EUROPEAN ACADEMIC RESEARCH
Vol. II, Issue 7/ October 2014

Impact Factor: 3.1 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Investigations on the charcoal rot caused by *Macrophomina phaseolina* (tassi) goid problem in sunflower and their management in Sindh, Pakistan

MUHAMMED AYAZ KEERIO
KHADIM HUSSAIN WAGAN
MANZOOR ALI ABRO[1]
MUHAMMED MITHAL JISKANI
Department of Plant Pathology
Sindh Agriculture University Tandojam, Pakistan
MUHAMMED ISHAQ MASTOI
National IPM Programme
Department of Plant and Environmental Protection
National Agriculture Research Centre, Islamabad
Pakistan

Abstract:

Charcoal rot of sunflower is one of the most important and dangerous disease threatening the crop throughout the world and reducing the yield. Therefore studies were conducted on the survey of different sunflower fields of Hyderabad and Nawabshah Districts was conducted to record the incidence of charcoal rot disease. The maximum disease incidence was recorded at Nasarpur (65.0%) followed by Tandojam (60.0%), Bhitshah (40.0%), Qazi Ahmed (30.0%) and minimum recorded at Pai forest sakrand (20.0%). Pathogenicity test was conducted on 2 weeks old seedlings of 10 commercial sunflower varieties, the mortality rate increased in Faisalabad-4, Suncross-42 and Baimisal-205 and significantly decreased in Hysen-37. Three isolates MP 10, MP5 and MP4 of M. phaseolina were identified on their growth pattern and colour and size of sclerotia (hard fruiting bodies of the fungus). Derosal and Topsin-M

[1] Corresponding author: mmanzoorabro@hotmail.com

significantly inhibited the colony growth of the ftingus as compared to Benlate and Copper oxychloride. The head diameter of inoculated plants of Faisalabad-4 was significantly decreased followed by Suncross-42, S-278 and Baimisal-205. The total seed weight per plant and 1000-seed weight also decreased in S-278, shams, Suncross-42, Baimisal-205 and Faisalabad-4 than the other sunflower varieties. Oil Percent significantly reduced in Faisalabad-4 followed by Baimisal-205 and Suncross-42 as compared to Hysen-33 and Hysen-37 respectively. Protein content was also lowest in Faisalabad-4 followed by Baimisal-205, Suncross-42 and Sham sunflower varieties.

Key words: charcoal rot, *Macrophomina phaseolina* (tassi) goid problem, sunflower, Sindh, Pakistan

Introduction

Sunflower (*Helianthus annuus* L.) has been considered as an important oilseed crop and widely grown in different countries of the world. Sunflower is the third major supplier of edible oil in the world after soybean and groundnut (Meric, 2003). Its production multiplied by approximately 1.8 times during the last 20 years (Pouzet & Delplancke, 2000). Sunflower was first introduced as an oilseed crop in the sixties in Pakistan to fill up the edible oil gap in the country (Mirza and Beg, 1984). Sunflower is short duration crop and can be grown as spring as well as autumn crop in major agro-ecological zones of irrigated and barani areas of Pakistan. (Samiullah, 2000) Sunflower has also recognized itself as major oilseed crop of the country and thus playing a vital role in the production and consumption of edible oil of the country (Bhatti and Soomro, 1996). The total area under sunflower cultivation in Pakistan was 844000 hectares in 2011-2012 with total production of 579000 metric tons (Pakistan statistical year book, 2011-12). In Sindh, the cultivation of sunflower as oilseed crop was done during 1964-

65 but it was commercially started in 1975. Since then the crop has been cultivated over an area of 533 87 hectares with an average yield about 1082 kg/hec.

As a part in the increase of sunflower acreage, there is potential increase in several pathological problems also that deteriorate the seed quality and market value of the crop (Ahmed, 1988). Ahmed *et al.* (1991) reported that there are about 16 major diseases found in sunflower crop in Pakistan including charcoal rot (*Macrophomina phaseolina),* leaf spots due to *Alternaria helianthi* and *Sepotoria helianthi,* head rot caused by *Rhizopus* sp. and *Sclerotinia sclerotiorum,* rust *(Puccinia helianthi)* and collar rot *(Sclerotinia rolfsii).* Most of the diseases caused by these pathogens are considered as seed borne in nature (Richardson, 1990). Bhutta *et al.* (1995) observed 9 fugal and 1 bacterial diseases on sunflower by surveying 45 locations in Pakistan.

