Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar

Spanish Journal of Agricultural Research 2011 9(3), 901-905
ISSN: 1695-971-X
eISSN: 2171-9292

Short communication. Management of *Macrophomina* root rot of mungbean using dry leaves manure of *Datura metel* as soil amendment

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Abstract

The antifungal activity of leaves of *Datura metel* for *in vitro* and *in vivo* management of *Macrophomina phaseolina*, the causal agent of root rot in mungbean [*Vigna radiata* (L.) R. Wilczek], was studied. In a laboratory bioassay, methanolic extracts of 0.5, 1.0, 1.5, 2.0 and 2.5 g/100 mL significantly reduced the fungal biomass by 36-47%. In a pot trial, dried powdered leaves of *D. metel* were mixed in soil at 0.5, 1.0 and 1.5% (w/w) with soil already inoculated with the target fungal pathogen. A negative control without fungal inoculation and leaf amendment, and a positive control with *M. phaseolina* inoculation only, were used for reference. Plant mortality due to *M. phaseolina* inoculation was significantly reduced by 42% and 80% due to 1.0 and 1.5% dry leaves amendments, respectively, as compared to positive control. There was a gradual increase in root and shoot growth of the plants with increase in dose of soil amendment. Generally, 1.5% dose significantly enhanced root and shoot growth as compared to positive control. *M. phaseolina* root rot in mungbean can effectively be managed by amending the soil with 1.5% dry leaf manure of *D. metel*.

Additional key words: leaf residue; medicinal plants; natural fungicides; *Vigna radiata.*

Resumen

Comunicación corta. Control de la pudrición carbonosa de la raíz causada por *Macrophomina* en judía mungo utilizando estiércol de hojas secas de *Datura metel* como enmienda para el suelo

Se ha estudiado la actividad antifúngica de las hojas de *Datura metel* para el control *in vitro* e *in vivo* de *Macrophomina phaseolina*, el agente causal de la pudrición carbonosa de la raíz en judía mungo [*Vigna radiata* (L.) R. Wilczek]. En un bioensayo de laboratorio, extractos metanólicos de 0,5, 1,0, 1,5, 2,0 y 2,5 g/100 mL redujeron significativamente la biomasa del hongo entre 36% y 47%. En un ensayo en macetas, se mezclaron hojas secas pulverizadas de *D. metel* al 0,5, 1,0 y 1,5% (p/p) con el suelo ya inoculado con el patógeno fúngico. Se utilizaron como referencia un control negativo y un control positivo inoculado solamente con *M. phaseolina*. La mortalidad de plantas debida a la inoculación con *M. phaseolina* se redujo significativamente en un 42% y 80% debido a las enmiendas de hojas secas al 1,0% y 1,5%, respectivamente, en comparación con el control positivo. Según aumentaba la dosis de la enmienda del suelo, hubo un aumento gradual en el crecimiento de la raíz y de los brotes de las plantas. En general, la dosis del 1,5% aumentó significativamente el crecimiento de la raíz y de los brotes en comparación con el control positivo. En conclusión, la pudrición carbonosa de la raíz causada por *Macrophomina* en judía mungo puede ser controlada por enmiendas en el suelo con estiércol de hojas secas al 1,5% de *D. metel.*

Palabras clave adicionales: extracto de hoja; fungicidas naturales; plantas medicinales; *Vigna radiata*.

Macrophomina phaseolina (Tassi) Goidanich, the charcoal rot fungus, occurs worldwide and is one of the most economically important fungal pathogens reported to infect about 500 plant species in more than 100 families, including many important crops such as alfalfa, chickpea, maize, sorghum, soybean and sunflower (Wyllie, 1993; Mihail and Taylor, 1995). Charcoal rot disease can be controlled by soil fumigation with methyl

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Received: 21-09-10. Accepted: 08-07-11.

Abbreviations used: MIC (minimum inhibitory concentration).

bromide-chloropicrin (Smith and Krugman, 1967); however, increased environmental concern has been a major factor in triggering regulatory restrictions on the use of soil fumigants, and in many countries, a phase-out of methyl bromide is underway (Gamlie *et al.,* 2000). Generally, crop rotation and irrigation are used to control the disease but these strategies are not always effective (Gaige *et al.,* 2010). Solar pasteurization has not been effective in controlling charcoal root rot disease (McCain *et al.,* 1982).

