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Kojic acid production by rhizospheric fungi recovered from Egyptian plants

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ABSTRACT

Soils are rich with various precious fungi that produce significant metabolites which could solve many medical issues of human life. Kojic acid represents a significant medical metabolite produced by fungi and used as anti-browning, anti-cancer, and anti-diabetic agent in medical products. In this study, a total of 31 species plus 3 varieties belonging to 14 fungal genera were recovered as rhizospheric fungi from the roots area of 30 samples of 27 different plants. The most common isolated genus was *Aspergillus* however; the most common isolated species were *Aspergillus parasiticus*, and *A. flavus*. Fifty two fungal isolates were tested for their ability to produce kojic acid, only 25 fungal isolates have the ability to produce kojic acid with various degrees and 27 isolates couldn't produce it. *Aspergillus flavus* var. *columnaris* Af-19, *A. parasiticus* Ap-48, *A. flavus* var. *columnaris* Af-7, *A. flavus* Af-52, and *A. flavus* Af-40 were the high producers of kojic acid giving (g/l) 42.68, 39.96, 39.85, 39.79, and 38.92 kojic acid, respectively. The percent of kojic acid yields based on consumed sugar by these five fungal isolates were 48.60, 44.84, 43.52, 45.00 and 43.62%, respectively. From the obtained data, it could be assumed that rhizospheric fungi represent a promising producers of kojic acid as significant fungal metabolite.

INTRODUCTION

Soils are consisting of minerals, gas, water, organic matter and microorganisms that support the plant life by their constituents. The soil microbial community consists of various organisms like; fungi, bacteria, yeasts, nematodes, and protozoa [1]. Microbes spread through all the soil parts; however they were found to be highest in the rhizosphere soil which is defined as the soil zone surrounding the plant roots. This microbial richness could be attributed to the nutrient richness of the plant root exudates that attract the microorganisms including sugars, fatty acids, amino acids, and organic compounds [2]. These microorganisms have special characters of secreting important metabolites and biologically active compounds like; antioxidants, immune-suppressive compounds, phyto-hormones, insecticides, and organic acids [3].

Kojic acid is an organic acid produced biologically as a secondary metabolite during the aerobic fermentation of different foods by various types of fungi. Its name is derived from the word (Koji) which means starter inoculum in the Japanese oriental food [4]. Chemically it consists of 5-hydroxy-2-hydroxymethyl- ɣ -pyrone with molecular formula $C_6H_6O_4$ and maximum peak of ultraviolet absorption spectrum between 280 -284 nm [5]. It is characterized by odorless crystalline needles with molecular weight of 141.1 and melting point 151°C-154°C [6 and 7]. It is soluble in ethyl acetate, ethanol, water, but less soluble in ether, and chloroform [8]. It forms salt in combination with nikal (Ni), zinc (Zn), cupper (Cu), sodium (Na), and calcium (Ca) [9].

Kojic acid can be synthesized biologically from glucose (which acts as precursor) through the action of cell enzymes [10 and 11]. The cell bound enzymes system includes glucose-6-phosphate dehydrogenase, gluconate dehydrogenase and hexokinase, that are involved in the synthesis of kojic acid directly from glucose [12]. Gluconic acid -ɣ-lactone and at least one of the three compounds 3 ketoglucose, 3-ketogluconic acid lactone and oxy- kojic acid are intermediates in kojic acid pathway [13]. Kojic acid is produced mainly by some fungal genera especially *Aspergillus, Penicillium* and *Mucor* [14]. However the most common species are *A. flavus, A. oryzae, A. tamari*, and *A. parasiticus* [4, 15 and 16].

