ANTIMICROBIAL ACTIVITIES OF *Tithonia diversifolia* AND *Kigelia africana* AGAINST *Fusarium oxysporum* IN TOMATOES

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ABSTRACT
Fusarium wilt disease caused by the soil-borne fungus *Fusarium oxysporum* Schlechtthe is a serious threat to tomato production worldwide. Chemical and cultural methods of management used are either ineffective or toxic to the environment. Plant secondary metabolites; therefore, pose a possible alternative because they are environmentally friendly and have minimal effect on non-target organisms. This study screened phytochemical compounds of two plants; *Tithonia diversifolia* and *Kigelia africana* and assessed their potency in controlling plant fungal pathogen *F. oxysporum*, the causative agent of Fusarium wilt disease in tomatoes. Leaf extracts of *T. diversifolia* and fruit extract of *K. africana* were used in this study becauses they are readily available and the plnts have been used in the treatment of varius diseases in humanbeings. The plant etract were concentrated in water and screened for phytochemical contents using standard procedures. Concentrations used were 25 g/L, 50 g/L and 100 g/L to sock the disc. *Fusarium oxysporum* was isolated from infected soil using potato dextrose agar media while the antifungal activity was evaluated by measuring the zone of inhibition against the test organism. In determining the antimicrobial properties of each of the plant extract, a 3×3 randomised complete block design was used, replication was done 3 times. The results showed that the mean inhibitory zones were highest at 100g/l in both plants, although *K. africana* fruit extract portrayed the higher inhibitory activity compared to *Tithonia africana*. The effect of the plant extracts and the positive control were statistically significant (pα0.05). This study indicates that *K. africana* and *T. diversifolia* possess the antifungal activity and can be used as a broad-spectrum fungicide against *F. oxysporum*. The effect of the plant extract was statistically significant ($p<0.05$). The Mean of inhibition ranged from 23.67 to 7.78 mm for *Tithonia diversifolia* and 23.67 to 11.75 for *Kigelia africana*. While for the combined extract the The Mean of inhibition ranged from 22.78 to 7.93 . The minimum reduction in sporulation was recorded in the positive control, which was significantly lower than the rest of the treatments.These plant extracts may provide an effective measure for the management of Fusarium wilt of tomatoes that may form an integral part of integrated pest management and become a prospect alternative to conventional fungicides.
Keywords: Plant extracts, *Fusarium, Kigelia africana,* Phytochemical effects, *Tithonia diversifolia*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae family and is grown and consumed globally. In Kenya, the production of tomato is 14% of the whole vegetable harvest and the whole horticultural crops account for 6.72% (Government of Kenya (GoK), 2016). According to Food and Agriculture Organization of the United Nations, (2018), tomatoes are either grown on fields or in greenhouses and its production in the field accounts for 95 % while greenhouse production accounts for 5 % of the entire tomato production in Kenya.

In Kenya, the production of tomatoes contributes to economic development and reduction of poverty. Tomatoes are a source of income, job creation and foreign exchange to the farmer leading to earning for economic development (Collins, 2012; Ongeri, 2014). However, its cultivation is faced by constraints such as adverse weather and environmental conditions, soil type and pathogenic microorganism causing diseases (Berrueta *et al*., 2012). *Fusarium oxysporum* is a pathogen that causes Fusarium wilt disease in tomatoes and it destroys the xylem (Ajilogba and Babalola, 2013). According to Agrios, (2005) control of *F. oxysporum* pathogen has become a problem since it persists in the soil and sustains infection in the farm.

