Cosmos Multidisciplinary Research E-Journal
Online Available at www.cmrj.in
Recognized International Peer Reviewed Journal
Impact Factor 4.94
ISSN No. 2456-1665

Antagonistic activity of *Trichoderma* Spp. against Neck rot disease of Galrlic (*Allium sativum* L.)

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Abstract:

Garlic (*Allium sativum* L.) is a most important vegetable, spice and medicinal crop produced on large scale throughout the world. In India garlic is most important commercial crop cultivated in various regions. There are number of fungal pathogens that attack on garlic crop throughout their development. The several fungi have been identified to be responsible for post-harvest decay of garlic bulbs during storage condition. These pathogens are responsible for to enormous loss of these bulbs not only in terms of quantity but also reduce its economic and nutritive value. The post-harvest diseases causes black mold, Soft rot, Purple rot, Brown rot, Neck rot, Basal rot, Internal rot and blue mold of garlic . The *Botrytis porri* is most important fungal pathogen which causes Neck Rot of garlic. In the present study tested *in vitro* antagonastic activity three species of *Trichoderma* i.e., *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma koningii* was tested against *Botrytis porri.* Out of these three *Trichoderma* species *Trichoderma viride* shows more effective inhibitory effect on *Botrytis porri* than *Trichoderma harzianum* and *Trichoderma koningii*.

Key words: Garlic, *Botrytis porri*, *Trichoderma sp.,* Neck rot.

Introduction:

Garlic (*Allium sativum* L.) Is an herbaceous plant belonging to family Alliaceae grown for its edible bulbs and leaves. It is a most important vegetable, spice and medicinal crop produced on large scale throughout the world. It is used as a flavoring agent, food and medicine. Garlic is the second most widely cultivated vegetable next to the onion. Garlic is valued for its flavor and has commercial importance because of its wide medicinal value and application in food and pharmaceutical preparations (Sharma and Prasad 2001). Garlic (*Allium sativum* L.) is among the oldest of all cultivated plants. In traditional the Ayurveda, Islamic medicine, folklore medicine and Chinese medicine system, several herbs and spices including garlic are described to possess medicinal properties e.g. antihypertensive, anti-thrombotic and hypolipidemic (Makheja 1990 and Moyers 1996). It has been used as a food, spice and folklore medicine for over 4000 years, and is the most widely researched medicinal plant (Milner 1996).

Garlic is most important vegetable and spice crop all over the world. And it attacks by several pathogens. Many soil borne fungi play a major role in causing several diseases such as bulb rot, root wilt, damping-off, root rot, collar rot, crown rot etc. Garlic is affected by various diseases caused by different types of microorganisms of which fungi are the most important pathogens. *Botrytis alli, Fusarium oxysporum, Aspergillus flavus, Aspergillus niger, Penicillium corymbiferum, Macrophomina Phaseolina, Rhizopus stolonifer, Chaetomium globosum* are reported on garlic bulbs from storage (Ghangaonkar 2013). The most important fungal pathogens of stored

temperate onion bulbs are *Botrytis alli* (Neck rot), *Aspergillus niger* (Black mold) and *Penicillium* sp., All this fungi occur in the pre harvest environment of onion crop. The post-harvest development of these pathogen is determined by temperature and relative humidity (Hayden et al. 1997). *Botrytis* spp. are necrotrophic pathogens causing pre- and postharvest diseases in many crops, such as bulb, tomato, and grapevine crops (Elad et al., 2004). Garlic bulbs are susceptible to *Botrytis* rots, *Fusarium* and *Penicillium* sp. Particularly if bulbs are damaged. The postharvest pathogens of garlic may be carried in seed cloves (Sumner 1995). The management of this disease is difficult owing to long saprophytic survival ability of pathogen in soil (Dey 2005). Neck rot of garlic, onion and shallot is one of the major bulbs destroying diseases which are caused by *Botrytis porri*, *Botrytis squamosa* and *Botrytis cinerea*. The fungus usually infects mature plants through the neck tissues or through wounds in the bulbs (R. K. Mishra et al., 2014). The biological control is the alternative method of the fungicides, that achieved remarkable success to control the plant pathogens by their rule and with their impact as antagonistic agents (Elad, Y., 2000).

