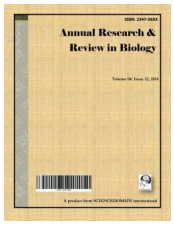

Annual Research & Review in Biology
9(4): 1-8, 2016, Article no.ARRB.23698
ISSN: 2347-565X, NLM ID: 101632869

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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

W. C. John[1], N. C. J. Anyanwu[2*] and T. Ayisa[3]

[1]Department of Pest Management Technology, Federal College of Forestry, Jos, Plateau State, Nigeria.
[2]Department of Biological Sciences, Faculty of Science and Technology, Bingham University, Karu, Nigeria.
[3]Department of Science Laboratory Technology, Federal Polytechnic, Bida, Niger State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors WCJ and NCJA designed the study, wrote the protocol and interpreted the data. Authors WCJ and NCJA anchored the field study, gathered the initial data and performed preliminary data analysis. Author TA managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2016/23698
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Miguel A. Sogorb, Universidad Miguel Hernandez, Spain.
(2) T. Anthoney Swamy, University of Eastern Africa, Baraton, Kenya.
(3) I. P. Tripathi, Mahatma Gandhi Chitrakoot Gramoday Vishwavidyalaya, Chitrakoot, India.
Complete Peer review History: http://sciencedomain.org/review-history/13116

Original Research Article

Received 17th December 2015
Accepted 18th January 2016
Published 29th January 2016

ABSTRACT

Aim: To evaluate the effects of different concentrations of ethanolic extract of *Vernonia amygdalina* on fungi associated with infected tomatoes (*Lycopersicon esculentum*) obtain from Jos North Local Government Area.
Place and Duration of Study: Jos Local Government Area markets; Microbiology Laboratory, Federal College of Forestry between May and August, 2015.
Materials and Methods: A total of thirty (30) infected tomatoes were collected. Three species of fungi were isolated, identified and observed in relation to their percentage of distribution. Isolates

**Corresponding author: E-mail: anyanwunneomaconfidence@gmail.com;*

obtained were subjected to *Vernonia amygdalina* ethanolic leaf extract to determine antifungal sensitivity at varying concentrations. 70% Ethanolic extract of *Vernonia amygdalina* at 12.5, 25, 50 and 100 mg/ml were used for sensitivity test with positive control using fluconazole (50mg/ml) and negative control using distilled water. The mean effects of the treatments on the isolates were determined at 24hours and 48hours, respectively.

Results: The percentage of distribution showed *Geotrichum candidum* (45.16%), *Rhizopus stolonifer* (22.58%) and *Fusarium oxysporum* (32.26%). After 24 hours, no inhibition was recorded on *Fusarium oxysporum* at all levels of concentration of the test plant, except the positive control which showed the highest inhibitory effect. However after 48 hours, there was inhibitory effect across all the isolates at all concentration levels of the test plant with significant differences between each level. 100 mg/ml of the extract had high inhibitory effect of 13.00 mm in comparison with other concentration levels. The lowest inhibitory effect of 1.00 mm was observed at 12.5 mg/ml concentration. The positive control showed no inhibitory effect on *Rhizopus stolonifer* within the duration of the observation. The inhibitory effects of the extract results shows significant difference among the three fungi tested (P<001).

Conclusion: This study revealed that natural products from higher plants are relatively broad spectrum, bio-efficacious, economical, and biodegradable and can be ideal for use as agro-chemicals. Among plants that are known to possess those qualities is *Vernonia amygdalina*.

Keywords: Lycopersicon esculentum; fungi; Fusarium oxysporum; Geotrichum candidum; Rhizopus stolonifer; Vernonia amygdalina; Jos.

1. INTRODUCTION

Vernonia amygdalina is a valuable medical plant that is widespread in East and West Africa [1], it is known as bitter leaf, due to its characteristics bitter taste and flavor, and may be used as an active anticancer [2], antibacterial, antimalarial and antiparasitic agent [3].

The leaves are used for human consumption and washed before eating to get rid of the bitter taste. They are used as vegetable and stimulate the digestive system, as well as they reduce fever. They are also used as local medicine against leech, which are transmitting bilharzias. Free living chimpanzees eat the leaves, if they had been attacked by parasites. *V. amygdalina* is also used instead of hops to make beer in Nigeria [4]. Furthermore, it is found in homes in villages as fence post and pot-herb [5].

