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Assessment of the fungal isolates interactions by laboratory culture technique with brown lentil seeds germination using vermicompost suspension

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ABSTRACT

This study focuses on the interactions among vermicompost fungi and vermicompost's ability to enhance seed germination by cultivatingbrown lentil seeds through using filter paper and pertri-dishes technique. Vermicompost prepared by using waste materials and earthworms was used for fungal isolation and lentil seed germination in the suspended form. The interaction among vermicompost fungi was observed during direct isolations using the serial dilution method and then using pure culture dual plate's plans. At the same time, brown lentil seed germination was occurred using vermicompost suspension and measuring seed germination percentage method. The results revealed that most isolated microorganisms were fungi. Interaction among vermicompost fungi varied from neutral to negative interaction. The brown lentil seedsachieved a high germination percentage (80%) using vermicompost suspension compared to a positive and negative control. The overall results confirmed that the vermicomposting fungi play an essential role as biological control and growth-promoting factor.

Keywords: Fungal isolates, brown lentil, seed germination, vermicompost suspension.

INTRODUCTION

The interactions among microorganisms occurbetween the same species, with different species, or even betweenentirely other genera and families. The interactive patterns within these webs are positive, negative, or neutral, where there is no effect on the interacting species (Faust and Raves, 2012).The microbial communities influence life extended in various disciplines; for example, humanassociated micro-biotaimpacts health, environmental microbes determine ecosystem sustainability, and microbe-driven industrial processes expand (Zaccaria *et al*., 2017).The production of synthetic fertilizer known as NPK (Nitrogen, Phosphorus, and Potassium) relies on mining phosphate rock's increasingly costly process. Global phosphate reserves are known to reach complete exhaustion within the next 50-100 years, hitting peak extraction by 2030 (Cordell *et al*., 2009). Since 2006 the price of phosphate has doubled, and between 2007-2008 the cost of synthetic fertilizer increased 800% (Tomlinson, 2010).

Vermicompost is a bio-oxidative process in which the earthworms interact with microorganisms and other fauna within the decomposer community, accelerating the stabilization of organic matter and significantly modify its physical and biochemical properties (Dominguez, 2004), which act as an essential biological process in the management and recycling of organic wastes, it provides a way to treat organic wastes more quickly (Lazcano *et al*., 2008). Although, microorganisms primarily accomplish the biochemical decomposition of organic matter, yet,

earthworms are crucial drivers of the process. They are involved in microbial populations' stimulation through ingestion and fragmentation of fresh organic matter, which results in a greater surface area available for microbial colonization, thereby drastically altering biological activity (Dominguez *et al*.,2010).

Earthworms modify microbial biomass and activitythrough stimulation, digestion, and dispersion in casts (Brown and Double, 2004; Aira *et al*., 2009; Monroy *et al*., 2009) and closely interact with other biological components of the vermicomposting system, affecting the structure of microflora and microfauna communities (Dominguez *et al*., 2003; Lores *et al*., 2006; Aira *et al*.,2007; Monroy *et al*., 2009). Vermicompost provides a stable mineral balance, improves the nutrient availability for plant growth,and could act as a complex bio-fertilizer rejuvenating the soil. The mutual uses of vermicompost with soil will build up high bacterial populations that improve plant growth and fall of pathogenic organisms else.

Lentilseeds add essential vitamins, minerals, and fiber to the diet, also provide protein, and are an excellent replacement for meat in meals; they decrease the risk of heart disease by reducing blood pressure naturally; also contains the carotenoids, lutein , zeaxanthin and polyunsaturated fatty acids (Zhang *et al*., 2014). The interaction between vermicomposting and isolated microorganisms such as fungi was evaluated and also using the vermicompost suspension for brown lentil seed germination was investigated.

MATERIALS AND METHODS

1. Vermicompost preparation

Vermicompost was prepared by mixing60 kgrabbits wastes (which have high protein content and less toxic effect), 40 Kgrice straw (as sheep plant source), andonekg worms (A mixture of earthworms, red wigglers, and tiger worms) for 75 days at pH7, 15-35˚Cand moderate moisturewas kept by continuously adding water every week (Figs. 1 & 2).Worms were obtained from Al monakh Lab, at the National Center for Land and Water Research, Cairo, Egypt.

