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EVALUATION OF SOME SUNFLOWER SEED HYBRIDS FOR OIL PROPERTIES AND FUNGAL INFECTION

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Abstract

Sunflower seed hybrids identified with the numbers: 403, 599, 4112 and 6480 were used for some analysis to study their specific properties for evaluation. The whole seeds were analyzed for moisture and oil contents percentage, and the detection of AFB_1 produced form the isolated fungi which were found to be able to grow on the seeds as seed-borne fungi.

The obtained results showed that the seeds (6480) contained the highest oil percentage (44.20%) and the highest infection of *A. flavus* (30 %) and *A. niger* (60%). On the other hand, the seeds (599) were found being infected with both *A. parasiticus* (7%) and *A. flavus* (10%). The frequent low growth could be due to the lower moisture and oil contents and consequently the produced aflatoxin AFB_1. The isolated fungal strain of *A. parasiticus* grown on the seeds (599) were the weakest strain for AFB_1 production on most substrates compared with *A. flavus*. The whole seed (6480) stimulated the high production of AFB_1 due to its high content of oil (44.20%), compared with the seeds (599) which contain 38% oil. The same trend was observed with the amount of AFB_1 produced in the pulp (dehulled seeds). However, the amount of AFB_1 in the meal (599) (defatted seeds with high protein remainings) were in high amount in contrary to the lower AFB_1 produced in the meal (6480) deffated from the high oil content with remaining lower protein.

The fatty acid composition of the seeds (6480) showed 90.60% of unsaturated fatty acids with a high amount of $C_{18:2}$ (43.50%) stimulated the production of AFB_1. Generally, sunflower seeds (403) could rank the best hybrid for its oil content (40.32%), high unsaponifiable matter (1.38%) which is the oil preservative component and the high oleic content (78.26%) which increases its stability.

INTRODUCTION

Sunflower ranks second to soybean among annual field crops grown in the world for production of edible soybean and sunflower oils; the production of soybean and sunflower oils were 20.8 and 9.8 million tons in 1997, respectively (FAO, 1997). Sunflower gets most of its economic value from the oil extracted from the seeds and the other value from the meal used in animal feeds for livestock and poultry; because of its high protein content 24% from hulled seeds to 40% from completely dehulled seeds (Robbelen *et al.*, 1989).

The oil extracted from the seeds (40-48%) contributes about 80% of the total value of the crop. This is in contrast to soybean, corn and cotton where the oil is a by-product of the meal, starch and fiber industries, respectively (Robbelen *et al.*, 1989). Sunflower oil is used as a salad and cooking oil. It is generally considered a premium oil because of its light colour, bland flavour, high smoke point, high level of oleic and linoleic acids (about 90%) and the absence of linolenic acid (Campbell, 1983).

Prasad and Singh (1983), found the presence of *A. flavus* and *Alternaria alternata* on the surface of sunflower seeds. Moreover, El-Maraghy and Maghraby (1986) reported that *A. flavus, A. nigher, Penicillium* and *Fusarium* were the most common fungal growth on 36 samples collected from different places in Egypt. Also, many investigators, Suryanarayanan and Suryanarayana (1990), Chulze *et al.* (1991), Dawar and Ghaffar (1991) and White and Jayas (1993), recorded the incidence of microfloral infection by *Aspergillus* and *Penicillium* species, and their growth affected directly the properties of the extracted oil.

Objectives in sunflower breeding vary with production areas, but generally emphasize high seed yield and high oil percentages. Breeding to improve seed and oil quality characteristics has generally been an important objective in developing improved cultivars, although significant genetic variation exists for the protein and lipid fractions of seeds (Robbelen *et al.*, 1989).

The aim of this work was to evaluate the extracted oils of four new hybrids of sunflower seeds for their oil contents, their chemical characteristics and the incidence of seed-born fungi producing aflatoxins, in order to choose the best hybrid for expansion in Egypt.

MATERIALS AND METHODS

1. Materials:

1.1. Sunflower seeds of four hybrids (403, 599, 6480 and 4112). Two kilograms of each, sample was obtained from Pioneer company for seed production, the experimental stations located at Moshtohor and Kaha.

1.2. Potato Dextrose Agar (PDA) medium was used for isolation, identification and propagation of the fungal strains; Yeast Extract Sucrose (YES) medium was used for fungal growth and aflatoxin, production. These media were prepared as described in Difco-Manual (1963).

1.3. Pure aflatoxin B1 was obtained from Sigma Chemicals Company, St. Louis, to be used for semi-quantitative detection of the produced aflatoxins.

