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Phytopathogenic Fungal Growth Inhibition by Actinomycetes Isolated from the Rhizosphere of *Cymbopogon citratus* (Lemongrass)

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Abstract

The search for natural antimicrobial substances to mitigate the deleterious effects of pathogens is on the increase due to the spread of resistance. In the agricultural sector, the current focus is on natural, eco-friendly, and sustainable approaches to improve crop yield. In this study, sixty actinomycetes isolates belonging to 3 genera; *Streptomyces*, *Nocardia,* and *Micromonospora*, obtained from 30 lemon grass rhizosphere samples, were screened for antifungal activity using three common fungi phytopathogens; *Fusarium solani, Aspergillus flavus* and *Alternaria alternata*. Preliminary screening involving the *in vitro* dual culture assay and the agar well diffusion techniques were employed to investigate the inhibitory activities of actinomycetes isolates and the ethyl acetate extract of their metabolites against the phytopathogens. Results obtained revealed that mycelial extensions in the fungi were significantly inhibited, though, to varying extents. Among the 60 actinomycetes isolates screened, 19 (31.37%) demonstrated significant inhibitory activity against at least one of the three test fungi. Out of the 19 isolates, 5(26.3%) suppressed mycelial growth in all three test fungi with percent mycelial inhibitions ranging from 51.63 – 69.29 %. Also, the crude extracts of the metabolites of these five actinomycetes isolates produced mycelial inhibitions ranging from 75.45 - 89.31 %. Analysis of the crude extracts using gas chromatography, revealed the production of five antifungal compounds which include: Benzylaldehyde,2-nitro-diaminomethylidene hydrazine, [1,2,4] triazolo [1,5] pyrimidine -6-carboxylic acid, 1,2, -benzenediol,3,5-bis(1,1-dimethylethyl), Benzo[h] quinolone,2,4-dimethyl-, and 2,4,6-cycloheptatrien-1-one, 3,5, bis-trimethylsilyl-. Therefore, the antifungal capabilities of these actinomycetes isolates make them efficient, natural, and ecologically friendly alternatives in the control of fungal phytopathogens.

Keywords: Actinomycetes, Lemon grass, rhizosphere, anti-fungal, phytopathogens, screening

Резюме

Търсенето на природни антимикробни вещества за намаляване на вредното въздействие на патогените се увеличава поради разпространението на тяхната резистентност. В селскостопанския сектор понастоящем вниманието е насочено към природни, екологични и устойчиви подходи за подобряване на добивите от култури. В това проучване, шестдесет изолата от *Actinomycetes*, принадлежащи към три рода - *Streptomyces*, *Nocardia* и *Micromonospora*, получени от 30 проби от ризосферата на лимонова трева са изследвани за антифунгална активност върху три често срещани гъбни фитопатогена: *Fusarium solani, Aspergillus flavus* и *Alternaria alternata*. За изследване на инхибиторната активност на изолатите от актиномицети и на етилацетатния екстракт от техните метаболити срещу фитопатогените са използвани предварителни скринингови методи, включващи *in vitro* тест с двойна култура и агар-дифузионен метод. Получените резултати показват, че развитието на мицела се инхибира значително в зависимост от вида на гъбите. Сред 60-те изследвани изолата на актиномицети 19 (31.37 %) демонстрират значителна инхибираща активност срещу поне един от трите моделни щама. От тях 5 изолата (26.3 %) проявяват активност срещу всички използвани щамове - от 51.63 до 69.29 %. Екстрактите от метаболитите на тези пет изолата също предизвикват инхибиране на мицелното развитие в диапазона от 75.45 до 89.31%. Чрез газово-хроматографски анализ в суровите екстракти от пет съединения с антифунгално действие е установено присъствието на бензилалдехид,

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2-нитро-диаминометилиден хидразин, [1,2,4]-триазоло [1,5]-пиримидин-6-карбоксилна киселина, 1,2,-бензендиол,3,5-бис(1,1-диметилетил), бензо[h]хинолон,2,4-диметил- и 2,4,6-циклохептатриен-1-он, 3,5,-бис-триметилизил. Получените резултати определят тези изолати от актиномицети като ефикасни, природни и екологично чисти алтернативи за контрол на гъбни фитопатогени.

