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Control of Green Rot Fungus of *Arachis hypogaea* L. in Orage Using Plant Extracts

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Abstract. The antifungal activity of the ethanolic and aqueous fruit extracts of *Zingiber officinale*, *Tetrapleura tetraptera*, *Garcinia kola* and *Cola nitida* on the green rot fungus (*Penicillium sp*.) isolated from infected groundnut (*Arachis hypogaea*) seeds in storage were investigated *in vitro*. Various concentrations of the aqueous and ethanolic fruit extract ranging from 5g/100ml, 10g/100ml, and 15g/100ml were separately added to Potato Dextrose Agar (PDA) media. The fungus was inoculated into the media and incubated for seven days. Results of the in vitro studies showed that the ethanolic extracts had a significant inhibitory effect ($p<0.05$) on the radial growth of *Penicillium sp*. at all levels of concentration tested. Complete inhibition of the fungus was at 15g/100ml of ethanolic extracts of *Garcinia kola* and *Tetrapleura tetraptera*. The aqueous extracts showed less inhibitory effect as compared to the ethanolic extracts. The effective inhibition of ethanolic extracts as compared to the aqueous extracts may be attributed to the efficiency of the extraction solvent on the phytochemical content of the plant extracts. The application of botanical extracts for disease management could be less expensive, easily available, non-polluting and eco-friendly. The ethanolic fruit extracts of *Garcinia kola* and *Tetrapleura tetraptera* at the higher concentrations can be produced in large quantities and used as spray in controlling the green rot fungus of *Arachis hypogaea* in storage.

Introduction

Groundnut *(Arachis hypogaea* L.) is a crop of global importance, belonging to the Fabaceae family, native to South America. It is cultivated extensively in the tropics and sub-tropics and requires an annual precipitation of about 100-120 cm, with the rainfall occurring in the growing season [1]. It thrives better in light, sandy loam soil that is slightly acidic with the pH range of 5.5-6.5 and high in organic matter contents. This light soil not only permits rapid root penetration and good water collation, but is also crucial for fruit development [2]. To develop well, groundnuts need warm weather throughout the growing season, as they do not tolerate frost. They can be grown with as little as 350 mm of water, but for best yields need at least 500 mm [3].

Groundnut is classified as both a grain legume and an oil crop (due to its high oil content). Like most legumes, they harbor symbiotic nitrogen-fixing bacteria in their root nodules making them valuable in crop rotation. When used in crop rotation, the yield of the groundnut crop itself is increased, through reduced diseases, pests and weeds [4]. The groundnut pods are developed underground thus earning its specific epithet '*hypogaea*'. It is possible that peanuts developed this growth habit as a method of protection from hot tropical sun, since they have thin pods [5].

Groundnut plants continue to produce flowers when pods are developed, therefore even when they are ready for harvest, some pods are immature. Groundnuts are harvested either traditionally or in mechanized systems but the timing of harvest is an important decision to maximize yield. If it is too early, too many pods will be unripe. If too late, the pods will snap off at the stalk, and will remain in the soil. Once the peanut pods are harvested, the plant is removed, and typically used for animal fodder. Groundnut are produced seasonally, their harvesting is done within a short period of 2-3 months, while their consumption is constant throughout the year, therefore making their storage necessary. Storage is important to ensure a uniform supply of food (for domestic uses or export) throughout the year, providing a reserve for contingencies such as: drought, flood and war [5].

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Groundnuts are usually dried properly and stored in dry conditions because if the groundnuts are too high in moisture, or if storage conditions are poor, they may become infected by the mould fungus *Aspergillus flavus*. The fungus releases a toxic and highly carcinogenic substance aflatoxin [6]. It is well known that peanuts can grow in different soil types, such as light sandy soil and heavier soils. Light sandy soil benefits for the rapid proliferation of *Aspergillus flavus*, particularly under dry conditions in the later growth period. On the contrary, heavier soil can reduce the level of aflatoxin contamination in peanut grown because of having a higher water-holding capacity [7, 8]. Cultivars of improved varieties increase the resistance of peanut to diseases, also significantly reducing the incidence of fungal contamination compared to the unimproved varieties [9].

