PHYTOHORMONES PRODUCTION BY FUNGI POLLUTED ONION (ALLIUM CEPA L.) AND MAIZE (ZEA MAYS L.) PLANTS

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Phytohormones are significant plant growth regulators produced by Plants and some microorganisms especially those related to the plant root partition. Auxins, and gibberellins are the most common phytohormones concentrated inside the plants. In this study, rhizosphere, rhizoplane and endophytic fungi were isolated from ten onion (Allium cepa L.) and maize (Zea mays L.) samples collected from Assiut Governorate on potato dextrose agar medium at $28\pm 2^{\circ}$ C. In the current study, Twenty species related to 13 genera were identified from onion (17 and 12) and maize (8 and 5) plants. The Highest occurrence genera on the examined plants were Aspergillus, Cochliobolus, and Fusarim, of which A. flavus, A. niger, A. terreus, C. spicifer, and F. oxysporum were the common species. Out of 58 fungal isolates tested, 42 isolates could produce indole acetic acid and the highest producer was Fusarium solani (No.148) which isolated from maize rhizosphere yielding 1249 µg/ml. Whereas, out of 55 fungal isolates, 52 isolates produce gibbrellic acid and the highest producer was endophytic fungus Aspergillus terreus (No.155) giving 203 µg/ml. The present study revealed higher diversity of rhizosphere fungi than rhizoplane and endophytes associated with the tested plants. Also, the colonization rate and diversity of fungi vary from plant to plant. The current work provides preliminary data for exploration into diverse bioactive natural products originated from fungi and prospects on ecosystem reconstruction. The study proved that two isolates (Fusarium No.148 and Aspergillus No.155) have a high ability to produce growth hormones that lead to increasing plant growth and improving its productivity. Future studies should also, consider isolating fungi from other plant parts and identification of their metabolites since these substances may contain potential novel properties.

Key words: Phytohormones, IAA, GA3, fungi, rhizosphere, rhizoplane, endophytes.

INTRODUCTION

Soil represents an essential portion for the sustenance of our life, composed of minerals and organic matter available to the plants [1]. It's a source of various organisms including; fungi, yeasts, bacteria, and protozoa [2]. Plant roots are heavily colonized with microorganisms due to the nutrient rich of the root exudates [3]. This microbial community that inhabits the soil zone surrounded the plant roots known as the rhizosphere, while rhizoplane microorganisms refer to the microbes on the root itself [4]. Endophyte microbes defined as microorganisms that inhibit the plant endosphere without any harming their hosts in asymptomatically living producing metabolites improving the plant development [5]. The rhizosphere part is nutrient-rich contains sugars, acids, fatty acids and organic compounds that amino attract microorganisms [6]. Root related microbes produce plant growth promoters, insecticides, antioxidants, and phytohormones which plays a vital role in the plant growth, and development [7, 8, 9]. These microbes also can improve the plant growth in stress conditions like nutrient, salinity, temperature, and heavy metal stress [10, 11].

Phytohormones could stimulate the plant growth, resistance to the stress factors, development, and nutrient acquisition [12]. Plant hormones or phytohormones represent naturally occurring organic molecules that effect on the growth and differentiation of plants. Phytohormones are classified into five categories; auxins, gibberellins, cytokinins, ethylene, and abscisic acid [13]. Most phytohormones activity occurred at low concentrations, while high concentrations could alter the plant growth in both positive and negative directions [14, 15].

Indole acetic acid (IAA) represented a natural auxin produced by higher plants, bacteria and fungi and playing critical role in the plant growth and even its development. It induces the plant cell elongation of stems and roots, stimulate the cell division, stimulate the initiation of lateral and adventitious roots, differentiation of vascular tissues, effects on the apical dominance, and phototropism [12]. It has been reported that microbial production of IAA can vary among different species and

strains, and it is also influenced by culture conditions, growth stage and substrate availability. Moreover, isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil [16]. Fusarium oxysporum recovered from the rhizoplane of onion plant recorded as high IAA producer [17]. Also, Alternaria alternata, Aspergillus fumigatus, A. Chaetomium niger, *Phanerochaete* chryosporium, globosum, Colletotrichum Chrysosporium acutatum, fructicola, Fusarium oxysporum, Fusarium sp., Paecilomyces sp., Penicillium citrinum, Phoma sp., Rhizopus sp. and Trichoderma harzianum were the highly common producers of IAA [17, 18, 19, 20, 21].

Gibberellins are a group of the most common phytohormones category, structurally are tetracyclic diterpenoid compounds produced by plants, bacteria and fungi that involved in a number of plant physiological processes [22]. The microbial production of gibberellins by root microorganisms could describe as secondary metabolites of microorganisms that participate as signaling factors for the host plant [23]. Gibberellic acid $(C_{19}H_{22}O_6)$ is a member of gibberellins used widely in plants and characterized as white crystals, 233-235 °C melting point, soluble in acetone, alcohols, ethyl acetate, while less soluble in petroleum ether, chloroform, and benzene [24]. Kobayashi et al [25] found that root fungi could produce gibberellins types as GA1, GA3, GA4 and GA20. Gibberellic acid is highly produced by genus Fusarium especially F. moniliforme [26]. Fusarium oxysporum, F. solani, F. incarnatum, F. chlamydosporum, and F. verticilloides recovered from Egyptian clover, maize, garlic, and onion plants were good producers of gibbrellic acid [27]. Also, the genera Penicillium, and Aspergillus recorded as GA3 producers [28]. The fungal species Aspergillus fumigatus, Penicillium janthinellum, Fusarium sacchari, Fusarium konzum, and Fusarium glutinans were endophytic resources for gibberellins production [29, 30].

The aim of the current work was planned to isolate rhizosphere, rhizoplane, and endophytic fungi from two cultivated plants, onion (*Allium cepa* L.) and maize (*zea mays* L.) collected from Assiut Governorate. Also, testing the ability of these isolated fungi for the

production of two common phytohormones, indole acetic acid (IAA) and gibbrellic acid (GA3) was evaluated.

