J. Soils and Crops 27 (1) 80-83, June 2017 ISSN 0971-2836

IN VITRO STUDIES ON MANAGEMENT OF COLLAR ROT CAUSED BY *Aspergillus niger* IN GROUNDNUT

R. B. Andge[1] , R. L. Parate[2] , H.R.Sawai[3] and R.R.Kalaskar[4]

ABSTRACT

Groundnut is an important and economical oil seed crop. It is subjected to variety of diseases like collar rot, root rot, leaf spots etc. which are threat in groundnut production. Among these diseases, collar rot caused by *Aspergillus niger* is important and major limiting factor for yield loss. The present investigation was aimed for isolation, identification, pathogenicity test. Experiments were conducted in Department of Plant Pathology Section, College of Agriculture, Nagpur during 2014-2015. Fungicides *viz.*, carbendazim, thiram, carboxin + thiram, carbendazim + thiram and carbendazim + mancozeb and bioagents *viz,, Trichoderma viride* and *Pseudomonas fluorescence* were tested *in vitro* against *Aspergillus niger* by poison food technique. Fungicides carboxin + thiram, carbendazim + thiram, carbendazim, thiram and carbendazim + mancozeb were found 100 per cent effective in controlling the radial mycelial growth of the test pathogen 7th days after inoculation.

(Key words: Groundnut, *Aspergillus niger, in vitro,* fungicides, bioagents)

INTRODUCTION

Groundnut is cultivated in tropical and subtropical countries of the world. The major groundnut producing countries are India, China, Brazil, America, Nigeria and Indonesia. India occupies second place in production contributing around 18.2 per cent of world share. India produced 9.67 mt from 5.53 mh area, with an average yield of 1274 kg ha^{-1} of groundnut. (Anonymous, 2014 a). It occupies first place in order of importance out of the all the oil seed crops growing in India. Maharashtra is one of the groundnut growing state in the country with an area of 0.27 million hectares with the total production of about 0.33 million tonnes during year 2014 (Anonymous, 2014 b). In Vidarbha, the total area under groundnut was about 17.90 thousand hectares with the total production of about 20.90 thousand tones during year 2014 (Anonymous, 2014 b). Among soil borne diseases Collar rot caused by *Aspergillus niger* is one of the most important disease. It causes severe loss to groundnut in terms of yield ranging from 10 to 50 per cent. Collar rot caused by *Aspergillus niger* van Tieghem is of considerable importance in warm and temperate groundnut growing areas. The fungus causes pre-emergence rotting of groundnut seed and the infected seed fails to germinate. In emerged young seedlings, *Aspergillus niger* infection results in sudden wilting (Middleton *et al*., 1994). It is known to create increased amount of pathogenicity in various species of plants, which can be treated by antibiotics, chemicals and antibiosis. Therefore, the present studies were particularly planned to carry out the *in vitro* studies on management of collar rot disease of groundnut.

MATERIALS AND METHODS

The present investigation on *In vitro* studies on management of collar rot caused by *Aspergillus niger* in groundnut was conducted at Plant Pathology Section, College of Agriculture, Nagpur during 2014-15. The culture of *Aspergillus niger* used in this study was isolated from infected collar rot of groundnut plant collected from the field of College of Agriculture, Nagpur. In order to isolate pathogens from the collar rot, infected parts were cut into 3 mm small bits and surface sterilized with 0.1 per cent $Hgcl_2$ solution for one minute and three subsequent washing with sterilized distilled water were given. The bits were placed in previously poured solidified PDA medium in petriplates. The plates were incubated at 27 ± 2 ^{0}C for 7 days. The isolated fungi were identified as *Aspergillus niger* on the basis of morphological characters and compared with published literature.

