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Activity of ethanolic extracts of spices grown in Tanzania against important fungal pathogens and early blight of tomato

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Abstract

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The nutritional and economic value of tomato is universally recognized yet its production in many regions is still low due to, among other reasons, fungal diseases. Farmers have desperately relied on synthetic chemical pesticides to manage the diseases but the chemicals, in spite of their efficacy, are associated with residual detrimental effects on human health and environment. The objective of this study was to evaluate the antifungal activity of selected spices against important fungal pathogens of tomato. Bioactive compounds from seven spices namely clove, black pepper, turmeric, ginger, lemongrass, cinnamon and cardamom powders were extracted in ethanol and tested for antifungal activity against *Alternaria solani, Fusarium oxysporum* f. sp *lycopersici* and *Pythium* sp. in poisoned food bioassay. The most active spice extracts were further tested for efficacy against early blight of tomato under field conditions. All extracts were significantly active against the tested fungal pathogens by about 85%. Clove extract was the most active against all the pathogens inhibiting their growth 100%. Ginger, black pepper and turmeric extracts inhibited growth of all the pathogens by between 74-80%. Lemongrass extract was the least active with an antifungal activity of about 49%. Under field conditions, clove remained active in reducing early blight disease load by about 36% compared to the negative control. The antifungal activity demonstrated by the spice extracts is an indication that they could be relied upon for disease control and this study recommends their consideration, especially clove, for formulation into a botanical fungicide for management of early blight of tomato.

Keywords: Alternaria solani; Botanical fungicide; *Fusarium oxysporum* f.sp. *lycopersici*; *Pythium; Solanum lycopersicum*

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop grown all over the world for its nutritious fruits and short production cycles that allow producers to grow the crop throughout the year (Mutayoba & Ngaruko, 2018). In Tanzania, tomato flourishes well due to good climatic and topographic conditions (Mtui et al., 2010). The most cited factors for low tomato production include poor access to certified seed, reliance on rainfall for production and infestation by insect pests and diseases which affect the crop throughout its productive period from seedling to post-harvest (Shenge et al., 2010; Chidege et al., 2016; Verma et al., 2020). Effort to curb the stresses caused by diseases to the crop such as developing resistant tomato varieties, cultural practices, use of synthetic chemicals and of recently, devel-

oping bio-based formulations have been suggested (Minja et al., 2011; Bais et al., 2019). Nevertheless, tomato farmers mainly rely on synthetic chemical pesticides to manage the diseases (Verma et al., 2020). Chemical pesticides are however a health hazard whether by inhalation or ingestion after consuming food produced under frequent applications of synthetic chemicals and or a pest management challenge that rises from resistance to the chemicals by the target pathogen (Kariathi et al., 2017). The gap between managing disease-causing microorganisms and meeting the demand for a healthy tomato produce with less chemical residues needs to be narrowed by research endeavors. Alternatives such as botanical preparations may offer solutions to both needs owing to their availability, activity and biodegradability (Mtui et al., 2010; Arzoo & Biswas, 2013).

Tanzania is a leading producer of spices in Africa with a tonnage of over 5500 per year (Maerere, 2014). Some of the spice plants grown in Tanzania include clove, pepper, cinnamon, cardamom, lemon grass, vanilla, ginger, coriander, turmeric and much more. These spices are not only used for their aromatic and flavoring aspect, but also for medicinal and beauty value (Dubey, 2017; Zadeh & Kor, 2014). Many spice plants contain bioactive compounds which render them useful as medicinal plants. The bioactive compounds are also responsible for the antibacterial, antifungal and antiviral properties of such plants (Dasgupta & Klein, 2014; Han et al., 2017).

Many studies have focused on the insecticidal properties of plant extracts with notable success (Stevenson et al., 2017; Isman, 2020). Failure of including a positive control in research studies has been cited as one of the limitations of adopting botanical pesticides. Unavailability of performance of botanical preparations under field conditions has also been listed as a limitation for registering botanical pesticides (Isman & Grieneisen, 2014). The objective of this study was to evaluate activity of ethanolic extracts of selected Tanzanian spices against *Alternaria solani*, *Fusarium oxysporum* f.sp. *lycopersici* and *Pythium* sp. *in vitro* and early blight of tomato under field conditions. The hypothesis tested is that the components that make the spices medicinal also makes them suitable for management of fungal diseases of crops.

