ISSN 2449-8955

European Journal of Biological Research

Research Article

Statistical optimization as a powerful tool for indole acetic acid production by *Fusarium oxysporum*

Ghada Abd-Elmonsef Mahmoud[1]*, Hassan H. A. Mostafa[2]

[1] Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut 71516, Egypt

[2] Central Laboratory of Organic Agriculture, Agricultural Research Center, Giza 12619, Egypt

*Corresponding author: Dr. Ghada Abd-Elmonsef Mahmoud; Tel.: 20 1010711661; Fax: 20 88 2342708;

E-mail: ghada_botany@yahoo.com; ghada.moukabel@science.au.edu.eg

Received: 18 August 2017; **Revised submission:** 04 October 2017; **Accepted:** 20 October 2017

DOI: http://dx.doi.org/10.5281/zenodo.1012348

ABSTRACT

Crop production is challenged in our world by increasing food demands, decrease natural resource bases and climatic change. Nowadays plant growth regulators works like fertilizers in increasing plant growth production efficiency and needed to produce in large industrial scale. Fermentation condition and medium constituents can significantly affect on the product production and designing an acceptable fermentation medium is critical importance. In this paper *Fusarium* sp. could be considered as promising indole-3-acetic acid producers with the ability to improve the production using statistical methods. The results showed that fermentation type, incubation temperature and L-tryptophan were the most influencing parameters on the production. Maximum IAA production by *Fusarium oxysporum* was 300.4 mg/l obtained under the fermentation conditions: temperature at 25^{o}C, incubation period 5 days, pH 7, inoculums size 2%, shaking rate at 150 rpm and medium constituents: Glucose 40 g/l, yeast extract 3 g/l, L-tryptophan 1 g/l, KH_2PO_4 2 g/l, $NaNO_3$ 4 g/l, $MgSO_4 \cdot 7H_2O$ 0.1 g/l with regression analysis (R^2) 99.67% and 2.12-fold increase in comparison to the production of the original level (142 mg/l).

Keywords: Auxin; Production; Plackett-Burman; *Fusarium.*

Abbreviations: Indole-3-acetic acid (IAA), potato dextrose agar medium (PDA).

1. INTRODUCTION

In the last few years by increasing population number every year the fulfilling food requirement remains a challenging task as climate changes affected on the agricultural production systems and there has been a growing interest in increasing crop plant yield [1]. 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 [2].

Several phytohormones control many physiological and bio-chemical processes like abscisic acid, gibberellins, ethylene, auxins, cytokinins, and brassinosteroids [3]. Indole-3-acetic acid extensively was the first identified plant hormone and the most important member of the auxins family of phytohormones [4]. Its play a vital role in physiological processes e.g. root initiation, production of longer roots, tissue differentiation, increase number of root hairs and lateral root which are involved in nutrient uptake [5-7]. In recent paper

by Takahashi [8] revealed that indole-3-acetic acid intracellular plant concentration is controlled by the biosynthesis and degradation process.

The presence of several microorganisms synthesizes IAA as secondary metabolites through tryptophan pathway are very important factor in soil fertility [9]. Several bacterial isolates could produce IAA however; most of the previous studies do not take into account of IAA production by filamentous fungi. Indole-3-acetic acid was produced by filamentous fungi like *Colletotrichum gloeosporioides*, *Colletotrichum acutatum*, *Dibotryon morbosum*, *Fusarium*, *Rhizopus suinus*, *Phoma glomerata*, *Penicillium*, *Taphrina deformans*, *Ustilago esculenta* and *Ustilago zeae* [10-18].

A classical method of optimizing the fermentation conditions and medium constituents depends on single parameter whilst all the other factors are maintained at a fixed level [19]. However, statistically based experimental designs proved to be most popular for production optimization as it enables us to obtain the physicochemical and factors influencing on the production process with less number of planned experiments. Plackett-Burman design is practical efficient when we screening large number of factors to produce optimal response [20].

The main objective of this paper is to test the ability of different *Fusarium* isolates to produce indole-3-acetic acid on glucose medium, secondly to improve IAA production by investigating the effect of several parameters on the production process and found the optimum fermentation conditions and medium constituents for the highest IAA production using statistical approach (Plackett-Burman design).

2. MATERIALS AND METHODS

2.1. *Fusarium* sp. isolation and identification

Fusarium species, isolated from different parts of Egyptian clover, faba bean, garlic, maize and onion plants on potato dextrose agar medium (PDA). The pure cultures were maintained aerobically on the same medium and stored at 4±1°C until using [21]. *Fusarium* sp. identified based on their macroscopic and microscopic characteristics [22].

