Archives of Phytopathology and Plant Protection

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gapp20

In vitro efficacy of botanical leaf extracts on *Fusarium oxysporum* causing cashew wilt disease in Tanzania

W. V. Mbasa

https://doi.org/10.1080/03235408.2021.1983386

In vitro efficacy of botanical leaf extracts on *Fusarium oxysporum* causing cashew wilt disease in Tanzania

W. V. Mbasa

Tanzania Agricultural Research Institute (TARI)-Naliendele, Mtwara, Tanzania

ABSTRACT

Management of *Fusarium oxysporum* causing cashew wilt disease is a current focus in cashew industry, in Tanzania. Limitations of practiced cultural and chemical management, triggered evaluation of *Azadirachta indica* and *Lantana camara* against *F. oxysporum* in this study. Fresh leaf crudes of botanicals were extracted, impregnated on PDA at three doses (0, 0.4 and 0.6 ml/20 ml of Medium) and then incubated for five days. The tested botanical extracts significantly inhibited growth of *F. oxysporum* after five days of incubation ($p \leq 0.05$). Application of *A. indica* (0.6 ml) and *L. camara* (0.4 ml) inhibited growth of *F. oxysporum* by 43.2% and 35.8% compared to control (0%). Significant correlation, revealed increased performance of botanical extract with increase of its doses ($p < 0.001$). The findings provide the promising efficacy of *A. indica* and *L. camara* on management of *F. oxysporum* on cashew. Further studies on *in-vivo* and field efficacy of botanicals are required.

ARTICLE HISTORY
Received 30 April 2021
Revised 7 August 2021
Accepted 7 September 2021

KEYWORDS
Cashew; botanical; *Fusarium oxysporum*; disease

Introduction

Fusarium wilt disease caused by soilborne pathogenic fungus, *Fusarium oxysporum* Schlechtend.: Fr., is the disease of economic importance to many agronomic and horticultural crops including cashew (Flood 2006; Ploetz 2015b). Cashew Fusarium Wilt Disease (CFWD) is the current destructive and devastating disease of cashew in Tanzania (Tibuhwa and Shomari 2016; Mbasa et al. 2021). *F. oxysporum* causes cashew wilt disease by entering the vascular system through the roots and root cortex (Agrios 2005; Tibuhwa and Shomari 2016). Post root cortex entrance, hypha grow, macro-microconidia develop and colonise the vascular vessel of roots/stem (Groenewald 2005; Warman et al. 2018).

CONTACT W. V. Mbasa billmbasa@gmail.com Tanzania Agricultural Research Institute (TARI)-Naliendele, P. O. Box 509, Mtwara, Tanzania

The colonised vascular vessel on root and stem become decolourised to brown/yellow streaks and dark spots (Pérez-vicente et al. 2014; Ploetz 2015a; Okungbowa and Shittu 2016; Mbasa et al. 2021). Initial external symptoms of yellow, brown leaf symptoms and stem gummosis appears, often localised on one side of cashew (Tibuhwa and Shomari 2016; Mbasa et al. 2021). Thereafter, a cashew tree completely wilt, die and ultimately leave cashew field bare and reduce income of both household and nation.

Management practices through cultural and chemical fungicides have been used against cashew Fusarium wilt disease in Tanzania (Tibuhwa and Shomari 2016; Majune et al. 2018; Mbasa et al. 2021). However, cultural practices by cutting and burning infected cashew trees and quarantine of the planting materials (seed and seedlings) from infested areas prevent the disease but not control the infected cashew trees. Chemical method is the best control measure of the Fusarium wilt diseases (Ajilogba and Babalola 2013; Bawa 2016), but the widespread use of chemical fungicides has been a focus of public concern due to their potential harmful effects on non-target organism in environment and human health (Basco et al. 2017; Du et al. 2017). Moreover, the cost implication of chemical fungicides is not affordable to smallholder farmers in many countries including Tanzania (Valentine 2014). Therefore, looking for an alternative control measure of chemical fungicides against cashew Fusarium wilt disease is a paramount objective.

