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Biocontrol of *Aspergillus* root rot by tripartite symbiosis in groundnut

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Abstract

Aspergillus niger is responsible for causing root rot of groundnut. Ten *A. niger* isolates were isolated and studied in the groundnut cultivated area of Madurai. Groundnut was challenged with *Aspergillus*, and the production of pectinase and cellulase enzymes was investigated to assess the virulence of the fungal isolates. Among the ten isolates *A. niger* CLR10 produced highest percentage (40%) of diseased plants. Based on the production of these enzymes in groundnut plants challenged with the pathogen *A. niger* CLR10 produced highest level of these enzymes so it was used for further studies. The treatment AT_{13} recorded minimum percentage mortality (15.9%) and the pathogen only inoculated control recorded 100% mortality. Application of AMF with rhizobial isolates decreased the mortality in groundnut. The biocontrol studies revealed that the combined inoculation of AMF and *Rhizobium* sp. reduced the pathogenesis of *A. niger* CLR10. However, the treatment AT_{13} recorded significantly ($p<0.05$ and 0.01) higher growth parameters than other treatments. The investigation suggested that inoculation of *Glomus mosseae* and *Gigaspora albida* along with *Rhizobium* sp.GPMK1 and GMDU1 can improve not only the growth but also render disease resistance to groundnut. It can be summarized that the practice of multiple inoculation is found more effective as compared to the traditional practice of single inoculation of inoculums.

Keywords: Root rot, *Aspergillus* sp.,Groundnut, *Rhizobium* sp., Biocontrol, Mycorrhiza.

Introduction

Groundnut is one of the major oilseed crops in India. It is cultivated under diversified agro climatic conditions in about 6.6 million hectare producing about 5.9 million tons (FAO, 2005). The yield is influenced by soil fertility, climatic condition, varieties grown, cultural practices, insect pests & diseases and environmental stresses.

In the early 1980s groundnut was grown in Uttar Pradesh on 0.3 million ha with a production of 0.19 million tons. Since then, both area and production have shown a steady decline due to various reasons. Groundnut is an exhaustive crop and depending upon the yield, it removes large amount of macro and micronutrients (Singh *et al*., 2008). Unfortunately, the groundnut production is hampered due to attack by different diseases. These include bacterial, fungal, nematode and viral diseases (Subrahmanyam *et al*., 1980). Rot diseases are mostly fungal disease, which are soil and seed borne. These cause disintegration of fruit and seed tissues and are covered with yellow or green spores. Fungal strains of *Aspergillus flavus* produce highly toxic aflatoxin. About 360 species of arthropod pests attack groundnut before harvest and cause direct damage as well as act as fungal and viral vectors (ICRISAT, 1987).

A number of diverse plant types of economic interest have AM activity. These include cereals and herbaceous legumes as well as vegetable crops, temperate fruit trees or shrups tropical plantation crops and ornamental horticultural crops. The main benefit of mycorrhiza is improved uptake of nutrients. They also benefit the host plant by supplying growth regulating substances and vitamins and protecting against pollutants and soil borne pathogens (Mitchell 1993). Interaction among mycorrhiza formation, nutrient uptake and soil microorganisms (N_2 fixing bacteria, phosphate solubilizing bacteria, plant growth promoting Rhizhobacteria (PGPR)) have been reported as beneficial (Azcon-Aguilar and Barea, 1992).

Competitive interaction between the mycorrhizal fungus and bacteria and other fungi have been observed and there may be allele-chemical interaction similar to antibiosis, which can be either stimulatory or suppressive (Tylka *et al.*, 1991). AM fungi thus interact with natural and introduced microorganisms in the mycorrhizosphere, hence affecting soil properties and quality. Soil microorganisms can produce compounds that increase root cell permeability of plants thereby increasing the rates of root exudation which stimulates the growth of hyphae of AMF in the rhizosphere and facilities root penetration and colonization by the fungus. (Miller and Jastrow, 2000).

Very little is known of the factors influencing soil borne fungal pathogens in colonization of root tissues, although root-infecting fungi are important as disease-causing agents (Deacon 1996). This lack of knowledge is in part a result of the difficulty of studying such processes below ground, and also because of the genetic intractability of many root-infecting organisms. *A. niger* causes crown rot disease of peanut the knowledge of the exact location of the pathogen in seed or the depth of seed infection by particular pathogen can be helpful in the control of seed borne infection.

