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Screening for antifungal activity of garlic (*Allium sativum*) powder against mycelia growth of three post-harvest pathogens

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ABSTRACT: Screening for antifungal activity of garlic powder against mycelia growth of three post-harvest pathogens (*Aspergillus*, *Rhizopus* and *Mucor* species) was investigated in this study. Five grams of malt extract agar (MEA) were poured into a conical flask, 100 ml of water and different weight of garlic powder (1, 3, 5 and 7 g) were separately added, stirred and later sterilized while MEA medium with no garlic added (0 g) served as control. The mycelia of each post-harvest pathogen was cut with 6mm cork borer and placed on the solidified medium in the Petri dish and incubated at 28±2^{o}C for 72 hours. Phytochemical screening of the garlic powder was also investigated. Results from this study showed that the different weights of the garlic powder apart from the control (0 g garlic) significantly inhibited the mycelia growth of the three post-harvest pathogens tested in the study and the order of antifungal activity of the garlic powder against mycelia growth of *Aspergillus*, *Rhizopus* and *Mucor* species was 7 g > 5 g > 3 g > 1 g > 0 g, 5 g > 7 g >1 g > 3 g > 0 g and 7 g > 5 g > 3 g > 1 g > 0 g respectively. The antifungal activity of the garlic powder may be related to the presence of active antimicrobial agents including alkaloids, saponins, tannins, flavonoids and cardiac glycosides that were detected in the powder.

Keywords: Garlic powder; Antifungal activity; Post-harvest; Pathogens; Phytochemicals.

1. INTRODUCTION

Post-harvest infection which develops on harvested parts of fruits and vegetables causes great damage to crops and high economic setback to countries that have poor storage system and are major producers of fruits and vegetables. Crop losses may result from physiological disorders such as superficial scald, pathological decays due to fungi activities and mechanical injury to fruit that occurs during transport and handling [1]. During export, postharvest pathogens such as *Botrytis cinerea*, *Penicillium expansum*, *Rhizopus* sp., *Aspergillus* sp., *Erwinia carotovora* and *Mucor* sp. etc. cause major economic losses due to postharvest latent infections that only manifest later on in the export chain [1]. The major postharvest pathogens associated with various fruits are green rot on orange caused by *Penicillium digitatum*, sour rot on tomato caused by *Rhizopus stolonifer*, soft rot on mango caused by *Aspergillus flavus*, blue mould caused by *Neofabraea* sp., which causes lenticel rot or bull's eye rot [2].

The control of postharvest pathogens is of great importance for the fruits industry. The use of chemical control on postharvest infection is widely used in developed countries where it is believed to be faster or a

better effective control over these pathogens. In fact, the use of fungicides for the control of plant diseases is a common practice all over the world [3]. However, the use of chemicals in control of plant diseases poised a risk to the survival of human race [4]. Consequently, the need to reduce the use of fungicides on export fruit has opened the door for innovative alternative measures to control postharvest diseases. The successful development of alternative measures for decay control would provide a more environmentally friendly and consumer-acceptable substitute over the current synthetic fungicides and would provide a competitive advantage to fruit producers and exporters in international markets. Such alternative measure is the use of plant extracts and essential oils which are safe for human consumption with no negative impact on the environment and besides, are natural sources of antimicrobials [5]. *Allium sativum* (garlic) is one of such plant species that is well documented for its value in improving human health and is readily available for consumption not just as a flavour component of food but also to be taken as a daily herbal diet supplement [6]. So many reports on the efficacy of garlic oils are available but there is dearth of information on the use of its powders. This study therefore screens antifungal activity of garlic powder against mycelia growth of three post-harvest pathogens.

2. MATERIALS AND METHODS

2.1. Isolation from infected fruits

Isolation of mycelia of species of *Aspergillus*, *Mucor*, and *Rhizopus* from infected fruits was made by cutting out the interface between the healthy and the disease issue and placing pieces of the affected fruits rind without surface sterilization on the plates of solidified malt extract agar (MEA). The plates were then incubated at 28±2^{o}C for 3 days. Subcultures of the isolates were prepared by transferring agar cut with distinct mycelium to sterilized Petri dishes containing solidified MEA and then incubated at 28±2^{o}C until pure cultures were obtained.

2.2. Morphological identification of fungal isolates

After incubation, identification of isolates was based mainly on the structural features as seen in the culture plates as well as microscopic characteristics. A drop of cotton-in-blue lactophenol solution was put on slide. Each isolate was put on a slide and was covered with a covert slip. Excess liquid was drained with a filter paper and the isolate was examined under microscope. Examination was with x40 objective lens of binocular microscope for the presence and type of hypae, mycelium either dark or clear and spore morphology and each isolate was identified using the text of Alexopoulos et al. [7].