Farmers prefer use of synthetic fungicides to control Fusarium wilt disease in tomato in Kenya (Mwangi *et al.,* 2015). However, synthetic fungicides and cultural practices that include crop rotation are not effective (Chandler *et al*., 2011; Chellemi et *al*., 2012; Palti, 2012; Machado *et al*., 2018). Crop rotation and shifting cultivation are no longer possible because of insufficient land due to increased human population (Bawa, 2016). Excessive uses of chemicals are environmentally toxic (Koutros *et al*., 2012; Malkhan *et al*., 2012; Onkendi *et al*., 2014). In addition, fungicide application to limit disease occurrence has lead to development of resistant pathogen strains (Akbar *et al*., 2009; Malkhan *et al*., 2012; Gliessman,

2014;Vincelli, 2016). Misuse of fungicidal synthetics has rendered them less effective in controlling the pathogen (Hadian *et al*., 2011). Further, fungicides due to complex production process have become expensive to farmers (Ngowi *et al*., 2007; Njoroge, 2014).

Due to limitations of synthetic fungicides and other methods used to control Fusarium wilt, there is need to explore and develop bio-fungicides from plant products (Gilligan, 2008). Plant products may be environmentally friendly with minimal effect on non target soil microorganisms (Njoroge, 2014; Lucia, 2017). *Tithonia diversifolia* and *K. africana* are widely distributed in Kenya, have medicinal value and high seed production and dispersal (Zarghani *et al*., 2015). Both are tolerant to harsh environmental conditions and therefore able to reproduce continuously even during the dry season. The two plants have allelopathic property facilitating their survival by minimising resource competition (Ajayi, 2017).

According to Dewole *et al*. (2013) and Saini *et al*. (2009) *Tithonia diversifolia* and *Kigelia africana* extracts have been used to control plant pathogens such as *Phytophthora nicotianae* and *Rhizoctonia solani,* However, information on their application and efficacy in control of *Fusarium oxysporum* sp lyperseci is scarce. Therefore, this study was carried out to evaluate the efficacy of the water extract of *Tithonia diversifolia* and *Kigelia africana* against *Fusarium oxysporum* mycelial growth under laboratory conditions.

MATERIALS AND METHODS

Experimental Site

All the samples for *T. diversifolia*, *K. africana* and *F. oxysporum* were collected in Kangai ward, Mwea west Sub County in Kirinyaga County. Kirinyaga county is located in the central highlands of Kenya. Kangai ward is located in the Northern part of Mwea west Sub County and is within the Upper Midland Zone 4 (UM4) Agro Ecological Zone (Fig 3).

The Latitude of the area lies between 0°60' south and Longitude 37°30' East. The altitude of the area lies at 1,050 Metres above sea level. Kangai ward has a soil made up of nitosols (Mwangi *et al*., 2015). The area experiences a bimodal rainfall with an average rainfall of about 850 mm and an average temperature of 22^{0}C. Antimicrobial studies were done at Chuka University in the Department of Biological Sciences Laboratories

Study Design

In determining the antimicrobial properties of each of the plant extract, a (3×3) Randomised complete block design was used, replication was done 3 times.

Plant Sample Collection

Purposive sampling was used to locate the sample collection points. Two plants were collected. For *Tithonia diversifolia*, the leaves were collected using a pair of scissors while for the *Kigelia africana* the fruits were collected using a knife. The samples were then chopped into small pieces on aluminum foil separately. The samples were transferred to a glass bottle whch was full to the brim separately. The plant samples were then placed in a bag and taken to the laboratory.

Preparation of Crude Extract Concentration

Preparation of crude extract concentration was done according to Hossain *et al*. (2013). The plant samples were washed and allowed to air dry under a shade separately. After a week, 100 g of fresh sample was crushed in a surface-sterilized pestle and mortar by adding 100 ml sterile water and allowed to stand for three days after which filtering was done using Whatman paper separately. Only the phytochemical constituents soluble in water dissolved to form a solution. Water was removed by freeze drying the solution. Concentrations of 2.5% (25 g/L) 5% (50 g/L) and 10% (100 g/L) were prepared separately by adding 2.5 g, 5 g and 10 g of the extract supernatant respectively with 5 ml of water to enhance dissolution and made up to 100 ml by adding distilled water. The plant extract was then placed into vials and stored in the refrigerator prior to antimicrobial assay.

