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α-Amylase, L-Asparaginase and Arginase Enzymes Production by Fungi Isolated from Rice Stored under Environmental Condition in Middle Egypt

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ABSTRACT: Rice is a cereal grain and is the main food of half the world's population. It is grown in many countries; Asia, Europe, America, Australia and Africa. The rice grain is often infected by a series of pathogens (fungi) during its storage, producing damages to the economy and health of humans.
It is considered a major risk to infect stored rice with fungi because the rice grain has been contaminated with toxigenic fungi, the source of which is mycotoxin.
Oryza sativa is a cereal food crop which belongs to family Poaceae of the plant kingdom. This crop can be more easily grown in tropics associated with humid climate (Yu *et al.* 2002). Oryza sativa is a cereal food crop which belongs to family Poaceae of the plant kingdom. This crop can be more easily grown in tropics associated with humid climate (Yu *et al*. 2002). Oryza sativa is a cereal food crop which belongs to family Poaceae of the plant kingdom.
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We studied the occurrence and predominance of mycotoxigenic fungi in stored rice in El-Miniagovernorate, Egypt and we detected the ability of these fungal isolates to produce alphaamylase, L- asparaginase and arginase enzymes. The study was conducted at Assiut university mycological centre (AUMC).
Sixty-two fungal isolates obtained from 51 rice grain samples, were screened for production of α-amylase, also 71 isolates for L-asparaginase and arginase production. The results confirmed that *Aspergillus* genus and *Trichurus spiralis* were the most active producers of α- amylase, while *Aspergillus* genera, *Penicillium* genera, *Lichtheimia corymbifera*, *Alternaria alternata, Cladosporium herbarum*, *Cochliobolus lunatus*, *Cochliobolus spicifer*, *Fusarium verticillioides*, *F. Semitectum, Gliocladium roseum, Mucor circinelloides, Nigrospora oryzae* and *Trichoderma harzianum* were the most active producers of L- asparaginase and *Aspergillus* genus, *Penicillium* genus, *L. corymbifera*, *A. alternata, F. semitectum*, *G. roseum* and *M. circinelloides* were the most active producers of arginase. Also we found the total fungal population and the dominant genera often increased with increasing humidity and storage period.

Keywords: *Aspergillus*, alpha amylase, arginase, L- asparaginase, Fungi and rice.

I. INTRODUCTION

Rice (*Oryzae sativa* L) is one of the record important main foods world-wide Rice contains nutrition compounds, starch, protein, fat, ash and fiber. Starch and protein are primary constituents and reflected as good substrates for fungi that contaminated rice [1]. Infection of stored rice with fungi is considered great problem, that rice grain was contaminated by toxigenic fungi which it is source of mycotoxins [2] and exhibited different activities in production of many enzymes [3]. In stored rice, the fungal flora is diverse from that in freshly harvested rice. *Aspergillus* spp. are common genus polluted stored rice [4-6] however species of *Alternaria* and *Penicillium* have also been stated [5,7]. Amylases are main enzymes active in the starch processing industries for the degrading of polysaccharides such as starch into glucose, maltose and other low molecular weight sugars [8], by hydrolysis 1-4 linkage of starch, it is also broadly used instarch liquefaction, food, pharmaceutical, paper industries and sugar industries. While amylases can be got from several sources, such as plant and animals (as human salvia), the enzyme from microbial sources usually meet industrial request [9, 10]. L-asparagine is a non-essential amino acid, was detected in a number of plants and animals. L-asparagine of rice offers fungi on rice with nitrogen. Fungi secrete L- as paraginase to hydrolyse L-asparagine into L-aspartate and ammonia [11]. L-asparaginase expected increased awareness in recent

years for its ant carcinogenic effect. The beneficial possible of this enzyme is in the dealing of acute lymphoblastic leukemia. Some tumors need the extra cellular sources of some amino acids, which are considered as non-essential in usual cells, due to metabolic deficiencies. Thus, enzymatic poverty of these amino acids can be an effective approach in the destruction of such tumors [12]. Asparaginase action is widely spread in plants, animals and microbes including bacteria, yeasts and fungi. The chief groups of microbes which produce asparaginase are Yeast comprises *Candida utilis and Rhodotorula sp,* then fungi as *Aspergillus nidulans, A. tamari, A. terreus, Penicillum sp, Fusarium sp,* and *Helminthosporium sp.* [13], also bacterial species contain; *Pseudomonas flourescens, P. ovalis, E. coli, Erwinia carotovora, Staphylococci, Mycobacterium phlei, Thermus aquaticus, Serratia marcescens and Tetrahymena pyriformis* secrete asparaginase. L-Arginase (arginine amidinase, canavanase, L-arginase, arginine transamidinase EC 3.5.3.1) is a manganese-containing enzyme that catalyzes the deamidation of L- arginine to l-ornithine and urea. Also arginine is an originator for the biosynthesis of polyamines, proline and agmatine as well as the cell-signaling molecules glutamate, aminobutyric acid, and nitric oxide [14,15,16]. Arginase existing in plants and in many mammals, also there are a wide range of microbial sources of arginase, including bacteria (many Bacilli, *Rhizobium* group, *Agrobacterium*-, cyanobacteria, *Proteus spp*.) [17]' protozoa (*Plasmodium falciparum* and *Entamoeba histolytica*), yeast (*Saccharomyces cerevisiae*) [18] and fungi (*Aspergillus nidulans, Neurospora crassa*, *Agaricus bisporus*) [19]. Improper packaging, increased humidity, poor ventilation and long storage in the warehouses exposed great effect on the occurrence of polluting fungi in stored rice grains [20]. The aim of the present study was to detect fungi production of some enzymes, for this drive, a number of fungal isolates were selected from many rice samples to get the strains which can produce amylases, asparginase sand arginine degrading enzymes. Also we planned the relationship between fungal populations and some storage environmental conditions such as moisture contents and storage periods. This study will aid to develop the strategies to switch the fungal contamination in rice grains, confirming food safety.