MATERIALS AND METHODS

1-Samples collection

Ten root samples from onion (*Allium cepa* L.) and maize (*Zea mays* L.) plants (5 samples each) were collected from Assiut University farm during the period 2018 to 2019. The plant roots were dislodged from the adhering soil and directly placed in clean and sterilized polyethylene bags and then transferred to laboratory for fungal analysis.

2- Medium used for fungal isolation

Potato dextrose agar medium (PDA) was used for isolation of rhizosphere, rhizoplane, and endophytes containing (g/l): potato (scrubbed and diced), 200; dextrose, 15.0 and agar agar, 20.0; distilled water, 1000 ml. The medium was supplemented with Rose-bengal (1/30000) and Chloramphenicol (250 mg/l) as bacteriostatic and bactericidal agents, respectively [31]. The pH of the medium was adjusted to 5.6. For preparation of the medium, potato was boiled for 1h and passes the mixture through a fine sieve (cloth chess), dextrose was added, stirred, and agar was mixed and boil until dissolving, then autoclaved at 121°C for 20 min [32].

3- Rhizosphere fungi

The plants were uprooted and gently shaken to remove superfluous soil. A known weight of roots adhering with soil was immersed in flask containing sterilized distilled water. After shaken, suitable dilutions were prepared [33]. One ml of the rhizosphere soil suspension was transferred to each sterilized Petri-dish (3 plates each sample) and covered with sterilized melted cooled medium. The plates were incubated at $28 \pm 2^{\circ}$ C for 7 days. The developing fungi were counted, isolated and identified. The counts were calculated as colony forming units (CFU) per g of rhizosphere soil.

4- Rhizoplane fungi

Roots were cut into equal segments size (approximately 1 cm²) and subjected to washing with dilute sodium hypochlorite (NaOCl) 5% for 3 min then objected to a series of washing by sterilized distilled water [33]. They were thoroughly dried between sterilized filter paper and five of them were placed on the surface of agar medium in each plate (3 plates each sample). The plates were incubated at $28 \pm 2^{\circ}$ C for 7 days. The developing fungi were identified, counted and calculated as colony forming units (CFU) per 15 segments of fresh roots each sample.

5- Endophytic fungi

The roots were washed with sterilized distilled water, then immersed in 70% alcohol for 5 min, washed three times with sterilized distilled water, immersed in NaOCl 5% for 2 min, washed with sterilized distilled water then dry between two sterilized filter papers [34]. Five segments size (approximately 1 cm²) were placed on the surface of agar medium in each plate. Three plates were used and incubated at $28 \pm 2^{\circ}$ C for 7 days and the endophytic fungi were identified, counted and calculated as colony forming units (CFU) per 15 segments of fresh roots each sample.

6- Identification of fungi

Purified fungal isolates in the present investigation were identified based on the morphological and microscopical characteristics. The following references were used; the genus *Aspergillus* [35], a revision of genus *Trichoderma* [36], dictionary of the fungi [37], fungi in agriculture soils [38], synoptic key to *Aspergillus nidulans* group and *Emericella* species [39], a laboratory guide to common *Penicillium* species [40], and the *Fusarium* laboratory manual [41].

7- Screening for indole acetic acid production by fungi

Fifty- eight fungal isolates belonging to 15 species related to 9 genera isolated in the present work were tested for indole acetic acid. The indole acetic acid production medium was Czapek's dextrose liquid medium supplemented with 0.2 g/l L-tryptophane, containing (g/l): glucose, 30.0; yeast extract, 5; NaNO₃, 3.0; KH₂PO₄, 1.0;

MgSO₄.7H₂O, 0.5; KCl, 0.5 and FeSO₄.7H₂O, 0.01; distilled water 1000 ml. pH adjusted to 5.5 and the medium was sterilized by autoclaving at 121°C for 20 min. Chloramphenicol (250 mg/l) was added as bacteriostatic agent. Incubation was carried out at $28\pm2^{\circ}$ C on a rotary shaking (150 rpm) for 7 days. After 7 days, flasks were examined for fungal dry weight and indole acetic acid production [17]. The fungal mycelium was obtained by filtration through dried and weighed filter paper, and then dried at 60°C overnight for dry weight determination. For IAA detection; 1 ml of supernatant was assayed using 2ml of Salkowaski reagent (2.02 g FeCl₃+ 500 ml distilled water and 300 ml conc. H₂SO₄) [16]. Absorbance was measured at 540 nm using spectrophotometer and development of pink coloration was checked [42] using two standard curve from (10-100 µg IAA/ ml) and (100-1000 µg IAA/ ml).

8- Screening for GA3 production by fungi

Fifty- five fungal isolates belonging to 16 species and 9 genera, isolated in the current study, were tested for GA3. The GA3 production medium was Czapek's dextrose liquid medium containing (g/l): glucose, 30.0; yeast extract, 5; NaNO₃, 3.0; KH₂PO₄, 1.0; MgSO₄.7H₂O, 0.5; KCl, 0.5 and FeSO₄.7H₂O, 0.01; distilled water 1000 ml. pH adjusted to 5.5 and the medium was sterilized by autoclaving at 121°C for 20 min. Chloramphenicol (250 mg/l) was added as bacteriostatic agent. Incubation was carried out at $28\pm2°$ C on a rotary shaking (150 rpm) for 7 days. After 7 days, flasks were examined for fungal dry weight and GA3 production [27]. For GA3 detection; the pH of the supernatant was adjusted at 2.5 by using 15% HCl. The filtrate was extracted with ethyl acetate (1:3 of filtrate: solvent ratio) and the extract was used for its GA3 determination using spectrophotometer at 254 nm absorbance [43] using two standard curve from (10-100 µg GA/ ml) and (100-1000 µg GA/ ml)

RESULTS

I-Fungi recovered from onion plant

Rhizosphere, rhizoplane and endophytes of onion (*Allium cepa* L.) plants were isolated on potato dextrose agar medium at $28\pm 2^{\circ}$ C. There is a remarkably high incidence of diverse fungi in the rhizosphere. The incidence of fungi was low in endophytes than rhizoplane and rhizosphere. A total of 17 species appertaining to 12 genera were isolated from the tested plant. The genera of the highest occurrence on onion plants were *Aspergillus, Cldosporium, Cohliobolus, Fusarim* and *Penicillium.* The species of the highest occurrence were *A. flavus, A. niger, C. cladosprioides, C. spicifer, F. oxysporum* and *P. chrysogenum* (Table 1).

