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Research Article

In Vitro Control of *Phytophthora infestans* and *Alternaria solani* Using Crude Extracts and Essential Oils from Selected Plants

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Tomato production is constrained by fungal diseases especially the early and late blight caused by *Alternaria solani* and *Phytophthora infestans*, respectively. Control of the two diseases is usually by use of synthetic fungicides which have a long residue effect and also contribute to environmental pollution. Innovative use of biocontrols may offer an eco-friendly and more sustainable solution. This study tested the in vitro efficacy of crude extracts and essential oils of ginger, garlic, tick berry, and Mexican marigold in inhibition of radial growth of *A. solani* and *P. infestans*. Extraction of the crude extracts was done using distilled water, ethanol, and methanol solvents, while essential oils were extracted using the dry steam distillation method. The extracts and essential oils were used to amend the growth media of the test pathogens before introducing the precultured pathogens. Sterile distilled water and synthetic fungicide, Ridomil Gold®, were used as positive and negative controls, respectively. Fungal growth inhibition was determined by measuring the radial growth of the test pathogens. Both the crude extracts and the essential oils portrayed some efficacy against the test pathogens. Garlic crude extracts were found to be the most effective, while ethanol was the most suitable extraction solvent. Essential oils were more effective in restricting the pathogen growth than crude extracts. Ginger and garlic oil was found to be as effective as the synthetic fungicide, and thus it was concluded that the two plants have strong antifungal properties with high potential of being utilized as biofungicides. However, effective utilization of these products in farmers' fields may require industrial formulation to improve their efficiency.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is the second most widely grown vegetable crop in East Africa and in the whole world after potato [1]. The crop is grown for its fruits which have high nutritive value rich in antioxidants such as lycopene, minerals such as potassium, magnesium, calcium, iron, and zinc as well as vitamins such as vitamins A, B group, C, and E [2]. In Kenya, the crop is one of the most important vegetable crops in the horticultural industry [3, 4]. Kenya is among Africa's leading producers of tomatoes and is ranked 6th in Africa with a total production of 410,033 tonnes [5]. Tomato production is a major source of income for small-scale holders and medium-scale commercial farmers [6]. Tomatoes enjoy a ready market in Kenya with considerably high demand throughout the year [7].

In Kenya, tomato production is mainly conducted in the open field or in the green houses [4]. However, production in open fields is highly constrained by pests and diseases [8] especially the early and late blight diseases caused by the fungus *Alternaria solani* and *Phytophthora infestans*, respectively [9]. The two diseases affect the leaves, stems, and fruits of tomato causing severe damage and high production losses of about 95.8% [10]. The symptoms of early blight on tomatoes include irregular black to brown spots that begins on older leaves where they enlarge to form lesions and may eventually lead to leaf fall [11]. The symptoms of late blight disease are black or brown lesions on leaves and stems. The lesions are water-soaked in appearance and are usually small

at the beginning and then expand to become necrotic [12]. The fungus attacks the fruit at the fruit stalk and cause large sunken areas with concentric rings and a black velvety appearance [11]. Infected stems and petioles collapse while the fruits become greasy and decay and fall off the plant [13].

In Kenya, late blight disease of tomato ranks among the top constraints of tomato production, and together with early blight, they cause 95.8% of all the preharvest tomato losses [14]. The pathogens are spread by wind, water, and weeds of the solanaceae family, volunteer and susceptible tomato plants [8]. Tomato plants can be infected by the early blight fungus at any stage of growth but becomes more severe at the fruit set [15]. The disease is more severe in areas with high humidity, low temperatures, and high rainfall [16]. The development of late blight disease on susceptible tomato plants is favoured by cool moist whether with high relative humidity and temperature ranging between 18–22°C [8]. The causal pathogen, *P. infestans*, has a sophisticated morphology which makes it difficult to manage in the field [17].

Tomato farmers have for a long time relied on the use of synthetic fungicides to control the early and late blight diseases in an effort to meet the high demand of tomato which is faced by seasonal shortages [18]. In Kenya, control of the two diseases use up to forty pesticide applications per crop season [14]. However, the chemicals are expensive and increases the production cost by 20% [19]. In addition, synthetic fungicides have many other detrimental effects. For example, they pollute the environment by contaminating the soil, water bodies, and food subsequently causing harm to the living things including humans and nontarget beneficial organisms such as micro-organisms and pollinators [20]. Some of the active ingredients in the agrochemicals are hormone disruptors and may cause infertility, carcinogenesis, and mutations that result in the resistance of the pathogens to the agrochemicals [21, 22]. Synthetic pesticides also pose problems within the markets due to their high chemical residue levels which makes some of the products rejected in the market resulting in losses [20, 23].

