

Antifungal Potential of *Curcuma longa* (Turmeric) and *Zingiber officinale* (Ginger) against *Alternaria alternata* Infecting Spinach in Kenya

Kirarei Ezra Kipkogei*, Kipsumbai Pixley Kiptui, Kiprop Ezekiel

Department of Biological Sciences, University of Eldoret, P.O Box 1125-30100, Eldoret, Kenya

*Corresponding author: ekirarei@gmail.com

Received September 11, 2019; Revised October 15, 2019; Accepted October 28, 2019

Abstract Spinach diseases caused by *Alternaria* spp. are one of the most significant devastating pathogens to spinach in Kenya and worldwide. *Alternaria alternata* has been associated with great losses in spinach both in total biomass yield and leaf quality. The pathogen has been traditionally controlled using synthetic fungicides which are expensive and harmful to both humans and environment. This study aimed at investigating the efficacy of the extracts of two plants; *Curcuma longa* (Turmeric) and *Zingiber officinale* (Ginger) against *Alternaria alternata* both in *in vitro* and *in vivo* conditions. Absolute ethanol, water, ethyl acetate and methanol were the solvents used in extraction of *Curcuma longa* and *Zingiber officinale* rhizome extracts. Decoctions were screened for antimycotic potential using the poisoned food technique. Results from this study revealed that *Curcuma longa* and *Zingiber officinale* extracts had varying degree of antifungal activity against the *Alternaria alternata* depending on the solvent used for the extraction and the concentration. Methanolic extracts of *Curcuma longa* and *Zingiber officinale* demonstrated the highest antifungal activity which was significant ($p \leq 0.05$) against the *Alternaria alternata* compared to ethanolic, ethyl acetate and aqueous extracts with percentage inhibition of 64% and 57%, respectively at the concentration of 50 mg/ml. At the concentration of 25 mg/ml and 50 mg/ml the percent inhibition on the fungal growth was not significant ($p \leq 0.05$) from the 8th day for all the solvents in both turmeric and ginger. Foliar spray with the extracts was found to be effective in lowering disease severity. *Curcuma longa* displayed the highest percent decrease index in comparison to *Zingiber officinale* with percent disease decrease index of 57.70% and 53.84%, respectively. The findings indicated methanol as the most suitable solvent for descending in the use of *Curcuma longa* and *Zingiber officinale* extracts in controlling *Alternaria alternata* associated with leaf spot of spinach in Kenya.

Keywords: spinach, plant extracts, antifungal, plant disease control, *Alternaria alternata*, *Curcuma longa*, *Zingiber officinale*

Cite This Article: Kirarei Ezra Kipkogei, Kipsumbai Pixley Kiptui, and Kiprop Ezekiel, "Antifungal Potential of *Curcuma longa* (Turmeric) and *Zingiber officinale* (Ginger) against *Alternaria alternata* Infecting Spinach in Kenya." *World Journal of Agricultural Research*, vol. 7, no. 4 (2019): 124-131. doi: 10.12691/wjar-7-4-2.

1. Introduction

Spinach (*Spinacia oleracea*) is an edible flowering plant in the family Amaranthaceae and is native to central and western Asia [1,2]. In Kenya, spinach is grown mostly by small scale holders for home consumption and for the market [3]. The total area under spinach production in Kenya was 5,615 hectares producing 75,563 tonnes in 2016 [4]. Spinach like other vegetables is infested by pests and diseases. Some of the major diseases infecting spinach are bacterial soft rot, cucumber mosaic virus, downy mildew, fusarium wilt, white rust and alternaria leaf spots which cause huge losses [5,6]. The genus *Alternaria* is an important plant pathogen causing foliage infections and consequently contributes to post-harvest loss of yield in crops and other plant products [7].

Alternaria leaf spot affliction on spinach caused by *A. alternata* has been noted to cause huge economic losses in the kingdom of Saudi Arabia [8], Poland [9] and Pakistan [10] where the mortality due to the disease was found to range between 20-80%. [8] Noted that leaf spot disease caused by *Alternaria alternata* is of economic importance as it results in significant loss in yield and reduction in quality of leaves. [7] noted that consumption of foodstuff contaminated with *Alternaria* toxins have increased incidence of esophageal carcinoma in humans. Further, the sensitivity to *Alternaria* sp. is a key agent in inducing asthma and allergic rhinitis on immune-depressed individuals [11].

Leaf spot diseases have generally been managed by synthetic fungicides. Continuous and wide use of these fungicides has caused serious threats to both human and environmental health [12]. Further, these pathogens have been known to develop resistance to these fungicides [13].

Due to increased awareness about the risk involved in the fungicides, the use of other alternative bio approaches in the management of plant diseases is gaining importance.

Several findings have noted the ability of decoctions from various sections of plant as an exceptional bio-control method in managing plant pathogenic fungi [14]. Ginger is known for its ethanomedicinal and nutritional values. [15] Reported ginger has antimycotoxigenic effects. [16] Noted that extracts from turmeric (*Curcuma longa*) have antifungal properties. [17] on their study found that *Curcuma longa* and *Zingiber officinale* were effective in controlling human dermatophytic fungi.

The extraction method, the type of plant material and the type of solvents used dictates the quality of plant extract [18]. [19] reported that a reliable solvent in plant extractions should have low toxicity, ease of evaporation at low heat, promotion of faster physiologic absorption of the extract, preservative potential and incapability to cause the extract to complex or dissociate. The main objective for this study was to test the antimycotic activity of ginger and turmeric in the control of *Alternaria alternata* causing leaf spot in spinach.