1-Rhizosphere fungi

Data in table (1) showed that, 11 species belonging to 9 genera were recovered on potato dextrose agar medium at $28\pm2^{\circ}$ C from onion samples. *Fusarium oxysporum* was the leader species isolated from 100% of the samples contributed 25.1% of total fungi. *Aspergillus* was the second higher genus, isolated from four samples (80%) in high occurrence comprising 24.4% of total fungi. It was represented by three species namely; *A. niger, A. flavus,* and *A. nidulans* giving 65.63%, 24.99%, and 9.38% of the total *Aspergillus* and 16%, 6.1%, and 2.2% of the total fungi, respectively.

The third higher incidence rate was represented by Cladosporium. From the genus one species was isolated namely C. cladosporioides isolated in moderate occurrence from three samples (60%) and occurred in 13% of total fungi. Emericella (A. nidulans) was occupied the fourth place in occurrence, it recovered in moderate occurrence from two samples (40%) and encountered in 20.6% of total fungi. Penicillium chrysogenum and Acremonium strictum, were isolated in moderate frequency of occurrence from two samples (40%) for each giving 4.5% and 2.2% of total fungi, respectively. Eupenicillium brefeldianum, Epicoccum nigrum, and Trichoderma harzianum were

isolated in low frequency of occurrence 20% of the samples for each and comprising 5.3%, 3.8%, and 0.7% of total fungi, respectively (Table 1).

2- Rhizoplane fungi

Using direct plating technique, 10 species belonged to 9 genera were recovered on potato dextrose agar medium at $28\pm2^{\circ}$ C from onion samples (Table 1). *Fusarium (F. oxysporum)* was the most prevalent genus, isolated from 100% of the samples matching 35% of total fungi. *Penicillium* and *Stachybotrys* genera were the second higher genera runner up *Fusarium*. They were occurred in 40% of the samples for each represented by two species namely, *P. chrysogenum* and *S. chartarum* and contributed 15.6% of total fungi for each.

Aspergillus represented by A. niger and A. oryzae occupied as the third higher incidence genus was recovered from 40% of the samples constituting 10.53% of total fungi. A. niger and A. oryzae contributed each 50% of the total Aspergillus for each and 5.2% of total fungi. Epicoccum nigrum was isolated low frequency 20% of occurrence and comprising 8.7% of total fungi. Acremonium strictum, Cladosporium herbarum, Cochliobolus spicifer and Macrophomina phaseolina were isolated in low frequency of occurrence 20% for each and comprising 3.6% of the total fungi for each (Table 1).

3- Endophytic fungi

Seven species belonged to 6 genera were recovered as endophytes on potato dextrose agar medium at $28\pm1^{\circ}$ C from onion samples (**Table 1**). *Fusarium (F. oxysporum)* was the most prevalent genus occurred in all root samples (100%) and constituting 33.3% of total fungi._*Cochliobolus spicifer* came behind *Fusarium* in frequency of occurrence, isolated from 80% of the samples having 22.2% of total fungi. *Penicillium (P. chrysogenum)* ranked third in moderate occurrence emerged in 40% of the samples matching 31.7% of total fungi. *Aspergillus* was occurred in moderate occurrence from two samples (40%) constituting 7.2% of total fungi. *Aspergillus oryzae* and *Aspergillus terreus* were isolated in low frequency of occurrence 20% of each and comprising 1.7% and 5.5% of

total fungi, respectively. *Acremonium strictum* and *Trichoderma harzianum* were isolated in low frequency of occurrence. They were encountered in 20% of each comprising 1.7% and 3.9% of total fungi, respectively (Table 1).

Table 1: Total count (TC, CFU/g soil, or 75 segments in all samples), percentage of total count (TC%), number of cases of isolation (NCI, out of 5 samples) and occurrence remark (OR) of rhizosphere, rhizoplane and endophyic fungi isolated from onion plants on potato dextrose agar medium at $28^{+}2^{\circ}$ C.

	Rhizoshere		Rhizoplane			Endophyte			
Fungal species	T.C	%TC	NCI& OR	T.C	%TC	NCI& OR	T.C	%TC	NCI& OR
Acremonium strictum	1000	2.3	2M	0.7	3.6	1L	0.3	1.7	1L
Aspergillus	10666	24.4	4H	2	10.4	2M	1.3	7.2	2M
A. flavus	2666	6.1	2M	-	-	-	-	-	-
A. nidulans	1000	2.3	1L	-	-	-	-	-	-
A. niger	7000	16	3M	1	5.2	2M	-	-	-
A. oryzae	-	-	-	1	5.2	2M	0.3	1.7	1L
A.terreus	-	-	-	-	-	-	1	5.5	1L
Cladosporium	5666	13	3M	0.7	3.6	1L	-	-	-
C. cladosporioides	5666	13	3M	-	-	-	-	-	-
C. herbarum	-	-	-	0.7	3.6	1L	-	-	-
Cochliobolus spicifer	-	-	-	0.7	3.6	1L	4	22.2	4H
Emericella nidulans	9000	20.6	2M	-	-	-	-	-	-
Epicoccum nigrum	1667	3.8	1L	1.7	9	1L	-	-	-
Eupenicillium brefeldianum	2333	5.3	1L	-	-	-	-	-	-
Fusarium oxysporum	11000	25.1	5H	6.7	35	5H	6	33.3	5H
Macrophomina phaseolina	-	-	-	0.7	3.6	1L	-	-	-
Penicillium chrysogenum	2000	4.5	2M	3	15.6	2M	5.7	31.7	2M
Stachybotrys chartarum	-	-	-	3	15.6	2M	-	-	-
Trichoderma harzianum	333	0.7	1L	-	-	-	0.7	3.9	1L
Total counts	43665	100		19.2	100		18	100	
No. of genera= 12	9			9			6		
No. of species $= 17$	11			10			7		

OR = Occurrence remark : H= High occurrence, more than 3 samples (out of 5 samples); M= Moderate occurrence, between 2-3 samples; L= Low occurrence, less than 2 samples.

