World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:11, No:8, 2017

Biological Control of Tomato Wilt Fungi Using Leaf Extracts of Bitter Leaf (*Vernonia amygdalina*)

Terna T. Paul, Agbara D. Onwoke

***Abstract*—**The antifungal potential of ethanolic leaf extracts of *Vernonia amygdalina* in the biological control of some common tomato wilt fungi was investigated. The experiment was set up in Completely Randomized Design (CRD) with eight treatments and three replicates. 5 mm diameter agar discs of 7 days old cultures of *Fusarium oxysporum* and *Sclerotium rolfsii* were obtained using a sterile 5 mm diameter cork borer and cultured on Potato Dextrose Agar (PDA) inoculated with 5 ml of various concentrations of *V. amygdalina* ethanolic leaf extracts in petri dishes, and incubated for 10 days at 28 ^{0}C. The highest radial growth inhibitions of *F. oxysporum* (34.98%) and *S. rolfsii* (31.05%) were recorded 48 hours post-inoculation, both at 75% extract concentration. The leaf extracts of *V. amygdalina* used in the study exhibited significant inhibition of radial growth of the test organisms ($P \leq 0.05$) and could be applied in the biological control of fungal wilt pathogens of tomato as a means of enhancing tomato yield and productivity.

***Keywords*—**Biological control, fungi, leaf extracts, tomato wilt, *V. amygdalina.*

I. Introduction

TOMATO (*Solanum lycopersicum* L.) belongs to the family Solanaceae, which includes more than 3000 species, occupying a wide variety of habitats [1]. It is considered one of the world's most popular vegetables [2]. It is also the most important tropical vegetable crop widely used throughout the world [3]. The dietary and nutritional relevance of the tomato crop is evidenced by its high demand among both rural and urban populations [4]. It is a key component in the so-called "Mediterranean diet", which is strongly associated with reduced risk of chronic degenerative diseases [5], [6]. Tomatoes are either consumed fresh or as processed products such as canned tomato, sauce, juice ketchup, stews and soup [7], [8].

Tomatoes contain high amounts of antioxidants such as β-carotene, a precursor of vitamin A, and mainly lycopene, which is largely responsible for the red color of the fruit; vitamins such as ascorbic acid and tocopherols, and phenolic compounds such as flavonoids and hydroxycinnamic acid derivatives [8], [9].

Wilts of the tomato crop caused by *S. rolfsii* and *F. oxysporum* are important limiting factors to tomato cultivation in the Savanna and forest zones of Nigeria [10], [11]. High prevalence of *Fusarium* wilt has been especially linked to high disease susceptibility of the commonly cultivated local tomato varieties [12]. Over the years, various studies on Tomato wilt have been carried out and results have shown that some fungi causing wilt of tomatoes pose a great threat to tomato yield and availability in several countries of the world. [13], [12], [14].

The problem of high cost of chemical pesticides and the resultant deleterious effect of excessive usage of synthetic chemical compounds in agriculture has called for the use of affordable and environmentally friendly approaches to plant disease control. In this regard, this study seeks to provide a readily available and environmentally friendly alternative to toxic synthetic chemicals in the control of Tomato wilts caused by fungi in the study area.

T.P. Terna is with the Department of Botany, Federal University Lafia, PMB 146 Lafia, Nasarawa State, Nigeria (Corresponding author, phone: +2347030350853; e-mail: ternapaul@yahoo.com; terna.paul@fulafia.edu.ng).

D.O. Agbara is an Undergraduate Student and Research Assistant in the Department of Botany, Federal University Lafia, PMB 146 Lafia, Nasarawa State, Nigeria (e-mail: ask4agbara@gmail.com).

II. Materials and Methods

A. Source and Maintenance of Test organisms

Pure cultures of *Fusarium oxysporum,* and *Sclerotium rolfsii* were obtained from the Plant Pathology Unit of Federal University Lafia and maintained on PDA slants at 27 ^{0}C.

B. Collection of Plant Materials

Healthy leaves of *Vernonia amygdalina* were handpicked from bitter leaf plants growing in farms in Lafia and conveyed in sterile polythene bags for further identification and bio assay in the Botany Laboratory of the Department of Botany, Federal University Lafia.

C. Identification of Plant Materials

Collected leaf samples were identified using morphological characteristics aided by identification keys as described by [15].

D. Preparation of Plant Materials

Prior to extraction, dried *Vernonia amygdalina* leaves were washed with distilled water, disinfected with 70% ethanol, chopped into small pieces, and crushed to finer particles using Mortar and Pestle [16].

