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Biological control and pathogenicity of the *Fusarium oxysporum* caused onion wilt in different localities of sindh and baltistan region

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Abstract
In arrange to verify the pathogenic character of isolated fungus *F. oxysporum* the (*f.sp Cepa*) the pathogenicity test was conductod on commonly growing onion varioty Hazari by root and shoot infestation method. In general these methods of inoculation showed substantial impact on disease development and plant growth. Onion germinalion was significanty reduced in pots either inoculated by shoot or root infestation method as compared to the un-inoculated plants (control). However, root infestation method caused maximum reduction in plant germmation (60%) followod by shoot infestation method (39% as comparod to control). Similar trend were also observd in plant mortality, where highest plant mortality were recorded in root infestation method as compared to control (28%). Significant differences were also observed between plant growth in inoculated and un- inoculated plants. The plant growth parameters were also significantly varied with method of inoculation. Minimum root length were recorded in shoot infestation method (5.34 cm) as compared to control (10.74 cm).Similarly minimum root weight were recordod in root infestation method (1776 mg) followed by shoot infestation method (1296 mg) as compared to control (0,22 mg). Reisolation of the inoculated fungus was confirmed as of pathogenic nature *F. oxysporum* was re-isolated significantly in higher frequency in root infestation method (84.78%) as compared to shoot infestation method also showed some infection of *F. oxysporum* (10%). It could be either due to the soil-bome nature of the *F oxysporum* or as a result of secondary infection from inoculated plants. 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 *Trichoderma verdii*.

Keywords: re-isolated, biological control, root and shoot infestation, pathogenicity

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 (Griffiths *et al*., 2002) [21].
According to an 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 8th position with 2.25% share in production (Anum *et al*., 2015) [6].
The Fusarium wilt disease is incited by *Fusarium oxysporium* and is a disease complex problem which is caused by the activity of more than one fungus like *F. oxysporium*, *F. solani*, *Rhizoctonia solani*, *Pythium* and *Macrophomina phaseolina spp* (Dar *et al*., 2011) [13].
Onion is attacked by many diseases ilke Purple blotch (*Alternaria porri*) Black mold (*Aspergillus Niger*) Neck rot (*Botrytis aclada*) Smudge (*Colletotrichum circinans*) Fusarium wilt *Fusarium oxysporum* f.sp. *Cepa* (Grooten. 2012) [22].
The biological control action of these species is chiefly attributable to a mixture of several mechanisms of action, which may affect the micropholora of the suppressiveness of compost the addition species of *Trichoderma* to dung is a common technique used to control different plant diseases to suppress Fusarium wilt evaluated by species of Trichoderma (Blaya *et al.*, 2013).
The antagonistic activity of two species of *Trichoderma*, *T. harzianum* and *T. viride* were tested on PDA against *Fusarium oxysporum* f.sp.*cepa* by dual culture inoculation technique among the bioagent, *Trichoderma viride* inhibited maximum growth as compare to *Trichoderma harzianum* (Ilhe *et al*., 2013) [2].
Therefore keeping in view the losses caused by the Fusarium wilt of onion, the aim of the study is to find out the most effective and eco-friendly approach to minimize the losses and manage the wilt disease in field of onion.

Materials and Methods
Survey of infected fields
A survey of onion fields of districts Hyderabad, Mirpurkhas, Tandoallahyar, Khrmang, Khpulo and Shigar from baltian region 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) [38], 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 al*.,*(*1983) [37] and with the help of characteristics of fungi mentioned in the book "The Isolation and Identification of Fungi" by Frank. M. Dugan (2005) [18].

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) [37] 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).

Biological control

This study was conducted to assess the efficacy bio-control agent *Trichoderma verdii* against *Fusarium oxysporum* f.sp *Cepa*, by using dual culture method as described by Bahareh *et al.* (2016) [8]. The five millimeter diameter mycelial colony disc of test antagonist *Trichoderma verdii* was taken from seven days old culture this was paired against same sized mycelial disc of *Fusarium oxysporum* at opposed ends of pettri plates. The pettri plates were incubated at the 25+2 ºC temperature for eight days to observe the colony grow inhibition percentage of the pathogen was calculated as explained by Anupama (2015) [7]. The inhibition growth of *Fusarium oxysporum* in the presence of *Trichoderma verdii* was calculated over control. The growth inhibition was calculated by following formula:

(RI-R2) x100

Where,

R1= Redial mycelial colony dual pathogen in control.