II- Fungi recovered from maize plants

Rhizosphere, rhizoplane and endophytes of maize (*Zea mays* L.) plants were isolated on potato dextrose agar medium at $28\pm2^{\circ}$ C. There is a remarkably of high incidence of diverse fungi in the rhizosphere and rhizoplane samples. While, the incidence of endophytes was low comparing with rhizoplane and rhizosphere samples. Eight species belonging to 5 genera were identified from maize plant. *Aspergillus* was the genus of the highest occurrence. The species of the highest occurrence were *A. flavus*, *A. niger* and *A. terreus* (Table 2).

1-Rhizosphere fungi

Data in table (2) showed that, 7 species belonging to 5 genera were recovered on potato dextrose agar medium at $28\pm2^{\circ}$ C from maize samples. *Aspergillus* was the leader genus. It was isolated from 100% of the samples contributed 75.5% of total fungi. From the genus three species were identified of which *A. flavus* and *A. niger* were the most prevalent species. They were isolated from four samples (out of 5 samples, 80%) constituting 37.5% of total *Aspergillus* for each and 28.3% of total fungi for each. The remaining species namely *A. terreus* was isolated from four samples each (out of 5 samples, 80%) constituting 25% of total *Aspergillus* and 18.8% of total fungi for each (Table 2).

Fusarium (represented by *F. oxysporum*) was the second higher genus. It was isolated from two samples (out of 5 samples, 40%) and comprising 9.4% of total fungi. *Penicillium* (represented by *P. oxalicum*) was isolated from one sample (out of 5 samples, 20%) and accounting for 9.4% of total fungi. *Phoma* (*P. herbarum*) was isolated from one sample (out of 5 samples, 20%) and encountered in 3.8% of total fungi. *Acremonium* (*A. strictum*) was isolated from one sample (out of 5 samples, 20%) and occurred in 1.9% of total fungi (Table 2).

2-Rhizoplane fungi

Using direct plating technique, 7 species belonged to 5 genera were recovered on potato dextrose agar medium at $28\pm2^{\circ}C$ from onion

samples (**Table 2**). Aspergillus was the most prevalent genus, isolated from 100% of the samples matching 63.9% of total fungi. Three species from the genus were identified (*A. flavus, A. niger* and *A. terreus*) of which *A. niger* was isolated in high frequency of occurrence 80%, counting 57.6% of the total *Aspergillus* and 36.8% of the total fungi. *A. flavus* was isolated in moderate frequency of occurrence 40%, giving 28.3% of the total *Aspergillus* and 18.1% of the total fungi. *A. terreus* was isolated in low frequency of occurrence 20%, giving 14.13% of the total *Aspergillus* and 9% of the total fungi. *Fusarium* (*F. verticillioides*) was the second higher genus runner up *Aspergillus*. It was occurred in 40% of the samples contributed 13.9% of total fungi. *Phoma herbarum* was recovered from 40% of the samples constituting 11.1% of total fungi. *Penicillium oxalicum* and *Acremonium strictum* was isolated low frequency of occurrence comprising 6.9% and 4.2% of total fungi, respectively (Table 2).

3-Endophytic fungi

Five species related to 3 genera were recovered as endophytes on potato dextrose agar medium at $28\pm2^{\circ}$ C from maize samples (**Table 2**). *Aspergillus (A. flavus, A. niger* and *A. terreus)* was the most prevalent genus occurred in all root samples 100% and constituting 58.4% of total fungi. From the three species identified of the genus *A. flavus* and *A. niger* were the common species. They were encountered in 40% and 60% of the samples comprising 26.8% and 23.2% of total fungi, respectively. However, *A. terreus* was isolated in low frequency of occurrence 20% and giving 9% of total fungi. *Acremonium (A. strictum)* ranked second in total count and in the number of cases of isolation. It was emerged in 60% of the samples matching 20.5% of total fungi. *Phoma herbarum* came behind *Acremonium* in frequency of occurrence, isolated from 40% of the samples and having 20.5% of total fungi.

Some fungi were isolated from onion plant and not encountered on maize plants such as: A. nidulans, A. oryzae, C. cladosprioides, C. herbarum, C. spicifer, E. nidulans, Epicoccum nigrum, Eupenicillium brefeldianum, Macrophomina phaseolina, P. chrysogenum, S. chartarum and T. harzianum. On the other hands, some species were occurred on

maize plant and not isolated from onion plant namely: *F.verticillioides*, *Penicillium oxalicum* and *Phoma herbarum* (Tables 1&2).

Table 2: Total count (TC, CFU/g soil or 75 segments in all samples), percentage of total count (TC%), number of cases of isolation (NCI, out of 5 samples) and occurrence remark (OR) of rhizosphere, rhizoplane and endophytic fungi isolated from maize plants on potato dextrose agar medium at $28+2^{\circ}$ C.

Fungal species		Rhizosh	ere		Rhizop	lane		Endophyte		
	T.C	%TC	NCI&	T.C	%TC	NCI&	T.C	%TC	NCI&	
			OR			OR			OR	
Acremonium strictum	333	1.9	1L	0.6	4.2	1L	2.3	20.5	3M	
Aspergillus	13333	75.5	5H	9.2	63.9	5H	6.6	59	4H	
A. flavus	5000	28.3	4H	2.6	18.1	2M	3	26.8	2M	
A. niger	5000	28.3	4H	5.3	36.8	4H	2.6	23.2	3M	
A. terreus	3333	18.9	4H	1.3	9	1L	1	9	1L	
Fusarium	1666	9.4	2M	2	13.9	2M	-	-	-	
F.oxysporum	1666	9.4	2M	-	-	-	-	-	-	
F.verticillioides	-	-	-	2	13.9	2M	-	-	-	
Penicillium oxalicum	1666	9.4	1L	1	6.9	1L	-	-	-	
Phoma herbarum	666	3.8	1L	1.6	11.1	2M	2.3	20.5	2M	
Total counts	17664	100		14.4	100		11.2	100		
No. of genera= 5	5			5			3			
No. of species= 8	7			7			5			

OR = Occurrence remark; H= High occurrence, more than 3 samples (out of 5 samples); M= Moderate occurrence, between 2-3

samples; L= Low occurrence, less than 2 samples.

