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Research Article

Biological Control of Collar Rot Disease of Groundnut Caused by *Aspergillus niger*

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ABSTRACT

The collar rot incidence in groundnut is one of the most severe diseases that reduce groundnut yield. The collar rot disease was reduced by applying Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens and plant extracts (Allium sativum, Ocimum sanctum, Tagetes erecta, Azadirachta indica and Datura stramonium). In vitro studies of fungal and bacterial antagonists, viz., Trichoderma spp. and Pseudomonas *fluorescens, indicated that T. harzianum was more effective in inhibiting A. niger. In pot culture experiment, the combined effect of seed treatment with T. harzianum resulted in significant reduction of collar rot. In vitro studies of plant extracts indicated that garlic extract was more effective in inhibiting A. niger. Combination of antagonist, fungicide and plant extracts also improved the growth parameters like length of the plant, biomass and yield, besides decreasing the disease incidence. Biological management of collar rot disease is effective and also eco-friendly.*

Keywords: *Biological control, Aspergillus niger, plant extracts, collar rot.*

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), is an important legume crop of tropical and subtropical areas of the world, described in 1753 by Linnaeus (Pattee & Young, 1982). It is a member of the genus *Arachis* in the sub-tribe *Stylosanthinae* of tribe *Aeschynomeneae* of the family *Fabaceae*. The different names in the world also know groundnut as peanut, earthnut, monkey-nut, goobernut, panda and marillnut. It is an economically important oilseed crop grown primarily for good quality edible oil and easily digestible proteins (Cobb & Johnson, 1973).

This crop is mainly cultivated as an oilseed, but considerable quantities are used directly for human consumption like other pulses. The kernels are widely known as a cheap source of vegetable proteins. It consists of 26 per cent protein, 48 per cent edible oil, 20 per cent carbohydrates and three per cent fibre and is also rich in calcium, thiamine and niacin (Haveri, 2017).

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Rajasthan stands in the second position in terms of area and production. The cultivation of groundnut is well adapted to the conditions prevailing in Rajasthan and is cultivated in about 7.34 lakh hectares with annual production 1.612 million tonnes and productivity of 2195 kg/hectare (Anonymous, 2019-20).

Several abiotic and biotic factors affect groundnut growth and development, leading to qualitative and quantitative yield losses. Diseases that are most damaging and major limiting factors that cause the largest economic losses in profitable cultivation of this crop in Rajasthan. The attack of several diseases primarily caused by fungi takes a heavy toll on the crop at all the stages of growth, from sowing to harvest and storage.

Similar to other crops, groundnut also suffers from various diseases caused by fungi and other microorganisms. The diseases, which affect the foliage, cause copious damage to tissues, interfere with the photosynthetic process and cause severe losses in yield.

Amongst fungal diseases, collar rot of groundnut also known as seedling blight caused by *Aspergillus niger* van. Teighem is one of the important seed and soil borne diseases. Collar rot of groundnut is prominently distributed in countries with tropical and sub-tropical climates where high temperature prevails during the rainy season, and it is present in most all the groundnut growing areas of the world. This disease was first reported by Jochem (1926) from Java. However, in India, Jain and Nema (1952) first recorded the *Aspergillus* blight of groundnut caused by *A. niger*. It is an important disease in the major groundnut growing states.

In Rajasthan, Bakhetia (1983) had reported disease incidence up to 50.00 per cent. Dighule et al. (2018) estimated yield losses in Maharashtra from 28.00 to 50.00 per cent due to collar rot of groundnut caused by *Aspergillus niger* van. Teighem. Most of the groundnut cultivars grown in our country are susceptible to this disease. This disease is prevalent in almost all groundnut-growing states of India *viz*., Punjab, Gujarat, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Maharashtra, Rajasthan, Karnataka and Odisha (Dighule et al., 2018).

MATERIALS AND METHODS

Efficacy of bio-agents against *Aspergillus niger* (*in vitro*)

The efficacy of bio-agents was studied by the dual culture technique (Dennis & Webster, 1971).

Table: 2.1 Bio-agents tested against *Aspergillus niger (in vitro)*

S.No.	Name of bio-agents
1.	*Trichoderma harzianum*
2.	*Trichoderma viride*
3.	*Pseudomonas fluorescens*

All the bio-agents (Table: 2.1) were obtained from the Department of Plant Pathology, RARI, Durgapura, Jaipur. A single colony of the isolate was sub-cultured in PDA and stored in the refrigerator to maintain their genetic purity. 20 ml of PDA medium was poured into a sterile Petri plate and allowed for solidification. 5 mm dia. disc from an actively growing colony of the pathogen was cut with a sterile cork borer and placed near the periphery of PDA plate. Similarly, the bio-agent was placed on the other side, i.e., at an angle of 180^0. Plates with no antagonists served as the control for the pathogen. The plates were incubated at 25 ± 1^0C for 7 days. Each treatment was replicated four times. The extent of antagonistic activity by bio agents, *i.e.*, the mycelial growth of bio-agent after contact with fungal plant pathogens was recorded after the incubation period by measuring mycelial growth of fungal plant

pathogens in the dual culture plate and on the control plate.

