Effect of Arbuscular Mycorrhizal Fungi in the Management of Black Bundle Disease of Maize caused by *Cephalosporium acremonium*

*Veerabhadraswamy A L and Rajkumar H Garampalli**
Department of studies in Botany, University of Mysore, Manasagangotri, Mysore – 570 006, Karnataka, India.
*rajkumarhg@yahoo.co.in

ABSTRACT

Three species of arbuscular mycorrhizal fungi (*Glomus fasiculatum, Glomus mossae* and *Acaulispora laevis*) were used as bio-agents to manage black bundle disease of maize caused by *C.acremonium*. The results revealed that colonization of arbuscular mycorrhizal fungi in root system of the host reduce the percentage of disease incidenceconsiderably.In the pots inoculated with *G. fasiculatum* no disease incidence (0.0%) was recorded whereas, in the pots inoculated with *A. leavis* and *G. mossae* 16.66 % of disease incidence was recorded and the pots treated with pathogen shows 66.66% of disease incidence compare to control. Among the three bio-agents, *Glomus fasiculatum*proved to be more effective in managing the disease followed by *G.mossae* and *A. laevis.* In addition, all the three AM fungi enhanced the plant growth when they are used alone as inoculum as compared to dual inoculation with the *C. acremonium* and overall control. This clearly suggests that, AM fungi if used, can serve dual purpose. It can be used as bio-control agent as it shows negative antagonistic interaction soil borne plant pathogens and used as growth promoter because of the ability to supply macro and micro nutrients to the host plants.

Key words: Arbuscular mycorrhizal fungi, Bio-control, Black bundle disease, *C.acremonium*and Maize.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important food crops of the world. It has a remarkable productive potential along with other members of the family Poaceae, such as wheat and rice (Kling and Edmeades 1997). The crop is being affected by various disease caused by bacteria, fungi, and viruses. Among these, fungal diseases contribute heavy loss in yield and diseases such as blight, stalk and ear rot and smut have been reported with localized yield losses of about 11–50% (Nankam 1991; Ngoko 1994; Cardwell *et al*. 1997). Black bundle disease is one such complex disease caused by *Cephalosporium acremonium* Corda, which was first reported by Reddy and Holbert (1924), is responsible for significant yield losses. The symptoms of diseased plants become more or less evident after the ears have reached the milk stage. The most conspicuous symptoms are the presence of blackened vascular bundles within the intermodal region of the stalk. Other symptoms of the disease are appearance of purple midribs of leaves, purple stalks, barrenness, nubbin ears and multiple ear formations (Kochler*et al.* 1925). The infected plant shows wilting symptoms, generally beginning form the top leaves, leaves become dull green, eventually loose colour and become dry. Disease kills the plant prematurely after flowering (Reddy and Holbert 1924).

Arbuscular mycorrhizal fungi play a key role in natural ecosystems and influence plant productivity, plant nutrition and plant resistance (Demir and Akkopru 2007). Biological control preserves environmental quality by a reduction in applying chemical inputs and is characteristic of sustainable management practices (Altieri 1994, Barea and Jeffries 1995). AMF have potential to reduce disease caused by fungal pathogens i.e., *Phytopthora, Sclerotinia, Rhizoctonia, Pythium, Verticillium* and *Aphanomyces* (Azcon-Aguilar and Barea 1996, Demir and Akkopru 2007 and Aysan and Demir 2009).

Effective management strategies have not been developed so far as the disease is considered to be more complex in nature. Biological control could be the best alternative and may be helpful, especially against soil borne pathogens (Hajieghrari *et al*. 2008). It is evident from several studies that arbuscular mycorrhizal (AM) fungi associations have been shown to reduce damage caused by soil-borne plant pathogens (Aguilar and Barea 1996). *Glomus fasiculatum* and *Gigaspora margarita* decrease root rot diseases caused by *Fusarium oxysporum* in Asparagus (Matsubara *et al.* 2001), *Glomus clarum*was able to reduce the root necrosis caused by *Rhizoctonia solani* in cow pea (Abdel-Fattah and Shabana 2002) and *Glomus mossae* was shown to systemically reduce disease infection caused by *Gaeumannomyesgraminis* in Barley (Khaosaad *et al.* 2007). Therefore, in the present study an effort was made to evaluate the effect of three AM fungi (*Glomus fasiculatum* (GF)*, Glomus mossae* (GM) and *Acaulispora laevis* (AL) on the management of black bundle disease caused by *C. acremonium*in in poly house condition.

