Review Paper

Production of plant growth regulators by some fungi isolated under salt stress

Abstract

Fifty-eight fungal isolates were isolated from salt soil, whey and salt fish on medium supplemented with 0.5% NaCl. Out of 58 isolates, 49 were capable to grow on medium supplemented with 1% NaCl. These halo-tolerant isolates were tested to produce plant growth regulators (PGR) on solid or in broth medium. On solid medium, 9 halo-tolerant isolates out of 58 isolates were gave indole acetic acid (IAA) which appeared red zone around growth zone with index ranged from 0.25 to 0.56. While in broth medium, 9 isolates were tried to produce IAA and gibberellic acid (GA) in presence of NaCl at 0.5 or 1%. The maximum IAA and GA production were obtained by FS12 isolate (4.32 and 4.52 mg/100ml) and by FW2 isolate (2.71 and 2.92 mg/100ml) at 0.5% and 1% NaCl, respectively. FS12 was selected as the most efficient isolate for PGR production and identified as *Aspergillus niger*. Carbon and nitrogen sources were studied for PGR optimization by the tested strain. Whey and peptone were used as a sole carbon and nitrogen source, where increased the IAA and GA production about 15.4% and 71.3% as compared to control (basal medium).

Key words: fungi, NaCl, phytohormones, synthesis, halophilic, carbon and nitrogen sources

Abbreviation: PGR: Plant growth regulator, IAA: Indol acetic acid, GA: Gibberellic acid, PDA: Potato dextrose agar, PGP: Plant growth promoters.

Introduction

Plant hormones are a group of naturally occurring, organic substances which influence physiological processes at low concentrations. The processes influenced consist mainly of growth, differentiation and development, though other processes, such as stomatal movement, may also be affected. Plant hormones have also been referred to as 'phytohormones' though this term is infrequently used **[1]**. There are several types of natural and synthetic plant growth promoters produced by some microorganisms as bacteria and fungi. Many of these regulators have interacted in

order to produce the final effect **[2]; [3]; [4]**. These substances are classified into two types; classical plant hormones (auxins, cytokinins, gibberellins, abscisic acid, ethylene and growth regulatory substances with similar biological effects and a more recently discovered natural substances that have phytohormonal roles as polyamines, oligosaccharins, salicylates, jasmonates, sterols, brassino steroids, dehydrodiconiferyl alcohol glucosides, turgorins, systemin, unrelated natural stimulators and inhibitors **[5]; [6].**

The ability of microorganisms to form phytohormones and phytohormone-like substances is also frequently used in the production of preparations for plant cultivation (in the majority of cases, the preparations in question are bacterial). Their additional advantages over expensive synthetic phytohormones include (1) broader spectrum of activity, (2) presence of phytohormones at optimum levels, and (3) presence of other biologically active compounds (e.g., vitamins), which are needed for normal plant development. Phytohormones have an important role in regulating of growth and development of plants.

Indol acetic acid (IAA) is the major and abundant plant growth promoter in plant. IAA plays an important role in the regulation and development of plant **[7]**. Auxin is one of the crucial molecules, regulating most plant processes directly or indirectly **[8]**. Gibberellins, classified with diterpenes, consist of isoprene residues that usually form four rings (A, B, C, and D). Gibberellic acids (GAs) like GA_3, GA_7, GA_1, and GA_4 are the best studied phytohormones of this group. They exhibit maximum biological activity and are the most widespread in nature. Gibberellins amount to more than 100 compounds, constituting the largest class of phytohormones, which are found in both plants and microorganisms. Certain compounds are classified with gibberellins based solely on their characteristic biological activity **[9]**.

The aim of the present study was to isolate halo-tolerant salinity fungi. Study the ability of the selected fungi to produce plant growth promoters (indol acetic acid (IAA) and gibberellic acid (GA)).

