffee across the globe. These problems make it essential to look for alternative strategies that can ensure competitive coffee production.

Many workers have reported antimicrobial activities of plant extract (phyotoproducts) and gaining due attention because of their proven attributes such as specificity, biodegradability and low toxicity (Okemo *et al.,* 2003; Saha *et al.,* 2005). In an approach toward the development of eco-friendly antifungal compounds for controlling major foliar fungal diseases of tea, ethanol and aqueous extracts of *Allium sativum* were tested against the pathogen *C. camelliae*. Ethanol and aqueous extracts of *A. sativum* show 100% inhibitory to spore germination of pathogen (Saha *et al.,* 2005). *In vitro* tests were carried out using extracts of *Maesa lanceolata* var. *goulungensis weir* against a broad range of fungal plant pathogens such as *Phytophthora cryptogea, Trichoderma virens, Aspergillus niger, Phoma* sp., *Fusarium oxysporium, Cochliobolus heterostrophus, Sclerotium rolfsii* and *Pyrenophora teres*. The extracts were very active against all the pathogens tested (Okemo *et al.,* 2003).

For instance, extract of some medicinal plants of Ethiopia for control of late blight (*Phytophtora infestans)* on tomatoes and potatoes have been investigated *in vitro* and *in vivo* (Mekuria *et al.,* 2005) and *Hagenia abyssinica, Lepidum sativum* and *Lippia adoensis* showed the strongest suppression of mycelium growth. In Ethiopia, information on antimicrobial activity of medicinal plants against fungal phytopathogens is very scanty. However, the use of medicinal plant extracts (e.g., *Allium sativum*) has a great potential in suppressing various plant pathogenic fungi (Okemo *et al.,* 2003; Satya *et al.,* 2005; Amin *et al.,* 2009) that may serve as better biological alternative in substituting the employment of chemical fungicides. Therefore, the objectives of this study were to evaluate the antifungal activities of the extracts of *Hagenia abyssinica, Allium sativum, Phytolacca dodcandera, Croton macrostachyus, Maesa lanceolata, Eucalyptus globules, Eucalyptus citriodera* and *Lippia adoensis* on the inhibition of the growth of the fungus *C. kahawae* and disease development on detached green coffee fruits and seedlings.

MATERIALS AND METHODS

Study area: The research was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), Jimma, Ethiopia, in Plant Pathology Laboratory and greenhouse from September 2009 to July 2010. JUCAVM is located at 7°42 N latitude and 36°50 E longitude and at altitude 1710 m a.s.l. The maximum and minimum temperatures of the area are 26.8 and 11.8°C, respectively, with relative humidity of 91% and the mean rainfall of 1500 mm per annum.

Preparation of fungal pathogen: Culture of *Colletotrichum kahawae* was obtained from Jimma Agricultural Research Center (JARC), Jimma, Ethiopia and sub-cultured on fresh PDA medium to obtain pure pathogen cultures for maintenance. Blocks of fungal agar were cut out with a sterile surgical blade from the leading edge of the actively growing portion and transferred to fresh agar medium and incubated at 25°C for 3-5 days. The fungal pathogen was maintained in two ways:

- By storing the pure culture of the pathogen in sterile distilled water
- Keeping the pathogen on susceptible coffee host plant (selection 370) on detached green berries (Van der Graaf, 1981)

Plant materials preparation: Ethiopian traditional medicinal plant sp. *H. abyssinica, P. dodcandera, C. macrostachyus, E. globules M. lanceolata* and *E. citriodera* were collected from natural habitats around Jimma, Ethiopia, while *A. sativum* and *L. adoensis* were purchased from the local market. The plant parts collected for extraction were leaf (*H. abyssinica, E. citriodera, E. globules* and *L. adoensis*), fruit (*R. dodcandera* and *M. lanceolata*), bark (*C. macrostachyus*) and bulb (*A. sativum*). The collected samples of each plant species was washed under tap water and surface sterilized with 5% sodium hypochlorite solution followed by thorough rinsing with sterile water. The plant samples were air dried at room temperature, ground and kept in bottles for subsequent activities.