Symptoms

In the field, symptoms are seen as stunted plants with dying or dead outer leaves. The infection develops at soil level in the stem (neck). Initially the affected tissues look water soaked which later become brown, dry and necrotic. Black sclerotia may be seen around the rotting neck. Sclerotia are hardened fungal structures resistant to the environment which allow the fungus to survive the winter. In storage, a gray mold can be seen on the surface of the bulbs or between the scales. Black sclerotia cluster around the neck and between the scales. On individual cloves, there may be extensive watersoaking extending beyond the obviously moldy area (Susan B. *et. al*., 2008).

Reduction or elimination of soil borne inoculums is the only effective solution to overcome the problem and this may be achieved through use of effective fungal antagonists. The present study was carried out to test the antagonistic activity of *Trichoderma spp.* against *Botrytis porri* a neck rot causing pathogen of garlic.

Material and Methods:

Isolation of Pathogen

Infected garlic plant were brought into the laboratory and cut the affected part into small pieces by sterilized blade. Then surface sterilized with 0.1% mercuric chloride for 30 seconds. The pieces washed with sterilized distilled water thrice. The pieces were transferred to solid potato dextrose agar medium (PDA) by sterile forceps. Inoculated plates kept at 27 ± 2^0 C. Pathogens were identified and isolated as *Botrytis porri.* The pathogens maintained on PDA slants for further study.

Dual culture Method

Dual culture method was used to determine the effect of *Trichoderma spp.* On mycelial growth of targeted pathogens. The dual culture of targeted pathogens and *Trichoderma viride, T. harzianum* and *T. koningii* were studied on PDA medium. 20 ml of PDA medium was poured in 9 cm diameter plates and allowed to solidify. 6mm diameter disc of mycelium cut from the margin of 6 days old culture of each *Trichoderma* spp. were placed at the periphery of each plate. Then the disc of 6mm diameter of mycelium cut from the growing edge of 7 days old culture of targeted

pathogens were placed opposite to the mycelial disc of *Trichoderma* spp. on each plates. Monoculture plates of both served as control. Three replicates were maintained for each treatment and incubated at 28± 2^0 C for 7 days. The radial growth of all fungi measured, when *Trichoderma* spp. in control plates show complete growth. The growth observed and compared with control. The growth inhibition calculated by using the following formula given by Vincent 1947.

% Inhibition = 100 x D1- D2 / D1

Where, D1 = Colony diameter in the control
D2 = Colony diameter in treated

Observation

Evaluation of *Trichoderma* spp. against targeted pathogens *in Vitro*

***Trichoderma* species**	**Inhibition %**
	Botrytis porri
Trichoderma harzianum	75 %
Trichoderma viride	80.60 %
Trichoderma koningii	72.40 %

Discussion

In the present study *Trichoderma viride* showed maximum antagonastic activity against *Botrytis porri* (80.60 %) followed by *Trichoderma harzianum* (75 %) and *Trichoderma koningii* (72.40 %). The observation showed that the species of *Trichoderma* can reduce the neck rot pathogens of garlic.

Results of the study showed that *T. viride* has highest inhibition against the causal pathogen which match the results reported by (*M.A.M. Hussein et. al., 2014*) studying *in vitro* antagonism between isolates of antagonistic fungi *T. harzianum* and *T. viride* against the used pathogenic isolate of *Botrytis porri* on potato dextrose agar medium *T. viride* caused inhibition of causal pathogen by different rates 86, 85, 84% this result refer to the inhibition by the different antagonistic isolates that affected on the growth of mycelia and the spores formations of the causal pathogen.

Trichoderma and *Gliocladium* have been observed to produce metabolites as gliotoxin which is antagonistic to *Botrytis cinerea*, causing *in vitro* inhibition for the fungal conidial germination (Elad and Stewart 2004).

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