2.2. Collection and preparation of garlic powder

Garlic (*Allium sativum*) bulbs were obtained at Southgate of Federal University of Technology, Akure in Ondo state, Nigeria and brought to the Department of Biology Laboratory, FUTA. The bulbs were crushed using a ceramic mortar and pestle into powder.

2.3. Antifungal activity of garlic powder against mycelia growth of the post-harvest pathogens

Five grams of malt extract agar (MEA) were poured into a conical flask and 100 ml of water was added. 1 g of powdered garlic was added and the mixtured stirred. The setup was corked with cotton wool and wrapped with aluminium foil and sterilized in an autoclave at 121^{o}C for 15 minutes. After sterilization, 250 mg of chloramphenol capsules was added to the mixture and stirred properly before pouring into the Petri dishes after cooling. The mixture was then allowed to cool and solidify. The procedure was repeated separately for 3, 5, and 7 g of powdered garlic while MEA medium with no garlic added (0 g) served as control. The mycelia of each isolated cultured pathogen (*Aspergillus*, *Rhizopus* and *Mucor* sp.) was separately

cut with 6 mm cork borer and placed on the solidified medium in the Petri dish. The plates were then incubated at 28±2°C for 72 hours.

2.4. Determination of phytochemical constituents of garlic powder

Garlic extract was prepared from the powered sample according to the method of Habourne [8], but with slight modification. Water was used for the extraction. For aqueous extraction, exactly 50 g of powered sample was soaked into 500 ml of water. The solution was allowed to stand for 24hours after which it was sieved with a clean muslin cloth and filtered with Whatman No.1 filter paper. The filtrate was then collected in a sterile clean beaker. The phytochemicals screened for were tannin, saponin, flavonoid, alkaloid and cardiac glycosides and the screening carried out according to the method described by Trease and Evans [9] but with little modification as described thus:

2.5.1. Tannin determination

About 5 ml of the garlic extract was stirred with 100 ml of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue - black green precipitate was taken as evidence for the presence of tannin.

2.5.2. Saponin determination

About 1 ml of the garlic extract was shaken with distilled water in a test tube, frothing which persisted on warming was taken as preliminary evidence for the presence of saponin.

2.5.3. Test for flavonoids

A piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid were added to 3 ml of the garlic extract. A pink red or red coloration of the solution indicated the presence of flavonoids.

2.5.4. Test for alkaloids

About 1 ml of the garlic extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. Then 1 ml of the filtrate was treated with Dragendorff's reagent. Turbidity or precipitation with the reagent was taken as evidence for the presence of alkaloids inthe extract.

2.5.5. Cardiac glycoside determination

The garlic powder was dissolved in pyridine and a few drops of 2% sodium nitro prusside together with a few drops of 20% NaOH were added. A deep red colour which faded to a brownish yellow indicated the presence of cardiac glycoside.

2.6. Data analysis

The data obtained for antifungal activity of different concentration of garlic was subjected to one way ANOVA; the means were compared at 95% confidence interval using Tukey's HSD Test (SPSS version 17).

3. RESULTS

3.1. Activity of different grams of garlic powder against mycelia growth of post harvest pathogens

Activity of different grams of garlic powder against mycelia growth of *Mucor* sp. after 72 hours of incubation was reported in Figure 1. Results showed that 7 g garlic powder in MEA had the highest inhibition zone of 25 mm when compared with the control having 0 mm inhibition zone. This was followed by 5 g garlic

with inhibition zone of 20 mm, then 3 g garlic with inhibition zone of 15 mm and the least was 1 g garlic having inhibition zone of 10 mm. Hence the order of antifungal activity was 7 g > 5 g > 3 g > 1 g> 0 g (Figure 1) and were significantly different ($p< 0.05$) from one another. Result was however different with mycelia growth of *Rhizopus* sp. 5 g garlic powder in MEA had the highest zone of inhibition of 17 mm against *Rhizopus* after incubation, followed by 7 g garlic having inhibition zone of 15 mm, then 1 g garlic with inhibition zone of 10 mm, then 3 g garlic with inhibition zone of 7 mm while 0 g garlic had no inhibition zone (Figure 2). Hence the order of antifungal activity was 5 g > 7 g > 1 g > 3 g > 0 g (Figure 2) and were significantly different ($p< 0.05$) from one another. Against mycelia growth of *Aspergillus* sp., results showed that 7 g garlic powder in MEA had the highest inhibition zone of 30 mm. This was followed by 5 g garlic with inhibition zone of 20 mm, then 3 g garlic with inhibition zone of 18 mm and the least was 1 g garlic having inhibition zone of 7 mm while 0 g garlic had no inhibition zone (Figure 3). Hence the order of antifungal activity was 7 g > 5 g > 3 g > 1 g > 0 g (Figure 3) and were significantly different ($p< 0.05$) from one another.

