Research Article

Management of *Fusarium* Basal Rot of Onion caused by *Fusarium oxysporum* f.sp. *cepae* through Desert Plants Extracts

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Abstract | Onion (*Allium cepa* L.) is a very economical and productive horticultural crop extensively cultivated worldwide. It is grown commercially throughout the country and contributes significantly to the economy of Pakistan. Onion is susceptible to many foliar, bulb and root fungal pathogens that badly destroy onion crops, greatly reducing its yield and quality. *Fusarium oxysporum* is a very common soil-borne fungus that causes basal rot onion and it has the ability to survive in the soil for many years without having a host. Many fungicides are available in the market to control the basal rot of onion which contain hazardous material that continuously destroys the environment and human health. The objective of the present study was to evaluate the fungicidal efficacy of desert plant extracts against basal rot of onion in the laboratory, greenhouse and field. Completely randomized design (CRD) was used for *in-vitro* study and randomized complete block design (RCBD) for greenhouse and field experiments. Three desert plants i.e. *Gossypium thurberi, Calotropis procera* and *Suaeda fruticose* were used at 50 ppm, 100 ppm and 150 ppm concentrations. Maximum basal rot disease reduction (78.6%) was recorded at 150 ppm concentration of wild cotton (*G. thurberi*) under *in-vitro* conditions. Whereas, wild cotton also gave the highest disease reduction under greenhouse (62.93%) and field conditions (52.56%) as compared with other two plant extracts and control. It is concluded that aqueous extract of wild cotton gave the best control of onion basal rot under lab, greenhouse and field conditions.

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Introduction

Onion (*Allium cepa* L.), family *Alliaceae,* is a very economical and productive horticultural crop cultivated worldwide (Griffiths *et al*., 2002). It took great importance worldwide due to its nutritional, medicinal and economic values (Nasri *et al*., 2012) also contain much anti-inflammatory, anti-cholesterol, anticancer, antioxidant properties. It is an important crop with a great source of phyto-constituents and is well known for its pleasant flavor. This crop is extensively used in many foods as pungency in soups, salads, sandwiches, meat dishes, and prepared as vegetable foods.

Fusarium oxysporum is a very common soil-born fungus. It has the capability to remain alive in the soil for numerous years without having a host. When a

susceptible host presents it attacks very badly. Fusarium wilt is caused by a huge number of extremely affectionate forms and races of this common soil-borne pathogen. *Fusarium* wilt is infected with many well-known nurseries, gardens and greenhouses. *F. oxysporum* overcomes the root system of the host, which results in the non-availability of water to the administrative tissues which causes wilting, leadings to the death of infected plants (Bayaa *et al.*, 1986). This fungus may infect the onion bulb at the basal stem plate and demoralize it; as the infection is preceded, it destroys the whole plant. Secondary infections occur in the dormant bulbs during storage (Cramer, 2000).

The most prominent symptoms of this rotting disease are yellowing of the floral part and dieback of leaf from the tips at early or middle periods of crop development. This infection starts from the bulb and advances from the basal portion in the direction of the neck of the bulb. Infected roots turn dusky brown to dusky pink, and sometimes white colour fungal growth is prominent at the basal part of diseased bulbs. The brown discoloration is clearly shown when an infected bulb is cut vertically to the stem plate's tissues. After on, the stem plates tissue turned rotted and the appearance of dry rotting is seen. Stem plates and drying the outer stretch split open underneath the dried conditions. This disease progresses in the storage conditions, and bulb mites rapidly inhabit the basal plates which are affected by the disease (UC IPM. 3453).

Many fungicides are available in the market to control the *Fusarium* basal rotting disease of onion, but synthetic fungicides have hazardous effects on the environment. For sustainable agriculture, biological control of plant pathogens is considered very effective and does not harm the environment (Al-Naemi, 2016; Youssef *et al.*, 2016). Desert plants (DP) are one of the best sources to control this fungal pathogen. In developed and developing countries, desert plants have been used to treat different fungal diseases (Jamshidi-Kia *et al.*, 2018; Rahimi-Madiseh *et al.*, 2017). In recent years, desert plants have gained much attention because they have had many benefits for their uses, such as reduces expenses and fewer side effects to the environment (Rahimi-Madiseh *et al.*, 2017).

