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Suppressive potential of chitosan and *Paenibacillus polymyxa* against *Fusarium verticillioides* isolated from maize (*Zea mays* L.) *in vitro*

Soad Ali Algam[1]✉, **Laila Amasaib**[1], **Elamin Hafiz Baillo**[2]

1 Department of Crop Protection, Faculty of Agriculture, University of Khartoum, Khartoum, Sudan.

2 Agricultural Research Corporation, Sudan.

✉ Corresponding authors: soadalgam@gmail.com

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Abstract

Ear rot, caused by *Fusarium verticillioides* (Sacc.), is one of the most destructive diseases affecting maize worldwide. This study aimed to evaluate the antifungal activity of chitosan and *Paenibacillus* strains as potential biocontrol agents against *F. verticillioides* under *in vitro* conditions. The pathogen was initially isolated from infected maize seeds and identified based on morphological and molecular characteristics. To assess the antifungal effects, three concentrations of chitosan (1, 3, and 5 mg/mL) and six *Paenibacillus polymyxa* strains (P1, P2, P4, P6, P7, and P8) were tested against the mycelial growth of *F. verticillioides*. The experiments were conducted using a completely randomized design, and the collected data were analyzed using an analysis of variance. Chitosan exhibited vigorous antifungal activity, achieving complete (100%) inhibition of mycelial growth at concentrations of 3 and 5 mg/ml and a 93.40% inhibition at 1 mg/ml. All *Paenibacillus* strains demonstrated inhibitory effects, with inhibition rates of 28.7%, 17.8%, 47.5%, 18.4%, 2.3%, and 42.6% for the strains P1, P2, P4, P6, P7, and P8, respectively. These findings suggest that chitosan, in particular, has strong antifungal properties and holds promise as an eco-friendly alternative to synthetic fungicides for managing *F. verticillioides* in maize.

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Introduction

Maize (*Zea mays* L.) is the third most important cereal crop globally, following wheat and rice. It is widely utilized for food, animal feed, and industrial applications. In Sudan, maize holds significant potential for food and feed production, ranking fourth among cereal crops after sorghum, wheat, and pearl millet (Mohammed et al., 2015). However, its cultivation in tropical and subtropical regions is hindered by various biotic stress factors, among which *Fusarium verticillioides* is a major concern (Akande & Lamidi, 2006). *Fusarium verticillioides* (Sacc.) is one of the most prevalent fungal pathogens affecting maize, causing root, stalk, and ear rot. It poses a significant threat in tropical and subtropical climates, leading to substantial losses in grain yield and quality (Olowe et al., 2018). Yield losses due to Fusarium stalk rot can range from 10% in areas with low disease incidence to as high as 30%–50% in severely affected regions. Fusarium ear rot, which is marked by grain discoloration and a reduction in grain number, not only decreases yield but also negatively impacts seed quality (Yates et al., 2003; Xu et al., 2023; Diniz et al., 2024). *Fusarium verticillioides* produces fumonisins, a group of mycotoxins which contaminate maize and are liked to various mycotoxicoses in humans and animals. Fumonisins consists of either 19 or 20 carbon backbones with hydroxyl, methyl, and tricarballylic acid moieties at different positions along the carbon backbone (Bryła et al., 2022). Biochemical analyses indicated that fumonisins are synthesized by a

polyketide synthase (PKS) gene known as FUM5 (Baird et al., 2008). Traditionally, the control of this disease has relied on chemical fungicides. However, increasing concerns over environmental toxicity and the development of fungicide-resistant strains have driven the search for safer, eco-friendly alternatives (Mclaughlin et al., 2023). Chitosan, a deacetylated derivative of chitin, has emerged as a promising biocontrol agent due to its biodegradability, non-toxicity, and potent antifungal properties. Recent studies have demonstrated that chitosan inhibits fungal growth by disrupting cell wall integrity, inducing oxidative stress, and downregulating virulence genes in *F. verticillioides*. Furthermore, formulations of chitosan have shown enhanced antifungal activity and an improved ability to trigger plant defense mechanisms (Algam & Elwagia, 2015; Debanjana et al., 2022). In parallel, *Paenibacillus* spp., a group of plant growth-promoting rhizobacteria (PGPR), exhibit broad-spectrum antimicrobial activity through the production of volatile organic compounds (VOCs), enzymes, and antimicrobial peptides. Notably, *P. polymyxa* has demonstrated efficacy in inhibiting *Fusarium* species and enhancing plant resistance (Zhai et al., 2021). Recent studies have explored the synergistic effects of combining *Paenibacillus* strains with chitosan, showing promising results in suppressing *F. verticillioides* and promoting maize root development in the rhizosphere (Zhai et al., 2021; Diniz, et al., 2024). The objective of this study was to,investigate the antifungal activity of chitosan and *Paenibacillus* spp. strains against pink ear rot, caused by *F. verticillioides* in maize *in vitro* conditions.