II. MATERIALS AND METHODS

Collection of rice samples. A total of 51 rice samples were collected from the market at diverse areas of El-Minia governorate, Egypt, including Abu-Qurqas, BniMazar, Deir Mawas, El-Edwa, Mattay, Maghagha, Minia, Mallawi, Samalott between March 2015 and March 2016at dissimilar environmental condition (moisture content and storage periods). These samples were transported directly to laboratory and reserved in plastic bags at 5-7 ℃ till mycological identification and enzymatic finding.

Isolation of fungi. The technique of seed-plate was used to found the seed borne fungi on the rice grains. The grains were formerly plated on a suitable isolation media at a plating rate of 5 rice grains each plate and four duplicates for each rice sample [50]. We used dichloran rose bengal chloramphenicol agar: DRBC, which contained (g/l of distilled water): glucose 10, peptone 5, potassium dihydrogen phosphate 1, magnesium sulphate 0.5, dichloran 0.002 (0.2% in ethanol 1ml), chloramphenicol 0.1, rose Bengal 0.025, agar 16, pH 5 [50]. All plates were incubated for 7-8 days at 30℃.

Identification of isolated fungi. Fungi isolated from rice grain samples were transported to new Czapek's Doxmediumin Petri plates for identification and slant media flasks for preservation. Formerly fungal colonies were exposed to microscopic identification allowing to [51-53].

Amylase production by fungi Growth medium. Fungi were full-grown in liquid yeast-starch medium of Emerson [54] which comprises (g/l): Difco milled yeast extract, 0.4g; K_2HPO_4, 0.1g;,$MgSO_4.7H_2O$, 0.5 and solvable starch, 15.0 g. The pH was accustomed to 7.0. Afore autoclaving, the medium was distributed in conical flasks (100 ml) having 50 ml of this medium. Next cooling the medium in flasks was injected with fungal strains then incubated for 7days at 28℃. Once incubation period, the fillings of each flask were filtered and the filtrate was used for detection of amylase activity, amylase action was noticed on yeast-starch agar medium [54] by means of the cup plate method. Cups (one/plate) of 10 mm diameter were made in the frozen yeast-starch medium. 0.1 ml of crude enzyme preparation were put into each cup. The plates were incubated for 24 hours at 30℃ tailed by submerging with 5 ml of (0.02N) iodine solution. The diameter of clear zone around holes were slow in mm and documented as a positive response.

Estimation of L - asparaginase activity. Modified Czapekdox's medium was used [37, 55], Ph 6.2, contained (g/l of distilled water): glucose, 0.2, l-asparaginase, 10.0, potassium dihydrogen phosphate, 1.52, kcl, 0.52, magnesium sulphate, 0.52, agar 20. Czapekdox's medium contained phenol red (%0.009) was used. This medium deprived of dye and deprived of asparagine (instead containing sodium nitrate as nitrogen source) was used as control. Variations in dye color was detected after development of fungal isolates.

Screening for arginase production. The technique founded on the combination of phenol red in a standard solution ready in ethanol (2.5% in ethanol 95%. pH 6.2). The medium contained (g/l): glucose, 2.0; Arginin, 10.0; $K_2PO_4$1.52; KCl, 0.52; $MgSO_4.7H_2O$ traces of $FeSO_4.7H_2O$. The last pH was adjusted to 6.2. Later inoculation cultures stayed incubated at 28℃ for 7 days next which results were recite [37, 55]. Production of arginase was detected as red colouring beneath the growing fungi owing to the release of ammonia subsequent from degradation of the amino acid arginin. Phenolred at alkaline is pink nevertheless in acidic is yellow.

Moisture content analysis. Each sample of rice grains was analysed for its moisture content subsequent the technique of 56. From each sample, 25 g of rice grains were occupied and located in before weighed crucibles and always dried at 105℃ in a hot air oven till the constant weight was achieved. Moisture content was resolute by calculating the variance between initial weight and dry weight of the sample.

III. RESULTS AND DISCUSSION

Amylase production by fungi Growth medium. In the present study, 62 fungal isolates, indicating 46 fungal species correlated to 15 genera were numerated allowing to AUMC as presented in Table 1. These strains were selected for their capacity to release extracellular enzyme amylase. Some tested strains have ability to this produce enzyme with different