Kojic acid is used as anti-browning agent with the combination of vitamin C, flavor enhancer of foods like soy sauce, miso, and sake [17 and 18]. Kojic acid and its derivatives have anti-inflammatory effect by inhibiting the proliferation of cancerous cells [19]. It is also used in pain relieving drugs and anti-inflammation [20], whitening agent in skin care products [21] and anti-diabetic agent [22]. Hussein-Al-Ali *et al*. [23] found that it acts as effective anti-microbial agent against lots of bacteria and fungi if compared with antibiotics like ceftazidime and nitrofurantoin because of its acidic nature. So, the aim of this study was to evaluate the ability of rhizospheric fungi isolated from the roots area of 30 plant samples for kojic acid production in glucose medium.

MATERIALS AND METHODS

1. Samples collection

Thirty samples of rhizospheric soil (500 g each) were collected from different places in Assiut Governorate. The samples were collected from the area that surrounding the roots of 27 different plants. The English and Scientific name of each plant were recorded in Table 1. The samples were transported to the mycological laboratory after placing them in clean plastic bags and utilized directly for the isolation of the rhizospheric fungi.

2. Isolation of rhizospheric fungi

Czapek's dextrose agar (CzDA) medium was used for the isolation of rhizospheric fungi consisting of (g/l): dextrose, 10.0; KH_2PO_4, 1.0; $NaNO_3$, 3.0, $MgSO_4$. $7H_2O$, 0.5; KCl, 0.5; $FeSO_4$, 0.07 and agar, 15.0 dissolved in 1000 ml distilled water. The medium was sterilized in autoclave for 20 min at 121°C and supplemented with 250 mg/l of chloramphenicol for suppressing the bacterial growth [24]. Dilution plate method was used for isolation of rhizospheric fungi as following; ten g of rhizospheric soil was transferred in flask contains 90 ml sterilized distilled water then serial dilutions were performed until dilution 10^{-3}. One ml of this dilution was introduced into each Petri dish, then 15-20 ml of CzDA were poured, and stirred well to dispense suspension in the medium. Three replicate plates were prepared for each sample, and incubated at 28±2°C for 7days [25 and 26]. The producing colonies were counted then calculated as colony forming unit (CFU) per 1 g of rhizosphere soil.

3. Phenotypic identification of fungi

The collected fungal isolates were identified based on macroscopic and microscopic features according to keys and descriptions of the following references [27 - 29].

4. Screening for kojic acid production by rhizospheric fungi

Modified Czapek's liquid medium was used for the production of kojic acid include (g/l): glucose, 100; KH_2PO_4, 1; $MgSO_4$, 0.5; yeast extract, 5 and distilled water,1000 ml with justifying initial pH to 3 then sterilized in autoclave for 20 min at 121°C and 1.5 atm pressure. Fungal inocula from five days old culture were transferred to conical flask containing 100 ml liquid medium, and incubated at 28± 2°C for 10 days [30- 32].

5. Analytical measurement

The supernatants were collected and used for determination of both kojic acid and residual sugars. Colorimetric method was used for quantitative determination of kojic acid as described by Zohri *et al*. [30] and Bentley [33] where 1 ml of diluted sample was mixed with 1 ml of ferric chloride ($FeCl_3$) reagent prepared in HCl (0.1N). The reaction between the functional group of hydroxyl in the samples and $FeCl_3$ produced red color, measured at wavelength 540 nm using spectrophotometer. Kojic acid values then calculated from KA 0.1 – 2.0 mg/ml standard curve. Residual sugar was determined using dinitrosalicylic acid (DNS) method as described by Miller [34]. One ml of supernatant was added to 1 ml of DNS reagent and left in room temperature for 15 min. Then the mixture was boiled in water bath for about 10 min. and cooled down for measuring the absorbance using spectrophotometer at wavelength 540 nm. Residual sugar values were calculated from glucose 1 - 10 g/l standard curve.