Charcoal rot of sunflower caused by *M. phaseolina* (Tassi) Goid was first time observed in USSR, but was not considered as an important disease of sunflower until 1957. Now, it has been as one of the most important and dangerous diseases threatening the crop throughout the world and reducing the yield around 20-30% (Sackston, 1981). The casual fungus *M. phaseolina* (Tassi) Goid. (Syn. *M. Phaseoli* (Manbl.) Ashby, *Rhizoctonia bataticola* (Taub) Butler, *Sclerotium bataticola* Taub, *Botryodiplodia phaseoli* (Maubl.) Thir.), attacks more than 500 hosts, including wide range of cultivated plants (Sinclair, 1982). The diseased plants are recognized by early maturing and showing black or charcoal type discoloration on stem from which the disease gets the name "charcoal root" CR. Very little work has been done on the performance of different sunflower verities against the fungus, *M. phaseolina.* The preliminary objectives of this study were to test the different existing sunflower varieties and to see their

existed resistance potential and to determine the effect of fungus on certain seed quality parameters and its management.

Materials and Methods

Survey and collection of disease samples

The survey of different sunflower fields that remained under sunflower cultivation for many years was conducted to estimate the incidence of charcoal rot disease. At least 50 plants from each field were randomly selected and notified clearly for further observations during the visit whenever required. The infected plants were also brought to the laboratory for isolation of the fungus.

Isolation of the fungus

i) Isolation from roots

Isolations were made from root samples of infected sunflower plants collected from different sunflower fields. The root portion of the plants was cut into small pieces with sterilized scissor. The root pieces were kept in 250 ml beaker and washed under running tap water for about 20-30 minutes then dried completely over blotting / tissue papers. The root pieces were rinsed in 0.01% $HgCl_2$ for 2-3 minutes and twice double washed with distilled sterile water for 2-3 minutes each and again dried completely. Five treated root pieces were placed in each Petri dish containing freshly prepared potato-dextrose agar medium. All the plates were kept at room temperature (30 ^{0}C) for 7 days. The same procedure was applied for isolation of fungus from seeds.

Pathogenicity test of *Macrophomina phaseolina*

Pathogenicity test of the fungus was conducted to determine its variability in Pathogenic reaction on 2 weeks old seedlings of

SF-187, Hysen-33, Shams, PR-3, S-278, Suncross-42, Faisalabad-4, Baimisal-205, Mehran-I and Hysens-37 sunflower varieties. Seedlings were transferred in 15 cm diameter earthen pots containing l kg steam sterilized soil already mixed in the fresh culture of *M. phaseolina*. The entire seedlings were observed daily for any reaction/symptoms produced by the fungus. The disease reaction/severity was determined after 12 days. After depotting, observations were recorded on mortality rate and infection (%) of the plants.

Characterization of isolates of *Macrophomina phaseolina*

M. phaseolina isolated from root tissues and seeds of soybean and sunflower. The isolations were done by immersing the root pieces and seeds in 0.01% $HgCl_2$ for 2 minutes, then passed through two washings of distilled sterile water (DSW) for 2 minutes each and placed to petridishes containing potato-dextrose and V-8 juice agar media. Growth pattern was observed daily for about 7 days at room temperature (30 + 1°C). The morphology of sclerotia was studied-under standardized microscope.

Effect of different fungicides on colony growth of the fungus

Four different fungicides Benlate, Derosal, Topsin-M and Copper oxychloride were used to see their effect on the mycelial growth of *M. phaseolina*. The fungicides mixed with potato-dextrose agar medium at the following rate.