3.2. Phytochemical constituent of the garlic extracts

Results of the phytochemical constituent of the garlic extracts were shown in Table 1. Tannins, alkaloids, cardiac glycosides, saponins and flavonoids were all detected in the extracts (Table 1).

Table 1. Phytochemical constituents of garlic (*Allium sativum*) powder.

Phytochemical constituents	Status
Alkaloids	+
Tannin	+
Flavonoids	+
Saponin	+
Cardiac glycosides	+

Key: + Present, - Absent.

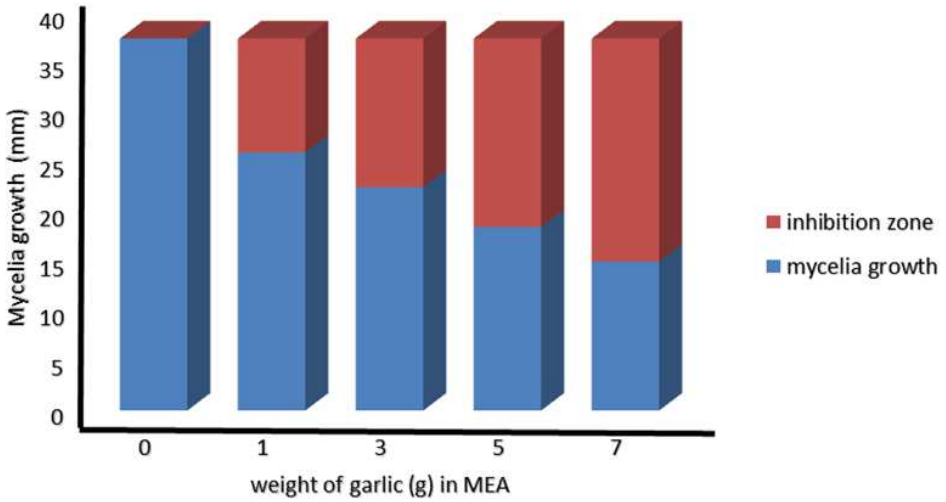

Figure 1. Activity of garlic powder against mycelia growth of *Mucor* sp. after incubation.

MEA - Malt extract agar.

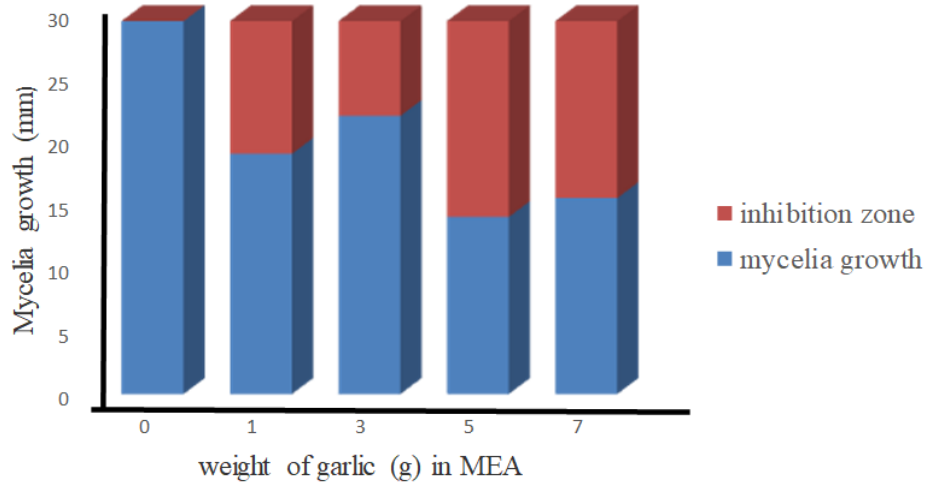

Figure 2. Activity of garlic powder against mycelia growth of *Rhizopus* sp. after incubation. MEA - Malt extract agar.