Current crop protection efforts focus on developing biopesticides that are environmental friendly and effective in managing the plant pests and diseases. Natural plant products have been shown to be good sources of agrochemicals since they are easily biodegradable and do not pollute the environment [24]. Various natural products of plant origin have been proven to be effective in controlling various foliar pests and pathogens in a similar way as the synthetic pesticides [24, 25]. The antimicrobial activity of several plant products against fungal pathogens have been studied under both in vitro and in vivo conditions [25]. Cold extracts of *Azadirachtin indica* were shown to inhibit the growth of *Fusarium oxysporum* which causes root and stem rot of cucumber [26]. This study aimed at evaluating the in vitro efficacy of crude extracts and essential oils of selected plants in inhibiting the growth of *A. solani* and *P. infestans*. The study also tested whether the extraction solvent had a significant influence on the efficacy of the crude extracts on the test pathogen.

2. Materials and Methods

2.1. Collection and Isolation of Fungal Pathogens. Tomato farms within Mwea Subcounty of Kirinyaga county in central Kenya were randomly sampled for collection of diseased plant samples. The area was selected because of its long history in tomato production and the high prevalence of early and late blight diseases. Diseased tomato leaves bearing symptoms of blights were identified by physical examination and collected randomly from identified farms. The diseased leaf samples were placed in cool boxes and taken to the Microbiology Laboratory at the University of Embu and stored at 4°C in a refrigerator awaiting further processing and analysis. The isolation of *A. solani* and *P. infestans* from the infected plant samples was conducted following the modified procedures of [27] using standard media of potato dextrose agar (PDA) and V8 juice agar, respectively. The infected tomato plant leaves were first washed under running water and then dipped into 1% sodium hypochlorite to surface sterilize them for three minutes and rinsed in three changes of distilled water. The cleaned samples were blotted dry using a sterilized blotting paper.

A sterilized scalpel was used to cut sections of infected leaf tissues measuring 3 mm × 3 mm towards the healthy portions where the pathogens were likely to be more active. The dried early and late blight infected tissues were directly plated on sterile PDA and V8 agar, respectively, and then incubated in the laboratory at room temperature (25°C) for 3 days. Single spore isolation was done to obtain pure cultures by isolating a section of hypha from the three-day old culture and introducing it into the growth medium and then incubating at room temperature. Fungal identification was done after 8 days using morphological characteristics and comparing with established keys [28] to confirm the identity of the target pathogens. Morphological identification was based on growth patterns, colour of mycelia, and microscopic examinations of vegetative and reproductive structures [28].

2.2. Preparation of the Test Plant Materials. The crude plant extracts and essential oils were obtained from garlic (*Allium sativum*) cloves, ginger (*Zingiber officinale*) rhizomes, tick berry (*Lantana camara*) leaves, and Mexican marigold (*Tagetes erecta*) leaves. Fresh tick berry and Mexican marigold leaves were collected from the field, while ginger rhizomes and garlic bulbs were obtained from the local open air market. For extraction of crude extracts, 1 kg each of tick berry and Mexican marigold leaves were washed under tap water, rinsed in three changes of distilled water, and dried using sterilized blotting paper. They were then separately oven-dried at 40°C for two days before being crushed to powder using a kitchen blender. Five-hundred grams each of garlic cloves and ginger rhizomes were peeled, washed under tap water, and rinsed in three changes of distilled water then dried using sterilized blotting papers. They were then cut into small pieces using a sterilized scalpel and oven-dried at 40°C for five days after which they were crushed to powder

using a kitchen blender. The dry powders were put in air tight dull bottles and stored for later use in the laboratory under room temperature. For extraction of essential oils, 1 kg each of tick berry and Mexican marigold leaves were washed under tap water, rinsed in three changes of distilled water, and then air-dried in the laboratory to evaporate the moisture content. One kilogram each of garlic cloves and ginger rhizomes were also washed with tap water and then rinsed with three changes of distilled water. They were also air-dried in the laboratory and later peeled. All the fresh materials were stored in the laboratory under room temperature awaiting the extraction of essential oils.

