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Influence of selected heavy metal on mycelial growth response of *Trichoderma* isolate

H.J. Abdullahi[1*], S.O. Alonge[1] and A.B. Zarafi[2]
[1]Department of Biology, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Crop Protection, Institute of Agricultural Research (IAR), Ahmadu Bello University, Zaria, Nigeria
abdullahihauwa61@yahoo.com

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Abstract

Heavy metal pollutants are increasingly being released into the environment. These metal ions in soil may influence mycelial growth of Trichoderma isolate which is a well-known bio control agent, this necessitated the evaluation of influence of copper, lead and cadmium on the mycelial growth response of Trichoderma isolate. Trichoderma species was isolated from the rhizosphere soil of uncultivated lands at four sampling sites; Hanwa lowcost, Kabama, Ahmadu Bello University Gymnasium and Bomo all in SabonGari L.G. A, Zaria, Nigeria. The isolate was then sent to International Mycological Institute (IMI), CABI BIOSCIENCE, UK for confirmation as Trichoderma longibrachiatum. The mycelial growth response of T. longibrachiatum on Potato Dextrose Agar (PDA) with six concentrations (0, 10, 100, 200, 500 and 1000ppm) of $CuSO_4$, Cd $(NO_3)_2$ and $Pb(NO_3)_2$and also their combinations were evaluated. These treatments in four repetitions were laid out in a completely randomized design (CRD). The results obtained in this study indicated that, Trichoderma longibrachiatum is present in soils collected from uncultivated lands of SabonGari Local Government Area of Zaria, Nigeria. Mycelial growth of T. longibrachiatum was observed to decrease generally with increase in concentrations of the heavy metals when compared with the control. It was further observed that, T. longibrachiatum, was unable to grow at 500 and 1000 ppm $CuSO_4$. The combination of $Cd(NO_3)_2$ and $Pb(NO_3)_2$ in the metal combinations Cd+Pb and Cd+Cu+Pb significantly reduced T. longibrachiatum mycelial growth. In conclusion, the presence of $CuSO_4$, $Cd(NO_3)_2$ and $Pb(NO_3)_2$ in growth media (Potato Dextrose Agar, PDA) significantly reduced mycelial growth of T. longibrachiatum.

Keywords: Trichoderma, mycelial growth, copper, cadmium and lead.

Introduction

Pollution by heavy metals is a major problem causing serious damage to the environment affecting the world at large. Heavy metals have been observed to affect relative abundance of soil micro-organisms such as soil bacteria and fungi. Their presence also causes changes in fungal community. *Trichoderma* species are fungi frequently isolated from soil[1]. The genus *Trichoderma* has been observed as a biocontrol agent against several plant pathogens[2,3]. Aside this, *Trichoderma* species play important role in bioremediation of pollutants such as heavy metals, tannery effluents, organometallic compounds, harmful chemicals like cyanide (CN) and agrochemicals[4,5].

Reports indicates that *Trichoderma* species is able to tolerate and accumulate several heavy metals compounds for example, cadmium, copper, arsenic and zinc in *invitro* conditions[4,6,7]. *Trichoderma harzianum* strains that are tolerant to more than one type of metals have also been reported[8]. Characterization of these isolated strains was carried out using UV mutagenesis for heavy metal resistance. Twenty four out of the one hundred and seventy-seven resistant mutants were observed to be resistant to one or more of the ten heavy metals ($CuSO_4 \cdot 5H_2O$: 1·6 mmol, $NiSO_4 \cdot 7H_2O$: 3mmol, $AlCl_3 \cdot 6H_2O$: 25mmol, $ZnSO_4 \cdot 7H_2O$: 2 mmol, $MnSO_4 \cdot 4H_2O$: 12mmol, $CoCl_2 \cdot 6H_2O$: 4mmol, $CdNO_3 \cdot 4H_2O$: 0·3 mmol, $PbNO_3$: 1mmol, $HgCl_2$: 0·4mmol, $FeSO_4 \cdot 7H_2O$: 1·5 mmol) tested. Generally, their success as a biological control agent has been directly through competition for nutrient and space, production of mycolytic enzymes and antibiotics and indirectly as induced resistance to host plant, plant growth enhancement and tolerance to stress[9-14].

Stress factors such as temperature, pH, drought, fertilizers and metal contaminants may affect their morphological characteristics as well as physiological functions. Aside the effect of low temperature and drought, heavy metal presence in soil is an important stress factor affecting *Trichoderma* strains[15,16,8].