Introduction

Worldwide, there is an increasing alarm about food insecurity borne out of the rise in the incidences of microbial resistance to agrochemicals due to the overuse of these chemicals in the agricultural sector (Fisher *et al*., 2018; Ramakrishnan *et al*., 2019; Sapkota *et al*., 2020; Miller *et al*., 2022). In the US alone, it has been reported that about 1.5 billion dollars is lost annually, due to the development of resistance in phytopathogens to pesticides (Pimentel and Burgess, 2014; Miller *et al*., 2022).

Fungi are important plant pathogens and food spoilage agents causing both low yields of crops as well as reduced quality of stored products. FAO report (2010), attributed 20 billion US dollars as a loss on agro-crops across the globe as a result of attacks by pesticide-resistant fungi and insect pests. At a global level, estimates of losses in annual yield of some staple crops due to resistant fungal pathogens include 21.5% (wheat), 30.0% (rice), 22.5% (maize), 17.2% (potato), and about 21.4% in soybean (Savary *et al*., 2019). Furthermore, the negative impact of plant diseases emanating from pesticide-resistant phytopathogens on the global economy is now about 220 billion dollars per year (FAO, 2017; Karkouri *et al.,* 2019). Consequently, the search for natural antimicrobial substances to mitigate the deleterious effects of plant pathogens is on the increase (Singh *et al.,* 2016; Miller *et al*., 2022).

Today, the use of chemicals as plant pathogen control agents is being discouraged as it has led to increased cost of food crops (de Cal *et al*., 2012; Rathi, *et al.,* 2012) as well as environmental persistence and toxicity resulting in harmful effects on non-target organisms (de Cal *et al*., 2012; Ubogu *et al*., 2017; Akponah, 2018; Fisher *et al*., 2018; Ramakrishnan *et al*., 2019). Hence, the current focus on maintaining plant health is on natural, eco-friendly, and sustainable approaches (Karkouri *et al.,* 2019; FAO, 2019; Ramakrishnan *et al*., 2019).

In various spheres including, agricultural, medical, pharmaceutical, and industrial sectors, the potential use of bioactive metabolites from microbial sources is increasingly gaining attention (Janardhan *et al.,* 2014). These compounds serve as important eco-friendly alternatives in combating pathogens (Nanjwade *et al.,* 2010; de Cal *et al*., 2012; Chaudhary *et al*., 2013; Sapkota *et al*., 2020). Thus, several bioactive molecules (over twenty-three thousand) have been isolated, characterized, and developed (Schweder *et al*., 2005; Nanjwade *et al.,* 2010; Brzezinska *et al*., 2014; Chaudhary *et al*., 2014; Gomes *et al*., 2018, Subbanna *et al*., 2018). Importantly, 80% of bioactive compounds that have been characterized are produced by actinomycetes (Raja and Prabakarana, 2011, Gomes *et al*., 2018). Actinomycetes have the genetic machinery to produce multiple secondary metabolites which may feature any of the following properties; antifungal, antibacterial, antioxidant, antitumor, anticancer, anti-inflammatory, immunosuppressive as well and insecticidal. (Omran and Kadhem, 2012; Chaudhary *et al*., 2013; Janardhan *et al.,* 2014). Therefore, the isolation and screening of actinomycetes for the potential of producing unique bioactive substances for the development of natural-based, safe products for the control of plant pathogens, cannot be over-emphasized.