III- Indole acetic acid (IAA) production by fungi

Fifty- eight fungal isolates related to 15 species belonging to 9 genera, recovered from different parts of onion and maize plants, were tested for their abilities to produce indole acetic acid on Czapek's dextrose liquid medium supplemented with L-typtophan (**Table 3**). The results showed that 42 fungal isolates (out of 58) have been grown and exhibited various degrees of indole acetic acid production.

Eleven (out of 42) indole acetic acid producing fungal isolates were considered as highly producers (IAA>300 μ g/ml), 15 isolates exhibited moderate indole acetic acid production (IAA150-300 μ g/ml), and 16 isolates achieved low indole acetic acid producers (IAA<150 μ g/ml). *Fusarium solani* (No.148) isolated from maize rhizosphere showed the highest indole acetic acid production on Czapek's dextrose liquid medium supplemented with L-typtophan yielding 1249 μ g/ml.

Also, the highest indole acetic acid producers (IAA>300 μ g/ml) are *Phoma herbarum* (134, 149), *Aspergillus flavus* (118, 146), *Cochliobolus specifier* (111), *Fusarium oxysporum* (77), *Penicillium rubens* (150), *Penicillium oxalicum* (120), *Macrophomina phaseoli* (86) and *Emericella nidulans* (85), gave 320, 1081.5, 500, 1050, 700.5, 497.5, 437.5, 371.5, 388.5 and 330 μ g/ml IAA; and 8.98, 13.07, 14.8, 4.6, 6.33, 5.77, 10.62, 4.72, 6.193 and 6.95 g/l Dry mass, respectively.

The second group contains 15 fungal isolates were considered as moderate producers of indole acetic acid (IAA150-300 μ g/ml). These isolates are *Penicillium oxalicum* (144), *Aspergillus flavus* (70, 74, 89, 106, 126, 133, 156), *Penicillium chrysogenum* (98), *Fusarim literatium* (157), *Aspergillus niger* (116, 151), *Fusarium oxysporum* (75), *Fusarium verticillioides* (125) and *Aspergillus oryzae* (54), yeilded 269.5, 162.5, 256.5, 225, 156, 143.5, 235.5, 268.5, 224.5, 224, 151, 197.5, 192.5, 170.5 and 137 μ g/ml IAA; and 7.06, 10.3, 5.61, 3.092, 7.24, 8.13, 10.51, 6.4, 6.86, 8.7, 9.11, 12.2, 8.098, 11.68, and 12.6 g/l Dry mass, respectively.

The remaining fungal isolates (16 out of 42) have low production of indole acetic acid (IAA<150µg/ml) including *Fusarium*

oxysporum (58,71, 73, 87, 88), Penicillium oxalicum (129), Aspergillus flavus (55, 83,97,104, 108), Aspergillus terreus (52), Aspergillus oryzae (100), Penicillium chrysogenum (81, 107) an Phoma herbarum (149), gave 147, 125.5, 58, 140.5, 30.5, 133, 42.5, 0, 92, 104.5, 57.5, 89.5, 86, 50.5, 26 and 37.5 μg/ml IAA; and 2.72, 6.6, 7.5, 4.333, 7.45, 7.08, 5.3, 0, 8.2, 9.86, 9.62, 12, 9.45, 5.81, 2.33 and 6.07g/l Dry mass, respectively.

Table 3: Screening for indole acetic acid production by fungi on L-typtophan Czapek's dextrose liquid medium

Fungal isolate	Isolate number	Isolation part	Isolation plant	IAA (µg/ml)	IAA remarks	Dry mass (g/l)
Aspergillus flavus	70	Rp	Allium cepa	162.5	М	10.3
A. flavus	84	Rs	Allium cepa	64.5	L	5.98
A. flavus	97	Rp	Allium cepa	92	L	8.2
A. flavus	126	Rp	Zea mays	143.5	М	8.13
A. flavus	106	Ep	Allium cepa	156	М	7.24
A. flavus	104	Ep	Allium cepa	104.5	L	9.86
A. flavus	74	Ep	Allium cepa	256.5	М	5.61
A. flavus	133	Rs	Zea mays	235.5	М	10.51
A. flavus	55	Rp	Allium cepa	42.5	L	5.3
A. flavus	108	Rs	Allium cepa	57.5	L	9.62
A. flavus	83	Rs	Allium cepa	0	-	0
A. flavus	146	Rs	Zea mays	1050	Н	4.6
A. flavus	156	Rp	Zea mays	268.5	М	6.4
A. flavus	154	Ep	Zea mays	0	-	0
A. flavus	118	Ep	Zea mays	500	Н	14.8
A. flavus	89	Rp	Allium cepa	225	М	3.092
A. niger	151	Rs	Zea mays	197.5	М	12.2
A. niger	80	Rs	Allium cepa	0	-	0
A. niger	135	Rs	Zea mays	0	-	0
A. niger	142	Ep	Zea mays	0	-	0
A. niger	116	Rs	Zea mays	151	М	9.11
A. niger	115	Rs	Zea mays	0	-	0
A. oryzae	100	Ep	Allium cepa	86	L	9.45
A. oryzae	54	Ep	Allium cepa	137	М	12.6
A. terreus	52	Rp	Allium cepa	89.5	L	12
A. terreus	155	Ep	Zea mays	0	-	0
A. terreus	145	Rs	Zea mays	0	-	0
Cochliobolus specifier	111	Rp	Allium cepa	700.5	Н	6.33
C. specifier	56	Ep	Allium cepa	0	-	0
Emericella nidulans	85	Rp	Allium cepa	330	Н	6.95
Eupenicillium brefeldianum	82	Rs	Allium cepa	0	-	0

