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Antifungal Efficacy of Garlic (*Allium Sativum* L.) on Selected Seed-borne Fungi

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Abstract

This study aimed to assess the antifungal properties of garlic (*Allium sativum* L) as a seed dressing substance and chemical on various crops, including cowpea, sorghum, maize, and groundnut, and to investigate its impact on associated seed-borne fungi. The assessment involved in vivo determination of fungal inhibition using garlic aqueous extract via seed culture bioassay. Additionally, the antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium chrysogenum* was examined through agar well diffusion method at different garlic extract concentrations. Findings revealed that seeds treated with 100% garlic extracts exhibited the lowest incidence of fungal attack, highlighting the potent inhibitory effect of garlic extracts on seed-borne fungi in vivo. In vitro findings showed significant inhibitory effects of 100% garlic aqueous extract on *A. flavus*, *P. chrysogenum*, and *A. niger*, with radial growth reductions of 29.0 mm, 24.3 mm, and 19.7 mm, respectively. Furthermore, garlic aqueous extracts + adjuvant demonstrated notable inhibition on radial growth, with reductions of 25.3 mm, 26.0 mm, and 24.7 mm for *P. chrysogenum*, *A. niger*, and *A. flavus*, respectively. However, garlic paste and Imidacloprid® treatments exhibited the highest inhibitory effects on fungal radial growth. Notably, the inhibition by garlic powder + molasses and the control was significantly lower ($p \leq 0.05$) than other treatments. This study suggests the potential of garlic extracts as natural antifungal agents against seed-borne fungi, presenting a possible source of organically based fungicide.

Keywords: Antifungal efficacy, Aspergillus flavus, Aspergillus niger, garlic, Penicillium chrysogenum

1.0 INTRODUCTION

Healthy seeds play a pivotal role as the most critical input in crop production, influencing the quality, yield, and productivity of crops. They encapsulate the genetic potential that determines desirable traits such as disease resistance, adaptability to climatic conditions, high yield potential, and improved nutrition. By utilizing improved and treated seeds, farmers can unlock the genetic potential of crops, ensuring uniformity within the crop, maximizing productivity, and facilitating efficient management of agricultural operations. The incorporation of pest and disease resistance traits in high-quality seeds reduces crop losses and minimizes the need for excessive pesticide use. Additionally, adapted seeds enable successful cultivation in diverse environments. Continuous research and development in the seed industry allows farmers to access advanced technologies such as GMOs, hybrid seeds, and precision breeding techniques. By recognizing the significance of seeds and utilizing high-quality varieties, farmers establish a strong foundation for successful and sustainable crop production (FAO. (2016), United Nations. (2020).

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 has heightened the scrutiny of organic seed quality. This has brought attention to concerns of seed-borne disease contamination. Seed-borne diseases are pathogens such as bacteria, fungus, or viruses, that live on the surface or interior of seed and have the potential to spread disease to the subsequent crop. Seed-borne fungal pathogens are 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). The choice of active ingredient, formulation, and method of application are critical factors that determine the effectiveness of seed dressing fungicides.

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). 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.

Seed-borne fungal pathogens are a major constraint to crop production worldwide, causing significant yield losses and reducing the quality of crops (Anjorin *et al.,* 2009). Synthetic fungicides have been widely used as seed dressing fungicides to control fungal diseases in crops, particularly those caused by seed-borne pathogens. The use of synthetic fungicides is being discouraged due to their adverse effects on human health and the environment (Shang *et al.,* 2019). Arising, 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 (*A. sativum* L.) 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 are scare in this area of study.