power, others didn't have this ability (Fig. 1). *Aspergillus terreus* isolate No.34, *A. tamarii* No.12, *A. flavus* 3 isolates No.14,38, 46, *A. versicolor* No.1, *A. flavus* No. 21 (AUMC11396), *A. parasiticus* No. 8 and *A. candidus* No.13 exhibited maximum action in the manufacture of amylase (21-29 mm). *A. fumigates* No. 32 (AUMC11372), *A. sydowii* No. 49, *A. flavus* 3 isolates No.9, 33, 42,*A. ochraceus* one isolate No. 4, *A. oryzae* No. 3, exhibited moderate activity in the production of amylase (15-20mm).Only one isolate of *A. ochraceus* No. 31(AUMC11382), *A. terreus* 2 isolates No. 33, 39 and *A. flavus* one isolate No. 29 were weak in the production of amylase (11-13.3mm). Also *A. aegyptiacus, A. cravats, A. flavus*, No.10, 47, A. *fumigatus No.*38, A. *niger* No. 31 (AUMC11385), *A. ochraceus* No. 34, *A. terreus* No. 45 and *A. wentii*No.39 (AUMC 11389) didn't have any activity in production of this enzyme. Allstrains of *Penicillium were* unable to produce amylase except *P. oxalicum*one isolate No.13 which has weak activity in production of this enzyme (12.6 mm).*Trichurusspiralis*No.22 (AUMC 11392) was showed high activity in production of amylase (22 mm). *Cochliobolusspicifer* No.5 and *A. rubrum* No.49 exhibited moderate activity in the production of amylase (15,16.6mm) respectively. *Lichtheimia corymbifera* No. 43, *Cladosporium cladosporioides* No.42(AUMC11381), *A. chevalieri* No. 28(AUMC11386) and *Fusarium verticillioides* No. 50(AUMC11388) were weak in the production of amylase (11.3-14mm). *Alternaria Alternata* No. 34(AUMC11379), *C. herbarum* No. 12,*C. sphaerospermum* No.21, *C. lunatus* No.41, *A. amstelodami* No.9 (AUMC11393), *A. montevidensis* No. 43 (AUMC11383),*Gliocladium roseum* No. 7(AUMC11374), *Mucor circinelloides* No.18, *Nigrospora oryzae* No.28, *Rhizopus oryzae No.*7, *Quambalaria cyanescens* No.41 (AUMC11376),*Trichoderma harzianum* No. 29 and *Wallemia sebi* No.49 didn't exhibit activity in production of this enzyme. There are many studies whose results correspond to ours where different fungal isolates show different abilities in the manufacture of amylase by many workers [21, 22, 23] and [57]. Abdel-Hafez *et al.,* [24] screened the aptitude of 50 fungal isolates to yield extra cellular hydrolytic enzymes in hard media concluded that *A. flavus*, *Cunninghamella echinulate, F. oxysporum, M. hiemalis* and *P. chrysogenum* showed maximum activity. Moharram *et al.,* [25] screened 44 fungal isolates from diverse parts off ababean plant, indicating 35 species and 2 varieties for amylase production. All fungal isolates tested had the ability to produce amylase. Chimata *et al.,* [26] found that the maximum manufacture of amylase was by *Aspergillus* sp. Erdal & Taskin [27] investigated the viability of loquat grains flour as substratein solid-state fermentation for α-amylase production by *P. expansum* MT-1, that he establish that production of -amylase by *P*. expansum was 6 days after incubation at 30ºC with culture medium composed of (LKF), starch as carbon source and peptone as nitrogen source and started by pH 6 with moisture content of 70%, particle size of 1 mm and 1 ml methanol as addition alcohol, the maximum enzyme production was 1012 U/g of LKF. De castro *et al.,* examined the production of amylases and also hydrolases (cellulases, xylanases, and proteases) by solid-state fermentation of babassucake, using the filamentous fungus *Aspergillus awamori* IOC-3914.

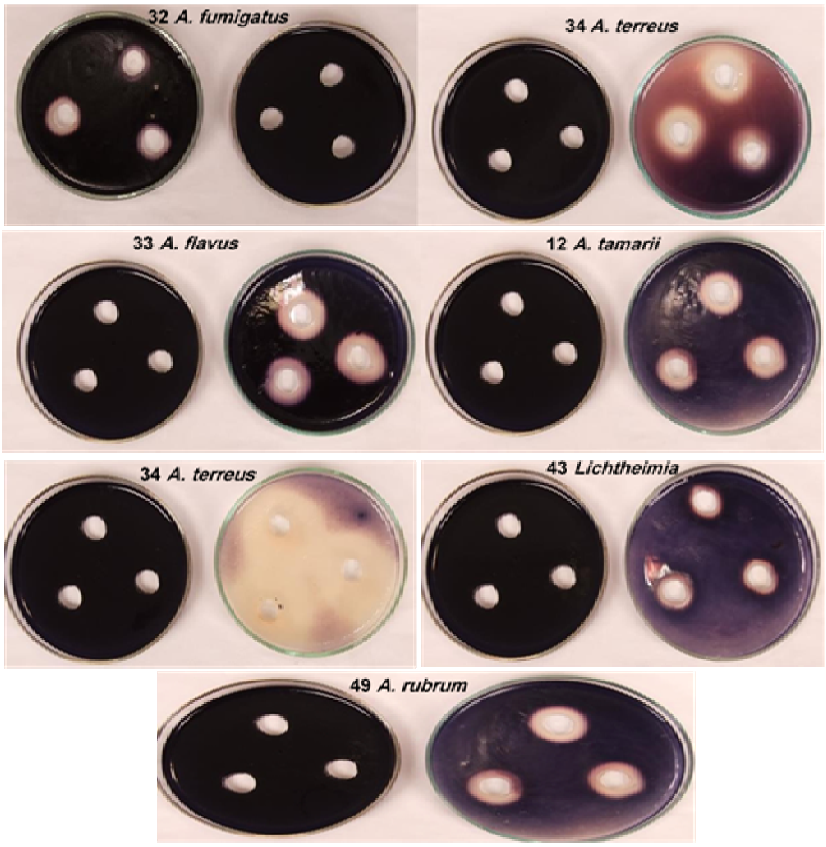

Fig. 1. Screening of amylase production by different fungi in different rice samples (comparing with control).