Fig.(1).Vermicompost preparation method at farm.

Fig.(2).Vermicompost.

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2. Physicochemical characteristics of vermicompost

The produced vermicompost was analyzed to detect the total elements, hormones, and other organic contents through the Agricultural research center, Soil Water, Environmental Research Institute, Giza, and Egypt.

3. Microbial isolation from vermicompost

a. Isolation technique

Microbial isolation was carried out using serial dilution methods (Benso, 2002), firstly after storage vermicompost at 4˚C for 30 minutes. After storage at room temperature for 30 minutes, 10 g of vermicompostsamplewas addedtoa 250 ml Erlenmeyer flask, each contains 100 ml of sterilized distilled water. Flasks were then shaken at a constant speed (150 RPM) for 15 minutes at room temperature. The flasks were left until complete sedimentation of compost. Serial decimal dilutions were prepared from an original concentrated to reach dilution $1/10^{-7}$.Then one ml aliquot from required dilutions was transferred to Petri-plates; three replicates were prepared for each dilution. The sterilized and cooled media were transferred to respective pre-labeled plates like potato dextrose agar media. After plating, plates were incubated at 27 ºC for 4 to 7 days,the pure culture was obtained by repeated streak plate method.

b. Identification of fungal isolates

Identification of fungal isolates was carried out at Mycology laboratory (Botany and Microbiology Department, Faculty of Science, Helwan University and it was based on morphological characteristics of each culture using the following references (Gilman, 1957; Johnand Pit, 1979; Thom an Raper, 1945; Toussoun and Nilson, 1983).

4. The interaction studies

I. Direct isolation from vermicompost sample (mixed culture)

1. Make a serial dilution of vermicompost sample (Benson,2002).
2. Inoculate 0.1 ml of each dilution into sterilized perti-dishes.
3. Pour the molten medium (45˚C) into the petri-dishes (use potato dextrose agar media).
4. Shake well and incubate at 28˚C for 3-5 days.
5. Examination of the plates. (Especially for 10^{-3} and 10^{-4}) and determine the interaction.

II. Determine the interaction between pure cultures isolated from the vermicompostsample

1. Pour the medium (use potato dextrose agar media) in sterilized petri-dishes and after solidification, inoculate thetested fungi near each other.

2.Incubate at 28˚C for 3-5 days.

4. Observe the manner of growth by thedual culture method (Skidmore and Dickenson, 1976).

5. Lentil seed germination using vermicompost suspension

The present study was carried out using petri-dishes (9 cm in diameter), which contained filter paper (Double ring 9cm) and Brown lentil (50 seeds of brown lentil), then adding 10 ml of vermicompost suspension (10 g vermicompost + 100 ml sterilized distilled water). For –ve control sets, 10 ml sterilizedistilled water was used, while for +ve control, 10 ml autoclaved vermicompost suspension was used. All treated sets were incubate at room temperature. Thegermination percentage, and length of the plumule and radicle were recorded over four days for all sets.

RESULTS AND DISCUSSION

1.Physicochemical characteristics of vermicompost

Analysis of vermicompost (Table 1) indicated that its physicochemical characteristics were greatly similar to the findings of Pasupathi and Devendiran (2018), who found that the pH ranged between 7.1-7.9, Organic Carbon 20-26 %, moisture content 22-66.7 %, nutrients (N= 1.16 %), (P= 0.04 %), (K= 0.34%), (Na = 2.89 %) and microbial colonies like Bacteria, Fungi, and Actinomycetes were also present in large numbers.

Table(1).The physicochemical characteristics of vermicompost.

