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Antifungal efficacy of garlic (*Allium sativum* L.) on selected crop seed-borne fungi

Toba Samuel Anjorin [1,*], Ezekiel Adebayo Salako [1], Elijah Ogenedoro Asarivwo [1], Joy Anwuri Edeh [1] and Wadzani Palnam Dauda [2]

[1] *Department of Crop Protection, Faculty of Agriculture, University of Abuja, P.M.B 117, Abuja Nigeria.*
[2] *Crop Science Unit, Department of Agronomy, Federal University, Gashua, Nigeria.*

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Abstract

This study aimed to assess the inhibitory effectiveness of garlic products on fungi associated with cowpea, sorghum, maize, and groundnut seeds. The *in-vivo* evaluation involved a seed culture bioassay treated with garlic aqueous extract, while the in vitro antifungal activity of the extract on the radial growth of isolated fungi was determined using the agar well diffusion method. The treatments included garlic powder, garlic paste, garlic powder + molasses, garlic aqueous extracts at 100%, garlic aqueous extracts + adjuvant, and Thiram® (a synthetic fungicide). On untreated cowpea seeds, *Aspergillus niger* was the most frequently isolated fungal species, with an incidence of 61.5%. Cowpea seeds treated with 100% garlic extracts showed zero incidence of fungal colonies, whereas sorghum seeds + garlic aqueous extract displayed only *A. niger*. The in vitro study revealed that the inhibitory effect of 100% garlic aqueous extracts + adjuvant on the radial growth inhibition of *A. niger, A. flavus*, and *P. chrysogenum* was 78.86%, 70.40%, and 67.36%, respectively. In contrast, the inhibition by garlic powder + molasses and the control was significantly lower ($p \leq 0.05$) than other treatments. In conclusion, this study suggests that garlic extracts could be a potential source of organically-based natural fungicides for safeguarding crop seeds.

Keywords: Agar Well; Antifungal Effect; Garlic; Seed-Borne Fungi; Seed Dressing; Seed Health

1. Introduction

Healthy seeds play a pivotal role as the most critical input in crop production, influencing the quality, yield, and productivity of crops. Many plant pathogens are seed-borne, which can cause enormous crop and yield losses. Recent increases in the production and sale of organic treated seed have heightened the scrutiny of organic seed quality. This has brought attention to concerns of seed-borne disease contamination. Seed-borne fungal pathogens such as bacteria, fungi, or viruses, live on the surface or interior of the seed and have the potential to spread disease to the subsequent crop (Jamal et al, 2014). This is one of the major constraints to crop production worldwide, causing significant yield losses and reducing the quality of crops (Himanshu *et al.,* 2022). Seed-dressing fungicides are one of the most effective means of controlling fungal diseases in crops caused by seed-borne pathogens. Seed-dressing fungicides are applied to the surface of seeds to control fungal diseases during germination and early seedling growth (Mehedi *et al.,* 2016).

Garlic (*Allium sativum* L.) is a widely cultivated bulbous plant that belongs to the family Amaryllidaceae. Garlic has been used traditionally for both culinary and medicinal purposes. Garlic is known to possess several bioactive compounds such as allicin, ajoene, and alliin which have been proven to possess antifungal properties against various fungal pathogens (Singh *et al.,* 2018; Anjorin *et al.,* 2022a &b.). These compounds exhibit inhibitory effects on the growth and development of fungal pathogens, making garlic a potential candidate for the development of seed-dressing fungicides.

* Corresponding author: Toba Samuel Anjorin; Email: toba.anjorin@uniabuja.edu.ng

The use of synthetic fungicides is being discouraged due to their adverse effects on human health and the environment (Shang *et al.,* 2019). There is a growing interest in the development of natural and eco-friendly seed dressing fungicides, such as those based on botanical sources like garlic extracts. Garlic extract has been studied extensively for its antifungal activity against various fungal pathogens, and its bioactive compounds have been found to exhibit inhibitory effects on the growth and development of fungal pathogens (Bayan *et al.,* 2014). Garlic extracts have been shown to inhibit the growth of seed-borne fungal pathogens, reduce disease severity, and improve seedling vigor (Sarfraz *et al.,* 2020). However, the potential of garlic extracts as a source of natural seed dressing fungicides has not been extensively investigated as literature is scarce in this area of study. The objectives of this study were to assess the antifungal efficacy of garlic extracts on cowpea, groundnut, maize, and sorghum seeds *in vivo,* and *a*ssess the antifungal efficacy of garlic extracts against *A. niger, A. flavus,* and *Penicillium chrysogenum,*

2. Materials and Methods

The study was carried out in the Crop Protection Laboratory, Faculty of Agriculture, University of Abuja, Nigeria.

