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Biological control of Aspergillus niger and Aspergillus flavus isolated from strawberry (Fragaria x ananassa Duch) by streptomyces sp.

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Abstract
Actinobacteria are major producers of antifungal metabolites especially for use in agriculture biocontrol. In this study, the identification of Streptomyces sp. isolated from saline soil was carried out using morphological and biochemical studies. Optimization the culture conditions to produce metabolite was done. Two fungal samples were isolated from decaying strawberry. Their molecular identity was determined. Effects of Antifungal activity of Streptomyces sp. activities was determined by agar dilution method and inhibitory percentage of growth evaluated. Results showed the strain of actinomyces isolate, strongly belongs to the genus Streptomyces. The highest metabolite wieght (1.4 gram) at 32 ^{0}C, at pH 6.0, an incubation time of 120h under aerobic conditions with culture medium compounds (yeast extract 0.4%, Dextrose 0.4 %, Pepton 0.5%, NaCl 0.5%) happened. Bioactive metabolite Streptomyces sp. effected on two isolates of Aspergilllus (*A. niger* and *A. flavus*) isolate. Concentration of 6 mg / ml of metbolites has 100% growth inhibition, In contrast, mancozeb fungicide had 100% growth inhibition at the same concentration too. Therefore, based on the results achived, metabolite produced by Streptomyces sp. can be used as a biological agent to control the fungi infecting strawberr y and it is a suitable alternative to chemical fungicides.

Keyword: Aspergillus, Biological fungicides, Streptomyces**,** strawberry

Introduction

Strawberry (*Fragaria* x *ananassa* Duch.) belongs to the family Rosaceae (Li,2023) and widely consumed worldwide [14]. The fruit of this plant becomes juicy over time and turns from acidic to sugary. Owing to its physiological characteristics and high respiration rate, strawberry is one of the most sensitive and easily spoilit fruits, and even if there are no spoilage substances in the environment, the metabolic activities of this fruit will start after harvesting [16]. Fungal contamination is a major challenge for the horticultural crops. Fungi are present all over in the environment and can easily infect horticultural products. Due to jusy texture and delicate nature, strawberries are one of the most decayable fruits, with a very limited storage time. Fungal infections are often the main cause of post-harvest erosion in strawberries. Several fungal strains commonly affect strawberries and causing decay and reducing fruit quality [4]. Microscopic filamentous fungi produce large amounts of extracellular enzymes like pectinases and hemicellulases, which are influencing the deterioration of fruits. Contamination of fruits by fungi not only causes high post-harvest waste, but also fruits can also be a source of toxic substances harmful to humans [2]. Common fungal infections species which contaminate strawberries are Aspergillus, Penicillium, Fusarium [14] and Botrytis [4]. These contaminations may not only compromise the organoleptic quality of strawberries, but also their safety, when mycotoxin production happen [14]. Aspergillus is one of the largest and most intensively investigated taxons of fungi. There are more than 180 species in this genus, including *A. fumigatus, A. flavus, A. niger,* and *A. terreu* [6]. Among the fungal species, *Aspergillus flavus* is of concern due to its aflatoxin-producing ability and posing health risks to consumers. *Aspergillus flavus* found all over the place that poses a significant threat to plants as well as humans. This fungi can produces pectinases, which accelerate spoilage and quality loss [14]. *Aspergillus niger* is a wide spread fungus in the world. Observing black mold on the fruits can be the reason for the growth of this fungus [18]. *Aspergillus niger* as a pathogen not only causes remarkalbe losses but also produces a large number of mycotoxins, making it one of the main agent of po-stharvest decay in berries [10]. In recent years, use of biological compounds to control contaminated fungal is notable. Research review revealed that streptomyces have an inherent potentiality to produce antimicrobial agents. Sustainable agriculture is increasingly related to biological agants as as alternatives to chemicals [19]. Soil bacteria have received major notice due to their extreme population diversity and production of a variety antifungal compounds. They mostly belong to the genera Streptomyces [1]. Streptomyces is the largest genus of Actinomycetota. Actinomycetes are the principal source of antifungal agents. The antagonistic activity of actinomycetes is used for the bio-control of fungal infections in herbs [7]. Streptomyces produces approximately 75-80% of the total antibiotics produced by microbes, such as nystatin, amphotericin B, natamycin, bafilomycin A_1, concanamycin, and 3-phenylpropionic acid [1]. The genus Streptomyces is famous for being a never-ending source of bioactive secondary metabolites belonging to various chemical classes such as