Botanical fungicides have been a current focus and universal accepted alternative control method of many crop diseases, including Fusarium wilt disease (Ibrahim et al. 2014; Du et al. 2017). These botanical fungicides have been safe, non-toxic, low cost and effective against different plant pathogens (Ibrahim et al. 2014). Essential oils and extracts (alkaloids and flavonoids) of many plants contain variety of volatile molecules ascribing to the antimicrobial activities of these botanicals in different crops diseases (Du et al. 2017; Jafar 2017). For instance, Bowers and Locke (2000) reported that pepper (*Piper nigrum*), cassia (*Cinnamomum cassia*) and clove (*Syzygium aromaticum)* extracts reduced population density of *F. oxysporum* f. sp. *chrysanthemi* by 99.9, 96.1 and 97.5%, respectively, compared with untreated control. Similarly, Shabana et al. (2017) reported the efficacy of neem (*Azadirachta indica* L.) on inhibition of spore germination (98.99%) and reduction of number of pustules/leaf (36.82%) compared with untreated control. Despite the effectiveness and importance of several botanical fungicides in managing *F. oxysporum* in different host crops, there is little information on botanical control measures against CFWD in Tanzania. Thus, this study presents the efficacy of *Azadirachta indica* and *Lantana camara* for management of the disease.

Materials and methods

Plant materials and plant extract preparation

Medicinal plant leaves of *Lantana camara* and *Azadirachta indica* were collected from Tanzania Agriculture Research Institute-Naliendele center at Mtwara, Tanzania. The mature leaves were collected by picking them from fresh and health lantana and neem plants before extraction following Hussain et al. (2012). The collected fresh leaves were thoroughly washed with running tape water then left 25 min for air drying before grinding. The used leaves were grinded, using sterile mortar and pestle. Ten grams of grounded sample was mixed with 100 ml of sterilised distilled water in a conical flask and kept for overnight (12 h) at room temperature. The filtrates were separated from the solid residue by filtering through Whatman no. 1 filter paper. The filtration process was repeated four times, and the obtained aqueous extracts were pooled and considered standard. A stock of aqueous solution of each extract was stored in the refrigerator at − 5 °C for ten days, then required experimental amount was autoclaved in 121 °C for 15 min before the use.

F. oxysporum *isolates preparation*

Pure culture of *Fusarium oxysporum* was obtained from the Botany Laboratory, Botany department, University of Dar es salaam, Tanzania (Isolated by Mbasa et al. 2021 and Tibuhwa and Shomari 2016). *F. oxysporum* isolate was maintained on potato dextrose agar (PDA) media plates at 25 °C in an incubator before being used.

Antifungal activities

The *in vitro* testing of botanical extracts was carried out at the Botany department, University of Dar es salaam, Tanzania. A poison food technique was deployed in which PDA medium was prepared, autoclaved and cooled to 50 °C, then impregnated with botanical extracts at different doses mentioned in Table 1. After mixing, the impregnated PDA with botanical extracts were cooled and solidified. Five days old culture of *F. oxysporum* was inoculated at the centre of PDA impregnated petri dishes using 5 mm cork borer before incubated at 25 ± 2 °C. The experiment was set using complete randomised design with three replications. The experiment was repeated three times, at each post incubation, radial growth was measured on underneath of petri dishes in perpendicular directions using zero error meter ruler after 3 and 5 days. Thereafter, percentage growth inhibition was calculated using the following formula (Das et al. 2010);

Table 1. Tested botanicals leaf extracts under *in vitro* condition.

Botanical extracts	Doses (ml/20 ml of medium)	2×2 Factorial treatments
Azadirachta indica	0.4	Azad 0.4 (T1)
	0.6	Azad 0.6 (T2)
Lantana camara	0.4	Lant 0.4 (T3)
	0.6	Lant 0.6 (T4)
Control	0	Control (T5)

$$\text{Growth Inhibition}\,(\%) = C - T/C \times 100$$

where C = Growth diameter of control and T = Growth diameter of treated fungal growth.

Data analysis

Collected growth inhibition data were subjected to ANOVA at 5% confidence level. The Turkey test for multiple comparison of treatments was used. The analysis was conducted using OriginPro Version 19 software.

Results

Efficacy of botanical extracts

Botanical leaf extract potentially inhibition the growth of *Fusarium oxysporum* as presented in Figure 1. The performed test revealed that both *Azadirachta indica* and *Lantana camara* inhibited the growth of *F. oxysporum* compared with control under *in vitro* condition. However, *A. indica* had wide distribution (within 5–50%) on the efficacy while *L. camara* had low distribution (25–45%) on the efficacy after three and five days of incubation. Control had lowest distribution within zero to five percent of growth inhibition. The performance of botanical extracts varied with applied doses on inhibition of *F. oxysporum* growth.

