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# COMBINED EFFECT OF ORGANIC AMENDMENT AND SOIL MOISTURE ON THE DECLINE IN NUMBERS OF SCLEROTIA OF *MACROPHOMINA PHASEOLINA*

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**Abstract**

Combined effect of organic amendment with either alfalfa, clover, mustard or wheat and soil moisture of 0,25,50,75 or 100% moisture holding capacity (MHC) on the viability of sclerotia of *Macrophomina phaseolina* at various periods of incubation was investigated. No significant change in population of sclerotia was noticed in dry soil. Sclerotial population declined rapidly in high soil moisture regimes of 75-100% MHC. Reduction in sclerotial numbers was more pronounced in wet amended soil than in non amended soil. In general organic amendment at 3% w/w was more effective in reducing sclerotial numbers than at 1% level. Alfalfa, clover and mustard amendments reduced sclerotial numbers by 86-96% and in wheat amendment 27-33% reduction was observed at 75-100% MHC after 20 days interval.

## Introduction

*Macrophomina phaseolina* (Tassi) Goid., [*Rhizoctonia bataticola* (Taub.) Butl.] causes seedling blight, charcoal rot, root rot, stem rot and pod rot of over 400 species of plants in tropical and subtropical countries of the world (Reichert & Hellinger, 1947; Young, 1949; Ghaffar *et al.*, 1964). The fungus persists in the form of small, black sclerotia (60-100x56-80 $\mu$m) which are produced in large numbers on infected host tissues and subsequently dispersed in soil during tillage operation (Cook *et al.*, 1973; Ghaffar & Akhtar, 1968; Smith, 1969a). Populations as high as 1000 sclerotial propagules $g^{-1}$ soil have been reported (Papavizas & Klag, 1975) and the importance of these sclerotia in the development of root rot has been stressed (Smith 1969a; Watanabe *et al*, 1967; Ilyas & Sinclair, 1974).

Most of the studies on survival of *M. phaseolina* have been made on recoveries of sclerotia made from infected host pieces in soil like soybean (Dhingra & Sinclair, 1974) corn and sorghum (Cook *et al.*, 1973), cucumber (Ghaffar & Akhtar, 1968). This approach is logical since pathogens are primarily associated with host crop residue but they are also released in soil during tillage. Ghaffar (1968) used sclerotia of *M. phaseolina* on fiber glass cloth pieces. It is not known as to the percentage of reduction in numbers

of viable sclerotia in nylon or host pieces.

In recent studies where tissue or soil formed sclerotia were used, viability of sclerotia gradually declined in soil but some sclerotia were still viable after 4 yr. of soil exposure (Watanabe, 1973). Freezing and thawing of moist soil reduced sclerotial germinability (Bristow & Wyllie, 1975). Dhingra & Sinclair (1975) showed that sclerotial populations declined 96-99% in soil at 60-100% MHC as compared with populations in dry soil, no reduction was observed at 0% MHC. In contrast, the biggest drop occurred in air dried soil (2-3% MHC) at 26°C and atleast 75% of sclerotia of *M. phaseolina* survived at 50-55% MHC (Papavizas, 1977). Similarly, of the organic amendments, alfalfa hay chitin and pine needles, only alfalfa hay at 0.8% w/w reduced survivability of sclerotia in soil by 75% in a year (Papavizas, 1977).

In the present paper the combined effect of organic amendment and soil moisture was studied on the decline in members of sclerotia of *M. phaseolina*.

**Materials and Methods**

Culture of *M. phaseolina* (K.U.M.H. Acc. No. 54) obtained from root rot of cotton was grown on corn meal sand medium (5%,w/w) for two weeks at 30°C and sclerotia separated by filtration through Whatman No. 42 filter paper after successive floatation in sterilized distilled water and decantation. The sclerotia were dried at room temperature and passed through 125 $\mu$m sieve before use.

