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#### Research

## Evaluvation of Various Biocontrol Agents (Plant Extracts) on Linear Colony Growth of the Fungus *fusarium Oxysporum* Causing Onion Wilt

Shahid Hussain<sup>1</sup>, Muhammad Ayub<sup>1\*</sup>, Muhammad Rasheed<sup>1</sup> and Faizullah Khan<sup>2</sup>

1Mountain Agriculture Resrarch Station PARC, Skardu, Pakistan

<sup>2</sup>PCSIR Laboratories Skardu, Pakistan

#### Abstract

Onion (Allium cepa L) is found in various regions now a day's used not only for culinary preparations but also for medicinal purposes. Onion with a variety of purposes is often used as a raw material in many dishes and accepts almost all of the traditions and culture. Owing to its storage characteristics and durability of shipping, onions have been traded more widely than most vegetables. Onion is attacked by many diseases like Purple blotch (Alternaria porri) Black mold (Aspergillus niger) Neck rot (Botrytis aclada) Smudge (Colletotrichum circinans) Fusarium wilt Fusarium oxysporum f. sp. cepa. The main objective is to assess the effect of various bio control agents on linear colony growth of the fungus Fusarium oxysporum causing onion wilt all the experiments were carried out under Completely Randomized Block Design (CRBD) layout system. The results of 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. Four different plant extracts were evaluated under invitro conditions to check the efficacy against Fusarium oxysporum at different doses. The botanical extracts used, were Neem (Azadirachta indica), (AKK) Calotropi (Calotropi sprocera), Tobacco (Nicotiana tabacum) and Dhttura (Datura stramonium). All the botanical extracts, significantly reduced the mycelia growth of Fusarium oxysporum (p<0.000). Among all the used botanical extracts, the Neem was found more effective in reducing the mycelia growth of the fungus at their highest dose (10.66 mm) and at lowest dose (20.00 mm) respectively, followed by Tobacco, which reduced the fungal growth at highest dose (37.00 mm) and at lowest dose (70.66 mm) whereas, the Datura reduces the mycelia growth of the fungus at its highest dose (65.66mm) and at lowest dose (72.33mm) respectively. Dhttura and Calotropi (AKK) were found less effective as compared to other three botanical extracts. All the botanical extracts at their highest doses significantly retarded the growth of fungus as compared to control (82.66 mm). Its concluded that wilt is actually responsible of basal rot in onions.

#### Introduction

Onion (*Allium cepa L*) is found in various regions now a day's used not only for culinary preparations but also for medicinal purposes. Onion with a variety of purposes is often used as a raw material in many dishes and accepts almost all of the traditions and culture. Owing to its storage characteristics and durability of shipping, onions have been traded more widely than most vegetables. The pungent fractions of garlic are mostly sulfur-containing moieties while its two chemical groups have marked effect on human health [1].

**Keywords:** Botanical; Reduced; Evaluvation; Plant Extracts

Processing and stabilizing onion wastes (residues and surpluses of onion) could solve the environmental problem derived from a great onion wastes disposal. Moreover, obtaining stabilized onion by-products as natural antioxidant food ingredients could be advantageous to food industry, not only to improve the use of onion wastes but also to obtain new natural and functional ingredients. The aim of this study was to characterize onion by-products – juice, paste and biogases – from two Spanish onion cultivars – 'Figures' and 'Recas' – that have been stabilized by thermal treatments – freezing, pasteurization and sterilization – in order to evaluate the effect of the

**\*Corresponding Author:** KMuhammad Ayub, Mountain Agriculture Resrarch Station PARC, Skardu Pakistan, E-mail: bsagripp@gmail.com

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processing and stabilization treatment on the bioactive composition, antioxidant activity and polyphenol oxidize (PPO) enzyme inhibition capacity [2].