Table 3(Cont.)									
Fusarim literatium	157	Rp	Zea mays	224	М	8.7			
F. oxysporum	77	Rs	Allium cepa	497.5	Н	5.77			
F. oxysporum	147	Rs	Zea mays	0	-	0			
F. oxysporum	75	Rs	Allium cepa	192.5	М	8.098			
F. oxysporum	71	Rp	Allium cepa	125.5	L	6.6			
F. oxysporum	72	Ep	Allium cepa	0	-	0			
F. oxysporum	88	Ep	Allium cepa	30.5	L	7.456			
F. oxysporum	87	Rp	Allium cepa	140.5	L	4.333			
F. oxysporum	58	Ep	Allium cepa	147	L	2.72			
F. oxysporum	73	Ep	Allium cepa	58	L	7.5			
F. solani	148	Rs	Zea mays	1249	Н	6.12			
F. verticillioides	140	Rp	Zea mays	0	-	0			
F. verticillioides	125	Rp	Zea mays	170.5	М	11.68			
Macrophomina phaseoli	86	Rp	Allium cepa	388.5	Н	6.193			
Penicillium chrysogenum	107	Rp	Allium cepa	26	L	2.33			
P. chrysogenum	98	Ep	Allium cepa	224.5	М	6.86			
P. chrysogenum	81	Rs	Allium cepa	50.5	L	5.81			
P. oxalicum	144	Rs	Zea mays	269.5	М	7.06			
P. oxalicum	120	Ep	Zea mays	371.5	Н	4.72			
P. oxalicum	124	Rp	Zea mays	0	-	0			
P. oxalicum	129	Ep	Zea mays	133	L	7.08			
P. rubens	150	Rp	Zea mays	437.5	Н	10.62			
Phoma herbarum	149	Ep	Zea mays	1081.5	Н	13.07			
P. herbarum	149	Ep	Zea mays	37.5	L	6.078			
P. herbarum	134	Rs	Zea mays	320	Н	8.98			
Trichoderma harzianum	65	Ep	Allium cepa	0	-	0			
T. harzianum	64	Rs	Allium cepa	0	-	0			

Rs= Rhizosphere; Rp= Rhizoplane; Ep= Endophyte; IAA= Indole acetic acid; H= high producer, $>300\mu g/ml$; M= moderate producer, 150-300 $\mu g/ml$; L;= low producer, $<150\mu g/ml$.

IV- Gibberellic acid (GA3) production by fungi

Fifty- five fungal isolates appertaining to 16 species belonging to 9 genera, recovered from different parts of onion and maize plants, were examined for their abilities to produce gibbrellic acid (GA3) on Czapek's dextrose liquid medium (**Table 4**). The results showed that 52 fungal isolates (out of 55) have been grown and exhibited various degrees of gibbrellic acid production,

Out of 52 gibbrellic acid producing fungal isolates 19 were considered as highly gibbrellic acid producers (GA3, $>50\mu$ g/ml), 25 isolates are moderate gibbrellic acid producers (GA3, 25-50 μ g/ml), and 8 isolates are low gibbrellic acid producers (GA3, $<25\mu$ g/ml). *Aspergillus terreus* (155) isolated as endophytes from maize plant showed the highest gibbrellic acid production on Czapek's dextrose liquid medium yielding 203 μ g/ml.

In addition, the highest gibbrellic acid producers (GA3, >50µg/ml) are *Penicillium rubens* (150), *Penicillium chrysogenum* (107), *Aspergillus niger* (80, 142, 153), *A. flavus* (55, 84, 154), *Trichoderma harzianum* (53), *Fusarium literatium* (157), *Eupenicillium brefeldianum* (82), *A. terreus* (52, 91, 145), *A. oryzae* (100), *Fusarium verticillioides* (140), and *Fusarium solani* (148), gave 95, 59, 51, 67.5, 82.5, 63.5, 74.5, 63, 70.5, 70, 66.5, 63.5, 55, 61.5, 53, 53 and 51.5 µg/ml GA3; and 12.469, 11.813, 10.494, 10.357, 17.714, 9.912, 10.251, 17.96, 14.539, 8.381, 15.547, 11.805, 15.103, 6.833, 9.807, 5.432 and 4.931 g/l Dry ma, respectively ss.

The second group contains 25 fungal isolates considering as moderate producers of gibbrellic acid (GA3, 25-50µg/ml) including *Aspergillus flavus* (74, 83, 89, 97, 104, 106, 108, 126), *Cochliobolus specifier* (56), *Fusarium oxysporum* (147), *Phoma herbarum* (134), *Aspergillus niger* (58, 116, 135), *Phoma herbarum* (149), *Fusarium oxysporum* (77), *Fusarium oxysporum* (87), *Penicillium oxalicum* (129), *Macrophomina phaseoli* (86), *Penicillium chrysogenum* (98), *Fusarium verticillioides* (125, 137), *Aspergillus oryzae* (54), *Penicillium chrysogenum* (81), and *Fusarium oxysporum* (109), gave 44.5, 50, 31.5, 38, 25, 33, 37, 47.5, 50, 48, 43, 41.5, 40, 26, 39.5, 38, 37.5, 35.5, 31.5, 31, 29.5, 26.5, 29, 28 and 27 µg/ml GA3; and 12.072, 16.994, 6.214, 10.915, 10.812, 8.333, 12.924, 10.134, 7.998, 14.027, 7.177, 6.735, 10.202, 11.581, 12.451, 4.604, 4.607, 10.224, 3.297, 10.472, 3.918, 4.945, 6.024, 11.015 and 9.771 g/l Dry mass, respectively.

The remaining fungal isolates (8 out of 52) have low production of gibbrellic acid (GA3, <25µg/ml) namely, *Fusarium oxysporum* (71,

72,75, 88), *Trichoderma harzianum* (64), *Aspergillus flavus* (70, 118), and *Penicillium oxalicum* (144) gave 22, 11, 24, 19.5, 20.5, 15, 18.5 and 12.5 μ g/ml GA3; and 6.011, 6.53, 6.353, 5.717, 4.202, 6.464, 7.11 and 13.156 g/l Dry mass, respectively.