It was observed that $CuSO_4$, $ZnSO_4.7H_2O$, $FeSO_4.7H_2O$, $CoCl_2.6H_2O$ and $MnSO_4.H_2O$ compounds were effective as mycelial growth inhibitors when tested individually while $CaCl_2.2H_2O$, $(NH_4)_2SO_4$ and $MgSO_4.7H_2O$ had a non toxic effect on *Trichoderma hamatum T614, T. harzianum*T969 and *T. virens*T525[17]. This study was therefore designed to investigate the individual and the combined effect of some heavy metal compounds (copper sulphate, cadmium nitrate and lead nitrate) on the mycelial growth of *Trichoderma* species.

Materials and methods

Source of experimental materials: All the metal compounds (copper sulphate, cadmium nitrate and lead nitrate) were purchased as salt form from Sigma Aldrich. *Trichoderma* species was isolated from the rhizosphere soils in SabonGari Local Government Area of Zaria, Nigeria.

Isolation, purification and identification of *Trichoderma* species: *Trichoderma* species was isolated using the serial dilution technique and purified using the single spore isolation. Preliminary identification for *Trichoderma* species was carried out through macroscopic (growth rate and colours of colonies were observed in the petri dishes) and microscopic (branching pattern of conidiophores and shapes of conidia were observed with the aid of a microscope) examinations. Macroscopic and microscopic characters were further compared to the key of *Trichoderma* species[18]. Pure cultures were sent to International Mycological Institute, CABI BIOSCIENCE, U.K. for identification confirmation.

Evaluation of *Trichoderma* species Response to Heavy Metals: *Trichoderma* mycelial growth response to individual metal concentrations testing: The mycelial growth response of *Trichoderma* to copper sulphate ($CuSO_4$) which supplied copper, lead nitrate (Pb $(NO_3)_2$) which supplied lead and cadmium nitrate (Cd $(NO_3)_2$) which supplied cadmium, was evaluated using the Poisoned medium method[17]. The medium (potato dextrose agar, PDA) was amended progressively with six concentrations (0, 10, 100, 200, 500, 1000ppm) of each metal, then sterilized (autoclaved at 121±1^{0}C for 18 minutes in a portable autoclave, Griffin and George), cooled to 40±1^{0}C and amended with 0.25g/l of chloramphenicol before 20ml was dispensed into each sterile petri dish. Each petri dish was inoculated at the centre with 3mm diameter mycelia plug of 3 days old *Trichoderma* colony grown on PDA, covered with cellotapes and then incubated in a growth chamber (gallenkamp cooled incubator, made in England) at 28±1^{0}C. The metal unamended PDA plates served as control. Mycelial growth was measured daily until all petri dishes were fully colonized at 5 days after inoculation (5DAI). The treatments which consisted of six concentrations (0, 10, 100, 200, 500 and 1000ppm) each of the three metals (Cu, Cd, Pb) were laid out in completely randomized design with four repetitions.

***Trichoderma* mycelia growth response to metal combinations testing:** The concentrations considered for the metal combination treatments were drawn from the individual metal concentrations that gave the highest mycelial growth (Cu-100 ppm, Cd-10ppm and Pb-100ppm). The four metal combinations evaluated were Cu/Cd, Cu/Pb, Cd/Pb and Cu/Cd/Pb. Each metal combination was prepared by amending the medium with 10ml of the individual metal components, after which it was sterilized at 121 ±1^{0}C for 18 minutes before being dispensed into sterile petri dishes. Each petri dish with combined metal amended PDA was inoculated centrally with 3mm diameter mycelia plug colony of *Trichoderma,* sealed with cellotapes and incubated in a growth chamber at 28±1^{0}C. The unamended PDA served as control. Mycelial growth of *Trichoderma* was measured daily with the use of a ruler until the plates were fully colonized. The four treatments (Cu/Cd, Cu/Pb, Cd/Pb and Cu/Cd/Pb) obtained from the combined concentrations as follows (100/10ppm, 100/100ppm, 10/100ppm and 100/10/100ppm) respectively were laid out in completely randomized design with four repetitions.

Data analyses: Data collected were analysed using SPSS version 20. Treatment means were calculated using Analysis of Variance (ANOVA) while Least Significant Difference (LSD) was used to separate means where significant differences were observed at 5% level of significance.

Results and discussion

Identification of *Trichoderma* isolate: *Trichoderma* species isolated was preliminary identified via macroscopic and microscopic observations by comparing its features to the key[18]. The confirmation was provided by the International Mycological Institute (IMI), CABI BIOSCIENCE, UK (with IMI number 604253). The isolate was processed and identified by ITS rDNA sequence analysis using the FASTA algorithm with the fungus data base as *T. longibrachiatum*.

Macroscopic characteristics of the isolated *Trichoderma* species: Mycelia of *Trichoderma* species were observed to be uniformly dispersed in concentric rings forming a continuous lawn, cottony, dark green core with completely visible older mycelia zones of fertile conidiophores while younger mycelia zones were white coloured (Figure-1). Yellow pigmentation diffused through the agar in the underside of Potato Dextrose Agar (PDA) cultures (Figure-2). No distinctive odour was observed. Colony radius on PDA at 28±1^{0}C was 6.48cm at 2 days after inoculation (DAI) while full colonization (9cm) was observed on the third day after inoculation (Figure-3).