MATERIALS AND METHODS

Source of inoculums

All the three species of AMF used in the present study were isolated form native rhizospheric soil of maize and identified based on morphological characters by consulting suitable keys (Schenck and Perez 1987) and visiting online INVAM identification website. *C.acremonium* was isolated form infected part of the maize plant tissues and identified based on morphological characters suing suitable keys (Nagamani *et al.* 2006). All the cultures were stored at 4°C till further use.

Pot experiment

The experiments were conducted in pots under greenhouse condition using sterile sandy, loamy soil to understand the potentiality of AM fungi in the management of black bundle disease. Pots (25cm in diameter) were filled with disinfected soil and sand mixture in the ratio of 3:1 at the rate of 20kg/pot. The experimental treatments were CAalone, CA+GF, GF alone, CA+GM, GM alone, CA+AL, AL alone and overall control (without any inoculum). All the treatments were maintained in triplicate.*C.acremonium* culture of 15 days old growth was added to all the pots.*Glomus fasiculatum*, *Glomus mossae*and *Acaulispora laevis*was added at the rate of 50 g of inoculum to each pot along with the carrier soil and root debris.

The AM fungi inoculum of the respective treatments and 15 days old cultures of pathogen were mixed in the soil before sowing the seeds. The pots were sown at the rate of six surface sterilized (with 2% sodium hypochloriteand washed in distilled water for 2-3 times) susceptible maize seeds (Renuka-G25) obtained from Agriculture research station, Arabhavi, Belgaum, Karnataka, India. After seedling emergence three plants were maintained in order to prevent competition.The pots were fertilized with NPK @ 1.0:1.5:0.5 gram pot^{-1} in 2 doses at the interval of 45 days of plant growth. Pots were irrigated regularly to maintain moisture and monitored to record disease symptoms till plant attained maturity (90 days). The disease incidence was calculated by using the following formula.

$$\text{Percentage of disease incidence} = \frac{\text{Total no. of plants showing disease symptoms}}{\text{Total no. of plants observed (sown in pots)}} \text{ X } 100$$

Assessment of AM fungal colonization

Percent root colonization of AM fungi in the representative root samples were evaluated by root clearing and staining technique (Phillips and Hayman1970) and percent association was calculated by slide technique (Giovanetti and Mosse 1980).

$$\text{Percent of root colonization} = \frac{\text{Total no.of root bits shows colonization}}{\text{Total no.of root bits observed}} \text{ X } 100$$

Estimation of dry weight: After 90 days of plant growth, the plants from all the treatments (including control) were uprooted, taking care not to damage the roots, the roots were washed in running water till the adhering soil particles were removed. The collected plant portions were oven dried at 72°C for 48 hours. The dry weight of the plants was recorded.

RESULTS AND DISCUSSION

The results of the present investigation clearly showed that *C. acremonium*which is causal agent of black bundle diseases of maize can be managed by AM fungi *G. Fasiculatum*.The experimental results clearly indicate that, in the pots inoculated with *G. fasiculatum* no disease incidence was recorded whereas, and in the pots inoculated with *A. leavis* and*G. mossae* 16.66 % of disease incidence was recorded (Table 1).Further, high degree of percent colonization was observed all the treated plants (Table 1).The growth parameters viz. dry weight and plant height were significantly increasedin mycorrhizal maize plants compared to non-mycorrhizal maize plants (Table 1). The dry weight of the plant wasrecorded high in *G. fasiculatum* treated plants (115.45 grams) followed by *G. mossae* (114 grams) and *A. leavis* alone (100 grams) treated. In case of dual inoculation (inoculated with AMF and CA), the plants inoculated with *G. fasiculatum,* reduced dry weight was recorded (70 grams) followed by *G. mossae* (89 grams) and *A. leavis* (112 grams).