2. Materials and Methods

2.1. Isolation and Identification of *Alternaria* Species

Spinach leaves infected with *Alternaria* leaf spot were collected from Nandi and Uasin Gishu Counties in Kenya, and taken to University of Eldoret laboratory for pathogen isolation. One centimeter portion of the diseased section with early levels of infection was cut from infected leaves and cleansed in running tap water for 30 seconds and thereafter surface sterilized using 1% sodium hypochlorite for 3 minutes. It was then rinsed in three changes of sterile distilled water and plated onto potato dextrose agar (PDA) media in a 90 mm diameter petri plate under aseptic lamina conditions. The inoculated petri dishes were incubated at $25 \pm 2^\circ\text{C}$ and observations made from 24 hours onwards. After 5 days, hyphal tip transfer was made onto newly prepared PDA plates. Pure cultures of sporulating fungi were obtained by single spore isolation technique and grown in PDA medium as described by [20].

Alternaria alternata, was identified using taxonomic, cultural and morphological reference as described by [21] and [22].

2.2. Pathogenicity Test

Approximately twenty five kilograms of loam soils were sterilized in the autoclave at 121°C and 15 Psi for 45 minutes, and left to cool for six days then transferred to the nursery at the greenhouse. *Alternaria alternata* susceptible spinach variety (Fort Hook) was grown in the nursery containing sterile soils. The seedlings were transplanted onto the plastic pots measuring sixteen centimeters (16 cm) in diameter containing 1.5kgs of sterile loam soil. The plants were inoculated when they had 3-5 well developed leaves.

2.2.1. Inoculum Preparation and Inoculation of the Spinach

The inoculum was yarked from the isolate by flooding 10 day old PDA cultures with 10 milliliters of sterile distilled water, gently rubbed with a sterile glass rod then put in sterile universal bottles and shaken for twenty minutes in a shaker (Aerotror Infoors AG CH-4103 Bottmingen, Switzerland) at 150 revolutions per minute (rpm) to dislodge the spores. The spore suspension was then filtered through two layers of sterile cheese cloth then conidial concentration estimated with the help of haemocytometer. A mean of five counts was calculated per colony and then adjusted to 1×10^7 conidia per milliliter.

Six weeks old plants (3-5 leaves) were inoculated with the adjusted (1×10^7 conidia per milliliter) conidial suspension by spraying with simple atomizer. Check plants were sprayed with sterile distilled water. Plants were enclosed for 24 hours with plastic bags to keep 100% relative humidity as described by [8]. Plants were kept in completely randomized design in the greenhouse at the University of Eldoret and monitored daily for the appearance of initial leaf spots symptoms.

2.3. Testing Antifungal Potential of Medicinal Plant Extracts for the Management of *Alternaria* Species

2.3.1. Collection of Medicinal Plants and Extraction of Turmeric and Ginger Plant Extracts

Plant samples were collected based on the guiding principle of the plant antifungal history from herbalists and documented findings by various researchers worldwide [23,24]. The plants tested for antifungal potential were rhizomes of turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*).

The collected plant parts were washed in running tap water to get rid of dirt, insects and any other unwanted foreign matter. They were chopped into small pieces then dried on the sun for three days up to moisture content of 13%. The dried plant parts were then ground into powder using an electric grinder. The ground parts were then preserved in sealed labeled glass bottles until use.

The extraction technique used was the modification of the homogenization in solvent as described by [8]. The solvent-to-sample ratio of 10:1 (v/w) solvent to dry weight ratio was used. Forty grams of each of the sun dried and ground powdered material from *Zingiber officinale* and *Curcuma longa* were dissolved with 400 ml of absolute solvents (methanol, ethanol, water and ethyl acetate) in sterile conical flasks with constant shaking on a shaker for 24 hours. After stirring, the solution was filtered through 2 layers of sterile cheese-cloth gauze and centrifuged at 5000 rpm for 20 minutes in a centrifuge (6000 series Centurion) to remove suspended fine particles before subjecting the filtrates to evaporation in Rotary Evaporator (Buchi Rotavapor R-3000, Switzerland) at 65°C , 79°C , 77°C and 100°C for solvent evaporation and purification of the extract respectively as per the boiling point of each solvent. The oily matter from the rotary evaporator was removed and dried in the water bath at 40°C for 12 hours. The dried material from the water bath was kept in small,

sterilized 5 ml screw-capped glass bottles and kept in the refrigerator (4°C) until further usage.

2.3.2. Evaluation of Antifungal Activity of Crude Plant Extracts

2.3.2.1. Preparation of plant extracts dilutions

The turmeric and ginger extracts were removed from the refrigerator. Aliquots of 0.05 g, 0.025 g and 0.0125 g of each extract (turmeric and ginger) were weight and kept until use.

2.3.2.2. *In vitro* screening of plant extracts against *Alternaria alternata* infecting spinach

Poisoned food technique as described by [25] was used. PDA media was weighted into fifty glass flasks of 60ml and autoclaved. After autoclaving, the media was cooled down to about 45°C. The 0.05 g, 0.025 g and 0.0125 g were aseptically diluted with PDA media to make dilutions 50 mg/ml, 25 mg/ml and 12.5 mg/ml for each turmeric and ginger specific solvent extract. Shaking was done gently for 1 minute to permit proper mixing of extract. Approximately 20 ml aliquots of the amended media were dispensed into sterilized 90 mm petri-dishes replicated thrice. A cork-borer was used to cut 10 mm agar discs from an actively growing *Alternaria alternata* cultures and placed at the center of the plate and incubated at room temperature (25±2°C). Control plates with media not amended with plant extracts was inoculated and incubated in the same conditions. Observations were made at day 4, 8, 12, 16 and 20 after plating as until control filled a 90 mm petri plate. The Petri-dish inoculated without the decoction concentrations, served as check. The experimental design was a completely randomized design with three replications.

2.3.2.3. Data collection and data analysis

Colony distance was determined by measuring the mean radial growth in millimeters (mm). The inhibition area (I), was calculated using the expression of [26] as follows:

$$\% \text{ Inhibition} = \frac{C - T}{C} \times 100$$

C= Pathogen radial growth in mm in control

T=Radial growth in mm in treated plates.

