

Management of dry root rot of greengram (*Vigna radiata*) caused by *Macrophomina phaseolina*

S CHOUDHARY¹, S PAREEK² and J SAXENA³

Banasthali University, Banasthali, Rajasthan 304 022

Received: 17 December 2009; Revised accepted: 11 August 2010

ABSTRACT

The experiment of screening was conducted during 2006 and 2007 to evaluate 25 greengram [*Vigna radiata* (L.) Wilczek] genotypes for resistance to root rot caused by *Macrophomina phaseolina* (Tassi.) Goid. Complete resistance to root rot could not be found, however, 'MSJ 118' genotype exhibited highest suppression of dry root rot, followed by the genotype 'KM 4-59' and appeared as moderately resistant genotypes. In compost experiment 4 different composts, viz banana (*Musa accuminata*), nadep, *Calotropis* and vermicompost have been used as soil amendments to determine their influence on disease incidence and soil microflora during rainy (*kharif*) 2007 and summer season 2007 and 2008 in greengram plants in pots (cv. 'RMG 492'). The composts in general enhanced microbial populations in amended soil resulting in reduction in the disease incidence. Banana and vermicompost amended treatments were found superior in the suppression of dry root rot, while *C. procera* compost-amended treatment showed the least suppression of the disease. Among composts, the counts of fungi, bacteria and actinomycetes were again higher in the vermicompost and also in the soil, amended with vermicompost, followed by banana compost amended soil, while *Calotropis* and *Calotropis* compost-amended soil had least counts of microflora.

Key words: Composts, Genotypes, Integrated Management, *Macrophomina phaseolina*, Root rot, Screening

Among pulses, greengram [*Vigna radiata* (L.) Wilczek] occupies an important position. Low and erratic rainfall, improper nutrient management and occurrence of many diseases are principal factors for its low productivity in semi-arid and arid regions of India. The crop suffers from various fungal, bacterial and viral diseases. Among the various fungal diseases, root rot caused by *Macrophomina phaseolina* (Tassi.) Goid. causes considerable yield loss by reducing the plant population in the field. The amendments of soil with decomposable organic matter is an effective method of changing the soil and rhizosphere environment. Amended soil held more water than non-amended sandy soil, which in turn reduced *M. phaseolina* population and its infection on the host plant due to enhanced antagonism or competition for sites (Sheikh and Ghaffar 1979, Filho and Dhingra 1980). The mechanism by which amendments of soil with compost suppresses plant pathogens is basically through the changes

brought in the soil, such as improvements in the water-holding capacity, soil aggregates, porosity and overall fertility. Composts enrich the soil with microflora potentially competitive or antagonistic to pathogens or release inhibitory substances or volatiles during decomposition. Considering the environmental unfriendly and uneconomic values of fungicides, attempts are being made to identify the sources of resistance to this disease and also 4 composts have been used as soil amendments to observe their influence on disease incidence and microbial population in amended soil.

MATERIALS AND METHODS

The experiment was conducted in the Department of Bioscience and Biotechnology, Banasthali University, Banasthali. The test pathogen, *M. phaseolina* was isolated from root rot infected plants of cv. 'RMG 492' greengram grown in Distt. Tonk, Rajasthan and multiplied on sand: maize meal medium (9 : 1 w/w) for 15 days at 28°C.

Twenty five genotypes were evaluated for resistance to root rot by adopting standard procedure during 2006 and 2007. Out of these 22 genotypes ('KM 4-21', 'KM 4-22', 'KM 4-24', 'KM 4-25', 'KM 4-27', 'KM 4-29', 'KM 4-32', 'KM 4-34', 'KM 4-35', 'KM 4-36', 'KM 4-38', 'KM 4-39', 'KM 4-41', 'KM 4-42', 'KM 4-44', 'KM 4-47', 'KM 4-51',

Based on a part of PhD thesis of the first author submitted to Banasthali University during 2009

¹Ph D scholar (e mail; sumi_15dec83@yahoo.co.in), ²Ex-Professor (e mail: savita_pareek@rediffmail.com), ³Reader (e mail: jyotissaxena2000@yahoo.co.in), Department of Bioscience and Biotechnology

'KM 4-55', 'KM 4-59', 'KM 4-60', 'KM 4-61', 'KM 4-63') were obtained from the Central Arid Zone Research Institute, Jodhpur, while 3 ('RMG 492', 'RMG 62' and 'MSJ 118') were procured from Krishi Vigyan Kendra, Banasthali University, Banasthali, Rajasthan.

