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RESEARCH ARTICLE

Isolation of Mycotoxin producing *Aspergillus Niger* from soil and its application as Pesticide

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ABSTRACT

Fungi associated with mycotoxin production are found worldwide and may produce toxins in almost any food source that will support their growth. Mycotoxins are the toxic secondary metabolite of the fungus kingdom and they can cause disease and death in both humans and other animals. In this study, we isolated the filamentous fungus *Aspergillus niger* from the soil and produced its mycotoxins. By using thin layer chromatography (TLC) the crude mycotoxins were characterized and used for testing their insecticidal and weed controller activity. The insecticidal activity tested by spraying mechanisms and pests are dead in a limited period of time and mycotoxins also showed good herbicide activity in selected weeds. The outcome of the present study demonstrates that extracted mycotoxins from *Aspergillus niger* can be used as a pesticide in agricultural fields.

Keywords: Filamentous fungi, *Aspergillus niger*, mycotoxins, microbial biopesticide.

1. INTRODUCTION

Fungi are able to producing secondary metabolites, they vary in production, function, and specific to a particular fungus [4]. The secondary metabolite mycotoxin's natural functions are unknown but they play a pivotal role in chemical defense and communication [5]. The production of a range of secondary metabolites depends on the physical and biological changes in the environment [7]. Fungal species have the potential to produce different secondary metabolites [8,9]. The Black Aspergilli, *Aspergillus niger* includes 27 species distributed worldwide, which are dark colonies and uniseriate or biseriate conidial heads [1]. *A. niger* species produce important mycotoxin ochratoxin A (OTA) found in foodstuffs, mainly in cereals and nuts products [2]. *Aspergillus niger* produces a very large number of secondary metabolites however, only a small number of fungal strains have the ability to produce this toxin [9] which includes isoflavones they are actually plant metabolites [10,11] and many volatiles and small organic acids [14-16]. *A. niger* also produce ochratoxin, fumonisin, and oxalic acid [12]. *A. niger* species contains a complete cluster of genes involved in OTA production [2,3]. Ochratoxins are the mycotoxins produced mainly by some *Aspergillus* (eg. *A. ochraceus*) and *Penicillium* (eg. *P. verrucosum*) species in different agricultural crops such as cereal grains, peanuts, dried beans, and coffee beans [13].

A variety of microbial pesticides, also known as biochemicals generated from microorganisms, are classified as biopesticides. It is advantageous to use plant pathogens for biological weed control in agroecosystems because they are a practical, safe, and environmentally friendly weed management technique. They have also been shown to be a workable, if small, part of contemporary integrated weed management systems [6]. Fungi are a significant source of food and natural products with diverse chemical structures and activities and apparently, the majority of fungi inhabiting the world have not yet been described. The main aim of the present study lies in the investigation of mycotoxins, which are isolated from soil filamentous fungi and their inhibitory activity against selected pests and insects.

2. MATERIALS AND METHODS

2.1. Fungal cultures and production media for mycotoxins

Black filamentous fungi, *Aspergillus niger* was newly isolated from soil near the Amaranthus garden and further evaluated in terms of macro and micro morphological features for confirmation before proceeding to the experimental protocols. Pure cultures were maintained on potato dextrose

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agar (PDB). *A. niger* was used for the production of ochratoxin. Specific production media used for the production of mycotoxin was by using sterile chopped corn wastes in conical flasks. Fungal spore suspensions of concentration 3x108/ml were inoculated to this specific media in conical flasks embedded with streptomycin to prevent the growth of bacteria. The cultures were then incubated for over a period of 21 days in an orbital shaker at 26ºC and 150 rpm.

2.2. Extraction of crude mycotoxins and characterization through thin-layer chromatography

Fungal cultures incubated for 21 days are subjected to vacuum pump filtration. Further, the filtrate solvent was extracted with ethyl acetate and chloroform in a ratio of 1:1. After evaporation, the residue was dissolved in DMSO at a concentration of 100µg/ml and characterized by using thin layer chromatography (TLC) using silica and specific eluents.

2.3. Characterization of crude mycotoxins by TLC

Thin glass plates were coated with a slurry of adsorbent silica and water in a ratio of 1:2 with 0.25 mm thickness. The TLC plates were developed in a chamber containing toluene: ethyl acetate: formic acid (30:6:0.5). The crude mycotoxin samples were applied as spots along with standards on the glass plates and placed in a chamber having solvent. TLC was performed till the solvent reached the top of the plates which were then removed and observed under UV Transilluminator for characterization of purified mycotoxins [21].

