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In-vitro Activity of Selected Plant Extracts on Post-Harvest Pathogens Causing Tomato Fruit Rot

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ABSTRACT

Most researchers have majored on research to improve tomato production while neglecting post-harvest issues. Control of the post-harvest diseases in tomato is by use of synthetic pesticides. However, current research shows that pesticides are toxic with long residue effect. Some of the products are rejected in the market due to high chemical residue levels resulting to losses. A sustainable solution to this problem can be obtained from bio-controls that are environmental friendly. In this study, three different crude plant extracts (ginger rhizomes, neem leaves and garlic bulbs) were evaluated *in-vitro* at different concentrations for the control of pathogens causing tomato post-harvest rots. The used concentrations were: 1, 2, and 3mg/ml. The isolated and identified pathogen species used in this study were *Fusarium*, *Rhizopus*, and *Geotrichum*. Pathogen growth media (Potato Dextrose Agar) were amended with the different concentrations of the selected crude plant extracts and the pathogens introduced into the media. Radial growth of the fungal pathogens was measured at an interval of twenty four hours after the second day for seven days and was compared with the control. Results showed that all extracts' concentrations had antimicrobial effect against the test pathogens with garlic having the highest bio-control activity. However, the antimicrobial effect varied with the concentration and the plant species. From the study it is evident that plant extracts can be used as safe alternatives for management of post-harvest rot causing pathogens in tomato fruits thus safeguarding the human health and the environment.

Keywords: Pathogens, Tomato fruit, Inhibition, Post-harvest, Plant extracts.

INTRODUCTION

Tomato is one of the most important vegetables that is widely produced in the world and consumed after potato [1]. It is an important commercial vegetable crop in Kenya which is grown both in green houses and open fields. Open field amounts to about 95% while greenhouse production averages 5% of the total production [2]. In Kenya tomato constitutes 7% of the total horticultural produce and 14% of the total vegetables produced [3]. Production in Kenya averages 410,033 tonnes of tomato fruits per year [3]. The crop is grown for its fruit that is widely used in households for stews, juices, soups, salads, sauce and pastes [4]. The fruit is rich in minerals such as iron, potassium, calcium, zinc etc, vitamins A, B, C and E and lycopene which is an antioxidant that prevents cancer, heart disease and muscular degradation [4]. The fruit is also cholesterol free, low fat content and a good source of fibre and proteins [5].

Despite the human need of tomato fruit, it is a very perishable fruit with a short shelf life and very prone to post-harvest pathogens which cause significant losses on harvested fruits during transportation and storage. These losses are either on-farm or off-farm losses. Developing countries of Sub-Saharan Africa experience the highest post-harvest tomato fruit losses in the fruit and vegetable supply chains [6]. This is because the developing countries allocate less than 5% of the resources on post-harvest while more than 95% of the resources are on the production [7]. Most scientists have focused research on production but neglecting post-harvest issues [7]. This result to increased production but this does not translate to profit because of post-harvest rots. In Kenya tomato post-harvest losses averages 55.3% every year [8]. Some of these losses are caused by fungi and bacteria [4]. Some of the common pathogens causing tomato post-harvest rots include, *Fusarium oxysporum* that causes fusarium rot, *Aspergillus niger* that causes aspergillus rot [9] and *Rhizopus stolonifer* that causes rhizopus rot [10]. Other fungal species known to cause post-harvest tomato fruit decay include *Aspergillus flavus*, *Penicillium* spp and *Fusarium solani* [4].

Reducing post-harvest losses improves the welfare of farmers and the consumers thus increasing food availability [6]. Successful control of post-harvest decay of vegetables and fruits is by use of different kinds of fungicides that are synthetic [11, 12]. However, use of the fungicides has been coupled with major concerns which include: consumers' complain over residues of pesticides on foods which may be poisonous and carcinogenic; fungi developing strains that are resistant to fungicides due to fungicides being used excessively; and pollution of the environment. The synthetic fungicides are health hazards to

farmers and consumers, toxic to non-target organisms such as pests' natural enemies and other beneficial organisms [13]. The consumer market has developed strategies in regard to maximum residue levels in fresh fruits and vegetables [14]. This has resulted to some of the products being denied lucrative market resulting to losses. The rejected products are brought back to the local market where they are sold at low prices. Since in the local markets there is nothing to measure the chemical residue levels in the fruits and vegetables, the consumers buy them because they are appealing to the eyes and therefore end up consuming the chemical residues. There is therefore the need to explore use of post-harvest disease management methods that are effective and pose less risk animal and human health and the environment.