E. Preparation of Extracts

Ethanol extraction of the plant material was carried out as described by [17], as follows: 50 g of crushed leaf samples were soaked in 200 ml absolute ethanol and allowed to stand for 72 hours, after which the mixture was placed in a water bath for a period of 6 hours to allow the ethanol evaporate leaving the original extracts.

F. Concentration of Extracts

100, 75, and 50 ml of leaf extract were respectively diluted

in 0, 25 and 50 ml sterile distilled water in a 100 cm^3 measuring cylinder to give 100%, 75%, and 50% concentrations respectively.

G. PDA-Extract Medium Preparation

The pour plate technique was used as described by [18]. 5 ml of each of various concentrations of plant extracts were dispensed aseptically into sterile petri dishes and 20 ml of sterile PDA gently mixed and allowed to solidify for about 30 minutes. The control plates comprised of 20 ml PDA mixed with 5 ml of sterile distilled water.

H. Bioassay

With the aid of a sterile cork borer, 5 mm diameter discs each of the test organisms were inoculated separately into petri dishes containing PDA-Leaf extracts and incubated at 27 ^{0}C for 48 hours. Radial growths of test organisms were measured every 42 hours for a total period of 10 days, with the aid of a meter rule. Percentage pathogen growth inhibition was calculated with respect to radial growth of pathogen on control plates (without leaf extracts) as reported by [19] as:

$$PRG = \frac{PC-P}{PC} X100 \quad (1)$$

where PRG = Percentage Radial Growth Inhibition of fungus; Pc = Radial growth of fungus in control plate; P = Radial growth of fungus in the presence of *V. amygdalina* leaf extracts.

I. Data Analysis

Data obtained from the study were subjected to Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) to compare differences among treatment means ($P \leq 0.05$) using SPSS software Version 17.

III. Results

TABLE I
EFFECT OF DIFFERENT CONCENTRATIONS OF *V. AMYGDALINA* LEAF EXTRACTS ON RADIAL GROWTH OF *SCLEROTIUM ROLFSII* AND *FUSARIUM OXYSPORUM*

	Radial Growth Inhibition (%)	
Concentration (%)	*S. rolfsii*	*F. oxysporum*
0	26.98[a]	40.04[a]
50	27.53[a]	34.07[a]
75	31.05[a]	34.98[a]
100	20.05[a]	28.11[a]

Means followed by same superscripts within same column are not significantly different.

The highest inhibition of radial growths of *S. rolfsii* (31.05%) and *F. oxysporum* (34.98%) were observed when 75% concentration of *V. amygdalina* leaf extracts were introduced (Table I). Differences in mean radial growth inhibitions of test fungi were not significant ($P \leq 0.05$).

Results of overall effect of different concentrations of leaf extracts of *V. amygdalina* on tomato wilt fungi (Table II) revealed that the highest radial growth inhibition of the test fungi was attained at 75% concentration of extracts. No inhibitions were observed at 0% concentration (Control). Differences in mean radial growth inhibitions were significant ($P \leq 0.05$).

TABLE II
OVERALL EFFECT OF EXTRACT CONCENTRATIONS ON RADIAL GROWTH INHIBITION OF TOMATO WILT FUNGI

% Concentration	% Inhibition
0	0.00[a]
50	28.22[b]
75	47.50[c]
100	46.70[c]

Values followed by same superscript within same column are not significantly different.

Values followed by different superscript within same column are significantly different ($P \leq 0.05$)

TABLE III
OVERALL RESPONSE OF TEST FUNGI TO INOCULATION OF *V. AMYGDALINA* LEAF EXTRACTS

Fungi	% Growth Inhibited
Fusarium oxysporum	34.30[a]
Sclerotium rolfsii	26.40[a]

Values followed by same superscripts within same column are not significantly different ($P \leq 0.05$)

Results of overall response of different tomato wilt fungi to growth inhibition by leaf extracts of *V. amygdalina* (as presented in Table II; Figs. 1 and 2) revealed that radial growth of *Fusarium oxysporum* was most inhibited (34.30%) compared to *Sclerotium rolfsii* (26.40%). Differences in mean radial growth inhibitions were not significant ($P \leq 0.05$).