It is considered a productive approach to use plant-based products to fight against fungal and parasitic diseases (Rafieian-Kopaei *et al.*, 2016). However, accomplished measures can be taken to make medicine by recognizing the active compounds of the plants. Some antifungal or other microbial active compounds are found in desert plants like Flavonoids, alkaloids, tannins, citronellol, geraniol, thymoquinone, and phenolic compounds (Alizadeh *et al.*, 2018). The uses of desert plants for the management of this disease yet not focused against basal rot of onion. The desert plant extracts may trigger the defense mechanism of the onion against fungal attack. The objective of the current study was to find out the best concentration of three desert plant extracts in-vitro against basal rot of onion, followed by greenhouse and field experiments.

Materials and Methods

Survey of Infected fields

The sampling was located at three onion producing districts (Faisalabad, Lodhran and Bahawalpur) of Punjab, Pakistan.

Collection of samples

Onion plant samples were collected showing disease symptoms such as rotting of the basal plate, curving, yellowing, wilting leaves and affected rot becomes dusky brown to dark pink, and a white colored fungal growth from standing crops in the field during summer using simple random sampling technique. Soil samples were also collected from rhizosphere soil of onion. Samples of the soil were taken with an auger (up to 10 cm depth) after removing three cm from soil surface. The diseased onion and soil samples were brought to Laboratory, Department of Plant Pathology, and University of Agriculture Faisalabad.

Disease severity

Throughout the analysis, observations were documented on the occurrence of the fusarium basal rotting in onion fields. According to the disease incidence formula, the severity of the disease was calculated (Anum *et al.*, 2015).

$$Disease\ severity(\%)\frac{No\ of\ rotted\ Plants}{Total\ no\ of\ plants}\times 100$$

Isolation, Identification and Maintenance of F. oxysporum

Infected onion bulbs with Fusarium basal rot disease were taken from the field of different district of the province Punjab, Pakistan. Collected samples were then carried into the lab aimed at isolation and identification procedure as suggested (Pathak, 1987).

First, samples were sterilized twice with the purified distilled water, and then sample were treated through 0.5% NaOCl (Sodium hypo chloride) or 70% ethanol for 2 min. For fungal isolation, potato dextrose agar (PDA) growth media was used. An infected portion of the sterilized surface of onion was plated on the PDA growth media in 90 mm in width. The petri dishes were incubated at 25 ± 1^{0}C for 7 days. The isolated fungi were sub-cultured on PDA media by means of a single colony of the fungus method for culture purification (Leslie and Summerell, 2006). The pure culture fungus was preserved at 4°C in a fridge-freezer. The isolated fungi were recognized as the *F. oxysporum* f.sp. *cepae* (Leslie and Summerell, 2006).

Preparation of desert plant extract

Desert plants were collected from Cholistan Institute of Desert Studies (CIDS), located in The Islamia University of Bahawalpur. It is very diverse desert area and has many different desert plants which contain medicinal values. Three desert plants were collected like Wild cotton, Aak and Kali lani and then taken into the laboratory. Different desert plant extracts were tested against *F. oxysporum*. The succulent extract was organized rendering to the formula labelled by (Steel *et al*., 1997). A round 70 gm fresh plant leaves of each were collected from the field and then with the help of mortar and pestle blended in 25 mL sterilized distilled water. The intermingled extracts were first passed through five layered muslin clothes and then filtered through What'sman filter paper.

The plant extract was considered predominant and stored in a freezer for further studies. With the help of a sterilized pipette, each dose of the DP extract was transferred into 100 mL PDA medium and the fungus of 5mm disk was inoculated into Petri plates. The fungus plug was taken from 8 days old culture of *F. oxysporum*. All these Petri plates were settled into the incubation chamber at 25 ± 1^{0}C and data of mycelia colony of fungi were noted after 24 h of inoculation and continue to eight days of incubation. The trial was conducted in a randomized complete block design (RCBD). Petri plates having only PDA medium without DP extracts was used as control.

