FUNGI ASSOCIATED WITH SEEDS OF SUNFLOWER (HELIANTHUS ANNUUS) CULTIVARS GROWN IN IRAQ

*S.K. Abdullah and **K.A. Al-Mosawi

Abstract

Mycobiota associated with seeds of nine sunflower cultivars/inbreed (Helianthus annuus) viz. ‘Akmar’, ‘Eurofflore’, ‘AS 508’, ‘Mannon’, ‘AS 615’, ‘Florasol’ and three unidentified local cultivars were studied. The seeds were associated with 48 species of fungi belonging to 19 genera. The broadest species spectrum on most cultivars consisted of the genera Aspergillus (nine species), Alternaria and Fusarium (six species each), followed by Penicillium (four species), Chaetomium, Trichoderma and Ulocladium (three species each). Aspergillus niger, A. flavus, Chaetomium globosum, Alternaria alternata, A. fumigatus, Ch. atrobrunneum, A. terreus, Penicillium expansum, P. brevicompactum, Fusarium oxysporum, F. solani, Rhizopus stolonifer, Mucor hiemalis and A. ochraceus were the most frequent species. The species composition, percentage of seed infection and seed germination percentage differed among cultivars. ‘Akmar’ cultivar showed the lowest number of detected species (17 species), whereas the highest number (48 species) was isolated from the unidentified local cultivar 3. The highest fungal infestation was recorded in unidentified local cultivar 3 (45%) and the lowest – in ‘Akmar’ (10%). Maximum seed germination was observed in ‘Akmar’ (100%) and minimum in unidentified local cultivar 3 (38%). Seed-borne pathogenic species Macrophomina phaseolina was detected in the three unidentified local cultivars with low percentage occurrence.

Key words: mycobiota, sunflower seeds, Iraq

Introduction

The cultivation of sunflower (Helianthus annuus) on a commercial level in Iraq was introduced at the middle of the twentieth century. The crop is cultivated widely in the middle and northern (Suluymania province) parts of Iraq and the
crop is principally used for production of edible oil as well as for seeds consumption (raw, roasted or salted; Al-Ansari 1982). Seeds used for cultivation of the crop were mostly imported cultivars or inbreeds. However, during the last decade attempts were made at several Iraqi scientific research foundations to produce sunflower genotypes adapted to the climate of different regions of Iraq.

Several phytopathogenic and saprotrophic fungal species have been reported on sunflower seeds. The most important seed-borne pathogens represented the genera *Alternaria* (*A. alternata, A. helianthi*), *Fusarium* (*F. chlamydosporum, F. solani, F. sporotrichioides, F. subglutinans and F. verticillioides*), *Macrophomina* (*M. phaseolina*), and *Verticillium* (*V. alboatrum and V. dahliae*) (Krishnapa and Shetty 1990, Bhutta et al. 1997 a, b, 1999, Lagopodi and Thanassoulopoulos 1998, Khan 2007, Sharfun-Nahar and Mushtaq 2007).

The economic value of sunflower seeds is greatly influenced by the associated saprotrophic fungi, which may reduce oil quality due to increase of free fatty acids amount in seeds during storage (McGee and Christensen 1970, Singh and Prasad 1977, Vijayalakshmi and Rao 1986, Bhutta et al. 1997 a) or produce mycotoxins (Shahnaz and Ghaffar 1991 a, Abdel-Malek et al. 1994, Abdullah and Al-Mosawi 2009).

Studies on the mycobiota associated with sunflower seeds and their significance have been made by researchers in different parts of the world (Roberts et al. 1986, Reddy 1989, Suryanarayanan and Suryanarayanan 1990, Mahajan and More 1991, Shahda et al. 1991, Shahnaz and Ghaffar 1991 b, Ataga and Akueshi 1996, Godika et al. 1996, Sharfun-Nahar et al. 2005). However, in Iraq only very limited studies have been performed on the subject (Moustafa et al. 1981 a, b, Kadhum et al. 1991, Ramadan and Mahmod 2003).

This study was undertaken to improve recognition of the mycobiota of sunflower seeds in Iraq and to assess the quality of seeds from several sources.

