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ISOLATION AND CHARACTERIZATION OF ACTINOBACTERIA WITH POTENTIAL FOR BIOLOGICAL CONTROL OF GRASSY AND BROAD LEAF WEEDS

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ABSTRACT

The management of grassy and broadleaf weeds in various ecosystems, including agricultural and natural landscapes, poses a significant challenge. This study investigated the biocontrol potential of metabolites produced by Actinobacteria on post-emergence of some weed species. Actinobacteria were isolated from different soil samples and characterized using physiological and biochemical methods. The secondary metabolites were extracted from the isolates, and the primary screening of the metabolites for herbicidal activity was conducted using Cucumis sativus assay, followed by post-emergent assay on grassy weeds (Pennisetum purpureum and Cynodon dactylon) and broad leaf weeds (Amaranthus spinosus and Tridax procumbens)under screen house conditions. Data on phytotoxicity of metabolites were collected and analyzed. In this study, twenty isolates of Actinobacteria were obtained and identified as species of Streptomyces (65%), Actinomyces (15%), Microbacterium (10%) and Micromonospora (10%). Eight (40%) of the isolates produced secondary metabolites that showed visual phytotoxic effects on Cucumis sativus with Streptomyces clavuligerus RSR2, S. clavuligerus RSD and Streptomyces griseus TRD exhibiting higher phytotoxicity.The study further showed that metabolites produced by S. clavuligerus RSR2, S. clavuligerus RSD and S. griseusTRDexhibited significantly ($p \leq 0.05$) higher phytotoxicity on post-emergent P. purpureum (55.8 – 68.0%), C. dactylon(55.0% - 60.0%), A. spinosus (58.0 – 69.80%) and T. procumbens(55.0 – 64.0%) in the screen house. The study, therefore, showed that the secondary metabolites of Streptomyces clavuligerus RSR2, Streptomyces clavuligerus RSD and Streptomyces griseus TRD could be developed as potential post-emergent bioherbicides for controlling some grassy and broad-leaved weeds.

Keywords: Biocontrol, Secondary metabolites, Actinobacteria, Post-emergence, Phytotoxicity

INTRODUCTION

Actinobacteria are Gram-positive bacteria containing a GC-rich linear genome with a biosynthetic potential to produce secondary metabolites of broad structural diversity and commercial importance (Barka *etal*., 2016). Actinobacteria have complex mycelial morphology including presence or absence of a straight or aerial mycelium, production of pigments that impart colour to the mycelium and sporulation pattern (Ranjani *et al*., 2016). Actinobacteria have been found in various ecosystems which include but not limited to saline soil, freshwater, sediments, sponges, animals and human guts, medicinal plants, deep forests and hot springs. Due to their various ecological functions, they have multiple applications in agriculture, biomedical, industrial and pharmaceutical (Demain *et al*., 2019) Apart from antibiotic production, the actinobacteria also produce secondary metabolites with herbicidal properties that are particularly significant in agricultural practice, some of which have been successfully developed as commercial herbicides (Shi *et al*., 2018).

Weeds compete with crops and forest plants for water, nutrients, and light, making them one of the most significant agricultural and environmental issues. These weeds have been successfully controlled using synthetic herbicides like glyphosate, dicamba, carfentrazone-ethyl, foramsulfuron, isoproturon and 2,4-D sodium salt but these chemicals have negative impacts on the environment and human health, contaminating the air, water, and soil. Some chemical herbicides end up in streams, killing aquatic life there and having the potential to evaporate into the air, polluting the atmosphere and deteriorating air quality. Additionally, inhaling chemical herbicides irritates the nose and nasal passages while contact to these chemicals causes skin irritation, leading to major health and environmental problems. Hence, developing alternative, sustainable strategies for weeds control is crucial to reduce reliance on synthetic herbicides. Biological control using actinobacteria-derived metabolites offers a natural product-based approach to weed control.

Biological weed control is the deliberate use of living organisms or their metabolites to control weeds without damaging non-target organisms and crops (Guo *et al*., 2020). It involves a mechanism to suppress the germination and growth of weed populations to an economic threshold level by utilizing natural enemies, natural substances, or biotic agents (Hasan *et al*., 2021). The microbial herbicides have many advantages over the chemical herbicides. They possess high degree of specificity for target weeds and not prone to producing herbicide resistance. They require a lesser amount per unit area, and are highly safe to humans, animals and beneficial insects, which minimizes their effect on the micro-ecological environment (Guo *et al*., 2020). In addition, bioherbicides may have both pre- and post-emergence effects on weed species, and usually have shorter half-life than synthetic herbicides (Sadia *et al*., 2016; Adetunji *et al*., 2017). In the past decades, actinobacteria and their secondary metabolites have been explored as biocontrol agents of weeds. Dhanasekaran *et al.* (2012) reported that *Streptomyces* sp. had the ability to inhibit the growth of *Echinochloa crusgalli*. Similarly, *Streptomyces* sp. KA1-3, *Streptomyces* sp KA1-4, *Streptomyces* sp KA1-7, and *Streptomyces* sp KA23A were found highly effective against *Cyperus rotundus*. The herbicidal activity of the bioactive compound N-phenyl-propanamide from *Streptomyces* sp. KA1-3 also showed 80% seed germination inhibition in *Cassia occidentalis* L. and rhizome *Cyperus rotundus* weeds (Priyadharsini *et al.*, 2013). *Streptomyces saganonensis* produces herbicidines and herbimycins that control monocotyledonous and dicotyledonous weeds. Anisomycin, which is produced by