2.2. Inoculums preparation

Prior to indole-3-acetic acid production experiments, *Fusarium* sp. were grown aerobically on potato dextrose agar medium at 28±1°C for 4 days. Homogeneous spore suspension of *Fusarium* sp. was prepared by scraping fungal hyphae from culture plates and suspended in sterilized distilled water containing 0.01% (v/v) tween 80 (2×10^6 spore/ml) and stirred for 30 min. One ml of the inoculums was transferred to an Erlenmeyer flask 250 ml containing 100 ml of the production medium.

2.3. Screening for IAA production by *Fusarium* sp.

Czapek's dextrose liquid medium supplemented with 0.2 g/l L-tryptophane, was used as production medium containing (g/l): glucose, 30.0; yeast extract, 5; $NaNO_3$, 3.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5 and $FeSO_4 \cdot 7H_2O$, 0.01. These contents were dissolved in 1000 ml distilled water with initial pH adjusted to 5.5 before autoclaving. After sterilization in an autoclave at 121°C and 1.5 atm pressure for 20 min. chloramphenicol, 250 mg/ml was sterilized separately by membrane filtration, using a membrane of pore size 0.22 mm and added as bacteriostatic agent. Incubation was carried out at 28±1°C on a rotary shaking (150 rpm) for 7 days. All the experiments were carried out independently in triplicates.

2.4. Optimization using Plackett-Burman design

Plackett-Burman design was used to screen the fermentation parameters that influenced indole-3-acetic acid (IAA) production with respect to their main effect and without interaction effects between various constituents of the medium [23]. Eleven trails carried out by Plackett-Burman design for screening the fermentation parameters under investigation is shown in Table 1. Each independent variable was tested at two levels, high (+1) and low (−1). In each column and row should contain equal number of negative and positive signs. The program Sigma XL (Version 6.12) was used to analyze this experiment.

Table 1. Plackett-Burman design for different variables screening in IAA production by *Fusarium oxysporum* (I).

Variable code	Variable	Unit	Level		
			Low (-1)	(0)	High (+1)
A	Incubation temperature	C°	25	30	35
B	Incubation time	D	5	7	9
C	Fermentation type		Shaking	Shaking	Static
D	Inoculums size	%	0.5	1	2
E	Initial pH		5	6	7
F	Glucose	gl^{-1}	20	30	40
G	Yeast extract	gl^{-1}	3	5	7
H	L-tryptophan	gl^{-1}	0.1	0.5	1
J	KH_2PO_4	gl^{-1}	0.5	1	2
K	$MgSO_4 \cdot 7H_2O$	gl^{-1}	0.1	0.5	1
L	$NaNO_3$	gl^{-1}	1	2	3

Plackett-Burman design was used to screen and evaluate the important medium components that influence the response. Indole-3-acetic acid yields are explained by the following polynomial equation:

$$Y=b_o + \sum b_i X_i + \sum b_{ij} X_i X_j + E_i \quad (1)$$

Where, Y: the variable dependent response; i: the regression coefficient; X: the independent variable level and E: the experimental error. The experimental data were statistically analyzed to determine the significant difference ($p \leq 0.05$) in response under different conditions. The response surface graphs were also plotted using the same software. The quality of fit for the regression model equation was expressed as R^2.

2.5. Analytical analysis

After the incubation period, *Fusarium* mycelium was recovered by filtration through dried and weighed Whatman filter paper (No. 113), washed with distilled water three times and then dried at 70°C overnight for dry mass (DM) determination. The supernatants were centrifuged at 4,000 rpm for 15 min. and sterilized by membrane filtration, using a membrane of pore size 0.22 mm to remove any remaining spores for quantitative determination of indole-3-acetic acid (IAA).

Indole-3-acetic acid was determined spectrophotometerically (Fig. 1) using Salkowski reagent containing 1ml of 0.5 M $FeCl_3 \cdot 6H_2O$ dissolved in 50 ml of 35% $HClO_4$ [24]. Two ml of Salkowski reagent was added to one ml of culture supernatant in 10 ml test tube leaves it in room temperature and read the color after 25 min. but before 3 h at 535 nm. The developed pink color measured using T60 UV with a split beam UV visible spectrophotometer covers a wavelength range of 190-1100 nm. The amount of IAA in the supernatant was measured quantitatively at 535 nm against substrate-free blank. The standard curve was prepared using pure IAA (1-100 mg/l).

