http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2019.206.213

Research Article
Inhibitory Effect of Aqueous and Ethanolic Extracts of Neem Parts on Fungal Rot Disease of *Solanum tuberosum*

Chukwuma Stephen Ezeonu, Verwiyeh Silas Tatah, Chinedu Imo, Emmanuel Mamma, Mida Habila Mayel, Ayobami Jahdahunsi Kukoyi and Ibrahim Adams Jeji

Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari, Nigeria

Abstract

Background and Objective: The processing and preservation of Irish potato tubers like many other crops has been affected by various pathogens like fungi (particularly storage rot) which causes a major constraint to Irish potato production in Nigeria resulting to enormous post-harvest spoilage. The inhibitory effect of aqueous and ethanolic extracts of neem leaves, stem bark and seeds on fungal rot disease of *Solanum tuberosum* (Irish potato) as an alternative treatment for fungal storage disease on Irish potato tubers cultivated in Nigeria has been determined in this study. **Materials and Methods:** Neem parts were harvested from Wukari, Nigeria and were processed for the experiment. Isolation of fungi and sub-culturing of the isolates was carried out to obtain pure culture. Pathogenicity study was carried out and effect of the plant extracts on mycelia growth of the test fungi was studied using the food poisoning techniques. The qualitative and quantitative phytochemical studies of the neem parts were carried out using standard methods. **Results:** Qualitative phytochemical screening of the selected parts of neem plant showed the presence of alkaloids, glycoside, flavonoids, carbohydrates, reducing sugar, steroids, tannins and saponins. The quantitative test revealed that the percentage quantity of phytochemicals in these plant parts ranged from 10.17-23.88%. Effect of the extracts on the test organisms was significant ($p<0.05$). Ethanolic extract of the seed (ESE) exhibited the highest inhibitory effect on *Aspergillus niger* (88.37%) followed by aqueous extract of stem bark (ABE) (87.21%), while aqueous seed extract (ASE), ethanolic leaves extract (ELE), aqueous leaves extract (ALE) and ethanolic stem bark extract (EBE) exhibited inhibition of 81.78, 77.52, 72.87 and 39.53%, respectively. The inhibitory effect of EBE was significantly ($p<0.05$) lower than that of other extracts, while there was no significant difference in the inhibitory effects of ESE, ASE and ABE compared to ketoconazole on *Aspergillus niger*. The ethanolic extracts of the seed and leaves had 100% inhibitory effect on *Fusarium oxyporium* with 87.60% inhibition by aqueous extract of the leaves. The seed and the leaves ethanolic extracts exhibited the highest inhibitory effects on *Pythium* spp. and *Fusarium oxyporium*, while the aqueous leaves extract showed the least inhibition on *Fusarium oxyporium*. **Conclusion:** The plant extracts in this study were found to be very effective in inhibiting fungal mycelia growth and hence can be potentially effective for preservation during storage of Irish potatoes to minimize post-harvest lost.

Key words: *Aspergillus niger*, *Fusarium oxyporium*, irish potato, inhibitory effect, neem, pathogenicity, *Pythium* spp.

Citation: Chukwuma Stephen Ezeonu, Verwiyeh Silas Tatah, Chinedu Imo, Emmanuel Mamma, Mida Habila Mayel, Ayobami Jahdahunsi Kukoyi and Ibrahim Adams Jeji, 2019. Inhibitory effect of aqueous and ethanolic extracts of neem parts on fungal rot disease of *Solanum tuberosum*. Pak. J. Biol. Sci., 22: 206-213.

Corresponding Author: Chinedu Imo, Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari, P.M.B. 1020, Wukari, Taraba State, Nigeria Tel: +2348037505543

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The third world's most pressing problem after poverty is food scarcity according to FOA. African farmers are faced with several constraints in the production of food and cash crops. Some of these constraints are poor soils, poor farm practices, land tenure and damages by diseases and pests[1]. In the quest for food and the struggle for human survival, Irish potato has historically played important role in food supply and thus instrumental in addressing problem of food insecurity, due to its yield per unit area (hectare) per unit time and its nutritional value[2].