According to [10] moisture content and several environmental factors such as temperature and relative humidity of the harvested groundnut prior to storage affects the occurrence of fungal mould. Therefore, groundnuts which are stored before sale or use should be kept dry and soil debris should be removed completely from the harvested groundnuts so as to avoid soil dwelling microbes such as *Fusarium sp.* from infecting the nuts and consequently causing spoilage in the warehouse. Hence, adequate drying of crops, prevention of moisture re-absorption and the general improvement of storage facilities at all levels is recommended as a safe guard against mould deterioration of groundnut.

Also, according to [11] fungal incidence might be due to increase in proliferation of microorganisms in the soil which synthesizes several enzyme proteins and sometimes causes rearrangement of nutritional constituents. This may be as a result of the presence of some fungi which release metabolites that inhibit the growth of other fungi which are capable of metabolizing carbohydrates. Also, the moisture content of freshly sundried groundnut but still containing 5.09±1.10g moisture could increase upon storage to 6.13±0.10g/100g. High moisture content helps in survival and growth of the moulds. Most of these fungi are able to grow at low water activities enabling them to initiate spoilage.

In the field, groundnut infections caused by fungi are mainly caused *by Rhizopus spp, Sclerotium rolfsii, Penicillium spp, Aspergillus niger, Aspergillus flavus, Puccina arachidia, Cercosporidium personatum, Pythium spp, Fusarium spp, Rhizoctonia solani, Cylindrocladium parasiticum, Diplodai spp,* etc causing diseases such as root rot, stem rot, wilts, blights, pod rot, rust, late leaf spot and early leaf spot. Plant diseases that occur in the field usually show up in stored products and subsequently reducing the monetary returns by increasing crop loss and reducing the desirable plant qualities produce [12].

These diseases are controlled mainly by application of pesticide, fungicide and other chemical control. However, the worldwide trend towards environmentally safe methods of plant disease control in sustainable agriculture calls for reducing the use of these synthetic chemicals. In an attempt to modify this condition, some alternative methods of the control have been adopted (biological control, improved cultivars etc). Recent efforts have focused on developing environmentally safe, long lasting and effective methods for the management of plant diseases [12].

Natural plant products are important sources of agrochemical for the control of fungal pathogens [13, 14]. Furthermore, botanical pesticides are biodegradable, non-phytotoxic and can contain multiple bioactive metabolites [15]-[17]. Plant products are now known to reduce population of fungal pathogens and control disease development [12].

Due to the menace caused by green rot fungus on groundnut in storage facilities of four major markets (Watt, Marian, Mbukpa and Akim) in Calabar Metropolis of Cross River State, Nigeria, it became necessary to isolate and identify the rot fungus as well evaluate the use of aqueous and ethanolic fruit extracts of *Cola nitida, Garcinia kola, Zingiber officinale and Tetrapleura tetraptera* in controlling the rot fungus *in vitro*.

Materials and Methods

Collection of plant materials

Dried fruits of *Cola nitida, Garcinia kola, Zingiber officinale* and *Tetrapleura tetraptera* were obtained and identified by a taxonomist in the Herbarium Unit of Department of Botany, University

of Calabar, Calabar, Cross River State, Nigeria. Infected groundnut seeds were collected from four major markets in Calabar Metropolis namely; Watt, Marian, Akim and Mbukpa. The seeds were packaged in sterile polythene bags and taken to the Mycology Laboratory of the Department of Botany, University of Calabar for further analysis. The experiment was carried out in the Laboratory of the Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria.

Isolation and Identification of green rot fungus

Infected groundnut seeds were surface sterilized with 70% sodium hypochlorite (bleach) solution for 1min and rinsed quickly in 3 changes of sterile distilled water, blotted dry on Whatman's No. 1filter paper and placed on Potato Dextrose Agar (PDA) in Petri dishes. Four (4) sections were inoculated per Petri dish. The plates were incubated at 28 ± 1°C until fungal growth was noticed. After 5 days, the isolate was sub-cultured on freshly prepared PDA to obtain their pure culture. Isolated fungus was microscopically (Olympus optical, Phillipines) identified as far as possible using the identification guides of the International Mycological Institute, Kew and of [18].