RESULTS

1. Rhizospheric fungi isolated in the current study

The total fungal count recorded in the thirty tested samples fluctuated between 79000 and 4500 CFU / g soil sample. The highest count was recorded in the soil sample collected from the area surrounded the root of Rocket 1 followed by Faba while the lowest count was recorded in the sample surrounded the root of Parsley 1 Table 1. Thirty one species and 3 species varieties belonging to 14 genera were isolated and identified Table 2. The number of genera in each sample fluctuated between 1 and 5. The highest number of genera was in Sorghum soil sample while the lowest number of genera was in Caraway and Spinach samples. Number of species in the samples ranged from 1 to 6, the highest number of species was in the soil surrounding onion roots while the lowest number of species was in sample from Parsley 1 root area Table 1.

The isolated fungi were classified according to their number of isolation (out of 30 samples) into four classes; high occurrence (H, isolated from 15 - 30 samples), moderate occurrence (M, from 7 - 14 samples), low occurrence (L, from 3 - 6 samples) and rare occurrence isolates (R, from only one or two samples) as shown in Table 2.

Aspergillus was isolated with high occurrence. It was represented by 50.93% of total fungal count and appeared in 28 samples. Fourteen species plus one species variety were recorded belonging to this genus. Only *A. niger* was isolated with high occurrence from 50 % of samples, representing 21.46 % of total *Aspergilli* and 11 % of total fungal count. Each of *A. flavus, A. oryzae* and *A. parasiticus* appeared with moderate occurrence. They were isolated from 36.66, 30 and 30 % of samples, matching 22.46, 12.81 and 16.47 % of total *Aspergilli,* and 11.44, 6.53 and 8.39% of total fungi, respectively. The remaining *Aspergillus* species (10 plus one variety) were recorded with rare occurrence and represented collectively 13.63 % of total count and 26.78 % of total *Aspergilli* Table 2.

Other five genera were isolated with moderate occurrence. These genera were *Emericella* (19.24 % of total fungal count) recorded in 9 samples and represented by *E. nidulans* var. *nidulans* (18.73 %) and *E. nidulans* var. *lata* (0.51 %), *Alternaria alternata* (3.64 %) appeared in 8 samples, *Rhizopus* (5.34 %) in 8 samples and represented by *Rhizopus stolonifer* (3.90 %) and *Rhizopus oryzae* (1.44 %), *Epicoccum nigrum* (5.68

%) in 7 samples and *Fusarium* (5.00 %) in 7 samples including *Fusarium solani* (3.14 %) , *Fusarium oxysporum* (1.10%) and *Fusarium lateritium* (0.76%). *Cochliobolus* (two species), *Botryotrichum* (one) and *Trichoderma* (two) were recorded with low occurrence, while the other five genera isolated with rare occurrence Table 2.

Table 1: Total counts (T.C., per g soil), number of genera (N.G.) and number of species & varieties (N.S. & V.) of rhizosphere fungi isolated from soil samples collected from the soil area surrounded 30 different plants from Assiut Governorate on Czapeks dextrose agar (CzDA) medium at 28 ±1ºC.

