International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Antifungal Properties of *Streptomyces* sp. against Aflatoxin Producing *Aspergillus* Species

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Abstract: ***The streptomyces tendae isolated from all five soil samples obtained from different locations of Gaya district exhibited antifungal activity against aflatoxin producing Aspergillus flavus and Aspergillus parasiticus, when examined after application of cell-free supernatant of Streptomyces sp. The cell-free supernatant concentrioin of Streptomyces tendae from 0.5% to 20% gradually increased the inhibitory action against both types of aflatoxin producing fungal species examined during present research work. Inhibition of fungal growth was achieved at optimum level after application of 20% concentration of cell-free supernatant of this bacteria. However, cell-free supernatant of this bacteria remained most effective against Aspergillus flavus as compared to Aspergillus parariticus. Hexa hydro 3-3 methyl propyle-2, H-4, 4-diazepin remained most effective against both types of aflatoxin producing species of Aspergillus at 26-27 µg/ml as minimum inhibitory concentration.4-(4 hydroxy pheny)-2-butanon remain least effective against both types of aflatoxin producing species of Aspergillus because minimum inhibitory concentration of this bioactive compound was observed as 92-89 µg/ml. However, all seven types of secondary metabolites showed inhibitory action against Aspergillus flavus and Aspergillus parariticus.***

Keywords: Antifungal, Streptomyces sp., Aflatoxin, Aspergillus flavus, Aspergillus parasiticus

1. Introduction

Presently, *Streptomyces* have been considered as safe alternative to chemical fungicides. One of the Actinomycetes such as *Streptomyces* produces bioactive secondary metabolites with different biological activitives including anti-fungal nature (Solecka, 2012). Thus, isolation of effective fungicides from new strains of *Streptomyces* is going on around the world, to reduce and control different fungal diseases (Donadio *et al*.2002).

Streptomyces sp. produces extra-cellular enzymes such as citrase, catalase and oxidase *Streptomyces* sp. hydrolyze lipid, gelatin, cellulose, starch, esculin, urea etc. (Sharma and Manhas, 2020). The actinomyces are known for production of useful antibiotics in the world as well as *Streptomyces* remain as major producers of these antibiotics (Nett *et al*., 2009). These microbes are most important natural resources showing effective biological activity against a wide range of pathogenic bacteria and fungi. These *Streptomyces* sp. naturally produces bioactive compounds to defend other microbes. The secondary metabolites extracellulary produced by *Streptomyces* are usually not required for their growth and development.

Thus, it is most suitable time to turn on eye to the microflora of the soil of Gaya district to find antagonistic *Streptomyces* sp. and metabolites active against aflatoxin producing fungi.

2. Materials and Method

Soil samples were collected from five locations of Gaya district and transported to laboratory for further experimentation. *Streptomyces* sp. was isolated and identified on the basis of morphological, physico-chemical and genomic characteristics, as *Streptomyces tendae.* The antifungal properties of cell-free supernatant and isolated antifungal secondary metabolites of *Streptomyces* sp. were examined against two aflatoxin producing fungi such as *Aspergillus flavus* and *Aspergillus parasiticus.* Both fungal species were cultured on PDA medium separately. Antifungal activity of 0.0%, 0.5%, 1.0%, 5.0%, 7.5%, 10.0% and 20.0% cell free supernatant of *Streptomyces* were applied on fungal colonies and percent inhibition of mycelial growth was observed. The MIC (µg/ml) value of all seven types of anti fungal secondary metabolites was also calculated against both species of aflatoxin producing fungi separately. Data obtained are presented in Tables-1 and 2.

The antifungal activity of the crude compounds (cell free supernatant) was tested using disc diffusion method. The filter paper discs of 6 mm in diameter containing different concentrations of compounds were placed on test fungal cultures. The anti fungal activity was observed by measuring the zone of inhibition (in mm) after incubation at 37°C temperature. The minimum inhibitory concentration of cell free supernatant was observed as 0.5%. At this concentration 13.68% and 15.57% inhibition of the mycelia growth of aflatoxin producing *A. flavus* and *A. parasiticus* were observed respectively during present research work.20% concentration of cell free supernatant of *Streptomyces tendae* was regarded as minimum fungicidal concentration because 94.10% and 85.68% inhibition of mycelia growth were observed for *A. flavus* and *A. parasiticus* during present research work (Table-1).

