

Original Article

ISOLATION AND ENZYMATIC CHARACTERIZATION OF STRESS-TOLERANT FUNGI ASSOCIATED WITH GROUNDNUT GERMINATION UNDER SALINE AND ALKALINE CONDITIONS

**Agba, M. O., Ngele B. A., Etim D. O. and Mboto, C. I.*

Department of Botany, University of Calabar,
Nigeria.

DOI: <https://doi.org/10.5281/zenodo.20749436>

Abstract: Salinity and alkalinity are major abiotic stressors that adversely affect seed germination and microbial activity in agricultural soils. This study investigated the fungi associated with the germination of groundnut (*Arachis hypogaea* L.) under saline and alkaline stress conditions and evaluated their oxidative enzyme activities as indicators of stress adaptation. Groundnut seeds were exposed to varying concentrations of sodium chloride (NaCl) and sodium carbonate (Na₂CO₃), after which the associated fungi were isolated and characterized using macroscopic and microscopic features. Pure fungal isolates were cultured and screened for oxidative enzyme activity, including peroxidase, catalase, polyphenol oxidase, and laccase. The fungal isolates exhibited morphological characteristics consistent with those of *Aspergillus* spp. Significant variations ($p \leq 0.05$) in enzyme activities were observed across treatments. The peroxidase activity was highest under Na₂CO₃ 125 mM treatment, whereas the catalase activity peaked at Na₂CO₃ 187 mM. The polyphenol oxidase activity showed moderate variation across treatments, whereas the laccase activity was highest under the Na₂CO₃ 62 mM treatment. Generally, alkaline stress induces stronger oxidative enzyme responses than saline stress. The findings indicate that fungi associated with groundnut germination possess adaptive oxidative enzyme systems that support their survival under saline and alkaline stress conditions. These enzymes may contribute to fungal stress tolerance and ecological adaptation in stressful germination environments.

Keywords: *Arachis hypogaea*, alkaline stress, salinity stress, fungal isolates, oxidative enzymes

Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most economically important leguminous crops cultivated globally for its edible seeds, oil production, livestock feed, and industrial applications. It is a major source of

Original Article

protein, carbohydrates, vitamins, and essential minerals, particularly in developing countries, where it significantly contributes to food security and household income (Syed *et al.*, 2021). Groundnut cultivation plays a crucial role in rural livelihoods and sustainable agricultural systems in many tropical and subtropical regions, especially across sub-Saharan Africa and Asia (Fukah *et al.*, 2024; Awoke *et al.*, 2025). However, groundnut productivity is increasingly threatened by environmental stressors, particularly soil salinity and alkalinity, which adversely affect seed germination, seedling establishment, and overall crop performance (Akter *et al.*, 2020; Manono, 2026).

Salinity and alkalinity are among the most destructive abiotic stresses limiting agricultural productivity worldwide. Soil salinization primarily results from the excessive accumulation of soluble salts, whereas alkaline stress involves both salt accumulation and elevated soil pH, which is often caused by the deposition of sodium carbonate and bicarbonate (Wang *et al.*, 2016; Cao *et al.*, 2022). These stress conditions reduce the ability of plants and microorganisms to absorb water, disrupt ion balance within cells, alter membrane permeability, and induce oxidative stress (Zhang *et al.*, 2018). During seed germination, such stress conditions can significantly alter the microbial communities associated with seeds and germinating tissues, thereby influencing both plant development and rhizosphere microbial interactions.

Fungi constitute an important component of the soil and seed-associated microbiota and play diverse ecological roles ranging from saprophytic decomposition and nutrient recycling to pathogenic and symbiotic interactions with plants (Frac *et al.*, 2018; Romão *et al.*, 2025). Some fungi possess remarkable physiological adaptability that enables them to survive and proliferate under extreme environmental conditions, including high salinity and alkaline pH. Under stress conditions, fungi activate several adaptive biochemical and metabolic pathways that enhance their survival and competitiveness in stressful environments (Lara-Martínez *et al.*, 2025). These adaptive responses often involve the secretion of oxidative and hydrolytic enzymes that assist in stress tolerance, nutrient acquisition, and cellular integrity maintenance.

Peroxidases, catalases, polyphenol oxidases, and laccases are among the important stress-responsive enzymes produced by fungi. These enzymes play essential roles in the detoxification of ROS, degradation of complex organic materials, stress signaling, and regulation of fungal physiological activities under stressful conditions (Baldrian, 2006; Park & Son, 2024). Peroxidases and catalases are important antioxidant enzymes that protect fungal cells against oxidative damage caused by stress-induced accumulation of hydrogen peroxide and other reactive oxygen intermediates. Polyphenol oxidases and laccases are involved in phenolic compound oxidation, lignin degradation, pigmentation, and detoxification processes that support fungal adaptation and survival (Revankar & Lele, 2006).

Some fungi develop adaptive biochemical mechanisms that enhance their survival and ecological competitiveness under saline and alkaline conditions. These include increased secretion of oxidative and hydrolytic enzymes such as peroxidases, catalases, polyphenol oxidases, and laccases, which help mitigate oxidative damage and facilitate nutrient cycling within stressed environments (Gessler *et al.*, 2007; Chaudhary *et al.*, 2022). Fungi isolated from saline or alkaline habitats often exhibit extremozymic properties, producing enzymes that retain activity and stability under extreme pH and high salt concentrations. Such adaptive responses significantly contribute to fungal persistence and metabolic activity under hostile environmental conditions.