Therefore, the research seeks to evaluate the effectiveness of garlic extracts as a seed dress chemical in controlling seed-borne fungal pathogens in crops. The objectives of these study are to: assess the antifungal efficacy of garlic extracts on cowpea, groundnut, maize and sorghum seeds *in vivo, a*ssess the antifungal efficacy of garlic extracts against *A. niger, A. flavus* and *Pennicillium chrysogenum,* and compare the efficacy of the garlic-based seed dressing fungicide with synthetic fungicides commonly used as seed treatments

2.0 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, and groundnut were purchased from the local market in Gwagwalada the Federal Capital Territory of Nigeria. Garlic cloves were obtained from the Ibrahim Badamasi Babangida market in Suleja, Niger State, Nigeria. The SDA (Sabouraud Dextrose Agar), distilled water, streptomycin sulfate, 70% ethanol, 1% sodium hypochlorite, ethanol for cleaning and sterilizing laboratory benches, sterile water for rinsing garlic bulbs and blending, sterile filter paper, sterile Petri dishes, sterile muslin cloth, sterile, airtight bottles, sterile swap, sterile inoculation loop, and sterile cork borer or sterile pipette tip, and sterile micropipettewere of analytical grade and were purchased from Domhealth Laboratories Pvt. Ltd., Suleja, Niger State. The vegetable oil (soya oil) and liquid soap (morning fresh) used as adjuvants were purchased from a local supermarket in Abuja, Nigeria, while molasses was obtained from a nearby sugar processing factory. The synthetic fungicide (imidacloprid) used in this study was procured from a local agricultural inputs dealer in Abuja, Nigeria.

2.1.1 Preparation of SDA media

Sabouraud Dextrose Agar (SDA) media was used for inoculation and culturing of fungal isolates. 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. The plates were stored at 4°C until use.

2.1.2 Isolation of Fungi from Seeds

Fungi species were isolated from cowpea, maize, sorghum, and groundnut seeds collected from different locations in Abuja. The seeds 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 25°C for 4 days.

2.1.3 Inoculation of Fungal Pathogens

Pure cultures of the isolated fungal pathogens from cowpea, maize, sorghum, and groundnut seeds, were streaked on the surface of the SDA plates using a sterile inoculation loop. The plates were incubated at 25°C for 4 days to allow the fungal pathogens to grow and form visible colonies on the agar surface. To culture the fungal isolates, a small amount of the fungal mycelium was transferred from the SDA plate to a fresh SDA plate using a sterile inoculation loop. The plates were incubated at 27°C for 4 days until a pure fungal culture was obtained. The SDA media were sterilized using an autoclave prior to use to avoid contamination of the media and samples. (Alshami, and Hussain (2019).

Identification and classification of the various isolates were based on macroscopic and microscopic examination (Alexopoulos *et al.,* 2002). The macroscopic examination was carried out by observing the colonial characteristics especially the colour formation of both the front and reverse sides of the plates while the microscopic examination was carried out by viewing the mycelia and conidia formation using a digital microscope at x40 magnification.

2.1.4 Preparation of Garlic Extracts

The laboratory benches were cleaned and sterilized with a swap of ethanol before starting the experiment. The 2 kg garlic bulbs were peeled and rinsed with sterile water to remove any dirt or debris and accurately weighed. The garlic 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 evenly. The garlic bulbs were blended thoroughly until a smooth and homogeneous garlic paste is 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 labelled accordingly including the concentration and the date of preparation.

2.1.5 Formulation of Garlic-based Seed Dressing Fungicide

Garlic-based seed dressing fungicide was formulated by mixing garlic extracts with inert/adjuvants materials such as vegetable oil (power oil), liquid soap (morning glory), molasses etc. The formulation was optimized by varying the concentration of garlic extracts and the ratio of garlic extracts to inert/adjuvants materials.

2.1.6 Evaluation of Antifungal Activity of Garlic Extracts

The antifungal activity of garlic extracts was evaluated against the isolated fungal pathogens using the agar well diffusion method. After inoculation, wells of approximately 6-8 mm in diameter were made on the agar surface using a sterile cork borer or a sterile pipette tip. The garlic extract was

added to the respective wells using a sterile micropipette and the plates were incubated at 27°C 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.

