

Effects of *Glomus aggregatum* on Lethal Yellowing Disease of Java Citronella Caused by *Pythium aphanidermatum*

N. RATTI*, M. ALAM, S. SHARMA, and K.K. JANARDHANAN
Central Institute of Medicinal & Aromatic Plants, P.O. CIMAP,
Lucknow 226015, India. Tel. +91-522-342683, Fax. +91-522-342666,
E-mail. root@cimap.sirnetd.ernet.in

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Abstract

Effects of the vesicular-arbuscular mycorrhiza (VAM), *Glomus aggregatum* Schenck and Smith emend Koske on the lethal yellowing disease of Java citronella (*Cymbopogon winterianus* Jowitt) caused by *Pythium aphanidermatum* (Edson) Fitzp. and its interaction with the pathogen affecting plant growth, biomass production, N, P and K concentrations and acid phosphatase activity were investigated under glasshouse conditions. Citronella plants infected by *P. aphanidermatum* (Pa treatment) were chlorotic and showed a reduction in biomass production from 39.8 to 18.2 g plant⁻¹, N, P and K concentrations in shoot from 14.7, 3.51 and 12.7 to 13.3, 1.74 and 5.4 mg g⁻¹ dry weight, respectively, and acid phosphatase activity from 17.52 to 11.08 $\mu\text{m p-nitrophenol min}^{-1} \text{mg}^{-1}$ fresh weight over untreated healthy control plants. Treatments of 15dGa+Pa and simGa+Pa reduced lethal yellowing by 80% and 60%, respectively as compared with Pa treatment. Further, 15dGa+Pa treatment increased the biomass by 163.74%, N, P and K concentrations in shoot by 51.88%, 152.29% and 157.41% and acid phosphatase activity in root by 80.96%, respectively, as compared with Pa treatment. Colonization by the VAM fungus (Ga treatment) also enhanced biomass, N, P and K concentrations and acid phosphatase activity over the non VAM control. It is concluded that *G. aggregatum* improves the biomass production and reduces the damaging effect of *P. aphanidermatum* on Java citronella.

Keywords: *Glomus aggregatum*, *Cymbopogon winterianus*, Java citronella, *Pythium aphanidermatum*, lethal yellowing, N, P and K concentration, phosphatase activity

*The author to whom correspondence should be sent.

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1. Introduction

Java citronella (*Cymbopogon winterianus* Jowitt), an aromatic grass, is cultivated for the production of citronella oil in tropical and subtropical regions of the world with moderately high summer rainfall. In India, it is commercially cultivated in different regions such as Uttar Pradesh, Himachal Pradesh, Karnataka, Kerala, and Assam. The oil is one of the chief sources of many important perfumery chemicals viz. citronellal, citronellol and geraniol, which are extensively used in the soap, perfumery, cosmetic and flavouring industries. Recently, the commercial cultivation of Java citronella has been reported to be severely affected by *Pythium aphanidermatum* causing lethal yellowing especially during the rainy season (Alam et al., 1992). The plants infected by *P. aphanidermatum* showed retarded early growth and chlorosis, which in the advanced stages of infection led to premature drying and death. Severe incidence of the disease has come to limit the production of citronella oil in India.