2.4. Collection of pests and testing the insecticidal activity of mycotoxin

Pests were randomly collected from Amaranthus garden such as *Epilachna ocellata, Olepa ricini, Aulacophora foveicollis, Nezara viridula, Acanthocoris scabrator, Epilachna vigintioctopunctata, Macrosiphum euphorbiae,* and *Antoba olivacea.* Tested its insecticidal activity by observing the time taken for the mortality of each pest upon spraying the mycotoxin in different concentrations in laboratory conditions.

2.5. Collection of weeds and testing the pesticide activity of mycotoxin

Commonly found weeds such as *Cynodon dactylon* and *Isachne miliacea* are collected from paddy fields with the help of an expert farmer and cultivated in lab conditions for one week. Mycotoxins solutions were sprayed to the test plants, and non-sprayed to the control plants. For each weed replicates were maintained and kept in this setup for 20 days and observe the number of days taken for wilting of weeds.

3. RESULTS

TLC analysis aided in the characterization of the mycotoxins isolated. Rf values of crude mycotoxins were calculated and compared with standards. The values obtained were nearly the same when compared to standards and represented a high degree of purity (Table 1).

Mycotoxins show good effectivity against pests such as *Epilachna ocellata, Aulacophora foveicollis, Macrosiphum euphorbiae,* and *Antoba olivacea* even under low concentrations of mycotoxin and all pests die upon the spraying at different concentrations (Table 2).

Mycotoxins show good effectivity against pests such as *Epilachna ocellata, Aulacophora foveicollis, Macrosiphum euphorbiae,* and *Antoba olivacea* upon spraying at different concentrations (Figure 2).

Both the test weeds are wilted in a few days, on continuous spraying of mycotoxin of different concentrations (Figure 3).

4. DISCUSSION

Mycotoxins are toxic metabolites characterized by small molecular weight, diverse toxicological activity, and unrelated chemical structure. Black aspergilli have been isolated as pathogens in onions, cereals maize, nuts, and other food crops, where they are responsible for the pre- and postharvest spoilage of many fruits and vegetables. The ochratoxins form a group of chemically related mycotoxins widely identified as a contaminant of cereal crops, which can serve as a major source of contamination for humans and animals [22].

Figure 1. Graphical representation of mycotoxin production

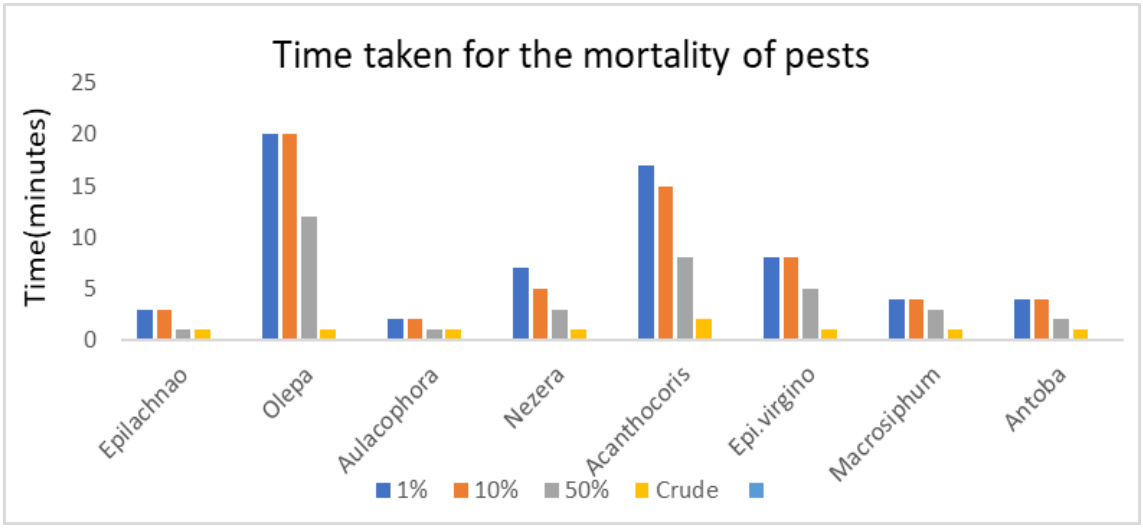

Figure 2. Time taken for the mortality of pests under spraying of mycotoxins at different concentrations

