*African Scientist Vol. 25, No. 4 December 31, 2024*
Printed in Nigeria

1595-6881/2023 $80.00 + 0.00

https://africansciientistjournal.org

afs2024047/25403

# Stress Alleviation Property of Two Fungi Isolates Applied as Soil Inoculants in the Growth of *Abelmoschus esculentus* (L.) Moench. in Arsenic ion ($As^{3+}$) Treated Soils

Emuejevoke Dennis Vwioko* and Success Obas Eguma

Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City.

*Corresponding author Email: emuejevoke.vwioko@uniben.edu; Tel: +234 (0) 805 596 6903

(Received October 10, 2024; Accepted in revised form December 13, 2024)

**ABSTRACT:** This study was conducted to evaluate the ameliorative effect of selected fungi *(Aspergillus sp*, and *Penicillium chrysogenum*) on arsenic-treated ($As^{3+}$) soils used for the cultivation of *Abelmoschus esculentus*. Stress alleviation property of the fungi *spp* was done in soils contaminated with different concentrations of $As^{3+}$ solution, and inoculation of fungi was done two weeks after planting. Vegetative parameters (number of leaves, branches, plant height, and girth circumference) and biochemical parameters (superoxide dismutase (SOD), catalase (CAT), and chlorophyll a and b) were also assayed for. This study was conducted for 5-6 weeks. The highest mean values of mycelial extensions were observed in *Aspergillus sp* 4 days after media inoculation at 0 ppm. The plant with the highest height was observed in okra that was inoculated with *P. chrysogenum* at 30 ppm. SOD and CAT analysis revealed that the highest percentage inhibition was recorded at 150 ppm and 100 ppm in *P. chrysogenum* inoculated soil respectively. Findings from this study revealed that these fungi exhibited an ameliorative effect on the growth of *Abelmoschus esculentus* in different concentrations of arsenic-contaminated soil samples and thus suggest their application in heavy metal soils stress environments.

**Keywords:** Fungi; Okra; Arsenic; Superoxide dismutase; Catalase, Stress.

## Introduction

Heavy metal (HM) pollution has become a major global problem as it tends to accumulate in agricultural soils in proportion to the pace of worldwide industrialization (Pant *et al*., 2011). Arsenic (As) poses serious environmental threats due to its toxicities (DalCorso *et al*., 2008; Bhattacharya *et al.,* 2012), and their contamination in soils can be due to natural (disintegration of rocks and minerals and lixiviation) and anthropogenic sources (Bolan *et al*., 2014). The Comprehensive Environmental Response, Compensation, and Liability Act permanently listed arsenic metal as number 1 out of 275 in its priority list of hazardous materials (ATSDR, 2007). It usually originates via volcanic action, erosion of rocks, and human activities such as the application of pesticides and wood preservatives, mining, and smelting operations (Wang and Neumann, 2010).
The contamination of As in groundwater used for irrigation and drinking is a worldwide problem as it not only affects crop productivity, but also accumulates in different plant tissues including grains, and contaminates the food chain (Verma *et al.,* 2016). Arsenic inhibits the growth of plants through loss of fertility, yield, and fruit production (Garg and Singla, 2011; Vwioko and Edobor, 2017). 'Recently, several studies have been carried out to investigate the physiological and molecular mechanism of As, accumulation, detoxification, and tolerance in various plants including rice, lettuce, spinach, and carrot (Kurma *et al.,* 2015*).* Inorganic arsenate (As V), and arsenite (As111), are two forms of As that exist in the environment. Both As (III) and As (V) are toxic and are regarded as major environmental pollutants based on the United States Environmental Protection Agency (USEPA) evaluation (Verma *et al.,* 2016). Arsenite is more toxic than As (V) and acts by interrupting biological functions in plants in different manners, for example, it binds to proteins with sulfhydryl groups, interfering with

their functions (Verma *et al.*, 2016). It also generates reactive oxygen species (ROS) which inhibits respiration by binding to vicinal thiols in pyruvate dehydrogenase and 2-oxo-glutarate dehydrogenase and acts indirectly as a mutagen by inducing intrachromosomal homologous recombination (Helleday *et al.*, 2000). On the other hand, in plants As (V) interferes with oxidative phosphorylation and ATP synthesis during energy metabolism (Verma *et al.*, 2016).

Numerous filamentous fungi belonging to the genera *Trichoderma, Penicillium, Aspergillus,* and *Mucor* have been described as having the ability to tolerate heavy metal stress (Ezzouhri *et al.*, 2009). Fungal cell walls have excellent metal binding properties due to the presence of negative charge on the different functional groups, e.g., carboxylic, amine or sulfhydryl, and phosphate, in different wall components (Ong *et al*., 2017). A study showed the interaction of *Aspergillus niger* var. tubingensis Ed8 with Cr (VI) mainly in a reduction process and also in a sorption process (Coreño-Alonso *et al.*, 2014). Previous studies reported a reduction in As-induced stress in chickpeas through *Trichoderma* sp. (Tripathi *et al*., 2013; Tripathi *et al*., 2017).

Arbuscular mycorrhizal fungi (AMF) are also one of the most prominent soil microorganisms. They establish a direct physical link between soil and plant roots, which increases root surface area and facilitates nutrient absorption by the plants (Saxena *et al*., 2017). Arbuscular mycorrhizal fungi also alleviate metal toxicity in the host plant (Meharg, 2003). The specific role of arbuscular mycorrhizae in the host plant on exposure to heavy metal depends on a variety of factors, including the plant species and ecotype, the fungal species and ecotype, the metal and its availability; soil edaphic conditions, including soil fertility; and plant growth conditions, such as light intensity or root density (Pawlowska and Charvat, 2004).

