Relative efficacy of on-farm weeds as soil-amendment for managing dry root rot of clusterbean in an arid environment

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Summary. The effectiveness of certain on-farm weeds as soil amendments was ascertained against *Macrophomina phaseolina*, a soil-borne pathogen causing dry root rot of crops grown under rainfed conditions in arid regions. Population changes in *M. phaseolina* were determined in soils amended separately with residues (1%, w:w) of *Aerva persica*, *Celosia argentea*, *Corchorus depressus*, *Euphorbia hirta*, *Heliotropium subulatum* and *Polycarpaea corymbosa*, for a period of 90 days. Significant reductions by 90.4–100% in the population of *M. phaseolina* were achieved with all the weed residues except *P. corymbosa*. *Celosia* and *Euphorbia* residues completely eradicated viable propagules of *M. phaseolina*. A strong increase (44–61%) in the population of antagonistic actinomycetes was also found in soil amended with *Corchorus* and *Euphorbia*. In field tests, soil amended (50 g m⁻²) with *Euphorbia*, *Aerva* and *Celosia* residues significantly reduced dry root rot incidence on clusterbean and also reduced *M. phaseolina* propagules in the soil. However, dry root rot incidence in *Polycarpaea*-amended soil (5.8–24.6%) was not significantly different from that in non-amended soil (4.3–25.3%) in both years of the experiment. *P. corymbosa* also increased the number of propagules of *M. phaseolina* in the soil. The results demonstrate that dry root rot of rainfed-cultivated annual crops in arid land can be managed with certain weeds as a soil amendment.

Key words: *Macrophomina phaseolina*, *Aerva persica*, *Celosia argentea*, *Polycarpaea corymbosa*, *Euphorbia hirta*.

Introduction

*Macrophomina phaseolina* (Tassi) Goid. is the most important soil-borne pathogen of arid regions. In such regions, it causes charcoal or dry root rot in many economically valuable plants (Lodha et al., 1986; Mihail et al., 1990). It survives in the soil as sclerotia formed in infected plant tissues (Cook et al., 1973). The population of sclerotia increases in the soil with each year of cultivation of susceptible crops (Lodha et al., 1990). In the hot arid regions of India, during the crop-free summer period, polyethylene mulching (soil solarization) increases the temperature in irrigated soil up to 58°C and reduces the density of viable propagules of *M. phaseolina* (Lodha, 1995). However, the high cost of polyethylene film restricts the use of this effective technique in the low-input agriculture of these regions. Combining cruciferous residues with one summer irrigation is another approach that was found highly effective to control *M. phaseolina*-induced diseases (Lodha et al., 1997; Mawar and Lodha, 2002). Cruciferous residues produce many biotoxic volatile compounds in the soil during decomposition (Brown et al., 1991) and the concentration of these volatile compounds is directly related to the temperature of the soil (Gamliel and Stapleton, 1993). However, adoption of this technique is limited in practice to certain pockets of the region where irrigation water and cruciferous residues are widely available. In-
corporation of organic amendments into the soil as crop residues, manure or compost is therefore the only practical alternative left in rainfed farming to reduce the soil population of *M. phaseolina* and the incidence of dry root rot (Hakeem and Ghaffar, 1977; Osunlaja, 1990).

In arid regions, the regular addition of organic amendments is of the utmost importance in maintaining the tilth, fertility and productivity of agricultural soils: protecting them from wind and water erosion and preventing nutrient losses through runoff and leaching. During the later part of crop growth in the rainy season it is frequently observed that certain weeds can withstand even deficient soil moisture. In traditional cultural practice, these weeds are left in the field to check soil erosion and are uprooted or ploughed down into the soil before planting the crop seeds in the next season. Not only commercially valuable plants, but also many on- and off-season weeds are susceptible hosts of *M. phaseolina* (Ghaffar and Zentmeyer, 1968; Young and Alcorn, 1984; Singh *et al*., 1990). Since sclerotia are formed during pathogenesis and are released into the soil after the disintegration of the host tissues, infected weeds may also contribute by increasing the inoculum density of *M. phaseolina* in the soil. Weeds can be important sites where pathogen populations increase and serve as inoculum reservoirs for adjacent cultivated crops (Mihail *et al*., 1987). Many of the weed flora of arid regions can be termed as on-farm wastes that can be utilized as a readily available cheaper source of organic matter, but their susceptibility to *M. phaseolina* prevent their unrestricted use. Their direct incorporation as organics may either increase or decrease the population of *M. phaseolina* in the soil. Information is therefore required on which of these weeds are suitable as a source of organic matter.