Materials and Methods

Isolation of the pathogen from infected maize seeds

Maize seeds (local varieties) were obtained from Khartoum North Central markets, Sudan for the purpose of pathogen isolation. Seeds were surface-sterilized using 3% (v/v) sodium hypochlorite for three minutes, followed by three rinses with sterile distilled water. The sterilized seeds were then dried under aseptic conditions on sterile Petri dishes lined with double layers of filter paper. Subsequently, the seeds were plated on potato dextrose agar (PDA, Difco, BD, USA) medium and incubated at 27±2 °C for seven days. White fungal mycelia that developed were transferred to fresh PDA plates to obtain pure cultures through sub-culturing. The purified fungal isolates were maintained on PDA slants for further studies (Hussain et al., 2013).

Identification of the pathogen

Morphological and cultural characteristics

Morphological identification was carried out based on colony growth patterns, pigmentation, arrangement of conidia and conidiophores, macroconidia and microconidia, formation of clusters and chains, septation, and presence/absence of chlamydospores of 7-day-old cultures grown on PDA at 27±2 °C. Spore slides were prepared from the cultures to examine spore shape, arrangement, and dimensions under a light microscope. Observations were compared with standard descriptions for *Fusarium* species (Leslie & Summerell, 2006; Hsuan et al., 2011).

Pathogenicity tests

The pathogenicity of the fungal isolates was tested on healthy maize seedlings (21-day-old). Seeds were germinated and grown in pots filled with a sterilized soil mixture composed of silt and sand at a 2:1 ratio. Four seeds were sown per pot. Inoculation was carried out using the soil inoculation method. The fungal inoculum was prepared by washing a 15-day-old pure *F. verticillioides* isolate with sterile distilled water. The resulting suspension was filtered through sterile cheesecloth to remove mycelial fragments. Spore concentration was adjusted to 10^4 spores/mL using a hemocytometer. Four pots were inoculated with the fungal spore suspension, while one pot was treated with sterile distilled water and served as the control. After 21 days, root and stem samples were collected from all treatments for fungal re-isolation and identification to confirm the presence or absence of *F. verticillioides*

Molecular detection

Genomic DNA extraction

Six-day-old isolates were used for DNA extraction at the Molecular Biology Unit at the National Center for Research, Khartoum. Genomic DNA was isolated using the Cetyltrimethyl-ammonium bromide (CTAB) method as described by Zhang et al. (2000) with some modifications. The fungal mycelia were frozen in liquid nitrogen and ground to a fine powder with a pestle and mortar. Fine powder was transferred to a 15 ml tube, in 5 ml of preheated (65˚C) 2XCTAB buffer containing 100 mM Tris (PH 8.0), 20 mM Ethylene diamin etetraacetic acid (EDTA) PH 8.0, 1.4 M Sodium Chloride (NaCl) and 1% Merceptoethanol. The mixture

was incubated at 60˚C for 1 h, and then mixed with an equal volume of chloroform: isoamyl alcohol (24:1), the tubes were inverted gently 10-15 times and then centrifuge at 10,000 rpm for 10 minutes, the chloroform isoamyl alcohol treatment was repeated when needed. The supernatant was transferred to a new tube to precipitate the DNA, 2/3 isopropanol and 7.5 M ammonium acetates were added and incubated for 30 minutes on ice, then centrifuged for 10 minutes at 5,000 rpm, 70% ethanol was added and centrifuged for 1 minute at low speed. The DNA pellets were then re-suspended in 100 µL of TE buffer, DNA quality was analyzed by agarose gel electrophoresis.

Polymerase Chain Reaction (PCR)

The extracted DNA was amplified by a PCR technique as described by Sambrook et al. (1989). PCR amplification reactions were carried out in 20 µl total volume of PCR mixture containing 3 µl of template DNA, 4 µl of the PCR master mix (Promega, USA) (50 unit/ml Tag DNA Polymerase in an appropriate reaction buffer pH 8.7, 400 µM of each dNTPs and 3 Mm $MgCl_2$ and 2 µl of each of the forward (FUM5F:5'TCC TAC GCG ATA CAT CCC ACC ACA AT-3') and reverse (FUM6R: 5'GAT CAA GCT CGGGGC CGT CGT TCA TAG 3') specific primer pair which amplified part of gene (419 pb.) (Proctor et al., 1999; Baird et al., 2008). The reactions were performed in a thermal cycler (ESCO, CHINA) programmed as follows: Initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 1 min, annealing at 64°C for 1 min., extension at 72°C for 1 min and a final extension step at 72°C for 7 min. The PCR products were examined by gel electrophoresis. A 1Kb DNA ladder (Solis Bio Dyne) was used as a molecular weight marker.