2.1. Collection of seeds and reagents

Seeds of cowpea, maize, sorghum, groundnut seeds, garlic cloves, and vegetable oil (soya oil), and liquid soap (morning fresh) used as adjuvant were purchased from the Gwagwalada-Abuja main market. The Sabouraud Dextrose Agar (SDA), lactophenol blue, and ethanol used were of analytical grade and were purchased from Domhealth Laboratories PVT. Ltd., Suleja, Niger State. The synthetic carbamate fungicide - Thiram® (50%WP, tetramethyltruram disulphide) used was procured from Bari Pharmaceutical store in Gwagwalada, Abuja.

2.2. Culturing and Isolation of Fungi from Seeds

Sabouraud Dextrose Agar (SDA) media was used for culturing the contaminated seeds. The media was prepared by dissolving 39 g of SDA powder in 1000 ml of distilled water and then autoclaved at 121°C and 15 psi pressure for 15 minutes. After cooling, 0.5 g of streptomycin sulfate was added to the media to suppress bacterial growth. The media was then poured into sterile Petri dishes and allowed to solidify and were stored in a refrigerator at 4°C until use. The collected seeds of cowpea, maize, sorghum, and groundnut were surface sterilized by dipping in 70% ethanol for 1 minute and 1% sodium hypochlorite for 3 minutes. The sterilized seeds were then rinsed in sterile distilled water and dried on sterile filter paper. The seeds were then plated on SDA media and incubated at 27±1°C for 4 days during which mixed culture of seed-borne fungi completely appeared

2.3. Isolation and identification of pure culture

The mycelia of the isolated fungal species from the plated seeds of cowpea, maize, sorghum, and groundnut were streaked on the surface of the SDA plates using a sterile inoculation loop. The plates were incubated accordingly to allow the pure culture to grow and form visible colonies on the agar surface. Identification of the various isolates was based on macroscopic and microscopic examination (Goko, 2021) which was by observing the colonial characteristics especially the colour formation of both the front and reverse sides of the plates The microscopic examination was carried out by viewing the mycelia and conidia formation using a digital microscope at x40 magnification, comparing with a using a standard fungi identification manual.

2.4. Preparation of Garlic Extracts and their formulation

The laboratory benches were cleaned and sterilized with a swap of ethanol before starting the extract preparation. The 2 kg garlic bulbs were peeled and rinsed with sterile water to remove any dirt or debris and accurately weighed. The bulbs were transferred into a blender (vitamix 7500) and a small amount of sterile water (less than 50 ml) was added to aid in blending the garlic thoroughly until a smooth and homogeneous garlic paste was formed. A muslin cloth which sieve size is 0.7 mm was used to separate the aqueous garlic from the garlic paste, by placing the sieve over a container to collect the filtrate. The prepared garlic extract was stored in a sterile airtight bottle to prevent cross-contamination. Each container was labeled accordingly including the concentration and the date of preparation.

The treatments were garlic powder, garlic paste, garlic powder + molasses, garlic aqueous extracts at 100%, garlic aqueous extracts + adjuvant, and Thiram®. Garlic-based seed dressing fungicide with adjuvant was formulated by mixing 1 kg of garlic extracts with two drops of vegetable oil (Power oil®) and liquid soap (Morning glory®) each. Also the treatment with molasses was 1kg garlic extract mixed with 10 g molasses with served a sticker.

2.5. Assessing the Antifungal Activity of Garlic Extracts

The efficacy of garlic-based seed dressing fungicides was evaluated by treating the seeds with different formulations of garlic seed dressing fungicides and synthetic fungicides. The treated seeds were plated and placed under stable conditions. The fungal infection was evaluated by visual inspection of the plants for disease symptoms and by counting the number of fungal colonies on the seeds.

The antifungal activity of garlic extracts on the radial growth of the isolated fungal pathogens using the agar well diffusion method. Wells of approximately 6-8 mm in diameter were made on the agar surface using a sterile cork borer (10 mm diameter). The garlic extract was added to the respective wells using a sterile micropipette and the plates were incubated accordingly for 48 hours. After inoculation, the plates were examined for the presence of inhibition zones around the wells, which indicated the antifungal activity of the garlic extracts against the fungal pathogens. The diameter of the inhibition zones was measured using a ruler and the measurements were recorded in mm.

2.6. Statistical Analysis:

The data collected from the study were analyzed using descriptive statistics and analysis of variance (ANOVA) to determine the level of significance of the data. The treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5 % level of significance.