The present investigation aims to determine how various readily available on-farm weeds affect the survival of *M. phaseolina* in the soil and the severity of dry root rot on a susceptible rainy-season clusterbean (*Cyamopsis tetragonoloba* [L.] Taub.) crop.

**Materials and methods**

**Study site**

Experiments were conducted in a field where *M. phaseolina*-susceptible legumes were under cultivation at the Central Arid Zone Research Institute, Jodhpur, India (26°18’N, 73°01’S). The soil of the experimental site was loamy sand with pH 8.1 and 0.25% organic matter. The soil textural fractions consisted of 85.0% sand, 8.9% clay and 5.5% silt. Twelve soil samples were collected randomly at 0–30 cm depth to estimate the population of *M. phaseolina*. The samples were incubated on a chloroneb-mercury-rose bengal (CMRA) medium highly selective for the enumeration of *M. phaseolina* colonies (Meyer *et al*., 1973). The soil was found to have an average native population of 540 sclerotia g⁻¹ soil, ranging between 481–562 sclerotia g⁻¹ soil.

**Field surveys**

During September–December 1997, regular surveys were made of the standing rainfed annual crops like clusterbean (*Cyamopsis tetragonoloba* [L.] Taub.), pearl millet (*Pennisetum glaucum* [L.] R. Br. emend. Stuntz.), cowpea (*Vigna unguiculata* [L.] Walp.) and moth bean (*Vigna aconitifolia* [Jacq.] Marechal.) grown in different experimental blocks at the Central Research Farm of the Institute to determine the sequential occurrence of the weed flora. These experimental blocks had a previous history of *Macrophomina*-induced diseases in severe form. In 1997 the incidence of dry root rot ranged from 13–46% on the crops in these blocks. During growth and after harvest of the crops, complete plants of eleven of the most common on-farm weeds, *Aerva persica* (Burm.f.) Juss. ex Schult; *Arnebia hispidissima* (Lehm.) D.C.; *Celosia argentea* (L.); *Corchorus depressus* (L.) Christensen; *Eragrostis ciliaris* (L.) R. Br; *E. poaeoides* P. Beauv; *Euphorbia hirta* (L.); *Heliotropium subulatum* Hochst. ex D C; *Polycarpaea corymbosa* (L.) Lamk; *Pulicaria crispa* (Cass.) Benth. & Hook. and *Volutarella divaricata* Benth. were randomly collected by gently uprooting them from the soil.

**Laboratory experiment**

Twenty roots of each weed species were cut at the root-stem junction and washed with sterile water. Five 1-cm pieces were cut from each root, washed in running tap water for 2 min., rinsed in distilled water for 2 min. and after surface sterilization with 0.1% mercuric chloride plated on potato dextrose agar Petri dishes. One Petri dish was
used to plate pieces from each root. Observations on the association of *M. phaseolina* with these weeds were recorded for the next 7 days. Five weeds, *A. persica*, *C. argentea*, *C. depressus*, *E. hirta* and *H. subulatum*, having no or only very slight (<10%) *M. phaseolina* infection, plus *P. corymbosa*, which had the highest (>80%) infection, were selected for further study.