2.1.7 Determination of Efficacy of Garlic-based Seed Dressing Fungicide in Controlling Seed-borne Fungal Pathogens

The efficacy of garlic-based seed dressing fungicide was evaluated by treating the seeds with different formulations of garlic seed dressing fungicide 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.

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

3.0 RESULTS

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

Table 1 presents the result on the incidence of fungal species isolated from untreated and treated seeds in a culture media at 4 days after inoculation (DAI). The isolated fungal species were *Aspergillus niger*, *Aspergillus flavus, Penicillium chrysogenum* (Plates 1-3), *Trichoderma* spp, and *Rhizopus nigricans*. From the Table3.1, it was observed that the untreated seeds of cowpea, groundnut, maize, and sorghum had incidence of multiple fungal species. *Aspergillus niger* was found to be present in all untreated seeds, indicating its high prevalence in the seeds.

On untreated cowpea seeds, *A. niger* was the most frequently isolated fungal species, with a frequency of 8 and an incidence of 61.5%. *Aspergilus. flavus* and *P. chrysogenum* had lower frequencies and incidences. *Trichoderma* spp. and *R. nigricans* were not isolated from the untreated cowpea seeds. On garlic aqueous Extract (GAE) treated cowpea seeds hadnone of the fungal species isolated, but on the untreated seeds, *A. niger, A. flavus,* and *P. chrysogenum* were each isolated with a frequency of 3. *Trichoderma* spp. and *R. nigricans* were not found on the untreated groundnut seeds. On GAE-treated groundnut seeds, *A. niger* and *P. chrysogenum* were each isolated but *A. flavus, Trichoderma* spp., and *R. nigricans* were not present.

On untreated maize seeds, one *A. niger* and *Trichoderma* spp. colonies were each isolated., while *P. chrysogenum* was found three times. *A. flavus* and *R. nigricans* were not detected on the untreated maize seeds. 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 *A. niger* colonies were found. while *Trichoderma* spp. was detected once. *A. flavus*, *P. chrysogenum*, and *R. nigricans* were not present on the untreated sorghum seeds. On the GAE-treated sorghum seeds, three *A. niger* colonies were found, while *A. flavus, P chrysogenum, Trichoderma* spp., and *Rhizopus nigricans* were not detected. The application of Garlic Aqueous Extract (GAE) generally had obvious effect on reducing the incidence of fungal species on each of the treated seeds (Plates 4a-h).

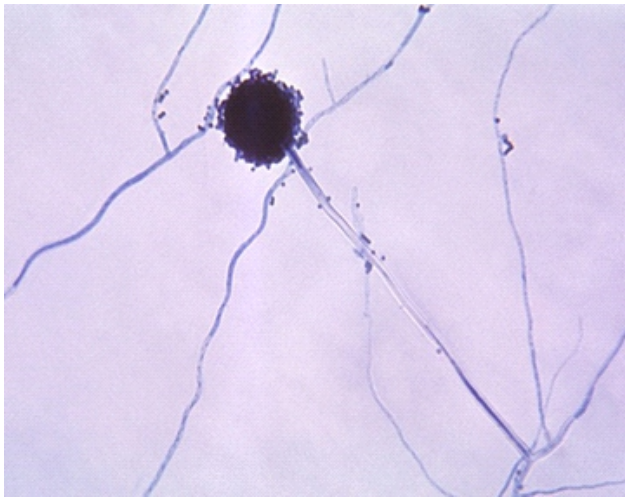


Plate 1: Photomicrograph of *Aspergillus niger:* Conidiophore present with short columnar conidia heads and a slightly visible mycelia (Mag. × 400)