Fungi associated with stressful soil environments possess enhanced oxidative defense systems that enable them to tolerate osmotic imbalance, ionic toxicity, and oxidative stress induced by salinity and alkalinity

Original Article

(Chandrashekar & Prasanna, 2011; Yakub *et al.*, 2022). Despite increasing interest in stress-tolerant fungi, limited information exists on fungi associated with groundnut germination under saline and alkaline conditions, as well as on their enzymatic adaptation mechanisms during the early stages of seed germination.

Understanding the enzymatic responses of fungi associated with germinating seeds under stress conditions is important for agricultural and ecological reasons. These fungi may influence seed health, stress tolerance, nutrient availability, and microbial competition within the germination environment. Therefore, investigating their enzymatic profiles could provide useful insights into fungal stress adaptation and ecological survival strategies under saline and alkaline conditions.

Therefore, this study was designed to isolate and characterize fungi associated with groundnut (*Arachis hypogaea* L.) germination under saline and alkaline stress conditions and evaluate their oxidative enzyme activities as indicators of physiological adaptation to environmental stress.

Materials and Methods

Study Area

This study was conducted in the Department of Botany, University of Calabar. Germination experiments were conducted under screen house conditions, while fungal isolation, culturing, and enzymatic analyses were performed in the Department of Botany's Undergraduate Research Laboratory.

Collection and preparation of groundnut seeds

Healthy seeds of groundnut (*Arachis hypogaea* L.) were purchased from the Watt Market, Calabar, Cross River State, Nigeria. Seeds were manually sorted to eliminate damaged, shriveled, and infected seeds. The selected seeds were surface sterilized by immersion in 70% ethanol for 1 min, then rinsed in sterile distilled water and air-dried under aseptic conditions before use.

Preparation of the stress treatment solutions

Deionized water was used as the control. Saline solutions were prepared using analytical-grade NaCl at concentrations of 62, 125, and 187 mM. Alkaline solutions were prepared using sodium carbonate (Na₂CO₃) adjusted to the corresponding concentrations. The pH of the treatment solutions was measured using a digital pH meter, and the conductivity and salinity were measured using a conductivity meter. Table 1 presents the physicochemical properties of the treatment solutions.

Germination experiment

Sterile germination trays lined with sterile tissue paper were used as the germination medium. Thirty seeds were placed in each tray and moistened with 150 mL of the designated treatment solution before planting. Subsequent watering was performed twice weekly with 50 mL of the respective treatment solution to maintain soil moisture. The experiment comprised seven treatment groups:

Control (distilled water), NaCl 62 mM, NaCl 125 mM, NaCl 187 mM, Na₂CO₃ 62 mM, Na₂CO₃ 125 mM, and Na₂CO₃ 187 mM

Each treatment was replicated three times and arranged in a completely randomized design (CRD). Germination trays were maintained under ambient laboratory conditions and seed germination and fungal growth were monitored daily.

Original Article

Isolation of fungi associated with seed germination

Fungi associated with germinating groundnut seeds were isolated using the potato dextrose agar (PDA) method. To inhibit bacterial growth, small portions of visibly colonized seed tissues and fungal mycelia emerging around germinating seeds were aseptically transferred onto sterile PDA plates supplemented with chloramphenicol. The inoculated plates were incubated at $28 \pm 2^\circ\text{C}$ for 5–7 days. To obtain pure cultures, emerging fungal colonies were repeatedly sub-cultured on fresh PDA plates. Pure isolates were maintained on PDA slants at 4°C for further characterization and enzymatic studies (Bassey *et al.*, 2025; Etim *et al.*, 2026).

Morphological and microscopic identification of fungal strains

The fungal isolates were identified based on macroscopic and microscopic characteristics following the standard fungal identification procedures described by Barnett and Hunter (1998). The colony morphology, including color, texture, margin characteristics, pigmentation, and growth pattern, was examined after incubation. Microscopic examination was performed using the lactophenol cotton blue staining technique. Small portions of fungal mycelia were mounted on clean glass slides, stained, and observed under a compound light microscope at $\times 400$ magnification. Morphological features, including hyphal structure, conidiophores, vesicles, phialides, and conidia arrangement, were used to identify fungal isolates.

Enzyme extraction

Enzymes were extracted in a mixed phosphate buffer (20 mM monobasic and dibasic potassium phosphate salts), pH 7.5, containing 1% of the phenolic absorbent polyvinylpyrrolidone (PVPP). Fresh fungal mycelium (0.2 g) was pulverized in 10 ml of extraction buffer and centrifuged at 6000 rpm for 10 min. The supernatant was stored in an ice chamber and used as a crude enzyme extract (Ngele *et al.*, 2024).

Determination of the peroxidase activity

The peroxidase activity was determined spectrophotometrically using guaiacol as the substrate. The reaction mixture consisted of 2.0 mL of phosphate buffer, 0.6 mL of enzyme extract, 0.1 mL of guaiacol, and 0.1 mL of hydrogen peroxide (H_2O_2). The increase in absorbance due to guaiacol oxidation was measured at 436 nm using a UV-visible spectrophotometer. Enzyme activity is expressed as $\mu\text{mol product L}^{-1} \text{ min}^{-1}$.

Determination of the catalase activity

Catalase activity was determined after further purification of the crude enzyme by precipitation with ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$ (80% saturation). In a cuvette containing 2 ml of assay buffer, 0.6 ml of purified enzyme was added, and the reaction was initiated by adding 0.2 ml of 10 mM H_2O_2 . The absorbance was measured at 240 nm after 10 s.