The most appropriate fermentation time was 144 hours, when exoamylase and endoamylase activities of 40.5 and 42.7 U g (-1) were achieved, r e s p e c t i v e l y [28]. Kim *et al.,* [29] isolated useful fungi with α-amylase activity from the Korean traditional *nuruk for* the quality of traditional Korean alcoholic beverage. Also, Kumar & Duhan [30] stated that five fungal strains, *A. candidus*, *A. terreus, A. flavus* and *A. allahabadi, A. niger* Mtcc-104 which were selected for manufacture of amylase were optimistic.

Estimation of L - asparaginase activity. In this study, it was possible to screen of 71 fungal isolates for their abilities to produce L-asparaginase and arginase. Out of 13 isolates of *A. flavus* No. 2 (AUMC 11399), 14, 19 (AUMC 11395), 20, 21(AUMC 11396), 31(AUMC 11398), 33, 42, 46, 47, 8, 38, 20(AUMC 11400), 3 isolates of *A. Ochraceus* No. 12, 34, 31(AUMC 11382), 2 isolates for *A. fumigates* No. 33, 32(AUMC 11372), one isolate for *A. oryzae* No. 3, *A. parasiticus* No.8*, A. sydowii* No.49, *A. tamarii* No.12, *A. versicolor* No. 12*, A. wentii* No. 39, *A. aegyptiacus* No. 30 and *A. candidus* No. 20 (AUMC 11378), *A. clavatus* No. 13, *A. flavipes* No.20 (AUMC11390) h a v e high activity in production of L-asparginase. Also, *Lichtheimia corymbifera* No. 43, *Alternaria alternate* No. 34(AUMC11379), *Cl. Herbarum* No. 21, *Cochliobolus lunatus* No.41, *C. spicifer* No. 5*, A. chevalieri* No. 28(AUMC 11386), *A. rubrum* No.49, *Fusarium verticillioides* No. 50 (AUMC 11388), *Fusarium semitectum* N o . 49, *Gliocladium roseum* No. 7 (AUMC 11374), *Mucor circinelloides* No. 18, *Nigrospora oryzae* No.42, *P. chrysogenum* No. 29 (AUMC 11373), *P. citrinum* No. 50(AUMC 11387), *P. corylophilum* No. 34(AUMC11380), *P. islandicum* No.49 (AUMC 11371), *P. pinophilum* No. 9 and *T. harzianum* No. 29 and (4 isolates) for *A. terreus* No. 45, 33, 34, 39 exhibited high activity in production of L–asparaginase.

Fig. 2. Screening of L-asparaginase Production by different fungi; A- *Penicillium oxalicum*, B- *P. corylophilum*, C- *P. duclauxii*, D- *Rhizopus oryzae*, E- *Quambalaria cyanescens*, F- *Aspergillus rubrum*, G- *Gliocladium roseum*, H- *Aspergillus flavus*, I- *Fusarium verticillioides*, J - *F. semitectum.*

Two isolates of *A. flavus* No. 10, 41 (AUMC 11394, 11397) and one isolate for *A. fumigates* No.38*, A. rubrum* No. 9 (AUMC 11377), *P. aurantiogriseum* No.40, *P. oxalicum* No.32 (AUMC 11375), *Quambalaria cyanescens* No.41 (AUMC 11376) and *Trichurus spiralis* No. 22 (AUMC 11392) presented temperate activity in the making of L-asparaginase (Table 1). Only one isolate for *Cl. cladosporioides, Cl. sphaerospermum, A. montevidensis, P. duclauxii, P. glabrum, P. thomii, Rhizopus oryzae* and *Wallemia sebi were* have low activity in production of this enzyme. One isolate of *A. niger* No. 33, *A. amstelodami* No.9 (AUMC 11393), *P. corylophilum* No. 35*, P. crustosum* No. 30 (AUMC 11384) and *P. oxalicum* No. 13 were unable to produce L-asparaginase, (Table 1 and Fig. 2). Several studies have been approved out on hydrolysis enzymes of the mycoflora related with numerous kinds of seeds and grains stored, by [3, 20, 31-36]. Also L-asparagine activity which recorded in our study was similar to that recorded in many previous studies, Asparaginase is formed by a diversity of microbial bases counting fungi [13, 37]. Sarquis *et al.,* [13] examined L-asparaginase manufacture in the filamentous fungi *A. tamari* and *A. terreus.* The fungi were cultured in medium having different nitrogen sources. *A. terreus* exhibited the maximum L-asparaginase (activity) production level (58 U/L) once cultured in a 2% proline medium. Both fungi existing the lower most level of L-asparaginase production in the occurrence of glutamine and urea as nitrogen sources. These outcomes propose that L-asparaginase production by filamentous fungi is in nitrogen rule.