Organic products originating from plants and their analogues have been evaluated as vital sources of agricultural microbicide which act as anti-microbials for control of pathogens affecting crops [15, 16]. Pesticides from plant origin "referred to as botanicals" are bio-degradable, environmentally friendly, cheap and easy to obtain. According to Arokiyaraj *et al.* [17], Shanmugavalli *et al.* [18], Swarnalatha and Reddy [19], new development of organic pesticides of plant origin can be done cheaply. Ramazani *et al.*, [20] reported that chemicals of plant origin are economical, broad spectrum and bio-efficacious. They degrade faster than chemical pesticides, are less likely to destroy beneficial organisms and therefore considered to be environmental friendly. According to Ilondu *et al.* [21] essential oils and phenolics from plants have restraint outcome on micro-organisms. Adaskaveg *et al.* [11] and Serrano *et al.* [22] documented that garlic can be an organic alternative pesticide for management of *Penicillium digitatum.*

Dushyent and Bohra [23] tested the impact of eleven different plant extracts on growth of *Alternaria solani* mycelia and discovered that exracts from *Salsola baryosma* and *Tamarix aphylla* leaves totally restricted pathogen growth *in vivo*. Reports by Stoll [24] and Oparaeke [25] indicated that ginger, neem and garlic extracts harbored some insecticidal properties that manage a broad scope of insect pests such as *Clavigralla tomentosicollis* and *Maruca vitrata*. This study aimed at evaluating the antimicrobial activity of selected crude plant extracts (ginger rhizome, neem plant leaf and garlic bulb) against post-harvest rot causing micro-organisms (*Fusarium* sp, *Geotrichum* sp, *Rhizopus* sp and *Erwinia* sp.) in tomato fruits to serve as an alternative to synthetic pesticides.

MATERIALS AND METHODS

Isolation of Pathogens from Infected Tomato Samples

Rotten tomato fruits were collected from farms and markets for pathogen isolation and were brought to the laboratory. Potato Dextrose Agar (PDA) was the standard media used for fungal isolation. The infected tomato samples were first washed in running tap water and then surface sterilized for 3 minutes using 1% sodium hypochlorite before rinsing them in three changes of sterilized distilled water. A sterilized blotting paper was used to blot the fruits dry. A disinfected scalpel was used to make a 3mm X 3mm cut moving from the healthy area to the rotten area where rot causing pathogens were likely to be more active. The tissues were dried of the juice using sterile blotting paper and directly plated on the sterile PDA petri-dishes and incubated for four days. To obtain pure cultures, single spore isolation was carried out. Sub-culturing was done to obtain pure cultures that were morphologically identified before being used in the experiment.

Preparation of Crude Extracts from Plants

The test crude extracts were attained from garlic (*Allium sativum*) cloves, neem (*Azadirachtin indica*) leaves and ginger (*Zingiber officinale*) rhizomes. The neem leaves were picked from Kenya Agricultural and Livestock Research Organization (KARLO) station in Embu while the ginger rhizomes and garlic bulbs were obtained from Mwea open air market. The process of extraction followed a revised procedure outlined by Handa *et al.* [26]. The ginger rhizomes, neem leaves, and peeled garlic cloves were first washed under tap water before rinsing in three changes of sterilized distilled water and a disinfected blotting paper was used to blot them dry. Garlic cloves and ginger rhizomes were first chopped into smaller pieces and all materials were dried oven separately for three days at a temperature of 40ºC. A kitchen blender was used to grind separately the plant parts into powder and placed in sterilized dull bottles at room temperature in the laboratory. Grinding was done to facilitate the mass transfer of active ingredients from the plant material to the solvent by maximizing the surface area.