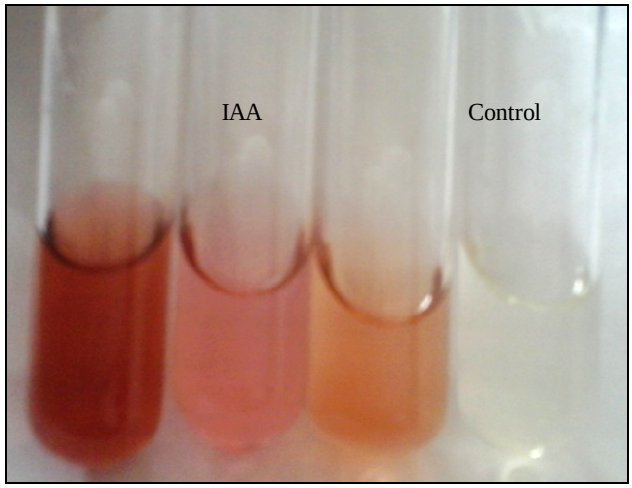

Figure 1. Different pink color degrees of IAA production after the reagent added in comparison with control (free IAA).

3. RESULTS

3.1. Isolation and identification of *Fusarium* sp.

Ten *Fusarium* isolates were isolated from different parts of Egyptian clover, faba bean, garlic, maize and onion plants on PDA medium. Based on *Fusarium* growth on the plate and the microscopic characters, *Fusarium* sp. were identified into seven species. *Fusarium solani*, *F. oxysporum*, *F. chlamydosporum*, *F. camptoceras*, *F. incarnatum*, *F. verticilloides* and *F. nygami*. The purified isolates were screened for their ability to produced indole-3-acetic acid (IAA) on fermented medium. Only one isolate was selected for further experiments based on the highest indole-3-acetic acid (mg/l) production.

3.2. Indole-3-acetic acid production by *Fusarium* sp.

All the isolates grown on the production medium and showed various degrees of dry mass and indole-3-acetic acid production. A wide variation in IAA production on the screening medium ranged from 18.37±1.04 to 142±6.46 mg/l and dry mass varied between 1.2±0.2 and 6.1±0.53 g/l. The highest fungus dry mass and indole-3-acetic acid producer was *Fusarium oxysporum* (I) isolated from onion rhizoplane giving 142±6.46 mg/l IAA (with productivity 23.14 mg/l/day) and 6.1±0.53 g/l dry mass so it was selected for the further experiments, the overall measurement results are summarized in Fig. 2.

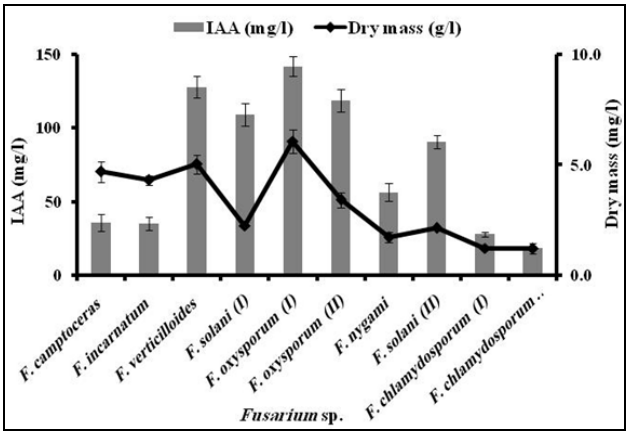

Figure 2. Screening for IAA production on glucose medium by different isolates of *Fusarium* species.

Brief description of indole-3-acetic acid highly producer *Fusarium oxysporum* (Schlechtendal) emend. Snyder & Hansen; Growth on PDA medium 50 mm in one week, Texture floccose becoming felted, Color white to pale apricot, usually with a purple tinge, Reverse purple. Conidiophores hyaline, simple, short, bearing spore masses at the apexes; two kinds of conidia: macroconidia boat-shaped, with slightly tapering apical cells and hooked basal cells, 4-celled; and microconidia ellipsoidal, 1-celled. Chlamydospores globose and usually solitary (Fig. 3).