Microscopic characteristics of the isolated *Trichoderma* species: Fertile conidiophores with several levels of branching were observed. Branches near the tip bore a single phialide and that did not rebranch. Branches of conidiophore distal to the tips were longer than branches closer to the tips. Solitary phialides were cylindrical and swollen at the middle terminating in a single cell and subtended by an intercalary phialide (Figure-4). Conidia were ellipsoidal, green and smooth (Figure-5).

Effect of copper, cadmium and lead salts on mycelial growth of *Trichoderma longibrachiatum:* The mycelial growth of *Trichoderma longibrachiatum* on PDA amended with varying concentrations of $Cd(NO_3)_2$, $CuSO_4$ and $Pb(NO_3)_2$ observed for four days after inoculation are presented in Table-2 and Figure-6-8.

The presence of $Cd(NO_3)_2$ at 2-4 days after inoculation (DAI) and $Pb(NO_3)_2$ at 2 DAI generally reduced mycelial growth of *T. longibrachiatum.* The control had the highest mycelial growth

under $CuSO_4$ and $Pb(NO_3)_2$ at 2 DAI. The least mycelial growth was observed at 1000 ppm under each metal at 2 DAI. *Trichoderma longibrachiatum* was unable to grow at 500 and 1000 ppm $CuSO_4$ treatments. At 3 DAI, Cd $(NO_3)_2$ at 100-1000 ppm and Pb $(NO_3)_2$ at 500-1000 ppm significantly reduced the mycelial growth of *T. longibrachiatum* compared with other treatments. Complete colonization of Plates was observed 3 DAI at 0-200ppm for $CuSO_4$ amended media while no growth was observed at 500 and1000 ppm even at 5 DAI (Table-1).

Complete colonization of PDA plates was observed at 4 DAI in all PDA amended with Pb $(NO_3)_2$ and PDA amended with 0-200 ppm $CuSO_4$ at 3 DAI. However, complete colonization was only observed in PDA amended with 0-100 ppm Cd $(NO_3)_2$ at 4 DAI. *Trichoderma longibrachiatum* mycelial growth was still significantly reduced at 4 DAI on PDA amended with Cd $(NO_3)_2$ at 200-1000ppm compared with the other treatments. The least mycelial growth, 6.68 and 8.09cm at 4 DAI and 5 DAI respectively was observed in 1000 ppm Cd $(NO_3)_2$ amended media. All Plates amended with the test metals $CuSO_4$, $Cd(NO_3)_2$ and $Pb(NO_3)_2$ were fully colonized at 5 DAI with the exception of $Cd(NO_3)_2$ at 1000ppm (8.09cm) while no growth was observed at 500 and 1000ppm for $CuSO_4$ amended media even at 5DAI (Table-1 and Figure-6-8).

Table-1: Influence of metal concentrations on the mycelial growth of *Trichoderma longibrachiatum.*

