Egypt. Acad. J. Biolog. Sci., 12(2):111-129(2020)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/

Biosynthesis of Plant Growth Hormones (Indol Acetic Acid and Gibberellin) By Salt-Tolerant Endophytic Fungus *Aspergillus terreus* SQU14026

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ARTICLE INFO

Article History
Received: 29/10/2020
Accepted:26/12//2020

***Keywords*:**
Indol Acetic Acid , Gibberellin, *Aspergillus terreus*, salt-tolerant plants.

ABSTRACT

Twenty-seven fungal isolates were isolated from salt-tolerant (halophytes) plants, which were collected randomly from the planted field in Wadi El-Natroun. All isolates screened for production of plant growth hormones (GA and IAA) production and salt tolerance. All isolates can tolerate salts and produce GA and IAA in varying degrees. The maximum IAA and GA production were obtained by isolating 19 (0.098 and 0.77 µg ml^{-1} respectively), which can tolerate salts (NaCl) up to 15% with MSC 20 mgl^{-1}. According to DNA and PCR amplification, isolate 19 was identified as *Aspergillus terreus* strain SQU14026. The optimum culture conditions for a maximum of production growth hormones by *Aspergillus terreus* strain SQU14026 were studied. The maximum yield was obtained on the 8th day of incubation at incubation temperature 30°C and pH6 on Czapeks Dox medium containing 10% NaCl, using glucose and ammonium sulphate concentrations (20g and 3g respectively). The high level of IAA and GA production attained by adding 0.2 mg / ml tryptophan to the growth medium under controlled conditions, the production increased by 124.7% and 48.19% respectively compared to their production at 8th day.

INTRODUCTION

Microbial endophytes colonize plant tissues without symptomatic behavior and consequently, they compete with other microbial pathogens on the same ecological niches. Therefore, the established plant-endophyte association improves plant health via different mechanisms displayed by endophytes and potentially contributes to the protection of plant hosts against microbial pathogenesis (Malhadas *et al.*, 2017). Plant growth-promoting endophytes (PGPE) produce various bioactive compounds with several biological activities which can be directly or indirectly described as plant growth-promoting (PGP) agents. Approximately most of the plants harbor endophytes interior their tissues; however, available information on PGPE and their biological activities is not equivalent to the high distribution of endophytes.

A superior comprehension of the native endophytes of plants may help clarify their capacities and potential in enhancing plant growth and establishing a sustainable system for crop production (Hassan, 2017). The presence of several microorganisms synthesizes IAA or GA as secondary metabolites through the tryptophan pathway are a very important factor in soil fertility (Mandal *et al.,* 2007). Plant growth-promoting endophytes (PGPE) inhabit plant tissues and the close linkage of endophytes inside plant tissues facilitates nutrient exchange and enzymes activity (Khan *et al.,* 2015). The distribution of growth-promoting hormones produced by endophytic microorganisms towards plant tissues positively promotes plant growth (Lin and Xu , 2013).

Phytohormones which could produce by microorganisms are known to play vital roles in plant growth and establishment by helping plants to acclimatize to varying environments (Fahad *et al.,* 2015). Khan *et al.,* (2015) established that two types of hormones are available to plants, one is endogenous production by plants and the second is exogenous production by microorganisms. Several phytohormones control many physiological and biochemical processes like abscisic acid, gibberellins, ethylene, auxins, cytokinins, and brassinosteroids (Iqbal *et al.,* 2014). Indole-3-acetic acid extensively was the first identified plant hormone and the most important member of the auxins family of phytohormones (Fahad *et al.,* 2015). It plays a vital role in physiological processes e.g. root initiation, production of longer roots, tissue differentiation, increase number of root hairs and lateral roots which are involved in nutrient uptake (Datta and Basu, *2000*).

Bajguz (2009) reported that a number of microbial strains produce a variety of phytohormones including IAA, gibberellic acid, proline, and zeatin (cytokinins). All these hormones play a pivotal role in the enhancement of plant growth and productivity not only under normal conditions but also under stress conditions especially salinity, drought, temperature, and oxidative and photogenic stresses (Cassan *et al.,* 2009).

Takahashi (2013) revealed that indole-3-acetic acid intracellular plant concentration is controlled by the biosynthesis and degradation process. Like plants, a number of microorganisms residing in the soil also produce phytohormones; however, their pathways for hormone production may be different from plants, a number of fungi present in the soil environment are also able to produce growth hormones (Hamayun *et al.,* 2010). Some fungi have demonstrated the ability to synthesize phytohormones that are directly associated with plant development, such as auxins and gibberellins (Contreras-Cornejo *et al.,* 2014). This phytohormone is known to act in the division and cell elongation process, since it activates enzymes that act on components of the bonds between cell wall cellulose microfibrils, increasing their plasticity (Castro *et al.,* 2001). Consequently, water enters the cells more easily and rapidly increases their size (Taghavi *et al.,* 2009).

Akhtar *et al.,* (2005), reported that 72 % of fungal strains isolated from wheat, maize, potato, and tomato, respectively, were able to produce phytohormone.

Khan *et al.,* (2011), Soil fungi and endophytes secrete plant growth-promoting substances such as indole acetic acid (IAA) and gibberellins Khan *et al.,* (2014) Indeed, the IAA produced by fungi that inhabit the rhizosphere have been shown to enhance root growth and nutrient availability by occupying large areas of fertile soil, leading to increased plant biomass production and disease resistance (Ahemad and Kibret, 2014). Additionally, certain endophytic fungi have been reported to secrete GAs and IAA into culture media (Redman *et al*., 2011).

MATERIALS AND METHODS

Sample Collection:

The halophytic plant's samples which were screened for their endophytic assemblages were collected from different areas in and around Wadi El-Natroun (a

north-westerly oriented desert depression about 60 kilometers long located in the Western Desert near the delta about 90 kilometers northwest of Cairo). Healthy plant samples were collected from saline soil. To acquire endophytes healthy plant samples from their natural habitat, separate, sterile polythene bags were used. The samples were brought into the laboratory and processed within 24 hours according to Fisher and Petrini (1987). The plants which were collected from different places were mixed together for random endophytic isolation. These halophyte plants are named: sugar beet plant (*Beta vulgaris)*, Aubergine (*Solanum melongen*a), zucchini (*Cucurbita pepo)*, boss (*Arundo*), halfabr1-halfabr2 (*Imperata cylindrica*), wheat (*Triticum aestivum*), Barley (*Hordeum vulgare).*

Culture Media:

Potato Dextrose Agar (PDA):

The medium contains (gl^{-1}): glucose, 20.0; agar-agar 20.0; potatoes filtrate from 1L. The potato filtrate was prepared by using 200.0 g of peeled potatoes that were then diced. 500 ml of distilled water was then added heated gently to boiling for 30 min. The medium was then filtrated and the volume of the filtrated was completed to 1.0 L to produce 1L of potato infusion. Glucose and agar were then added and mixed thoroughly. The medium was then heated gently to boiling. The medium was autoclaved at 121˚C for 15 min (Paterson and Bridge, 1994).