Determination of PPO activity

The activity of polyphenol oxidase was assayed using phloroglucinol as a substrate. The reaction mixture included 1.4 mL of mixed phosphate buffer, 0.5 mL of 10 mM phloroglucinol, and 0.6 mL of enzyme extract; another mixture was prepared as described but without enzyme extract, which served as a blank. The mixture was incubated in the dark for 10 min at 30°C , after which absorbance was measured at 475 nm. The absorbance reading was used to calculate polyphenol oxidase activity ($\mu\text{mol product/L/min}$) using an extinction coefficient of $1433 \text{ M}^{-1} \text{ cm}^{-1}$ for the quinone product.

Determination of laccase activity

The laccase assay was performed using guaiacol as the substrate, and the reaction mixture was prepared by adding 1 mL of 2 mM guaiacol to a test tube, 3 mL of assay buffer, and 1 mL of enzyme extract. Water was added to a

Original Article

second test tube instead of the enzyme extract to serve as a blank. The mixture was incubated at room temperature for 15 min, and the absorbance was measured at 450 nm using a UV-VIS spectrophotometer.

Absorbance readings were used to calculate enzyme activity as follows:

$$E.A = A \times V / t \times e \times v$$

Where E. A ($\mu\text{g}/\text{ml}$) = enzyme activity, A = absorbance, V = total mixture volume (ml), v = enzyme volume (ml), t = incubation time (min), e = extinction coefficient: $0.6740 \mu\text{M}/\text{cm}$ for guaiacol and $43.6 \mu\text{M}/\text{cm}$ for hydrogen peroxide.

Statistical analysis

All experiments were conducted in triplicate, and the obtained data are expressed as mean $\pm$ standard deviation. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0. One-way analysis of variance was used to determine significant differences among treatment means, while the least significant difference test was used for mean separation at $p \leq 0.05$.

Results

Morphological and microscopic characteristics of the fungal isolates

Fungal isolates associated with germinating groundnut seeds exposed to saline and alkaline stress (Plate 1) were successfully cultured and characterized using macroscopic and microscopic features. The isolates exhibited rapid colony growth on PDA, with distinct variations in colony texture, pigmentation, and sporulation patterns across treatments.

Macroscopically, the fungal colonies initially appeared whitish and later developed dark green to black pigmentation as sporulation progressed (Plate 2). The colonies were circular with dense aerial mycelia and powdery surfaces characteristic of *Aspergillus* species. Reverse colony coloration ranged from pale yellow to brownish, depending on the culture age and treatment conditions. In addition, the isolates exhibited extensive radial growth and dense conidial formation, suggesting active fungal metabolism under stress conditions.

Microscopic examination of the fungal isolates using lactophenol cotton blue staining revealed septate hyphae with well-developed conidiophores arising from specialized foot cells. The conidiophores terminated in globose vesicles bearing biserial phialides and conidial chains radiating outward from the vesicle surface. The conidia were spherical to sub-spherical and densely arranged in characteristic radiating conidial heads typical of *Aspergillus* species.

Photomicrographs of fungal cultures further confirmed the presence of morphological features consistent with those of *Aspergillus spp.* (Plate 3). Variations in conidial density, pigmentation intensity, and hyphal branching patterns were observed among isolates exposed to different saline and alkaline treatments. Fungal isolates cultured under alkaline stress conditions exhibited denser mycelial networks and more pronounced sporulation than those under saline stress. The observed morphological characteristics are consistent with the descriptions provided by Barnett and Hunter (1998) and Blackwell (2011) for stress-tolerant *Aspergillus* species commonly associated with soil and seed environment

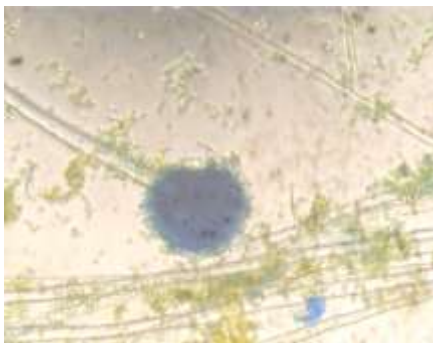
Original Article



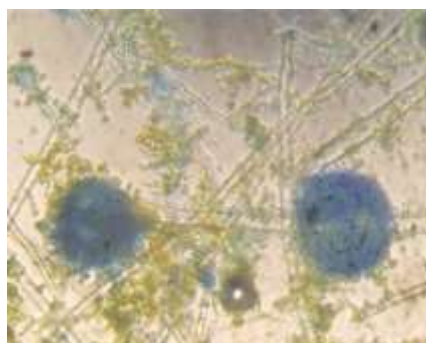
Plate 1: Germination setup under stress treatment



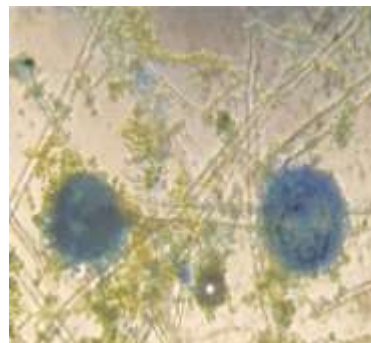
Plate 2: Pure culture of fungal isolate on Potato Dextrose Agar (PDA).