Screening for arginase production. Also in our study, 71 isolates were tested for their ability to secrete arginase, *Aspergillus genus* (11 isolates) include *A. amstelodami* No. 9 (AUMC 11393), A. chevalieri No. 28(AUMC 11386), *A. fumigatus* No. 32 (AUMC 11372), 33, 38, *A. ochraceus* No. 12, 31 (AUMC 11382), *A. rubrum* No. 9 (AUMC 11377), 49, *A. sydowii* No. 49 and *A. versicolor* No.12 showed high activity in production of arginase while one isolate for *A. aegyptiacus* No. 30. *A. candidus* No. 20 (AUMC 11378), *A. clavatus* No.13, *A. niger* No. 33, *A. oryzae* No. 3 and *A. terreus* No. 39 and 2 isolates for *A. flavus* No. 19 (AUMC 11395), 20 cannot produce this enzyme. One isolate for *A. flavipes* No. 20 (AUMC 11390), *A. ochraceus* No. 34, 2 of *A. flavus* No.10, 20(AUMC11394, 11400) showed moderate activity for production of arginase. *A. flavus* No. 2 (AUMC 11399), 14, 21 (AUMC 11396), 31 (AUMC 11398), 33, 41(AUMC 11397), 42, 46, 47, 8, 38 and one isolate for *A. parasiticus* No. 8, *A. tamari* NO. 12, *A. Wentii* NO. 39 (AUMC 11389) and *A. montevidensis* NO. 43 (AUMC 11383) showed low activity in production of arginase (Table 1) and (Fig. 3). Four isolates of *Penicillium* genus *P. chrysogenum* NO. 29 (AUMC 11373) *P. corylophilum* NO.34 (AUMC 11380), *P. glabrum* NO.40 (AUMC 11370) and *P. pinophilum* NO. 9 showed high activity except one isolate for *P. aurantiogriseum* NO. 40, *P. duclauxii* No. 38 (AUMC 11369) and *P. oxalicum* NO.32 (AUMC 11375) exhibited weak activity but *P. thomii*one isolate No. 40 AUMC11391) showed moderate activity while one isolate for *P. citrinum* No. 50) AUMC 11387), *P. crustosum* No. 30 (AUMC 11384), *P. islandicum* No. 49(AUMC 11371)*, P. oxalicum* No.13 and *P. corylophilum* No. 35 had no activity for arginase production. *Lichtheimia corymbifera* No. 43, *Alternaria alternate* No.34 (AUMC 11379), *Fusarium semitectum* No.49, *Gliocladium roseum* No. 7 (AUMC 11374) and *Mucor circinelloides* No. 18 exhibited maximum activity for this enzyme. *C. cladosporioides* No. 42(11381), *C. herbarum* No. 21, *Quambalaria cyanescens* No. 41(AUMC 11376) and *Trichurus spiralis* No. 22(AUMC 11392) showed low activity and *F. verticillioides* No. 50 (AUMC 11388) showed moderate activity, one isolate for *Cl. Sphaerospermum* No. 21,

Cochliobolus lunatus No.41, *Cochliobolus spicifer No.*5, *Nigrospora oryzae* No.42, *Rhizopus oryzae* No.7*, T. harzianum* No. 29 and *Wallemia sebi* No. 49 did n't show any activity for arginase production. For the production of arginase, there were few previous literatures about arginase, butour results were in accordance with Al hussaini [3], Moharram *et al.,* [38]. Arginase has been stated to be purified and described from *Neurospora crassa* by Borkovich & Weiss [39]. Arginine specific carbamylphosphate matabolism in mitochondria of *Neurospora crassa* was planned by Davis & Ristow [40] wherever it was stated that arginine efficiently feedback- inhibits intra mitochondrial ornithine production. Two forms of Arginase expressed by *Neurospora crassa* as stated by Marathe *et al.,* [41] is the only reported example of multiple forms of arginase in a microbial organism. The higher form was made by mycelia increasing in arginine-supplemented medium. *Aspergillus nidulans* yields 22 arginases which allows the fungus to use arginine as the sole nitrogen source [42] Mycelial extracts of *Trichoderma* sp. were described to be a basis of arginase by El-Meleigy & Khattab [43]. A broad study of advanced fungi accepted out by Wagemaker *et al.,* [44] shown that the occurrence of arginase in members of family Agaricaceae containing *Agaricus bisporus* that led to the increase of urea in its fruit groups.

Moisture content analysis. We found, On DRBC medium, mainly the difference in moisture contents and storage periods of the rice samples is consistent with the difference in total count of fungi. The highest total count of fungi was observed in sample no. 2 with highest moisture content (15.75% (and storage period (12 months), it was 38 colonies/ 20 grains, also 2 samples No 50, 17 with moisture contents (13.72%, 13.70%) respectively and storage periods 7 months showed high total count of fungi (Table 2).

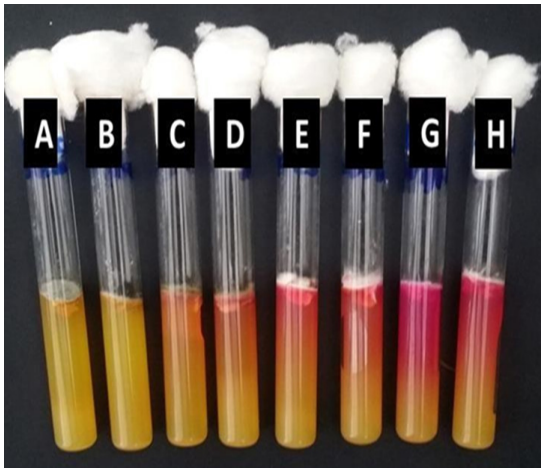

Fig. 3. Screening of Arginase Production by different fungi; A- *Penicillium crustosum* 30, B- *Aspergillus clavatus* 13, C- *A. flavus* 42, D- *A. flavus* 33, E- *A. flavipes* 20, F- *Fusarium verticilliodes* 50, G- *P. corylophilum* 34, H- *Gliocladium roseum* 7.