Streptomyces sp., is a growth inhibitor for annual grassy weeds such as barnyard and common crabgrass, as well as some broad-leaved weeds (Harir *et al*., 2018). Anisomycin can destroy the ability of the plants to synthesize chlorophyll. Its synthetase may accumulate ammonia and control photosynthetic phosphorylation, causing plant death. Similarly, bialaphos, a metabolite of *Streptomyces viridochromogenes*, is widely used to control annual and perennial grassy weeds and broad-leaved weeds by inhibiting glutamine synthesis. *Streptomyces hygroscopicus* produces carbocyclic coformycin and hydantocidin, which can decrease synthetase of adenylosuccinate by increasing the content of ATP and delays the synthesis of protein (Pillmoor, 1998).

The present study was, therefore, conducted to isolate, characterize, and determine the phytotoxic effects of their secondary metabolites on post-emergence of some grassy and broadleaf weeds under greenhouse conditions.

MATERIALS AND METHODS

Collection of Soil Samples

A total number of ten samples of soil were obtained within the depth of 0-10cm from the refuse dump site, mechanic workshop, stream water sediments, quarry site and farm land in Odeda Local Government area, Ogun state. The soil samples were collected aseptically in sterile zip-lock bags, transported to the laboratory on ice box and stored at 4ºC for further microbiological analysis.

Isolation and Characterization of Actinobacteria

Isolation of actinobacteria was carried out using the method described by Balachandar *et al*. (2018) and El-Hadi *et al*. (2019) with slight modifications. Firstly, the soil samples were air-dried for 7 days at room temperature (25±2°C), followed by pre-heating at 45°C for two hours to eliminate the residents of Gram-negative bacteria (Valan Arasu *et al*., 2008). Five grams (5.0 g) of each soil sample was aseptically suspended in 45.0 ml of sterile peptone and shaken vigorously on a rotary shaker for 5 minutes. The suspension was left for about 10 minutes and serially diluted to 10^{-4}. Then, 0.1ml of 10^{-4} dilution was inoculated on sterile starch casein agar plates and allowed to solidify. The incubation of the plates was carried out at 30°C for 7 days. Pure cultures of actinomycetes were obtained by series of sub-culturing of colonies on starch casein agar plates, and incubating at 30°C for 7 days. The pure actinobacterial isolates were maintained on starch casein agar slants at 4°C.The characterization of actinobacterial isolates was done by observing their macroscopic characteristics (aerial mycelium, submerged mycelium, colour, and diffusible pigments), Gram's staining and series of biochemical tests. The actinobacterial isolates were identified using Bergey's Manual of Systemic Bacteriology.

Extraction of Metabolites from Actinobacterial Isolates

Extraction of metabolites from actinobacterial isolates was carried out using submerged fermentation method described by de Souza *et al*. (2017) and Bo*et al*. (2019) with slight modifications. Each actinobacterial isolate was cultured in starch casein broth at 30˚C for 14 days. The inoculum was then transferred into 500 mL Erlenmeyer flask containing 250.0 mL of fermentation medium (glucose, 10.0 gL^{-1}; yeast extract, 7.5 gL^{-1}; peptone, 10.0 gL^{-1}; $(NH_4)_2SO_4$, 2.0 gL^{-1}; $FeSO_4{\cdot}7H_2O$, 1.0 gL^{-1}; $MnSO_4{\cdot}H_2O$, 1.0 gL^{-1}; $MgSO_4$, 0.5 gL^{-1}; and pH 6.0) and incubated at 30ºC for 14 days with shaking at 150 rpm. After fermentation, the culture medium was centrifuged at 10,000 rpm for 10 minutes to obtain the supernatant. The supernatants were then filtered through a 0.45µm microporous membrane to obtain cell-free filtrates. Each culture filtrate was evaluated for herbicidal activity.