Fusarium wilt is economically significant disease caused by Fusarium oxysporum. It was first reported by Erwin F. Smith in the year of 1894 in the South Eastern constituency of the United States at present. It is cruel in California, mid-western states, central Wisconsin and lowers Ontario. Though, the infection is now widespread throughout most regions in the world [3]. The pathogen attacks the plants in all stages of its life cycle from nursery to flowering. Fusarium wilt is caused by a number of highly dedicated forms and races of the common soil borne fungus Fusarium oxysporum which can persist for many years in the soil without a host. Fusarium wilt diseases attacks many popular garden, greenhouse and nursery. Fusarium oxysporium invades the root system of the host and causes an obstruction of the water conducting tissues ensuring wilting and ultimate death of infected plants [4]. Once the fungus enters a garden, nursery, greenhouse or field it can live in determinately in several of soil types autonomous of any kind of host plant. This eliminates ability to endure an efficient controller determines any standard rotation platform or universal hygiene [5].

Fusarium wilt which is caused by Fusarium oxysporum is a complex problem, various fungi have been isolated from the diseased parts of infected young nursery plants viz., Fusarium oxysporum, Lasiodiplodia theobromae,Botryodiplodia theobromae [6], Botryodiplodia ribis, Fusarium equiseti, Colletotrichum sp., Curvularia sp [7]. Phomopsis sp, Colletotrichum gloeosporioides, Rhizoctonia solani, Pestalotia mangiferae, as cause of wilt [8]. During the identification process of plant pathogenic fungi morphological identification is basic and the most important one. Thus, TEF has become the marker of selection as a single-locus identification tool in Fusarium. F. oxysporum is very active and show the best differences in cultural, morphological physiological and races characters in other Fusaria, identification is usually based on morphological criteria such as outline of micro and macroconidia, arrangement of the micro conidiophores and the configuration and temperament of chlamydospores [9]. It has been observed by both onion producers and a plant protection onion is attacked by a number of insect pests like Onion Maggot and Cutworm, Leek moth (Acrolepiopsis assectella) Wireworms (Agriotes spp.) Weevil (Ceutorhynchus suturalis) Onion flies (Delia antiqua) Onion leaf miner (Liriomyza cepa) and Thrips (Thrips tabaci) which are responsible for production losses of onion among these Thrips are very distractive in field condition [10]. Onion is attacked by many diseases like Purple blotch (Alternaria porri) Black mold (Aspergillus niger) Neck rot (Botrytis aclada) Smudge (Colletotrichum circinans) Fusarium wilt Fusarium oxysporum f.sp. cepa [11] Blue mold (Penicillium spp.) Downy mildew (Peronospora destructor) White tip (Phytophthora porri), Rust (Puccinia allii), Pink root (Pyrenochaeta terrestris), White rot (Sclerotium cepivorum), Stemphylium (Stemphylium vesicarium), Onion smut (Urocystis cepulae) and Fusarium basal rot (Fusarium oxysporum) [10]. Among this Fusarium oxysporum occur in frequently in onion field [12,13]. concluded that Fusarium oxysporum has long occurred at low levels on most onion farms in New York. At following commercial acceptance in the mid-1960's of certain varieties noted to be much less susceptible to wilt disease Fusarium oxysporum is a well-known phytopathogenic fungus that affects hundreds of crops worldwide. Most studies of the fungus focus on the ability of F. oxysporum to cause vascular wilt on a particular host. However, environmental surveys of a wide range of habitats frequently find F. oxysporum in the absence of plant disease. Isolation of Fusarium solani, Fusarium monillforme, Fusarium oxysporum and F. oxysporum f. spp Cepa from rotted onion bulbs, sets, seedlings and soil from New York F. oxysporum f. sp Cepa and F. solanl were found the most virulent inducing damping-off were equally powerful disintegration of onion sets in-vitro experiments [14]. introduced that more than 30 isolates fusarium spp were obtained from different onion fields. The servay was done in different regions of Japan. These isolates included Fusarium oxysporum, Fusarium solani, Fusarium vertcilioides. After confirmation of pathogenicity of 5 local cultivar of onions in geen house 4 cultivars susibliable and one is tolerant against onion wilt. Fusarium spp are commonly found in onions roots and shoots.