One hundred and fifty (150 mls) of methanol was used to soak 50 gms of each of the powder in different conical flasks making sure that the all the powder was completely immersed into the solvent. The mixture was allowed to stand at room temperature but shaking vigorously at different intervals for two days. The extract was filtered through a filter paper and the filtrate poured into sterile universal bottles. The crude extract was concentrated by evaporating the solvent using a vacuum evaporator. After evaporation a concentrate was obtained which was later dried in an oven at a temperature of 40°C for two days to evaporate the remaining methanol. The concentrate was stored in air tight bottles in the refrigerator at 4°C.

Experimental Design and Layout

The experiment was carried out at Kenyatta University Department of Agricultural Science and Technology Laboratory. The experiment was laid out as a completely randomized design consisting of four treatments replicated four times. The treatments included: three concentrations of the crude plant extracts (1mg/ml, 2mg/ml and 3mg/ml) dispensed into four petri-dishes and a control. To make the required concentrations, the crude extracts were weighed separately 1 mg, 2 mg and 3 mg of ginger, neem and garlic concentrates respectively. 1ml of dimethyl sulfoxide (DMSO) was used to dissolve each of concentrate and then in 1ml of sterilized distilled water to form different concentrations of the solutions. Hundred (100) ml of PDA was amended with 3 ml of each of the concentrations of the different extracts respectively and dispensed into four Petri-dishes with four replicates. For negative controls, 3 ml of water was mixed with the media. The media were allowed to cool and solidify. One week old 5 mm fungal culture discs of *Fusarium* sp., *Rhizopus* sp. and *Geotrichum* sp. were cultured at the centre of each Petri-dish per replicate and incubated at room temperature. Data on fungal growth was collected from the second day to the seventh day by measuring the radial growth from each of the treatment at an interval of 24 hr. The mean of the fungal growth on the amended media was compared with the control.

Data Analysis

The antimicrobial activity of the plant extracts to the test pathogens and effects of the concentrations was analysed using SAS one way ANOVA and separation of means done using Students-Newman-Keuls Test (SNKT $p<0.05$).

RESULTS

Efficacy of Different Crude Extracts Concentrations on Radial Growth of

Fusarium sp.

The study revealed that all the plant extracts had antimicrobial effects on the test pathogen by causing radial growth inhibition as compared to control but their anti-microbial activity varied significantly (< 0.001) with concentration and the plant type. Growth of *Fusarium sp.* was inhibited in all neem extract concentrations. At 1mg/ml concentration Fusarium sp growth was noted in ginger and garlic but it was far much lower than the untreated control (Table 1). At 2 mg/ml garlic concentration, growth of *Fusarium* sp. was also completely inhibited but the pathogen exhibited a slow growth on the PDA amended with ginger extract. At 3gm/ml concentration there was 100% *Fusarium* growth inhibition in all the extracts. There was a significant difference (p<0.001) in the bio-control activity of the first two concentrations of ginger and garlic on *Fusarium* sp.

Table 1: Efficacy of crude extracts on radial growth (mm) of *Fusarium* spp.

Concentration (mg/ml)	Neem meanRG±SD	Ginger meanRG±SD	Garlic meanRG±SD	p-value
0	42.85±2.68^{D}	42.85±2.68^{D}	42.85±2.68^{D}	-
1	5.00±0.00^{A}	6.40±0.50^{Cb}	5.40±0.82^{Bb}	0.01
2	5.00±0.00^{A}	5.20±0.41^{Ba}	5.00±0.00^{Aa}	0.012
3	5.00±0.00^{A}	5.00±0.00^{Aa}	5.00±0.00^{Aa}	-
		<0.001	0.012	

Mean values followed by the same lower case within the same column are not significantly different (One way ANOVA, Students-Newman-Keuls test, α=0.05) while mean values followed by the same upper case within the same row are not significantly different (One way ANOVA, Students-Newman-Keuls test, α=0.05)
RG refers to radial growth and it includes inoculum disc which was 5mm

Efficacy of Different Crude Extracts Concentrations on Radial Growth of

Geotrichum sp.