Screening of Metabolites for Herbicidal Activity

The secondary metabolites extracted from actinobacterial isolates were screened for herbicidal activity using cucumber (*Cucumis sativus* L.cv.Ashley) model technique described by Schein *et al*. (2022) with little modifications. The seeds of *C. sativus* were pre-germinated for 2 days at 25±2°C without treatment. Two seedlings were sown in each pot containing sterilized soil (62.4% sand, 17.6% silt, 20% clay and pH 7.5), and later thinned to one plant per pot of comparable height 5 days after sowing. With the aid of manual sprayer, a total of 5.0 mL of each culture filtrate was sprayed directly on the shoot of each plant. Control plants were sprayed with the same volume of sterile fermentation medium instead of culture filtrate. All pots were maintained in a screen house for 12 days after metabolite application at a 12 h photoperiod, relative humidity of 70 to 90% and temperature of 25 to 30°C. The plant injury was visually observed and the phytotoxicity of the metabolites was assessed where 0 represented no effect/injury and 100 represented total plant death. Based on this primary screening, three most effective actinobacterial bioherbicides (*Streptomyces clavuligerus* RSR2, *S. clavuligerus* RSD and *Streptomyces griseus* TRD*)* were selected for weed assay.

Phytotoxic Effects of Metabolites from Actinobacteria on Post-emergence of Selected Grassy and Broad leaf Weeds

The post-emergence herbicidal effect of actinomycetes culture filtrate was carried out as described by Singh *et al.* (2018) with slight modifications. Five seeds from each plant species (*Pennisetum purpureum,Cynodon dactylon, Amaranthus spinosus* and *Tridax procumbens*) were pre-germinated for 2 days on 0.9% water agar. The seedlings were then sown in each pot containing sterilized soil, and thinned to three plants (4 – 6 leaf stage) per pot 5 days after sowing. For each weed, the experiment was conducted in a completely randomized design with four treatments and five replicates. The treatments were: T_1 = weed plant sprayed with sterile fermentation medium, T_2 = weed plants sprayed with *Streptomyces clavuligerus* RSR2metabolite, T_3 = weed plants sprayed with *S. clavuligerus* RSD metabolite and T_4 = weed plants sprayed with *Streptomyces griseus*TRDmetabolite. A total of 5.0 mL of each culture filtrate was sprayed directly on the shoot of each weed plant. The same volume of sterile fermentation mediumwas sprayed on the weed plants that served as control (T_1 group). Third day after metabolites application, visual assessment of phytotoxic symptoms like burns, curls, yellowing, chlorosis, withering/wilting, necrosis and total death was done till 12 days after application. The phytotoxicity of the metabolites on the weed plants was determined based on symptoms of mortality as follows: 0 (no symptom/activity), 1 (slight activity), 2 (moderate activity), 3 (strong activity), and 4 (total plant death) (Bo *et al*., 2019).

Statistical Analysis

The data obtained were analyzed using One-way Analysis of Variance (ANOVA), followed by separation of treatment means by Duncan's multiple range test at $p < 0.05$.

RESULTS AND DISCUSSION

Results

Isolation of Actinobacteria from Soil Samples

A total number of 20 isolates of actinobacteria were obtained from different soil samples, and then characterized by

macroscopic, microscopic and biochemical methods. The isolates were identified to belong to the genera *Streptomyces* (65%), *Actinomyces* (15%), *Microbacterium* (10%) and *Micromonospora* (10%). *Streptomyces* was found as the most frequently occurring genus with four species (*Streptomyces clavuligerus*, *S. griseus*, *S. olivaceous* and *S. albidoflavus*), followed by *Actinomyces* with two species. Macroscopically, there were variations in the colonial characteristics of the actinobacterial isolates. The aerial and substrate mycelium were mostly creamy and white, with entire, raised, rough and opaque colonies. The morphological and biochemical characteristics also revealed that all the isolates were Gram positive, non-motile, indole negative and fermented glucose. Some species were catalase and citrate positive (Table 1). Similarly, the percentage occurrence of actinobacterial isolates showed that *Streptomyces clavuligerus* had the highest occurrence (25%) while *Actinomyces gerencseriae* had the least occurrence (5%) (Figure 1).

Bioherbicidal Potentials of Actinobacterial Metabolites

The results of the primary screening of the secondary metabolites produced by actinobacterial isolates revealed that the secondary metabolites of 8 (40%) out of 20 isolates showed visual phytotoxic effects on model plant (*Cucumis sativus)* with varying degree of activity ranging from dark necrotic lesions on the leaves, yellowing to total death of some plants. Significant differences existed in the phytotoxicity of metabolites, however, the phytotoxic activities of *Streptomyces clavuligerus* RSR2, *S. clavuligerus* RSD and *S. griseus* TRD metabolites were significantly higher than other metabolites. The effects of the metabolites of these three isolates (*S. clavuligerus* RSR2, *S. clavuligerus* RSD and *S. griseus* TRD) started appearing on the plants within 3 days after application; resulting in total death of at least one of the plants sprayed with these metabolites and reached about 60% to 70% phytotoxicity at 12 days after application. On the other hand, the metabolites of 5 isolates induced low to moderate phytotoxicity while no phytotoxic effect was observed in any of *C. sativus* plants sprayed with the metabolites of remaining 12 isolates and sterile fermentation medium. The results therefore revealed that secondary metabolites produced by *Streptomyces clavuligerus* RSR2, *S. clavuligerus* RSD and *S. griseus* TRD possess strong herbicidal activity (Table 2).