Whole plants of these 6 weeds were uprooted from the field at their vegetative stage of growth. The weeds were air-dried for at least 4 weeks and then ground up separately in a sample mill (Cyclotec 1093, Tecator, Hoganas, Sweden). Powdered weed residues were stored at room temperature (28–34°C) in polyethylene bags until further use. A pathogenic strain of *M. phaseolina* isolated from diseased roots of clusterbean was multiplied on a 5% cornmeal:sand medium for 15 days at 30±2°C. The sclerotia of *M. phaseolina* so produced were passed through a 300 mesh (53 µm) sieve. The infested material left on the sieve was examined under the microscope to confirm that it contained only sclerotia free on the sieve was examined under the microscope mesh (53 µm) sieve. The infested material left on the sieve was examined under the microscope to confirm that it contained only sclerotia free on the sieve was examined under the microscope

Another experiment was conducted in the field to study the association of *M. phaseolina* with these weeds in the soil. A pathogenic strain of *M. phaseolina* was multiplied on a 5% cornmeal:sand medium for 8 days at 28±1°C and then placed in the centre of each Petri dish and incubated for three more days. Four replications were used for each strain of actinomycetes. Strains were considered antagonistic if they induced an inhibition zone of at least 2 mm. Six Petri dishes of each medium were used to evaluate each category from each soil sample. The means of 6 Petri dishes were considered one determination per replicate of each treatment.

**Field experiment**

During laboratory evaluation the three most promising weeds in reducing *M. phaseolina* propagules (*A. persica*, *C. argentea*, *E. hirta*), and the one weed (*P. corymbosa*) that maintained *M. phaseolina* propagules in the soil were selected for further field testing. Air-dried residues of all these weeds were chopped into small pieces, 2–3 cm in size. In the last week of June, 3 kg field soil per pot was placed in 30×60 cm porcelain pots. Five kg of chopped residue per pot was placed on this soil, which was covered with a further 2 kg of field soil and 10% of water (w:w) was provided at once. After 10 days, in the second week of July, the partially decomposed weed residues and soil were taken from the pots and separately dug to a depth of
samples were digested with H$_2$SO$_4$ and distilled ferrous ammonium sulphate. For N determination, the excess chromic acid was back titrated with nitric acid in the presence of H$_2$SO$_4$ (Jackson, 1958). Carbon was determined by oxidizing it with chromic acid along with non-amended soils was estimated from these samples. As well as the C:N ratio of both amended and non-amended soil.

Three soil samples were randomly collected by a tubular probe (2.5 cm) at 0–30 cm depth from the five replication of all treatments 15 days after the harvest of the clusterbean crop in both years. The samples were bulked to form one sample for each set of replications. Bulked samples were air-dried and ground to pass through a 2 mm sieve. These were then processed to determine the M. phaseolina population each year. In addition, after the harvest of clusterbean in 1999, the total microbial and antagonistic actinomycetes population as well as the C:N ratio of both amended and non-amended soils was estimated from these samples. Carbon was determined by oxidizing it with chromic acid in the presence of H$_2$SO$_4$ (Jackson, 1958). The excess chromic acid was back titrated with ferrous ammonium sulphate. For N determination, samples were digested with H$_2$SO$_4$ and distilled water using the Kjeltec Auto System II (Tecator, Höganäs, Sweden).

Statistical analysis
The data on the microbial population, the M. phaseolina population, and final plant mortality from dry root rot were subjected to analysis of variance (ANOVA) and the treatment means were compared by LSD ($P=0.05$). The data on plant mortality were converted to angular transformed values before analysis. The data on population changes of M. phaseolina in amended and non-amended soil, and disease progression in the clusterbean crop were subjected to pooled analysis of variance for measurements over time by considering the time of observation as an additional factor (Gomez and Gomez, 1983). Correlation analysis was also done to determine the relationship between the M. phaseolina population and the incidence of dry root rot on clusterbean.

Results

Laboratory experiment
Of the eleven weeds screened to determine their association with M. phaseolina, the roots of E. hirta, C. depressus, H. subulatum and A. persica were completely free of M. phaseolina infection, and with A. hispidissima, V. divaricata and C. argentea at least one out of the ten root bits was infected. The other weeds had a greater frequency of root-infection with M. phaseolina, with a maximum being achieved by P. corymbosa (>80%).