Czapeks Dox Medium:

The medium contained (gl^{-1}): Sucrose, 30.0; Sodium Nitrate ($NaNO_3$), 3.0; Di-potassium Hydrogen Orthophosphate (K_2HPO_4), 1.0; potassium chloride (KCL), 0.5; Magnesium Sulphate ($MgSO_4.7H_2O$), 0.5; Ferrous Sulphate ($FeSO_4.5H_2O$), 0.001. Medium components were dissolved in one liter of distilled water and autoclaved at 121˚C for 20 min (Atlas and Barther, 1997). The medium was supplemented with sterilized 0.1 gl^{-1} tryptophan for IAA and GA production.

Isolation and Purification of Endophytic Fungi:

Endophytic fungi were isolated from endemic plants by the procedure of standardized and modified method described by Hallman *et al.,* (2007). Stems, roots and leaves of healthy and naturally grown salt-tolerant plants were processed within a few hours to reduce the chances of contamination. Plant explants after proper washing were cut into 0.5-1 cm long sections, and leaves were cut into 0.5 cm x 0.5 - 1cm sections without midrib under aseptic conditions. Explants were then surface sterilized by dipping them in 70% ethanol for 30 seconds, then submerged in 13% commercial bleach solution sodium hypochlorite solution (Clorox) for 3 min, followed by three rinses with sterile distilled water. Explants were then placed in sterilized Petri dishes containing sterilized potato dextrose agar (PDA) supplemented with chloramphenicol (50 mg l^{-1}) to suppress bacterial growth**.** All the dishes were sealed with par film and incubated at 28 °C for up to 7 days, and emerging fungi were purified until single uniform colonies were obtained then transferred into potato dextrose agar slants and preserved (Suryanarayanan *et al.,* 2003).

Identification of Fungal Isolates:

One of the total 27 fungal isolates was identified at the Regional Center for Mycology and Biotechnology (RCMB) Al-Azhar univ. Cairo, Egypt using image analysis system (Leica CTR 5000, 280 DFC). The endophytic fungus was transferred to malt extract media and incubated with shaking (180 rev min^{-1}) at 25°±2C for 7 days. Mycelia were collected by centrifugation and DNA was extracted. Purified DNA was subjected to PCR amplification using primers ITS1 and ITS4. Fungal identification methods were based on their internal transcribed spacer ribosomal DNA (ITS-rDNA rRNA) sequences. A pair of primers ITS1 (5′-TCC GTAGGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) was used for ITS-rDNA (rRNA) amplification (White *et al.,* 1990), which produces an amplicon of approximately 550 bp of the ITS region. Sequence data was analyzed in the Gene Bank database by using the BLAST program available on the National Center for Biotechnology Information

website (www.ncbi.nlm.nih.gov). The unknown sequence was compared to all of the sequences in the database to assess the DNA similarities (Altschul *et al.*, 1997). Alignment and molecular phylogeny were evaluated using Bio Edit software. Purification and sequencing of PCR products for the isolate under study were performed in the Sigma Company of Scientific Service.

Preparation of Liquid Culture (Fermentation condition):

Plates of PDA medium were inoculated by fungal isolates and incubated at 28°±2C for 7 days. One disk of 1 cm diameter 7days old cultures of each isolate (hold about 1.1×10^6) was used for the inoculation of 100 ml Erlenmeyer flasks each contained 50 ml of sterile potato dextrose broth medium and incubated in a rotary shaker incubator at 150 rpm and 28°C for 4 days.

Quantitative Determination of Plant Growth-Promoting Hormones In Culture Media Of Fungal Isolates:

The fungal broth (30 ml) was adjusted to pH 8.6 with 1% Na OH and extracted 3 times with equal volumes of ethyl acetate. The aqueous phases were adjusted to pH 2.8 with 1% HCL and extracted 3 times with equal volumes of ethyl acetate and the remaining aqueous phase was discarded. While the acidic ethyl acetate fractions were used for the determination of acid hormones such as indol acetic acid (IAA)and Gibberellin (GAA) (Shindy and Smith, 1973). Determination of indol acetic acid (IAA) and Gibberellin (GA) according to the method described by Glickman and Dessoux (1995) and Udagwa and Kinoshita (1961), respectively.

Determination of Tolerance Index of Na Cl:

The isolated fungi were grown separately on Dox media supplemented with different concentrations of sodium chloride (1-20%). the plates were inoculated with a 5 mm disc of pre-grown 7 days isolate culture. Three replicates of each concentration and control without salt were used. The inoculated plates were incubated at 28±2°C for 7 days. The lowest salt concentration that allows isolating growth will be detected as its Minimum I Salt Concentration (MSC) tolerated by the fungal isolate. The growth was estimated by measuring the radius of the colony extension (mm) against the control and the determination of the index of tolerance.

Parameters Controlling Indol Acetic Acid and Gibberellin Production by Selected Endophytic Fungi:

Effect of Different NaCl Concentration:

The selected fungal isolate was cultivated on Czapeks Dox broth medium by adding sodium chloride (NaCl) with concentrations of 5,10,15 and 20 % (w/v). The medium was supplemented with 0.1 g l^{-1} tryptophan (the precursor of IAA) and incubated for 7 days at 28±2°C in a shaker incubator adjusted at 150 rev min^{-1}.

Effect of Different Incubation Periods:

In order to determine the time at which maximum production of hormones by selected fungal isolates, the culture was allowed to grow on 50ml Czapek's Dox broth medium supplemented with 0.1 g l^{-1} tryptophan and incubated at 28±2°C under shaking condition at 150 rev min^{-1}. The hormones production was detected at different periods of incubation (1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 14 days).

Effect of Different Incubation Temperature:

The influence of different incubation temperatures on hormones production by the selected fungus was carried out by incubating the isolate under shaking conditions at 150 rev min^{-1} at different temperature ranges (20, 25, 30, 35 and 40 °C).

Effect of Different pH:

The optimum pH at which maximum yield of hormones by selected fungal isolate was determined by adjusting the medium at different pH values. Six pH values (3, 4, 5, 6, 7, 8, 9) were selected for this study.

Effect of Different Carbon Sources:

In this experiment, the effect of the addition of different carbon sources to C'zapeks Dox broth medium on the production of hormones was estimated. The medium was supplemented with 30 g l^{-1} of

each carbon source separately. The tested carbon sources were glucose, starch, sucrose, fructose, maltose, lactose, xylose, mannose, galactose, cellulose, and mannitol.