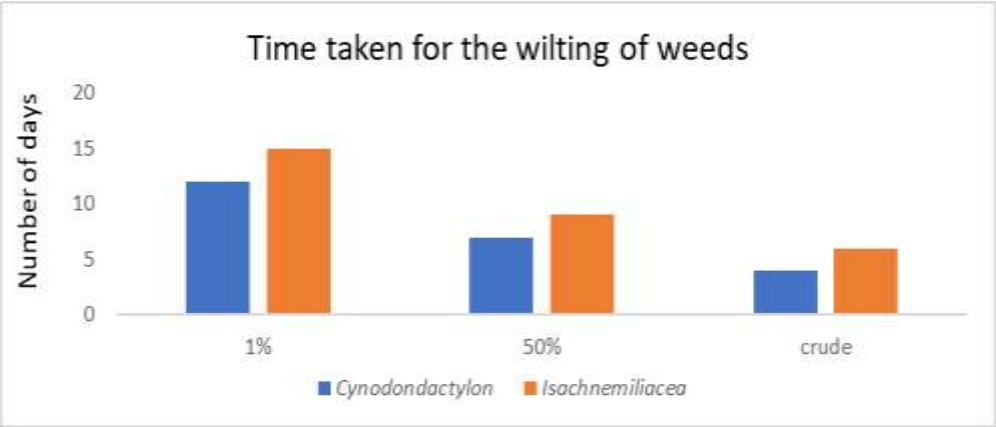

Figure 3. Number of days taken for the wilting of weeds

Table 1. Rf values of crude and standard samples of mycotoxins

S. No.	Name of the mycotoxins	Rf value of standards	Rf values of crude sample
1	Aflatoxins	0.57	0.83 ± 0.3
2	Patulin	0.32	0.83 ± 0.3
3	Ochratoxins	0.89	0.83 ± 0.3

Values are given as *Mean±SD

Table 2. Time is taken for the mortality of pests in different concentrations

S.No.	Name of Pests	Concentration of mycotoxin			
		1%	10%	50%	Crude
1	*Epilachnao*	3 mts	3 mts	1 mts	>1 mts
2	*Olepa*	20 mts	20 mts	12 mts	>3 mts
3	*Aulacophora*	2 mts	2 mts	>1 mts	>1 mts
4	*Nezera*	7 mts	5 mts	3 mts	>1 mts
5	*Acanthocoris*	17 mts	15 mts	8 mts	>2 mts
6	*Epi.virgino*	8 mts	8 mts	5 mts	>3 mts
7	*Macrosiphum*	4 mts	4 mts	3 mts	>3 mts
8	*Antoba*	4 mts	4 mts	2 mts	1 mts

Table 3. Time is taken for the mortality of pests in different concentrations

NAME OF WEED	Number of days		
	1%	50%	Crude
Cynodon dactylon	12	7	4
Isachne miliacea	15	9	6

Several studies were reported previously about the poisonous and *hazards* of mycotoxin in food and animals. According to Dohlman the different perceptions of tolerable health risks of mycotoxins at the global level, mainly associated with the level of development and susceptibility of crops to contamination in different countries, have led to a lack of consensus about standards for regulated mycotoxins in food and feed [18]. Adeye postulates that the main source of ochratoxin A in cereal grains, primarily those grown in northern temperate areas. Ochratoxin A (OTA) is suspected as a chief etiological agent responsible for human Balkan Endemic Nephropathy (BEN) [17]. Alcaide-Molina reported that mycotoxins including trichothecenes, fumonisins, ochratoxins, and zearalenone are the broad and major toxins that occur in food grains and other important agricultural commodities, such as peanuts. However here we isolated *A. niger* species from soil and produced mycotoxins by a very cheap method and used those mycotoxins for further studies [19]. Despite of there being no studies about mycotoxins as a pesticide till now, here we used this poisonous effect of mycotoxins in a useful way. Here we used the poisonous effect of mycotoxins against some selected pests and common weeds as pesticides. It also showed effective results. We might conclude on the basis of the evidence obtained from the different analyses carried out in this work the filamentous fungi *A. niger* isolated from soil and their potential ability to produce mycotoxins, particularly ochratoxin and it shows an effective pesticide nature. Hence the present study suggests that these metabolites and fungal strains can be further utilized for biotechnological applications in industry and agriculture.

5. CONCLUSION

Based on our studies, *A. niger* isolated from soil could produce mycotoxins, which contain ochratoxin as mycotoxins and can be used as a potential insecticide as well as a weed controller. To reduce the use of chemical insecticides, we can use bioinsecticides. However, when we used mycotoxins in agricultural fields against pests, there is a chance to get excessive accumulation in the environment. Fortunately, significant progress has been made in identifying microorganisms and microbial enzymes that can effectively degrade mycotoxins in an environmentally-friendly manner, which offers hope for solving the problem of such accumulation.

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