Jain & Neema (1952) reported that *A. niger* produced circular brownish spot on the cotyledon and this discoloured area rapidly rotted and spread to the stem and hypocotyls apparently it exerts its effect in substantial part by production of oxalic acid.

Microorganisms play an enormously important role in plant disease control. As naturally occurring resident antagonists, they can be managed or exploited to achieve the desired results.

The objective of studying these AM fungi –*Rhizobium* sp. interactions has focused upon obtaining an additive effect and biocontrol action against the pathogen *A.niger* on plant growth by microbial inoculants either singly or in combination.

Materials and Methodology

Isolation of AM spores from soil samples by wet sieving & decanting method:

The spores / sporocarps present in the root zone soil were isolated following the decanting and wet sieving technique of Gerdemann and Nicolson (1963). Fifty grams of soil samples were suspended in water and were allowed to settle down for some time. The suspension was passed through a series of sieves with 22, 36, 60, 150, 200 and 350µm mesh. The spores in the soil suspension were then collected.

The spores collected were placed on the filter paper and examined under a binocular microscope, transferred to a clean microscopic slide with the help of a fine needle and mounted in PVLG (Polyvinyl alcohol + lactic acid + Glycerol). Microscopic observations were made under high magnification for quantitative and qualitative characters of spores. The spores were identified from spore characteristics as detailed in the manual of Schenck and Perez (1988).

Cultivation and inoculation of AM fungi:

The AM spores and sporocarps were multiplied in onion pulb (*Allium cepa*) plants in sterile soil under potted conditions. One hundred gram of soil inoculums containing 300 to 800 spores and sporocarps was spread over the lower layer of sand/soil 5.0g in each pot. Then 1kg of sand/soil was layered over the inoculums before sowing.

Funnel technique:

This technique is commonly used to inoculate plants in the green house. The funnel pore root to grow near the spores and assures infection even when few spores are used. Funnels can be formed from aluminum foil and simply peeled away to allow access to roots or for easy transplanting of the inoculated seedling.

Isolation of Rhizobium from root nodules

Rhizobium was isolated from the root nodules of *Arachis hypogaea* L by following the technique of Vincent (1970). The root nodules were washed in

running water to remove grass surface contaminants, surface sterilized in 0.1% $HgCl_2$ for 5 to 7 min and then washed thoroughly in sterile distilled water. Using a sterile glass rod, the nodules were gently crushed in a sterile yeast extract mannitol broth. An aliquot was plated onto yeast extract mannitol agar containing 0.025% Congored (Hahn, 1966) and incubated at 25±1°C. The white, translucent, glistening, elevated and small colony with entire margin was transferred to YMA slants.

Maintenance and inoculation of *Rhizobium* sp.:

The Rhizobium culture was maintained on YMA slants with 0.3% calcium carbonate (Vincent *et al.*, 1980). The purity of the culture was periodically ascertained using congo red reaction (Hahn, 1966). Seven days old seedlings of *Arachis hypogaea* L. were inoculated with 2 ml thick suspension of peanut *Rhizobium* (approximately $1x10^9$ cells / ml) prepared from 72hr old cultures. The rhizobial suspensions were prepared in 5% sucrose solution and introduced around the base of each seedling.

Microscopic observation of *Aspergillus* fungi:

The preparations of slides were carried out firstly; the glass slides were sterilized in an oven at 160°C for 1 hour and later allowed to cool (Bayer *et al*., 1966). Then, small portion of mycelium of the organism was collected from stock in Mc Cartney bottle with the help of sterilized loop and observed under the microscope. Then, the characters such as size, shape and colour of conidia, conidiophores were studied as well as colony size observed from culture. They were subsequently identified using the criteria adopted by Raper and Fannel (1965).

Isolation of pathogenic fungi:

During investigation different area were used and in each area, five groundnuts were inspected to determine the rate of spread of storage rot disease. Infected seeds were obtained and wrapped in polythene bags and brought to the laboratory for further investigation.