Collection of *Fusarium oxysporum* in the Farm

Soil samples were collected where tomatoes were grown in the farm using random sampling. The farm was then structured into different homogenous units based on visual observation. Soil samples were collected using the zigzag method. The surface litter was removed from the sampling spot. A ‘V’ shape cut of 15 cm depth was dug from the sampling spot using a spade. In each of the sampling units, Ten soil samples were collected. The thick slices of soil were removed from top to bottom of the exposed face of the ‘V’ shape cut and placed in a clean container.

The soil sample was mixed thoroughly and the foreign materials like stones, roots, and pebbles were removed. The bulk of the soil sample was reduced to about half to one kilogram by the use of quartering. Quartering was done by dividing the soil sample after mixing thoroughly into four equal parts on a piece of paper. The two opposite quarters were discarded while the other remaining quarter was mixed and the process was repeated until a reasonable sampling size of 30 g was obtained. The sample was placed in a clean piece of aluminium foil. The sample was labelled with the location of the farm and the date of collection.

Preparation of Potato Dextrose Media

Thirty-nine grams of Potato Dextrose Agar (PDA) media were placed in 1000 ml distilled water and dissolved by heating over a bunsen burner. Sterilization was done by autoclaving at 15 lbs. pressure (122°C) for 15 minutes. It was then mixed well before dispensing (Sheringham and Brightwell, 2012).

Preparation of *Fusarium oxysporum* Cultures

Ten grams of the soil sample was spread on the surface of the PDA media. The soil sample was then inoculated into the PDA media and then kept in the incubator at 28^0C (Latiffah *et al*., 2010), to allow growth. After growth, the fungus was sub cultured to obtain a pure culture. Koch's postulate was used to confirm that the pathogen was indeed *Fusarium oxysporum*.

Identification of *Fusarium oxysporum* Cultures

Identification and isolation of *F. oxysporum* was done according to De Carolis *et al*. (2012) through observation of growth and microscopic traits. For the growth traits identification was done by observing the colour in the nutrient medium because *Fusarium oxysporum* tends to be pink or purple in colour. For the microscopic characteristics, chlamydospores were observed. First, the formation of microconidia spores were observed. Observation was based on the presence of unicellular spores with an oval shape.

Preparation of Standard Inocula of Test Pathogens

This was done according to Alaniz *et al*. (2011). Spore suspension of *F. oxysporum* was prepared from young 10-day old cultures grown on PDA. The spore suspensions were obtained using a sterile cork-borer to pick a colony on the cultured surface to remove the spores from the surface of the PDA nutrient medium.

Preparation of Antimicrobial Test Discs

The discs were prepared following Arunkumar (2009) procedures. Test disc was made using a paper punch. Paper discs of 6 mm diameter were cut off from a sheet of Whatman paper 3. The paper discs were then sterilized in the oven at 150ºC. The bottle containing the paper disc was stored in a cool, dry cupboard. Antimicrobial test disc was made by taking two ml of each of the concentration of the stock solution of each plant extract and pipetting on a sterile paper disc of 6 mm diameter in a sterile petri- dish, this was to give a concentration of 2 mg/disc. The preparation of antimicrobial test disc was done in triplicate three.

Antimicrobial Testing of *Fusarium oxysporum*

The antimicrobial test was done according to Assob et al. (2011). Eighty-one plates of PDA media plates were prepared. Pure isolates of *F. oxysporum* were cultured by transferring a loop of culture into sterile nutrient media on each plate. The antimicrobial test discs or the Whatman paper discs socked with plant extracts were placed on to the media; the media was placed in an inverted position and incubated at 28ºC for 1 week to allow for growth. Benomyl was used as a positive control in the experiment.

Data Analysis

Statistical Analysis Software version 9.4 was used to analyze the measured data of zone of inhibition. Two-way ANOVA was performed to determine differences in zones of inhibition between the plant extracts exposed to *F. oxysporum*. Means were determined using Least Significance Difference test with $\alpha=0.05$.