Materials and Methods

Preparation of the extracts

Powders of pure spices of clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum* sp.), black pepper (*Piper nigrum*), lemongrass (*Cymbopogon citratus*), turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), cardamom (*Elletaria cardamomum*) were purchased from a local market in Arusha, Tanzania. Half a kilo of each of the powders was separately soaked in one and a half liters of ethanol for a week. The mixture was kept in constant shaking and then filtered through two layers of cheese cloth and Whatman No. 1 filter paper. Under vacuum, the alcohol in the filtrate was evaporated at 40°C using a rotary evaporator. The ethanolic spice extracts were divided into two portions, one part for antifungal tests and the other for field experiments (Rizwana, 2016).

Evaluation of extracts for antifungal activity

Modified procedures described by Muthomi et al. (2017) were adopted for this activity. Pure cultures of *Alternaria solani, Pythium* and *Fusarium oxysporum* f. sp. *lycopersici* were obtained from the Plant Pathology Laboratory, University of Nairobi. Ten grams of each of the extracts were individually weighed and added into vials containing 10 mL of ethanol to make a stock solution. Aliquots of 100 µL were drawn from the stock solution and diluted in 900 µL of ethanol where 500 µL were further drawn and added to 15 mL of prior prepared potato dextrose agar media and cooled to a temperature of about 45°C. This temperature was ideal to allow evaporation of ethanol while allowing incorporation of the spice extracts into the media. The media amended with spice extracts was poured in 90 mm Petri plates and allowed to set. A corkborer with a diameter of three millimeters was used to bore mycelia of each of the fungi from actively growing zones of seven-day old cultures and placed centrally in the petri plates. Negative control plates contained unamended media while positive control plates contained media amended with a synthetic fungicide, Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg). The experiment was laid out in completely randomized design in four replicates. Radial growth of the pathogens was measured every two days after incubation for 20 days. The growth measurements were used to calculate the inhibition percentage of the spice extracts in comparison to the negative control plates as per the following formula:

$$\%\text{Inhibition} = \frac{\text{Colony diameter (Control)} - \text{Colony diameter (Treatment)}}{\text{Colony diameter (Control)}} \times 100$$

Assessment of the efficacy of extracts in managing early blight

Field experiments were conducted at the Field Station of College of Agriculture and Veterinary Sciences, University of Nairobi, Kenya. The location has good climatic and topographic conditions, ideal for tomato production (Jaetzold et al., 2006). The area has a long history of tomato production which guaranteed availability of early blight inoculum. Seedlings of Money Maker® tomato variety were raised in spindling trays and transplanted into plots in a prior prepared

field. The plots sizes were 3 m x 3 m and with a spacing of 60 cm x 60 cm. Fertilizer application was done prior to planting with diammonium phosphate (DAP) at the rate of 200 kg/ha. After establishment, five plants were selected from the middle rows of the plots and tagged for the application of treatments and monitoring of disease development.

Based on laboratory *in vitro* experiments, four most active extracts from clove, black pepper, turmeric and ginger were selected for field trials. For each spice extract, a stock solution was prepared by adding 10 g of extracts in 10 mL of ethanol. Five milliliters of each of the four crude plant extracts' stock solution were separately added to one liter of water together with three drops of Tween 80, acting as a surfactant. The solution was uniformly applied on the foliage of the tagged plants using a hand sprayer. The positive control was a commercial chemical fungicide, Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg) and an insecticide, Confidor® (Imidacloprid (chloro-nicotinyl) 700 g/kg) diluted according to manufacturers' recommendation. Negative control plots had no foliar application. The experiment was laid out in randomized complete block design in triplicate, conducted over two cropping cycles. Application of the treatments commenced two weeks after transplanting and was done once every week until fruiting.

