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Biological control of *Aspergillus flavus* infection and growth promotion of peanut seedlings by *Lactiplantibacillus plantarum* and *Levilactobacillus brevis*

Quoc-Duy Nguyen[1] and Anh Duy Do[2*]

Abstract

Background *Aspergillus* spp. infection might induce negative effects on peanut seeds, including decreased germination rates and suppressed seedling vigor. Furthermore, *A. flavus* can secret aflatoxin, regarding food safety and human health. The prolonged use of fungicides for treating mold infections has raised concerns regarding the emergence of fungicide-resistant strains, environmental pollution, and adverse effects on human health. The usage of lactic acid bacteria, including *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* for the management of plant diseases, has garnered increasing attention in recent years as a viable alternative to chemical-based therapies. This study aimed to investigate the efficacy of LABs in pre-treating peanut seeds as a biological solution against *A. flavus* infection before cultivation.

Results *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* have demonstrated the ability to suppress *A. flavus* in vitro. In the in vivo investigation, pre-treatment of peanut seeds with cell-free supernatant derived from *L. plantarum* (LP-CFS) and *L. brevis* (LB-CFS) significantly reduced *A. flavus* infection levels. The conidial count decreased from 8.63 log conidia/g in the untreated group to 5.35 log conidia/g with LP-CFS treatment and 4.59 log conidia/g with LB-CFS treatment. Additionally, *A. flavus* infection reduced the germination rate of peanut seeds to only 20.4% compared to 63.6% in the control group. In comparison, pre-treatment with LP-CFS and LB-CFS increased the germination rate to 75.6% and 76.8%, respectively, and further improved the vigor index in *A. flavus*-infected peanut seeds.

Conclusion The present findings indicated that bioactive compounds derived from *L. plantarum* and *L. brevis* emerge as promising candidates for treating peanut seeds, effectively protecting them against *A. flavus* infection. Moreover, these compounds facilitate the growth of seedlings, which could be a potential alternative to chemical fungicides, and contribute to sustainable agricultural development.

Keywords *Aspergillus flavus*, Fungicide, Germination rates, *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, Vigor index

Background

Peanuts (*Arachis hypogaea* L.) serve as both an essential oil crop and a staple food source, contributing significantly to food security in the world. In Vietnam, the peanut cultivation area is over 0.18 million hectares, yielding approximately 0.24 million tons annually (Kim et al. 2020). Peanuts are susceptible to mold infection in both pre- and post-harvest, as well as during storage.

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Aspergillus flavus frequently present in peanuts presents a concern by inducing storage-related harm, leading to seed deterioration and reducing the germination ability of peanut seeds (Norlia et al. 2019). Furthermore, this fungus might produce aflatoxin, which is classified as a type I carcinogen, thereby posing negative effects on human health (Marchese et al. 2018). To mitigate the negative effects of mold infection, cultivators commonly employ strategies such as applying calcium carbonate to soil, using fungicides, or subjecting seeds to heat treatment at 50 °C before planting. However, the sustainability of these methods remains a concern, as no approach has proven to be entirely effective and environmentally friendly. Additionally, the usage of fungicides to manage *A. flavus* might increase the risk of developing fungicide-resistant mold strains, environmental pollution, and negative effects on food safety (Malik et al. 2021).

The increasing demand for food safety and sustainable agricultural practices requires the application of environmentally friendly pest management strategies as an alternative strategy for chemical fungicides. Biological control involves decreasing plant diseases by employing biological agents such as beneficial microorganisms and their secondary metabolites, et cetera (Sundin et al. 2016). Lactic acid bacteria (LAB) have been shown a history of usage in the food industry, thereby being considered generally regarded as safe (GRAS) (Goldstein et al. 2015). Recently, LAB have emerged as potential biological control agents in the management of various plant pathogens. *Lactiplantibacillus plantarum* KB2 have been shown to produce a wide range of antifungal substances, such as reuterin and fatty acids, which might prevent the infection of several potato pathogens infestation, including *Alternaria solani, A. tenuissima, A. alternata, Phoma exigua, Rhizoctonia solani,* and *Colletotrichum coccodes* (Steglińska et al. 2022). Furthermore, *Weissella confusa* and *Pediococcus pentosaceous* have been demonstrated to prevent the growth of various fungal diseases in fruit, including *Penicillium* spp., *Aspergillus* spp., *Fusarium* spp., (Crowley et al. 2013). Additionally, LAB might release phytohormones and metabolites that enhance the stress resistance in plants. According to Mussa et al. (2018), *Enterococcus* spp., might solubilize phosphate, while (Higdon et al. 2020), indicated that *Lactococcus lactis* isolated from Sierra Mixe maize showed nitrogen metabolism ability and synthesis of siderophores, which contributes to enhancing plant growth. *Leuconostoc* sp., and *Lactobacillus* sp., have been demonstrated to produce gibberellin and indole-3-acetic acid (IAA), which contribute to the elongation of roots, enhance stress resistance, and stimulate the flowering in plants (Abhyankar et al. 2021). In addition, pre-treatment of peanut seeds with a mixture of IAA-producing LAB strains has been shown to improve seed germination, with the vigor index showing a 22.4% increase compared to the control group (Nguyen et al. 2021).

In Vietnam, corn, rice, peanuts, and sesame are susceptible to *A. flavus* infection, leading to aflatoxin contamination (Do et al. 2020). Actually, not much research has been done on the use of biological agents for pre-treating peanut seeds before sowing to mitigate mold infection and enhance plant growth. This study aimed to evaluate the efficacy of LAB strains for the pre-treatment of peanut seeds to prevent *A. flavus* infection and further enhance germination and seedling development.

Methods

Microorganisms and peanut seeds

The LAB strains used in this study including *Lactiplantibacillus plantarum* (accession number: PP515266), *Levilactobacillus brevis* (accession number: PP116083), *Leuconostoc lactis* (accession number: PP515272), *Weissella cibaria* (accession number: PP515280) were obtained from the Food laboratory, Department of Food Technology, Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh city, Vietnam. *Lactobacillus rhamnosus* ATCC 53103, *Lactobacillus casei* ATCC 393, *Aspergillus flavus* isolated from peanut seeds were obtained from Microbiology laboratory, Department of Biotechnology, NTT Hi-tech Institute, Nguyen Tat Thanh University, Ho Chi Minh city, Vietnam. *A. flavus* was maintained in potatoes dextrose agar (Himedia, India), LABs were maintained in De Man–Rogosa–Sharpe (MRS) agar (Himedia, India). Peanut seeds (*Arachis hypogaea* L.) variety GV10 were purchased from cultivation farms in Binh Duong province, Vietnam.