A



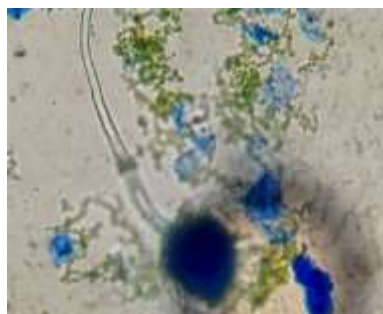
B



C



D



E



F



G

Plate 3: Fungal isolate photomicrograph showing conidiophores and conidia of *Aspergillus* spp. ($\times 400$). A=NaCl 187 mM, B=NaCO₃ 62 mM, C=NaCO₃125 mM, D=NaCO₃ 187 mM, E=NaCl 62 mM, F=NaCl 125 mM, G = control

Oxidative enzyme activities of fungal isolates under saline and alkaline stress conditions

The activities of peroxidase, catalase, polyphenol oxidase, and laccase produced by fungal isolates associated with groundnut germination under saline and alkaline stress conditions are presented in Figs. 1-4. Significant

Original Article

variations ($P \leq 0.05$) were observed among treatments, indicating differential enzymatic responses of the fungal isolates to stress levels.

Peroxidase activity

The peroxidase activity increased significantly with increasing saline and alkaline stress conditions compared with the control treatment. The results in Fig. 1 show that the highest peroxidase activity was recorded in fungi exposed to Na_2CO_3 125 mM treatment ($383.410 \pm 1.155 \mu\text{mol product L}^{-1} \text{ min}^{-1}$), followed by Na_2CO_3 187 mM ($224.830 \pm 0.595 \mu\text{mol product L}^{-1} \text{ min}^{-1}$) and Na_2CO_3 62 mM ($179.936 \pm 0.531 \mu\text{mol product L}^{-1} \text{ min}^{-1}$) Fig. 1. Among the NaCl treatments (Fig. 1), peroxidase activity increased progressively with salt concentration, ranging from $82.420 \pm 0.693 \mu\text{mol product L}^{-1} \text{ min}^{-1}$ at 62 mM NaCl to $137.657 \pm 1.014 \mu\text{mol product L}^{-1} \text{ min}^{-1}$ at 187 mM NaCl. The control treatment recorded the lowest peroxidase activity ($47.730 \pm 0.133 \mu\text{mol product L}^{-1} \text{ min}^{-1}$).

Catalase activity

The catalase activity varied significantly among the treatments (Fig. 2). The highest catalase activity was observed in fungal isolates subjected to Na_2CO_3 187 mM treatment ($59.940 \pm 0.023 \mu\text{mol product L}^{-1} \text{ min}^{-1}$), while the lowest activity was recorded in the control treatment ($14.340 \pm 0.052 \mu\text{mol product L}^{-1} \text{ min}^{-1}$). Under saline stress, catalase activity was highest at NaCl 62 mM ($47.310 \pm 0.058 \mu\text{mol product L}^{-1} \text{ min}^{-1}$) and decreased with increasing salt concentration. Similarly, alkaline treatments showed progressive variation, with Na_2CO_3 125 mM recording $36.390 \pm 0.040 \mu\text{mol product L}^{-1} \text{ min}^{-1}$ and Na_2CO_3 62 mM recording $29.373 \pm 0.464 \mu\text{mol product L}^{-1} \text{ min}^{-1}$. The elevated catalase activity observed under stress conditions indicates enhanced oxidative stress defense mechanisms in the fungal isolates.

Polyphenol oxidase (PPO) activity

The polyphenol oxidase activity exhibited moderate variation across treatments. The highest activity was observed in the 125 mM NaCl treatment ($1.303 \mu\text{mol product L}^{-1} \text{ min}^{-1}$), followed closely by the control treatment ($1.260 \pm 0.023 \mu\text{mol product L}^{-1} \text{ min}^{-1}$). Fungal isolates exposed to NaCl 187 mM and Na_2CO_3 125 mM recorded similar activities of $1.240 \pm 0.001 \mu\text{mol product L}^{-1} \text{ min}^{-1}$. Lower polyphenol oxidase activities were observed under NaCl 62 mM ($1.113 \pm 0.003 \mu\text{mol product L}^{-1} \text{ min}^{-1}$) and Na_2CO_3 62 mM ($1.103 \pm 0.464 \mu\text{mol product L}^{-1} \text{ min}^{-1}$). The lowest activity was recorded under Na_2CO_3 187 mM treatment ($0.449 \pm 0.004 \mu\text{mol product L}^{-1} \text{ min}^{-1}$).

Laccase activity

The laccase activity significantly differed among treatments. The highest laccase activity was observed under Na_2CO_3 62 mM treatment ($0.860 \pm 0.003 \mu\text{mol product L}^{-1} \text{ min}^{-1}$), indicating that moderate alkaline stress strongly enhanced laccase production in the fungal isolates. The second-highest activity was recorded under Na_2CO_3 187 mM treatment ($0.448 \pm 0.004 \mu\text{mol product L}^{-1} \text{ min}^{-1}$), while the control treatment showed moderate activity ($0.323 \pm 0.003 \mu\text{mol product L}^{-1} \text{ min}^{-1}$). Lower activity was observed in NaCl treatments, particularly in NaCl 125 mM, which recorded the lowest laccase activity ($0.129 \pm 0.001 \mu\text{mol product L}^{-1} \text{ min}^{-1}$). These findings indicate that alkaline stress conditions are stronger inducers of laccase synthesis than saline stress.