Solanum tuberosum L. (Irish potato) is an annual plant that grows about 60 cm high, depending on variety[3]. Irish potato was introduced in Nigeria in the later part of 19th and early 20th century by Europeans, notably the tin miners in Jos Plateau. Most important potato producing areas in Nigeria are Jos Plateau, Kano, Zaria, Mambila, Biu and Obudu highlands. About 95% of the total production comes from Jos Plateau. The current Irish potato production in Nigeria is 800,000 t per annum[4]. Irish potato is ranked fourth in importance after rice, wheat and maize. Potatoes are eaten boiled, fried and with stews and also grown for livestock feed and industrial purposes[5].

The processing and preservation of Irish potato tubers like many other crops has been affected by various pathogens like fungi, bacteria and viruses, that results to various disease states. The greatest of these constraints is the post-harvest spoilage of farm produce, that affect several tons of harvested produce, allowing only a fraction to be utilized while most of the crops are lost to post harvest diseases, especially rot diseases[6]. Storage rot constitutes a major constraint to Irish potato production in Nigeria. The storage rot is caused by fungi, bacteria and viruses[7]. Several fungi have been isolated from rotten Irish potato tubers[8]. Research findings have reported that *Alternaria solani*, *Phytopthora infestans* and *Rhizopus stolonifer* caused post-harvest rot of irish potato tubers in Egypt[7,8]. Fungi such as *Rhizoctonia bataticola*, *Butry diplodia theobromae*, *Fusarium redolens*, *F. oxysporum*, *Penicillium* species and *Rhizopus oryzae* were associated with post-harvest rot of irish potato tubers in south-east and south-west, Nigeria[9]. These pathogens cause great losses and reductions in the economic and nutritional value of crops. However, a good system of control and management can play a key role in their eradication. The use of chemicals (fungicides) has helped in control of rot but identifiable problems associated with their use (e.g., chemical residues, bio-degradation, phytotoxicity, pollution, development of resistance in target organism, high cost, at times non-availability and hazard to man and his environment) has rendered them either slow to adopt by farmers or farmers have totally failed to adopt them, for one cultural reason or the other[10], hence the need for alternative control methods. There has been great research in developing plant products as alternatives to chemical fungicides that are more human and eco-friendlier and act directly on the pathogens by inhibiting their pathogenic effect or indirectly by inducing resistance in plant products.

Azadirachta indica, commonly known as neem, nimtree or Indian lilac is a tree in the mahogany family Meliaceae[11]. It is one of two species in the genus *Azadirachta* and is native to the Indian subcontinent. It is typically grown in tropical and semi-tropical regions. Neem trees also grow in islands located in the southern part of Iran and the northern part of Nigeria. Its fruits and seeds are the source of neem oil. The neem tree is noted for its drought resistance[12]. Neem can grow in different types of soil but it thrives best on well drained deep and sandy soils[12]. Plant extracts have exclusive antimicrobial properties, which act in holistic mode. *Azadirachta indica* (Neem) is one of the most promising medicinal plants, having a wide spectrum of biological activity and well known for its insecticidal properties. Every part of neem tree has been known to possess a wide range of pharmacological properties, especially as anti-bacterial, anti-fungal, anti-ulcer, anti-feedant, repellent, pesticidal, inhibitor and sterilant and is thus commercially exploitable and hence, traditionally used to treat large number of parasitic infections[13,14].

Neem leaves are dried in India and placed in cupboards to prevent insects from eating clothes and also in tins where rice is stored[15]. Neem oil is also used for healthy hair, to improve liver function, detoxify the blood and regulate blood sugar levels. Neem leaves have also been used to treat skin diseases like eczema, psoriasis. Neem is a key ingredient in non-pesticidal management (NPM), providing a natural alternative to synthetic pesticides. Neem seeds are ground into a powder that is soaked overnight in water and sprayed onto the crop. To be effective, it must be applied repeatedly, at least every 10 days. Neem does not directly kill insects on the crop. It acts as an anti-feedant, repellent and egg-laying deterrent, protecting the crop from damage. The insects starve and die within a few days. Neem also suppresses the hatching of pest insects from their eggs. Neem-based fertilizers have been effective against the pest southern army worm. Neem cake is often sold as a fertilizer[16]. Neem oil

has been shown to avert termite attack as an eco-friendly and economical agent.

This study was aimed at determining the inhibitory effect of aqueous and ethanolic extracts of neem leaves, stem bark and seeds on the fungal rot disease of Irish potato to serve as a potential alternative treatment for fungal storage disease on Irish potato tubers cultivated in Nigeria.