Isolation of *Aspergillus* fungi associated with the infected groundnut seed in each area was made. The sections of the seeds affected with rots were cut with a sharp and sterilized knife. These were surface sterilized in 0.5% sodium hypochloride. They were then placed in potato dextrose agar in petridishes. The dishes were incubated at room temperature and relative humidity 68-75% under continuous light for five days. The experiments were repeated 3 times and culture was incubated at room temperature up to 3 weeks. All the isolated species were later subcultured and transferred into Mc Carteney bottles with PDA for storage at 4°C in a refrigerator. Three replicate plates were prepared from each dilution. One ml of each sample was poured into sterilized petridishes containing 20 ml sterilized medium (Czapek-Dox-Agar). The plates were incubated at 28 °C and fungi count was estimated after 5 days and again after 8 days to count the slow growing fungal species. The isolated fungi were identified according to their morphological characters (Barnet, 1960) and were presented on Plates 1 and 2.

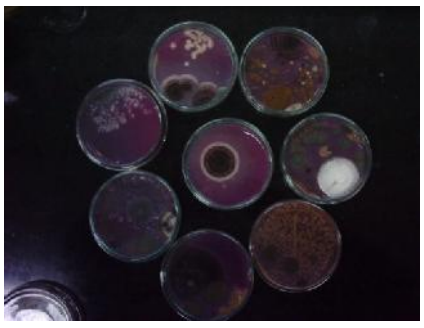

Plate 1: Isolation of *Aspergillus* sp. on Martin's Rose Bengal

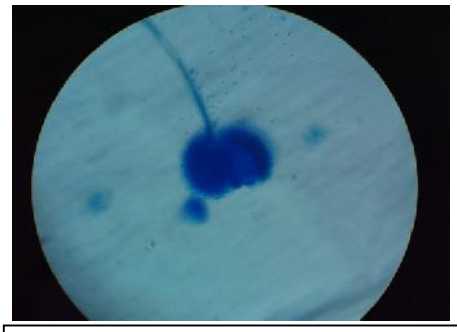

Plate 2: Microscopic observation of *A. niger* mycelium

Evaluation of pathogenecity of *Aspergillus niger* on groundnut

The pathogenecity of the *Aspergillus* sp. isolated from various locations were evaluated by two methods. First was their ability to cause rot in groundnut seedlings. Secondly, their ability to produce enzymes such as pectinase and cellulase.

Enzymatic studies to evaluate the pathogenecity of isolated strains of *A. niger*

Czapek's medium devoid of sucrose with 3% pectin for pectinolytic enzyme or 1% cellulose powder for cellulolytic enzyme was prepared. Czapek's medium with sucrose acted as control. The media were dispensed in 50ml aliquots in 250 ml Erlenmeyer flasks and inoculated with 8mm culture disc of the *Aspergillus niger* isolated from different locations. For each treatment 3 replicates were maintained. After 7 days of incubation at room temperature (28 $\pm 2^{o}$ C) the mycelium was removed. The filtrates were retained for enzyme study.

Pectinase activity

The activity of pectinase was determined by changes in viscosity of citrus pectin. To 4 ml of freshly prepared 1% citrus pectin dissolved in boric acid buffer at pH 8.6, 1 ml of Tris acetate buffer and 2ml of the culture filtrate at 8.6 were added. The mixture was transferred to an Oswald-Fenste Viscometer and loss in viscosity was determined after 2 hours of incubation.

Cellulolytic enzymes

Cellulose (Cx) (Hussain and Diamond, 1960)

The ability to reduce the viscosity of carboxy methyl cellulose (CMC) in an Oswald-Fenste Viscometer, at 30^{o} C, was used to assay the activity of enzyme Cx. CMC at a 0.5% concentration was prepared in sodium acetate – acetic acid buffer (pH 4.8). To 4 ml of CMC solution, 1ml of buffer at pH 4.8 and 2 ml of the culture filtrate was added, then transferred to an Oswald-Fenste Viscometer placed in a waterbath at 30 ± 1^{o} C and the viscosity losses determined as described earlier. In all experiment 1ml of Chloramphenicol- cyclohexamide solution (25 mg in 20 ml water) was added to prevent the microbial contamination.

Pot culture study to evaluate the pathogenecity of isolated strains of *Aspergillus niger* on groundnut.

Groundnut CO2 variety was obtained from Tamilnadu Agriculture University, Coimbatore, which seedlings were raised at a rate of 3 seedlings/ plastic cup in the laboratory up to 10 days. Then, the seedlings were challenged with a loopful of various strains of *A. niger* collected from different locations. For each strain 3 samples were maintained. The pathogen virulence in causing collar rot symptoms was observed with various strains on groundnut plants.