Antagonistic activity of LABs against *A. flavus* in vitro using dual culture assay

Dual culture assay was employed to preliminary evaluate the antagonistic activity of LAB strains against *A. flavus* (Dugassa et al. 2021). Each of LAB strains was cultured in two lines on yeast extract–peptone–dextrose (YPD) medium, positioned 3 cm away from the center of the plate. Subsequently, a 5-mm-diameter mycelial plug of *A. flavus* was inoculated into the plate's center. The group that only inoculated *A. flavus* was referred to as an untreated group. These plates were incubated at 28 °C, and the radial growth of *A. flavus* was measured after 7 days. The inhibition of *A. flavus* growth attributable to LAB presence was quantified as the reduction in mycelial radial growth on plates containing LAB compared to the untreated group.

Indole-3-acetic acid (IAA) production assay

IAA production was quantified following the method outlined by Gang et al. (2019). LAB strains were inoculated into MRS broth and incubated at 37 °C for 24 h. Subsequently, the supernatant was collected by centrifugation at 10,000×*g* for 5 min. One hundred µl of the supernatant derived from each LAB strain was mixed with 200 µl of Salkowski's reagent (Sigma Aldrich, USA) and incubated in the dark for 15 min. The absorbance of the reaction mixture was measured at a wavelength of 530 nm using C-7000UV spectrometer (Peak instrument, USA). The IAA content was determined using the IAA standard curve equation in the concentration range of 0–100 µg/ml.

Preparation of the cell-free supernatant derived from *L. plantarum* and *L. brevis* cultures

Lactiplantibacillus plantarum and *Levilactobacillus brevis* were individually cultured in YPD medium at 37 °C for 24 h. The cell-free supernatant (CFS) derived from each LAB was prepared by centrifugation at 10,000×*g* for 5 min, then filtrated using a 0.22-µm polycarbonate membrane filter (Poornachandra Rao et al. 2019). The CFSs derived from *L. plantarum* and *L. brevis* were referred to as LP-CFS and LB-CFS, respectively. The pH-neutralized CFSs were prepared by adjusting each CFS to pH 7, using 1N NaOH. Heat-treatment CFSs were prepared by incubating each CFS at 95 °C for 15 min (Danial et al. 2021).

Effect of CFSs derived from LAB cultures on the growth of *A. flavus* in vitro

A. flavus conidia were harvested from a 7-day-old PDA culture medium and then resuspended in YDP broth and the conidia concentration was determined, using a hemocytometer. The fresh CFSs, pH-neutralized CFSs, and heat-treated CFSs derived from *L. plantarum* and *L. brevis* cultures were individually supplemented to the 50 ml YPD broth at the concentration of 20%. *A. flavus* conidia were inoculated in YPD broth containing each CFS at a concentration of 5×10^6 conidia/ml. The group that only inoculated *A. flavus* without any CFS supplement was referred to as a control group. All treatment cultures were incubated at 28 °C on a rotary shaker. *Aspergillus flavus* mycelial was collected after 7 days of cultivation, using centrifugation at 10,000×*g*, and then were dried at 50 °C to a moisture content of less than 10%. The biomass of *A. flavus* was determined by weighting the dried mycelia (Shakeel et al. 2018).

Antagonistic activity of CFS derived from *L. plantarum* and *L. brevis* against *A. flavus* on peanut seeds

The peanut seeds underwent surface sterilization by sequential soaking in 70% alcohol for 30 s, followed by 20% NaOCl for 30 s, and rinsing with sterile distilled water. Subsequently, peanut seeds wash water was cultured on PDA and tryptone soya agar (TSA) (Himedia, India) to confirm the absence of bacterial and fungal contaminants. After surface sterilization, the peanut seeds were soaked in LP-CFS or LB-CFS individually for 30 min, followed by the removal of the CFSs. Fifty pre-treated peanut seeds were then placed in sterile petri plates, and then *A. flavus* conidia were introduced at a density of 1×10^5 conidia/g. The group neither infected with *A. flavus* nor pre-treated with CFSs was referred to as a control group. The group that only infected *A. flavus* without pre-treating with CFSs was indicated as the infection group. The positive control was conducted by treating the *A. flavus*-infected peanut seeds using carbendazim 0.3% (v/w) following the manufacturer's instructions. The fungal biomass presence on seed surfaces was harvested, performing serial dilutions in phosphate-buffered saline (PBS), plated on PDA, and counted the colonies formed after 48–72 h incubation at 28 °C (Trinh et al. 2023).

Effect of LP-CFS and LB-CFS pre-treatment on the germination and the growth of seedlings in *A. flavus*-infected peanut seeds

The peanut seeds underwent surface sterilization and were subsequently soaked in sterile warm water at 40 °C for 4 h (Hung and Chen 2022). Then, the seeds were soaked in LP-CFS or LB-CFS for 30 min. *Aspergillus flavus* conidia were introduced at a density of 1×10^5 conidia/g. The control group included seeds neither infected with *A. flavus* nor pre-treated with CFSs, while the infection group consisted of seeds solely infected with *A. flavus* without CFS pre-treatment. Additionally, a positive control was included, wherein *A. flavus*-infected peanut seeds were treated with carbendazim 0.3% (v/w). Fifty peanuts from each treatment were then transferred to individual sterile plastic boxes measuring 30×30 cm, lined with moist cotton. The germination percentage (GP%) were investigated after 3 days, and the disease incidence (DI), seedling disease severity index (DSI) (Dieme et al. 2018), and seedling vigor index (VI) (Singh et al. 2019) were investigated after 7 days as follows:

$$\mathrm{GP} = \frac{\text{Total germinated seeds}}{\text{Total seeds were sown}} \times 100\%$$

$$\mathrm{DI} = \frac{\text{Total of seedlings showing mold infection}}{\text{Total seeds were sown}} \times 100\%$$

$$\mathrm{DSI} = \frac{(n0 \times 0) + (n1 \times 1) + (n2 \times 2) + (n3 \times 3) + (n4 \times 4) + (n5 \times 5)}{\text{Total seeds were sown}}$$

where n (0–5) denotes total number of seedlings with the same disease symptoms at each disease level.

