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To assess the efficacy of different fungicides against linear colony of the fungus *Fusarium Oxysporum in- vitro* conditions, the casual agent of onion wilt

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Abstract
Onion (*Allium cepa* L.) is a queen of kitchen found all over the world. Fusarium wilt is economically significant disease caused by *Fusarium oxysporum*. It was first reported by Erwin F. Smith in 1894 in the United States. Though, the infection it is now widespread throughout most regions in the world the main objective is to assess the efficacy of different fungicides against linear colony of the fungus *Fusarium oxysporum*. All the experiments were carried out according to the Completely Randomized Block Design (CRBD) layout system. The results of the study are summarized as under Results regarding the survey of different onion field of district Hyderabad, Tandialyar and Mirpurkhas showed that the Fusarium wilt disease is frequently found in all the districts surveyed with less or more incidence. The maximum disease incidence was recorded from Hyderabad. The results of different fungicides indicated that the Fusarium wilt of onion can be managed through certain broad spectrum systematic fungicides. The fungicides used against the fungus significantly reduce the colony growth of the fungus. Among them, Topsin-M was more effective in reducing the colony growth of the fungus followed by Carbendazim whereas; Ridomil and Acrobat were found very less effective in arresting the colony growth of the fungus as compared to Topsin-M and Carbendazim.

Keywords: Significant, fusarium wilt, fungicides

Introduction
Onion (*Allium cepa* L.) is herbaceous crop found all over the world. At world Laval onion production has improved by at least 25 to 30% over the past 10 years with recent production being around 45 million tonnes make it the second most essential crop after tomatoes. Onions are rich in two chemical groups that have perceived benefits to human health. These are the flavonoids and the alk (en) yl cysteine sulphoxides (ACSOs) (Griffiths *et al*., 2002) [4].
The asthatic value of Alliun Cepa is very important. resurch dow that it has been use as a food remidy which contain large amound of flavonoid chemicals. These flavonoid protect both human and plant cells from cancer and Gastrointestinal disease (Sampath *et al*., 2010) [11].
Total area and production of important vegetable crops were 611.7 thousand hectare and 8478.8 thousand tonnes, respectively. According to an estimate, Pakistan annually produces about 3.5 mt of potatoes. According to another estimate, the area under onion and potato crops during 2013- 14 was 127.8 and 169.9 thousand hectares, respectively, whereas production of onion and potato crops was 1,661.3 and 3,507.1 thousand tonnes, respectively. It is estimated that the total world production of onion was about 86.34 mt and Pakistan occupied 8 position with 2.25% share in production (Anum *et al*., 2015) [1].
Symptoms of the Fusarium wilt are slender, elongated, redish to brown color lesions on the stems; laterally crack often build up lesions expand downward to the main taproot which may shrink, decay or may die. In some case symptoms and sign widen up the hypocotyls further to outside. Various Clusters of fibrous roots are usually developed over shriveled taproot. Which can balance the ideal growth conditions they will bound beyond ground symptoms. Symptoms shwing plants are repeatedly under developed, growing slowly and are light green to yellow when compared to health ones. Deprived roots occupation deprives plants of minerals and water (Brooth *et al.*, 1977).
The cultivation area of Pakistan is 143.7 T/h with a production of 1892 Tn during 2010-11.

There are a number of biological factors that are considered to cause deterioration of onion bulbs for instance respiration and pathogen which attacks and make the bulbs unfit for marketing (Imran *et al*., 2014).

The plan was to find out the efficacy of some fungicides against the present isolates, fungicides (i.e., Carbendazim, Topsin-M, Mancozeb, Antracol,) Ore of sulpher groups were selected for *in-vitro* assessment against the distructive pathogens. These fungicides were extensively repressed mycelial expansion at different concentration and incubation time correspondingly (Shahnaz, 2010) [7].

Materials and Methods

Survey of infected fields

A survey of onion fields of districts Hyderabad and Mirpurkhas was carried out to record the occurrence of Fusarium wilt.

