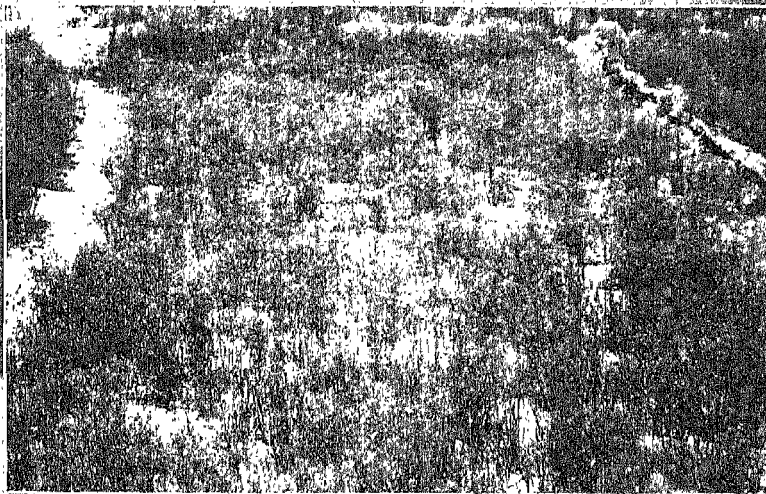


TOWARDS MANAGING SOIL-BORNE PLANT DISEASES IN ARID REGION



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Research bulletin

Towards Managing Soil-borne Plant Diseases in Arid Region

by

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Abstract : In this bulletin salient findings of several laboratory and field experiments conducted on management of soil-borne plant diseases particularly those caused by *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *cumini* are presented and discussed. Utilizing natural resources of the Indian arid region, host of technologies were developed for managing these diseases in rainfed and irrigated agriculture.

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Cover : Healthy and Wilt infected cumin field

Back cover : Packets of biocontrol agents

Foreword

In Indian arid region, soil pests, such as fungi, nematode, bacteria, parasitic plants, arthropods and other organisms frequently cause heavy losses, by affecting both quantity and quality of agricultural production. In severe cases, total devastation forces affected farmers to either abandon the land or shift to less susceptible but often less profitable crops. Due to limitation of suitable lands in arid region, crops are frequently or even continuously planted on same piece of land, leading to rapid build up of host specific pest population confounding the problems. There is, therefore, a need to evolve effective management strategies to ensure crop productivity and yield stability under harsh arid climate. These strategies must be technologically sound, economically viable and environmentally safe.

Realizing the importance of this problem and need of the region, dedicated efforts of a team of multi-disciplinary scientists in controlling soil-borne pathogens utilizing natural resources of the region like intense solar radiation, on-farm wastes and native bio-control agents have culminated in this bulletin.

The findings presented in the bulletin will be quite informative and useful for those engaged in dissemination of low external input sustainable technologies for the management of soil-borne diseases in Indian arid region. My compliments to the team of scientists for this useful publication.

Dr. Pratap Narain
Director

Preface

In the arid regions of India, besides weather aberrations, diseases and pests also become a limiting factor for successful crop production and are often responsible for crop failures. Among the diseases, those caused by soil-borne pathogens are more prevalent due to favourable agro-climatic conditions for their development and repeated cultivation of susceptible hosts on the same piece of land. Yield losses due to wilt alone in cumin, a cash crop, often reach to the extent that growers are left with no alternative except to abandon its cultivation. Several folk songs depict the plight of hapless desert dwellers in this regard.

These soil-borne pathogens survive in the form of resting structures and their populations increase in the soil with each year of cultivation. To reduce the population of these pathogens below the economic threshold level, efforts have been made at the Central Arid Zone Research Institute, Jodhpur to evolve cost effective, environmentally sound methods of management utilizing natural resources of the region. In this bulletin, an attempt has been made to compile the results of various experiments conducted on biology and pathology of soil-borne plant pathogens. We hope that low external input sustainable technologies developed for the management of these diseases would certainly be useful for increasing productivity of the valuable crops. This will provide an additional tool in the hands of research and extension workers to benefit the resource constrained farmers of the arid region.

We express our sincere thanks and gratitude to Dr. Pratap Narain, Director, CAZRI, Jodhpur for constant encouragement. We are grateful to Prof. J. Katan, The Buck Family Professor of Plant Pathology, The Hebrew University, Rehovot, Israel for offering valuable scientific suggestions for many experiments. Scientific assistance rendered by Dr. Ritu Mawar, Dr. Sunil Israel, Mrs. Meenu Bareja and Mr. J.S. Meena is gratefully acknowledged. Thanks are also due to Mr. Rajan Lal, Mr. Arjun Singh and Smt. Rukma devi, staff of Plant Pathology laboratory of the Institute.

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Introduction

The Indian arid zone covers 12 per cent of country's geographical area occupying 31.8 million hectares of hot desert. Pearl millet, clusterbean, moth bean, cowpea, sesame and sorghum are the major crops grown under rainfed conditions while wheat, cumin, mustard and isabgol are the principal crops grown in winter season where assured irrigation is available due to ground water resources or inception of canal. With the increasing biotic pressure, most of the arid and semi-arid regions are confronted with the challenges of producing more per unit land with uncertain and dwindling supplies of water.

In this region, besides weather aberrations, diseases caused by certain soil-borne pathogens are responsible for low productivity of agricultural crops (Lodha and Singh, 1983; Lodha *et al.*, 1986). In addition to losses caused to annual crops, occurrence of these diseases in forestry and pastures takes away a major share (Lodha, 1983; Lodha *et al.*, 1994). Because of the requirement for free water, diseases of aerial plant parts are much less common than those involving roots when plants are grown in dry regions where humidities are low and rains are infrequent and/or of short durations. Under these conditions, pathogens like *Macrophomina phaseolina*, *Ganoderma lucidum*, *Cylindrocarpon lichenicola*, *Neocosmospora vasinfectum*, *Sclerotium rolfsii* and species of *Fusarium* (*F. solani*, *F. equiseti* and *F. oxysporum*) causes dry root rot and wilts in many commercially valuable and perennial plants (Akem and Lodha, 2000 ; Bohra and Lodha, 1999 a, b; Lodha, 2000 a, b; Singh and Lodha, 1983) (Table 1).

Table-1. Important soil-borne diseases of crops in arid region

Pathogen	Disease	Crop (s)
<i>Macrophomina phaseolina</i>	Leaf, Stem blight, Web blight, Root rot, Charcoal rot	Mungbean, Mothbean, Clusterbean, Cowpea, Chickpea, sesame
<i>F. oxysporum</i> f. sp. <i>Ciceri</i>	Wilt	Chickpea
<i>F. oxysporum</i> f. sp. <i>Ricini</i>	Wilt	Castor
<i>F. oxysporum</i> f. sp. <i>corianderi</i>	Wilt	Coriander
<i>F. oxysporum</i> f. sp. <i>Cumini</i>	Wilt	Cumin
<i>Sclerotium rolfsii</i>	Collar rot, Root rot	Chickpea
<i>Fusarium solani</i>	Root rot	Chick pea, Jojoba
<i>Rhizoctonia solani</i>	Root rot	Chickpea
<i>Ganoderma lucidum</i>	Root rot	Shrubs and trees
<i>Neocosmospora vasinfectum</i>	Slow wilt	Clusterbean, Cowpea

Certain agro-climatic factors of arid region are attributed for the development of few specific diseases caused by soil-borne pathogens. Low organic matter and microbial

population coupled with poor moisture retention capacity of soils favour survival and multiplication of the main source of inoculum of soil-borne pathogens (Fig. 1).