Vesicular-arbuscular mycorrhizal (VAM) fungi are associated with almost all crop plants (Bolan, 1991). Beneficial effects of VAM fungi on plant growth have been well documented (Mosse, 1973; Gianinazzi-Pearson et al., 1981). It has been shown that VAM treatment reduced the severity of many diseases due to adverse effects on the development of fungal root pathogens (Linderman, 1992; Datnoff et al., 1995; Torres-Barragan et al., 1996). VAM fungi enhance resistance in citrus seedlings to diseases caused by *Phytophthora parasitica* (Davis and Menge, 1980) and in tobacco and cotton by *Thielaviopsis basicola* (Baltruschat and Schonbeck, 1975; Schonbeck and Dehne, 1977). There are several hypotheses for reduction of disease symptoms by VAM fungi. One is that reduction of disease in VAM plants is due to increased P nutrition provided by the VAM fungus, which makes the plant more resistant or able to compensate for the effects of the disease (Graham and Menge, 1982; Trotta et al., 1996). A second explanation for reduced diseases in VAM plants is that the fungus enhances populations of microorganisms antagonistic to plant pathogens (Meyer and Linderman, 1986; Caron et al., 1986). A third hypothesis is that VAM fungi alter host physiology, thereby making the root more resistant to pathogens (Wick and Moore, 1984). Dehne et al. (1978) observed increased antifungal chitinase in VAM roots and suggested that increased arginine accumulation in VAM roots suppressed *Thielaviopsis basicola* sporulation. Chitinase, chitosanase and β -1,3-glucanase activities were enhanced in *Allium* and *Pisum* roots inoculated with *Glomus versiforme* than in non mycorrhizal roots (Dumas-Gaudot et al. 1992). Pre-establishment of VAM fungus suppressed pathogen invasion efficiently, thereby reduced the severity of the disease (Dehne and Schonbeck, 1975; Rosendahl, 1985).

There is no information available pertaining to the effect of VAM fungi on the root borne diseases of Java citronella. Therefore, we determined the influence of a VAM fungus (*Glomus aggregatum*) on lethal yellowing disease of Java citronella caused by *P. aphanidermatum*.

2. Materials and Methods

Planting

Healthy slips were taken out from fully green clones of Java citronella growing in the experimental fields of the Central Institute of Medicinal & Aromatic Plants (CIMAP), Lucknow. Their roots were removed and the slips were washed thoroughly in tap water to remove soil particles completely. They were then surface-sterilized with 0.5% sodium hypochlorite for 5 min, washed thoroughly with sterile water and two slips were planted in earthen pots filled with 8 Kg autoclaved soil (pH 7.5, Ec 0.825 dsm⁻¹, available P 15.60 µg g⁻¹ soil, total N 0.08%, Na 0.96 mg g⁻¹ soil, K 3.65 mg g⁻¹ soil and organic carbon 0.25% (Jackson, 1973). The soil was sandy-loam, collected from the CIMAP experimental fields. The pots were kept in the glasshouse and irrigated with sterile water, as needed.

Method of inoculation

A soil culture of *Glomus aggregatum* Schenck and Smith emend Koske maintained on palmarosa (*Cymbopogon martinii* (Roxb) Wats var. *motia*) in sterile soil (Gupta and Janardhanan, 1991) was used as inoculum. The inoculum contained 50 spores g⁻¹ of soil and was spread 2 cm below the surface of the soil. The inoculum of *P. aphanidermatum* was prepared by growing it on corn meal agar (CMA) in Petri dishes at 25°C±2°C under fluorescent light for 7 days. The culture was then cut into 1 cm strips and flooded with 20 ml of sterile distilled water. Twenty millilitre inoculum containing 1×10⁷ zoospores ml⁻¹ was applied in each pot to the exposed roots around the base of the plants to be treated by *P. aphanidermatum*.

Treatments

The five treatments were: Inoculation with *G. aggregatum* alone = Ga treatment; *G. aggregatum* inoculation 15 days prior to inoculation with *P. aphanidermatum* = 15dGa+Pa; simultaneous inoculation of *G. aggregatum* and *P. aphanidermatum* = simGa+Pa; inoculation with *P. aphanidermatum* alone =

Pa treatment; and untreated control. Each treatment had two plants with five replications.

Evaluation of VAM infection on plant traits

Observations on chlorosis and mortality were recorded weekly, while root colonization by VAM, biomass production, N, P and K concentrations, phosphatase activity and oil content were analysed at the time of harvest. Herbs and roots yield were determined after drying at 40°C for 7 days. Mycorrhizal infection was observed by microscopic examination of root samples by the method of Phillips and Hayman (1970). Per cent root colonization was recorded by the grid-line intersection method of Giovannetti and Mosse (1980). VAM spores were isolated from the soil samples by wet sieving and decanting method of Gerdemann and Nicolson (1963).