Anti-fungal effect of chitosan on mycelial growth *in vitro*

Chitosan extracted from shrimp shells (degree of N-deacetylation 75%; Sigma-Aldrich, U.S.A.), obtained from the Plant Pathology Laboratory, Faculty of Agriculture, University of Khartoum, was solubilized in 1% acetic acid to obtain a concentration of 10 mg/mL (stock solution). The solution was alkalized to pH 5.6 with NaOH and autoclaved at 121°C for 15 min (Algam et al, 2010). To evaluate the antifungal effect of chitosan on *F. verticillioides* mycelial growth, sterilized chitosan solutions were incorporated into sterilized, cooled (50 °C) molten PDA to obtain final concentrations of 1, 3, and 5 mg/mL, following the method of Muñoz et al. (2009). Under aseptic conditions, the amended media were thoroughly mixed in 250 mL conical flasks and poured into sterilized 9 cm Petri dishes, with four replicate plates prepared for each concentration. Plates without chitosan served as the control. After solidification, each plate was inoculated at the center with a 6 mm diameter mycelial plug taken from the actively growing margin of a 7-day-old *F. verticillioides* culture. The plates were incubated at 27±2 °C, and colony radial growth was measured daily for 10 days (Pillai et al., 2005; Hanlon et al., 2007; Algam et al., 2015). The percentage of mycelial growth inhibition was calculated using the following formula: Inhibition (%) = [(Xc – Xi) / Xc] × 100, where Xc is the mean colony diameter in the control plates, and Xi is the mean colony diameter in the chitosan-treated plates (Abd-Elkareem et al., 2006; Guo et al., 2006).

Antifungal activity of *Paenibacillus polymyxa* inoculum preparation

Six strains of *Paenibacillus polymyxa* (designated as P1, P2, P4, P6, P7, and P8) were obtained from the Plant Pathology Laboratory, Faculty of Agriculture, University of Khartoum. The isolates were cultured on Nutrient Agar (NA) medium. Subculturing of the *Paenibacillus* strains was carried out by streaking onto NA plates, followed by incubation at 28±2°C for 48 h, as described by Osman et al. (2016).

Effect of *P. polymyxa* on mycelial growth of *F. verticillioides*

The antagonistic activity of six *P. polymyxa* was evaluated against *F. verticillioides* using the dual culture technique. PDA medium was used, with four replicate plates prepared for each bacterial strain and three plates served as control. Each plate was divided into four sections and inoculated at the center with a 6 mm mycelial plug taken from a 7-day-old culture of *F. verticillioides*. The *Paenibacillus* strains, obtained from 3-day-old cultures, were streaked along the margin of each section. Control plates were left without bacterial inoculation. All plates were incubated at 27±2 °C, and the radial growth of fungal colonies was measured daily for 10 days. The percentage of mycelial growth inhibition was calculated as previously described.

Statistical Analysis

A completely randomized design (CRD) with four

replicates was employed in this study. The collected data were analyzed using analysis of variance (ANOVA), and mean comparisons were performed using Duncan's Multiple Range Test (DMRT) $P > 0.05$. All statistical analyses were conducted using SPSS version 21 software.

Results

Cultural and morphological identification

Six representative strains exhibiting typical *Fusarium* colony growth patterns on PDA were selected for morphological characterization. The colonies displayed white maycelia with pink to violet pigment with age (Fig.1A). Microscopic examination revealed that macroconidia were infrequent, while microconidia were abundant and formed in a chain and cluster (Fig.1B), furthermore, the microcondia are oval with a flattened base with no septa, borne from monophialides which occur in V-shaped pairs, chlamydospores were not produced, which are the identifying feature of *F. verticillioides*.