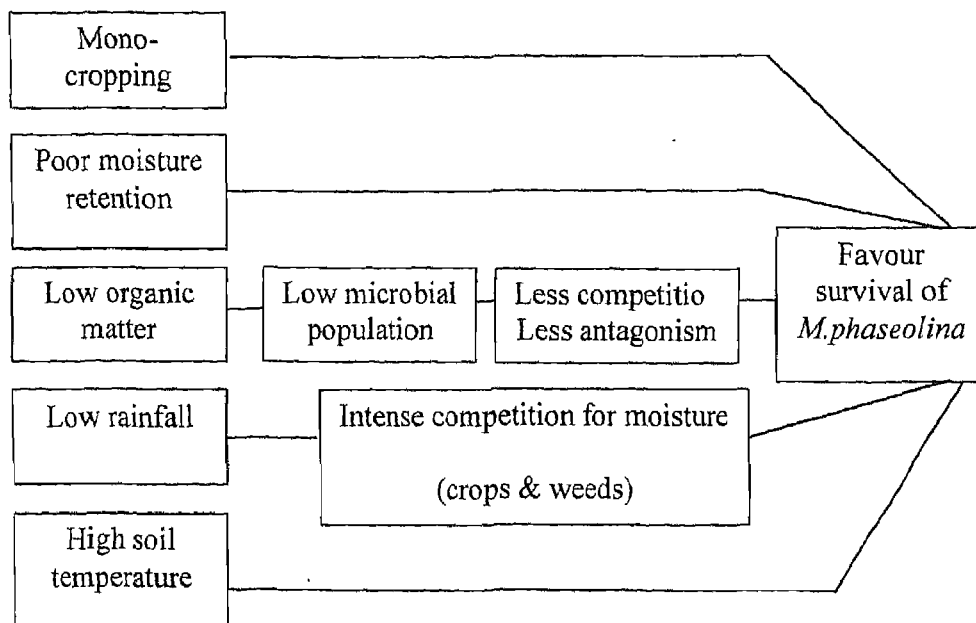


Fig. 1. Factors influencing survival of *M. phaseolina* in arid soils

Inoculum of these pathogens increases in the agricultural lands with increased years of cultivation of susceptible crops and the inoculum density in the soil is directly proportional to disease intensity in the field. Studies conducted on loss estimation over a period of eight years revealed that amongst soil-borne pathogens, *M. phaseolina* and *Fusarium* are the major cause of serious crop losses (Table 2). Losses due to wilt (*Fusarium oxysporum* f.sp. *cumini*) in cumin alone may reach 40%. Frequently growers are left with no alternative except to abandon its cultivation after a few successive years of cropping (Lodha and Mawar, 2000a). Several folk songs depict the plight of hapless desert dwellers that suffer enormously when wilt takes away their expected rich harvest within a fortnight. Thus, occurrence of *M. phaseolina* under rainfed conditions and *F.o. f. sp. cumini* in winter season in the same fields has become a serious limiting factor for the profitable production of oilseeds/legumes and cumin, respectively (Lodha and Mawar, 2000 a, 2002).

Table 2. Maximum disease severity and yield losses due to important soil-borne pathogens of arid crops during 1977-84.

Crops	Variety	Disease	Mortality (%)	Yield Losses (%)
Clusterbean	FS-277	Dry root rot	31.00	32.11
Cowpea	V-8	Dry root rot	64.50	-
Cumin	Local	Wilt	40.07	36.35
Sesame	T-13	Dry root rot	43.89	50.15
Pearl millet	HB-3	Downy mildew	57.20	50.15

Studies on biology and pathology of *M. phaseolina* and *F. oxysporum* have been done world wide with a view to evolve a number of management strategies to reduce yield losses. These studies have broadened the horizon of knowledge that particular agro-climatic region requires specific host of technologies to suit the socio-economic conditions of the farming community of that region. The beneficial effects of these should form an integral part of low-input sustainable agriculture (LISA). During past two decades, research efforts were concentrated at the Central Arid Zone Research Institute, Jodhpur to evolve management strategies against major soil-borne plant pathogens of the region (Lodha, 1993;1997). Concerned with the conservation of fast deteriorating environment, efforts have been diverted to evolve eco-friendly management strategies to avoid dependence on expensive and hazardous chemical means of control because biology and ecology rather than chemistry governs the sustainability in arid region.

Resistance

Breeding

Studies demonstrated that maximum expression of dry root rot required for screening germplasm can be secured by inoculations of seedlings grown in *M. phaseolina* infested soil at the time of moisture stress (Singh and Lodha, 1986; Lodha 1998). This procedure was used to screen 28 elite genotypes rigorously to identify resistant sources. In the absence of complete resistance, least susceptible genotypes 'RGC 471' and 'Kutch 8' were categorized moderately resistant while 'HG 75' and 'AG 111' as highly susceptible. These genotypes were used for making three crosses i.e. 'Kutch 8 x RGC 471'; 'Kutch 8 x HG 75' and 'HG 75 x AG 111'. Progenies were subsequently advanced to F₅ and F₆ generations (Lodha and Solanki, 1993).

There was a wide range of variation for dry root rot resistance among the F₁'s produced by these crosses. The F₁ from Kutch 8 x RGC 471 showed resistance close to either of the parent. The susceptibility value (33.1%) of F₁ was almost equal to the mid-parent value (33.4%) indicating absence of dominance. Scaling test revealed the presence

of epistasis in 'Kutch 8 x RGC 471' and 'HG 75 x AG 111' crosses and appropriateness of additive dominance model for 'Kutch x HG 75'. In gene effect studies, additive, additive x additive and dominance x dominance were more pronounced in R x S cross. These findings are in close agreement with those of Rao and Shinde (1985) who reported inheritance of resistance to be polygenic in sorghum for *M. phaseolina* induced charcoal rot and found that non-allelic interaction played an important role.

It emerged from this study that in the absence of complete resistance, only alternative left for the improvement of the population was to select plants possessing more resistance to dry root rot. Thus, few strains combining field resistance as well as high yield were selected (Lodha and Solanki, 1993). Of these, strain CAZG 27-1 ranked first in all India testing in gum content (32.8%). In case of cowpea, of the 33 genotypes evaluated against *M. phaseolina*, 26/4/1, V16, K39, 25/8/2 and CO₃ were found moderately resistant (Singh and Lodha, 1986).

Relative tolerance of crops/genotypes

During screening of the germplasm of arid legumes against dry root rot, it was observed that under similar conditions of soil moisture stress, genotypes of a crop or different crops respond differently to *Macrophomina*. When cowpea, clusterbean and moth bean were grown in grass-legume intercropping system, the effect of soil moisture stress was earliest discernible on cowpea, which got heavy infection due to *Macrophomina* (Table 3). With the onset of severe moisture stress, most of the cowpea plants expressed dry root rot symptoms leading to high mortality (Lodha and Singh, 1984). However, under similar situations, clusterbean and mothbean, in that order, was less infected. This indicated that there exist a strong correlation between water stress in host and dry root rot incidence. Subsequently, relationship between water relation of host before and after *Macrophomina* infection and susceptibility of cowpea, clusterbean and mothbean genotypes were worked out in detail.

Table 3. *M. phaseolina* population and mortality of legumes in grass-legume inter-cropping.