Efficacy of plant extracts against *Aspergillus niger (in vitro)*

The experiment was carried out to test the fungi toxicity of five plant extracts (Table 2.2) against the collar rot pathogen. 100 grams leaves/cloves of the plant were collected and washed 2-3 times with water and allowed to dry at room temperature (25 ± 1 ^{0}C). Before extracting, leaves/cloves of each plant (100 g) were crushed separately with 100 ml sterilized water. The extract was filtered through muslin cloth and centrifuged at 5000 rpm for 30 min. the extracts were sterilized.

The extract of each plant was diluted in order to achieve three concentrations viz., 5, 10 and 15 per cent. Petri plates containing PDA supplemented with different plant extracts, each with three concentrations and replicated three times, were inoculated with 7 days old culture (5 mm dia. disc). A suitable control (without plant extract) was also maintained. The fungal colony was measured after 7 days of incubation at 25 ± 1 ˚C.

Table 2.2 Plant extracts tested against *Aspergillus niger* (*in vitro*)

S.No.	Common Name	Botanical name	Plant part used	Concentration (%)
1.	Garlic	*Allium sativum*	Clove	5,10,15
2.	Tulsi	*Ocimum sanctum*	Leaf	5,10,15
3.	Marigold	*Tagetes erecta*	Leaf	5,10,15
4.	Neem	*Azadirecta indica*	Leaf	5,10,15
5.	Datura	*Datura stramonium*	Leaf	5,10,15

RESULTS

3.1 Efficacy of bio-agents against *Aspergillus niger (in vitro)*

The effectiveness of Trichoderma harzianum, Trichoderma viride and Pseudomonas fluorenscens, has been tested against A. niger with dual culture technique. After 7 days of incubation at 25 ± 1 0C inhibition of mycelium growth was recorded.

The results presented in (Table 3.1, Figure 3.1) showed that all bio agents namely, T. harzianum, T. viride and P. fluorescence were opposed to the growth of A niger. The magnitude (81.66%) inhibition of mycelial growth of the pathogen was recorded in T. harzianum followed (66.94%) in T. Viride and inhibition of small mycelial growth were recorded in P. fluorenscens (43.14%).

Table 3.1 Efficacy of bio-agents against the growth of *Aspergillus niger* by dual culture technique *(in vitro)*

Bio-agents	Growth inhibition (%)
Trichoderma harzianum	81.66 (65.00)
Trichoderma viride	66.94 (54.90)
Pseudomanas fluorescens	43.14 (41.06)
Control	0.00 (0.00)
SEm±	2.11
CD (p = 0.05)	6.51