1. Derosal	i.	50 mg in 100 ml of the medium
(Carbendazim)	ii.	100 mg in 100 ml of the medium
	iii.	150 mg in 100 ml of the medium
2. Topsin-M	i.	40 mg in 100 ml of the medium
(Thiophanate Methyle)	ii.	90 mg in 100 ml of the medium

	iii.	140 mg 100 ml of the medium
3. Benlate	i.	50 mg in 100 ml of the medium
(Benomyle)	ii.	100 mg in 100 ml of the medium
	iii.	150 mg in 100 ml of the medium
4. Copper oxychloride	i.	100 mg in 100 ml of the medium
	ii.	150 mg in 100 ml of the medium
	iii.	200 mg in 100 ml of the medium

All the plates inoculated with 5 mm disk of the fungus. The linear colony growth of the fungus (in mm) was recorded after 10 days. The untreated inoculated petridishes were served as control.

Field planting

Seeds of 10 commercial sunflower varieties, SF-187, Hysen-33, Shams, PR-3, S-278, Suncross-42, Faisalabad-4, Baimisal-205, Mehran-I and Hysen-37 was sown at experimental area of Oilseed Research Section A.R.I. Tandojam. The experiment was arranged in a randomized complete block design with three replications having plot size of measuring 3x5m.

Field inoculation

Field inoculations were done by preparing the suspension of *M. phaseolina* isolated from infected seed for sunflower varieties. The suspension of the fungus was prepared from 1-2 weeks old culture containing mycelial growth and viable sclerotia in 250 ml of distilled sterile water in a haring blender at very low speed. Plants were inoculated at flowering (60 days after emergence). Plants were harvested after maturity and sampled separately. The following parameters were studied.

Total seed weight / plant, head diameter, 1000-grain weight, oil and protein content.

Results and Discussion

Incidence of charcoal rot disease in different sunflower fields

The maximum disease incidence % was recorded at Nasarpur (65.0%) followed by Tandojam (60.0%), Bhitshah (40%) and Qazi Ahmed (30%). The minimum disease incidence occurred at Pai forest sakrand (20%). The results are given in (Table-1). Infected plants show reddish brown discoloration at the emerging portion of the hypocotyls and discoloration is very much evident at the soil line and above. Infected plants ripen prematurely and discolored areas turn dark brown to black.

Table-1. Incidence of charcoal rot disease in different sunflower fields.

Locality	No. of samples studies	Infected plants	Disease incidence (%)
Nasarpur	50	32	65.0
Tando Jam	50	30	60.0
Bhitshah	50	20	40.0
Qazi Ahmed	50	16	30.0
Hala	50	16	30.0
Bhanot	50	14	25.0
Sakrand (A.R.S.S)	50	14	25.0
Mehrabpur	50	12	24.0
Moriolakho	50	12	24.0
Pai Forest Sakrand	50	10	20.0

Pathogenicity test of *Macrophomina phaseolina*

Pathogenicity test of *M. phaseolina* was done by artificially inoculating the plants of Faisalabad-4, Suncross-42, Baimisal-205, S-278, Shams, SF-187, Hysen-33, PR-3, Mehran-I, and Hysen-37 sunflower varieties. The typical symptoms appeared within 48hr after inoculation. The mortality rate was high in'Faisalabad-4, followed by Suncross-42, and Baimisal-205 (Table-2). The varieties, Hysen-33, PR-3 Mehran-1 and Hysen-37 did not show significant response to the disease infection

(Table-2).Chandra et al. (1985) also found pre and post emergence mortality in sunflower plants due to *Alternaria helianthi* and other seed borne pathogens. Gul et al. (1989) reported that *M. phaseolina* was more pathogenic to early maturing varieties. Ahmed et al. (1994) conducted pathogenicity test and found that *M. phaseolina* was more pathogenic serve during post-emergence phase while *Sclerotium rolfsii* caused pre and post - emergence damping-off. The results are also in conformity with day and MacDonald (1995) and Leghari (1998). Tossi and Zazzerini (1998) also reported the similar results by inoculating sunflower results varieties with *M. phaseolina* and *Phoma* sp. Hafeez and Ahmed (1997) tested 17 different sunflower genotypes and found that SF-187 was highly resistant while PTH-1 and SMT were resistant to the disease.