Table 4:- Screening for	gibbrellic acid production by fungi on Czapek's
dextrose liquid medium	

Fungal isolate	Isolate number	Isolation part	Isolation plant	GA3 (µg/ml)	GA3 remarks	Dry mass (g/l)
Aspergillus flavus	70	Rp	Allium cepa	15	L	6.464
A. flavus	55	Rp	Allium cepa	63.5	Н	9.912
A. flavus	74	Ep	Allium cepa	44.5	М	12.072
A. flavus	84	Rs	Allium cepa	74.5	Н	10.251
A. flavus	126	Rp	Zea mays	47.5	М	10.134
A. flavus	97	Rp	Allium cepa	38	М	10.915
A. flavus	104	Ep	Allium cepa	25	М	10.812
A. flavus	106	Ep	Allium cepa	33	М	8.332
A. flavus	133	Rs	Zea mays	55	Н	9.398
A. flavus	154	Ep	Zea mays	63	Н	17.96
A. flavus	118	Ep	Zea mays	18.5	L	7.11
A. flavus	89	Rp	Allium cepa	31.5	М	6.214
A. flavus	108	Rs	Allium cepa	37	М	12.924
A. flavus	83	Rs	Allium cepa	50	М	16.994
A. niger	58	Ep	Allium cepa	41.5	М	6.735
A. niger	135	Rs	Zea mays	26	М	11.581
A. niger	116	Rs	Zea mays	40	М	10.202
A. niger	142	Ep	Zea mays	67.5	Н	10.357
A. niger	80	Rs	Allium cepa	51	Н	10.494
A. niger	153	Rp	Zea mays	82.5	Н	17.714
A. oryzae	54	Ep	Allium cepa	29	М	6.024
A. oryzae	100	Ep	Allium cepa	53	Н	9.807
A. terreus	91	Ep	Allium cepa	55	Н	15.103
A. terreus	52	Rp	Allium cepa	63.5	Н	11.805
A. terreus	155	Ep	Zea mays	203	Н	14.952
A. terreus	145	Rs	Zea mays	61.5	Н	6.833

Table 4(Cont.)									
Cochliobolus specifier	56	Ep	Allium cepa	50	М	7.998			
Emericella nidulans	85	Rp	Allium cepa	0	-	0			
Eupenicillium brefeldianum	82	Rs	Allium cepa	66.5	Н	15.547			
Fusarim literatium	157	Rp	Zea mays	70	Н	8.381			
F. oxysporum	77	Rs	Allium cepa	38	М	4.604			
F. oxysporum	76	Rs	Allium cepa	0	-	0			
F. oxysporum	147	Rs	Zea mays	48	М	14.027			
F. oxysporum	109	Rs	Allium cepa	27	М	9.771			
F. oxysporum	75	Rs	Allium cepa	24	L	6.353			
F. oxysporum	71	Rp	Allium cepa	22	L	6.011			
F. oxysporum	72	Ep	Allium cepa	11	L	6.53			
F. oxysporum	88	Ep	Allium cepa	19.5	L	5.717			
F. oxysporum	87	Rp	Allium cepa	37.5	М	4.607			
F. oxysporum	73	Ep	Allium cepa	0	-	0			
F. solani	148	Rs	Zea mays	51.5	Н	4.931			
F. verticillioides	125	Rp	Zea mays	29.5	М	3.918			
F. verticillioides	137	Rp	Zea mays	26.5	М	4.945			
F. verticillioides	140	Rp	Zea mays	53	Н	5.432			
Macrophomina phaseoli	86	Rp	Allium cepa	31.5	М	3.297			
Penicillium chrysogenum	98	Ep	Allium cepa	31	М	10.472			
P. chrysogenum	81	Rs	Allium cepa	28	М	11.015			
P. chrysogenum	107	Rs	Allium cepa	59	Н	11.813			
P. oxalicum	144	Rs	Zea mays	12.5	L	13.156			
P. oxalicum	129	Ep	Zea mays	35.5	М	10.224			
P. rubens	150	Rp	Zea mays	95	Н	12.469			
Phoma herbarum	134	Rs	Zea mays	43	М	7.177			
P. herbarum	149	Ep	Zea mays	39.5	М	12.451			
Trichoderma harzianum	53	Rs	Allium cepa	70.5	Н	14.539			
T. harzianum	64	Rs	Allium cepa	20.5	L	4.202			

Rs= Rhizosphere; Rp= Rhizoplane; Ep= Endophyte; GA3= gibbrellic acid; H= high producer, >50µg/ml; M= moderate producer, 25-50µg/ml; L= low producer, <25µg/ml.

DISSCUSION

Plant roots are rich part in colonization with various rhizosphere, rhizoplane, and endophytic microorganisms for the nutrient rich of the root exudates [3, 4, 44]. The rhizosphere part specially is nutrient-rich zone with sugars, fatty acids, amino acids, and organic compounds which attract the fungi [6]. This could explain our results in which high incidence of diverse fungi in the rhizosphere zone comparing with the incidence of rhizoplane and endophytic fungi in both plants. Aspergillus flavus, A. niger, A. terreus, and Fusarium oxysporum were the leader species of onion and maize rhizosphere, rhizoplane and endophytes recovered from 100% of the samples. In agreement with our results; Fusarium represent one of the basic genera of fungi constituents the rhizosphere and rhizoplane of various Egyptian plants [45], Also, Alternaria alternata, Aspergillus flavus, A. niger, C. spicifer, Fusarium oxysporum, Humicola grisea, and Penicillium chrysogenum were isolated as rhizosphere fungi from Egyptian plants [46], Aspergillus ochraceus, Fusarium solani, Emericella quadrilineata, and Penicillium purpurgenum were recovered from rhizosphere and rhizoplane samples of cultivated plants from Assiut Governorate [47]. Aspergillus flavus, F. solani, Fusarium oxysporum, and Macrophomina phaseolina were dominant species of soybean endophytes [48]. Aspergillus flavus, A. terreus F. oxysporum, F. nygamia, and F. solani were the most common fungal species recovered from the 25 rhizosphere samples of the Egyptian maize, while Fusarium and Cochliobolus were the most common fungal genera isolated from the 25 rhizoplane samples of the Egyptian maize [33].