Mass multiplication of *Aspergillus niger,* Pathogenicity test (Koch's Postulate) and Re-isolation:

Sorghum sand medium was prepared by mixing 100 g sorghum, 50 g dry sand and 50 ml distilled water in 1000 ml capacity conical flask and autoclaved at 15 Ibs psi for 15 minutes for two consecutive days. It was inoculated with pure cultures of *Aspergillus niger* separately in isolation chamber and were incubated at room temperature for two weeks. The prepared inoculum was mixed in pot soil to test the efficacy of fungicides and bio-agents against *Aspergillus niger*. The pots were disinfected with 5 per cent formalin solution. Soil sterilization was done with the help of 10 per cent formalin solution. Two weeks old growth of

1, 3 & 4. P.G. Students, Plant Pathology Section, College of Agriculture, Nagpur
2. Asstt. Professor, Plant Pathology Section, College of Agriculture, Nagpur
3. Asstt. Professor, Entomology Section, College of Agriculture, Nagpur

soil borne pathogen of *Aspergillus niger* on sorghum sand medium were mixed separately in upper 15 cm layer of soil, 72 hours prior to sowing. The pots containing sterilized soil without inoculation served as control. Before sowing, seeds of groundnut were surface disinfected with 2 per cent sodium hypochloride solution for 2 minutes followed by three washings with distilled sterile water. Ten seeds were sown in pot containing *Aspergillus niger* and the pots without inoculum served as control treatment. The infected collar rot plants were used for re-isolation as per the standard procedure. The culture obtained was confirmed for growth with original culture from which isolations were made to prove pathogenicity.

***In vitro* study:** To test the efficacy of fungicides and bio-agents, poisoned food and dual culture technique was used against *Aspergillus niger*. Potato Dextrose Agar medium was prepared and distributed at the rate of 100 ml in 250 ml conical flasks, autoclaved at 15 Ibs psi for 15 minutes then before solidification of medium different fungicides with desired concentration were incorporated aseptically in different flasks. These flasks were shaken thoroughly and poured in petriplates @ 20 ml plate^{-1} likewise three plates for each treatment were poured. One set of three plates was poured without any fungicide to serve as control. After solidification of medium, plates were inoculated with eight days old pathogens separately. The five mm mycelial discs selected from peripheral growth of the test pathogen by cork borer were used for inoculating the plates by keeping one disc plate^{-1} in the centre in inverted position so as to make the mycelial growth touch the surface medium. The inoculated plates were incubated at room temperature for seven days. The dual culture technique with two bio-agents was conducted. The treatment was replicated thrice. Observations on colony diameter of fungus were recorded. Per cent inhibition of growth of test fungus was calculated.

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

C = Mycelial growth in control (cm)

T = Mycelial growth in treatment (cm)

RESULTS AND DISCUSSION

The association, isolation and pathogenicity of *Aspergillus niger* was proved. The *Aspergillus niger* was isolated from infected groundnut plants showing symptoms like pre-emergence rotting of seed, rotting of hypocotyls by using tissue segment method. The isolate fungi of infected sample namely *Aspergillus niger,* were identified on the basis of morphological characters (Plate 1). A typical black mycelium conidia growth of *Aspergillus niger* was observed after 72 hours of incubation, at 28 ± 2° C, in an incubator. *Aspergillus niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles and other decaying vegetation. Microscopically, its conidiophores are smooth walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5.0 um in diameter), dark brown to black and rough-walled. The similar type of cultural and morphological characters of fungus was observed by Gajera *et al*. (2011). This pure culture was maintained throughout the study by periodical transfer on PDA medium under aseptic condition, to keep the culture fresh and viable.