2. Methods:

Two kilograms of each of the sunflower seed hybrids were used for the following experiments:

1. Isolation and purification of the fungal flora present on the dry seed samples was performed according to Quasem and Christensen (1958).

2. The purified fungi were identified according to Barrnett and Hunter (1972).

3. Aflatoxins in the experimental seed samples (100 gm ground seeds) were detected as described by Schuller and Van Egmond (I 983).

4. The seed-born fungi of *A. flavus* and *A. parasiticus* isolated from the seed samples were propagated by incubation on PDA medium for 10 days at 28-30°C then tested for their ability to produce aflatoxin by contaminating of the sterilized samples of the sane seed sources with their different fractions as hulls, pulp (dehulled seeds), meal (defatted ground seed) and compared with the YES medium. The samples were incubated at 28-30°C for 2 weeks; then aflatoxin B_1 was detected as described by Schuller and Van Egmond (1983).

5. Moisture and oil percentages in the different sunflower seed hybrids were identified according to methods described in AOCS (1985).

6. The different sunflower seed samples were crushed and grounded using Osterizer mill. The oil was extracted for 24 hrs with hexane. The oil samples were kept in dry dark glasses at -10°C for the oil analyses.

7. Oil analyses (refractive index, acid value, peroxide number and unsaponifiable matter) were carried out according to the methods described in AOCS (1985).

8. The aforementioned experiments and analyses were carried out in triplicates and the average was calculated and tabulated for discussion.

9. Chromatographic analysis of fatty acid composition of the extracted oils was performed according to the method of Farag *et al.* (1986).

RESULTS AND DISCUSSION

Sunflower seed hybrids identified with the numbers 403, 599, 4112 and 6480 were used for some analysis to study their specific properties for evaluation. The whole seeds were analyzed for moisture and oil contents percentage, and the detection of aflatoxin B1 (AFB1) produced from the isolated fungi which were found be able to grow on the seeds as seed-born fungi. The obtained results tabulated in Table 1 show that the lowest moisture content was 6.86% in the seeds 599 and the highest was 8.76% in seeds 4112. This high moisture content above 8% is not recommended especially for storage of seeds for a period of 12 months; as reported by White and Jayas (1993) who mentioned that sunflower seeds could be stored safely without fungal growth and consequently without increasing their free fatty acids for 12 months at 6% moisture content and 30°C; 7% moisture content and 20°C or 8% moisture content and 10°C. Therefore it is preferred for these sunflower seeds to be stored in a cooler place between 10 and 20°C or at level less than 75% RH (relative humidity) to avoid the fungal growth of the seed-born fungi. It is clearly observed from Table 1 that (6480) hybrid sects contained the highest oil percentage (44.20%) and the highest infection of the fungal growth of both *A. flavus* (30%) and *A. niger* (60%). And due to their growth in the sane medium the higher growth (A. niger) inhibited the function of *A. flavus* to produce AFB1 (15.32 µg/kg) which is considered lower than the safe limit (20 µg/kg). This result is in agreement with the findings proved by Paster *et al.* (1992). Another fungal growth was the infection of hybrid seeds (403) with 30% *A. niger,* but this was not considered dangerous because such species is not aflatoxin producer. On the other hand, the hybrid seeds (599) were found infected with both *A. parasiticus* (7%) and *A.flavus* (10%). The less growth could be due to the lower moisture and oil contents which inhibited their higher growth and consequently the produced aflatoxin (Table 1). These results could be in good correlation with the other data tabulated in Table 3; which showed the high amounts of AFB_1 produced in the hybrid seeds 6480 containing higher moisture and oil contents.

The properties of the different extracted sunflower oils are presented in Table 2. The acid value ranged from 0.42 in the hybrid seeds (4112) to 0.55% in the seeds (599). Also, the peroxide numbers were the lowest 1.80 meq/kg in the seeds (4112) and the highest 6.36 meq/kg in the seeds (599). Moreover, the unsaponifiable matters were in the lowest (0.95%) in the seeds (4112) and highest (1.72%) in the seeds (599). These observations were correlated with the fungal infestation. Once the fungal growth, on the lipid content of the seeds, the consequence results are the increase of the free fatty acids, the peroxide number and the accumulation of the metabolites and

pigments excreted in the oils. These observations were found by Farag *et al.*, (1986).