Wilt disease which causes economical losses in different plants have been also known for onion for a long time. Several countries it was reported that among wilt disease agents, Fusarium oxysporum f. sp. cepa caused pre-and post emergence death and basal rot (Ozer, 1995). Fusarium wilt also causes damage to many crops including Chili, potato, tomato, and pepper [15]. Fusarium oxysporum, wellknown as formal specials (f.spp.) on the basis of their host specificity, cause vascular wilts ,root rot and Damping off on many essential crops. Fusarium oxysporum f. sp. Cepa is predominantly problematic to onion growers worldwide and is rising in occurrence in the UK [16]. The diseased onion plants showed both above and below ground symptoms. The affected plants were characterized by general wilting, stunted growth with profound yellowing, followed by browning of leaves. Later on, these leaves became dry and plants showed die-back. The above ground symptoms comprise of rotting of roots and basal plate discoloration. Affected tissues appeared brown and watery. The bulbs become soft, exhibited semi watery decay initiated from the basal plate and move upwards. F. oxysporum f. sp. cepae predominantly isolated from diseased bulb and root pieces and identified with the help of keys developed by various workers as stated in materials and methods. Fungus produced the fluffy white colonies on the surface of the medium. The micro conidia are abundant, mostly non-septet, ellipsoidal or cylindrical, straight or curved. The macro conidia are fusiform, slightly curved, pointed at the tip, mostly three septet [17].

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Wilt caused by Fusarium oxysporum is a devastating disease of different cops in Pakistan including cehpae tometo potatoes and onions. In the present study 321 genotypes from diverse sources were estimated under controlled circumstance to recognize genetic sources of tolerant against this disorder at seedling and regeneration phase. Infection reaction at two phases revealed significant dissimilarity along with the genotypes. At seedling stage disease incidence varied from 0 to 29.3% whereas at reproductive stage ranged from 0 to 57%. 173 genotype were found in small plants in which 55 were resistant and 93 were infected by pathoen. In reproductive phase 34 were tolerant genotypes and 102 were tolerant 183 were very susceptible some of them showed very resistant in all stages. These genotypes may be oppressed for the development of resistant cultivars adjacent to wilt (Muhammad, 2010). The total losses of onion in the Pakistan due to Fsarium wilt and basal rot of onion is 6 million rupees [18]. Fusarium wilt pathogen may cause epidemic and indemic disorder spread from crops to crops causing widespread death of the seedlings in the nursery and fields. Many tree species of the tropical regions including mango are susceptible to diseases of fungal origin. The fungal invasion of the succulent root tissues causes the young tree seedlings to dieback; their leaves become discolored, wilted and eventually dead. The fungus can also be multiply over long distances either in contaminate transplants or in soil. Though the fungus can occasionally contaminate the fruit and contaminate its seed, The extend of the fungus by way of the seed is extremely rare. It is also probable that the spores are broading by wind [19].

In the case of soilless growing, the sources of primary infectivity are microconidia transferred from air. The disease develops quickly in cool soil 18 °C (Kim et al., 2001). Fusarium oxysporum can stay alive in soil for many years as a resistant spore. The pathogen is stirred about the field by diffrent source which includ air, water, tools and soil residiuse etc Other agents which contaminate the soil and water capable the pathogen to inhance disease infection [20]. The Fusarium wilt disease of mango nursery caused by F. oxysporium is characterized by a long phase of incubation [21] reported that fungus can be isolated in the vicinity of the lesions and does not extend systemically. Infection occurs throughout the wounds and natural holes shaped by the recently produced root [22].

