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Efficacy of some chemical fungicides against fusarium wilt of sunflower *in-vtro* condition Couse by *Fusarium oxysporum*

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Abstract
Sunflower (*Helianthus annuus* L) is an important member of the family *Asteraceae*. Presently sunflower has become the most important oil crop of Pakistan after canola. The results of studies showed that, different fungi were associated with the wilted parts (viz., roots, stems leaves and flower heads) of sunflower. Most frequent and pre-dominant fungi were isolated and identified as *Fusarium oxysporum*, *Fusarium moniliforme*, *Alternaria helianthi* and *Aspergillus niger*. Among all the isolates, *Fusarium oxysporum* remains most frequent and pre-dominant fungus and was identified on the bases of their morphological characteristics. Statistical analysis of the data reveals that most of the used fungicides significantly reduced the mycelial mass of *Fusarium oxysporum* ($p<0.0000$). Among all tested fungicides, Nativo was found more effective in reducing the linear colony growth of the fungus at its highest and lowest doses (7.00mm), followed by Alliete (16.1 mm), whereas, Cabriotop and Romeo found less effective as compared to Nativo and Alliete. Dragon was found very less effective as compared to other four fungicides which reduce the linear colony growth of the tested fungi, at its highest dose (64.467 mm). All the fungicides at their respective doses significantly retarded the growth of fungus as compared to control (81.00mm).

Keywords: chemical fungicides, *helianthus annuus*

Introduction
Sunflower (*Helianthus annuus* L.) is main source of oil in the world. The oil has excellent nutritional properties and free of toxic components. It contains important nutrients like vitamins, A, B and E, selenium, copper, and zinc as well (Gonzalez *et al*. 2002) [12]. Sunflower oil is liked more for the Anti-cholesterol action in human beings. linoleic and oleic are considered most important fatty acids in sunflower oil (about 90 percent unsaturated fatty acids), along with remaining stearic and palmitic saturated fatty acids (Mukhtar, 2009) [16].

The sunflower crop was introduced in Pakistan with the objective of fulfilling the gap between production and consumption of oil seed during the year 1960. In Pakistan there is a serious shortfall of vegetable oil, hence 28% of our needs are full filled with other native sources, whereas the remaining 72% is met by importing the oil from other countries. The budget of imported edible oil is increasing in billions of dollars; it is becoming a huge burden on our foreign exchange reserves. Oilseeds and edible oil import cost were 1045 million USD during 2006-07. The edible oil demand was 3,094,000 tons in the same year. This included 2,237,000 tons of imported sources (oil and oil seeds). Local production stood at 857,000 tons. The demand for edible oil is growing at a rate of 5.4 % per year. According to this rate, after five years our per year demand would be 4,168,000 tons and if local production is not enhanced quickly annual import bill will increase to 2.593 billion USD (Anonymous, 2008).

The sunflower cultivation is distributed throughout districts of the Punjab but major areas of cultivation includes Faisalabad, Sargodha, Sialkot, Sheikhupura, Sahiwal, Okara, Gujranwala, Multan, Vehari and Rahimyar Khan. However, in Gujrawala division sunflower planting is implemented as rice based farming system and in Multan division in the cotton based farming system. However, in Sindh, it is cultivated mostly in district Badin (61%). Whereas, other main districts of sunflower cultivation in Sindh are Hyderabad, Thatta, Tharparkar and Sukkar (PAR, 2015). In consonance with federal ministry of national food security and research (MNFS) figure, Pakistan's total sunflower acreage stood at 236,001 ha in 2012, with considerable contributions about 80 % with regard to production provided by Sindh. About 66.2% production of Sindh comes from area of Badin, Tando Muhammad Khan, Hyderabad, Mirpurkhas, Thata, Nawabshah, Golarchi and Sukkur (PAR, 2015). However, during 2013, the area harvested increased to 283000 ha with production of 378000 tons of seed in Pakistan (FAOSTAT, 2013) [9].

More than 30 diseases have been reported to attack on the sunflower crop and cause heavy damages to the yield and production (Anonymous, 2007) [3]. These diseases are mainly caused by the four types of infectious microorganisms like, Bacteria, Viruses, Nematodes and Fungi. Among all, the fungal diseases are widely spread throughout the world. The major diseases of the sunflower are Blight, Rust, Wilt, Powdery mildew, Downy mildew, Mosaic virus, Charcoal rot, Sclerotina stalk and Head rot (Mukhtar, 2009) [16]. Among them, the Fusarium wilt is one of the serious disease which cause heavy losses to yield of the crop (Masirevic and jasnic, 2006) [15].