Actinomycetes are a group of gram-positive, facultatively anaerobic, filamentous bacteria belonging to the order Actinomycetales (Bhatti *et al*., 2017). They have a very high Guanine-cytosine content, form asexual spores, and are widely distributed in both aquatic and terrestrial environments (Balagurunathan *et al.,* 2010). Although a few members of this heterogenous group are pathogenic, many species are harmless, beneficial, and sources of a wide array of industrial and medically relevant compounds. Also, they have been established as plant growth-promoting rhizobacteria (Khamna *et al.,* 2009) and are associated with plant protection and improvement of soil health (Singh *et al.,* 2016).

According to Brzezinska *et al*. (2014) and Gomes *et al.* (2018), only about 150 (< 1%) of the already characterized bioactive compounds have been put into use in agricultural fields. Hence, there is a critical need to screen and develop more of these compounds for enhanced and sustainable agriculture. The aim of this study is to screen a previously unexplored ecological niche (lemon grass rhizosphere) for actinomycetes with anti-fungi capability using three common fungi phytopathogens

Materials and Methods

Collection of rhizosphere samples

Thirty lemon grass samples were uprooted carefully from three locations (gardens designat-

ed as Stations A, B, and C respectively) in Abraka, Delta State. The method described by Ubogu *et al*. (2019), was adopted for the collection of rhizosphere samples. The rhizosphere of each grass sample was obtained by initial shaking off of all loose soils bound to the root. Soils that were firmly attached to the root surfaces were then shaken vigorously into sterile black polyethylene bags. All samples were immediately transported to the laboratory for further analysis.

Isolation of actinomycetes from Cymbopogon citratus rhizosphere

The rhizosphere samples obtained were diluted using the ten-fold serial dilution technique and then subsequent 0.1 mL of appropriate dilution that produced 30-300 colonies were inoculated using the pour plate method on Starch Casein agar [with composition: starch 10, casein; 0.3, KNO_3; 2.0, $MgSO_4.7H_2O$; 0.05, K_2HPO_4; 2.0, NaCl; 2.00, $CaCO_3$; 0.02, $FeSO_4.7H_2O$; 0.01 and agar 15 (g/L)]. Incubation at ambient temperature for 48-72 h followed immediately. Discrete colonies that developed were sub-cultured for identification and subsequent assay.

Also, bulk soils were collected at about 30 cm away from the point where each lemon grass sample was uprooted for the determination of actinomycetes load and subsequent derivation of lemon grass rhizosphere effect on the density of actinomycetes.

The rhizosphere effect was calculated by adapting the method of Zheng *et al*. (2019) as follows:

Rhizosphere effect on actinomycetes density =

$$= \left(\frac{LAL - LAB}{LAB}\right) 100$$

Where, LAL = Count of actinomycetes in lemon grass rhizosphere

LAB = Count of actinomycetes in bulk soil

Identification of actinomycetes

Identification of actinomycetes isolates was done following observations of cultural and microscopic characteristics and further biochemical characterization as stipulated in Bergey's Manual of Determinative Bacteriology (Goodfellow *et al*., 2012).

Isolation/ Identification of plant pathogenic fungi isolates.

Three plant pathogenic fungi including *Alternaria alternata*, *Fusarium solani* and *Aspergillus flavus* were sourced and isolated from the soil of cassava farmland using a culture-based method. Subsequent identification based on cultural and microscopic attributes followed with particular reference to Barnett and Hunter (1998).

Preliminary screening for anti-mycotic substance production by actinomycetes

Sixty isolates of actinomycetes obtained from the rhizosphere of lemon grass were screened for the production of anti-fungal substance following the dual culture method as reported by (Ohike *et al*., 2018). Seven days old actinomycetes culture plug of 6 mm was aseptically inoculated onto a freshly prepared Potato Dextrose Agar plate at 5 mm from the periphery of the Petri dish. Incubation at ambient temperature for seven days followed immediately. On the seventh day, the test fungus plug (6 mm) was inoculated on the plate at the opposite end to the actinomycetes plug and 5mm from the opposite end of the plate. Further incubation at room temperature, followed. On Day 1, 2, 4, and 7, measurements of fungal mycelial extension in the direction of the actinomycetes plug was taken. Control plates involved PDA plates with only fungal plugs without prior inoculation of actinomycetes. Percent mycelial extension inhibition was calculated thus:

$$\% \text{ MEI} = \left(\frac{\text{MEC}-\text{MET}}{MEC}\right) 100$$

Where, % MEI = percent mycelial extension inhibition.