Data on percentage inhibition of fungal culture by plant extracts were analysed by ANOVA procedures using the GenStat computer Software 14th Edition, release 14.10.5943, 2013 (VSN International Ltd). Means separation was by using Fishers unprotected least significant difference (LSD) at 0.05.

2.3.2.4. *In-vivo* evaluation of plant extracts against the *Alternaria* leaf spot of spinach

Susceptible spinach (Fort Hook) plants in pots placed in the greenhouse were sprayed with *Alternaria alternata* at the concentration of 1×10^7 conidia per milliliter. After the appearance of the first symptoms (9th day), methanol extracts of turmeric and ginger were sprayed at the concentration of 50 mg/ml. Control plants were left untreated/unsprayed. The experiment was conducted in a complete randomized design with three replications.

2.3.2.5. Establishment of disease reduction and data analysis

To study the disease severity a scorecard technique following a 5 point scale (0-5) for scoring leaf spot disease as described by [27] was adopted. From each plant, 3 leaves were selected randomly and marked. Disease scoring was done at 7, 14, 21 and 28 days after planting. The rating scales used for the study of disease severity were as follows;

Disease grade	Disease scale (%)	Description
0	0	No symptoms
1	1-10	Leaf area covered
2	11- 25	Leaf area covered
3	26 - 50	Leaf area covered
4	51 - 75	Leaf area covered
5	>75	Leaf area covered

The percent disease index (PDI) was calculated by the techniques of [28].

$$PDI = \frac{\text{Sum of all numerical ratings}}{\left(\frac{\text{Number of leaflets observed}}{\times \text{maximum rating used}} \right)} \times 100.$$

Percent decrease in PDI

The percent decrease in PDI was calculated by using the formula given by [27]

$$\frac{C - T}{C} \times 100$$

Where; C- PDI observed in control treated
T-PDI observed in different treatments.

3. Results

3.1. Pathogenicity of *Alternaria alternata*

Alternaria leaf spot susceptible spinach variety was used to confirm the pathogenicity of the isolate of *A. alternata* to cause leaf spot. The initial symptoms of *Alternaria* leaf spot on Fort Hook variety appeared on the ninth day and the pathogen re-isolated onto PDA medium from infected plants after 20 days. The resulting fungus from the inoculated plants was identical to that originally noted on the spinach leaves, confirming the Koch's postulates.

3.2. *In-vitro* Antifungal Activities of Ginger (*Zingiber officinale*) Rhizome Extract against *Alternaria alternata*

Extracts of ginger (*Zingiber officinale*) showed varying anti-fungal potential against *Alternaria alternata* which depended on the solvent used during extraction and the concentration of the extract at which the fungi was exposed (Plate 1a to d). There was significant difference ($P \leq 0.05$) among the extracts of *Zingiber officinale* inhibition activity on *Alternaria alternata* (Figure 1).

Methanolic extracts of ginger rhizomes showed the highest antifungal potential against the *Alternaria alternata* with a percentage inhibition of between 40.1% and 57.4% in the concentration of 12.50 mg/ml and 50mg/ml of the extract. Aqueous extract of ginger rhizomes showed moderate antifungal potential against the *Alternaria alternata* with percentage inhibition of between 31.2% and 56.6% in the concentration of 12.50 mg/ml and 50 mg/ml of the extract, similarly ethyl acetate extract of ginger demonstrated moderate antifungal activity against the *Alternaria alternata* with percentage inhibition of between 22.8% to 52.3% in 12.50 mg/ml and 50.00 mg/ml of the extract respectively (Figure 1). The lowest antifungal potential against the *Alternaria*

alternata was shown by ethanolic extracts which ranged between 25.4% and 43.0% on 12.50 mg/ml and 50 mg/ml respectively.

There was noticeable difference ($P \leq 0.05$) among the extracts in the ginger inhibition activity against *Alternaria alternata* (Table 1). Methanolic extract of *Z. officinale* demonstrated the highest antifungal activity which was significant ($P \leq 0.05$) against *Alternaria alternata* compared to ethanolic, aqueous and ethyl acetate extracts, but the percent inhibition was not significant ($P \leq 0.05$) from day 8th in all the solvents of the extraction for ginger. Percentage inhibition was not significantly different in aqueous and ethyl acetate extracts from day 4 to day 12 and from day 4 to day 16 respectively.