Materials and methods

Sample collection

Salt soil sample was collected from rhizosphere region in Sinai Peninsula, Egypt, whey sample was obtained from milk factory at 6-October city and salt fish sample was collected from super market in Shoubra.

Isolation of halo-tolerant fungi:

The tested samples (salt soil, whey and salt fish) were used for isolation of halo-tolerant fungi on potato-dextrose agar medium (PDA) **[10]**, its composition was as follow (g/L): potatoes extract, 200; dextrose, 20; agar agar, 20; and adjusted pH to 5. This medium supplemented with NaCl 0.5 or 1.0%. The isolation was performed using serial dilution technique **[11]**. The plates were inoculated with suitable dilution of the collected samples and incubated at 28 ºC for 5 days. The growing colonies were picked under aseptic conditions, purified and stored at 4 ºC.

Comment [TI1]: What is molarity and/or electrical conductivity of these NaCl concentrations?

Fungal culture preparation

Fungal isolates grown on PDA slants for 48 h at 30 ± 1 °C were used to prepare the suspension by adding 10 mL of sterile tap water to each fungal agar slant and gently scraping with sterile inoculation loop. The obtained suspension was used to inoculate 50 mL of medium 1, incubated at 29 ± 1 °C for 24 h and shacked (100 rpm) for activation.

Screening of plant growth promoters (PGP) producing fungi and fermentation process:

On solid medium

The halo-tolerant fungal isolates were tested to produce indole acetic acid (IAA) as PGP on Czapex Dox agar medium **[12]**, its composition was as follow g/L; sucrose, 30.0; Na NO_3, 3.00; K_2HPO_4, 1.0; KCl, 0.50; Fe SO_3; Mg $SO_4.7H_2O$, 0.50; agar, 20.0; and adjusted pH to 5. This medium composition was modified by addition of NaCl (0.5 or 1.0 %) and tryptophan 0.21 g/L. The growth was detected on plates with added 5 mL solution composed of 150 mL sulphoric acid (95%) and 7.5 mL ferric chloride (0.5 M) to observe the red color around the growth. The diameter of red zone and growth zone was measured, and IAA index was calculated.

On broth medium

Batch culture experiments were performed in 250 mL plugged Erlenmeyer flasks. Each containing 150 mL sterile Czapex Dox broth medium supplemented with 0.5 or 1.0% NaCl and tryptophan 0.21 g/L and then, inoculated with 4.0 mL of standard inoculum from the tested fungal isolates which incubated at 28 °C on rotary shaker at 150 rpm for 5 days. At the end of fermentation, samples (10 mL) were taken and filtered. Cell dry weight was determined in the pellets, and the IAA and gibberellic acid (GA) were assayed in supernatant.

Identification of the pioneer isolate

The most active fungal isolate was identified based on the morphological appearance under the microscope (shape and color of conidia) and culture properties according to **[13].**

Optimal carbon and nitrogen sources investigation for plant growth promoters:

Carbon sources

Influence of carbon sources on plant growth promoter's production by the selected fungal strain was investigated. The appropriate carbon source was selected by replacing the original carbon source of the medium (glucose) with equivalent carbon amount of each of the tested carbon source (glucose, sucrose, fructose, black strap molasses and whey) to eliminate errors which may occur as a result of differences in carbon concentration in each source.

Nitrogen source

Effect of organic and inorganic nitrogen sources on production of plant growth promoters by the tested strain. Therefore, the appropriate nitrogen source was selected by replacing the original one of the used medium with equivalent nitrogen amount of each of the tested nitrogen source to eliminate errors, which may occur as a result of differences in nitrogen concentration in each source. Organic nitrogen sources applied were mixture of peptone and yeast (as control), peptone, yeast extract, beef extract, soy bean extract and tryptone. Inorganic nitrogen sources being (ammonium chloride, Tri ammonium citrate, Tri ammonium orthophosphate and ammonium nitrate).