MATERIALS AND METHODS

Duration and year of study: This study was carried out from the month of March-September, 2017 at Biochemistry laboratory and Biology laboratory of Federal University Wukari, Nigeria.

Sample collection: Tubers of Irish potato with symptoms of rot were obtained from Mangu Market in Jos, Plateau state, Nigeria. The diseased tubers were packaged in polyethylene bags and taken to the laboratory at Federal University Wukari, Nigeria, where they were assessed for fungal presence for analysis. Neem seeds, leaves and stem bark were collected from the same growing tree plantation at Mission quarters, Wukari, Taraba state. The matured healthy seeds and green leaves were sorted out. The seeds, leaves and stem bark were washed under running tap water to eliminate dust and other foreign particles and air dried.

Sample extraction (Microwave assisted extraction): The air-dried neem leaves, stem bark and seeds were ground separately using pestle and mortar to the smallest possible size. Each sample (20 g) was measured and added into separate extraction containers for ethanolic and aqueous extraction. Ethanol (200 mL) and distilled water (200 mL) were then added to each container, tightly covered and placed in the microwave oven, set between 400-680 Watts and allowed to run for 3 min and stopped. The set up was carefully removed and allowed to cool before re-introducing it for another period of 3 min. This process was repeated for 45 min, allowed to cool and filtered. The filtrate was used for the experiment.

Preparation of culture medium and isolation of fungi: Throughout the study, the assayed culture medium employed was LAB M Potato Dextrose Agar (PDA). The Petri dishes that contained the medium were incubated for 24 h at room temperature (28°C) to check for sterility before use as described by Cheesbrough[17]. Isolation of fungi was done according to the method described by Oniyike and Maduewesi[18]. Fungi associated with the Irish potato rot affected tissue were observed and the frequency of isolation determined using method of Okigbo and Ikediugwu[19]. Sub-culturing was done to obtain pure cultures of the isolates.

Identification of fungal isolates: Sub-culturing of the isolates was carried out to obtain pure culture. The colonies growing on the plates were identified macroscopically and microscopically. Colony colour, type (compact, loose, aerial hyphae), texture (velvety, cottony, coarse) shape and growth pattern were observed. Direct observation of culture under the light microscope (low power) by careful preparation of slides, staining with cotton blue-in-lactophenol was done. Detailed drawings of the diagnostic features and identification manual and guides according to Rippon[20], Nelson *et al.*[21], Samson *et al.*[22] and Snowdon[23] were used.

Pathogenicity test and anti-fungal activity of the extracts: The pathogenicity test was carried out to establish which of the fungal isolates caused the rot and to determine whether they could induce similar symptoms on inoculation and be re-isolated, thus fulfilling Koch's postulates. The method of Okafor[24] and Okigbo and Ikediugwu[19] were adopted for the pathogenicity study. Effect of plant extract on mycelia growth of the test fungi was studied using the food poisoning techniques[25]. Each treatment consists of three replicates. The negative control set up contained a blank agar plate (no extract) inoculated with the test fungi. All the plates were incubated at 28°C for 5 days and examined daily for growth and presence of inhibition. Colony diameter was taken as the mean growth along two directions on two pre-drawn perpendicular lines on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition, which was calculated according to the method described by Whipps[26]:

$$\text{Percentage inhibition} = R1 - R2 \times \frac{100}{R1}$$

where, R1 is the farthest radial distance of pathogen in control plate while R2 is the farthest radial distance of pathogen in extract incorporated agar plates.

Qualitative phytochemical studies on *Solanum tuberosum* leaves, seeds and stem bark: The qualitative phytochemical studies were carried out according to the methods described by Horbone[27] and Tiwari *et al.*[28]. The tests were carried out to determine the presence of active chemical constituents such as alkaloids, glycosides, phenolic compounds, terpenoids and steroids, flavonoids, reducing sugar and tannin.