The height of the plants varied in different treatments when compared to control. In CA+GF treatment showed 110.05 cm height which is very less when compared to all other treatments. The highest height was recorded in dual treatment CA+GM pots (137.16cm) followed 136.52cm which is recorded in negative control. Remaining all treatment showed considerably high when compared to positive control (Table 1). This clearly suggests that, in treatment CA+GF there is reduction of disease incidence, plant dry biomass and plant height. Plant height was recorded very high in negative control treatment when compared to other treatments, as it was evident from the earlier reports that CA increases the plant height (Reddy and Holbert 1924). Further, the presence of CA enhanced the percent colonization of AM fungi.

Table1. Illustration of the effects of various treatments of AM fungi on maize plants.* Values are the means of three replicates

Sr. No.	Treatments	Percent colonization of AM fungi*	Percent disease incidence*	Dry weight in grams*	Plant height in cm*	Total No. of leaves*
1	*Cephalosporium acremonium* (CA) (Negative control)	_	66.66%	± 81	± 136.52	6.3
2	*CA + G. fasiculatum*	100%	Nil	± 70	± 110.05	7.3
3	*G. fasiculatum*	98.75%	_	±115.45	± 121.41	7.3
4	*CA +G. mossae*	100%	16.66%	± 89	±137.16	7
5	*G. mossae*	96.25%	_	± 114	± 124.46	7.4
6	*CA +A. leavis*	100%	16.66%	± 112	± 130.37	7
7	*A. leavis*	95.00%	_	± 100	± 124.86	7.3
8	Control (without any inoculum) (Positive Control)	_	_	± 117.5	± 117.98	7.6

This may be attributed to the fact that, plants depends more on AM fungi during stress conditions (Table 1 and Fig.1 A - I) which is evident from earlier reports (Akthar and Siddiqui 2008). The resultsof the present study indicate that, potential benefits could be obtained from the AM fungi in the management of black bundle disease of maize.

ACKNOWLEDGMENT

The authors are grateful to Dr. M.C Wali, Agriculture research station, Arabhavi, Belgaum, Karnataka, Indiafor providing black bundle disease susceptible seed variety.

LITERATURE CITED

Abdel-Fattah GM and Shabana YM. 2002. Efficacy of the arbuscular mycorrhizal fungus *Glomus clarum* in protection of cowpea plants against root rot pathogen *Rhizoctoniasolani. J. Plant Dis. Prot.* **109**:207-215.

Akthan MS and Siddiqui ZA. 2008. Arbuscular mycorrhizal fungi as potential Bioprotectants against plant pathogens. *In: Mycorrhizae, Sustainable Agriculture and Forestry*, Siddiqui ZA, Akhtar MS and Futai K (Eds). Springer Netherlands, Dordrecht, TheNetherla.

Al-Askar AA and Rashad YM. 2010. Arbuscular mycorrhizal fungi, A biocontrol agent against common bean *Fusarium* root rot disease. *Plant Pathology Journal.* **9**(1):31-38.