R2= Redial mycelial colony growth of pathogen in dual culture experiment with antagonists.

Pathogenicity Test of the Fungus

The Pathogenicity Test of most predominant fungus *Fusarium oxysporum* was done by mixing of the fresh inoculums of the fungus with 1kg of sterilized soil in pots. Thirty onion plants sown in pots containing infested and un-infested soil. The data was recorded on number of healthy and infected plants and disease incidence severity and disease mortality after 35 days. The experiment was conducted in completely randomized block design (RCBD), with 3 treatments and 3 replications.

Conidial suspension

For injection, condial solusion was ready from 2 weeks old culture of *Fusarum oxysporum*. Clean water was added in each culture plate of the test fungus order to harvest fungus conidia; the culture was gently rubbed with spatula. The spore solution was collect in an uncontaminated glass beaker and strained through muslin cloth. Conidial concentration (conidia/ml) were determined with the help of hemocytometer and adjusted by adding water.

Root Infestation Method

An onion root was dipped in 100 ml of spore suspension for five minutes. The un- infested onion plants were used as control. Five seedlings of both infested and un-infested control plants were grown per pot (containing 2 kg /per pot). Four replications of every inoculated and un-inoculated were carry out. These pots were kept in unwrap air and watered as per requirement. After 35 days of sowing, the plants were uprooted carefully. The data were recorded plant mortality, plant growth and root infection.

Shoot Infestation Method

The already prepared fungal suspension iject in shoots of onion two kg soil was filled in each earthen pot. Three plants were sown in each infested and un-infested (control pots). The experiment was arranged as Randomized complete Block Design with three replications. These pots were kept in open air and watered as prequirement. Later than 45 days of sow, the plants were uprooted cautiously. The statistics were recorded on, i, e mortality and plant growth.

Re-isolation of the inoculated fungus

Re-isolation was made from inoculated and uninoculated plants. For this principle, the roots and shoots of the plant was washed carefully with tap water and cut into small pieces after surface sterilization with alchohal solution, pieces were placed on PDA plates. The recovery of inoculated fungus was recorded and disease percent was calculated with the help of following formula:

$$\text{Infection \%} = \frac{\text{NO. Of spp colonized by the fungus}}{\text{Tot no. of sp. studied}} \text{x} 100$$

Results

1. Survey of different onion fields

A Survey of different onion fields of district Hyderabad, Tandolayar and Mirpurkhas, Kharmang Shigar and Khapulo 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*) (Grooten, 2012) [22]. 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).

2. Isolation and identification of the fungus causing Fusarium wilt of onion

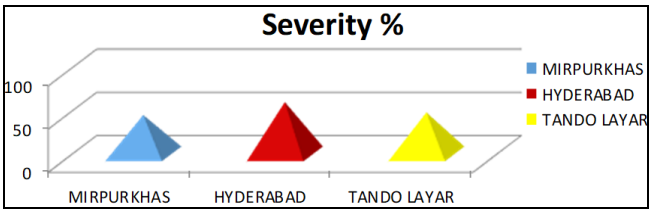
The collected infected specimens were then brought to laboratory for isolation and identification process of the fungus caused. The isolation and identification process reveals the association of different kinds of the pathogens with the wilted parts of the Onion shoots. Among all the isolated fungi, *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 Frank M. Dugan (2005) [18] and with the help of electronic microscope, help from the senior Professors of the department was also taken in this regard.

Evaluvation of *Trichoderma verdii* against *Fusarium oxysporum in vitro* conditions.

Trichoderma verdii was tested *in-vitro* conditions against *Fusarium oxysporum* f.sp *Cepa*, for this purpose dual-culture method was used and both the fungus the *Trichoderma verdii* and *Fusarium oxysporum* were placed in same plates at opposed from each other *Trichoderma verdii* extensively reduced the mycelia colony growth of *Fusarium oxysporum* at ($P< 0.05$) 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).

Table 1: Disease incidence of Fusarium wilt of Onion fields in three districts of Sindh during servay.

Locality	No. Of Fields visited	Samples taken/per Fields	Samples studied			
			Diseased	Healthy	Total	Severity %
Mirpurkhas	04	25	45	55	100	45.00
Hyderabad	04	25	60	40	100	60.00
Tando Layar	04	25	48	52	100	48.00
Kharmang	04	25	30	40	100	30.00
Shigar	04	25	30	70	100	30.00
Khapulo	04	25	40	45	100	40.00



Graph 1: Highest Disease incidence of Fusarium wilted fields in three districts during servay.