Out of 58 fungal isolates 42 isolates could produce indole acetic acid (in range 26 to 1249 μ g/ml IAA) and the highest producer is *Fusarium solani* (No. 148) which isolated from maize rhizosphere yielding 1249 μ g/ml. Also, *Aspergillus flavus*, *Fusarium oxysporum*, *Cochliobolus specifier*, *Penicillium rubens*, *Macrophomina phaseoli*, *Emericella nidulans*, and *Phoma herbarum* recorded as high IAA producers. Hasan [49] stated that *Fusarium oxysporum* isolates could produce IAA from 100 to 140 mg/l. According to Ahmad et al [50] IAA is highly produced by *Rhizopus*, *Aspergillus niger*, *Herbaspirillum seropedicae*, and *Erwinia* sp. Bilkay et al [51] found that *Aspergillus*

niger could produce IAA from 1.28 to 6.8 µg/ml. Yadav et al [19] obtained IAA from *Aspergillus niger* (85 µg/ml), *Penicillium citrinum* (52 µg/ml), and *Trichoderma harzianum* (68 µg/ml). While, Sugiharto [52] found that *Aspergillus, Penicillium, Trichoderma,* and *Rhizopus* could produce IAA in range 2.5 to 10.3 ppm. Whereas, *Chaetomium globosum, Alternaria alternata, Chrysosporium pseudomerdarium, Aspergillus fumigatus, Paecilomyces* spp., *Fusarium* spp., *Phoma* spp., *Penicillium* spp., and *Tulasnella* sp. recorded as IAA producers [20]. Mahmoud and Mostafa [17] reported that *Fusarium oxysporum* recovered from onion rhizoplane giving 142 mg/l IAA. But, Khan [53] obtained about 31 µg/mL IAA from *Fusariun oxysporum*.

Out of 55 fungal isolates 52 isolates could produce gibbrellic acid (in range 11 to 203 µg/ml GA3) and the highest producer was endophytic Aspergillus terreus (No. 155) giving 203 µg/ml. Also, Aspergillus flavus, Aspergillus niger, Aspergillus oryzae, Aspergillus terreus, Eupenicillium brefeldianum, Fusarium literatium, Fusarium verticillioides, Fusarium solani, Penicillium rubens, and Trichoderma harzianum recorded as high GA3 producers. In this respect the genera of Alternaria. Aspargillus, Cylindrocarpon, Neurospora, Fusarium. Penicillium, Rhizopus, and Trichoderma were listed as gibberellins producers [54]. Paecilomyces formosus isolated from sorghum could produce 1.1 ng/ml GA3 [55]. Fusarium moniliforme isolated from rice gives 0.66–600 mg/l GA [56]. According to Khan et al [57] GA3 was found to be produced by Aspergillus fumigatus, Gibberella fujikuroi, Neurospora crassa, Penicillium citrinum, Chrysosporium pseudomonarium, and Penicillium funiculosum. Also.. Cladosporium sp. recovered from Glycine max roots gives 5.18 ng/ml GA3 [58]. Fusarium monilifome, and Gibberella fujikoroi represents high producers of GA, could produce until 0.299 g/l [59]. The endophytic fungi namely, Aspergillus niger, Fusarium oxysporum, Aspergillus flavus, Paecilomyces formosus, Penicillium corylophilum, and Rhizopus stolonifer were found as GA3 and IAA producers [60]. Mohamed and Mahmoud [27] reported that Fusarium oxysporum and Fusarium solani recovered from onion rhizoplane giving 268.9 and 252.17 mg/l GA3.

CONCLUSION

The present study revealed higher diversity of Rhizosphere fungi than Rhizoplane and endophytes associated with the tested plants. Also, the colonization rate and diversity of fungi vary from plant to plant. The current work provides preliminary data for exploration into diverse bioactive natural products originated from endophytes and prospects on ecosystem reconstruction. The study proved that two isolates of *Fusarium solani* and *Aspergillus terreus* have a high ability to produce growth hormones that lead to increasing plant growth and improving its productivity. Future studies should also, consider isolating fungi from other plant parts and identification of secondary metabolites since these substances may contain potential novel properties.

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إنتاج الهرمونات النباتية (Allium cepa L.) والذرة (Zea mays L.) والذرة (Zea mays L.) بالفطريات الملوثة لنباتى البصل

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تم إختبار قدرة ٥٨ عزلة فطرية على إنتاج إندول أسيتيك أسيد ووجد أن ٤٢ عزلة لها القدرة على إنتاج الحمض وكانت فطرة Fusarium solani (رقم ١٤٨) والمعزولة من جذور نبات الذرة أعلى إنتاجية للحمض حيث أنتجت ١٢٤٩ ميكرو غرام / مل بينما من بين ٥٥ عزلة فطرية تم أختبارها لإنتاج حمض الجبريليك لوحظ أن ٥٢ عزلة لها القدرة على إنتاج الحمض وكانت أعلاها إنتاجية هى فطرة Aspergillus terreus (رقم ١٥٥) ويعطى ٢٠٣ ميكرو غرام / مل.

أوضحت الدراسة الحالية عن تنوع أكبر في فطريات Rhizosphere مقارنة بفطريات Rhizoplane and Endophytes المرتبطة بالنباتات المختبرة. كما يختلف معدل الإنتشار وتنوع الفطريات من نبات لآخر. يوفر العمل الحالي بيانات أولية للتعرف على العديد من المنتجات الطبيعية النشطة بيولوجيًا والمنتجة بواسطة الفطريات المرتبطة بالنباتات وإعادة بناء النظام البيئي. أثبتت الدراسة أيضاً أن عزلتين (Aspergillus No. 155) ، (Fusarium No 148) لهما قدرة عالية على إنتاج هرمونات النمو التي تؤدي إلى زيادة نمو النبات وتحسين إنتاجيته. يجب أن تنظر الدراسات المستقبلية أيضًا في عزل الفطريات من أجزاء النبات الأخرى وتحديد المركبات الثانوية لأن هذه المواد قد تحتوي على خصائص جديدة.