**Materials and methods**

**Seed sources**

Sunflower seeds of nine cultivars/inbreed were obtained from official sources or purchased from local markets at Basrah, Iraq as shown in Table 1.

**Isolation of fungi**

A working sample (600 seeds) of each cultivar was taken according to the „International rules for seed testing” (1966). Seeds were surface disinfected with 1% sodium hypochlorite in a beaker for 10 min and then rinsed three times in sterile distilled water. Surface disinfected seeds were placed on water soaked blotters in sterilized aluminium trays (32 × 16 × 2 cm) as described by Abdullah and Kadhum (1987). One hundred seeds were placed in each tray. The trays were cove-
red by autoclavable cellophane sheets and the seeds in the trays were incubated for 7–10 days at 25°C (regime: 12 h of darkness and 12 h of cool white fluorescent light).

Seeds were examined individually under a dissecting microscope. The frequency of occurrence (PF) for each fungus species was calculated by applying the following formula:

\[
PF\% = \frac{\text{number of seeds on which a fungus appeared}}{\text{total number of seeds}} \times 100
\]

The percentage of seeds infected by fungi was calculated. Seeds which germinated into healthy seedlings were counted. The fungi growing from the seeds were either identified directly from isolation trays or were subcultured onto other media for identification. Culture media used for identification included potato dextrose agar (PDA), malt extract agar (MEA) and Czapek agar (CZ). The media were prepared according to Pitt and Hocking (1997).

The isolated fungi were identified following Ellis (1971, 1976), Domsch et al. (1980), Sivanesan (1987), Pitt and Hocking (1997) and Klich (2002).

### Results and discussion

A total of 48 fungal species were isolated from seeds of nine sunflower cultivars using blotter method (Table 2). The common genera were *Aspergillus* (nine species), *Alternaria* and *Fusarium* (six species each) and *Penicillium* (four species). *Chaetomium*, *Trichoderma* and *Ulocladium* were represented by three species each. *Bipolaris* and *Cladosporium* were represented by two species each. Other genera were represented by a single species.

The most common species were *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. terreus*, *Chaetomium atrobrunneum*, *Ch. globosum*, *Fusarium*...
Table 2

Percentage frequency of occurrence of fungi on sunflower seeds determined by blotter method and their cultivar source

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Percentage of infected seeds of the particular cultivar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>'Akmar'</td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>1.2</td>
</tr>
<tr>
<td>Alternaria chlamydospora</td>
<td>0.0</td>
</tr>
<tr>
<td>Alternaria helianthi</td>
<td>0.0</td>
</tr>
<tr>
<td>Alternaria longipes</td>
<td>0.0</td>
</tr>
<tr>
<td>Alternaria raphani</td>
<td>0.0</td>
</tr>
<tr>
<td>Alternaria tenuissima</td>
<td>0.0</td>
</tr>
<tr>
<td>Aspergillus candidus</td>
<td>0.0</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>1.3</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>0.7</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>2.0</td>
</tr>
<tr>
<td>Aspergillus nivens</td>
<td>0.0</td>
</tr>
<tr>
<td>Aspergillus ochraceus</td>
<td>0.2</td>
</tr>
<tr>
<td>Aspergillus parasiticus</td>
<td>0.0</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>0.3</td>
</tr>
<tr>
<td>Aspergillus versicolor</td>
<td>0.0</td>
</tr>
<tr>
<td>Bipolaris hawaiensis</td>
<td>0.0</td>
</tr>
<tr>
<td>Bipolaris spicifera</td>
<td>0.0</td>
</tr>
<tr>
<td>Chaetomium atrobrunneum</td>
<td>0.5</td>
</tr>
<tr>
<td>Chaetomium elatum</td>
<td>0.0</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>0.8</td>
</tr>
<tr>
<td>Cladosporium cladosporoides</td>
<td>0.0</td>
</tr>
<tr>
<td>Cladosporium herbarum</td>
<td>0.0</td>
</tr>
<tr>
<td>Curvularia lunata</td>
<td>0.0</td>
</tr>
<tr>
<td>Doratomyces microsporus</td>
<td>0.0</td>
</tr>
<tr>
<td>Emericella quadrilineata</td>
<td>0.0</td>
</tr>
<tr>
<td>Fusarium culmorum</td>
<td>0.0</td>
</tr>
<tr>
<td>Fusarium graminearum</td>
<td>0.0</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>0.3</td>
</tr>
<tr>
<td>Fusarium semitectum</td>
<td>0.2</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>0.5</td>
</tr>
<tr>
<td>Fusarium verticillioides</td>
<td>0.0</td>
</tr>
<tr>
<td>Macrophomina phaseolina</td>
<td>0.0</td>
</tr>
<tr>
<td>Mucor hiemalis</td>
<td>0.3</td>
</tr>
<tr>
<td>Myrothecium roridum</td>
<td>0.0</td>
</tr>
</tbody>
</table>
oxysporum, F. solani, Mucor hiemalis, Penicillium brevicompactum, P. expansum, Rhizopus stolonifer and Ulocladium chartarum which were isolated from almost every cultivar. Aspergillus flavus, A. fumigatus and A. niger showed the highest incidence on the nine cultivars with the frequency of occurrence 1.3–11.3, 0.7–8.7 and 2.0–12.3%, respectively. High frequency of species such as Alternaria alternata (1.2–7.0%), Chaetomium atrobrunneum (0.5–7.0%), Ch. globosum (0.8–9.0%) and Fusarium oxysporum (0.3–4.2%) was also recorded.