Varied performance of botanical extracts on different doses was noted as presented in Figures 2 and 3. *Azadirachta indica* at 0.4 and 0.6 ml had significant inhibition potential of 25% and 29.2%, respectively compared to control (0.0%) after three days of incubation ($p < 0.01$, df = 4). After, five days of incubation, *A. indica* at 0.6 ml had significant inhibition potential of 43.3% compared to control (0.0%) ($p < 0.001$, df = 4). On *L. camara* at 0.4 and 0.6 ml both had significant growth inhibition of 34.7 and 27.9%, respectively compared to control with 0.0% after three days of incubation ($p < 0.01$, df = 4). On day five from incubation, *L. camara* at 0.4 and 0.6 ml both had significant growth inhibition of 35.8 and 32.5% of *F. oxysporum* compared to control ($p < 0.001$, df = 4).

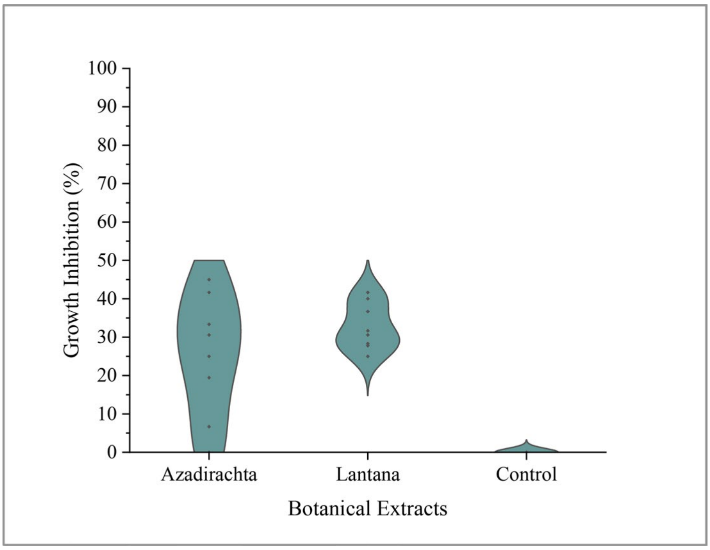

Figure 1. Violin plot presenting potential growth inhibition of botanical fungicides on *Fusarium oxysporum* causing cashew wilt disease.

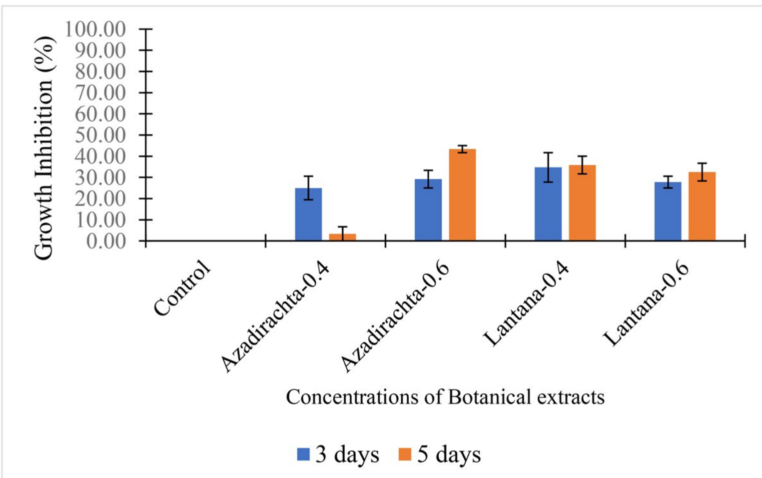

Figure 2. Inhibition potential of different doses of botanical fungicides on *Fusarium oxysporum* causing cashew wilt disease.

Correlation analysis of botanical fungicide doses against growth inhibition

Significant Pearson correlations were noted between botanical fungicide doses and growth inhibition as presented in Table 2. Strong and significant positive correction was noted between botanical extract doses and growth inhibition after 3 days of incubation (Pearson corr. = 0.80457, $p = 0.001$). Also, strong and significant positive correlation was recorded between botanical extract doses and growth