Assessment of blight intensity

Assessment of early blight severity was done after every seven days, commencing one week after the first application of the treatments. Blight severity was assessed using a 1-5 disease severity scale as follows: 0 = no disease symptoms, 1 = < 20% leaf area infection, 2 = 21-40% leaf area infected, 3 = 41-60% leaf area infected, 4 = 61-80% leaf area infected, 5 = 81-100% leaf area infected (Barratt and Horsfall, 1945). Area under the disease progress curve (AUDPC) was used to compute the disease load present during the two cropping cycles as per the following formula:

$$\text{AUDPC} = \sum_{i=1}^{Ni-1} \frac{(yi+yi+1)}{2}(ti + t1 - ti)$$ (Shaner & Finney, 1977).

Data analysis

One way analysis of variance was adopted using Genstat® 15th edition, VSN International, to determine the effect of ethanolic spice extracts on the growth of the three fungal pathogens as well as development of early blight. Means were separated using Tukey's Test at 5%.

Results

Activity of spice extracts against tomato pathogenic fungi in vitro

There were significant ($P \leq 0.05$) differences among the extracts in the activity against the test fungal pathogens. Clove extract was superior in inhibiting growth of all the pathogens by about 85%. Its activity was followed by black pepper, turmeric and ginger all which were more active compared to the commercial fungicide, Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg). Cinnamon and lemongrass were the least active spice extracts registering an inhibition of below 50%. Cardamom, cinnamon and lemongrass seemed to stimulate growth of *Alternaria solani.* Among the test pathogens, *Pythium* was the most susceptible and clove and black pepper inhibited its growth completely (Table 1).

Clove remained active and completely inhibited growth of *Alternaria solani* over 20 days of incubation. The activity of clove was superior to the chemical fungicide while the

Table 1. Percentage inhibition of colony radial growth of fungal pathogens of tomato cultured on PDA amended with ethanolic spice extracts at 20 days after incubation

Treatments	*Alternaria*	*Pythium*	*Fusarium*	Mean
Clove	100.0a	100.0a	100.0a	85.64a
Black pepper	40.0c	100.0a	56.2b	81.47ab
Ginger	32.9c	78.9ab	62.7b	74.46bc
Turmeric	44.3bc	98.4a	61.0b	80.85ab
Cardamon	-1.4d	55.1bc	27.4d	57.9d
Cinammon	-5.7d	44.2c	19.2de	48.4e
Lemongrass	-12.1d	24.0c	15.8e	49.64de
Fungicide	58.6b	97.1a	42.5c	71.85c
s.e.m	3.9	7.0	2.1	1.9
s.e.d	5.5	9.8	2.9	2.69
L.S.D ($P \leq 0.05$)	11.2	20.2	6.0	5.34
CV (%)	22.9	18.0	8.4	10.9

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

activity of turmeric decreased over time and was comparable to the activity of the fungicide. The activity of cinnamon and lemongrass extracts against *A. solani* was below 20% throughout experimental period (Figure 1).

Extracts from clove remained highly active and completely inhibited growth of *Fusarium,* except at 16 days of incubation. Turmeric remained in the activity range of the fungicide and both their activity also decreased at 16 days after incubation. The low activity of cinnamon against *Fusarium* zeroed at 18 through 20 days after incubation. Lemongrass showed minimal activity against *Fusarium* and the treated cultures were outgrown by pathogen mycelium from start of incubation to about four days after incubation (Figure 2).

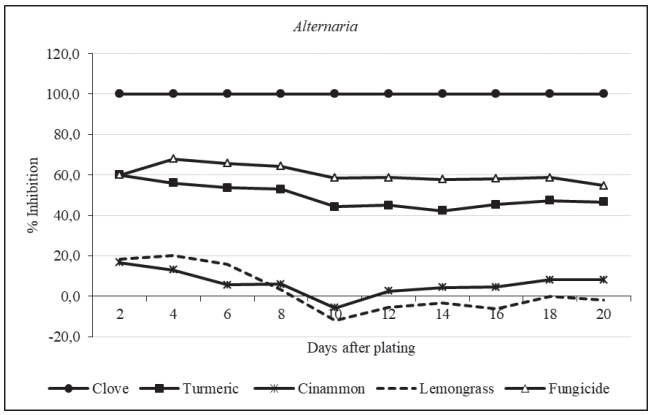

Fig. 1. Percentage inhibition of colony radial growth of *Alternaria solani* cultured on media amended with ethanolic spice extracts over time