2.3. Extraction of Test Plant Crude Extracts. The extraction of test plant crude extracts was done following a modified procedure described by Handa et al. [29]. Two-hundred (200) grams dry powder of each of the four plant products were soaked separately in 500 ml of sterile distilled water, analytical grade ethanol (95%), and methanol (95%) for 72 hrs. The content was then filtered using Whitman's (No.2) filter paper. For water extraction, the filtrate formed the stock solution and was refrigerated at 4°C for further use in testing the efficacy on the fungal pathogens. Ethanol and methanol filtrates were evaporated using a rotary evaporator, and the remaining small amounts of the alcoholic solvents were evaporated in the oven at 40°C. The remaining contents were stored in the refrigerator at 4°C and later used to test their efficacy on the growth inhibition of the fungal pathogens.

2.4. Extraction of Essential Oils from the Test Plants. Extraction of essential oils was done following a modified procedure described by Adams [30]. The vertical steam distillation unit, consisting of a hot plate, boiling flask, biomass flask, still head, condenser, and receiving flask (separating funnel), was used for the dry steam distillation of plant material. The fresh materials of each plant were put separately in the biomass flask, and the distillation unit was switched on. Steam was produced in the boiling flask by heating distilled water with the hot plate. The steam moved upward into the biomass flask where essential oils and other water-soluble plant compounds were moved as vapour through the still head, condensed in the water-cooled condenser, and collected in the receiving flask (separating funnel). The receiving flask separated the heavier-than-water oils from the lighter-than-water oils while allowing excess water-soluble compounds to be drained out and collected separately.

2.5. Experimental Design and Layout. The experiment was conducted at the University of Embu Microbiology Laboratory. There were two sets of factorial in vitro experiments that were laid out in a completely randomized design replicated four times. The first experiment consisted of crude extracts from the four test plants extracted using the three different solvents (sterile distilled water, ethanol, and methanol). The second set of experiment consisted of four essential oils extracted from the four test plants. All the biocontrol products were tested against *A. solani* and *P. infestans*.

The experiment was conducted following the modified procedure of Al-samarrai et al. [31, 32]. The methanol and ethanol crude plant extracts were first diluted with 2 ml dimethyl sulfoxide (DMSO) before diluting with water in the ratio of 1 : 1. Five-hundred (500) ml each of PDA and V8 agar were amended separately with 5 ml each of the four different crude extracts and 1 ml each of the four essential oils separately and dispensed into separate Petri dishes. Five ml of distilled water and 1.5 g of Ridomil Gold® (metalaxyl-M and S-isomer, Mancozeb) dissolved in 5 ml of distilled water were mixed with the media as the negative and positive controls, respectively. In the second experiment, 1 ml of distilled water and 1.5 g of Ridomil Gold® dissolved in 5 ml of distilled water were mixed with the media as the negative and positive controls, respectively. Fungal culture discs of 5 mm were cut from one week old cultures of *A. solani* and *P. infestans* using a sterilized cork-borer and cultured at the centre of each Petri dish per replicate and incubated at room temperature (25°C ± 2) for seven days in order to monitor the growth of the test pathogens.

2.6. Data Collection and Analysis. Fungal colony diameter of each treatment was measured using a pair of dividers and a ruler after every two days up to the seventh day. Data obtained were subjected to analysis of variance (ANOVA) using XLSTAT version 2019, and separation of means was conducted using Student Newman's Keul (SNK) test at a 95% level of confidence.

3. Results

3.1. Effect of Water-Extracted Crude Extracts on the Fungal Pathogens. The results obtained from the study showed that all the water-extracted plant extracts were effective in inhibiting the growth of the test pathogens as compared with the negative control (Table 1). However, the rate of inhibition varied significantly ($p < 0.0001$) among the different test plant extracts as compared with the positive and negative controls within a period of 7 days. Both pathogens recorded incremental growth from day one to day seven in all the treatments except the positive control with synthetic fungicide where no pathogen growth was recorded. Varied effects of the different extracts were recorded in all the sampling days, but a clear separation was observed on the 5^{th} and 7^{th} days where all the treatments were significantly different from each other for both pathogens. Garlic extracts were consistently the most effective in inhibiting the growth of both pathogens followed by ginger, tick berry, and Mexican marigold in that order (Table 1).

3.2. Effect of Methanol-Extracted Crude Extracts on the Fungal Pathogens. The results showed that all the methanol-extracted plant extracts were effective in inhibiting the growth of the test pathogens as compared with the negative control, but their efficacy against both pathogens varied

Table 1: Antimicrobial activity of water-extracted plant extracts on the growth of the fungal pathogens.