Plants	Fungi	T.C. ($x10^2$)	N. G.	N.S. & V.	Common species
Eng. Name	Sci. Name				
Roselle	*Hibiscus sabdarriffa* L.	105	2	4	*Alternaria alternata* & *A. parasiticus*
Maize	*Zea mays* L.	85	4	4	*Epicoccum nigrum* & *R. oryzae*
Faba	*Vicia faba* L.	345	4	5&1	*Alternaria alternata* & *Epicoccum nigrum*
Nasturtium	*Tropaeolum majus* L.	155	3	3&1	*A. parasiticus* & *R. stolonifer*
Lentil	*Lens culinaris* L.	220	4	5&1	*A. niger* & *E. nidulans* var. *nidulans*
Flax	*Linum usitatissimum* L.	270	3	6	*A. niger* & *F. solani*
Hollyhock	*Alcea rosea* L.	225	4	6	*A. carbonaris* & *A. parasiticus*
Jute	*Corchorus olitorius* L.	85	2	2	*A.awamori & F. lateritium*
Pepper	*Capsicum annuum* L.	250	3	3&1	*A. oryzae* & *E. nidulans* var. *nidulans*
Sorghum	*Sorghum bicolor* L.	205	5	5&1	*A. niger* & *R. oryzae*
Cabbage 1	*Brassica oleracea var. capitate* L. *1*	205	2	3	*A. flavus* & *R. stolonifer*
Cabbage 2	*B. oleracea var. capitate 2*	165	4	4	*A. parasiticus* & *R. stolonifer*
Okra	*Abelmoschus esculentus* L.	170	2	4	*A. flavus* & *A. niger*
Zucchini	*Cucurbita pepo* L.	130	3	3	*Botryotrichum piluliferum* & *T. longibrachiatium*
Guava	*Psidium guajava* L.	135	2	4	*A. fumigatus* & *A. oryzae*
Mulberry	*Morus alba* L.	130	2	2&1	*A. flavus* & *A. flavus var. columnaris*
Pomegranate	*Punica granatum* L.	90	2	2	*A. oryzae & C.sativus*
Cauliflower	*Brassica oleracea var. botrytis* L.	235	3	3	*A. fumigatus* & *Alternaria alternata*
Tomato	*solanum lycopersicum* L.	225	4	4	*Rhizocotonia solani* & *Stachybotrys chartarum*
Onions	*Allium cepa* L.	160	4	6	*A. flavus* & *Botryotrichum piluliferum*
Rocket 1	*Eruca* Sativa L.1	790	3	3&1	*E. nidulans* var. *nidulans* & *A. niger*
Rocket 2	*Eruca* Sativa L.2	120	3	4	*A. flavus* & *F. oxysporum*
Parsley 1	*Petroselinum* crispum L.1	45	3	1&2	*E. nidulans* var. *nidulans & C. lunatus*
Parsley 2	*Petroselinum* crispum L.2	90	2	4	*A. niger & Fusarium oxysporum*
Dill	*Antheum graveolens* L.	265	3	2&1	*A. flavus* & *E. nidulans* var. *nidulans*
Coriander	*Coriandrum sativum* L.	185	3	4	*A. niger* & *A. oryzae*
Caraway	*Carum carvi* L.	125	1	3	*A. flavus* & *A. niger*
Radish	*Raphanus sativus* L.	225	3	5	*A. carneus* & *A. parasiticus*
Lettuce	*Lactuca sativa* L.	200	2	2&1	*A. flavus* & *E. nidulans* var. *nidulans*

Spinach	*Spinacia oleracea* L.	250	1	3	*A. niger* & *A. parasiticus*

Table 2: Total counts (T.C., per 30g soil, one g of each sample), percentage of fungal counts (T.C. %, calculated as % of total counts), number of cases of isolation (NCI, out of 30 samples) and occurrence remarks (OR: H= high, M= moderate, L= low & R= rare) of rhizosphere fungi isolated from soil samples collected from the area surrounded 30 different plants from Assiut Governorate on Czapeks dextrose agar (CzDA) medium at 28 ±1ºC.

Fungal species	T.C	T.C%	NCI	OR
Absidia corymbifer	2000	0.34	2	R
Alternaria alternata	21500	3.64	8	M
Aspergillus awamori	6000	1.02	2	R
A. candidus	500	0.08	1	R
A. carbonaris	14500	2.46	2	R
A. carneus	13500	2.29	2	R
A. flavus	67500	11.44	11	M
A. flavus* var. *columnaris	5500	0.93	2	R
A. fumigatus	24500	4.15	2	R
A. japonicus	2500	0.42	1	R
A. niger	64500	11	15	H
A. oryzae	38500	6.53	9	M
A. parasiticus	49500	8.39	9	M
A. sydowii	6000	1.02	2	R
A. tamarii	500	0.08	1	R
A. ustus	3500	0.59	1	R
A. versicolor	3500	0.59	1	R
Botryotrichum piluliferum	13000	2.20	3	L
Cochliobolus lunatus	4000	0.68	3	L
C. sativus	6000	1.02	3	L
Emericella. nidulans* var. *lata	3000	0.51	1	R
E. nidulans* var. *nidulans	110500	18.73	8	M
Epicoccum nigrum	33500	5.68	7	M
Fusarium lateritium	4500	0.76	1	R
F. oxysporum	6500	1.10	2	R
F. solani	18500	3.14	4	L
Humicola grisea	3000	0.51	1	R
Mucor circinelloides	2500	0.42	1	R
Rhizocotonia solani	12500	2.12	2	R
Rhizopus oryzae	8500	1.44	3	L
R. stolonifer	23000	3.90	5	L
Stachybotrys chartarum	7500	1.27	1	R
Trichoderma harzianum	4500	0.76	2	R
T. longibrachiatium	5000	0.85	1	R
Total count	590000	100		
No of genera + species & species varieties		14 + 31 & 3		

