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In vitro evaluation of bio control agents against black spot of rose caused by *Diplocarpon rosea*

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Abstract

The *in vitro* screening of *Trichoderma* species was to evaluate the inhibition and control the disease of black spot of rose caused by the pathogen *Diplocarpon rosea*. Which was a severe problem in rose gardens. Treatment with these biocontrol agents against the pathogen in dual culture method in *in vitro* conditions. We have taken three antagonistic fungi of *Trichoderma* species in which amongst the *Trichoderma viride* is effective in controlling the pathogen against *D.rosea* in aseptic conditions.

Keywords: Rose, *Trichoderma* species, control.

Introduction

Rose is preferred as the most beautiful flower in the world amongst the nature's creation. In India it has the high potential to export the cut flowers. Rose is considered as "QUEEN OF FLOWERS". It shows the symbol of love, adoration and innocence it is also used in the decorative, worship, bouquets, loose flowers. From the rose oil can also be extracted and it can be utilised in preparation of cosmetic industries and for fragrance's, perfumes. Rose is popular crop for commercial and domestic purposes. In order to encourage the floriculture sector the equal attention to be given to the production/productivity availability and quality of flowers.

Black spot of rose is a fungal disease and the most severe effected disease in rose. It is caused by the pathogen *Diplocarpon rosea* (F. A. WOLF 1912) [17]. The fungal disease appears on the upper surface of the leaves. The appearance of lesions are basically dark brown to purple colour with the circular spots on the leaf of which followed by yellowing and defoliation of leaves. The fungal pathogen occurs in two stages i.e. during perfect stage & it is commonly known as *Diplocarpon rosea* (wolf 1912) [17] and during the imperfect stage *Marssonina rosea* (Lib. Lund (Baker, 1948) and other synonyms included during imperfect stage was *Actinonema rosea*, *Marssonina rosea*, *Asteroma rosea*. (Baker, 1948; Horst, 1983) [7] Some other 25 different names. The fungus during winters dormant stage as seen in in thorns, buds & fallen leaves (Lyle 1943, cook 1981) [10, 3]. Among this the conidia are formed during the spread of natural occurring's of wind, rain splashing and certainly through insect vectors and arachnids (palmer *et al.* p1948) [12]. The apothecia containing 8 ascospores was very and the genetic variation generated by sexual recombination was not clear (Walker, 1995) [16]. The formation of apothecia is recorded in various countries as Great Britain (Knight & Wheeler, 1977, Cook 1981) [9, 3]. This disease was first described in European countries and some other countries during ancient times Africa, China, Australia, North America. Some of ocean occurring Island's, Philippines & Hawaii (Horst, 1983, Drewes-Alvarer 2003) [7].

Collection of diseased samples

The diseased leaves of rose plant with the symptoms of black spot were collected from A.P. & dehradun in some localities of the area. The isolation of the sample technique (Ricker & Ricker. 1936) [13]. The diseased leaf part were cut into small pieces (0.5) the infected cut part of leaves were immersed in 70% ethyl alcohol solution for 1/2 minutes & rinsed thrice in distilled water. Potato Dextrose Agar (PDA) was prepared, autoclaved and poured in sterilised petri plates and incubated at 25°C,

after 5-10 days the mycelia of fungus pathogen appears & subculture by transferring 5mm mycelial disc by sterilised cork borer & incubated at 25 ± 2 °c for 5-10 days to get the pure culture.

In vitro screening of *Trichoderma* species

The three antagonistic fungi of *Trichoderma* species which includes i.e. *Trichoderma asperillum*, *Trichoderma viride*, *Trichoderma harzianum* were tested against the *Diplocarpon rosea* under invitro conditions through the dual culture technique. The data % growth inhibition by different antagonists of *Trichoderma* species for this experiment PDA plates were used among this each petri dish is divided into two halves, the forest was inoculated with disc (0.6 cm in diameter) of tested antagonist fungus on the second half was inoculated with the similar disc of the pathogenic fungus. The plates without the *Trichoderma* species and with fungus are acted as control plate each treatment was taken in three replications. And all the petriplates/dishes were incubated at 25 ± 2 °c in the BOD incubator & and observed the radial growth in the plate. After 7days of incubation the pathogenic fungi almost covered the surface of the medium in control treatment. The result were expressed as percent inhibition of growth over control. The percentage of inhibition (I%) was collected according to Vincent (1927).

Table 1: The plates without the *Trichoderma* species and with fungus are acted as control plate each treatment was taken in three replications.

<i>Trichoderma</i> species	% of Inhibition Day 7 th (168 hrs)
<i>Trichoderma harzianum</i>	42.38
<i>Tichoderma viride</i>	68.09
<i>Trichoderma asperillum</i>	59.07
C.D.	2.913
SE(m)	0.826

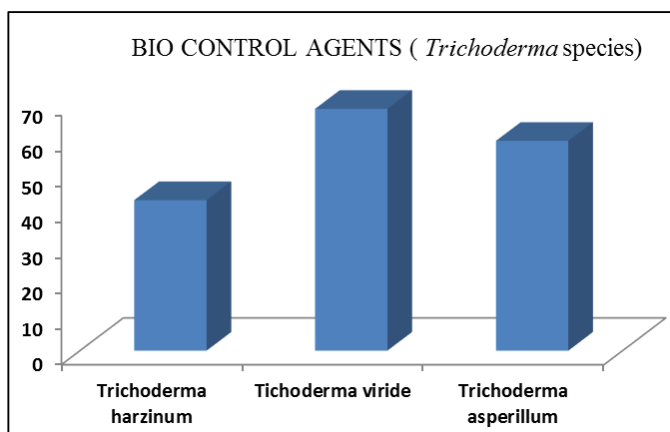


Fig 1: In vitro evaluation of *Trichoderma* species against *Diplocarpon rosea*.

Result and Discussion

Three antagonist fungi of *Trichoderma* species were tested against the *Diplocarpon rosea* under in vitro conditions through dual culture technique the inhibition growth percent by different antagonist of *Trichoderma* species the experimental study revealed that inhibition responses of different antagonist of *Trichoderma* species were found more inhibitory against the test pathogen irrespective of different *Trichoderma* species were screened against growth of *Diplocarpon rosea*. Chandra Sekhar J, et al, 2020 [2] who also used same biocontrol agents of *Trichoderma* species against

the pathogen and results were observed. *Trichoderma viride* (68.09%) is the most effective in providing growth inhibition %. M. Karthikeyan. et al. 2007 [11] also stated that *T. viride* is effective against *D. rosea*. Followed by *Trichoderma asperillum* (59.07%) and it was recorded minimum in *Trichoderma harzianum* (42.38%) invitro.



Fig 2: In vitro evaluation of *Trichoderma* species against *Diplocarpon rosea*.



Fig 3: In vitro evaluation of *Trichoderma* species against *Diplocarpon rosea*.

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