Quantitative determination of phytochemical constituents of *Solanum tuberosum* leaves, seeds and stem bark

Determination of total alkaloids: About 5 g each of seed, leaves and stem bark of the sample (Neem) was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed:

$$\text{Alkaloid (\%)} = \frac{W_2}{W_1} \times 100$$

Where:

W_2 = Weight of dried end product

W_1 = Weight of powdered sample taken for the analysis

Determination of total flavonoids: Five grams of plant sample was repeatedly extracted with 100 mL of 80% methanol at room temperature. The mixture was filtered through a Whatman No. 1 filter paper into a pre-weighed 250 mL beaker. The filtrate was transferred into a water-bath and allowed to evaporate to dryness and weighed according to Krishnaiah *et al.*[29]:

$$\text{Flavonoid (\%)} = \frac{W_2}{W_1} \times 100$$

Where:

W_2 = Weight of dried end product

W_1 = Weight of powdered sample taken for the analysis

Determination of saponin: Estimation of saponin was done according to slightly modified standard method. Ten grams of ground sample was dispensed into 250 mL conical flask and 100 mL of 20% ethanol was added to it. The mixture was heated in a hot water bath of 55°C for 5 h with continuous stirring. The mixture was filtered through Whatman No. 1 filter paper. The residue was mixed with 20% ethanol and heated in a similar way for about 5 h. The solution was filtered and mixed with previously filtered solution. The combined filtered solution was placed on a hot water bath at 90°C and heated until the volume was reduced to 20% of its initial volume. The concentrated sample was transferred into a 250 mL separating funnel and 10 mL of diethyl ether was added to it and shaken vigorously. The aqueous layer was separated carefully after setting down the solution. The purification process was repeated again and the samples washed twice with 10 mL of 5% NaCl solution. The remaining solution was heated in a water bath at 50°C until the solvent evaporated and the solution turned into semi-dried form. The sample was then dried in an oven. This saponin content was calculated using the following equation:

$$\text{Saponin (\%)} = \frac{W_2}{W_1} \times 100$$

Where:

W_2 = Weight of oven dried end product

W_1 = Weight of powdered sample taken for the analysis

Statistical analysis: Statistical analysis was carried out on the selected results using One-Way Analysis of Variance (ANOVA) and further with Duncan multiple comparisons with the use of Statistical Package for Social Sciences (SPSS), version 21. The result means were compared for significance at $p \leq 0.05$ and the group results were then presented as mean ± standard deviation.

RESULTS

Aqueous extract of neem leaf was found to have maximum number of phytochemicals like glycoside, flavonoids and carbohydrates, reducing sugar, steroids, tannins, saponins and alkaloids but proteins was absent. Ethanolic extract of neem leaf also recorded the presence of glycoside, flavonoids and carbohydrates, reducing sugars, steroids, tannins and saponins. However, proteins and steroids were absent.

Aqueous extract of neem seed recorded the presence of alkaloid, flavonoid, saponin, carbohydrate, reducing sugar and steroid. Glycosides, tannins and protein were not present. Similarly, ethanolic extract of neem seed showed the presence of alkaloids, flavonoids, saponin, carbohydrate, reducing sugar and steroids. As with the aqueous extract, tannin, glycoside and protein were not recorded.

Ethanolic and aqueous extract of stem bark of neem showed the presence of alkaloids, flavonoids, tannins, saponins, carbohydrate and reducing sugars. However, glycosides and proteins were not recorded.

Table1: Qualitative phytochemical analysis of *Solanum tuberosum* leaves

		Results	
Constituents	Test	ALE	ELE
Alkaloids	Mayer's test	-	+
	Wagner's test	+	+
	Hager's test	+	+
Flavonoids	Alkaline test	+	+
	Lead Acetate test	+	+
Tannins	Ferric Chloride test	+	+
	Lead Acetate test	+	+
	Iodine solution test	+	+
Saponins	Froth test	+	+
Carbohydrates	Molisch's test	+	+
	Barfoed's test	+	+
Reducing sugars	Fehling's test	+	+
	Benedict's test	+	+
Glycosides	Borntrager's test	+	-
	Killer-Killiani test	+	+
Protein	Biuret test	-	-
	Ninhydrin test	-	-
Steroids	Salkowski test	+	-

ALE: Aqueous leaves extract, ELE: Ethanolic leaves extract, +: Present, -: Absent

Table 2: Qualitative phytochemical analysis of *Solanum tuberosum* seed

		Results	
Constituents	Test	ASE	ESE
Alkaloids	Mayer's test	+	+
	Wagner's test	+	+
	Hager's test	+	+
Flavonoids	Alkaline test	+	+
	Lead acetate test	+	+
Tannins	Ferric chloride test	-	-
	Lead acetate test	-	-
	Iodine solution test	-	-
Saponins	Froth test	+	+
Carbohydrates	Molisch's test	+	+
	Barfoed's test	+	+
Reducing sugars	Fehling's test	+	+
	Benedict's test	+	+
Glycosides	Borntrager's test	-	-
	Killer-Killiani test	-	-
Protein	Biuret test	-	-
	Ninhydrin test	-	-
Steroids	Salkowski test	+	+