Phosphatase activity was measured using a method of Andersch and Scyptinski (1947) as modified by Bergmayer (1974). Phosphorus concentration was estimated using a Tecator flow injection analyser FIA Star Model 5010, while N content was estimated using a Tecator Kjeltac Auto 1030 Analyser. Oil was extracted from the leaves by steam distillation using a Clavanger apparatus and was estimated quantitatively.

Statistical analysis

Treatments were compared for statistical significance using Duncan's multiple range test (Duncan, 1955) for all characters under study. For the purpose of analysis of variance, the lay out and analysis of a randomized complete block design was followed. The correlation of P concentration in stem with biomass and of phosphatase activity with P concentration and biomass have been studied using linear correlation coefficient (r).

3. Results

Effect of G. aggregatum on lethal yellowing and root rot caused by P. aphanidermatum

Although the plants inoculated by *P. aphanidermatum* started showing mild yellowing in new emerging leaves after one week, the chlorosis was distinctly produced after two weeks (Fig. 1, upper B). Both VAM and control plants were free from chlorosis (Fig. 1, upper A&D). *P. aphanidermatum* caused necrosis on the roots (Fig. 1, lower A), while *G. aggregatum* permitted

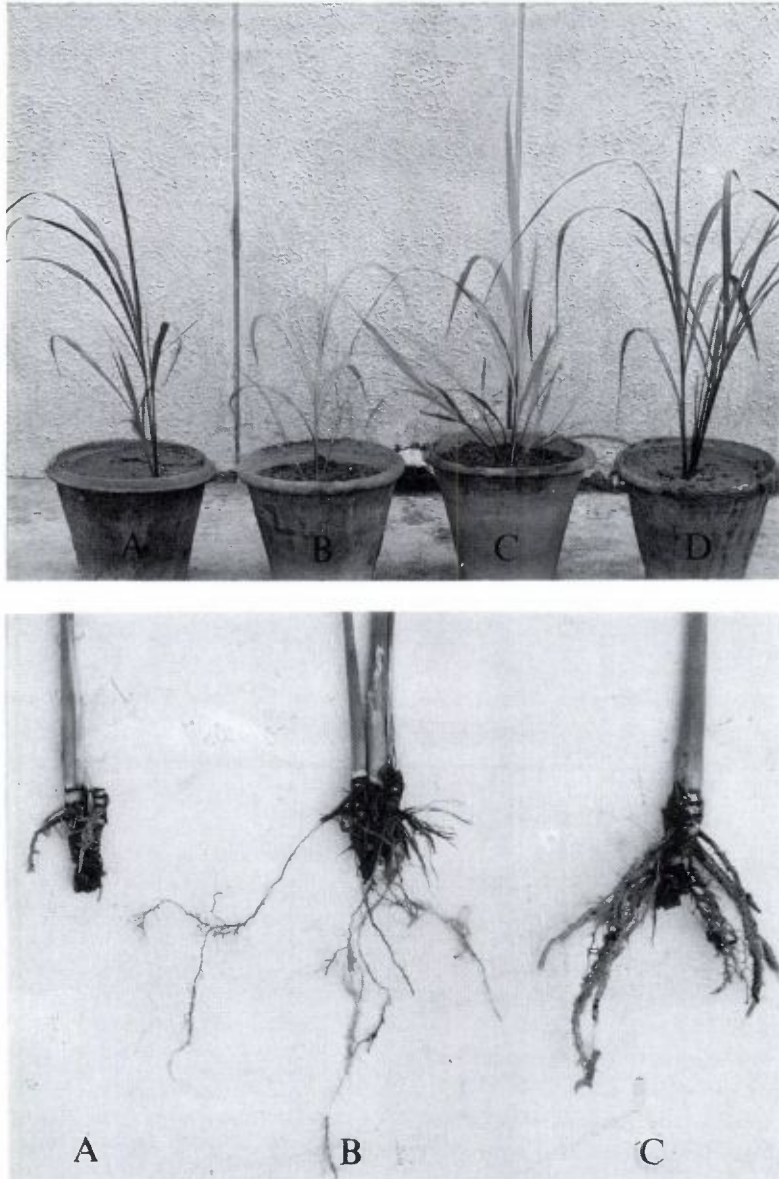


Figure 1. Effect of *Glomus aggregatum* on lethal yellowing disease of Java citronella caused by *Pythium aphanidermatum*. Upper: Uninoculated control (A); Pa treatment (B); simGa+Pa treatment (C), and Ga treatment (D). Lower: Roots infected by *P. aphanidermatum* (A) showing complete rotting; roots infected by *G. aggregatum* inhibiting the rotting caused by *P. aphanidermatum* (B) and healthy roots infected by *G. aggregatum* (C).

