Short Research Article

Efficacy of Fungicides and Plant Extracts against *Fusarium oxysporum*f. sp. *batatas* Incitant of *Fusarium* Wilt of Sweet Potato (*Ipomeae batatas l.*)

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ABSTRACT

In vitro experiments were conducted to evaluate the effect of some fungicides and some plant extracts against *Fusarium oxysporum*f. sp. *batatas* (*Fob*). The experiments were conducted in the Plant Pathology Laboratory of the Department of Crop Protection, Bayero University Kano. Three fungicides (Mancozeb, Hexaconazole and Metalaxyl + coprous oxide) and sterile distilled water as control were arranged in a completely randomized design. The treatments were repeated five times. Mancozeb as the most promising fungicide against the fungus was used as check, other treatments include; 25% aqueous neem leaf extract (ANLE), 50% ANLE, 25% aqueous garlic bulb extract (GABE), 50% GABE, 25% aqueous Callotropis leaf extract (CALE), 50% CALE and control. The treatments were arranged in a completely randomized design and repeated thrice. Data on radial growth (RG) and percent growth inhibition (PGI) of the fungus were recorded and subjected to analysis of variance using GenStat 17th Edition. Among the fungicides tested against *Fob*, Mancozeb significantly had the least RG (13mm) and inhibited growth of the pathogen by 71.1% compared to the other fungicides. Bioassay on plant extracts revealed least RG of the fungus (36.7mm) and highest PGI (43.5%) when 50% ANLE was used. This differed significantly with the use of 25% ANLE which caused 39.3 mm RG of the fungus and inhibited growth by 38.5%. This was followed by 50% and 25% CABE, respectively with lower fungal growth and higher PGI compared to GABE at the different concentrations, exhibiting similar effect on the growth of the fungus and its inhibition. Aqueous neem leaf extract (50%) contained higher phytochemicals than the other botanical extracts at the different concentrations. These phytochemicals are responsible for better suppression of *Fob*. Application of 50% ANLE could be further evaluated as potential bio-pesticide to supplement the use of fungicide against Fusarium wilt of sweet potato.

Note: Review paper may have different types of subsections.

Keywords: *CallotropisDanchana, Fusarium oxysporum*f. sp*. batatas, garlic, neem, percent growth inhibition,phytochemicals, sweet potato*

INTRODUCTION

Sweet potato is one of the most important food crops in the world and has made great contributions to our food security [1]. The crop is the world`s seventh most important food crop after wheat, rice, maize, potato, barley and cassava [2]. The tuber is highly nutritious and widely consumed in human diet due to its good taste [3]. It is an important secondary crop that plays an important role in house hold food security in many countries [4, 5]. The production of sweet potato is constrained by numerous biotic and abiotic stresses among which Fusarium wilt is one of the severest [1]. The disease is caused by *Fusarium oxysporum*f. sp. *batatas* (Fob). Although, the disease is primarily caused by *F. oxysporum*f. sp. *batatas*, sometimes strains of the tobacco pathogen *F. oxysporum*f. sp. *nicotianae* can cause wilt in susceptible sweet potato cultivar [6]. All strains of *F. oxysporum*are saprophytic and able to grow and survive for long periods on organic matter in soil and in the rhizosphere of many plant species [7]. In some cases, infected plants may survive and produce storage roots that are infected. If such tubers are used as seed tubers, they can transmit the fungus to the fresh sprouts which may will in plant beds. Yield losses may be up to 50 percent and are more likely under warm weather and in dry soils [8]. Plants normally die within a few

days after visible symptoms appear on the plant [9]. Control methods include use of disease resistance variety, crop rotations, planting diseases free plants and fungicides applications [10]. Ansari [11] reported that chemical fungicides could be effective in the management of fungal diseases; but the attendant problem of indiscriminate use of fungicides is not only hazardous to human but disrupts the natural ecological balance by killing the beneficial soil microbes.