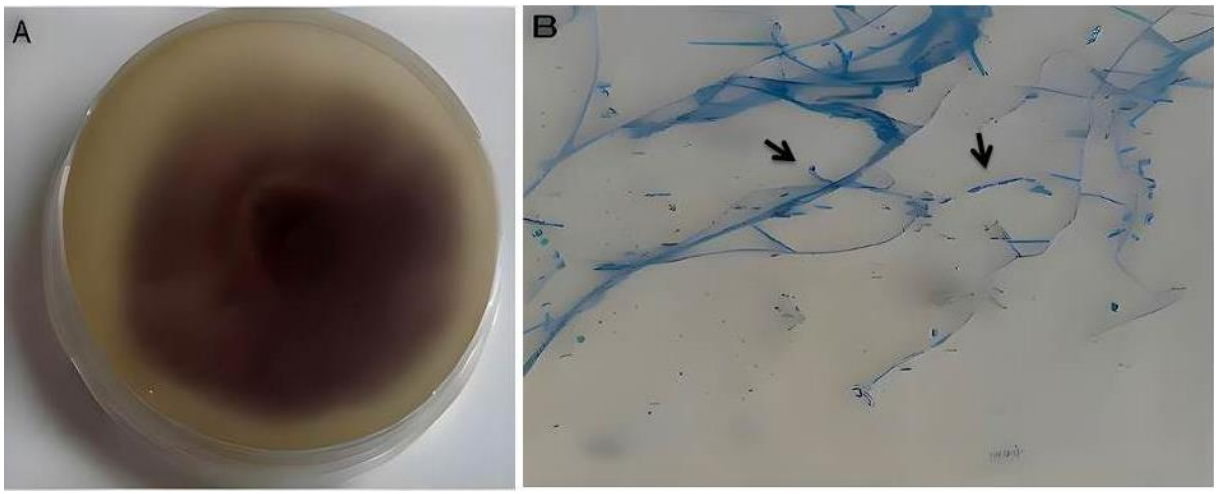

Fig. 1. Cultural and morphological characteristic of *Fusarium verticillioides*: *(*A) on PDA, (B) microconidia in chains and clusters.

Pathogenicity tests

Twenty-one days after planting, the infected seedlings showed reduced seedlings vigor symptoms and mortality in severe infection, the fungus was successfully re-isolated from both the roots and stems of the infected plants. Morphological examination, along with PCR analysis, confirmed the identity of the re-isolated pathogen as *F. verticillioides*.

Molecular detection

The identity of the *F. verticillioides* isolates was confirmed through PCR amplification of the internal transcribed spacer (ITS) region using species-specific primers. The PCR analysis yielded a product of approximately 419 bp, consistent with the expected size for *F. verticillioides* (Fig. 2).

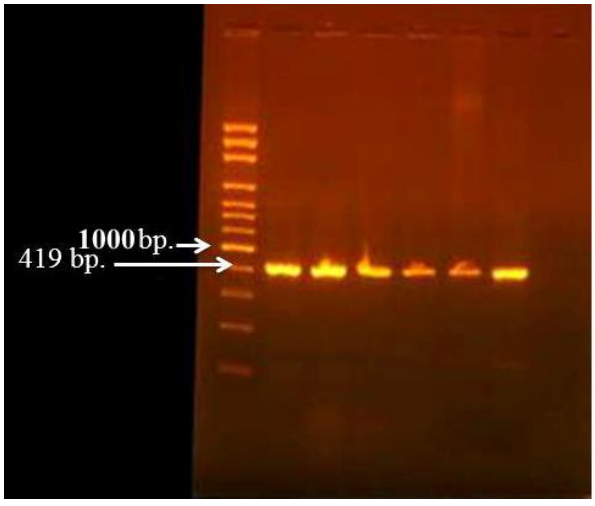

Fig. 2. PCR amplifications (419 bp.) using species-specific primers on DNA extracts from *Fusarium verticillioides* on agarose gel.

Antifungal effect of chitosan on mycelial growth of *F. verticillioides*

The growth of *F. verticillioides* was inhibited to varying degrees by chitosan at different concentrations. A clear dose-dependent response was observed, with higher concentrations exhibiting greater inhibitory effects. Chitosan at concentrations of 3 and 5 mg/mL resulted in complete inhibition (100%) of *F. verticillioides* mycelial growth. At 1 mg/ml, fungal mycelial growth was inhibited by 93.4% (Table 1).

Table 1: Effect of chitosan concentrations on the mycelial growth of *Fusarium verticilliodes.*

Chitosan concentration (mg/ml)	Mycelia growth (cm)	Inhibition %
5	0[a]	100
3	0[a]	100
1	0.37[b]	93.4
Control	5.61[c]	0
Probability	.000**	
SEM	0.89	

Means followed by the same letter(s) were not significantly different at $P<0.05$.

Statistical analysis revealed no significant difference between the inhibitory effects of 3 and 5 mg/ml concentrations of chitosan on mycelial growth of *F. verticillioides*. However, both concentrations showed highly significant differences ($P < 0.01$) when compared to the control. The impact of different chitosan concentrations on the mycelial growth of *F. verticillioides* is visually represented in Fig. 3.

