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STUDY ON PATHOGENICITY OF SEED-BORNE FUNGI OF SUNFLOWER IN PAKISTAN

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SUMMARY

Pathogenicity of twenty fungi isolated from sunflower seed intended for sowing, was tested on three sunflower cultivars (Ho-1, SMH-13, Suncome-110) seedlings and plants by appropriate recommended methods for different fungi under test and Kock's postulate's proved. Out of the twenty fungi studied, 15 fungi, *Alternaria alternata, A. helianthi, A. zinniae, Curvularia lunata, Fusarium culmorum, F. moniliforme, F. semitectum, F. oxysporum, F. solani, Macrophomina phaseolina, Myrothecium roridum, Phoma oleracea, Phomopsis helianthi, Stemphylium healianthi* and *Verticillium dehliae* were found to be pathogenic on sunflower. Of the sunflower cultivars used in the pathogenicity test, Ho-1 was found most susceptible to the pathogens whereas Suncome-110 showed variable trend to different pathogens. *Fusarium culmorum, F. moniliforme* and *F. semitectum* were found to be less agressive as compared with *F. solani* and *F. oxysporum*.

Key words: Sunflower seed, pathogenicity, seed-borne fungi, Pakistan.

INTRODUCTION

From seed germination to harvest, sunflower is attacked by a number of infectious microorganisms mostly fungi, which under certain climatic conditions reduce the yield and quality significantly. Chandra *et al.*, (1985) detected nine seed-borne fungi on oilseed crops such as *Aspergillus fumigatus, A. nigar, Alternaria, Cladosporium, Curvularia, Fusarium* and *Penicillium* spp. All these fungi individually and in combination induced high mortality at the pre-emergence stage. Štraser (1988) isolated a number of fungi in seeds of different sunflower hybrids from 1984 to 1986 in Yugoslavia. These fungi were *Alternaria tenuis* with frequency up to 100 percent, *Aspergillus flavus* (0.01-1.60%), *Botrytis cinerea* (up to 8.31%), *Diplodia nitelensis* (0.1%), *Phomopsis helianthi* (0.1%) and *Sclerotinia sclerotiorum* (0.31-

21.4%). These disease-causing organisms are known to decrease the quantity and quality of seed (Singh and Prasad, 1977).

In Pakistan, fourteen fungi are reported to be seed-borne in sunflower (Ahmad *et al.*, 1992; Khan *et al.*, 1974 and Bhatti & Muhammad, 1984). Dawar and Ghaffar (1991) isolated 20 different genera and 36 species of fungi associated with sunflower seeds. The pathogenic species included *Rhizoctonia solani, Macrophomina phaseolina, Fusarium moniliforme, F. semitectum, F. equisetti, F. solani, Drechslera specifer, Alternaria alternata* and *A. tenuissima*. The principal author recorded 20 fungi, considered to be seed-borne pathogens in major oilseed crops (unpublished).

Although a large number of seed-borne fungi were found to be associated with sunflower seed, the pathogenicity of most of the fungi associated with sunflower seeds are not reported. Therefore, pathogenicity tests were carried out for 20 seed-borne fungi to learn the pathogenic nature of fungi associated with sunflower seeds. The results may be used for better planning disease control strategies for sunflower crop.

MATERIALS AND METHODS

Pathogenicity tests

Cultures of the following 20 seed-borne fungi, detected and isolated from the sunflower seeds intended for sowing during 1991-92, were used for pathogenicity studies; *A. alternata, A. helianthi, A. zinniae, Curvularia lunata, Drechslera hawaiiensis, D. longirostrata, D. tetramera, Emericellopsis terricola, Fusarium culmorum, F. moniliforme, F. oxysporum, F. semitectum, F. solani, M. phaseolina, Myrothecium roridum, M. verrucaria, Phoma oleracea, Phomopsis helianthi, Stemphylium helianthi* and *Verticillium dehliae*. Disinfected seeds of three local cultivars, Suncome-110, Ho-1 and SMH-133, were used for the pathogenicity test all of fungi under study. Severity of disease was expressed on area basis in leaf and length basis in stem and roots on the following disease rating scale (James, 1971).