Disease level: 0—absent of mycelium appears (0%), 1—mycelium begins to present on the surface of seedling (1–20%), 2—mycelium presence on the surface of seedling (20–40%), 3—spores begin to present on the surface of seedling and the growth of secondary roots were suppressed (40–60%), 4—the seedling root were soft, and completely covered with spores (60–80%), 5—the seedlings are rotten and cannot grow (80–100%)

$$\mathrm{VI} = \text{Germination percentage (\%)} \times \text{Seedling length (cm)}$$

Superoxide dismutase and catalase activities in peanut seedlings

Peanut seedlings aged 7 days in each group were ground in liquid nitrogen using mortars and pestles to damage the cellulose cell wall (Blainski et al. 2018). The lysed samples were used to measure SOD and catalase activity using assay kits from Cayman Chemical, Inc., Ann Arbor, MI, USA, following the manufacturer's instructions. The results were quantified as fold-change relative to the control group.

Statistical analysis

Each treatment was replicated three times using a completely randomized methodology. The data were analyzed using SAS 9.4 software (SAS, Inc., Cary, NC, USA) and were given as the mean ± standard error of the mean of triplicate readings. The statistical significance among groups was assessed using Tukey's test, with a significance level of $p < 0.05$.

Results

Effect of lactic acid bacteria on the growth of *A. flavus* in vitro using dual culture assay

The potential inhibition of lactic acid bacteria on the growth of *A. flavus* was screened using a dual culture assay. Among 6 LAB strains, *L. plantarum* and *L. brevis* indicated a significant inhibitory effect on the radial growth of *A. flavus* mycelium (Fig. 1A, B). Additionally, the preliminary screening demonstrated that, *L. plantarum* and *L. brevis* showed significant potential for plant growth enhancement by secretion of phytohormone as indole-3-acetic acid (IAA) in vitro (Fig. 1C). Therefore, *L. plantarum* and *L. brevis* were selected for further investigation.

Effects of cell-free supernatant derived from *L. plantarum* and *L. brevis* cultures on the growth of *A. flavus* mycelia in vitro

The antifungal activity of LP-CFS and LB-CFS was evaluated in YPD broth. The results in Fig. 2A demonstrated a positive relationship between the concentration of CFSs and the inhibition of *A. flavus* mycelium growth. In the untreated group, the *A. flavus* biomass was approximately 1055.6 mg/flask (Fig. 2A). However, the presence of 20% CFSs in the culture medium led to low *A. flavus* mycelia growth, with dry biomass was 258.6 mg/flask and 230.5 mg/flask in the LP-CFS and LB-CFS treatment groups, respectively (Fig. 2A). Besides that, the inhibitory effects were still maintained even after neutralizing the pH (Fig. 2B). However, heat treatment at 95 °C for 15 min decreased the mold inhibition efficacy of CFSs, compared to the fresh CFSs (Fig. 2B). These findings suggested that the inhibitory effects of *L. plantarum* and *L. brevis* on the growth of *A. flavus* mycelia were not attributable to the physical contact or lowering pH value, but rather to the release of bioactive molecules into the culture medium.

In-planta antifungal and seed germination-promoting CFSs derived from *L. plantarum* and *L. brevis*

The efficacy of LP-CFS and LB-CFS in preventing *A. flavus* infection on peanut seeds was evaluated, with carbendazim serving as a positive control. As shown in Fig. 3A, C, the infection group demonstrated a high density of *A. flavus* conidia characterized by the yellow color, with the density of 8.63 log conidia/g peanut seeds. Interestingly, the growth of *A. flavus* was significantly suppressed in the peanut seeds which pre-treated with CFSs derived from *L. plantarum* (5.35 log conidia/g) and *L. brevis* (4.59 log conidia/g), even overcoming carbendazim treatment (6.63 log conidia/g) (Fig. 3A, C).

The germination ability of peanut seeds treated with LP-CFS and LB-CFS was further investigated. It was observed that *A. flavus* infection resulted in a significant decreased in the germination rate of peanut seeds (20.4%) compared to the control group (63.6%) (Fig. 3B, D). Interestingly, pre-treatment with LP-CFS and LB-CFS significantly improved the germination rate to 75.6 and 76.8%, respectively (Fig. 3B, D). These results highlight the potential effectiveness of both *L. plantarum* and *L. brevis* in preventing the contamination of *A. flavus*