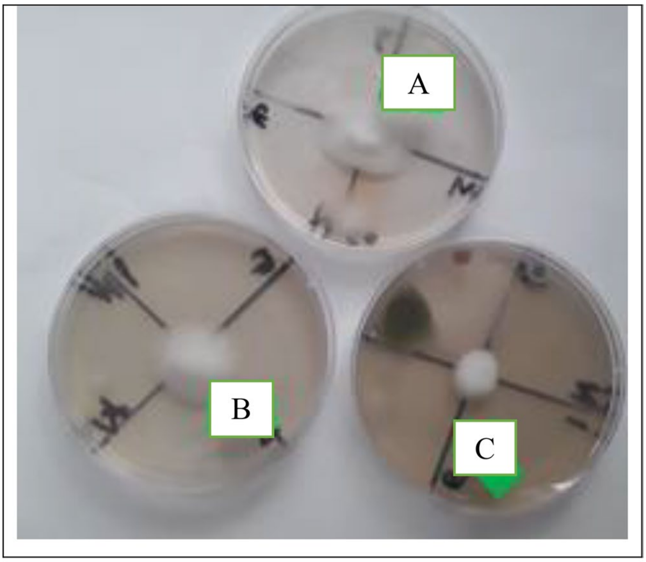

Figure 3. Growth inhibition of botanical fungicides on *Fusarium oxysporum* after 5 days of incubation. A). Control, B). Botanical fungicide at 0.4 ml and C). Botanical fungicides at 0.6 ml.

Table 2. Pearson correlation between botanical extracts doses and growth inhibition of *F. oxysporum* causing cashew wilt disease.

Treatments		Doses	G.I-3 days	G.I-5 days
Doses	Pearson Corr.	1		
	p-value	–		
G.I-3 days	Pearson Corr.	0.80457	1	
	p-value	0.005	–	
G.I-5 days	Pearson Corr.	0.76616	0.7193	1
	p-value	0.00976	0.01904	–

G.I, Growth Inhibition.

inhibition after five days of incubation (Pearson corr. = 0.76616, $p < 0.001$).

Discussion

This study tested the potential efficacy of botanical extracts on the management of *Fusarium oxysporum* causing cashew wilt disease in Tanzania. The tested botanical extracts were *A. indica* (Neem) and *L. camara* (Lantana). All botanical extracts managed the growth of *F. oxysporum*, despite varied performance among its doses. *A. indica* was the most effective botanical since it had high percent of growth inhibition followed by *L. camara*. The performance of botanical extracts congruent with Ghante et al. (2019) who reported the promising efficacy of different botanicals. The performance variation among botanicals extracts might have been attributed by their inherent antifungal nature.

Potential efficacy of *A. indica* has been reported from various host crops of *F. oxysporum*. These include control of *F. oxysporum* in beans (Obongoya 2009), onion (Hussain et al. 2019) and tomato (Sreenu and Zacharia 2017). *A. indica* also controlled *Aspergillus flavus* (Rasad et al. 1993), *Aspergillus niger* and *Colletotrichum sp*. (Toan et al. 2018). The growth inhibition potential of *A. indica* varied with the doses and time of incubation. *A. indica* at 0.6 ml had good inhibition potential at both three and five days of incubation compared to 0.4 ml. Antifusorisis of *A. indica* ascribed by containment of antifungal compounds including azadirachtin, azadiradione, nimbin, salanin and others, which are systemic and broad spectrum (Sadeghian and Mortazaienezhad 2007; Hossain et al. 2013).

L. camara, inhibited the growth of *F. oxysporum* under *in vitro* condition. The potential inhibition was noted from both 0.4 and 0.6 ml after 3 and 5 days of incubation. Different studies indicate the efficacy of *L. camara* on management of *F. oxysporum* in different crops. These crops including banana (Rasad et al. 1993), tomato (Sreenu and Zacharia 2017) and pigeon pea (Ghante et al. 2019). Nonetheless, *Lantana camara* has broad antifungal activities such as control of *Puccinia triticina* in wheat (Draz et al. 2019), *Colletotrichum gloeosporioides* Penz in Mango (Bashir et al. 2019), *Alternaria alternata* in Potato (Singh and Srivastava 2012). The antifungal activities of *L. camara* are attributed by the presence of bioactive and phytochemical such as alkaloids, flavonoids, glycosides, phytosterols, phenols, saponins, tannins, naphthoquinones, terpenoids and coumarins (Singh and Srivastava 2012; Fayaz et al. 2017; Bashir et al. 2019).

Strong positive correlations between botanical extracts doses and growth inhibition, indicates the potential influence of doses. The performance of botanical extracts increased with an increase of its doses and time of incubation. Implying that, for the good efficacy of *A. Indica* and *L. camara*, application of high dosage with increased time of incubation is unavoidable. The influence of doses and time of incubation on antifusorisis support the findings of Hossain et al. (2013) and Toan et al. (2018) on their studies in onion and orange crops, respectively. This study support the findings of Sreenu and Zacharia (2017), who revealed that, application of botanical fungicides is the safe, and effective intervention against pathogens causing vascular wilt such as *F. oxysporum*.