In the soil incubation studies, the population of M. phaseolina was significantly reduced in all the weed residues except P. corymbosa as compared with non-amended soil (Fig. 1). C. argentea and Euphorbia-amended soils reduced viable propagules of M. phaseolina by 94 and 80% respectively in 30 days, which rose to 100% in 90 days. In Aerva-amended soil the viable population of M. phaseolina was reduced by 88% in 30 days, with no significant change at the next sampling date, but at the final sampling date the population of M. phaseolina was down by 94%. Similarly, in soils amended with Corchorus and Heliotropium residues, viable M. phaseolina propagules were reduced by 94 and 87% respectively in 90 days. By contrast, in Polycarpaea-amended soil there was only a 24.6% reduction in viable counts of M. phaseolina after 30 days. At this stage, M. phaseolina reduction in non-amended soil was significantly higher (48%) than that in Polycarpaea-amended soil, and this unfa-vourable trend continued on the second sampling date. However, by 90 days, the reduction in viable M. phaseolina propagules in Polycarpaea amended soil was 75.3% compared with 70% in non-amended soil.

The total number of bacteria, fungi and actinomycetes was significantly higher in all the residue amended soils compared to the non-amended soil after 90 days of incubation, except in Euphorbia amended soil where the actinomycetes did not differ significantly from their number in the non-amended soil (Table 1). A dramatic increase of 44 and 61% in the antagonistic actinomycetes population was found in soil amended with Corchorus and Euphorbia residues respectively. There was a significant increase in antagonistic actinomycetes populations also with other weed residues as compared with non-amended soil, except in soil amended with Aerva and Heliotropium-residue.
Fig. 1. Population changes of *Macrophomina phaseolina* in weed residue amended and non-amended soils recorded at 30-day intervals (LSD \( P=0.05 \)): Treatment 9.3; Interval 9.0; Treatment \( \times \) Time in days (of a particular amendment) 23.8; and Treatment \( \times \) Time in days [at a fixed interval] 21.6.
Table 1. Effect of weed residues (1%) on total numbers of bacteria, fungi, actinomycetes and antagonistic actinomycetes (g⁻¹ soil) after 90 days of soil incubation at 28±2°C under laboratory conditions.

<table>
<thead>
<tr>
<th>Amendment weed</th>
<th>Bacteria (×10⁵)</th>
<th>Fungi (×10⁴)</th>
<th>Actinomycetes (×10⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Aerva</td>
<td>21</td>
<td>14.5</td>
<td>102</td>
</tr>
<tr>
<td>Celosia</td>
<td>14</td>
<td>13.5</td>
<td>123</td>
</tr>
<tr>
<td>Corchorus</td>
<td>22</td>
<td>12.2</td>
<td>81</td>
</tr>
<tr>
<td>Euphorbia</td>
<td>16</td>
<td>21.3</td>
<td>54</td>
</tr>
<tr>
<td>Heliotropium</td>
<td>12</td>
<td>9.5</td>
<td>79</td>
</tr>
<tr>
<td>Polycarpaea</td>
<td>21</td>
<td>14.2</td>
<td>113</td>
</tr>
<tr>
<td>Control (non-amended)</td>
<td>8</td>
<td>3.9</td>
<td>52</td>
</tr>
<tr>
<td>LSD (P=0.05)</td>
<td>3</td>
<td>1.7</td>
<td>8</td>
</tr>
</tbody>
</table>

Field experiment

Development of dry root rot

Weather conditions varied during the two years of field experimentation. The seasonal rainfall was 205 in 1998 and 178 mm in 1999, compared to a normal rainfall of 298 mm. In spite of a sufficient inoculum load of M. phaseolina in the soil, six well distributed rainfall events in 1998 did not favour the occurrence of dry root rot in severe form because the clusterbean crop did not experience long durations of soil moisture stress. However, in 1999, after an initial spell of good rainfall, the crop began to experience mild moisture stress 15 days after planting, which became severe moisture stress after 25 days. Symptoms of dry root rot became conspicuous from the first week of September and variations in weekly mortality due to this disease were quite discernible with the different treatments.

In Aerva-amended plots, clusterbean plants remained virtually disease free till harvest in 1998, and in Euphorbia and Celosia-amended soils, dry root rot incidence was significantly lower than that in the non-amended control soil (Table 2). The highest plant mortality from dry root rot was recorded in the Polycarpaea-amended plots. In 1999, though the lowest mortality from dry root rot was again recorded in the Aerva-amended plots, it was not significantly different from plots amended with Euphorbia. Clusterbean mortality from dry root rot in Celosia and Polycarpaea amended plots followed a similar trend as in 1998.