Analytical method:

Dry weight determination

Determination of fungal cell dry weight by filtrated using filter paper No.1. Then the biomass was washed twice with distilled water and dried at 80 °C until constant weight.

IAA determination

On solid medium

After observing a colonies growth of the tested isolates on agar plates containing 0.21 g tryptophane/L medium, these plates were covered with filter paper Whatman No.1 saturated with Salkowski' S reagent for 30 min at room temperature in dark place. A pink color was appeared on filter paper and surrounding the colony **[14].** Therefore, the IAA production index may be calculated as a follows equation reported by **[15]:**

IAA production index =

$$\frac{\text{(Red zone formation (mm) – Growth zone (mm))}}{\text{Growth zone (mm)}}$$

On liquid medium

IAA amount was estimated using the method described by **[14]**. One milliliter of cell-free supernatant was mixed vigorously with 4 mL Salkowski's reagent, then incubated at room temperature in dark place for 20 min till pink color appeared. This color was measured at 535 nm by using spectrophotometer (Unico S2100 series UV/Vis). The concentration of IAA was calculated from the regression equation of standard curve prepared in the range of 0.1 to 1.00 mg/100 mL of tryptophan.

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Gibberellic acid (GA) determination

Gibberellic acid concentration was estimated by colorimetric method suggested by **[16].** The determination was passed with several step being:

1st step was propagation

Mixed 15 mL of cell free supernatant with 10 mL of alcohol 95 % in volumetric flask (100 mL) and completed the volume to 40 ml with dDistilled water. Added 2 ml of zinc acetate solution to the sample and agitated well then allowed to stand for 2 min. Then added 2 mL of potassium ferrocynaid solution, agitated well and completed the volume to 50 mL with distilled water and allowed to stand at room temperature for 5 min. the solution was filtrated using filter paper Whatman

No.1. Absolute alcohol (8 mL) was added to 10 mL of supernatant (filtrated liquor) and then complete the volume to 100 mL with dilute hydrochloric acid (30%).

2nd Step of extraction

Took 5 mL of the filtered sample in extraction funnel and completed the volume to 10 mL with distilled water then adjusted pH to 2 with hydrochloric acid. Added 20 mL of ethyl acetate to reaction then mixed well for 1 min. Took the bottom layer and then repeated the extraction with 20 ml of ethyl acetate and mixed well for 1 min. then took again the bottom layer and re-extraction in phosphate buffer and added 20 mL of phosphate buffer and extract with mixed well for 1 min. Then added 15 mL of phosphate buffer and extract with mixed well for 1 min. Added 10 mL of phosphate buffer and extract with mixed well for 1 min.

3rd Step of determination

Collected the bottom layer in standard flask and completed the volume to 50 mL with phosphate buffer. Mixed the extracted sample with phosphate buffer in two standard flasks each one contains 20 mL of extract. Added to each one of two flasks 10 mL of absolute alcohol and agitated well. *The first flask (sample) completed to 100 mL with dilute hydrochloric acid (35%) and added 35 mL of dilute hydrochloric acid (5%) to** the second flask (control) and completed the volume to 100 mL with distilled water. Allowed the two standard flasks to stand at room temperature for 80 min. Absorbance was read at 254 nm by using spectrophotometer (Unico S2100 series UV/Vis) against distilled water in the blank cell.

Statistical analysis

The collected data were statistically analyzed using IBM® SPSS® Statistics software (2011).

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Which means test was used?
Describe how many treatments and replicates were used for each treatment.