Among the nine species of Aspergillus reported, A. flavus, A. niger and A. fumigatus showed the highest incidence. A high incidence of A. flavus and A. niger was reported in sunflower seeds from different locations in Pakistan (Shahnaz and Ghaffar 1991 b, Sharfun-Nahar et al. 2005). A high incidence of A. flavus was reported on sunflower seeds from India (Vijayalakshmi and Rao 1986), while Ataga and Akueshi (1996) reported A. niger among the dominant fungi associated with sunflower seeds in Nigeria. Godika et al. (1996) reported A. flavus and A. niger among the dominating fungi on sunflower seeds grown in Rajasthan, India. In a recent study Banu and Muthumary (2005) reported predominant A. flavus in sunflower seed samples used in vegetable oil refinery in Tamilnadu, India. Isolates of A. flavus and A. parasiticus detected from sunflower seeds have been well documented as potentially aflatoxigenic (Shahnaz and Ghaffar 1991 a, Abdel-Malek et al. 1994). In a recent study on the aflatoxigenic potential of Aspergillus section Flavi, strains detected in sunflower seeds in Iraq proved aflatoxigenic: 55% of A. flavus isolates and 100% of A. parasiticus isolates (Abdullah and Al-Mosawi 2009).

Aspergillus spp. were followed by Alternaria and Fusarium, represented by six species each. Alternaria alternata was the most frequent among those of the former and recovered from almost all samples, while A. chlamydospora and A. longipes were de-
ected in eight cultivars. However, *A. helianthi* and *A. tenuissima* were detected in seven and six cultivars, respectively. *Alternaria alternata, A. helianthi* and *A. tenuissima* have been well documented causing characteristic leaf spots on sunflower in several parts of the world (Krishnapa and Shetty 1990, Godika et al. 1996, Kumar and Sing 1997 and Bhutta et al. 1999).

Six *Fusarium* species were identified (Table 2). Among these, *F. oxysporum* and *F. solani* were found common in seeds of all cultivars, whereas *F. culmorum, F. semitectum* and *F. verticillioides* were common in seven cultivars.

All the reported *Fusarium* species are known to be pathogenic to sunflower, causing various symptoms, in particular wilting and seedling rot (Bhutta et al. 1997 b, 1999, Shamin et al. 2003, Sharfun-Nahar and Mushtaq 2007).

Four species of *Penicillium* were detected viz. *P. brevicompactum, P. chrysogenum, P. expansum* and *P. oxalicum*. *Penicillium brevicompactum* and *P. expansum* were common in all cultivars, whereas *P. chrysogenum* and *P. oxalicum* were detected in five and seven cultivars, respectively. *Penicillium* species are commonly detected on sunflower seeds (Shahnaz and Ghaffar 1991 b, Reddy 1989, Abdel-Malek et al. 1994).