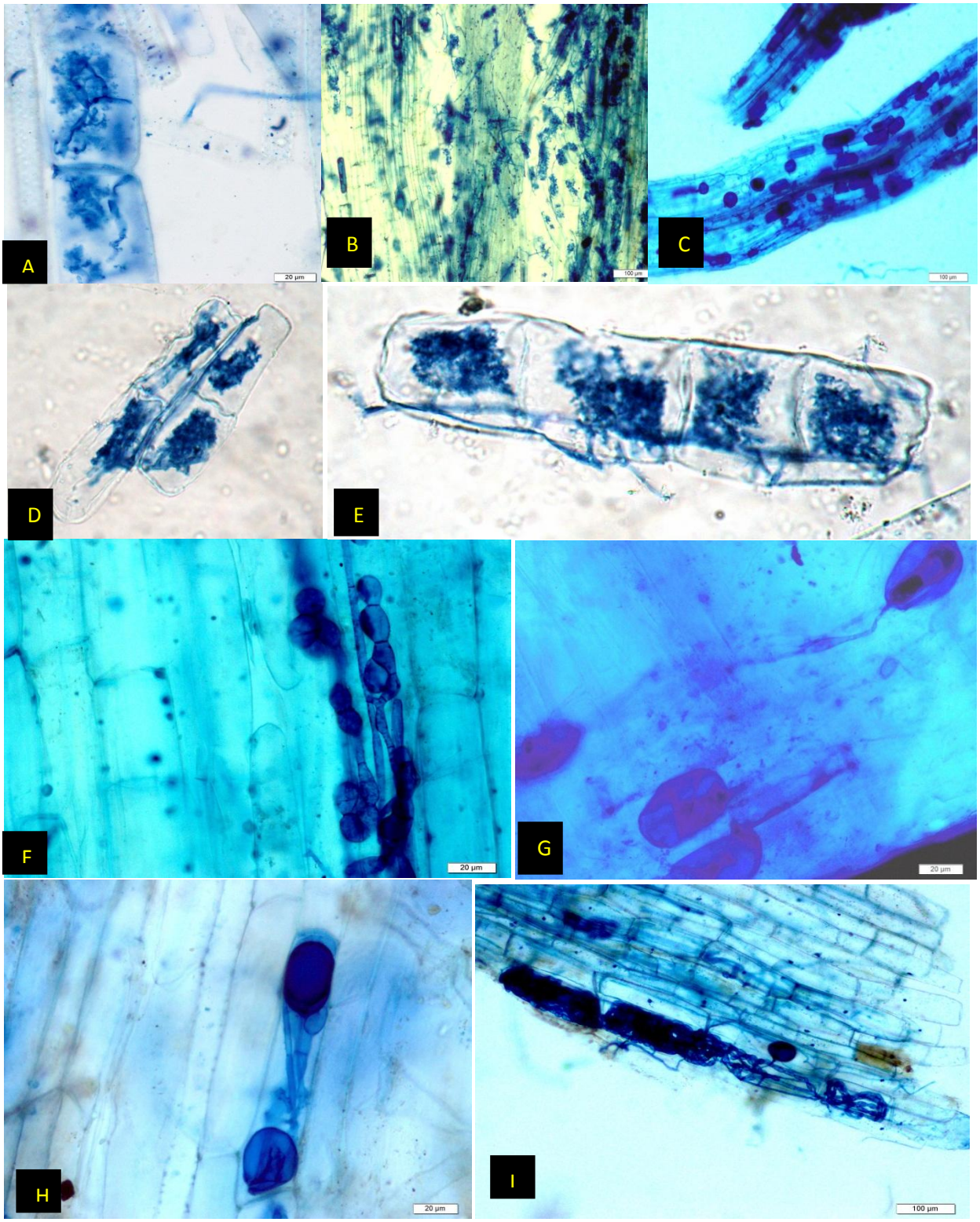

Fig. 1: *Acaulispora leavis*-arbuscles, vesicles and hyphae (A-E).*Glomus fasiculatum*-vesicles and hyphae (F and G).*Glomus mossae*-vesicles and hyphae (H and I).

Altieria MA. 1994. Sustainable agriculture*. Encyl. Agric. Sci.***4**:239-247.

Azcon-Aguilar C and Barea JM. 1996. Arbuscular mycorrhizas and biological control of soil borne plant pathogens – an overview of the mechanisms involved. *Mycorrhiza* **6**:457-464.

Azcon-Agailor C and Barea JM. 1996. Arbuscular mycorrhizal fungi and *Rhizobium leguminosarum* Biovaar. *Phaseoli* against *Sclerotinia sclerotiorum* de berry in the common Bean (*Phaseolus vulgaris*). *Plant pathology Journal*. **87**:74-78.

Borea JM and Jefferies P. 1995. Arbuscular mycorrhizas in sustainable soil plant systems*. In: Hock B. Varma A (Eds) mycorrhizal structure function*, *Molecular biology and biotechnology*. Springer, Heidelberg.Pp521-559.

Cardwell KF, Schuthless F and Ndemah Rand Ngoko Z. 1997.A systems approach to assess crop health and maize yield losses due to pests and diseases in Cameroon. *Agriculture, Ecosystems and Environment.***65**: 33**–**47.