Characterization of isolates of ***Macrophomina phaseolina***

The three growth patterns were observed i.e. dense growth, feathery spreading growth and restricted growth in potato-dextrose medium. In general, sclerotia isolated from Faisalabad-4, were highest in size followed by Shams, S-278 and SF-187 sunflower varieties. It showed the presence of the three isolated of *M. phaseolina* (MPIO, MP5 and MP4) respectively). The smallest size of sclerotia obtained from soil, HO-1 and Hysen-33 sunflower varieties (Table-2).

Pearson et al. (1986) using selective lima bean medium to isolate and identify the isolates of *M. phaseolina* from corn, soybean and soil samples. Asad et al. (1992) found the variation among sunflower genotypes and pathogen response. They also confirmed the occurrence of 3 isolates MPIO, MPS and MP4 isolates of *M. phaseolina* from different growing areas of Pakistan. The similar results have been reported by Maqbool et al. (1992). Desai (1998) found that *M. phaseolina* isolated from

sunflower different locations was also virulent in 13 sorghum genotypes.

Table-2.Growth response of *Macrophomina phaseolina* isolates from Sunflower soil on potato-dextrose agar medium

Source	Size of Sclerotia				Growth on Medium	Isolate
	Length (µm)	Rang	Width (µ m)	Range		
S-278	126.38	94.2-157.76	91.45	62.80-117.75	Dense	MP-10
HO-I	77.32	54.95-117.75	63.03	51.03-94.20	Feathery	MP-5
SF-18	109.11	86.35-150.00	91.84	70.65-126.00	Dense	MP-10
Faisalabad-4	140.51	102.05-172.70	119.32	100.00-149.00	Dense	MP-10
Shams	115.86	58.86-164.85	96.55	54.95-141.30	Feathery	MP-5
Soil	54.95	47.10-70.65	49.45	45.00-62.80	Restricted	MP-4
Hysen-33	86.95	60.95-145.00	65.45	49.07-141.00	Restricted	MP-4

Effect of different fungicides on colony growth *of Macrophomina phaseolina*

The fungicides, Derosal and Topsin-M significantly inhabited the radial colony growth of the fungus at its all doses (Table-3). Benlate was found as the third fungicide by reducing the growth. The growth was not more significantly different from copper oxychloride at 100 mg / 100 ml medium (32.62mm) as compared to control (37.37mm). The results are given in (Table-3). Solanke et al. (1997) observed that the combination of both Thiram and Carbendazim reduced mycoflora on seed and also increased germination of the plants. Bhutta et al. (2001) used Tecto, Benlate, Baytan, Topsin-M and Carbendazim for control of *M. phaseolina*. They obtained best results with Tecto and Benlate.

Table-3. Effect of different Fungicides on liner colony growth of *Macrophomina Phaseolina*.

Fungicide	Dose (mg) 100ml medium	Average Colony Growth (mm)
Derosal	50 100 150	5.56g 4.43d 3.75c
Topsim-M	40 90 140	6.25f 4.62h 3.66i
Benlate	50 100 150	7.56e 5.68fg 4.50h
Copper oxychloride	100 15 200	32.62b 18.16c 11.16d
Control (-)	-	3.37a
LSD (P<0.05)	**-**	**0.675**

Effect of *Macrophomina phaseolina* on plant growth and seed quality parameters

a) Head diameter

The head diameter in inoculated plants was significantly decreased in Faisalabad-4 (7.40 cm), Suncross-42 (7.80 cm) followed by S-278 (8.00 cm) and Baimisal-205 (8.40 cm) as compared to other varieties and all un-inoculated varieties (Table-4). There was no significant difference in head diameter of Hyasen-33, Sf-187 and Shams and Baimisal-205 respectively (Table-4).