Systems	Population (g ⁻¹ soil)		Mortality (%)
	At symptom initiation	At harvest	
<i>Cenchrus ciliaris</i> (Cc)	6	16	-
Cc + clusterbean	96	100	43.8
Cc + cowpea	148	98	71.4
Cc + moth bean	40	64	28.5
Clusterbean	36	47	28.6
Cowpea	46	60	41.6
Moth bean	28	38	14.2

Water relation parameters of the healthy and diseased plants of six cowpea genotypes were studied at the time of mild and severe moisture stress (20 to 40 days after sowing). It was observed that susceptibility of cowpea genotypes to *Macrophomina* increased with increasing moisture stress. At 20 days after sowing, when seedlings experienced mild stress (-8 bars), mortality due to dry root rot ranged from 5.3 to 11.3%. While, at severe moisture stress (-13 bars), 22-35% plants of cowpea genotypes experienced root rot mortality. Shoot water potential decreased significantly in the healthy and diseased plants of susceptible genotypes 'ARS Durgapura' compared to resistant 'V-265' (Burman and Lodha, 1996). At severe moisture stress, susceptible genotype reflected greater magnitude of changes in leaf transpiration, turgescence and temperature compared to those of resistant genotype (Burman and Lodha, 1996). At mild soil moisture stress, vulnerability of 'ARS Durgapura' to *M. phaseolina* infection could be partly attributed to low Ψ shoot of healthy seedlings. Poor root biomass and plugging of vessels (Ilyas and Sinclair, 1974) under severe stress conditions reduced water uptake in 'ARS Durgapura', concurrent higher transpiration rate made this genotype further susceptible to *M. phaseolina* infection. Thus, increased susceptibility of a genotype to *Macrophomina* infection could be assigned to impairment of water transport system.

Studies on different arid legumes demonstrated that mothbean is the most tolerant to *M. phaseolina* infection. Cowpea plants became predisposed to fungal infection only after its shoot water potential declined below -14 bars (Burman and Lodha, 2000). However, clusterbean and mothbean reached that stage at -18 and -20 bars, respectively (Table 4). In spite of experiencing greater water deficit compared to clusterbean and cowpea, moth bean maintained higher leaf turgescence by lowering its transpiration rate more than other studied legumes. Lowest leaf transpiration rates (0.27 to 0.29 $\mu\text{g H}_2\text{O cm}^{-2} \text{Sec}^{-1}$) were recorded in moth bean leaves while cowpea leaves registered maximum (0.41 to 0.61 $\mu\text{g H}_2\text{O cm}^{-2} \text{sec}^{-1}$). *Macrophomina* infection reduced both transpiration and leaf turgescence probably due to impairment of water uptake process. However, genotype of a crop could be differentiated on the basis of leaf RWC. Thus, the findings of this study demonstrated that leaf RWC can be used as a parameter to screen genotypes against *Macrophomina*. This parameter has also been suggested to screen drought resistance in cowpea (Walker and Miller, 1986). If the apparent relationship, that mechanism of drought tolerance also confers resistance to *Macrophomina* holds true (Pastor-Corrales and Abawi, 1988), identification of a common parameter should facilitate evaluation of drought tolerance as well as resistance against *Macrophomina*.

Table 4. *M.phaseolina* induced changes in water relation parameters of some arid legumes.

Crop & genotypes	Sclerotia (g ⁻¹ soil)	Mortality (%)	Shoot water potential (-bars)		Transpiration (µg cm ² sec ⁻¹)		Leaf turgescence(%)	
			H ^a	D ^b	H	D	H	D
Cowpea								
V-265	280	27.9	14.0	17.3	1.78	1.18	64.8	28.0
AD	200	36.2	13.3	19.3	1.21	0.97	61.5	41.8
Clusterbean								
RGC-471	80	9.3	17.0	21.7	0.43	0.41	67.0	54.9
HG-75	186	27.5	18.0	21.0	1.27	0.71	61.3	54.5
Moth bean								
RMO-40	14	7.0	19.7	22.0	0.81	0.37	73.1	62.9
Maru moth	6	12.8	19.0	23.7	1.12	0.47	70.6	70.0

^a Healthy ^b Diseased

Chemical Control

M. phaseolina and *Fusarium* are predominantly soil-borne. However, importance of seed-borne infection in the transmission of disease in newer areas of cultivation and onset of the seedling disease from germination itself can not be overlooked (Lodha, 1984; Singh *et al.*, 1972). Studies were, therefore, conducted to identify effective fungicide for use as seed treatment. Fifteen fungicides viz., Fytolan, Thiride, Brassicol, Agrosan GN, Dithane M-45, Cuman L., Dithane Z-78, Panoram, Calixin, Bavistin, Benlate, Kitazin, Ridomil and Captan were evaluated at concentrations ranging from 10 to 2000 ppm. Bavistin, Benlate and Thiride appeared to be the most effective as they could check the growth of pathogen even at 10 ppm concentrations (Lodha 1984; 1986). Promising fungicides were evaluated for their efficacy as seed dressers (0.2%) during 1981 and 1982 under green house conditions. Vitavax (0.2%) appeared as the most effective in reducing pre-emergence (6.94%) followed by Bavistin (11.38%). However, lowest post-emergence mortality was recorded with Thiride (17.62%) and Brassicol (18.34%) compared to control (28.25%).

These fungicides alongwith streptocycline were also evaluated in the field. Bavistin (0.2%) appeared significantly superior in reducing mortality (9.1%) followed by Thiride (16.7%) and streptocycline + Bavistin (17.5%). Maximum yield was, however, recorded with Dithane M-45 (435 kg ha⁻¹) followed by streptocycline + Bavistin (425 kg ha⁻¹). In the subsequent study on dry root rot of cowpea, Bavistin, Benlate and Agrosan GN appeared as the most promising seed dressers in reducing disease incidence (Singh and Lodha, 1986).

Physical Control – basic aspect

Fusarium oxysporum f. sp. *cumini*

Knowledge of quantitative and qualitative aspects of inoculum dynamics is necessary for improving the efficacy of physical and biological methods in controlling *Fusarium* wilts. Studies on population dynamics of *Fusarium* are also required to understand the suppressiveness of soils and to isolate specific antagonists from these soils as bio-control agents. Therefore, investigation was undertaken to study effects of various biotic and abiotic factor influencing population dynamics of *F.o.* f. sp *cumini* in presence and absence of cumini crop in arid soil.

For survival studies, inoculum of *Fusarium* was mixed with the field soil (2 Kg soil) and ploughed uniformly to 30 cm depth by a hand spade in experimental plots (1 x 1 m) comprised of either of the two treatments: 1 – cumini crop (cv. RZ 19) and 2 – fallow. The soil samples were collected regularly every month from 3 different depths, i.e. 0-5 cm, 6-15 cm and 16-25 cm from sowing until the harvest of cumini crop for moisture determination and microbial analysis. In general, soil moisture was minimum at 0-5 cm soil depth and increased with increasing soil depth throughout the experimental period in both the treatments (Israel, 2002).

In presence of crop, maximum population of *Fusarium* was estimated at 0-5 cm depth, but the population density tends to decline progressively with distance from the surface

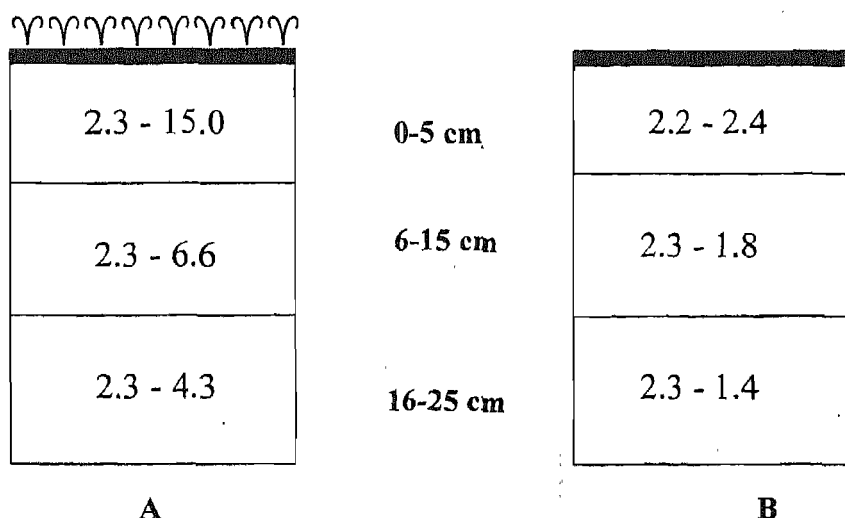


Fig 2. Vertical distribution of *Fusarium* ($10^3 \times \text{CFU g}^{-1}$) in (A) presence and (B) absence of crop

Significant positive correlations of *Fusarium* population were established with maximum soil temperature ($r = 0.50$), bacteria ($r = 0.51$) and microbial population ($r = 0.53$). Highest population observed at top soil and its positive correlations with maximum soil temperature and total bacterial population in the study indicate that lesser competition due to actinomycetes and total fungi supported greater survival of *Fusarium* propagules at this depth. Path coefficient analysis also supported these findings where highest direct effect on *Fusarium* population was of total bacteria followed by microbial population and maximum soil temperature. Maximum indirect effect of microbial population was also via total bacteria (Table 5).