There were considerable differences in dry root rot progression on clusterbean plants between residue amended and non-amended soils. In Aerva and Celosia-amended soils, plants did not die from dry root rot even after 7 days of dry root rot occurrence with other amendments. After 14 days, in both these soils clusterbean had less than 5% mortality, but subsequently a steep increase in mortality occurred in Celosia-amended plots (Fig. 2). However, in the Aerva-amended plots, disease incidence began to increase only after 21 days from initiation. In Euphorbia-amended plots, there was a linear increase in the development of dry root rot soon after initiation, but at maturity disease incidence in Euphorbia-amended soil was not significantly different from that in Aerva-amended soil. In Polycarpaea-amended plots mortality from dry root rot increased sharply after just 14 days, to levels significantly higher than with any other amended soil. In the non-amended control, dry root rot incidence increased linearly for the first 21 days, after which there was a sudden increase, bringing mortality to the level of Polycarpaea residue soils.

M. phaseolina and the total microbial population

An initial population of 504 sclerotia g⁻¹ soil was drastically reduced in all the weed residue amended plots and in the non-amended plots in 1998, as shown by samples analysed after the harvest of the crop. The reduction was greatest in Celosia-amended plots, and smallest in Polycarpaea-amended soil. In 1999, after the harvest of the crop, the population of M. phaseolina was greatest in Polycarpaea-amended soil, which was not significantly different from that in the non-amended control soil. In all the other weed amended plots the population of M. phaseolina was significantly lower than in the Polycarpaea-amended plots, with
maximum reduction in *M. phaseolina* being in *Celosia*-amended plots. However, a significant correlation between final plant mortality and the *M. phaseolina* population could not be established.

The total bacterial population was significantly higher in *Aerva* and *Celosia*-amended plots than in *Polycarpaea*-amended soil and in the non-amended control (Table 3). However, counts of fungi and actinomycetes were significantly higher in all the amended soils, with maximum counts being in *Polycarpaea*-amended soil for fungi and *Celosia* amended soil for actinomycetes. Antagonistic actinomycetes occurred in both amended and non-amended soil but their population was encouraged more by *Aerva* and *Euphorbia* amended soil. Of the various amended and non-amended soils, the lowest C:N ratio was estimated in *Celosia*-amended soil after the harvest of clusterbean crop in 1999, while *Euphorbia* amended soil had the highest C:N ratio (Table 2).

**Discussion**

Incorporation of selected weeds after partial decomposition reduced the viability of the sclerotial population of *M. phaseolina* in the soil and the incidence of dry root rot in clusterbean in the field under rainfed conditions.

Preliminary screening to determine the relative susceptibility of the weed residues to *M. phaseolina* helped in selecting field-resistant weeds only. A sharp reduction in the *M. phaseolina* counts within 30 days of incorporation of *Aerva* and *Celosia* residues into the soil could be attributed to the toxic nature of the weed residues and the release of biotoxic volatiles during initial decomposition. Further, the complete eradication of *M. phaseolina* propagules in *Celosia* or *Euphorbia*-amended soil within 90 days suggested that different types of volatiles were released during the process of decomposition. The anti-microbial activity of extracts of *Aerva* against *M. phaseolina* and certain viruses has been well documented (Verma and Srivastava, 1985; Gehlot and Bohra, 1998). *Euphorbia* is considered a natural source of certain hydrocarbons and many phyto-chemicals (Sekar and Francis, 1998). *Euphorbia* extract is reported to possess bioactivity against many other pathogens as well, including *Fusarium* (Mohamed et al., 1996). In a preliminary examination, the amount of sulphide and di-sulphides released by decomposing *Euphorbia* residues (74.6% precipitation) was greater than