Greengram seeds were sown on 24 July 2006 and 7 August 2007 and harvested on 26 September 2006 and 9 October 2007, respectively. These were sown in plots of 4 m × 8 m. Twenty-five rows were replicated thrice in the plot. Ten seeds of each genotype were sown in each row having 1 m row length. Inoculum multiplied on media was placed in row at 5–10 cm depth @ 200 g/m row length to increase the disease pressure. Data on disease incidence was recorded from 15 randomly selected plants from each row of plot at 60 days and the reaction of the genotypes to the disease was determined by the score chart, ie Free-no disease Resistant. 0.1 to 5.0, Moderately resistant-5.1 to 10.0, Moderately susceptible-10.1 to 25.0, Susceptible-25.1 to 50.0, Highly susceptible-50.1 to 100.0 (Agrawal 1993).

Fully dried residues of banana (*Musa accuminata*) and *Calotropis* (*Calotropis procera*) were used for the preparation of composts. The process of composting was initiated under partially anaerobic conditions in separate plastic containers according to the principles of the Indore method (Howard and Ward 1931). In each plastic container, 2 kg of residue enriched with 20 g gypsum (1%) and 40 g urea (2%) was filled layer by layer. Each layer was provided with sufficient moisture and then covered with a mixture of 800 g cowdung and 1200 g field soil. Three such layers of residues with soil-dung mixture were placed in each container. These containers were finally covered with plastic lid and sealed. The content was mixed at every 7–8 days interval.

Fresh cowdung was collected at one place. For cooling and removal of methane gas, sufficient water was sprinkled for 8–10 days. Mixing was done twice. Such prepared cowdung was spread uniformly on shaded bed (12 m × 3 m × 2 m). Approximately, 3000 worms were released in equal layer on prepared bed. After spraying the water, the bed was covered with jute cloth. First inversion was done after 25 days of filling of bed. The bed was covered again with jute cloth and water was sprinkled. Second inversion was done after 25 days of first inversion. After 70 days, the desired vermicompost was available for use.

Fully dried residue of farmwaste in sufficient quantity was used for the preparation of nadep compost (20 : 80). The process of composting was initiated in pits (4.5 m × 1.8 m × 0.9 m). Firstly, farm waste layer was spread at the base of the pit and covered with cowdung. Five such layers of farm waste with cowdung were placed one after the other in the pit. This pit was finally covered with the mixture of cowdung and black soil and there after water was sprinkled. It takes around 90 days for nadep compost to mature.

These composts (each 10 tonnes/ha) were filled to a depth

of 3 cm by a spade in separate pots (22.5 cm) containing *M. phaseolina* infested soil 2 days before sowing. Pots without any amendments served as control. Eight replications were used for each treatment. 'RMG 492' greengram seeds were sown @ 10 seeds/pot. Data on disease incidence was recorded after 60 days of sowing.

For biological assays, soil samples were collected from each pot after 5–6 days of mixing of compost and at the time of harvesting. These were bulked to form a composite sample from each of the eight replications. This sample was processed for the determination of microbial populations. Colony forming units (cfu) of fungi were estimated by the standard dilution plate method on Potato Dextrose Agar (PDA) and for bacteria and actinomycetes Thornton's Agar was used.

All the data were subjected to analysis of variance (ANOVA) and the treatment means were compared with CD ($P=0.05\%$).

RESULTS AND DISCUSSION

Disease incidence in greengram genotypes

On the basis of per cent dry root rot incidence, the greengram genotypes were categorized free, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible. Evaluation of 25 genotypes under artificial inoculation condition revealed that none of the genotypes was completely free from the disease (Table 1). Incidence of dry root rot ranged between 6.67–86.67 and 6.67–80% in 2006 and 2007, respectively. Maximum and minimum incidence of disease was recorded in the control genotype 'RMG 62' and 'MSJ 118', respectively during both the years of study. In 2006, all the genotypes were significantly superior over the control, while in 2007 except 3 genotypes 'KM 4-24', 'KM 4-27' and 'KM 4-36', all the others were significantly superior in resisting the attack of dry root rot pathogen. The genotypes 'MSJ 118', 'KM 4-44' and 'KM 4-59' appeared as moderately resistant consistently in both the seasons with significantly equal disease incidence (5.1–10%). However, 8 genotypes, viz 'KM 4-21', 'KM 4-22', 'KM 4-25', 'KM 4-29', 'KM 4-35', 'KM 4-42', 'KM 4-47' and 'KM 4-63' were categorized as moderately susceptible having disease incidence of 10.1–25%, while 10 genotypes, viz 'RMG 492', 'KM 4-32', 'KM 4-34', 'KM 4-38', 'KM 4-39', 'KM 4-41', 'KM 4-51', 'KM 4-55', 'KM 4-60' and 'KM 4-61' were classified as susceptible recording a disease incidence of 25.1–50% during both the years of study. Rest of the genotypes, ie 'KM 4-24', 'KM 4-27' and 'KM 4-36' exhibited highly susceptible (50.1–100%) reactions to the pathogen.