3. Results

3.1. Incidence of Fungal species isolated from Untreated and Treated Seeds in a Culture Media at 4 DAI

Table 1presents the incidence of fungal species isolated from garlic-treated and untreated seeds in a culture media at 4 days after inoculation (DAI). The identified isolated fungal species found in all untreated seeds were *Aspergillus niger*, *A.flavus, Penicillium chrysogenum* (Figures 1-3), *Trichoderma* spp, and *Rhizopus nigricans,* and *A. niger.*

Table 1 Incidence of Fungal species isolated from Untreated and Treated Seeds in a Culture Media at 4 DAI

Treatment (Seeds)	**Frequency and incidence (%) of fungal species on garlic-treated and untreated seeds at 4 DAI**				
	Aspergillus niger	*Aspergillus flavus*	*Penicilliun chrysogenum*	*Trichoderma spp*	*Rhizopus nigrican*
Cowpea seeds	8 (61.5)	2 (15.4)	1 (7.7)	2 (15.4)	0 (0.0)
Cowpea + GAE	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Groundnut seeds	3 (30.0)	3 (30.0)	1 (10.0)	0 (0.0)	3 (30.0)
Groundnut + GAE	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0)
Maize seeds	1 (10.0)	0 (0.0)	3 (30.0)	6 (60.0)	0 (0.0)
Maize + GAE	1 (20.0)	0 (0.0)	0 (0.0)	4 (80.0)	0 (0.0)
Sorghum seeds	3 (50.0)	0 (0.0)	0 (0.0)	3 (50.0)	0 (0.0)
Sorghum + GAE	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

GAE = Garlic Aqueous Extract

On untreated cowpea seeds, *Aspergillus niger* was the most frequently isolated fungal species, with an incidence of 61.5%. *Aspergillus flavus, Trichoderma* spp and *P. chrysogenum* had lower occurrence. On Garlic Aqueous Extract (GAE) treated cowpea seeds, no fungal growth was observed. On GAE-treated groundnut seeds, *A. niger* and *P. chrysogenum* (50% each) were observed.

On untreated maize seeds, one *A. niger* (10%), six *Trichoderma* spp. colonies (60%) and *P. chrysogenum* (30%) were found. On the GAE-treated seeds, one colony each of *A. niger* and *P. chrysogenum* were isolated from the seeds, while *Trichoderma* spp., *A. flavus* and *R. nigricans* growths were not found. On untreated sorghum seeds, three each *A. niger* and *Trichoderma* spp colonies were found(50% each)On the GAE-treated sorghum seeds, only three *A. niger* colonies (100%) were found, while all other fungi species were not detected. The application of Garlic Aqueous Extract (GAE) generally had an obvious effect on reducing the incidence of fungal species on each of the treated seeds as shown in Figures 4a-h.

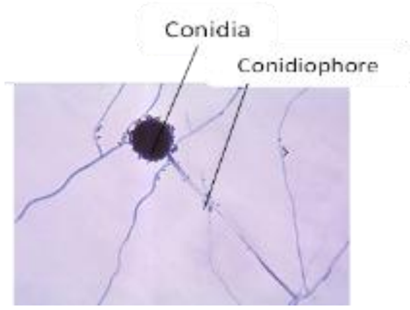

Figure 1 Microscopic view Aspergillus niger (Mag. x200)

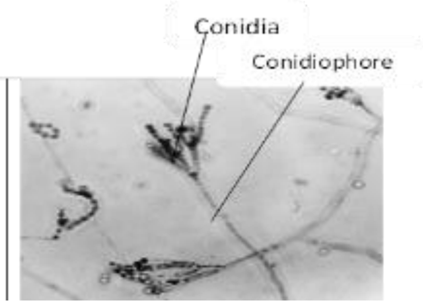

Figure 2 Microscopic view Penicillium chrysogenum (Mag. x200)

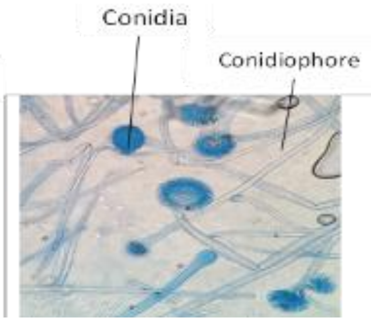

Figure 3 Microscopic view Aspergillus flavus (Mag. x200)

Figure 4a Untreated cowpea seeds (4 DAI)

Figure 4b Cowpea + GAE 100% (4DAI)

Figure 4c Untreated Groundnut seeds (4 DAI)