Results and discussion

Isolation of halo-tolerant microorganisms

Fifty-eight fungal isolates were obtained from different sources, i.e., salt soil, whey and salt fish under salinity stress on potato-dextrose agar (PDA) medium supplemented with NaCl with concentration of 0.5% and 1%. Results are represented in **Table 1.** Clearly showed that all 58 fungal isolates have ability to grow on medium supplemented with 0.5% NaCl. While, 49 isolates among 58 microbial isolates were able to grow on medium supplemented with 1–% NaCl, it i's mean that 9 isolates were loose growth at high concentration of NaCl (1%). At 0.5% NaCl, the numbers of isolates obtained from salt soil, whey and salt fish were 25, 17 and 16 fungal isolates, respectively. Whereas, at 1% NaCl the number of fungal isolates being 23, 14 and 12 were collected from salt soil, whey and salt fish, respectively. In addition, **[17]** reported that plant response to salinity is one of the most widely researched subjects in plant physiology. It comes second only to photosynthesis in popularity. These results are in disagreement with **[18]** who reported that ions that contribute to soil salinity include Cl^-, SO_4^{2-}, HCO_3^-, Na^+, Ca^{2+}, Mg^{2+}, and, rarely, NO_3^- or K^+. The salts of these ions occur in highly variable concentrations and proportions. They may be indigenous, but more commonly they are brought into an area in the irrigation water or in waters draining from adjacent areas. Natural drainage is often so poorly developed in arid regions that salts collect in inland basins rather than being discharged to the sea. Moreover, **[19]** reported that soil salinization adversely affects plant growth and has become one of the major limiting factors for crop productivity worldwide. The conventional approach, breeding salt-tolerant plant cultivars, has often failed to efficiently alleviate the situation. In contrast, the use of a diverse array of microorganisms harbored by plants has attracted increasing attention because of the remarkable beneficial effects of microorganisms on plants.

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Screening the most efficiency growth promoters producing isolates

Qualitative estimation of growth promoters IAA produced by halo-tolerant isolates

Results are tabulated in **Table (2)** clearly showed that out of 58 isolates, 9 fungal isolates gave a red zone around the microbial growth under salinity stress on Czapex Dox agar medium. These results exhibited that 6 fungal isolates namely FS12, FS14, FS16, FW1, FW2 and FF3 among 58 isolates were capable to produce IAA on solid medium under salinity stress at 0.5% NaCl which gave red zone diameter ranged from 3.2 to 4.4 mm with IAA production index ranged from 0.25 to 0.56. Whereas, 3 fungal isolates (FW1, FW2 and FF3) among 9 isolates were tolerated to grow on 1% NaCl. These isolates recorded that diameter of red zone being 3.8, 4.0 and 3.8 mm with IAA production index being

0.47, 0.38 and 0.47, respectively. Another tested isolates have not ability to hydrolyze tryptophan into IAA under salinity stress. From these results, it was observed that FS12 isolate gave the highest red zone diameter of IAA being 4.1 mm with IAA production index reached to 0.56.

From previous result, it could be concluded that 9 halo-tolerant isolates were capable of producing IAA growth promoters on solid medium. Where, six fungal isolates only gave IAA on solid medium containing 0.5% but also all isolates cannot grow at 1.0%. Furthermore, three fungal isolates with codes FS12, FS14 & FS16 were preferred to IAA production on solid medium with 0.5% NaCl, so these halo-tolerant isolates were selected for next investigation.

These results are in agreement with **[20]** who reported that relatively few attempts have been made to evaluate yeasts as plant growth promoters and even fewer as biocontrol agents for the management of soil-borne fungal plant pathogens. However, **[21]** suggested that IAA, GA3 and GA4 as metabolites potentially involved in plant growth responses toward root colonizing fungi. These compounds were found to be widely produced by plant-associated fungi.

Quantitative determination of plant growth promoter's production by the selected isolates

Data in **Fig. 1** revealed that the tested fungal isolates were tested for IAA and gibberellic acid production (GA) in presence of NaCl at 0.5 or 1-% in Czapex Dox broth medium. At 0.5-% NaCl, the highest significant production of IAA (4.32 mg/100 mL~~l~~) and GA (4.52 mg/100 mL~~l~~) were achieved by FS12 isolate with cell dry weight being 1.20 g/L. However, at 1% NaCl, the maximum significant of IAA (2.71mg/100 L~~m~~l) and GA (2.92 mg/100 m~~l~~L) production were recorded by FW2 isolate, with cell dry weight of 0.89 g/L.