Item	Unit	Value
Density	Kg/m3	585
Moisture content	%	62
pH(1:10)	%	7.9
Total nitrogen	%	1.82
Ammonical nitrogen	PPM	76
Nitrate nitrogen	PPM	38
Organic matter	%	47.14
Organic Carbon	%	27.34
Ash	%	52.86
C/N Ratio		15:1
Total phosphorus (P_2O_5)	%	1.13
Total Potassium (K_2O)	%	0.80
Weed seeds		Nd
Nematoda	Larva/200g	Nd
Germination index	%	90
Total potassium humate	%	0.95
Humic acids	%	0.82
Fulvic acids	%	0.11
Total coliform bacteria	Cfu/g	Nd
Fecal coliform bacteria	Cfu/g	Nd
Salmonella and shigella bacteria	Cfu/g	Nd
Total count of bacteria	Cfu/g	$15x10^5$
Total count of fungi	Cfu/g	$20x10^5$
Total count Actinomycetes	Cfu/g	$4x10^5$

Previous determinations were calculated on dry weight basis except for moisture content and density. Nd=not detected CFU=Colony Forming Unit.

2. Fungal isolates

The results in Table (3) show that seven fungal species have been isolated from vermicompost, and the highest fungal population was recorded in vermicompost which storaged at 4˚C for 30 minutes, compare to vermicompost storaged at room temperature for 30 minutes. These fungal species were identified as *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus tamarii*, *Rhizopus stolonifer*, *Alternaria sp*, *Penicillumsp* and

Aspergillus nidulas (Table 3).This result may be due to temperature plays an essential role in the growth of microbial colonies during composting of municipal solid waste as has been mentioned by Pathaka *et al*. (2012).

Table(2). Brown lentil seed germination

Parameters	Treatments		
	Negative control (Dist.H_2O)	Positive control(Autoclaved vermicompost suspension)	Vermicompost suspension
Germination (%)	50%	60%	80%
Plumule length (cm)	2	3	4
Radicle length (cm)	1.3	1.5	2

Table(3). Morphological identification of the fungal isolates.

Fungal isolates number		Identification
After storage at 4˚C	After storage at room temperature	
1	1	***Aspergillus niger*Van Tieghem**
2	-	***Aspergillus tamari* Kita**
3	-	***Aspergillus flavus* Link**
4	-	***Aspergillus nidulans* (Eidam) Wint**
5	2	***Penicillum chrysogenum* Thom**
6	-	***Rhizopus stolonifer* (Ehrenberg)Vuillemin**
7	3	***Alternaria alternate* (Fr)Keissl**

3. The interaction studies

The results in Figures (3,4&5) show the interaction between fungi of vermicompost which varies from negative interaction to neutral one.This result agrees with that of Belen *et al*. (2020) and helps to understand the impact of fungi of vermicomposton the plant. Also, explainstheir ability to prevent the growth of pathogenic fungi such as *Apergillus niger*as part of biological control strategies. On the other hand, Pei-Hua *et al*. (2018) indicated that antagonistic yeast strains inhibited *B. cinerea* and those with high potential for sustainable strawberry production.

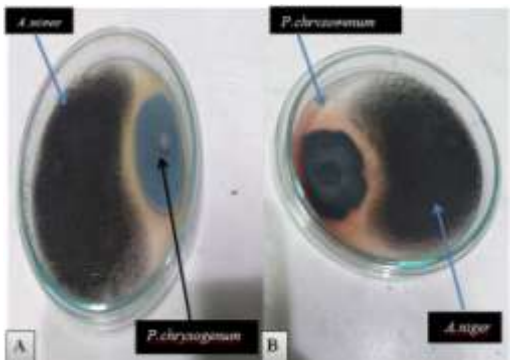

Fig.(3).Deviation of growth (negative interaction) using pure culture of *Aspergillus niger* and *penicillum chrysogenum*Thom.

