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Inhibitory Effect of *Vernonia amygdalina* Leaf Powder on *Rhizopus stolonifer* and *Fusarium* sp of Tomato Plants in a Greenhouse

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Authors' contributions

This work was carried out in collaboration between all authors. Author WCJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TAI and MOM managed the analyses of the study. Author MI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was conducted to determine the effect of different concentration of *Vernonia amygdalina* leaf powder against the fungal disease of Tomato plants in a greenhouse. The research was carried out in a greenhouse at the Federal College of Forestry in Jos, from February to April 2018. 2 × 3 factorial in completely randomised design was used. A total of 20 diseased tomato plants and 120 healthy tomato seedlings were collected from 3 different farms in Farin gada, Jos. Tomato seedlings were grown on sterile soil packaged in polythene bags mixed with *Vernonia amygdalina* leaf powder at different concentrations of 100, 150, and 200 g and inoculated with *Rhizopus stolonifer* and *Fusarium* specie isolated from diseased tomato plants. the isolates were pre-grown in sterile peptone water for 72 hours and replicated thrice. Inoculated non-amended soil served as control. The Effect of *Vernonia amygdalina* leaf powder against inoculated fungi was determined by tomato plant heights, root length and number of the leaf after eight weeks of transplanting. Data were analysed using one way ANOVA at P = 0.05. The result showed an inhibitory effect at

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different concentrations with significant difference among all treatment. 50 g of *Vernonia amgydalina* powder against *Fusarium* specie gave the highest activity which is revealed in plant height =54.33, number of leaf =31.11 and root length = 19.50. The result obtained indicated that *Vernonia amgydalina* leaf powder could serve as a biological agent in prevention and control of the tomato plants soil-borne disease.

Keywords: Fusarium; Rhizopus; Vernonia amygdalina; Jos.

1. INTRODUCTION

Tomatoes (*Lycopersicon esculentum* M.) is an important crop in Nigeria and plays a major role in food industries all over the world. It is very nutritious and a rich source of Vitamin A, B, C and minerals [1]. The cultivated crops are infected by one or more fungal pathogens causing economic losses. The majority of these diseases are caused by fungi which usually results in fruit loss or death of crop plants [2].

Soil-free from diseases are needed to achieve desired germination, emergence, healthy seedlings and plant population [3]. Fungal pathogens result in heavy losses in crop yield and seed quality. Seed-borne fungal species are ubiquitous and include both plant pathogenic and saprophytic species that may damage crops in the field and cause post-harvest decay. Pathogenic fungi, such as *Alttternaria, Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, and Trichoderma* have been implicated in some crop spoilage [4]. *Rhizopus stolonifer* is the causal agent of *Rhizopus* rot disease in various fruits and vegetables such as peach (*Prunus persica* Batsch.), papaya (*Carica papaya* L.) and tomato (*Lycopersicon esculetum* M) [5–7]. *Rhizopus stolonifer* is a good coloniser of plant debris and infects harvest fruits, often destroying the entire contents within a few days by hydrolysis with the tissue-macerating ability [8]. Fungi contamination of many agricultural products, including tomatoes starts in the fields [9]. *Fusarium* is a causal agent of tomato wilt. The fungus which is a soil-borne pathogen can exist many years in the soil without a host. Most infections originate from the population associated with infected tomato debris. Healthy plants can become infected by *Fusarium* if the soil in which they are growing is infested with the pathogen [10,11]. However, species of the genus *Fusarium* that are the causal agents of tomato wilt also cause root and basal stem deterioration resulting in the wilting of vegetable plants.

Vernonia amygdalina is a tropical plant belonging to the family Compositae and is used widely as vegetable and medicinal plant. It has the common name bitter leaf [12]. *Vernonia amygdalina* had been reported to exert antibiotic action against drug-resistant microorganisms and possess antioxidant, anticancer, antiviral, anti-helminthic and anti-inflammatory activities [13, 14]. Botanical control of plant pathogens is preferred in comparison with synthetic products. More use of fungicides like organo-mercurials, carbamates etc. have posed serious health problems to human and environment [2]. There is an urgent need to search for the natural biodegradable source of bio-fungicides. Botanical-based pesticides have been shown to be one of the better alternatives for the control and prevention of fungal diseases, as they are known to have minimal environmental problems and less negative effects to consumers in contrast to chemical pesticides [15]. Study on a more sustainable and eco-friendly agriculture system is the need of the time, as there is a growing concern on the deteriorating quality of the environment as a result of intensive agriculture. Based on this, the present research was conducted to use *Vernonia amygdalina* in the development of a new approach for the control of pathogenic fungi associated with tomato seedlings based on due to its economic importance.