MEC = mean of final mycelial extension in the control plate

MET = mean of final mycelial extension in the test plate

All plates were in triplicates. Also, Actinomycete isolates that produced a percent mycelial inhibition that was significantly higher than that of the control plate was considered inhibitory.

Submerged fermentation for the production of an anti-mycotic bioactive substance by isolates of actinomycetes

Five isolates of actinomycetes that significantly inhibited mycelial extension in the three test fungi species obtained as described in the preceding section were further selected for this experiment. Each isolate was inoculated at a concentration of 1.0 x 10^7 spores/ml into 100 mL of Starch Casein broth contained in a 250 mL Erlenmeyer flask. Then, incubation followed immediately at room temperature for 7 days under shaken conditions (120 rpm), using a shaker incubator (SEARCHTECH). At the end of the incubation period, cells were harvested

by centrifugation (using 800 D centrifuge) at 5000 rpm for 30 minutes. Crude extracts of the bioactive compounds produced were then extracted from the supernatant using 1% v/v ethyl acetate following the method described by Dhananjeyan *et al*. (2010). The supernatant obtained was mixed with ethyl acetate in a ratio of 1:1. The mixture was allowed to stand for 24 h, after which the upper layer of ethyl acetate was separated and evaporated to dryness at 40°. Concentrated bioactive compounds were reconstituted in sterile deionized water for further analysis.

Determination of the anti-fungal activity of crude extract of bioactive molecules

Crude extracts obtained as described in the preceding section, were weighed, and reconstituted in sterile distilled water to make a concentration of 0.01 mg/L. The inhibitory effects of each extract on fungal mycelial growth were determined using the agar well diffusion method. Each reconstituted extract (0.5 mL) was introduced into a well of 6 mm in diameter which was aseptically made at one end on a sterile Potato Dextrose agar plate. This was allowed to stand for 72 h, after which, the fungus plug (6mm in diameter) was inoculated at the opposite end of the plate. Incubation followed immediately at ambient temperature for 7 days. Fungal mycelial extensions were then measured at the end of the incubation period. Control plates were inoculated with 0.5 mL sterile distilled water (equivalent of crude extract aliquot introduced into test plate) and the appropriate test fungus. All plates were replicated in triplicates and the percent inhibitory effect of the crude extract on mycelial extension was derived as follows:

$$\text{CEI (\%)} = \left(\frac{\text{FMEC} - \text{FMET}}{FMEC}\right) 100$$

Where CEI = Percent mycelial inhibition by crude extract

FMEC = mean of final mycelial extension in the control plate

FMET = mean of final mycelial extension in test plate.

Identification of bioactive anti-mycotic substance

The bioactive substances responsible for the inhibition of fungal mycelial extension were identified using gas chromatography coupled with a mass spectrometer detector (Agilent Technologies). The ethyl-acetate extract obtained as described above was utilized for the analysis. Identification of the bioactive compounds present in the extract was based on a reference to the mass spectra database in the National Institute of Standard and Technology (NIST) library.

Statistical analysis

Microsoft Excel Analysis tool Pak was used in the statistical analysis of the data obtained. Mean, standard deviation, t-test, and analysis of variance were done.

Results

The results of the total actinomycetes load of the rhizosphere of lemon grass is as presented in Table 1. It was observed that actinomycetes were associated with all the samples of lemon grass rhizosphere with counts ranging from 3.0 x10^4 to 1.02 x 10^5 CFU/g. There was no significant difference at the 95% confidence limit in the actinomycetes population of the rhizosphere of lemon grass obtained from various stations.