Plant materials extraction: Plant samples were extracted using maceration techniques following standard procedures of Amadioha (2002) by some modification. Aqueous extracts were prepared 100 g/500 mL (w/v) of sterilized distilled water and shaken on orbital shaker (130 rpm) for one hour. The mixture was allowed to stand for 48 h and filtered using cheese cloth followed by filter paper (Whatmann No. 1). A 70% ethanolic extract was prepared by the method of Alade and Irobi (1993) by some modification, 100 g of each dried powder plant was soaked in 500 mL of 70% ethanol for 48 h. The extracts were filtered through cheesecloth followed by Whatmann filter paper No. 1. The organic solvents were evaporated under oven 30-40°C at room temperature. The remaining extract (2 mL) was diluted by adding appropriate quantity of sterilized distilled water to make 20% extract. The stock extracts were transferred to labeled sterile screw capped bottles and stored at 4°C for further use.

***In vitro* antifungal assays:** The effect of the plant extracts on the radial growth of *C. kahawae* was determined using the method described by Amadioha (2002). One milliliter of the respective extract was separately spread on the surface of the pre-solidified PDA contained in the petri dishes. The control was not inoculated. Five-millimeter fungal agar block was cut with a sterile scalpel from

the 7 to 10 days old culture at the actively growing portion on PDA and placed at the center of 9 cm diameter petri dish. The plates were incubated at room temperature (20-22°C). The radial growth of fungus for each treatment was measured at right angles for each colony every 48 h after 5 days of inoculation for 21 days. The experiments were carried out with three replications for each treatment.

Percentage radial growth inhibition by a given concentration of plant extracts was calculated as:

$$\text{Inhibition \%} = \frac{\text{Gowth of fungus in extract}}{\text{Gowth of fungus in control}} \times 100$$

Based on the initial results obtained in *in vitro*, *A. sativum* bulb aqueous extract that showed the most suppressive effect on fungal radial growth was further tested against *C. kahawae* at different concentrations to determine the Minimum Inhibitory Concentrations (MIC). Accordingly, four concentrations [200, 150, 100 and 50 mg mL^{-1} (w/v)] of *A. sativum* bulb extracts were evaluated.

***In vivo* antifungal assays:** *In vivo* antifungal potential of the extracts was examined on detached green coffee berries and on seedlings under greenhouse conditions. For the *in vivo* test on detached green berries, coffee berries were inoculated following standard methods (Van der Graaf, 1981). Berries having the same size and developmental stage were collected from the same line of coffee selection 370. The berries were harvested and surface sterilized with 5% sodium hypochlorite for 2 min and rinsed 3 times for 3 min with sterile water. Thereafter, the berries were placed on clean plastic petri dish and covered with sponge soaked in sterile distilled water to obtain 100% humidity. A total of 20 berries were used for each three replications. For inoculation of the detached green berries, conidial suspension (2×10^6 conidial mL^{-1}) was used. The concentration of conidia in the suspension was determined using a haemocytometer. Twenty-five microliter conidia in the suspension was dropped at the center of the berries at different times with the medicinal plant extracts that showed high inhibitory effects in the *in vitro* test. The selected plant extracts were sprayed on berries by using an air-pressurized hand sprayer per the following schedule: (1) 48 h before spore inoculation, (2) 48 h after spore inoculation and (3) at the same time. The interaction between medicinal plant crude extracts and inoculated pathogen were recorded starting from 15 day after inoculation every 2 day until 21 days after inoculation. The disease assessments on green berries were performed using 0-5 scale (Table 1).

Disease Index (DI) on berries was calculated using the following equation:

$$DI = \frac{100\ (b1+2b2+3b3+4b4+5b5)}{5(b0+b1+b2+b3+b4+b5)}$$

where, b0, b1, b2, b3, b4, b5 are No. of berries in class 0, 1, 2, 3, 4 and 5, respectively.