Pathogenicity test

Pathogenicity test was ascertained using Koch's postulates. To test pathogenicity of the isolated fungal pathogen, some pieces of spores were re-inoculated into healthy groundnut seeds. The healthy groundnut seeds were washed with distilled water and surface sterilized with 70% ethanol. For the spore inoculation, a spore suspension was produced using the method of [19]. With a sterilized hypodermic needle, approximately 2 x 10-5 spore was inoculated on the groundnut seeds by spraying to run-off level. The groundnut seeds was put into transparent polyethylene bags and allowed to stay for 24hours. Spores measurement was done with a haemocytometer. The control experiment was carried out with sterile distilled water without spores. All the experimental setup was observed for symptom development. The experiment was carried out in three replicates.

Preparation of plant materials

Dried fruits used in the study were separately washed thoroughly using distilled water and surface sterilized with 70% ethanol and sun-dried for 3 days. The dried plant fruits were blended separately using a sterile electric blender to obtain 200grams of fine powder of each fruit. Aqueous extracts of fruits were obtained by adding the dried powder (blended) of plant material to distilled water at room temperature 28±1˚C. Four levels of concentrations were obtained by dissolving 5g, 10g and 15g of each sample with 100ml of distilled water. This was vigorously stirred and allowed for 24 hours. The solution was then filtered through four-folds of sterile cheese cloth for all the plant materials. The filtrates obtained were used as aqueous extracts of the test plants and stored in reagent bottles for further use. Ethanolic extracts of plant materials were obtained by adding the powdered sample at different concentrations, 5g, 10g and 15g to 100mls of ethanol. This was stirred vigorously and allowed for 24 hours at room temperature 28±1˚C. The solution was then filtered through four-folds of sterile cheese cloth for all the plant materials. The filtrates obtained were used as ethanolic extracts of the test plants and stored in reagent bottles for further use.

Susceptibility test

The extracts percentage concentrations were prepared at 5g/100ml, 10g/100ml and 15g/100ml with ethanol and water as solvent.

In vitro antifungal assay

1ml of each concentration of both the aqueous and ethanol extracts was first poured into different Petri-dishes using sterile syringe. The Sterile Potato Dextrose Agar (PDA) was also poured into the plates containing the solvent extracts after which the plates containing the solvent extracts were gently swirled to ensure mixing. The media was allowed to solidify and with a sterilized cork borer (5mm in diameter), a disc of the matured culture was punched out from advancing margin of a four- day old pure culture and inoculated at the center of plates and incubated at room temperature (28±10c) for 7 days. The experiment was replicated thrice. Area of inhibition was measured daily for 7days using a meter rule and recorded.

Results

Isolated green rot fungus and morphological description of the isolate

The green rot fungus isolated and identified as the causative agent of storage rot of groundnut in this study was *Penicillium sp* (Plate 1).

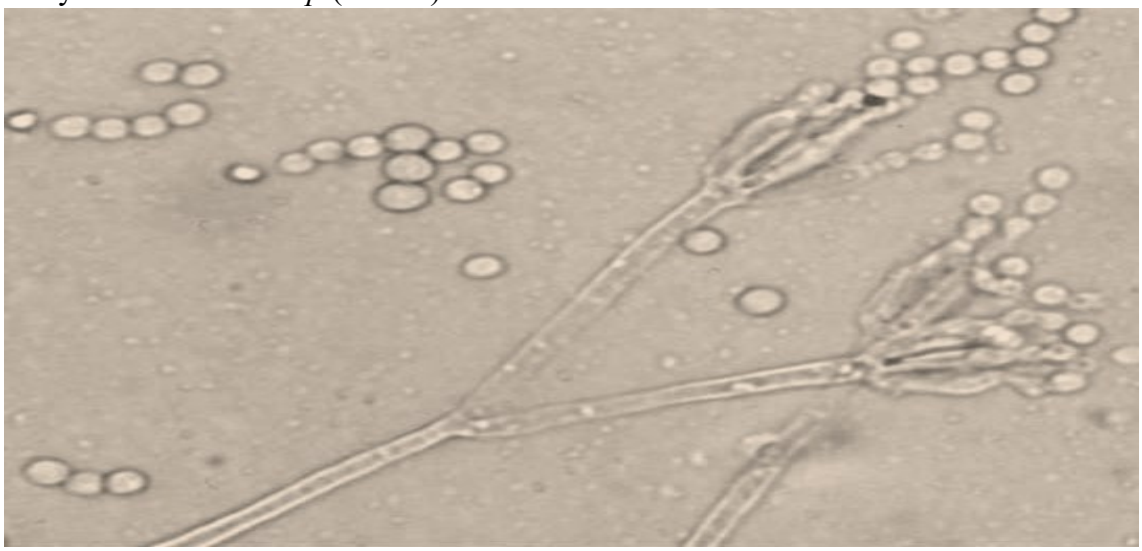


Plate 1: Photomicrograph of *Penicillium sp.* × 40 showing conidiophores and spores.