Survey of fungi related with postharvest decline of onion bulbs in four chief markets in Kumasi were conduct. Infected onion shoots obtained from the different markets. Abinchi, Anloaga, Kwadasi and middle markets were contaminated by five fungal species: *Aspergillus sp, Aspergillus sp, Penicillium sp., Rhizopus stolonifer* and *Fusarium oxysporum.* Efficacy of aqueous leaf extracts of Pawpaw (Carica papaya), Neem (Azadirachta indica), Moringa (Moringa oleifera), Cassia (Cassia alata) and Tobacco (Nicotiana tabacum) in managing seed-borne fungi of Bawku Red were studied in vitro and in vivo. All the aqueous leaf extracts significantly (P < 0.05) inhibited the radial mycelia growth of the test *fungi* (*Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Botrytis sp. and Fusarium oxysporum*) *in vitro.* The highest percentage growth inhibition was achieved with aqueous Pawpaw leaf extract [23]. Management of the soil with 10% water emulsions of the formulated extract of a chili pepper extract and essential oil of garlic mixture, a cassia tree extracts, and clove oil reduced the populations of Fusarium to 98.9, 97.1, and 96.5%, respectively, 3 days after soil treatment. These same formulations also ensored disease progress in the greenhouse and resulted in 90 to 99% plant stand after 5 weeks. The experimental reductions in the spors population in soil and the enhance in plant stand in the greenhouse indicates that these usual plant yield may have important roles in antagonastic based management strategies for manage of Fusarium wilt diseases (John, 2000).

### **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 in onion fields. The Disease in severity of the disease was calculated according to the disease incidence formula [24]

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

### Isolation and Identification of The Disease Causing Fungus

Samples were taken from infected shoots, bulbs, and roots of infected onions. Collected samples were then brought to the laboratory for isolation and identification process as described by [25], where, the samples were first surface sterilized twice with distilled sterilized water and then were treated with 0.5% Naocl (Sodium hypo chloride) 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  $25 \pm 1^{\circ}$ C for seven days. After seven days of inoculation the fungi isolated, were then identified with the help of keys for identification of fungi by [26] and with the help of characteristics of fungi mentioned in the book "The Isolation and Identification of Fungi" by [27].

### Identification of Fusarium Spp

*Fusarium spp*. isolated from infected tissues of roots and shoots were then identified by studying their colony characteristics and conidial

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morphology using the keys described by [26] and with the help of characteristics of fungi mentioned in the book "The Isolation and Identification of Fungi" by Frank M Dugan.

#### Consequence of Different Leaf Extracts on The Linear Colony Growth of *Fusarium Oxysporum*

Four different botanical extracts were tested in-vitro condition against *Fusarium oxysporum*. The botanical extracts used were, Neem (*Azardichta indica*), Datura (*Datura stramonium*), Tobacco (*Nicotiana tabacum*) and AKK (*Calotropisprocera*). The aqueous extract was prepared according to the formula described by [28]. There are 70 gm fresh leaves of each plant were collected from field and then blended in 25 ml sterilized water with the help of mortan and pestle. The bledded 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

5. No	Name of Botanical extracts		Dose in ml
		i.	1.0 ml in /100 ml medium
1	Neem (Azadirachta indica)	ii.	2.0 ml in /100 ml medium
		iii.	3.0 ml in /100 ml medium
		i.	1.0 ml in /100 ml medium
2	Tobacco (Nicotiana tabacum)	ii.	2.0 ml in /100 ml medium
		iii.	3.0 ml in /100 ml medium
		i.	1.0 ml in /100 ml medium
3	Akk (Calotropi sprocera)	ii.	2.0 ml /in 100 ml medium
		iii.	3.0 ml in /100 ml medium
		i.	1.0 ml in /100 ml medium
4	Datura (Datura stramonium)	ii.	2.0 ml in /100 ml medium
		iii.	3.0 ml in /100 ml medium
mm).			

of sterilized pipette and 5mm disk of the fungus was inoculated into Petri plates. The disc of the fungus was taken from 8 days old culture of *Fusarium oxysporum*. All these Petri plates were then transferred to incubator at  $25 \pm 1^{\circ}$ C and data of mycelia expansion of the fungus was record once 24 hours of inoculation till 8 days of inoculation. The trial was conducted in completely randomized block design (RCBD), with 4 treatments and 3 replications. Petri dishes containing only PDA medium without botanical extracts were used as control.

### Results

#### Survey of Different Onion Fields

A Survey of different onion fields of district Hyderabad, Tandolayar 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 spp.*) Downy mildew (*Peronospora destructor*) Onion smut (*Urocystis cepulae*). 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 Layar, 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 predominant fungus and was recognized on the bases of their size and shapes mentioned in the book "identification of the fungi" written by [27] and with the help of electronic microscope, help from the senior Professors of the department was also taken in this regard (Figure 2).

