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Research Article

Inoculation Techniques of *Fusarium Oxysporum* on Onion

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Abstract

The onion crop reported to be suffered from several fungal diseases in nurseries, field or in storage. Among these, basal rot caused by *Fusarium oxysporum* is a wide spread disease observed in nurseries as well as in field, which causes losses at all the stages of crop growth right from germination to till harvest and also in storage. Information on inoculation technique of *Fusarium oxysporum* on onion is not available; therefore the present study was carried out. Different inoculation techniques viz., seed, soil and seedling inoculation were used to produce basal rot of onion caused by *Fusarium oxysporum*. Studies revealed that maximum per cent disease incidence was observed in seedlings inoculation techniques (72.0% and 68.0% disease incidnece respectively, in seedling inoculated by dipping them in culture and seedling transplanted in inoculated soil) followed by soil inoculation technique (66.1%) and seed inoculation technique (56.7%).

Keywords: Inoculation techniques, seedling, disease, *Fusarium oxysporum*

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Introduction

Onion (*Allium cepa* L.) is a unique vegetable among *Alliums* grown in India, which is consumed by almost all the sections of society throughout the year, not only at maturity, but also at different stages of growth. It is an underground bulbous vegetable grown on commercial scale due to its wide adaptability and comparatively high production potentiality. Onion bulb and greens both are rich in minerals, proteins and ascorbic acid. The pungency in onion is due to sulphur bearing compound in the volatile oil allyl propyl disulphide. The red colour of the outer skin of onion bulbs is due to protocatechoic and catechoic a phenolic factor present in red onions which has antifungal properties also [1, 2]. Onion is used throughout the year in the form of salad or condiment or for cooking with other vegetables [3]. Onion is also used for making pickles in vinegar or brine. It's use in soups and sauce is very common. It has several medicinal uses, it's is very useful to ward-off sunstroke in summer [4].

Among biotic factors, diseases are the major cause of loss to onion crop throughout the world. Disease not only cause serious damage to onion in field, but onion bulbs do not keep well in storage leading to post harvest losses as well. The onion crop reported to be suffered from several fungal diseases in nurseries, field or in storage. Among these, basal rot caused by *Fusarium oxysporum* is a wide spread disease observed in nurseries as well as in field, which causes losses at all the stages of crop growth right from germination to till harvest and also in storage. Information on inoculation technique of *Fusarium oxysporum* on onion is not available therefore the present study was carried out.

Materials and Methods

The present studies were carried out in the laboratory, Department of Plant Pathology, S.K.N. College of Agriculture, Jobner, Jaipur during 2010-11. The fungus (*Fusarium oxysporum*) isolated from diseased roots adjoining to bulbs of onion purified by single spore technique, was tested for its pathogenicity. Pathogenicity of *Fusarium oxysporum* was tested by following seed, soil and seedling inoculation techniques.

Seed inoculation technique

One hundred apparently healthy onion seeds were surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) solution for 1-2 minutes followed by three to four subsequent washing in sterilized distilled water. These seeds were rolled on 7 days old culture of *Fusarium oxysporum.* Inoculated seeds were sown in 30 cm earthen pots filled with

pre- sterilized soil. Twenty seeds per pot were sown with five replications. Uninoculated surface sterilized onion seeds sown in pots filled with sterilized soil served as the check. Both inoculated and check pots were then kept in cage house for observations and were irrigated as and when required. Observations on germination, number of seedling showing symptom and per cent disease incidence were recorded after 30 days of sowing. Reisolations were made from the infected onion bulbs and the culture so obtained was compared with original one.

Soil inoculation technique

Soil inoculation technique was employed under pot-house conditions. Inoculum was multiplied on pre- soaked (Over-night) sterilized sorghum grains in flasks by inoculating with 7 days old culture of *Fusarium oxysporum* and incubated at 25 $\pm$ 1ºc for 7 days. This inoculum was applied in above 4 cm layer of sterilized soil by layering method. Inoculated pots were left for 5 days and irrigation was given on every alternate days. Sowing of one hundred onion seeds were sown in these inoculated pots. Twenty seeds per pot were sown with five replications. Uninculated pots were maintained as check. Observations on germination, number of seedling showing symptoms and per cent disease incidence were recorded after 30 days of sowing. The fungus was reisolated from the artificially inoculated plants and resulting cultures were compared with the original ones [5].

Seedling inoculation technique

Seedling inoculated by dipping

Seedlings were inoculated by seedling dip technique under pot – house conditions. One month old one hundred apparently healthy seedling raised from local susceptible genotype were inoculated by dipping in 7 days old culture of *Fusarium oxysporum* for 30 minutes. Inoculated seedlings were transplanted in 30 cm earthen pots filled with pre-sterilized soil. 10 seedlings/pot × 2 were transplanted with five replications. Uninoculated seedling served as check. Observations on number of seedlings showing symptoms and per cent disease incidence were recorded after 30 days of sowing. The fungus was reisolated from the artificially inoculated plants and resulting cultures were compared with original ones [6].