There are number of useful plants compounds that have antifungal properties with less environmental risk that could be used against fungal pathogens [12]. Such compounds being biodegradable and selective in their toxicity are considered valuable for controlling different plant diseases [13). Several researchers have reported antifungal activity of different plant extracts against wide range of plant fungi. These plants extracts have been reported to be safe, non-toxic to man, but effective against plant pathogens [14]. Agbenin and Marley [15] reported the used of extracts of neem and garlic in inhibiting mycelial growth of *Fusarium oxysporum*f. sp*. lycopersici*. However, the use of plant products for the control of *Fusarium* wilts of sweet potato in Nigeria is not extensively studied. Therefore, this experiment was carried out to evaluate the most promising fungicide and plant extract in suppressing *Fusarium oxysporum*f. sp. *batatas*

2. MATERIAL AND METHODS

2.1 Preparation of Aqueous Plant Extracts

Fresh and healthy leaves of neem and callotropis were collected from the new site Bayero University Kano, f*resh garlic cloves were purchased from Janguza market, Kano.* The leaves of the two plants and the peeled *garlic cloves* were thoroughly washed under running tap water and rinsed with distilled water and then shade dried for a week at room temperature 2± 37°C. The dried leaves and peeled garlic were separately grinded with pistil and mortar and a 25-mesh diameter sieve was used to obtain a fine powder. Hundred grams of each powder was mixed with 300ml of *distilled water* in one litrer conical flask. The mixture were stirred for 30 min and left for 48h. The extracts were then filtered separately through a fine cloth and again through filter paper (Whatman No. 1). *The extracts were considered to be 100% concentrate and were diluted down to make up the 25% and 50%. They were then* preserved in tightly corked labeled bottles as 25% and 50% aqueous neem leaf extract, garlic clove extract and callotropis leaf extract, respectively. The extracts were stored in a refrigerator at 4°C for preservation before use.

2.2 Phytochemical Screening

Concentrated plant extracts were used for the phytochemical screening using standard procedures to identify the constituents as described by Riaz [16] in Table 1. *Phlobotannins*was evaluated using the method of Taoheedet al. [17] by adding 2 mL of each of the the extract with aqueous hydrochloric acid (1%) and boiled. Deposition of a red precipitate is evidence for the presence of phlobatannins. For Anthraquinones, 5 ml of the extracts were mixed with Benzene (10 mL), filtered and 10% ammonia solution (5 mL) was added to the filtrate. The mixture was shaken and the presence of pink, red or violet colour in the ammoniacal phase indicated the presence of anthraquinones [17].

2.3 *In vitro* Evaluation of Three Fungicides against *Fusarium oxysporum* f. sp. *batatas*

The experiment was conducted in the Plant Pathology laboratory in the Crop Protection Department, Faculty of Agriculture, Bayero University Kano, to assess the efficacy of some fungicides on growth and inhibition of *Fusarium oxysporum*f. sp. *batatas*. Three fungicides

were used and tested on the test pathogen isolated from infected sweet potato. Treatments were arranged in a completely randomised design (CRD) which was repeated four times. The treatments includes: Hexaconazole 5% SC (HATRICK), Mancozeb 80% WP (RAKSHA), Cuprous Oxide +Metalaxyl (BLUE BOLTS) and control. Petri dishes containing potato dextrose agar (PDA) were separately amended with the respective fungicides, while control treatments contained only PDA.

Food poisoning technique was used, following the procedures described by Ngono *et al.* (2000). The inhibitory activity of the fungicides on mycelial growth of the pathogens was determined by growing the fungal isolate *Fusarium spp* on potato dextrose agar amended separately with the different treatments. Potato dextrose agar (PDA) was autoclaved at 121°C for 15 minutes, 15 ml of the molten PDA medium were added to 15 Petri dishes (9-cm diameter) amended with 0.1 ml from each of the respective treatments excluding the control which was left un-amended. After solidification, each plate was inoculated with a 5 mm mycelial disc mat of the pathogen *Fusarium* sp. in the centre and incubated at room temperature.

The radial growth of the pathogen was measured separately after seven days of inoculation and the percent growth inhibition (PGI) of the pathogen was calculated using the formula $(D_c - D_t) / D_c \times 100$, where D_c is the average diameter increase of fungal colony with control and D_t is the average diameter increase of fungal colony with treatment.

2.4 Bioassay on the Effect of Aqueous Extracts of Garlic Cloves, Callotropis and Neem Leaves Extracts against*Fusarium oxysporum*f. sp. *batatas*

The experiment was laid out in a completely randomized design consisting of seven treatments repeated three times. The treatments includes: 25% ANLE, 50% ANLE, 25% GABE, 50% GABE, 25% CALE, and 50% GALE, Mancozeb as check and Control. Petri dishes containing potato dextrose agar (PDA) were separately amended with the respective plant extracts, while check contained PDA amended with the fungicide and control treatments contained only PDA. The radial growth of the pathogen was measured separately after seven days of inoculation and the percent inhibition of the pathogen was also calculated. Data on radial growth and growth inhibition were measured and analyzed using Genstat 17th Edition, and treatment means were separated using LSD at 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The phytochemical analysis revealed the presence of alkaloids, saponins, tannins,phylobtannins, flavoids, cardiac glycocides and steroids in garlic, neem, and callotropis leaf extracts (Table 2).These plant-derived compounds werereported to inhibit pathogen growth