Table-4. Effect of *Macrophomina haseolina* on head diameter of different sunflower varities

Variety	Head Diameter (cm)	
	Inoculated	Un-inoculated
Hysen-37	10.40a	18.20 a
Mehran – I	11.40.ab	18.00a
Hysen-33	9.00bcd	17.40a
PR-3	9.4bc	16.80ab

SF-187	9.00bcd	15.80bc
Shams	8.80cde	15.40bcd
Baimisal – 205	8.40cde	15.4bcd
S-278	8.00cde	15.00cd
Suncross-42	7.80de	14.20de
Faisalabad-4	7.40e	13.40e
LSD (P<0.05)	**1.586**	**1.400**

b) Mortality and infection (%)

The mortality rate and infection percentage were increased in Faisalabad-4 (15.00 and 62.80%). The mortality rate and infection percentage were also high in Suncross-42 and Baimisal-205 sunflower varieties. The morality rate and infection were significantly decreased in Hysen-37 (3.60 and 12.4%) and Mehran-I (7.60 and 12.60%) sunflower varieties (Table-5).

Table-5. Effect of *Macrophomina Phaseolina* on infection% and mortality rate in different sunflower varities

Variety	Infection (%)	Morality (%)
Faisalabad-4	62.80a	15.00a
Suncross-42	44.00b	12.00b
Baimisal-205	37.00c	11.20b
S-278	33.00d	11.00bc
Shams	24.20e	10.80bcd
SF-187	22.20ef	9.40cd
Hysen-33	20.00f	9.40cd
PR-3	15.40g	9.20dc
Mehran-1	12.60h	7.60e
Hysen-3	12.40h	3.60f
LSD (P<0.05)	2.268	1.752

c) Effect on seed weight

The total seed weight per plant was reduced in inoculated plants of Hysen-33 (15.28), S-278 (17.61 g) followed by Shams (19.98 g), Suncross-42 (23.96 g), Baimisal (25.18 g) and Faisalabad-4 (26.1 Ig). The weight was significantly highest in Mehran-I, SF-18 and other varieties (Table-6). The maximum seed weight (57.98 g) was occurred in un-inoculated plants of

Hysen-37 variety. The lowest 1000 seed weight was found in FaislabadT4 (27.61 g) followed by Suncross-42 (31.69 g) and S-278 (32.26 g) as compared to SF-187 and Mehran-I (Table-6). The varieties Faisalabad-4, Suncross-42, S-278 and Baimisal-205 also showed the similar trend in controls (Table-6).

Table-6. Effect of *Macrophomina Phaseolina* on seed wight (g) of different sunflower varieties

Variety	Total Seed wt./plant (g)		1000-Seed Weight (g)	
	Inoculated	Un-inoculated	Inoculated	Un-inoculated
Hysen-37	22.68d	57.98a	34.94e	49.90d
PR-3	24.71b	54.48b	38.5c	68.72a
Mehran-1	26.66a	54.04bc	40.80b	58.62d
Hysen-33	15.28g	51.38c	37.28d	51.07cd
SF-187	25.10abc	47.85d	46.36a	52.94cd
Shams	19.98d	46.44d	31.19f	69.50a
Baimisal-205	25.18abc	43.5le	32.43f	54.62bc
S-278	17.61f	40.45f	32.26f	55.13bc
Suncross-42	23.98cd	38.96f	31.69f	41.48e
Faisalabad-4	26.11ab	29.96g	2.61g	54.29c
lSD (P<0.05)	**1.857**	**2.859**	**1.627**	**4.245**

Effect on oil and protein content

Oil percent significantly decreased in Faisalabad-4 (25.56) followed by Baimisal-205 (29.75) and Suncross-42 (29.88) as compared to inoculated plants of Hysen-33 and Hysen-37 sunflower varieties (Table-7). There was no significant difference in oil percent in un-inoculated plants Mehran-I, PR-3 and SF-187 varieties (Table-7).

Protein content was also low in Faisalabad-4 (9.13) followed by Baimisal-205 (1 1.02), Suncross-42 (1 1.36) and Shams (12.10) respectively (Table-7). The protein content was significantly high in un-inoculated plant of Hysen-33, SF-187, Hysen-37 and Shams (Table-8). Chohan and Kaur (1975) observed the significant reduction in sunflower yield due to seed and soilborne diseases. Sackston (1981) found that the

charcoal rot is the most destructive diseases for major yield losses in sunflower growing areas throughout the world. Similar results have been reported by Prasaad and Singh (1983) and Beg (1984). The' plant height and 1000-seed weight were significantly reduced by *M. Phaseolina* (Rauf, 1985). Arun et al. (1994) also obtained the significant reduction in 1000-seed weight, germination percentage, seedling vigor and oil content due to *M. phaseolina*. Khanam (1995) recorded the maximum germination in SC-83 (75%) and minim in HO-I (60%) sunflower varieties.