2. MATERIALS AND METHODS

2.1 Study Area

The experiment was carried out at Jos North Local Government Area, Plateau State is located on latitude 9°55’N longitude 8°54'5 at an altitude of 1200 m above sea level. The area falls under natural region 11 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 Collection of Sample

Apparently fresh healthy *Vernonia amygdalina* leaf samples (about 2000 g) was purchased from Farin gada Market in Jos North Local Government Area. Sixty healthy UTC tomato seedlings and twenty diseased tomato plants

(showing loss of green colour and wilting) were collected from Farin gada vegetable farm from different farmers at a different location of the farm area.

2.3 Preparation of Bitter Leaf Powder

Vernonia amygdalina leaf was air dried and pulverised into powder using mortar and pestle, and then followed by sterilisation using hot air oven at 160°C for 1 hour.

2.4 Isolation of Fungal Pathogen of Tomato

Potato Dextrose Agar (PDA) was prepared according to the manufacturer's instructions. The fungal isolation was done following the techniques described by John et al. [16]. 80 mg of Gentamycin, an antibiotic was added to each 500 ml preparation of the medium to inhibit bacteria growth. The diseased portion of the tomato plants was cut under aseptic conditions into small bits into a sterile Petri dish with the aid of scissors which was flamed over a Bunsen burner flame and dipped inside methylated spirit. The cut diseased part was sterilised with 70% ethanol and placed on Petri dishes containing solidified potato dextrose agar (PDA). The solidified plates were incubated at room temperature (28 plus or minus 2°C) 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. The percentage occurrence of the fungi isolated was determined by the following formula:

$$\text{Percentage occurrence} = \frac{\text{Number of isolate}}{\text{d number of total isolates}} \times 100 \quad (1)$$

2.5 Identification of Fungal Organism

Macroscopic and Microscopic examination were used to determine the morphological characteristic of the fungal isolates. For macroscopic identification, colony characteristics such as appearance and change in colour were observed on the Petri plates. For microscopic examination, the sterile inoculating needle was used to pick a little portion of five-day-old isolate and placed on a sterile glass slide, and the slide was stained with lactophenol cotton blue, mixed and covered with a slip. The slide was then viewed under the microscope, using x10 and x40 magnification. Shapes of the conidia and conidiophores were taken note of. These features were matched with standards described by Barnett and Hunter [17] and Booth [18] and identified by the help of an expert.

2.6 Soil Amendment

Hot air oven sterilised soil was amended using *Vernonia amygdalina* powder. 4000 g of sandy loam soil samples were amended by incorporating 0 g, 100 g, 150 g and 200 g of *Vernonia amygdalina* leaf powder. Each concentration were replicated thrice. Inoculated and non-amend soil samples served as the control.

2.7 Inoculum——Preparation and Soil Infection

The suspension for inoculation was prepared by pouring 250 ml of nutrient broth into a bottle containing 10-day-old isolate broths, then stirring the mixture with a sterile glass stick, and pouring it into a glass. An estimated 1x10^6 conidia/ml each of the isolated fungal isolate were inoculated into a 250 ml nutrient broth. Each of the preparation was sprinkled over the soil sample.

2.8 Seedling Planting

Five three weeks old seedlings were used. The plant roots were washed with sterile distilled water and then dipped in 70% ethanol for 2 minutes and carefully transplanted without thinning in 20-inch diameter pot at 12 inches deep, The plants were water twice daily by drip irrigation.

2.9 Experimental Design

The experiment was layout using 2 x 3 factorial in complete random design (CRD), whereby, the four (4) treatments were replicated three times (0, 100, 150 and 200 g).

Treatment combinations = 2 × 4 = 8
Replications = 3
Total plots = 3 × 8 = 24.

2.10 Data Collection and Statistical Analysis

Data were collected 8 weeks after seedling transplanting to the soil, the number of leaves, length of root, and length of the shoot were determined. The data were analysed using one-

way analysis of variance (ANOVA) and T-test to compare means. Treatment means were separated using Duncan Multiple Range Test (DMRT) at $p = 0.05$.

3. RESULTS

Rhizopus stolonifer and *Fusarium* sp were isolated from diseased tomato plant root. The percentage occurrence of fungi isolates revealed *Rhizopus stolonifer* have percentage occurrence of 44.91%, with *Fusarium* sp giving the highest occurrence of 59.09%. The percentage distribution results showed a significant difference at $p = 0.05$. The activity of *Vernonia amgydalina* against *Rhizopus stolonifer*, *Fusarium* sp and consortium have taken after 8 weeks of transplanting showed that the *Vernonia amgydalina* powder had an inhibitory effect against the fungi tested.