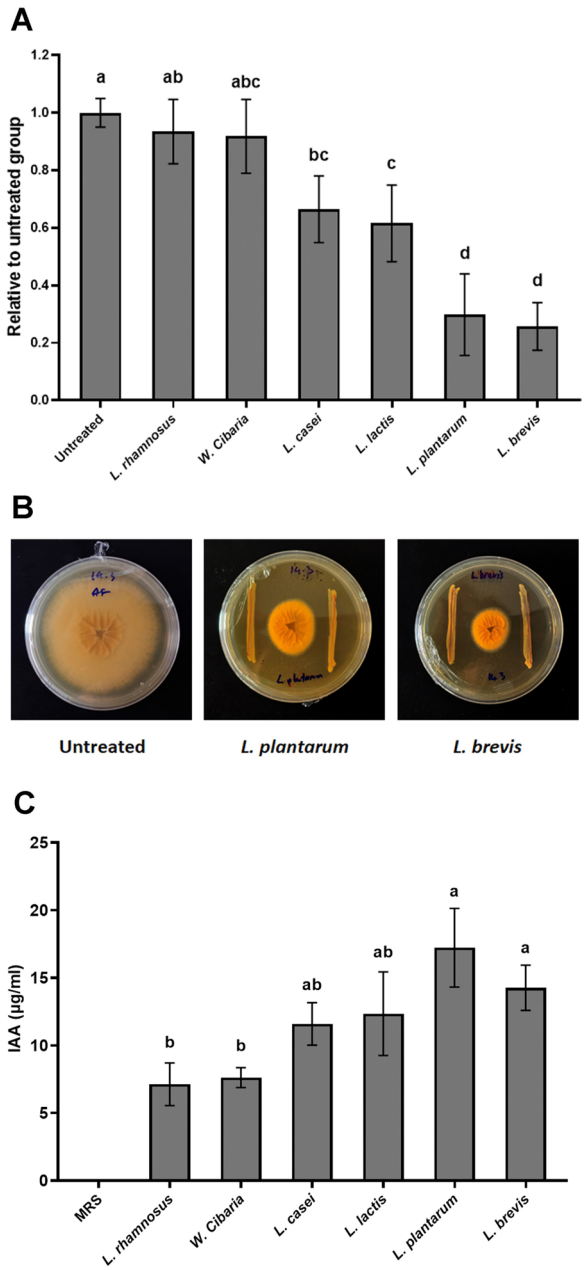

Fig. 1 **A** Inhibitory effect of lactic acid bacteria (LAB) against *Aspergillus flavus* in dual culture on PDA culture medium after 7 days of cultivation. **B** Representative image of the effect of LAB strains on decreasing the radial expansion of *A. flavus* mycelia. **C** The phytohormone indole-3-acetic acid (IAA) content in LAB cultures supernatant after 24 h of cultivation. Data are presented as the means of triplicate analysis ± standard deviation. The statistical analysis was conducted using ANOVA followed by Tukey's test ($p < 0.05$), with lowercase letters (a–c) indicating significant differences between groups

on peanut seeds and further enhancing the germination ability of *A. flavus*-infected peanut seeds.

In-planta plant-promoting ability of CFSs derived from *L. plantarum* and *L. brevis*

The efficacy of LP-CFS and LB-CFS in promoting peanut seed growth was assessed after 7 days of sowing, using parameters such as DI, DSI, VI, and ROS stress. The results in Fig. 4A indicated that pre-treatment with LP-CFS and LB-CFS significantly decreased the DI and DSI in *A. flavus*-infected peanut seeds, which overcame the carbendazim treatment. Additionally, *A. flavus* infection resulted in a decrease in the SOD and catalase activity

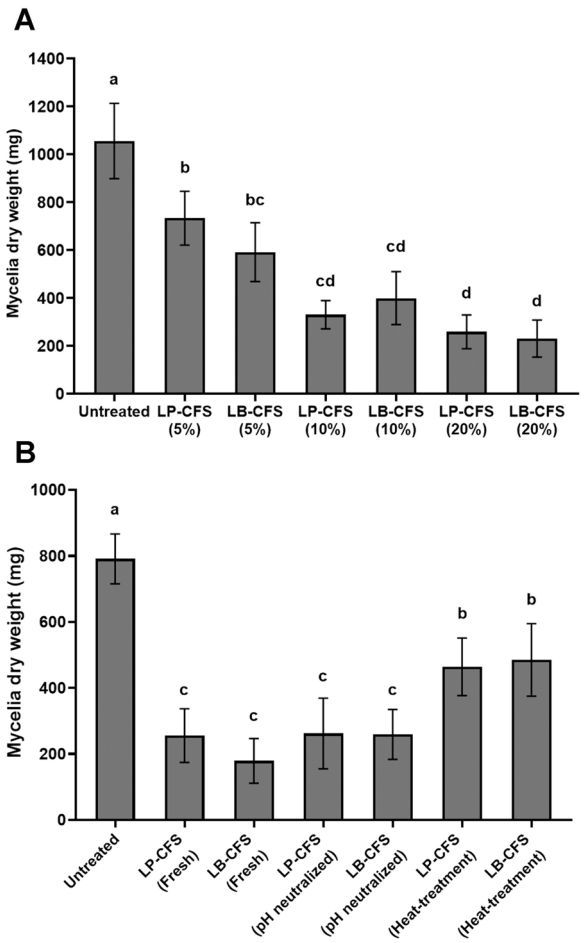

Fig. 2 **A** Effect of cell-free supernatant derived from *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* cultures at different concentrations on the growth of *A. flavus* mycelia after 7 days of cultivation. **B** Effect of pH-neutralized and heat treatment of cell-free supernatant derived from *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* cultures on the growth of *A. flavus* mycelia after 7 days of cultivation. Data are presented as the means of triplicate analysis ± standard deviation. The statistical analysis was conducted using ANOVA followed by Tukey's test ($p < 0.05$), with lowercase letters (a–c) indicating significant differences between groups. LP-CFS, cell-free supernatant derived from *Lactiplantibacillus plantarum* culture; LB-CFS, cell-free supernatant derived from *Levilactobacillus brevis* cultures