Results obtained revealed that the extracts from the three plant inhibited radial growth on *Geotrichum* sp. as compared to the control but the fungicidal ability varied significantly (p<0.001) with extracts' concentrations and the type of the plant extract (Table 2). At 1 mg/ml concentration, effects of the three plant extracts on *Geotrichum* spp. differed significantly (p<0.001). At 2mg/ml concentration, the effects of the extracts on *Geotrichum* sp. also differed significantly (p<0.001). The most effective concentration in reducing *Geotrichum* sp. radial growth in all extracts was the 3 mg/ml. The antifungal activity of neem differed significantly (p<0.001) at varying concentrations with 3mg/ml showing highest growth inhibition. The antimicrobial activity of ginger also differed significantly (p<0.001) at 1mg/ml and 2mg/ml concentrations. The 3 mg/ml concentration was the best in reducing the radial growth of *Geotrichum* sp. Garlic had the highest efficacy in restraining the growth of *Geotrichum* sp. among the tested extracts and it excellently inhibited the growth of the test pathogen in all the concentrations.

Table 2: Efficacy of crude extracts on radial growth (mm) of *Geotrichum* sp.

Concentration (mg/ml)	Neem meanRG±SD	Ginger meanRG±SD	Garlic meanRG±SD	p-value
0	34.80±2.42^{Dd}	34.80±2.42^{Dd}	34.80±2.42^{Dd}	-
1	7.70±2.08^{Cc}	6.10±1.07^{Bb}	5.00±0.00^{A}	<0.001
2	6.20±1.20^{Cb}	5.60±0.50^{Ba}	5.00±0.00^{A}	<0.001
3	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00	-
p-value	<0.001	<0.001	-	

Mean values followed by the same lower case within the same column are not significantly different (One way ANOVA, Students-Newman-Keuls test, α=0.05) while mean values followed by the same upper case within the same row are not significantly different (One way ANOVA, Students-Newman-Keuls test, α=0.05)
RG refers to radial growth and it includes inoculum disc which was 5mm.

Efficacy of Different Crude Plant Extracts Concentrations on Radial

Growth of *Rhizopus* sp.

The results obtained from the study revealed that the three plants extracts portrayed varying antifungal effects on *Rhizopus* sp. as compared to the control although their antifungal property differed significantly with the concentration (Table 3). All the concentrations of garlic extract completely inhibited the growth of the *Rhizopus*. The 1 mg/ml and 2 mg/ml concentration of neem and ginger extracts did not differ significantly in their fungicidal activity against *Rhizopus* sp. In ginger the bio-control activity in 1mg/ml and 2mg/ml concentrations did not differ significantly against the test pathogen. The antimicrobial activity of neem varied with the concentrations. At 3 mg/ml concentration radial growth of *Rhizopus* was completely inhibited in all the extracts respectively.

Table 3: Efficacy of crude extracts on radial growth (mm) of *Rhizopus* sp.

Concentration (mg/ml)	Neem meanRG±SD	Ginger meanRG±SD	Garlic meanRG±SD	p-value
0	80.15±0.89^{C}	80.15±0.89^{C}	80.15±0.89^{C}	-
1	7.20±1.77^{Bc}	6.70±3.57^{Bb}	5.00±0.00^{Aa}	0.010
2	5.85±0.88^{Bb}	5.55±1.15^{Bb}	5.00±0.00^{Aa}	0.007
3	5.00±0.00 $^{A a}$	5.10±0.11^{Aa}	5.00±0.00 ^{Aa}	0.012
p-value	<0.001	0.028	-	

Mean values followed by the same lower case within the same column are not significantly different (One way ANOVA, Students-Newman-Keuls test, α=0.05) while mean values followed by the same upper case within the same row are not significantly different (One way ANOVA, Students-Newman-Keuls test, α=0.05)
RG refers to radial growth and t includes inoculum disc which was 5mm.