Similar to Plant growth-promoting fungi (PGPR), several mechanisms have been hypothesized for toxic metal direction and allocation in plant roots in the presence of AMF including (a) heavy metals bound to cell walls are deposited in the vacuoles of AMF, (b) metal sequestration by the help of siderophores in the soil or into root apoplasm, (c) metals bound to metallothioneins or phytochelatins inside the fungal or plant cells, (d) metal transporters at the tonoplast of both plants and fungi catalyze the transport of metals from cytoplasm (Jan and Parray, 2016).

Okra or okro (*Abelmoschus esculentus* (L.) Moench. Is a flowering plant in the mallow family. It is valued for its edible green seed pods. The Okra species have a short growth cycle, where flower and fruit production are completed within 2-3 months. The plant is cultivated in tropical, subtropical, and warm temperate regions worldwide (Gemede *et al.*, 2015). It is grown commercially in India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malaysia, Brazil, Ghana, Ethiopia, Cyprus, and the Southern United States. India ranks first in the world with 3.5 million tons (70% of the total world production) of okra produced from over 0.35 million hectares of land (FAOSTAT, 2008). Many local names in different parts of the world know okra. It is called lady's finger in England, gumbo in the United States of America, guino-gombo in Spanish, ikhievbho in Edo, Okworo in Igbo, guibeiro in Portuguese, and bhindi in India. It is quite popular in India because of its ease of cultivation, dependable yield, and adaptability to varying moisture conditions. Even within India, different names have been given in different regional languages (Chauhan, 1972). Its pods are rich in fiber and vitamins (Andras *et al.*, 2005). The objective of this study was to determine the growth of *Abelmoschus esculentus* in arsenic ion-treated soil, supplemented with fungi isolates.

## Materials and methods

*Plant material*: *Abelmoschus esculentus* L. (Moench) seeds used in this study were obtained from a local farmer at Uselu, Benin City, Edo State.

*Collection of soil samples from car spraying painting workshop (March, 2018)*: Topsoil samples (0-10 cm) containing suspected fungi species that inhabit soil environments contaminated with heavy metals like arsenic were collected from a car spray painting workshop at Ikpoba Hill (6.35258 ° N, 5.64152 °E), Ikpoba-okha local government area, Edo state.

*Isolation of fungi*: One gram of soil sample was weighed into a 25 ml conical flask. Nine milliliters of sterilized water were added and shaken together. One milliliter was collected using a sterile syringe into a test tube containing 9 ml of sterilized water. By serial dilution of up to $10^{-6}$ ml was used to inoculate Petri dishes containing PDA (potato dextrose agar) as media. Incubation was done at room temperature (30° ±1). Seventy-two hours after inoculation, the growth of different fungi was seen on the petri dishes. A subculturing process to obtain pure culture was carried out (Osaigbovo *et al.*, 2022).

*Identification of fungi*: The fungal isolates were morphologically identified and characterized by observing their morphological characteristics using lactophenol stain, spore, and hyphae as well as the presence or absence of septa, etc., and colored monographs were recorded and compared with those that appeared in (St Germain and Summerbell, 1996), (Eillis and Eillis, 1996) and (Barrett and Barry, 1998)

*Screening for tolerance of fungal isolates in $As^{3+}$ion supplemented media*: After obtaining the pure culture of fungi isolates, identified, the isolates were grown in different concentrations of $As^{3+}$ion supplemented media (0, 30, 50, 100, and 150 ppm $As^{3+}$). Three replications of each replicant of each concentrations were used per fungi isolate. A total of thirty petri dishes supplemented with $As^{3+}$ion were prepared for *Aspergillus sp* and *Penicillium chrysogenum*. The record of the mycelial extension was used to determine the tolerating capacity of fungi species.

*Preparation of Broth*: Two fungi isolates with higher mycelia extension were selected, from the result of the screening growth on the media supplemented with $As^{3+}$ion and were used for inoculation in the greenhouse experiment, located in the botanical garden faculty of life sciences, University of Benin two weeks after planting broth culture were prepared by inoculating PDA broth with the fungi and incubating the culture for 3 to 5 days at 32 °C. Hemocytometer was used to determine the number of spore. Spore suspension was standardized to $16\times10^5$ spores/ml by adding 10 ml of the culture to 90 ml of sterile distilled water.

*Application of fungi isolates (broth) to experimental pots*: The standardized spore suspensions of *Aspergillus* sp and *Penicillium chrysogenum* were used to inoculate the soils in the experimental pots. This was made possible by using a syringe to collect 2ml of the standardized spore suspension which was applied to each pot of $As^{3+}$ ion treated soil and control soils. *Aspergillus* sp was applied to twenty pots and *Penicillium chrysogenum* to another 20 pots. The application of the fungi isolates was done four days after the seeds had germinated.

*Preparation of Arsenic ion ($As^3$) solution*: The arsenic solution was prepared by dissolving different masses of the arsenic III trioxide ($As_2O_3$) in a fixed volume of deionized water (4L) to get the different concentrations of 30 ppm, 50 ppm, 100 ppm and 150 ppm, and a concentration tagged 0 ppm was also used as a control in the course of the experiment (Vwioko and Edobor 2017).

*Test of viability*: Before planting, the Okra seeds were tested for viability using the floatation technique. This was done by allowing the seeds to stand in a water bowl for 5-10 minutes. Seeds that remained afloat were considered non-viable and then discarded. The viable seeds were used for this study.

*Preparation of experimental pots*: Three kilograms of dried sieved soils were weighed into an experimental pot, prepared, and taken to the field for acclimatization. The pots were watered and allowed to stand overnight in the field.

*Application of arsenic ion ($As^{3+}$) treatment to the experimental pots containing the soil*: Two hundred milliliters of different concentrations of $As^{3+}$ solution are used to wet the experimental pots each concentration has eight experimental pots. The pots were left to stand in the field for two days.

*Sowing of seeds*: The sowing of seeds was carried out after the pots had received the $As^{3+}$ion treatments. This was done early hours of between 6:30 am – 7:30 am at a depth of 2-3 cm deep. The seeds were seen in each experimental pot and watered.