Studies have shown that soil-borne plant diseases can effectively be managed by amending the soil with different types of plant materials (Bailey and Lazarovits, 2003; Riaz *et al.,* 2007 and 2010). Ndiaye *et al.* (2007) reported that *M. phaseolina* in cowpea (*Vigna unguiculata*) can be reduced significantly by amending the soil with millet [*Pennisetum glaucum* (L.) R. Br.] residues. *Datura metel* L (syn. *Datura alba* Nees) is a popular medicinal plant of family Solanaceae that it is known for its antibacterial activity (Gnanamani *et al.*, 2003), antifungal activity against phytopathogens (Dabur *et al.,* 2004) and herbicidal activity against parthenium (*Parthenium hysterophorus* L.) weed (Javaid *et al.,* 2010a). The aim of the present study was to investigate the effect of soil amendment with dry leaf manure of *D. metal* on development of root rot disease in mungbean.

Mungbean plants infected with root rot were collected from University of the Punjab Lahore, Pakistan in March 2010. Diseased portions of roots were cut into 5 mm pieces and surface sterilized by 0.1% sodium hypochlorite solution for 1 min and then thoroughly rinsed with sterilized water. These pieces were aseptically placed on Petri plates containing malt extract agar medium and incubated at 25 ± 2°C under 12 hours light period daily for 7 days. After that time, the fungal colonies developed on root pieces were transferred to fresh malt extract agar plates. The color of fungal colony was grey that darken with age. Characteristic black oblong microsclerotia were observed. On the basis of these characteristics, the isolated fungus was identified as *Macrophomina phaseolina* (Wyllie, 1993; Watanabe, 2002).

One hundred grams of dried and powdered leaf material from *D. metel* were soaked in 600 mL of methanol for 7 days. Afterwards, material was filtered through muslin cloth followed by a filter paper. The filtrates were evaporated under vacuum in a rotary evaporator at 45°C which yielded 5.09 g of crude methanolic leaf extract.

Six milliliters of stock solution were prepared dissolving 4.5 g of the crude methanolic leaf extract form *D. metel* in sterilized distilled water. Six 250-mL conical flasks containing 58 mL of malt extract medium each were autoclaved and then cooled at room temperature. Five concentrations of leaf methanolic extract (0.5, 1.0, 1.5, 2.0 and 2.5 g/100 mL) were made by adding 0.4, 0.8, 1.2, 1.6 and 2.0 mL of the stock solution, and 1.6, 1.2, 0.8, 0.4 and 0.0 mL of sterilized distilled water, respectively, to each flask to make a total volume of 60 mL in each flask. This volume for each treatment was divided into three equal portions and dispensed into 100-mL conical flasks to serve as replicates. For control treatment, 2 mL of sterilized distilled water were added to 58 mL of malt extract medium.

Mycelial discs from the hyphal tips of a 7-days old *M. phaseolina* fungal culture were cut using a sterilized 5-mm diameter cork borer and transferred to each flask. Each treatment was replicated three times. Flasks were incubated at room temperature for 7 days. After that time the fungal biomass in each flask was filtered, dried at 60ºC to constant weight in an electric oven and weighed.

Chickpea (*Cicer arietinum* L.) seeds were purchased from a local market. Seeds were thoroughly washed with tap water and soaked for 2 hours. After that time, seeds were boiled for about 30 min to make them soft. One kilogram of boiled chickpea seeds was autoclaved at 121°C for 30 min in transparent plastic bags. Then chickpea seeds were inoculated with a 14-days old culture of *M. phaseolina.* Inoculated chickpea seeds were incubated at room temperature for 14 days and stored in refrigerator at 4°C until use (3 days).

Plastic pots 6-cm in diameter and 10-cm deep were filled with soil at 350 g pot^{-1}. Pot soil was inoculated by thorough mixing of 5 g of *M. phaseolina* inoculum prepared on chickpea and watered. Pots were left for 1 week under natural environmental conditions (mean minimum and maximum temperatures 16°C and 27°C, respectively; relative humidity 57% and total bright sunshine duration 237.5 h during this month) in March 2010 for the establishment of fungal inoculum. After that time, pot soil was amended with 0.5, 1.0 and 1.5% (w/w) dry powdered leaf material of *D. metel.* Pots for positive control were prepared by inoculating with fungus only, while the negative control treatment did not include either fungal inoculum or dry leaf amendment. Each treatment was replicated three times with five pots in each replicate. Each pot was irrigated with 50 mL of tap water and left for 15 days under natural environmental conditions as mentioned above. Pots

were regularly watered whenever required to keep the pot soil moist.

Mungbean seeds were surface sterilized with 1% sodium hypochlorite solution for 2 minutes followed by three thorough washings with sterilized water. Five surface-sterilized seeds were sown in every pot in April 2010, when the temperature was moderately high. After 3 days of sowing, pots were placed under natural environmental conditions (mean minimum and maximum temperatures were 23°C and 36°C, respectively; relative humidity 29% and total bright sunshine duration 288.0 h during this month), in the Herbal Heritage Garden at the Institute of Agricultural Sciences, University of the Punjab, Lahore. Pots were irrigated with tap water in such a way that some water stress conditions were there for the development of disease.