Sandy loam, pH 8.1 and moisture holding capacity (MHC)42% obtained from cotton field at Karachi University experimental farm was air dried to 2% moisture and passed through 2 mm screen before use. Soil was artificially infested with 50 mg of dry sclerotia of *M. phaseolina* per Kg of soil and thoroughly mixed. The addition of sclerotia to soil gave a population of 47 sclerotial propagules $g^{-1}$ soil. Infested soil was amended wih i) alfalfa *(Medicago sativa)* ii) Clover *(Trifolium alexandrinum)*, iii) mustard *(Brassica compestris)* leaf and stem fraction (low C:N ratio) and iv) wheat *(Triticum aestivum)* straw (high C:N ratio). All plant material were air dried, ground in a mill and passed through 20-mesh screen before incorporation in to soil. These were added singly to infested soil at the rate of 1 and 3% w/w, amedment· soil. Non amended soils served as control. Soil samples were transferred to 90 mm diameter Petri dishes, 100g in each, and adjusted and maintained at 0.25, 50.75 and 100% MHC (Keen & Raczkowski, 1921) by periodic watering. There were 5 replicates of each treatment and the dishes were incubated at 28°C ± 5.

At 0. time and at intervals of 10,20,40, and 80 days, 20g soil samples were removed from each replicate Petri dish, air dried for 48 hr. and sclerotial population determined (Sheikh & Ghaffar, 1975). Ten g of artificially infested soil were dispersed in water in a beaker and wet sieved through 120 mesh on to a 300 mesh screen. The 300 mesh fraction was washed with running water for 1 min and transferred into a beaker containing