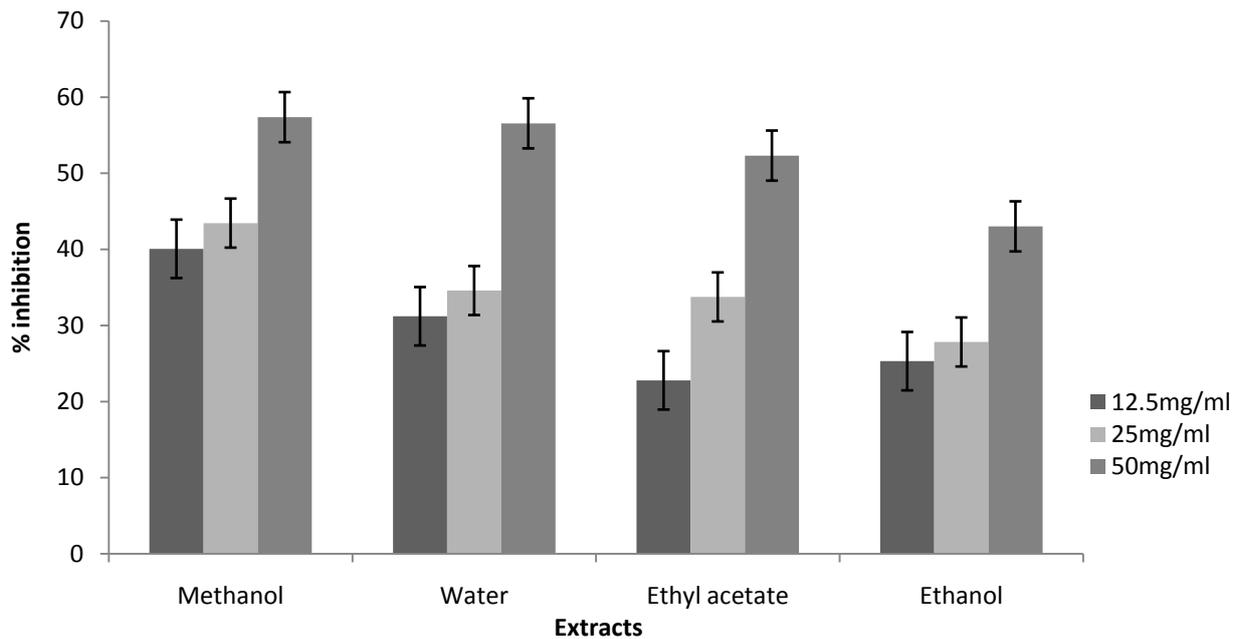


Figure 1. Percentage inhibition by ginger extracts at various levels of concentration at day 20

Table 1. Antifungal effect of ginger rhizomes against *Alternaria alternata* at different days

Days	Ginger			
	Methanol	Ethanol	Water	Ethyl acetate
Day 1	0.0a	0.00a	0.0a	0.0a
Day 4	22.88b	14.9b	19.75b	20.86b
Day 8	42.17c	24.61c	28.87bc	31.77bc
Day 12	43.61c	24.74c	30.2bc	32.13bc
Day 16	45.76c	27.8c	34.37c	33.64bc
Day 20	46.97c	32.06c	40.79c	38.68c
LSD (0.05)	9.668	8.988	14.181	15.668
CV	15.8	23.9	30.4	32.9

Values with the same letter (s) along the same column are not significantly different ($P \leq 0.05$).



Plate 1. The suppressed colonies displayed by different ginger extracts at the concentration of 50mg/ml, (a) Methanolic extract, (b) ethanolic extract, (c) aqueous extract and (d) ethyl acetate extract

3.3. *In-vitro* Antifungal Activities of Tumeric (*Curcuma longa*) Rhizome Extract against *Alternaria alternata*.

Extracts of turmeric (*Curcuma longa*) showed varying levels of anti-fungal potential depending on the solvent used for extraction and the concentration at which the *Alternaria alternata* was exposed (Plate 2a to d). There was significant difference ($P \leq 0.05$) among the turmeric decoctions against *Alternaria alternata* (Table 2). Methanolic extracts of turmeric rhizomes showed the highest antifungal potential against the *Alternaria alternata* with a percentage inhibition of between 46.3% and 64.56% in the concentration of 12.50 mg/ml and 50 mg/ml of the extract. Turmeric rhizomes extracted by ethyl acetate showed moderate antifungal potential against the *Alternaria alternata* with percentage

inhibition of between 33.74% and 62.88% in 12.50 mg/ml and 50 mg/ml of the extract respectively. Similarly, ethanolic extract of turmeric demonstrated moderate antifungal activity against the *Alternaria alternata* with percentage inhibition of between 36.27% and 59.92% in 12.50 mg/ml and 50 mg/ml of the extract respectively (Figure 2). The lowest antifungal potential against the *Alternaria alternata* was shown by aqueous extracts which ranged between 42.56% and 54.82% on 12.50 mg/ml and 50 mg/ml respectively as shown in Figure 2.

It was established that from day 4 to day 8 the inhibition percentage showed noticeable difference ($P \leq 0.05$) in all the decoctions against *Alternaria alternata*. However, from day 8 onwards there was no significant difference except in the water extracts of turmeric as shown in (Table 2).

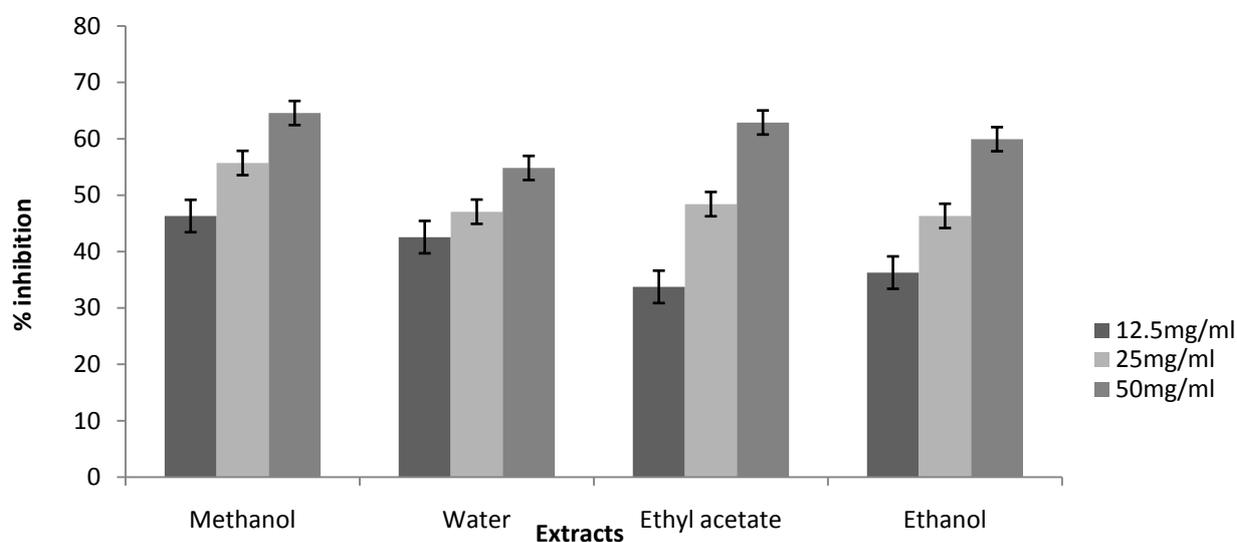


Figure 2. Percentage inhibition by tumeric extracts at various levels of concentration at day 20

Table 2. Antifungal effect of tumeric rhizomes against *Alternaria alternata* at different days.