An initial population of 2.3×10^3 CFU g^{-1} gradually increased in the presence of cumin crop and reached 8.6×10^3 CFU g^{-1} in April after harvest of the crop. After sowing of second cumin crop in November, population of *Fusarium* further increased and reached 15×10^3 CFU g^{-1} soil at the end of the second season. Thus, a 6-fold increase in the population was estimated after two successive cumin crops in the same piece of land at top soil layer. A 2.5-fold increase in population of *Fusarium* after one crop and 3.7-fold increase after second successive crop of cumin explains why disease intensity increases in the field with increased years of cultivation of a susceptible crops in the same piece of land. Increase in population of *Fusarium* has been significantly correlated with wilt incidence (Mawar and Lodha, 2002).

Similar trend of increase in *Fusarium* population was also estimated at 6-15 cm soil depth during crop season of even in the absence of crop. But the rate of increase was significantly lower than that estimated at top soil layer. Population of all the three groups of members also increased during first season of cumin but maximum increase was estimated in total fungi. However, in the absence of crop total actinomycetes and bacteria increase considerably at this depth and remained almost same even after the harvest of second cumin crop except in case of total bacteria where a sharp decline was estimated.

Positive correlation of maximum as well as minimum soil temperature at 6-15 cm soil depth in presence of crop is in close agreement with the earlier findings that wilt incidence was maximum between third week of January and first week of February (Mathur and Mathur, 1966). The rate of increase in *Fusarium* population in the presence of first and second cumin crop at 16-25 cm soil depth was relatively slower than that estimated at 0-5 and 6-15 cm soil depths. However, a high residual factor estimated in path coefficient analysis along with no significant correlation of any of the factors studied indicated that survival of *Fusarium* propagules at 16-25 cm soil depth is influenced by some other factors. Similar findings obtained through correlations and path coefficient

analysis in treatment combination 2 further support that in arid soils studied factors could not influence survival of *Fusarium* propagules.

Table 5. Direct and indirect effects of certain factors influencing *F.o. f. sp. cumini* population at different soil depths in the presence of crop

Factors	Soil temperature		Soil moisture	Bacteria	Fungi	Actinomycetes	Microbial population
	Maximum	Minimum					
0 – 5 cm							
Maximum	2.06	1.76	- 1.61	0.61	- 0.08	0.58	0.66
Soil temperature							
Minimum	- 1.73	- 2.0	1.65	- 1.06	- 0.31	- 0.21	- 1.07
Soil temperature							
Soil moisture	0.29	0.30	- 0.37	0.07	- 0.03	0.09	0.07
Bacteria	7.03	12.43	- 4.53	23.81	13.08	0.39	23.68
Fungi	- 0.04	0.17	0.11	0.64	1.16	0.54	0.70
Actinomycetes	0.49	0.18	- 0.44	0.02	0.81	1.75	0.21
Microbial Population	- 7.59	- 12.47	4.91	- 23.59	- 14.25	- 2.89	- 23.72
<i>Residual factor = 0.41</i>							
6 – 15 cm							
Maximum	0.91	0.84	- 0.61	0.37	0.30	0.14	0.38
Soil temperature							
Minimum	- 0.71	- 0.77	0.54	- 0.49	- 0.27	- 0.08	- 0.48
Soil temperature							
Soil moisture	0.19	0.21	- 0.29	0.15	0.08	- 0.02	0.14
Bacteria	- 35.47	54.89	42.87	- 85.23	- 44.12	- 25.87	- 84.80
Fungi	- 0.02	- 0.02	0.02	- 0.04	- 0.08	- 0.05	- 0.04
Actinomycetes	- 1.45	- 0.95	- 0.88	- 2.71	- 6.17	- 8.92	- 3.54
Microbial Population	37.19	56.15	- 42.25	88.38	51.03	35.28	88.82
<i>Residual factor = 0.39</i>							
16 – 25 cm							
Maximum	- 1.13	- 1.12	0.65	- 0.63	- 0.54	0.16	- 0.61
Soil temperature							
Minimum	0.47	0.47	- 0.26	0.25	0.22	- 0.07	0.24
Soil temperature							
Soil moisture	0.04	0.04	- 0.08	0.03	0.02	- 0.01	0.02
Bacteria	- 42.99	- 40.38	28.94	- 76.70	- 62.58	11.61	- 75.88
Fungi	- 0.73	- 0.70	0.51	- 1.24	- 1.52	0.79	- 1.14
Actinomycetes	1.80	1.86	- 2.77	1.83	6.34	- 12.12	0.06
Microbial Population	42.25	39.53	- 26.90	76.63	58.10	- 0.42	77.46
<i>Residual factor = 0.82</i>							

Bold letters denote direct effects.

To quantify the relative contribution of each factor on *Fusarium* population, regression analysis was carried out for different soil depths. The multiple regression equations so constructed are as under:

$$\text{0-5 cm: } Y = -13.62 + 0.91x_1 - 0.74x_2 - 3.36x_3 + 22.15x_4 + 0.81x_5 + 1.74x_6 - 2.17x_7 \quad (R^2 = 0.83)$$

$$\text{6-15 cm: } Y = 0.84 + 0.81x_1 - 0.14x_2 - 0.29x_3 - 36.43x_4 - 0.04x_5 - 3.65x_6 + 3.64x_7 \quad (R^2 = 0.57)$$

$$\text{16-25 cm: } Y = 2.72 - 0.32x_1 + 0.31x_2 + 0.03x_3 - 3.08x_4 - 0.07x_5 - 0.35x_6 + 0.31x_7 \quad (R^2 = 0.72)$$

where,

Y, x_1 , x_2 , x_3 , x_4 , x_5 , x_6 and x_7 are estimated values of *F. o. f. sp. cumini*, maximum soil temperature, minimum soil temperature, soil moisture, total bacteria, total fungi, total actinomycetes and total microbial population, respectively.

In absence of crop also, population of *Fusarium* decreased with increasing depth. However, in general, these population ranges were considerably lower than that estimated in the presence of crop. The population remained almost stationary throughout the experimental period at 0-5 and 6-15 cm soil depth. In path coefficient analysis, minimum soil temperature had highest direct effect on *Fusarium* population followed by maximum soil temperature (Table 6). In general, a stationary population maintained by *Fusarium* in the absence of wilt susceptible cumin crop is the clear indication that our soils are deficit of adequate quantity of antagonists making it conducive for the occurrence of wilt in severe form.

Further, increase in inoculum level in winter months of first season and then subsequent decline in the absence of crop suggests that this species of *Fusarium* cannot maintain its population for prolonged periods without some parasitic or saprophytic activity. Several reports document on reduction in the population of *Fusarium* sp. in the absence of host (Hall, 1996). In the absence of crop only 59 and 42 % of the variations in the population of *Fusarium* is explained by various factors for 0-5 and 6-15 cm soil depths. However, at 16-25 cm soil depths these factors could explain only 13 % of the variations.