Table 1: Amylase, L-asparaginase, and Arginase activity of fungal strains isolated from rice grains samples.

S. No.	Fungal genera, species	Fungal source/ AUMC No. if present	L-asparaginase Degree	Amylase Diameter of clear zone(mm)	Arginase degree
43	*Lichtheimia corymbifera*	Mallawi DRBC (un numbered by AUMC)	+3H	11.3 L	+3H
34	*Alternaria alternate*	El-Minia DRBC (11379)	+3H	-	+3H
30	*Aspergillus aegyptiacus*	El-Minia DRBC (un numbered by AUMC)	+3H	-	-
9	*A. amstelodami*	Maghagha DRBC (11393)	-	-	+3H
13	*A. candidus*	Bni-Mazar DRBC (un numbered by AUMC)	-	29 H	-
20	*A. candidus*	Mattay DG18 (11378)	+3H	-	-
28	*A. chevalieri*	El-Minia DRBC (11386)	+3H	12 L	+3H
13	*A. clavatus*	Bni-Mazar DRBC (un numbered by AUMC)	+3H	-	-
20	*A. flavipes*	Mattay DG18 (11390)	+3H	-	+2M
2	*A. flavus*	El-Edwa DRBC (11399)	+3H	-	+1L
8	*A. flavus*	Maghagha DRBC (un numbered by AUMC)	+3H	-	+1L
9	*A. flavus*	Maghagha DG18 (un numbered by AUMC)	-	20 M	-
10	*A. flavus*	Maghagha DRBC (11394)	+2M	-	+2M
14	*A. flavus*	Bni-Mazar DRBC (un numbered by AUMC)	+3H	22 H	+1L
19	*A. flavus*	Mattay DRBC (11395)	+3H	-	-
20	*A. flavus*	Mattay DRBC (un numbered by AUMC)	+3H	-	-
20	*A. flavus*	Mattay DG18 (11400)	+3H	-	+2M
21	*A. flavus*	Samalott DRBC (11396)	+3H	23.3 H	+1L
29	*A. flavus*	El - Minia DRBC (un numbered by AUMC)	-	12.6 L	-
31	*A. flavus*	El-Minia DRBC	+3H	-	+1L

S. No.	Fungal genera, species	Fungal source/ AUMC No. if present	L-asparaginase	Amylase	Arginase
			Degree	Diameter of clear zone(mm)	degree
		(11398)			
33	*A. flavus*	El-Minia DRBC (un numbered by AUMC)	+3H	16.6 M	+1L
38	*A. flavus*	Abu-Qurqas DRBC (un numbered by AUMC)	+3H	24 H	+1L
42	*A. flavus*	Abu-Qurqas DRBC (un numbered by AUMC)	+3H	16 M	+1L
46	*A. flavus*	Mallawi DG18(un numbered by AUMC)	+3H	24 H	+1L
47	*A. flavus*	Mallawi DRBC (un numbered by AUMC)	+3H	-	+1L
32	*A. fumigatus*	El -Minia DRBC (11372)	+3H	15.3M	+3H
33	*A. fumigatus*	El-Minia DG18 (un numbered by AUMC)	+3H	-	+3H
38	*A. fumigatus*	Abu-Qurqas DG18 (un numbered by AUMC)	+2M	-	+3H
43	*A. montevidensis*	Mallawi DG18 (11383)	+1L	-	+1L
31	*A. niger*	El -Minia DRBC (11385)	-	-	-
33	*A. niger*	El-MiniaDRBC (un numbered by AUMC)	-	-	-
4	*A. ochraceus*	El-Edwa DG18 (un numbered by AUMC)	-	19 M	-
12	*A. ochraceus*	Bni-Mazar DG18(un numbered by AUMC)	+3H	-	+3H
31	*A. ochraceus*	El -Minia DRBC (11382)	+3H	11 L	+3H
34	*A. ochraceus*	El -Minia DG18 (un numbered by AUMC)	+3H	-	+2M
3	*A. oryzae*	El-Edwa DG18 (un numbered by AUMC)	+3H	20 M	-
8	*A. parasiticus*	Maghagha DRBC (un numbered by AUMC)	+3H	24 H	+1L
9	*A. rubrum*	Maghagha DRBC (11377)	+2M	-	+3H
49	*A. rubrum*	DeirMawas DG18 (un numbered by AUMC)	+3H	16.6M	+3H
	A. sydowii	DeirMawas DRBC (un numbered by AUMC)	+3H	16 M	+3H
12	*A. tamarii*	Bni-Mazar DRBC (un numbered by AUMC)	+3H	21 H	+1L
45	*A. terreus*	Mallawi DG18 (un numbered by AUMC)	+3H	-	+1L
	A. terreus	El- Minia DG18 (un numbered by AUMC)	+3H	11.3L	+1L
34	*A. terreus*	El- Minia DRBC (un numbered by AUMC)	+3H	15 M	+1L
39	*A. terreus*	Abu-Qurqas DRBC (un numbered by AUMC)	+3H	13.3L	-
1	*A. versicolor*	El-Edwa DG18 (un numbered by AUMC)	-	23 H	-
12	*A. versicolor*	Bni-Mazar DRBC (un numbered by AUMC)	+3H	-	+3H
39	*A. wentii*	Abu-Qurqas DRBC (11389)	+3H	-	+1L
42	*Cladosporium cladosporioides*	Abu-Qurqas DG18 (11381)	+1L	12 L	+1L
12	*C. herbarum*	Bni-Mazar DRBC (un numbered by AUMC)	-	-	-
21	*C. herbarum*	Samalott DG18 (un numbered by AUMC)	+3H	-	+1L
21	*C. sphaerospermum*	Samalott DG18 (un numbered by AUMC)	+1L	-	-
41	*Cochliobolus lunatus*	Abu-Qurqas DRB*C* (un numbered by AUMC)	+3H	-	-
5	*Cochliobolus spicifer*	El-Edwa DRBC (un numbered by AUMC)	+3H	15 M	-
50	*Fusarium verticillioides*	DeirMawas DRBC (11388)	+3H	14 L	+2M
49	*F. semitectum*	DeirMawas DG18 (un numbered by AUMC)	+3H		+3H
7	*Gliocladium roseum*	Maghagha DRBC	+3H	-	+3H