Original Article

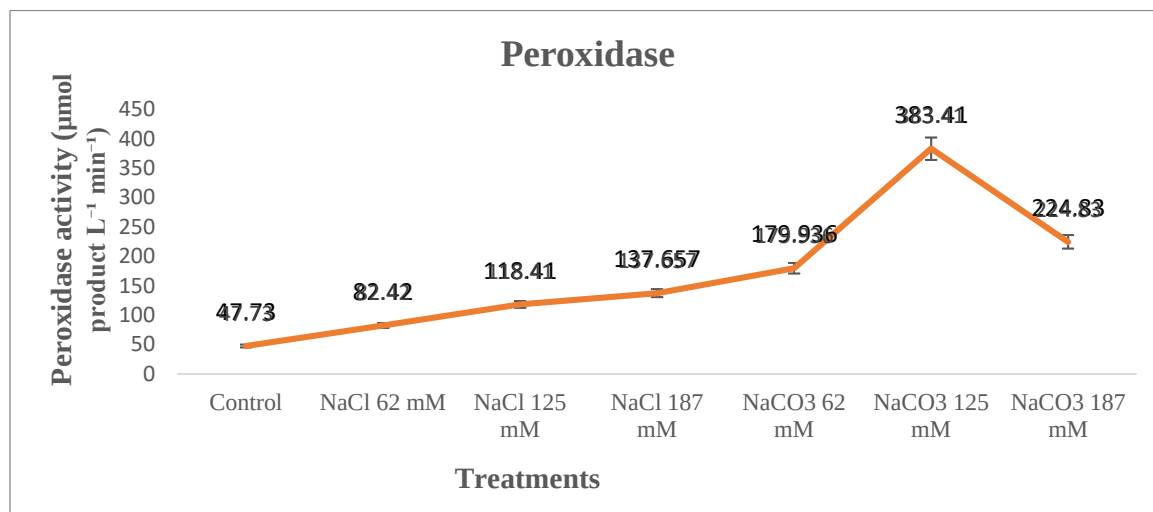


Fig 1: Peroxidase activity of groundnut germination-associated fungal isolates under saline and alkaline stress conditions

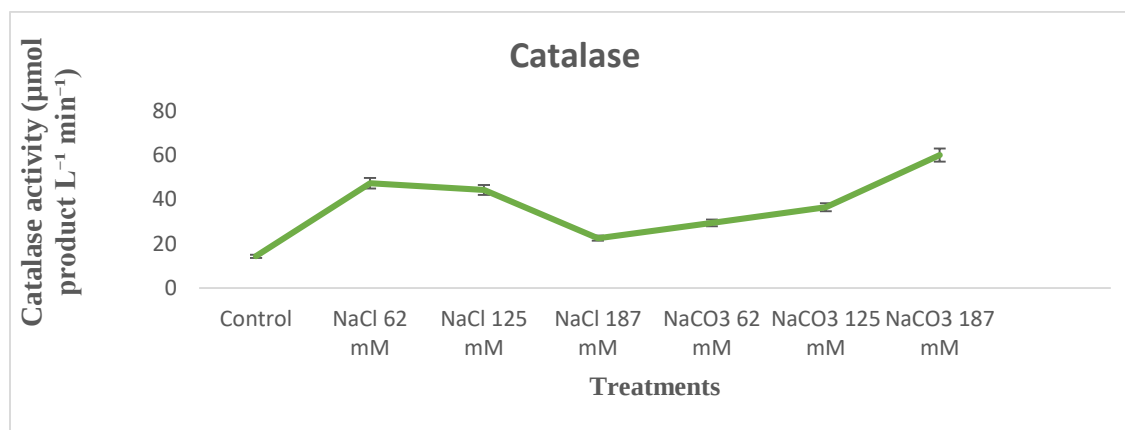
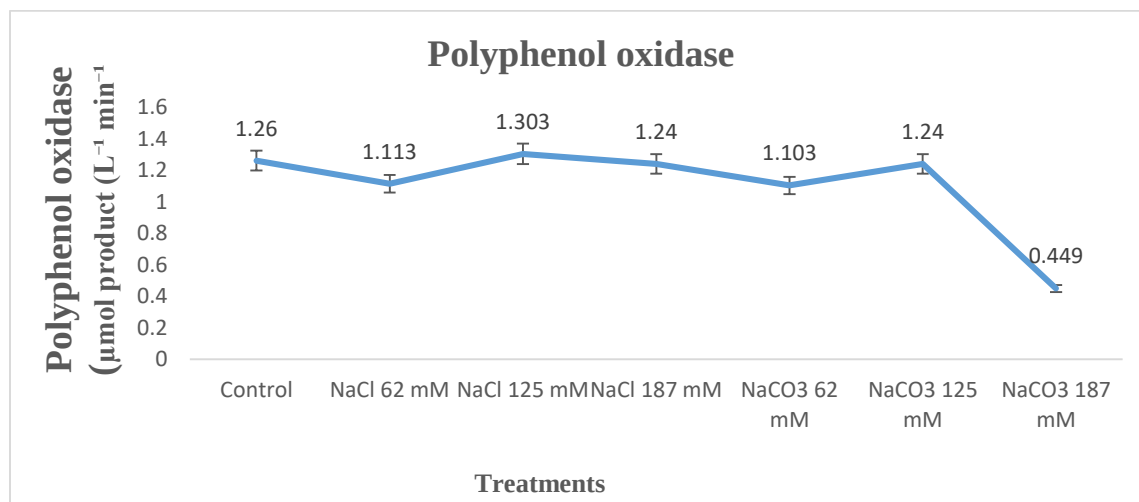
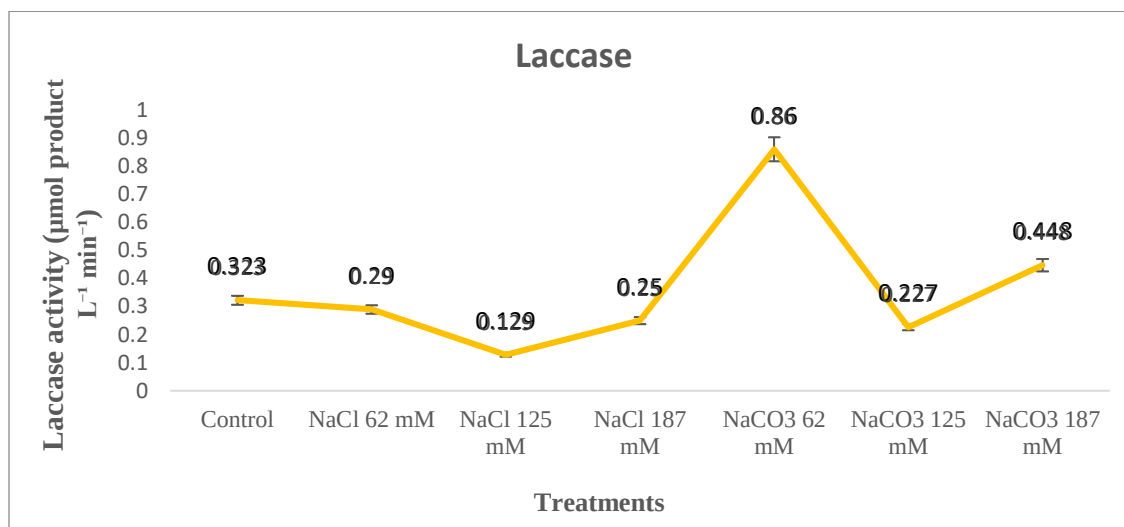