Effect of Different Nitrogen Sources:

In order to investigate the influence of different organic and inorganic nitrogen sources on hormones production by selected fungi, the medium supplemented with different nitrogen sources separate in amounts equivalent to the amount of nitrogen in sodium nitrate (the original nitrogen of the medium). The tested nitrogen sources were sodium nitrate, potassium nitrate, ammonium nitrate, ammonium oxalate, ammonium sulphate, L-glutamic, L-aspartic, L-serine, L-glutamine, L-aspargine, peptone and yeast extract.

RESULTS AND DISCUSSION

Endophytic Colonization Frequency:

The Colonization Frequency (CF) percentage and the dominant endophytic fungal isolates showed in table (1). The highest colonization Frequency percentage (60%) was obtained from the stem section. On the other hand, the leaf section revealed the lowest number of fungal isolates with a CF percentage of 10%.

Minimum Salt Concentration (MSC) for Isolated Endophytic Fungi:

Data in the table (2) represented the different minimum inhibitory salt concentrations (MISC) against the isolated endophytic fungi, isolates No. 1, 5, 11, 18, 19, 23, 24 25 and 27, revealed the highest MISC (20 mgl^{-1}). This high MISC is related to the resistance of those isolates towards NaCl impact (Stone *et al.*, 1994).

Effect of Different Tryptophan Concentrations:

The fact that tryptophan was the precursor of hormones, made it necessary to study the effect of different concentrations of tryptophan on the synthesis of hormones. Tryptophan was added to the medium in a concentration ranging from 0.05 - 1.0 gml^{-1}. The sterilized medium was inoculated with one disk (5 mm diameter) of 72 hrs old of selected fungi and incubated shaker adjusted at 150 rev min^{-1}.

Production of Indol Acetic Acid (IAA) and Gibberellin by the Isolated Endophytic Fungi:

All isolates of endophytic fungi (27 isolates) are tested for their indol acetic acid and gibberellin production (Table 3). Results showed variable amounts of indol acetic acid and gibberellin production in tryptophan-supplemented medium whereas no indol acetic acid production was observed in the medium devoid of tryptophan. The production of indol acetic acid by the isolates only in the presence of L-tryptophan indicates that the tested isolates utilize L-tryptophan as a precursor for indol acetic acid production. Maximum indol acetic acid and gibberellin production were obtained by isolate 19. So, this endophytic fungus will be used for further studies.

Table 1: Endophytic fungal colonization frequency

Site of isolates	No. of fungal isolates	Colonization Frequency (CF) %
Stem	15	60
Root	7	30
Leaf	5	10
Total	27	100

Table 2: Sodium chloride tolerance by isolated endophytic fungi)

Organism code	MSC (mgl^{-1})	Organism code	MSC (mgl^{-1})
1	20	15	10
2	15	16	10
3	15	17	15
4	15	18	20
5	20	19	20
6	15	20	10
7	15	21	10
8	10	22	10
9	10	23	20
10	15	24	20
11	20	25	20
12	15	26	10
13	10	27	20
14	10		

Table 3: Production of Indol acetic acid and Gibberellin by the isolated endophytic fungi

Organism code	GA (µg ml^{-1})	IAA (µg ml^{-1})	Organism Code	GA (µg ml^{-1})	IAA (µg ml^{-1})
1	0	0	15	0.01	0.01
2	0.01	0.01	16	0.01	0.01
3	0.01	0.01	17	0.006	0.006
4	0.006	0.01	18	0.01	0.01
5	0.07	0.01	19	0.77	0.098
6	0.26	0.015	20	0.06	0.001
7	0.006	0.006	21	0.005	0.04
8	0.01	0.01	22	0.01	0.012
9	0.01	0.006	23	0.01	0.012
10	0.01	0.01	24	0.01	0.006
11	0.57	0.01	25	0.01	0.57
12	0.006	0.01	26	0.006	0.01
13	0.53	0.006	27	0.01	0.006
14	0.01	0.006			

Identification of the Selected Endophytic Fungal Isolated:

Isolate 19 was selected to be identified since it was the best isolate for hormones production. The yellowish-brown to cinnamon colonies were spreading rapidly consisting of a dense felt of conidiophores (Fig. 1a). Conidial heads densely columnar, conidiophore stipe smooth-walled, hyaline. Vesicles subspherical, 10 - 20 µm in diameter. Conidiogenous cells biseriate. Metulea as long as the phialides. Conidia smooth-walled, hyaline, spherical to proudly ellipsoidal, 1.5-2.5 µm in diameter (Figure 1b). According to the Sigma technique the isolate is defined *Aspergillus terreus* (Table 4 and Fig.2) and was identified according to DNA and PCR amplification as strain *Aspergillus terreus* strain SQU14026 (Figs. 2, 3 and 4).

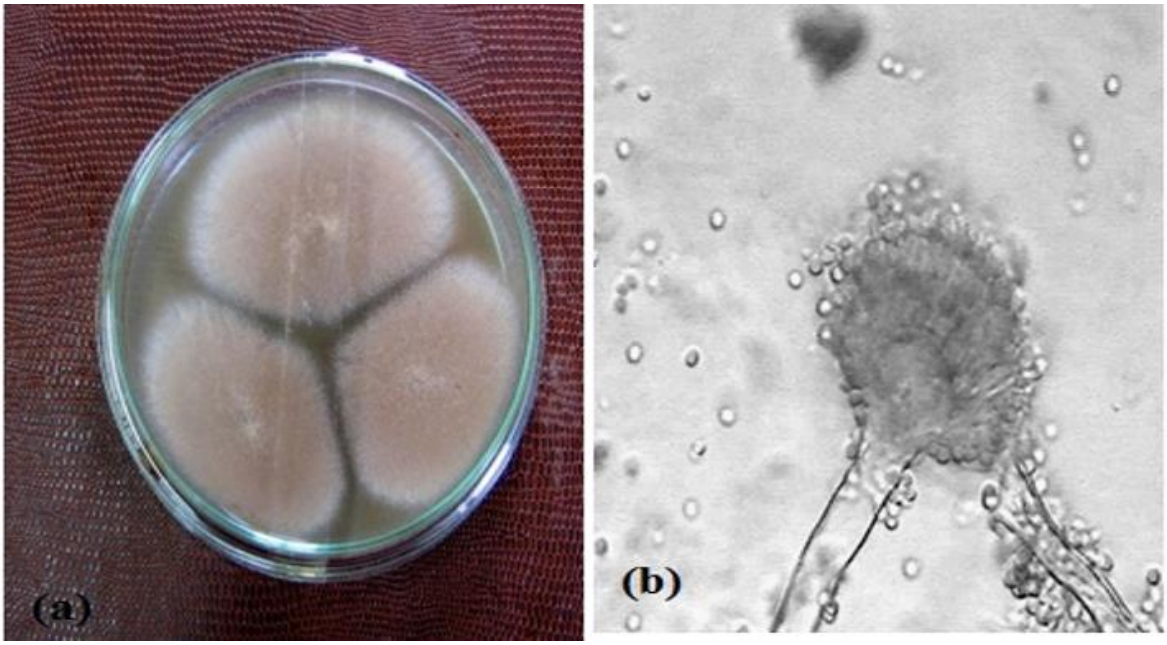

Fig. 1: (**a**) colonial growth of *A. terreus*SQU14026 (**b**) L.M. micrographs showing conidiophore and conidia of *A. terreus*SQU14026.