**Vegetative measurements:**

*Germination*: The appearance of the plumule above the surface was taken as germination. Germination record was taken for 14 days after sowing. The percent germination was calculated using the formula.

$$\textbf{Germination Percentage (\%)} = \frac{\text{Number of Seeds germinated}}{\text{Number of seeds sown}} \times 100$$

*Plant heigh*t: This was measured using a calibrated meter rule from the base to the tip of the plant. This was done once in two weeks throughout the experiment from 2nd week.

*Number of leaves*: The leaves of each plant were counted and recorded once every 2 weeks and from the second week after planting

*Number of branches*: The number of branches of each plant were counted and recorded once every 2 weeks and from the second week after planting.

*Stem circumference*: This measurement was carried out by using a thread to tie the base of the stem, the length of the thread was measured using a meter rule to determine the plant circumference. This was done once every two weeks.

*Statistical analysis*: Data collected were used to calculate mean and standard error. One-way analysis of variance was carried out. Mean difference were done using the Duncan multiple range (DMR).Test at $\alpha = 0.05$

**Biochemical analysis**

*Estimation of superoxide dismutase (SOD) activity*: This was determined according to the methods of (Misra and Fridovich, 1972).

*Principle***:** Adrenaline undergoes auto-oxidation rapidly to adrenochrome whose concentration can be determined at 420 nm with the aid of a spectrophotometer. The auto-oxidation of adrenaline depends on the presence of superoxide anions. Superoxide dismutase inhibits the auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420 nm.

*Reagents and their preparation*: Carbonate buffer (0.05 M) pH 10.2: This was prepared by dissolving 0.2014 g of $Na_2CO_3$, 0.2604g of $NaHCO_3$, and 0.0372g of EDTA in 100ml of distilled water. The pH was adjusted to 10.2 using sodium hydroxide.
*Hydrochloric acid:* This was prepared by adding 0.044 concentration HCl to 99.96 mls of distilled water.
Adrenaline solution (0.3 mM): This was prepared by dissolving 0.01098 g of adrenaline in 100 ml of 0.005 M HCl solution.
A plasma volume of 0.2 ml was mixed with 2.5 ml of carbonate buffer and 0.3 ml of adrenaline solution. 0.2 ml of distilled water was mixed with 2.5 ml of carbonate buffer and 0.3 ml adrenaline as reference sample. These were mixed and absorbance was read at 420 nm.

$$\textbf{\% inhibition} = \frac{\text{O.D.test} - \text{O.D.ref}}{\text{O.D.test}} \times 100$$

Enzyme concentration can thus be calculated

$$\textbf{Unit/mg protein} = \frac{\text{\% inhibition}}{50 \times Y}$$

where Y = mg of protein in the volume of sample used.
*Estimation of catalase activity*: This was determined by the method of (Cohen *et al.*, 1970)
*Principle***:** Catalase is present in almost all plants, animals, and bacteria cells. It acts to prevent the accumulation of noxious $H_2O_2$ which is converted to $H_2O$ and $O_2$.
*Reagents*: 30mM $H_2O_2$; 6M $H_2S0_4$; 0.01M potassium permanganate
**Preparation of reagents:**
0.01M potassium permanganate was prepared by dissolving 0.158g of $KMnO_4$ in 100 ml of distilled water. Phosphate buffer (pH 7.4). 0.426g of $Na_2HPO_4$ and 0.240g of $NaH_2PO_4$ was weighed and dissolved in 100ml of distilled water.
6 M $H_2SO_4$ = 32.3 ml of conc. $H_2SO_4$ was added to 66.7 ml of distilled water.
30 mM $H_2O_2$ solution: This was prepared by measuring 0.34ml of 30% of $H_2O_2$ in 100ml of phosphate buffer.
*Procedure:* To a known volume of plasma (0.5ml), 5.0 ml of $H_2O_2$ added. This was mixed by inversion and allowed to stand for 30 min. The reaction was stopped by adding 1.5 ml of 6M $H_2SO_4$ and 7 ml of 0.01 $KMnO_4$. Distilled water was used as blank. These were mixed by inversion and allowed to stand for 30 min. The reaction was stopped by adding 6M $H_2SO_4$. The absorbance was taken at 480 nm within 30 – 60 seconds against distilled water.
*Calculation*:

$$\textbf{Activity} = \frac{\text{O.D/min} \times Vt}{M \times V \times L \times Y}$$

OD = Absorbance, L = Light path =1cm, $V_t$ = Total volume of reaction sample, M = Molar extinction coefficient of $H_2O_2$ (40/M/cm), Y = mg of protein in the sample, V = volume of sample homogenate used
*Estimation of chlorophyll a and b*: This was investigated according to methods described by (Arnon, 1949) and (Maxwell and Johnson, 2000).
*Procedure:* One gram of the leaf samples was cut into small pieces, macerated with 80% (v/v) acetone, with minute sand and a pinch of calcium carbonate. The homogenate was then centrifuged at 3000g for 10 minutes and the supernatant was determined at 645 nm and 663 nm in a spectrophotometer, against 80% acetone blank. All the procedures were carried out in dim light. The chlorophyll a and b content were determined using the formulas below. *pe equation here.*

$$\textbf{Chlorophyll a (mg/g FW)} = \frac{(12.7 \times A663) - (2.69 \times A645) \times V}{100 \times W \times a} \quad \ldots\ldots\ldots\ldots (8)$$

$$\textbf{Chlorophyll b (mg/g FW)} = \frac{(22.9 \times A645) - (4.88 \times A633) \times V}{100 \times W \times a} \quad \ldots\ldots\ldots\ldots (9)$$

where: W= Fresh weight of sample (g)
A = Absorbance at a specific wavelength
V =Volume of light path in the cell (1cm)
Total chlorophyll content= chlorophyll a (mg/g FW) + chlorophyll b (mg/g FW)

## Results

The results obtained from this study are shown in Tables 1-6 and Figures 1-3 below. Morphological identification of isolated fungi was examined based on microscopic features which include; colour, spore, hypha structure, colony texture, and shapes of these fungi (Table 1).