Identification of the isolate was based on macroscopic and microscopic observation. The macroscopic observation showed greenish black colored colonies with white border having concentric ring. The microscopic view showed single celled spores in chains, sterigma rising from the metula of the conidiophore, branching conidiophores arise from a septate mycelium.

In vitro effect of ethanolic extracts of *Zingiber officinale*, *Tetrapleura tetraptera*, *Garcinia kola* and *Cola nitida* on *Penicillium sp*. at the different concentrations.

The *in vitro* effect of the ethanolic plant extracts at different concentrations on the radial growth of the fungal isolate is presented in (Tables 1-4). Results from the study showed that extracts of *Zingiber officinale, Tetrapleura tetraptera, Garcinia kola* and *Cola nitida* had significant effect on the isolated fungal pathogen at all levels of concentration (5g/100ml, 10g/100ml and 15g/100ml) tested as compared with the aqueous extracts. Results (Table 1) showed that at 5g/100ml and 15g/100ml concentrations, *Zingiber officinale* completely inhibited the radial growth of *P*enicillium *sp*. in the first to third day of incubation while at 10g/100ml the growth was inhibited in the first to fourth day of incubation when compared with the aqueous extracts. Results (Table 2) showed that *Tetrapleura tetraptera* extracts at 5g/100ml and 10g/100ml concentrations completely inhibited the radial growth of *Penicillium sp.* in the first to third day and first to fourth day of incubation respectively while at 15g/100ml concentration, the radial growth of *Penicillium sp.* was completely inhibited throughout the incubation period as compared with the aqueous extracts. Results (Table 3) showed that *Garcinia kola* extracts at 5g/100ml and 10g/100ml concentrations completely inhibited the radial growth of *Penicillium sp.* in the first to third day and first to fourth day of incubation respectively while at 15g/100ml concentration, the radial growth of *Penicillium sp.* was completely inhibited throughout the incubation period as compared with the aqueous extracts. Results (Table 4) showed that at 5g/100ml, 10g/100ml and 15g/ml concentrations the extracts of *Cola nitida* completely inhibited the radial growth of *Penicillium sp.* in the first to third day of the incubation when compared with the aqueous extracts.

Table 1: *In vitro* effect of ethanolic *Zingiber officinale* extracts on *Penicillium sp.*

Concentrations	Days of incubation and radial growth (cm)						
	1	2	3	4	5	6	7
5g/100ml	0.0	0.0	0.0	0.2	0.8	1.4	1.6
10g/100ml	0.0	0.0	0.0	0.0	0.1	0.2	0.8
15g/100ml	0.0	0.0	0.0	0.1	0.1	0.1	0.2
LSD	0.49						

Note: Values are means of three replicates.

Table 2: *In vitro* effect of ethanolic *Tetrapleura tetraptera* extracts on *Penicillium sp.*

Concentrations	Days of incubation and radial growth (cm)						
	1	2	3	4	5	6	7
5g/100ml	0.0	0.0	0.0	0.2	0.6	0.8	1.9
10g/100ml	0.0	0.0	0.0	0.0	0.6	0.8	1.9
15g/100ml	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSD	0.65						

Note: Values are means of three replicates

Table 3: *In vitro* effect of ethanolic *Garcinia kola* extracts on *Penicillium sp.*

Concentrations	Days of incubation and radial growth (cm)						
	1	2	3	4	5	6	7
5g/100ml	0.0	0.0	0.0	0.0	0.1	0.1	0.3
10g/100ml	0.0	0.0	0.0	0.1	0.2	0.3	0.3
15g/100ml	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSD	0.12*						