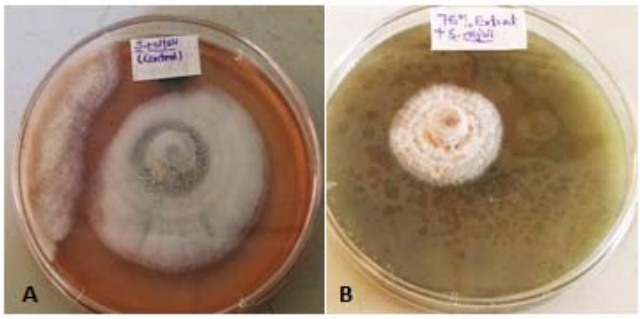

Fig. 1 Radial Growth of *S. rolfsii* in (A) Control Plate, and (B) in the presence of 75% *V. amygdalina* leaf extracts

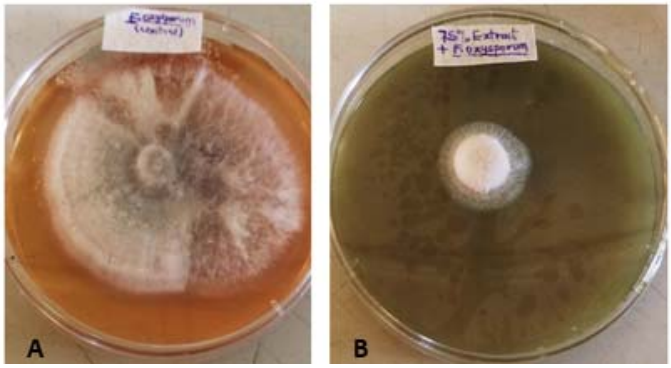

Fig. 2 Radial Growth of *Fusarium oxysporum* in (A) Control Plate, and (B) in the presence of 75% *V. amygdalina* leaf extracts

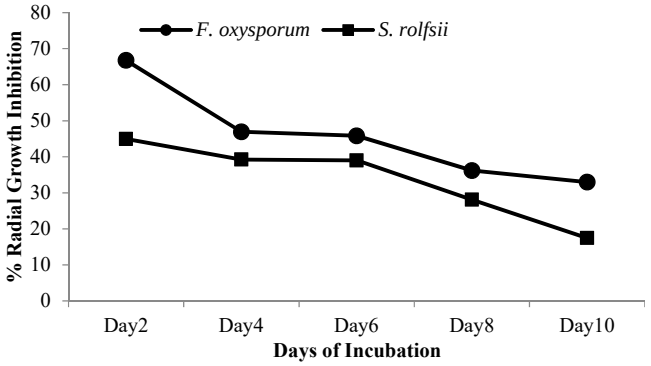

Fig. 3 Growth Inhibition Progress Curve for Effect of *Vernonia amygdalina* leaf extracts on Tomato Wilt Fungi

Results of effect of post-inoculation time of *V. amygdalina* leaf extracts on radial growth inhibition of *S. rolfsii* and *F. oxysporum* (Fig. 3) revealed that the highest inhibitions of the radial growths of test fungi were observed 2 days after inoculation of leaf extracts and began to decline afterwards. The least inhibition of test fungi was recorded on the 10^{th} day after inoculation of leaf extracts. Differences in mean effect of post-inoculation time on radial growth inhibition of test fungi by the evaluated plant extracts were not significant ($P \leq 0.05$).

IV. Discussion

Ethanolic extracts of *V. amygdalina* used in the study demonstrated significant antifungal activity. In a similar work [20], it was reported that extracts of *V. amygdalina* inhibited radial growth of *Fusarium solani* causing tuber rot on cassava. Furthermore, [21] reported the inhibitory effect of *V. amygdalina* against *Rhizopus stonolifer*. The inhibition of the mycelial growth may be attributed to the presence of fungi-static compounds in the plant extracts used. This is further supported by [22], which also mentioned that the presence of certain allele-chemicals in plant extracts account for their detrimental effects on microbial cell division, cell elongation and nutrient uptake. References [23]-[25] also reported that such chemicals either dissolve the cytoplasm or render it inactive, and are also capable of penetrating the microbial walls and distorting routine microbial metabolic processes.

The variation in antifungal activity of different concentrations of the leaf extracts on the test fungi observed in the study is in consonance with the observations of [26] and [27] who also reported significant differences in the inhibitory effect of various concentrations of leaf extracts of *Azadirachta indica* and *Allium sativum* on different rot causing fungi of *Dioscorea* species respectively. References [28] and [29] also observed that test extracts at increasing concentrations significantly inhibited the radial growth of fungal pathogens. The increase in fungal growth inhibition with increased extract concentrations could be linked to the buildup of toxic materials which tend to inflict more inhibitory reactions on affected fungal cells in higher concentrations.