**Table 1:** Morphological identification of fungi in PDA media supplemented with different concentrations of $As^{3+}$ concentration solutions.

| Fungi | Colour | Spore | Hyphae structure | Colony texture | Shapes |
|---|---|---|---|---|---|
| *Aspergillus* sp | colourless | Conidia | 3-6µm in diameter and septate have acute-angle branching | 60-70mm with white initial growth colour and black coloration due to the production of spore | Glubose |
| *Penicillium chrysogenum* | Blue-green | Conidia | 2-5µm in diameter a branch conidia with secondary branches | 30-45mm appears as blue-green with a yellow pigment | Brush-like(penicillus) |

Mycelia extension or growth in the PDA media supplemented with $As^{3+}$ solution by *Aspergillus sp and Penicillium chrysogenum* were recorded in all concentrations considered. The highest growth observed in *Aspergillus sp and Penicillium chrysogenum* were 4.00±0.00 cm and 3.94±0.06 cm respectively, at 0ppm $As^{3+}$ media, 4 days after inoculation (see Table 2). Significant differences were recorded for mycelial growth as the $As^{3+}$ concentration in media increased. The lowest mean values for mycelial extension were obtained in 150ppm supplemented media 4 days after inoculation.

**Table 2:** Mycelial extension (cm) in PDA media supplemented with different concentrations of arsenic trioxide solutions

| Treatments | Organism | 1DAI | 2 DAI | 3 DAI | 4 DAI |
|---|---|---|---|---|---|
| 0 ppm | *Aspergillus sp* | $1.70^{b}$±0.12 | $3.50^{d}$±0.00 | $3.51^{bc}$±0.00 | $4.00^{c}$±0.00 |
| | *Penicilium chrysogenum* | $2.22^{bc}$±0.07 | $2.93^{bcd}$±0.26 | $3.03^{bd}$±0.27 | $3.94^{c}$±0.06 |
| 30 ppm | *Aspergillus sp* | $2.43^{bc}$±0.29 | $3.23^{bc}$±0.17 | $3.32^{d}$±0.16 | $3.89^{c}$±0.11 |
| | *Penicilium chrysogenum* | $3.08^{bc}$±0.70 | $2.77^{bd}$±0.29 | $2.91^{bd}$±0.24 | $3.64^{bc}$±0.18 |
| 50 ppm | *Aspergillus sp* | $2.43^{a}$±0.54 | $3.07^{bc}$±0.17 | $3.20^{b}$±0.15 | $3.93^{c}$±0.06 |
| | *Penicilium chrysogenum* | $2.35^{bc}$±0.42 | $2.73^{bc}$±0.12 | $2.87^{bd}$±0.06 | $3.61^{bc}$±0.06 |
| 100 ppm | *Aspergillus sp* | $2.03^{bc}$±0.27 | $2.63^{bc}$±0.46 | $2.73^{b}$±0.47 | $3.14^{ab}$±0.46 |
| | *Penicilium chrysogenum* | $1.95^{bc}$±0.13 | $2.48^{bc}$±0.08 | $2.56^{b}$±0.07 | $2.99^{a}$±0.01 |
| 150 ppm | *Aspergillus sp* | $2.32^{bc}$±0.14 | $2.43^{bc}$±0.18 | $2.62^{ab}$±0.16 | $3.09^{ab}$±0.07 |
| | *Penicilium chrysogenum* | $0.38^{a}$±0.19 | $1.28^{a}$±0.02 | $1.56^{bd}$±0.18 | $2.57^{a}$±0.24 |

DAI= day after inoculation. Values = mean±S.E. Values in the same column with similar alphabets as superscript are not significantly different using Duncan multiple range test at 5% level of significance.

Height obtained for *Abelmoschus esculentus* plants sown in $As^{3+}$ treated soils which were supplemented in *Aspergillus sp and Penicillium chrysogenum* were recorded once in every two weeks for 4 weeks. Four weeks after planting (4WAP), the highest height of *Abelmoschus esculentus* seedlings recorded in soil inoculated with fungi isolates were 28.00±1.68 cm at 30ppm and 25.30±1.38 cm at 50ppm in *Aspergillus sp and Penicillium chrysogenum* respectively (Table 3). The least mean value was obtained in 0ppm concentration 4 weeks after planting.

**Table 3:** Height (cm) of *Abelmoschus esculentus* plants grown in $As^{3+}$ treated soils inoculated with fungi species two weeks after planting

| Treatments | Organism | 2WAP | | 4WAP | |
|---|---|---|---|---|---|
| 0ppm | *Aspergillus sp* | $16.30^{ab}$ | ±5.68 | $20.00^{ab}$ | ±6.70 |
| | *Penicilium chrysogenum* | $9.75^{a}$ | ± 5.72 | $13.40^{a}$ | ± 4.04 |
| 30ppm | *Aspergillus sp* | $22.80^{b}$ | ±0.63 | $28.00^{c}$ | ±1.68 |
| | *Penicilium chrysogenum* | $13.00^{ab}$ | ± 4.36 | $14.80^{a}$ | ± 5.28 |
| 50ppm | *Aspergillus sp* | $19.30^{ab}$ | ±0.48 | $24.30^{bc}$ | ±2.25 |
| | *Penicilium chrysogenum* | $19.80^{ab}$ | ± 0.85 | $25.30^{bc}$ | ± 1.38 |
| 100ppm | *Aspergillus sp* | $17.50^{ab}$ | ±1.44 | $21.80^{bc}$ | ±1.60 |
| | *Penicilium chrysogenum* | $19.00^{ab}$ | ± 0.71 | $22.30^{bc}$ | ± 1.03 |
| 150ppm | *Aspergillus sp* | $18.80^{ab}$ | ±1.38 | $24.00^{bc}$ | 1.08 |
| | *Penicilium chrysogenum* | $17.80^{ab}$ | ± 0.75 | $21.30^{bc}$ | ± 2.10 |

WAP= week after planting. Values = mean±S.E. Values in the same column with similar alphabets as superscript are not significantly different using Duncan multiple range test at 5% level of significance.