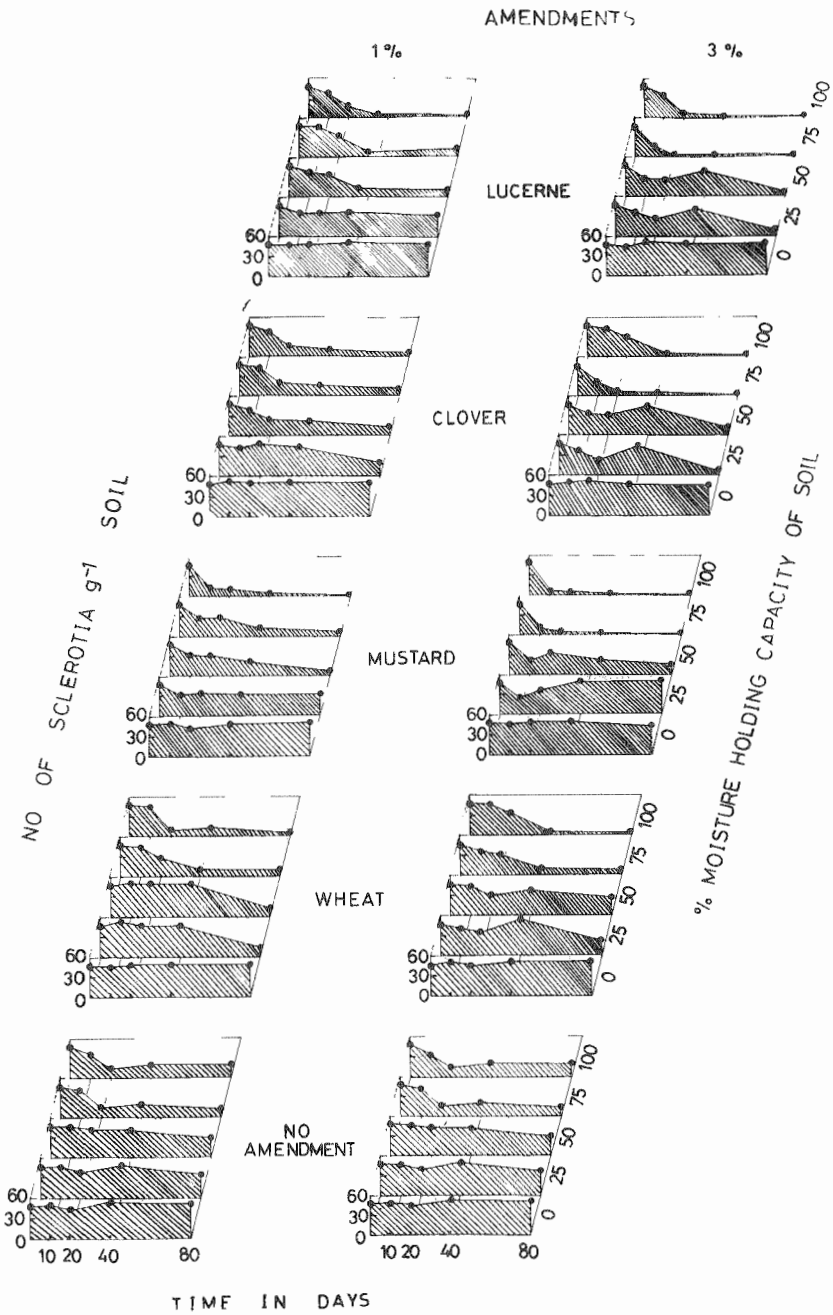


Fig. 1. Population changes in sclerotia of *Macrophomina phaseolina* as affected by organic amendments, soil moisture and length of incubation.

Table 1. ANOVA table for the recovery of sclerotia of *M. phaseolina*..

| Source of variation | SS | df | M.S. | F ratio |
|---|---|---|---|---|
| Water holding capacity (W) | 141483.3 | 4 | 35370.82 | 389.8040*** |
| Amendment (A) | 4753.3 | 3 | 1584.43 | 17.4612*** |
| Amendment level (L) | 11972.0 | 2 | 5986.00 | 65.9687*** |
| Time (T) | 101723.3 | 4 | 24530.82 | 280.2603*** |
| *First order interactions* | | | | |
| W X A | 3090.0 | 12 | 257.50 | 2.8378** |
| W X L | 5974.7 | 8 | 746.84 | 8.2305*** |
| W X T | 68593.4 | 16 | 4287.09 | 47.2458*** |
| A X L | 2846.7 | 6 | 474.45 | 5.2287*** |
| A X T | 4636.7 | 12 | 386.39 | 4.2582*** |
| L X T | 9464.7 | 8 | 1183.09 | 13.0382*** |
| *Second order interactions* | | | | |
| W X A X L | 5556.0 | 24 | 231.50 | 2.5512*** |
| W X A X T | 6070.0 | 48 | 126.46 | 1.3936* |
| A X L X T | 4521.3 | 24 | 188.39 | 2.0761** |
| L X W X T | 9093.6 | 32 | 284.17 | 3.1317** |
| *Interaction of all factors* | | | | |
| W X A X L X T | 12311.00 | 96 | 128.24 | 1.4133* |
| Residual | 108894.00 | 1200 | 90.74 | – |
| Total | 500984.00 | 1499 | – | – |

Levels of significant
*P < 0.05, **P < 0.01, ***P < 0.001

sclerotial population was more and rapid after amendments of soil with alfalfa, clover, mustard or wheat. Our results corroborates the findings of Dhingra & Sinclair (1975) who found rapid decline in sclerotial population at 60-100% MHC than in dry soil and in soils amended with glucose and $NaNO_3$ in different C/N ratios but they did not study the combined effect of amendements and soil mositure. These results would suggest a practi-

0.5% CaCl0 and made up to 50 ml to obtain 1:5 dilution. The sclerotia and soil suspension was continuously agitated by a magnetic stirrer at slow speed and a 1 ml aliquot was evenly spread on 3 day old potato dextrose agar (PDA) pH 5.4, containing penicillin and streptomycin (each 60 mg/liter), Demosan (300 mg/liter) and Rose bengal (100 mg/liter). There were 5 replicates for each treatment. The plates were incubated at 30°C for 5 days after which greyish to black colonies of *M. phaseolina* were easily detected.