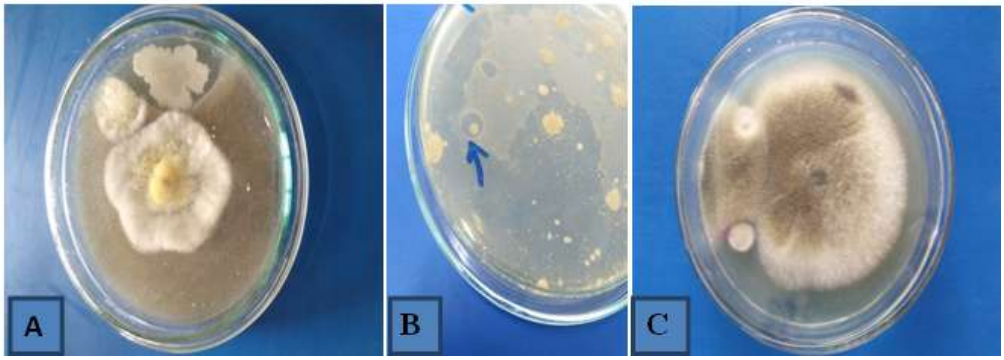

Fig. (4).A, C deviation of growth and B, clear zone using direct isolation (negative interaction).

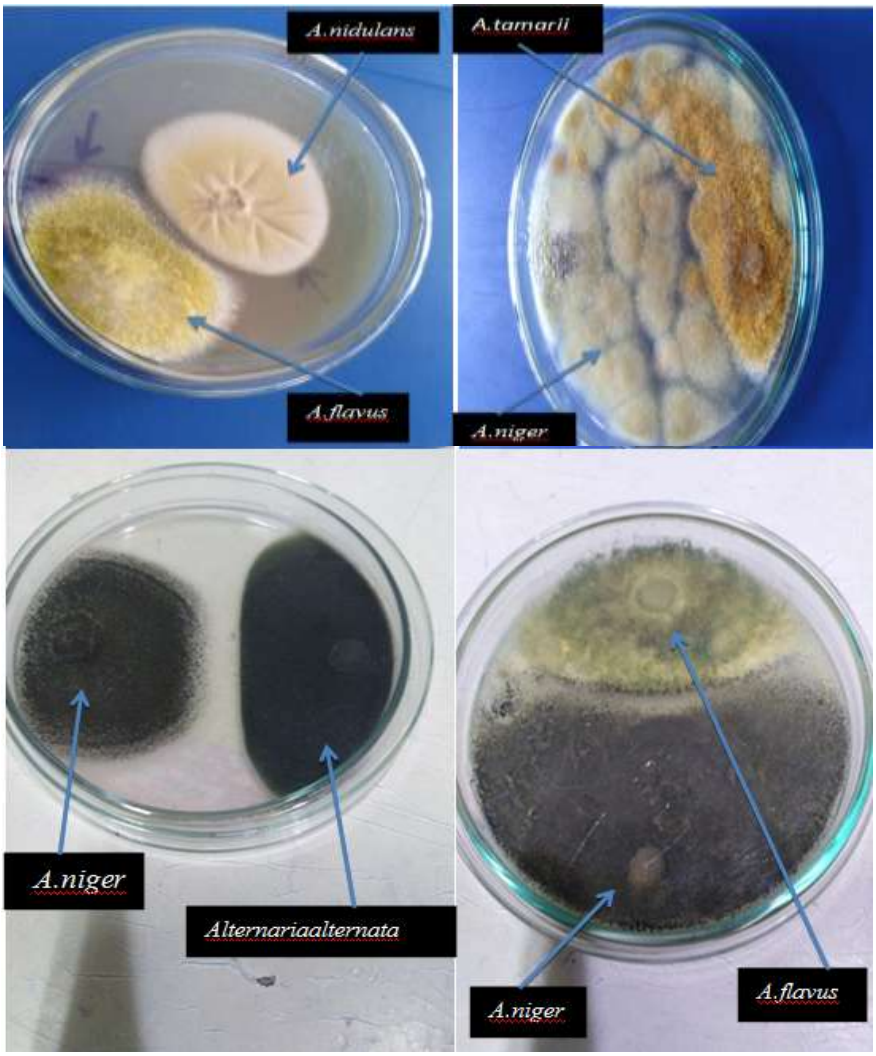

Fig.(5).Neutral interaction using pure culture of *Aspergillus niger, Apergillus tamarii, Aspergillus flavus, Apergillus nidulas* and *Alternaria alternata*.