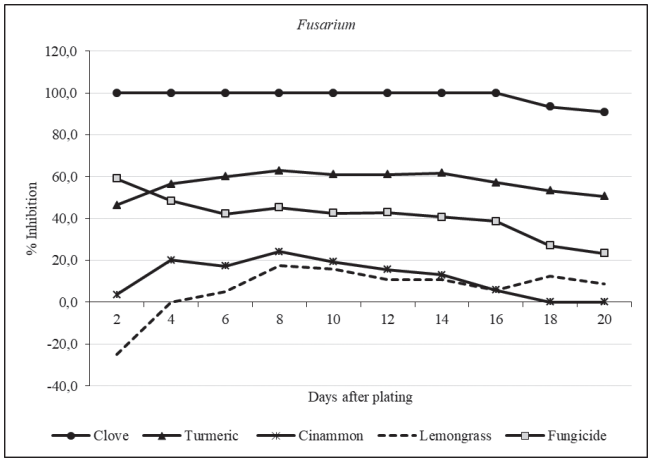

Fig. 2. Percentage inhibition of colony radial growth of *Fusarium oxysporym* f. sp. *lycopersici* cultured on media amended with ethanolic spice extracts over time

The activity of the extracts from clove and turmeric against *Pythium* was comparable to that of the fungicide in completely inhibiting growth of the causal agent of damping off over 20 days of incubation (Figure 3). Cinnamon and lemon grass extracts were also active starting at about 60% then decreasing to below 10% at 18 days after incubation. Plates containing media amended with extracts of black pepper, cardamom and ginger showed altered rosette patters of *Pythium* compared to the negative control (Figure 4).

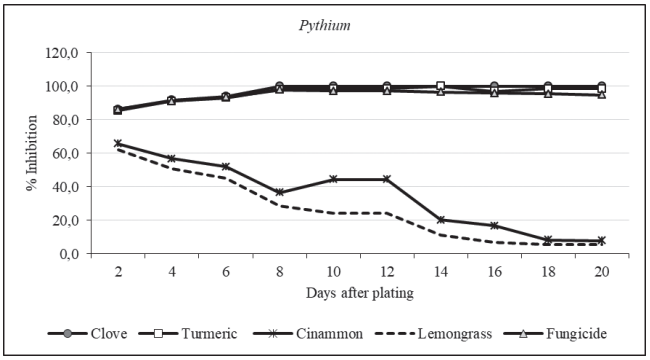

Fig. 3. Percentage inhibition of colony radial growth of *Pythium* cultured on media amended with ethanolic spice extracts over time

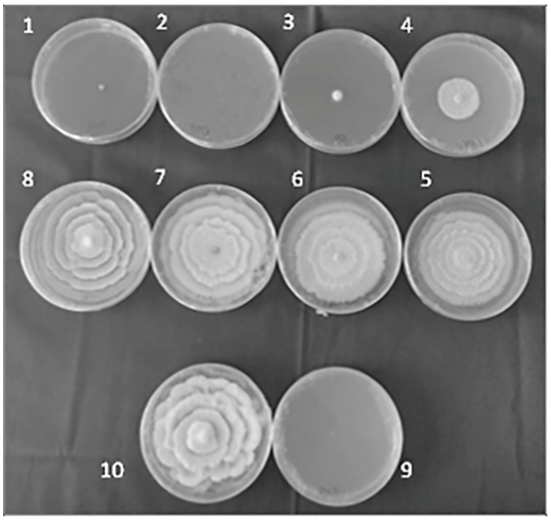

Fig. 4. *In vitro* activity of ethanolic spice extracts against *Pythium* at eight days after incubation: 1- Clove, 2 and 3- turmeric, 4- black pepper, 5- cardamom, 6- ginger, 7- cinnamon, 8- lemongrass, 9- fungicide 10- negative control

Efficacy of ethanolic spice extracts in managing early blight of tomato

There was low severity of early blight in the first cropping cycle. Effects of the treatments on reducing the disease had no significant ($P \leq 0.05$) differences. During the first week after application, the lowest disease was observed in plots treated with clove and the negative control. In the second week after application no disease was observed among all the treatments. On the third week after application, symptoms of early blight reappeared on plots treated with ginger and the positive control. At the fourth week after application, there was disease in plots treated with black pepper, turmeric, ginger and fungicide, while all other plots had no symptoms. After week six there was disease in all plots with no significant ($P \leq 0.05$) differences among the treatments. High severity was recorded in plots treated with black pepper, ginger and the positive control (Figure 5). Activity of all the treatments in reducing the severity of early blight fluctuated throughout the first cropping cycle.