Fig 2: Catalase activity of fungal isolates associated with groundnut germination under saline and alkaline stress conditions



Original Article

Fig 3: Polyphenol oxidase activity of fungal isolates associated with groundnut germination under saline and alkaline stress conditions**Fig 4: Laccase activity of fungal isolates associated with groundnut germination under saline and alkaline stress conditions****Discussion**

Fungi associated with germinating groundnut (*Arachis hypogaea* L.) seeds subjected to saline and alkaline stress conditions were successfully isolated and characterized based on their macroscopic and microscopic features. The fungal isolates exhibited morphological characteristics typical of *Aspergillus* spp., including dense aerial mycelia, powdery colonies, septate hyphae, and well-developed conidiophores bearing conidia chains. The observed variations in sporulation intensity and mycelial density across different treatments indicates that the fungi responded differently to saline and alkaline stress. Several authors have reported similar morphological characteristics of stress-tolerant *Aspergillus* species (Nyongesa *et al.*, 2015; Awad *et al.*, 2023).

The predominance of *Aspergillus*-like isolates in this study may be linked to the adaptive capacity of the genus to stressful environmental conditions. Previous studies have shown that fungi exposed to osmotic and oxidative stress activate protective mechanisms, including enhanced sporulation, oxidative enzyme secretion, and cellular metabolism regulation, to improve survival under adverse conditions (Gharaghani *et al.*, 2022; Khan *et al.*, 2025). This study also revealed significant variations in oxidative enzyme activities among the fungal isolates under saline and alkaline stress conditions. The increased activities of peroxidase, catalase, polyphenol oxidase, and laccase indicate that the fungal isolates activated oxidative defense systems in response to environmental stress. Similar responses have been reported in fungi exposed to saline and alkaline environments, where oxidative enzymes play important roles in stress adaptation and cellular protection (Rivera-Hoyos *et al.*, 2013; Kiviniemi *et al.*, 2023).

Peroxidase activity was highest under the Na₂CO₃ treatments, indicating that alkaline stress imposed greater oxidative pressure on the fungal isolates than saline stress. Peroxidases are involved in the detoxification of reactive oxygen species generated under environmental stress and contribute to maintaining fungal cellular stability (Gostinčar *et al.*, 2022; Abrashev *et al.*, 2025).

Catalase activity also increased significantly under stress, particularly under high alkaline conditions. Catalase is an important antioxidant enzyme that decomposes H₂O₂ produced during oxidative stress. The elevated catalase

Original Article

activity observed in this study demonstrates the activation of fungal antioxidant defense mechanisms necessary for maintaining cellular homeostasis under stress conditions. Kapoor and Mukerji (1999), Bahrami-Rad and Hajiboland (2017), and Gharaghani *et al.* (2022) reported similar observations.

The activity of polyphenol oxidase showed moderate variation across treatments, although severe alkaline stress considerably reduced enzyme activity. Polyphenol oxidases are involved in phenolic compound oxidation and stress-related metabolic processes (Mayer, 2006). The reduced activity observed under highly alkaline conditions may indicate the suppression of fungal metabolic processes due to excessive stress (Khan *et al.*, 2025). Prasad *et al.* (2015) reported similar reductions in polyphenol oxidase activity under alkaline conditions.

Laccase activity was highest under moderate alkaline stress conditions, demonstrating that alkaline environments stimulated laccase production in fungal isolates. Laccases are oxidative enzymes involved in fungal adaptation and stress responses (Baldrian, 2006). The findings of this study are consistent with those of Revankar and Lele (2006), who observed enhanced laccase production in fungi cultured under alkaline conditions.