Table 1: Antifungal activity of cell-free supernatant of *Streptomyces* sp. as Minimum Inhibitory Concentration (% inhibition of fungal mycelia growth)

Sl. No.	Concentration of cell-free supernatant	Aflatoxin Producing Fungi	
		Aspergillus flavus	*Aspergillus parasiticus*
1.	0.00 (Control)	0.00	0.00
2.	0.5%	13.68+0.14	15.57+0.00
3.	1.0%	31.30+0.11	34.83+0.10
4.	5.0%	45.46+0.09	40.18+0.09
5.	7.5%	61.80+0.10	49.72+0.13
6.	10.0%	73.14+0.16	70.11+0.19
7.	20.0%	94.10+0.20	85.68+0.16

Each value is the mean of 3 replicates with + SD

Table 2: Effectivity of isolated and purified secondary metabolites produced by *Streptomyces* sp. on *Aspergillus* sp. (MIC→µg/ml)

Sl. No.	Anti- fungal compounds	Fungal Species	
		A. flavus	*A. parasiticus*
1.	Acetic Acid Phenylester	48	83
2.	1-H-indole-3-Carboxylic acid	41	22
3.	2-Dihydroxy-5 methyl benzaldehyde	45	20
4.	Hexadecoic Acid (methyl ester)	30	36
5.	Hexahydro-3-3-methyl propyl-2, H-1, 4-diazepin	26	27
6.	4-(4-hydroxy phenyl)-2-butanon	92	89
7.	Octa-deconoic Acid	88	61

MIC value for crude compounds isolated from *Streptomyces* was observed by dilution method. The minimum inhibitory concentration was recorded as the lowest concentration of the crude extract that inhibited the visible growth of fungal members.

The lowest minimum inhibitory concentration of hexahydro-3-2-methyl propyl-2, H-1, 4, diazepin as 26 µg/ml was observed among other secondary metabolites examined against *Aspergillus flavus.* The lowest minimum inhibitory concentration of 2-dihydroxy-5 methyl benzaldehyde as 20 µg/ml was recorded among other secondary metabolites produced by *Streptomyces tendae* against *A. parasiticus*. The minimum inhibitory concentration of all seven anti-fungal secondary metabolites against *Aspergillus flavus* was observed in range of 26-92 µg/ml and against *Aspergillus parasiticus* in range of 20-89 µg/ml. Thus it became evident that all seven antifungal secondary metabolities obtained from *Streptomyces tendae* individually showed anti-fungal antagonistic activity against both aflatoxin producing fungal species examined during present research work (Table-2).

Sharma and Manhas (2020) observed that increase in concentration of cell free supernatant of *Streptomyces* M4 increases the rate of inhibition of fungal growth and thus fungal biomass decreases. They observed that upto 95.09% inhibition of fungal growth taken place at a concentration of 20% (V/V) cell free supernatant of *Streptomyces* sp. They observed different types of morphological abnormalities in fungal colony near the zone of inhibition such as inhibition of sporulation, thinning of hyphae, discolouration, granular cytoplasm as well as leakage of cellular material. They also observed bulbous structure of the spores of *Aspergillus* sp. near the zone of inhibition. The results obtained during their research work indicates that both purified antifungal compound and crude extract showed significant anti-fungal impact against tested fungal isolates and MIC values were observed in range of 31.25-100 µg/ml (purified compound) and 50-250 µg/ml (Crude extract).

The results obtained during present research work remain in partial conformity with results obtained by Sharma and Manhas (2020).