All lemon grass rhizosphere harbored a substantial number of actinomycetes. Also, a significant difference was noticed in the population of actinomycetes in lemon grass rhizospheres and bulk soils with rhizosphere effects on actinomycetes' densities as shown in Table 1. Results obtained indicated that the density of actinomycetes in the rhizosphere samples was significantly higher than in bulk soils. A total of 60 isolates belonging to three genera of actinomycetes were obtained as also represented in Table 1. The genera included *Streptomyces*, *Nocardia*, and *Micromonospora* with percentage occurrence of 81.67, 10.00, and 8.33 respectively. Thus, analysis of the diversity of actinomycetes associated with the rhizosphere of

Table 1. Diversity of actinomycetes obtained from lemon grass rhizosphere from various stations.

Station	Number of lemon grass obtained	Range of Total actinomycetes Count (CFU/g)	Tentative actinomycetes genera (Frequency of occurrence)	Rhizosphere Effect on actinomycetes density (range)
A	10	3.0 – 4.7 x10^4	*Streptomyces* (14) *Nocardia* (3) Micromonospora (3)	50 - 135
B	10	5.1 -8.9 x10^4	*Streptomyces* (16) Nocardia (2) *Micromonospora* (2)	45.71 - 154.29
C	10	4.1 – 7.2 x 10^4	*Streptomyces* (19) *Nocardia* (1) *Micromonospora* (0)	10.81 - 94.59

lemon grass revealed that *Streptomyces* spp. were predominant.

Preliminary investigation of the anti-fungi activity of the actinomycetes isolates obtained, (60), revealed that 19 (31.67 %) demonstrated inhibitory activity against at least one of the test fungi as depicted in Fig. 1. Morphological observation and biochemical characterization revealed that 17 among the 19 isolates designated IS1-IS17 were *Streptomyces* spp. while isolates designated as IS18 and IS19 were *Nocardia* spp.

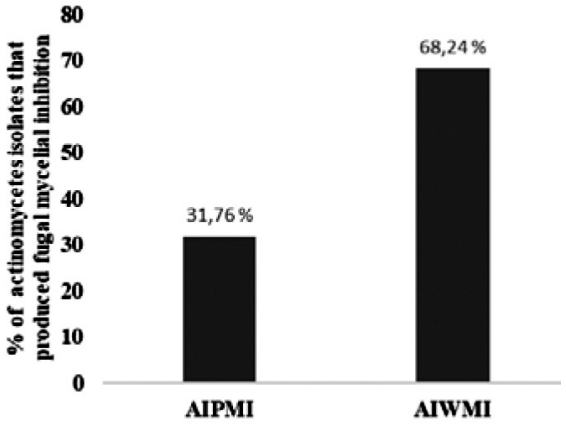

Fig. 1. Fungal mycelial growth inhibition capabilities of total actinomycetes isolates.
*AIPMI = actinomycete isolates that produced mycelial growth inhibition; AIWMI = Actinomycete isolates with no inhibition effect.

The results of the inhibitory effects of the 19 actinomycetes isolates on the mycelial extension of the test fungi (*F. solani*, *A. flavus,* and *A. alternata*) are represented in Table 2. It was observed that among these 19 actinomycetes isolates, 5 (26.3%) were able to produce inhibitory effects against mycelial growth in all the three test fungi with percent mycelial inhibition ranging from 51.63 – 69.29 % while another 5 had inhibitory effects on the mycelial growth of only two of the three test fungi. Analysis of variance showed that there were significant differences in the responses (mycelial extensions) of the various test fungi to the inhibitory activities of the Actinomycete isolates. In *F. solani*, the percentage of mycelial inhibition by the various actinomycetes isolates ranged from 0 - 69.29 %. While 0 - 59.49 % and 0 - 66.67 % were the range of percent mycelial extension inhibitions produced by the actinomycetes against *A. flavus* and *A. alternata* respectively (Table 2).