Table 4: Sequence of ITS-rDNA producing significant alignment for fungal isolate

Description	Max score	Total score	Query cover	E value	Ident	Accession
Aspergillus terreus strain SQU14026 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene	891	891	100%	0.0	99%	KY684268.1
Uncultured fungus clone Asc2-18 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.	891	891	100%	0.0	99%	KX462902.1
Aspergillus sp. PE-2014 strain GM1532 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA g	891	891	100%	0.0	99%	KP175270.1
Aspergillus sp. PE-2014 strain GM837 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge	891	891	100%	0.0	99%	KP175265.1
Aspergillus terreus strain PKKS1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, cc	891	891	100%	0.0	99%	KJ729483.1
Aspergillus terreus isolate BA6 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, com	889	889	100%	0.0	99%	MG725681.1
Aspergillus terreus isolate S28 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i	889	889	100%	0.0	99%	MG654693.1
Aspergillus terreus isolate S27 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i	889	889	100%	0.0	99%	MG654692.1
Aspergillus sp. strain KG_34 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int	889	889	100%	0.0	99%	MG647866.1
Aspergillus tubingensis strain KG_31 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen	889	889	100%	0.0	99%	MG647863.1
Aspergillus sp. strain KG_20 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int	889	889	100%	0.0	99%	MG647852.1
Aspergillus terreus strain NIOSN M-65 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge	889	889	100%	0.0	99%	MG589549.1
Aspergillus terreus strain NIOSN M-44 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge	889	889	100%	0.0	99%	MG589538.1
Aspergillus terreus strain NIOSN M-43 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge	889	889	100%	0.0	99%	MG589537.1
Aspergillus terreus isolate NIHHS305 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	889	889	100%	0.0	99%	KY554980.1
Aspergillus terreus strain NIOSN-SK56-C46 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RN	889	889	100%	0.0	99%	MG584976.1
Aspergillus terreus strain NIOSN-SK56-C14 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RN	889	889	100%	0.0	99%	MG584961.1

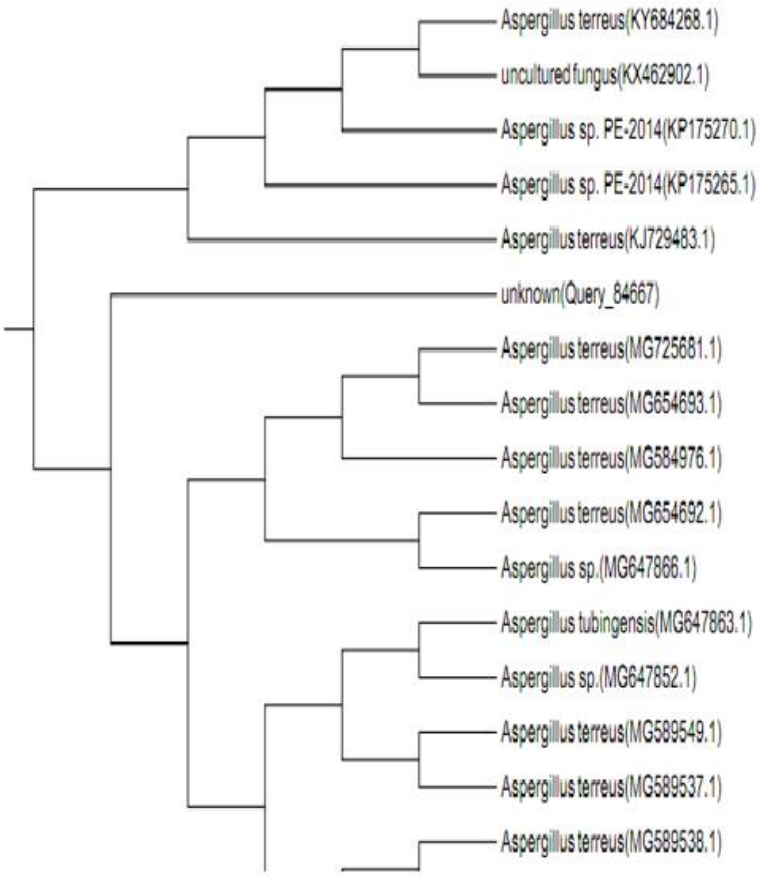

Fig. 2: Phylogenetic tree illustrating the genetic relationship of *Aspergillus terreus* strains and the isolated strain SQU14026.

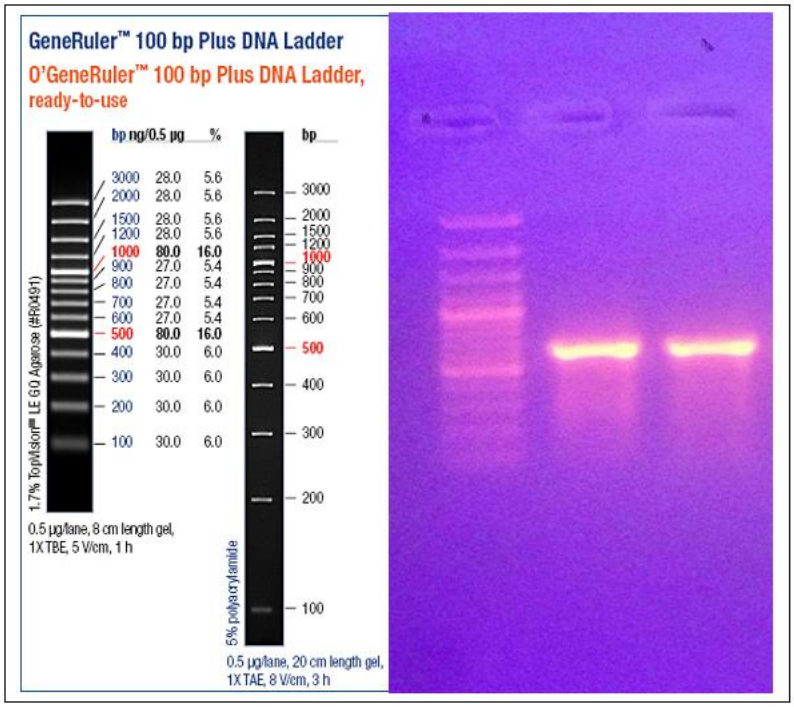

Fig. 3: (**a**) Separation of PCR product of DNA on 1% agrose gel and the sharp band produced by the strain DNA 100bp marker used is shown. and (**b**) Electrophoresis marker.