Result indicated that the production of IAA and GA by the best fungal isolates in presences of 0.5% NaCl were preferred than production at 1% NaCl, it might be due to high concentration of NaCl delayed IAA production have been reported by **[22]** who confirmed that the tested strains of *Pseudomonas* produced IAA in present of NaCl with concentration ranged from 0 to 0.75 % and the production of IAA was delayed above 0.75 % NaCl. Moreover, it was observed that the production of growth promoters by the tested fungal isolates was better than bacterial isolates. So, the fungal isolate of FS12 was selected as a high significant growth promoter producing isolate for further studied. Furthermore, **[23]** stated that gibberellin (GA) and indol-acetic acid (IAA) are secondary metabolites, which are important biotechnological products, produced commercially from fungi for the agriculture and horticultural industry. Moreover, the contents of GA and IAA were significantly increased at 0.5

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and 1% NaCl after 5 days, but they were lowered at 4% NaCl. In addition, the salinity represents one of the most important factors exerting stress on fungal as well as plant cell metabolism.

Identification of the selected fungal isolate (FS12)

According to the morphological properties (microscopic shape and color of conidia) of fungal isolate FS12 which were subjected to the preliminary classification to be the genus *Aspergillus* according to **[13]**. This isolate showed granular colonies on Czapek's Dox agar. The colonies were flat, with radial grooves. Microscopic observation of the fungal isolate indicated erect conidiophores with globose vesicles bearing chains of conidia **(Fig. 2)**. In this respect, different species of *Aspergillus* have been reported as efficient plant growth promoters' production **[24]**.

Effect of carbon and nitrogen sources on PGP Produced by *Aspergillus Niger* FS12

Carbon sources

Five carbon sources (glucose, fructose, sucrose, black strap molasses and whey) were tested to produce IAA and GA by *A. niger* FS12 in presence of 0.5% NaCl. Data in **Table 3** indicate that the highest production of IAA and GA by the tested strain being 7.16 mg/100 mL and 5.93 mg/100 mL and were recorded in presence of whey with cell dry weight of 2.40 g/L followed by black strap molasses and fructose, respectively. While added sucrose in production medium, the strain gave the lowest production of growth promoters of IAA (2.59 mg/100 mL) and GA (3.22 mg/100 mL).

Furthermore, it could be stated that the production of IAA and GA by *A. niger* FS12 in presence of whey as by-product was preferred than glucose which increased up to 17.5 folds and 3.1 folds, respectively. The tested fungus was preferred whey because it is as a nutrient source for fungal development due to the low protein and high carbohydrate levels that result in a carbon to nitrogen ratio of approximately 5:1 **[25].**

Nitrogen sources

Effect of 10 different nitrogen sources, 4 inorganic sources (ammonium chloride, tri-ammonium citrate, tri-ammonium orthophosphate and ammonium nitrate) and 6 organic sources (mixture of beef extract and~~&~~ peptone, beef extract, tryptone, peptone and malt extract) on the production of IAA and GA by *A. niger* FS12 were presented in **Table 4.** Data revealed that the maximum production of IAA (8.26 mg/100 mL~~l~~) and GA (15.31 mg/100 mL~~l~~) were recorded in medium supplemented with peptone followed by tryptone then soybean extract and ammonium nitrate. Whereas, beef extract and ammonium chloride were giving the lowest production of growth promotors being 5.77 and 5.79 mg/100 mL~~l~~ of IAA and ~~(~~10.47 and 10.64 mg/100 mL~~l~~~~)~~ of GA.

So, *A. niger* FS12 used peptone as an organic nitrogen source for IAA and GA production which increased about 15.4% and 71.3% more than production in control medium containing mixture of beef extract and peptone.