Phytotoxic Potentials of Actinobacterial Metabolites on the Post-emergence of Selected Weeds

The results of the post-emergent assay revealed that spraying of selected grassy and broadleaved weeds (*Pennisetum purpureum,Cynodon dactylon*, *Amaranthus spinosus* and *Tridax procumbens*) with secondary metabolites produced by *Streptomyces clavuligerus* RSR2, *S. clavuligerus* RSD and *S. griseus* TRD showed significant differences in their potentials to cause injuries to the weeds under screen house conditions. The phytotoxic symptoms, including leaf curling, burns, necrosis and wilting appeared on broadleaved weeds (*Amaranthus spinosus* and *Tridax procumbens*) within 3 days after application and increased rapidly, reaching 58.0 – 69.80% and 55.0 – 64.0% killing at 12 days post-application, respectively (Figures 2 and 3). The highest phytotoxic effects on the post-emergent *A. spinosus* and *T. procumbens* were observed in metabolites produced by *S. clavuligerus* RSR2 and *S. griseus* TRD, respectively. Similarly, little curls, wilts and burns were observed on grassy weeds (*Pennisetum purpureum* and *Cynodon dactylon*) from day 3 after application of metabolites which progressed, and by 12th day, there was observation of full chlorosis, wilts, burns and plant death resulting in 55.8 – 68.0% and 55.0% - 60.0% phytotoxic activities, respectively (Figures 4 and 5). The secondary metabolite produced by *S. griseus* TRD exhibited the highest phytotoxic activity on post-emergence of *P. purpureum* while that of *S. clavuligerus* RSD induced the highest phytotoxic effect on post-emergent *C. dactylon* at 12 days post-application (Plates 1 and 2).

Table 1: Morphological and Biochemical Characteristics of Actinobacteria Isolated from Different Soil Samples

Gram reaction	Catalase	Citrate	Starch hydroly	Indole	Oxidase	MR	VP	H_2S	Motility	Glucose	Lactose	Sucrose	Organisms
+	+	+	+	-	-	-	+	-	-	+	-	-	*Microbacterium oxydans*
+	+	+	+	-	+	+	-	-	-	+	-	-	*Streptomyces clavuligerus*
+	+	+	+	-	+	-	+	-	-	+	-	-	*Streptomyces griseus*
+	+	+	+	-	-	-	-	-	-	+	+	+	*Streptomyces olivaceous*
+	+	+	-	-	+	+	+	-	-	+	+	+	*Streptomyces albidoflavus*
+	+	-	+	-	-	+	-	-	-	+	+	+	*Actinomyces gerencseriae*
+	-	+	+	-	+	-	-	+	-	+	-	+	*Actinomyces israelii*
+	+	+	+	-	+	+	-	-	-	+	-	-	*Micromonospora chalcea*

Keys: MR: Methyl red VP: Voges-proskauer H_2S: Hydrogen sulphideproduction + Positive - Negative