Challenging with *A. niger* in pots

After 30, 60 and 90 DAS the plants were inoculated with collar rot pathogen *A. niger* at the collar region with the inoculum multiplied on PDA for 5 days at 28 ± 2^{0} C. the mixture of mycelium and spores was put around the collar region and covered with wet cotton to keep the inoculum moist (Dange and Saradeva, 1986).

Treatments details of pot culture studies with *Aspergillus niger*

Following were the AMF and *Rhizobium* sp. treatments given to the groundnut (CO_2) variety in pot culture studies. In total there were 15 treatments with 3 replications for each treatment. The treatment details are presented here under

S.no	Name of the treatment	Treatment number
1	Control	C
2	*A. niger* only	CLR10 (T0)
3	*Rhizobium* sp GPMK1+T0	T1
4	*Rhizobium* sp GMDU1+T0	T2
5	*Glomus mosseae*+T0	T3
6	*Gigaspora albida*+T0	T4
7	*Glomus mosseae* + *Gigaspora albida*+T0	T5
8	*Rhizobium* sp GPMK1 + *Rhizobium* sp GMDU1+T0	T6
9	*Rhizobium* sp GPMK1 + *Glomus mosseae*+T0	T7
10	*Rhizobium* sp GMDU1 + *Glomus mosseae*+T0	T8
11	*Rhizobium* sp GPMK1 + *Rhizobium* sp GMDU1 + *Glomus mosseae*+T0	T9
12	*Rhizobium* sp GPMK1 + *Gigaspora albida*+T0	T10
13	*Rhizobium* sp GMDU1 + *Gigaspora albida*+T0	T11
14	*Rhizobium* sp GPMK1 + *Rhizobium* sp GMDU1+ *Gigaspora albida*+T0	T12
15	*Rhizobium* sp GPMK1+*Rhizobium* sp GMDU1+*Glomus mosseae* + *Gigaspora albida*+T0	T13

Statistical Analysis

The data recorded were subjected to analysis of variance (ANOVA) using AGRES statistical package. SED and CD at 5% and 1% level of significance are given in Tables. The input data were given manually and computed output results obtained from software designates whether the differences between the treatments are significant or non significant CD at 5% and 1% levels. The treatments which show insignificant differences are grouped on par ($p<0.01$).

Results

Enumeration of soilborne *Aspergillus niger* in different locations

Population of fungal flora in soil was estimated from different locations viz., Thirumangalum, Thirunagar, Villacherry, Pzhanganantham, K.Puthur, Karrupaurani,Annupanadi,N.P.Kottai,Pasumalai and Chellur. Number of colonies per plate (at 10^3 CFU) among the total number of fungal colonies, total number of *Aspergillus* colonies and number of *Aspergillus niger* colonies were estimated and their results are presented in Table 1.

Table 1: Assessment of the soilborne *Aspergillus niger* in different locations

S. No	Location	Total no. of fungal colonies($\times 10^3$CFU/g rhizosphere soil)	Total number of *Aspergillus* species ($\times 10^3$ CFU/g rhizosphere soil)	Number of *Aspergillus niger* ($\times 10^3$ CFU/g rhizosphere soil)
1.	Thirumangalum	42±2.0	26±3.7	3±2.0
2.	Thirunagar	39±1.0	22±2.0	6±2.0
3.	Villacherry	26±6.1	14±2.0	3±1.0
4.	Pazhanganantham	43±1.0	17±3.0	2±1.0
5.	K.puthur	28±5.3	10±2.7	1±0.0
6.	Karrupaurani	34±2.6	16±2.7	4±1.0
7.	Annupanadi	25±2.0	15±2.7	4±2.0
8.	N.P.Kottai	47±3.6	24±1.0	5±1.0
9.	Pasumalai	55±2.6	27±4.0	7±2.0
10.	Chellur	57±4.4	45±6.3	12±2.7

Mean value of three replicates

The location Chellur recorded the highest total number of fungal colonies per plate with 57 followed by Pasumalai with 55 whereas the least was noticed in Annupanadi as 25 colonies per plate. The highest total number of *Aspergillus* colonies per plate was noticed in Chellur with 45 followed by Pasumalai with 27 and the least was observed in the location K.Pudur as 10. The location Chellur recorded the highest 12 number of *Aspergillus niger* colonies per plate followed by Pasumalai with 7. The least number of *Aspergillus niger* colonies per plate was noticed in location K.Pudur as 1.