Fig. 4: *Aspergillus terreus* strain SQU14026 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**.**

Parameters Controlling Indol Acetic Acid IAA and Gibberellin Production by Selected Endophytic Fungus:

1-Effect of Different NaCl Concentrations on IAA and Gibberellin Production by the Selected Endophytic Fungus *Aspergillus terreus* SQU14026:

As shown in figure (5) the lowest production of IAA and GA were at 15% NaCl (1.22 and 10.31µg ml^{-1} respectively), while the best production was at 10 % NaCl with 4.33 and 33.7 µg ml^{-1} for IAA and GA production, respectively**.**

The metabolite biosynthesis in microbes is tightly controlled by regulatory mechanisms to avoid overproduction; yet, these regulatory mechanisms often sometimes process to undesirably low levels. The yield of bioactive compounds can sometimes be substantially increased by the optimization of physical (temperature, salinity, pH and light) and chemical factors (media components, precursors, and inhibitors) for the growth of microbes Miao *et al.,* (2006); Kumara and Rawal, (2008); Thakur *et al.,* (2009); Gautam *et al.,* (2011); Sudarkodi *et al.,* (2012).

Moreover, Singh (2003) reported that the accumulations of metabolic products were strongly influenced by the medium compositions such as carbon source, nitrogen source, and other growth factors. The influence of NaCl on the biomass and bioactive compound production by *Aspergillus terreus* has been reported by Bugni and Ireland (2004) that the maximal activity in bioactive compounds from obligate marine fungi can reach in the media containing 25-50% seawater.

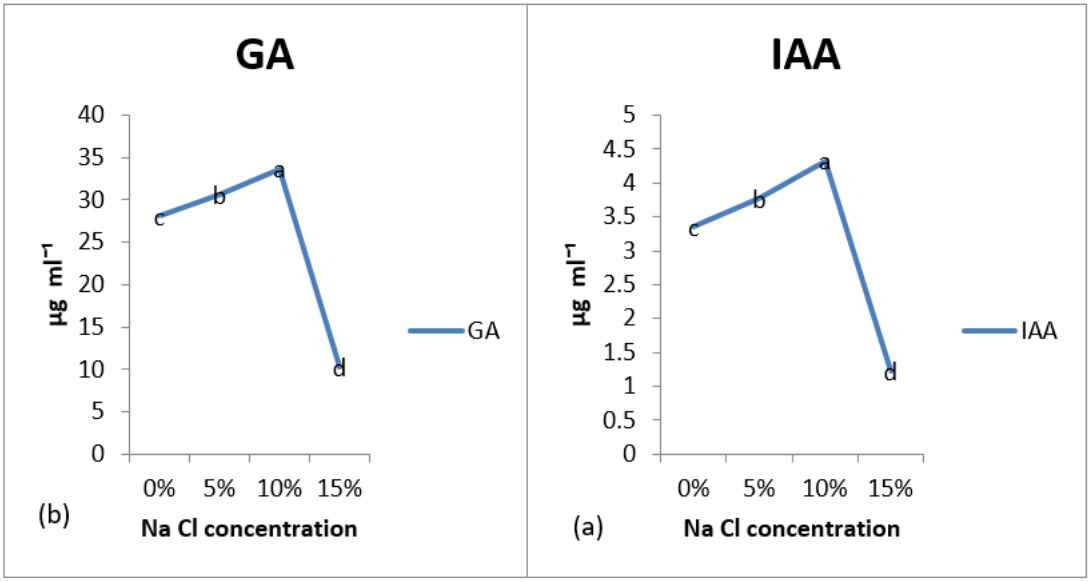

Fig. 5: Effect of different concentration of NaCl on (a) IAA, (b) Gibberellin production by selected fungus *Aspergillus terreus* SQU14026

2-Effect of Different Incubation Periods on IAA and Gibberellin Production by Selected Fungus *Aspergillus terreus* SQU14026:

To investigate the effect of incubation periods (1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14 days) on IAA and GA production, the samples were withdrawn from the production media at 24h. interval up to 14 days. The data obtained illustrated that maximum production was significantly detected at the 8th day which was 6.5 and 40.3 µg ml^{-1} for IAA and GA, respectively (Fig. 6). The results indicated that L-tryptophan from the medium is taken up at a more or less constant rate and transformed to IAA. After the 8th day, both IAA and GA production gradually decreased.

These findings are supported by the previous study of Swain *et al.*, (2007) demonstrated that IAA production increased linearly from 2 to 8 days and decreased later with a decrease in the growth of organisms in L-tryptophan-supplemented medium. Patten and Glick (2002) reported an increase in IAA production up to 96 h and attributed to the greater availability of the precursor.

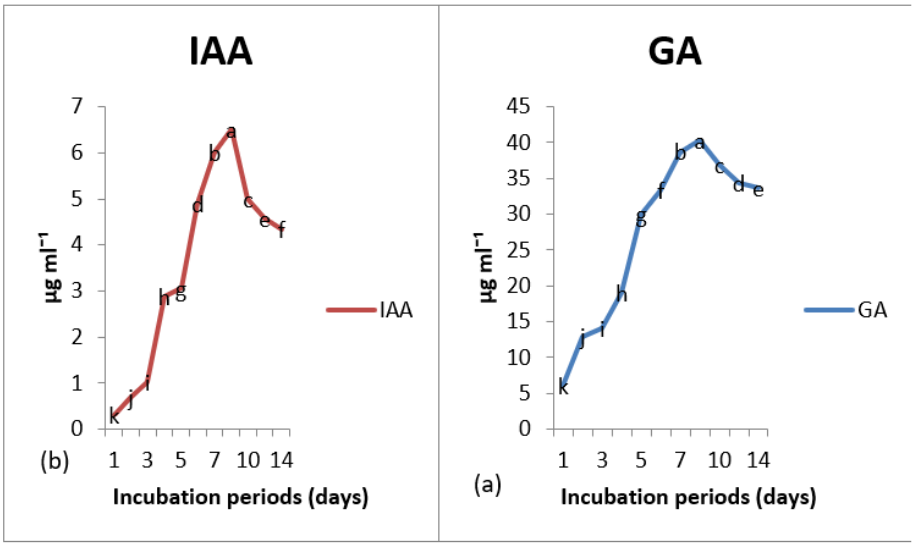

Fig. 6: Effect of different incubation periods on (a) Gibberellin (b) IAA production by selected fungus *Aspergillus terreus* SQU14026

3-Effect of Different Incubation Temperature Degrees on IAA and Gibberellin Production by The Selected Fungus *Aspergillus terreus* SQU14026:

To assess the effect of temperature on IAA and GA production, culture flasks were incubated with different temperature degrees at 20, 25, 30, 35 and 40°C. Drastic reductions of IAA and GA production were at 20°C and 40°C (Fig. 7). The best production for both IAA and GA was at 30ºC (12.07 and 64.41 µg ml^{-1} respectively). Temperature degrees above or below the optimum (30 °C) exhibited a reduction in IAA and GA induction.