Based on the result of the preliminary investigation, the five *Streptomyces* isolates that were inhibitory to the three test fungi were further selected for the production of bioactive substances (secondary metabolites).

Table 2. Inhibition of mycelial extension in test fungi by various actinomycetes isolates

Isolate	Mycelial growth inhibition (%)		
	F. solani	*A. flavus*	*A. alternata*
IS1	52.75	3.92	3.92
IS2	55.11	10.45	10.45
IS3	59.84	58.82	58.82
IS4	66.93	0.00	0.00
IS5	55.11	56.86	56.86
IS6	59.84	51.63	51.63
IS7	69.29	54.90	54.90
IS8	62.20	0.00	0.00
IS9	66.93	56.23	56.23
IS10	59.84	0.00	0.00
IS11	59.84	53.59	53.59
IS12	0.78	54.25	54.25
IS13	2.36	6.00	6.00
IS14	1.56	59.47	59.47
IS15	66.14	58.82	58.82
IS16	64.56	0.00	0.00
IS17	48.02	0.00	0.00
IS18	0.00	0.00	0.00
IS19	4.72	0.00	0.00
Control	0.00	0.00	0.00

*IS1 – IS17 = Various *Streptomyces* spp.
IS18 & IS19 = *Nocardia* spp.

The results of inhibitory effects of the crude extracts of the bioactive substances recovered from the five actinomycetes isolates (IS 3, IS 5, IS 7, IS9, and IS 11) against the test fungi are presented in Table 3.

Fungi mycelial extensions were significantly lower in test plates than in control plates indicating suppressiveness of fungi growth by the extracts. However, there was no significant difference in the responses of the three test fungi to the inhibitory effects of the crude extracts of bioactive compounds obtained from the five selected actinomycetes. As a result of the similarity in the inhibitory property of the crude extracts assayed, only the crude extract from one of the five actinomycetes isolates (IS7) was subjected to gas chromatographic analysis.

The analysis of the crude extract of the bioactive substances produced by IS7 revealed the production of 25 bioactive compounds (peaks) as illustrated by Fig. 2.

Five of the twenty-five bioactive compounds produced have been established to possess antifun-

Table 3. Inhibitory effects of crude extracts of bioactive substance on fungal mycelial growth

Crude extract of bioactive substance	Mycelial Extension (mm)		
	F. solani	*A. flavus*	*A. alternata*
IS3	7.33±2.08	6.67±1.15	6.00±1.00
IS5	9.33±1.15	9.67±1.53	4.67±1.53
IS7	6.67±0.5B	9.00±1.00	8.33±2.52
IS9	5.00±1.00	8.00±1.00	7.67±2.08
IS11	7.00±1.00	8.67±1.53	5.67±1.15
Control	38.00±2.65	49.67±2.52	43.67±1.53