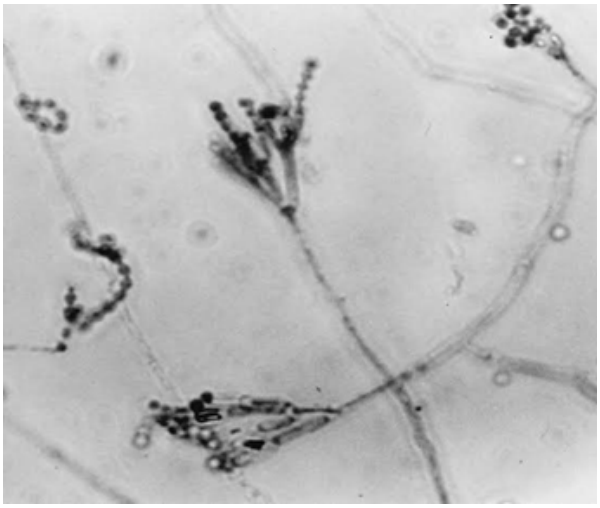


Plate 2: Microscopic view of *Penicillium chrysogenum:* Conidiophore and conidia appeared smooth with septate hyphae (Mag. × 400)

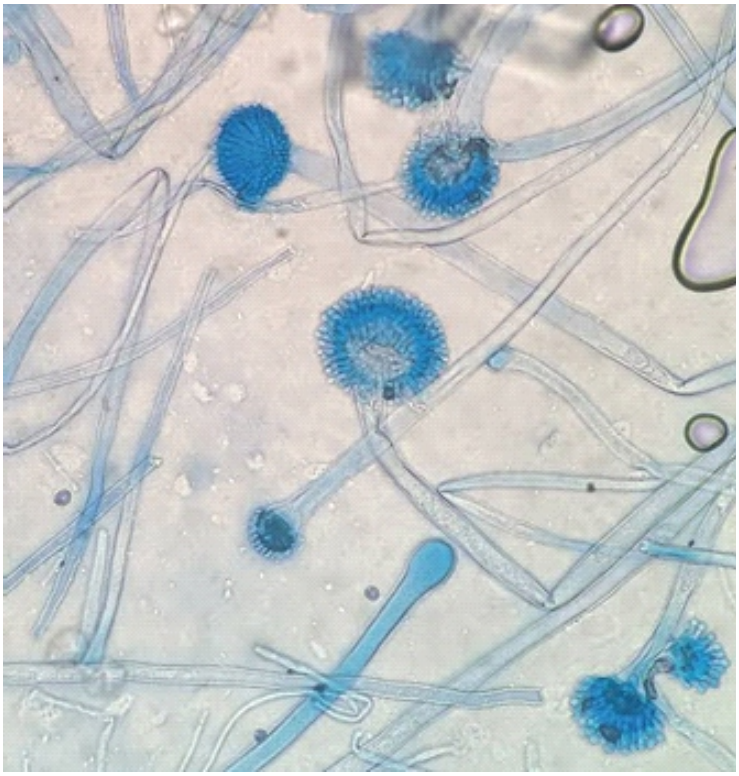


Plate 3: Microscopic view of *Aspergillus flavus*

Table 3.1: 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	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	3 (30.0)	3 (30.0)	1 (10.0)	0 (0.0)	3 (30.0)
Groundnut + GAE	1 (33.3)	0 (0.0)	1 (33.3)	0 (0.0)	1 (33.3)
Maize	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	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

Plate 4a: Cowpea 4 DAI (control)

Plate 4b: Garlic + Cowpea 4DAI

Plate 4c: Groundnut 4 DAI

Plate 4d: Garlic + Groundnut 4 DAI

Plate 4e: Maize 4 DAI (control)