Table 1: Phytochemical evaluation methods of the aqueous extract of garlic bulb, neem and calotropis leaves extracts

Tests	Description

Alkaloids test	5ml of the ginger extracts were accurately measured and transferred into a flask and stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath. Then 1ml of that filtrate was treated with few drops of Dragen dorff's reagent. A color change to blue black was the evidence of presence of alkaloids.
Saponins test	5ml of the botanical extracts and 5ml distilled water in a test tube. Appearance of Frothing on shaken with water showed the presence of saponins.
Tannins test	5ml of the botanical extracts along with 100ml distilled water and filtered, then ferric chloride reagents was added, blue-black or blue green precipitate appeared which showed the presence of Tannins.
Phlobotannins test	When an aqueous extract of the test sample was boiled with 1% hydrochloric acid, disposition of red precipitate had confirmed the presence of phlobotannins.
Flavonoids test	When 5ml of diluted ammonia solution was added to aqueous filtrate of the test sample followed by the addition of concentrated H2SO4, a yellow coloration was observed which determined the presence of flavonoids.
Cardiac glycosides (keller-killiani test)	When 5ml of the ginger extracts dissolved in 2ml glacial acetic acid containing a drop of ferric chloride solution was underplayed with 1ml concentrated H2SO4. A brown ring appeared at interface indicated adeoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a green ring may form just gradually spread throughout this layer.
Steroids test	When 2 ml of acetic anhydride was added to 0.5 g of ginger extract and 2 ml of sulphuric acid was added by the sides of the test tube a color change was observed to violet or blue-green which showed the presence ofsteroids.
Terpenoids test	When 2 ml of chloroform was added to 1ml of the extract, and Concentrated H2SO4 (3 ml) was added to form a layer, a reddish brown coloration at the interface indicated the presence of terpenoid.

Source: Riaz *et al.* [16]

and/or induce plant resistance [18]. This is similar to the findings of Usman *et al*. [19] and Mainasaraet al.[20] who reported their presence in callotropis leaf extract. Similarly, Pandey *et al*. [21] and Muhammad and Idris [22] also reported their presence in neem leaf extract and garlic bulb extract, respectively. The presence of phytochemicals in the aqueous extracts of the botanicals inhibited the growth of the fungus. This results correlate with the finding of Prohp and Onoagbe [23]who attributed growth inhibition of many fungi, bacteria and viruses by tannins, and also with the findings of Abaoab*et al*. [24] and Muhammad and Idris [22] who reported growth inhibition of bacteria and fungi due to the presence of

saponin, tannin, flavonoid and terpenoid found in clove extracts. The phytochemical analysis of the botanicals showed presence of alkaloid, saponins, flavonoids, glycoside, anthraquinones, tannin and terpenoids. Presence of alkaloids, steroids and flavonoids were better in neem leaf extracts than in garlic and callotropis extracts and this could be the reason for least mycelial growth of the fungus and highest growth inhibition.

3.2 *In vitro* Evaluation of Three Fungicides against *Fusarium oxysporum*f. sp. *batatas*

Result in Table 3 shows the effects of three commercially available fungicides on mycelial growth and inhibition of *Fusarium oxysporum*f. sp. *batatas.* Generally, all the three fungicides exhibited least mycelial growth and higher percent growth inhibition than the control. Mancozeb significantly (P≤0.05) had the lowest radial growth of the fungus (13 mm) and highest growth inhibition (71.1%) than the other fungicides. This was followed by Copper oxide + Metalaxyl with 21 mm radial growth and 50.7% inhibition of *Fusarium* sp., which differed significantly with Hexaconazole (26.7 mm radial growth and 36.7% inhibition. The pathogen in the control treatment recorded zero growth inhibition and longest radial growth (42.7 mm) compared to the other treatments.