Disease severity

During the survey observations were recorded on the incidence of the Fusarium wilt of onion fields. The Disease in severity of the disease was calculated according to the disease incidence formula.

$$\text{Disease severity (\%)} \frac{\text{No. of wilted plants}}{\text{Total No. of plants}} \text{ x } 100$$

Isolation and identification of the disease causing fungus

Samples were taken from infected shoots, bulbs and roots of infect onions. Collected samples were then bring to the laboratory for isolation and identification process as described by Pathak, (1987), where, the samples were first surface sterilized twice with distilled sterilized water and then were treated with 0.5% NaOCl (Sodium hypochlorite) for 2 minutes. After surface sterilization the samples were dried on sterilized blotter papers and placed in pettriplates containing sterilized potato dextrose agar medium. All the petridishes 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 a.,* (1983) [8] and with the help of characteristics of fungi mentioned in the book "The Isolation and Identification of Fungi" by Frank, (2005) [3].

Identification of *Fusarium* spp

Fusarium spp. isolated from infected tissues of roots and shoots were then identified by studying their colony characteristics and conidial morphology using the keys described by Nelson *et al.* (1983) [8] and with the help of characteristics of fungi mentioned in the book "The Isolation and Identification of Fungi" by Frank. M. Dugan.

General characteristics of *Fusarium oxysporum*

Mycelium appeared just like yellow, reddish-brown or blue-black in color. Macroconidia short, straight, or 40-75 x 25.5-5 micron and long 5-8 septate. Microconidia consisit of 1-3 septate, with a short beak and 22-48 x 3.4 microns (Salam *et al*., 2015) [9].

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

Four different fungicides were tested under *in-vitro* conditions for their efficacy against predominant fungus *Fusarium oxysporum* (Carbendazim, Ridomil, Acrobat and Topsin -M). 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 4 treatments and 4 replications briefly by Steel *et al.*, (1997) [10]. The doses of the fungicides were kept same (10, 100, 1000, 10,000 ppm) one of the each dosses of the fungicide was mixed with 100 ml media and poured into petridishes 5 mm disk of the fungus was taken from the growing margin of 8 days old culture of *Fusarium oxysporum* with the help of sterilized cork borer. Petridishes containing only PDA medium without fungicides were used as control. All the petridishes were then transferred to incubator on 25 ± 1^0C 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: Doses of different fungicides on the linear colony expansion of *Fusarium oxysporum.*

S. No.	Name of Fungicides	Dose in ppm
1.	Carbendazim	i. 10 ppm ii. 100 ppm iii. 1000 ppm iv 10,000 ppm
2.	Ridomil	i. 10 ppm ii. 100 ppm iii. 1000 ppm iv. 10,000 ppm
3.	Topsin- M	10 ppm ii.100 ppm iii.1000 ppm iv. 10,000 ppm
4.	Antracol	10 ppm ii.100 ppm iii.1000 ppm iv. 10,000 ppm

Results

Survey of different onion fields

A Survey of different onion fields of district Hyderabad, Tando Allahyar and Mirpurkhas was carried out to observe the incidence of Fusarium wilt in the onion fields of these districts. During the survey it was observed that almost all the onion fields were suffering from some severe diseases like, Purple blotch *Alternaria porri*, *Aspergillus niger*, Neck rot (*Botrytis aclada*) Smudge (*Colletotrichum circinans*) Fusarium wilt *Fusarium oxysporum* f.sp. *cepa*. Blue mold (*Penicillium* sp) Downy mildew (*Peronospora destructor*) Onion smut (*Urocystis cepulae*) (Grooten, 2012) [5]. Fusarium basal rot (*Fusarium oxysporum*). Among all of them, the Fusarium wilt was found most dominating disease throughout all the onion fields visited. The maximum disease incidence was recorded from Hyderabad (60.0%) whereas, the disease incidence in district Mirpurkhas was bit low as compared to district Tando Allahyar, maximum incidence of Fusarium wilt recorded from district Hyderabad was (60.0%) (Table 1).