3.3. Optimization of IAA production using Plackett-Burman design

The Plackett-Burman design was an effective way to improve IAA production. The highly IAA producer (*Fusarium oxysporum* (I)) was chosen for screening the effects of different parameters on IAA production using Plackett-Burman design. Each variable was studied at two levels (-1, 1) as declared in Table 1. Relationship between the response and the screened variables was expressed by the following polynomial equation:

IAA (mg/l) = (91.483) + (-40.02) * A: Incubation temperature + (-22.15) * B: Incubation time + (-49.82) * C: Fermentation type + (6.82) * D: Inoculum size + (2.95) * E: Initial pH + (18.35) * F: Glucose + (-1.75) * G: Yeast extract + (26.48) * H: L-tryptophane + (0.95) * J: KH_2PO_4 + (-13.78) * K: MgSO4 + (17.65) * L: $NaNO_3$ (2)

The results obtained in Table 2 indicated that there was a wide variation in IAA production from (13.6 to 300.4 mg/l) and dry mass varied between 3 and 9.1 g/l. This indicates the important effect of the medium components and environmental factors on growth and production of IAA. The ANOVA results are shown in Tables 3 showed that among the eleven variables, G (yeast extract) and J (KH_2PO_4) were found to be non-significant ($p>0.05$). Among the tested parameters, fermentation type, incubation temperature and L-tryptophan were the most effective parameters plays a crucial role in IAA production with 49.82%, 40% and 26.48% coefficient effect as shown in Pareto-Plot (Fig. 4).

All the predicted values of Plackett-Burman design were located in close proximity to the experimental values. This supports the hypothesis

that the model Eq. (2) is sufficient to describe the response of the experimental observations of IAA production (Fig. 5). Three-dimensional response surface curves were generated to study the interaction between each two variables (Fig. 6A-F). The Model F value of 324.6 ($p<0.05$) implies that the model is significant. Model F value is calculated as ratio of mean square regression and mean square residual due to the real error. The R^2 value was 99.67% indicated that the entire variation was explained by the model. The adjusted R^2 value was 99.36%.

Maximum IAA production (300.4 mg/l) by *Fusarium oxysporum* (I) obtained under the fermentation conditions: temperature at 25°C, incubation period 5 days, pH 7, inoculums size 2%, shaking rate at 150 rpm and medium constituents: Glucose 40 g/l, yeast extract 3 g/l, L-tryptophan 1 g/l, KH_2PO_4 2 g/l, $NaNO_3$ 4 g/l, $MgSO_4 \cdot 7H_2O$ 0.1 g/l.

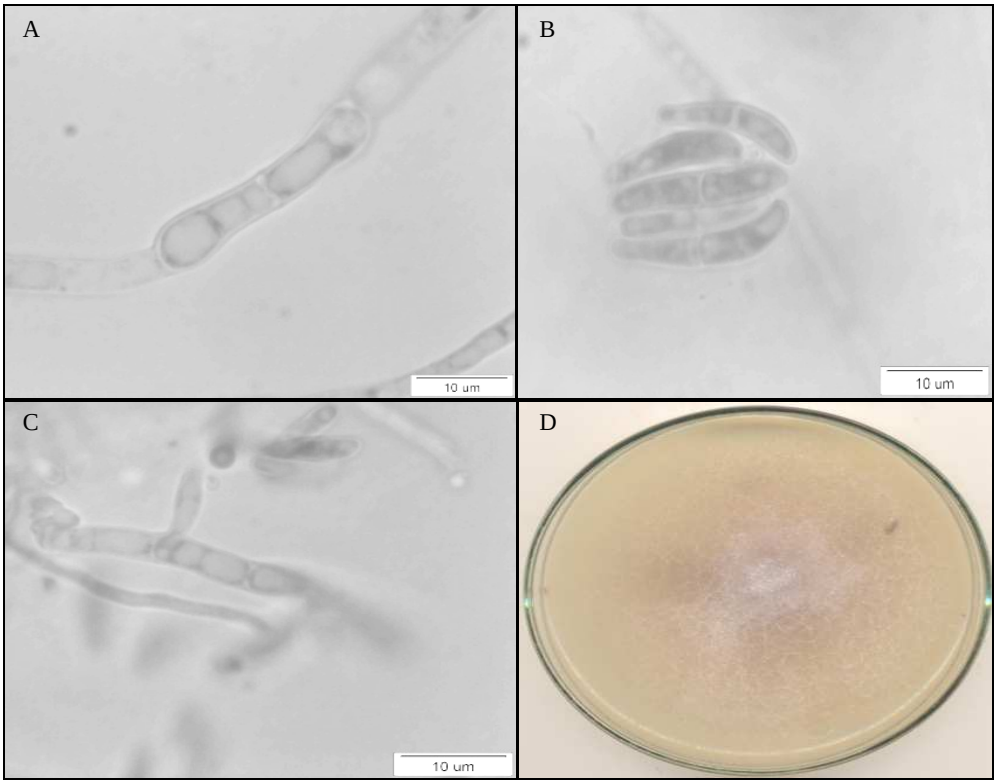

Figure 3. *Fusarium oxysporum* (I) Sch., A: Chlamysospores (Ch); B: Monophilaidic conidiogenous cell (Ph) and hypha (Hy); C: Macroconidia (Ma); Bars, 10 µm; D: Fungus growth on potato dextrose agar medium.