Conclusion and recommendation

The efficacy test of botanical fungicides revealed that all tested botanicals inhibited the growth of *Fusarium oxysporum* under *in vitro* condition. Moreover, *Azadirachta indica* at 0.6 ml and *Lantana camara* at both 0.4

and 0.6 ml had promising management potential of *F. oxysporum* causing cashew wilt disease. Further studies on efficacy of botanicals under both *in-vivo* and field conditions are required. In addition, further studies on characterisation of specific active leaf extract compound and different methods of extraction are required.

Acknowledgements

The authors acknowledge TARI-Naliendele Centre and the University of Dar es Salaam for providing support of research facilities. Many thanks go to Mr. Selemani Libuburu for collection and preparation of fresh plant leaves. Also, thanks to Commission of Science and Technology (COST ECH) for provision of scientific writing skills.

Disclosure statement

No potential conflict of interest was reported by the author.

References

Agrios GN. 2005. Plant pathology. 5th ed. In: Sonnack KD, editor. Dana Dreibelbis Kelly. California, USA.

Ajilogba CF, Babalola OO. 2013. Integrated management strategies for tomato Fusarium wilt. Biocontrol Sci. 18(3):117–127.

Basco M, Bisen K, Keswani C, Singh H. 2017. Biological management of Fusarium wilt of tomato using biofortified vermicompost. Mycosphere. 8(3):467–483.

Bashir S, Jabeen K, 1qbal S, Javed S, Naeem A, 2019. *Lantana camara*: Phytochemical analysis and antifungal prospective. *Planta Daninha*. 37(3):e019193526.

Bawa I. 2016. Management strategies of Fusarium wilt disease of tomato incited by *Fusarium oxysporum* f. sp *lycopersici* (Sacc.): a review. Int J Adv Acad Res Sci Technol Eng. 2(5):32–42.

Bowers JH, Locke JC. 2000. Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil and control of Fusarium wilt in the greenhouse. Plant Dis. 84(3):300–305.

Das K, Tiwari RKS, Shrivastava DK. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: current methods and future trends. J Med Plants Res. 4(2):104–111. https://doi.org/10.5897/JMPR09.030.

Draz IS, Elkhwaga AA, Elzaawely AA, El-Zahaby HM, Ismail A-WA. 2019. Application of plant extracts as inducers to challenge leaf rust of wheat. Egypt J Biol Pest Control. 29(1):4–11.

Du R, Liu J, Sun P, Li H, Wang J. 2017. Inhibitory effect and mechanism of *Tagetes erecta* L. fungicide on *Fusarium oxysporum* f. sp. *niveum*. Sci Rep. 7(1):14442–14413.

Fayaz M, Hussain Bh M, Fayaz M, Kumar A, Kumar Jain A. 2017. Antifungal activity of *Lantana camara* L. leaf extracts in different solvents against some pathogenic fungal strains. Pharmacologia. 8(3):105–112.

Flood J, Ukc CB, Lane B. 2006. Fusarium-induced diseases of tropical perennial crops: a review of Fusarium wilt of oil palm caused by *Fusarium oxysporum* f. sp. *elaeidis*. Phytopathology. 96(6):660–662.

Ghante PH, Apet KT, Kanase KM, Daunde AT, Chavan PG. 2019. *In vitro* efficacy of fungicides against *Fusarium oxysporum* f. sp. *udum* causing wilt disease of pigeonpea. J Pharmacogn Phytochem. 8(1):1927–1931.

Ghante PH, Kanase KM, Markad HN, Suryawanshi AP, Chavan PG. 2019. *In vitro* efficacy of phyto-extracts against *Fusarium oxysporum* f. sp. *udum* causing wilt disease of pigeonpea. J Pharmacogn Phytochem. 8(2):19–21.

Groenewald S. 2005. Biology, pathogenicity and diversity of *Fusarium oxysporum* f. sp. *cubense* [Master thesis]. University of Pretoria Etd. South Africa.

Hossain MA, Al-Toubi WAS, Weli AM, Al-Riyami QA, Al-Sabahi JN. 2013. Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. Integr Med Res. 7(4):181–188.