Pathogenicity of fusarium wilt

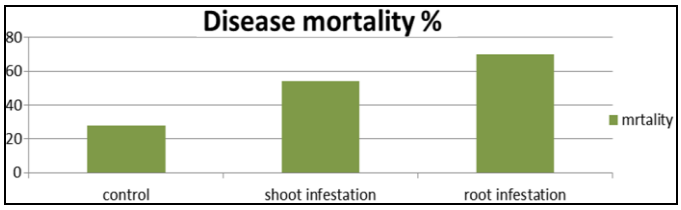
In arrange to verify the pathogenic character of isolated fungus *Fusaruim oxysporum* the *f.sp*, *cepa*) the pathogenicity test was conductod on commonly growing onion varioty Hazari by root and shoot infestation method.In general these methods of inoculation showed substantial impact on disease development and plant growth.

Onion germinalion was significanty reduced in pots either inoculated by shoot or root infestation method as compared to the un-inoculated plants (control). However, root infestation method caused maximum reduction in plant germmation (60%) followod by shoot infestation method (39% as comparod to control) (Fig 1). Similar trend were also observd in plant mortality, where highest plant mortality were recorded in root infestation method as compared to control (28%).

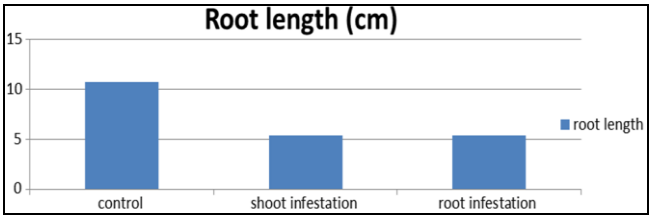
Significant differences were also observed between plant growth in inoculated and un- inoculated plants. The plant growth parameters were also significantly varied with method of inoculation. Minimum root length were recorded in shoot infestation method (5.34 cm) as compared to control (10.74 cm) (Fig. 2),

Similarly minimum root weight were recordod in root infestation method (1776 mg) followed by shoot infestation method (1296 mg) as compared to control (0,22 mg) (Fig 4).

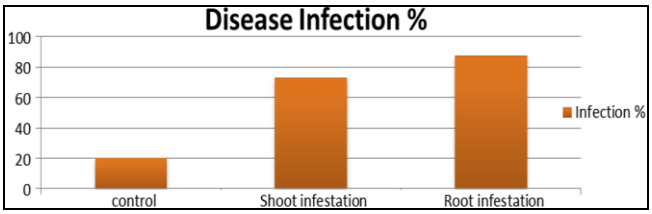
Reisolation of the inoculated fungus was confirmed as of pathogenic nature *F. oxysporum* was re-isolated significantly in higher frequency in root infestation method (84.78%) as compared to shoot infestation method also showed some infection of *F. oxysporum* (10%). It could be either due to the soil-bome nature of the *F oxysporum* or as a result of secondary infection from inoculated plants.



Graph 4: Showing Disease mortality of onions

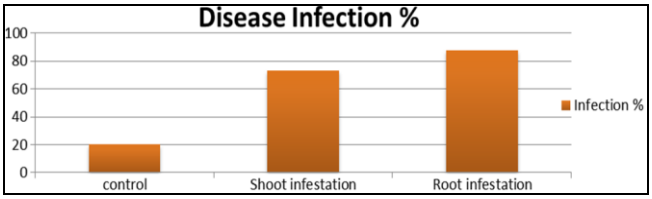


Graph 5: showing root length of onions

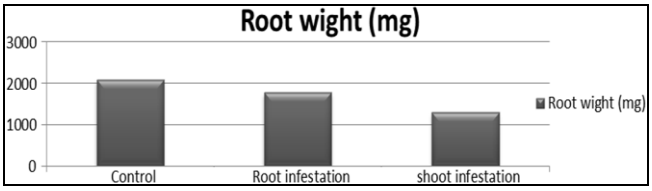


Effect of *Fusarium oxysporum* on (1) disease mortality and (2) Root length of onion plant

Graph 6: Showing Disease infection of onions



Graph 7: Showing root weight of onions



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

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

(A) District Hyderabad (Tandojam) Tando alyar District Mirpurkhas

Fig 2: the pure culture the fungus *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 Wrather and Phipps, (2002) [53] and Montomery, (2009) [36], who found 50-70% and 9.4 % infection on cotton due to *Fusarium spp*. Moreover Khanzada *et al.*, (2004) [10] reported 60% mango decline intensity due to soil borne microflora accompanied with *Fusarium oxysporum* also.