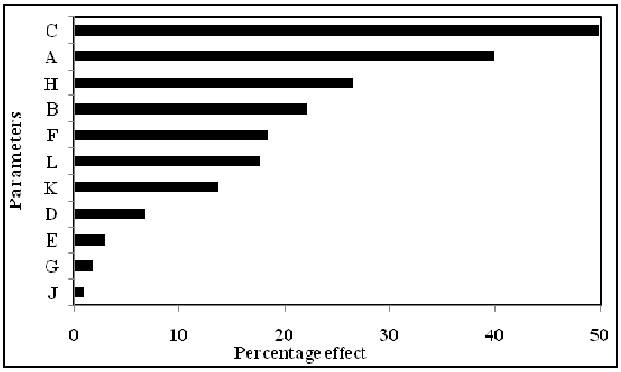

Figure 4. Pareto-Plot for Plackett-Burman parameter estimates the effect of each parameter on IAA produced by *Fusarium oxysporum* (I).

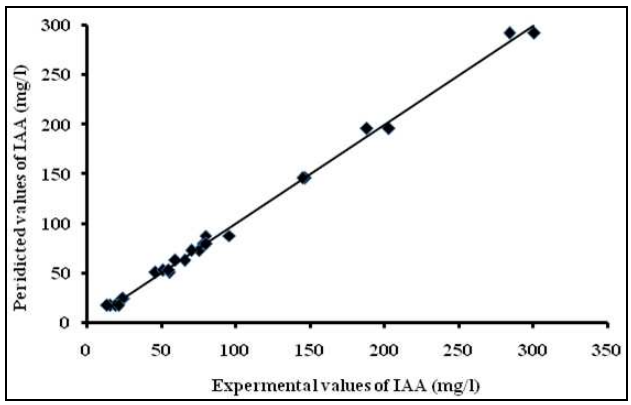

Figure 5. Comparison between IAA (mg/l) experimental and predicted values of the Plackett-Burman design.

Table 2. Plackett-Burman design variables with IAA production by *Fusarium oxysporum* (I) as response.

Trials	A	B	C	D	E	F	G	H	J	K	L	IAA (mgl^{-1})	Dry mass (gl^{-1})
1	-1	-1	1	1	1	-1	1	1	-1	1	-1	95.6	4.8
2	-1	1	1	1	-1	1	1	-1	1	-1	-1	46	6.82
3	1	1	-1	1	-1	-1	-1	1	1	1	-1	65.6	3.66
4	1	-1	1	1	-1	1	-1	-1	-1	1	1	24.8	6
5	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	146.8	4
6	1	1	-1	1	1	-1	1	-1	-1	-1	1	75.6	9.1
7	1	1	-1	1	1	-1	1	-1	-1	-1	1	70	8.4
8	1	1	1	-1	1	1	-1	1	-1	-1	-1	15.6	3.88
9	1	-1	1	-1	-1	-1	1	1	1	-1	1	50.8	3.6
10	-1	1	-1	-1	-1	1	1	1	-1	1	1	188	7.99
11	1	1	1	-1	1	1	-1	1	-1	-1	-1	19.2	3.81
12	1	-1	1	1	-1	1	-1	-1	-1	1	1	23.6	5.4
13	1	1	-1	1	-1	-1	-1	1	1	1	-1	59.2	3.68
14	-1	1	1	1	-1	1	1	-1	1	-1	-1	55.2	8.83
15	-1	-1	-1	1	1	1	-1	1	1	-1	1	284	4.8
16	-1	1	1	-1	1	-1	-1	-1	1	1	1	13.6	5.22
17	-1	1	-1	-1	-1	1	1	1	-1	1	1	202.8	8.21
18	1	-1	-1	-1	1	1	1	-1	1	1	-1	78.4	6.8
19	-1	-1	1	1	1	-1	1	1	-1	1	-1	79.6	4.4
20	1	-1	1	-1	-1	-1	1	1	1	-1	1	54.8	4.2
21	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	144.8	3
22	1	-1	-1	-1	1	1	1	-1	1	1	-1	80	7.2
23	-1	-1	-1	1	1	1	-1	1	1	-1	1	300.4	4.6
24	-1	1	1	-1	1	-1	-1	-1	1	1	1	21.2	5.51

The sign +1 and −1 represent the two different levels (high and low) of the independent variable under investigation. A: Incubation temperature, B: Incubation time, C: Fermentation type, D: Inoculums size, E: Initial pH, F: Glucose, G: Yeast extract, H: L-tryptophan, J: KH_2PO_4, K: $MgSO_4 \cdot 7H_2O$ and L: $NaNO_3$.