Note: Values are means of three replicates

Table 4: *In vitro* effect of ethanolic *Cola nitida* extracts on *Penicillium sp.*

Concentrations	Days of incubation and radial growth (cm)						
	1	2	3	4	5	6	7
5g/100ml	0.0	0.0	0.0	0.7	1.7	2.7	2.9
10g/100ml	0.0	0.0	0.0	0.2	0.4	0.6	1.9
15g/100ml	0.0	0.0	0.0	0.1	0.1	0.2	1.3
LSD	0.99						

Note: Values are means of three replicates

In vitro effect of aqueous extracts of _Zingiber officinale_, _Tetrapleura tetraptera_, _Garcinia kola_ and _Cola nitida_ on the _Penicillium sp_. at the different concentrations.

The *in vitro* effect of the aqueous plant extracts at different levels of concentration on the radial growth of the fungal isolate is presented in (Tables 5-8). Results from the study showed that extracts of *Zingiber officinale, Tetrapleura tetraptera, Garcinia kola and Cola nitida* had little or no significant inhibitory effect on the isolated rot fungus (*Penicillium sp.*) at all levels of concentration (5g/100ml, 10g/100ml and 15g/100ml) tested when compared with the ethanolic extracts.

Table 5: *In vitro* effect of aqueous *Zingiber officinale* extracts on *Penicillium sp.*

Concentrations	Days of incubation and radial growth (cm)						
	1	2	3	4	5	6	7
5g/100ml	2.7	3.4	3.6	4.2	4.2	4.3	4.4
10g/100ml	2.3	2.9	3.3	3.8	4.3	4.3	4.3
15g/100ml	2.1	2.8	3.9	4.2	4.5	4.5	4.5
LSD	0.90						

Note: Values are means of three replicates

Table 6: *In vitro* effect of aqueous *Tetrapleura tetraptera* extracts on *Penicillium sp.*

Concentrations	Days of incubation and radial growth (cm)						
	1	2	3	4	5	6	7
5g/100ml	2.4	3.4	4.5	4.5	4.5	4.5	4.5
10g/100ml	2.1	3.6	4.5	4.5	4.5	4.5	4.5
15g/100ml	2.3	3.2	4.0	4.5	4.5	4.5	4.5
LSD	0.96						

Note: Values are means of three replicates

Table 7: *In vitro* effect of aqueous *Garcinia kola* extracts on *Penicillium sp.*

Concentrations	Days of incubation and radial growth (cm)						
	1	2	3	4	5	6	7
5g/100ml	2.7	3.0	4.5	4.5	4.5	4.5	4.5
10g/100ml	2.4	2.9	4.5	4.5	4.5	4.5	4.5
15g/100ml	1.7	2.9	4.2	4.3	4.4	4.5	4.5
LSD	1.07						

Note: Values are means of three replicates

Table 8: *In vitro* effect of aqueous *Cola nitida* extracts on *Penicillium sp.*

Concentrations	Days of incubation and radial growth (cm)						
	1	2	3	4	5	6	7
5g/100ml	2.6	4.5	4.5	4.5	4.5	4.5	4.5
10g/100ml	2.2	3.1	4.2	4.5	4.5	4.5	4.5
15g/100ml	2.1	3.7	4.0	4.5	4.5	4.5	4.5
LSD	0.96						