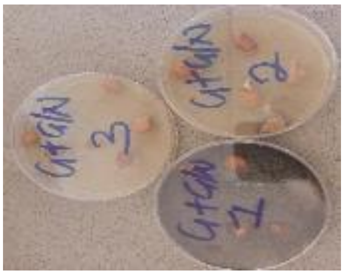

Figure 4d Groundnut+ GAE 100% (4DAI) (4DAI)

Figure 4e Untreated Maize seeds (4 DAI)

Figure 4f Maize + GAE100% (4DAI)

Figure 4g Untreated Sorghum seeds (4 DAI)

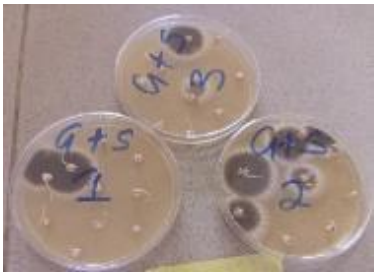

Figure 4h Sorghum + GAE 100% (4DAI)

Figures 4a-h Incidence of fungi growth on the cultured treated and untreated seeds at 4DAI

3.2. Radial Growth Inhibition (mm) of *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium chrysogenum* by Garlic Extracts at 48 HAI

The result of *in vitro* study on the inhibition of radial growth of *A. niger, A. flavus, and P. chrysogenum* applied with various garlic extracts at 48 Hours After Inoculation (HAI) is presented in Table 2. Among all the formulations, GAE 100% with or without adjuvants exhibited the highest significant inhibitory effect ($p \leq 0.05$) on the radial growth of the tested fungi.

Table 2 Radial Growth Inhibition (mm) of *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium chrysogenum* by Garlic Extracts at 48 HAI

Treatment	**Radial growth inhibition**					
	A. niger		*A.flavus*		*P. chrysogenum*	
	Radial inhibition(mm)	*% inhibition*	Radial inhibition (mm)	*% inhibition*	Radial inhibition (mm)	*% inhibition*
Garlic powder	11.0^{bc}	25.0	5.0^{ab}	11.4	12.0^{b}	27.27
Garlic paste	15.0^{cd}	34.09	15.0^{c}	34.09	20.67^{c}	47.04
Garlic powder + molasses	7.7^{b}	15.9	5.7^{b}	12.89	14.33^{bc}	32.57
Garlic aqueous extract 100%	19.67^{d}	44.7	29.0^{d}	65.90	24.33^{d}	55.29
Garlic aqueous extract + adjuvant	26.0^{e}	59.09	24.7^{d}	56.07	25.33^{d}	57.56
Thiram®	34.7^{f}	78.86	31.0^{d}	70.44	27.0^{d}	61.36
Control	0.5^{a}	1.14	0.0^{a}	0	0.0^{a}	0

Means followed by the same letter(s) within a vertical column are not significantly different using Duncan Multiple Range Test at 5% level of probability; HAI = Hours After Inoculation

It was observed that 100 % garlic aqueous extract had an inhibitory effect on *A. niger, A. flavus, P. chrysogenum* radial growths by 44.7%, 65.9% and 55.29% respectively. Also, the inhibitory effect of garlic aqueous extracts + adjuvant on radial growth inhibition of *A. niger, A. flavus, P. chrysogenum* were by 78.86, 70.40 and 67.36% respectively. However, the Thiram® fungicide treatment, displayed a relatively higher inhibitory effect on the fungi while the control showed no inhibition. Generally, garlic powder and garlic+ molasses showed relatively lower inhibition than garlic paste or extract with or without adjuvant.

Figure 5a Radial growth Inhibition by garlic aqueous extract on *P. chrysogenum* (front view)

Figure 5b Radial growth Inhibition by garlic paste on *P. chrysogenum* (front view)

Figure 5c Radial growth Inhibition by Thiram® on *A. niger* (front)

Figure 5a-c Radial Growth Inhibition of Fungi Species at 48 HAI

4. Discussion

The results showed that *A. niger* was the most prevalent fungal species in all untreated seeds of the cowpea, groundnut, maize, and sorghum, indicating its high occurrence and possible potential negative impact on seed health. Recent studies reported the presence and significance of *Aspergillus* species in seed-borne fungal diseases (Ghosh *et al.,* 2018). Seed-borne fungi such as *Alternaria* and *Aspergillus* spp infection could damage seeds at varying degree, causing seed shrinkage or colour change (Zafar et al., 2014). Pathogenic seed-borne fungi are either present on the seed coat or inside the seed tissue (Chohan et al., 2017). Morphologically detected fungi species could be further molecularly confirmed by PCR-based identification (Kumar et al 2020) High incidence of *Aspergillus tamari* and *Aspergillus niger* were recently reported in Nigeria in groundnut seeds but varied considerably from one location to another (Aberagi et al.,).