		Mycelial growth (cm)		
Days After Inoculation (DAI)	Metal concentration (ppm)	$CuSO_4$	$Cd(NO_3)_2$	$Pb(NO_3)_2$
2	0	6.48 (± 0.27)^a	6.48 (± 0.27)^a	6.48 (± 0.27)^a
	10	6.33 (±0.10) ^{a}	6.81 (±0.05) ^{a}	5.70 (± 0.10) ^{b}
	100	6.40 (±0.04)^a	4.78 (±0.10) ^{b}	6.33 (± 0.06) ^{a}
	200	5.82 (±0.05)^a	4.17 (± 0.07)^c	5.68 (± 0.20) ^{b}
	500	0 (±0.00)^b	3.27 (± 0.14)^d	4.75 (± 0.08) ^{c}
	1000	0 (±0.00) ^{b}	2.56 (± 0.11)^e	4.16 (± 0.06) ^{d}
	Mean SE±	0.08	0.12	0.13
3	0	9.00 (± 0.00)^a	9.00 (±0.00) ^{a}	9.00 (± 0.00) ^{a}
	10	9.00 (± 0.00) ^{a}	9.00 (± 0.00) ^{a}	9.00 (± 0.00) ^{a}
	100	9.00 (± 0.00) ^{a}	7.58 (± 0.12) ^{b}	9.00 (± 0.00) ^{a}
	200	9.00 (±0.00) ^{a}	6.31 (± 0.17) ^{c}	8.67 (± 0.21) ^{a}
	500	0 (± 0.00) ^{b}	5.75 (± 0.14) ^{d}	7.63 (± 0.06) ^{b}
	1000	0 (± 0.00) ^{b}	4.70 (± 0.10) ^{e}	6.12 (± 0.38) ^{c}
	Mean SE±	0.00	0.09	0.11
4	0	9.00 (±0.00) ^{a}	9.00 (± 0.00) ^{a}	9.00 (± 0.00) ^{a}
	10	9.00 (± 0.00) ^{a}	9.00 (± 0.00) ^{a}	9.00 (± 0.00) ^{a}
	100	9.00 (± 0.00) ^{a}	9.00 (± 0.00) ^{a}	9.00 (± 0.00) ^{a}
	200	9.00 (± 0.00) ^{a}	8.20 (±0.28) ^{b}	9.00 (± 0.00) ^{a}
	500	0 (± 0.00) ^{b}	7.62 (± 0.10) ^{c}	9.00 (± 0.00) ^{a}
	1000	0 (± 0.00) ^{b}	6.68 (±0 .10) ^{d}	9.00 (±0.00) ^{a}
	Mean SE±	0.00	0.08	0.00
5	0	9.00 (±0.00) ^{a}	9.00 (±0.00) ^{a}	9.00 (±0.00) ^{a}
	10	9.00 (±0.00) ^{a}	9.00 (±0.00) ^{a}	9.00 (±0.00) ^{a}
	100	9.00 (±0.00) ^{a}	9.00 (±0.00) ^{a}	9.00 (±0.00) ^{a}
	200	9.00 (±0.00) ^{a}	9.00 (±0.00) ^{a}	9.00 (±0.00) ^{a}
	500	0 (±0.00) ^{b}	9.00 (±0.00) ^{a}	9.00 (±0.00) ^{a}
	1000	0 (±0.00) ^{b}	8.09 (± 0.08) ^{b}	9.00 (±0.00) ^{a}
	Mean SE±	0.00	0.01	0.00

Values having the same superscripts along the column at each day after inoculation (DAI) are not significantly different at P=0.05, using Least Significance Difference (LSD), The standard error of the mean is represented in parentheses.

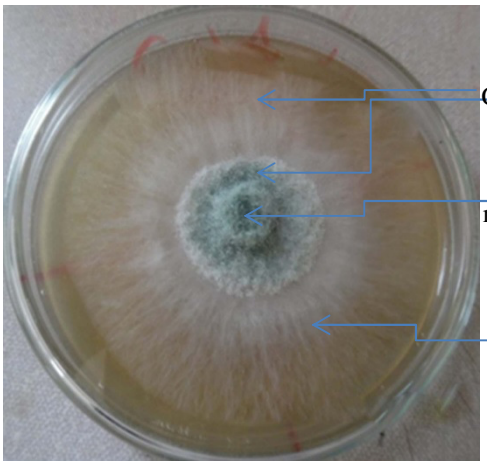

Figure-1: Cultural growth habit of two day old *Trichoderma longibrachiatum* on PDA.

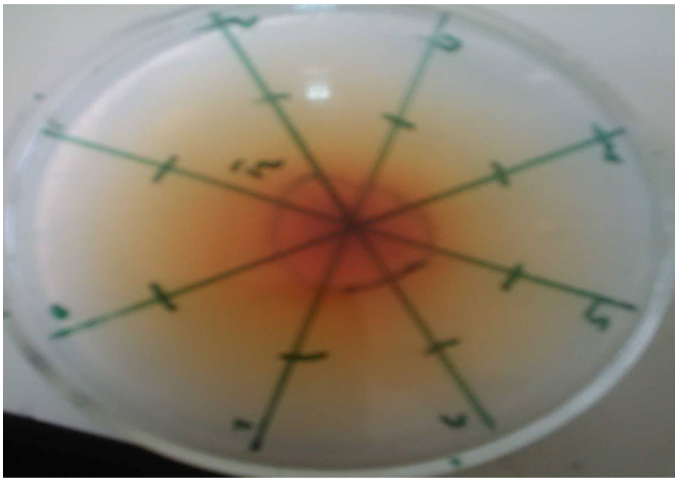

Figure-2: Underside view of three day old *Trichoderma longibrachiatum* culture on PDA.

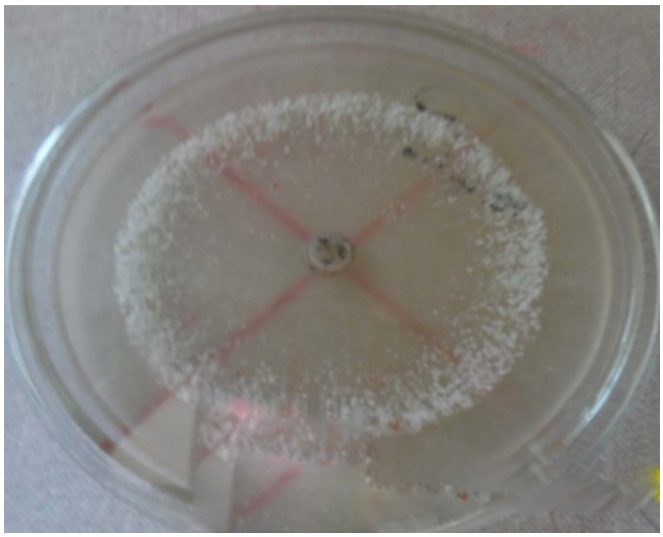

Figure-3: Front view of three day old *Trichoderma longibrachiatum* culture on PDA.