In the reported study, radial growth of *Fusarium oxysporum* was most inhibited compared to *S. rolfsii*. Higher resistance to leaf extracts by *S. rolfsii* was also reported by [30]. The presence of thickened cell layer and melanin pigmentation in the sclerotia and mycelia of *S. rolfsii* has been reported as a means of resistance to environmental stress and could account for the higher resistance to various concentrations of the leaf extracts used in the study.

Highest inhibition of radial growth of test fungi was observed after 2 days of extract inoculation. In a similar study, [31] reported that azadirachtin isolated from *Azadirachta indica* acted effectively as fungicide after 48 hours of inoculation and became less effective during the later time of inoculation. This is likely due to the ability of the test organisms to readjust their adaptive mechanisms to the initial environmental stress caused by the release of antifungal active ingredients into the growth medium by the leaf extracts.

V. Conclusion

The ethanolic leaf extracts of *Vernonia amygdalina* used in the study exhibited potent antifungal ability and could be further explored *in vivo* for biological control of tomato wilt fungi especially *Fusarium oxysporum* and *Sclerotium rolfsii.*

References

[1] S. Knapp, "Solanum section Geminata", *Fl Neotrop*, vol. 84, 2002, pp. 1-405.

[2] P. Pritesh and R.B. Subramanian, "PCR based method for testing *Fusarium* wilt resistance of Tomato", *African Journal of Basic and Applied Sciences,* vol. 3, no. 5, 2011, pp. 222.

[3] W.C. John, N.C.J. Anyanwu, and T. Ayisa, "Evaluation of the Effects of the Extract of *Vernonia amygdalina* on Fungi Associated with Infected Tomatoes (*Lycopersicon esculentum*) in Jos North Local Government Area, Plateau State, Nigeria**",** *Annual Research and Review in Biology,* vol. 9, no. 4, 2016, pp. 1-8.

[4] M.M. Waiganjo, N.M. Wabule, D. Nyongesa, J.M. Kibaki, I. Onyango, S.B. Webukhulu and N.M. Muthoka, "Tomato production in Kiriyanga District, Kenya. A baseline survey report. KARI/IPM-CRSP Collaborative project, 2006.

[5] A. Agarwa and A.V. Rao, "Tomato lycopene and its role in human health and chronic Wilt Disease", *International Resource Journal of Microbiology*, vol. 3, No. 1, 2000, pp. 14-23.

[6] A.V. Rao, and S. Agarwal, "Bioavailability and *in vivo* antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer", *Nutr. Cancer,* vol. 31, 1998, 199-203.

[7] M.S. Lenucci, D. Cadinu, M. Taurino, G. Piro and G. Dalessandro, "Antioxidant composition in cherry and high-pigment tomato cultivars", *J. Agric. Food Chem.,* vol. 54, 2006, pp. 2606-2613.

[8] R. Borguini, and E. Torres, "Tomatoes and tomato products as dietary sources of antioxidants", *Food Rev. Intern*., vol. 25, 2009, pp. 313–325.

[9] Z. Kotkov, J. Lachman, A. Hejtmnkov and K. Hejtmnkov, "Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants", *LWT - Food Sci. & Technol.,* vol. 44, 2011, pp. 1703-1710.

[10] D. Mardi, B. Janet, and W. Paul, *Organic Greenhouse Tomato Production*. Fayetteville, 2002.

[11] I.D. Erinle, *Tomato Diseases in the Northern States of Nigeria*. Ahmadu Bello University, Zaria: Extension Bulletin 11, Agricultural Extension and Research Liaison Services, 1986.

[12] A.R. El-Shanshoury, S.M. Abu El-Sououd, O.A. Awadella, and N.B. El-Bandy, "Effect of *Streptomyces corchorusii, Strptomyces mutabilis, Pendimethalin* and *Metricuzin* on the control of bacterial and *Fusarium* wilt of tomato". *Can. J. Bot*., vol. 74, 1996, pp. 1016-1022.

[13] M.S. El-Abyad, M.A. El-Sayed, A.R. El-Shanshoury, and S.M. El-Sabbagh, "Towards the biological control of fungal and bacterial diseases of tomato using antagonistic *Streptomyces* spp", *Plant and Soil,* vol. 149, 1993, pp. 185-195.