Conclusions

Nine halo-tolerant fungal isolates out of 58 isolates gave IAA and GA on~~-~~/in solid or broth medium under salt stress. FS12 isolate was chosen from nine~~9~~ isolates as the pioneer isolate produce a high amount of PGR at 0.5 % NaCl. This isolate was classified as *Aspergillus niger* strain. So, the PGR product and halo-tolerant strain will be applied in the agriculture field in future.

Comment [TI4]: Use complete name

Reference

Comment [TI5]: Put the references with the same font of letters. Standardize.

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Table 1. Isolation of halo-tolerant fungal isolates from different slat sources on PDA medium supplemented with 0.5 and 1-% NaCl.

Sources of isolation	Isolate code	Growth on NaCl concs. of		Isolate code	Growth on NaCl concs. of	
		0.5-%	1-%		0.5-%	1-%
Salt Soil	FS1	+	+	FS14	+	+
	FS2	+	+	FS15	+	+
	FS3	+	+	FS16	+	+
	FS4	+	+	FS17	+	+
	FS5	+	+	FS18	+	+
	FS6	+	+	FS19	+	+
	FS7	+	+	FS20	+	+
	FS8	+	+	FS21	+	+
	FS9	+	-	FS22	+	+
	FS10	+	-	FS23	+	+
	FS11	+	+	FS24	+	+
	FS12	+	+	FS25	+	+
	FS13	+	+			
Whey	FW1	+	+	FW10	+	+
	FW2	+	+	FW11	+	+
	FW3	+	-	FW12	+	+
	FW4	+	+	FW13	+	+
	FW5	+	+	FW14	+	+
	FW6	+	-	FW15	+	+
	FW7	+	+	FW16	+	+
	FW8	+	+	FW17	+	-
	FW9	+	+			
Salt fish	FF1	+	-	FF9	-	+
	FF2	+	+	FF10	+	+
	FF3	+	+	FF11	+	+
	FF4	+	+	FF12	+	+
	FF5	+	+	FF13	+	+
	FF6	+	-	FF14	+	-
	FF7	+	+	FF15	+	+
	FF8	+	+	FF16	+	-

concs. = concentrations, + = growth, - = no growth

Table (2) Qualitative estimation of growth promoters IAA produced by halo-tolerant isolates on solid media containing 0.5% and 1.0% NaCl.