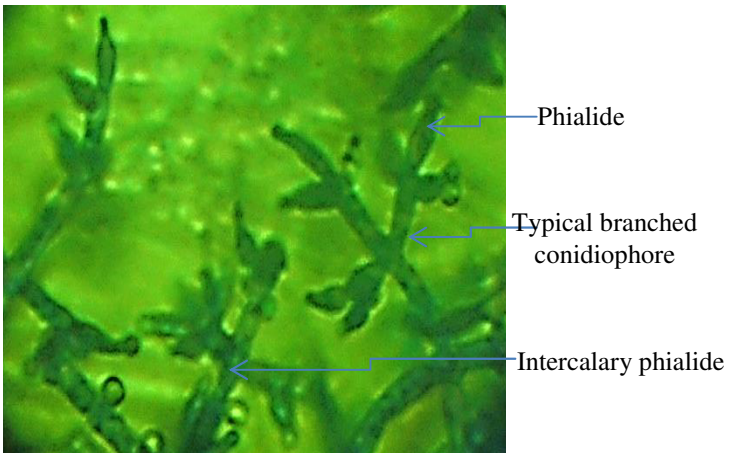

Figure-4: Branched conidiophores of *Trichoderma longi-brachiatum* bearing phialides (X 400).

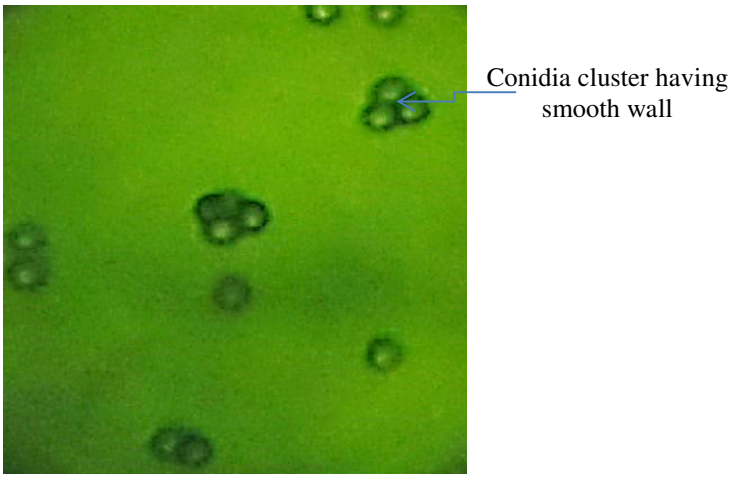

Figure-5: Conidia of *Trichoderma longibrachiatum* (X 400).

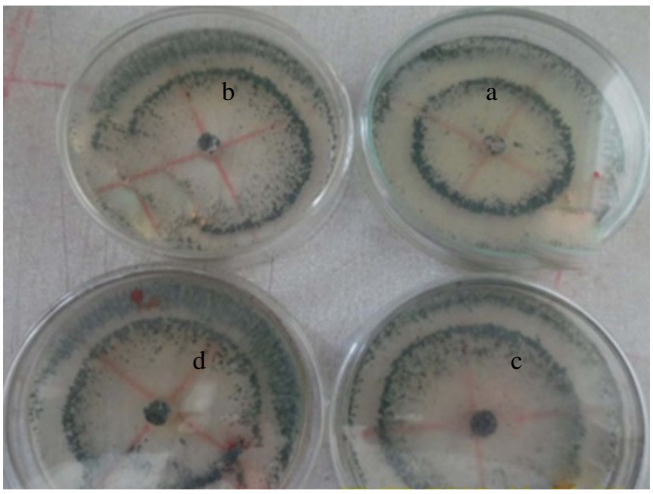

Figure-6: Cultures of *Trichoderma longibrachiatum* on PDA amended with varying concentrations of copper sulphate which supplied copper. a=Control, b=10ppm, c=100ppm, d=200ppm.