0.00% (No diseases symptom noticed)

1 = 10% (10% of leaf, stem and root area with disease symptom)

2 = 25% (25% of leaf, stem and root area with disease symptom)

3 = 50% (50% of leaf, stem and root area with disease symptom)

4 = 75% (75% of leaf, stem and root area with disease symptom)

5 = 100% (100% of leaf, stem and root area with disease symptom)

Emericellopsis terricola, Fusarium spp. and *Verticilium dehliae*

Three-layered plateforms of blotter paper were placed in each test tube (size 2.5 x 20cm) filled with 10 ml of water. The plateforms were punctured by fine nee-

dle to provide a number of holes before putting into the base of test tube so that water level remained below the blotter paper plateform. Test tubes were plugged with cotton roll and sterilized at 15 lbs/sq inch pressure for 20 minutes. Sunflower seeds were disinfected by one percent sodium hypochlorite for 15 min. and then washed with distilled water thrice. Seed were dropped aseptically in individual sterilized test tubes, on the blotter paper plateform. These test tubes were kept in incubation room at 25°C (± 2) under day/night flourescent light. After 5-7 days, visually healthy seedlings in test tubes were selected having no spore/mycelium growht on seed, plumule and radicle.

Five test tubes with healthy young seedlings were selected from each four replicates in randomized complete block (RCB) design. One week old cultures of *F. terricala*, *F. culmorum*, *F. moniliforme*, *F. oxysprum*, *F. semitectum*, *F. solani* and *V. dehliae* were prepared on potato dextrose agar. Cork borer was used to cut disc of culture which was dropped with the help of inoculating needle in each test tube near plumule and radicle emerging out (Hill *et al.*, 1983). These test tubes were then placed at 25°C for 2-3 week. No culture was added in control. Roots and stems showing brown and discoloured appearance were counted along with post mortality of seedlings. Data were statistically analysed.

Macrophomina phaseolina

Ten sterilized sunflower seeds per plate were placed on the culture plate, full of grayish-white mycelium and covered with a 1/2 cm thick layer of sterilized sand. Then these plates were incubated at 24°C (±2°C) under 16 hours photoperiod. RCB design with four replicates along with control treatment was used. Percent emergence damping off of seedlings was recorded after seven days (Ahmad, 1992).

***Alternaria, Drechslera* and *Curvularia* spp.**

Fifty disinfected seeds were plated on three layers of moistened blotter papers at the rate of 5 seeds per petri dish and incubated at 25°C under twelve hours daily artificial daylight tubes. Eight apparently healthy seedlings with no fungal growth on seeds etc. were selected and transplanted in pots filled with sterilized sand. After two-leaf stage, only five seedlings were sprayed using plastic atomizer @ 10^5 spores per ml of fungus culture and covered with polyethene paper. The control plants were sprayed with distilled water and pots were kept at 25°C for 25-30 days. Hoagland solution was added to plants in pots and every day plants were also sprayed with distilled water to maintain humidity (about 85%). A separate set of experiments was conducted for each fungus, *i.e.*, *A. alternata*, *A. helianthi*, *A. zinniae*, *Curvularia lunata*, *D. hawiiensis*, *D. longirostrata* and *D. tetramera* (Neergaard, 1945; Srinivasan, 1971; Maden *et al.*, 1975; Seung *et al.*, 1982 and Thanassoulopoulos and Kolokousi, 1988). Inoculating fungus was isolated from diseased portions and identified with reference to stock culture sprayed on seedlings in these experiments.

Myrothecium roridum* and *M. verracaria

Twenty-five healthy-looking seedlings were raised in sterilized sand in four replicates (5 seeds/replicate) in a complete randomized design. Spore suspension @ 10^7 spores per ml was inoculated on the young seedlings at four-leaf stage by spraying with a mechanical plastic atomizer and the pots were kept at 25°C. Relative humidity was maintained from 80-90 percent by keeping plants in plastic-made tent and Hoagland solution was added during experiment. Minute circular brown to black leaf spots were observed within two week but final observation was made after 25 days (Nguyen *et al.*, 1993).