Pathogenicity of *Aspergillus niger* isolate was carried out by soil inoculation technique using TAG-24 variety of groundnut. The soil was made sick by incorporating the inoculum, ten seeds were sown in each pot (30 cm size pots). In this way 3 pots were maintained along with other 3 pots which were without inoculum serve as control, to record the observations.The association, isolation and pathogenicity of *Aspergillus niger* was proved and observed the mortality to the extent of 80.00 per cent due to *Aspergillus niger* (Table 1). Initially *Aspergillus niger* causes rotting of seed, pre-emergence soft rot of hypocotyls and post –emergence collar rot of seedlings. Pre-emergence rotting was observed at 7 DAS. Similarly, collar rot was observed at 15 DAS and disease progress. Germinated seeds were first covered with masses of black conidia, rapid drying of plants. At seedlings circular brown spots on cotyledons were observed. The symptoms were spread later to hypocotyl and stem. Brown colour spot appeared on collar region. The affected portion became soft and rotten, resulting in collapse of seedling. In fact when occur in adult plant showed crown rot symptoms. Large lesions developed on the stem below the soil and spread upward along the branches causing drooping of leaves, wilting of plants (Plate 2). The pathogen was re-isolated and confirm as *Aspergillus niger.*

Pathogenicity test and symptomatology of collar rot was earlier studied by many workers. Sharma (2012) reported root curling and top deformation in *Aspergillus* rot. Sheela and Packiaraj, (2000) showed *Aspergillus niger* was associated with almost all the tested seeds of groundnut. This indicated that pathogen was seed borne. These results were similar with the present finding.

Some fungicides *viz.,* carbendazim, thiram, carboxin + thiram, carbendazim + mancozeb, carbendazim + thiram and bioagents *Trichoderma viride, Pseudomonas fluorescens* were evaluated at recommended concentration in the laboratory for their efficacy against *Aspergillus niger* by applying poison food technique and dual culture test and the data were presented in table 2 and plate 4. Fungicides and bioagents significantly inhibited the growth of *Aspergillus niger.* Fungicides *viz.,* carbendazim, thiram, carboxin + thiram, carbendazim + mancozeb, carbendazim + thiram completely inhibited the radial growth of *Aspergillus niger* at 3rd, 5th, and 7th DAI with 100 per cent growth inhibition. Prabhu and Patil (2004) and Mani *et al.* (2012) also observed inhibition of growth of *Aspergillus niger* at 7th DAI. Among the bioagents combination with *Trichoderma viride and Pseudomonas fluorescens*

treatment recorded minimum radial mycelial growth at 3rd DAI with 27.6 mm. There was slight increase in the radial mycelial growth from 5th to 7th DAI with minimum per cent inhibition in both the bioagent treatments. The beneficial effect due to *Trichoderma viride, Pseudomonas fluorescens* in controlling the *Aspergillus niger* have been reported by Vyas (1994), Gaikwad and Nimbalkar (2003), Lokesha and Benagi (2007), Devi and Prasad (2009), Patale and Mali (2009), Kumar *et al.* (2011), Gajera *et al.* (2011), Mani *et al.* (2012) and Pandav *et al.* (2013).

In the present investigation it was proven that collar rot was caused by *Aspergillus niger* through pathogenicity test. Fungicides and bioagents were tested against pathogen *in vitro* by poison food technique and observed that all the fungicides completely inhibited the radial growth of *Aspergillus niger* at 3, 5, and 7th DAI with 100 per cent growth inhibition. Among bioagents *Trichoderma viride* showed 27.6 mm, 28.16 mm, 31.75 mm mycelial growth of pathogen at 3rd, 5th and 7th DAI respectively with 87.51 per cent growth inhibition. While *Pseudomonas fluorescens* showed 20.5 mm, 24.8 mm, 28.00 mm mycelial growth of pathogen at 3rd, 5th and 7th DAI respectively with 73.33 per cent growth inhibition.