Test material	Growth of fungal isolates							
	Alternaria solani				*Phytophthora infestans*			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
Control	3.59^{a}	20.08^{a}	41.92^{a}	61.67^{a}	6.00^{a}	27.25^{a}	45.25^{a}	64.42^{a}
Marigold	3.67^{a}	19.00^{b}	37.25^{b}	56.17^{b}	3.42^{b}	18.59^{b}	37.67^{b}	56.17^{b}
Tick berry	2.92^{ab}	17.00^{c}	34.75^{c}	51.00^{c}	4.00^{b}	18.34^{b}	36.33^{c}	54.50^{c}
Ginger	2.42^{b}	16.25^{c}	33.42^{d}	47.75^{d}	3.25^{b}	16.75^{c}	31.92^{d}	49.83^{d}
Garlic	1.75^{c}	16.84^{c}	31.59^{e}	45.17^{e}	2.17^{c}	14.75^{d}	28.25^{e}	37.67^{e}
Ridomil	0.00^{d}	0.00^{d}	0.00^{f}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{f}	0.00^{f}
P > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SE	0.212	0.255	0.400	0.414	0.335	0.358	0.315	0.433
DF	5	5	5	5	5	5	5	5

Mean values followed by the same letter within the same column are not significantly different at $p = 0.05$.

significantly ($p < 0.0001$) between the treatments (Table 2). The positive control with Ridomil Gold® synthetic fungicide recorded 100% growth inhibition of both pathogens throughout the 7 days' testing period. On the other hand, the negative control exhibited the highest pathogen growth which was significantly ($p < 0.05$) different from the other treatments throughout the testing period. The general trend exhibited near the end of the 7 days testing period showed that garlic extracts were the most effective in inhibiting the growth of both pathogens followed by ginger, tick berry, and Mexican marigold in that order (Table 2).

The plant extracts portrayed varied effects on specific pathogens over the testing period. Ginger extracts performed better than the garlic extracts in controlling the growth of *A. Solani* on the 1st and 3rd days of testing but the two switched positions on the 5th and 7th day. The efficacy of tick berry and Mexican marigold against *A. solani* were similar on day 1 and day 5, but tick berry performed significantly better than Mexican marigold on the 3rd and 7th day. On the other hand, garlic extracts were consistently the most effective in inhibiting the growth of *P. infestans* followed by ginger extracts. Mexican marigold performed significantly better than tick berry against *P. infestans* at the initial testing stages (days 1–3) but the two switched positions in the later stages (days 5–7).

3.3. Effect of Ethanol-Extracted Crude Extracts on the Fungal Pathogens. All ethanol-extracted plant extracts were effective in inhibiting the growth of the test pathogens, but their efficacy varied significantly ($p < 0.0001$) among them and from the positive and negative controls (Table 3). The positive control maintained 100% inhibition of the pathogen growth, while the negative control recorded the highest pathogen growth. The four crude extracts demonstrated varied reactions to the pathogens at the initial testing stages. Unlike the trend observed with water and methanol-extracted products, tick berry appeared to be more effective than ginger in controlling the growth of *A. solani* from day 1 to day 5. However, the latter became more effective than the former on day 7. The response of *P. infestans* to ethanol-extracted plant products assumed a similar trend to that observed with water and methanol solvents. By the end of the testing period, it was evident that garlic was still the most effective biocontrol against both pathogens followed by ginger and tick berry. Mexican marigold was the least effective in the control of both pathogens.

3.4. Comparative Effect of Extraction Solvents on the Fungal Pathogens. The type of solvent used in extraction of the crude plant extracts was found to have a significant influence on the efficacy of the extracts (Figure 1). The results showed that ethanol-extracted products recorded the best ($p < 0.05$) efficacy against both pathogens by the end of the testing period (day 7), while water-extracted products were the least effective. On the other hand, the growth of the test pathogens did not vary significantly ($p > 0.05$) within the same extraction solvent.

3.5. Comparative Response of the Two Fungal Pathogens to the Crude Extracts. The average growth of the two pathogens varied significantly under the control treatment and when subjected to ginger and garlic extracts but did not vary significantly ($p > 0.05$) when treated with both tick berry and Mexican marigold extracts (Figure 2). The growth of *A. solani* was significantly lower than that of *P. infestans* when treated with ginger extracts. However, garlic extracts had a significantly higher growth inhibition on *P. infestans* as compared with that in *A. solani*.

