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PHYTOCHEMICAL REMEDIAL REACTION OF BLACK PEPPER (*Piper nigrum*) EXTRACT AGAINST FUNGAL TUBER ROT PATHOGEN OF SWEET POTATO (*Ipomoea batata*)

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ABSTRACT

Tubers of sweet potato serve as a staple food in Nigeria and also used for industrial production of starch, sugar and alcohol. However, production of this valuable tuber crop suffers from several constraints especially poor tropical storage conditions and postharvest microbial deterioration. An experiment was carried out at the research Laboratory of Plant Pathology of Crop Science and Horticulture, Nnamdi Azikiwe University, Awka to investigate the phytochemical effect of black pepper (*Piper nigrum)* extract on fungal tuber rot pathogen of sweet potato (*Ipomoea batata*). The experiment was a 2 x 4 factorial laid out in a Completely Randomized Design (CRD) and replicated three times. The factors were 2 extracting solvents: ethanol and N-hexane, while the other factors were four concentration levels of the two plant extracts (0%, 50%,75% and 100%). A poison method was applied where a 5cm discs of mycelia of the test fungus collected from the interface of the point of active growth of the fungus of a 7-day pure culture and placed in the center of petri dishes containing 5mls of the *Piper nigurum* extracts of various concentrations fortified with few drops of lactic acid. Plates were incubated at room temperature (28ºC ± 2) on laboratory benches and observed for eight days during which radial inhibition was measured and recorded. Results showed that fungal organism such as *Fusarium solani*, *Fusarium oxysporum* and *Mucor circinelloides* were implicated as the causal agents of sweet potato tuber rot. The phytochemical used was found to be very effective in controlling the test organism, where N-hexane at 100% concentration produced 93.67% inhibition of *F. oxysporum* in culture, followed by 83.33% inhibition value obtained in ethanol at 100% concentration levels. It could be concluded that fungi were among the organisms responsible for tuber rot of sweet potato. Therefore, it could be recommended that phytochemicals from indigenous plants like *piper nigrum* as used in this experiment be adopted as replacement to synthetic chemicals as a remedy for postharvest disease.

Keywords: Reaction, Phytochemical, Concentration, Black Pepper, Organisms

INTRODUCTION

Sweet potato (*Ipomoea batatas*) is a dicotyledon and belongs to the Convolvulaceae family, ranked as the seventh most important food crop and the fifth essential crop in the world (Neela and Fanta, 2019). There are more than 115 nations that cultivate sweet potatoes, and the annual production of sweet potatoes is over 120 million tons, with China, Nigeria and Tanzania being the global production leaders in production (FAOSTAT, 2020). In tropical and subtropical regions, sweet potato is the primary crop as it requires low agriculture input and labor intensity, a short maturation period, and can be harvested several times each calendar year (Laurie *et al*., 2018; Neela and Fanta, 2019). The variety of cultivars is mainly distinguished by the color of their flesh (yellow, white, red, orange, purple), physical properties, and chemical composition. For example, orange-fleshed sweet potatoes are high in carotenoids, and purple-fleshed sweet potato contains high levels of anthocyanins (Neela and Fanta, 2019; Obomeghei *et al*., 2020). The color and sweet taste also contribute to the high sensory acceptability of sweet potato, commonly cooked and prepared by boiling, frying, steaming, and baking in Western countries (Hadero and Nigusse, 2018). In addition, some novel food products have been developed using sweet potatoes as a composite with wheat and soybean, such as noodles and cookies (Hadero and Nigusse, 2018; Kolawole *et al*., 2020). The tuber is also used for the industrial production of starch, sugar and alcohol (Belay, 2018). Sweet potato plays critical roles in rural diets in certain areas during shortage of grain crops like maize, when drought occurs. Most African household plant sweet potatoes as a food security or famine prevention crop. Sweet potatoes are viewed in this instance as a form of insurance in the event of drought, political turmoil, or other food supply-threatening events (Belay, 2018).