Evaluation of pathogenecity of *A. niger* strains on *Arachis hypogaea* L.

In this study, we have applied 10 different *A. niger* strains isolated from 10 different locations. The ten strains were isolated from various locations and their disease causing status of collected plants is given in Table 2. The percentage of diseased plants varied from 10 to 40. Among the ten *A. niger* strains, the highest (40%) diseased plants were noticed in 10th location Chellur (CLR). Hence the isolate was named as *Aspergillus niger* CLR10 and used for further study. The percentage of diseased plants in Chellur was significantly ($p<0.05$ and 0.01) higher than those observed in other locations. Study results were represented on Plates 3 and 4.

Table 2: Evaluation of pathogenecity of *A. niger* strains isolated from various locations on *Arachis hypogaea* L.

S. no	Location	Isolates	% of diseased plants
1.	Thirumangalam	TMGM1	30
2.	Thirunagar	TNGR2	10
3.	Villacherry	VCRY3	20
4.	Pazhanganantham	PGNM4	20
5.	K.Pudur	KPR5	30
6.	Karruppaurani	KPRI6	30
7.	Anuppanadi	APNI7	10
8.	N.P.Kottai	NPK8	10
9.	Pasumalai	PMLI9	20
10.	Chellur	CLR10	40

SED	2.6565
CD(0.05)	5.5811
CD(0.01)	7.6477

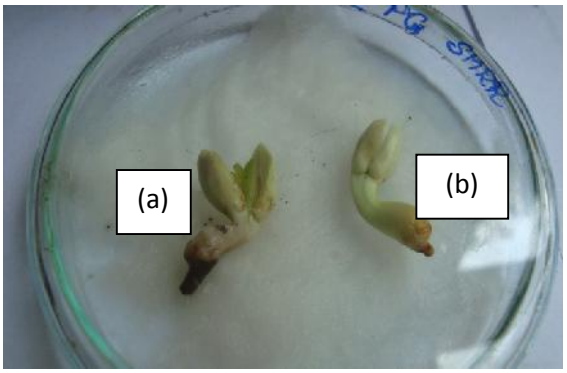

Plate 3. *Aspergillus niger* CLR10 inoculated groundnut seed (a) and (b) uninoculated seed

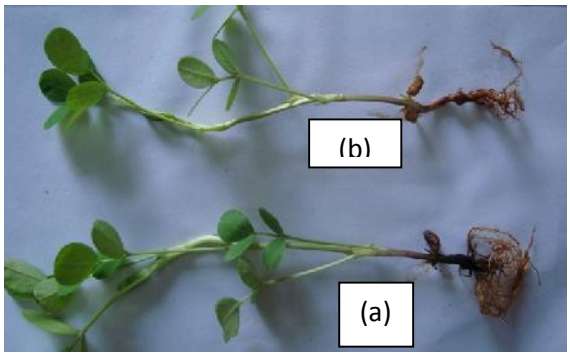

Plate 4: *Aspergillus* root rot symptom in groundnut on 30 DAS (a) and uninoculated groundnut plant (b)

Pectinase and cellulase activity of the isolated *A. niger* strains

Enzyme study of *Aspergillus niger* isolates was detected and their results are tabulated in Table 3 and depicted in the Figure 1. Two enzymes related with pathogenicity were studied and their activity was expressed as percent reduction in viscosity.

Table 3 : Pectinase and Cellulase activity of the isolated *A. niger* strains.

S. No	Isolates	Pectinase*	Cellulase*
1.	TMGM1	49.7	26.9
2.	TNGR2	39.0	12.5
3.	VCRY3	59.1	34.2
4.	PGNM4	51.4	31.5
5.	KPR5	30.3	13.3
6.	KPRI6	53.8	24.9
7.	APNI7	42.5	22.5
8.	NPK8	45.7	26.7
9.	PMLI9	55.5	28.8
10.	CLR10	61.2	34.2
SED		1.8857	1.9282
CD (0.05)		3.9617	4.0510
CD (0.01)		5.4287	5.5510

*Enzyme activity expressed as percent reduction in viscosity

Pectinase activity: Pectinase activity of *A. niger* isolates varied from 30.3 to 61.2 percent reduction in viscosity. CLR10 isolate produced highest 61.2 percent reduction in viscosity followed by VCRY3 isolate as 59.1 and the least was observed in KPR5 isolate as 30.3. The pectinase activity of isolates CLR10 and VCRY3 were significantly ($p<0.05$ and $p<0.01$) higher than other isolates.