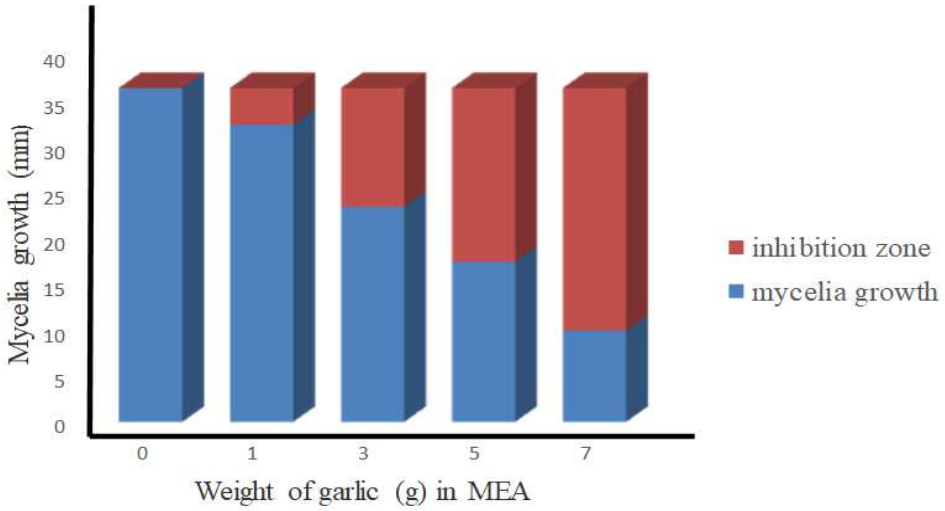

Figure 3. Activity of garlic powder against mycelia growth of *Aspergillus* sp. after incubation. MEA - Malt extract agar.

4. DISCUSSION

Results from this study showed that the different weights of the garlic powder apart from the control (0 g garlic) significantly inhibited the mycelia growth of the three post-harvest pathogens tested in the study. This is in consonance with the work of Abulazis et al. [10] who reported that garlic is a spice with global recognition and has been shown to inhibit the growth of fungi when tested. Also, the antifungal effect of garlic on plant pathogens has been shown by Russel and Mussa [11] for the control of *Fusarium oxysporum* f.sp. *phaseoli.* Investigations have also shown inhibitory effects of garlic against *Penicillium digitatum* [12]. The antifungal activity of garlic extracts may be related to the presence of active antimicrobial agents including alkaloids, saponins, tannins, flavonoids and cardiac glycosides which were the phytochemical constituents of garlic extracts [13]. Interestingly, these phytochemicals were equally detected in the garlic powder used in this study. Alkaloids have been shown to display antifungal activity against eleven agronomically important fungi including *Aspergillus* sp., *Rhizopus* sp., *Fusarium* sp. and others [14]. Marjorie

[15] also reported the presence of some classes of compound in plant extracts identified as alkaloids and flavonoids to possess antifungal activity.

Results further showed that the higher quantity of the garlic powder produced a corresponding better control. This could be buttressed by the work of Atia [16] who reported that increase in concentration of garlic resulted in reducing percentage of mycelia growth of the tested fungi. This cannot but be connected with the fact that effectiveness of plants extracts depend on the nature and amount of biologically active ingredients it contains. Hence, increasing the concentrations of the plant seed, bulb, and leaves extracts will correspondingly decrease radial growth of the tested pathogens in a dose - response effect. This was observed by Carson et al. [17] that low concentrations of garlic result in changes of the cell structure, inhibiting respiration and changing the permeability of the cell membrane of the tested fungi whereas high concentrations lead to severe membrane damage, loss of homeostasis and cell death. Hence, increasing the weights of garlic powder probably leadsto an increase in its active ingredient and several researchers have attributed the antimicrobial action of garlic to allicin, which is present as the main active component [18]. The formation of allicin is followed by its rapid decomposition into sulphur-derived compounds such as diallyldisulphide, diallylsulphide, diallyltrisulphide, sulphur dioxide, allyl propyl disulphide and diallyltetrasulphide which are strong antimicrobial and antifungal compounds.

5. CONCLUSION

Results of this study have shown that garlic powder significantly inhibited the mycelia growth of *Aspergillus*, *Rhizopus* and *Mucor* species and increase in weights of the garlic powder have a better inhibitory effect on these pathogens *in vitro*. Thus, garlic powder can be used as natural fungi-toxicants to control the growth of storage moulds and thus reduce dependence on synthetic fungicides. However, *in vivo* study can be further carried out on fruits attacked by these pathogens.

Conflict of Interest: The author declares no conflict of interest.

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