Ghassan et al. [6] evaluated eco-friendly botanicals (natural plant extracts) as alternatives to synthetic fungicides and reported that fungicides are widely used in conventional agriculture to control plant diseases. Prolonged usage often poses health problems as modern society is becoming more health-conscious. *Penicillium digitatum*, the cause of citrus green mould, is an important postharvest pathogen which causes serious losses annually. The disease is currently managed with synthetic fungicides. There is, however, a growing concern globally about the continuous use of synthetic chemicals on food crops because of their potential effects on human health and the environment.

The problems caused by synthetic pesticides and their residues have increased the need for effective biodegradable pesticides with greater selectivity. Alternative strategies have included the search for new types of pesticides, which are often effective against a limited number of specific target species, m [7]. Applied chemical pesticides are one of the effective and fast means for reducing the loss of post-harvest diseases. Nevertheless, the excessive use of these chemicals for controlling mould fungi in fruit has been counterproductive, causing damage to the environment and humans, with increased demands to reduce the use of these chemicals that accumulate in fruits and vegetables [8].

The use of these chemicals has increased significantly with improper use and have being left to grow globally, leading to the use of these chemicals that accumulate in fruits and vegetables in circulation. It is claimed that these fruits were major health problems after they became a crop of major commodities exported to different countries worldwide. As persistent hazardous chemicals, the use of these pollutants, such as Thiabendazole (TBZ) and Imazalil to control a wide range of fungi has led to an imbalance in the natural enemies in the environment [9,10].

Natural plant products derived from plants effectively have enormous potential to influence modern agrochemical research. When extracted from plants, these chemicals are referred to as botanicals. The use of botanical pesticides is now emerging as one of the prime means to protect crops and their products and the environment from pesticides [11]. Botanicals degrade more rapidly than most chemical pesticides, and therefore are considered to be eco-friendly and less likely to kill beneficial pests than synthetic pesticides with longer environmental retention. Most of the botanical pesticides generally degrade within a few days, and sometimes even within a few hours [12].

Tomato is one of the widely consumed fresh fruit worldwide since it contributes to a healthy well balanced diet which is rich in vitamins such as vitamin A, B, C and E. Carbohydrates such as fructose and glucose; Minerals which include phosphorus, sodium, potassium, calcium, magnesium and trace elements like iron, copper, zinc and dietary fibres [13]. The deep red colouration of ripened tomato is due to the presence of lycopene, a form of B-carotenoid pigment and a powerful antioxidant that help to protect against prostate cancer, cardiovascular disease and diabetes [14], thus there is an appeal and demands of the fruits by consumers as a result of their knowledge that they are healthy, tasty, convenient and fresh [15].

Vegetable crops including tomatoes are widely cultivated in most parts of Sub Sahara Africa, particularly by small scale farmers in most states of Nigeria [16,17]. Generally, global production of fruits and vegetables tripled from 396 million metric tonnes in 1961 to 1.34 billion metric tonnes in 2003 [18]. Global production of tomatoes is about 89.8 million metric tonnes from an area of about 3,170.000 ha [19]. Adegbola et al. [20] stated that Nigeria is undeniably the 14th largest producer of tomatoes, second to Egypt in Africa at 1.51 million metric tonnes valued at N87.0 billion with a cultivated area of 254,430 ha being the biggest producer in Sub-Sahara Africa. The aim of the research is to evaluate the effects of different concentrations of ethanolic extract of *Vernonia amygdalina* on fungi associated with infected tomatoes (*Lycopersicon esculentum*) obtain from Jos North Local Government Area.

2. MATERIALS AND METHODS

2.1 Study Area

The experiment was carried out at Jos North local government area, Plateau state. Located on latitude 9°55´N and longitude 8°54´E, at an altitude of 1200 m above sea level. The area falls under Natural Region II of Nigeria's agro-ecological zones, the climate of the area is humid with an average annual rainfall and temperature between 140-1480 mm and 10°-32°C respectively.

2.2 Samples Collection

Infected tomato fruits with symptoms of softness were randomly procured locally from Farin gada market, Terminus market and Jarawa Tomato market in Jos between May and August 2015. Ten (10) samples were collected randomly from three different sellers at the different markets (a total of thirty), placed in sterile polythene bags and conveyed into the laboratory for fungal isolation and subsequent identification. 100 g of *Vernonia amygdalina* leaf were collected from Bauchi Ring Road, Jos, placed in a polythene bag and taken to the Pharmacognosy laboratory of pharmaceutical department, University of Jos for plant extraction and concentration preparation.

2.3 Isolation of Fungal Organisms

Potato Dextrose Agar (PDA) was prepared according to the manufacturer's instructions, following the techniques described by Arora and Arora [21]. 80 mg of Gentamycin, an antibiotic was added to each 500 ml preparation of the agar to inhibit probable bacteria growth.