Table 3. Statistical analysis of Plackett-Burman design of each variable at two levels for IAA production by *Fusarium oxysporum* (I).

Variable code	Variable	Coefficient	*t* value	*P* value
A	Incubation temperature	91.48	69.58	<0.0001*
B	Incubation time	-40.02	-30.44	<0.0001*
C	Fermentation type	-22.15	-16.85	<0.0001*
D	Inoculums size	-49.82	-37.89	0.0002*
E	Initial pH	6.82	5.19	0.0445*
F	Glucose	2.95	2.24	<0.0001*
G	Yeast extract	18.35	13.96	0.2079 N
H	L-tryptophan	-1.75	-1.33	<0.0001*
J	KH_2PO_4	26.48	20.143	0.4838 N
K	$MgSO_4.7H_2O$	0.95	0.72	<0.0001*
L	$NaNO_3$	-13.78	-10.48	<0.0001*

t – student's test, *p* – corresponding level of significance,* Significant at p ≤0.05, N, non-significant at p≥0.05.

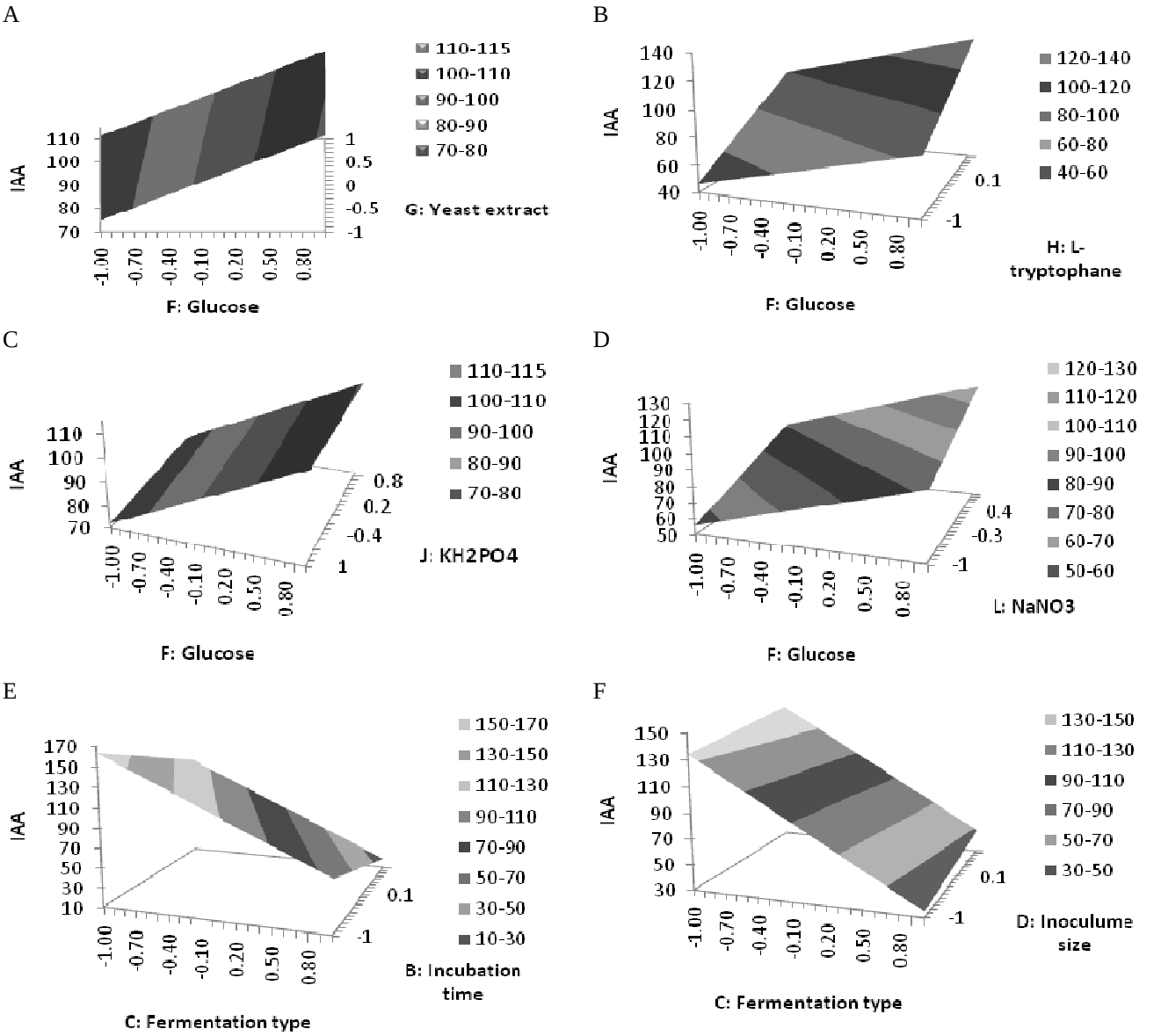

Figure 6. Response surface plots of IAA production by *Fusarium oxysporum* (I) showing the effect of two variables (other variables were kept at zero in coded unit): (A) Glucose and yeast extract, (B) Glucose and L-tryptophane, (C) Glucose and KH_2PO_4, (D) Glucose and $NaNO_3$, (E) Fermentation type and incubation time, (F) Fermentation type and inoculums size.

4. DISSCUSSION

Indole-3-acetic acid produced by all *Fusarium* isolates on the production medium with various degree of production giving maximum value by *Fusarium oxysporum.* Lynch [25] suggested that indole-3-acetic acid is a common product of L-tryptophan metabolism which produced by several microorganisms including plant growth-promoting rhizobacteria, other bacterial types and fungi. Hasan [15] found that all isolates of *Fusarium* oxysporum which isolated from different plant seeds could produce IAA (100-140 mg/l).

After screening the effect of eleven parameters on IAA we found that fermentation type, incubation temperature and L-tryptophan were the most effective parameters play a crucial role in IAA production. Thuler [26] revealed that IAA production is oxygen dependent, so agitation during production seems to be preferable when compared with a static condition. When incubation performed by agitation the production medium homogeneous better and the oxygen supplies increase, which increase both biomass and production in the medium [27]. From later researches, the optimum incubation temperature for IAA production was