Since in our study, there was no evidence of active destruction of pathogenic propagules, enhancement of specific antagonists like non-pathogenic strains of *Fusarium* (Alabouvette and Conteaudier, 1992), fungal biocontrol agents like *Trichoderma harzianum*, etc. (Elad *et al.*, 1982), fluorescent *Pseudomonas* (Duijff *et al.*, 1999) and lytic bacteria (Greenberger, *et al.*, 1987) holds promise through manipulation of soil environment by the application of effective amendments. Thus, next step in research will be to look at specific antagonists and their interactions with the pathogen (Larkin *et al.*, 1993; Larkin and Fravel, 1999) for their use in integrated disease management programme for enhancing yield of this high value crop.

Table 6. Direct and indirect effects of certain factors influencing *F.o. f. sp. cumini* population at different soil depths in fallow dry plots

Factors	Soil temperature		Soil moisture	Bacteria	Fungi	Actinomycetes	Microbial population
	Maximum	Minimum					
0 – 5 cm							
Maximum	0.75	0.62	-0.09	0.09	-0.06	-0.08	0.08
Soil temperature							
Minimum	-1.10	1.32	0.27	-0.52	0.07	0.58	-0.46
Soil temperature							
Soil moisture	0.01	0.01	- 0.07	0.00	-0.01	-0.00	0.00
Bacteria	0.04	0.14	-0.03	0.35	0.22	-0.04	0.35
Fungi	0.06	0.00	-0.01	-0.04	- 0.07	-0.03	-0.04
Actinomycetes	0.01	0.05	-0.00	-0.01	-0.05	- 0.13	-0.02
Microbial	0.02	0.09	-0.02	0.26	0.17	0.05	0.02
Population							
<i>Residual factor = 0.80</i>							
6 – 15 cm							
Maximum	0.21	0.19	-0.10	0.09	0.13	-0.04	0.09
Soil temperature							
Minimum	0.21	0.23	-0.14	0.12	0.17	-0.06	0.12
Soil temperature							
Soil moisture	-0.09	0.11	0.19	-0.08	-0.09	0.01	-0.08
Bacteria	-2.60	3.18	-2.75	6.08	2.31	1.51	6.06
Fungi	0.16	0.19	-0.13	0.10	0.27	-0.00	0.10
Actinomycetes	-0.18	-0.25	0.07	0.22	-0.01	0.90	0.26
Microbial	-2.67	-3.25	2.85	-6.29	-2.53	-1.89	-6.31
Population							
<i>Residual factor = 0.75</i>							
16 – 25 cm							
Maximum	0.77	0.76	-0.37	0.04	-1.48	-0.20	0.45
Soil temperature							
Minimum	-0.83	- 0.84	0.41	-0.51	1.27	0.22	-0.48
Soil temperature							
Soil moisture	0.13	0.13	- 0.28	-0.12	0.09	-0.00	0.09
Bacteria	6.07	5.86	-3.28	- 20.16	9.58	0.56	9.50
Fungi	0.00	0.00	-0.02	-0.21	0.02	0.08	0.03
Actinomycetes	-0.31	-0.32	0.01	1.06	0.07	1.20	0.22
Microbial	-5.94	-5.72	3.39	19.77	-9.98	-1.91	- 10.06
Population							
<i>Residual factor = 0.93</i>							

Bold letters denote direct effects

Macrophomina phaseolina

Specific factors influencing survival of *M. phaseolina* in aridisols were studied during absence of a crop in order to develop effective management strategies. A field experiment was conducted for 9 months to study factors influencing the survival of *M. phaseolina* population at different soil depths (Lodha *et al.*, 1990 a).

Highest *M. phaseolina* population (233 sclerotia g⁻¹ soil) in November was estimated at the 0-5 cm depth followed by that at the 20-30 cm soil layer (150 g⁻¹ soil), the 10-20 cm layer (93 g⁻¹ soil) and the 5-10 cm layer (63 g⁻¹ soil). The presence of a relatively high population of *M. phaseolina* in the surface soil after harvest could be attributed to more than one factor. During the later part of the preceding crop season, rapid decomposition of the fallen infected-plant residues released a large number of sclerotium inoculum in the top soil layer. Until harvest, this inoculum could survive because of high soil temperature (35-40°C) and low soil moisture content (0.5 to 1%). Short *et al.*, (1980) have also reported higher *M. phaseolina* population in surface soils.

A sharp decline in *M. phaseolina* population and a sudden upsurge of actinomycetes was observed in subsequent months i.e. December and January. A significantly high negative correlation ($r = -0.91$) between actinomycetes and *M. phaseolina* population was established. Path coefficient analysis also supported these findings. Minimum soil temperature had the highest negative direct effect on *M. phaseolina*. The direct effect of actinomycetes population was also considerably high.

At 5-10 cm soil depth, *M. phaseolina* population was negatively correlated with minimum and maximum soil temperature ($r = -0.88$ and -0.75) and positively correlated with total fungi ($r = 0.96$). Increase in actinomycetes and decrease in *M. phaseolina* population was more conspicuous only after February. Path analysis also showed the highest negative effect of minimum soil temperature and the highest positive direct effect of total fungi on *M. phaseolina*. Reduction in *M. phaseolina* and increase in actinomycetes population, at 10-20 cm soil depth was quite conspicuous during the winter months. From January, rise in bacterial population and decline in actinomycetes was noted. However, *M. phaseolina* was negatively correlated only with minimum and maximum soil temperature ($r = -0.75$ and -0.73 , respectively). The highest negative direct effect of total bacteria followed by total actinomycetes on *M. phaseolina* further demonstrated the antagonistic role of these factors.

At 20-30 cm soil depth, actinomycetes population declined after March and consequently no further reduction in *M. phaseolina* population was observed. Soil temperatures were not found to be significantly correlated with *M. phaseolina* population, as there was no conspicuous difference between the two at this depth. Total bacterial

population showed direct negative effect (-0.86) on *M. phaseolina* population indicating their antagonistic role.

The antagonistic effects of some soil bacteria, actinomycetes and fungi on *M. phaseolina* are well documented (Dhingra and Sinclair, 1975; Ghaffar *et al.*, 1969). In our experiment, changes in bacterial and fungal populations had individually shown little influence on the sharp reduction in *M. phaseolina* population in winters. Ghaffar *et al.*, (1969) also observed negligible effects of fungal population on *M. phaseolina*.

To quantify relative contribution of each factor on *M. phaseolina* population, regression analysis was carried out for different soil depths. The multiple regression equations so constructed are as under:

$$\hat{Y} = 112.30 + 0.18x_1 - 43.48x_2 - 12.13x_3 + 3.76x_4 - 5.23x_5 + 6.72x_6 \dots\dots\dots(0-5 \text{ cm}, R^2 = 0.987)$$

$$\hat{Y} = 41.63 + 2.80x_1 + 1.31x_2 - 0.36x_3 - 0.15x_4 - 0.63x_5 + 0.60x_6 \dots\dots\dots(5-10 \text{ cm}, R^2 = 0.995)$$

$$\hat{Y} = 33.332 + 3.24x_1 - 1.19x_2 - 6.27x_3 - 0.20x_4 + 0.81x_5 - 5.31x_6 \dots\dots\dots(10-20 \text{ cm}, R^2 = 0.957)$$

$$\hat{Y} = -160.94 + 45.23x_1 - 13.32x_2 + 6.4x_3 - 0.53x_4 - 52.40x_5 + 56.92x_6 \dots\dots\dots(20-30 \text{ cm}, R^2 = 0.952)$$

where,

\hat{Y} , x_1 , x_2 , x_3 , x_4 , x_5 and x_6 are estimated values of *M. phaseolina*, total fungi, total bacteria, total actinomycetes, total microbial population, minimum and maximum soil temperature, respectively. Thus, all the regression equations accounted for 95.2 to 99.5% variability in the population of *M. phaseolina*. This together with the low residual factors calculated by path coefficient analysis confirmed that during fallow no other factor could be of greater importance than those studied. These studies demonstrate that among various bioecological factors, microbial antagonism primarily by actinomycetes and soil temperature had the maximum influence on the survival of *M. phaseolina*.