Isolates code	NaCl concentrations						Isolates code	NaCl concentrations					
	0.5%			1%				0.5%			1%		
	GD (mm)	RZD (mm)	IAA Index	GD (mm)	RZD (mm)	IAA Index		GD (m)	RZD (mm)	IAA Index	GD (mm)	RZD (mm)	IAA Index
FS1	2.7	0.0	0.00	2.2	0.0	0.00	FW5	1.8	0.0	0.00	2.8	0.0	0.00
FS2	2.0	0.0	0.00	1.8	0.0	0.00	FW6	2.6	0.0	0.00	-	0.0	0.00
FS3	3.6	0.0	0.00	1.4	0.0	0.00	FW7	2.0	0.0	0.00	2.0	0.0	0.00
FS4	4.8	0.0	0.00	2.5	0.0	0.00	FW8	2.5	0.0	0.00	2.8	0.0	0.00
FS5	2.1	0.0	0.00	1.9	0.0	0.00	FW9	1.8	0.0	0.00	2.8	0.0	0.00
FS6	1.6	0.0	0.00	2.0	0.0	0.00	FW10	2.6	0.0	0.00	2.8	0.0	0.00
FS7	1.9	0.0	0.00	2.5	0.0	0.00	FW11	0.9	0.0	0.00	-	0.0	0.00
FS8	2.1	0.0	0.00	1.7	0.0	0.00	FW12	2.8	0.0	0.00	1.5	0.0	0.00
FS9	1.6	0.0	0.00	-	0.0	0.00	FW13	2.5	0.0	0.00	1.8	0.0	0.00
FS10	1.5	0.0	0.00	-	0.0	0.00	FW14	2.3	0.0	0.00	1.8	0.0	0.00
FS11	1.6	0.0	0.00	2.0	0.0	0.00	FW15	2.1	0.0	0.00	1.5	0.0	0.00
FS12	1.8	4.1	0.56	2.4	0.0	0.00	FW16	2.9	0.0	0.00	1.6	0.0	0.00
FS13	1.0	0.0	0.00	2.1	0.0	0.00	FW17	2.7	0.0	0.00	NG	0.0	0.00
FS14	2.0	3.5	0.43	1.4	0.0	0.00	FF1	2.1	0.0	0.00	2.0	0.0	0.00
FS15	1.7	0.0	0.00	1.0	0.0	0.00	FF2	2.5	0.0	0.00	2.0	0.0	0.00
FS16	2.4	3.2	0.25	2.1	0.0	0.00	FF3	2.9	4.4	0.34	2.0	3.8	0.47
FS17	3.1	0.0	0.00	2.4	0.0	0.00	FF4	2.3	0.0	0.00	1.6	0.0	0.00
FS18	2.5	0.0	0.00	2.5	0.0	0.00	FF5	2.1	0.0	0.00	NG	0.0	0.00
FS19	2.0	0.0	0.00	2.2	0.0	0.00	FF6	2.2	0.0	0.00	NG	0.0	0.00
FS20	2.8	0.0	0.00	1.4	0.0	0.00	FF7	1.5	0.0	0.00	1.9	0.0	0.00
FS21	2.1	0.0	0.00	1.3	0.0	0.00	FF8	2.8	0.0	0.00	1.4	0.0	0.00
FS22	1.1	0.0	0.00	1.2	0.0	0.00	FF9	2.2	0.0	0.00	2.5	0.0	0.00
FS23	1.1	0.0	0.00	2.2	0.0	0.00	FF10	2.6	0.0	0.00	2.1	0.0	0.00
FS24	2.0	0.0	0.00	2.0	0.0	0.00	FF11	1.9	0.0	0.00	NG	0.0	0.00
FS25	2.0	0.0	0.00	2.1	0.0	0.00	FF12	2.3	0.0	0.00	1.5	0.0	0.00
FW1	2.0	3.3	0.39	2.0	3.8	0.47	FF13	2.0	0.0	0.00	2.2	0.0	0.00
FW2	2.0	3.5	0.43	2.5	4.0	0.38	FF14	2.2	0.0	0.00	1.6	0.0	0.00
FW3	2.0	0.0	0.00	NG	0.0	0.00	FF15	1.6	0.0	0.00	1.7	0.0	0.00
FW4	1.5	0.0	0.00	7.0	0.0	0.00	FF16	1.9	0.0	0.00	1.9	0.0	0.00

GD = Growth diameter, RZD = Red zone diameter, IAA = - = No growth.

Comment [TI6]: Put name

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Table 3. Impact of carbon sources on biomass and plant growth promoters production by *A. niger* FS12 on medium supplemented with 0.5% NaCl.

Carbon sources	*A. niger* FS12		
	CDW (g/L)	IAA conc. (mg/100 mL)	GA conc. (mg/100 mL)
Glucose (control)	1.20^{d}	4.32^{c}	4.52^{b}
Fructose	1.60^{c}	5.41^{bc}	3.22^{d}
Sucrose	1.18^{d}	2.59^{d}	2.49^{e}
Black strap Molasses	2.27^{b}	6.35^{ab}	3.77^{c}
Whey	2.40^{a}	7.16^{a}	5.93^{a}

conc. = concentration, CDW = cell dry weight

Means in the same column followed by the same letter do not significantly differ from each other at 5 % level.

Comment [TI7]: Which means test was used?

Table 4. Effect of nitrogen sources on biomass and plant growth promoters production by *Aspergillus niger* FS12 on medium supplemented with 0.1% NaCl.