The different stains of *A. flavus* and *A. parasiticus* were found to be grown on sunflower seeds, were tested for their ability to produce aflatoxin B_1 in the sterilized samples of their same substances (sources) with the different fractions of the seeds. The obtained results in Table 3, show that *A. parasiticus* (599) was the weakest fungal strain producing AFB_1 in most substrates compared with *A. flavus*. The whole seed (6480) stimulated the high production of AFB_1 due to its high content of oil percentage (44.20%), compared with the production of AFB_1 in the hybrid seeds (599) which contain 38% oil. The same trend was observed with the amount of AFB_1 produced in the pulp (dehulled seeds). However, the amount of AFB_1 in the meal 599 (defatted seeds and high protein remaining) were in high amount in contrary to the lower AFB_1 produced in the meal (6480) defatted from the high oil content with remaining lower protein.

The hulls proved to be a non-nutritive substrate for the experimented fungal strains. Moreover, the results showed that the YES medium increased the potential of AFB_1 production for the experimented seed-born fungi of *Aspergillus flavus* and *Parasiticus*, due to the presence of all the nutritive needs for fungal growth and the production of aflatoxins. It could be added that the sunflower seeds with their amounts of moisture, oil content or oil composition could affect the fungal growth and aflatoxin production.

The fatty acid composition in Table 4 show the effect of hybridization among the four sunflower seed samples and the standard in references 1 and 2. Chulze e*t al.*, (1991) proved that the increase of unsaturated fatty acids stimulated aflatoxin production in sunflower seeds. Therefore, upon their findings, the seeds (403) containing 93.55% unsaturated fatty acids should be a good substrate for *Aspergillus;* but this was not found in the present study, that could be due to the lower amount of $C_{18:2}$ and $C_{18:3}$ in comparison with references 1 and 2. This could be also explained positively with the seeds (6480) containing 90.6% unsaturated fatty acids with a high amount of $C_{18:2}$ (43.50%) and in which the amount of polyunsaturated fatty acids stimulated the production of aflatoxin (Table 1), together with the high amount of oil. The aforementioned conclusion could not be suitable with the fatty acid composition of the seeds (599) which were found infected with the *Aspergillus* strains; but the reason of infection could be due to the low oil compensated with the high protein content which enhanced the fungal invasion and aflatoxin production.

The fatty acid composition in Table 4 of the uninfected sunflower seeds (403) comparing with (4112) seeds; it could be considered that the sample (403) rank first for its high content of $C_{18:1}$ (78.26%) and low $C_{18:2}$ (14.86%) which increase the oil stability; but the oil seed (4112) has a high amount of $C_{18:2}$ (62.48%) and a low amount of $C_{18:1}$ (22.69%) which decrease the oil stability due to its easily oxidation. Therefore, it could be concluded that sunflower seeds (403) rank the best hybrid for its oil content (40.32%), high unsaponifiable matter (1.38%) which is the oil preservative component and the high oleic content (78.26%) which increases its stability.

Table 1. Properties of different sunflower seed hybrids.

Sunflower samples	Moist. (%)	Oil (%)	Isolated fungi	Det. AFB_1 µg/kg
403	7.74	40.32	*A. niger* (30%)	-
599	6.86	38.01	*A. parasiticus* (7%), *A. flavus* (10%	21.66
4112	8.76	40.11	- - -	15.32
6480	7.19	44.20	*A. flavus* (30%)	
			A. niger (60%)	

Table 2. Properties of the oil extracted from different sunflower seed hybrids.

Oil Samples	Ref. Index	Acid value (%)	Peroxide value (meq./kg)	Unsap. Matter (%)
403	1.471	0.46	4.38	1.38
599	1.4760	0.55	6.36	1.72
4112	1.4700	0.42	1.80	0.95
6480	1.4750	0.48	6.10	1.46

Table 3. Ability of seed-born fungi to produce AFB_1 in seeds, hulls, pulp, meal and culture medium (YES).

Sterilized samples		AFB_1 µg/kg		
		A. parasiticus (599)	*A. flavus* (599)	*A. flavus* (6480)
seeds	599	150.10	195.60	160.50
	6480	210.60	240.10	250.20
Hulls	599	13.80	10.80	traces
	6480	10.50	10.50	traces
Pulp	599	189.50	215.10	288.60
	6480	210.30	242.60	295.50
Meal	599	260.45	275.60	255.70
	6480	210.10	250.10	190.10
Medium	YES	355.60	386.70	375.80

Table 4. Fatty acid composition of sunflower seed hybrids and the comparison with standard references.