Processing of plant material

The collected plant materials (bark, wood and stem) were sterilized with 0.1% NaOCl and washed three times with sterile distilled water. Properly cleaned plant materials were dried in the shade. Seeds were separated by decupling and powdered after proper cleaning and drying. Powdered plant materials were stored in sterile cellophane bags in a cool and dry place till further use.

Extraction of plant material

The ground plant materials (bark, wood and stem) weighed 70g fresh plant parts were extracted with methanol in a soxhlet extractor for 8 to 10 h. The process was run till the decolourisation of the solvent, after which the extract was filtered with Whatman filter paper (No.1) and the filtrates were concentrated using a rotary evaporator. Then the extracts were evaporated to dryness over water bath and solvent free extracts of respective parts were obtained. Extracts of wood, bark and stem of plant materials were weighed and kept in labeled sterile specimen bottles.

Evaluation of Desert Plant extract under Greenhouse condition

A Greenhouse experiment was taken for bio-control of *Fusarium* basal rot of onions through altering the seed and soil through desert plant extract according to the technique of (Javaid and Rauf, 2015) with various amendments. The surface of the seed was purified in Sodium hypo-chlorite (NaOCl) sol. 0.5% for 3 min then washed for five minutes in sterile distilled water then dried. In soil inoculation a disinfected soil combination of 1/3 field soil + 1/3 manure + 1/3 sand was taken and mixed thoroughly than dried the soil and place into the greenhouse. Without inoculation seeds and soil were considered for controls. Three treatments and three replications of 40 seeds were sown in plastics pots. Pots were kept in the greenhouse. After 25 days of sowing the inoculation of *F. oxysporum* was done with different concentrations of desert plant extract like Wild cotton (*G. thurberi*), Aak (*C. procera*) and Kali lani (*S. fruticose*) with 50 ppm, 100 ppm and 150 ppm, respectively was sprayed. After 10 days of inoculation, the growth inhibition of *F. oxysporum* was observed with different % respectively.

Evaluation of medicinal desert plant extract in field condition

For the field survey, several trials were conducted on the onion crop to estimate the effectiveness of *F. oxysporum* on the prevalence of *Fusarium* basal rot (FBR) disease in the field. Three trials were conducted in the field. The size of the plot in which the treatment is contained is 4x3m and individually, treatment was replicated three times of interval in

Complete Randomized Block Design (RCBD). Two onion seedlings were used for all the experiments and bulb treatment with different DP extract@ 10g/ kg was done before planting. The onion seedlings were planted in the edges @ 240 seedlings/ plot (20/m^2) with a distance of 45cm among the ridges and 10cm between the plants. Three desert plant extracts were used, like Wild cotton (*G. thurberi)*, Aak *(C. procera)* and Kali lani (*S. fruticose*).

Three different concentrations of desert plants extracts were used to control this fungal disease. Soil application of different DP extract was done after planting onion seedlings. Plots which obtain the above treatment at different concentration of DP extract was mixed with 50 g of soil and applied homogeneously as a group along the ridges. The examination was recorded on the occurrence of seedlings rot on 30 and 60 days after planting and at the time of harvest based on the percentage of bulb infected. Onion bulb yield was also being recorded. It was noted at 150ppm Wild cotton is much active to overcome this disease was52.56% followed by Aak and Kali lani at their high concentration was 46.63% and 38.5% respectively. All the data was investigated statistically as per (Gomez and Gomez, 1984).

Statistical analyses

Growth Parameter: The existence of *Fusarium* basal rot per pot was noted based on the disease signs (like rotting, leaf dieback, yellowing and browning of leaves, chlorosis and bulb mites) for several days from the initial arrival of the disease. By the period of harvest per plant, the severity of basal rotting will be noted on 1–5 scale, whereas 1 means deprived of any kind of decline symptom, two means more than ten percent rotted roots, three means ten to thirty percent rotted roots with more than ten percent rotting basal plates, four means entirely rotting roots and ten to thirty percent rotting basal plates & five means wholly rotting roots and further thirty percent rotting basal plates (Rengwalska and Simon, 1986). FBR harshness outcome will be transformed into % age severity index (PSI):

$$PSI = \frac{Sum\ of\ numerical\ ratings}{No.of\ plants\ scored \times Maximum\ score\ on\ scale} \times 100$$

Combined data from persistent trials were exposed to the examination of alteration and mean segregation was fulfilled by both CRD and RCBD. All values were directed by means of the Statistical Analysis Systems version 9 (SAS Institute, Cary, NC).