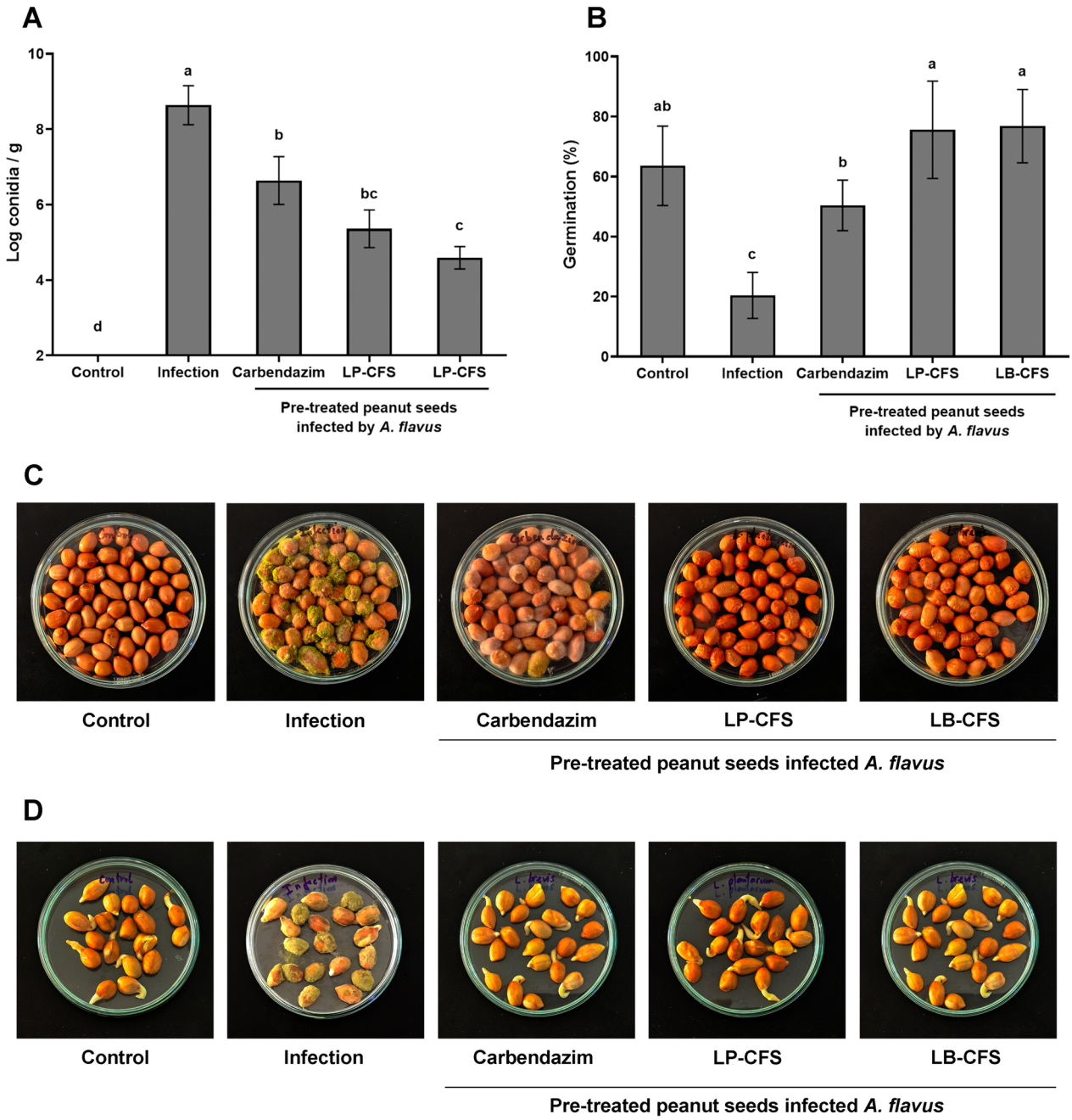

Fig. 3 **A** Effect of cell-free supernatant (CFS) derived from *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* cultures on inhibition of *Aspergillus flavus* infection on pre-treated peanut seeds. **B** Effect of the pre-treatment using CFS derived from *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* cultures on the germination rates of *Aspergillus flavus*-infected peanut seeds after 3 days of sowing. **C** Representative image of the peanut seed pre-treated with CFSs derived from *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* cultures and infected with *A. flavus* conidia. Data are presented as the means of triplicate analysis ± standard deviation. The statistical analysis was conducted using ANOVA followed by Tukey's test ($p < 0.05$), with lowercase letters (a–c) indicating significant differences between groups. LP-CFS, cell-free supernatant derived from *Lactiplantibacillus plantarum* culture; LB-CFS, cell-free supernatant derived from *Levilactobacillus brevis* cultures

(Fig. 4B), which are key enzymes involved in lowering ROS stress within cells. While carbendazim treatment could diminish DI and DSI (Fig. 4A), it failed to activate SOD and catalase (Fig. 4B). In contrast, pre-treatment with LP-CFS and LB-CF restored the SOD and catalase levels comparable to the control group (Fig. 4B).

Furthermore, the seedling length showed significant enhancement in the group treated with LP-CFS and LB-CFS compared to the control, whereas *A. flavus* infection resulted in a dramatic reduction of seedling length (Figs. 4C, 5). The seedling weight and the formation were express in the same pattern (Fig. 4D, E). This trend is further supported by the VI, where the group treated with LP-CFS and LB-CFS surpassed the efficacy of the carbendazim-treated group (Figs. 4F, 5). Interestingly, this finding aligns with the data presented in Fig. 1C, indicating that both *L. plantarum* and *L. brevis* showed the ability to IAA, a phytohormone has been known for its role in promoting root formation and elongation.

Discussion

Aspergillus species are soil fungi that cause significant damage to peanut seeds, leading to both pre- and post-harvest deterioration and causing considerable agricultural losses. *Aspergillus* species have been indicated to negatively affect peanut yield, decreased germination rates, lowered seed formation, and stem rot development (Nji et al. 2023). In addition, *A. flavus* is widely recognized for its production of aflatoxin, a hepatotoxin that can cause liver damage and lead to the development of liver carcinoma (Valencia-Quintana et al. 2020). Fungicides such as boscalid and isopyrazam are commonly used for the management of *A. flavus*. However, the widespread consumption of chemical agents has raised concerns about developing fungicide-resistant strains (Masiello et al. 2020). Therefore, it is necessary to investigate alternative management strategies to control *A. flavus.*

LABs have gained significant attention as a promising strategy for biological control. Previous studies indicated that LABs are effective in the management of bacterial and fungal plant pathogens (Daranas et al. 2019). In this study, *L. plantarum* and *L. brevis* showed strong inhibition against the growth of *A. flavus* mycelium in dual culture conditions. Furthermore, LP-CFS and LB-CFS dramatically suppressed the growth of *A. flavus* mycelium, which indicated the inhibitory effects were not attributed to physical interactions between LABs and *A. flavus*. The inhibitory effect remained even after neutralizing the pH and heat treatment, suggesting that it was not primarily dependent on pH lowering caused by lactic acid. These findings indicated the presence of biologically active compounds in the CFSs that contribute to fight against pathogenic fungi. Previous studies indicated that, *L. plantarum* has been demonstrated to produce lactic acid along with antibacterial compounds such as hydrogen peroxide, diacetyl, and bacteriocins to fight against various pathogens (Arena et al. 2016). In addition, *L. reuteri* might produce reuterin, a potent fungicide that disrupts DNA replication, thereby against several fungi strains such as *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp., (Vimont et al. 2019). Further investigation into the biological nature of the released compounds is required to understand the mechanisms by which *L. plantarum* and *L. brevis* contribute to the inhibition of phytopathogenic fungi.