Comparative Response of Varying Extracts on the Test Fungal Pathogens

From the results, it was evident that all the extracts from the test plants contained antimicrobial properties against the test fungi, although the bio-control activity of the extracts varied with type of the plant (Table 4). The test pathogens responded differently to the different crude extracts. The efficacy of the extracts differed significantly (p<0.001) as compared to the control on all the test

pathogens. On *Geotrichum* sp, the fungicidal activity of the extracts differed significantly with garlic being the most effective followed by ginger. The most effective crude plant extract on *Fusarium* sp. was neem crude extract, followed by garlic and last was ginger. However, neem extract was less effective on *Geotrichum* sp. The antimicrobial properties of ginger and neem extracts did not differ significantly on the *Rhizopus* pathogen. However, garlic extract portrayed a higher antifungal activity against all the pathogens as compared to ginger and neem. *Fusarium* spp. was the most susceptible pathogen whose radial growth was mostly restricted by all the extracts used in the study compared to other pathogens (Table 4). There was no significant difference between the susceptibility of *Geotrichum* spp. and *Rhizopus* spp. on ginger and garlic extracts. The unamended control differed in growth as compared to other treatments.

Table 4: Efficacy of crude extracts on the test fungi

	Geotrichum	***Fusarium***	***Rhizopus***
Treatment	**Mean RG±SE**	**Mean RG±SE**	**MeanRG±SE**
Control	34.80±2.42^{d}	42.85±2.68^{d}	80.15±0.89^{c}
Neem	6.30±0.23^{c}	5.00±0.00^{a}	6.02±0.19^{b}
Ginger	5.57±0.10^{b}	5.60±0.09^{c}	5.75±0.29^{b}
Garlic	5.00±0.00^{a}	5.13±0.06^{b}	5.00±0.00^{a}
p-value	<0.001	<0.001	<0.001

Mean values followed by the same lower case within the same column are not significantly different (One way ANOVA, Students-Newman-Keuls test, α=0.05)
RG refers to colony radial growth and it includes inoculum disc which was 5mm

DISCUSSION

The study revealed that all the different concentrations of crude plant extracts used in the experiment had antimicrobial properties against the tested post-harvest loss causing pathogens although in all the cases their effectiveness varied with the concentration. The results obtained proof that ginger, neem and garlic extracts contain anti-bacterial and anti-fungal properties by their ability to inhibit mycelial growth of the test fungi. However the antimicrobial properties of the extracts varied with the type of the plant that the extracts were obtained from. The results showed that the most effectual crude extract in hindering the growth of all fungal pathogens used in the study was garlic and was used in lower concentration as compared to neem and ginger. In addition, all evaluated concentrations of garlic had significant reduction of mycelial growth of fungi hence could be considered as the best alternative for the management of post-harvest pathogens in tomato. The results are in consonance with those of other authors such as Dutta *et al*. [27], who cited that garlic at a concentration of 10 % caused total reduction in sclerotial production and excellent mycelial inhibition of *Rhizoctonia solani* that causes rice sheath blight was inhibited by garlic at a concentration of 20%. Studies carried by Anjorin *et al.* [28] reported that garlic portrayed antifungal activity against *Fusarium* sp.

Bhuiyan *et al*. [29], reported garlic extracts at a concentration of 20% to have fungicidal properties against *Colletotrichum dematium*. Sowjanya and Manohara [30] also documented that amongst five plant extracts tested, garlic extract at 10% concentration portrayed a higher antifungal activity by completely inhibiting the mycelial growth of *Microsporum gypseum*. Garlic had been evaluated together with ocimum and neem. This indicates that higher plants are unexploited reservoirs of several vital chemical compounds that are anti-fungal. Reports by Paradza *et al.* [31] indicated that when neem and garlic extracts were used in management of bacterial soft rot, garlic portrayed higher antibacterial activities in reducing bacterial maceration of the potato tissue. It was established that the tested crude plant extracts demonstrated different antibacterial and fungicidal ability against the tested pathogens which could be attributed to differences in degree of susceptibility of each of the test pathogen to the varying dosage of the extracts. *Fusarium* sp. proofed to be the most sensitive pathogen in all the plant extracts tested with neem leaf extract showing the most antifungal activity against *Fusarium* sp.