Diseased portion of the tomato fruits were cut under aseptic conditions into small bits into a sterile dish with the aid of sterile scissors (flamed over a Bunsen burner flame) and dipped inside methylated spirit [22]. The bits were sterilized with 70% ethanol and placed on Petri dishes containing already prepared solidified potato dextrose agar (PDA). The solidified plates were incubated at room temperature (28±2°C) in the dark until visible growths were seen on the plates. The fungal colonies grown from the incubated plates were sub-cultured into fresh medium until pure cultures were obtained.

2.4 Identification of Fungal Organisms

Microscopic examination was used for examining the colony characteristics. A sterile needle was used in taking a little portion of the colony, placed on the sterile glass slide, stained with lactophenol cotton blue and examined under the microscope for morphology and culture characteristics of fungal structures.

2.5 Preparation of Plant Extracts

Vernonia amygdalina leaves were air dried and pulverized into powder using blender. 40g of the plant powder was weighed into 500ml conical flasks and was soaked in 70% ethanol. This was left to stand overnight (24hrs), then shook for 3hrs on a mechanical shaker. The content was filtered using a non-absorbent cotton wool on a Buchner funnel-flask using a vacuum pump. The residue was subjected to several parts of rinsing and filtration with fresh solvents to attain some level of maceration (extraction). The collective filtrate was evaporated to dryness using a rotary evaporator and a drying cabinet. The percentage yield of the extract was determined and the extract was transferred into a stirrer sample container and preserved in the refrigerator.

$$\% \text{ Yield} = (\text{Weight of extract} / \text{Weight of crude powder}) \times 100$$

2.6 Antifungal Sensitivity Testing

The Broth dilution method was adopted for the antifungal sensitivity by subjecting the isolate to a fresh culture in a nutrient broth for 24hours, and then a sterile swab stick was used to inoculate the isolate from the broth on an already prepared potato dextrose agar plate. Six holes were bored on the plate afterwards and 0.2mls of the ethanolic extracts and controls were introduced separately in each hole. The set up was incubated at room temperature for 48hours and daily measurements of the fungi growth of the cultures were determined by measuring culture along two diameters with plain metre rule. Fungi growth inhibition was taken and recorded. Four concentrations level of the ethanolic extract (12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml) were used. Fluconazole (50 mg/ml) was used as positive control while sterilized distilled water served as negative control. Three replicates were done for each of the organisms including the concentration levels and controls.

2.7 Statistical Analysis

One way Analysis of Variance (ANOVA) was used to analyze the results obtained to determine if the effects of the extract on the fungi isolate was linked to the different concentrations of the extract use. Each test was conducted at 95% confidence interval, $P<0.05$ at the appropriate degree of freedom (d.f.). A P-value of $P<0.05$ was considered significant. The data were analyzed using the program IBM SPSS version 22.

3. RESULTS

Table 1 illustrates the culture and morphological characteristics of the identified fungi. Implicated isolates were *Geotrichum candidum*, *Rhizopus stolonifer* and *Fusarium oxysporum*. Table 2 shows the percentage distribution of the fungi isolated from infected tomatoes in the different markets. The percentage distributions were estimated; *G. candidum* (45.16 %), *R. stolonifer* (22.58%) and *F. oxysporum* (32.26%). Tables 3 and 4 show the zones of inhibition of the extracts and controls observed on the media. The results (measured at 24 hours and 48 hours) show that increase in concentration is significantly proportional to increase in zone of inhibition ($P<0.001$). After 24 hours, there was inhibitory effect of the extract on *Rhizopus stolonifer* and *Geotrichum candidum* while *Fusarium oxysporum* showed no measurable effect. The result obtained at 24 hours shows a mean effect of 10.00 mm at 100 mg/ml and 4.50 mm at 12.5 mg/ml, a considerably significant difference ($P<0.001$). However after 48 hours, the three organisms showed increasing inhibitory effect. The positive control inhibited *Geotrichum candidum* and *Fusarium oxysporum* while the in the negative control there was no zone of inhibition. Tables 5 and 6 showed the mean effect of concentrations across all isolates. At concentration 100 mg, the isolates showed more susceptibility than other concentrations use after 24 hours and 48 hours respectively. Tables 7 and 8 showed the mean effect of extract on Isolates across all concentrations. *Rhizopus stolonifer* shows to be more sensitivity to the extract at all concentrations after 24 hours and 48 hours. The *Rhizopus stolonifer* was more susceptible to the treatment (plant extract) in all concentration level with mean effect of 4.75 mm after 24 hours and 7.25 mm after 48 hours, followed by *Geotrichum candidum* and then *Fusarium oxysporum* which was least susceptible. They all showed significant differences ($P<0.001$).