The pathogenicity and chemical control of fungal agents of wilt disease in onion were investigetad fungal agents of wilt disease, *F. oxysporum*. 42.9I% and 53.55 %, *Botrytis allii* Munn 23.88% and 10.07 % *Fusarium acuminatum (Ellis) Everh*. 16.05 % and 10.82 % were determined both in onion roots and bulbs respectively (Ozer, 1995).

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 discribed by Nelson *et al.*, (1983) [37] and with help of a hand book" isolation and identification of fungi" written by Frank. M. Dugan (2005) [18]. These results are in link with the studies of Grooten (2012) [22] 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.

Keeping in view onions has a great economic importance, its export value losses caused by the Fusarium wilt, different management practices like botanical, and biological were carried out under *in-vitro* conditions against the *Fusarium oxysporum* to find out the most effective eco-friendly cost effective and easily available control measure to manage the

Fusarium wilt disease of onion Behrani (2015) [10].

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 biocontrol agents *Trichoderma* sp were positive as substitute to chemical control of the onion basal rot and to better growth and yield of onion and inhibtted the calony growth of *Fusarium oxysporum* (Malathi, 2015) [34].

Conclusions

The present studies were conducted to report the disease incidence pathogenicity and biological control 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 in sindh region as compare to GB. 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 *Trichoderma verdii*.

Suggestions and Recomendation

Keeping in view the results of present research work, it is suggested *Trichoderma verdii* should be recommended against this disease. Fungicides are hazardous for human health and there is problem of resistance against fungicides, so alternative control is needed, for this purpose *Trichoderma verdii* could be used. These studies also showed that the disease can also be control through botanical extracts and Bilological control like *Trichoderma verdii*.