Fusarium wilt is economically important disease which caused

by *F. oxysprum*. Erwin F. Smith in 1894 [10] first reported this disease in the area of Southeastern United States, at present it is most sever in lower Ontario, Central Wisconsin, California and the Midwest. Nevertheless the disease has frequently spread mostly all over Areas of the world (Larkin *et al*. 1996) [14]. The pathogen attacks the plants in all stages of its life cycle from seedlings to flowering. Fusarium wilt of sunflower is caused by the most prominent soil borne pathogen *Fusarium* spp, mainly by *Fusarium oxysporum*. The pathogen has ability to persist many years in the soil without a host. *Fusarium oxysporum* invades the root system of the host and causes a blockage of the water conducting tissues resulting in root rot and eventual death of affected plants (Bayaa *et al.* 1986) [7]. Once the fungus introduced into the field then it has ability to survive into the various kinds of soil without a host for an unspecified period. Hence it is difficult to handle the disease but it can be manage through normal sanitation and any general crop-rotation program (Forsyth *et al.* 2006) [10].

Fusarium wilt is one of the desperate threats to the sunflower throughout the world caused by the various species of fusarium (Nahar *et al*. 2005).While the most prominent fungi of Fusarium are, as *F. solani, F. oxysporum,* and *F. moniliforme.* Among all species, the *Fusarium oxysporum* is active in causing the Fusarium wilt in most cases (Masirevic and Jasnic, 2006) [15].

The wilted plant shows the symptoms of reduction of growth as compared to healthy plants. The leaves of the infected plants become pale yellow and eventually fall down and death of Leaves margins are also take placed (Wu *et al.* 2009) [21]. Typical symptoms of *Fusarium oxysporum* includes wilting, stunting, chlorosis, necrosis, drooping of young leaves, damping-off and brown to blackish strips of vascular tissues. Fusarium Wilt begin to show their initial symptoms like a vein clearing appearance on young leaves, discoloration and drooping of older leaves commence from the base and progress upward to the upper parts of the plant followed by defoliation and finally plant death. Brown to blackish strips found in the vascular tissues when cut stem of infected plant at base. While tuberous roots, bulbs and corn of flowering plants show a dark discoloration within underground parts (Jones *et al*. 1982) [13].

Sultana and Abdul, (2013) [20] used fungicides, micro biological antagonists and oilcakes *in-vitro* and *in-vivo* to control *Fusarium oxysporum*, the cause of Fusarium wilt of sunflower. Aliette, Benlate and Carbendazim completely inhibit the colony growth of *F. oxysporum* at 100 ppm whereas Mancozeb, Ridomil, Topsin-M and Vitavax completely inhibited the colony growth at 100 ppm. Fungicidal treatment of bottle gourd and cucumber seeds artificially infested with *F. oxysporum* significantly reduced seedling mortality and root infection. Benlate, Carbendazim and Topsin-M totally checked seedling death in bottle gourd.

Materials and Methods

Collection of diseased specimens

For the collection of the diseased specimens of the Fusarium wilt of sunflower, a survey of the experimental field of oil seed section A.R.I Tandojam near the Nuclear Institute of Agriculture tandojam was carried out during the academic year 2015. The collected specimens were labelled properly and brought to the Mycology laboratory, Plant Pathology department, Faculty of Crop Protection, Sindh Agriculture University Tandojam for further processing (Fig. 1).

Isolation and identification of the disease causing fungus

The collected specimens were brought to the laboratory for the purpose of isolation and identification, where, the samples were first surface sterilized twice with distilled sterilized water and then treated with 5% NaOCl (Sodium hypochlorite) for 2 minutes. After surface sterilization the samples were dried on sterilized blotter papers and placed in Petri plates containing sterilized potato dextrose agar medium. All the Petri dishes were incubated at 25 ± 1^0C for about seven days. After seven days of inoculation the fungi isolated, were then identified with the help of keys for identification of fungi by Nelson *et al.* (1983) [18].

Identification of *Fusarium* spp

Fusarium spp. isolated from rotted tissues of infected roots of sunflower then identified by studying their colony characteristics and conidial morphology using the keys described by Nelson *et al.* (1983) [18] and with the help of characteristics of fungi mentioned in the book "The Identification of Fungi" (Dugan, 2006) [11].