Plate 4f: Garlic + Maize 4DAI

Plate 4g: Sorghum 4 DAI (control

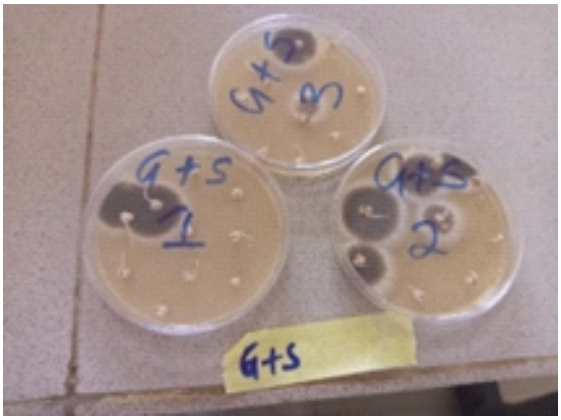


Plate 4h: Garlic + sorghum 4DAI

Plates 4a-h: Incidence of fungi growth from the media prepared at 4DAI

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

Table 3.2 presents the results of the inhibition of radial growth of *A. niger, A. flavus, and P. chrysogenum* by various garlic extracts at 48 hours after inoculation (HAI).

The garlic extracts showed varying degrees of inhibition against the tested fungal species. Garlic paste exhibited the significant inhibitory effect on the growth of *A. niger* (15.0 mm), *A. flavus* (15.0 mm), and *P. chrysogenum* (20.67 mm). Imidacloprid, a synthetic pesticide, displayed the strongest inhibition of radial growth among all the treatments. It significantly inhibited the growth of *A. niger* (34.67 mm), *A. flavus* (11.0 mm), and *P. chrysogenum* (27.0 mm). Garlic aqueous extract at 100% concentration demonstrated strong inhibitory activity against the fungal species, with radial growth inhibition values of 19.67 mm for *A. niger*, 29.0 mm for *A. flavus,* and 24.33 mm for *P. chrysogenum*. Garlic aqueous extract 100% + adjuvants also exhibit strong inhibitory activity against the fungi specie, with radial growth inhibition values of 26.0 mm for *A. niger,* 24.67 mm for *A.flavus*, and 25.33 mm for *P. chrysogenum*.

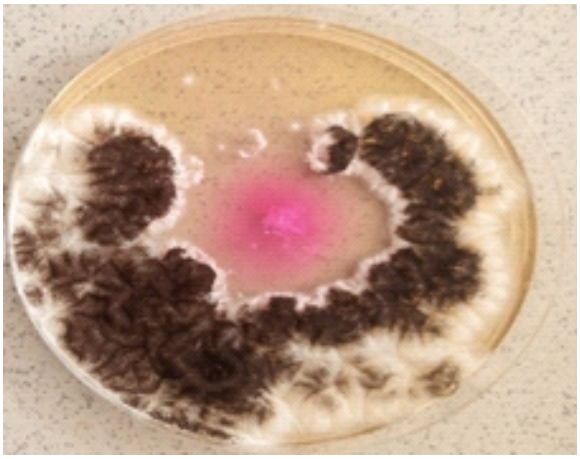
Table 3.2 Radial Growth Inhibition (mm) of *Aspergillus niger*, *Aspergillus flavus*, and *Pennicillium chrysogenum* by Garlic Extracts at 48HAI

Treatment		Radial growth inhibition (mm)		
		A. niger	*A. flavus*	*P.chrysogenum*
1	Garlic powder	11.0^{bc}	5.0^{ab}	12.0^{b}
2	Garlic paste	15.0^{cd}	15.0^{c}	20.67^{cd}
3	Garlic powder + molasses	7.67^{b}	5.67^{ab}	14.33^{bc}
4	Imidacloprid	34.67^{f}	11.0^{bc}	27.0^{d}
5	Garlic aqueous extract 100%	19.67^{d}	29.0^{d}	24.33^{d}
6	Garlic aqueous extract + Adjuvant	26.0 ^{e}	24.67^{d}	25.33^{d}
7	Control plate	0.00^{a}	0.0^{a}	0.0^{a}

Means followed by the same letter(s) within a v ertical column are not significantly different using Duncan Multiple Range Test at 5% level of probability. HAI = hours after inoculation

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

5b. Radial growth Inhibition by garlic aqueous extract on *P. chrysogenum* (reverse view)



5c.Radial growth Inhibition by imidacloprid on *A. niger* (front view

5d. Radial growth Inhibition by garlic paste on *P.chrysogenum* (front view

Plate 5a-d. Radial Growth Inhibition of Fungi Species at 48 HAI

4.0 DISCUSSION

The results showed that untreated seeds of cowpea, groundnut, maize, and sorghum were colonized by multiple fungal species. *Aspergillus niger* was the most prevalent fungal species in all untreated seeds, indicating its high occurrence and potential negative impact on seed health. Previous studies have also highlighted the significance of *Aspergillus* species in seed-borne fungal diseases (Abarca *et al.,* 2004; Leslie and Summerell, 2006).

