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Evaluation of different inoculation techniques on Pathogenecity of *Sclerotium rolfsii*

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Abstract
Collar rot is one of the most destructive diseases of lentil. Yield losses can be attributed to fungal growth at collar region near soil line which leads to smaller and fewer seeds, premature ripening, shattered pods. The stem rot fungus overwinters as sclerotia in the soil, stubble or mixed with seed. Sclerotia can remain viable for five years or more when conditions are suitable sclerotia will germinate myceliogenically and directly infect plants near soil line. The present investigations were carried out the efficacy of various inoculation techniques of Sclerotium fungus in lentils. Maximum disease severity 44.5% were recorded in wheat inoculums method followed by agar disc method (40%) and the minimum disease severity 12% was observed in toothpick method.

Keywords: Sclerotia, Inoculation techniques and Sclerotium

Introduction
Pulses are major sources of proteins among the vegetarians in India having significant nutritional and health benefits and also known to reduce several non-communicable diseases such as colon cancer and cardiovascular diseases (Yude *et al*, 1993; Jukanti *et al*, 2012) [4, 5]. Pulses can be grown on range of soil and climatic conditions and play important role in crop rotation, mixed and inter-cropping, maintaining soil fertility through nitrogen fixation, release of soil-bound phosphorus. India is the largest producer and consumer of pulses in the world. Chemically Lentil seed is a good source of protein, Carbohydrates, Fiber, Protein, Calcium, Iron, Potassium and Vitamins (Canadian Diabetes Association 2010). Lentil is affected by a wide range of fungal diseases among them collar rot of lentil is an important seedling disease which is very destructive particularly under high moisture and high temperature conditions. It is caused by the fungal pathogen *Sclerotium rolfsii*. The pathogen has an extremely wide host range at least 500 species in 100 families are susceptible. The most common hosts are the legumes, crucifers, and cucurbits. *S. rolfsii* produces sclerotia, which can survive in the soil for many years. Infected young seedlings show damping-off symptoms. Plants infected at an advanced stage gradually turn pale, droop and dry. Although its symptoms are similar to Sclerotinia white mold, it can be differentiated by its cord-like mycelial strand and smaller sized sclerotia. It occurs under warm weather, whereas Sclerotinia disease occurs under cool weather conditions. Collar rot of lentil caused by *Sclerotium rolfsii* Sacc. is a major disease of lentil in all the lentil growing areas of the country and causes up to 50% loss at farmer's field. The disease is also widely prevalent in Utter Pradesh and causing 25 to 35% loss (Khare *et al*., 1979) [3]. Prevailing environmental conditions and amount of inoculum affect the disease development.

Methods and Materials
Experiment was carried out in Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology Meerut, U.P. Inoculum for the agar disk technique was prepared using sclerotia produced on plates of PDA. Sclerotia of *Sclerotium rolfsii* were dipped in 95% ethanol for 1 minute, placed on PDA plates 2 cm apart, and allowed to germinate for 48 hr. A one cm diameter agar disk with the sclerotium in the center was cut with a sterile cork borer and appressed to the base of the central stem of designated plants (Shokes *et al*., 1996) [1].
Wheat inoculum was prepared by sterilization of water soaked wheat seed with on each of two successive days. The seed then were distributed into sterile conical flask under a laminar air

flow chamber and inoculated with three day old cultures of *S. rolfsii* grown on PDA. Mycelia were allowed to grow for Three weeks to thoroughly infested seed and then 40 g of wheat inoculums were placed in each pot near the central stems (Besler *et al*., 1997) [2]. Toothpick inoculum was produced by boiling wooden toothpicks in water twice to remove tannins. They were then impregnated with Potato Dextrose Broth by autoclaving together in beakers. The fungus was grown on the substrate for 1 week before use. Toothpicks were inserted into central stems about 1 cm above the soil surface in toothpick stem technique or placed upright in soil, 1 cm deep and 1 cm from the central stems in toothpick soil technique. Non infested toothpicks were inserted into stems as an untreated check for comparison against toothpick stem technique, since this technique is a form of wound inoculation. Appropriate without inoculation also were used in each experiment (Shokes *et al*., 1996) [1].

Experimental results

Three inoculation techniques consistently resulted in significantly high disease severity. Final disease severity was very similar to the agar disk and wheat grain inoculum techniques in pot culture tests. Tooth Pick method caused slightly lower disease severities than the agar disk and wheat inoculum techniques. Lower and more variable levels of disease resulted with the toothpick methods. Rating for the agar disk, wheat inoculum, and toothpick techniques varied from test to test but indicated a similar increase in disease over time. Wheat inoculum and agar disk techniques were significantly greater in comparison to the toothpick techniques. No collar rot was noted on the check pots. The maximum disease severity 44.5% were recorded in wheat inoculums method followed by agar disc method (40%) and the minimum disease severity 12% was observed in toothpick method and no disease was observed in control pots.

Table 1: Evaluation of different inoculation techniques on per cent infection and disease severity

S.N.	Inoculation techniques	No. of Seed Sown	No. of seed Germinated	No. of plant Inoculated	No. of plant Infected	Percent of Infection	Disease Severity
1.	Wheat inoculum method	50	50	50	40	80	44.5
2.	Agar Disc Method	50	50	50	25	50	40
3.	Tooth Pick method	50	50	50	28	56	12
4.	Control	50	50	00	00	00	0

Discussion

Pathogenecity test were carried out on healthy Lentil plants and seedlings grown in pathogen- infected soil to examine the pathogenic behaviour of the isolated pathogen. In pathogenecity tests it produced typical symptoms of collar rot on lentil plants and thus satisfied the Koch's postulates.

Three inoculation techniques *viz*. Agar Disc, Wheat grain inoculum and Tooth Pick method were applied for artificial inoculation of *S. rolfsii* Sacc. in lentil plants. Wheat grain inoculum technique was found to cause more disease severity. It was very similar to the agar disk technique in pot culture tests. Tooth Pick method caused slightly lower disease severities than the agar disk and wheat inoculum techniques. Lower and more variable levels of disease resulted with the toothpick methods. The maximum disease severity 44.5% was recorded in wheat grain inoculum method followed by agar disc method (40%) and the minimum disease severity 12% was observed in toothpick method. The more or less similar results were reported by Shokes *et al.* (1996) [1] they observed that wheat grain inoculum in groundnut crop has greater inoculum potential then agar disks, and it is rapid and reliable.

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