Isolation and identification of the fungus causing Fusarium wilt of onion

The collected infected specimens were then brought to laboratory for isolation and identification process of the fungus caused. The isolation and identification process reveals the association of different kinds of the pathogens with the wilted parts of the Onion shoots. Among all the isolated fungi, *Fusarium oxysporum* remains most frequent and pre-dominant fungus and was recognized on the bases of their siz and shaps mentioned in the book "identification of the fungi" written by Dugan (2005) and with the help of

electronic microscope, help from the senior Professors of the department was also taken in this regard.

Efficacy of different fungicides on the mycelial growth of the fungus (*Fusarium oxysporum* f.sp. *cepa*)

During the study, four different fungicides were tested against *Fusarium oxysporum* the casual organism of Fusarium wilt of onion 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. Data 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<0.000). Among them, Topsin-M was found more efficient in reducing the linear colony growth of the fungus at their highest dose (9.33 mm) as compared to its lowest dose (19.66 mm) respectively, followed by Carbandazim which reduce the radial colony growth of the test fungus at its highest dose (9.33 mm) and at lowest dose (29.66 mm) whereas, the Acrobat and Ridomil was less effective in arresting the linear colony growth of the test fungus as compared to Carbendazim and Topsin- M which reduced the fungal growth at highest dose (54.00 mm) and at lowest dose (70.33mm), while, Ridomil at its highest dose reduce the fungal growth (59.66mm) and at the lowest dose (74.33mm). Ridomil was found less effective as compared to other three fungicides which reduce the fungal growth at highest and lowest dose (20.33 and 74.33 mm) respectively. All the fungicides at their respective doses significantly retarded the growth of fungus as compared to control (81.66 mm).

Only Topsin-M was totally inhibitted the colony growth of F. *oxysporum* at 10000 ppm. Where as, Carbandazim, modratly inhibited the colony growth at 10000 ppm. Fungicidal treatment artificially infested with *F. oxysporum* significantly reduced root infection Behrani (2015).

Table 2: Disease incidence of Fusarium wilt of Onion fields in three districts of Sindh during survey.

Locality	No. of Fields visited	Samples taken/per Fields	Samples studied			
			Diseased	Healthy	Total	Incidence %
Mirpurkhas	04	25	40	60	100	44.00
Hyderabad	04	25	60	40	100	60.00
Tando Allah Yar	04	25	40	60	100	40.00

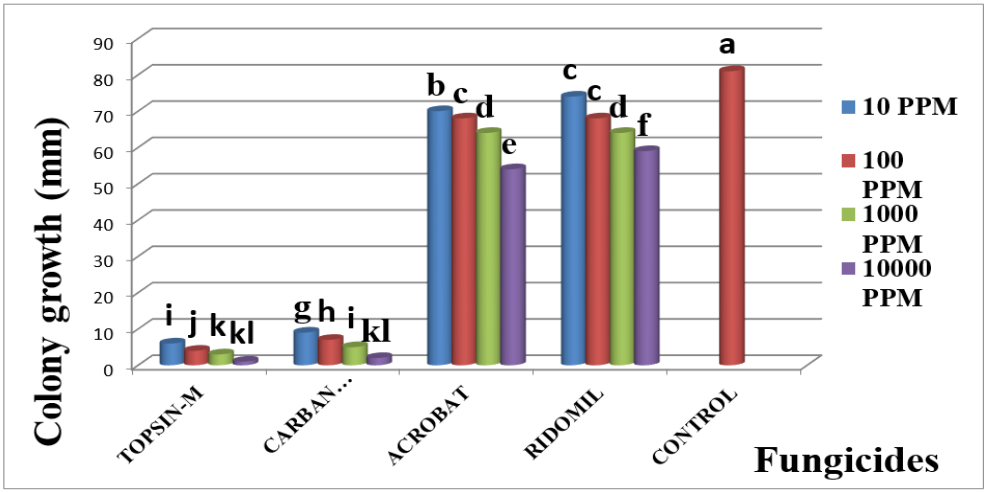

Fig 1: Effect of selected fungicides on the mycelial colony growth of *Fusarium oxysporum*