For the *in vivo* seedling test in greenhouse, the plant extracts with the highest inhibition percentage under *in vitro* test were further tested in greenhouse. In this test was performed

Table 1: Classification for Coffee Berry Disease (CBD) assessment on detached green berries

Class	Symptom	Code for disease index (DI)
0	no symptom	b0
1	minute brown spot lesion of 1 mm in diameter	b1
2	black lesion of 1-5 mm in diameter	b2
3	black lesion of 5-10 mm in diameter	b3
4	black lesion of 10-20 mm in diameter	b4
5	black lesion of greater than 20 mm in diameter	b5

following standard procedures (Tegegne *et al*., 2008) with some modifications on susceptible coffee seedlings to evaluate their ability to control coffee berries disease two days before, after and at the same time of inoculation. Plant extracts (200 mg mL^{-1}) were sprayed on the seedling plants. Data were taken after 15 days 2 to 3 times with 2 day intervals until 21 days.

For inoculation of coffee seedlings, susceptible coffee seedlings were raised in greenhouse from freshly harvested seeds of 370 cultivar. To obtain seedlings, ripe cherries were picked from Jimma Agriculture Reseach Center and dried under shade after removing the pulp by hand. Seeds of 370 coffee cultivar were prepared by removing the parchment and soaked in sterile distilled water and kept for 48 h. Thereafter, seeds were sown (12 seeds pot^{-1}) in heat sterilized and moistened forest soil,compost and sandy soil (2:1:1 ratio) in disinfected plastic pots arranged on benches and covered with mulch in greenhouse. Six weeks after sowing, the emerging seedlings were kept at 20-22°C. Two days before inoculation, the hypocotyls of the seedlings were sprayed with sterile distilled water and covered with plastic sheet for 48 h to obtain 100% relative humidity.

Plastic pots containing 10 seedlings pot^{-1} of 370 coffee cultivar were inoculated with 1 mL fungal spore suspension in different time of applications by stem brushing procedure with fine camel hairbrush (Van der Graaf, 1981). Fourty eight hours before inoculation, the seedlings were treated with medicinal plant extracts (aqeoues and 70% ethaol), respectively at the rate of 200 mg mL^{-1} by using an air pressurized hand sparyer.

Mycelia colonies of fungal pathogen was carefully removed with a sterile scalpel from the PDA medium while washing with sterile distilled water to harvest conidia from 10 days old cultures. The suspension was stirred with magnetic stirrer for 10-15 min and filtered through double layers of cheese cloth. After repeating the procedure, the spore concentration in the suspension was adjusted to 2×10^6 conidia mL^{-1}. Completely Randomized Design (CRD) was employed to see the interactions of medicinal plant extrats and the fungal pathogen on 370 coffee seeedlings cultivar. The seedlings were maintained wet with sponge soaked in sterile distilled water for additional 48 h under plastic cover. Temperature and relative humidity were adjusted by using digital sling psychrometr (Gm Nievw-vennep the Netherlands model 8706) to 80-85%, 20-22°C, respectively for 3 weeks. The reaction of each seedling of coffee cultivar against the fungal pathogen was assessed 15 and 21 days after inoculation using the symptom classifications (0-4 scale).

Experimental design and statistical analysis: The experiment was run using complete randomized design with three replications and repeated. Data were subjected to analysis of variance using SAS version 9.2. Single and interaction effect of factors were determined using the GLM procedure of SAS. Whenever, significant interactions were observed between factors, the level of one factor was compared at each level of other factors. Mean values among treatments were compared by the Tukey's test (Montgomery, 2008) at $\alpha = 0.05\%$ level of significance.

RESULTS

***In vitro* antifungal activity:** The *in vitro* antifungal activity of aqueous and 70% ethanol extracts of 8 medicinal plants at 20% (w/v) against *Colletotrichum kahawae* was studied through measurement of radial growth of the fungus. There was significant ($p<0.0001$) interaction effect between type of medicinal plants used and methods of extraction in inhibiting radial growth of *C. kahawae*. As a result, the effect of medicinal plants on radial growth of the pathogen was presented for each method of extraction. Generally, extracts of all the tested medicinal plants except aqueous extracts of *R. dodcandera* significantly inhibited the mycelia growth of *C. kahawae* compared to the untreated control (Table 2). The inhibitory effect of aqueous extracts of the 8 medicinal plants ranged from 13 to 100% with the highest inhibition by *A. sativum* (100%) followed by *C. macrostachyus* (88%). With the ethanol extract, inhibition of the mycelia growth of *C. kahawae* ranged from 41 to 83%. The highest inhibition showed by *A. sativum* (83%) followed by *R. dodcandera* (76%), *E. citriodera* (70%) and *C. macrostachyus* (68%). Overall, *A. sativum* and *C. macrostachyus* had significantly higher inhibitory effect on *C kahawae* with both aqueous and ethanol extracts (Fig. 1). While, *R. dodcandera* had significantly higher inhibitory effect only with ethanol extract (Table 2).