General characteristics of *Fusarium oxysporum*

Mycelium appeared yellow or reddish-brown or blue-black. Macroconidia straight, short or long 5-8 septate and 40-75 x 25.5-5 micron. Microconidia are 1-3 septate, with a short beak and 22-48 x 3.4 microns.

Effect of different fungicides on the linear colony growth of *Fusarium oxysporum*

Five different fungicides were tested under *in-vitro* conditions for their efficacy against predominant fungus *Fusarium oxysporum* (Native, Cabrio@top, Aliette, Dragon and Romeo). The standard (aqueous) solution of these fungicides was prepared according to their active ingredients. The layout of the experimentation was conducted in complete randomized block design (RCBD) in 5 treatments and 3 replications briefly by Steel *et al*. (1997). The doses of the fungicides were kept same (50, 100, 150 ppm) one of the each dosses of the fungicide was mixed with 100 ml media and poured into Petri dishes 5 mm disk of the fungus was taken from the growing margin of matured culture of the fungus at least seven days old. Petri dishes containing only PDA medium without fungicides were used as control. All the Petri dishes were then transferred into incubator on 25 ± 1 ^{0}C for about 8 days. Mycelial growth of the fungus was recorded in mm after 24 hours of inoculation till 8 days of inoculation

Table 1

S. No	Name of Fungicides	Dose in ppm
1	Nativo	i) 50 ppm in 100 ml medium. ii) 100 ppm in 100 ml medium. iii) 150 ppm in 100 ml medium.
2	Cabrio@top	i) 50 ppm in 100 ml medium. ii) 100 ppm in 100 ml medium. iii) 150 ppm in 100 ml medium.
3	Aliette	i) 50 ppm in 100 ml medium. ii) 100 ppm in 100 ml medium. iii) 150 ppm in 100 ml medium.
4	Dragon	i) 50 ppm in 100 ml medium. ii) 100 ppm in 100 ml medium. iii) 150 ppm in 100 ml medium.
5	Romeo	i) 50 ppm in 100 ml medium. ii) 100 ppm in 100 ml medium iii) 150 ppm in 100 ml medium.

Results

The field experiment was conducted at plant pathology department under the title of "efficacy of some chemical fungicides and botanical extracts against Fusarium wilt of sunflower" during the academic year 2015. The experiment was conducted under *in-vitro* conditions through the Randomized Complete Block Design layout system with five treatments and three replications of each treatment. The diseased specimens were collected from experimental field of oil seeds section A.R.I. Tandojam. The samples were collected from the root, stem and leaves of the sunflower and brought to the mycology laboratory for *in-vitro* studies.

1. Isolation and identification

The diseased specimens were treated at the mycology laboratory, for the isolation and identification of the disease causing organisms. For this purpose the specimens were cultured on the artificial nutrient media (PDA) and kept under observation for eight days. The isolated fungi were then sub-cultured for purification of the actual cause of the disease. The isolation and identification process reveals the association of different fungi from the infected parts of the sunflower i,e, *Fusarium oxysporum*, *Fusarium moniliforme*, *Alternaria helianthi*, *Aspergillus niger*. Among all the isolated fungi, *Fusarium oxysporum* remains most frequent and pre-dominant fungus and was identified on the bases of their morphological characteristics with the help of electronic microscope and the senior Professors of the department was also taken in this regard (Table. 1).

2. Effect of different fungicides on the mycelial growth of the fungus (*Fusarium oxysporum*)

During the study, five different fungicides were tested against *Fusarium oxysporum* the casual organism of Fusarium wilt of sunflower, for their efficacy under *in-vitro* conditions at different doses. Data was recorded on the regular basis from 24 hours of inoculation till 8 days and was analysed by using the Statistics 8.1 application of the computer which shows that all the used fungicides significantly reduced the linear colony growth of *Fusarium oxysporum* ($p<0.0000$). Among all, Nativo was found more effective in reducing the linear colony growth of the fungus at their highest dose and lowest doses (7.00mm) followed by Aliette which reduce the radial colony growth of the tested fungus at its highest dose (16.1 mm) and at lowest dose (52.5 mm) whereas, the Cabrio@top was less effective in arresting the linear colony growth of the test fungus as compared to Nativo and Aliette. Cabrio top reduced the fungal growth at highest dose (48.1 mm) and at lowest dose (61.6 mm), while, Romeo at its highest dose reduce the fungal growth (51.8 mm) and at the lowest dose (62.0 mm) and Dragon was found less effective as compared to other four fungicides which reduce the fungal growth at highest and lowest dose (64.467 and 76.333 mm) respectively. All the fungicides at their respective doses significantly retarded the growth of fungus as compared to control (81.00mm) (Table. 2, Fig. 2).