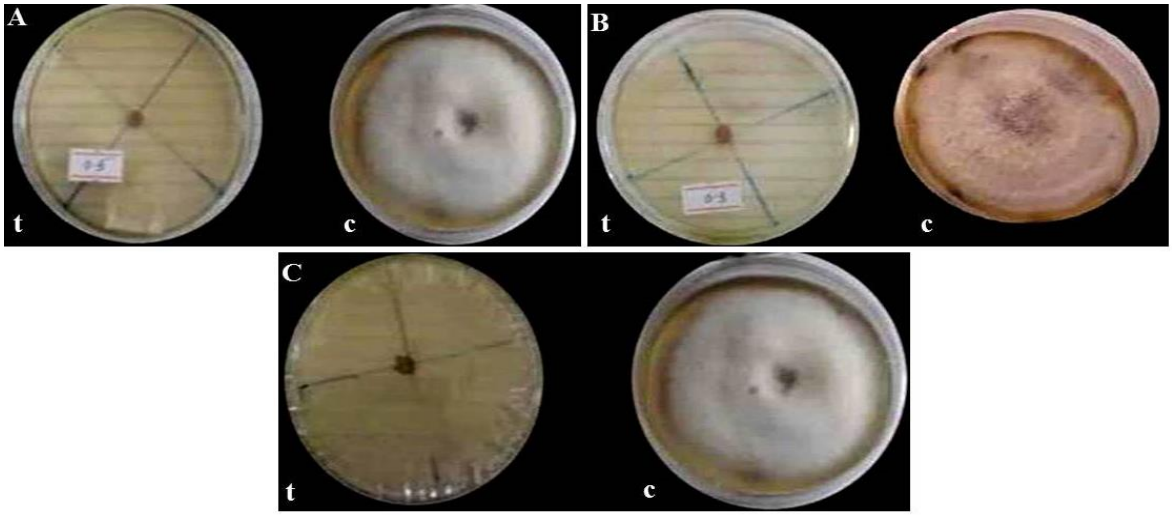

Fig. 3. Inhibitory effect of chitosan. (A) 5 mg/ml, (B) 3 mg/ml and (C) 1 mg/ml concentrations on the mycelia growth of *Fusarium verticillioides* (t: tratments, c: controls).

Effect of *Paenibacillus* strains on the mycelial growth of *F. verticillioides*

The results presented in Fig. 4 demonstrate that all tested strains of *P. polymyxa* exhibited inhibitory effects on the mycelial growth of *F. verticillioides*, although the percentage of inhibition varied among the strains. Strains P4, P7, and P8 showed the highest antagonistic activity (Fig. 4 D, F and G), with inhibition rates of 47.6%, 42.3%, and 42.0%, respectively. In contrast, strains P2, P6, and P1 exhibited lower inhibition rates (Fig. 4 C, E and B) of 17.2%, 18.0%, and 28.7%, respectively (Table 2). Statistical analysis revealed highly significant differences ($P < 0.01$) between the treatments and the control.

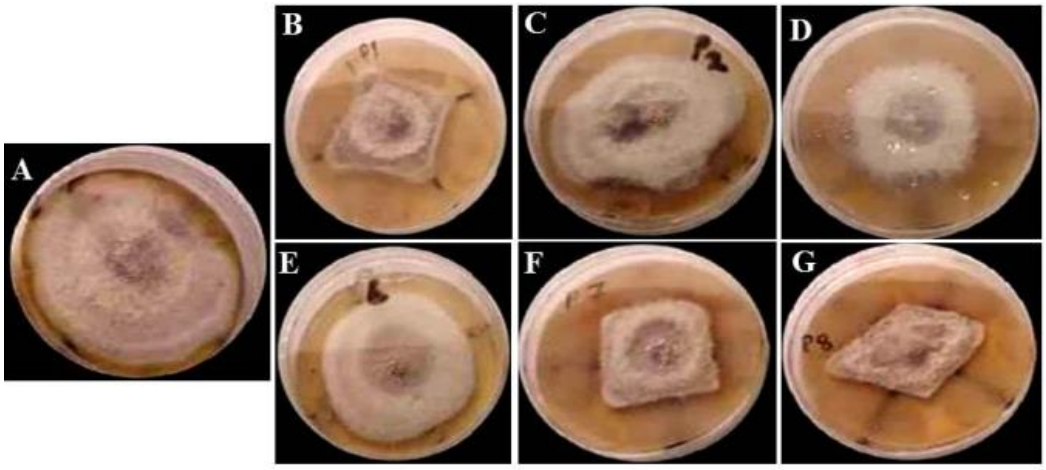

Fig. 4. Inhibitory effects of *Paenibacillus polymyxa* strains on the mycelial growth of *Fusarium verticillioides*. **(**A) Control, (B) Strain P1, (C) starin P2, (D) strain P4, (E) strain P6, (F) strain P7 and (G) strain P8.

Table 2: Inhibitory effects of *Paenibacillus polymyxa* strains on the mycelial growth of *Fusarium verticillioides*.

Bacterial strains	Mycelial growth (cm)	Inhibition (%)	Significance
Control	6.52[a]	0	***
P1	4.65[bc]	28.7	***
P2	5.36[b]	17.8	NS
P4	3.42[d]	47.5	NS
P6	5.34[b]	18	
P7	3.76[cd]	42.3	
P8	3.74[cd]	42.32	
SEM	0.4847		

***= significantly different at P < 0.01, NS= not significantly different.

Means followed by the same letter(s) were not significantly different , P<0.05

Discussion

Fusarium verticillioides is widely distributed throughout the world and is particularly associated with maize disease where it can cause stalk rot and ear rot that results in significant yield losses and reductions of grain quality (Omotayo & Babalola, 2023). The use of chemical fungicides in the field is a risk to environment and human, therefore, alternative biocontrol agents are needed (Vanessa et al., 2019). In this study, *F. verticillioides* was isolated from infected maize seed and identified based on fungal morphological characteristics. For further confirmation, the isolated fungus was targeted for PCR-based identification. The expected PCR product with approximately 419 bp. was obtained when DNA was amplified with species-specific primer.

