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Research Article

Use Powder of Earthworm to Increase Growth and Spores Numbers of Two Species of Fungi (*Aspergillus niger* & *Pencillium expansum*)

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ABSTRACT

The present study prepars various concentrations of dried earthworm powder from (200 – 250 – 300 – 350 – 400 – 450 - 500) mg / ml used to increase the growth of *Aspergillus niger*&*Pencillium expansum* and the spore number , the result show the growth of *A.niger* and the spore number more than *P. expansum*in all concentrations along the period of experiment and analysis show differences significant (p< 0.05) among the different concentrations of earthworm powder.

Keyword: Powder of earthworm, Earthworm, Fungi.

INTRODUCTION

Earthworm play functional role in the soil ecosystem, increase the soil fertility so the referred a farmer friend and considered protein source[1]. Earthworm powder used to food fish because contain high percentage of protein,Powder of earth worm important to health of human because have large amounts of zinc, iron, manganese and copper[2]. Earthworm utilized as a part of pharmaceutical for different cures since 1340 AD and utilized conventional drug as calming, pain relieving and antipyretic operator. Earthworms are long living beings in a situation rich with smaller scale creatures, parasites and other potential pathogens, and manage of against microbial movement. Earthworm powder utilized as a hostile to coagulation impact and unwinding impact for the vascular framework as a thrombotic treatment[3,4]. Its tonic properties makes it valuable help for the liver and other organ systems[5] *Aspergillus niger* use in the sustenance business for the generation of numerous compounds, for example, cellulases, amylase, pectinases, lactase , and acid proteases[6,7]. Likewise, the yearly creation of citric acid by fermentation is currently about 350,000 tons[8]. *Pencillium expansum*, the most well-known foodborne growths on apple natural product, is a standout amongst the most considered species in the variety since it is a reliable maker of poisonous metabolites particularly patulin. *Pencillium expansum* is accounted for to create no less than 50 distinctive auxiliary metabolites, for example, citrinin, roquefortine C, ochratoxin A, penitrem A, PR-poison, chaetoglobosins A and C[9].

MATERIAL AND METHODS

Collection the samples and preparing powder of earthworm

Earthworm were collected from house garden in Alsilaykh region (north of Baghdad) digging the soil and take the worm to the laboratory washed them in tap water to remove dirt from the body surface. The earthworms were soaked in distilled water for 6-8 hours to allow the soil in its tract to be excreted, then earthworms were washed with distilled water and collected in Petridish that kept in an –incubator for 24h. at 55C° after that removed and pounded to make it into powder , stored in refrigerator of normal temperature[10] then used eight concentrations of powder to see any one good to increase the growth and calculated the spore numbers of *A. niger* and *P.expansum*.

Preparing spore suspension

The method used slant containing PDA medium were inoculated with isolate of *A.niga*,*P. oxysporium* then each slant was incubate of 30 for 7 days and kept at 4 C^0 in refrigerator spore suspension was prepared 5 ml of sterilized distilled water (containing 0.1% tween80) to aid wetting and separation of spores, the suspension was filtered through sterile cotton wool . The filtrate was centrifuged at 300 rpm for 5 minutes. The supernatant was removed and the spore washed twice by re suspending in sterile distilled water and further centrifuged then 5 ml of sterile distilled water was added to the precipitate and mixed one drop of suspension was added to the haemocytometer by pastor pipette. Number of spore calculated under high power (40X)using the following equation Concentration of spores=Z*4*^{4}10

Determination fungal activity

the agar well diffusion methods modified, used in the drilling to observed the effect powder earthworm on the growth fungi *Aspergillus niger & Pencillium expansum* Vaccinate as the center of (PDA) by sterile swab from fungi stuck working digging by pastor pipette, then transferred concentrations Preperated (200 – 250 – 300 –

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Table 1: Effect of earthworm extract on SporsNumber [x 10^4] of *A.niger* after second and fourth day.

Concentration	Mean ± SD		LSD value
	2 day	4 day	
Control	0.00 ± 0.00	2.15 ± 0.15	0.461 *
200	3.55 ± 0.05	6.10 ± 0.10	0.326 *
250	4.10 ± 0.10	6.60 ± 0.10	0.409 *
300	5.15 ± 0.15	8.05 ± 0.05	0.228 *
350	6.55 ± 0.05	9.95 ± 0.05	0.271 *
400	7.95 ± 0.05	11.15 ± 0.15	0.297 *
450	9.15 ± 0.15	12.25 ± 0.25	0.378 *
500	11.90 ± 0.10	15.15 ± 0.14	0.451 *
LSD value	0.311 *	0.453 *	----

* (P<0.05).