The in vivo antifungal activity suggested that the LP-CFS and LB-CFS have the potential to protect peanut seeds against mold infection and promote germination and seedling growth. The mold contamination was significantly reduced from 8.63 log conidia/g to 5.35 and 4.59 log conidia/g, respectively, which was more effective than carbendazim treatment (6.63 log conidia/g). These results are consistent with previous study that have shown *Streptomyces yanglinensis* 3–10 demonstrated effectiveness in preventing the contamination of *A. flavus* on peanut seeds up to 89.4% (Shakeel et al. 2018). *A. flavus* is known for causing yellow mold, which leads to seed softening, root rot, and reduced seed germination, ultimately suppressing plant growth (Pal et al. 2014). However, there was a significant decrease in DI and DSI values in the peanut seeds pre-treated with LP-CFS and LB-CFS, which indicated that the biological activity compound presence in the CFSs derived from *L. platarum* and *L. brevis* cultures might prevent *A. flavus* infection in peanut seeds. Obtained results align with the study of Le et al. (2019) showed that pre-treating peanut seeds with *Bacillus*

(See figure on next page.)

Fig. 4 Effect of the pre-treatment of cell-free supernatant (CFS) derived from *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* cultures on the disease incidence (DI) and disease severity index (DSI) (**A**), superoxide dismutase and catalase activity (**B**), seedlings length (**C**) seedlings weight (**D**), number of secondary root (**E**), and vigor index (VI) (**F**) on *Aspergillus flavus*-infected peanut seeds after 7 days of sowing. Data are presented as the means of triplicate analysis ± standard deviation. The statistical analysis was conducted using ANOVA followed by Tukey's test ($p < 0.05$), with lowercase letters (a–c) indicating significant differences between groups. LP-CFS, cell-free supernatant derived from *Lactiplantibacillus plantarum* culture; LB-CFS, cell-free supernatant derived from *Levilactobacillus brevis* cultures

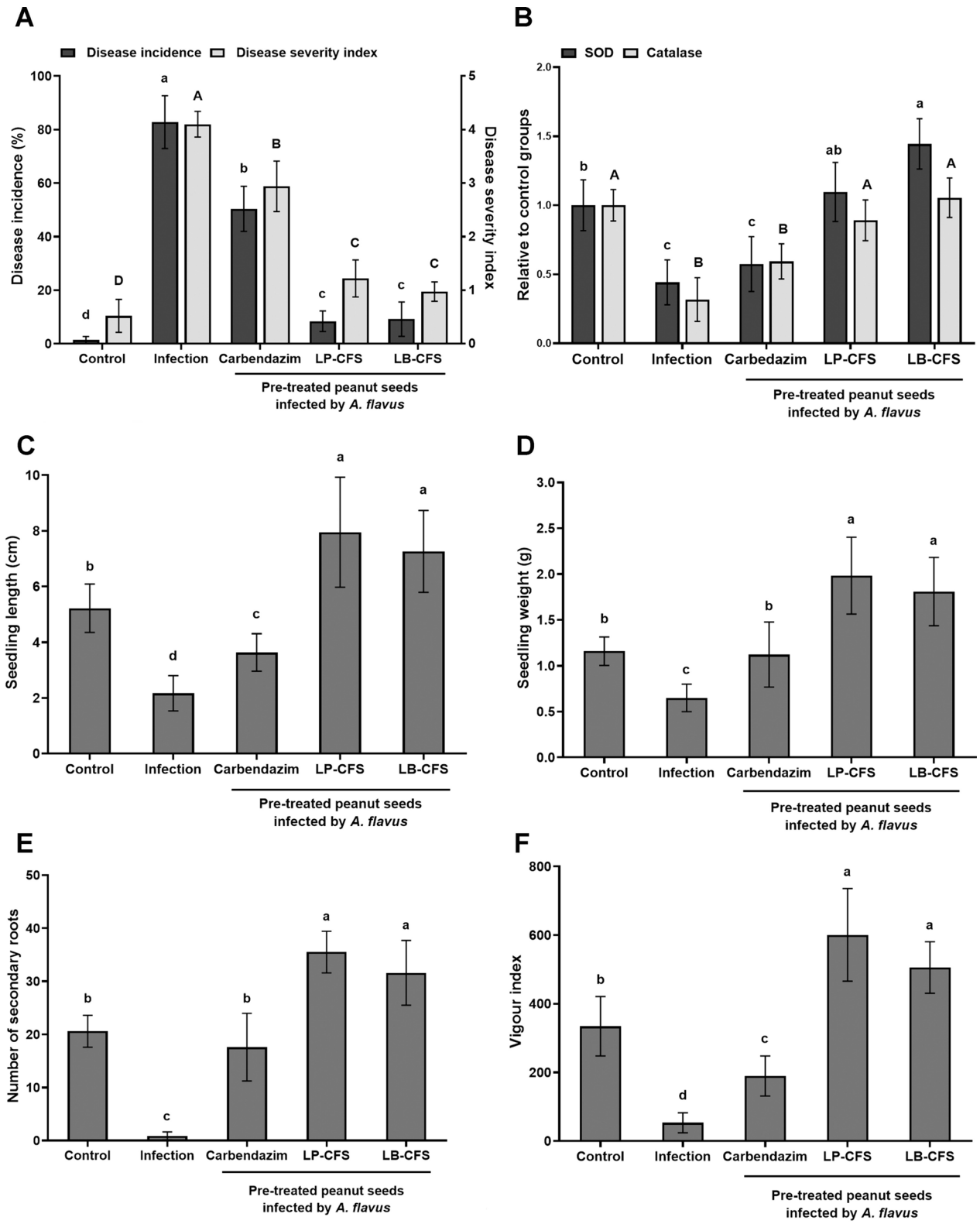

Fig. 4 (See legend on previous page.)