Results in the present study revealed that extract of *A. sativum* gives significantly higher inhibition zone compared to all the tested medicinal plants. Thus, the antifungal activity of *A. sativum* aqueous and ethanol extracts at different concentrations was tested against *C. kahawae* to determine Minimum Inhibitory Concentration (MIC). The results showed that there was an interaction effect between methods of extraction and concentration of extracts used indicating that the antifungal effect of *A. sativum* depends on the method of extraction used and the concentration level. Generally, both aqueous and ethanol extracts at 10, 15 and 20% significantly reduced the radial growth of *C. kahawae* compared to the control, while there was no significant difference

Table 2: Effect of medicinal plant extracts and methods of extraction (aqueous and ethanol) on radial growth of *Colletotrichum kahawae in vitro* test

Methods of extraction	Medicinal plants	Radial growth (mm)*	Inhibition (%)
Aqueous Extract	*Allium sativum*	0^{h}	100.0
	Croton macrostachyus	4^{g}	88.0
	Phytolacca dodcandera	29.6^{a}	12.9
	Eucalyptus globules	13.6^{d}	57.0
	Eucalyptus citriodera	19^{b}	44.0
	Hagenia abyssinica	21^{b}	60.0
	Maesa lanceolata,	16^{cd}	50.0
	Lippia adoensis	10^{ef}	67.0
	Control	32^{a}	0.0
Ethanol extract	*Allium sativum*	5^{fg}	83.0
	Croton macrostachyus	10.6^{e}	68.0
	Phytolacca dodcandera	7^{f}	76.0
	Eucalyptus globules	18^{bc}	64.0
	Eucalyptus citriodera	7^{f}	76.0
	Hagenia abyssinica	18^{bc}	41.0
	Maesa lanceolata,	17^{c}	44.0
	Lippia adoensis	14.3^{d}	56.0
	Control	32.1^{a}	0.0

*Means with the same letter are not significantly different ($\alpha = 0.05$)

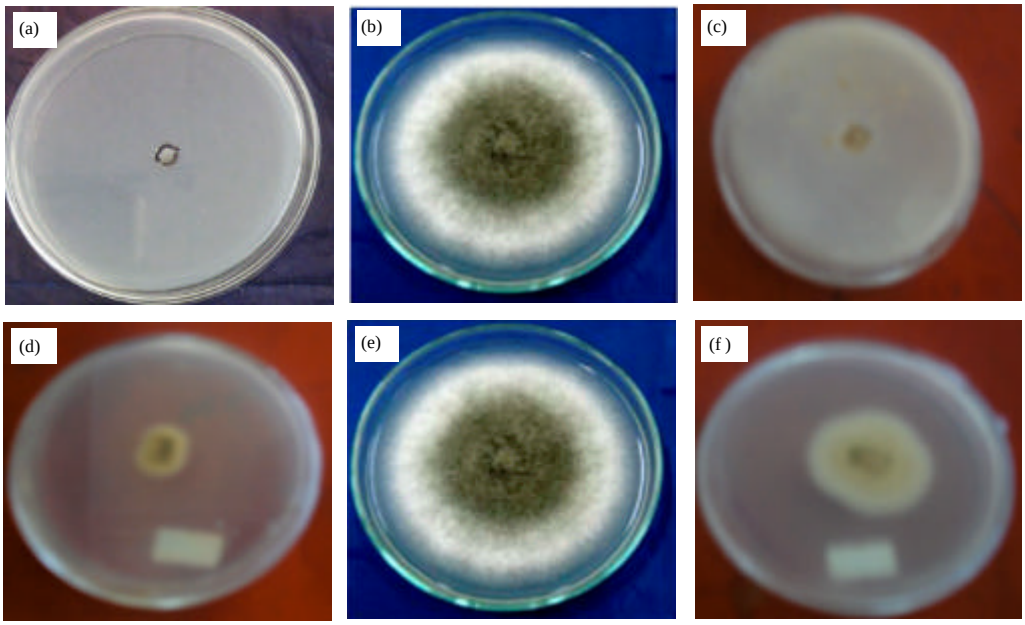

Fig. 1: *In vitro* antifungal activity of the most effective medicinal plant extracts against *Colletotrichum kahawae* pathogen. (a) *Allium sativum* aqueous extract; (b) and (e) untreated plates; (c) *Croton macrostachyus* aqueous extracts; (d) *Allium sativum* 70% ethanol extract and (f) *Croton macrostachyus* 70% ethanol extract)