Table 2: Effect of earthworm extract on growth of *A.niger* (cm) after second and fourth day.

Concentration	Mean ± SD		LSD value
	2 day	4 day	
Control	0.60 ± 0.10	1.45 ± 0.05	0.257 *
200	3.05 ± 0.05	3.30 ± 0.10	0.125 *
250	3.25 ± 0.05	2.55 ± 0.05	0.278 *
300	3.45 ± 0.05	3.85 ± 0.05	0.209 *
350	3.65 ± 0.05	4.05 ± 0.05	0.185 *
400	3.95 ± 0.05	4.25 ± 0.05	0.227 *
450	4.20 ± 0.10	4.55 ± 0.05	0.194 *
500	4.45 ± 0.06	4.85 ± 0.05	0.205 *
LSD value	0.215 *	0.191 *	----

* (P<0.05).

Table 3: Effect of earthworm extract on Spors Number [x 10^4] of *Pencillium expansum* after second and fourth day.

Concentration	Mean ± SD		LSD value
	2 day	4 day	
Control	0.00 ± 0.00	1.45 ± 0.05	0.315 *
200	1.15 ± 0.15	2.05 ± 0.05	0.259 *
250	2.55 ± 0.05	3.20 ± 0.10	0.261 *
300	3.10 ± 0.10	5.15 ± 0.15	0.275 *
350	4.15 ± 0.15	6.55 ± 0.05	0.195 *
400	6.05 ± 0.05	7.55 ± 0.15	0.224 *
450	7.65 ± 0.15	8.95 ± 0.07	0.285 *
500	9.15 ± 0.15	12.15 ± 0.16	0.304 *
LSD value	0.373 *	0.341 *	----

** (P<0.05).

350 – 400 – 450 - 500) (mg/ml) of extracts to drilling and size (50 microlitter) in each of drilling and found one drilling containing distilled water (Control). Then incubating petri Temperature 25ºC during 48, 72 hrs, and determined activity of extracts measured diameter growth zone around the each drilling by centimeter[11].

Statistical Analysis

ANOVA test was used to compare the result with among groups according to[12].

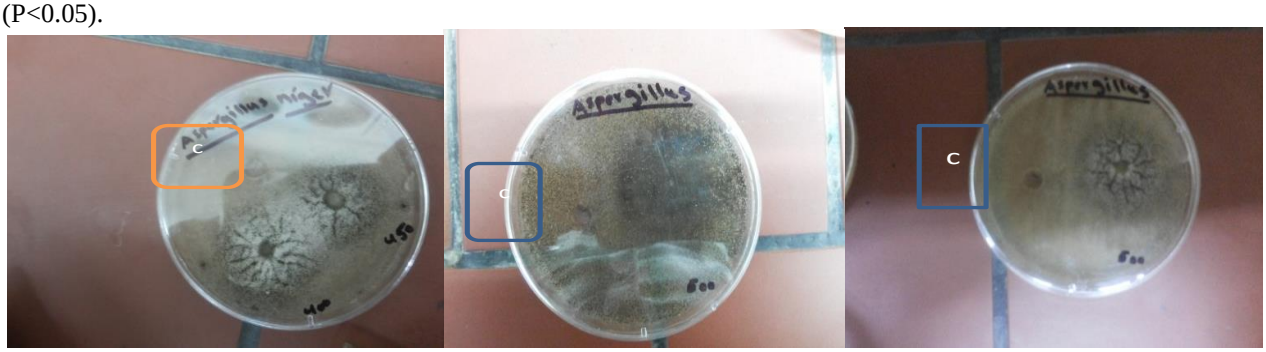
RESULT AND DISSCUSION

Tables 1, 2, 3, 4 show significant differences (p< 0.05) After two days of experiment in control the growth(cm.)of *A.niger*was 0.5 and the spores number (spo.n.) was zero while the growth of *P.expansum* in the same concentration was 1 but the spores number zero, in concentration 200 mg/ml the growth was 3 and the (spo.n.) 3.5 $_x10^4$that to *A.nager*. while to *P.expansum*the growth was 1.7 and (spo.n.) 1 $_x10^4$and in 250 mg /ml concentration to *A.niger* the growth number was 3.2, the (spo.n.) 4_x10^4 while to *P.expansum* .The values were 1.9 and 3 $_x10^4$respectively, in 300 mg /ml growth to *A.niger* 3.3 and the (spo.n.) 5 $_x10^4$while to *P.expansum* 2.1 and the(spo.n.) 3 $_x10^4$, the growth of *A.niger* Was 3.6 and the(spo.n.)6. 5 $_x10^4$ and to *P.expansum* growth was 2.2 and the (spo.n.) 4 $_x10^4$that in concentration 350 mg / ml while in concentration 400 mg /ml the growth of *A.niger* was 3.9 while in *P.expansum*2.5 while the (spo.n.) 8 $_x10^4$ and 6_x10^4 respectively, in concentration 450 mg /ml the growth was 4.1 and 2.8 to *A.niger*and *P.expansum* While the (spo.n.) 9 $_x10^4$ to *Asp.* and 7.5_x10^4 t0 *Pen.* Finally the in concentration 500 mg/ ml the growth of *A.niger* was 4.3 while in *P.expansum*3.4 while the (spo.n.) 12 $_x10^4$ and (spo.n.) 9 $_x10^4$ to both genesis respectively .In fourth day of experiment and in control the growth (cm.) of *A.niger* was 1.5 and the (spo.n.) 2 $_x10^4$and in the same