Furthermore, chitosan concentrations (1, 3, and 5 mg/ml) were screened in vitro against *F. verticillioides*. The application of chitosan gave promising results as strong antifungal activity against *F. verticillioides*, the mycelia growth was inhibited by chitosan treatments, varying from partial inhibition at low concentration to full inhibition at high concentrations. Moreover, approximately similar results were obtained when seeds of maize were treated with *F. verticillioides* and seeded in different concentration of chitosan, these results were consistent with Algam et al. (2010) who stated that chitosan exhibited strong antibacterial activity against *Ralstonia sonlanacearum*. Osman et al. (2016) demonstrated that chitosan was also exhibited good antibacterial effect against the *Xanthomonas malvacearum* the causal agent of black arm disease of cotton. Similar results were also obtained by Muñoz & Moret (2010) who demonstrated that chitosan exhibited strong antifungal activity against different plant pathogenic fungi. Algam & Elwagia (2015) found that chitosan had strong antifungal activity against *Alternaria alternata* which cause early blight of tomato.

Taken together, the mycelia growth inhibition increase as the concentration of chitosan increases, and the inhibition caused by the chitosan might be due to interfering with enzyme's activities. Similar results were reported by Guerra-Sánchez et al. (2009) who found that chitosan oligomers diffuse inside hyphae interfering on the enzyme's activity responsible for the fungus growth. Furthermore, the intensity of degradation action of chitosan on fungal cell walls is also dependent upon the concentration and local pH (Stössel et al., 1984). It has been stated that the antifungal mechanism of chitosan involves cell wall morphogenesis with chitosan molecules interfering directly with fungal growth (El Ghaouth et al., 1992). As we demonstrated in this study, a highly significant difference was observed between the control and the tasted strains of *P. polymyxa*. The inhibition varied from one strain to another, our results are inconsistent with result reported by Figueroa-López et al. (2016), the author highlighted that the growth of *Fusarium* sp. was inhibited by using *Paenibacillus* spp that possess significant antifungal properties against *Fusarium* pathogens through the production of diffusible and volatile compounds, especially lipopeptides like fusaricidin, as well as enzymatic activities. Moreover, it has been found that *Paenibacillus* spp. inhibited the growth of *Ralstonai sonlanacearum* (Algam et al., 2010). Furthermore, it was found that *Paenibacillus* strains inhibit the growth of *Xanthomonas malvacearum* (Osman et al., 2016).

Conclusion and Recommendation

This study confirmed the identity of *F. verticillioides*, the causal agent of ear rot disease in maize. It also demonstrated the antifungal potential of chitosan and six strains of *P. polymyxa* against *F. verticillioides* under *in vitro* conditions. Among the tested treatments, chitosan at concentrations of 1, 3, and 5 mg/mL effectively inhibited both mycelial growth and spore germination of *F. verticillioides*, with higher concentrations exhibiting complete inhibition. Furthermore, chitosan treatments were found to be more effective than the tested *P. polymyxa* strains in suppressing fungal growth. These findings highlight the potential of chitosan as a promising and environmentally safe alternative to synthetic fungicides for controlling *F. verticillioides* in maize. Given its dual function as both an antimicrobial and plant defense inducer, chitosan represents a sustainable tool for integrated disease management.

However, further research is recommended to evaluate its efficacy under field conditions, assess its broad-spectrum activity against other plant pathogens, and optimize its application methods. Future studies on different crops will be valuable to fully explore the potential of chitosan as a natural agrochemical for plant disease control

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Conflict of interest

The authors have no conflicts of interest to declare.

CRediT author statement

S. A. Algam: Supervision, writing & reviewing. **L. Amasaib:** Laboratory works **& E. H. Baillo:** reviewing.

References

Abd-Elkareem, F., Elmougi, S. N., Elgamal, N. G., & Fatouhy, Y. O. (2006). Use of chitin and chitosan against tomato root rot disease under greenhouse condition. *Research Journal of Agricultural and Biological Sciences*, *2*(4), 147–152.

Akande, S., & Lamidi G. (2006). Performance of quality protein maize varieties and disease reaction in the derived-savanna agro-ecology of South-West Nigeria. *African Journal of. Biotechnology*, *5*(19), 1744–1748.