The result revealed an increase in *Vernonia amgydalina* powder concentration increased inhibition leading to the better growth of tomatoes plant. 200 g of *Vernonia amgydalina* powder against *Fusarium* sp gave the highest activity which was revealed in plant height =54.33, No. leaf =31.11 and Root length = 19.50. The result showed there was a significant difference between the various treatments and the control. The result of inhibitory activity *Vernonia amgydalina* powder against the consortium fungi gave a reduced effect. 200 g of *Vernonia amgydalina* powder against consortium fungi gave the highest inhibitory activity of 14.33, 5.57 and 3.57 which is revealed in plant height, number of leaves, and root length respectively.

4. DISCUSSION

Twenty-six *Fusarium* spp. and eighteen *Rhizopus stolonifer* were isolated from 40 diseased tomatoes root from Farin gada, Jos North LGA. The occurrence distribution of the 44 isolates revealed that *Fusarium* sp. had the highest percentage of occurrence of 59.09%, thus agreeing with the work of Fatih et al. [19] who stated that *Fusarium* sp and *Rhizopus stolonifer* was commonly observed in tomato plants. In a similar work done by Dimphna and Eneke, [1] on fungi associated with postharvest decay of tomatoes in Abakalike, Nigeria where *Fusarium* sp and *Rhizopus stolonifer* were implicated. Fungi have been shown to contaminate many agricultural products including tomato plants coming from the field [15]. A large amount of water and soft endocarp make tomatoes more susceptible to fungi attack [20].

Table 1. Percentage distribution of fungi species isolated from diseased tomatoes

Isolates	No of fungi occurrence	Percentage occurrence
Fusarium sp	26	59.09^{a}
Rhizopus stolonifer	18	44.91^{b}
SE±	-	4.22
LSD	-	*
Total	**44**	**100**

a, b, c = Means separations indicating the level of significance.
Means within a column followed by the same letters are not significantly different (P = 0.05) using Duncan Multiple Range Test.
** = Significant at 95% level of probability.*
LSD = Least significant difference.

Table 2. Morphological and microscopic characteristic of fungi isolates

Culture characteristic	Microscopic characteristic	Isolate
Clustered growth, appears creamy on the surface	Oval to kidney-shaped micronidia. Sickle-shaped, thin-walled, Microconidia produce in the false head.A single, terminal chlamydiospore	*Fusarium Sp*
Distinct colonies, whitish became greyish brown	Hyphae broad, scarcely separate rhizoids and stolons present, sporangiospore brown, diverging from the point at which the rhizoids formed. Ovoid sporangiospores	*Rhizopus stolonifer*

Table 3. Morphological views of fungi isolates

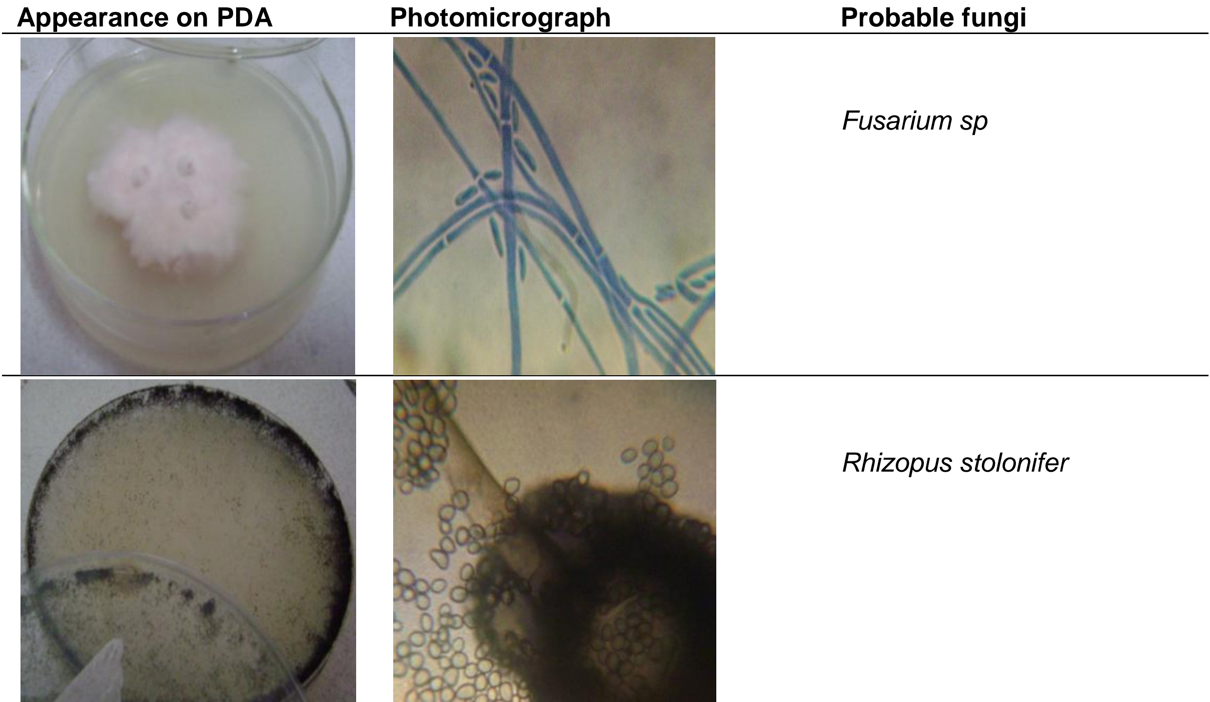
Appearance on PDA	Photomicrograph	Probable fungi
		*Fusarium sp*
		Rhizopus stolonifer