Hussain AI, Chatha SAS, Noor S, Khan ZA, Arshad MU, Rathore HA, Sattar MZA. 2012. Effect of extraction techniques and solvent systems on the extraction of antioxidant components from peanut (*Arachis hypogaea* L.) Hulls. Food Anal Methods. 5(4):890–896.

Hussain S, Ayub M, Rasheed M, Khan F. 2019. Evaluvation of various biocontrol agents (plant extracts) on linear colony growth of the fungus *Fusarium oxysporum* causing onion wilt. Int J Environ Agric Sci. 3(2):23.

Ibrahim M, Tafinta IY, Imam UA, Ibrahim M. 2014. Efficacy of some plant extracts on growth and germination of *Rhizopus stolonifer* and *Fusarium oxysporum* isolated from rotten Irish potato tubers. Ann Biol Sci. 2(3):63–67. Retrieved from http://abiosci.com/archive.html 66.

Jafar FN. 2017. Effect of clove extract (*Eugenia caryophllus*) on *Fusarium oxysporum* f. sp. *lycopersici* the causative of tomato wilt disease. J Chem Biol Phys Sci. 7(1):114–121.

Majune DJ, Masawe PA, Mbega ER. 2018. Status and management of cashew disease in Tanzania. IJEAB, 3(5):1590–1597.

Mbasa WV, Nene WA, Kapinga FA, Lilai SA, Tibuhwa DD. 2021. Characterization and chemical management of Cashew Fusarium Wilt Disease caused by *Fusarium oxysporum* in Tanzania. Crop Prot. 139(2021):105379.

Obongoya BO, Wagai SO, Odhiambo G. 2009. Fungitoxic properties of four crude plant extracts on *Fusarium oxysporum* Schl. f. sp. *Phaseoli*. Afr J Food Agric Nutr Dev. 9(8):1652–1666.

Okungbowa FI, Shittu HO. 2016. Fusarium wilts: an overview. Environ Res J. 6(2):83–102. Retrieved from https://www.researchgate.net/publication/292243135.

Pérez-Vicente L, Dita MA, Martinez E. 2014. Technical manual prevention and diagnostic of Fusarium wilt (Panama disease) of banana caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (TR4). Food and Agriculture Organization of the United Nations. 4(May):1–74.

Ploetz RC. 2015a. Fusarium wilt of banana. Phytopathol Rev. 105(12):1512–1521.

Ploetz RC. 2015b. Management of Fusarium wilt of banana: a review with special reference to tropical race 4. Crop Prot. 73(2015):7–9.

Rasad MF, Linn A, Linn F, Thuja L, Linn A, Linn A. 1993. Efficacy of leaf extracts of some medicinal plants against disease development in banana. Lett Appl Microbiol. 17:269–271.

Sadeghian MM, Mortazaienezhad F. 2007. Investigation of compounds from *Azadirachta indica* (Neem). Asian J of Plant Sciences. 6(2):444–445.

Shabana YM, Abdalla ME, Shahin AA, El MM, Draz IS, Youssif AW, … Youssif AW. 2017. Efficacy of plant extracts in controlling wheat leaf rust disease caused by *Puccinia triticina*. Egypt J Basic Appl Sci. 4(1):67–73.

Singh P, Srivastava D. 2012. Biofungicidal or biocontrol acticity of *Lantana camara* against phytopathogenic *Alternalia alternata*. Int J Pharm Sci Res. 3(12):4818–4821. Retrieved from www.ijpsr.com.

Sreenu B, Zacharia S. 2017. *In vitro* screening of plant extracts, *Trichoderma harzianum* and carbendazim against *Fusarium oxysporium* f. sp. *Lycopersici* on Tomato. Int J Curr Microbiol Appl Sci. 6(8):818–823.

Tibuhwa DD, Shomari S. 2016. Fusarium wilt disease: an emerging threat to cashew nut crop production in Tanzania. Asian J of Plant Pathol. 10(4):36–48.

Toan T, Ky T, Hoang H. 2018. Evaluation of two eco-friendly botanical extracts on fruit rot pathogens of orange (*Citrus sinesis* L.) Osbeck. J Vietnames Environ. 10(2):107–112.

Valentine V. 2014. Analysis of factors affecting agricultural output in Tanzania [Master thesis]. Makelele University.

Warman NM, Aitken EAB, Dita M, Grant M. 2018. The movement of *Fusarium oxysporum* f. sp. *cubense* (sub-tropical race 4) in susceptible cultivars of banana. Front Plant Sci. 9(174):1–9.