Table 3: Effect of extraction method and concentration of *Allium sativum* on radial growth of *Colletotrichum kahawae in vitro* test

Method of extraction	Concentration % (w/v)	*Radial growth (mm)
Ethanol extract	5	26^{ab}
	10	23^{b}
	15	15.6^{d}
	20	6.3^{e}
	Control	32^{a}
Aqueous extract	5	26^{ab}
	10	21^{c}
	15	0^{f}
	20	0^{f}
	Control	32^{a}

*Means with the same letter are not significantly different ($\alpha = 0.05$)

between 5% concentration and the control in radial growth (Table 3). The minimum inhibitory concentration for radial growth of *C. kahawae* under *in vitro* condition was 10% for both aqueous and ethanol extracts.

***In vivo* antifungal activity:** The aqueous and ethanol extracts of *A. sativum* and *C. macrostachyus* were tested on detached coffee berries to evaluate their *in vivo* antifungal activities against *C. kahawae* because of their high antifungal effect observed under *in vitro* conditions. There was significant ($p<0.0001$) interaction effect between type of medicinal plants used, extraction method and time of application of the extracts. Application of aqueous extracts on

Table 4: Effect of type of extraction methods, plant species used and time of application on severity of coffee berry disease on detached green berries

		*Disease severity (%)		
Method of extraction	Plant species	48 h before	48 h after	Same time
Aqueous extract	*A. sativum*	29^{f}	45de	27^{f}
	C. macrostachyus	78^{ab}	67^{b}	50^{d}
	Control	94^{a}	94^{a}	94^{a}
Ethanol extract	*A. sativum*	37^{e}	45de	47^{d}
	C. macrostachyus	77^{ab}	79^{ab}	62^{c}
	Control	94^{a}	94^{a}	94^{a}

*Means with the same letter are not significantly different ($\alpha = 0.05$)

Table 5: Effect of methods of extraction, plant species used and time of application on coffee berry disease severity on seedlings in greenhouse

		*Disease severity (%)		
Method of extraction	Plant species	48 h before	48 h after	Same time
Aqueous extract	*A. sativum*	4.1^{d}	27^{c}	30^{c}
	C. macrostachyus	28^{c}	37^{b}	35^{bc}
	Control	83^{a}	83^{a}	83^{a}
Ethanol extract	*A. sativum*	24^{c}	46^{b}	24^{c}
	C. macrostachyus	34^{bc}	46^{b}	42^{b}
	Control	83^{a}	83^{a}	83^{a}

*Means with the same letter are not significantly different ($\alpha = 0.05$)

green berries 48 h before and after inoculations of the test organism significantly ($p<0.001$) reduced severity of coffee berry disease on the berries (Table 4). For *C. macrostachyus*, its aqueous extract significantly reduced severity of the disease when applied at the time of inoculation of the test organism and 48 h after inoculation but not when applied 48 h before inoculation of the pathogen. However, its ethanol extracts reduced severity of the disease significantly only when the extract was applied at the time of inoculation of the pathogen (Table 4).

In order to evaluate the antifungal activity of *A. sativum* and *C. macrostachyus* against coffee berry disease, their aqueous and ethanol extracts were tested on coffee seedlings by applying the extracts at the time of inoculation of the test pathogen and 48 h before and after inoculation and measuring severity of the disease on the seedlings. The results showed that there was significant ($p<0.0001$) interaction effect among methods of extraction, plant species used and time of applications of the extracts on the seedlings. Aqueous and ethanol extracts of *A. sativum* significantly reduced CBD severity on coffee seedlings when applied at the time of inoculation of the pathogen and 48 h after and before inoculation (Table 5). However, in *C. macrostachyus* the aqueous and ethanol extracts consistently reduced the disease severity only when applied 48 h before inoculation of the pathogen.