RESULTS AND DISCUSSION

Isolation of *Fusariumo xysporum*

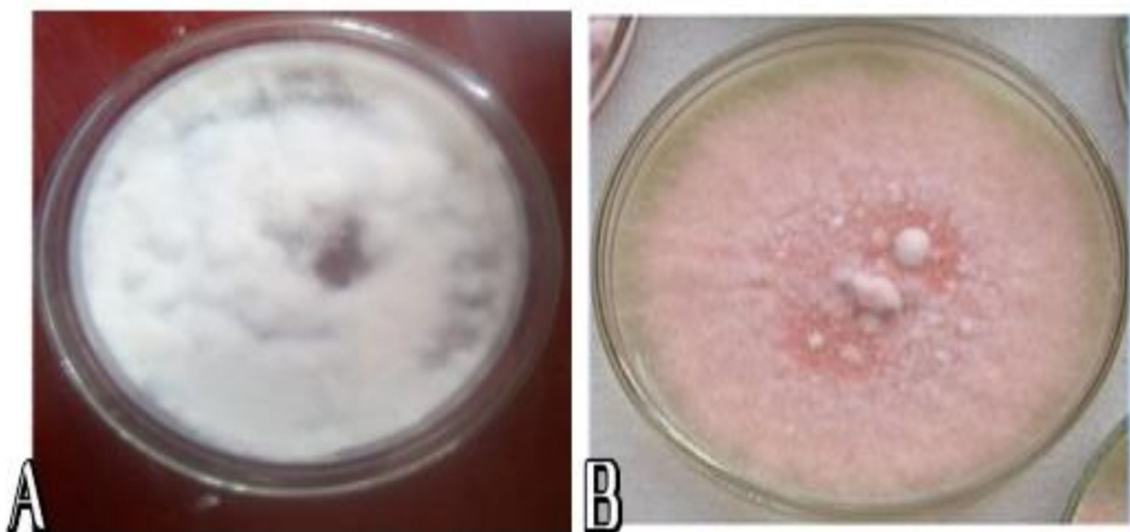
Fusarium oxysporum was recovered from the soil. The microscopic features that were observed during identification were septation and shapes of microconidia and macroconidia and the structures of the chlamydospores. *Fusarium oxysporum* grown in the PDA media produced white mycelia, with a cotton appearance that was pink in colour on the reverse side of the plate. Microscopic characteristics: presence of oval non-septate microconidia and macroconidia with a slight curvature, septate and pointed apical cell confirm that the isolates were of *Fusarium oxysporum.*

Antimicrobial Activity of Plant Extracts

Antimicrobial activity of *T. diversifolia* and *K. africana*

As the plant extract concentration was increased, there was a reduction in the fungal mycelia growth. The effect of the plant extract was statistically significant ($p<0.05$). The Mean of inhibition ranged from 23.67 to 7.78 mm for *T. diversifolia* and 23.67 to 11.75 for *K. africana*. The growth inhibition of *F. oxysporum* increased linearly with an increase in the concentration of the botanicals (Tables 1 and 2).

The zone of inhibition on the mycelial sizes on the control plates were significantly higher ($P=0.05$) than for the plant extract. The statistical analysis showed that both *T. diversifolia* and *K. africana* extract at different concentrations significantly affected radial growth and sporulation of *F. oxysporum*. From the LSD test, the mycelia growth rate for concentration 100 g/ml was lower than the growth rate of the control.