S. No.	Fungal genera, species	Fungal source/ AUMC No. if present	L-asparaginase	Amylase	Arginase
			Degree	Diameter of clear zone(mm)	degree
		(11374)			
18	*Mucor circinelloides*	Mattay DG18 (un numbered by AUMC)	+3H	-	+3H
28	*Nigrospora oryzae*	El- Minia DG18 (un numbered by AUMC)	-	-	-
42	*Nigrospora oryzae*	Abu-Qurqas DRBC (un numbered by AUMC)	+3H	-	-
29	*Penicillium arantiogrisem*	El- Minia DRBC (un numbered by AUMC)	-	-	-
40	*P. aurantiogriseum*	Abu-Qurqas DRBC (un numbered by AUMC)	+2M	-	+1L
29	*P. chrysogenum*	El-Minia DRBC (11373)	+3H	-	+3H
40	*P. chrysogenum*	Abu-Qurqas DRB*C* (un numbered by AUMC)	-	-	-
50	*P. citrinum*	DeirMawas DRBC (11387)	+3H	-	-
34	*P. corylophilum*	El-Minia DRBC (11380)	+3H	-	+3H
35	*P. corylophilum*	El-Minia DG18 (un numbered by AUMC)	--	-	-
43	*P. corylophilum*	Mallawi DG18 (un numbered by AUMC)	-	-	-
30	*P. crustosum*	El- Minia DRBC (11384)	-	-	-
38	*P. duclauxii*	Abu-Qurqas DG18 (11369)	+1L	-	+1L
38	*P. glabrum*	Abu-Qurqas DG18 (11384)		-	
40	*P. glabrum*	Abu-Qurqas DRBC (11370)	+1L		+3H
49	*P. islandicum*	DeirMawas DRBC (11371)	+3H	-	-
13	*P. oxalicum*	Bni-Mazar DRBC (un numbered by AUMC)	-	12.6 L	-
32	*P. oxalicum*	El- Minia DG18 (11375)	+2M	-	+1L
9	*P. pinophilum*	Maghagha DRBC (un numbered by AUMC)	+3H	-	+3H
40	*P. thomii*	Abu-Qurqas DRBC (11391)	+1L	-	+2M
7	*Rhizopus oryzae*	Maghagha DG18 (un numbered by AUMC)	+1L	-	-
41	*Quambalaria cyanescens*	Abu-Qurqas DRBC (11376)	+2M	-	+1L
29	*Trichoderma harzianum*	El Minia DG18 (un numbered by AUMC)	+3H	-	-
22	*Trichurus spiralis*	Samalott DRBC (11392)	+2M	22 H	+1L
49	*Wallemia sebi*	DeirMawas DRBC (un numbered by AUMC)	+1L	-	-

Amylase activity was calculated as average diameter of clear zone in mm) of the tested fungal isolates. In case of asparginase and arginase; Degree +1 means low production of enzyme (L), +2 means moderate production (M) and +3 means high production of enzyme (H).Diameter of clear zone from 21 to 30 means high activity of enzyme (H), from 15 to 20 = moderate activity of enzyme (M) and less than 15 = low activity of enzyme (L)