However, production of this valuable tuber crop suffers from several constraints especially poor tropical storage conditions and hygiene, and postharvest microbial deteriorations (Nwanja *et al*., 2017). For these reasons fresh sweet potato tubers have been reported to store for about three weeks only after harvest if left untreated (Maranzu, 2019). Loses due to postharvest fungal attacks on stored tubers ranging 20- 100 % have been reported because of inadequate farm and village-level storage (Nwanja *et al*., 2017). These fungal pathogens cause spoilage and postharvest spoilage of sweet potato by producing various types of mycotoxins. Several fungi have been implicated in the spoilage of sweet potatoes. Nwaneri *et al,* (2023). reported *Aspergilus niger, Aspergilus flavus, Penicilum expansum, Rhalstonia solanacearum, Rhizopus stolonifer, Fusarium oxysporium* and *Fusarium solani* responsible for the postharvest decay of sweet potato.

Chemicals have proved helpful in the control of sweet potato tuber rot diseases but one of the major problems is that frequent use of chemicals predisposes target organisms to resistance and also chemical control leaves behind residual effects which are not eco-friendly. These toxic chemical residues have been reported to trail treatment of stored agro-products with synthetic pesticides. In most instances consumption of such toxic chemical residues-contaminated products leads to harmful effects such as teratogenicity, allergies and even death on mammals (Enyiukwu *et al*., 2014). Many plants are known to produce phytochemicals that exhibit antimicrobial, antifungal, and antioxidant properties,

which can be exploited for natural disease management. One such plant is black pepper (*Piper nigrum*), widely used in traditional medicine and known for its bioactive compounds. Black pepper [*Piper nigrum* (L.), family: Piperaceae is used traditionally for the treatment of various diseases including; cough, cold, dyspnea throat diseases, intermittent fever, dysentery, stomachache, worms and piles. The pharmacological potential of black pepper is due to the presence of metabolites like phenolic compounds, alkaloids, flavonoids, carotenoids, terpenoids, etc. Phytochemical analyses have described the main chemical constituents of black pepper, including carbohydrates, proteins, calcium, magnesium, potassium, iron, vitamin C, tannins, flavonoids and carotenoids (Ashokkumar *et al.,* 2021). The volatile oil content ranges from 0.4 to 7 % in dried berries. The major constituents of black pepper essential oil are sabinene, 3-carene, D-limonene, α-pinene, caryophyllene, β-phellandrene, α-phellandrene, α-thujene, and β-bisabolene. Additionally, piperine is the naturally occurring and principal bioactive alkaloid constituent of black pepper owing to its potential therapeutic properties, including cerebral brain functioning and increased nutrient absorption. The black pepper essential oil has several biological roles, including antioxidant, anti-inflammatory, anticancer, anti-obesity, antidepressant, antidiabetic, antimicrobial, gastroprotective, and insecticidal activities (Ashokkumar *et al.,* 2021). Akthar *et al.* (2014) investigated the antimicrobial activity of leaf extract of *P. nigrum* against the foodborne pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli, Salmonella typhimurium*, and *Pseudomonas aeruginosa* and fungi (*Aspergillus* spp. and *Candida albicans*). Another study by Basak and Guha (2017) described the effect of essential oil of *Piper* spp. on spore inactivation and the cell viability of *Aspergillus flavus* and *Penicillium expansum*. These authors reported that the cells of *A. flavus* and *P. expansum* lose viability when treated with the essential oil. Since spore inactivation or inhibition of spore germination is necessary in order to restrict fungal infection and mycotoxin production in food. The use of *P. nigrum* extract as a natural antifungal agent in tuber rot disease of sweet potato is very promising. The objective of this research is to evaluate the phytochemical effect of black pepper (*Piper nigrum)* extract on fungal tuber rot pathogen of sweet potato (*Ipomoea batata*).

MATERIALS AND METHODS

Experimental site

The experiment was carried out in the Plant Pathology Laboratory of the Department of Crop Science and Horticulture of the Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Anambra State. The area is located between latitude 06º15N and longitude 07º08E. It has a minimum and maximum average temperature of the planting period 25.3ºC and with average annual rainfall of 1828 cm.

Materials Used

Potato tubers showing signs of rot, black pepper seeds, ethanol, N-hexane, methylated spirit, Agar material, cotton wool, inoculating loop.