Fusarium oxysporum *grown in the PDA media, (A) showing a white mycelia, with a cotton appearance and (B) showing a pink colour on the reverse side of the plates*

Table 1: Zone of inhibition recorded on petri dishes treated with different concentrations of *Tithonia diversifolia* against *Fusarium oxysporum*

Treatment	Zones of inhibition
p1benomyl	23.67 [a]
p2benomyl	23.00 [a]
p3benomyl	21.67 [b]
c3t3	10.63 [c]
c2t3	10.40 [cd]
c1t3	10.22 [cd]
c2t2	9.90 [cde]
c1t2	9.67 [de]
c3t2	9.25 [e]
c2t1	8.2 [f]
c3t1	7.88 [f]
c1t1	7.78 [f]
$LSD_{(0.05)}$	0.44
Mean	10.68
CV (%)	6.18

[a]Means followed by the same letter are not significantly different. P1 benomyl; P was pathogen and benomyl positive control, C3T3, C2T2 and C1T2;(C) was *Fusarium oxysporum* pathogen and (T) *Tithonia diversifolia*

Comparison for the Best Performance between the Plant Extract and Positive Control

The Mean of inhibition ranged from 22.78 to 7.93 (Table 3). The minimum reduction in sporulation was recorded in the positive control, which was significantly lower than the rest of the treatments. The *Fusarium oxysporum* fungal mycelial diameters on media with the control (benomyl) were significantly lower compared with the plates that contained *Kigelia africana* and *Tithonia diversifolia*. Higher inhibition of fungal hyphal growth was recorded in media treated with benomyl. Hyphae on media incorporated with *Kigelia africana* had a bigger diameter than in media treated with *Tithonia diversifolia* though there was no significant difference ($P < 0.05$). The findings showed that the fungicides tested against Fusarium oxysporum were significant in inhibiting the fungal mycelial growth. Benomyl was significantly superior to the

plant extract. The positive control was effective thus producing zones of inhibition on the plate cultured with Fusarium oxysporum, however, *Kigelia africana* was more effective at a high concentration whereas, *Tithonia diversifolia* was comparatively less effective compared to *Kigelia africana*. The sporulation of the *Fusarium oxysporum* varied greatly with plant extract used at different concentrations. The sporulation decreased linearly with an increase in concentrations and the type of plant extract. The effect of the plant extracts and the positive control was statistically significant ($p<0.05$).

Table 2: Zone of inhibition recorded on petri dishes treated with different concentrations of *Kigelia africana against Fusarium oxysporum*

Treatment	Zones of inhibition
p1benomyl	23.67 [a]
p2benomyl	23.00 [ab]
p3benomyl	21.67 [b]
c1k3	16.00 [c]
c3k3	15.38 [c]
c2k3	15.30 [c]
c3k2	13.50 [d]
c1k2	13.22 [de]
c2k2	13.10 [de]
c2k1	12.30 [de]
c1k1	12.13 [de]
c3k1	11.75 [e]
$LSD_{(0.05)}$	0.75
Mean	14.56
CV (%)	7.71

[a]Means followed by the same letter are not significantly different. P1 benomyl; P was pathogen and benomyl positive control, C1k3, C3k3 and C2k3:(C) was *Fusarium oxysporum* pathogen and (K) *Kigelia africana* extract.

Table 3: Comparison for the best performer between the plant extract and the positive control

Treatment	Zone of inhibition
benomyl	22.78[a]
K3	15.56[b]
K2	13.30[c]
K1	12.07[cd]
T3	10.44[cd]
T2	9.63[e]
T1	7.93[f]
LSD	0.59
Mean	12.08
CV	8.05

[a]Means followed by the same letter are not significantly different by two way ANOVA followed by LSD test at 5% probability level. Benomyl was the positive control, (K3,K2,K1; 2.5% (25 g/L) 5% (50 g/L) and 10% (100 g/L) was *Kigelia africana* and (T1, T2, T3; 2.5% (25 g/L) 5% (50 g/L) and 10% (100 g/L) was *Tithonia diversifolia*.