3.6. Antimicrobial Activity of the Essential Oils from the Test Plants on the Test Pathogens. Essential oils extracted from the four test plants varied significantly ($p < 0.05$) in their antimicrobial activity against the growth of the test pathogens (Table 4). Ginger and garlic oils produced similar results to the positive control treatment with synthetic fungicide where no growth of the test pathogens was observed throughout the testing period. The antimicrobial activity of essential oils obtained from Mexican marigold and tick berry were also significantly ($p < 0.05$) different from the negative control in inhibiting the growth of both pathogens. Comparative microbial activity of Mexican marigold and tick berry essential oils against the growth of *P. infestans* was not significantly different except on day 1

Table 2: Antimicrobial activity of methanol-extracted plant extracts on the growth of the fungal pathogens.

Test material	Growth of fungal isolates							
	Alternaria solani				*Phytophthora infestans*			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
Control	5.00[a]	27.25[a]	47.75[a]	64.92[a]	5.33[a]	31.58[a]	62.08[a]	76.75[a]
Marigold	3.67[b]	20.17[b]	36.42[b]	52.50[b]	3.83[c]	20.25[c]	38.67[b]	54.25[b]
Tick berry	3.08[b]	19.00[c]	36.09[b]	50.25[c]	4.75[b]	26.09[b]	37.25[c]	50.33[c]
Ginger	1.92[c]	12.42[e]	30.00[c]	42.67[d]	2.50[d]	18.09[d]	36.42[c]	47.83[d]
Garlic	2.17[c]	15.83[d]	28.00[d]	39.75[e]	1.50[e]	13.09[e]	24.67[d]	35.08[e]
Ridomil	0.00[d]	0.00[f]	0.00[e]	0.00[f]	0.00[f]	0.00[f]	0.00[e]	0.00[f]
Pr > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SE	0.262	0.297	0.575	0.286	0.192	0.618	0.449	0.270
DF	5	5	5	5	5	5	5	5

Mean values followed by the same letter within the same column are not significantly different at $p = 0.05$.

Table 3: Antimicrobial activity of ethanol-extracted plant extracts on the growth of the fungal pathogens.

Test material	Growth of fungal isolates							
	Alternaria solani				*Phytophthora infestans*			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
Control	5.17[a]	24.50[a]	39.67[a]	61.25[a]	5.25[a]	26.75[a]	53.42[a]	69.59[a]
Marigold	5.00[a]	22.83[b]	40.67[a]	51.25[b]	4.84[a]	22.25[b]	40.83[b]	50.00[b]
Tick berry	2.67[b]	13.50[d]	28.50[c]	47.58[c]	2.67[b]	14.67[c]	32.33[c]	49.34[b]
Ginger	5.50[a]	19.75[c]	30.75[b]	40.59[d]	2.42[b]	11.00[d]	26.42[d]	42.25[c]
Garlic	1.42[c]	9.42[e]	22.83[d]	34.50[e]	0.00[c]	5.00[e]	17.59[e]	29.75[d]
Ridomil	0.00[d]	0.00[f]	0.00[e]	0.00[f]	0.00[c]	0.00[f]	0.00[f]	0.00[e]
Pr > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SE	0.203	0.300	0.428	0.404	0.169	0.287	0.568	0.344
DF	5	5	5	5	5	5	5	5

Mean values followed by the same letter within the same column are not significantly different at $p = 0.05$.

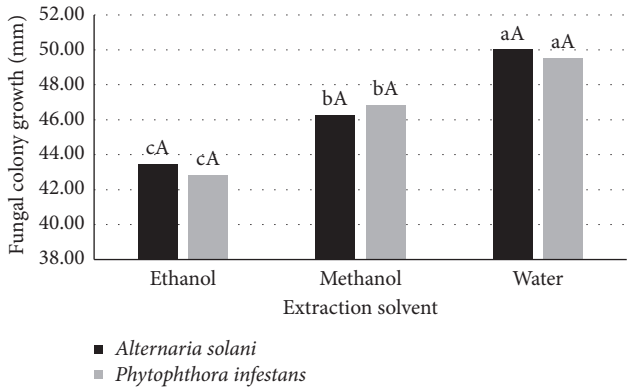

Figure 1: Comparative effect of extraction solvent of crude extracts on their efficacy against the fungal pathogens at day 7. Means with the same lowercase letter on different extraction solvents are not significantly different while those with the same upper case letter on the same extraction solvent are not significantly different at $p = 0.05$.

where tick berry essential oils appeared to be more effective than Mexican marigold. However, the two plants varied significantly in controlling the growth of *A. solani* where essential oils from Mexican marigold were found to be more effective than those from tick berry (Table 4).