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alkaloids, aminoglycosides, terpenoids, glycopeptides, tetracyclines, polyketides, β-lactams, macrolides, and others [3]. According to what has been stated, in this study,we evaluate the antifungal potential of metabolites obtained from Streptomyces spp against *A. niger* and *A. flavus* isolated from strawberries In the following, the results obtained from Streptomyces metabolite as a biological compound have been compared with mancozeb poison as a chemical compound.

Results Discussion

In this study, two fungal strains, *Aspergillus niger* and *Aspergillus flavus*, were isolated from decaying strawberry fruits. Macroscopic and microscopic characteristics of fungal isolates were examined "Figure 1".

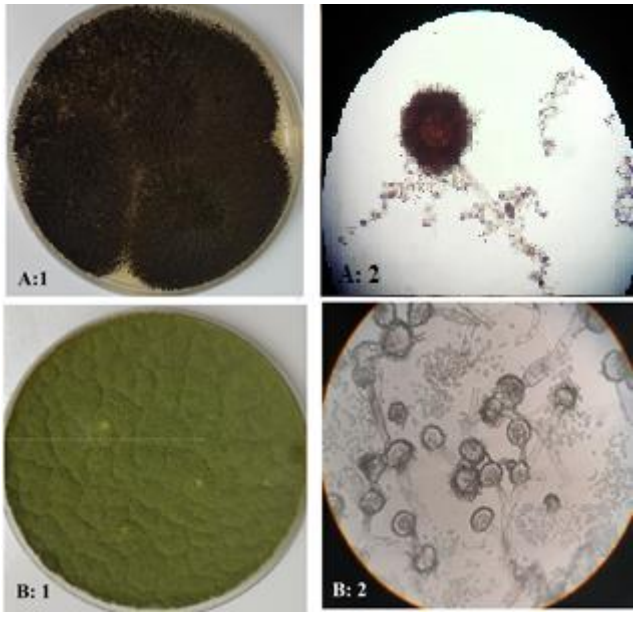

Figure (1) A:1 and A:2 Macroscopicac and microscopic picture of *A. niger*, B:1 and B:2 Macroscopicac and microscopic picture of *A. flavus*

To identify molecular identity, polymerase chain reaction (PCR) using ITS primers was done [15]. Based on ITS gene sequence analysis, the closest phylogenetic neighbour of isolates were *Aspergillus niger* "Figure 2" and *Aspergillus flavus* "Figure 3"

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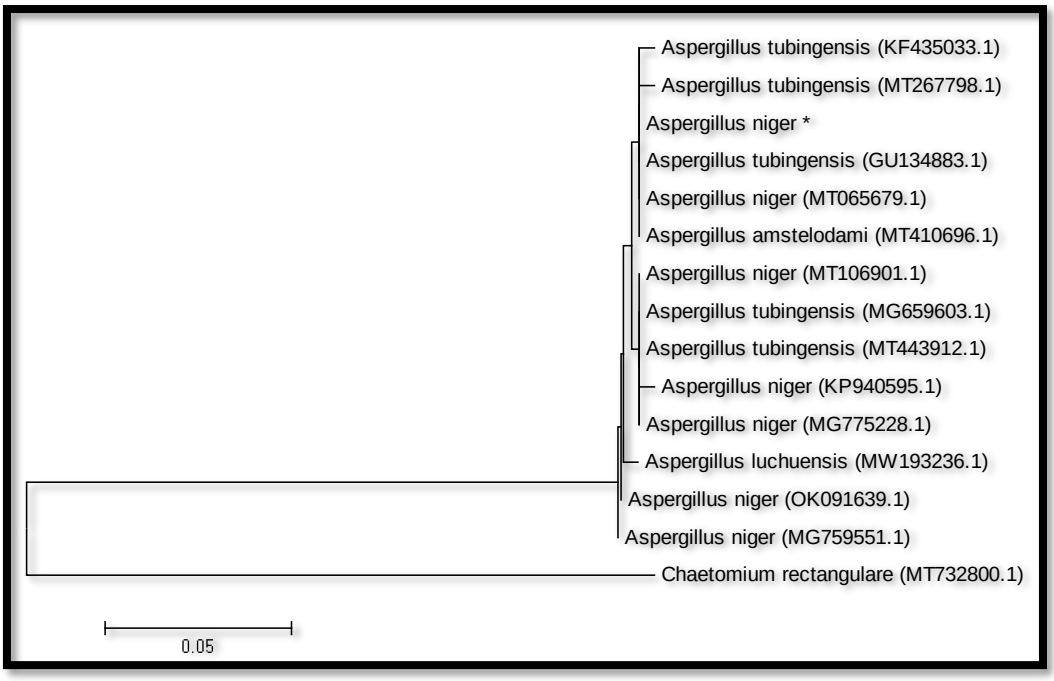