Days	Tumeric			
	Methanol	Ethanol	Water	Ethyl acetate
day 1	0.00a	0.00a	0.00a	0.00a
day 4	26.77b	20.3b	28.94b	32.22b
day 8	44.96c	38.92c	42.23c	44.96c
day 12	45.43c	39.63c	44.07c	45.76c
day 16	47.61c	40.75c	45.6cd	46.66c
day 20	50.92c	43.17c	50.49d	47.67c
LSD (0.05)	9.709	11.173	4.968	11.076
CV	14.8	20.2	7.8	16.8

Values with the same letter(s) along the same column are not significantly different ($P \leq 0.05$).

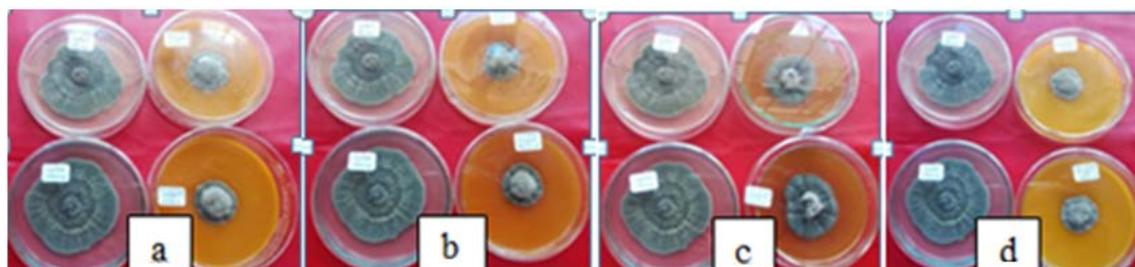


Plate 2. The suppressed colonies displayed by different tumeric extracts at the concentration of 50mg/ml. (a) Methanolic extract, (b) ethanolic extract, (c) aqueous extract and (d) ethyl acetate extract

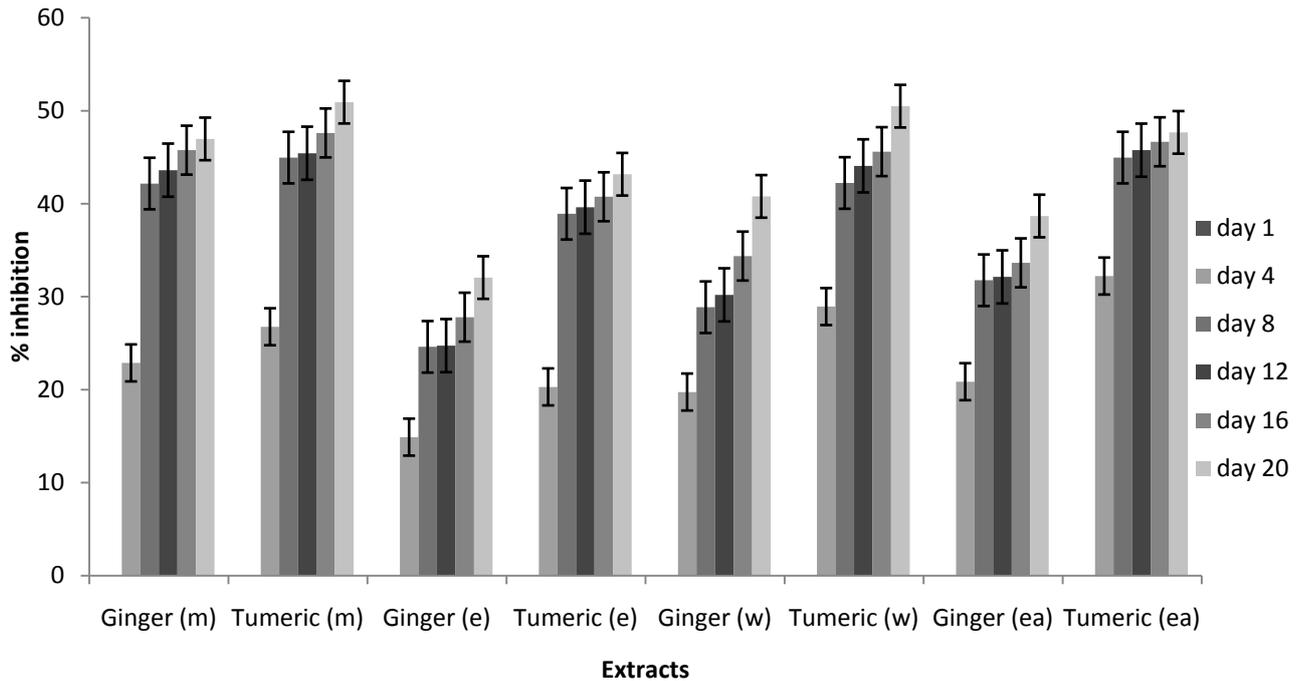


Figure 3. Comparison of antifungal activities of *Curcuma longa* and *Zingiber officinale*

3.4. Comparison of Anti-fungal Activities of Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale*)

For all the plant extracts (Methanolic extract, Ethanolic extract, Acqueous extract and Ethyl acetate extract) at different levels of concentrations the activity of extracts on the growth of the *Alternaria alternata* was found to be active from day 8 of inoculation (Figure 3). There was significant difference ($P \leq 0.05$) among the extracts of ginger and turmeric inhibition activity on *Alternaria alternata*. The growth of the *Alternaria alternata* was actively inhibited with almost constant growth inhibition from day 8 onwards. With increasing concentration of the plant extract, the inhibition percentage increased. Methanolic extract of turmeric showed the highest inhibition rate at the concentration of 50 mg/ml followed by aqueous extract of turmeric. Ethanolic extract of ginger had the least inhibition percentage on the *Alternaria alternata* in all the plant extracts tested. Extracts of *Curcuma longa* performed better than the *Zingiber officinale* extracts (Figure 3).