Nitrogen Source	A niger (FS12 0.5%)		
	CDW (g/L)	IAA conc. (mg/100mL)	GA conc. (mg/100mL)
Ammonium choloride	1.92^{a}	5.79a	10.45b
Tri-ammonium citrate	1.98^{a}	6.84a	10.56b
Tri-ammonium orthophosphate	1.98^{a}	6.64a	10.76b
Ammonium nitrate	2.51^{a}	7.11a	10.64b
Beef extract	1.93^{a}	5.77a	10.47b
Tryptone	2.62^{a}	7.61a	10.79b
Peptone	2.72^{a}	8.26a	15.31a
Soybean extract	2.58^{a}	7.11a	10.63b
Yeast extract	1.89^{a}	7.27a	10.73b
Beef extract + Peptone (control)	2.40^{a}	7.16a	8.93c

conc. = concentration, CDW = cell dry weight

Comment [TI8]: Standardize the letters of the means test

Values in the same column followed by the same letter do not significantly differ from each other at 5 % level.

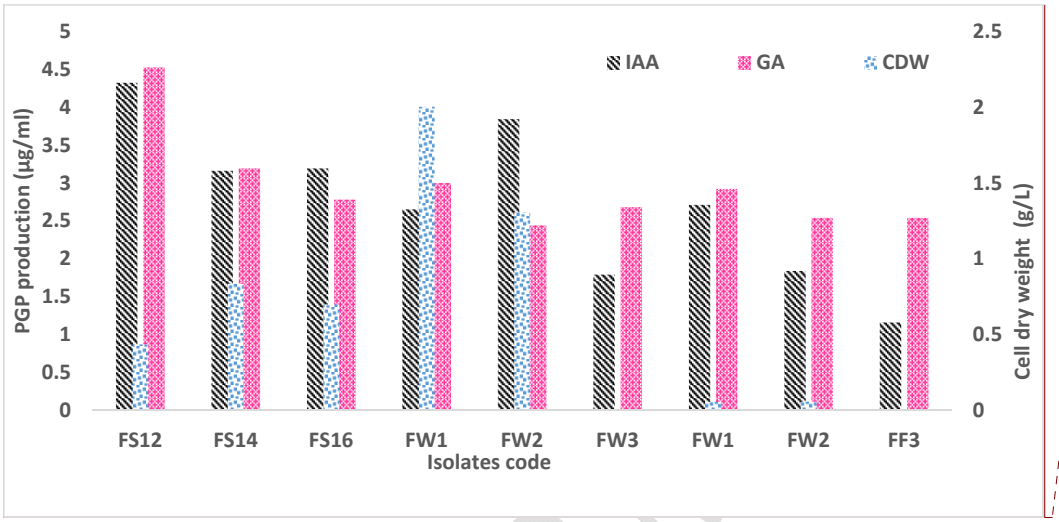

Fig. 1. Cell dry weight, IAA and GA production by halo-tolerant fungal isolates in presence of 0.5 % and 1% NaCl.

PGP = plant growth promoters, IAA = indol aceticacid, GA = Gibberellic acid-, CDW= cell dry weight.

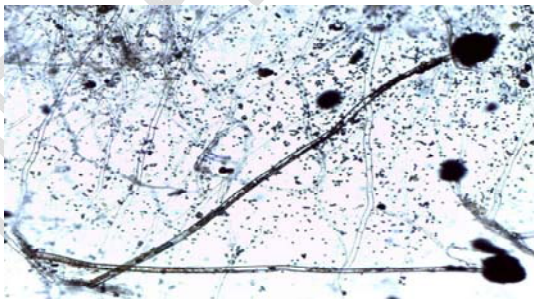

Fig. 2. Morphological of the most active plant growth promoter's production fungal isolate.

Comment [TI9]: Which means test was used?

Comment [TI10]: Standardize

Do a means test to compare the isolates.

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