range of 25-30°C by several microorganisms [15, 28, 27].

Maximum IAA production (300.4 mg/l) by *Fusarium oxysporum* (I) obtained under the fermentation conditions: temperature at 25°C, incubation period 5 days, pH 7, inoculums size 2%, shaking rate at 150 rpm and medium constituents: Glucose 40 g/l, yeast extract 3 g/l, L-tryptophan 1 g/l, KH_2PO_4 2 g/l, $NaNO_3$ 4 g/l, $MgSO4{\cdot}7H_2O$ 0.1 g/l. In agreement with our results, indole-3-acetic acid synthesis by ectomycorrhizal fungi was maximized after 30 days of incubation [29]. Indole-3-acetic acid production by *Fusarium oxysporum* maximized on 15 days and 10 for mycelium [15]. Indole-3-acetic acid production *Aspergillus niger* give maximum production after 6 days of incubation [27]. From the outcome of our investigation it is possible to conclude that genus *Fusarium* can be highly recommended in industrial production of indole-3-acetic acid. Also using statistical method in optimization for improving the production has a great potential for applications and was very effective in our study as the production of IAA (300.4 mg/l) in this paper increase with 2.12-fold in comparison to the production of original level (142 mg/l) using Plackett-Burman design.

5. CONCLUSION

From the outcome of our investigation it is possible to conclude that genus *Fusarium* can be highly recommended in industrial production of indole-3-acetic acid. Also using statistical method in optimization for improving the production has a great potential for applications and was very effective in our study as the production of IAA (300.4 mg/l) in this paper increase with 2.12-fold in comparison to the production of original level (142 mg/l) using Plackett-Burman design

AUTHORS' CONTRIBUTION

Both authors contributed in the success of this research and the final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare that has no conflict of interest.