Our findings are in close agreement with those of other workers who had reported that in most of the crops, complete resistance against *M. phaseolina* is not available. (Kraft *et al.* 1994). In other rainfed arid legumes also, many efforts have been made to locate resistance sources, 'Kutch 8' and 'RGC 471' genotype of guar [*Cymopsis tetragonoba* (L.) Taub.] were found to be moderately resistant (Lodha and

Table 1 Evaluation of 25 genotypes against dry root rot incidence on greengram (2006 and 2007)

Germplasm	Disease incidence (%) ^a	
	2006	2007
'MSJ 118'	6.67 (8.86)	6.67 (8.86)
'RMG 492'	26.67 (30.79)	33.33 (35.01)
'KM 4-21'	20.00 (26.57)	13.33 (13.08)
'KM 4-22'	13.33 (17.71)	20.00 (21.93)
'KM 4-24'	53.33 (46.92)	60.00 (51.14)
'KM 4-25'	13.33 (13.08)	13.33 (17.71)
'KM4-27'	53.33 (49.92)	60.00 (50.77)
'KM 4-29'	13.33 (17.71)	13.33 (13.08)
'KM 4-32'	40.00 (38.86)	26.67 (30.79)
'KM 4-34'	40.00 (38.86)	46.67 (43.08)
'KM 4-35'	20.00 (16.92)	13.33 (13.08)
'KM 4-36'	53.33 (46.92)	66.67 (60.00)
'KM 4-38'	46.67 (43.08)	40.00 (39.23)
'KM 4-39'	46.67 (43.08)	26.67 (26.15)
'KM 4-41'	46.67 (43.08)	26.67 (30.79)
'KM 4-42'	13.33 (17.71)	20.00 (26.57)
'KM 4-44'	6.67 (8.86)	6.67 (8.86)
'KM 4-47'	20.00 (21.93)	13.33 (17.71)
'KM 4-51'	26.67 (21.14)	26.67 (30.79)
'KM 4-55'	26.67 (26.15)	33.33 (30.00)
'KM 4-59'	6.67 (8.86)	6.67 (8.86)
'KM 4-60'	33.33 (34.64)	26.67 (30.79)
'KM 4-61'	26.67 (30.79)	33.33 (34.64)
'KM 4-63'	13.33 (17.71)	20.00 (21.93)
'RMG 62' (control)	86.67 (72.29)	80.00 (68.07)
CD (P=0.05%)	28.53	27.33

Figures in parentheses are Arc-sin transformed values

^aAverage of 3 replications, each having 5 plants

Solanki 1993). Absence of dominance of resistance in F₁ plants of cross between 'Kutch 8' × 'RGC 471' showed that inheritance of resistance is polygenic in nature.

Nageshwar Rao (2008) reviewed the available information on source of resistance against *M. phaseolina* in sorghum (*Sorghum bicolor* L. Moench) germplasm. Many tolerant and resistant sources have been listed. In sorghum also, drought tolerance and resistance restricts charcoal rot development.

Effect of composts on disease incidence

Table 2 depicts that in summer 2007, 2008 and *kharif* 2007, all the compost-amended treatments were significantly effective in reducing the incidence of dry root rot compared to non-amended control. Least incidence of dry root rot was observed in the banana and vermicompost amended treatments in summer 2007, while in summer 2008, banana compost-amended treatment appeared superior in suppressing the dry root rot incidence. On the other hand, in *kharif* 2007, vermicompost-amended treatments had highest suppression of dry root rot, followed by banana and nadeop compost amended treatments.