3.3 Bio-efficacy of some plant extracts against *Fusarium oxysporum* f. sp. *batatas*

Table 4 shows the effect of some plant extracts at different concentrations on radial growth of*Fusarium oxysporum*f. sp*. batatas* and PGI. Plates containing PDA amended with Mancozeb showed least growth of the pathogen (17 mm), exhibiting highest growth inhibition (73.8%) than the other treatments.Plates containing PDA amended with Mancozeb showed least growth of the pathogen (17 mm), exhibiting highest growth inhibition (73.8%) than the other treatments. Radial growth of the fungus was significantly (P≤0.05) lower on plant extracts amended PDA which recorded highest percent inhibition in comparison with the control. Aqueous neem leaf extract at 50% produced 36.7 mm radial growth and reduced growth of the test pathogen by 43.5%, compared to 25% ANLE which caused 39.3 mm radial growth of the fungus and inhibited growth by 38.5%. This was followed by 50% and 25% CABE, respectively with lower fungal growth and higher growth inhibition than GABE at the different concentrations, exhibiting similar effect on the growth of the fungus and its inhibition. Among the fungicides tested against the pathogen, Mancozeb was the most effective in reducing radial growth and inhibiting the growth of the fungus. This conforms to the findings of Obagwu et al. [25] who reported Mancozeb as the most effective fungicide ininhibiting the growth of *Colletotrichum capsici*responsible for brown blotch of Bambara nut. Similar result was presented by Osunlaja and Alamutu [26] who also reported the effectiveness of Mancozeb in inhibiting the growth of *Physoderma maydis* (brown spot).
Neem was found to be significantly superior in lowering the radial growth of the pathogen and growth inhibition, followed by callotrophis and garlic, respectively. This agrees with the findings of Amadioha et al. [27] who reported significant growth reduction of *Pyriculariaoxyzae*due treatment with Neem seeds and leaf extracts. Several researchers also used different extracts from neem to inhibit growth of many fungi such as *Fusarium oxysporum*,

Table 2: Phytochemical screening of aqueous garlic bulb extract, neem leaf and calotropis leaves extracts

Phytochemical	ANLE	GABE	CALE
Alkaloids	+ +	+	+

Saponins	+ +	+	+
Tannins	+	+	+
Phylobtannins	+	+	+
Flavoids	+ +	+	+
Cardiac glycocides	+	+	+
Steroids	+	+	+
Tarpenens	+	+	+

Table 3: *In vitro* effect of fungicides on and of *Fusarium oxysporum*f. sp. *batatas*

Treatment	Mycilial growth	Percent growth inhibition
Mancozeb	13[d]	71.1[a]
Hexaconazole	26.7[b]	36.7[c]
Metalaxyl + Coprous oxide	21.0[c]	50.7[b]
Control	42.7[a]	0.0[d]
SE±	0.28	0.14

Table 4: *In vitro* effect of plant extracts against *Fusarium oxysporum*f. sp. *batatas*

Phytochemical	Mycilial growth	Percent growth inhibition
ANLE 25%	39.3f	39.5c
ANLE 50%	36.7e	43.5b
GABE 25%	58.0b	10.8f
GABE 50%	56.3b	13.4f
CALE 25%	53.3c	18.0e
CALE 50%	49.3d	24.2d
Mancozeb	17.0g	73.8a
Control	65.0a	0.0g
SE±	0.70	0.58

ANLE= Aqueous neem leaf extract, GABE = Aqueous garlic bulb extract, CABE = Aqueous calltropis leaf extract

Macrophinabphaseolina, *Aspergillus flavus* [15, 28, 29 and 30].The result conforms to the findings of several researchers also used different extracts from neem to inhibit growth of fungi such as *Fusarium oxysporum*, *Pyriculariaoxyzae, Macrophinaphaseolina*, *Aspergillus flavus, Cochliobolusmiyabeanus*[27, 15 and 31]. Bio-efficacy of 50% aqueous neem leaf extracts is attributed to higher quantity of phytochemicals presence compared to those found in the other two botanicals. The use of 50% neem leaf extracts could be further studied to supplement the use of fungicides under screenhouse and field conditions.

4. CONCLUSION

The three botanicals at the different concentrations were tested against the fungus *Fusariumoxysporum* f. sp.*batatas* and the results revealed least radial growth and growth inhibition when 50% ANLE was tested against the pathogen. Aqueous neem leaf extract (50%) contained phytochemicals such as Alkaloids, cardiac glycocides, flavoids, phylobtannins, saponins, steroids, tannins, tarpenensmore than the other botanical extracts.. These phytochemicals are responsible for better suppression of

Fusarium oxysporum f. sp. *batatas*. Application of 50% ANLE could be further evaluated in the screen house and field conditions as potential bio-fungicide to be used in an integrated management package on Fusarium wilt of sweet potato.

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