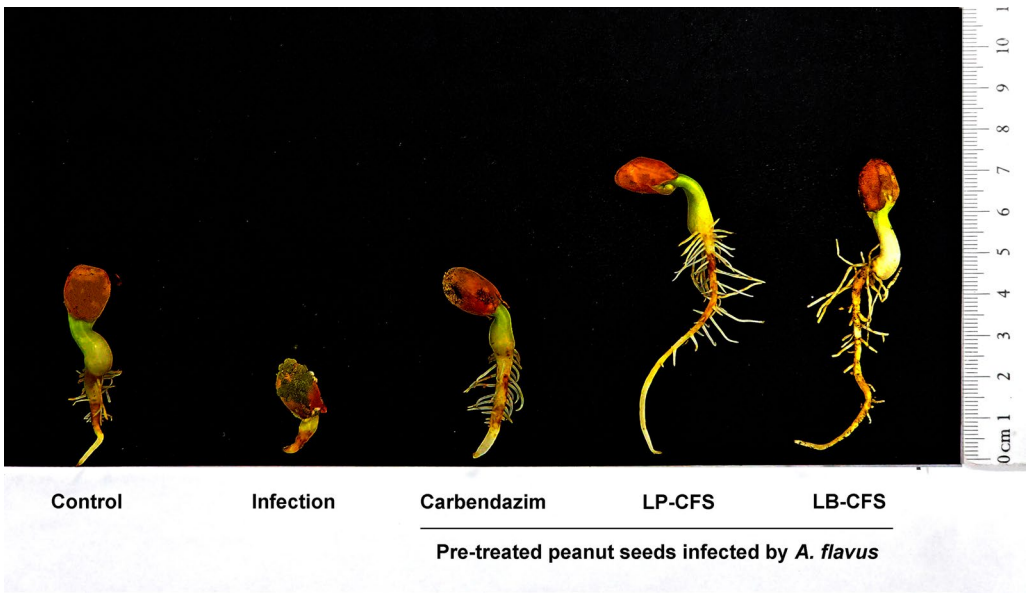

Fig. 5 Representative images indicate the protective ability of cell-free supernatant derived from *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* cultures against *Aspergillus flavus* infection and promoting the growth of peanut seedlings after 7 days of sowing. LP-CFS, cell-free supernatant derived from *Lactiplantibacillus plantarum* culture; LB-CFS, cell-free supernatant derived from *Levilactobacillus brevis* cultures

spp., *Pseudomonas* spp., and *Chryseobacteria* spp., for 30 min increased germination yield and reduced wilting symptoms in seedlings caused by *Sclerotium rolfsii*.

The increasing dependence on chemical control agents has resulted in unintended repercussions for agricultural quality, environmental degradation and human health. As an alternative to these chemical agents, LAB is gaining recognition as a feasible option for controlling plant pathogens (Jaffar et al. 2023). The present results indicated that *L. plantarum* and *L. brevis* had the potential as strategies for peanut seed treatments to mitigate diseases caused by mold infections. However, the precise nature of these antifungal compounds remains undefined and requires additional investigation for field application in the future. However, like other biological control agents, a primary challenge in using LAB for agricultural applications is their viability and produce sufficient quantities of bioactive compounds under optimal conditions. Further studies are required to optimize the culture condition for these LAB strains in industrial production to achieve antifungal compounds and phytohormones for field application investigation.

Conclusion

In this study, the antagonistic activity of LP-CFS and LB-CFS against *A. flavus* infection in peanut seeds was effectively demonstrated. Briefly, *A. flavus* infection resulted in seed damage, reduced germination ability, and induced oxidative stress in peanut seedlings, inhibiting root formation and seedling vigor. LP-CFS and LB-CFS showed the ability to impede *A. flavus* infection in peanut seeds and further reducing disease incidence (DI) and disease severity index (DSI) in seedlings. Additionally, both strains produced the phytohormone IAA, enhancing seedling resistance to oxidative stress and promoting germination rate and vigor index. Further investigations are needed to elucidate the antifungal mechanisms of these biological compounds and optimize their industrial-scale production for field applications. In summary, the bioactive compounds derived from CFSs of *L. plantarum* and *L. brevis* could serve as promising pre-planting treatments for peanut seeds, providing an environmentally friendly strategy in the management of phytopathogenic, and contributing to sustainable agricultural development.

Abbreviations

DI	Disease incidence
DSI	Disease severity index
GP	Germination percentage
IAA	Indole-3-acetic acid
LAB	Lactic acid bacteria
LB-CFS	Cell-free supernatant derived from *L. brevis* culture
LP-CFS	Cell-free supernatant derived from *L. plantarum* culture
MRS	De Man–Rogosa–Sharpe
PDA	Potato dextrose agar
SOD	Superoxide dismutase
VI	Vigor index
YPD	Yeast extract–peptone–dextrose

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Author contributions
ADD designed this study; QDN and ADD performed experiments; ADD and QDN wrote the paper. All authors approved this final manuscript. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Consent for publication was taken from the co-authors.

Competing interests
The authors have no competing interests to declare.