Data regarding root and shoot growth was recorded in terms of length (cm), and fresh and dry biomass (g) after 15 days of seed germination. Number of dead plants was recorded and a mortality incidence was calculated. Means of three replicates of every treatment and their standard errors were calculated using the software Microsoft Excel 2003. Data were analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test using COSTAT version 4.

The methanolic leaf extract of *D. metel* caused a significant reduction in biomass of the target fungal pathogen relative to control; reductions of 36 to 47% were caused by the methanolic leaf extract (Fig. 1). Earlier, Rajesh and Sharma (2002) reported antifungal activity of *D. metel* extracts against three *Aspergillus* species namely *A. fumigatus*, *A. flavus* and *A. niger* with minimum inhibitory concentration (MIC) values of 625 µg mL^{-1}. Ma *et al.* (2006) isolated five withanolide glycosides named daturataturin A, daturametelins H-J and 7,27-dihydroxy-1-oxowitha-2,5,24-trienolide from the methanolic extract of *D. metel* aerial parts. In addition, Gupta *et al.* (1991) reported a hexacyclic withanolide namely Withametelin B from leaves of *D. metel*. Withanolides are known to exhibit antifungal activities **(**Choudhary *et al.,* 1995).

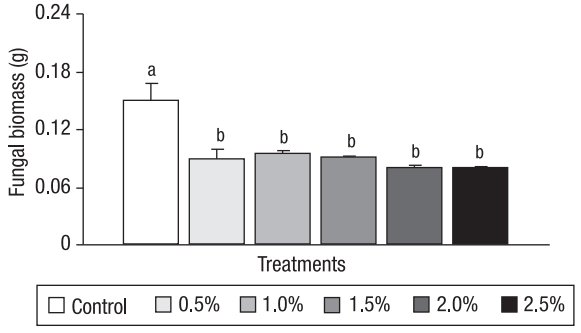

Figure 1. Effect of different concentrations of methanol leaf extract of *Datura metel* on biomass of *Macrophomina phaseolina* in laboratory bioassay. Vertical bars show standard error of means of three replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by Duncan's Multiple Range Test.

The highest shoot length was recorded in negative control (neither fungal inoculum nor *D. metel* leaf amendment). *M. phaseolina* inoculation (positive control) significantly reduced shoot length by 82%. Different soil amendments had an effect on shoot length, with longer shoots as leaf manure dose increased. However, there were non-statistical differences between lower concentrations (0.5% and 1.0%) and the positive control. The highest dose of leaf manure (1.5%) significantly enhanced shoot length as compared to positive control mean value and did not differ significantly to the negative control shoot length (Fig. 2a). Response of shoot fresh weight to amendments on soil inoculated with *M. phaseolina* was similar to that of shoot length (Fig. 2b). For shoot dry weight, although the effect of soil amendments in alleviating the biotic stress was not significantly different from the positive control, there were pronounced increases of 3.5 and 4.2-fold due to 1% and 1.5% manure dose over the positive control (Fig. 2c). The enhanced plant growth may be due to the alleviating effect leaf manure over the biotic stress posed by *M. phaseolina* (Rajesh and Sharma, 2002). Moreover, according to Javaid *et al.* (2010a,b), lower doses of 1 and 2% leaf manure of *D. metel* and other medicinal plants of the family Solanaceae are known to enhance plant growth, while higher doses generally suppress plant growth.

Root length was significantly reduced due to *M. phaseolina* inoculation. There was a gradual increase in root length as the manure dose was increased. The effect of 1.0% and 1.5% manure doses was statistically significant over the positive control (Fig. 2d). Both fresh and dry root biomass were significantly enhanced by 1.5% manure dose as compared to positive control and was statistically similar to the root biomass for the negative control treatment (Fig. 2e and f).

Data regarding the effect of dry leaf manure of *D. metal* on plant mortality of mungbean in soil inoculated with *M. phaseolina* is presented in Figure 2g. In negative control treatment pots, none of the plants were affected by the disease. In positive control pots, mortality reached 87%. Soil amended with 0.5% leaf manure exhibited non-significant effect, however, further