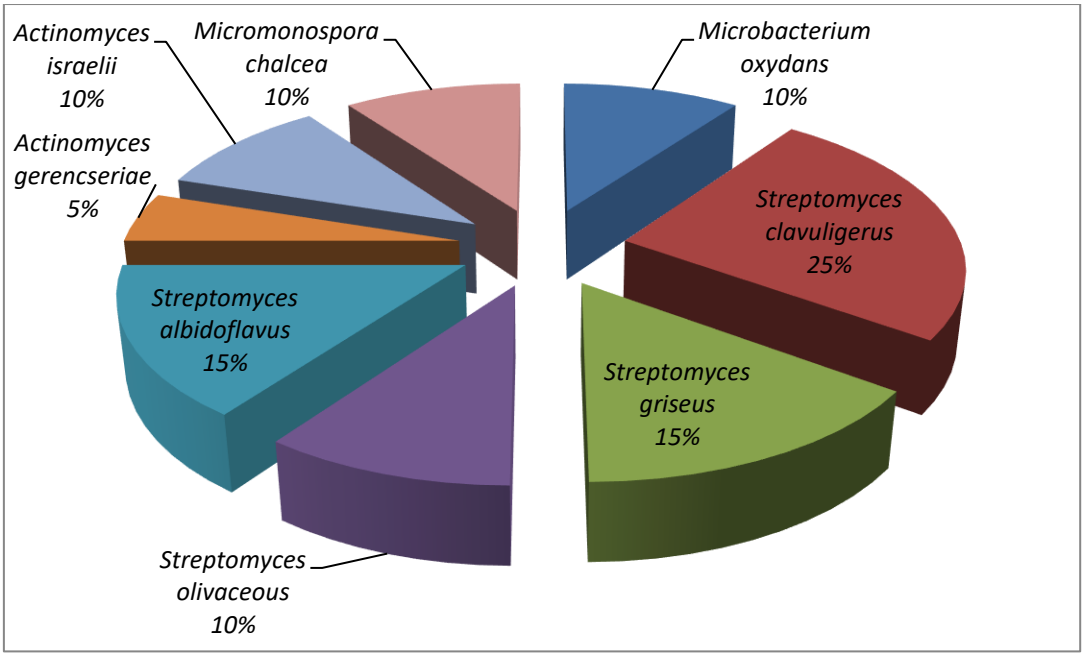

Figure 1: Percentage Occurrence of Actinobacteria Isolated from Different Soil Samples

Table 2: Herbicidal Activity of Actinobacterial Metabolites on *Cucumis Sativus*

Actinobacterial isolates	**Herbicidal activity (%±S.D.)**
Actinomyces gerencseriae RSD	40.0±0.20^{c}
Actinomyces israelii TRD	0.00±0.00^{f}
Actinomyces israelii RSR	0.00±0.00^{f}
Streptomyces clavuligerus RSR1	0.00±0.00^{f}
Streptomyces clavuligerus RSR2	70.00±1.40^{a}
Streptomyces clavuligerus TRD	0.00±0.00^{f}
Streptomyces clavuligerus MWS	0.00±0.00^{f}
Streptomyces clavuligerus RSD	60.00±1.10^{b}
Streptomyces griseus TRD	66.00±0.80^{ab}
Streptomyces griseus WSD	36.50±0.40^{c}
Streptomyces griseus RSR	0.00±0.00^{f}
Streptomyces olivaceous WSD	28.00±0.20^{d}
Streptomyces olivaceous TRD	0.00±0.00^{f}
Streptomyces olivaceous RSR	15.80±0.50^{e}
Streptomyces albidoflavus MWS	0.00±0.00^{f}
Streptomyces albidoflavus RSR	38.00±0.50^{c}
Microbacterium oxydans TRD	0.00±0.00^{f}
Microbacterium oxydans WSD	0.00±0.00^{f}
Micromonospora chalcea RSR	0.00±0.00^{f}
Micromonospora chalcea RSD	0.00±0.00^{f}
Control	0.00±0.00^{f}

values in columns followed by the same letter are not significantly different at $p \leq 0.05$

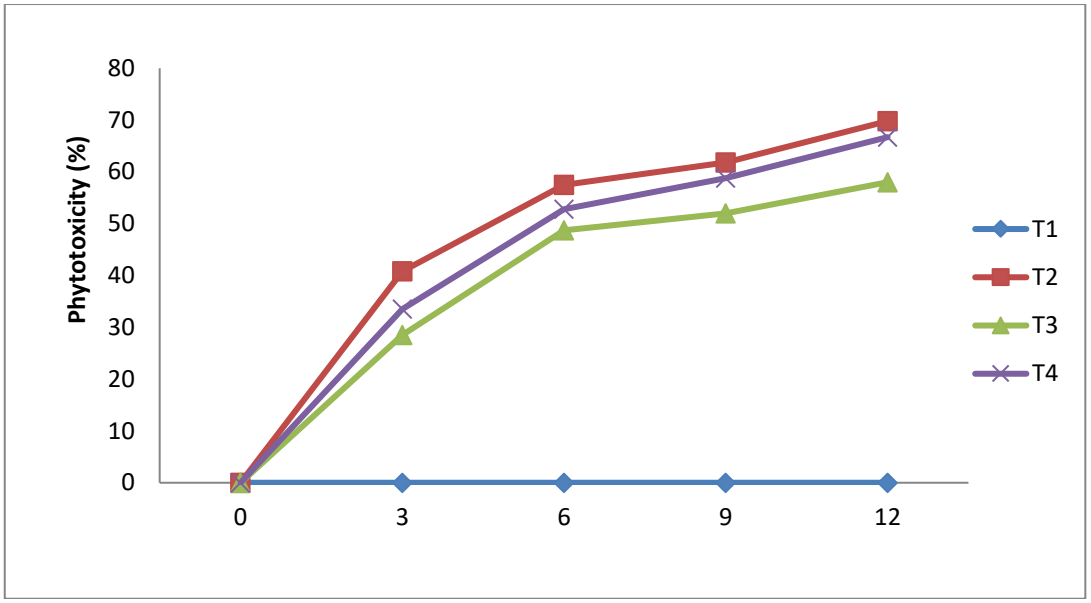

Figure 2: Phytotoxic Activity of the Actinobacterial Metabolites on *Amaranthus Spinosus*