On the basis of pooled average, least incidence of dry

Table 2 Effect of composts on root rot incidence on greengram plants (cv. RMG 492) grown in *Macrophomina phaseolina* inoculated soil at 60 days

Treatment ^a	Disease incidence (%) ^c		
	Summer 07	<i>Kharif</i> 07	Summer 08
<i>M. phaseolina</i> inoculum ^b			
Vermicompost	37.50 (33.75)	25.00 (26.25)	37.50 (33.75)
Banana compost	37.50 (33.75)	37.50 (37.50)	25.00 (26.25)
Nadeop compost	50.00 (45.00)	37.50 (37.50)	37.50 (37.50)
<i>Calotropis</i> compost	62.50 (52.50)	50.00 (41.25)	50.00 (45.00)
Non-amended soil + inoculum	87.50 (75.00)	75.00 (60.00)	75.00 (60.00)
CD (P=0.05%)	17.07	15.85	12.92

Figures in parenthesis are Arc-sin transformed values

^aComposts were amended @ 10tonnes/ha in soil, ^b*M. phaseolina* inoculum was added @ 20g/kg soil

^cAverage of 8 replications

root rot was observed in the banana and vermicompost-amended treatments, while highest incidence was observed in the *Calotropis* compost amended treatment. Mathur *et al.* (2003) observed that vermicompost was significantly superior in reducing the incidence of *Fusarium oxysporum* causing wilt of fenugreek in field conditions. Ratnoo and Bhatnagar (1993) also reported a reduction in the incidence of *M. phaseolina* in cowpea by wheat (*Triticum aestivum* L. emand. Fioris Paol.) straw amendment, whereas Sharma *et al.* (1995) recorded the same by mustard cake and cauliflower residues. Lodha *et al.* (2002) not only noticed lower disease incidence in clusterbean but also found significant increase in yield. Release of *M. phaseolina* inoculum from infected plant residues was significantly reduced by organic composting.

Microbial populations in compost

Among 4 composts, maximum population of total fungi and bacteria was significantly higher in vermicompost. However, actinomycetes population remained significantly at par in banana, nadeop and vermicompost (Table 3). Least microbial population was estimated in *Calotropis* compost.

Amended soil at initial level and at harvest

It is clear from Table 3 that amending soil with different composts significantly improved the total fungal and bacterial population compared to non-amended soil. An initial fungal population in the soil raised from 2×10^3 to $5-8 \times 10^3$ cfu/gm and bacterial population from 7.5×10^5 to $14-22.17 \times 10^5$ cfu/g after incorporation of different composts in field soil. Significant improvement in fungal and bacterial population was estimated in the soil, which was amended with

Table 3 Microbial population in compost and compost-amended soil at initial and at harvesting stage

Type of compost	Fungi ^a ($\times 10^3$ cfu/g compost)			Bacteria ^a ($\times 10^5$ cfu/g compost)			Actinomycetes ^a ($\times 10^5$ cfu/g compost)		
	Compost	At initial	At harvest	Compost	At initial	At harvest	Compost	At initial	At harvest
Vermi-compost	10.50	8.00	8.50	29.50	22.17	23.33	1.83	1.50	1.33
Banana compost	7.17	6.00	7.33	27.00	17.50	18.50	1.33	1.17	1.00
Nadep compost	8.00	5.83	7.00	24.67	19.17	20.33	1.33	0.67	1.17
<i>Calotropis</i> compost	6.33	5.00	5.67	16.50	14.00	14.33	1.00	0.67	0.67
Soil		2.00	2.50		7.5	8.83		0.50	0.83
CD ($P=0.05\%$)	1.47	0.78	0.88	1.15	1.52	1.72	0.63	0.61	0.53

^aAverage of 6 replications for each samples

vermicompost compared to all other compost amended soil, while *Calotropis* compost-amended soil showed least improvement. Only banana and vermicompost amended soil had significant improvement in total actinomycetes population compared to non-amended soil. An initial actinomycetes population in the soil raised from 0.50×10^5 to $0.67-1.50 \times 10^5$ cfu/gm after incorporation of different composts in field soil. Vermicompost-amended soil had maximum actinomycetes population, which was significantly at par with banana compost-amended soil.

Table 3 shows that, amending soil with different composts had significant improvement in the total fungal and bacterial population compared to non-amended soil. Fungal population in the soil raised from 2.5×10^3 to $5.67-8.50 \times 10^3$ cfu/gm and bacterial population from 8.83×10^5 to $14.33-23.33 \times 10^5$ cfu/gm after incorporation of different composts in field soil. Maximum improvement in the fungal and bacterial population was estimated in the vermicompost-amended soil which was significantly superior than all other amended treatments, while *Calotropis* compost-amended soil showed least improvement. None of the compost-amended soil had significant improvement in actinomycetes population. An actinomycetes population in the soil raised from 0.83×10^5 to $0.67-1.33 \times 10^5$ cfu/gm after incorporation of different composts in field soil.