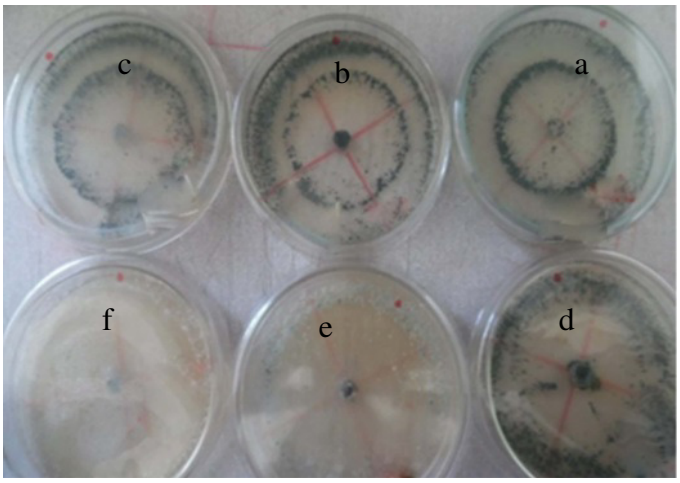

Figure-7: Culture of *Trichoderma longibrachiatum* on PDA amended with varying concentrations of lead nitrate which supplied lead. a=Control, b=10ppm, c=100ppm, d=200ppm, e=500ppm, f=1000ppm.

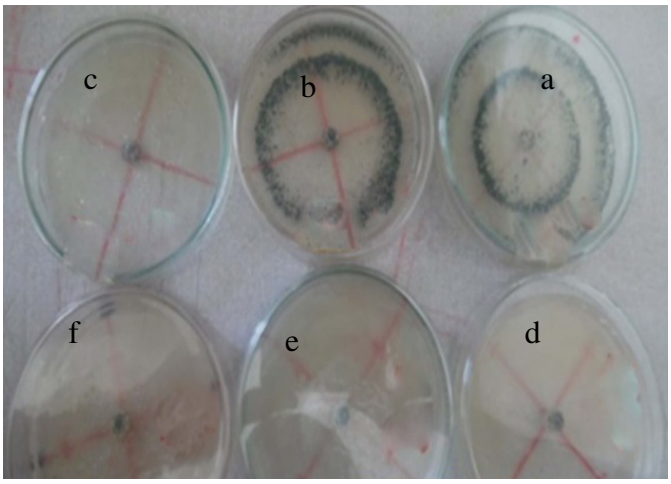

Figure-8: Culture of *Trichoderma longibrachiatum* on PDA amended with varying concentrations of cadmium nitrate which supplied cadmium. a=Control, b=10 ppm, c=100 ppm, d= 200 ppm, e= 500 ppm, f=1000 ppm.

Effect of salts combinations of copper, cadmium and lead on mycelial growth of *Trichoderma longibrachiatum:* At day 1 and 2 after inoculation, mycelial growth in Cu/Cd (100/10ppm respectively) and Cu/Pb (100/100ppm respectively) were highest and did not differ significantly from unamended control. However, they were both significantly (*P*=0.05) higher than *Trichoderma* mycelial growth in Cd/Pb (10/100ppm respectively) and Cu/Cd/Pb (100/10/100 respectively) metal combination treatments. On the other hand, the combination of Cu/Cd/Pb (100/10/100 respectively) resulted in the least mycelial growth (2.73cm) of *Trichoderma* at 1 DAI when compared to the control, on the other hand combination of Cd/Pb had the least growth (6.56cm) at 2 DAI (Table-3 and Figure-9). At 3 DAI, no significant difference was observed in the mycelial growth of *Trichoderma* on all the metal treatments.

Table-3: Influence of metal combinations on the mycelial growth of *Trichoderma longibrachiatum.*

Days After Inoculation (DAI)	Metal combinations	Mycelial growth (cm)
1	Control	3.37(±0.04)[a]
	Cu/Cd	3.47(±0.08)[a]
	Cu/Pb	3.52(±0.08)[a]
	Cd/Pb	2.80(±0.04)[b]
	Cu/Cd/Pb	2.73(±0.09)[b]
	Mean SE±	0.07
2	Control	7.97(±0.07)[a]
	Cu/Cd	8.09(±0.13)[a]
	Cu/Pb	7.93(±0.12)[a]
	Cd/Pb	6.56(±0.08)[b]
	Cu/Cd/Pb	6.78(±0.18)[b]
	Mean SE±	0.12
3	Control	9.00(±0.00)[a]
	Cu/Cd	9.00(±0.00)[a]
	Cu/Pb	9.00(±0.00)[a]
	Cd/Pb	9.00(±0.00)[a]
	Cu/Cd/Pb	9.00(±0.00)[a]
	Mean SE±	0.00

*Values having the same superscripts along the column at each DAI are not significantly different at P=0.05, using Least Significance Difference (LSD), *The standard error of the mean is represented in parentheses, *Mean SE- Mean Standard Error, *Cu-Copper, Cd-Cadmium and Pb-Lead.