Cellulase activity: Cellulase activity of *A. niger* isolates varied from 12.5 to 34.2 percent reduction in viscosity. Both CLR10 and VCRY3 isolates produced highest cellulase activity as 34.2 percent reduction in viscosity followed by PGNM4 isolate as 31.5.

The least was observed in isolate TNGR2 as 12.5. However, the cellulase activity in isolates VCRY3, PGNM4, PMLI9 and CLR10 were on par.

Groundnut plants challenged with root rot pathogen – *Aspergillus niger* isolate CLR10

In this study, *A. niger* CLR10 isolate was inoculated on *Arachis hypogaea* L. along with AMF and *Rhizobium* sp. and the percentage mortality was studied and the results are tabulated (Table 4). The percentage mortality of groundnut varied from 15.9 to 100. The lowest percentage mortality indicates the highest biocontrol activity against *A. niger* CLR10 strain. According the treatment AT_{13} shows the lowest percentage mortality with 15.9±0.49 followed by treatment AT_7 with 19.3±1.83. Evidently, the treatment AT_{13} was best among other treatments.

Table 4: Groundnut challenged with the selected *Aspergillus niger* strain CLR10

Treatments	% Mortality of groundnut
CLR 10 only	100.0±0.0
AT_1	29.6±1.11
AT_2	22.2±1.09
AT_3	39.6±0.57
AT_4	38.3±0.83
AT_5	46.2±0.99
AT_6	32.6±1.90
AT_7	19.3±1.83
AT_8	22.2±1.38
AT_9	43.1±2.12
AT_{10}	35.1±2.01
AT_{11}	30.9±0.32
AT_{12}	37.4±1.56
AT_{13}	15.9±0.49

Mean value of three replicates

Study on the plant growth parameters of *Arachis hypogaea* L. as influenced by different treatments along with *Aspergillus niger* CLR10 on 90 DAS.

In this study the pathogenic strain *A. niger* CLR10 was inoculated with AMF and *Rhizobium* sp. on *Arachis hypogaea* L. to analyze their biocontrol activity and the results are tabulated in Table 5. No growth parameters were observed in pathogen *Aspergillus niger* CLR10 only inoculated control treatment.

Table 5: Biological control of *Aspergillus* root rot disease of groundnut by AMF and *Rhizobium* sp. and their effect on the growth parameters of groundnut on 90 DAS.

Treatments	Shoot length (cm)	Root length (cm)	Plant fresh weight (g)	Plant dry weight (g)	Nodules Plant $^{-1}$	Nodules (mg)	
						Fresh wt	Dry wt
CLR 10	-	-	-	-	-	-	-
C	24.95	12.23	10.0	0.84	17.0	0.109	0.032
AT_1	27.89	13.29	13.2	1.38	32.3	0.156	0.048
AT_2	27.88	14.02	14.8	1.66	30.7	0.190	0.055
AT_3	26.96	13.54	11.7	1.30	19.0	0.114	0.048
AT_4	27.66	14.54	12.4	1.84	17.3	0.120	0.042
AT_5	29.65	16.10	14.8	1.98	38.0	0.161	0.083
AT_6	31.83	16.97	14.8	2.54	46.3	0.229	0.091
AT_7	33.47	16.72	15.7	3.10	40.0	0.166	0.096
AT_8	32.89	16.54	16.0	3.60	38.7	0.161	0.087
AT_9	39.04	18.37	17.6	3.90	51.3	0.248	0.102
AT_{10}	30.99	18.12	15.3	3.60	23.0	0.124	0.069
AT_{11}	31.06	18.13	13.9	3.70	27.3	0.156	0.082
AT_{12}	38.06	17.46	14.5	3.90	25.7	0.130	0.062
AT_{13}	41.35	19.62	19.2	4.70	55.7	0.285	0.128
SED	1.0058	0.5627	0.5855	0.2614	2.3270	0.0080	0.0048
CD (0.05)	2.0675	1.1567	1.2034	0.5372	4.7833	0.0165	0.0098
CD (0.01)	2.7949	1.5637	1.6269	0.7263	6.4664	0.0224	0.0133

Discussion

Biocontrol activities against root rot disease - Enumeration of soilborne pathogen *Aspergillus niger*