The application of GAE showed promising results in inhibiting the growth of fungal species on the treated seeds. Garlic extract with or without adjuvant exhibited a significant inhibitory effect on the growth of all tested fungal species. This aligns with previous studies that have reported the antifungal activity of garlic extracts against various fungal pathogens (Anwar *et al*., 2009; Shafi *et al.,* 2012). This might be due to the antifungal properties of garlic which have been reported in various studies (Castro et al., 2014; Zohri *et al.,* 2017;). The choice of active ingredient, formulation, and method of application are critical factors that determine the effectiveness of seed dressing fungicides. The addition of adjuvant such as surfactants and crop oil often boost pesticide efficiency (Teixeira *et al.,* 2023).

Garlic extract was reported to have elongated food shelf life by inhibiting bacterial and fungal growth in the treated food samples. The extract possesses antimicrobial properties that can suppress the growth of fungi due to the presence of active sulfur-containing bioactive compounds such as allicin (Bayan *et al.,* 2014). These compounds act by interfering with fungal cell metabolism and inhibit spore germination (Redondo-Blanco et al., 2020)

Daniel *et al.,* (2023) reported from their investigations that garlic extracts showed more potent antifungal activity against conidial germination than against mycelial growth of the test fungi. Sittisart et al. (2017) observed from the evaluation of percentage inhibition of mycelial growth that there was no significant difference ($p \geq 0.05$) in the inhibition effect of chili and garlic extract and the garlic extracts had the lower inhibitory effect. Application of garlic essential oils (1:6:6, v/v/v) on *A. niger* cultured in SDB had strong inhibitory effects against the growth of the test fungus. *A, niger* when enumerated by using serial dilution (Irkin et al., 2007). Thiram®, showed the strongest inhibition of radial growth among all the treatments. It significantly inhibited the growth of *A. niger A. flavus* and *P. chrysogenum*. The effectiveness of Thiaram® against fungal pathogens is well-known and has been widely used in crop protection for its potent fungicidal properties (Liu *et al.,* 2013).

Sagarika et al. (2023) reported the field efficacy of seed treatment of Carboxin® + Thiram® against *Macrophomina phaseolina* in Soybean. Also, the fungicide thiram® gave better pathogen control in groundnut, resulting in good seed development (Barbosa et al., 2013). The fungicides carbendazim + thiram , fludioxonil + metalaxyl-m and fludioxonil + mefenoxam + thiabendazole gave satisfactory pathogen control but did not allow the peanut seeds to develop properly. this might be due to phytotoxicity effect.

The combination of garlic aqueous extract at 100% concentration with adjuvants also showed strong inhibitory activity against the fungal species, Adjuvants such as soap and sticker agents can enhance the efficacy of botanical pesticides by improving their penetration and retention on the target surfaces (Castro *et al.,* 2014).

Both garlic powder and garlic powder with molasses showed inhibition of radial growth for the tested fungal species, although to a lesser extent compared to garlic paste and other garlic treatments. Garlic powder contains bioactive compounds that can inhibit fungal growth (Chakrapani *et al*., 2020), and the addition of molasses might have contributed to enhancing its antifungal activity. This aligned with the findings of previous studies that have reported the antifungal activity of garlic extracts against various plant pathogens (Li et al., 2019; Mohamed et al., 2021).

5. Conclusion

The results of the study demonstrate the efficacy of garlic and its formulation as seed dressing fungicides against *Aspergillus niger* and *A flavus*, as well as *Penicillium chrysogenum.* Garlic paste, garlic aqueous extract at 100 %, and garlic aqueous extract with or without adjuvant showed strong inhibitory effects on the radial growth of the tested fungi, next to Thiram® synthetic fungicide. Garlic powder and garlic powder with molasses exhibited lower inhibitory effects. The evidence of antifungal effects observed in this study provides evidence of its potential as a natural antifungal agent for seed-borne fungal disease management, however, the effect of the treatment on seed germination and vigour should be further investigated.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest to be disclosed.

Author's Declaration Form

All of the authors declared that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version. Additionally, there are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

Author Contributions Statement

Anjorin T.S. conceived and designed the study project and drafted the manuscript, Wadzani D analyzed and interpreted the data collected. Asarivwo, E.O. collected the materials needed, Salako E.I critically revised the manuscript for intellectual content and corrected the reviewed paper. All authors agreed to be accountable for all aspects of the work.

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