ASE: Aqueous seed extract, ESE: Ethanolic seed extract, +: Present, -: Absent

DISCUSSION

This study showed the presence of some phytochemicals in the neem parts. The presence of saponins, tannins, glycosides, alkaloids, terpenes and flavonoids in aqueous leaf extracts of neem in this study (Table 1) is in line with the reports of Biu *et al.*[30]. Qualitative phytochemical analysis of *Solanum tuberosum* seed and stem bark are shown in Table 2 and 3, respectively. Grayer and Harborne[31] reported that glycosides and saponins have anti-fungal activity, while Osbourn[32] also reported that many saponins exhibit potent anti-fungal activity and are often present in high levels in healthy plants and as a result have been implicated as determinants of a plants resistance to fungal attack. These reports are in agreement with the result of this study.

The quantitative test revealed that the quantity of the selected phytochemicals in the neem parts ranged from 10.17-23.88% (Table 4). The highest quantitative yield of flavonoid was obtained in neem seed (19.72%) followed by the stem bark (11.94%), with the least in leaves (10.17%). Stem bark of neem recorded the highest quantitative yield of alkaloid (23.88%) followed by neem seed (22.42%) while the least was leaves of neem (16.60%). More Saponin was detected in stem bark (23.84%) preceded by seed extract

Table 3: Qualitative phytochemical analysis of *Solanum tuberosum* stem bark

		Results	
Constituents	Test	ABE	EBE
Alkaloids	Mayer's test	+	+
	Wagner's test	+	+
	Hager's test	+	+
Flavonoids	Alkaline test	+	+
	Lead acetate test	+	+
Tannins	Ferric chloride test	+	+
	Lead acetate test	+	+
	Iodine solution test	+	+
Saponins	Froth test	+	+
Carbohydrates	Molisch's test	+	+
	Barfoed's test	+	+
Reducing sugars	Fehling's test	+	+
	Benedict's test	+	+
Glycosides	Borntrager's test	-	-
	Killer-Killiani test	-	-
Protein	Biuret test	-	-
	Ninhydrin test	-	-
Steroids	Salkowski test	+	+

ASE: Aqueous stem bark extract, ESE: Ethanolic stem bark extract, +: Present, -: Absent

Table 4: Selected quantitative phytochemical composition of *Solanum tuberosum* leaves, seeds and stem bark (%)

Constituents	Seed	Stem bark	Leaves
Flavonoid	19.72	11.94	10.17
Alkaloid	22.42	23.88	16.60
Saponin	20.67	16.09	23.84

Flavonoid was highest in the seed, followed by the stem bark. Alkaloid was highest in stem bark, followed by the seed and lowest in the leaves. Saponin was highest in leaves, followed by the seed and lowest in the stem bark

Table 5: Macro and micro-features of fungi isolates

Isolates	Colony characteristics	Microscopy
Pythium spp.	Colonies appear compact which is grey-black and surrounded by a clear white zone	Mycelium consists of slender, non-septate hyphae. The sporangia are globose and are terminal on somatic hyphae. It has a large regularly shaped, branched sporangia
Fusarium oxyporium	Growth on PDA is rapid. White aerial mycelium tinged with pink purple colour	Micro and macro conidia are present. Macro conidia slightly sickle celled with apical cell and foot shaped basal cell, chlamydospores are present, single and some in pairs
Aspergillus niger	Growth on PDA is rapid and fast with black powder almost covering the plates after 72 h	Non-septate conidiophores a rising from thick-walled foot cells. Each conidiophore ends in a terminal enlarged spherical swellings Conidia borne by phialides arising from a terminal swelling on the conidiophores

(20.67%), the least in Saponin being neem leaves (16.09%). These results are higher than those reported by Anukworji *et al*.[33]. They recorded 3.840% flavonoid, 6.480% alkaloid and 2.280% saponin in neem leave. This difference may be attributed to difference in soil type or other environmental factors.