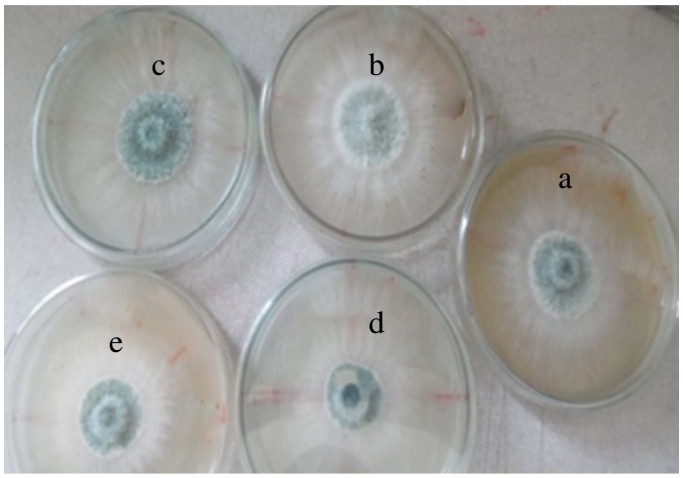

Figure-9: Culture of *Trichoderma longibrachiatum* on PDA amended with combinations of copper sulphate, lead nitrate and cadmium nitrate which supplied copper, lead and cadmium respectively. a=Negative control, b=Cu/Cd, c=Cu/Pb, d=Cu/ Cd/Pb, e= Cd/Pb.

Discussions: Isolated and identified *Trichoderma* species: *Trichoderma longibrachiatum* was isolated from soils collected from uncultivated lands of SabonGari Local Government Area of Zaria, Nigeria. Although, other soil inhabiting fungi may be present in the study sites, the *Trichoderma* selective media used in this study have eliminated the possibility of isolating other soil borne fungi. In a similar study, different species of *Trichoderma* was isolated from soil collected from environs of Keffi Metropolis, Nasarawa Nigeria[19]. In the study, ten fungi species including *Cladosporium herbarum, Absidia corymbifera, Aspergillus fumigatus, A.flavus, A. niger, Curvularia lunata, Alternaria alternata, Rhizopus stolonifer, Penicillium* species and *Trichoderma viride* were isolated. The species difference observed in this study might be due to differences in the environmental conditions where the soil samples were taken from. Environmental factors such as temperature and rainfall are important determinants in distribution of microorganisms in natural ecosystem. Adaptation to specific soil conditions such as soil temperature and water potential may restrict species distribution[20]. In another study, eleven *Trichoderma* isolates were obtained from rhizosphere soils, humus and compost in Kampar and Penang region of Malaysia[21]. These isolates were characterized and identified by morphological characterization and sequence analysis of 5.85-ITS region. The *Trichoderma* isolates were five *T. harzianum*, four *T. asperellum*, one *T. virens* and one *T. strigosum*. Again, these species difference may be due to differences in environmental conditions which are as a result of differences in geographical location. In a study carried out at Ogun state, Nigeria, ten species of *Trichoderma* were isolated from maize plants and its rhizosphere. These include: five strains of *T. pseudokoningii*, three strains of *T. harzianum*, *T. hamatum* and *T. longibrachiatum*[22]. The environmental conditions present in Ogun State, Nigeria (rainforest zone) are actually different when compared with the present study location (Zaria). This might have influenced the level of occurrence of *Trichoderma* species observed in this study location (Zaria) which represents a typical savanna zone. This may possibly be the reason why other *Trichoderma* species found in the western part of Nigeria were absent in the present study location which had only *T. longibrachiatum*.

Influence of cadmium,copper and lead salts on the mycelial growth of *Trichoderma longibrachiatum:* *Trichoderma longibrachiatum* mycelial response to the different metal compounds differed. This might be due to differences in it's sensitivity to the individual metal salts. This further suggest that, the sensitivity of *T. longibrachiatum* is dependent on metal type and it's concentration. Similar variation in fungal sensitivity was reported on four fungi species (*Acremonium pinkertoniaea*, *Trichophaea brunnea*, *Paxillus involutus* and *Rhizopogon roseolus*) isolated from industrial wastes. It was also observed that, *A. pinkertoniaea* was highly tolerant to copper when compared to the other fungi isolates[23]. Growth inhibition of 50% was observed at 250mg/kg, a concentration of copper that was lethal to other species. *Acremonium pinkertoniaea* was observed to be the only species able to grow at concentrations as high as 600 mg/kg.