Similarly, our results were found in agreement with the previous report, that temperatures in the range 25- 30°C suitable for growth and IAA production by different isolates (Khamna *et al.*, 2010 and Harikrishnan and Shanmugaiah, 2014). The increase of the incubation temperature from 25°C to 30°C enhanced the growth of the cells and production of bioactive metabolite in *Aspergillus* strain (Bhattacharyya and Jha, 2011).

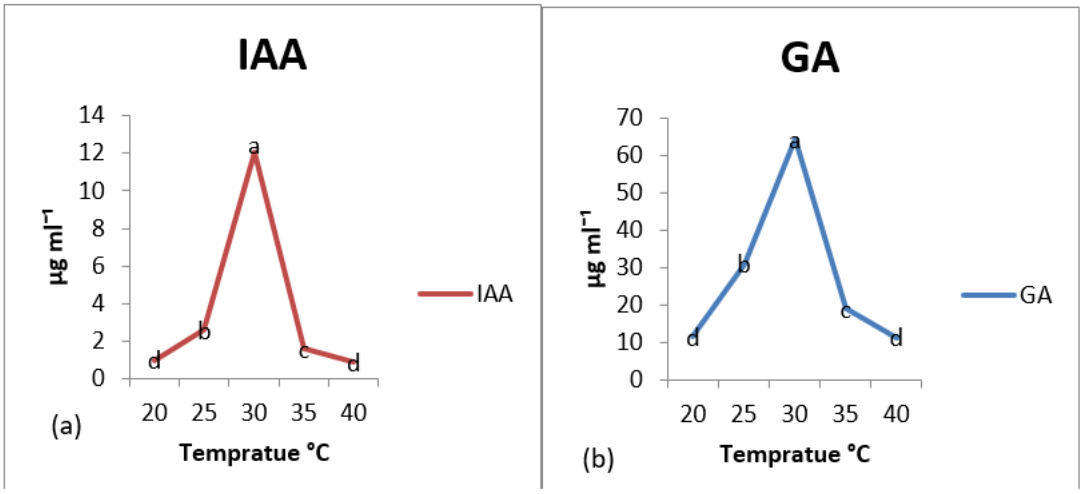

Fig. 7: Effect of different incubation temperature degrees on (a) IAA (b) Gibberellin production by the selected fungus *Aspergillus terreus* SQU14026**.**

4-Effect of different pH on IAA and GA production by the selected fungus *Aspergillus terreus* SQU14026

pH is one of the most physicochemical conditions of the media that affect directly on the biosynthesis of bioactive compounds and this clearly appear in the obtained results (**Fig.8**). The production of IAA and GA was not affected at low pH from 3 -5, but maximum production attained at pH 6, reached 12.07 and 64.40 µg ml^{-1} for IAA and GA respectively. By increasing the pH of the culture medium ranging from 7 to 9, the level of IAA and GA production decreased.

Physicochemical conditions of the media used are always specific for the organisms to biosynthesize the products. One of the most important parameters for the IAA and GA producing organisms and their metabolic activity is the pH of the production media. (Yuan *et al.*, 2011) they studied the effect of different fertilization treatments on soil which leads to change in the pH of soils and in turn affects the IAA production by soil microorganisms. Santi *et al.*, (2007) demonstrated that maximum IAA production was found at pH 7.2 by *Rhizobium sp* isolated from the root nodules of *Vigna mungo* (L.).

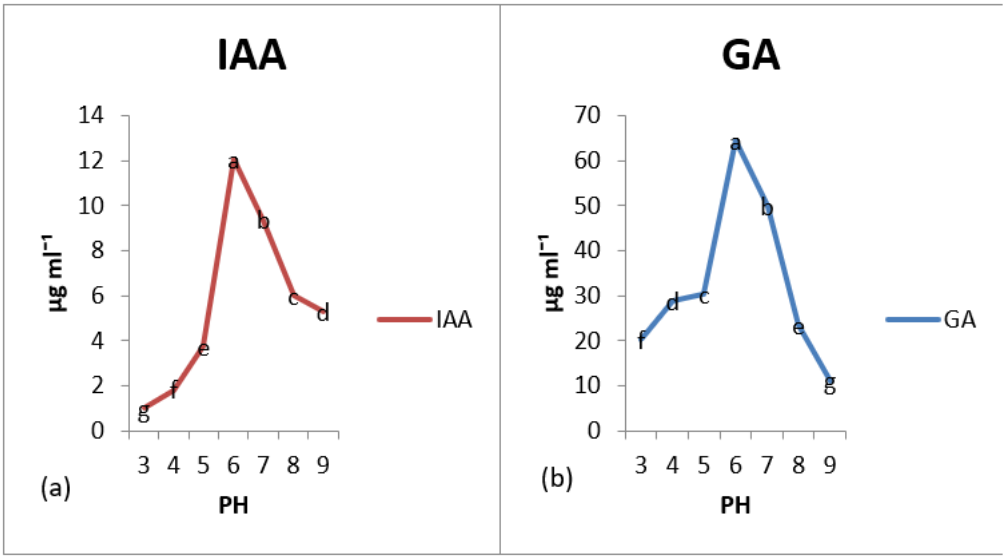

Fig. 8: Effect of different pH on (a) IAA (b) Gibberellin production by selected fungus *Aspergillus terreus* SQU14026

5-Effect of Different Carbon Sources on IAA and Gibberellin Production by Selected Fungus *Aspergillus terreus* SQU14026:

The carbon sources that are used in the biosynthesis of plant growth hormone indol acetic acid and Gibberellin by the experimental fungus during its growth in liquid culture media contribute to the overall efficiency of biosynthesis.

Glucose in the medium exhibited the maximum production of hormones as compared to other carbon sources (14.2 and 73.35 µg ml^{-1} for IAA and GA production respectively) followed by sucrose (12.07 and 64.41 µg ml^{-1} for IAA and GA production, respectively) as shown in figure (9).

On the other hand, the lowest production level of both hormones was found in the presence of cellulose (0.04 and 10.01µg ml^{-1}) mannitol (0.1 and 8.99 µg ml^{-1}) for IAA and GA respectively as a carbon source in the production medium.

These findings of the current study were supported by that of Basu and Ghosh (2001) who reported that biomass to carbon source ratio plays a very important role in cell yield and related IAA and GA production where the preferred carbon source by different soil isolates was glucose.

On the other hand, the results of the present study were contrary to that of Sridevi *et al.*, (2008) who revealed that mannitol and L-glutamic acid were the best promoters for IAA and GA production by different isolates. Bhattacharyya and Jha (2011) reported that *Aspergillus* sp grew on all the carbon sources, and the maximum growth and bioactivity of the strain was noted when the sucrose was used as a sole carbon source.