2. kojic acid (KA) production by fungal isolates

Fifty two fungal isolates, collected from the rhizospheric samples, were tested for their ability to produce kojic acid. The results showed that only 25 isolates had the ability to produce kojic acid with variable degrees Table 3. kojic acid concentration ranged from 42.68 – 1.90 g/l matching 48.60 – 2.15 % of consumed sugar.

The producers of kojic acid were classified into 3 categories, the first category produced more than 30% kojic acid from the consumed sugar and represented by 6 species and two species varieties. The highest producer was *Aspergillus flavus* var. *columnaris* Af-19 followed by *A. flavus* Af-52, *A. flavus* Af-48, *A. flavus* Af-40**,** *A. flavus* var. *columnaris* Af-*7, A. oryzae* Ao-37, *A. flavus* Af-43 and *A. oryzae* Ao-47. Their yields of kojic acid ranged from 30.49 to 48.60 % of consumed sugar Table 3.

The second category yield kojic acid with rang from 20 to 30% of consumed sugar including the moderate producers. This group was represented by 8 species, the first one was *Aspergillus flavus* Af-11 followed by *A. niger* An-28, *A. niger* An-49, *A. parasiticus* Ap-32, *A. parasiticus* Ap-10, *Epicoccum nigrum* En-27, *A. tamarii* At-33 and *A. parasiticus* Ap-39. Members of this group formed 21.13 to 29.91 % kojic acid from consumed sugar Table 3.

Kojic acid yields by the third category (low producers) was less than 20% of consumed sugar. It was represented by 9 species; the first one was *Aspergillus oryzae* An-29 followed by *A. oryzae* Ao-42, *A. oryzae* Ao-25, *A. flavus* Af-14, *A. parasiticus* Ap-51, *A. oryzae* Ao-15, *A. flavus* Af-44, *Trichoderma harzianum* Th-36, and *Epicoccum nigrum* En-50 Table 3.

On the other side, a total of 27 out of the 52 tested rhizospheric fungal isolates in this study could not to produce kojic acid. These isolates were omitted from Table 3. They were belong to *Alternaria alternata* (3 isolates), *Aspergillus awamori (1) A. flavus* (2), *A. fumigatus* (2), *A. niger* (6), *A. parasiticus* (1), *Botryotrichum piluliferum (1), Cochliobolus lunatus* (1), *C. sativus (1), Emericella nidulans* var. *nidulans* (1), *Fusarium lateritium (1), F. oxysporum* (1), *F. solani (1), Humicola grisea (1), Mucor circinelloides* (1), *Rhizocotonia solani (1), Stachybotrys chartarum (1) and Trichoderma longibrachiatium* (1).

Table 3: Kojic acid producer isolates out of the tested rhizospheric fungi.