Generally, the results demonstrated that saline and alkaline stress conditions significantly influenced oxidative enzyme activities in groundnut germination-associated fungi. Alkaline treatment generally induced stronger enzyme responses than saline treatment.

Conclusion

This study revealed that saline and alkaline stress conditions influenced the occurrence and oxidative enzyme activities of fungi associated with groundnut germination. Fungal isolates morphologically consistent with *Aspergillus* spp. survived under varying stress conditions and exhibited significant oxidative enzyme responses, particularly under alkaline conditions. The increased activities of peroxidase, catalase, polyphenol oxidase, and laccase indicate that these enzymes play an important role in fungal adaptation to environmental stress. The findings highlight the ecological adaptability of fungi associated with germinating groundnut seeds under saline and alkaline conditions.

References

- Abrashev, R., Miteva-Staleva, J., Gocheva, Y., Stoyancheva, G., Dishliyska, V., Spasova, B., Krumova, E., & Angelova, M. (2025). Cell response to oxidative stress in Antarctic filamentous fungi. *Applied Sciences*, 15(9), 5149. <https://doi.org/10.3390/app15095149>
- Akter, S., Jharna, D. Y., Mali, S. K., & Sayeed, A. (2020). Salinity induced deleterious effects on germination, growth, physiological and biochemical process of two varieties of groundnut (*Arachis hypogaea* L.). *Archives of Agriculture and Environmental Science*, 5(2), 144–150. <https://doi.org/10.26832/24566632.2020.050209>
- Awad, M. F., Albogami, B., Mwabvu, T., Hassan, M. M., Baazeem, A., Hassan, M. M., & Elsharkawy, M. M. (2023). Identification and biodiversity patterns of *Aspergillus* species isolated from some soil invertebrates at high altitude using morphological characteristics and phylogenetic analyses. *PeerJ*, 11, e15035. <https://doi.org/10.7717/peerj.15035>
- Awoke, R. W., Kimurto, P. O., & Okello, D. (2025). Determinants of adoption of climate-smart agricultural technologies and practices in groundnut production: a gender-disaggregated analysis in arid and semi-arid lands of Kenya. *Discover*, 3: 238. <https://doi.org/10.1007/s44279-025-00406-1>

Original Article

- Bahrani-Rad, S., & Hajiboland, R. (2017). Effect of salt stress on germination and early seedling growth of peanut (*Arachis hypogaea* L.). *J Plant Nutr.* 2017;40(15):2180-2192.
- Baldrian, P. (2006). Fungal laccases—occurrence and properties. *FEMS Microbiol Rev*, 30(2), 215–242. <https://doi.org/10.1111/j.1574-4976.2005.00010.x>
- Barnett, H. L., & Hunter, B. B. (1998). *Illustrated genera of imperfect fungi* (4th ed.). New York: APS Press.
- Bassey, R. A., Etim, D. O., Agba, M. O., & Ndarake, E. I. (2025). Investigating the morphological characteristics and host range of three pathotypes of *Choanephora cucurbitarium* using 15 commonly grown vegetable crops. *Researchers Journal of Science and Technology*, 5(3), pp. 57–76. doi:10.1016/j.resjst.2025.05.016
- Blackwell, M. (2011). The fungi: 1, 2, 3... 5.1m species? *Am. J. Bot.*, 98(3), 426-438.
- Cao, Y., Song, H., & Zhang, L. (2022). New insight into plant saline-alkali tolerance mechanisms and application to breeding. *International Journal of Molecular Sciences*, 23(24), 16048. <https://doi.org/10.3390/ijms232416048>
- Chandrashekar, P., & Prasanna, K. (2011). Production and characterization of peroxidase by *Aspergillus flavus*. *Asian Journal of Plant Science and Research*, 1(2), 57–64. <https://doi.org/10.1016/j.ajpsres.2011.09.007>
- Etim, D. O., Agba, M. O., Bassey, R. A., Owan, K. O., & Bassey, G. A. (2026). Integrated use of poultry manure and *Trichoderma reesi* for the control of (*Melioidogyne arenaria*) on Indian spinach (*Basella alba*) in Calabar. *Global Journal of Pure and Applied Sciences*, 32, 139-147. <https://doi.org/10.1016/j.gjps.2012.01.013>.
- Frąc, M., Hannula, S. E., Bełka, M., & Jędryczka, M. (2018). Fungal biodiversity and their role in soil health. *Frontiers in Microbiology*, 9, 316246. <https://doi.org/10.3389/fmicb.2018.00707>
- Fukah, F. K., Magubika, A. J., Tryphone, G. M., & Nassary, E. K. (2024). Meta-analysis of legumes and groundnut production trends and variability in the Global South. *Journal of Agriculture and Food Research*, 18, 101501. <https://doi.org/10.1016/j.jafr.2024.101501>
- Gessler, N.N., Aver'yanov, A.A., & Belozerskaya, T.A. (2007). Reactive oxygen species in regulation of fungal development. *Biochemistry (Moscow)*, 72(10), 1091–1109. <https://doi.org/10.1016/j.biochem.2007.09.010>
- Gharaghani M, Mahmoudabadi A, Rezaei-Matehkolaei, A. (2022). Antioxidant enzyme responses of environmental fungal isolates under osmotic and saline stress conditions. *Current Medical Mycology*, 8(1), 45–53. <https://doi.org/10.18502/cmm.8.1.8932>
- Gong, B., Wen, D., VandenLangenberg, K., Wei, M., Yang, F., Shi, Q., & Wang, X. (2013). Comparative effects of NaCl and NaHCO₃ stress on photosynthetic parameters, nutrient metabolism, and the antioxidant system in tomato leaves. *Scientia Horticulturae*, 157, 1-12. <https://doi.org/10.1016/j.scienta.2013.03.032>