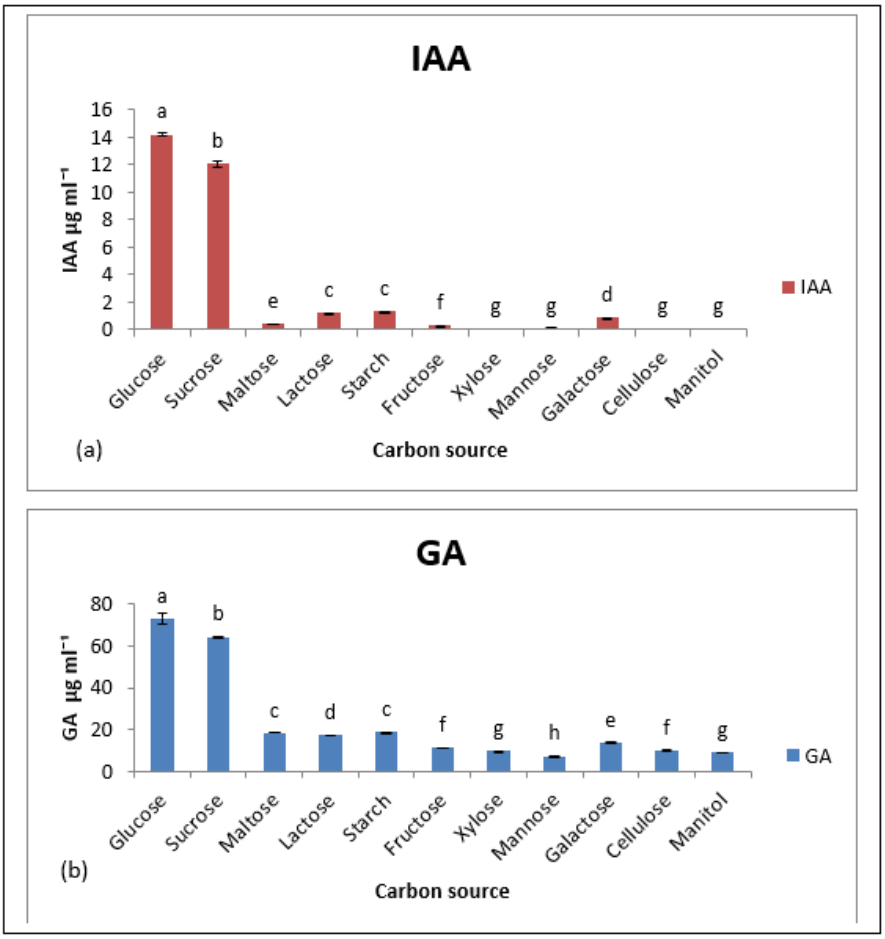

Fig. 9: Effect of different carbon sources on (a) IAA (b) Gibberellin production by selected fungus *Aspergillus terreus* SQU14026

6-Effect of different nitrogen sources on IAA and Gibberellin production by the selected fungus *Aspergillus terreus* SQU14026:

The impact of nitrogen sources on IAA and GA production was studied by addition of various nitrogenous compounds. Different nitrogen sources exhibited variable concentrations of IAA and GA production. As shown in figure (10), ammonium sulphate was the best nitrogen source for IAA and GA production (15.4 and 76.93µg ml^{-1} respectively) followed by sodium nitrate (7.087and 41.98µg ml^{-1} for IAA and GA, respectively).

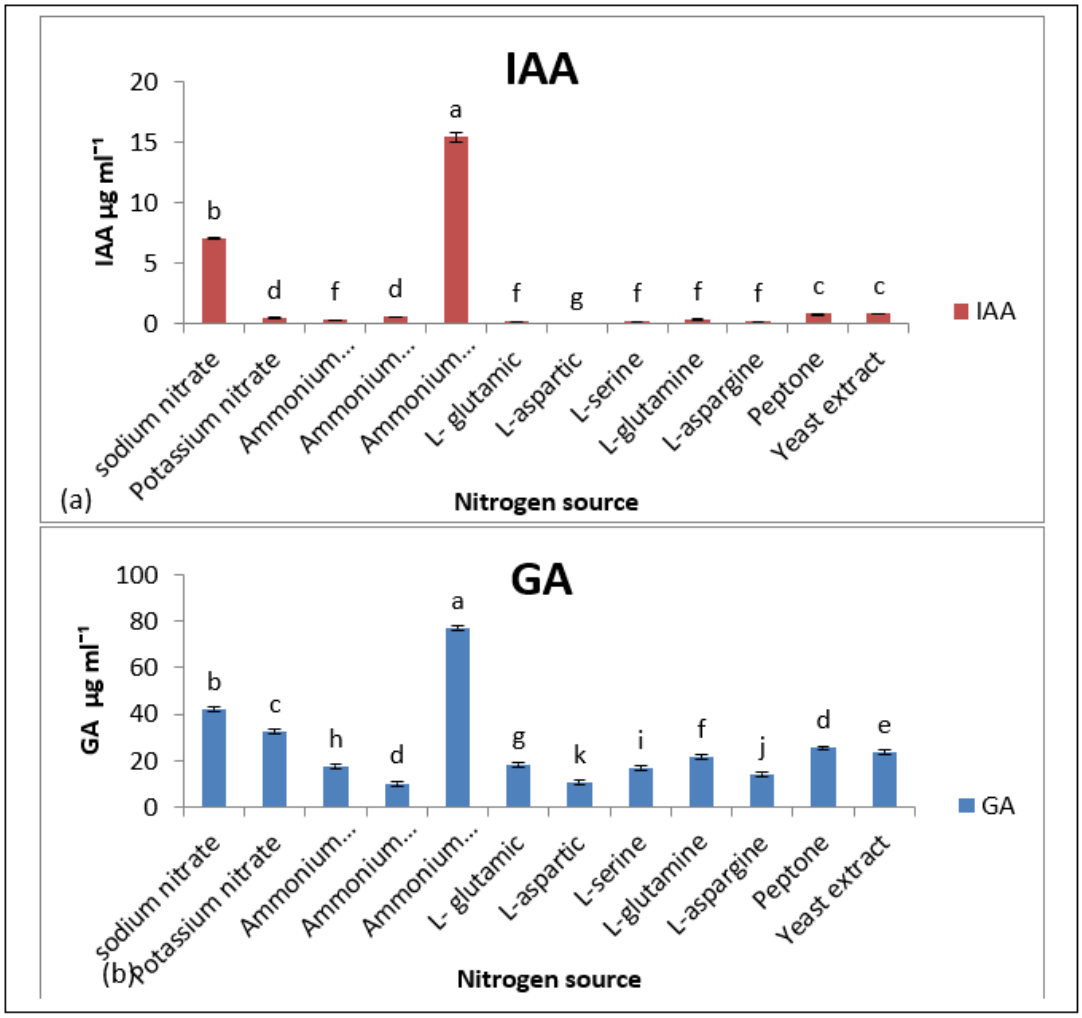

Fig. 10: Effect of different nitrogen sources on (a) IAA (b) Gibberellin production by selected fungus *Asperigillu sterreus*SQU14026

The results of the present study were supported by Bhattacharyya and Jha (2011) who reported that the *Aspergillus* sp. grew on all the nitrogen sources and the maximum growth and bioactivity of the strain was noted when ammonium sulphate was used as a sole nitrogen source. Thakur *et al.*, (2009) showed the effect of different nitrogen sources on biomass and bioactive metabolite IAA and GA) production by *Aspergillus terreus*, and the maximum biomass production was observed in culture supplemented with $(NH4)_2SO_4$.