Phoma oleracea

Hundred surface-disinfected seeds of each cultivar were plated in petri plate @ 5 seeds per plate using blotter paper method. After eight days, twenty-five healthy looking seedlings were selected and inoculated with fungal suspension (Tuite, 1967). Five plants were similarly immersed in distilled water as control. Pots were arranged in CRB design. The plants were kept at 25°C for two months. Premature death of seedlings was observed at early stage and at later stage. Black lesions at the base of stalk were also observed.

Stemphylium helianthi* and *Phomopsis helianthi

Fungal inoculum was prepared by chopping 2-3 week old culture of *S. helianthi* and *Ph. helianthi* in distilled water along with medium. This suspension was filtered through a double layer of cheese cloth and conidial concentration was adjusted up to 10^5 with a haemocytometer. Five seedlings at four-leaf stage in each replicate were raised as in previous experiment and inoculated by spraying the leaves. Plants were kept in growth room at 25-28°C for 30 days and covered with polyethene bags. Control plants were sprayed similarly but with sterilized water. Plants were sprayed daily with distilled water and Hoagland solution was added to plants in pots to maintain the general health of the plants (Aćimović, 1988 and Madjidieh, 1988).

RESULTS AND DISCUSSIONS

Out of the twenty fungi tested for pathogenicity on three sunflower cultivars, *i.e.*, Ho-1, SMH-133 and Suncome-110, fifteen were found pathogenic (Table 1). Severity of disease was expressed on area basis in leaf and length basis in stem and roots on standard disease rating scale.

***Alternaria, Curvularia* and *Drechslera* spp.**

All the species of *Alternaria* produced symptoms. None of the *Drechslera* species could produce any symptom on the three cultivars of sunflower under test. *A. alternata*, *A. helianthi*, *A. zinniae* and *Curvularia lunata* gave almost similar

Table 1: Pathogenicity of seed mycoflora of sunflower in Pakistan

S.No.		Fungus	Pathogenicity
1.		*Alternaria alternata* (Fr.) Kelssler	+
2.	*	*A. helianthi* (Hansf) Tubaki and Nishihara	+
3.		*A. zinniae* Pape and MB. Ellis	+
4.		*Curvularia lunata* (Wakker) Boed	+
5.	*	*Drechslera hawaiiensis* (Bugnicourt) Subram + Jain	-
6.	*	*D. longirostrata* (Drechsler) Richardson and Frasier	-
7.		*D. tetramera* (Mckinney) Sub + Jain	-
8.		*Emericellopsis terricola* Van Beyma	-
9.	*	*Fusarium culmorum* (W.G.Sm.) Sacc.	+
10.		*F. moniliforme* Sheldon	+
11.	*	*F. oxysporum* Schlecht	+
12.		*F. semitectum* Berk and Rav	+
13.		*F. solani* (Mart) App and Wr.	+
14.		*Macrophomina phaseolina* (Tassi) Goid	+
15.	*	*Myrothecium roridum* Tode ex. Fr.	+
16.	*	*M. verrucaria* Dith. ex. Fr.	-
17.	*	*Phoma oleracea* Sace.	+
18.	*	*Phomopsis helianthi* Munt. Cvet. *et al.*,	+
19.	*	*Stemphylium helianthi* (Aćimović)	+
20.	*	*Verticillium dehliae* Kleb.	+

\+ Pathogen: - No pathogen
* New reports on sunflower seeds in Pakistan

results (Figures 1,2,3,4). *A. alternata* and *C. lunta* were found to be more aggressive on the open pollinated variety Ho-1. In Pakistan, *Alternaria* leaf spot (*A. alternata*) is reported as the major leaf spot disease of sunflower (Bhutta *et al.*, 1993).

Emericellopsis terricola, Fusarium spp. and *Verticillium dehliae*

Emericellopsis terricola did not cause any infection and seedlings remained healthy. *F. moniliforme, F. oxysporum, F. semitectum* and *F. solani* produced symptoms in varying degree on Ho-1, SHM-133 and Suncame-110 (Figures 5,6,7,8,9). *F. culmorum* proved pathogenic only on the open pollinated variety (OPV) Suncome-110. The cultivar Suncome-110 showed higher susceptibility to *Fusarium* spp. than Ho-1 and SMH-133. *Verticillium dehliae* produced symptom in varying degree on all cultivars used for pathogenicity test but Ho-1 showed an increased susceptibility to this pathogen (Figure 10).