[14] F. Suarez-Estrella, M.C. Varga-Garcia, M.J. Lopez, C. Capel and J. Moreno, "Antagonistic activity of bacteria and fungi from horticultural compost against *Fusarium oxysporum* f.sp. melonis", *Crop Protection*,

vol. 26, 2007, pp. 46-53.
[15] J.G. Harris and M.W. Harris, *Plant Identification Terminology, 2nd Edition,* Utah: Spring Lake Publishing, 2001, 216pp.
[16] M. Momoh, M. Adikwu, and A.R. Oyi, "*Vernonia amygdalina* and CD4+ cell counts: An immune mutual interaction between antioxidants", *LWT-Food Sci. and Technol.,* vol. 44, 2010, pp. 1703-1710.
[17] T.T. Epidi, A. Alamene, B.A. Onuegbu, "Influence of some plant extracts on yield and insect pests of cowpea (*Vigna unguiculate* (L.) Walp), *Nigerian Journal of Plant Protection,* vol. 22, 2005, pp. 65-76.
[18] E.H. Kass and C.H. Norden, "Bacteriuria of pregnancy- a critical appraisal, *Amer. Rev. Med.,* vol. 19, 1968, pp. 431-470.
[19] T.P. Terna, A.C. Odebode, J.I. Okogbaa and J.I. Waya, "*Invitro* screening of *Trichoderma* isolates for biological control of a post-harvest rot fungus", *IOSR Journal of Agriculture and Veterinary Science*, vol. 9, no. 4, 2016, pp. 46-51.
[20] K.I. Ugwuoke, C.C. Onyeke and N.G.R. Tsopmbeng, "The efficacy of botanical protectants in the storage of cocoyam (*Colocasia esculenta* (L.) Shott)", *Agro-Science Journal of Tropical Agriculture Food Environment Extension*, vol. 7, no. 2, 2008, pp. 93-98.
[21] C.A. Onyeani, S.O. Osunlaja, O.O. Oworu, and A.O. Joda, "Evaluation of effect of aqueous plant extract in the control of storage fungi", *International Journal of Science Technology Research*, vol. 1, no. 6, 2012, pp. 76-79.
[22] N.E. Blake, "Effect of allelochemicals on mineral uptake and associated physiological processes" in *The Chemistry of Allelopathy: Biochemical Interactions among the plants,* A.C. Thompson, Ed. Washington: American Chemical, 1985, pp. 161.
[23] J.B. Harborne, *Methods in Plant Biochmistry Volume 1. Plant phenolics,* London: Academic Press LTD, 1989, pp. 552.
[24] H. Wiendenfield, and E. Roder, "Pyrozidine alkaloids from Agenrantum conyzoides", *Planta Med.,* vol. 57, no. 6, 1991, pp. 578-579.
[25] A.K. Singh, L.M. McIntyre and L.A. Sherman, "Microarray analysis of the genome-wide response to iron deficiency and iron reconstitution in the cyanobacterium *Synechocystis* sp. PCC 6803", *Plant Physiol* vol. 132, 2003, pp. 1825-1839.
[26] M.N. Suleiman, "Fungitoxic activity of neem and paw paw leaves extracts on *Alternaria solani,* casual organism of yam rots", *Global Journal of Biotechnology and Biochemistry,* vol. 5, 2010, pp. 92-96.
[27] R.N. Okigbo, U.E. Eme, R. Aseidu and P. Ramesh, "Effect of crude extracts of *Allium sativum* Linn, *Cymbopogon citratus* C.D. Stapf and *Terminalia* catappa on rot causing fungi of *Dioscorea* species*", Nig. J. Biol.,* vol. 22, no. 2, 2009b, pp. 359-369.
[28] A. Banso, S.O. Adeyemo and P. Jeremiah, "Antimicrobial properties of *Vernonia amygdalina* extract", *J. Appl. Sci. Manage*., vol. 3, 1999, pp. 9-11.
[29] N. Martins, L. Barros, C. Santos-Buelga, M. Henriques, S. Silva and I.C. Ferreira, "Evaluation of bioactive properties and phenolic compounds in different extracts prepared from *Salvia officinalis* L.", *Food Chem.,* vol. 170, 2015, pp. 378-85.
[30] K.D. Sharma, U.P. Singh, and K.P. Singh, "Variability in Indian isolates of *Sclerotium rolfsii", Mycologia,* vol. 946, 2002, 1051-1058.
[31] R.C. Dubey and R. Kumar, "Efficacy of azadirachtin and fungicides on growth and survival of sclerotia of *Macrophomina phaseolina* causing charcoal rot in soyabean", *Indian Phytopathology,* vol. 56, 2003, pp. 216-217.