roots to grow normally (Fig. 1, lower C). *G. aggregatum* reduced the rotting of roots by *P. aphanidermatum* in simGa+Pa treatment (Fig. 1, lower B). Chlorosis and mortality were decreased by 80% after the 8th week of 15dGa+Pa treatment (Figs. 2 and 3). Pre-establishment of *G. aggregatum* was more effective than its simultaneous inoculation with the pathogen.

Effect of G. aggregatum and P. aphanidermatum on the growth of Java citronella

Ga treatment produced an average of 8.6 tillers plant⁻¹ and a biomass of 67.2 g plant⁻¹, which was significantly higher than in those of the other treatments (Fig. 4 and Table 1). The Pa treatment produced an average of 2.8 tillers plant⁻¹ and a biomass of 18.2 g plant⁻¹ which was 54.3% less than untreated control. 15dGa+Pa treatment produced an average of 6 tillers plant⁻¹ and a biomass of 48.0 g plant⁻¹, which was significantly higher over Pa treatment, but simGa+Pa treatment had no significant effect on tillering and biomass production (Fig. 4 and Table 1).

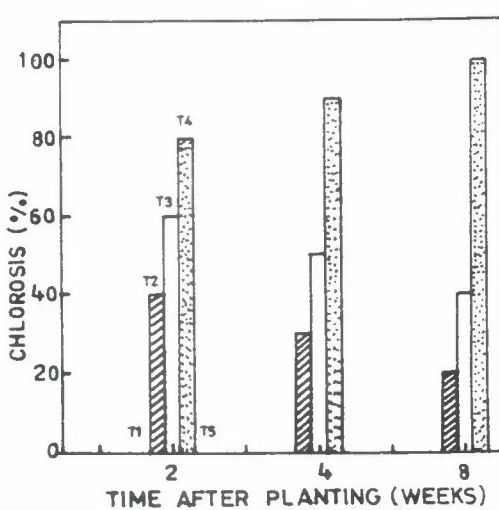


Fig. 2.

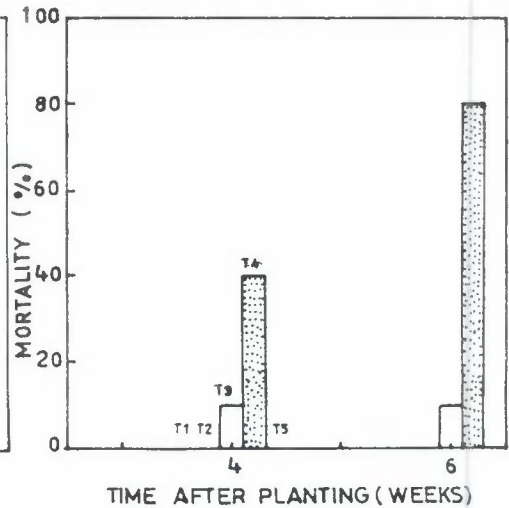


Fig. 3.

Figure 2. Effect of *G. aggregatum* on chlorosis (%) caused by *P. aphanidermatum* in Java citronella after 2nd, 4th and 8th week. Ga treatment (T1); 15dGa+Pa treatment (T2); simGa+Pa treatment (T3); Pa treatment (T4); and untreated control (T5).

Figure 3. Effect of *G. aggregatum* on mortality (%) caused by *P. aphanidermatum* in Java citronella after 4th and 8th week. Ga treatment (T1); 15dGa+Pa treatment (T2); simGa+Pa treatment (T3); Pa treatment (T4); and untreated control (T5).