Incidence of CBD on coffee seedlings varied depending on type of medicinal plant used methods of extraction and time of application of extracts. The study showed that *A. sativum* extracts significantly reduced CBD incidence on the seedlings except when its ethanol extract was applied 48 h after inoculation of the seedlings with the pathogen inoculums (Fig. 2). Whereas, in

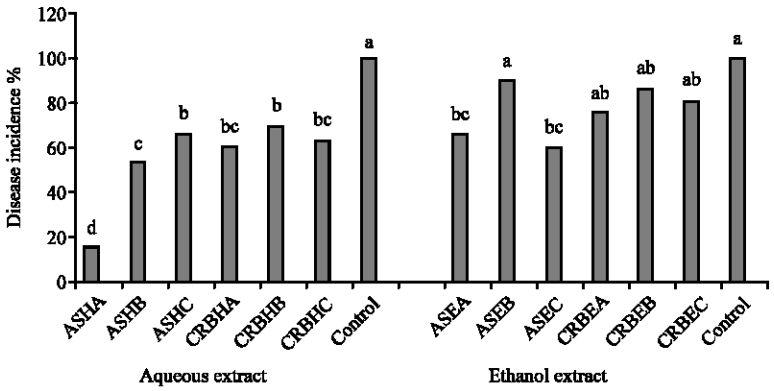

Fig. 2: The effect of method of extraction (aqueous and ethanol extracts) and plant species used on coffee berry disease incidence on seedlings 21 days after inoculation with pathogen in greenhouse. (ASHA) *Allium sativum* aqueous extract applied 48 h before pathogen inoculation; (ASHB) *A. sativum* aqueous extract applied 48 h after pathogen inoculation; (ASHC) *A. sativum* aqueous extract and the pathogen spore treated at the same time; (CRBHA) *Croton macrostachyus* bark aqueous extract applied 48 h before pathogen inoculation; (CRBHB) *C. macrostachyus* bark aqueous extract applied 48 h after pathogen inoculation; (CRBHC) *C. macrostachyus* bark aqueous extract and the spore treated at the same time; (ASEA) *A. sativum* ethanol extract applied 48 h before pathogen inoculation; (ASEB) *A.sativum* ethanol extract applied 48 h after pathogen inoculation; (ASEC) *A.sativum* ethanol extract and pathogen spore treated at the same time; (CRBEA) *C.macrostachyus* bark ethanol extract applied 48 h before pathogen inoculation; (CRBEB) *C. macrostachyus* bark ethanol extract applied 48 h after pathogen inoculation; (CRBEC) *C. macrostachyus* bark aqueous extract and pathogen spore treated at the same time and (Control) Seedlings inoculated only by the pathogen spore. Means followed by different letters are significantly different ($\alpha = 0.05$)

C. macrostachyus its aqueous extract significantly reduced CBD incidence at the three times of applications but its ethanol extract did not show significant difference at all the three times of applications.

DISCUSSION

Plants are rich source of potentially useful antimicrobial products for the development of new chemotherapeutic agents (Mousavi *et al.,* 2009; Musyimi *et al.,* 2008; Ferreira *et al.,* 2009; Safary *et al.,* 2009). Many reports are available on the antifungal, antibacterial, antiviral, anthelmintic, antimolluscal and anti-inflamatory properties of plants (Dey and De, 2010; Mahesh and Satish, 2008; Samy and Ignacimuthu, 2000; Palombo and Semple, 2001). Some of these observations have helped in identifying the active compounds responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, not many reports are available on the exploitation of antifungal or antibacterial property of plants for developing commercial formulations for applications in crop protection.