Macrophomina phaseolina

Pre-emergence mortality of seedlings was observed on all sunflower cultivars used in this study. All cultivars showed almost similar reaction with respect to disease incidence and severity (Figure 11).

Figures 1.-15.
Pathogenicity test for different pathogens:
a) incidence (infected plants)
b) severity of disease

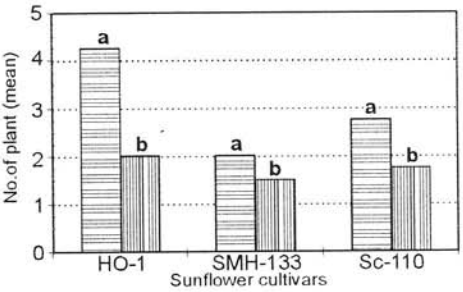

Figure 1. Alternaria alternata

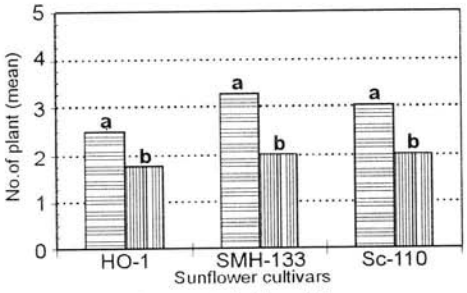

Figure 2. Alternaria helianthi

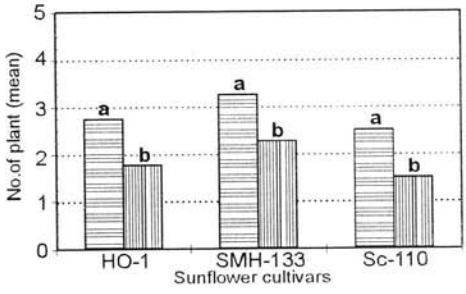

Figure 3. Alternaria zinniae

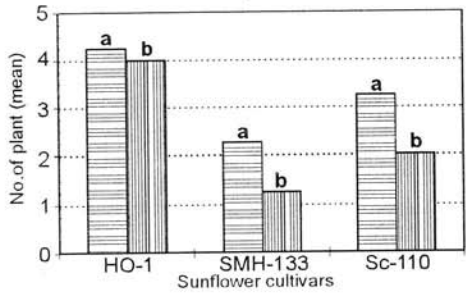

Figure 4. Curvularia lunata

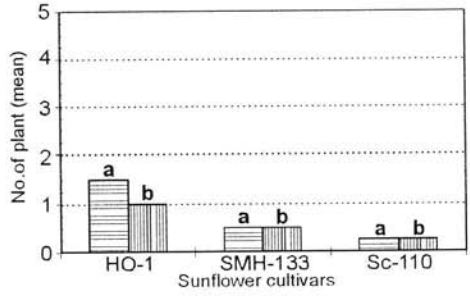

Figure 5. Fusarium culmorum

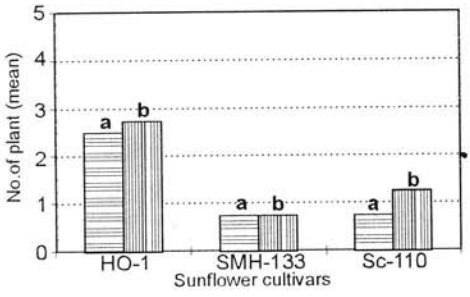

Figure 6. Fusarium moniliforme

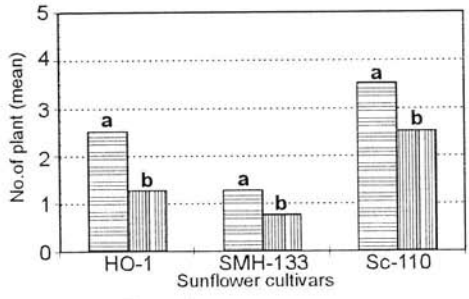

Figure 7. Fusarium oxysporum

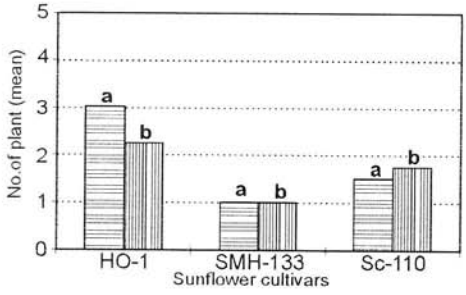

Figure 8. Fusarium semitectum

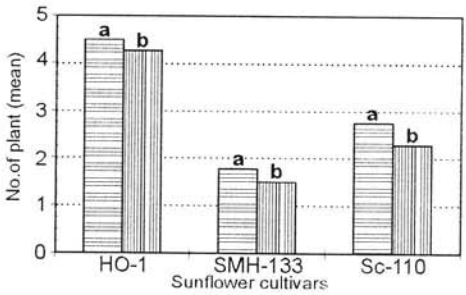

Figure 9. Fusarium solani

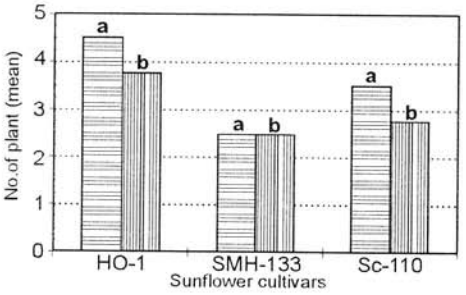