3.7. Comparative Response of the Fungal Pathogens to Essential Oils and Crude Extracts. From the results of the study, it was evident that the test plants had varying biocontrol potential against the two test pathogens as compared with the negative and positive controls. However, the essential oils were found to be more effective than the crude extracts in inhibiting the growth of the test pathogens (Figure 3). Essential oils from ginger and garlic caused 100% growth restriction of the two pathogens just like the synthetic fungicide. Likewise, the efficacy of Mexican marigold and tick berry was found to improve when essential oils were used as compared with raw extracts.

3.8. Effect of the Extraction Method of the Plant Product on the Test Pathogens. The growth inhibition on the test pathogens was found to be significantly ($p < 0.0001$) influenced by the method used to extract the biocontrol products (Table 5). The essential oils were more effective in pathogen growth inhibition than crude extracts extracted using extraction solvents. The three extraction solvents were also found to significantly influence the efficacy of the extracts in pathogen growth inhibition. For *A. solani,* water extraction produced relatively better results than ethanol and methanol on the 1stand 3rd day, respectively. However, towards the end of the testing period, ethanol extraction produced the best results

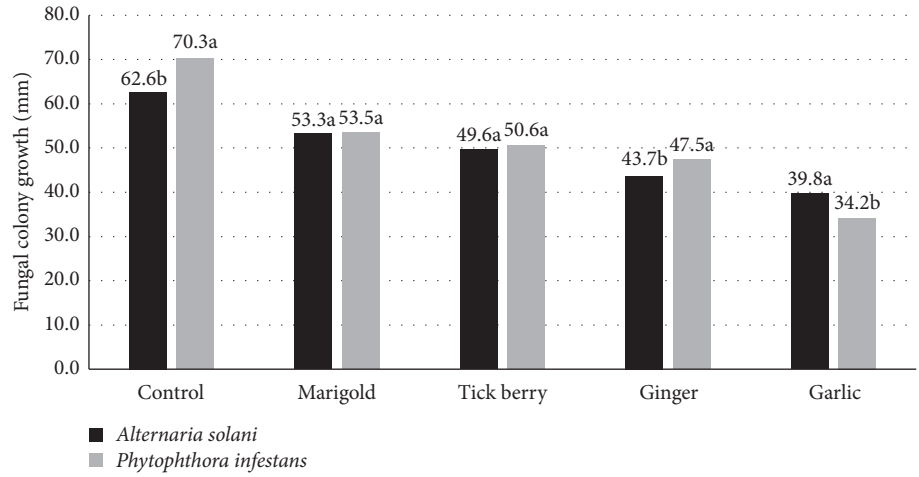

Figure 2: Comparative response of the two fungal pathogens to the crude extracts at day 7. Means with the same letter on the bars are not significantly different under the same test plant at $p = 0.05$.

Table 4: Antimicrobial activity of essential oils on the growth of the fungal pathogens.

Test material	Growth of fungal isolates							
	Alternaria solani				*Phytophthora infestans*			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
Control	4.67[a]	25.33[a]	43.42[a]	59.67[a]	4.75[a]	27.17[a]	50.75[a]	68.67[a]
Marigold	1.84[c]	7.17[c]	21.59[c]	31.67[c]	1.83[b]	8.92[b]	21.33[b]	28.58[b]
Tick berry	2.92[b]	10.25[b]	23.25[b]	35.25[b]	1.25[c]	8.83[b]	22.09[b]	29.00[b]
Ginger	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[c]	0.00[c]	0.00[c]
Garlic	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[c]	0.00[c]	0.00[c]
Ridomil	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[c]	0.00[c]	0.00[c]
Pr > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SE	0.228	0.372	0.441	0.644	0.115	0.357	0.484	0.624
DF	5	5	5	5	5	5	5	5

Mean values followed by the same letter within the same column are not significantly different at $p = 0.05$.