T_1 = weed plant sprayed with sterile fermentation medium
T_2 = weed plants sprayed with *Streptomyces clavuligerus* RSR2metabolite
T_3 = weed plants sprayed with *S. clavuligerus* RSD metabolite
T_4 = weed plants sprayed with *Streptomyces griseus* TRD metabolite

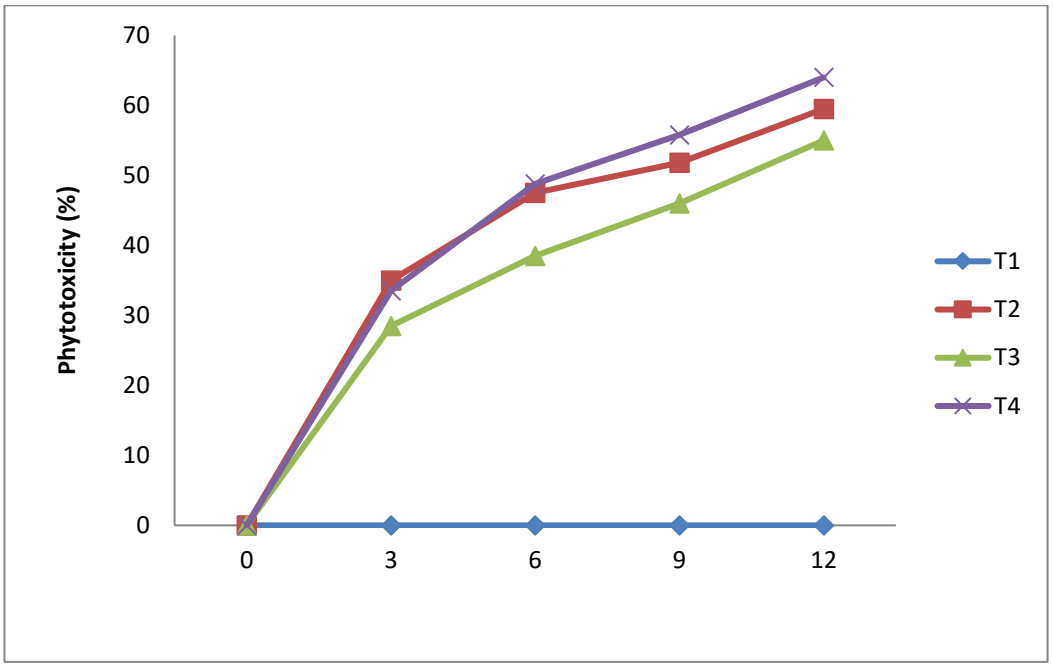

Figure 3: Phytotoxic Activity of the Actinobacterial Metabolites on *Tridax Procumbens*

T_1 = weed plant sprayed with sterile fermentation medium
T_2 = weed plants sprayed with *Streptomyces clavuligerus* RSR2metabolite
T_3 = weed plants sprayed with *S. clavuligerus* RSD metabolite
T_4 = weed plants sprayed with *Streptomyces griseus* TRD metabolite