Table-7 Effect of *Macrophomina Phaseolina* on oil percent and protein content of different sunflower varieties

Variety	Oil (%)		Protein Content	
	Healthy	Infected	Infected	Healthy
Hysen-33	44.83	40.33a	15.66a	17.50a
Hysen-37	42.58b	37.41d	12.50c	14.75c
Mehran-1	41.33c	32.58d	11.15e	14.06de
PR-3	40.98c	35.58c	12.43cd	14.43cd
SF-187	40.66c	28.58g	13.29b	15.41b
Shams	40.33c	30.66e	12.10d	14.75c
Baimisal-205	39.58d	29.75f	11.01e	13.90ef
S-278	36.90e	30.00ef	12.25cd	13.8ef
Suncross-42	35.37f	29.88ef	11.36e	13.50f
Faisalabad-4	33.35g	25.56h	9.13f	11.5g
lSD (P<0.05)	**1.019**	**0.889**	**0.368**	**0.503**

Conclusions

The incidence of charcoal rot of sunflower was highest at Nasarpur (65.0%) and lowest was at Pai forest sakrand (20.0%). All the three doses of Derosal and Topsin-M fungicide greatly inhibited linear colony growth by *M. phaseolina* followed by Benlate as compared to Copper oxychloride and control plates in which fungicide was not used. The head diameter of inoculated sunflower plants was reduced in Faisalabad-4, Suncross-42, S-278 and Baimisal-205 varieties. The total seed weight per plant and 1000-seed weight were also decreased in

Faisalabad-4, Suncross-42 and Shams varieties. The oil percent was highest in Hysen-33 (44.330), Hysen-37 (37.417) and PR-3 (35.583) as compared to Faisalabad-4 and Suncross-42. Protein content also increased in Hysen-33, Hysen-37 and PR-3 as compared to other varieties.

REFERENCES

Ahmed, I. 1988. Occurrence of sunflower rots in Pakistan. PARC Cargill Joint Intnl. Conf. On Sunflower disease. Lahore, Pakistan.l:ll.

Ahmed, T.K., I. Burney and S. Asad. 1991. Current status of sunflower diseases in Pakistan Natl. Symp. Plant Pathology. Univ. of Karachi. 53pp.

Ahmed, K. G. M., S.I.A. El-Said, R.N. Fawzy, A.E. Badr and M.A. Abd-Allah. 1994. Pathological study on sunflower plant, chemical and biological control and seed oil content. Annals of Agril. Sci. Moshtohar 32:1529-1543.

Arun, K., CD. Kaushik, S.K. Gupta and A. Kumar. 1994. Effect of charcoal rot (Rhizoctonia *bataticola)* in sunflower. Annals of Biology-Ludhiana 10:59-60.

Asad, S., K. Saifullah, M.A. Rana and I. Ahmed. 1992. Differential reaction of sunflower genotypes to isolates of Macrophomina phaseolina. Proc.13'" Intnl. Sunflower Conf., Pisa, Italy. 2:977-979.

Beg, A. 1984. The average oil content in sunflower seed. Progressive Farming. 4:5-9.

Bhatti, I.M. and A.H. Soomro. 1996. Agricultural inputs and field crop production in Sindh. 2ND Ed. 310 pp.

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

Bhutta, A.R., M.H.R. Bhatti, I. Ahmed and S. Ishrat. 2001. Chemical control of seed borne fungal pathogens of sunflower. Helia 24:67-72.

Bianco-Lopez, M.A. and R.M. Jimenez-Diaz. 1983. Effect of irrigation on susceptibility of sunflower to Macrophomina phaseolina. Plant Disease 67:1214-1217.

Chandra, S., M. Narange and R.K. Sirivastava. 1985. Studies on mycoflora of oilseed in India. Seed Sci. 2 Technol. 13: 537-541.