Macro and micro-features of the fungi isolates are recorded (Table 5). Pathogenicity tests revealed that *Aspergilllus niger*, *Pythium* spp. and *Fusarium solani* caused rot. The nature of rot varies between the inoculated cocoyam tubers with various selected fungi. *Aspergillus niger* shows dry and soft rotting, *Pythium* spp. caused dry rot. Among the fungi isolated, *Aspergillus niger* and *Fusarium oxyporium* were the most pathogenic, while *Pythium* spp. is the least pathogenic as evident from the weight loss (Table 6).

Effect of the extracts on the test organisms was significant ($p<0.05$). Ethanolic extracts of the seed (ESE) gave the highest inhibitory effect on *Aspergillus niger* by 88.37% followed by aqueous extract of stem bark (ABE) (87.21%), while aqueous seed extract (ASE), aqueous leaf extract (ALE), ethanolic leaf extract (ELE) and ethanolic stem bark extract (EBE) showed inhibition of 81.78, 72.87, 77.52 and 39.53%, respectively (Table 7). Shrivastava and Swarnkar[34] also reported that methanol and ethanol extracts of *Azadirachta indica* showed growth inhibition against *Aspergillus flavus*, *Alternaria solani* and *Clados porium.* The inhibitory effect of EBE was

Table 6: Pathogenicity test result

Isolates	Symptoms of infection after 14 days	Pathogenicity
Aspergillus niger	Dry rot	+++
Pythium spp.	Dry rot	++
Fusarium oxyporium	Dry rot	+++

+++: Highly pathogenic, ++: Moderately pathogenic

Table 7: Mean zone of inhibition (%) of *Solanum tuberosum* leaves, seeds and stem bark extracts on fungi isolates (%)

Parameters	*Aspergillus niger*	*Pythium* spp.	*Fusarium oxyporium*
Ethanolic seed extract	88.37±1.17^{a}	100.00±0.00^{a}	100.00±0.00^{a}
Aqueous seed extract	81.78±2.92^{ab}	93.41±2.42^{b}	88.76±1.78^{b}
Ethanolic leaves extract	77.52±1.35^{b}	100.00±0.00^{a}	100.00±0.00^{a}
Aqueous leaves extract	72.87±3.55^{b}	94.96±1.78^{b}	87.60±4.40^{b}
Ethanolic stem bark extract	39.53±8.39^{c}	94.18±1.16^{b}	95.34±2.33^{a}
Aqueous stem bark extract	87.21±1.16^{ab}	94.18±1.16^{b}	94.96±1.38^{a}
Ketoconazole	89.92±1.35^{a}	100.00±0.00^{a}	100.00± 0.00^{a}

Values are Means±Standard deviations of triplicate determinations. Means in the same column with the same letter of the alphabet as superscript are not significantly different ($p<0.05$)

significantly ($p<0.05$) lower than that of other extracts, while there was no significant difference in the inhibitory effect of ESE, ASE, ABE and ketoconazole on *Aspergillus niger*. The ALE and ELE did not show any significant inhibitory difference on *Aspergillus niger*. Meanwhile, ketoconazole which was used as the positive control showed the highest inhibition on the growth of the fungus, apart from ELE and ESE which had 100% inhibitory effect on *Phythium* spp. and *Fusarium oxysporium* (Table 7). There was no significant difference ($p>0.05$) in the inhibitory effects of the ethanolic seed extract, aqueous seed extract and aqueous stem bark extract on the *Aspergillus niger*.

The anti-fungal activities of the seed, leaf and stem bark extracts correlates with the phytochemical results that indicated the high concentrations of glycosides and saponins which exhibit potent anti-fungal activity. The report of Nwachukwu and Umechuruba[35] that the extract of neem was observed to be promising for protecting Irish potato and beans against major seed borne fungi agrees with this present investigation. Amadioha and Obi[9] and Natarajan *et al*.[36] also reported that aqueous extracts of various parts of neem such as neem oil and its chief principles have antifungal activities. However, this study has also demonstrated that neem extracts may be selective in their anti-fungal activity. In the future, it is possible that neem parts will be a good alternative to certain antifungal agents used in the storage of some tubers.

CONCLUSION

This study has demonstrated that ethanolic and aqueous extracts from different parts of neem plant can be promising for protecting Irish potato and other related tubers against major seed borne fungi. The plant extracts in this study were found to be very effective in inhibiting fungal mycelia growth and hence can be potentially effective for preservation during storage of Irish potatoes to minimize post-harvest lost.