*Average of four replications

Figures in parentheses are angular transformed values

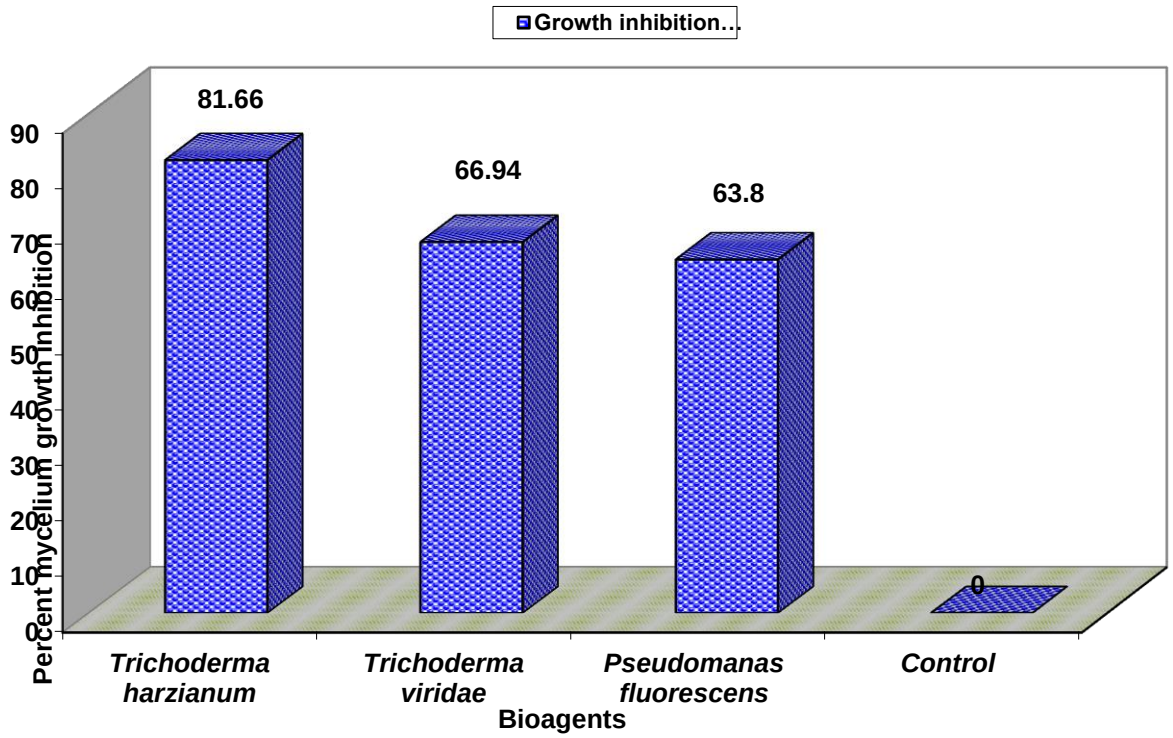

Fig. 3.1 Efficacy of bioagents on mycelial growth of *Aspergillus niger (in vitro)*

3.2 Efficacy of plant extracts against *Aspergillus niger* (*in vitro*)

The performance of five plant extracts, namely, Garlic, Tulsi, Marigold, Neem and Datura was tested in vitro for three concentrations (5, 10 and 15%) against A. niger in PDA in the form of poisonous food. Of the five plants extracted, Garlic clove extract was found to be very effective in inhibiting mycelial growth (77.11, 84.55 and 90.00%) of A. niger at 5, 10 and 15%, respectively, followed by Neem leaves extract (70.36, 76.36 and 80.76% 80.76%) over control. Marigold and Tulsi leaf extracts have been found to be less effective in inhibiting mycelial A-growth. niger over control. The total concentration (5, 10 and 15%) of garlic clove extract was found to be significantly higher than other therapies. Data is presented in (Table- 3.2, Fig.-3.2).

Table 3.2 Efficacy of plant extracts against *Aspergillus niger* by poisoned food technique (*in vitro*)

Plant extracts	Plant part Used	Percent mycelium growth inhibition at various Concentration			Average*
		5%	10%	15%	
Garlic	Clove	77.11 (61.42)	84.55 (66.85)	90.00 (71.57)	83.89
Tulsi	Leaf	61.80 (51.83)	64.90 (53.67)	68.53 (55.88)	65.08
Marigold	Leaf	50.40 (45.23)	52.80 (46.61)	58.53 (49.91)	53.91
Neem	Leaf	70.36 (57.01)	76.36 (60.91)	80.76 (63.98)	75.83
Datura	Leaf	65.48 (54.02)	68.83 (56.06)	71.48 (57.72)	68.60
Control		0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
		SEm+	CD (p = 0.05)		
Plant extract (P)		0.88	2.43		
Concentration (C)		1.24	3.43		
P x C		2.15	5.95		