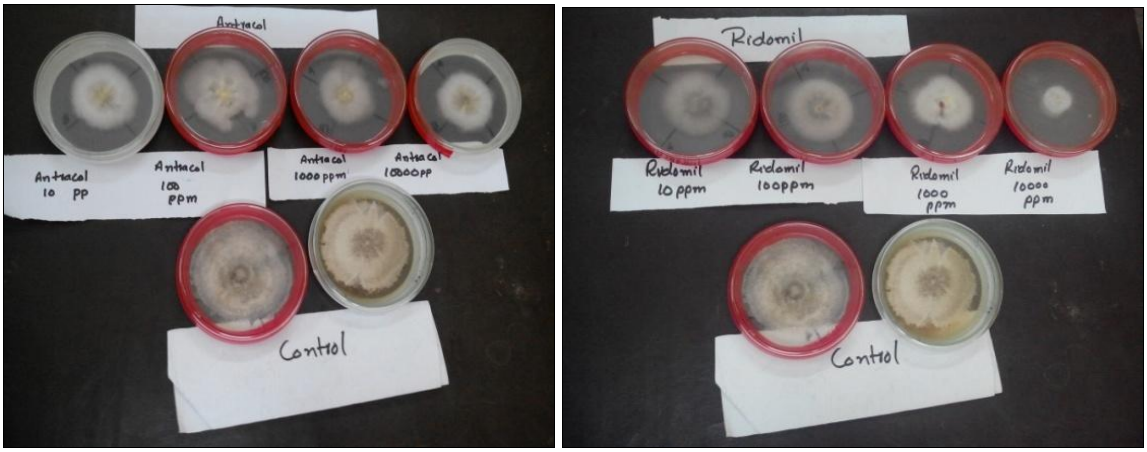

Fig 2: Figures showing efficacy of different fungicides on the radial colony growth of *Fusarium oxysporum*

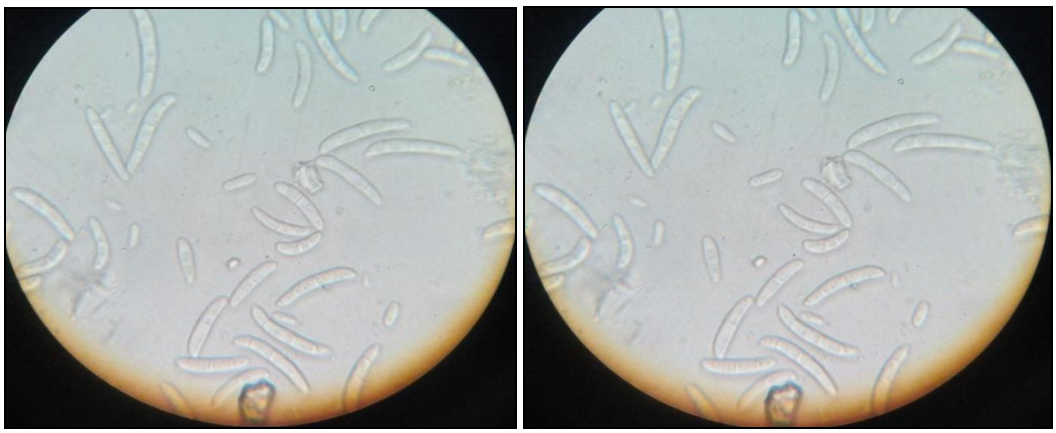

Fig 3: Conidai growth of *Fusarium oxysporum*

Conclusions

The present studies were conducted to report the disease incidence of Fusarium wilt disease of onion fields in three districts of Sindh namely Hyderabad Tandoalyar and Mirpurkhas to find out the more effective measures to manage the onion wilt disease. Our studies showed that Fusarium wilt disease of onion is one of the major serious threats to onion fields. 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 Biological and botanical extracts.

Suggestions and Recommendation

Keeping in view the results of present research work, it is suggested that certain fungicides such as Topsin-M and Carbandazim should be recommended against this disease. Efficacy of these fungicides should be evaluated at field conditions level at different onion fields at different localities.

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