Original Article

- Gostinčar, C., Zalar, P., & Gunde-Cimerman, N. (2022). No need for speed: Slow development of fungi in extreme environments. *Fungal Biol. Rev.*, 39, 1-14.
- Kapoor, R., & Mukerji, K. G. (1999). Changes in antioxidant enzymes during arbuscular mycorrhizal colonization of *Zea mays* L. *Journal of Plant Physiology*, 154(5-6), 691–696. doi:10.1016/j.jpphysio.1999.010
- Khan, M. A., Hussain, A., Ali, S., Ahmad, R., & Rahman, H. (2025). Physiological and biochemical adaptation mechanisms of fungi under saline and alkaline stress environments. *Microorganisms*, 13(2), 214. <https://doi.org/10.3390/microorganisms13020214>
- Kiviniemi, E., Mikkola, A., Mattila, H., Wahlsten, M., & Lundell, T. (2023). Oxidative stress and culture atmosphere effects on bioactive compounds and laccase activity in the white rot fungus *Phlebia radiata* on birch wood substrate. *Current Research in Microbial Sciences*, 7, 100280. <https://doi.org/10.1016/j.crmicr.2024.100280>
- Lara-Martínez, D., Tristán-Flores, F. E., Cervantes-Montelongo, J. A., & Silva-Martínez, G. A. (2025). Fungal stress responses and the importance of GPCRs. *Journal of Fungi*, 11(3), 213. <https://doi.org/10.3390/jof11030213>
- Leng, S., & Liu, C. H. (2026). Environmental adaptation strategies of deep-sea fungi. *Journal of Fungi*, 12(3), 176. <https://doi.org/10.3390/jof12030176>
- Manono, B. O. (2026). Effects of salinity on seed germination: mechanisms, impacts, and mitigation strategies. *Seeds*, 5(1), 1. <https://doi.org/10.3390/seeds5010001>
- Mayer, A. M. (2006). Polyphenol oxidases in plants and fungi: Going places? A review. *Phytochemistry*, 67(21), 2318–2331. <https://doi.org/10.1016/j.phytochem.2006.09.016>
- Ngele, B. A., Agba, R. A., Bassey, M. O., & Egeh, E. A. (2024). Effect of irrigation with household detergent on germination, activities of oxidative stress enzymes, and chlorophyll content of pod maize. *Pakistan Journal of Agricultural Research*, 37(3), 290–299. <https://doi.org/10.1016/j.pjar.2024.04.009>
- Nyongesa, B., Okoth, S. & Ayugi, V. (2015). Identification key for *Aspergillus* species isolated from maize and soil of Nandi County, Kenya. *Advances in Microbiology*, 5, 205–229. doi: 10.4236/aim.2015.54020.
- Park, J., & Son, H. (2024). Antioxidant systems of plant pathogenic fungi: functions in oxidative stress response and their regulatory mechanisms. *Plant Pathology Journal*, 40(3), 235. <https://doi.org/10.5423/PPJ.RW.01.2024.0001>
- Prasad, R., Kumar, S., & Yadav, K. D. S. (2015). Polyphenol oxidase activity in fungi isolated from different soil types. *International Journal of Advanced Research*, 3(12), 1051–1056. <https://doi.org/10.1016/j.ijaar.2015.09.010>

Original Article

- Revankar, M. S., & Lele, S. S. (2006). Enhanced production of laccase using a new isolate of white-rot fungus WR-1. *Process Biochemistry*, 41(3), 581–588. <https://doi.org/10.1016/j.pbio.2006.09.010>.
- Rivera-Hoyos, C. M., Morales-Álvarez, E. D., Poutou-Piñales, R. A., Pedroza-Rodríguez, A. M., Rodríguez-Vázquez, R., & Delgado-Boada, J. M. (2013). Fungal laccases. *Fungal Biology Reviews*, 27(3-4), 67-82. <https://doi.org/10.1016/j.fbr.2013.07.001>
- Romão IR, do Carmo Gomes J, Silva D, Vilchez JI. The seed microbiota from an application perspective: an underexplored frontier in plant-microbe interactions. *Crop Health*. 2025 Jun 4;3(1):12. doi: 10.1007/s44297-025-00051-6.
- Syed, F., Arif, S., Ahmed, I., & Khalid, N. (2021). Groundnut (Peanut) (*Arachis hypogaea*). In M. A. Talhaoui & D. Smith (Eds.), *Oilseeds: Health attributes and food applications* (1st ed., Chap. 4). Springer Nature. <https://doi.org/10.1007/978-981-15-4194-0>
- Wang, Q. J., Sun, H., Dong, Q. L., Sun, T. Y., Jin, Z. X., Hao, Y. J., & Yao, Y. X. (2016). The enhancement of tolerance to salt and cold stresses by modifying the redox state and salicylic acid content via the cytosolic malate dehydrogenase gene in transgenic apple plants. *Plant Biotechnology Journal*, 14:1986–1997. doi: 10.1111/pbi.12556.
- Zhang, F., Zou, Y., & Wu, Q. (2018). Quantitative estimation of water uptake by mycorrhizal extraradical hyphae in citrus under drought stress. *Scientia Horticulturae*, 229, 132-136. <https://doi.org/10.1016/j.scienta.2017.10.038>