Equipment used

Soxhlet extractor, Compound microscope, Conical flasks, Beakers, Measuring Cylinders, Petri dishes, Scalpel, inoculating needle, Spirit Lamp,

Preparation of Sarbroud Dextrose Agar (SDA)

Twenty (20) grams of Sarbroud Dextrose Agar (SDA) was weighed with the electronic weighing balance and was mixed in 500 ml of distilled water in a conical flask. The mixture was stirred vigorously until it became homogenous. It was then corked, using cotton wool wrapped with foil before being placed into the autoclave. The conical flask containing SDA was placed into the autoclave and was properly sealed. The autoclave was boiled at a temperature of 120°C and pressure of 15 ± 1 Psi for 20 min and allowed to cool after which it was used.

Isolation of fungal pathogens from rot potato tubers using PDA method

The working bench was surface sterilized with methylated spirit and cotton wool so as to prevent contamination. Some selected tubers of potatoes showing signs of rot were surface sterilized by washing in 10% ethanol and rinsed two time in sterile distilled water and dried in between four layers of blotter paper. Some sections of the rot potato tubers of size 10mm were carefully cut from between healthy and spoilt portions of the tuber with flame sterilized scalpel. The cut sections were plated onto already prepared SDA and allowed to incubate for 5 days on the laboratory bench at a room temperature of 28 ± 2°C (Iwuagwu *et al* 2022). Daily observation was made for fungal growth. Fungal growths were later sub-cultured.

Subculturing of fungal isolates using SDA

The initial culture was sub-cultured three times to obtain a pure culture. A sterile inoculating loop was used to place the infection from the seeds into sterile Petri dishes containing 10 ml of SDA with two drops of lactic acid. The lactic acid was added to inhibit the growth of bacteria. The Petri dishes was properly sealed and labeled. The plates with 3 replicates were incubated at temperature 28 ± 2°C and left for seven days. The resulting pure cultures were used for subsequent identification and characterization of the fungi isolates with the aid of a compound microscope and identification guides (Iwuagwu *et al.,* 2019).

Identification of isolated fungal pathogen

A compound microscope with model (Olympus–XN 50) was used to view the organisms. A sterilized slide was used to place the organisms for viewing. A drop of distilled water was placed on the slide and a small portion of the culture from the seven-day culture was collected from the growth using a sterile needle, it was then covered with the slide cover and placed under the microscope for viewing

Soxhlet extraction method using methanol and ethanol

Using Soxhlet extraction method Azwanida (2015) the ground Black pepper was soaked in ethanol and N-hexane, in a white plastic container for with vigorous shaking at intervals during this period. The extracts were filtered with white cheese cloth first and then filtered again with the use of Buckner funnel. Then methanol was evaporated to dryness by placing it in water bath. The black pepper was weighed out and placed into the thimble. Paper thimble was used in the Soxhlet apparatus, using ethanol as the extracting solvent. Black pepper extract was mixed with distilled water into a measuring cylinder to produce percentage concentrations of the extracts. The Soxhlet extraction process heats the solvent (ethanol) to boiling temperature (>78°C). The evaporated ethanol is contained within the apparatus by the condenser unit; however, the apparatus should be placed under a fume hood in case of escape. The solvent (250ml of ethanol) was added to a round bottom flask, which is attached to a Soxhlet extractor and condenser on an isomantle. The crushed plant material is loaded into the thimble, which was placed inside

the Soxhlet extractor. The side arm was lagged with glass wool. The solvent is heated using the isomantle and will begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 16 hours.

Effect of plant extracts on fungal growth

Effect of plant extract on mycelia growth of the isolated fungi was studied using the poisoned food technique (Sangoyomi, 2004). Five milliliters of plant extract of various concentrations (0, 50, 75 and 100% v/v) was poured into different Petri dishes containing 10mls of molten SDA fortified with lactic acid and carefully spread evenly over the plate, this gave rise to SDA-extract mixture. The plate was gently rotated to ensure even dispersion of the extracts. The agar extract mixture was allowed to solidify and then inoculated at the center with a diameter disc obtained from the colony edge of a 7-day old pure culture of the test fungi. Each treatment consists of three replicates. The control consists of blank agar plate (no extract) inoculated with the test fungi as described above. Petri dishes dispensed with molten SDA at different concentrations, inoculated with the test fungus served as the commercial fungicide. All the plates were incubated and examined daily for growth inhibition. Colony diameter was taken as the mean growth along on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition, was calculated according to the method described by Whipps (1987).

$$\% \text{ radia inhibition} = \frac{Do-Dt}{Do} \times \frac{100}{1}$$

Where D_0 is the distance of radial spread in control plate while D_t is the distance of radial spread in extract incorporated agar plates.