Algam, S., Xie, G., Yu, S., Su, T., & Lersen, J. (2010). Effect of *Paenibacillus* strains and chitosan on growth promotion and control of Ralstonia wilt of tomato. *Journal of Plant Pathology*, *92* (3), 593–600. DOI: 10.4454/jpp.v92i3.303.

Algam, S., & Elwagia, M. (2015). Evaluation of chitosan efficacy on tomato growth and control of

early blight disease. *Jordan Journal of Agricultural Sciences*, *11*(1), 27–36.

Baird, R., Hamed, K., Abbas, H., Windham, G., Williams, P., Baird, S., Ma, P., Kelley, R., Hawkins, L., & Scruggs, M. (2008). Identification of select fumonisin forming *Fusarium* species using PCR applications of the polyketide synthase gene and its relationship to fumonisin production *in vitro*. *International Journal of Molecular Sciences*, *9*, 554–570. DOI: 10.3390/ijms9040554.

Booth, C. (1972). The Genus *Fusarium*. *Taxon*, *21*(1), 180. https://doi.org/10.2307/1219251.

Debnath, D., Samal, I., Mohapatra, C., Routray, S., Kesawat, M. S., & Labanya, R. (2022). Chitosan: An autocidal molecule of plant pathogenic fungus. *Life*, *12*(11), 1908. https://doi.org/10.3390/life12111908.

El Ghaouth, A., Arul, J., Grenier, J., & Asselin, A. (1992). Antifungal activity of chitosan on two post-harvest pathogens on strawberry fruits. *Phytopathology*, *82*, 398–402.

Figueroa-López, A. M., Cordero-Ramírez, J. D., Martínez-Álvarez, J. C., López-Meyer, M., Lizárraga-Sánchez, G. J., Félix-Gastélum, R., Castro-Martínez, C., & Maldonado-Mendoza, I. E. (2016). Rhizospheric bacteria of maize with potential for biocontrol of *Fusarium verticillioides*. *Springerplus*, *15*(5), 330. DOI: 10.1186/s40064-016-1780-x.

Leslie, J., & Summerell, B. A. (2006). The *Fusarium* Laboratory Manual. DOI:10.1002/9780470278376.

Guerra-Sánchez, M. G., Vega-Pérez, J., Velázquez-del Valle, M. G., & Hernández-Lauzardo, A. N. (2009). Antifungal activity and release of compounds on *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. by effect of chitosan with different molecular weights. *Pesticide Biochemistry and Physiology*, *93*(1), 18–22. DOI: 10.1016/j.pestbp.2008.09.001.

Diniz, G. F. D., Figueiredo, J. E. F., Canuto, K. M., Cota, L. V., Souza, A. S. Q., Simeone, M. L. F., Tinoco, S. M. S., Ribeiro, P. R. V., Ferreira, L. V. S., Marins, M. S., de Oliveira-Paiva, C. A., & Dos Santos, V. L. (2024). Chemical and genetic characterization of lipopeptides from *Bacillus velezensis* and *Paenibacillus ottowii* with activity against *Fusarium verticillioides*. *Frontiers in Microbiology*, *15*, 1443327. DOI: 10.3389/fmicb.2024.1443327.

Guo, Z., Chen, R., Xing, R., Liu, S., Yu, H., Wang, P., Li, C., & Li, P. (2006). Novel derivatives of chitosan and their antifungal activities in vitro. *Carbohydrate Research*, *341*(3), 351–354. DOI: 10.1016/j.carres.2005.11.002.

Hanlon, A., Taylor, M., & Dick, J. D. (2007). Agar dilution susceptibility testing. In R. Schwalbe, L. S. More, & A. C. Goodwin (eds.), *Antimicrobial Susceptibility Testing Protocols*, (pp. 91–97), Taylor & Francis Group Boca Raton London New York.

Hussain, W., Hussain, A., Ishtiaq, M., Azam, S., & Hussain, T. (2013). Pathogenicity of two seed-borne fungi commonly involved in maize seeds of eight districts of Azad, Jammu and Kashmir, Pakistan. *African Journal of Biotechnology*. *12*(12), 1360–1370.

Hsuan, H., Salleh, B., & Zakaria, L. (2011). Molecular Identification of *Fusarium* species in *Gibberella fujikuroi* species complex from rice, sugarcane and maize from Peninsular Malaysia. *International Journal of Molecular Sciences*, *12*, 6722–6732. https://doi.org/10.3390/ijms12106722.

Kiranjot, K., Jaspal, K., Anita, P., & Karmjit, S. (2020). Cultural, morphological and molecular

characterization of *Fusarium verticillioides* causing maize ear rot under Punjab condition. *International Journal of Current Microbiology and Applied Sciences*. *9*(10), 1698–1706. https://doi.org/10.20546/ijcmas.2020.910.205.

