



## To evaluate different botanical extracts against *Fusarium oxysporum in-vitro* condition, the casual agent of sunflower wilt

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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. Its contribution reached up to 16 % in domestic edible oil production. The crop is being attacked by several insect pests and diseases, among all these, the Fusarium wilt caused by *Fusarium oxysporum* is one of the major damaging disease, which cause heavy losses in terms of quality and quantity of the crop. Therefore, keeping in mind the importance of sunflower in the country's economy, the present studies were conducted *in-vitro* conditions at laboratory of Department of Plant Pathology, Faculty of Crop Protection, Sindh Agriculture University Tando Jam, during the year 2015. The major aims of the study were to isolate and identify the wilt causing fungi associated with the wilt of sunflower and to find out the most appropriate and eco-friendly approach to manage the wilt of Sunflower under *in-vitro* conditions. 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 of botanical extracts i.e. Neem, Jimsonweed, Apple of Sodom, Tobacco and Bitter apple were also used. Among all the used botanical extracts, the Neem was found more effective in reducing the mycelial growth of the fungus (25.50 mm), followed by Jimson weed and Apple of Sodom, which reduced the fungal colony growth (34.16 mm) and (40.00 mm) respectively. Whereas, Tobacco also reduce the linear colony growth of the fungus (43.83 mm) while, the Bitter apple was found very less effective to reduce the mycelial growth of the tested fungus (53.16 mm). All the botanical extracts at their highest doses significantly showed the anti-fungal properties as compared to control (87.56 mm).

**Keywords:** *Fusarium oxysporum*, sunflower, *in-vitro*, botanical extracts

### Introduction

Sunflower (*Helianthus annuus* L) belongs to family *Asteraceae*. It is an important oilseed crop which is recognized worldwide for its beauty and source of food. Sunflower is reported to be originated from Southwest of the USA, or someplace in Mexico. It was introduced in the sixteenth century in Europe and imported into Russia from the Netherlands in the eighteenth century. As an improved variety of crop, it had been reintroduced in the United States from Russia in 1893. Nowadays the major sunflower producing countries of the world are Ukraine, Russia, Argentina, China, and United States (NDSU, 2007). The sunflower was introduced in Pakistan as a crop of oilseeds in 1960s (Mukhtar, 2009) [10].

In Pakistan there is a serious shortfall of vegetable oil, hence 28 percent of our needs are full filled with other native sources, whereas the remaining 72 percent 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 million 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 (Mukhtar, 2009) [10]. While in Pakistan, Due to the oil production and economic value of the sunflower, several research and investigation on different aspects of the crop were started in 1964. In 1970-71, sunflower crop was cultivated over an area of 666 ha in Punjab and 04 ha in Sindh with the total production of 482 tons. This area under cultivation was increased during 1987-88, as 33,122 hectares in Punjab, 9648 hectares in Sindh and 342 hectares in KPK, with overall production of 42,531 tons. Hence the Punjab contributes the 76 percent of the production of the country followed by Sindh 21 percent and KPK 3 percent (Anonymous, 2001-15). In 1991-92, sunflower was cultivated in 63328 hectares with production of 83312 tons. However, during 2013, the area harvested increased to 283000 hectares

with total production of 378000 tons of seed (FAOSTAT, 2013). Presently sunflower has become the most important oil crop of Pakistan. Its contribution reached to 16 % in domestic edible oil production.

The oil of the sunflower is famous due to its light color, flavor, lowest levels of saturated fats and also ability to stand at high temperatures. Sunflower varieties available in the market contains 40-49 percent oil in the form of seed which accounts for 80 percent value of this crop as compared with soya bean which contains most of its oil in the form of meal. Main fatty acids in the oil are linoleic and oleic (about 90 percent unsaturated fatty acids), and the remainder consists of saturated fatty acids stearic and palmitic (Mukhtar, 2009) <sup>[10]</sup>. The Oil of sunflower is also useful for making lubricants, candles and soaps. Sunflower seeds are also available in the market for edible purpose and can be eaten as roasted, cooked, raw, or dried. They are most famous and nutritious, containing 40 to 50 percent oil and 23 percent protein. They possess remarkable amount of unsaturated fats, crude protein, fiber and major nutrients such as vitamins A, B, and E, selenium, copper, and zinc as well (Gonzalez *et al.*, 2002) <sup>[8]</sup>.