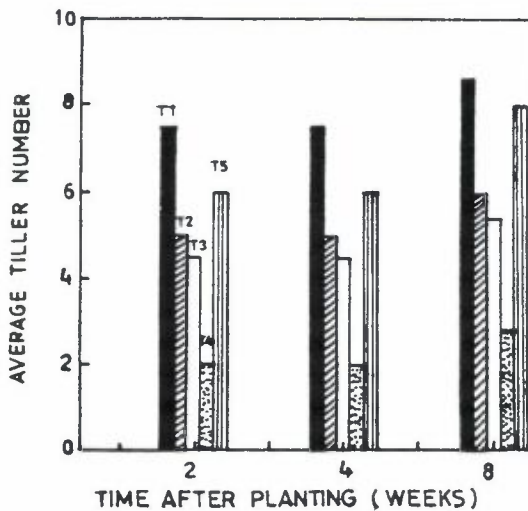


Figure 4. Effect of *G. aggregatum* on average tillers plant⁻¹ in Java citronella after 2nd, 4th and 8th week. Ga treatment (T1); 15dGa+Pa treatment (T2); simGa+Pa treatment (T3); Pa treatment (T4); and untreated control (T5).

VAM root infection and spore population in soil

In Ga treatment chlamydospore formation and root colonization were 532 spores 100 g⁻¹ soil and 72.5%, respectively which were significantly reduced to 465 spores 100 g⁻¹ soil and 57.1% colonization in simGa+Pa treatment. The 15dGa+Pa treatment had no significant effect on spore formation, but colonized roots significantly over the simGa+Pa treatment (Table 1).

Effect of G. aggregatum and P. aphanidermatum on N, P and K concentration and acid phosphatase activity

Nitrogen, P and K concentrations in shoot and root were highest in the Ga treatment (Table 2). There was more N (24.3, 16.7 mg g⁻¹), P (7.28, 1.82 mg g⁻¹) and K (14.6, 8.3 mg g⁻¹) in the shoots and roots of VAM plants than in untreated control. Acid phosphatase activity was also significantly higher in VAM roots than in non VAM roots. The enzyme activity was positively correlated with the shoot P content ($r=0.89$, $P<0.05$) and shoot dry weight ($r=0.98$, $P<0.01$), respectively. The P concentration in shoot and biomass were also significantly correlated ($r=0.96$, $P<0.01$).

Table 1. Effect of *G. aggregatum* and *P. aphanidermatum* interaction on growth, oil content and VAM infection of Java citronella.

Treatments	Height (cm)	Herb yield (g)	Root yield (g)	Total biomass (g)	Oil content ml 100 g ⁻¹ fw	Root coloniz. (%)	Spores 100 g ⁻¹ soil
Ga (T1)	126.4 a	35.0 a	32.2 a	67.2 a	1.44 a	72.5 a	532 a
15dGa+Pa (T2)	114.2 a	27.6 b	20.4 b	48.0 b	1.35 a	65.6 b	502 ab
simGa+Pa (T3)	99.6 b	16.2 c	8.0 c	24.2 d	0.98 c	57.1 c	465 b
Pa (T4)	76.2 c	12.6 c	5.6 c	18.2 d	0.92 c	—	—
Control (T5)	96.8 b	22.6 b	17.2 b	39.8 c	1.18 b	—	—

Values are the means of five replications. Means followed by the same letter do not differ significantly ($P < 0.05$) as determined by Duncan's multiple-range test. fw = fresh weight.

Table 2. Effect of *G. aggregatum* and *P. aphanidermatum* interaction on N, P and K concentration and acid phosphatase activity of Java citronella.