During the second cropping cycle, severity of early blight remained low in all the treatments. The disease was however still high in the positive control. There were no significant ($P \leq 0.05$) differences among treatments in the first and second weeks after application (Figure 6).

AUDPC however indicated that clove was the most effective in reducing the disease load of early blight in both cropping cycles by up to 36% in comparison to the negative control. The activity of clove was followed by that of turmeric (18.4%) after which the synthetic fungicide followed. In the second cropping cycle black pepper reduced the disease load by 1.7%. The highest disease load was recorded in plots treated with ginger followed by the negative control. The trend observed in the first cropping cycle was relatively similar to that of the second cropping cycle (Figure 7).

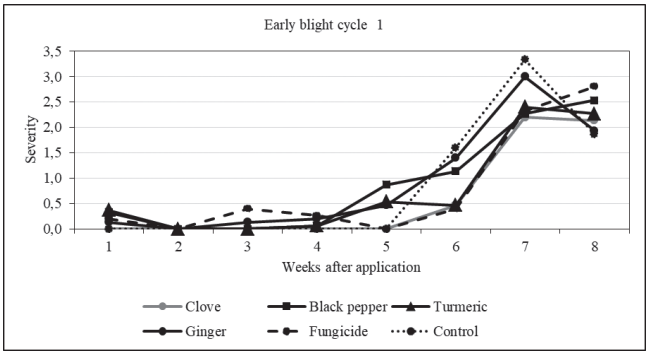

Fig. 5. Severity of early blight disease on tomatoes sprayed with ethanolic spice extracts during cropping cycle 1

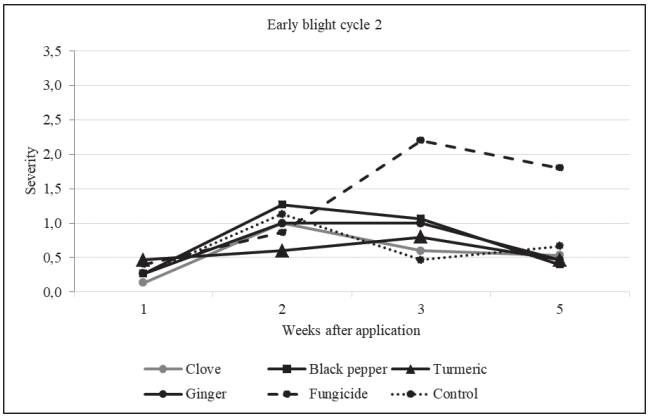

Fig. 6. Severity of early blight disease on tomatoes sprayed with ethanolic spice extracts during cropping cycle 2

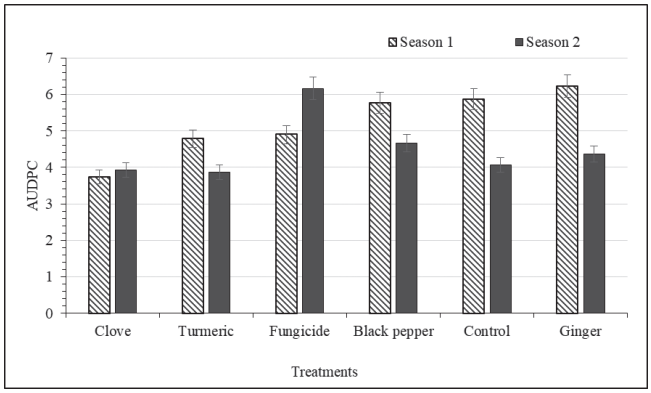

Fig. 7. Area under disease progress curve of early blight disease on tomatoes sprayed with ethanolic spice extracts for two cropping cycles