DISCUSSION

Isolation of *Fusarium oxysporum*

The colony had a mass of white colour from the upper part of the plate. Cultural characteristics of the pink colour confirmed that the isolate was *F. oxysporum*. The findings were similar to those of Shobha and Kumudini (2012). Pink colour was seen on the lower surface of the PDA plate. This finding is consistent with that of El Kichaoui (2016) and Bedasa (2018)

Antimicrobial Activities of the Plant Extracts

Antimicrobial activities of *T. diversifolia* and *K. africana*

The findings revealed that *Tithonia diversifolia* had the lowest antifungal activity. The study also revealed that as the concentration of *Tithonia diversifolia* extract was increased, the mean mycelia diameter decreased. This was attributed to the various phytochemical constituents that contributed to antifungal activity, these phytochemical components of *Tithonia*

diversifolia are the ones that either have direct inhibitory effects on pathogens, exhibiting bio fungicidal or bio fungistatic properties (Tagne *et al*., 2018). However in this study, *Tithonia diversifolia* showed the least zones of inhibition. This could be due to the variation on the quantity of each of the phytochemicals used.

Enyiukwu *et al*. (2014) also reported a similar finding that the leaf extracts of *Tithonia diversifolia*is toxic to *Fusarium oxysporum* spelaeidis showing a complete inhibition of mycelial growth and spore germination. According to Enyiukwu *et al*. (2014), they did report similar findings that *Tithonia diversifolia* plant extract was very effective in inhibiting the growth and the sporulation of selected fungal pathogen causing leaf spot disease.

Tagne *et al*. (2018) also reported that the fungicidal spectrum of *Tithonia diversifolia* has been attributed to various compounds such as stigmasterol and sitosterol. Spore yield among fungicides treatments was as a result of the antimicrobial activities on the mycelia growth. He further reported that *Tithonia diversifolia* antimicrobial activity was very high in some fungal pathogens and the mycelia count was low compared to the other plates in which *Tithonia diversifolia* was not used thus the fungus was able to actively sporulate.

The finding revealed that the crude extract of *Kigelia africana* resulted in a significant inhibition in radial mycelia growth. The study revealed that as the concentration of *Kigelia africana* extract was increased, the mean mycelia diameter decreased. This was attributed to the various phytochemical constituents for instance; kigelinone, ferulic acid and iridoids that could be causing the zone of inhibition (Rahmatullah *et al*., 2010).

These findings were similar to the ones reported by Rejeki and Addy (2017) and Zofou *et al*. (2013) who reported the anti-fungal activity of *Kigelia africana* against *Fusarium oxysporum* spcubense which is a pathogen that attacks bananas. Similar results were also reported by Itonga (2011) who reported that *Kigelia africana* extract was effective against eighteen different fungi species.

This finding was similar to the one reported by Al-Mujammaa'e (2008) who observed that *Kigelia africana* extracts induce the disruption of fungal cell metabolism, increased permeability of fungal plasma membrane and destruction of the conidial wall structure. The more the *Kigelia africana* extract is concentrated the higher the inhibition level.

The positive control plates treated with fungus alone had the highest mycelial diameter (no zone of inhibition) which confirms that *Kigelia africana* has antifungal compounds that inhibited *Fusarium oxysporum* mycelial growth.

According to Ibrahim *et al*. (2015), the antifungal activities of acetone extract of *Kigelia africana* were effective against various fungal pathogen. Moreover, he reported that the antimicrobial activity of the plant extracts was due to the phytochemical constituents for instance tannins and other polyphenols present in the plant extracts.

Comparison of the Best Performance between the Plant Extract and Positive Control.

Fungicides are an effective and straight method for fungal disease management because they are easily obtained in the agro vet shops. The study revealed that the fungicide (Benomyl) had the largest zone of inhibition. This could be a result of the interaction of Metalaxyl and Mancozeb contained in Ridomil making it more effective. Although, the pesticide could be more effective, the consequences of debris of chemicals have caused problems to human beings, livestock and the environment.