7-Effect of different L-Tryptophan concentrations on IAA and Gibberellin production by the selected fungus *Aspergillus terreus* SQU14026:

L-tryptophan is considered as a precursor for IAA production because its addition to the medium increases IAA production (Ahmad *et al.*, 2005; Sridevi *et al.*, 2008; Santi *et al.*, 2007).

Different concentrations of L-tryptophan from 0.05 to 1 mg ml^{-1}) were selected to investigate their effect on IAA and GA production. The spectrophometric analysis (Fig.11) showed gradual increase in the IAA production with the increase in L-tryptophan concentration. 0.2 mg ml^{-1} of L-tryptophan concentration in the medium showed maximum IAA and GA production (9.73 and 49.94 µg ml^{-1} respectively).

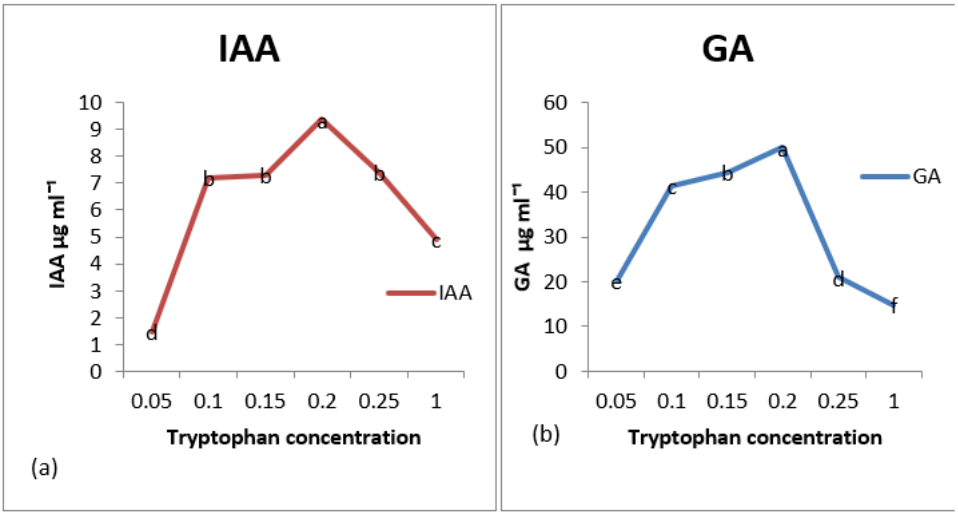

Fig. 11: Effect of different L-Tryptophan concentrations on (a) IAA and (b) Gibberellin production by selected fungus *Aspergillus terreus* SQU14026

Our results are in agreement with the work of Lee *et al.*, (2004) who concluded that in presence of tryptophan, the microbes release greater quantities of IAA and related compounds. Khalid *et al.*, (2004) studied the effect of L-tryptophan concentrations on the production of IAA and observed that L-tryptophan-derived auxin biosynthesis was enhanced several folds.

Ahmad *et al.*, (2005) reported that rhizosphere bacteria *Azoctobacter sp.* and *Pseudomonas sp.* produced to a high level of IAA when this bacterium was cultured in a nutrient broth amended with 2 to 5 µg ml^{-1} of tryptophan. The L- tryptophan as a physiological precursor for IAA production by microorganisms, because microorganism such as *Streptomyces*, *Pseudomonas and Bacillus* is capable of synthesis IAA by utilizing L- tryptophan through indole -3-pyruvic acid pathway (Shanmugaiah *et al.*, 2008; Harikrishnan and Shanmugaiah, 2014; Charulatha *et al.*, 2013).

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ARABIC SUMMARY

التكوين الحيوى لهرمونات نمو النبات (حامض الاندول والجبريلين) بواسطة فطر الاسبرجلس تيرس المتحمل للملوحة

اجلال عبد الله غنيمى - مى أحمد يونس الخواجة - مروة مصطفى عبد العزيز - هند اسماعيل ابو ليله
1-قسم النبات والميكروبيولوجي - كلية العلوم بنات - جامعة الأزهر
2-المركز الاقليمى للفطريات - جامعة الأزهر

تم عزل سبع وعشرين عزلة فطرية من النباتات المتحملة للملوحة، والتي جمعت عشوائياً من النباتات المزروعه في منطقه وادي النطرون. تم اختبار جميع العزلات لإنتاج هرمونات النمو (حامض الجبريلين والاندول) وايضا مدي تحملها للملوحه. وقد أظهرت النتائج قدرة كل العزلات على تحمل الملوحه وإنتاج كلا من حامض الجبريلين والاندول بدرجات متفاوتة. تم الحصول على الحد الأقصى لإنتاج الجبريلين والاندول بالعزله رقم 19 التي اعطت (0.098 و 0.77 ميكروغرام ملتر على التوالي) ، والتي يمكن أن تتحمل الأملاح(كلوريد الصوديوم) حتى 15٪. وفقا لتعريف الحمض النووي و تفاعل البلمرة المتسلسل ، تم تحديد العزلة 19 على أنها سلالة اسبريجللس تيرس(SQU14026) و تمت دراسة الظروف المثلى لتحقيق أفضل إنتاج لهرمونات النمو بواسطة سلالة اسبريجللس تيرس (SQU14026) تم الحصول على اعلي معدل في اليوم الثامن من التحضين عند درجة حرارة الحضانة 30 درجة مئوية و درجه الاس الهيدروجيني 6 على وسط بيئي يحتوي على 10 ٪ كلوريد الصوديوم ، والجلوكوز وكبريتات الأمونيوم (20جم و 3جم) كمصدر للكربون والنيتروجين ارتفع مستوى إنتاج الجبريلين والاندول بإضافة 0.2 ملغم / لتر من التربتوفان إلى وسط النمو ،ووصل الانتاج الي 124.7٪ و 48.19٪ على التوالي مقارنة بإنتاجهما في اليوم الثامن.