Discussion

The susceptibility of *Alternaria solani* exhibited in this study varied according to the type of spice extracts. Varied sensitivity of *A. solani* to clove extract has been reported widely by various studies (Rahmatzai et al., 2017; Sarfraz et al., 2018). Activity of clove is not limited to *A. solani* causing early blight of tomato but also to other species such as *A. alternata* causing diseases in other crops (Castro et al., 2017; Gadhi et al., 2018). *Alternaria* sp. was also susceptible to turmeric, a finding further supported by Rex et al. (2019) and Amsaraj et al. (2020). However, Shingne et al. (2020) reported a reduced activity of turmeric than what has been reported in the current study. Turmeric also has

an effect on growth of other species of *Alternaria* affecting other crops such as cumin, brinjals and cabbage (Nagaraju et al., 2020; Pun et al., 2020). Despite the average activity recorded, cinnamon also affected the growth of *A. solani* in this study as similarly reported by Rahmatzai et al. (2017). This finding is highly disabused by a study report (Yeole et al., 2014) who reported complete inhibition of *Alternaria solani* by cinnamon extracts. The activity of lemongrass seemed to stimulate growth of *A. solani* with reduced inhibitory activity, a finding supported by Tzortzakis & Economikas (2017). The activity of ginger in this study was above average, a finding supported by various studies (Naik et al., 2020; Rao et al., 2020a) and contrasted by others (Bhalerao et al., 2019; Rex et al., 2019). The cell wall of *Alternaria* is made up of chitin and glucan which are responsible for development of the fungi (Fernandes et al., 2014). *Alternaria* produce primary and secondary conidia, melanin as well as mycotoxins (Thomma et al., 2003), features that make the activity of plant bioactive compounds less effective. The complete inhibition of growth reported after using clove extract is an indication that the bioactive compounds present in the spice have the ability to break down the cell wall components of *A. solani* and hinder growth and development of the pathogen.

Every spice extract tested in this study had an effect on the growth of *Fusarium oxysporum* f. sp *lycopersici.* Turmeric extracts inhibited the pathogen by about 63%. This finding and one by Wongkaew & Sinsiri (2014) who reported a 64% activity of turmeric against the same pathogen rhyme. Other species of *Fusarium* have also been affected by turmeric extracts in separate studies (Shukla & Dwivedi, 2012; Yerukala et al., 2018). Rao et al. (2020 b) however contrasts the findings herein by reporting a reduced activity of about 35% by turmeric against *Fusarium oxysporum* f. sp. *lycopersici*. Ginger inhibited growth of *Fusarium* by up to 63% in the current study and other studies have reported varied activity of ginger against the wilt pathogen (Prasad et al., 2018; Azman et al., 2020; Rao et al., 2020 a,b). Cinnamon extracts had a low activity against *Fusarium* in the current study, a finding highly contrasted by many studies involving other species of *Fusarium* (Park et al., 2017; Sarkosh et al., 2018; Clerck et al., 2020). Clove extract was still superior against *Fusarium*, an outcome supported by other study reports (El-Samawaty et al., 2013; Hamad et al., 2015; Sharma et al., 2017). The chemical fungicide was averagely active against *Fusarium* because it is designed for late blight caused by an oomycete, *Phytophthora infestans*. The cell wall of *Fusarium* is majorly made up of chitin and glucan (Schoffelmeer et al., 1999) a feature that makes it complicated for many plant extracts to attack the pathogen. In the current study, clove extract was able to completely inhibit the growth of the wilt pathogen. This activity may be attributed to presence of certain bioactive compounds in clove with ability to dissolve the chitinous cell walls of *Fusarium oxysporum* f.sp *lycopersici*. Bioactive compounds present in turmeric, black pepper and ginger may also assumably have the ability to digest chitin though only to a given extent.