Influence of cropping sequences

Studies conducted over four years revealed that continuous and sequential clusterbean cropping increased the population of *M. phaseolina* (Lodha *et al.*, 1990a). Significant variations were recorded with sequences involving fallow in the second or third year. In clusterbean-fallow systems, *M. phaseolina* population declined markedly after the fallow. It increased after the second crop of clusterbean but remained less than that under continuous cropping (Lodha 1995a). In sequences involving clusterbean, moth bean and pearl millet, differences among *M. phaseolina* populations were not significant (Table 7). However, lowest population recorded in the sequences involving moth bean indicated its relative tolerance compared to clusterbean (Lodha and Singh, 1984). During fallow, absence of a crop and increase in soil moisture in the rainy season might have hastened the decomposition of pearl millet residues resulting in a rapid increase of competitive microorganisms which directly or indirectly reduced the population of *M.*

phaseolina (Lodha *et al.*, 1990a). This study suggests that crop rotation with less susceptible crop will restrict the increase in *M. phaseolina* population in the soil.

Table 7. Influence of different cropping sequences on *M. phaseolina* (g⁻¹ soil)

Year			
1980	1981	1982	1983
Grass	Guar	Guar	Guar
6	38	49	64
		Moth bean	Guar
		42	56
		Pearl millet	Guar
		35	58
		Fallow	Guar
		19	45

Thermal inactivation of *F. o. f. sp. cumini*

Extensive studies by many workers worldwide have shown that 30 minute exposure at 65°C would kill most of the important plant pathogens, insects and weeds (Bollen, 1985; Bollen *et al.*, 1989). However, no information was available concerning exposure times necessary to kill chlamydospores of *Fusarium* in the sub-lethal temperature ranges (45-50°C) in amended and in lethal ranges (<60°C) in unamended soil. In order to determine effectiveness of sub-lethal heating in combination with cruciferous residues, information was needed on the effects of soil temperatures and exposure time on survival of *Fusarium* propagules.

Chlamydospores infested soil was exposed at 45°C, 50°C, 55°C, 60°C, 62°C and 65°C under wet and dry conditions for different time intervals separately in a thermostatically controlled water bath. Population of *Fusarium* was estimated at the initial level and after exposure on selective medium. In another set, infested soil was amended with 1% mustard pod residue and then exposed at 40, 45 and 50°C in open and closed culture tubes. Mortality of *Fusarium* propagules was calculated through probit analysis.

Studies revealed that viability of chlamydospores was severely affected with increase in temperature and duration of exposure (Israel and Lodha, 2003a). After 45°C, with every increment of 5°C, the time required for complete inactivation of chlamydospores reduced considerably. Similarly, the time taken under moistened conditions was significantly less than that estimated under dry conditions. Thus LD₉₀ calculated for 45°C, 50°C, 55°C and 60°C were 207, 64, 43 and 19 minutes, respectively under moist conditions and for 60°C, 62°C and 65°C, LD₉₀ were 31 minutes, 9.7 sec. and

3.4 seconds, respectively under dry conditions (Table 8). These were calculated by computer-assisted programme using probit analysis (Fig. 3).

Table 8. Time-temperature-death relationship for *Fusarium* propagules at different moisture levels.

Temperature (°C)	Moisture level	Equations	LD ₉₀
45	Wet	$\hat{Y} = -5.2089 + 4.962 x$	207 minutes
50	”	$\hat{Y} = -0.4845 + 3.747 x$	64 ”
55	”	$\hat{Y} = 3.1514 + 1.919 x$	43 ”
60	”	$\hat{Y} = 3.0540 + 2.500 x$	19 ”
60	Dry	$\hat{Y} = 2.2335 + 2.705 x$	31 ”
62	Wet	$\hat{Y} = 3.2608 + 4.273 x$	5 seconds
62	Dry	$\hat{Y} = 3.9105 + 2.403 x$	9.7 ”
65	”	$\hat{Y} = 3.2608 + 4.273 x$	3.4 ”
		$\hat{Y} = 3.9105 + 2.403 x$	
		$\hat{Y} = 3.9520 + 4.404 x$	

Where \hat{Y} =mortality of *Fusarium* propagules and x = time required in minutes/seconds.

Addition of mustard pod residue in soil at sub-lethal temperatures (40-50°C) was found effective in reducing the viability of *Fusarium* propagules. Further, heat treatment to amended soil both in open and closed environment showed significant variations in eradication of viable *Fusarium* propagules. LD₉₀ calculated for 40°C, 45°C and 50°C were 169, 126 and 44 minutes, respectively under open conditions compared to 127, 88 and 34 minutes under closed conditions (Table 9). Survival of *Fusarium* chlamyospores even upto 65°C in dry soils is a clear evidence of the heat tolerant nature of chlamyospores, which might have well adapted to high soil temperature conditions prevailing in hot arid regions. Our results on thermal inactivation under simulated conditions, where LD₉₀ calculated for 55°C was 43 minutes are in close agreement with pronounced reduction in viable *Fusarium* propagules due to elevated temperatures (55-58°C) achieved under polyethylene mulching in arid conditions (Lodha, 1995b).

These observations were further confirmed by the findings of present studies where LD₉₀ calculated for 60°C under moist conditions was 19 minute. With an additional increment of 2°C, only 5 seconds were required to eliminate 90% of viable *Fusarium* propagules indicated that temperature range of 60-62 °C is the most critical for inactivating *Fusarium* under moist conditions but under dry conditions this range was found to be 62-65°C. Significantly low LD₉₀ estimated at 50 °C in amended compared to unamended soil explains why higher *Fusarium* control was achieved in amended soils with only one irrigation in our frequent field experiments (Lodha, 1995b; Mawar and Lodha, 2002).