Experimental design

The experimental design used was a 2x4 factorial laid out in a Complete Randomized Design (CRD). The factors include two extracting solvents with four concentration levels: 0, 50, 75, and 100%) which were replicated three times

Data Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using GenStat statistical package and means were separated using Least Significant Difference (LSD) at 5% probability level.

RESULTS AND DISCUSSION

Isolation and identification of fungal organisms from rot tubers of sweet potato.

The results of isolation of fungal organism associated with tuber rot of sweet potato showed that three fungi from two genera were identified: *Fusarium solani*, *Fusarium oxyspormn* and *Mucor circinelloides*

Main Phytochemical effect of Black pepper (*Piper nigurum)* extracts on radial inhibition of Fusarium sp isolated rot tubers of sweet potato (*Impomea batata*)

Table 1 shows that there was a significant difference in the effect of Black pepper extract extracted with Ethanol and N-hexane, where ethanol extract gave significantly higher radial inhibition of *Fusarium sp* in culture in days 3-4 except in day 7. The effect of the plant extract extracted by the two solvents had statistically similar effect on days 2,5,6 and 8. Table 1 also reveal that the highest (83.83%) was produced in both ethanol and N-hexane extracts in day 8 followed by 83.46% obtained in day 6 by ethanol extraction, while the least (17.08%) was produced by ethanol extraction on day 2. Moreso, the Table also shows that fungitoxic effect of the Black pepper extract increased with time in culture.

Table 1: Main Phytochemical effect of Black pepper (*Piper nigurum)* extracts on radial inhibition of Fusarium sp isolated rot tubers of sweet potato (*Impomea batata*)

Treatment	Radial inhibition of *Fusarium sp* in SDA culture (%)						
	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Ethanol	17.08	49.21	69.33	79.79	83.46	82.67	82.83
N-hexane	17.60	46.83	65.42	79.78	83.27	82.83	82.83
LSD $_{(0.05)}$	NS	0.82	0.35	NS	NS	0.16	NS

Main effect of concentration of Black (*Piper nigurum)* pepper extracts on radial inhibition of Fusarium sp in SDA culture (%)

Results in Table 2 shows that there was a significant difference in the main effect of carious concentration Black pepper extracts on radial inhibition of Fusarium spp in culture, where 100% concentration level consistently produced highest inhibition values in days 2 to 5 in both N-hexane and ethanol extracts. The highest inhibition percentage (83.93%) was observed on day 5 at 100% concentration level followed by 83.60% on the sixth day at 100% concentration level while the least (11.00%) occurred on second day at 50% concentration level. It was also observed that fungitoxic effect of the different concentration increase with time in culture. Generally, all the concentration levels performed better than control.

Table 2: Main effect of concentration of Black pepper (*Piper nigurum)* extracts on radial inhibition of Fusarium sp in SDA culture (%)

Cons. (%)	Radial inhibition of *Fusarium sp* in SDA culture (%)						
	Day 2	day 3	day 4	day 5	day 6	day 7	day 8
N-hexane							
100	19.67	53.67	71.67	81.83	83.60	83.00	82.67
75	14.67	37.33	61.67	81.67	83.33	82.83	83.00
50	11.33	36.83	61.00	72.33	83.50	81.83	82.33
Ethanol							
100	19.73	48.00	64.67	83.93	62.82	82.33	82.00
75	17.00	43.00	65.33	82.70	83.00	83.00	83.00
50	11.00	27.3	48.67	70.33	82.67	83.00	83.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD $_{(0.05)}$	1.24	1.57	1.57	1.57	1.57	1.57	1.57

Interactive effect of Black pepper (*Piper nigurum)* extracts and concentration on radial inhibition of *Fusarium sp* in culture (%)

Table 3 shows that there were significant interactive effect pf Black pepper extracts on radial inhibition of *Fusarium sp* in culture where N-hexane x 100% extract consistently produced highest values than other interactive levels. The highest inhibition percentage (93.67%) being significantly higher than others was obtained in N-hexane x 100% interaction, except in days 4,5 and 7 followed by 83.33% while the least (11.00%) observed on day 2 at N x 50 % interaction level. Toxicity of the plant extracts increased with time in culture. All the interactive level did better than control