3.5. In-vivo Evaluation of Plant Extracts

There was a percent disease index (PDI) of 18.3% and 20% on spinach treated with turmeric crude methanolic plant extracts and ginger crude methanolic plant extracts, respectively (Table 3). This was significantly different ($P \leq 0.05$) with the non-treated infected plant. The disease decrease when compared with non-inoculated control was 57.7% for turmeric crude extracts and 53.8% reduction in disease from spinach treated with ginger plant extracts. However from these results there was no significant difference ($P \leq 0.05$) in disease reduction between the crude extracts of the two plants in controlling *Alternaria* leaf spot affliction in spinach.

Table 3. *Alternaria* leaf spot PDI and percent disease decrease from crude plant extracts treatment

Treatment (50 mg/ml)	PDI	Percent disease decrease
Turmeric	18.33%	57.70%
Ginger	20.00%	53.84%
Control	43.33%	

4. Discussion

4.1. Antifungal Potential of Ginger and Turmeric against *Alternaria alternata*

These results are in conformity with the earlier findings of [29] who reported that extracts from *Z. officinale* and *Curcuma longa* had significantly inhibited mycelial growth of *Alternaria alternata* infecting different crops. They reported 73.6% by *Zingiber officinale* and 60.2% by *Curcuma longa* where the mycelial radial growth of *Alternaria alternata* decreased drastically with increment in concentrations of the trial bioagents from 10 to 20 percent. [30] Studied the phytochemical contents of *Zingiber officinale* and reported that it contains hydrocarbons such as α -seliene, α -pinene, ar-curcumene, camphor- α -farnesene, tricyclene, neral, elemol, zingiberenol and octane. On subjection to *Alternaria alternata* at different concentrations, they noted a percentage growth inhibition of 45%, 66% and 73% at the concentrations of 5%, 10% and 20% respectively. Similar findings had earlier been documented by [31], [32] and [33] attributed the activity of ginger to the presence of phenolic amalgams; gingerol, cendrene, zingiberene, phellandrene, geranial, neral, β -bisabolene, alkaloids, saponins, tannins, flavonoids and terpenoids while *Curcuma longa* is rich in phenolic compounds; alkaloids, terpenoids, steroids, flavonoids, tannins, saponins, secondary metabolites such

as curcuminoids and hydrophobic essential oils [34] which could be the active ingredients causing the present results.

The present results are in tandem with those of [35] who noted ethanolic infusions of *Curcuma longa* are active against *Alternaria alternata* and *Phythium* sp. [36] documented similar findings on garlic, neem and turmeric extracts against *Alternaria porii*. From a study by [37], ethanolic extracts of *Momordica charantia* showed 44% inhibition against the mycelial growth of *Alternaria alternata* and 100% on spore germination at the concentration of 60 mg/ml. In our study, methanolic, ethanolic, aqueous and ethyl acetate extracts of both *Curcuma longa* and *Zingiber officinale* demonstrated that 25mg/ml minimum inhibitory concentration against *A. alternata* was obtained. [16] Reported efficacy of a number of plant decoctions against important pathogens of *Lycopersicon esculentum*. Amid the tested plant extracts, *Curcuma longa*, *Allium sativum*, *Citrus limon* and *Zingiber officinale* were the most effective extracts. They reported that *Curcuma longa* was the most brisk on the tested fungal phytopathogens decreasing their radial growth by 72% on *Alternaria solani*. *Curcuma longa* was sensitive against both young and old cultures of fungal pathogens. Extracts from *Zingiber officinale* and *Rosmarinus officinalis* showed high antifungal activity on young cultures of the fungal pathogens. There was difference in the antifungal ability of the plant decoctions at various levels of development of the fungal phytopathogens similar to the findings from the present study where variation in radial growth of *Alternaria alternata* was observed from day 1 to day 8 but henceforth the percent inhibition was not significantly different. Similarly, [38] reported that ethanolic extracts of *Zingiber officinale*, *Allium cepa* and *Allium sativum* at concentrations of 50%, 75% and 100% showed high inhibition activity against the mycelial growth of *Alternaria solani* whereas the plant extract concentration level increased, it resulted to direct decrease in mycelial growth. [13] Compared the ethanolic fractions of *Zingiber officinale* viz; ethyl acetate, chloroform, petroleum ether, n-butanol and aqueous extracts against the *Alternaria alternata* causing leaf spot of spinach. The findings showed that fraction of ethyl acetate displayed highest antimycotic inhibition on the *Alternaria alternata* succeeded by petroleum ether and chloroform while aqueous decoction and n-butanol showed no inhibition effect, which differed with present study where methanolic extracts of both ginger and turmeric at higher concentrations demonstrated higher antifungal activity. Similar to the present findings, [39] observed that increasing the concentrations of ginger oil extract results to more suppression of the fungi. Researchers attributed the higher antifungal activity of *Zingiber officinale* to the high level of phytoconstituents of both non-volatile and volatile chemicals like Shogaols, zingerone, sesquiterpenoids, ingerols, monoterpenoids, α -curmene, cedrene and gingerol [32,40].

The difference in the antimycotic actions of the studied plant decoctions could be as a result of extraction solvent, the method of extraction, origin and nature of the plants [41,42]. Further, the efficacy of the plant is controlled by responsiveness of the trial phytopathogen and the strength of the decoctions [43,44]. [45] Noted that the type and amount of active phytochemicals in a plant depends on the

origin and nature of the plant. [46] Observed that aqueous decoctions were more effective than organic decoctions while [43] noted that the use of water in extraction raises the levels of additives and this could negatively affect the purity of extracts. [47] Observed from their study that for effective efficacy from different plant decoctions against target pathogens then different level of concentrations are applied.