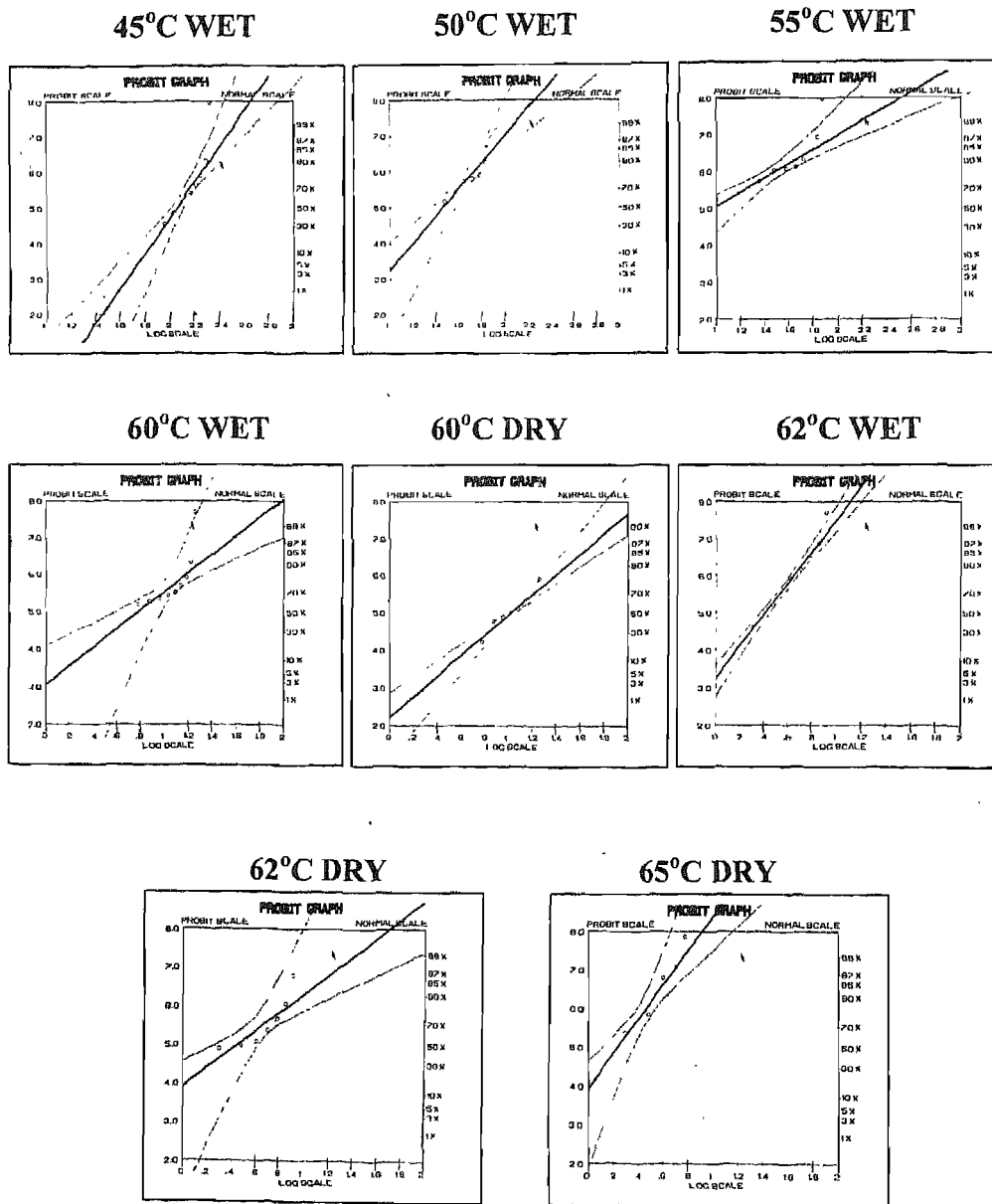


Fig 3. Probit graphs for percentage survival of *Fusarium oxysporum* f. sp. *cumini* propagules in soil in relation to temperature and time of exposure under wet and dry conditions.

The information collected on thermal inactivation periods thus can serve as the basis for estimation of time needed after summer irrigation of residue amended soil for control of *Fusarium* (Fig. 3).

Table 9. Time-temperature-death relationship of *Fusarium* in mustard residue amended soils.

Temperature (°C)	Environment	Equations	LD ₉₀
40	Open	$\hat{Y} = -4.7208 + 4.936 x$	169 minutes
40	Closed	$\hat{Y} = -2.6868 + 4.265 x$	127 minutes
45	Open	$\hat{Y} = -6.7696 + 6.216 x$	126 minutes
45	Closed	$\hat{Y} = -7.8779 + 7.288 x$	88 minutes
50	Open	$\hat{Y} = 0.8996 + 3.276 x$	44 minutes
50	Closed	$\hat{Y} = 1.8561 + 2.888 x$	34 minutes

Where \hat{Y} = mortality of *Fusarium* propagules and x = time required in minutes

Physical Control – applied aspect

Soil Solarization

Solar heating (soil solarization) achieved through mulching the moistened soil with transparent polyethylene sheet during hot summer days has been found highly effective for the control of many soil-borne pathogens throughout the world (Katan *et al.*, 1976; Katan, 1981; Pullman *et al.*, 1981). This technique is especially effective in the regions where climatic conditions favour adequate heating of the soil. Since intense solar irradiations and high temperatures are characteristic features of Indian arid region (Table 10), series of field experiments were conducted to evaluate this method of pathogen control (Lodha, 2001).

Table 10. Maximum temperatures (°C) recorded during experiments on solar heating in different years

	1986	1987	1989	1993
Ambient	45	44	45	47
Dry soil	63	61	63	60
Irrigation (SI)	53	51	55	51
SI + Solarization	58	58	58	61

Macrophomina phaseolina

A field experiment was conducted during summer of 1986 for 15 days (6-20 June). The treatments comprised of wet and dry plots with and without mulch. Mulching was done with transparent polyethylene sheet (50 μm). In wet plots, one irrigation was given 2 days before mulching. Soil temperatures were recorded at 2 h interval from 10.00 AM to 4.00 PM daily. Propagules of *M. phaseolina* were determined quantitatively, using wet sieve, serial dilution and selective medium of Papavizas and Klag (1975).

Studies revealed that polyethylene mulching during hot days considerably increase the soil temperature (Lodha, 1989; Lodha and Solanki, 1992). The maximum soil temperature at 2.00 PM in mulched plots was 58°C (wet) and 69°C (dry) at 5 cm depth, which in non-mulched plots did not exceed 53°C (wet) and 63°C (dry).

In 15 day mulching period, soil temperature reached 65°C or more for atleast 6 days while in other parts of world it never exceeded 65°C in dry mulched soils (Katan *et al.*, 1980; Sheikh and Ghaffar, 1984). These temperature ranges were higher than the thermal death time-temperature (50°C for 90 minutes) reported for *M. phaseolina* (Bega and Smith, 1962). Elevated soil temperature and a shift in favour of antagonistic microorganisms reduced the population of *M. phaseolina* from 350 to 7 sclerotia g^{-1} soil at 5 cm soil depth but reduction decreased with increase in soil depth (Fig. 4). After 15 days, 149 and 275 viable sclerotia g^{-1} soil were present in wet and dry mulched treatments, compared with 279 and 335 sclerotia g^{-1} soil in the corresponding controls at 30 cm depth (Lodha and Solanki, 1992). The decrease in population in the top soil is of considerable importance because highest population and survival rate of *M. phaseolina* sclerotia is at 0-5 cm soil depth (Lodha *et al.*, 1990a).

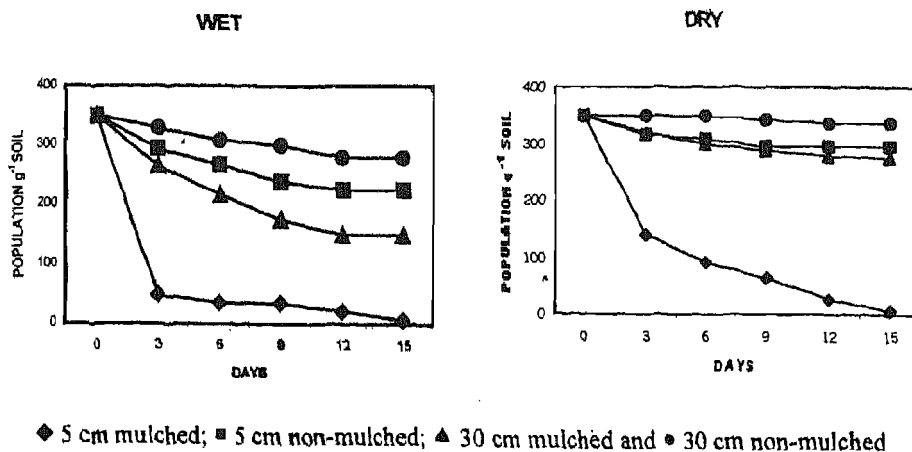


Fig. 4. Effect of soil solarization on *M. phaseolina* population under wet and dry conditions

After solarization, a shift in favour of bacterial population antagonistic to *M. phaseolina* indicates that biological control mechanism also played a significant role in the ultimate reduction of *M. phaseolina* propagules. Sclerotia are parasitized by soil bacteria at high soil moisture (Kovoor, 1954). One beneficial consequence of the microbial shift in mulched soil would be its protection from reinfestation.