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References

1. Abdelrahman Abdel-Motaal, El-Sayed Jogaiah, Shigyo Tran. Dissection of *Trichoderma longibrachiatum*-induced defense in onion (*Allium cepa* L.) against *Fusarium oxysporum* f. sp. *cepa* by target metabolite profiling. 2016; 246(128):38.
2. Adongo CK Kwoseh, Moses. Storage rot fungi and seed-borne pathogens of onion, Journal of Science and Technology (Ghana), 2015, 35(2).
3. Agrios GN. Plant Pathology, 3rd. ed. Academic press, In: New York, 1988, 803pp.
4. Agrios George N. Plant pathology. 5th edi. Amsterdam: Elsevier Academic, 522. From Wikipedia, the free encyclopedia, 2005.
5. Al Adawi AO, Deadman ML, Al Rawahi AK, Khan AJ, Al Maqbali YM. *Diplodia theobromae* associated with sudden decline of mango in the sultanate of Oman. Plant Pathology. 2003; 52:419-419.
6. Anum Fatima, Saleem Abid, Sobia Naheed. Trends in wholesale prices of onion and potato in major markets of pakistan: a time series analysis, Pakistan J Agric. Res. 2015; 2(8):2.
7. Anupama Sonawane, Manali Mahajan, Sonali Renake. Antifungal Activity of a Fungal Isolates against Pomegranate Wilt Pathogen Fusarium. Int. J Curr. Microbiol. App. Sci. 2015; 2:48-57.
8. Bahareh Ghanbarzadeh, Naser Safaie, Ebrahim Mohammadi, Younes Rezaee Danesh, Fatemeh Khelghatibana. Biological control of *Fusarium* basal rot of onion using *Trichoderma harzianum* and Glomus mosseae, J Crop Prot. 2016; 5(3):359-368.
9. Bayaa B, Erskine W, Khoury L. Survey of wilt damage on lentils in northwest, Arab J pl. prot. 1986; l:l19-11g.
10. Behrani RN, Syed MA, Abro MM Jiskani, Khanzada. 2015. Pathogenicity and chemical control of basal rot of onion caused by F*usarium oxysporum* f. sp. *Cepa*, Pak. J. Agri., Agril. Engg. Vet. Sci. MA. 1986; 31(1):60-70.
11. Booth C. The genus *Fusarium*. Common wealth mycological institute, kew, survey, England, 1971, 237. pp.
12. Brayford D. IMI descriptions of fungi and bacteria set 127., Mycopathologia, 1996, 133(1).
13. Dar GH, Beig MA, Ahanger FA, Ganai NA, Ahanger MA. Management of wilting caused by *Rhizoctonia solani* and *Fusarium oxysporum* in Blue pine (*Pinus wallichiana*) through use of fungal antagonists. Asian. J Plant Pathol. 2011; 5(2):62-67.
14. Eduvigis Rolda, Concepcio´n Sa´nchez-Moreno. Characterisation of onion (*Allium cepa* L.) by-products as food ingredients with antioxidant and antibrowning properties, Food Chemistry. 2008; 108(7):916.
15. FAO. Agricultural Statistics of Pakistan, Ministry of Food, Agriculture and Livestock, Government of Pakistan, Islamabad, 2013, pg 122-40.
16. FAO. Food and Agriculture Organization of the United Nations Pakistan, 2014.
17. Forsyth LM, Smith LJ, Aitken EAB. Identification and characterization of nonpathogenic *Fusarium oxysporum* capable of increasing and decreasing Fusarium wilt severity. Mycological Research. 2006; 30:1-7.
18. Frank M Dugan. The identification of fungi, APS PRESS, 2005, (4):109.
19. Fravel C Olivain, Alabouvette C. *Fusarium oxysporum* and Its Biocontrol Special Issue: Soil Microbes and Plant Production. 2003; 157(3):493-502.
20. Gordon. The evolutionary biology of *Fusarium oxysporum* Annual Review of Phytopathology. 1997; 111(35):128.
21. Griffiths G, Trueman L, Crowther T Thomas. Onions: a global benefit to health Phytotherapy Research. 2002; 17(7):603-615.
22. Grooten Slot BV, Westelijke Randweg. Major pests and diseases in onion, (booklet). 2012; 42(10):06.
23. Gupta RK, Bansal RK. Comparative efficacy of plant leaf extracts and fungicides against *F. oxysporum* Schlecht inducing fenugreek wilt under pot house conditions.