Chohan, J.N. and S. Kaur. 1975. Seed mycoflora sunflower and control of seed borne pathogens. India J. Mycology and P. Pathol. 5:210-211.

Day, J.P. and M.V. MacDonald. 1995. Plant-pathogen interactions of sunflower and Macrophomina phaseolina in vitro and in vivo. P. Pathol. 44: 216-269.

Desai S, A, 1998. Virulent isolate Macrophomina phaseolina (Tassi) Goid from sunflower infecting sorghum. Karnataka J. Agril. Sci. 11:1094-1095.

Gul, Z., S. Hassan and I. Ahmed. 1989. Pathogenic variations in Macrophomina phaseolina and differential response of some important sunflower varieties to charcoal rot resistance. Sarhad J. Agriculture 5:659-663.

Hafeez, A. and S. Ahmed. 1997. Screening of sunflower germplam for resistance of charcoal rot. Pak. J. Phytopathol. 9:74-76

Jimenez-Diaz, R.M., M.A. Bianco-Lopez and W.E. Sackston. 1983. Incidence and distribution of charcoal rot of sunflower caused by Macrophomina phaseolina. Plant Disease 67:1033-1036.

Khanam, M. 1995. Seed borne fungi of sunflower varieties, their effect on germination and yield. Sarhad J. Agriculture 1 1:369-372.

Leghari, H.B. 1998. Incidence and distribution of sclerotia of Macrophomina phaseolina (Tassi) Goid in different

cultivated sunflower soil. M.Sc. Thesis Sindh Agriculture University, Tandojam. 62 pp.

Maqbool, A., S.S. Mehdi and M. Ahmed, 1992. Oil content and fatty acids of exotic sunflower inbred lines as influenced by different charcoal rot isolates. 13TH Intnl. Sunflower Conf. Pisa, Italy 2:946-956.

Mirza, M.S. and A. Beg. 1984. Disease of sunflower in Pakistan FAO Bull. Helia 6:55-56.

Meric, C.,F. Dane and G. Olgun. 2003. Histological aspects of anther wall in male fertile and cytoplasmie male sterile Sunflower (Helianthus annuus L.). Helia, 26: 7-18.

Pakistan Statistical Year Book. 2012. Federal Bureau of Statistics, Statistics Division, Govt. Pakistan.14 pp.

Prasaad, T. and B,K. Singh. 1983. Effect of relative humidity on oil properties of fungal infected sunflower seeds (Abstr.) Rev. PI. Pathol. 63:34-35.

Pearson. C.A.S,, J.F. Leslie and F.W, Schwenk. 1986. Variable chlorate resistance in Macrophomina phaseolina from corn, soybean and soil. Phytopathology. 76:646-649.

Pouzet A. and D. Delplancke. 2000. Evolution comparee dela production etde la competitivite du tournesal dars differentes aires de production. Proc. 15th int. Sunflower conf. Toulouse, France. Tomel: A 1-9.

Raut, J.G. 1985. Effect of charcoal rot caused by Macrophomina phaseolina on sunflower plants. India phytopathol. 38:345-346.

Richardson, 'M.J. 1990. An annolated list of seed borne diseases. 4th Ed. Inst. Seed Test Ass. Switzerland 164 pp.

Sackston, W.E. 1981. The sunflower crop and diseases progress, problems and prospects. Plant Disease 65:643-648.

Samiullah, K. 2000. Heat units requirement of sunflower hybrids. MS Thesis, pp .2.

Sinclair, J.B.- 1982. Compendium of soybean diseases. 2nd Ed. American Phytopathol society. St. Paul. MN. 104 pp.

Solanke, R.B., M.M. Hussaini, L.N. Jawale and W.J. Sonde. 1997. Effect of fungicidal seed treatment on seed health of sunflower under strong conditions. J. Maharashtra Agri. Univ. 22:349-350.

Tossi, L. and A. Zazzerini. 1998. Comparative phytopathological survey of varieties of sunflower Agril. information. 54:73-V5.

Young, D.J. and S.M. Alcon. 1982. Soil population of *Macrophomina phaseolina* in Arizona (Abstr.) Phytop.