Figure 10.Verticillium dehliae

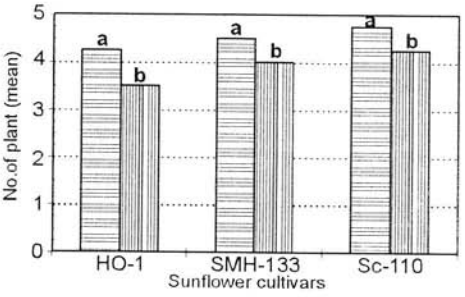

Figure 11.Macrophomina phaseolina

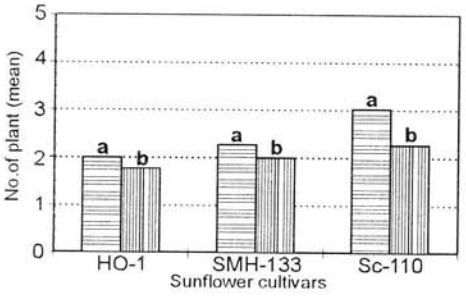

Figure 12.myrothecium roridum

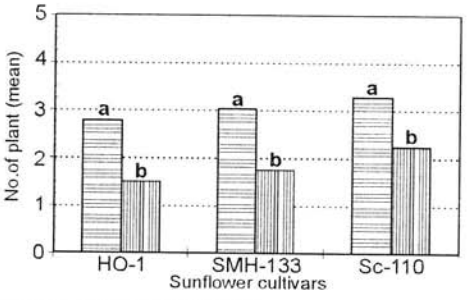

Figure 13.Phoma olerace

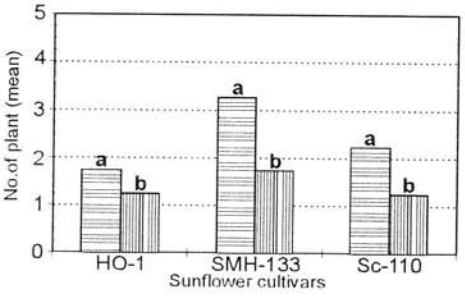

Figure 14.Phomopsis helianthi

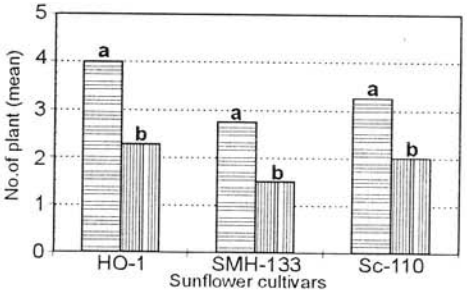

Figure 15.Stemphylium helianthi

Myrothecium roridum* and *M. verrucaria

Within two weeks, *M. roridum* produced minute circular brown to black spots on leaves of all cultivars (Figure 12). Although *M. roridum* has not been reported as a field disease of sunflower in Pakistan, Myrothecium leaf spot (*M. roridum*) has been reported to be prevalent in almost all cotton growing areas effecting mainly cotton leaves; bolls are also affected under favourable conditions (Tanweer and Akhtar, 1974). In the present study this fungus showed weak reaction to sunflower plant and it could be the reason that fungus may not be capable to produce field disease under local conditions.