Seedling sown in inoculated soil

Soil inoculation technique was employed under pot-house conditions. Inoculum was multiplied on pre- soaked (overnight) sterilized sorghum grains in flasks by inoculating with 7 days old culture of *Fusarium oxysporum* and incubated at 25 $\pm$ 1ºC for 7 days. This inoculum was applied in above 4 cm layer of sterilized soil by layering method. Inoculated pots were left for 5 days and then moistend as and when required. On 6th day one hundred one month old seedling raised in surface sterilized from seeds of local susceptible genotype in pots containing sterilized soil were transplanted in inoculated pots @ 10 seedling / pot × 2 with five replications. Uninoculated pots were maintained as check. Observations on number of seedlings showed symptoms and per cent disease incidence were recorded after 30 days of transplanting. The fungus was reisolated from the artificially inoculated plants and resulting cultures were compared with the original ones [6].

Observations were recorded on seed germination, no. of seedling showed symptoms and per cent disease incidence after 30 days of showing.

Results and Discussion

Results indicated that inoculation of seed, soil and seedling with *Fusarium oxysporum* caused reduction in seed germination and increase in disease incidence of basal rot. Minimum seed germination (56.0 %) and maximum disease incidence (66.7 %) was recorded when seeds were sown in soil inoculated with *Fusarium oxysporum*. Among the seedling inoculation test maximum infected seedlings (72.0 %) were recorded when the seedling were dipped in culture of *Fusarium oxysporum* of for 30 minutes, before transplanting. Thus, it was evident that *Fusarium oxysporum* was a strong pathogen of onion when inoculated by seed, soil and seedling method (**Table 1, Figure 1)**.

Further, it was observed that after 30 days of inoculation leaves of the infected plants turned yellow and get stunted & wilted. The roots of the affected plants turns pinkish to brown colour and bulbs become soft and semi watering decay developed from the base of the scale leaves [7-9]. No symptoms were observed in uninoculated pots. Reisoled culture of the fungus identicaly same to the original culture. The reisolated culture was again found to produce the disease on the artificial inoculation. The pathogenicity test results were conformity to Gupta and

Srivastava [10] and Christopher [6]. They observed that all the inoculated plant showed typical symptoms of the disease after 25-30 days in seed, soil and seedlings inoculation method.

Table 1 Effect of inoculation techniques on the per cent disease incidence (PDI) of basal rot (BR) of onion under pot conditions

S.No.	Treatment	Germination (%)	No. of seedling tested	No. of seedling showing symptoms of BR	PDI
1.	**Seed inoculation**				
(i)	Seed inoculated	60	-	34	56.7
(ii)	Seed uninoculated (check)	76	-	00	0.0
2.	**Soil inoculation**				
(i)	Soil inoculated	56	-	37	66.1
(ii)	Soil uninoculated (check)	74	-	00	0.0
3.	**Seedling inoculation**				
(i)	Seedling inoculated by dipping	-	100	72	72.0
(ii)	Seedling transplated in inoculated soil	-	100	68	68.0
(iii)	Seedling uninoculated (check)	-	100	00	0.0

Average based on 100 seeds/ or 100 seedling used

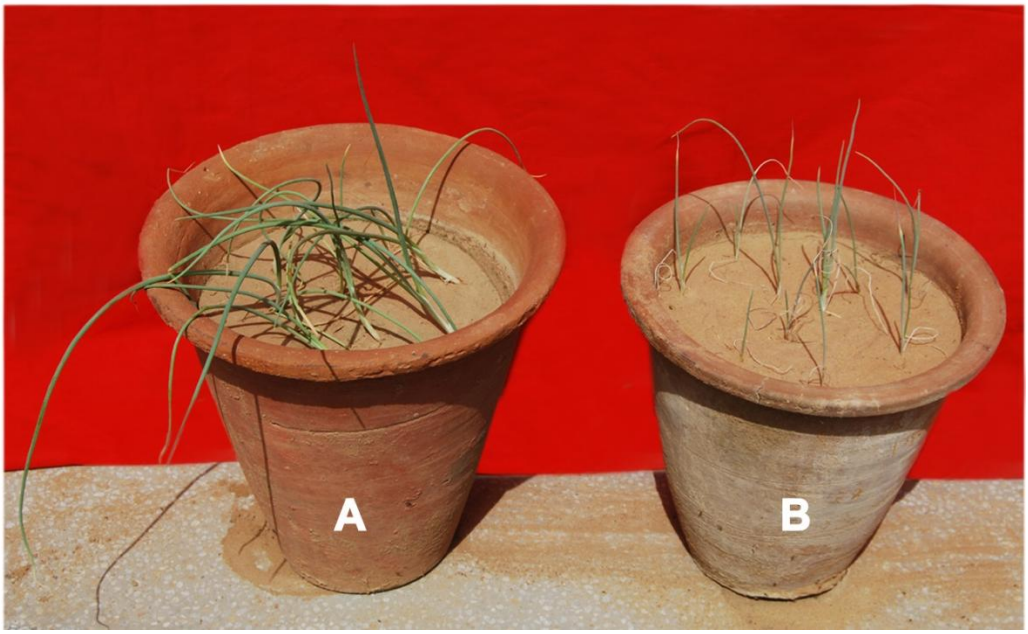

Figure 1 Seedlings of onion, uninoculted (A) and dip inoculated (B)

Conclusion

In Inoculation techniques maximum per cent disease incidence was observed in seedlings inoculation techniques (72.00% and 68.00% disease incidnece in seedling inoculated by dipping and seedling sown in inoculated soil, respectively) followed by soil inoculation technique (66.07%) and seed inoculation technique (56.66%) disease incidence.

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