Aspergillus root rot commonly results in seedling blight, but also may affect older plants from mid to late-season. The fungus that causes root rot, *Aspergillus niger*, is different from the species that causes aflatoxin contamination of harvested kernels. *A. niger* is present in most soils with groundnut cultivation and is a common contaminant of groundnut seed. However, outbreaks of the disease are sporadic and appear to be related to the prior occurrence of one or more stresses. Extreme heat or fluctuations in soil moisture during the seedling stage, poor seed quality, seedling damage from pesticides or cultivation, and feeding by root and stem boring insects are stresses thought to aggravate the disease. The economic importance of *Aspergillus* root rot is difficult to assess. Generally scattered plants are affected, although stand losses of 50% have been reported in isolated fields (John and Hassan, 2008).

In the present study, the enumeration of *Aspergillus niger* from the groundnut cultivated areas of Madurai soil was undertaken. The location Chellur recorded the highest number of *A. niger* colonies per plate followed by the location Pasumalai and the least was noticed in location K.Pudur. Similar work on the distribution and the capacity of fungi to invade living tissues of wounded groundnut seeds directly from the soil of Mexico had been done by Bruce, 2005.

Evaluation of pathogenecity of *A. niger* isolates on groundnut

Ten *A. niger* isolates isolated from various locations were inoculated on groundnut, among them *A. niger* CLR10 produced highest percentage (40%) of pathogenicity.

The production of pectinase and cellulase enzymes has been investigated in a wide range of plant pathogens and they play a key role in pathogenesis (Collmer and Keen, 1986). In the present work, both pectinase and cellulase in high levels were found to be secreted in groundnut plants infected with pathogen *A. niger* CLR 10. Groundnut plants were challenged with *Aspergillus niger* CLR 10 in fifteen treatments, the treatment AT_{13} recorded minimum percentage mortality (15.9%±0.49) and the pathogen only inoculated control recorded 100% mortality. The growth inhibition of *Aspergillus* fungi by dual culture study could be due to its fast growing nature, secretions of harmful extra-cellular compounds like antibiotics, cell wall degrading enzymes such as gluconases, endochitinases and chitinases (Nakkeeran *et al.*, 2002).

Application of AMF with rhizobial isolates decreased the percentage mortality in groundnut and it is in line with the findings of Osamu *et al*. (2006) on *Fuzarium* sp. causing root rot in blackgram. Tarekegn *et al.* (2007) studied with groundnut seed treatment with *Rhizobium* sp. and mycorrhiza has shown higher emergence, reduced plant mortality, and increased yield compared to untreated controls.

Study of plant growth parameters of *Arachis hypogaea* L. by different treatments along with *Aspergillus niger* CLR10.

Plant diseases may be suppressed by the activities of one or more plant associated microbes, researchers have attempted to characterize the organisms involved in biological control. Historically, this has been done primarily through isolation, characterization, and application of individual organisms. By design, this approach focuses on specific forms of disease suppression. Specific suppression results from the activities of one or just a few microbial antagonists. This type of suppression is thought to be occurring when inoculation of a biocontrol agent results in substantial levels of disease suppressiveness (Weller *et al.* 2002).

In this study, the shoot length, root length, plant fresh and dry weight, number of nodules, nodules fresh and dry weight reduction were observed in treatments when compared to the study without pathogens inoculation. It revealed that the combined inoculation of AMF and *Rhizobium* sp. reduced the pathogenesis of *A. niger* CLR10. However, the treatment AT_{13} recorded the highest growth parameters on 90 DAS.

Siddiqui and Akhtar (2007) observed that the application of phosphate-solubilizing microorganisms alone and in combination increased plant growth, pod number, and chlorophyll, nitrogen, phosphorus and potassium contents, and reduced gall formation, nematode multiplication and root-rot index of chickpea. Combined inoculation of these microorganisms caused the greater increase in plant growth and reduction in the root-rot index more than individual inoculations.

Most pathogens will be susceptible to one or more biocontrol strategies, but practical implementation on a commercial scale has been constrained by a number of factors. Cost, convenience, efficacy, and reliability of biological controls are important considerations, but only in relation to the alternative disease control strategies. Cultural practices and host resistance can go a long way towards controlling many diseases, so biocontrol should be applied only when agronomic practices are insufficient for effective disease control (Pal and McSpadden, 2006).

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