Stem circumference or girth of *Abelmoschus esculentus* grown on soil treated with $As^{3+}$ solution supplemented with *Aspergillus* sp and *Penicillium chrysogenum* were recorded in all concentrations. The highest stem circumference of okro plants observed in *Aspergillus* sp and *Penicillium chrysogenum* inoculated soils were 0.67±0.07 cm at 50ppm and 0.68±0.07 cm at 30ppm $As^{3+}$ respectively, 4 weeks after planting (Table 4). The least mean values of stem circumference were obtained for plants grown in 100ppm arsenic-treated soil, 4 weeks after planting.

**Table 4:** Stem circumference (cm) of *Abelmoschus esculentus* plants grown in $As^{3+}$ treated soils inoculated with fungi species two weeks after planting

| Treatments | Organism | 2WAP | | 4WAP | |
|---|---|---|---|---|---|
| 0ppm | *Aspergillus sp* | 0.42[a] | ±0.13 | 0.50[a] | ±0.15 |
| | *Penicilium chrysogenum* | 0.55 [a] | ± 0.06 | 0.65 [a] | ± 0.06 |
| 30ppm | *Aspergillus sp* | 0.52[a] | ±0.04 | 0.62[a] | ±0.04 |
| | *Penicilium chrysogenum* | 0.50 [a] | ± 0.04 | 0.68 [a] | ± 0.06 |
| 50ppm | *Aspergillus sp* | 0.56[a] | ±0.05 | 0.67[a] | ±0.07 |
| | *Penicilium chrysogenum* | 0.45 [a] | ± 0.03 | 0.60 [a] | ± 0.04 |
| 100ppm | *Aspergillus sp* | 0.44[a] | ±0.16 | 0.54[a] | ±0.19 |
| | *Penicilium chrysogenum* | 0.30 [a] | ± 0.18 | 0.33 [a] | ± 0.19 |
| 150ppm | *Aspergillus sp* | 0.46[a] | ±0.05 | 0.59[a] | ±0.06 |
| | *Penicilium chrysogenum* | 0.48 [a] | ± 0.09 | 0.60 [a] | ± 0.07 |

WAP= week after planting. Values = mean±S.E. Values in the same column with similar alphabets as superscript are not significantly different using Duncan multiple range test at 5% level of significance.

*Abelmoschus esculentus* plants grown in soils treated with $As^{3+}$ solution supplemented with *Aspergillus* sp and *Penicillium chrysogenum* separately produced leaves in all concentrations studied. Number of leaves were recorded for all plants at 2 WAP and 4 WAP. The highest mean values for number of leaves produced by okra plant grown in $As^{3+}$ treated soil supplemented with *Aspergillus sp and Penicillium chrysogenum* were 5.25±0.29 at150ppm and 4.50±0.29 at150ppm respectively. Differences of mean values obtained for the number of leaves of okra plants grown in $As^{3+}$treated soils were significant. The least mean value was recorded for number of leaves okra plants grown in 0ppm $As^{3+}$ treated soil 4WAP.

**Table 5:** Number of leaves of *Abelmoschus esculentus* plants grown in $As^{3+}$ treated soils

| Treatments | Organism | 2WAP | | 4WAP | |
|---|---|---|---|---|---|
| 0ppm | *Aspergillus sp* | 4.00[a] | ±0.48 | 3.75[ab] | ±1.31 |
| | *Penicilium chrysogenum* | 2.25[a] | ± 1.31 | 2.25 [a] | ± 1.15 |
| 30ppm | *Aspergillus sp* | 4.75[a] | ±0.48 | 5.00[b] | ±0.00 |
| | *Penicilium chrysogenum* | 3.50 [a] | ± 1.19 | 4.25 [a] | ± 1.44 |
| 50ppm | *Aspergillus sp* | 3.00[a] | ±0.00 | 3.50[ab] | ±0.50 |
| | *Penicilium chrysogenum* | 4.00 [a] | ± 0.00 | 4.00 [a] | ± 0.00 |
| 100ppm | *Aspergillus sp* | 3.75[a] | ±0.48 | 5.00[b] | ±0.41 |
| | *Penicilium chrysogenum* | 3.75[a] | ± 0.48 | 4.50 [a] | ± 0.29 |
| 150ppm | *Aspergillius sp* | 4.25[a] | ±0.25 | 5.25[b] | ±0.25 |
| | *Penicilium chrysogenum* | 3.25 [a] | ± 0.25 | 4.50 [a] | ± 0.29 |

WAP = week after planting. Values = mean±S.E. Values in the same column with similar alphabets as superscript are not significantly different using Duncan multiple range test at 5% level of significance.

The record for number of branches per plant is shown in Table 6. *Abelmoschus esculentus* plants grown in $As^{3+}$soils treated supplemented with *Aspergillus sp and Penicillium chrysogenum* separately produced branches. Number of branches were recorded for all plants at 2WAP and 4WAP. The highest mean value for the number of branches per plant observed for plants grown in $As^{3+}$ treated soils supplemented with *Aspergillus sp and Penicillium chrysogenum* separately were 5.25±0.25 and 4.50±0.29 respectively at 150 ppm $As^{3+}$ treated soils. The least mean was recorded at 0ppm week 4 after planting.