Table 4. Inhibitory Mean effect of *V. amygdalina* on the *Rhizopus stolonifer* growth

Concentration	Plant height (cm)	No. leaf	Root length (cm)
Control	25.10^{c}	4.33^{d}	8.40^{d}
100	33.06^{b}	16.70^{c}	10.03^{c}
150	41.33^{a}	26.17^{b}	11.23^{b}
200	34.53^{b}	29.67^{a}	14.50^{a}
SE±	1.74	0.45	1.39
LSD	*	*	*

a, b, c = Means separations indicating the level of significance.
Means within a column followed by the same letters are not significantly difference (P = 0.05) using Duncan Multiple Range Test.
** = Significant at 95% level of probability.*
LSD = Least significant difference.

Table 5. Mean inhibitory effect of *V. amygdalina* on the *Fusarium* specie growth

Concentration	Plant Height (cm)	No. Leaf	Root Length (cm)
Control	14.10^{d}	7.83^{d}	10.90^{c}
100	23.70^{c}	19.00^{c}	17.31^{b}
150	41.63^{b}	26.66^{b}	16.33^{b}
200	54.23^{a}	31.11^{a}	19.50^{a}
SE±	1.74	0.45	1.39
LSD	*	*	*

a, b, c = Means separations indicating the level of significance.
Means within a column followed by the same letters are not significantly different (P = 0.05) using Duncan Multiple Range Test.
** = Significant at 95% level of probability.*
LSD = Least significant difference.

Table 6. Inhibitory Mean effect of *V. amygdalina* on the fungal consortium growth

Concentration	Plant Height(cm)	No. Leaf	Root Length (cm)
Control	15.00^{b}	3.33^{c}	7.20^{b}
100	23.00^{a}	6.09^{a}	5.33^{a}
150	21.33^{a}	6.67^{a}	5.33^{a}
200	14.33^{b}	5.11^{b}	3.50^{c}
SE±	1.74	0.45	1.39
LSD	*	*	*

a, b, c = Means separations indicating level of significance.
Means within a column followed by the same letters are not significantly different (P = 0.05) using Duncan Multiple Range Test.
** = Significant at 95% level of probability.*
LSD = Least significant difference.

This study observed inhibition after 8 weeks of transplanting, using a different concentration of V. *amygdalina* leaf powder of 100, 150 and 200 g respectively. The result of the inhibitory effect of V. *amygdalina* leaf powder against *Fusarium* sp. and *Rhizopus stolonifer* in Tables 3 and 4 showed that powder of V. *amygdalina* leaf powder had antifungal activities which resulted in the significant difference between the plant length, a number of leaves and root length of the treatment and the control. 200 g of V. *amygdalina* leaf powder gave the highest activity as revealed in plant height =54.33, number of leaf =31.11 and root length = 19.50. This is similar to a study conducted by Saroja, [21], who reported that *Chromolaena odorata* (leaf), *Tridax procumbent* (leaf) activity which is revealed in plant height =54.33, number of leaf =31.11 and root length = 19.50 Ogundare et al. [22] revealed that *V. amygdalina* leaf powder containing sapronin, flavonoid, tannin were found to have very potent antifungal activities.

The inhibitory effect of *V. amygdalina* leaf powder against consortium fungi as revealed in Table 5 giving a reduced effect. 200 g of *Vernonia amgydalina* powder against consortium fungi gave the highest activity of 14.33, 5.57 and 3.57 which is revealed in plant height, number of leaves, and root length respectively. The obtained result showed lesser activity comparing to that in Tables 3 and 4. This could be attributed to the synergic response of the consortium fungi resulting in the degradation of the plant powder.

5. CONCLUSION

Vernonia amgydalina leaf powder showed to possess bioactive ingredient which was inhibitory against fungi in tomatoes field and suggests that crude powder of the plant leaf should be used in soil amendment against fungi and fungal disease.

6. RECOMMENDATION

Further research should be carried out to ensure whether continuous use of *Vernonia amygdalina* as pesticide could cause fungi to develop resistance against it or not.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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