Fungi	Isolate number	KA (g/l)	RS (g/l)	Consumed sugar (g/l)	Yield %	Yield Index
Aspergillus flavus	**Af-14**	**13.60± 1.94**	**10.04±2.21**	**89.96**	**15.11**	**L**
A. flavus	**Af-40**	**38.92± 2.61**	**10.78±1.16**	**89.22**	**43.62**	**H**
A. flavus	**Af-43**	**33.65± 2.61**	**10.29± 0.80**	**89.71**	**37.50**	**H**
A. flavus	**Af-52**	**39.79± 4.57**	**11.58±0.52**	**88.42**	**45.00**	**H**
A. oryzae	**Ao-37**	**33.92± 4.41**	**12.25± 0.23**	**87.75**	**38.65**	**H**
A. flavus	**Af-44**	**5.15± 0.68**	**10.84± 1.07**	**89.16**	**5.77**	**L**
A. flavus	**Af-11**	**26.25± 0.21**	**12.25± 0.15**	**87.75**	**29.91**	**M**
A. flavus* var. *columnaris	**Af-19**	**42.68± 2.32**	**12.19±0.44**	**87.81**	**48.60**	**H**
A flavus* var. *columnaris	**Af-7**	**39.85± 2.37**	**8.45±0.35**	**91.55**	**43.53**	**H**
A. niger	**An-28**	**25.50± 1.39**	**11.42±1.34**	**88.58**	**28.78**	**M**
A. niger	**An-49**	**25.35± 0.26**	**9.92± 0.61**	**90.08**	**28.14**	**M**
A. oryzae	**Ao-15**	**9.66± 1.48**	**12.58± 1.10**	**87.42**	**11.05**	**L**
A. oryzae	**Ao-42**	**15.51± 1.78**	**10.52± 1.25**	**89.48**	**17.33**	**L**
A. oryzae	**Ao-47**	**27.36± 1.08**	**10.29± 1.36**	**89.71**	**30.49**	**H**
A. oryzae	**Ao-29**	**17.05± 0.18**	**11.18± 0.76**	**88.82**	**19.19**	**L**
A. oryzae	**Ao-25**	**14.86± 0.88**	**13.37± 1.25**	**86.63**	**17.15**	**L**
A. flavus	**Af-48**	**39.96± 2.05**	**10.90±0.60**	**89.1**	**44.84**	**H**
A. parasiticus	**Ap-10**	**21.40± 2.02**	**12.43±1.83**	**87.57**	**24.43**	**M**
A. parasiticus	**Ap-32**	**22.95± 1.14**	**12.03± 0.35**	**87.97**	**26.08**	**M**
A. parasiticus	**Ap-39**	**22.08± 1.77**	**12.14± 0.83**	**87.86**	**21.13**	**M**
A. parasiticus	**Ap-51**	**12.61± 1.21**	**11.67± 0.59**	**88.33**	**14.27**	**L**
A. tamarii	**At-33**	**18.80± 2.14**	**12.18±0.42**	**87.82**	**21.40**	**M**
Epicoccum nigrum	**En-27**	**19.75± 2.03**	**10.54± 0.83**	**89.46**	**22.07**	**M**
E. nigrum	**En-50**	**1.90± 0.94**	**11.89± 0.31**	**88.11**	**2.15**	**L**
Trichoderma harzianum	**Th-36**	**4.51± 1.32**	**9.71± 0.72**	**90.29**	**4.99**	**L**

KA, kojic acid; RS, residual sugar; yield, calculated as % of consumed sugar; yield index H (> 30%), M (20 – 30%), L (<20%).

DISCUSSION

Plant roots are surrounded by narrow area of soil called rhizosphere. It has high diversity of microbes and it is expected that its community structure is different from that found in the bulk of soil [35]. Roots of plant release high amount of compounds that contain carbon known as rhizodeposits (exudates, nutrients,…) that makes the rhizosphere zone more attractive for microorganisms than the other parts of soil where rhizosphere is rich area with nutrients as fatty acids, sugars, organic compounds, and amino acids that attract plenty of microorganisms [36].