The application of Garlic Aqueous Extract (GAE) showed promising results in inhibiting the growth of fungal species on the treated seeds. This aligns with the antifungal properties of garlic, which have been reported in various studies (Zohri *et al.,* 2017). The active compounds in garlic, such as allicin and other sulfur-containing compounds, possess antimicrobial properties that can suppress the growth of fungi (Bayan *et al.,* 2014). The effectiveness of GAE in controlling fungal infections may be attributed to its bioactive compounds, which interfere with fungal cell metabolism and inhibit spore germination (Yin *et al.,* 2019). However, further research is necessary to understand the specific mechanisms involved and optimize the application of GAE in seed treatment protocols.

Garlic paste exhibited a significant inhibitory effect on the growth of all tested fungal species. It displayed radial growth inhibition values of 15.0 mm for *A. niger,* 15.0 mm for *A. flavus*, and 20.67 mm for *P. chrysogenum*. These results suggest that garlic paste contains potent antifungal compounds that can effectively suppress the growth of the tested fungi. 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). Imidacloprid, a synthetic pesticide, showed the strongest inhibition of radial growth among all the treatments. It significantly inhibited the growth of A. niger

(34.67 mm), *A. flavus* (11.0 mm), and *P. chrysogenum* (27.0 mm). The effectiveness of imidacloprid against fungal pathogens is well-known and has been widely used in agricultural settings for its potent fungicidal properties (Liu *et al.,* 2013). Garlic aqueous extract at 100% concentration demonstrated strong inhibitory activity against the fungal species. It exhibited radial growth inhibition values of 19.67 mm for *A. niger*, 29.0 mm for *A. flavus*, and 24.33 mm for *P. chrysogenum*. This indicates that the aqueous extract of garlic contains active compounds that can effectively inhibit the growth of these fungi. The combination of garlic aqueous extract at 100% concentration with adjuvants also showed strong inhibitory activity against the fungal species, with radial growth inhibition values of 26.0 mm for *A. niger*, 24.67 mm for *A. flavus*, and 25.33 mm for *P. chrysogenum*. Adjuvants can enhance the efficacy of botanical pesticides by improving their penetration and retention on the target surfaces (Isman, 2006).

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 (Rees *et al.,* 2005), and the addition of molasses might have contributed to enhancing its antifungal activity. The control plate, as expected, showed no inhibition of fungal growth, confirming the validity of the experimental setup. Overall, the results demonstrate that garlic extracts, particularly garlic paste, possess potent antifungal properties and can effectively inhibit the radial growth of *A. niger, A. flavus*, and *P. chrysogenum*. This aligned with the findings of previous studies that have reported the antifungal activity of garlic extracts against various plant pathogens (Anwar *et al.,* 2009; Shafi *et al.,* 2012).

5.0 CONCLUSION

The results of the study demonstrate the efficacy of garlic and its derivatives as seed dressing fungicides against *Aspergillus niger and Aspergillus flavus*, as well as *Penicillium chrysogenum.* Garlic paste, garlic aqueous extract at 100 %, and garlic aqueous extract with adjuvant showed strong inhibitory effects on the radial growth of the tested fungi, followed by imidacloprid, a synthetic fungicide. Garlic powder and garlic powder with molasses exhibited lower inhibitory effects. This study provides evidence for the potential use of garlic extracts as a natural antifungal agent for plant disease management.

6.0 ACKNOWLEDGMENT

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