Table 4: Effect of earthworm extract on growth of *Pencillium expansum* (cm) after second and fourth day.

Concentration	Mean ± SD		LSD value
	2 day	4 day	
Control	0.950 ± 0.05	1.45 ± 0.05	0.194 *
200	1.75 ± 0.05	2.15 ± 0.05	0.205 *
250	1.95 ± 0.05	2.45 ± 0.05	0.137 *
300	2.15 ± 0.05	2.70 ± 0.10	0.186 *
350	2.40 ± 0.10	3.25 ± 0.05	0.207 *
400	2.65 ± 0.05	3.65 ± 0.05	0.225 *
450	2.95 ± 0.05	3.95 ± 0.04	0.281 *
500	3.50 ± 0.10	4.00 ± 0.00	0.178 *
LSD value	0.215 *	0.182 *	---

* (P<0.05).



A B C

Figure A: Effect of earthworm extract (400,450)mg on growth of *A.niger* after second day
Figure B: Effect of earthworm extract (500)mg on growth of *A.niger* after fourth day.
Figure C: Effect of earthworm extract (500)mg on growth of *A.niger* after fourth day.

A B C

Figure A: Effect of earthworm extract (300,350)mg on growth of *Pencillium expansum* (cm) after second day.
Figure B: Effect of earthworm extract (500mg) on growth of *Pencillium expansum* (cm) after Fourth day.
Figure C: Effect of earthworm extract (500)mg on growth of *Pencillium expansum* (cm) after second day

concentration to *P.expansum* the growth was 1.4 and (spo.n.) 1.5 $_{x}10^{4}$ in the concentration 200 mg / ml to *A.niger* the growth was 3.4 while (spo.n.) 6 $_{x}10^{4}$.The growth to *P.expansum*2.1 and (spo.n.) 2 $_{x}10^{4}$, in 250 mg/ml concentration the growth of *A.niger*3.6 and (spo.n.) 6.5 $_{x}10^{4}$ to *P.expansum* the growth was 2.3 and (spo.n.) 3 $_{x}10^{4}$, the growth of *A.niger* 3.8 and to *P.expansum*3.6 while the (spo.n.) was 8 $_{x}10^{4}$ to *A.niger* and 5 $_{x}10^{4}$ to *Pen.* respectively all that in concentration 300 mg /ml, while in concentrations 350 – 400 – 450 - 500 mg/ml the growth of *A.niger* was (3.9) (4.1) (4.3)(4.8) while to *P.expansum* in the same concentrations the growth was (2.8)(3.1)(3.6)(3.9) respectively and the (spo.n.) to *A.niger* in the same concentrations was (10 $_{x}10^{4}$)(11 $_{x}10^{4}$)(12.5 $_{x}10^{4}$)(15 $_{x}10^{4}$) and to *P.expansum* (6.5$_{x}10^{4}$)(7 $_{x}10^{4}$)(9 $_{x}10^{4}$)(12 $_{x}10^{4}$) respectively (Table, 1,2,3,4). The results show that the growth of *A.niger* and the spores numbers more than *P.expansum*in all concentrations along the period of experiment because that powder contains on numbers of nutrition elements, that important in growth of Fungi like carbohydrates, proteins, nitrogen, phosphors, potassium, iron, magnesium, manganese, zinc, copper and calcium that's play an important role in energy metabolism also potassium is important for DNA, protein synthesis and cell volume regulation[13]. Experiment contradict all studies in that place because studies shows the powder of earthworm that has antifungal properties. *Eisenia foetida*

powder have antifungal properties that inhibiting the growth of *Candida albicans* and tested with water is more effective than when tested with acetone[14,15] founded the same concentrations of earthworm powder was show antifungal against *Candida albicans* and antibacterial agent against *Aeromonas hydrophila*. Earthworm have largely been used internally as strong aphrodisiacs[16], Anti-inflammatory activist[17] and anti-pyretic[18].

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