Among the seven anti-fungal secondary metabolites isolated from *Streptomyces* sp., 4-(4-hydroxy phenyl)-2-butanon showed strong inhibition against both fungal species examined during present research work. But all seven identified bioactive compounds from crude extract of *Streptomyces* isolates remained active against *Aspergillus flavus* and *Aspergillus niger* (Table-2).1-H-indole-3-carboxylic acid was obtained as light-brown amorphous compound soluble in DMSO.3-dihydroxy-5-methyl benzaldehyde was observed as a pale yellow compound soluble in DMSO.

Kavitha *et al.* (2010) also observed that the secondary metabolite as 4-(4-hydroxy phenyl)-2-butan-2-one isolated from *Streptomyces* sp. TK-VL-333 shows anti microbial activity against pathogeni fungi. Thus the results obtained during present research work remain in conformity with results obtained by Kavitha *et al.* (2010).

Indole-3-carboxylic acid was isolated from *Streptomyces* sp. Act 80115 by Poumale *et al.* (2006). This anti fungal secondary metabolite was also isolated by Motoshashi *et al.* (2008) from *Streptomyces* sp. MS239. El-Raheem *et al.* (2022) isolated Octadeconoic acid, an antifungal bioactive compound from *Streptomyces exfoliatus.* El-Sabbagh *et al.* (2013) also obtained effective antifungal compounds from *Streptomyces exfoliates.*

The antifungal profile of bioactive secondary metabolites in terms of MIC is presented in Table-2, 4-14-hydroxy phenyl)-2-butanon showed good anti-fungal activity against *Aspergillus flavus,* followed by Octa-deconoic acid, Acetic acid phenyl ester; 2-Dihydroxy-5-methyl benzaldeyde, 1-H indole-3-carboxylic and hexa hydro-3-2-methyl propyl-2, H-1, 4-diazepin. The anti fungal metabolite 4-14-hydroxyl phenyl)-2-butanon also showed good activity against *Aspergillus parasiticus* followed by Acetic acid phenyl, ester, Octa-deconoic acid, hexa decoic acid (methyl ester), hexahydro-3-2-methyl propyl-2, H-1, 4-diazepin, 1-4-indole-3-carboxylic acid and 2-dihydroxy-5 methyl benzaldehyde. Thus it became evident that both fungal species remained most sensitive to 4-(4-hydroxyl phenyl)-2-butanon as MIC 92 µg/ml and 86 µg/ml against *A. flavus* and *A. parasiticus* respectively. Octa-deconoic acid and acetic acid phenyl ester showed superior antifungal activity against. *A. flavus* and *A. parasiticus* respectively. Bioactive compounds obtained from *Streptomyces* sp. are products of secondary metabolic processes. Thus the results obtained during present research work shows conformity with results obtained by Poumale *et al.* (2006) and Motoshashi *et al.* (2008).

3. Conclusion

All strains of *Streptomyces tendae* obtained from different soil samples showed anti fungal activity against aflatoxin producing *Aspergillus flavus* and *Aspergillus parasiticus.* When examined after application of cell-free supernatant. Minimum inhibitory concentration of cell-free supernatant remained as 0.5% and inhibited 13.68% and 15.57% growth of the mycelicom of *Aspergillus flavus* and *Aspergillus parasiticus* respectively. The minimum fungicidal concentration remained as 20% at which 94.10% and 85.68% of the mycelial growth of *A. flavus* and *A. parasiticus* were inhibited respectively. However, 20% concentration remained most effective against aflatoxin producing *A. flavus* as compared to *A. parasiticus.* When effectivity of each secondary metabolite against aflatoxin

producing fungi was observed, it became evident that minimum inhibitory concentration of hexahydro-3-2-methyl propyl-2, H-1, 4-diazepin remained lowest (26 µg/ml) as compared to other bioactive compound against *Aspergillus flavus.* But minimum inhibitory concentration of 2-dihydroxy-5-methyl benzaldehyde remained lowest as compared to other secondary metabolites when examined against *Aspergillus parasiticus.* Minimum inhibitory concentration of 4-(4-hydroxy phenyl)-2-butanon remained highest as compared to other secondary metabolites as 92 µg/ml and 86 µg/ml against *Aspergillus flavus* and *Aspergillus parasiticus* respectively.

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