**Table 6:** Number of branches per plant of *Abelmoschus esculentus* grown in $As^{3+}$ treated soil

| Treatments | Organism | 2WAP | | 4WAP | |
|---|---|---|---|---|---|
| 0ppm | *Aspergillus sp* | 4.00[a] | ±0.48 | 3.75[ab] | ±1.31 |
| | *Penicilium chrysogenum* | 2.25[a] | ± 1.31 | 2.25 [a] | ± 1.15 |
| 30ppm | *Aspergillus sp* | 4.75[a] | ±0.48 | 5.00[b] | ±0.00 |
| | *Penicilium chrysogenum* | 3.50 [a] | ± 1.19 | 4.25 [a] | ± 1.44 |
| 50ppm | *Aspergillus sp* | 3.00[a] | ±0.00 | 3.50[ab] | ±0.50 |
| | *Penicilium chrysogenum* | 4.00 [a] | ± 0.00 | 4.00 [a] | ± 0.00 |
| 100ppm | *Aspergillus sp* | 3.75[a] | ±0.48 | 5.00[b] | ±0.41 |
| | *Penicilium chrysogenum* | 3.75[a] | ± 0.48 | 4.50 [a] | ± 0.29 |
| 150ppm | *Aspergillius sp* | 4.25[a] | ±0.25 | 5.25[b] | ±0.25 |
| | *Penicilium chrysogenum* | 3.25 [a] | ± 0.25 | 4.50 [a] | ± 0.29 |

WAP= week after planting. Values = mean±S.E. Values in the same column with similar alphabets as superscript are not significantly different using Duncan multiple range test at 5% level of significance.

The total chlorophyll contents of the leaves of *Abelmoschus esculentus* plants grown in different concentrations of $As^{3+}$treated soils supplemented with fungi inoculants are presented in Figure 1. The presence of inoculants (*Aspergillus* sp = organism 1 and *Penicillium chrysogenum* = organism 2) separately in $As^{3+}$ treated soils to improve the chlorophyll contents of the leaves of okro plants at 100 ppm $As^{3+}$ treated soil sample (*Penicillium chrysogenum*) and 150 ppm $As^{3+}$ treated soil sample (*Aspergillus* sp.)

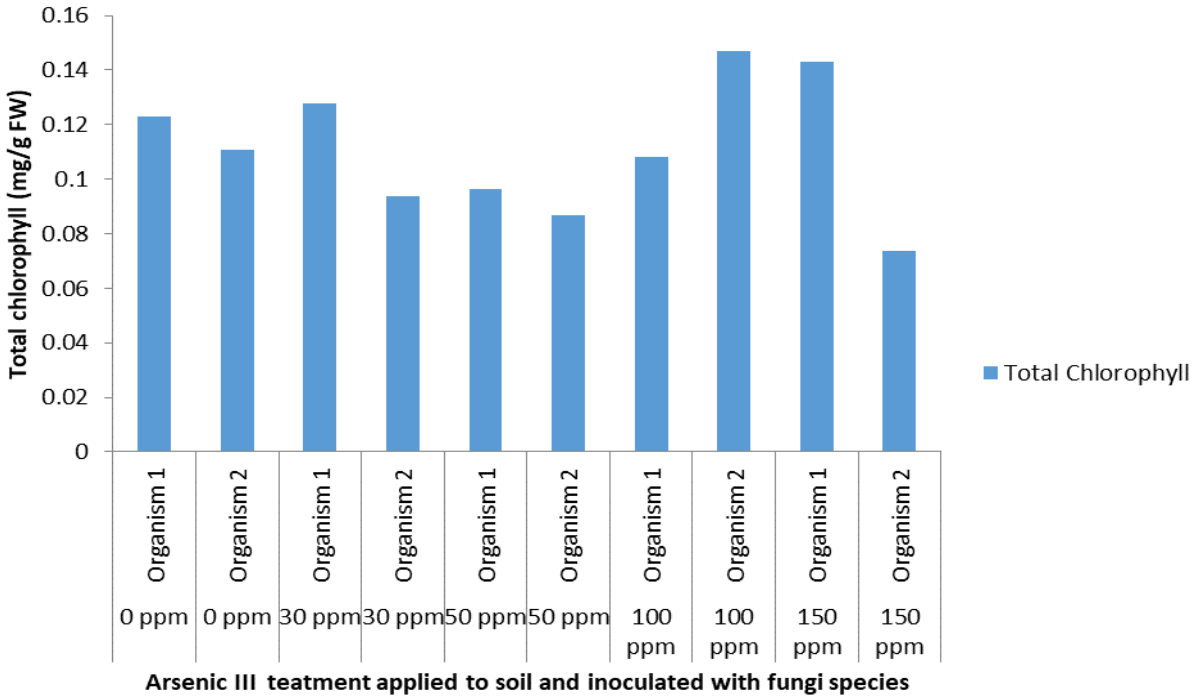


**Figure 1:** Total chlorophyll contents of *Abelmoschus esculentus* plants grown in $As^{3+}$ treated soils inoculated with fungi species (*Aspergillus* sp = Organism 1 and *Penicillium chrysogenum* = Organism 2, each bar of the graph is an average of values obtained from two separate analysis).

The estimation of superoxide dismutase (SOD) contents in roots and leaves of *Abelmoschus esculentus* plants grown in $As^{3+}$ treated soils and supplemented with *Aspergillus* sp (organism 1) and *Penicillium chrysogenum* (organism 2) separately are shown in Figure 2. Generally, SOD contents were observed to be higher in leaves than in roots of *Abelmoschus esculentus* plants. Plants grown in control soil treatments (0 ppm $As^{3+}$) exhibited the least SOD contents. SOD contents increase as $As^{3+}$ ions concentrations increased in soils. The highest values

for SOD contents were recorded for plants grown in 150ppm $As^{3+}$ treated soils. The SOD values obtained for roots of okra plants grown in $As^{3+}$treated soils augmented with *P. chrysogenum* were higher than those obtained for soils augmented with *Aspergillus* sp.