Results and Discussion

Effect of plants extract on the radial growth of F. oxysporium on PDA

Different desert plant extracts were evaluated under *in-vitro* circumstances to determine the efficacy against *F. oxysporum* at different concentrations of 50, 100 and 150 ppm.

Wild cotton (*Gossypium thurbrei*) significantly reduced fungal growth (78.6%) at 150 ppm whereas Aak (*Calotropis procera*) and Kali Lani (*Suaeda fruticose*) 150 ppm concentration reduced up to 65.44% and 56.83%, respectively (Table 1).

Table 1: *Compressions of mean values of different desert plants extracts after 3 and 7 days % growth inhibition of Fusarium oxysporum.*

Sr. No.	Desert plant extracts tested	Dose ppm medium	After 3 Days Radial colony growth (mm)	After 7 Days Radial colony growth (mm)
1	Wild cotton	50 ppm 100 ppm 150 ppm	32.73 c 48.56d e 70.63 b	40.8 c 57.73 e 78.6 b
2	Aak	50 ppm 100 ppm 150 ppm	27.7 e 44.5 h 60.06 b	34.36 f 52.63 i 65.44 d
3	KALI LANI	50 ppm 100ppm 150 ppm	24.2 c 38.3 f 50.63 b	30.43 d 43.03 h 56.83 c
4	CONTROL	3.31a		4.04b c
LSD (P<0.000)		P = 0.0000		

Greenhouse experiment

In greenhouse conditions, the results were recorded for bio-control of Fusarium basal rot of onion. Seed and soil amendments were taken using three concentrations i.e. 50, 100 and 150 ppm of desert plant extract. Results showed that there was a significant reduction in basal rot disease of onion. Disease frequency was reduced meaningfully with the application of plant extract of wild cotton (*G. thurberi*), and there was 30.53%, 41.73% and 52.56% disease incidence at application of 50, 100 and 150 ppm, respectively (Figure 1). *G. thurberi* was most effective among other desert plant extract, while Aak (*C. procera)* and Kali Lani (*S. fruticose)* at 150 ppm concentration gave 46.63% and 38.5% disease reduction, respectively.

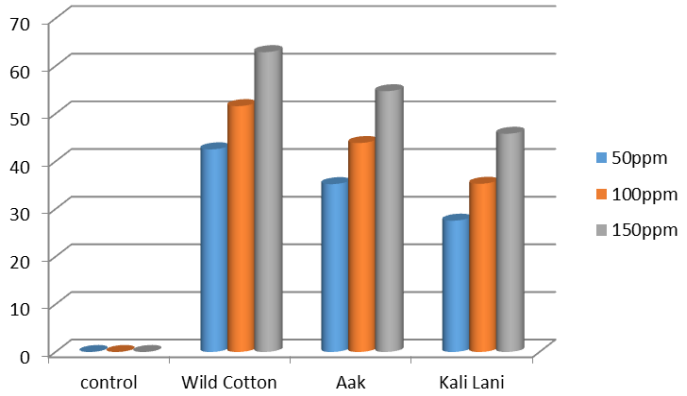

Figure 1: *Graphical representation of desert plant extract under greenhouse conditions.*

Field experiment studies

Under field conditions, the application of wild cotton *(G. thurbrei)* at 150 ppm concentration reduced the basal rot incidence by 52.56%, which is the greatest among the other desert plant extract. The application of Aak (*C. procera*) and Kali lani (*Suaeda fruticosa*) also showed the highest incidence 46 .63% and 38.5% at 150 ppm, respectively (Figure 2).