Pythium was the most susceptible pathogen to all the spice extracts in the current study and clove, black pepper and turmeric completely inhibited its growth. Similar growth inhibition activity of clove against species of *Pythium* has been reported (Kareem et al., 2009; McMaster et al., 2013). While Zagade et al. (2012) reported a similar activity of turmeric of about 82%, Gholve et al. (2014) recorded a 24% inhibition activity of turmeric against *Pythium* species. The activity of ginger reported herein is widely supported by various reports involving different species of *Pythium* causing damping off and wilts in numerous crops (Suleiman & Emua, 2009; Zagade et al., 2012; Ravi et al., 2017). Cooper & Aranson (1967) mechanically isolated the cell wall of *Pythium debaryanum* and reported that it constituted mainly of glucan and cellulose. The activity of clove and black pepper that completely inhibited growth of *Pythium* in the current study may be attributed to the ability of major compounds of the spices to dissolve the cellulosic cell walls of the pathogen. The compounds in clove and black pepper may also be responsible for the altered look of the mycelia of *Pythium* from the rosette appearance that distinguishes the pathogen. *Pythium* was also highly susceptible to the chemical fungicide which was designed for management of late blight caused by *Phytophthora infestans*, an oomycete, a family where *Pythium* belongs.

The extracts in this study were extracted in ethanol and their activity against fungal pathogens was significant. The choice of ethanol was made after several studies have reported its effectiveness as an extractant of bioactive compounds in various plants (Dirar et al., 2018). The activity of the spice extracts discussed herein is attributed to presence of bioactive compounds possessed by each of the spice plants. The abundance and activity of those compounds is however dependent on the plant species, extraction system, the geographical location where the plants were grown, susceptibility of the pathogens and species variability of the spice plants (Nikolić et al., 2015).

Eugenol, a major compound found in clove is highly associated with the antifungal activity of the spice and its oil due to its lipophilic nature which interferes with fungal membranes (Olea et al., 2019). *Ar*-turmerone is one of the dominant compounds in turmeric and its antifungal activity has been reported against *Phytophtora infestans* and *Erysip-*

he graminis (Lee et al., 2003). Piperine is a major compound in black pepper and its antifungal activity has been reported against *Candida albicans* by binding to the ergosterol which interferes with the integrity of the membrane, eventually killing the fungus (Moraru et al., 2019). Individual plant compounds may or not be significantly antifungal on their own and may require to be combined with other compounds for synergy.

Plant extracts evaluated in this study reduced the severity of early blight under field conditions. Clove was the most effective in reducing the disease compared to the rest and even to the commercial fungicide. Activity of clove extract and oil against early blight and improvement of vegetative growth of tomato has been previously reported (Derbalah et al., 2012; Rahmatzai et al., 2017). While black pepper was not as effective in reducing the early blight disease in the current study, Pattnaik et al. (2012) reported that *Piper nigrum* reduced severity of early blight by about 36%. Despite the reduced antifungal activity of lemongrass in the *in vitro* studies of the current study, Abd-El-Khair & Haggag (2007) reported that water extracts of lemongrass reduced severity of early blight by about 81%.

The different antifungal activity levels exhibited by plant extracts in this and other studies under field conditions is due to environmental and technical factors. Plant extracts in their basic form, without any form of formulation, are subject to rapid degradation due to environmental conditions such as temperature, light and precipitation (Luiz de Oliveira et al., 2018). The bioactive constituents in the plant extracts are also highly volatile and are vulnerable to processes such as oxidation which render them partially active (Pavela & Benelli, 2016). This challenges their efficacy against plant diseases under natural conditions. The solace of effectiveness of botanical preparations lies in formulation of the bioactive compounds for the sake of stability and persistence especially under field conditions (Phuna et al., 2020).

Conclusion

The spices evaluated for antifungal activity in this study showed a significant degree of effectiveness against selected fungal pathogens of tomato. The variances in their antifungal activity are due to differences in the abundance and concentration of the bioactive compounds present in those plant species. The reduced antifungal activity under field conditions is influenced by factors such as stability of the extracts, environmental conditions and severity of the disease under natural conditions. The outstanding activity of clove extracts both *in vitro* and in the field gives a lead to consideration for formulation into a botanical fungicide for management of early blight of tomato.

All the spice plants used in this study were grown and processed in Tanzania. Their reported antifungal activity is an indication of their richness as lead sources for botanical fungicides. Availability of such plants within the local reach guarantees a steady supply should they be considered as sources of plant protection products. It also creates confidence among farmers who would use them as crop protection products since they already interact with the plants either as food additives or as medicine. Therefore, introducing a botanical fungicide made from the same plants they are used to would not be met with utmost resistance. Adoption of the spices studied herein as sources of botanical fungicides would create a new market for spices thereby increasing the income of respective farmers and producing countries such as Tanzania.

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