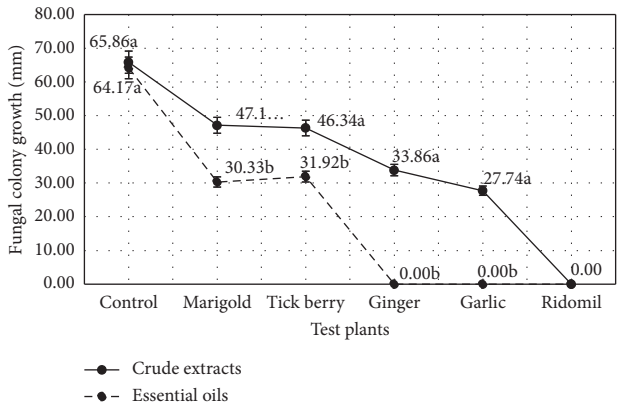

Figure 3: Comparative response of crude extracts and essential oils on the fungal pathogens. Means with the same letter under the same test plant are not significantly different based on SNK test at $p = 0.05$. Means on the same curve whose error bars are overlapping are not significantly different.

by recording the lowest growth of *A. solani* fungus. For *P. infestans*, a clearer trend was exhibited where ethanol extraction consistently recorded the lowest pathogen growth followed by methanol. However, methanol and water extraction methods recorded similar results on the 1st and 7th days of testing (Table 5).

4. Discussion

From the study, it was evident that the crude extracts from all the plants (ginger, garlic, tick berry, and Mexican marigold) that were tested were effective in inhibiting the growth of *A. solani* and *P. infestans* as compared with the control. However, the inhibition varied with the type of the plant with garlic showing the highest rate of growth inhibition on the two test pathogens. This was an indication that the tested plants contain certain antifungal compounds. This observation supports other previous reports from different authors on the antifungal properties of various plants. Reports of Daniel et al. [33] showed that garlic effectively inhibited *Botrytis cinerea* in vitro. Tick berry extracts were reported to effectively inhibit the growth of *P. infestans* [34, 35] and *Colletotrichum falcatum* that causes red rot disease of sugar cane [36]. Mexican marigold extracts were also reported to significantly suppress the root-knot nematodes that infect tomato [37] and to have antibacterial

Table 5: Effect of the extraction solvents and essential oils on the growth of the test pathogens.

Extraction method	Growth of fungal isolates							
	Alternaria solani				*Phytophthora infestans*			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
Water	2.39^{b}	14.86^{b}	29.82^{a}	43.62^{a}	3.14^{a}	15.94^{b}	29.90^{b}	43.76^{a}
Methanol	2.64^{b}	15.78^{a}	29.71^{a}	41.68^{b}	2.99^{a}	18.18^{a}	33.18^{a}	44.04^{a}
Ethanol	3.29^{a}	15.00^{b}	27.07^{b}	39.19^{c}	2.53^{b}	13.28^{c}	28.43^{c}	40.15^{b}
Essential oils	1.57^{c}	7.12^{c}	14.71^{c}	21.10^{d}	1.31^{c}	7.49^{d}	15.69^{d}	21.04^{c}
Pr > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Mean values followed by the same letter within the same column are not significantly different at $p = 0.05$.

effects against *Escherichia coli* and *Staphylococcus aureus* [38]. Extracts from ginger were found to have fungicidal properties against *Fusarium oxysporum* f.sp. *lycopersici* [39]. Sumira et al. [40] reported that crude extracts of *Origanum vulgare* had significant antimicrobial activity against 39 bacteria, 2 yeast, and 16 fungi species.

Different scientists have been applying different concentrations of crude extracts in efficacy studies. In this study, 5 ml of each plant extracts were used to amend 500 ml each of PDA and V8 agar. Sifat and Monjil [41] used a 10% concentration of crude garlic extract which caused 95.50% growth inhibition of *Rhizoctonia* spp, while 20% concentration showed excellent mycelial inhibition of *Rhizoctonia solani* causing sheath blight of rice. Bhuiyan et al. [42] found a 20% concentration of garlic extracts to be effective in controlling the growth of *Colletotrichum dematium*. Garlic and ginger extracts inhibited growth of *Penicillium* spp and *Aspergillus* spp in in vitro [43]. Ginger essential oil was found effective in inhibiting the growth of *Fusarium oxysporum, Rhizoctonia solani,* and *Botryodiplodia theobromae* causing decline in the disease of guavas [44].

From the study, it was evident that the two test pathogen species responded differently to different crude extracts. Ginger extracts were more effective against *A. solani* than *P. infestans* and vice versa for the garlic extracts. This observation can, therefore, be assumed to indicate that the efficacy of the crude extracts may be determined by the sensitivity of the target pathogens to the extracts. This study did not analyse the actual compounds that were responsible for antifungal growth inhibition in the selected test plants. However, analysis of the biochemical compounds in the test plants showed that the chief constituent compounds in ginger, garlic, Mexican marigold, and tick berry were gingerols, allicin, phenols, tagetone, and lantacin, respectively (data not presented). The active antifungal compounds in these plants have been reported in some previous studies. Vidyasagar and Tabassum [45] reported that Mexican marigold plant species contain compounds such as piperitone and piperitenone which inhibit fungal mycelial growth by modifying the structure of the mycelia. Sarfraz et al. [46] reported that allicin from garlic effectively controlled *Alternaria brasicicola*, *Verticillium dahlia*, *Verticillium longisporum*, and *B. cinerea*. Reports of Mahadyet al. [47] showed that ginger has shogaols that contain both antifungal and antibacterial properties. Tick berry reportedly contains flavonoids, alkaloids, and tannins [48] that have been shown to inhibit the growth of *Alternaria alternata* [49].