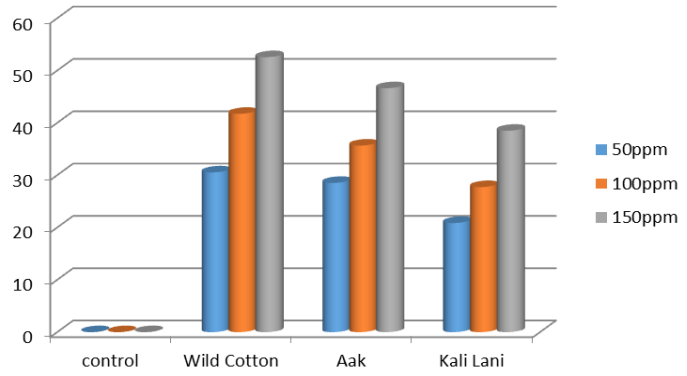

Figure 2: *Graphical representation of desert plant extract in field condition.*

Fusarium basal rot is the most severe and shattering disease of onion fields all over the world, including Pakistan, especially in the province of Punjab district Bahawalpur, which causes severe damages to young plants parts and leads to their death. In this study, it was noted that *Fusarium* Basal rot of onion was found more aggressively in almost all the onion fields of the three districts with high or less disease prevalence. The highest occurrence was noted from Bahawalpur district (65%) however; the disease incidence from the Lodhran district up to (50.0%) was recorded. Studies were examined by (Wrather *et al.*, 2002; Montomery, 2009), who found that 50-70% and 9.5 % infection occurred in cotton due to *Fusarium* spp. Furthermore, Khanzada *et al.* (2004) stated that 60% mango decline strength is owing to soil-borne micro flora also complemented with *F. oxysporum*.

Our investigation, isolation and identification of infected samples revealed that the presence of fungi, *F. oxysporum* from rotted plant parts roots and bulb. The *F. oxysporum* was originated in maximum incidence among all the associated fungi. Identification of isolated fungal organisms was made on the foundation of morphological characteristics & colonies color of fungi. With the help of taxonomical keys, identification also was done, which is defined by Nelson *et al.* (1983) and also with the assistance of a hand book" isolation and identification of fungi" presented by Frank (2005). Results are related to the findings of Grooten and Westelijke, (2012) isolated species of fungal-microbes from the roots and bulb of infected onion plants result that *F. oxysporum* were more aggressive related with onion fields.

The use of chemical fungicides is prohibited by some countries. Such chemicals contain serious health hazardous effects on human health as well as on the environment and also are very costly so, the substitutes of these chemical fungicides were tried to control the *Fusarium* basal rot of onions. For this purpose, we have used three different desert plant extracts to control this devastating disease against *F. oxysporum* at different concentrations (50, 100 and 150 ppm). Among all the tested desert plant extracts, wild cotton was considered highly significant in the inhibition of mycelial colony growth of the fungus, followed by Aak. Although, Kali lani was considered least effective in the inhibition of the mycelia development of fungi as compared to control.

The results are related to the previous research which revealed that latex extracts of medicinal plant *G. thurbrei* have fungicidal activity (Jaliwala *et al.*, 2011; Emeka, 2009). Results are also concerned with many other research works, as medicinal plant *C. procera* had shown anti-fungal activity (due to the presence of biologically active constituents in ethanolic extract of *Calotropis* latex) (Gupta *et al.*, 2000; Verma *et al.*, 2012).

Conclusions and Recommendations

The present study was carried out to evaluate the effect of desert plants extracts such as wild cotton, Aak and Kali lani against basal rot disease. It is concluded that 150 ppm concentration, wild cotton was the most effective against basal rot of onion followed by

aak and kali lani. The growers are recommended to use desert plant extracts against fungal diseases rather than fungicides as fungicides are dangerous for human health and there is a problem of resistance against fungicides. So, alternative control is needed, for this purpose, desert plants extracts could be used.

Novelty statement

The use of desert plant extracts in the management of *F. oxysporum* on onion was demonstrated for the first time in this study.

Authors Contribution

Muhammad Madni Afzal: Conceptualization, research execution.
Shahbaz Talib Sahi: Data interpretation, Manuscript proofreading.
Amer Habib: Bench work, Manuscript writing.
Waqas Ashraf: Research laboratory support.
Muhammad Ahmad Zeshan: Data analysis and interpretation.
Muhammad Raheel: Manuscript writing and proofreading.
Qaiser Shakeel: Research design, sample preparation.

Conflict of Interest
The authors have declared no conflict of interest.

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