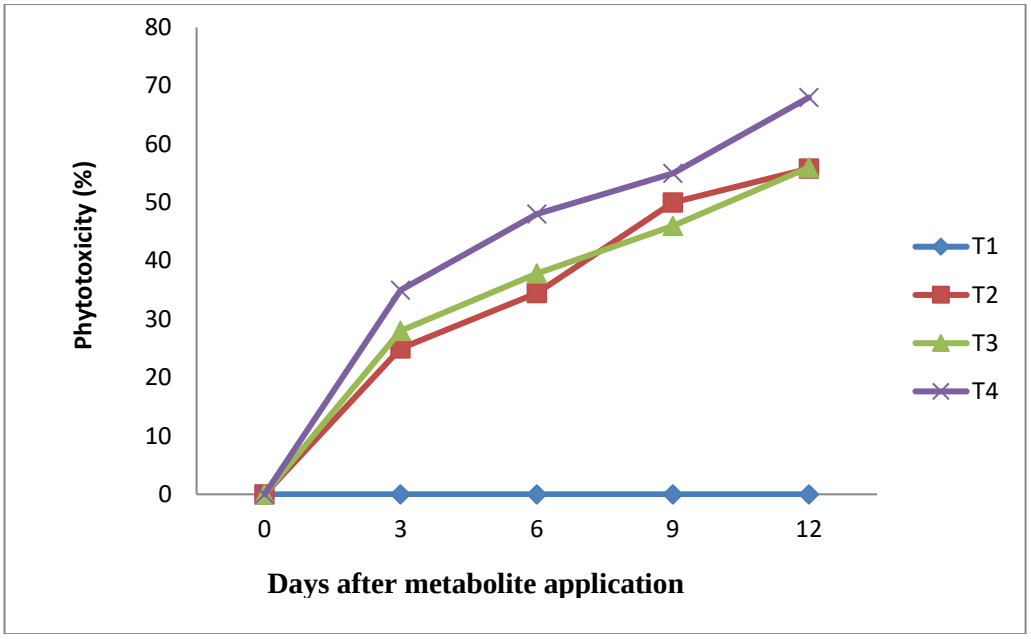

Figure 4: Phytotoxic Activity of the Actinobacterial Metabolites on *Pennisetum Purpureum*

T_1 = weed plant sprayed with sterile fermentation medium
T_2 = weed plants sprayed with *Streptomyces clavuligerus* RSR2 metabolite
T_3 = weed plants sprayed with *S. clavuligerus* RSD metabolite
T_4 = weed plants sprayed with *Streptomyces griseus* TRD metabolite

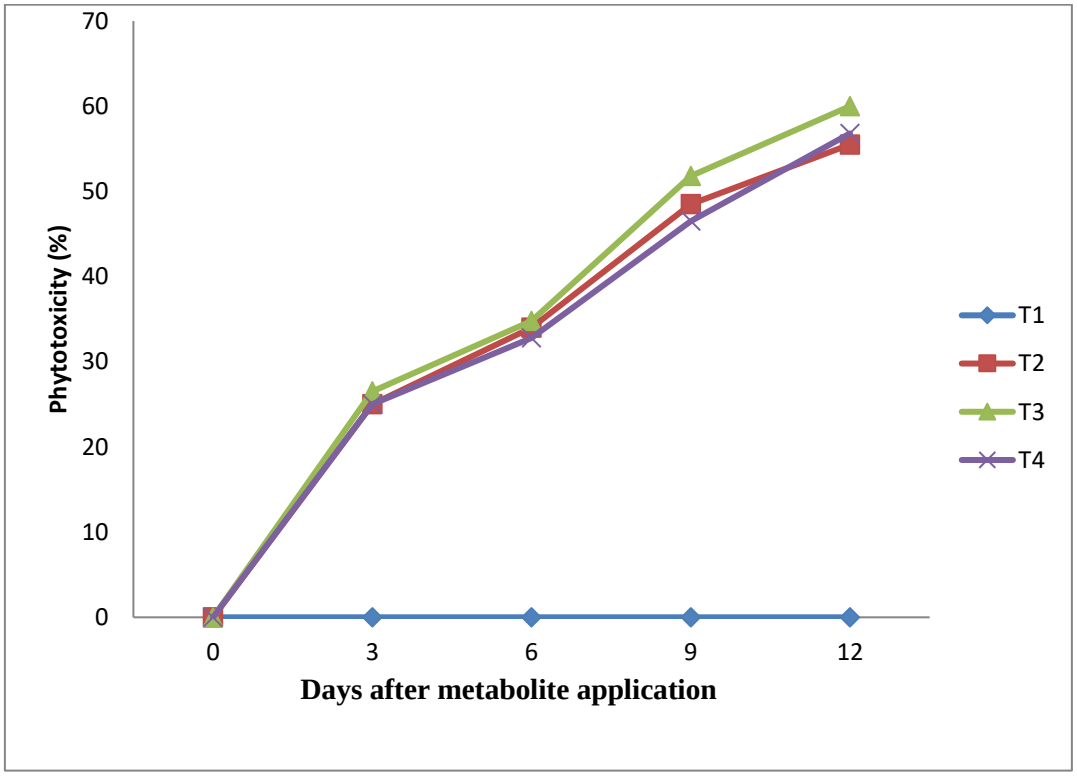

Figure 5: Phytotoxic Activity of the Actinobacterial Metabolites on *Cynodon Dactylon*

T_1 = weed plant sprayed with sterile fermentation medium
T_2 = weed plants sprayed with *Streptomyces clavuligerus* RSR2 metabolite
T_3 = weed plants sprayed with *S. clavuligerus* RSD metabolite
T_4 = weed plants sprayed with *Streptomyces griseus* TRD metabolite