5. Conclusions

Tumeric and ginger plant decoctions studied at *in vitro* and *in vivo* were noted to have antifungal potential against *A. alternata*. Methanolic extracts of both ginger and turmeric demonstrated the highest antifungal activity against the *A. alternata*. However, turmeric extracts showed higher antifungal activity against *A. alternata*.

The inhibition levels observed at *in vitro* and *in vivo* revealed the ability to control and manage plant phytopathogens and consequently could be used instead of synthetic pesticides upon purification and formulation thereby minimizing risks and hazardous effects of the synthetic fungicides.

Statement of Competing Interests

The authors have no competing interests.

References

- [1] Sato, A., Takeda, H., Oyanagi, W., Nishihara, E. & Murakami, M. (2010). Reduction of cadmium up take in spinach, *Spinacia oleracea* L. by soil amendment with animal waste compost. *181*, 298-304.
- [2] Wachira, M. J., Mshenga, M. P. & Saidi, M. (2014). Comparison of profitability of small scale green house and open-field tomato production systems in Nakuru North District, Kenya. *Asian Journal of Agricultural Sciences* 6, 54-61.
- [3] MoA. (2016). External Evaluation of Horticulture and Food Security Program. Ministry of Agriculture, Kenya.
- [4] FAOSTAT. (2016). Crops/ Regions/ World list of Production Quality of Spinach. The Food and Agricultural Organisation of the United Nations. Rome, Italy.
- [5] MoA. (2000). Local and Export Vegetables: growing Manual. Ministry of Agriculture, Rural Development, Kenya & Japan International Cooperation Agency, Printed by Agricultural Information Resource Centre, Nairobi, Kenya.
- [6] Singh, V., Vhrivastava, A., Jadon, S., Wahi, N., Singh, A. & Sharma, N. (2015). *Alternaria* Diseases of Vegetable Crops and its Management Control to Reduce the Low Production. *International Journal of Agriculture Sciences*, 13, 834-840.
- [7] Thomma, B. (2003). *Alternaria* spp. from general saprophyte to specific parasite. *Mol. Plant Pathol*, 4, 226-236.
- [8] Marraiki, N. N., Siddiqui, I., Rizwana, H. & Javaid, J. (2012). First report of *Alternaria alternata* leaf spots on spinach in Saudi Arabia. *The Journal of Animal & Plant Sciences*, 22 (1), 247-248.
- [9] Czajka, A., Czubatka, A., Sobolewski, J. & Robak, J. (2015). First Report of *Alternaria* Leaf Spot Caused by *Alternaria alternata* on Spinach in Poland, 99 (5), 729-738.
- [10] Aslam, S. H., Aslam, M. U., Abbas, A., Ali, M. A., Alam, M. W., ... Amrao, L. (2019). First Report of Leaf Spot of Spinach Caused by *Alternaria alternata* in Pakistan. *Plant Disease*, 103 (6), 1430-1442.
- [11] Kuna, P., Kaczmarek, J. & Kupezyk, M. (2011). Efficacy and safety of immunotherapy for allergies to *Alternaria alternata* in children. *J. Allerg. Clin. Immunol*, 127, 502-508.