Table 2: Effect of different chemical fungicides on the mycelia growth of *oxysporum*

S. No	Fungicides tested	Dose (PPM) / 100 ml. Medium	Radial colony growth (mm)
1	Nativo	i) 50.0 ii) 100.0 iii) 150.0	7.000 l 7.000 l 7.000 l
2	Alliete	i) 50.0 ii) 100.0 iii) 150.0	52.500 h 33.500 j 16.167 k
3	Cabriotop	i) 50.0 ii) 100.0 iii) 150.0	61.667 e 59.333 f 48.167 i

4	Romeo	i) 50.0 ii) 100.0 iii) 150.0	62.000 e 57.333 g 51.833 h
5	Dragan	i) 50.0 ii) 100.0 iii) 150.0	76.333 b 72.600 c 64.467 d
6	Control	-	81.000 a
	Lsd (P<0.0000)		0.8787

Fig 1: Survey of experimental field of oil seed section A.R.I Tandojam for the collection of samples of Fusarium wilt

Discussion

The *in-vitro* studies were conducted at Plant Pathology Department, Faculty of Crop Protection, Sindh Agriculture University Tandojam based on the objectives of finding out the most appropriate and eco-friendly approach to manage the Fusarium wilt of the sunflower. All the studies were carried out under Randomized Complete Block Design layout system. The collected data was statistically analyzed and showed significant impact on the development and growth of the *Fusarium oxysporum.*

Keeping in view the importance of sunflower in Pakistan and the losses caused due to the fungal origin specially Fusarium wilt of the sunflower, the *in-vitro* studies were conducted on the management of the Fusarium wilt through chemical fungicides and botanical extracts. The results of chemical fungicides showed that all the used fungicides significantly reduced the linear colony growth of *Fusarium oxysporum* ($p<0.0000$). Among all fungicides, the Nativo was found more effective in reducing the linear colony growth of the fungus followed by Alliete and Cabriotop corresponding. Whereas, Dragon and Romeo were found less effective in reducing the mycelia growth of fungus as compared to the control. These results are consistent with the studies by Afzal *et al*. (2010) [1] who worked on four fungicides viz., capton, topsin, vitavax and bayleton against seven fungi viz., *Fusarium oxysporum*, *Aspergillus niger*, *Alternaria alternata*, *Dreschlera tetramera* and *Rhizopus* spp isolates from seeds of the sunflower. For their efficacy Bayleton and Topsin were found the effective in shortening the linear colony growth of the tested pathogens.

Sultana and Abdul (2013) [20] used different microbial agents, oilcakes and fungicides *in-vitro* and *in-vivo* to control *Fusarium oxysporum* the cause of seed rot, seedling and root infection of bottle gourd and cucumber. Alliette, Benlate and Carbandazim totally inhibit the colony growth of *Fusarium oxysporum* at 100 ppm. Whereas Mancozeb, Ridomil, Topsin-M and Vitavax completely inhibited the colony growth at 100 ppm. Fungicidal treatment of bottle gourd and cucumber seeds artificially infested with *F. oxysporum* significantly reduced seedling dead and root infection. Benlate, Carbendazim and Topsin-M completely checked seedling mortality in bottle gourd. Gupta and Bansal, (2003) tested Carbendazim, Mancozeb, Captan, Thiram, and Topsin M at 0.2% concentration against *F. oxysporum* inducing fenugreek wilt under pot conditions. Carbendazim was found significantly effective followed by Mancozeb. Buchvarova *et al*. (1989) [8] found that Vitavax 200 HP showed best control *in- vivo* but under *in-vitro* conditions, the best were Mugibon, Vitavax 200-NP and Homai 80 WP against *Fusarium oxysporum*.

Conclusions and Suggestions

During the studies regarding the management it was found that the disease can be managed significantly through certain management strategies like by the use of chemical fungicides.

Keeping in view the results of present research work, it is suggested that certain fungicides such as Nativo and Alliete should be recommended against this disease. Efficacy of these fungicides should be evaluated at different fields of sunflower at different localities.

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