*Average of three replications

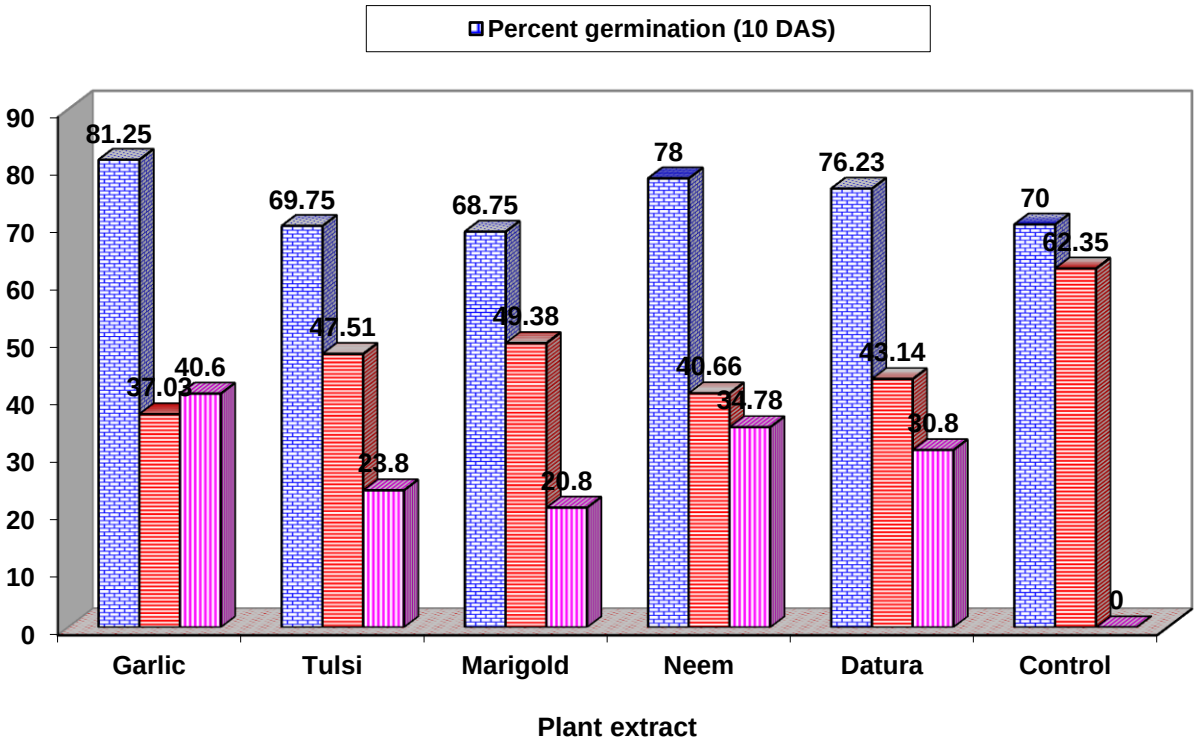

Fig. :3.2 Efficacy of plant extract against collar rot caused by *Aspergillus niger*

DISCUSSION

Bio-control agents have been proven to be particularly effective against soil-borne pathogens. The current investigation *tested T. harzianum, T. viride* and *P. fluorescens* using a dual culture method. The results show that high inhibition of mycelial A growth. niger by *T. harzianum* and (81.66%) followed by T. viride by (66.94). What we have seen is consistent with the results of Gajera et al. (2011), Nathawat and Partap (2014) and Krishna Kishore (2005). They reported that *T. harzianum* is very high in reducing the mycelial growth of A. niger.

The various bio-agents used in the current investigation were tested under pot conditions. *T. harzianum* + P. fluorescens found to be very effective with (52.21%) disease control followed by *T. harzianum* and *T. viride* by (45.73%) and (38.28%), respectively. P. fluoresens found to be malfunctioning. We have seen consistent with Kishore et al. (2007); read about the effects of preventing myco-parasitism of T. harzianum, in the growth of the collar rotting nuts (A. niger) under the field conditions. Sharma et al. (2012) report that seed coatings with *T. harzianum* has been found to be very effective in reducing collar rot in nuts.

Devi and Parsad (2009) reported seed treatment with *T. viride* at 4 g / kg of seeds + incorporation of *T. viride* at 2.5 kg / ha reduced cola rot to nuts. *Trichoderma* species are active mycoparasites and cold producers of secondary metabolites, some of which have clinical significance. Many species produce elicitors and form plant resistance through the root colon (Mukherjee et al., 2013).

Five plants extracted Garlic, Tulsi, Marigold, Neem and Datura were tested at 5, 10 and 15 percent against *A. niger* with inhibition of mycelial growth in vitro. Garlic provided the highest (90.00%) inhibition of mycelial growth @ 15% focus followed by Neem with (80.76%). Similarly, the results have been observed by Avasthi et al. (2010) and Nathawat and Partap (2009) while working with A. niger in vitro. Garlic is the most effective in controlling (40.60%) diseases following Neem, Datura, Tulsi and Marigold. Similarly, the results have been observed by

Mahapatra and Tewari (1994), Yadav et al. (2007) and Patel et al. (2008).

CONCLUSION

To avoid the risk of environmental pollution due to agrochemical misuse and to prevent the growth of pathogenic fungi from commonly used fungicides, the use of bio-control agents in controlling plant diseases has increased in recent years. All countries need to adopt, introduce, and enforce environmental protection legislation. Successful implementation of these proactive, effective preventive methods is a major step toward maintaining a healthy environment, national reconciliation, sustaining ethnic harmony, and optimizing human health. All of the plants as mentioned above used in the current study have been used for seed treatment. All extracellular plants can reduce the incidence of disease out of control significantly.

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Conflict of interest:
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Author Contribution:
All authors contributed equally to establishing the research and design experiment topic.

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