The mycelial growth of *T. longibrachiatum* was observed to decrease with increasing concentration for all individual metal treatments. This might be that, the influence of these metals on *T. longibrachiatum* mycelial growth is concentration dependent, with higher reduction of mycelial growth observed as metal concentration increases. A similar finding was observed in a study on the influence of some metal compounds on mycelial growth of *Trichoderma* species[17]. It was reported that, mycelial growth of the *Trichoderma* species generally decreased with increased concentrations of manganese sulphate ($MnSO_4.H_2O$), cobalt chloride ($CoCl_2.6H_2O$) and calcium chloride ($CaCl_2.2H_2O$). The study showed that, the highest mycelial growth (56.11, 51.11 and 46.56cm respectively) was recorded under the control of each metal while the least mycelial growth (20.56, 8.72 and 7.89cm respectively) was observed at 1000 ppm when the test metals were incorporated in the growth media of *Trichoderma* species.

The absence of mycelial growth observed when $CuSO_4$ was incorporated in the growth media at 500 and 1000ppm, might be due to the inhibitory effect of copper ions on *Trichoderma* species mycelial growth response which could be attributed to copper ion catalysis. Copper ions are able to catalyze the production of highly hydroxyl radicals leading to damage of lipids, DNA, proteins, and other biomolecules thereby affecting its morphology and physiological functions. Inhibitory effect of $CuSO_4$ on the growth of *T. harzianum* was reported while it was observed that, *T. harzianum* T_1 grew well in malt extract agar[24]. In the same report, mycelial growth of *T. harzianum* T_1 was reduced when $CuSO_4$ or $CaSO_4$ were added in the media at high concentration of $300mgL^{-1}$, although no reduction in mycelial growth was recorded at lower concentrations of $0\text{-}250mgL^{-1}$. However, it was capable of mycelial growth at concentrations as high as $500mgL^{-1}$ of zinc, iron and manganese, but no mycelial growth was recorded at $350mgL^{-1}$ of $CuSO_4$. This finding is also in line with an investigation that determined the mycelial growth of three biocontrol agents including *T. virens* T525, *Trichoderma harzianum* T969 and *T. hamatum* T614 in the presence of 0, 50, 91.2, 166, 302, 550 and 1000 mg/l of $CuSO_4$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $MnSO_4.H_2O$, $MgSO_4.7H_2O$, $CoCl_2.6H_2O$, $(NH_4)_2SO_4$ and $CaCl_2.2H_2O$[18]. It was observed that, $CuSO_4$ added to the growth media of the *Trichoderma* species, consistently reduced mycelial growth as concentration increased from 91.2 to 302ppm, while no growth was observed at 302-1000ppm excluding *T. harzianum* T969 with slight mycelial growth at 302 ppm $CuSO_4$.

The full colonization observed at 4-5 DAI showed that after the initial reduction in mycelial growth, *Trichoderma longibrachiatum* possibly became tolerant or adapted to the presence of all the test metal salts by 4-5 DAI. Fungi species can show different level of tolerance towards different metal compounds. In a study that investigated the tolerance level of

some fungi isolated from soil, *Aspergillus niger,* isolated from soil samples of Faisalabad showed the highest tolerance level to all the test metals ($PdNO_3$, $ZnSO_4$, $NiSO_4$, $CdSO_4$)[25]. *A. niger* isolated from soil samples of Rawalpindi district showed the highest tolerance followed by *Aspergillus* sp. and *Penicillium* species while *Fusarium* species from soil samples of Rawalpindi showed the highest minimum inhibitory concentration against $ZnSO_4$. However *Fusarium* sp. of Jappaywala, Faisalabad showed this order of tolerance: $PdNO_3$>$ZnSO_4$>$NiSO_4$>$CdSO_4$.

Influence of the combinations of cadmium, copper and lead salts on the mycelial growth of *Trichoderma longibrachiatum:* The significant difference in the metal combination of Cd/Pb and Cu/Cd/Pb when compared with the control and all other treatments at 1-2 DAI might be due to the synergism effect of cadmium and lead on *Trichoderma longibrachiatum* mycelial growth. However, report on the effect of manganese on the total amount of mineralized carbon was inhibited in the presence of cadmium[26]. Several researches have shown negative effect of heavy metals on soil biological properties[27,28]. In another report, no relationship was observed between high metal concentration and some soil microbial properties[29]. These suggest that, a particular heavy metal presence may affect the availability of another in the soil and hence the microorganisms and higher plants found there. In other words, antagonistic and synergism behaviour exist among heavy metals.

Conclusion

In conclusion, this study showed that, *Trichoderma longibrachiatum* is present in soils collected from uncultivated lands of SabonGari Local Government Area of Zaria, Nigeria. It also indicates that, the presence of increased concentrations (0-100 ppm) of $CuSO_4$, Cd $(NO_3)_2$ and Pb $(NO_3)_2$ in growth media (Potato Dextrose Agar, PDA) whether in individual or combination significantly reduced *T. longibrachiatum* mycelial growth with no mycelial growth observed at 500-1000ppm of $CuSO_4$.

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