In this study the antifungal effect of the aqueous and ethanol (70%) extracts of 8 medicinal plants were evaluated against *Colletotrichum kahawae* under both *in vitro* and *in vivo* conditions. The findings showed that the effect of plant extracts on *C. kahawae* radial growth and disease

development vary depending on the type of plant species used, method of extraction and concentration of the extracts applied. *A. sativum* and *C. macrostachyus* were found to be the most effective plants extracts in inhibiting the radial growth of the pathogen *in vitro* and reducing disease development on detached green berries and seedlings *in vivo* condition. Overall, however, *A. sativum* is the most effective plant extract in reducing the radial growth of the pathogen *in vitro* and in reducing severity and incidence of the disease *in vivo* with both aqueous and ethanol extracts consistently. This indicates that *A. sativum* possess antifungal activity against *C. kahawae*. The result of this study corresponds with work done by William (2000) who reported that sprays made from aqueous garlic extracts have antibiotic and antifungal properties and will suppress a number of plant diseases, including powdery mildew on cucumbers and to some extent black spot on roses. Similar results were reported by Slusarenko *et al.* (2008) who tested the effectiveness of garlic juice against a range of plant pathogenic bacteria, fungi and oomycetes *in vitro*. Another investigation by Charimbu *et al.* (2009) on the antifungal activity of garlic extracts against *Phaeoisariopsis griseola* in common bean also showed that the aqueous and methanolic crude extracts of *A. sativum* showed highest antifungal activity compared to commercial fungicide Ridoml.

In an approach towards the development of eco-friendly antifungal compounds for controlling major foliar fungal diseases of tea, (Saha *et al.*, 2005) tested ethanol and aqueous extracts of *A. sativum* against the pathogen *C. camellia* for evaluation of antifungal properties. Results showed that aqueous extracts of *A. sativum* was 100% inhibitory to spore germination (Saha *et al.*, 2005). Garlic extracts were tested against three pathogenic fungi namely *A. flavus, C. lunata* and *F. moniliforme*. The results of phytochemical screening showed that water and ethanol extracted more components from garlic extract. In most traditions, decoctions or infusions of herbs are usually made with either alcohol or water as the solvent (Olusanmi and Amadi, 2010). This may be related to their efficiency in extracting most of the active principles in plants. At times, marked differences exist between the phytochemical profile of alcoholic and aqueous extracts of plants. For *A. sativum,* the aqueous extract is recommended because no vital phytochemical constituent seemed to be left out and also because of probable unwanted effects that alcohol which is another drug on its own may produce (Olusanmi and Amadi, 2010).

Aqueous extracts of *A. sativum,* has been reported to inhibit the growth of *Alternaria alternata*, *A. brassicola* and *Myrothecium roridum* (Khan *et al.,* 1998). Su and Cheng (2009) also reported total inhibition of mycelia growth of *Phytophthora capsici* at different concentrations. In general, voluminous number of literature reported the effectiveness of *A. sativum* against most pathogenic microorganisms. A crude extract of *A. sativum*, however, showed the lowest inhibitory effect (<16%) at 10% concentration against *Botryodiplodia theobromae* Pat and *Macrophomina phaseolina* (Tassi) Goidanich. among six tested fungal pathogens isolated from rotted cassava roots (Okigbo *et al.*, 2009).

The effects of the antifungal compounds may be on spore germination leading to its inhibition or may be due to effect of these compounds on the cell wall altering its permeability (William, 2000). The antifungal compounds may also suppress the early stages of mycelia growth leading to inhibition of the fungus.

The antifungal activity of *A. sativum* may be due to sulfur containing compounds such as ajoene allicin found in it. Allicin is produced in garlic when the tissues are damaged (Slusarenko *et al.,* 2008) and it has been found to effectively control seed-borne *Alternaria* sp. in carrot, *Phytophtora* leaf blight of tomato, tuber blight of potato and downy mildew of *Arabidopsis*.

CONCLUSION

The present study suggested that aqueous and ethanol extracts of *A. sativum* bulb parts have the potential to be applied as a control measure against infection of coffee berry disease caused by *C. kahawae*. The application of aqueous extract of *A. sativum* bulb seems promising as it is easy, effective and cheap alternative means of *C. kahawae* management for the majority of Ethiopian subsistence farmers, who cannot afford synthetic chemicals. Moreover, the risk associated with synthetic chemicals as well as consumers' resistance, towards its application in agriculture make the product more attractive natural product for organic agriculture. This study provides new scientific information on antifungal activity of *A. sativum* against *C. kahawae*. The extracts should be tested against the disease under field conditions.

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