Treatments	N content (mg g ⁻¹ dw)		P content (mg g ⁻¹ dw)		K content (mg g ⁻¹ dw)		Acid phosphatase act. ($\mu\text{m p-nitroph.}$ $\text{min}^{-1} \text{mg}^{-1} \text{fw}$)
	Shoot	Root	Shoot	Root	Shoot	Root	
Ga (T1)	24.3 a	16.7 a	7.28 a	1.82 a	14.6 a	8.3 a	21.72 a
15dGa+Pa (T2)	20.2 b	14.1 b	4.39 b	1.64 b	13.9 b	4.4 c	20.05 a
simGa+Pa (T3)	14.3 c	12.8 c	3.02 d	1.60 b	10.4 d	4.0 c	12.59 c
Pa (T4)	13.3 d	10.1 d	1.74 e	1.41 c	5.4 e	3.4 d	11.08 c
Control (T5)	14.7 c	13.2 c	3.51 c	1.64 b	12.7 c	6.3 b	17.52 b

Values are the means of five replications. Means followed by the same letter do not differ significantly ($P < 0.05$) as determined by Duncan's multiple-range test. fw = fresh weight, dw = dry weight.

Effect of *G. aggregatum* on oil content of Java citronella

The results shown on Table 1 indicated that the oil content decreased significantly by 22% in Pa treatment as compared with the untreated control. In

Ga treatment the oil content increased by 22% and 56.5% over control and Pa treatment respectively, while in 15dGa+Pa treatment an increase of 46.7% was recorded over Pa treatment. The simGa+Pa treatment had no significant effect on oil content over Pa treatment.

4. Discussion

Lethal yellowing of Java citronella is reported to be caused by *P. aphanidermatum* (Alam et al., 1992). In the present study, it has been demonstrated that infection by *P. aphanidermatum* reduced the growth of Java citronella and caused severe chlorosis leading to death. The results indicate that *G. aggregatum* inhibits root rot caused by *P. aphanidermatum*, reduces the intensity of the disease, and leads to substantial recovery from chlorosis after 8 weeks (Fig. 2). Torres-Barragan et al. (1996) found significant protection in onion plants against *Sclerotium cepivorum* causing white rot, as compared to nonmycorrhizal controls. They found pre-inoculation with the VAM fungus more effective than simultaneous inoculation with the pathogen. Stewart and Pfleger (1977) reported that pre-inoculation of *Poinsettia* plants with VAM fungi gave significant control in root rot disease caused by *Pythium* and *Rhizoctonia*. The reduced intensity of the disease in VAM plants indicates that Java citronella infected by VAM fungi was less susceptible to damage by the pathogen. Other workers have also reported that in VAM plants infection by fungal root pathogens is inhibited (Volpin et al., 1994; Newsham et al., 1995; Dugassa et al., 1996). Root colonization by *G. aggregatum* was higher when it was inoculated 15 days prior than simultaneously with *P. aphanidermatum*. This provides evidence that in the presence of *P. aphanidermatum*, root colonization by VAM is adversely affected. Cordier et al. (1996) reported that pre-inoculation of *Glomus mosseae* significantly reduced the number of hyphae of *P. nicotianae* var. *parasitica* in the cortex of tomato plants as compared to non VAM plants. Early VAM colonization may be responsible for change in host physiology which is responsible for resistance against the pathogen attack (Linderman, 1992).

Plants treated with *G. aggregatum* often have shown higher P concentrations and acid phosphatase activities than non VAM plants. Reduction of disease in VAM plants was due to increased P nutrition provided by the VAM fungus, which might make the plant more resistant or able to compensate for the effects of the disease as reported earlier (Gianinazzi-Pearson et al., 1981; Graham and Menge, 1982). It appears that *G. aggregatum* enhanced the uptake of P in the plant resulting in vigorous growth and the synthesis of more oil than in non VAM plants. Dodd et al. (1987) reported that an increase in acid

phosphatase activity in VAM plants depends on the species of the VAM fungus. Trotta et al. (1996) suspected combination of mechanisms including the role of P nutrition by *Glomus mosseae* for reduction of root rot infection in tomato by *Phytophthora nicotianae* var. *parasitica*.

Based on our findings, it has been concluded that *G. aggregatum* acts as biocontrol agent against *P. aphanidermatum*. The treatment of Java citronella slips with *G. aggregatum* prior to planting can reduce the occurrence of lethal yellowing disease under field condition.

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