4. Brown lentil seed germination

The present results indicated the ability of lentil seeds to achieve the highest germination percentage(80%) using vermicompost suspension compared to negative and positive control one (Fig. 6 and Table 2). This in agreement with Norman *et al*. (2012)who observed thatgermination percentage increased when seeds were soaked in a

vermicompost extract from chicken manure compared with seeds soaked in water. This suggests that factors beyond physical alteration of seed coats were responsible for earlier and better germination. Spaccini *et al*. (2008) reported that aerated compost extracts contain low-molecular-weight bioactive microbial origin compounds. Arancon *et al.* (2007) demonstrated that applying a vermicompost extract to growth media enhanced seed germination and seedling growth of tomatoes and cucumbers. Lazcano *et al*. (2010) reported positive effects of vermicompost extracts on the germination and early development of *Pinus pinaster*.

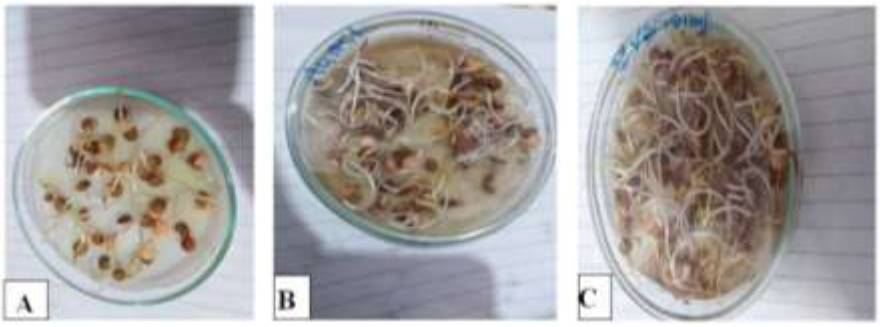

Fig.(6). Brown lentil seed germination, A (-ve control), B (+ ve control) and C (vermi-compost suspension).

Conclusions

Vermicompost prepared using rabbit wastes and rice straw as a source for worm breeding was suspended for fungal isolation to germinate the lentil seeds. The results indicated the ability of vermicompost fungi to interact using different laboratory techniques through petri-dishes. Also, indicated the ability of vermicompost suspension to promote seed germination of brown lentil.

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تقييم تفاعل العزلات الفطرية بتقنية الاستزراع المخبري مع إنبات بذور العدس البني باستخدام معلق السماد الدودي

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المستخلص

تركز هذه الدراسة على التفاعلات بين فطريات السماد الدودي وقدرة السماد الدودي على تعزيز إنبات البذور عن طريق زراعة بذور العدس البني باستخدام ورق الترشيح وتقنية الأطباق البيترى. تم استخدام السماد الدودي المحضر باستخدام المخلفات وديدان الأرض للعزل الفطري وإنبات بذور العدس في شكل معلق. لوحظ التفاعل بين فطريات السماد الدودي أثناء العزلات المباشرة باستخدام طريقة التخفيف التسلسلي ومن ثم استخدام طرق المزرعة ثنائية الاطباق النقية. وفي نفس الوقت تم إنبات بذور العدس البني باستخدام معلق السماد الدودي وطريقة قياس نسبة إنبات البذور . أظهرت النتائج أن معظم الكائنات الدقيقة المعزولة كانت فطريات. تفاوت التفاعل بين فطريات السماد الدودي من تفاعل متعادل إلى سلبي. حققت بذور العدس البني نسبة إنبات عالية (80٪) باستخدام السماد الدودي المعلق مقارنة بالكنترول الإيجابي والسلبي للعينات. أكدت النتائج الإجمالية أن فطريات السماد الدودي تلعب دورًا أساسيًا كعامل تحكم بيولوجي وايضا معزز للنمو.