These results of this study are also in support of the results of Hassanein *et al.* [32], where neem extracts was evaluated in four concentrations and the lowest concentration (20 %) excellently restrained (100%) *Fusarium oxysporum* mycelial growth. The results also support the findings of Tijjani *et al.* [4] who reported that antifungal properties of neem and moringa extracts inhibited mycelia growth of *R. stolonifer* that causes potato wet rot. Report of Amadi and Olusanmi [33], extracts from neem and garlic have anti-microbial properties against a vast range of plant disease causing micro-organisms with garlic having the highest antimicrobial activity. Garlic extract was also found to have a higher fungicidal activity against *C. tropicalis*, *C. albicans* and *S. paratyphi* [34]. According to Nahed [35] cold extracts of *Azadirachtin indica* reduced growth of *Fusarium oxysporum*, a causal agent of rots in cucumber. *C. gloesporides*, a pathogen that causes cassava anthracnose was managed using neem extracts [36].

Aqueous and methanolic garlic extracts were reported to have antibacterial activity against three bacteria isolates (*E. coli*, *S. aureus* and *P. aeruginosa*) in an *in-vitro* study [37]. Udo *et al.* [38] reported mycelial growth restriction and low spore production in fungal pathogens of *Ipomea batatas* by extracts from garlic. Stangarlin *et al.* [39] showed that sclerotial production and mycelial growth of *Sclerotina sclerotium in-vitro* was inhibited by aqueous extract of ginger at varying concentrations. The anti-fungal properties of ginger in inhibiting the growth of the mycelia of fungal pathogens is in agreement with the results of this study. The inhibitory effect was directly proportional to the crude extract concentration: the higher the concentration the higher the inhibitory effect. Ijato [40] documented that extracts of *Ocimum gratissimum* and *Zingiber officinale* were toxic to *Fusarium oxysporum*, *Aspergillus flavus* and *Aspergillus niger* that cause post-harvest rot of yam tubers and that the extracts efficacy increased with increase in concentration as it was noted in this study. The findings of Chuku *et al.* [41] indicated that ginger extract at a concentration of 3 gm/20ml of water excellently restrained the growth of fungi that cause tomato fruit rots *invitro*. Tijjani *et al.* [42] reported that tomato fruits coated with aqueous extracts from neem seeds, moringa seeds and garlic bulbs inhibited growth of fungi and maintained the fruit quality.

The antimicrobial activity of the plant extracts on the test pathogens differed between the plant species. This could be due to variation in chemical compounds present in different plants. This observation is supported by Miron *et al.* [43] and Daniela *et al.* [44] who reported that allicin which is a compound found in garlic is readily membrane-permeable and undergoes thiol-disulphide exchange reactions with free thiol groups in proteins and the compound is anti-viral, anti-fungal and anti-bacterial. When garlic bulbs are cut a substrate alliin combines with alliin-lyase enzyme and forms a volatile compound which is anti-fungal and disrupts fungal cell metabolism due to oxidation of proteins [45]. These properties may have formed the basis for its anti-microbial action. Chiejina and Ukeh [46] documented that ginger has high quantities of alkaloids and this may have formed the

basis of its efficacy against pathogens. According to Okwu [47] the most efficient, significant and therapeutic plant substances are alkaloids. Fresh ginger contains high concentrations of oxygenated compounds such as geranial, neral, borneol, 1,8-cineole and α-terpineol with high antifungal and antibacterial properties [48]. Hoque *et al*. [49] cited Mahmoodin compound found in neem a very active against gram-negative and gram-positive bacteria.

CONCLUSION

In conclusion, the evaluated plant extracts were effective against the test pathogens but the efficacy was dependent on the concentration. Crude extracts can substitute synthetic fungicides as alternative means of controlling rots in tomato fruits by farmers. Results of this study provide an important step in developing bio-pesticides of plant origin the control of fruit rots because the plants are affordable and readily available. However, there is need for *in-vivo* trials and then formulations that may be easy and efficient for farmers and consumers to use.

Conflict of Interests

The author state that there is no conflict of interest.

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