Annals of Biology. 2003; 19:35-37.
24. Haapalainena Latvalab, Kuivainena Qiua, Segerstedtb Hannukkala. *Fusarium oxysporum*, *F. proliferatum and F. redolens* associated with basal rot of onion in Finland, Plant Pathology10.1111/ppa.12521, 2016.
25. Harun Bayraktar, Fatma Sara Dolar. Molecular identification and genetic diversity of Fusarium species associated with onion fields in Turkey. 2010; 10(11):1439-0434.
26. Hedges, Lister. The nutritional attributes of *Allium* species Crop & Food Research Confidential Report No. 2007; 18(14):7.
27. Ilhe Musmade, kawade SB. Management of basal bulb rot of onion (*Allium cepa* L.) International journal of plant protection. 2013; 6(2):349-352.
28. Katan Rotem, Finkel Daniel. Solar heating of the soil for the control of pink root and other soil borne diseases in onions, J Agr. Sci. Te. 1980; 8(1):39-50.
29. Khanzada M, Lodhi AB, saleem Shehzad. Phtyopathogenecity of lasidiodiplodia threobromae and *Fusarium oxysporum* on mangoes. Pak. J Bot. 2004; 36(1):181-189.
30. Kuldau GA, Yates IE. Evidence for Fusarium endophytes in cultivated and wild plants, In Bacon CW, White JF, Jr (ed), Microbial endophytes. Marcel Dekker, New York, 2000, 85-117.
31. Lager Sara. Survey of *Fusarium* species on yellow onion (*Allium cepa*) on Öland Department of Forest Mycology and Plant Pathology, Uppsala Swedish University of Agricultural Science, Jordbruksverket, 2011, 06-10.
32. Larkin RP, Hopkins DL, Martin FN. Suppression of Fusarium Wilt of water melon by *Fusarium oxysporum* and other microorganisms recovered from a disease-suppressive soil. Phytopathology, 1996, p.812-819.
33. Maha Laksha, Chandrika. Pathogenic variation and molecular characterization of Fusarium species isolated from wilted Welsh onion in Japan, Journal of General Plant Pathology, Volume. 2009; 75(1):37-45.
34. Malathi. Biological control of onion basal rot caused by*Fusarium oxysporum* f. sp. *cepae*, AJBS/10.1/21-26, 2015.
35. Matice srpske za prirodne nauke. Faculty of Agriculture, Novi Sad, Serbia 2 Maize Research Institute “Zemun Polje", Beograd—Zemun, Serbia Nauåni institut za ratarstvo i povrtarstvo, Novi Sad, Serbia. 2008; 114:135-148.
36. Montomery Jannella. Reducing cotton disease risk. Cotton Tales Gwypir valley 20-07-2009.
37. Nelson PE, Tousson TA, Marasas WFO. "*Fusarium* species”. An Illustrated manual for identification.' The Penn. State University Press, University Park, USA, 1983, p. 123.
38. Pathak VN. Laboratory manual of Plant pathology. 2nd edition. Oxford IBH Publication Company. New Dehli. Page number, 1987, 23-25.
39. Pestizid Aktions. Fusarium wilt." Pan Germany. - Netzwerk. Web. 23 and wikipedia.org. Plant disease Reporter. 2010; 6:1080-1084.
40. Ploetz RC. Malformation: a unique and important disease of mango, *Mangifera indica* L. In B. A. Summerell, J. F. Leslie, D. Backhouse, W. L. Bryden and L. W. Burgess (eds), *Fusarium: Paul E. Nelson Memorial Symposium* (St Paul: APS Press), p. 233 247, Malformation, Indian Society of Mycology and Plant Pathology News. 2001; 9(3):2.
41. Ramos LJ, Davenport TL, McMillan RT, Lara SP. The resistance of mango (*Mangifera indica*) cultivars to tip dieback disease in Florida. Plant. Disease. 1997; 81:509-514.
42. Rizk MA. Phytotoxic effect of *Calotropi sprocera* extract on seedling development and rhizosphere mycoflora of tomato plants grown in soil infested with *Fusarium oxysporum f. sp. Lycopersici*. World Applied Sciences Journal. 2008; 3(3):391-397.
43. Rukhsana B. Antifungal activity of allelopathic plant extracts VI: *in-vitro* control of fungal pathogen by aqueous leaf extracts of Eucalyptus. Mycopath. 2005; 3(1-2):7-12.
44. Sampath Kumar, Debjit Bhowmik, Chiranjib Biswajit, Pankaj Tiwari. *Allium cepa*, A traditional medicinal herb and its health benefits, J Chem. Pharm. Res. 2010; 2(1):283-291.
45. Sharifi Tehrani, Ramezani. Biological control of *Fusarium oxysporum*, the causal agent of onion wilt by antagonistic bacteria. Commun Agric Appl Biol Sci. 2003; 68(4):543-7.
46. Sharma I. A note on population dynamics and etiology of die back of mango in Himachal Pradesh. New Agriculturist. 1993; 2(2):229-230.
47. Steel RGO, Torrie JH, Dickey D. Principles and procedures of Statistics. Abiometrical approach, 3rd edition. Mc Graw - Hill, New York, USA, 1997, 336-352.
48. Steinkellner S, Mammerler R, Vierheilig H. Microconidia germination of the tomato, 2005, 65-63.
49. Suleria HA, Butt MS, Anjum FM, Saeed F, Khalid N. Onion nature protection against physiological threats. Crit Rev Food Sci Nutr. 2015; 55(1):50-66.
50. Sultana N, Abdul G. Effect of fungicides, microbial antagonists and oil cakes in the control of *Fusarium oxysporum*, the cause of seed rot and root infection of bottle gourd and cucumber. Pak. J Bot. 2013; 45(6):2149-2156.
51. Taskeen-Un- Nisa, Wani A, Mohd Yaqub Bhat. In vitro inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. Journal of Biopesticides. 2011; 4(1):53-56.
52. Taylor Vágány, Jackson Harrison, Rainoni A Clarkson. Identification of pathogenicity-related genes in *Fusarium oxysporum* f. sp. *cepae*. Mol Plant Pathol. 2015; 26(18):1111.
53. Wrather, Phipps. Fungi associated with post emergence cotton seedling disease in Missouri. Plant Health Progress DOI-1094/PHP-2002-0772-01-RS yellow in Gladiolus species. J. Fruit and ornamental in plant research. 2002; 18(2):361-380.