In a study carried out by Kesavachandran *et al*. (2009), he reported that about one million persons die due to the chronic diseases caused by pesticide poisoning. Although, the pesticide is a source of employment to those who formulate and manufacture the chemical (Tornero and Hanke, 2016), it poses a threat to their lives because they handle harmful solvents and chemicals during the manufacture of these chemicals. Farmers are also at risk because they inhale the toxic substances during spraying and mixing of the pesticide.

The inhalation of the pesticides is likely to cause implications on human health. The pesticides disrupts the endocrine system by antagonizing the production of the hormones from the endocrine glands, exposure of the pesticides to different dose rates has implication to the immune system, reproductive gametes and eventually resulting into cancer.

Similar findings were also recorded by Iyer and Makris (2010) who reported that there was increased death rate due to the destruction of the cardiovascular and respiratory diseases caused by pesticides in human beings. He also reported that cancer and death cases had increased. He did observe gastrointestinal and the lymphatic tissues upon exposure to pesticides were at risk of having mutation thus leading to cancer.

This finding was similar to the one by Amini and Sidovich (2010) who reported that bromuconazole and Prochloraz proved to be antimicrobial and were effective against the fungal Fusarium oxysporum in an invitro study done under the glasshouse conditions.

According to Yin *et al*., (2011) different fungicides were more efficient against the control of various fungi and inhibited multiplication of the cells (cell division) and mitosis in fungi. However, Singh *et al*. (2017) reported that the bromuconazole and Mancozeb component of ridomil was found to be present in the pawpaw fruit. Ridomil was sprayed to the pawpaw to reduce the postharvest losses.

Due to the lack of awareness from the consumer and the famer, they are exposed to these synthetics when they consume food crops that pesticides are used in the management of pest.

Previous researches revealed the fungicides inhibitory property usually is a result of polymerization of β-tubulin in microtubules reducing their proliferation and dynamic instability (Ding *et al*., 2016). Further, methylbenzim-idazolecarbamate fungicides suppress the meeting of spindle microtubules owing to disturbed chromosomal alignment and microtubule-kinetochore interactions at the metaphase plate causing chromosome loss and chromatid loss, in pathogenic fungi (Lebeda *et al*., 2010).

Difenoconazole fungicide exhibits antimicrobial activities against different plant fungal pathogens for instance ascomycetes, basidiomycetes and deutromycetes (Kuck *et al*., 2012). However this pesticide has caused more implications to the environment. In a study carried out by Close (2018) he reported that the difenoconazole fungicide that was used to control disease in grapes greatly polluted the environment, in addition it also killed some insects, birds, fish and other non target organisms.

The plant extract had a variation in the zone of inhibition. The differences might be due to the difference in nature, quality and quantity of the inhibitory substances present in the botanicals (Ceylan and Fung, 2004). It is evident from results that the zones of inhibition of the *Fusarium oxysporum* pathogens by the plant extracts depend upon plant species and extracts concentration.

Kumaran (2003) did report comparable findings that the fungal susceptibility toward a plant extract was due to plant species, the solvent used for extraction and extract concentration, as well as the organism tested. The activity of plant extracts and essential oils as anti-sporulant agents have been revealed against a large number of fungal diseases as reported by several other workers (Singh *et al*., 2017).

CONCLUSIONS

The *T. diversifolia* and *K. africana* plant extract studied provides a solution to the farmers seeking better bio fungicides from natural sources that are perceived to be more effective and with minimal negative effects than synthetic chemicals that are used.

The current study validated the utilization of *T. diversifolia* and *K. africana* in plant disease control. The plants possessed different phytochemicals with the presence of terpenoids, phenols, steroids, tannins, flavonoids, and saponins. Therefore, these plants may substitute for the supply of anti fungal.

RECOMMENDATIONS

There is a need to create awareness to farmers that *T. diversifolia* and *K. africana* have a potential benefit in the control of *F. oxysporum* and other plant pathogens. The government should advocate and consider this work to produce the phytochemicals in large quantities to control and manage *F. oxysporum* pathogen especially in tomatoes.

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