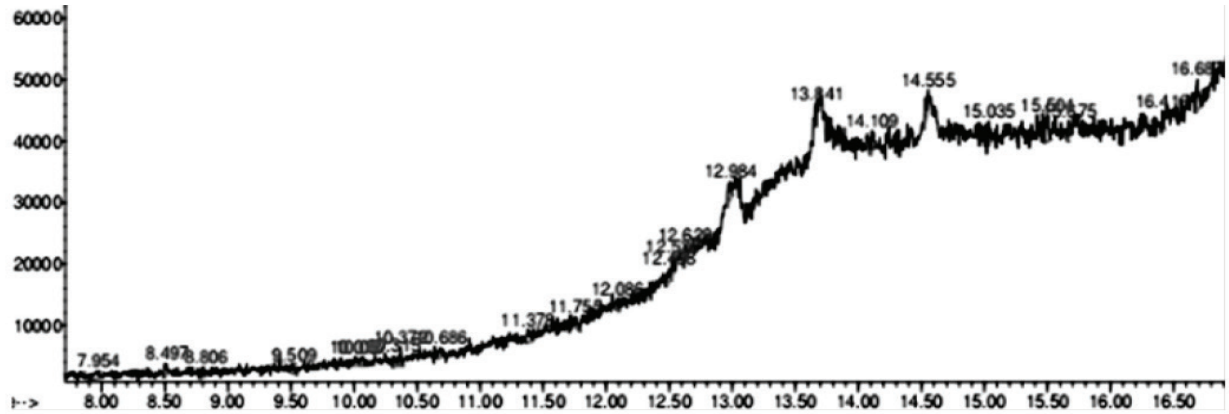

Fig. 2. Chromatogram of compounds present in *Streptomycetes* spp fermentation broth

gal properties. They include; Benzylaldehyde,2-nitro-diaminomethylidene hydrazine, [1,2,4] triazolo [1,5] pyrimidine -6-carboxylic acid, 1,2, -benzenediol,3,5-bis(1,1-dimethylethyl), Benzo[h]quinolone,2,4-dimethyl- and 2,4,6-cycloheptatrien-1-one, 3,5, bis-trimethylsilyl-. The retention time and concentrations of these compounds eluted from the crude extract as well as their percent resemblance or correlation to compounds as enlisted in the search library (NIST.14. L) are presented in Table 4.

Discussion

The study demonstrates that the rhizosphere of lemon grass induces several species of actinomycetes belonging to different genera including *Streptomyces*, *Nocardia,* and *Micromonospora*. It has been reported that each plant influences the kind and population of actinomycetes occurring in its roots through its exudates and slough-off root materials (Khamna *et al.,* 2009; Alimuddin *et al*., 2011). Previous studies have also shown that the diversity of actinomycetes in rhizosphere soils correlates positively with the level of organic matter or root exudates. (Sapkota *et al.*, 2020). Probably, this accounts for the dominance of *Streptomyces* spp. among the various genera of actinomycetes seen as indigenous to the rhizosphere of lemongrass.

Table 4. Antifungal compounds eluted from crude extracts of various *Streptomyces* spp. fermentation broth

S/N	Antifungal compound	Retention time (minutes)	% Correlation with compound in the library	Concentration (mg/L)	Peak height
1	Benzylaldehyde,2-nitro-, diaminomethylidene hydrazine	7.954	68.15	4.16	1229
2	[1,2,4] triazolo [1,5] pyrimidine -6-carboxylic acid	10.686	66.61	2.73	1506
3	1,2, -benzenediol,3,5-bis(1,1-dimethylethyl)	12.984	71.84	6.20	2783
4	Benzo[h]quinolone,2,4-dimethyl-	14.555	79.89	6.89	2931
5	2,4,6-cycloheptatrien-1-one, 3,5, bis-trimethylsilyl-	15.504	95.53	8.24	6460

There were marked differences in the capabilities of the actinomycete isolates in suppressing the growth of the various test fungi, as only 19 (31.7%) of the 60 actinomycetes isolates screened, demonstrated fungi growth inhibition. This suggests strongly, that all actinomycetes are at variance in the possession of machinery(ies) necessary for fungal inhibition and hence may not play similar roles in their ecological niches. Similar observations have been made by Khamna *et al*. (2009), Balagurunathan *et al.* (2010), Janardhan *et al.* (2014), and Gomes *et al.* (2018).

Among the 19 isolates that were able to suppress fungi growth, 17 were *Streptomyces* spp. Nanjwade *et al.,* 2010, asserted that *Streptomyces* alone account for the production of over 60% of known antibiotic substances produced by the actinomycetes. Similar remarks have also been documented hitherto by other authors (Hayakawa, 2008; Alimuddin *et al*., 2011; Chaudhary *et al*., 2013; Gomes *et al*., 2018).