Figur (2) Phylogeny tree of *Aspergillus niger* isolates

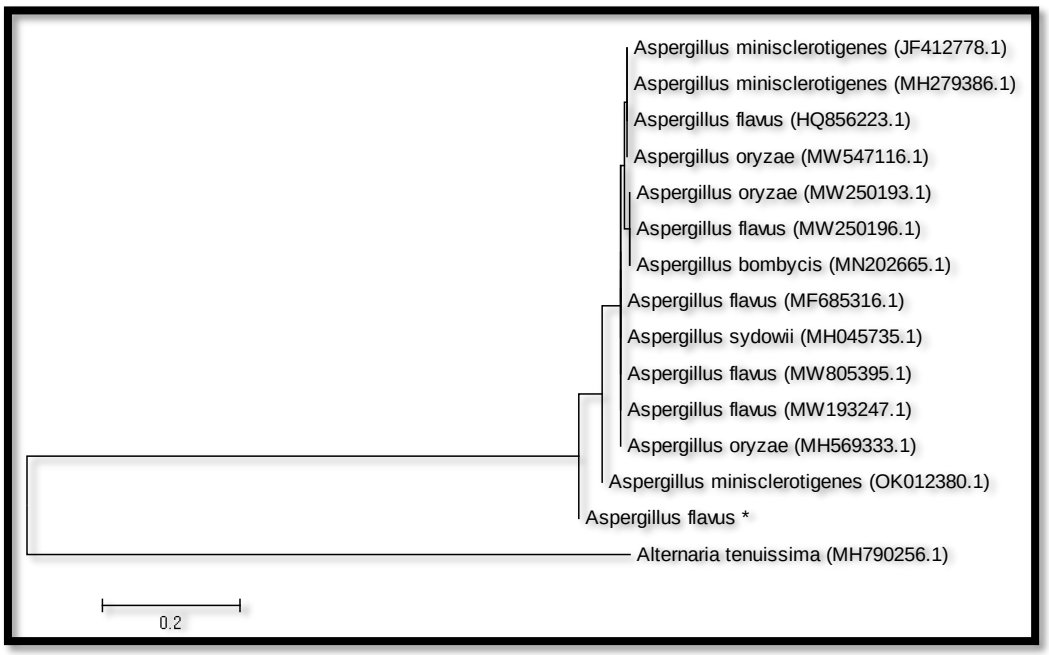

Figure (3) Phylogeny tree of *Aspergillus flavus* isolates

In the current study, an aerobic, Gram-positive, acid-fast negative (Figure 4) and non-motile actinomycete was isolated from a salty soil sample collected in the Kerman,Iran. Morphological and biochemical characteristics showed a high similarity to the genus Streptomyces.

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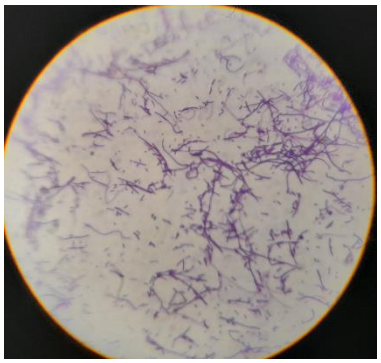