Demir S and Akkopru. 2007. Using of arbuscular mycorrhizal fungi (AMF) for biocontrol of soil borne fungal plant pathogens. *In: Biological control of plant diseases*, Chincholkar SB and Mukerji KG (Eds). Haworth press, USA. Pp17-37.

Gerdemann JW and Nicolson TH. 1963. Spores of mycorrhizal endogene species extracted from the soil by wet sieving and decanting. *Transaction of British Mycological society.***46**:235-244.

Giovanetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots.*New Phytologist.* **84**:489-500

Hosamani PA, Lakshman HC, Sandeepkumar K, Kadam MA and Kerur AS. 2011. Role of arbuscular mycorrhizae in conservation of *Withaniasominifera*. *Bioscience Discovery.* **2**(2):201-206.

Khaosaad T, Garcia-Garrido JM, Steinkellner S and Vierheiling H. 2007. Take-all disease is systemically reduced in roots of mycorrhizal barley plants*. Soil. Biol. Biochem*. **39**:727-734.

Kling JG and Edmeades G. 1997. Morphology and growth of maize IITA/CIMMYT.Research guide 9 Training programme. *International institute of tropical agriculture IITA*, Ibadan, Nigeria, Pp31.

Kochler B, Dungan GH and Holbert JR. 1925. Factors influencing lodging in corn Bulltin No. 266.University of Illinois, Agricultural Experiment station.Urbana, Illinois, Pp311-371.

Liasu MO and Shosanya O. 2007. Studies of microbial development on mycorrhizosphere and rhizosphere soils of potted maize plants and the inhibitory effects of rhizobacteria isolates on two fungi*. African journal of biotechnology*. **6**(5):504-508.

Matsubara Y, Ohba N and Fukui H. 2001. Effect of arbuscular mycorrhizal fungus infection on the incidence of *Fusarium* root rot in Asparagus seedlings*.J.Jap.Soc. Hortic. Sci.***70**:202-206.

Maya C and Lakshman HC. 2009. Interaction between arbuscular mycorrhizal fungi (AM fungi) and *Rhizobium*: And their effects on *Cassia occidentalis*Linn. *National journal of life science.***6**(1):25-30.

Nagamani A, Kunwar IK and Manoharachary C. 2006. *Handbook of Soil Fungi,* I.K. International Pvt. Ltd., New Delhi, India, Pp106.

Nankam JC.1991. Incidence of blight and race of *Exserohilumturcicum*in Cameroon. Proceedings of the SAFGRAD Inter-Network Conference in Niamey, 7–14 March 1991, Niger,Pp 257–261.

Ngoko Z 1994. *Maize Diseases in the Highlands of Cameroon.* Technical Bulletin, Institute of Agricultural Research,Pp22.

Phillips JM and Hayman DS. 1970. Improved procedures for cleaning roots and staining parasite and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Transaction of British mycological society*.**55**:158-160.

Pradeepkumar Singh, Meenakshi Singh and Deepak Vyas. 2010. Biocontrol of *Fusarium* wilt of chickpea using arbuscular mycorrhizal fungi and *Rhizobium leguminoserum* Biovar. *Caryologia.***63**(4):349-353.

Reddy CS and Holbert JR. 1924. The black bundle disease of corn.*Journal of Agriculture research.***27**: 177-205.

Schenck NC and Perez Y. 1987. *Manual for the identification of VA Mycorrhizal Fungi***,** 1453 Fifield Hall University of Florida, Gainesuille, Florida, Pp1-245.

Tahat MM, Kumaruzaman, Sijam and Othman R. 2010. Mycorrhizal fungi as a biocontrol agent. *Plant Pathology Journal.* **9**(4):198-207.

Vierheiling H, Steinkellner S, Khaosaad T and Garcia-Garrido JM. 2008. The biocontrol effect of mycorrhization on soil borne fungal pathogens and autoregulation of the AM symbiosis.One mechanism, two effects. *Mycorrhiza*.Pp307-320.

Vigo C, Norman JR and Hooker JE. 2000. Biocontrol of the pathogen *Phytopath parasitica* by arbuscular mycorrhizal fungi is a consequence of effects on infection loci. *Plant pathology.***49**:509-514.