# Effect of Different Botanical Extracts on The Mycelial Growth of The Fungus (*Fusarium Oxysporum F.* Sp Cepa,).

Four different plant extracts were evaluated under in-vitro conditions to check efficacy against *Fusarium oxysporum* at different doses. The botanical extracts used, were Neem (*Azadirachta indica*), (AKK) Calotropi (*Calotropi sprocera*), Tobacco (*Nicotiana tabacum*) and Dhttura (*Datura stramonium*). All the botanical extracts, significantly

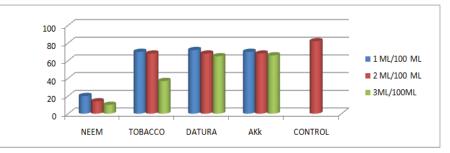
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reduced the mycelia growth of *Fusarium oxysporum* (p<0.000). Among all the used botanical extracts, the Neem was found the more effective in reducing the mycelia growth of the fungus at their highest dose (10.66 mm) and at lowest dose (20.00 mm) respectively, followed by Tobacco, which reduced the fungal growth at highest dose (37.00 mm) and at lowest dose (70.66 mm) whereas, the Datura reduces the

mycelia growth of the fungus at its highest dose (65.66mm) and at lowest dose (72.33mm) respectively. Dhttura and Calotropi (AKK) were found less effective as compared to other three botanical extracts. All the botanical extracts at their highest doses significantly retarded the growth of fungus as compared to control (82.66 mm).

S. NO	<b>Botanical extracts tested</b>	Dose (100ml). medium	Radial colony growth (mm)
1	NEEM	1ml	20.000f
		2ml	14.000g
		3ml	10.667g
2	TOBACCO	1ml	70.667bc
		2ml	68.667bcd
		3ml	37.000e
3	DATURA	1ml	72.333b
		2ml	68.667bcd
		3ml	65.667d
4	AKk	1ml	70.667bc
		2ml	68.000cd
		3ml	66.000d
5	CONTROL		82.667a
	LSD (P<0.000)		P = 0.000

Ghraph 1 shows Efficacy of different botanical extracts on linear colony growth of Fusarium oxysporumcausal agents of wilt in Onions



Effect of Fusarium oxysporum on (1) disease Infection % and (2) Root wight of onion plant



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(A)District Hyderabad (Tandojam)



(B) Tando alyarTypicl symptoms of Fusarium wilt in onion in fields .



Figure 1. Photograph showing the survey of different infected onion firlds of Fusarium wilt

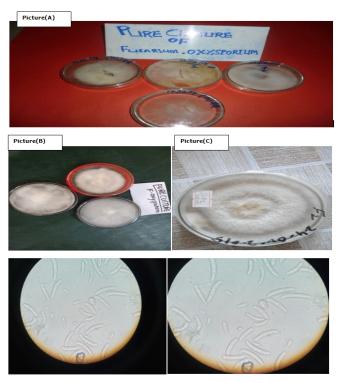


Figure 2. Picture showing the pure culture the fungus *Fusarium oxysporum* 

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#### Fusarium oxysporum after 8 days of inoculation

Figure 3. Effect of different botanical extracts on the linear colony growth of Fusarium oxysporum after 8 days of inoculation

#### Discussion

Fusarium wilt is most serious and devastating disease of onion field throughout the world including Pakistan especially in Sindh province district Hyderabad, which causes serious losses to young plants and may lead to their death. So, therefore keeping in view the incidence and the losses caused by the disease in onion field, the survey of three, districts of Sindh, e.g. Hyderabad, Tandolayar and Mirpurkhas was carried out to record the incidence of the disease in different onion fields of these three districts. During the survey it was observed that the Fusarium wilt of onion was found frequently in all the onion fields of the three districts with more or less incidence. The maximum incidence was recorded from Hyderabad district (60.0%) whereas, the incidence of the disease from the Mirpurkhas district was recorded up to (45.0%). These studies were investigated by [29] and [30], who found 50-70% and 9.4 % infection on cotton due to Fusarium spp. Moreover [31] reported 60% mango decline intensity due to soil borne micro flora accompanied with Fusarium oxysporum also.