Phoma oleracea

The fungus produced black lesions at the base of the stalk and developed high incidence and severity on SMH-133 followed by Suncome-110 and Ho-1 (Figure 13).

Phomopsis helianthi* and *Stemphylium helianthi

The fungus *S. helianthi* developed necrotic symptoms on the leaves, while *Ph. helianthi* also produced necrotic areas but leaves showed burning appearance. Both fungi gave higher incidence and severity on Ho-1 and SMH-133 as compared with Suncome-110 (Figures 14, 15). Disease caused by *S. helianthi* has not been reported so far on sunflower in Pakistan but *Phomopsis* leaf spot has been reported upto 20 percent incidence in Punjab area which is known to be the main sunflower growing area in Pakistan (Bhutta *et al.*, 1985).

CONCLUSIONS

In the present study, *F. culmorum, F. moniliforme and F. semitectum* were found to be less aggressive as compared with *F. solani* and *F. oxysporum. A. alternata* and *Curvularia lunata* were found more aggressive than *A. helianthi, A. zinniae* and *Myrothecium roridum. Macrophomina phaseolina* and *V. dehliae* gave high incidence on all cultivars tested. *P. oleracea, Ph. helianthi* and *S. helianthi* showed similar reaction to some extent. Ho-1 was found susceptible to most of the pathogens whereas SMH-133 and Suncome-110 showed variable trend towards different pathogens. Findings of this study may help in fixing priority in disease control strategies in the production of disease free seed of sunflower.

REFERENCES

Aćimović, M., 1988. The observation on the development of the perithecial stage in *Diaporthe helianthi*. Munt. Cvet. Proceedings of the 12th International Sunflower Conference Novisad, Yugoslavia (July, 25-29, 1988).

Ahmad, I., Iftikhar, S. and Bhutta, A.R., 1992. Seed-borne micro-organisms in Pakistan - Checklist, 1991. Published by PARC, pp-32.

Ahmad, Z., 1992. *In-vitro* selection of sunflower for resistance to *Macrophomina phaseolina*, cause of charcoal. M.Sc. Thesis. Uni. of Agri. Faisalabad. pp-79.

Bhatti, M.A.R. and Muhammad, F., 1989. Fungi occuring on sunflower seed in Pakistan. Pak. J. Agric. Res. 22(#) : 251-252.

Bhutta, A.R., Rahber-Bhatti, M.H., Solangi, G.R., Ahmad, I. and Rehman, M.H., 1993. Sunflower production and pathological problems in Pakistan. Science Technol. and Development. 2(2): 51-53.

Bhutta, A.R., Rahber-Bhatti, M.H., Ahmad, S.I. and Ahmad, I., 1995. Prevalence and incidence of sunflower diseases in Pakistan. Pak. J. Phytopathol. 7(2): 135-139.

Chandra, S., Narange, M. and Srivasava, R.K., 1985. Studies on mycoflora of oilseeds in India. Mycoflora in relation to pre- and post emergence mortality. Seed Sci. & Technol. 13: 537-54.

Dawar, S. and Ghaffar, A., 1991. Detection of seed-borne mycoflora of sunflower. Pakistan J. Botany. 23(2): 173-178.

Hill, J.P., Frenandez, J.A. and Mcshane, M.S., 1983. Fungi associated with common root rot of winter wheat in Colorado and Wyoming. Plant Disease. 795-797.

James, W.C., 1971. An illustrated series of assesment keys for plant diseases, their preparation and usage. Canadian Plant Dis. Surv. 51(2) : 39-65.

Khan, S.A., Mathur, S.B. and Neergaard, P., 1974. Survey on new seed-borne organisms of Pakistan. Seed Sci. & Technol. 2(4): 477-479.