A. *Pennisetum purpureum* Sprayed with Fermentation Medium

B. *P. Purpureum* Sprayed with *Streptomyces Griseus* TRD Metabolite

C. *P. purpureum* Sprayed with *S. Clavuligerus* RSR2 Metabolite

D. *P. Purpureum* Sprayed with *S. Clavuligerus* RSD Metabolite

Plate 1: Effects of actinobacterial metabolites on *Pennisetum purpureum*

A: *Cynodon Dactylon* Sprayed with *Streptomyces Clavuligerus* RSD Metabolite

B: *Cynodon Dactylon* Sprayed with *S. Clavuligerus* RSR2 Metabolite

C: *Cynodon Dactylon* Sprayed with *Streptomyces Griseus* TRD Metabolite

D: *Cynodon Dactylon* Sprayed with Fermentation Medium

Plate 2: Effects of Actinobacterial Metabolites on *Cynodon Dactylon*

Discussion

The present study revealed that *actinobacterial* strains can be found ubiquitously in soil. The actinobacterial genera obtained from different soil samples were *Streptomyces*, *Actinomyces*, *Microbacterium* and *Micromonospora*, with *Streptomyces* occurring as the most dominant genus. These results corroborate several studies that reported the isolation of different genera of actinobacteria from diverse soil habitats including forest, farmland, grassland and coastal soils (Dhanasekaran *et al*., 2010; Das *et al.*, 2018; Bo *et al*., 2019). Also, *Streptomyces* was found as the most occurring genus and this agrees with the study of Yu *et al*. (2015) who isolated one hundred and seventy two actinobacterial strains and 70.93% of the isolates belonged to the genus *Streptomyces.*

Isolation and purification of the bioactive compounds or secondary metabolites produced by microorganisms possessing herbicidal activity on weeds could be a feasible technique for the production of effective bioherbicides for weed control. It is an acceptable weed management strategy due to its environmentally friendly nature. The present study showed that the secondary metabolites produced by some actinobacterial strains could exhibit low to high phytotoxic activity on the seedlings of *Cucumis sativus*. The visual phytotoxic effects ranging from dark necrotic lesions on the leaves or leaf spots, yellowing to total death were noticed on the target plants and these symptoms had been previously observed on *Cucumis sativus* seedlings by de Souza *et al*. (2017) and de Almeida *et al*. (2020) when evaluating the phytotoxicity of some microbial secondary metabolites. Daniel *et al*. (2018) also reported necrosis and chlorosis in 70% of foliar area of *Cucumis sativus* and *Sorghum bicolor* seedlings when assessing the phytotoxic potential of metabolite produced by *Fusarium fujikuroi*. Schein *et al*. (2022) also observed phytotoxic symptoms like necrosis, yellowing and leaf spots on *Cucumis sativus* seedlings when screening some microbial strains for herbicidal activity.

In the same vein, the present study further showed that the secondary metabolites produced by *Streptomyces clavuligerus* RSR2, *S. clavuligerus* RSD and *S. griseus* TRD induced strong phytotoxic effects on post-emergent *Pennisetum purpureum*,*Cynodon dactylon*, *Amaranthus spinosus* and *Tridax procumbens*with symptoms ranging from leaf curling, burns, necrosis and wilting appearing on the weeds within 3 days after application of metabolites to plant death. These results are in agreement with the report of Singh *et al*. (2018) who also observed leaf curling, wilting and burning on *Parthenium hysterophorus*, *Bidens biternata* and *Ageratum conyzoides* seedlings sprayed with culture filtrates of some endophytic actinomycetes. Bo *et al*. (2019) also observed serious injuries such as yellowing, leaf spots and blight on the foliage of *Digitaria sanguinalis*, which eventually resulted to the plant death, when determining the phytotoxic potential of bioherbicides produced by some species of *Streptomyces*. The results also agree with the observation of Oloyede *et al*. (2025) who demonstrated that secondary metabolites of *Fusarium oxysporum*, *Fusarium incarnatum-equiseti* and *Alternaria alternata* possessed strong phytotoxic effects on the post-emergence of *Ageratum conyzoides* plants by causing series of phytotoxic symptoms ranging from leaf curling, burns, necrosis, wilting/withering, leaf yellowing to total plant deaths. The metabolites could cause injuries to the weed plants through toxification of weed cells. The phytotoxic metabolites might interfere with weed plant metabolism or destroy the plants by acting on their cell membranes, mitochondria and chloroplasts (Shang *et al.*, 2016; Oloyede *et al*., 2025). The metabolites could also directly or indirectly affect the expression of genes, inhibiting the synthesis of proteins and nucleic acids in weed plants and eventually lead to plant death (Stergiopoulos *et al*., 2013; Daguerre *et al*., 2014; Zeilinger *et al.*, 2016).

CONCLUSION

This study showed that the secondary metabolites produced by *Streptomyces clavuligerus* RSR2, *S. clavuligerus* RSD and *S. griseus* TRD exhibited desirable phytotoxic effects on pre-emergence of some weed plants, which could be developed as potent post-emergent bioherbicides for weed control to reduce the risk of health hazards caused by chemical herbicides on both human and animal at large. However, further studies need to be carried out to evaluate the safety, appropriate formulations, and mechanism of action of these phytotoxic metabolites.

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