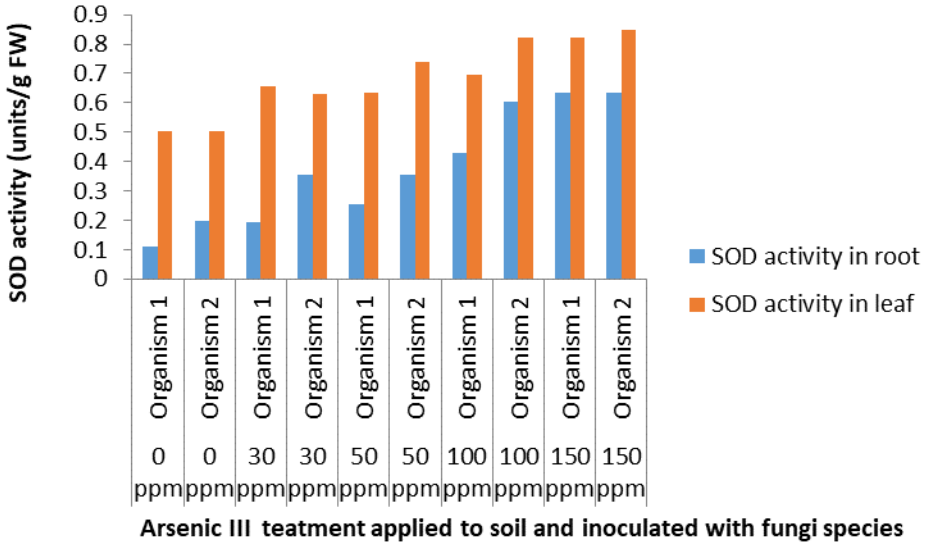


**Figure 2:** Superoxide dismutase (SOD) activity in *Abelmoschus esculentus* plants grown in $As^{3+}$ treated soils inoculated with fungi species (*Aspergillus* sp = Organism 1 and *Penicillium chrysogenum* = Organism 2, each bar of the graph is an average of values obtained from two separate analysis).

The activity of catalase (CAT) in the roots and leaves of *Abelmoschus esculentus* plants grown in $As^{3+}$ treated soils inoculated with fungi species, *Aspergillus* sp (organism 1) and *Penicillium chrysogenum* (organism 2) separately, are shown in Figure 3. Generally, CAT activity was observed to be higher in the leaves than the roots of the *Abelmoschus esculentus* plants. Plants grown in control soil treatment (0ppm $As^{3+}$) exhibited the least CAT activity. CAT activity increases as $As^{3+}$ ion concentration increases in the soils. The highest values for CAT activity were recorded for plants grown in 150ppm $As^{3+}$ treated soils for *Penicillium chrysogenum*. The CAT values obtained for roots and leaves of okra plants grown in $As^{3+}$treated soils augmented with *P. chrysogenum* and *Aspergillus* sp increase progressively as the $As^{3+}$concentration increases in soils.

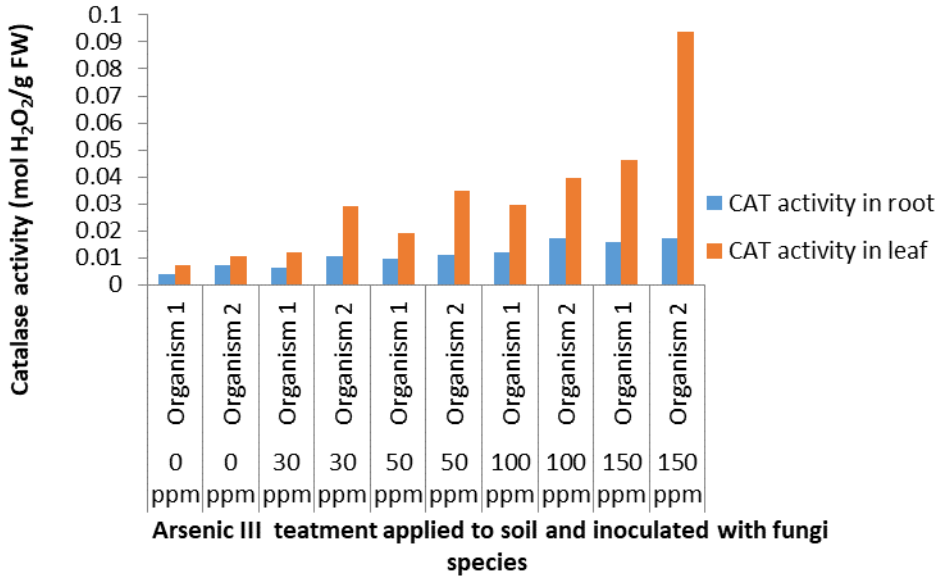


**Figure 3:** Catalase (CAT) activity in *Abelmoschus esculentus* plants grown in $As^{3+}$ treated soils inoculated with fungi species. (*Aspergillus* sp = Organism 1 and *Penicillium chrysogenum* = Organism 2, each bar of the graph is an average of values obtained from two separate analysis).

## Discussion

Heavy metal bioaccumulation disrupts the biological function of all living kingdoms (Plantae, Animalia, Monera, fungi, and Protista,) which is an important issue for environmental health and safety. Arsenic is generally considered phytotoxic and is expected to negatively affect plant growth (Kabata-Pendias and Pendias, 1991). As a result of many negative effects on plants, arsenic causes reduced growth of plants. Reactive oxygen species can directly damage proteins, amino acids, and nucleic acids and cause peroxidation of membrane lipids (Dat *et al*., 2000). This study investigated the role of fungi in reducing stress imposed on *Abelmoschus esculentus* plants in arsenic-contaminated soils. From this study, it was shown that the presence of fungi inoculants (*Aspergillu*s sp and *Penicillium chrysogenum*) in $As^{3+}$ treated soils exhibited an ameliorative effect on *Abelmoschus esculentus* plants; as the highest value for height was recorded for plants grown in soils treated with 30 ppm $As^{3+}$.