Bryła, M., Pierzgalski, A., Zapaśnik, A., Uwineza, P. A., Ksieniewicz-Woźniak, E., Modrzewska, M., & Waśkiewicz, A. (2022). Recent research on *Fusarium* mycotoxins in maize—A review. *Foods*, *11*(21), 3465. https://doi.org/10.3390/foods11213465.

McLaughlin, M., Roy, M., Abbasi, P., Carisse, O., & Yurgel, S., Ali, S. (2023). Why do we need alternative methods for fungal disease management in plants? *Plants* (Basel). *1012*(22), 3822. https://doi.org/10.3390/plants12223822.

Mohammed, M., A., Hassan, A., & Fatih, E. (2015). A proposal for the release of two introduced maize hybrids for Central and Northern Sudan. A paper was submitted to the Variety Release Committee. Khartoum, Sudan.

Muñoz, Z., & Moert, A. (2010). Sensitivity of *Botrytis cinerea* to chitosan and Acibenzolar-S-methyl. *Pest Management Science*, *66*, 974–979. DOI: 10.1002/ps.1969.

Muñoz, Z., Moret, A., & Garcés, S. (2009). Assessment of chitosan for inhibition of *Colletotrichum* sp. on tomatoes and grapes. *Crop Protection (Guildford, Surrey)*, *28*(1), 36–40 https://doi.org/10.1016/j.cropro.2008.08.015.

Olowe, O. M., Olawuyi, O. J., Sobowale, A. A., & Odebode, A. C (2018). Role of arbuscular mycorrhizal fungi as biocontrol agents against *Fusarium verticillioides* causing ear rot of *Zea mays* L. (maize). *Current Plant Biology*, *15*, 30–37. DOI: 10.1016/j.cpb.2018.11.005.

Omotayo, O. P., & Babalola, O. O. (2023). *Fusarium verticillioides* of maize plant: Potentials of propitious phytomicrobiome as biocontrol agents. *Frontiers in Fungal Biology*, *7*(4), 1095765. DOI: 10.3389/ffunb.2023.1095765.

Osman, T. M., Algam, S. A., Ali, M. E., Osman, E. H., & Mahdi, A. A. (2016). In vitro screening of some biocontrol agents against *Xanthomonas axonopodis* pv. *malvacearum* isolated from infected cotton plants *International Journal of Agriculture, Forestry and Plantation*, *2*, 270–278.

Proctor, R., Desjardins, A., Plattner, R., & Hohn T. M. (1999). A polyketide synthase gene required for biosynthesis of fumonisin mycotoxins in *Gibberella fujikuroi* mating population A. *Fungal Genetics and Biology*, *27*, 100–112. DOI: 10.1006/fgbi.1999.1141.

Pillai, S., Moellering, R., & Eliopoulos, G. (2005). Antimicrobial combinations. In V. Lorian (ed.) *Antibiotics in Laboratory Medicine*, (pp. 365–440), (15th ed.). Lippincott Williams and Wilkins, Philadelphia, Pa, USA.

Sambrook, J., Fritschi, E. F., & Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.

Stössel, P., & Leuba, J. L. (1984). Effect of chitosan, chitin and some aminosugars on growth of various soilborne phytopathogenic fungi. *Journal of Phytopathology*, *111*(1), 82–90. DOI: 10.1111/j.1439-0434.1984.tb04244.x.

Vanessa, G., Zachetti Eugenia, C., María , J., Sofía, N., & María, L. (2019). Preliminary study on the use of chitosan as an eco-friendly alternative to control *Fusarium* growth and mycotoxin production on maize and wheat. *Pathogens*, *8*(1), 29. DOI: 10.3390/pathogens8010029.

Xu, Y., Zhang, Z., Lu, P., Li, R., Ma, P., & Wu, J. (2023). Increasing *Fusarium verticillioides* resistance in maize by genomics-assisted breeding: methods, progress, and prospects. *Crop Journal*, *11*, 1626–1641. https://doi.org/10.1016/j.cj.2023.07.004.

Yates, I., Arnold, J., Hinton, D., Basinger, W., & Walcott, R. (2003). *Fusarium verticillioides* induction of maize seed rot and its control. *Canadian Journal of Botany*, *81* (5), 422–428. DOI: 10.1139/B03-034.

Zhai, Y., Zhu, Jx., & Tan, T. (2021). Isolation and characterization of antagonistic *Paenibacillus polymyxa* HX-140 and its biocontrol potential against Fusarium wilt of cucumber seedlings. *BMC Microbiology*, *21*, 75. DOI: 10.1186/s12866-021-02131-3.

Zhang, D., Cervantes, J., Huamán, Z., Carey, E., & Ghislain, M. (2000). Assessing genetic diversity of sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars from tropical America using AFLP. *Genetic Resources Crop Evolution*, *47*, 659–665. DOI: 10.1023/A:1026520507223.