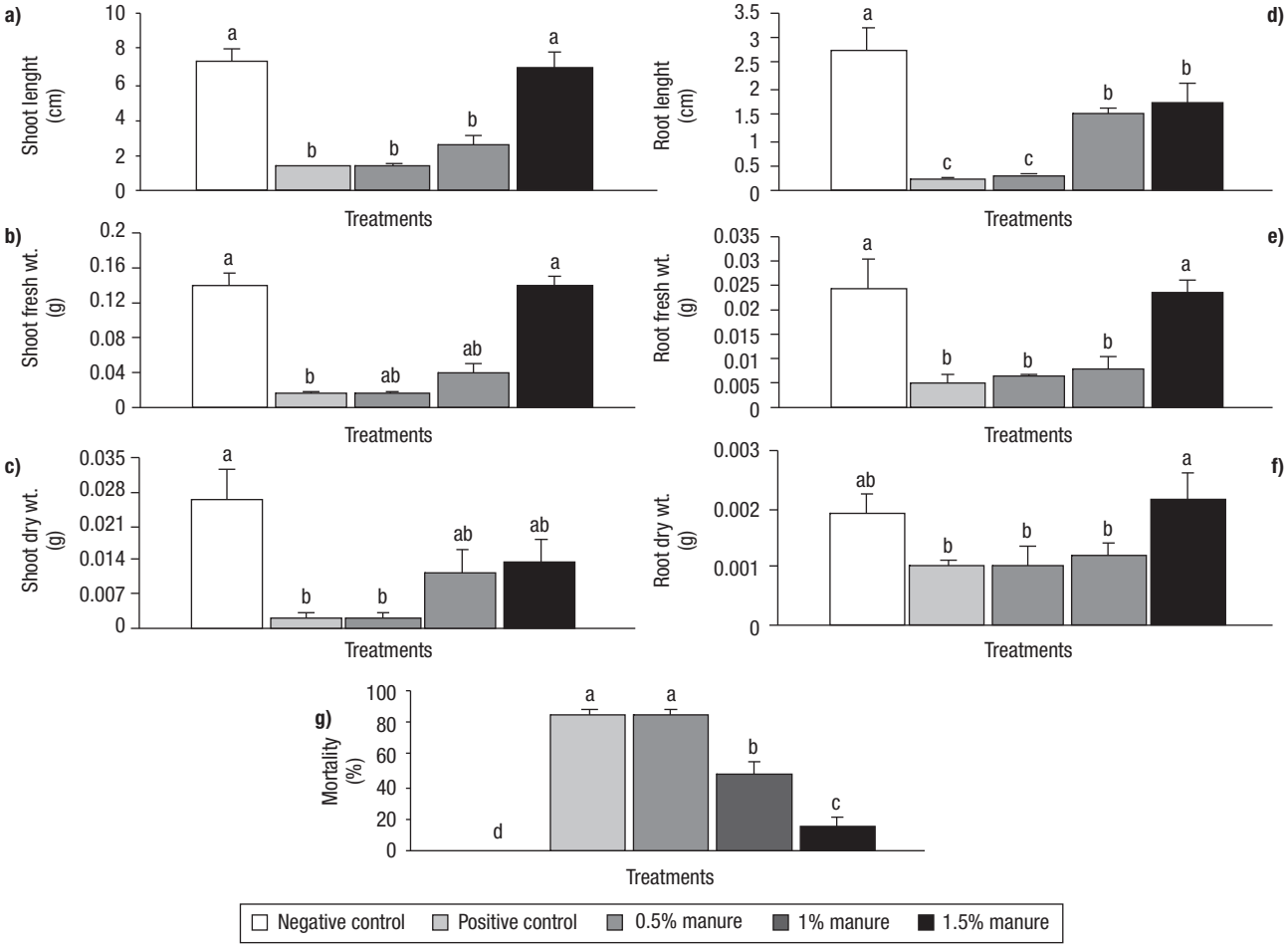

Figure 2. (a-g). Effect of *Macrophomina phaseolina* inoculation at different doses of dry leaf manure of *Datura metel* on shoot growth (a-c), root growth (d-f) and plant mortality (g) of mungbean. Vertical bars show standard error of means of three replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by Duncan's Multiple Range Test. Negative control: soil non inoculated with *M. phaseolina* and non-amended with leaf manure. Positive control: soil inoculated with *M. phaseolina* and non-amended with leaf manure. 0.5%, 1% and 1.5% manure: soil inoculated with *M. phaseolina* inoculation and with the corresponding percentages of leaf manure.

increase in manure dose gradually and significantly reduced disease incidence. There were 42 and 80% reduction in plant mortality due to 1.0 and 1.5% manure dose, respectively, in comparison to the positive control (Fig. 2g). It is possible that various types of withanolide present in leaves of *D. metel* (Gupta *et al.,* 1991; Ma *et al.,* 2006), would have been released in the rhizospheric soil through extraction in the irrigation water or during decomposition of residues. This would lead decreased plant infection and mortality (Choudhary *et al.,* 1995; Rajesh and Sharma, 2002). The present study concludes that *M. phaseolina* root rot in mungbean can be effectively managed by amending the soil with 1.5% dry leaf manure of *D. metel* without any significant losses in plant growth.

Acknowledgements

We are grateful to University of the Punjab, Lahore, Pakistan for providing financial assistance to complete this project.

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