From this study, it was found that microbial count was maximum in the vermicompost and also in the soil amended with vermicompost, followed by banana compost-amended soil. These results, therefore, indicate that addition of composts to the soil, in general, and especially of vermicompost and banana compost increased the soil microflora by supporting higher population of fungi, bacteria and actinomycetes as well as causing more reduction in the soil population of *M. phaseolina*. The antagonists/saprophytes, which compete for the nutrients with the soil-borne pathogens thus might have affected the multiplication of mungbean root pathogen in soil adversely, which could be one of the reasons of low build up of pathogen in compost amended soils. Our observations get further support from the findings of Gilbert *et al.* (1968) who were of the opinion that the rise in the population of total fungi

and bacteria ultimately suppressed the growth of the pathogenic forms. Gugino *et al.* (1973) also reported that increase in total population of fungi, bacteria and actinomycetes was associated with decrease in population dynamics of *Pythium irregulare* in 100% pine bark media. The high microbial activity and biomass caused by the 'general soil microflora' in compost-amended soil prevented germination of pathogenic propagules and infection of the host, presumably through microbiostasis (Hoitink and Boehm 1999).

Our studies conclusively demonstrated that various on-farm wastes like those of banana and *Calotropis* can be effectively recycled for their use in preparation of composts. Banana peels has been considered for preparation of composts with a view to utilize this waste from vegetable market, hotels and hostels. This is an easily decomposable material.

Growing of moderately-resistant genotypes of mungbean in soil amended with these composts hold a great promise in rainfed as well as in irrigated agriculture for improving the soil fertility. There exists a great scope of utilizing composts for suppression of soil-borne plant pathogens.

REFERENCES

- Agrawal S C. 1993. *Diseases of Greengram and Blackgram*, pp 321. International Book Distributors, Dehradun.
- Filho E S and Dhingra O D. 1980. Population changes of *Macrophomina phaseolina* in amended soil. *Transactions of British Mycological Society* **74**: 471-81.
- Gilbert R G, Menzies J D and Griebel G E. 1968. The influence of volatile substances from alfalfa on growth and survival of *Verticillium dahliae*. *Phytopathology* **58**: 1051.
- Gugino JL, Pokoorny FA and Hendrix FF. (Jr.) 1973. Population dynamics of *Pythium irregulare* Buis in container plant production as influenced by physical structure of media. *Plant and Soil* **39**: 591-602.
- Hoitink HAJ and Boehm M J. 1999. Biocontrol within the context of soil microbial communities: A substrate dependent phenomenon. *Annual Review of Phytopathology* **37**: 426-7.
- Howard A and Ward Y D. 1931. *The Waste Products of Agriculture and Their Utilization as Humus*, 167 pp, Oxford University Press, London.

- Kraft J M, Haware M P, Jimnez-Diaz R M, Bayaa B and Harrabai M. 1994. Screening techniques and sources of resistance to root rots and wilts in cool season food legumes. *Euphytica* **73**: 27–9.
- Lodha S and Solanki K R. 1993. Inheritance of dry root rot resistance in clusterbean. *Indian Phytopathology* **45**: 430–3.
- Lodha S, Sharma S K and Aggarwal R K. 2002. Inactivation of *Macrophomina phaseolina* propagules during compost and efficacy of composts on dry root rot and seed yield of clusterbean. *European Journal of Plant Pathology* **108**: 253–61.
- Mathur K, Bansal R K and Gurjar R B S. 2003. Organic management of wilt of fenugreek. A seed spice. *Journal of Mycology and Plant Pathology* **33**: 491.
- Rao Nageshwar T G. 2008. Sorghum. (in) *Disease Management in Arid Land Crops*, pp 43–80. Lodha (Ed.) Scientific Publishers (India) Jodhpur.
- Ratnoo R S and Bhatnagar M K. 1993. Effect of straw, oil cakes on Ashygrey stem blight of *Macrophomina phaseolina* (Tassi.) Goid. of cowpea. *Indian Journal of Mycology and Plant Pathology* **23**: 186–8.
- Sharma S K, Aggarwal R K and Lodha S. 1995. Population changes of *Macrophomina phaseolina* and *Fusarium oxysporium* f. sp. *cumini* in the oil cake and crop residue amended sandy soils. *Applied Soil Ecology* **2**: 281–4.
- Sheikh A H and Ghaffar A. 1979. Relation of sclerotial inoculum density and soil moisture to infection of field crops to *Macrophomina phaseolina*. *Pakistan Journal of Botany* **11**: 185–9.