Table 1. Pathogenicity test with isolate of *Aspergillus niger* on groundnut

Pathogen	No. of seeds sown	No. of affected seedlings	Per cent collar rot
Aspergillus niger	30	24	80.00
Control	30	0	00.00

Table 2. *In vitro* efficacy of fungicides and bioagents against *Aspergillus niger* causing collar rot of groundnut

Tr No.	Treatment details	Mycelial growth of pathogen (mm)*			Growth inhibition over control (7 DAI)
		3rd DAI	5th DAI	7th DAI	
T1	Carbendazim	00.00*	00.00*	00.00*	100
T2	Thiram	00.00	00.00	00.00	100
T3	Carboxin + Thiram	00.00	00.00	00.00	100
T4	Carbendazim + Mancozeb	00.00	00.00	00.00	100
T5	Carbendazim + Thiram	00.00	00.00	00.00	100
T6	*Trichoderma viride*	27.6	28.16	31.75	87.51
T7	*Pseudomonas fluorescens*	20.5	24.8	28.00	73.33
T8	Control	62.4	81.16	90.00	
	SE ± (m)	**0.384**	**0.380**	**0.372**	**4.316**
	CD (P=0.01)	**1.143**	**1.130**	**1.107**	**12.826**

* mean of three replications

REFERENCES

Anonymous, 2014a. Fourth Advance Estimates, Deptt. of Economics and Statistics, GOI, New Delhi.

Anonymous, 2014b. Agricultural statistics at a glance.

Devi, M. C. and R. D. Prasad,2009. Biointensive management of collar rot of groundnut caused by *Aspergillus niger.* J. Biol. Control, **23**(1): 21-24.

Gaikwad, A. P. and C. A. Nimbalkar, 2003. Management of collar and root rot (*Aspergillus niger*), *(Rhizoctonia solani)* of bell pepper with bioagent *(Trichoderma* spp.*)* and fungicides. J. Maharashtra agric. Univ. **28**(3): 270-273.

Gajera, H., K. Rakholiya and D. Vakharia, 2011. Bioefficacy of *Trichoderma* isolates against *Aspergillus niger* inciting collar rot in groundnut (*Arachis hypogaea* L.). J. Pl. Protc. Res. **51**(3): 240-247.

Kumar, S., S. Sharma, D. V. Pathak and J. Beniwal, 2011. Integrated management of collar rot caused by *Aspergillus niger.* J. Tropical Forest Sci. **23**(1): 35–41.

Lokesha, N. M. and V. I. Benagi, 2007. Biological management of pigeonpea collarrot caused by *Aspergillus niger.* Karnataka J. Agric. Sci. **20**(1): 54-56.

Mani, T. G., A. Chetan., S. Janawad and V. Hosamani, 2012. Efficacy of fungicides and biocontrol agents against *Sclerotium rolfsii* causing foot rot disease of finger millet, under *in vitro* conditions. G.J.B.A.H.S., **1**(2): 46-50.

Middleton, K.J., S. Pande, S. B. Sharma and D. H. Smith, 1994. Diseases. In the groundnut crop: a scientific basis for improvement. Edited by J. Smartt. Chapman and Hall, London, U. K. pp. 336-394.

Pandav, S.M., J. J. Kadam, P. M. Zagde, M. S. Joshi, R. A. Karande and N. P. Gurav, 2013. *In vitro* bio-efficacy of fungicide and bioagents against *Sclerotium rolfsii* sacc. causing collar rot disease in gerbera. J. Pl. Dis. **8**(2): 215-217.

Patale, S. S. and V. P. Mali, 2009. Efficacy of *Trichoderma* in management of collar rot of groundnut. Bioinfolet. **6**(2): 154-156.

Prabhu, H. V. and P. V. Patil, 2004. Efficacy of chemical and biological seed dressers and host resistance in the management of collar rot of soybean caused by *Sclerotium rolfsii* Sacc. Soybean Res. **2**: 41-45.

Sharma, R. 2012. Pathogenecity of *Aspergillus niger* in plants. Cibtech. J. Microbiol. **1**(1): 47-51.

Sheela, J. and D. Packiaraj, 2000. Management of collar rot of groundnut by *Pseudomonas fluorescens.* IAN **20**: 50-51.

Vyas, S. C. 1994. Integrated biological and chemical control of collar rot of groundnut. Indian. J. Mycol Pl. Pathol. **24**(2): 132-134.

Rec. on 25.06.2016 & Acc. on 30.07.2016