The results of this study showed that the extraction solvent had a significant influence on the efficacy of the crude extracts. This corraborates the findings of other scientists who reported that the activity of crude extracts varies depending on the plant material, the method of extraction, and the solvent used [50–52]. In this study, the most effective crude extracts were those extracted with ethanol followed by methanol extracts and then water extracts. Similar results were reported by Singh and Srivastava [49] where ethanolic extracts of *Lantana camara* were the most effective in inhibiting the growth of *Alternaria alternata* followed by methanolic and water extracts. In vitro antibacterial activity of ethanolic extracts of tick berry was also reported to be more effective than of water extracts in inhibiting the growth of both Gram-negative and Gram-positive bacteria [53]. The higher level of effectiveness in ethanol as a solvent is attributed to the fact that ethanol is a polar solvent and therefore it produces a higher concentration of phenolics [54]. The low efficacy of aqueous plant extracts is as a result of water not extracting nonpolar active compounds in plant materials [55]. The use of water in extraction also increases the amount of impurities that lower the quality of the extracts [50]. However, hot water extracts from ginger were found to inhibit the growth of *Fusarium oxysporum*, *Aspergillus Niger*, and *A. Flavus* and reduced rotting of yam tubers [56]. Dabur et al. [57] obtained better results with tick berry water extracts than with organic solvents.

From the results of this study, essential oils from the four test plants were also effective in inhibiting the growth of the test pathogens as compared with the negative and positive controls. Similar results have been reported in other previous studies. Essential oils of tick berry were reported to exhibit significant antibacterial activities against different strains of Gram-positive and Gram-negative bacteria [58–60]. Tick berry essential oils also exhibit insecticidal, antifeedants, and antihelminthic properties [61]. Garlic essential oils are reported to exhibit insecticidal properties [62, 63] and nematicidal properties [64]. Mahmoud et al. [65] reported extensive inhibitory effect of garlic essential oil on *F. solani*, *M. phaseolina, R. solani*, and *S. Rolfsii*. Essential oils of ginger were reported to have a substantial fungicidal effect on the *Penicillium expansum*, *A. flavus*, *A. alternata*, *Fusarium oxysporum* [66], *A. parasiticus*, and *A. flavus* [67]. According to Saha et al. [68], essential oils of Mexican

marigold portrayed antifungal properties against *Pyricularia grisea*, *Sclerotium rolfsii*, *A.solani*, and *F. oxysporum*.

The essential oils were found to have a higher efficacy against the test pathogens than the crude extracts. Garlic and ginger essential oils caused total growth restriction in both pathogens just like the synthetic fungicide, Ridomil. These findings were in agreement with those of Sumira et al. [40] who reported that essential oils of *Origanum vulgare* species had a significantly higher antimicrobial activity against 39 bacteria, 2 yeast, and 16 fungi species as compared with crude extracts.

5. Conclusion and Recommendations

From the findings of this study, it was evident that garlic, ginger, tick berry, and Mexican marigold have antifungal properties against *A. solani* and *P. infestans*. The four plants are therefore recommended for industrial utilization for production of less cumbersome fungicidal products that are efficient for use under field conditions. The antimicrobial scope of these test plants can also be expanded by testing their efficacy on a wide range of disease causing micro-organisms. The efficacy of tick berry and Mexican marigold which was found to be lower than that of ginger and garlic can be improved through integrated utilization. Since ginger extracts were found to be more effective against *A. solani* than *P. infestans* and vice versa for the garlic extracts, this study recommends further analysis between these two plants to ascertain this observation before issuing a specific recommendation. In addition, since this study was conducted in vitro, there is also need to conduct a confirmatory in vivo experiment in a greenhouse. This study also recommends ethanol as the most effective extraction solvent as compared with methanol and water.

Data Availability

Most of the data used to support the findings of this study are included within the article. Additional data are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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