Samples No.1, 13, 15, 16, 19, 20, 21, 22, 23,24, 25, 28, 30, 32, 34, 35, 37, 38, 41, 43, 45, 46, 47 showed low fungal total count with moisture content %0.7-%7.10 and storage periods between 10 days- 3 months (Table 2). The lowest cases 24 and 25 samples in the number of fungal colonies (one colony) at the lowest moisture content (1.01) and one-month storage, also two colony isolated from the sample 46 at less moisture content 0.7 and10 days storage. *Aspergillus, penicillium* and *Fusarium* are the most common genera in rice samples with increased humidity and storage period. In addition to the presence of *Alternaria* and *Cladosporim* isolated a few times from some rice samples that have a moisture content and length of storage period. The relationship of moisture content and storage period to fungal population, seed germination, grain whiteness are very important. Various fungal species predominated at different moisture conditions and storage periods. We concluded, the greater the moisture content, the higher the fungal total count. The increase in the number of fungi is significant when the humidity is greater than 14%, As well as increasing the storage period increases the rate of colonization of rice fungi, it is noticeable after six months. This finding corresponding with previous studies recorded by [45, 46], Only 9 of 5117.64%) of samples with moisture contents above the commended

level for safe storing of rice grains % 0.14they were samples No. 2, 3, 4, 6, 8, 12, 18, 29, 42 with moisture contents ranged between 14.24% - %15.57 % and storage periods ranged from 7-12 months, they showed high total count of fungi. Many studies have been approved out on the mycoflora related with several types of seeds stored under moisture content conditions in many parts of the world. As suggested by [47] cereals are conserved by decrease of moisture content to fewer than 13.5% and oil seeds to fewer than 7.8%, because storage fungi such as *Aspergillus* spp. or *Penicillium spp.* cannot mature at these low moisture content. Unluckily low energy means of aeration seeds are not continuously applied as sort fungi can grow earlier the seed dry, especially in parts where the relative humidity is high next yield [48]. When gathered, rice moisture contented shows a significant role in fungal growth and AF manufacture. Rice with great moisture content wants to be dry directly in order to escape fungal and mycotoxin production. It has been recognized that *A. Flavus* can contaminate rice grains only when moisture content is upper than 12% [49].

Table 2: Effect of moisture content and storage periods on fungal total counts and common fungal genera (on DRBC medium).

Sample number	Moisture content	Storage periods months	Total count (TC) (colonies / 20 grains)					
			TC	*Aspergillus*	*Penicellium*	*Fusarium*	*Alternaria*	*Cladosporium*
1	7.00	3	10	9	1	-	-	-
2	15.57	12	38	23	12	-	-	2
3	14.92	8	29	20	1	-	-	1
4	14.26	7	22	19	2	-	-	-
5	13.70	7	18	9	9	-	-	-
6	14.80	8	27	17	-	-	-	3
7	13.00	6	16	7	7	-	-	-
8	14.60	8	25	3	20	-	-	-
9	10.02	5	13	10	3	-	-	-
10	10.05	5	13	1	6	-	4	1
11	10.02	6	15	10	-	-	-	-
12	14.32	7	22	1	19	2	-	-
13	5.03	3	6	5	-	-	-	-
14	13.50	7	18	11	3	-	3	-
15	7.01	3	8	3	4	-	-	-
16	1.01	1	2	1	-	-	-	-
17	13.70	7	20	4	14	1	-	-
18	14.50	7	23	14	3	5	-	-
19	1.01	1	3	2	1	-	-	-
20	7.01	3	9	9	-	-	-	-
21	1.01	1	2	2	-	-	-	-
22	1.01	1	2	2	-	-	--	-
23	5.00	3	7	7	-	-	-	-
24	1.01	1	1	1	-	-	-	-
25	1.01	1	1	1	-	-	-	-
26	10.07	4	11	7	4	-	-	-
27	10.07	3	12	8	1	1	1	1
28	7.03	3	9	4	2	-	1	1
29	14.24	7	22	22	-	-	-	-
30	1.01	1	4	4	-	-	-	-
31	13.01	6	16	8	8	-	-	-
32	6.07	3	8	8	-	-	-	-
33	13.50	7	18	18	-	-	-	
34	7.01	3	9	8	-	-	-	-

Sample number	Moisture content	Storage periods months	Total count (TC) (colonies / 20 grains)					
			TC	*Aspergillus*	*Penicellium*	*Fusarium*	*Alternaria*	*Cladosporium*
35	1.01	1	3	3	-	-	-	-
36	10.02	5	15	15	-	-	-	-
37	7.10	3	9	7	2	-	--	-
38	6.80	3	10	9	-	1	-	-
39	10.10	5	14	14	-	-	-	-
40	13.69	7	17	12	-	-	1	2
41	7.01	3	10	3	-	-	1	4
42	15.30	8	30	24	-	-	-	5
43	1.01	1	3	1	1	-	-	-
44	10.00	5	14	8	6	-	-	-
45	7.03	3	9	5	4	-	-	-
46	0.7	10 days	2	-	2	-	-	-
47	4.8	1	7	-	2	-	2	-
48	10.02	4	11	6	1	-	-	4
49	13.10	6	16	13	3	-	-	-
50	13.72	7	21	11	2	-	3	2
51	1.01	8	3	3	-	-	-	-

IV. CONCLUSION

We studied the contamination of markets rice by fungi, many of the isolated genera and species have the capacity to create **α-** Amylase, L-Asparaginase and Arginase. *Aspergillus* and *Penicillium* genera showed remarkable activity in producing these enzymes. This study is an attempt has to offer a contaminated rice fungus as basis of enzymes for medicinal and manufacturing requirements such as applications in food, detergent, pharmaceutical, paper, textile, industries and production of ethanol. Though, other complete study is necessary to describe these enzymes, which may be used in the important manufacture for viable determination in future. It was observed that moisture contents and storage periods encouraged fungal growth. Hence, to preserve the safety of rice storage, it must be preserved in environmental condition unsuitable for fungal growth.

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Conflict of interest. The authors confirm that there are no known conflicts of interest associated with publication of this paper.

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