The inhibitory activity of the 19 actinomycete isolates (*Streptomyces* spp. and *Nocardia* spp.) as noticed, may be a result of the production of one or more metabolites. Such metabolites could be capable of any of the following: degrading fungi chitin, dissociation of cell membrane/nuclear material, and inhibition of protein synthesis (Velickovic *et al*., 2012; Govender *et al*., 2016; Sulaiman *et al*., 2020). In addition, several authors have demonstrated that various strains of *Streptomyces* inhibit the development of fungi pathogens by producing degradative enzymes (Khamna *et al.,* 2009; Kun *et al.,* 2012; Omran, 2012; Mohite, 2013; Janardhan *et al.,* 2014; Sapkota *et al*., 2020). Some or all these mechanisms may have led to the fungal growth suppressiveness displayed by the various actinomycetes isolates studied.

Furthermore, results obtained in this study revealed that 5 *Streptomyces* isolates designated as IS3, IS5, IS7, IS9, and IS11 possessed relatively equal and the widest spectrum of inhibition. The similarity, in the spectrum of their inhibitory activity, is probably due to the production of the same bioactive compounds which included benzylaldehyde,2-nitro-diaminomethylidene hydrazone, 2,4,6-cycloheptatrien-1-one3,5, bis-trimethylsilyl-and [1,2,4] triazolo [1,5] pyrimidine-6-carboxylic acid among others. Benzylaldehyde derivatives had been shown to possess antifungal activity against *A. flavus, A. niger,* and *Candida albicans* by disruption of the cell wall, shrinking of cytoplasmic size, coagulation of cell proteins and precipitation of mineral crystals (Alamri *et al*., 2012; Ullah *et al*., 2015). However, the mechanism of action of 2-nitro-, diaminomethylidene hydrazone is by the inhibition of enzyme function (Popiolek, 2017; Kratky *et al*., 2021). Also, there has been a report on the inhibition of *in vitro* spore germination and mycelial growth in *Botrytis cinearea* and *A. alternata* by troponoids such as 2,4,6-cycloheptatrien-1-one 3,5-bis-trimethylsilyl (Fallik and Grinberg, 1992; Saniewski *et al*., 2014). The triazole derivative ([1,2,4] triazolo [1,5] pyrimidine-6-carboxylic acid) as was characterized in the extract assayed in this study, may have contributed to the inhibition of mycelial growth of the various test fungi. Recently, the inhibitory effects of chemically synthesized 1,2,4 triazoles to various fungi including *A. fumigatus and C. albicans* was reported (Kazeminejad *et al*., 2022).

The five bioactive compounds identified in the crude extract (Benzylaldehyde,2-nitro-diaminomethylidene hydrazine, [1,2,4]triazolo [1,5] pyrimidine -6-carboxylic acid, 1,2,-benzenediol,3,5-bis(1,1-dimethylethyl), Benzo[h]quinolone,2,4-dimethyl- and 2,4,6-cycloheptatrien-1-one, 3,5,bis-trimethylsilyl-) to which the antifungal property of the *Streptomyces* spp. isolated in this study are likely linked, and have all been previously implicated as potent compounds against fungal mycelial growth (Antoci *et* al., 2021; Devi *et al*., 2021; Ojeda-Hernandez *et* al., 2023). However, these compounds have only been chemically synthesized or isolated from either plants or rare actinobacteria. Therefore, the production of these antifungal compounds by strains of Streptomyces which can be easily grown in the laboratory, is quite promising.

Conclusion

Lemon grass rhizosphere has been herein, demonstrated as a rich reservoir of diverse actinomycetes with great potential for use in the biocontrol of plant fungi pathogens. This is quite critical to being a readily available source of cheap and ‘’green’’ alternatives in the management of crop health and sustainability of agroecosystems with an overall improvement in global food supply. Finally, this study pave the way for the determination of the conditions for optimized production of the antimycotic compounds produced by the strains of these Streptomyces as well as the further extraction, purification, and commercialization of the products.

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