F.A. Comp.	Ref.[1]	Ref.[2]	403	599	4112	6480
C:14						0.20
16	5.5	6.9	6.45	19.60	3.43	5.40
16:1	0.1					0.3
18:0	4.7	3.9		4.31	11.36	3.80
18:1	19.5	30.1	78.26	76.09	22.69	46.50
18:2	68.5	53.8	14.86		62.48	43.50
18:3	0.1	5.3	0.43		0.04	0.30
20:0	0.3					
20:1	0.1					
22:0	0.9					
24:0	0.2					
Total sat.	11.7	10.8	6.45	19.60	14.79	9.4
Unsat	88.3	89.2	93.55	80.40	85.21	90.6

Ref.[1] = Gunstone *et al.*, (1994).

Ref.[2] = Sanchez-Muniz *et al.*, (1993).

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تقييم بعض أصناف بذور هجين عباد الشمس لصفات الزيوت والتلوث الفطرى

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تم فى هذا البحث إجراء مجموعة من التحاليل بغرض تقييم بعض بذور هجين عباد الشمس التى تحمل الأرقام (٤٠٣)، (٥٩٩)، (٤١١٢) و(٦٤٨٠) – تم تحليل البذور الكاملة لتقدير النسبة المئوية للرطوبة والزيت وكذلك تقدير نسبة افلاتوكسين ب١ الناتج من النمو الفطرى عليها.

أوضحت النتائج أن البذور (٦٤٨٠) تحتوى على أعلى نسبة زيت (٤٤,٢٠٪) وكذا أعلى نسبة تلوث بفطرى اسبرجلس فلافس *A. Flavus* (٣٠٪) واسبرجلس نيجر *A. niger* (٦٠٪). بينما احتوت البذور (٥٩٩) على نسبة تلوث فطرى (٧٪) لفطر اسبرجلس بارازيتيكس *A. parasiticus* و(١٠٪) لفطر اسبرجلس فلافس *A. flavus* وهذه النموات الفطرية لم تعد فى مستوى النمو المرتفع نتيجة انخفاض نسبة كل من الرطوبة والزيت التى أدت إلى تثبيط النموات الفطرية وبالتالى قل إنتاج افلاتوكسين ب١ AFB_1. كذلك لوحظ أن الفطر اسبرجلس بارازيتيكس *A. parasiticus* النامى على البذور (٥٩٩) كان ضعيف فى إنتاج افلاتوكسين ب١ على معظم البيئات المغذية مقارنة بالفطر اسبرجلس فلافس *A. flavus*. وفى ظروف إنتاج افلاتوكسين ب١ وجد أن البذور (٦٤٨٠) كانت أكثر ملائمة للنشاط والنمو الفطرى وأعطت نسبة مرتفعة منه ويرجع ذلك لاحتواء هذه البذور على نسبة زيت مرتفعة (٤٤,٢٠٪) مقارنة بكمية الافلاتوكسين الناتج فى البذور (٥٩٩) والتيتحتوى على (٣٨٪) من الزيت. وقد تكررت هذه الظاهرة أيضاً بإنتاج كمية مرتفعة من الافلاتوكسين ب١ فى البذور المنزوعة القشرة والمحتوية على نسبة زيت مرتفعة. وكانت النتائج على عكس الظاهرة السابقة فى حالة نمو الفطريات على الكسب الخالى من الزيوت فكان افلاتوكسين ب١ المتكون فى كسب البذرة (٥٩٩) أعلى من الافلاتوكسين المتكون فى كسب البذرة (٦٤٨٠) نتيجة فصل نسبة الزيت المرتفعة وبقاء نسبة البروتين المنخفضة التى تقلل من إنتاج التوكسين – وبتحليل الأحماض الدهنية للبذور لوحظ أن البذور (٦٤٨٠) تحتوى على نسبة ٩٠,٦٪ من الأحماض الدهنية الغير مشبعة ومنها ٤٣,٥٠٪ من الحامض الدهنى $C_{18:2}$ وأدى ذلك إلى زيادة ملائمة هذه البذور لإنتاج افلاتوكسين بها.

والخلاصة أن بذور عباد الشمس (٤٠٣) ترقى إلى أفضل بذور الهجين لاحتوائها على نسبة ٤٠,٣٢٪ زيت ونسبة مرتفعة من المواد الغير متصبنة ١,٣٨٪ التى تعتبر من المركبات التى تحافظ على صفات الزيوت وكذلك احتوائها على نسبة مرتفعة من الحامض الدهنى أوليك (٧٨,٢٦٪) الذى يعمل على زيادة ثبات الزيوت أيضاً.