SIGNIFICANCE STATEMENT

This study discovered the inhibitory effect of aqueous and ethanolic extracts of neem leaves, stem bark and seeds on the fungal rot disease of Irish potato that can be beneficial for the effective storage and preservation of Irish potatoes. This study will help researchers to uncover alternative agents to the commonly used anti-fungal substances in the storage and preservation of Irish potatoes.

REFERENCES

1. FAO., 1997. Nigeria food balance sheet 1997. Basic Data Branch, Statistic Division FAO., Violedelle, Terme delle,Terme di Cairo Calla 00100, Rome, Italy.
2. Tewe, O.O., F.E. Ojeniyi and O.A. Abu, 2003. Sweet potato production utilization and marketing in Nigeria. Social Science Department, International Sweet Potato Centre (CIP), Lima, Peru.
3. Kudi, T.M., J.G. Akpoko and P. Yada, 2008. Resource productivity profitability analysis of irish potato production in Plataeu state, Nigeria. Savanna J. Agric., 35: 47-52.
4. Mirocha, C.I., S. Wxie and E.R. Filha, 2003. Chemistry and Detection of *Fusarium myscotoxin*. In: *Fusarium* Head Blight of Wheat and Barley, Leonard, J. and Buch (Eds.)., Vol. 6, The American Phytopathology Society, USA., pp: 144.
5. Odebunmi, E.O., O.O. Oluwaniyi, A.M. Sanda and B.O. Kolade, 2007. Nutritional compositions of selected tubers and root crops used in Nigerian food preparations. Int. J. Chem., 17: 37-43.