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References

Abhyankar P, Gunjal A, Kapadnis B, Ambade S (2021) Potential of lactic acid bacteria in plant growth promotion. Bhartiya Krishi Anusandhan Patrika 36(4):326–329
Arena MP, Silvain A, Normanno G, Grieco F, Drider D, Spano G, Fiocco D (2016) Use of *Lactobacillus plantarum* strains as a bio-control strategy against food-borne pathogenic microorganisms. Front Microbiol 7:464
Blainski JML, da Rocha Neto AC, Schimidt EC, Voltolini JA, Rossi MJ, Di Piero RM (2018) Exopolysaccharides from *Lactobacillus plantarum* induce biochemical and physiological alterations in tomato plant against bacterial spot. Appl Microbiol Biotechnol 102:4741–4753
Crowley S, Mahony J, van Sinderen D (2013) Broad-spectrum antifungal-producing lactic acid bacteria and their application in fruit models. Folia Microbiol 58:291–299
Danial AM, Medina A, Magan N (2021) *Lactobacillus plantarum* strain HT-W104-B1: potential bacterium isolated from Malaysian fermented foods for control of the dermatophyte Trichophyton rubrum. World J Microbiol Biotechnol 37:1–11
Daranas N, Roselló G, Cabrefiga J, Donati I, Francés J, Badosa E, Spinelli F, Montesinos E, Bonaterra A (2019) Biological control of bacterial plant diseases with *Lactobacillus plantarum* strains selected for their broad-spectrum activity. Ann Appl Biol 174(1):92–105
Dieme RMA, Faye I, Zoclanclounon YAB, Fonceka D, Ndoye O, Diedhiou PM (2018) Identification of sources of resistance for peanut *Aspergillus flavus* colonization and aflatoxin contamination. Int J Agron 2018:1–7
Do TH, Tran SC, Le CD, Nguyen H-BT, Le P-TT, Le H-HT, Le TD, Thai-Nguyen H-T (2020) Dietary exposure and health risk characterization of aflatoxin B1, ochratoxin A, fumonisin B1, and zearalenone in food from different provinces in Northern Vietnam. Food Control 112:107108
Dugassa A, Alemu T, Woldehawariat Y (2021) *In-vitro* compatibility assay of indigenous *Trichoderma* and *Pseudomonas* species and their antagonistic activities against black root rot disease (*Fusarium solani*) of faba bean (*Vicia faba* L.). BMC Microbiol 21:1–11
Gang S, Sharma S, Saraf M, Buck M, Schumacher J (2019) Analysis of indole-3-acetic acid (IAA) production in Klebsiella by LC-MS/MS and the Salkowski method. Bio-protocol 9(9):e3230–e3230
Goldstein EJ, Tyrrell KL, Citron DM (2015) *Lactobacillus* species: taxonomic complexity and controversial susceptibilities. Clin Infect Dis 60(suppl_2):S98–S107
Higdon SM, Huang BC, Bennett AB, Weimer BC (2020) Identification of nitrogen fixation genes in *Lactococcus* isolated from maize using population genomics and machine learning. Microorganisms 8(12):2043
Hung C-H, Chen S-D (2022) Study of inducing factors on resveratrol and antioxidant content in germinated peanuts. Molecules 27(17):5700
Jaffar NS, Jawan R, Chong KP (2023) The potential of lactic acid bacteria in mediating the control of plant diseases and plant growth stimulation in crop production—a mini review. Front Plant Sci 13:1047945
Kim C, Alvarez C, Sattar A, Bandyopadhyay A, Azzarri C, Moltedo A, Haile B (2020) Production, Consumption, and Food Security in Viet Nam diagnostic overview. IFPRI & FAO, Hanoi
Le CN, Thai T, Nguyen X, Nguyen T, Tran T, Tran T (2019) Biological control of groundnut stem rot by *Bacillus* sp. strain S20D12. Arch Phytopathol Plant Prot 52(7–8):625–638
Malik MT, Rehman AU, Naqvi SA, Hasnain A, Umar UU, Azeem H, Shahid M, Umair M (2021) Biological mediated management of bacterial diseases in crop plants: a review. Pak J Phytopathol 33(1):217
Marchese S, Polo A, Ariano A, Velotto S, Costantini S, Severino L (2018) Aflatoxin B1 and M1: biological properties and their involvement in cancer development. Toxins 10(6):214
Masiello M, Somma S, Haidukowski M, Logrieco A, Moretti A (2020) Genetic polymorphisms associated to SDHI fungicides resistance in selected *Aspergillus flavus* strains and relation with aflatoxin production. Int J Food Microbiol 334:108799
Mussa A, Million T, Assefa F (2018) Rhizospheric bacterial isolates of grass pea (*Lathyrus sativus* L.) endowed with multiple plant growth promoting traits. J Appl Microbiol 125(6):1786–1801
Nguyen HH, Tram B, Le T (2021) Use of lactic acid bacteria in peanut seed treatment. J Technol Innov 1(1):20–22
Nji QN, Babalola OO, Mwanza M (2023) Soil *Aspergillus* species, pathogenicity and control perspectives. J Fungi 9(7):766
Norlia M, Jinap S, Nor-Khaizura MAR, Radu S, Samsudin NIP, Azri FA (2019) *Aspergillus* section Flavi and aflatoxins: occurrence, detection, and identification in raw peanuts and peanut-based products along the supply chain. Front Microbiol 10:2602
Pal KK, Dey R, Tilak K (2014) Fungal diseases of groundnut: control and future challenges. In: Goyal A, Manoharachary C (eds) Future challenges in crop protection against fungal pathogens. Springer, New York, pp 1–29
Poornachandra Rao K, Deepthi B, Rakesh S, Ganesh T, Achar P, Sreenivasa M (2019) Antiaflatoxigenic potential of cell-free supernatant from *Lactobacillus plantarum* MYS44 against *Aspergillus parasiticus*. Probiotics Antimicrob Proteins 11:55–64
Shakeel Q, Lyu A, Zhang J, Wu M, Li G, Hsiang T, Yang L (2018) Biocontrol of *Aspergillus flavus* on peanut kernels using Streptomyces yanglinensis 3–10. Front Microbiol 9:1049
Singh SB, Gowtham H, Murali M, Hariprasad P, Lakshmeesha T, Murthy KN, Amruthesh K, Niranjana S (2019) Plant growth promoting ability of ACC deaminase producing rhizobacteria native to Sunflower (*Helianthus annuus* L.). Biocatal Agric Biotechnol 18:101089
Steglińska A, Kołtuniak A, Motyl I, Berłowska J, Czyżowska A, Cieciura-Włoch W, Okrasa M, Kręgiel D, Gutarowska B (2022) Lactic acid bacteria as biocontrol agents against potato (*Solanum tuberosum* L.) pathogens. Appl Sci 12(15):7763
Sundin GW, Castiblanco LF, Yuan X, Zeng Q, Yang CH (2016) Bacterial disease management: challenges, experience, innovation and future prospects: challenges in bacterial molecular plant pathology. Mol Plant Pathol 17(9):1506–1518
Trinh LL, Le Nguyen AM, Nguyen HH (2023) Root-associated bacteria *Bacillus albus* and *Bacillus proteolyticus* promote the growth of peanut seedlings and protect them from the aflatoxigenic Aspergillus flavus CDP2. Biocatal Agric Biotechnol 47:102582
Valencia-Quintana R, Milić M, Jakšić D, ŠegvićKlarić M, Tenorio-Arvide MG, Pérez-Flores GA, Bonassi S, Sánchez-Alarcón J (2020) Environment changes, aflatoxins, and health issues, a review. Int J Environ Res Public Health 17(21):7850
Vimont A, Fernandez B, Ahmed G, Fortin H-P, Fliss I (2019) Quantitative antifungal activity of reuterin against food isolates of yeasts and moulds and its potential application in yogurt. Int J Food Microbiol 289:182–188

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