Note: Values are means of three replicates

Discussion

In the storage of groundnut (*Arachis hypogaea* L.), fungal incidence is a major limiting factor and account for crop loss in great quantity. The fungal rot diseases are a major problem in the production, storage and distribution of this cash crop worldwide. The cash crop loss during pre and post-harvest processes and the cost of controlling these diseases constitute a significant burden in the agricultural sector of the producing countries. Thus, there is an urgent need for production of effective and sustainable controls of these diseases. The use of botanical pesticides to control fungal incidence are cost-effective, accessible, non-phytotoxic and environmentally friendly. In this study, the rot fungus isolated as the causative agent of the rot diseases of groundnut was *Penicillium sp*. Results of this study revealed that this fungus was responsible for the rot disease of stored groundnut obtained from four major markets (Watt, Marian, Akim and Mbukpa) in Calabar Metropolis of Cross River State as evidenced by the pathogenicity test. The fungal spores of this pathogen could be spread by water and insects and can land on susceptible groundnut seeds [20]. This agrees with the work of [21] who reported the isolation of *Penicillium chyrsogenum*, *Aspergillus niger and Aspergillus flavus* from *Arachis hypogaea, Sorghum bicolor, Lens esculentus, Vigna sinensis, Vicia faba, Oryza sativa and Triticum aestivum*. However, [22] reported the isolation of *Aspergillus sp*. from stored groundnut seeds while [23] reported *Rhizoctonia solani* as the causative agent of fungal rot diseases in groundnut which is in disagreement with the findings of this study. The antifungal activity of the four plant extracts was tested *in vitro* on the fungal isolate of the stored groundnut. Results (Tables 1-4) showed that the ethanolic plant extracts significantly ($p<0.05$) inhibited the radial growth of *Penicillium sp*. at all levels of concentration tested. Results (Tables 5-8) showed that the aqueous extracts of the same plants showed less inhibitory effect on the radial growth of *Penicillium sp* as compared to the ethanolic extracts.

However, the inhibitory effects of both the ethanolic and aqueous extracts were more effective with increase in concentration of the extracts and the rate of inhibition varied between the plants extracts tested. The differences in the fungitoxic potency of the different plants extracts may be attributed to the susceptibility of the fungus to the different concentrations of each plant extract. This agrees with the work of some researchers like [24] who reported that some essential oil and extracts of various plants have shown remarkable antifungal effect exhibited by retardation in mycelial growth and sporulation of fungal pathogens. Complete inhibition of *Penicillium sp.* was achieved with the ethanolic extract of *Tetrapleura tetraptera* and *Garcinia kola* at 15g/100ml concentration. Also, all the tested ethanolic concentrations inhibited significantly the radial growth of the *Penicillium sp.* while the aqueous extracts of the tested plants showed less inhibitory effect on the radial growth of *Penicillium sp*.

The inhibitory potency of all the plant extracts tested may be attributed to the phytochemical compounds like phenolics, polyphenols, alkaloids, terpenoides, polypeptides, cardiac glycosides, reducing compounds, and anthraquinones. This agrees with the work of [25] and [15] who reported the bioactive compounds of plant extracts in the inhibition of spores, mycelial elongation, development and spread of fungal pathogens. The ethanolic extracts of *Zingiber officinale, Tetrapleura tetraptera, Garcinia kola and Cola nitida* were also observed to be more effective than the aqueous extracts of the plants at all levels of concentration tested. The inhibitory potency of the ethanolic extracts may be attributed to the efficiency of the extraction solvent on the phytochemical content of the plant extracts. This agrees with the works of some researchers like [26, 24] who reported that methanol and ethanol were good extracting solvents of the bioactive compounds of the various plant extracts used in their studies. The greater efficiency of the ethanolic solvent as compared to the aqueous solvent may be due to its constituents, polarity and the high solubility of the active compounds contained in the ethanol solvent. This agrees with the work of [27] who reported that of the four (crude, hexane, ethyl acetate and aqueous) solvents used in extracting the bioactive antifungal properties of *Cosmos caudatus* against *Phytophthora palmivora, Pythium sp. Collectrichum gloesporoides, Collectrichum capsici* and *Pestaloptiopsia sp.* isolated from *Theobromae cacao*, aqueous extracts were the least effective.

Conclusions

The rot fungus isolated and identified from this study as the causative agent of rot disease of stored groundnut obtained from four major markets (Watt, Marian and Mbukpa) in Calabar Metropolis of Cross River State was *Penicillium sp*. The efficacy of the plant extracts (*Zingiber officinale, Tetrapleura tetraptera, Garcinia kola and Cola nitida*) against groundnut rot fungus was tested *in vitro*. The *in vitro* results showed that the extracts significantly inhibited the radial growth of the fungal pathogen at all levels of concentration tested using ethanol as solvent than the aqueous solvent. However, the plant extracts significantly ($p<0.05$) inhibited the radial growth of the fungal isolate at higher concentrations tested and the rate of inhibition varied from one plant extract to the other.

Conclusively, the ethanolic plant extracts showed significant inhibition of the radial growth of *Penicillium sp*. and as such can be used in the control of fungal rot disease of stored groundnut (*Arachis hypogaea*).

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