Petals of sunflower are also edible and can be roasted and eaten as artichokes. They are not only humans that get taste of sunflower because sunflower leaves are also used as feed for livestock. Soil and water radiation can be removed by sunflower roots. Plant roots are also used to manufacture a traditional herbal medicine to cure the snake and spider bites. Tea of the sunflower leaves has been proved better to treat fevers, diarrhea and pulmonary diseases. Stem can be used to make clothes and papers (Nasreen *et al.*, 2011) <sup>[11]</sup>.

The Sunflower crop is also infected by various infectious microorganisms, which decrease the quality and yield of the sunflower remarkably (Mirza and Beg, 1983) <sup>[9]</sup>. Plant of sunflower is mainly attacked by various fungi, bacteria and nematodes (Bhutta *et al.*, 1997) <sup>[7]</sup>. Severeness of losses depends upon the infectious microorganisms and crop growth stage. Occasionally, if the disease level passes from economic injury level to economic threshold level when then yield of the crop is not impaired (Zadoks, 1985).

There are many important sunflower diseases caused by fungi and the most economic threats caused by these fungi to sunflower crop are wilt, downy mildew, rust, head rot, sclerotinia stalk, charcoal rot, leaf spot and phoma black stem (Mukhtar, 2009) <sup>[10]</sup>.

Among all these, wilt is one of the major threats to all the stages of crop growth and responsible for the heavy yield losses. Many *Fusarium* spp are reported to cause sunflower wilt (Nahar *et al.*, 2005). Recently the most devastating disease of sunflower is caused by the *Fusarium* spp (*F. oxysporum*, *F. soloni*, *F. moniliforme*, *F. helianthi* and *F. eques*) particularly *Fusarium oxysporum* (Masirevic and Jasnica, 2006).

## Materials and Method

### 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 Tando Jam near the Nuclear Institute of Agriculture Tando Jam 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 Tando Jam 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  $25 \pm 1^{\circ}\text{C}$  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) <sup>[12]</sup>.

### 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) <sup>[12]</sup> and with the help of characteristics of fungi mentioned in the book "The Identification of Fungi" (Dugan, 2005).

### Effect of different botanical extracts on the linear colony growth of *Fusarium oxysporum*

Five different botanical extracts were tested *in-vitro* condition against *Fusarium oxysporum*. The botanical extracts used were, Neem (*Azardichta indica*), Bitter apple (*Citrullus cololcynthis*), Jimson weed (*Datura stramonium*), Tobacco (*Nicotiana tabacum*) and Apple of sodom (*Calotropis procera*) (Rukhsana, 2005). The aqueous extract was prepared according to the formula described by Steel *et al.* (1997). There are 75 gm fresh leaves of each plant were collected from field and then grinded in 25 ml sterilized water with the help of mortar and pestle. The grinded extracts were first passed through five layered muslin clothes and then filtered through what's man filter paper. The extract got was considered standard and was stored in freezer for further studies. Each dose of the botanical extract was poured into 100 ml PDA medium with the help of sterilized pipette and 5mm disk of the fungus was inoculated into Petri plates. The disk of the fungus was taken from 7 days old culture of *Fusarium oxysporum*. All these Petri plates were then transferred to incubator at  $25 \pm 1^{\circ}\text{C}$  and data of mycelial growth of the fungus was recorded after 24 hours of inoculation till 8 days of inoculation. The experiment was conducted in completely randomized block design (RCBD), with 5 treatments and 3 replications. Petri dishes containing only PDA medium without botanical extracts were used as control.

**Table 1:** Different botanical extracts and Dose in ml.

S. No	Name of Botanical extracts	Dose in ml	
1	Neem ( <i>Azadirachta indica</i> )	1	1.0 ml in 100 ml medium
		2	2.0 ml in 100 ml medium
		3	3.0 ml in 100 ml medium
2	Bitter apple ( <i>Citrullus cololcynthis</i> )	1	1.0 ml in 100 ml medium
		2	2.0 ml in 100 ml medium
		3	3.0 ml in 100 ml medium
3	Tobacco ( <i>Nicotiana tabacum</i> )	1	1.0 ml in 100 ml medium
		2	2.0 ml in 100 ml medium
		3	3.0 ml in 100 ml medium
4	Apple of sodom ( <i>Calotropis procera</i> )	1	1.0 ml in 100 ml medium
		2	2.0 ml in 100 ml medium
		3	3.0 ml in 100 ml medium
5	Jimson weed ( <i>Datura stramonium</i> )	1	1.0 ml in 100 ml medium
		2	2.0 ml in 100 ml medium
		3	3.0 ml 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. Tando Jam. 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).