4. DISCUSSION

Deductions from the frequency of occurrence of these organisms (*Geotrichum candidum, Rhizopus stolonifer* and *Fusarium oxysporum*) that were isolated and identified revealed that *Geotrichum candidum* had higher prevalence of contamination, followed by *Fuarium oxysporum*, then *Rhizopus stolonifer* had least prevalence. Sensitivity test using different concentrations of extract of *Vernonia amygdalina* showed they

significantly affected all the identified organisms with *R. stolonifer* showing the highest inhibitory effect (P<0.001).

This study resulted into a mixed culture of fungal species, after several stages of sub culturing, three fungi were isolated and identified, then subjected to further experimentation. The identified isolates were *Geotrichum candidum, Rhizopus stolonifer* and *Fusarium oxysporum.* This finding is similar with the reports of previous investigations carried out by Chuku et al. [23] and Ugwu et al. [24]. Chuku et al. [23] work on fungal spoilage of tomato and isolated *Geotrichum candidum, Rhizopus stolonifer* and *Fusarium moloniforme*. The report of Ugwu *et al*. [24] isolated 6 fungal species which were *Candida tropicalis, Penicillium notatum, Aspergillus niger, Fusarium oxysporum, Absidia corynbifera and Rhizopus stonolifer*.

Table 1. Culture, morphological characteristics and Identification of fungal species

Culture characteristics	Microscopic characteristics	Identification
Clustered growth, appears creamy on the surface	Macro-conidia sparse in some strains, borne on phialides on branched conidiospores, septate, fusiform, more or less curved, pointed at both ends with a pedicellate basal cell	*P Fusarium oxysporum*
Distinct colonies, whitish became grayish brown	Columnella globose, subglobose or ovoid. Zygospores brownish-black, warted, with unequal suspensors	*Rhizopus stolonifer*
Mouldy surface covered the plate	Advancing hyphae septate, dichotomously branched (forked). Conidia cylindrical, barrel-shaped or ellipsoidal, formed by breaking up fertile hyphae, chains mostly aerial, erect or decumbent.	*Geotrichum candidum*

Table 2. Percentage distribution of fungal organisms isolated from infected tomatoes

S/No	Organisms	Number of organisms Isolated	Percentage (%)
1	*G. candidum*	14	45.16
2	*R. stolonifer*	7	22.58
3	*F. oxysporum*	10	32.26
Total		**31**	**100.00**

Table 3. Mean effect (inhibitions) of extract on the fungal growth after 24 hrs

Concentration	A	B	C
12.5 mg/ml	4.50±0.57^{d}	1.00±0.00^{e}	0.00±0.00
25.0 mg/ml	6.00±1.15^{c}	2.00±0.00^{d}	0.00±0.00
50.0 mg/ml	8.00±0.00^{b}	3.50±0.57^{c}	0.00±0.00
100.0 mg/ml	10.00±1.82^{a}	5.00±1.21^{b}	0.00±0.00
Pc	0.00±0.00^{e}	15.00±1.63^{f}	16.00±1.82
Cn	0.00±0.00^{e}	0.00±0.00^{a}	0.00±0.00
SE±	0.26	0.12	0.00

Means in the same column having the same letters are not significantly different (P<0.001)
Keys: A – Rhizopus stolonifer; B – Geotrichum candidum; C – Fusarium oxysporum;
Pc – Positive control (Fluconazole) 60 mg/ml; Cn – Negative control (Distilled water)

Table 4. Mean effect (inhibitions) of extract on fungal growth after 48 hrs

Concentration	A	B	C
12.5 mg/ml	8.50±0.577^{d}	2.00±0.00^{e}	1.00±0.00^{e}
25.0 mg/ml	10.00±1.15^{c}	3.00±0.00^{d}	2.50±0.51^{d}
50.0 mg/ml	12.00±0.00^{b}	5.00±0.00^{c}	3.50±0.53^{c}
100.0 mg/ml	13.00±0.00^{a}	6.50±0.00^{b}	5.00±0.00^{b}
Pc	0.00±0.00^{e}	15.00±0.00	16.00±0.00^{a}
Cn	0.00±0.00^{e}	0.00±0.00^{f}	0.00±0.00^{f}
SE±	0.26	0.12	0.17

Means in the same column having the same letters are not significantly different (P<0.001)
Keys: A – Rhizopus stolonifer; B – Geotrichum candidum; C – Fusarium oxysporum;
Pc – Positive control (Fluconazole) 60 mg/ml; Cn – Negative control (Distilled water)

Antifungal effectiveness of some tropical plants extracts in controlling several plant pathogens have been reported by several researchers [25,26,27,28,29,30].