## Results

Fig. 1 shows the combined effect of various organic amendments (at different levels) and different moisture regimes (percentage of MHC) on the sclerotial populations of *M. phaseolina* at various periods of incubation. The ANOVA for the same data is presented in Table 1. The sclerotial population in various treatments (excluding controls) declined singnificantly with progressive periods of incubation ($p < 0.001$). The reduction in the population of sclerotia was most pronounced in soils maintained at 75 and 100% MHC ($p < 0.001$). Organic amendments also markedly reduced the sclerotial population and the sclerotial population responded differentially to various amendments ($p < 0.001$) as well as to different levels of amendments ($p < 0.001$). The effect of alfalfa, lucerne, clover and mustard amendments on the reduction of sclerotial population was more or less of similar order but that of wheat appeared to be less pronounced. In general, sclerotial population greatly declined at 3% level of amendment that at 1% level, particularly in alfalfa, clover and mustard amendments (AxL, $p < 0.001$).

No significant change in the population of sclerotia was noticed in dry soil (0%MHC). From an initial population of 47 sclerotial propagules $g^{-1}$ soil the population denisty at 25 and 50% MHC declined by 23-25% upto 80 days (TxW, $p < 0.001$). The sclerotial population at 75 and 100% MHC, however, declined by 65-68% upto 20 days and thereafter remained more or less constant. Similarly the decline in population density was relatively more rapid in alfalfa, clover and mustard amendments compared to that of wheat (TxA, $p < 0.001$). A rapid decline in sclerotial population occurred at 3% level of alfalfa, clover and mustard amendments in which the population density declined by 31-51% at 25-50% MHC and by 86-96% at 75-100% MHC after 20 days interval. At 1% level of these amnedments the decline was relatively gradual (LxT, $p < 0.001$). Various organic amendments exhibited pronounced effect on the reduction in sclerotial population at 75 and 100% MHC and this interaction was more evident in case of alfalfa, clover and mustard amendments (AxW, $p < 0.001$), particularly at 3% level (LxW, $p < 0.001$) in which it was reduced to zero after 80 days interval.

## Discussion

Our results showed that population of sclerotia of *M. phaseolina* can be considerably reduced by subjecting soil to high soil moisture of 50% and above. This reduction in

cal control of *Macrophomina* infection by flooding and the effect enhanced by organic amendments since the decline in number of sclerotia would influence the amount of disease (Sheikh & Ghaffar, 1979). This observed phenomenon would also explain the reduction in charcoal rot in pine seedling (Hodges, 1962) and in cotton (Ghaffar & Erwin, 1969) by frequent irrigation or as observed in a rainy period. Adverse effects of high soil moisture is similarly reported for *Fusarium roseum* (Cook & Papendick, 1970) and for *Theilaviopsis basicola.* (Papavizas & Lewis,1971).

In contrast to our results Papavizas (1977) found that low soil moisture (2-3% MHC) and high temperature (25 and 35°C) were detrimental to sclerotial survival and that sclerotia survived best in moist soil at 26°C and exposure to 78% RH caused high loss of germinability.

The means by which sclerotial population are reduced through organic amendments and or high soil moisture are unclear. High soil moisture might result in a lack of $O_2$ an excess of $CO_2$ or in production of toxins. One or more of these factors may affect soil microflora or their interactions. Although high concentration of $CO_2$ from increased microbial activity can check growth of *Rhizoctonia solani* (Papavizas & Davey, 1962) it had no effect on *M. phaseolina* (Vasudeva, 1937). Addition of organic amendments are known to stimulate microbial activity in soil and suppression of *Macrophomina* infection of cotton was related to an increase in populations of antagonistic bacteria and actinomycetes in amended soils (Ghaffar *et al.*, 1969). The reduction in sclerotial population was significantly corelated with increase in total microbial population (Dhingra & Sinclair, 1975). Although in the present study microbial analysis of soil after amendment was not carried out, however, our high moisture and or soil amendments which caused a reduction in sclerotium populations may have i) dissipated sclerotia in soil due to increase in microbial activity (Papavizas, 1977) or ii) stimulated sclerotial germination (Ayanru & Green, 1974; Smith, 1969b) after which the fungus becomes vulnerable to competition from soil microflora with the germ tube and hyphae being attacked by bacteria and actinomycetes (Kavoor, 1954; Norton, 1953; Ghaffar, 1968; Dhingra & Khare, 1973). Further studies will be necessary to determine the exact mechanism involved.

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