REFERENCES

1. Hussain S, Peng S, Fahad S, Khaliq A. Rice management interventions to mitigate greenhouse gas emissions: a review. Environ Sci Pollut Res Int. 2015; 22(5): 3342-3360.
2. Fahad S, Hussain S, Matloob A, Khan FA. Phytohormones and plant responses to salinity stress: a review. Plant Growth Regul. 2015; 75(2): 391-404.
3. Iqbal N, Umar S, Khan NA, Khan MIR. A new perspective of phytohormones in salinity tolerance: regulation of proline metabolism. Environ Exp Bot. 2014; 100: 34-42.
4. Fahad S, Hussain S, Bano A, Saud S. Potential role of phytohormones and plant growth-promoting rhizobacteria in a biotic stresses: consequences for changing environment. Environ Sci Pollut Res. 2015; 22(7): 4907-4921.
5. Datta C, Basu P. Indole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub *Cajanus cojan*. Microbiol Res. 2000; 155: 123-127.
6. Teale WD, Paponov IA, Palme K. Auxin in action: signaling, transport and the control of plant growth and development. Mol Cell Biol. 2006; 7: 847-859.
7. Eyidogan F, Oz MT, Yucel M, Oktem HA. Signal transduction of phytohormones under a biotic stresses. In: Khan NA, Nazar R, Iqbal N, Anjum NA, eds. Phytohormones and a biotic stress tolerance in plants. Springer, Berlin, 2012: 1-48.
8. Takahashi H. Auxin biology in roots. Plant Root. 2013; 7: 49-64.
9. Mandal SM, Mondal KC, Dey S, Pati BR. Optimization of cultural and nutritional conditions for indole-3-acetic acid (IAA) production by a *Rhizobium* sp. isolated from root nodules of *Vigna mungo* (L.) Hepper. Res J Microbiol. 2007; 2: 239-246.
10. Thimann KV. Hydrolysis of indole acetonitrile in plants. J Biol Chem. 1953; 109: 279-291.
11. Wolf FT. The production of indole acetic acid by *Ustilago zeae* and its possible significant in tumor formation. Biochem. 1952; 38: 106-111.
12. Crady EE, Wolf FT. The production of indole acetic acid by *Dibotryon morbosum* and *Taphrina deformans.* Physiol Plant. 1959; 12: 526-533.
13. Perley JE, Stowe BB. On the ability of *Taphrina deformans* to produce indole acetic acid from tryptophan by way of tryptamine. Plant Physiol. 1966; 41: 234-237.

14. Riov RMJ, Sharon A. Indole-3-acetic acid biosynthesis in *Colletotrichum gloeosporioides* f. sp. *Aeschynomene*. Appl Environ Microbiol. 1998; 64: 5030-5032.
15. Hasan HA. Gibberellin and auxin-indole production by plant root-fungi and their biosynthesis under salinity-calcium interaction. Acta Microbiol Immunol Hung. 2002; 49: 105-118.
16. Shilts CK, Timmer EL, Ueng PP. Indole derivatives produced by the fungus *Colletotrichum acutatum* causing lime anthracnose and postbloom fruit drop of citrus. FEMS Microbiology Lett. 2003; 226: 23-30.
17. Chung KR, Tzeng DD. Biosynthesis of indole-3-acetic acid by the gall-inducing fungus *Ustilago esculenta*. J Bio Sci. 2004; 4(6): 744-750.
18. Waqas M, Khan AL, Kamran M, Hamayun M. Endophytic fungi produce gibberellins and indole acetic acid and promotes host-plant growth during stress. Molec. 2012; 17: 10754-10773.
19. Xu C, Kim S, Hwang H, Choi J. Optimization of submerged culture conditions for mycelial growth and exobiopolymer production by *Paecilomyces tenuipes* C240. Process Biochem. 2003; 38(7): 1025-1030.
20. Aravindan R, Viruthagiri T. Sequential optimization of culture medium composition for extracellular lipase production by *Bacillus sphaericus* using statistical methods. J Chem Tec Bio. 2007; 82: 460-470.
21. Booth C. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England; 1971.
22. Leslie JF, Summerell BA. The *Fusarium* Laboratory Manual. Blackwell Publishing; 2006.
23. Plackett RL, Burman JP. The design of optimum multifactorial experiments. Biometrika. 1947; 33: 305-325.
24. Gordon SA, Weber RP. Colorimetric estimation of indole acetic acid. Plant Phys. 1951; 26: 192-195.
25. Lynch JM. Origin, nature and biological activity of aliphatic substances and growth hormones found in soil. In: Vaughan D, Malcom RE, eds. Soil organic matter and biological activity. Martinus Nijhoff /Dr. W. Junk Publishers. Dordrecht, Boston, Lancaster, 1985: 151-174.
26. Thuler DS, Floh EI, Handro W, Barbosa HR. *Beijerinckia derxii* releases plant growth regulators and amino acids in synthetic media independent of nitrogenase activity. J Appl Microbiol. 2003; 95: 799-806.
27. Bilkay IS, Karako S, Aksöz N**.** Indole-3-acetic acid and gibberellic acid production in *Aspergillus niger*. Turk J Biol. 2010; 34: 313-318.
28. Yalçınkaya Y. Effects of some physiological conditions on indole-3-acetic acid production by *Gibberella fujikuroi* G5, MSc, Hacettepe University Institute of Science and Technology; 2007.
29. Gopinathan S, Raman N. Indole-3-acetic acid production by ectomycorrhizal fungi. Indian J Exp Biol. 1992; 30: 142-143.