From the present study; *Aspergillus* was the most dominant genus recovered from most samples followed by *Alternaria, Fusarium* and *Rhizopus.* While the most common isolated species were *Aspergillus parasiticus* and *A. flavus* followed by *Alternaria alternata, Aspergillus oryzae, Emericella nidulans* var. *nidulans, Rhizopus stolonifer* and *Fusarium solani*. In agreement with these data; Domsch and Gams [37] stated that *Penicillium, Aspergillus, Chaetomium, Fusarium, Myrothecium, Cochliobolus* and *Trichoderma* were recovered from plants rhizosphere. Abdel-Hafez *et al.* [38] recorded *Fusarium* as one of the basic genera of fungi isolated from rhizosphere soil of Egyptian plants. Mandeel [39] found that ascomycetous fungi are the dominant fungal group in the rhizosphere soil. *Aspergilllus niger, Aspergillus flavus, Alternaria alternata, Fusarium oxysporum, Penicillium chrysogenum* and *Humicola grisea* isolated from Egyptian plants as rhizosphere fungi by Abdel-Hafez *et al*. [40]. Jamiolkowska and Wagner [41] stated that *Trichoderma* species was recovered with high incidence from the plants rhizosphere and was utilized as bio-control agent. *Aspergillus flavus, A. terreus, A. niger, F. oxysporum, F. chlamydosporum, F. nygamia,* and *F. solani* were recovered as dominant fungal species from the 25 rhizosphere soil of the Egyptian maize [42]. *Aspergillus* sp., *Fusarium* sp., *Emericella* sp., and *Penicillium* sp. were recovered as rhizosphere and rhizoplane fungi from soil surrounding different cultivated plants in new reclaimed soil, Upper Egypt [43]. *Fusarium* species was isolated from various rhizosphere soils of onion and garlic plants [44].

Only 25 out of 52 tested fungal isolates had the ability to produce variable degrees of kojic acid ranging from 1.9 to 42.68 g/l (equal to 2.15 - 48.60 % of consumed sugar). The highest producers were belonging to genus *Aspergillus* including *Aspergillus flavus* var. *columnaris, A. parasiticus, A. flavus* and *A. oryzae.* In convenience with these data; Machida *et al.* [45] mentioned that *Aspergillus oryzae* and *Aspergillus flavus* were the main producers of kojic acid. Wan *et al.* [46] found that *A. oryzae* produced 41g/l kojic acid on glucose medium. Wakisaka *et al.* [47] stated that *A. oryzae* could give 24 g/l kojic acid. Immobilized cells of *A. oryzae* can give 80 g/l kojic acid [48], also El-Sharkawy [49] obtained 60 g/l kojic acid by *A. flavus* ATCC 9179 using immobilization. Ogawa *et al.* [50] obtained 20g/l kojic acid from the immobilized cells of *A. oryzae* NRRL 484.

Manabe *et al.* [51] stated that after optimization, *A. flavus* gives 40 g/l kojic acid, however, Liu *et al.* [52] after optimization of *Aspergillus oryzae* obtained 83.47 g/l kojic acid. Hazzaa *et al.* [53] produced 25.5 g/l kojic acid from *A. oryzae* using glucose as carbon source. Yan *et al.* [54] produced 33.1g/l kojic acid from *A.oryzae* M866 using the corn stalk. Mahmoud *et al.* [32] stated that kojic acid production by *Aspergillus flavus* enhanced by zinc complexes to 26.63 ± 0.04 g/l. Mahmoud and Zohri [16] reported that the concentrations of kojic acid produced by some strains belonging to *A.flavus* and *A.oryzae* isolated from Egyptian sources fluctuated between 0.091 and 66.18 g/l on glucose medium. Our research emphasized that rhizosphere soil is a rich isolation source of kojic acid producing fungi particularly *Aspergillus* species. Also, the kojic acid producing isolates are promising candidates for large scale production.

CONCLUSION

High concentrations of kojic acid can be produced by *Aspergillus* species especially *A. flavus*. Kojic acid has many applications among them in cosmetics as whitening agent, in medicine as anticancer, in agriculture and in chemical industries. The next studies will concern with optimization and applications of kojic acid in many fields.

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