6. Alexandratos, N., 1995. World Agricultural: Towards 2010. John Wiley, Chichester, UK.
7. Abiodun, O.S. and O.P. Olumide, 2007. Thermal control of some post-harvest rot pathogens of Irish potato (*Solanum tuberosum* L.). J. Agric. Sci., 52: 17-31.
8. Kabeil, S.S., S.M. Lashin, M.H. El-Masry, M.A. El-Saadani, M.M. Abd-Elgawad and A.M. Aboul-Einean, 2008. Potato brown rot disease in Egypt: Current status and prospects. Am. Eurasian J. Agric. Environ. Sci., 4: 44-54.
9. Amadioha, A.C. and V.I. Obi, 1998. Fungitoxic activity of extracts from *Azadirachta indica* and *Xylopia aethiopica*on *Colletotrichum lindemuthianum* in Cowpea. J. Herbs Spices Med. Plants, 6: 33-40.
10. Okigbo, R.N. and A.N. Ajalie, 2005. Inhibition of some human pathogens with tropical plants extracts *Chromolaena odorata*, *Citrus aurantifolia* and some antibiotics. Int. J. Mol. Med. Adv. Sci., 1: 34-40.
11. Sidhu, O.P., V. Kumar and H.M. Behl, 2003. Variability in neem (*Azadirachta indica*) with respect to azadirachtin content. J. Agric. Food Chem., 51: 910-915.
12. Prakash, G., S.S. Bhojwani and A.K. Srivastava, 2002. Production of azadirachtin from plant tissue culture: State of the art and future prospects. Biotechnol. Bioprocess Eng., 7: 185-193.
13. Girish, K. and S.S. Bhat, 2008. Neem-A green treasure. J. Biol., 4: 102-111.
14. Kumari, A., R. Kumar, S. Maurya, J.S. Choudhary and S. Kumar, 2013. Antifungal efficacy of aqueous extracts of neem cake, karanj cake and vermicompost against some phytopathogenic fungi. Bioscan, 8: 671-674.
15. Srivastava, S. and A.K. Srivastava, 2013. Production of the biopesticide azadirachtin by hairy root cultivation of *Azadirachta indica* in liquid-phase bioreactors. Applied Biochem. Biotechnol., 171: 1351-1361.
16. Ghorbanian, M., M. Razzaghi-Abyaneh, A. Abdolamir, S.G. Masoomeh and M. Qorbani, 2007. Study on the effect of neem (*Azadirachta indica* A. Juss) leaf extract on the growth of *Aspergillus parasiticus* and production of aflatoxin by it at different incubation times. Mycoses., 51: 35-39.
17. Cheesbrough, M., 2004. Medical Laboratory Manual for Tropical Countries Microbiology. Oxford University, Linacre house Jordan Hill, Oxford, UK., Pages: 260.
18. Oniyike, R.C.I. and J.N.C. Maduewesi, 1985. Variability in pathogenicity of isolates and sensitivity of dithane M-45 and PCNB on cocoyam rot Fungi. Niger. J. Plant Prot., 9: 74-81.
19. Okigbo, R.N. and F.E.O. Ikediugwu, 2000. Studies on biological control of post harvest rot of yam (*Dioscorea* spp.) with *Trichoderma viride*. J. Phytopathol., 148: 351-355.
20. Rippon, J.W., 1988. The Pathogenic Fungi and Pathogenic Actinomycetes. In: Medical Mycology: The Pathogenic Fungi and the Pathogenic, Rippon, J.W. (Ed.)., 3rd Edn., Sanders Co., Philadelphia, pp: 163.
21. Nelson, P.E., T.A. Toussoun and W.F. Marasas, 1983. *Fusarium* sp.: An Illustrated Manual for Identification. University Park Press, Pennsylvania, pp: 193.
22. Samson, R.A., E.S. Hockstra and A.N. Van-Orschot, 1984. Introductory to Food Borne Fungi. The Netherlands Academy of Arts and Science, The Netherlands, pp: 1-25.
23. Snowdon, A.L., 1991. A Color Atlas of Post-Harvest Diseases and Disorders of Fruits and Vegetables. Vol. 2, CRC Press, Boca Raton, FL., Pages: 416.
24. Okafor, N., 1966. Microbial rotting of stored yams (*Dioscorea* spp.) in Nigeria. Exp. Agric., 2: 179-182.
25. Sangoyomi, T.E., E.J.A. Ekpo and R. Asiedu, 2009. Fungitoxic effects and attributes of *Allium sativum* and *Occimum gratissimumex* tracts on *Rhizoctonia solani*, the causal organism of yam (*Dioscorea rotundata* (Poir) rot disease. Niger. J. Mycol., 2: 166-167.
26. Whipps, J.M., 1987. Effect of media on growth and interactions between a range of soil borne glasshouse pathogens and antagonistic fungi. New Phytol., 107: 127-142.
27. Horbone, J.B., 1998. Phytochemical Methods. 1st Edn., Chapman and Hall, London, pp: 60-66.
28. Tiwari, P., B. Kumar, M. Kaur, G. Kaur and H. Kaur, 2011. Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sciencia, 1: 98-106.
29. Krishnaiah, D., R. Sarbatly and A. Bono, 2007. Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnol. Mol. Biol. Rev., 1: 97-104.
30. Biu, A.A., S.D. Yusufu and J.S. Rabo, 2010. Acute toxicity study on neem (*Azadirachta indica*, Juss) leaf aqueous extract in chicken (*Gallus gallus domesticus*). Afr. Scient., 11: 241-244.
31. Grayer, R.J. and J.B. Harborne, 1994. A survey of antifungal compounds from higher plants, 1982-1993. J. Phytochem., 37: 19-42.
32. Osbourn, A., 1996. Saponins and plant defence-a soap story. Trends Plant Sci., 1: 4-9.
33. Anukworji, C.A., R.R. Putheti and R.N. Okigbo, 2012. Isolation of fungi causing rot of cocoyam (*Colocasia esculenta* (L.) Schott) and control with plant extracts: (*Allium sativum* L., *Garcinia kola*, Heckel, *Azadirachta indica* L. and *Carica papaya* L.). Global Adv. Res. J. Agric. Sci., 1: 33-47.
34. Shrivastava, D.K. and K. Swarnkar, 2014. Antifungal activity of leaf extract of neem (*Azadirachta indica* Linn). Int. J. Curr. Microbiol. Applied Sci., 3: 305-308.
35. Nwachukwu, E.O. and C.I. Umechuruba, 2001. Antifungal activities of some leaf extracts on seed-borne fungi of African yam bean seeds, seed germination and seedling emergence. J. Appl. Sci. Environ. Management, 5: 29-32.
36. Natarajan, V., P.V. Venugopal and T. Menon, 2003. Effect of *Azadirachta indica* (neem) on the growth pattern of dermatophytes. Indian J. Med. Microbiol., 21: 98-101.