### Effect of different botanical extract on the mycelial growth of the fungus (*Fusarium oxysporum*)

Five different plant extracts were experienced under *in-vitro* conditions to check efficacy against *Fusarium oxysporum* at different doses. The botanical extracts used, were Neem (*Azadirachta indica*), Apple of Sodom (*Calotropis procera*), Bitter apple (*Citrullus cololcynthis*), Tobacco (*Nicotiana tabacum*) and Jimson weed (*Datura stramonium*). All the botanical extracts, significantly reduced the mycelial growth of *Fusarium oxysporum* ( $p < 0.0000$ ). Among all the used botanical extracts, the Neem was found more useful in lessening the linear colony growth of the fungus at their highest dose (25.50 mm) and at lowest dose (43.50 mm) respectively, followed by Jimson weed, which reduced the fungal growth at highest dose (34.16 mm) and at lowest dose (55.33 mm) whereas, the Apple of Sodom reduces the mycelial growth of the fungus at its highest dose (40.00 mm) and at lowest dose (61.33 mm) respectively. Tobacco and Bitter apple were found less effective as compared to other three botanical extracts. Tobacco reduced the colony growth of the fungus at highest dose (43.83 mm) and at the lowest dose (62.33 mm) while, the Bitter apple reduced the mycelial growth of the fungus at highest dose (53.16 mm) and lowest dose (75.00 mm) respectively. All the botanical extracts at their highest doses significantly retarded the growth of fungus as compared to control (87.56 mm) (Table. 3, Fig. 3).

**Table 2:** Effect of different botanical extracts on the mycelia growth of the *Fusarium oxysporum*.

S. No	Botanical extracts tested	Dose (100ml). Medium	Radial colony growth (mm)
1	Neem (Neem)	i. 1.0	43.500 g
		ii. 2.0	35.167 i
		iii. 3.0	25.500 j
2	Jimson Weed (DATURO)	i. 1.0	55.333 e
		ii. 2.0	43.333 g
		iii. 3.0	34.167 i
3	Apple of Sodom (AKK)	i. 1.0	61.333 c
		ii. 2.0	51.833 f
		iii. 3.0	40.000 h
4	Tobacco (TOMAK)	i. 1.0	62.333 c
		ii. 2.0	58.500 d

		iii. 3.0	43.833 g
5	Bitter Apple (TOOH)	i. 1.0	75.000 b
		ii. 2.0	56.667 de
		iii. 3.0	53.167 f
6	Control	-	87.567 a
	LSD (P<0.0000)		1.8406



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

### Discussion

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., captan, 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. During our studies on the anti fungal potential of certain botanical extracts, five different plant extracts were experienced under *in-vitro* conditions to check efficacy against *Fusarium oxysporum* at different doses. All the

botanical extracts, significantly reduced the mycelial growth of *Fusarium oxysporum* ( $p < 0.0000$ ). Among all the used botanical extracts, the Neem was found more effective in lessening the linear colony growth of the tested pathogens followed by Jimson weed and Apple of Sodom. Whereas, less effective botanical extracts were Tobacco and Bitter apple. These results are consistent with the studies by Afzal *et al.* (2010) [1] who worked on the antifungal activity of four botanical extracts viz., Neem (*A. indica*), Garlic (*A. sativum*) Jimson weed (*D. stramonium*) and Rhizomes of ginger (*Z. officinale*) against the seed mycoflora of sunflower and found that Neem and Garlic revealed the supreme antifungal feature against all fungi tested and therefore stand for helpful substitutes for the control of dangerous fungicides

### Conclusions

During the studies regarding the management it was found that the disease can be managed significantly through this management strategie like by the use of botanical extracts.

### Suggestions

Keeping in view the results of present research work, it is suggested that Fungicides are hazardous for human health and there is problem of resistance against fungicides, so alternative strategie is needed, for this purpose botanical extracts could be used. These studies also showed that the disease can also be control through botanical extracts like Neem and Jimson weed above 98%.

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