Three species of *Chaetomium* were identified viz. *Ch. atrobrunneum, Ch. globosum* and *Ch. elatum*. The former two species were common in all cultivars, whereas *Ch. elatum* was detected in eight cultivars. Their frequencies were much higher in the three unidentified local cultivars as compared to identified cultivars (Table 2). Sharfun-Nahar et al. (2005) detected four species of *Chaetomium*, including *Ch. globosum*, in sunflower seeds in Pakistan. *Chaetomium globosum* was also isolated from sunflower seeds in Northern Nigeria (Ataga and Akueshi 1996) and was earlier reported from sunflower seeds in Iraq (Kadhum et al. 1991).

*Curvularia lunata* (teleomorph *Cochliobolus lunatus*) was detected in seven cultivars. The species was frequently isolated from sunflower seeds in different parts of the world (Shahda et al. 1991, Ataga and Akueshi 1996, Sharfun-Nahar et al. 2005). *Bipolaris hawaiiensis* (teleomorph *Cochliobolus hawaiiensis*) and *B. spicifera* (teleomorph *C. spicifer*) were detected only in unidentified local cultivars. The latter species was reported among the dominating pathogenic fungi on sunflower, causing losses in seed germination and seedling symptoms in India and Pakistan (Godika et al. 1996, Bhutta et al. 1997 b, 1999).

It is interesting that in our survey the most important pathogen of sunflower plant, *Macrophomina phaseolina*, was detected only on unidentified cultivars purchased from the local markets (Table 2). The pathogen is worldwide distributed and is responsible for several diseases of sunflower, including seedling blight, damping-off, root rot, basal stem rot and charcoal rot (Khan 2007).

It is evident from Table 2 that the number of fungi (47–48) recovered from unidentified local cultivars (sample numbers 7–9) were much higher than those isolated from identified cultivars (17–38 species). This may be attributed to the fact that the seeds of known (identified) cultivars were collected immediately after harvest and hence they were infested only by field fungi. On the other hand, those unidentified cultivars (of unknown harvest dates) were obtained from local markets, and so they might have been harbouring both field and storage fungi. Similar suggestion was made by Abdullah and Kadhum (1987) who recovered 30 species from un-
identified cultivars of *Sorghum bicolor* collected from local markets, against 23 species from cultivars with known harvest date. Ten fungal species were obtained only from unidentified cultivars. These were *Aspergillus candidus*, *A. parasiticus*, *Doratomyces microsporus*, *Bipolaris hawaiiensis*, *B. spicifera*, *Emericella quadrilineata*, *Macrophomina phaseolina*, *Myrothecium roridum*, *Oedocephalum glomerulosum* and *Trichoderma viride*.

The degree of seed infestation ranged from 10 to 45% and the share of germinated seeds ranged from 38 to 100% (Table 3). Three unidentified sunflower cultivars which have been collected from local markets displayed the highest fungal infestation (from 38 to 45%) and the smallest share of germinated seeds, ranging from 38 to 68%. Fungal species composition and percentage of fungal infestation varied among sunflower cultivars. Such variations may be attributed to the differences in geographical locality of cultivation, storage condition or to differences in physico-chemical nature of different sunflower genotypes. This is in line with the results obtained by Reddy (1989) and Godika et al. (1996).

*Alternaria chlamydospora*, *A. longipes*, *A. raphani*, *Bipolaris hawaiiensis*, *B. spicifera*, *Chaetomium atrobrunneum*, *Ch. elatum*, *Doratomyces microsporus*, *Emericella quadrilineata*, *F. culmorum*, *F. graminearum*, *F. semitectum*, *Myrothecium roridum*, *Oedocephalum glomerulosum*, *Trichoderma hamatum* and *Ulocladium botrytis* were reported for the first time as seed borne fungi on sunflower in Iraq.

This study indicates that there is a large number of fungal species associated with seeds of sunflower cultivars grown in Iraq. The greatest numbers of fungi were recovered from seeds purchased from local markets. The majority of the detected species are reported for the first time on sunflower seeds in Iraq and some of them are well known as seed-borne potentially pathogenic fungi.
Streszczenie

GRZYBY WYSTĘPUJĄCE NA NASIONACH RÓŻNYCH ODMIAN SŁONECZNIKA (HELIANTHUS ANNUUS) UPRAWIANYCH W IRAKU


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