Table 5. Mean effect of concentrations on all isolates after twenty four hours (24 hrs)

Concentrations	Inhibition after 24 hrs
12.5 mg/ml	1.83±2.03[e]
25 mg/ml	2.67±2.67[d]
50 mg/ml	3.83±3.43[c]
100 mg/ml	5.00±4.26[b]
Pc	10.33±7.64[a]
Cn	0.00±0.00[f]
SE±	0.10

Means in the same column having the same letters are not significantly different (P<0.001)
Keys: Pc – Positive control (Fluconazole) 60 mg/ml; Cn – Negative control (Distilled water)

Table 6. Mean effect of concentrations on all Isolates after forty eight hours (48 hrs)

Concentrations	Inhibition after 48 hrs
12.5 mg/ml	3.83±3.48[e]
25 mg/ml	5.17±3.63[d]
50 mg/ml	6.83±3.88[c]
100 mg/ml	8.17±3.63[b]
Pc	10.33±7.64[a]
Cn	0.00±0.00[f]
SE±	0.11

Means in the same column having the same letters are not significantly different (P<0.001)
Keys: Pc – Positive control (Fluconazole) mg/ml; Cn – Negative control (Distilled water)

Table 7. Mean effect of Isolates inhibitions after twenty four hours (24 hrs)

Organisms	Inhibition after 24 hrs
A	4.75±3.87[a]
B	4.42±5.11[b]
C	2.67±6.09[c]
SE±	0.07

Means in the same column having the same letters are not significantly different (P<0.001)
Keys: A – Rhizopus stolonifer; B – Geotrichum candidum; C – Fusarium oxysporum

Table 8. Mean effect of isolates inhibitions after forty eight hours (48 hrs)

Organism	Inhibition after 48 hrs
A	7.25±5.45[a]
B	5.25±4.93[b]
C	4.67±5.44[c]
SE±	0.08

Means in the same column having the same letters are not significantly different (P<0.001)
Keys: A – Rhizopus stolonifer; B – Geotrichum candidum; C – Fusarium oxysporum

This study observed inhibition after 24 and 48 hours using 70% ethanolic extract of *Vernonia amygdalina,* the work showed that the extract is effective against fungi associated with tomato infection and this is similar with Ijato et al. [31] who reported that *Chromolaena odorata* (leaf), *Tridax procumbens* (leaf), *Venonia amygdalina* (leaf) and *Azadirachta indica* (leaf) had inhibitory effect on the radial growth of rot fungi; after 72 hours observation with 1 ml of 15% ethanolic extract of *Venonia amygdalina* achieved an inhibitory effect of 54.58% on the radial growth of rot fungi. Report by Ugwuoke et al. [32] shows that *Venonia amygdalina* was use to control *Fusarium solani* causing tuber rot on cassava. He also reported the use of *Azadirachta indica* in the control of *Fusarium oxysporum.* Onyeani et al. [33] showed the inhibitory effect of *Venonia amygdalina* against *Rhizopus stonolifer* in his work.

At 24 hours, there was no measurable effect of the extract on *Fusarium oxysporum* until after 48hours. After 48 hours, there was increase in the effect of the extract but the positive control (Fluconazole at 50 mg/ml) had the same inhibitory effect as it did at 24 hours. However, *Rhizopus stonolifer* showed resistance to the positive control. The mean effect of the levels of concentration (12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml) and the controls (fluconazole and distilled water) were significantly different ($P \leq 0.001$) at both 24 hours and 48 hours.

5. CONCLUSION

The effect of extract of *Venonia amygdalina on* fungi isolated from tomatoes at Jos North LGA markets shows that the plant leaves have significant inhibitory effects on the growth of the fungi. The effect of the extract increases with increasing concentrations and time of exposure to the plant leaf powder. This suggests that the plant extract contains some chemical component that has fungicidal activity.

6. RECOMMENDATIONS

i. Other parts of the plant (stem and root) can also be subjected to study to check for probable higher fungicidal activities than the leaf part.
ii. Further research work should explore the botanicals to determine a higher effective extracts that can control fungi rot on tomatoes.
iii. Further research work may also seek to do a comparative study on the effect of more than one plant extract.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13116