Figure (4) Acid-fast staining of streptomyces isolate

Optimazation of growth condition for maximum production of metabolite by Streptomyces Sp. were examined. Testing different solutions to optimal biomass and metabolite product, the streptomyces Sp, exhibited maximum metabolite wieght (1.4 gram) at 32 ^{0}C, at pH 6.0, an incubation time of 120h under aerobic conditions with culture medium compounds (yeast extract 0.4%, Dextrose 0.4 %, Pepton 0.5%, NaCl 0.5%). Almost Similar results were obtained in the study of Oskay (2011) in the effects of environmental conditions on biomass production by Streptomyces Sp. Which showed the highest antimicrobial activities under aerobic conditions at temperature 30°C and pH at 7.5. Kavitha and Vijayalakshmi (2009) in the determination of the effect of various cultural parameters on the metabolite activity of *Nocardia levis*, found medium containing 2% sucrose supported high levels of biomass and bioactive metabolite production by the strain. In this research, antifungal test of metabilite was investigated by agar dillution method. The concentration used for test were 6 mg/ml, 3 mg/ml and 2 mg/ml. Then, the inhibitory percentage of growth was evaluated by the following formula [11]. Finally, The results of test compared with Moncozeb chemical fungicide which used in agriculture.

I= 100 (C-T)/C
I: Inhibitory percentage of growth
C: Diameter of control colony
T: Diameter of treartment colony

The test results showed metabolite of streptomyces Sp. has the ability for the inhibition of *A. niger* and *A. flavus* growth in 6 mg /ml concentration "Table 1" and "Table 2". Both metabolite (biological antifungal) and mancozeb (chemical fungicide) at concentration of 1 mg /ml had no antifungal effect on any Aspergillus isolates.

Table 1- Percentage inhibition of *A. niger* growth

	Inhibitory percentage of growth		
	6 mg /ml (Concentration)	**3 mg /ml (Concentration)**	**1 mg /ml (Concentration)**
Metabolite	**100 %**	**0**	**0**
Mancozeb	**100 %**	**50 %**	**0**

Table 2- Percentage inhibition of *A. flavus* growth

	Inhibitory percentage of growth		
	6 mg /ml (Concentration)	**3 mg /ml (Concentration)**	**1 mg /ml (Concentration)**
Metabolite	**100 %**	**0**	**0**
Mancozeb	**100 %**	**67 %**	**0**

Related studies have confirmed that many biological factors have been found to control fungi causing spoilage of agricultural products. Giacomelli Ribeiro (2024) confirmed actinobacteria from genus *Amycolatopsis*, *Curtobacterium*, *Kocuria*, *Nocardioides*, *Nocardiopsis*, *Saccharopolyspora*, *Streptoverticillium* and especially *Streptomyces* showed a broad antifungal spectrum through several antibiosis mechanisms such as the production of natural antifungal compounds, siderophores, extracellular hydrolytic enzymes and activation of plant defense system. Park (2024) founad a strain of Streptomyces with remarkable antifungal activity against multiple phytopathogenic fungi. This strain not only inhibited seven phytopathogenic fungi including *Fusarium oxysporum* and *Aspergillus niger* and but also showed a control effect against *F. oxysporum* infected red pepper, strawberry, and tomato in the in vivo test. Shen (2024) worked on antifungal bacterium,*Streptomyces graminearus* STR-1, which showing antagonistic activity to diverse fungal pathogens including *Magnaporthe oryzae*, *Rhizoctonia solani*, *Fusarium graminearum*, *Ustilaginoidea virens*, and Bipolaris maydis. Its antifungal activity was relatively stable and less affected by temperature and pH.

Conclusions

Postharvest decay is one of the main factors that determine losses and reduce food quality of strawberry. Aspergillus is a group of fungi that easily grow on strawberries and cause them to spoil quickly. Chemical fungicides resulted in environmental pollution, expanded risk of health problems, development of pathogenic fungi resistance, reduce soil quality, and produced hazardous agricultural products. It is evident from the results of this investigation that bioactive metabolite *Streptomyces* sp. effected on two isolates of Aspergilllus (A. niger and A. flavus) isolated from strawberries. Concentration of 6 mg / ml of metbolites has 100% growth inhibition. In contrast, mancozeb fungicide had 100% growth inhibition at the same concentration too.Based on what was obtained from the results of this research, it seems logical to use the metabolite *streptomyces* sp. as a biological agent to control and eliminate fungi that could corruption.

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