In our studies, isolation and identification of the collected diseased specimens showed the association of three fungi i-e., *Fusarium oxysporum, Aspergillus niger, Botrytis* from rotted roots and shoot tissues. Among them Fusarium oxysporum was found in highest frequency from all the associated fungi. The isolated fungi were then identified on the basis of morphological characteristics and color of

the colonies of fungi. The identification was also done with help of taxonomical keys described by [26] and with help of a hand book" isolation and identification of fungi" written by [27]. These results are in link with the studies of [32] isolated 3 different species of fungal microbes from roots and shoots of diseased onion plant and found that the *Fusarium oxysporum* were frequently associated with fields of onion.

As the chemical fungicides are much expensive and have some health hazardous effects on human health and environment, so, alternatives of chemical fungicides were tried to control the Fusarium wilt of onions. For this purpose, we have used four different botanical extracts and biological control agents under in-vitro conditions for their efficacy against Fusarium oxysporum at different doses (1ml, 2ml, 3ml). Among all the tested botanical extracts, the Neem was sought highly significantly in reducing mycelia colony growth of the fungus followed by Akk and Datura. Whereas, Tomak were sought less effective in reducing the mycelia colony growth of the fungus as compared to control. These studies are in line with the studies of [33] who also reported different anti pathogenic activity of botanical extracts of different varieties of Eucalyptus and also described that the Eucalyptus camaldulensis showed highly antipathogenic activity. Jha et al., (2004) tested the efficacy of four botanicals such as Bel (Aegle marmelos), Neem (Azadirachta indica), Onion (Allium cepa.) and Garlic (Allium sativum) and evaluated separately or in

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integrations of different concentrations against spore germination of *Helminthosporium maydis*, *lycopersici* found groundnut and mustard at a 2% concentration were most effective in reducing the pathogen population more than (70%). However, groundnut was superior to mustard as it not only reduced a higher disease index (77.1%) but it also improved plant growth, cotton seed cake was the least effective [34]. studied the effect of various treatments of *Calotropi sprocera* (AKK) and *Fusarium oxysporum* inoculation on tomato seedling development and rhizosphere micro flora and reported the suppressive effect of the pathogenic fungus and improved the percentage emergence, root and shoot lengths of tomato seedling. The mycelia growth, percentage spores germination and germ tube extension in *Fusarium oxysporum* decreased when Calotropis procera extract concentration increased.

Bio-control agent was tested in-vitro conditions against Fusarium oxysporum f.sp Cepa, In the dual- culture the growth of Trichoderma verdii was proved (48mm) and Fusarium oxysporum (23mm) compared to control Trichoderma verdii (84 mm) and control Fusarium oxysporum (86mm). The bio control agents Trichoderma sp were positive as an substitute to chemical control of the onion basal rot and to better growth and yield of onion and inhibited the colony growth of Fusarium oxysporum [35,36,37]. The results of the botanical extracts showed that the Fusarium wilt of onion field can also be managed through the use of certain botanical extracts instead of chemical fungicides [38,39,40,41,42,43,44]. All the botanical extracts significantly reduced the colony growth of the fungus Fusarium oxysporum. Among of them [45,46,47,48,49], Need was sought more significantly in reducing mycelia colony growth of the fungus [50]. Followed by AKK and Tobacco whereas; Datura were found very less effective in arresting the colony growth of the fungus [51,52,53,54].

### 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 Tandoalvar 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 Biological and botanical extracts need was found more effective as compare to other extracts. Keeping in view the results of present research work, it is suggested that certain botanical extracts such as need and Tobacco should be recommended against this disease. Efficacy of these botanical extract should be evaluated at field conditions level at different onion fields at different localities. Fungicides are hazardous for human health and there is problem of resistance against fungicides, so alternative control is needed, for this purpose botanical extracts could be used. These studies also showed that the disease can also be control through botanical extracts and Biological control like Need and *Trichoderma verdii*.

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