Maden, S., Singh, S., Mathur, S.B. and Neergaard, P., 1975. Detection and location of seed-borne inoculum of *Ascochyta rabiei* and its transmission in chick pea (*Cicer arietinum*). Seed Sci. & Technol. 3: 667-681.

Madjidieh, G.S., 1988. A new sunflower disease in Iran caused by *Phomopsis helianthi*. Proceedings of the 12th International Sunflower Conference, Novi Sad, Yugosalavia (July, 25-29) P: 108-109.

Mirza, M.S., Beg, A. and Ahmad, Y., 1983. First report of *Alternaria helianthi* on sunflower from Pakistan. FAO Plant Prot. Bull. 32(4) : 147.

Neergaard, P., 1945. Danish species of *Alternaria* and *Stemphylium*. Taxonomy, parasitism, economic significance. Einar Munksgaard, Copenhagen, Denmark.

Nguyen, T.H., Neergaard, P. and Mathur, S.B., 1973. Seed-borne species of *Myrothecium* and their pathogenic potential. Trans. Brit. Mycol. Soc. 61-347-354.

Singh, B.K. and Parasad, T., 1971. Effect of seed-borne fungi on the physio-chemical properties of sunflower oil. Phytopath. Z. 90: 337-341.

Srinivasan, M.C., Chidambambaran, C.P., Mathur, S.B. and Neergaard, P., 1971. A simple method for inducing sporulation in seed-borne fungi. Trans. Brit. Mycol. Soc. 56: 31-35.

Štraser, N., 1988. Frequency index distribution of sunflower seed mycopopulation. Proceedings of the 12th International Sunflower Conference. Novi Sad. Yugoslavia (July, 25-29, 1988), p: 144-149.

Tanweer, M. and Akhtar, A.S., 1974. Myrothecium leaf spot of cotton in Pakistan. The Pakistan Cotton. 18(4): 165-167.

Thanassoulopoulos, C.C. and Kolokousi, A.T., 1988. A new *Alternaria* diseases of sunflower in Greece. Publ. in Proc. of the 12th International Sunflower Conference, Novi Sad, Yugoslavia (July 25-29, 1988), p: 126-130.

ESTUDIO DE LA PATOGENICIDAD DE HONGOS DE LA SEMILLA DE GIRASOL EN PAKISTAN

RESUMEN

La patogenicidad de 20 hongos de la semilla fue evaluada en plántulas y plantas de tres cultivares de girasol (Ho-1, SMH-13, Suncom-110) por los métodos apropiados recomendados para hongos diferentes bajo los postulados de Kock. En 20 hongos, 15 fueron encontrados patogénicos en girasol. *Drechslera hawaiiensis, D. longirotrata, D. tetramera, Emericellopsis terricola* y *Myrothecium verrucaria* fallaron en inyectar el girasol en condiciones experimentales. De los cultivares de girasol utilizados en test de patogenicidad, Ho-1 fue encontrada más susceptible a la mayoría de los patógenos donde Suncome-110 mostraron una tendencia variable a diferentes patógenos. *F. culmorum, F. moniliforme* y *F. semitectum* fueron encontrados menos agresivos en comparación con *F. solani* y *F. oxysporum*.

ETUDE DE LA PATHOGÉNICITÉ DES CHAMPIGNONS TELLURIQUES DE LA GRAINE DE TOURNESOL AU PAKISTAN

RÉSUMÉ

La pathogénicité de 20 champignons telluriques de la graine a été testée sur les semis et les plantes de 3 cultivars de tournesol (Ho-1, SMH-13, Suncome-110) par les méthodes habituelles de test des différents champignons et selon le postulat de Kock. Parmi Les vingt champignons testés, 15 ont montré de la pathogénicité sur le tournesol. *Drechela hawaiiensis, D. longirotratra, D. tetramera, Emericellopsis terricola*, and *Myrothecium verrucaria* n'ont pas infecté le tournesol dans les conditions expérimentales. Parmi les cultivars de tournesol utilisés pour le test de pathogénicité, Ho-1 s'est révélé plus sensible à la plupart de pathogènes tandis que Suncome-110, présentait une réponse variable vis à des divers pathogènes. *F. culmorum, F. moniliforme* et *F. semitectum* ont été les moins agressifs par rapport à *F. solani* et *F. oxyssporum*.