Table 3: Interactive effect of plant extracts and concentration on radial inhibition of Fusarium sp in culture (%)

Treatments	Radial inhibition of *Fusarium sp* in SDA culture (%)						
	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Ethanol x 50	14.67	37.33	61.67	81.67	83.33	82.83	83.00
N-hexane x 50	11.00	27.30	48.67	70.33	82.67	81.83	83.00
Ethanol x 75	11.33	36.83	61.00	72.33	83.50	82.33	82.33
N-hexane x 75	17.00	43.00	65.33	81.83	83.00	83.00	83.00
Ethanol x 100	19.67	48.00	71.67	82.47	83.00	93.67	82.33
N-hexane x 100	19.73	53.67	64.67	83.00	83.43	83.00	82.00
Ethanol x 0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N-hexane x 0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$LSD_{(0.05)}$	1.409	1.629	1.505	1.423	1.246	0.692	0.847

Discussion

The results on isolation of fungus associated with tuber rot of sweet potato showed that fungal organisms such as *Fusarium solani, Fusarium oxysporum* and *Mucor circinelloides* were implicated. This finding is in agreement with the report of Iwuagwu *et al*. (2020), who reported that *Aspergillus sp* was responsible for yam tuber rot in Awka Anambra state which led to depreciation in nutritional content of the rot portions compared to intact and uninfected tubers. The result is also corroborating the finding of Nanaware *et al.* (2024), who reported that fungal organisms were responsible for the spoilage of potato tubers and deposition of mycotoxin. Agu *et al.* (2015) have also reported that sweet potato tubers are susceptible to soft rot disease caused mainly by fungi such as *Botryodiplodia theobromae, Cerato cystis, Rhizopus oryzae, Aspergillus flavus, Fusarium solani and Sclerotium rolfsii.* An important reason for high susceptibility of potato tubers rot could be as some enzymes contained in the tubers that are involved in breaking down starch into sugar during storage making storage of fresh tubers to be very difficult (Akpam *et al*., 2024)

Control of fungal organisms associated with spoilage of fresh potato tubers with *Piper nigurum* seed extract.

From the results it was discovered that the use of black pepper (*Piper nigurum*) seed extract was very effective in controlling the spoilage fungal organism (Fusarium oxysporum) isolated from rot sweet potato tubers in an in-vitro method. This is similar to works by Gwa and Nwankiti, (2017), Basak and Guha (2017), Iwuagwu *et al.* (2022) and Iwuagwu *et al*. (2023) who reported that plant extracts have been used to control post-harvest diseases of crops including yam, African Yam Bean and Cucumber. These authors reported that the cells of *A. flavus* lose viability when treated with the essential oil. Since spore inactivation or inhibition of spore germination is necessary in order to restrict fungal infection and mycotoxin production in food.

It was also discovered that the N-hexane extracting solvent performed better than extracts extracted with ethanol in inhibiting the growth of Fusarium oxysporum in culture. This is in line with the findings of Yeo *et al.* (2014), reported that Solvents with low to intermediate polarity used such as hexane, petroleum ether and ethyl acetate demonstrated better antimicrobial activity as compared to other solvents used. This is also similar to Iwuagwu *et al.* (2018), who revealed that Soxhlet extraction utilizing Pet ether as the extracting solvent had the maximum level of inhibition of cocoyam leaf necrotic fungi in Nigeria.

CONCLUSION

We have seen from this research that fungal organisms such as *Fusarium solani, Fusarium oxysporum* and *Mucor circinelloides* were responsible for postharvest tubers rot of sweet potato. Also, the use of plant extracts is very effective for the control of fungal organisms associated with tuber rot of sweet potato. Both ethanol and N-hexane did very well as extracting solvents for phytochemicals, which could serve as a replacing alternative to synthetic chemicals in the management of postharvest diseases. It could therefore be recommended that *Piper nigurum* extract should be adopted by farmers for the control of postharvest tuber rot of sweet potato instead of synthetic fungicides which are not readily available as well as not being friendly to both human, animals and the environment. Further researches should be carried out to understand how to standardize the bioactive compounds present in the indigenous plants so as to commercialize the use of them.

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