The fungi used in this study were isolated from soil samples collected at an automotive painter's workshop. Soils found around that region are believed to contain heavy metals. (Thorpe and Harrison, 2008) reported that soils within the automobile shop receive varying inputs of heavy metals from a wide range of mobile and stationary sources such as indiscriminate disposal of carbide, fuel combustion, panel-beating, vehicular emission, spray painting, spilling of spent fuel, and discarding lead plates on the soil. Two fungi isolates, *Aspergillu*s sp and *Penicillium chrysogenum*, were identified using their shape, colour, conidia, hyphae texture, and colony structure (Ellis and Ellis, 1996; Barnett and Barry, 1998). The two isolates exhibited good tolerance by surviving growth in PDA $As^{3+}$ supplemented media *in vitro*. If these two isolates could show mycelial extension up to 150ppm axenically the ability of isolates to cope in soil media contaminated with $As^{3+}$ cannot be in doubt. The two fungi isolates possess the arsenic ion-resistant ability to survive and also assist plants to grow in metal-contaminated soil environment. Fungi can absorb $As^{3+}$ ion due to its oxidizing ability, this is made possible by the secretion of an enzyme called Arseniclloxidase which catalyzes this oxidative process (William and Magpantay, 2024). (Zafar *et al.*, 2007) have observed that fungi can tolerate, biosorb, and detoxify metals by several mechanisms including valence transformation, extra and intracellular precipitation, and active uptake.

These fungi as used in this study assisted plant growth positively as shown in Tables 3-6. Other vegetative parameters affected positively marginal were stem circumference, number of leaves, and number of branches. Values were observed to increase significantly as $As^{3+}$ concentrations in soils increased significantly, indicating the positive impact of both fungi on soil conditions. A similar study conducted by (Kaushik *et al.*, 2013) indicated that two arsenic-resistant fungal strains, *Aspergillus flavus*, and *A. niger*, obtained from some polluted sites of Kolkata, India, exhibited the potential of removing 50% -76 % of arsenic from different arsenic enriched media and as well exhibiting tolerance to different other heavy metals (Cd, Pb, Hg, Zn, and Cr).

Chlorophyll content is often measured to assess the impact of environmental stress on plants, as changes in chlorophyll content can be associated with symptoms of plant disease and changes in photosynthetic productivity (Zengin and Munzuroglu, 2005). The accumulation of arsenic in the leaf biomass has been observed to reduce the rate of photosynthesis in plants (Marques and Anderson, 1986; Caporale *et al.*, 2014). It was observed from the result of this study that plant-inoculated fungi showed an increase in chlorophyll content predominantly in contaminated soil treated with *Penicillium chrysogenum*. This is attributed to the ability of the fungi to help the plant tolerate the $As^{3+}$ contaminated soils. It was observed plants grown in 100ppm $As^{3+}$treated soil inoculated with *P. chrysogenum* gave the highest chlorophyll content in leaves. (Caporale *et al*., 2014) reported that the inoculation of *Chrysopogon zizaniodes* with *Glomus* spp. significantly increased the amount of chlorophylls a and b in the leaf biomass of plants.

Superoxide dismutase (SOD) is an antioxidant enzyme that converts superoxide ($O_2^-$) into hydrogen peroxide (Younus, 2018.), which is also another oxidizing agent (Gunes *et al.*, 2009). It is the first line of defense against reactive oxygen species. Superoxide dismutase and catalase are enzymes that are induced when plants are exposed to stress (e.g. metallic stress from the presence of arsenic ions), this enzyme acts on superoxide free radicals (Alscher and Erturk, 2002). The activities of SOD were induced and found to be higher in plants grown in $As^{3+}$ treated soils. SOD activity in the plant roots and leaves increased as arsenic concentrations in the soil increased. The higher concentration of arsenic ions in soils did not impact very high inhibitory effect on plants grown because of the presence of inoculants. Among the influences exhibited by the presence of inoculants in arsenic ions treated soils was to ameliorate the toxicity of arsenic ions to the plants by limiting its uptake into the plants or creating a detoxification process to support plant growth. In similar studies, the arsenic application increases SOD (Srivastrava *et al.*, 2005; Al-Ani *et al.*, 2015) content. (Kanwal *et al*., 2016) reported that the SOD contents in plants were high in plants with mycorrhizal-associated treatments when compared to non-mycorrhizal-associated treatments.

The induction of catalase (CAT), a hydrogen peroxide decomposing enzyme that converts hydrogen peroxide into water and molecular oxygen, by okra plants growing in arsenic-contaminated soil media was analyzed in this study. It is a tetrameric, heme-containing enzyme found in the peroxisomes, glyoxisome, cytosol,

mitochondria, and root nodules (Sharma, 2012). Catalase activity in the leaves and roots of plants followed a similar pattern as SOD activity. From this study, catalase activity was induced and found to be higher in plants grown in $As^{3+}$ treated soils. CAT activity increases as the concentration of $As^{3+}$ increases (Figure 3). It has been reported that CAT activity increases in *Zea mays* during arsenic exposure (Mylona *et al*., 1998). The increase in concentration of arsenic in soils did not inhibit plant growth because of the presence of the inoculant. The highest CAT activity was observed in plants grown in 150ppm $As^{3+}$ concentrations in soils inoculated with fungi isolates, and the plant throve well under that condition. (Osaigbovo *et al.*, 2022) stated that 150 ppm arsenic ions in soil negatively affected plant height in the absence of any fungi isolate. Whereas, plant height was promoted in the presence of *Aspergillus terreus* in 150 ppm $As^{3+}$-treated soil environments.

## Conclusion

This study deduced that fungi helped ameliorate the soil contaminated with arsenic ion concentration and help alleviate heavy metal stress in okra plants as seen in plant height, number of leaves, number of branches, and antioxidant activity. From this study, it can be understood that the use of fungi inoculants can be introduced in soils contaminated with arsenic to ameliorate the toxic effect of its activity. I suggest the introduction of this biological process to bush burning as poisonous gasses are not released during this process and soil composition is maintained.

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