- [12] Mizubuti, G. S. E., Junior, V. L. & Forbes, G. A. (2007). Management of late blight with alternative products. *Pest Technol.* 1(2), 106-116.
- [13] Humaria, R. (2015). Exploiting antifungal potential of ginger for the management of *Alternaria alternata*, the cause of leaf spot disease of spinach. *Mycopathology*, 13 (2), 97-104.
- [14] Shafique, S., Shafique, S., Bajwa, R., Akhtar, N. & Hanif, S. (2011). Fungitoxic activity of aqueous and organic solvent extracts of *Tagetes erectus* on phytopathogenic fungus-*Ascochyta blighti*. *Pak. J. Bot.* 43, 59-64.
- [15] Tatsadjieu, N. L., Dongmo, P. M. J., Ngssoum, M. B., Etoa, F. X. & Mbofung, C. M. F. (2009). Investigation on the essential oil of *Lippia arugosa* from Cameroon for its potential use as antifungal agent against *Aspergillus flavus*. *Food Cont.*, 20, 161-166.
- [16] Muthomi, J. W., Geraldin, M. W. L., Maina, J. W. & Rama, D. N. (2017). *In vitro* activity of plant extracts against some important plant pathogenic fungi of tomato. *Australian journal of crop science*, 11(06), 683-689.
- [17] Pankaj, S. & Richa, S. (2017). Antifungal screening of *Curcuma longa* and *Zingiber officinale* against dermatophytes causing superficial Mycosis. *Int. J. Pharm Res Health Sci.* 5, 1980-1983.
- [18] Malkhan, S. G. Shahid, A., Masood, A. & Kangabam, S. S. (2012). Efficacy of plant extracts in plant disease management. *Journal of Agricultural Sciences*, 3 (3), 425-433.
- [19] Ncube, N., Afolayan, S.A.J. & Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. *African Journal of Biotechnology*, 7, 1797-1806.
- [20] Noman, E., Al-Gheethi, A. A., Rahman, N. K., Talip, B., Mohamed, R. and Kadir, O. A. (2018). Single Spore Isolation as a Simple and Efficient Technique to obtain fungal pure culture. *IOP Conf. Series: Earth and Environmental Science*, 140, 012-055.
- [21] Ellis, M. B. (1971). *Dematiaceae Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England. 464-497.
- [22] Simmons, E. G. (2007). *Alternaria*. An Identification Manual. In: Biodiversity No 6. CBS fungal diversity centre, Utrecht, The Netherlands.
- [23] Al-Samarrai, G., Singh, H. & Syarhabil, M. (2012). Evaluating eco-friendly botanicals as alternatives to synthetic fungicides. *Annals of Agriculture and Environ. Medicine*, 9, 673-676.
- [24] Goufo, P., Fontem, A. & Ngnokam, D. (2010). Evaluation of plant extracts for tomato late blight in Cameroon. *NZ. J. Crop Hortic. Sci.* 38, 171-176.
- [25] Grover, R. K. & Moore, J. D. (1962). A toximetric study of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. *Phytopathol.* 52, 876-880.
- [26] Francisco, D. H. (2010). *Lippia graveolens* and *Carya illinoensis* Organic Extracts and their *in vitro* Effect against *Rhizoctonia Solani* Kuhn. *American Journal of Agricultural and Biological Sciences*, 5 (3), 380-384.
- [27] Biswas, M. K. & Tanmay, G. (2018). Evaluation of Phyto-extracts, Biological Agents and Chemicals against the Development of *Alternaria brassicae* *in vitro* and *in vivo* *European Journal of Medicinal Plants*, 22(3), 1-9.
- [28] McKinny, H. H. (1923). A new system of grading plant diseases. *Agric. Res.* 26, 95-98.
- [29] Vasudha, A., Kadam, D. N., Dhutraaj, D. V. P. & Patil, D. D. (2018). Bio Efficacy of Bio Agents and Botanicals against *Alternaria alternata* (Fr.) Keissler Causing Leaf Spot of Pomegranate. *Int. J. Curr. Microbiol. App. Sci.* 7(11), 1146-1155.
- [30] Sajad, A. M., & Abid, H. Q. (2017). Antifungal activity of *Zingiber officinale* oil against plant pathogenic fungi isolated from solanaceous vegetable fruits. *Asian Journal of Pharmacy and Pharmacology*, 3(4), 121-124.
- [31] Bansod, S. & Rai, M. (2008) Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *Aspergillus niger*. *World Journal of Medical Sciences*, 3(2):81-88.
- [32] Rahmah, A. N., Mostafa, A. A., Abdel-Megeed, A., Yakout, S. M. & Hussein, S. A. (2013). Fungicidal activities of certain methanolic plant extracts against tomato phytopathogenic fungi. *Afr. J. Microbiol Res.*, 7, 517-524.
- [33] Olubunmi, A. A., Felix, A. A., Daniels, J. A., Oloswol, O. O. & Ayodele, B. O. (2018). Phytochemical screening and antifungal activities of *Zingiber officinale* on mycotoxygenic fungi associated with the deterioration of *Pennisetum glaucume* grains. *Journal of Advances in Microbiology*, 13(1), 1-11.
- [34] Mamarabadi, M., Abbas, T. & Younes, R. (2018). Antifungal activity of recombinant Thanatin in Comparison with two plant extracts and a chemical mixture to control fungal plant pathogens. Open Access.
- [35] Wongkaew, P. & Sinsiri, W. (2014). Effectiveness of ringworm cassia and turmeric plant extracts on growth inhibition against some important plant pathogenic fungi. *Am. J. Plant Sci.*, 5, 616-626.
- [36] Chethana, B. S., Ganeshan, G., Rao, A. S. & Bellishree, K. (2012). *In-vitro* evaluation of plant extracts, bioagents and fungicides against *Alternaria porri* causing purple blotch diseases of onions, Pest Management in Horticultural Ecosystems, 18 (2), 194-198.
- [37] Madhu, G., Sushil, S. & Rekha, B. (2017). Phytotoxicity of *Momordica Charantia* extracts against *Alternaria Alternata*. *J. Pharm. Sci. & Res.* 9 (1), 28-34.
- [38] Mudyiwa, R. M., Chiwaramakanda, S., Manenji, B. T. & Takawira, T. (2016). Anti-*Alternaria solani* Activity of Onion (*Allium cepa*), Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) *in vitro*. *International Journal of Plant & Soil Science*, 10(4), 1-8.
- [39] Neeta, S., Richa, T. & Madhu, P. S. (2013). *Zingiber officinale* Roscoe. Oil: A preservative of stored commodities against storage mycoflora. *Int. J. Curr. Microbiol. App. Sci.* 2(7), 123-134.
- [40] Grzanna, R., Lindmark, L. & Frondoza, C. G. (2005). Ginger an herbal medicinal product with broad anti-inflammatory actions. *J. Medical. Food*, 8, 125-132.
- [41] Odhiambo, A. J., Siboe, G. M., Lukhoba, C. W. & Dossaji, F. S. (2009). Antifungal activity of crude extracts of selected medicinal plants used in combination in Lake Victoria basin, Kenya. *Plant Product Res J.* 13, 35-43.
- [42] Mahlo, S. M., Chauk, H. R., McGaw, L. J. & Eloff, J. N. (2013). Antioxidant and antifungal activity of selected plant species used in traditional medicine. *J. Med Plants Res.* 7(33), 2444-2450.
- [43] Bandor, H., Hijazi, A., Ramma, H., Hachem, A., Saad, Z. & Badran, B. (2013). Techniques for the extraction of bioactive compounds from labanese *Urticadiotica*. *Journal of Phytomedicine Clinical Therapeutics*, 1(6), 507-513.
- [44] Brusotti, G., Cesain, I., Dentamaro, A., Caccialanza, G. & Massolin, G. (2013). Isolation and characterization of bioactive compounds from plant resources: the role of analysis in the ethnopharmacological approach. *J. Pharm Biomed Anal.* 87, 218-228.
- [45] Sumitra, A., Kanojia, A. K., Kumar, A., Mogha, N. & Sahu, V. (2012). Biopesticide formulation to control tomato lepidopteran pest menace. *Curr Sci.* 102 (7), 1051-1057.
- [46] Dabur, R., Gupta, A., Mandal, T. K., Singh, D. D., Bajpai, V., ... & Guvar, A. M. (2007). Antimicrobial activity of some Indian medicinal plants. *Afr. J. Tradit. Complement Altern. Med.* 4 (3), 313-318.
- [47] Javaid, A. & Rehman, A. H. (2011). Antifungal activity of leaf extracts of some medicinal trees against *Macrophomina phaseolina*. *J. Med Plants Res.* 5(13), 2858-2872.