<table>
<thead>
<tr>
<th>Amendment weed</th>
<th>Final plant mortality (%)</th>
<th><em>M. phaseolina</em> population (g soil$^{-1}$)</th>
<th>C:N ratio of soil after harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aerva</em></td>
<td>0.5</td>
<td>13.00</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>(4.05)*</td>
<td>(20.50)</td>
<td></td>
</tr>
<tr>
<td><em>Celosia</em></td>
<td>1.86</td>
<td>17.80</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td>(7.71)</td>
<td>(22.53)</td>
<td></td>
</tr>
<tr>
<td><em>Euphorbia</em></td>
<td>1.43</td>
<td>13.10</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>(6.92)</td>
<td>(21.12)</td>
<td></td>
</tr>
<tr>
<td><em>Polycarpaea</em></td>
<td>5.80</td>
<td>24.59</td>
<td>269</td>
</tr>
<tr>
<td></td>
<td>(13.90)</td>
<td>(29.91)</td>
<td></td>
</tr>
<tr>
<td>Control (non-amended)</td>
<td>4.33</td>
<td>25.33</td>
<td>248</td>
</tr>
<tr>
<td></td>
<td>(11.56)</td>
<td>(30.37)</td>
<td></td>
</tr>
<tr>
<td>LSD ($P=0.05$)</td>
<td>3.67</td>
<td>6.11</td>
<td>7</td>
</tr>
</tbody>
</table>

* 0.5 ton ha$^{-1}$.
* After 15 days of harvesting in 1999. Initial population was 504 sclerotia g$^{-1}$ soil in July 1998.
* Angular transformed values.
that released by Aerva (59.7%) and Celosia (66.2%). However, the greatest amount of hydrogen sulphide (20.9% precipitation) and ketonic/aldehyde derivatives (21.3%) was released by decomposing Celosia and Aerva residues respectively (data not shown). The reduction of viable *M. phaseolina* propagules by 70% even in non-amended moistened soil after 90 days indicated that soil moisture alone was a factor in reducing the population of *M. phaseolina* (Dhingra and Sinclair, 1975; Lodha, 1996). The C:N ratio of the soil after incorporation of the amendment may also have played an important role in influencing the *M. phaseolina* population (Filho and Dhingra, 1980).

Amending the soil with plant residues reduces the inoculum density of certain plant pathogens.
by changing the general microbial balance in the soil. This reduction would be expected to persist if the pathogen has a low saprophytic and sporulating ability in the soil. The increased microbial population along with the antagonistic actinomycetes recorded in the present study might also have contributed to the ultimate reduction of the *M. phaseolina* counts. A strong correlation between higher levels of the microbial population and a decrease in *M. phaseolina* has already been established (Filho and Dhingra, 1980; Lodha, 1996). Antagonistic actinomycetes have been found to reduce the *M. phaseolina* population even in arid soils (Lodha et al., 1990). Since *M. phaseolina* has a low saprophytic ability, a permanent reduction in the inoculum density of this pathogen in soil amended with weed residues would be expected when the microbial population of the soil is increased.

Our field results correlated well with the soil incubation findings. A significant reduction in the severity of dry root rot for two successive years in all the weed-amended plots except *Polycarpaea* signified that organic amendments were important in the control of *M. phaseolina* (Osunlaja, 1990). The soil moisture in the residue-amended plots generally remained 9–14% higher than that in the non-amended control plots during the different stages of crop growth. Thus, the slow initial progress of dry root rot in the *Aerva* and *Celosia* amended plots could be a combined effect of the release of biotoxic volatiles and better soil moisture conditions, leading to a lower population of *M. phaseolina* propagules. Soil moisture may also have helped to maintain a high water potential of the plants at a vulnerable stage, which did not express symptoms of *Macrophomina* infection (Burman and Lodha, 2000). In the resource-deficient farming of arid regions, where crop residues are not widely available, incorporating selected on-farm weeds into the soil can be one component of an integrated management of dry root rot. *Aerva* and *Celosia* plants appear in the later part of the crop season in the agricultural fields and wastelands of this region. Their profuse growth even under moisture stress provides an adequate biomass. The increased levels of *M. phaseolina* inoculum and disease incidence when *Polycarpaea* was incorporated into the soil suggests that such weeds should be frequently uprooted in agricultural fields to reduce *M. phaseolina* incidence.

### Literature cited


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