Polyethylene mulching was also effective in controlling the population of weeds in standing crop; reduction being greater in the wet-mulched plots. Common weed species reduced due to solar heating were *Cenchrus biflorus*, *Prosopis juliflora*, *Heliotropium subulatum*, *Cyperus rotundus*, *Cynodon dactylon*, *Boerhaavia diffusa* and *Gisekia pharnaciodes*. Control of weeds was also beneficial because weed compete with principal crop for moisture and nutrients. Presences of weeds create soil moisture stress, favourable for the multiplication and infection of *M. phaseolina*; and some weed species also play host to *M. phaseolina*.

Clusterbean crop raised at mulched plots remained disease free till harvest, whereas that grown at non-mulched plots showed 12-18% mortality (Lodha *et al.*, 1990b). Reduction in both the weed population and incidence of dry root rot could increase the seed yield in wet-mulched plots, which was 77 and 30% higher than that in the dry and wet non-mulched plots, respectively. This study established that hot arid climate is most appropriate for soil solarization and elevated soil temperatures reduced the viability of a heat-resistant pathogen *M. phaseolina* at top soil layer.

Fusarium solani

F. solani is a root rot pathogen of several economically important plants like Jojoba, Gayule, *Eucalyptus*, etc. (Lodha and Singh, 1983; Lodha and Bohra, 1995; Kumar and Vishwanath, 1988). Further, its interaction with *M. phaseolina* caused more diseases in jojoba (Bohra and Lodha, 1998). An experiment was performed in June 1986 for a period of 15 days (9-23) to ascertain efficacy of soil solarization on the viability of chlamydo spores of *F. solani*. The treatments consisted of two moisture levels, viz. dry and wet with and without mulch. Mulching was done with a transparent polyethylene sheet (50 μ m). Soil temperatures were recorded by inserting thermometers at 5, 15 and 30 cm depth.

Studies revealed that polyethylene mulching increased the soil temperature and resulted in pronounced reduction in the population of *F. solani* (Lodha and Vaidya, 1990). At the 5-cm depth, the maximal temperature difference was 12°C (dry) and 9°C (wet) at 2.00 PM between mulched and non-mulched plots. As a result, an initial population of 250 propagules g⁻¹ soil declined to 13 in the dry soil and 7 in the moist soil, during a period of 15 days, leading to a net reduction of 71.3 and 68.4% in dry and wet mulched treatments, respectively. Soil temperature elevation at 15 cm depth was relatively low, which resulted in 53.4% (dry) and 60.8% (wet) reduction in viable

Fusarium propagules in mulched plots. An increase of 4 and 3°C soil temperature, however, could reduce the number of propagules by only 23 and 39% in dry and wet mulched soils, respectively at the 30-cm depth.

The reduction in number of *Fusarium* propagules even in wet non-mulched soils provides a new avenue of research in hot arid regions of India, where temperatures of the top soil layer (0-5 cm) often reaches 60°C. Apparently, in a dry soil this heating is ineffective for the control of fungus since the soil remains dry during the summer. Application of just one irrigation in the summer months (without polyethylene mulching) can enhance soil thermal conductance and the thermal sensitivity of resting structures of soil-borne plant pathogens. Katan (1981) has also suggested that merely moistening the soil during summer may result in disease control in certain arid zones. In such region, this may be termed as summer irrigation.

Cylindrocarpon lichenicola

Efficacy of soil solarization was tested for controlling dry root rot of jojoba (*Simmondsia chinensis* (Link.) Schneider) seedlings caused by *Cylindrocarpon lichenicola* in nursery soils. Transparent polyethylene mulching during May increased the soil temperature by 10 and 7°C at 5 cm depth in dry and wet soils, respectively. In solarized pits, the viable propagules of *C. lichenicola* were almost eradicated (Bohra *et al.*, 1996). In general, propagules of this weak pathogen showed sensitivity to high soil temperatures prevailing during the experimental period even in the absence of plastic mulch. Soil solarization further improved the sensitivity, eradicating the pathogen completely in both the solarized pits except at 20 cm in dry mulched pits, where almost 90% reduction in the viable propagules occurred. The mortality caused by this pathogen in the seedlings raised in solarized plots decreased significantly. Jojoba seedlings in solarized plots were more vigorous in growth and the disease symptoms appeared only 4 months after planting. Solarization significantly increased the lytic bacterial density against *C. lichenicola*.

Extending efficacy of soil solarization

Our studies on soil solarization showed that a sizable proportion of *M. phaseolina* propagules survived below 15 cm soil depth. This increased the chances of rebuild of inoculum density after 2-3 years of soil solarization. Since repeated mulching with polyethylene was expensive, it was thought worthwhile to integrate other non-chemical methods of management for extending benefits of soil solarization for long-term.

Field tests were carried out to examine efficacy of soil solarization in conjunction with urea (20 kg N ha⁻¹) and farmyard manure (10 ton ha⁻¹) for the simultaneous control of dry root rot of clusterbean and wilt (*F.o. f. sp. cumini*) of cumin in the same field. During the solarization period (1-15 June, 1987), maximum soil temperatures were

always higher in the solarized than non-solarized plots at all the depths (Lodha, 1995b). Increase in temperature coupled with amendments greatly reduced the population of *Fusarium* in the samples buried in the soil. The fungal population declined from the initial 400 propagules (P) g⁻¹ soil to 14 P g⁻¹ in dry mulched and further to 9 P g⁻¹ soil in wet mulched plus N treatments at 5 cm soil depth amounting to a net reduction of 94.7 and 92.2%, respectively. Reductions at the 15 cm depth were 92.5% (wet) to 73.8% (dry). At 30 cm depth, this reduction ranged from 74% in wet to 56.3% in dry soil for solarized plots compared to non-solarized plots. The population of *Fusarium* remained suppressed in solarized plots despite the cultivation of susceptible cumin crops (Table 11). After the harvest of the second crop of cumin, the *Fusarium* population remained significantly lower only in wet plus N plus manure compared to dry mulched plots. Solarization greatly decreased the mortality of cumin in both seasons, which resulted in increased yields. Incidence of wilt in all the treatments having moistened soil was also significantly lower than that of dry soil. Maximum reduction in mortality was recorded in the wet solarized plots amended with N plus manure. As a result, the cumin seed yield also increased by 36-52% in all the solarized plots and was the highest in solarized wet plus N plus manure plots. The per cent increase in the yield was more conspicuous in the second year of cumin cultivation, where it ranged from 77-121% in all the solarized plots. (Table 11)

There was a pronounced reduction in the population of *M. phaseolina* also due to solarization. The native population of 54 sclerotia g⁻¹ soil declined by 9.7% in non-solarized dry plots, 68.5% in dry solarized and further to 85.2% in wet plus N plus manure solarized plots. The population also remained at low levels in all the mulched treatments after the harvest of the clusterbean in 1987 and after fallow in 1988. The population in wet plus N plus manure and wet plus N non-solarized treatments was significantly lower than those of dry non-mulched plots after fallow. Clusterbean raised in the wet and dry solarized plots remained disease free until harvest, whereas there was 19 and 23% mortality due to dry root rot in the non-mulched wet and dry plots. This resulted in an increase in seed yield (55.2-71.6%) in the solarized plots (Table 11).

Polyethylene mulching had pronounced effects on weed growth during the cropping seasons. In the cumin crop, it reduced weed growth with respect to population and dry weight by 68 and 75%, respectively compared to non-solarized controls. The corresponding reduction in weed growth in the clusterbean crop was 75 and 60%. Soil solarization also enhanced the available contents of K⁺ considerably (8.8 ppm in non-solarized soils to 17.5 ppm in solarized soils) and that of Ca⁺⁺ to a marginal extent.

Table 11. Effect of soil solarization and amendments of urea (N) and farmyard manure (FYM) on soil population of *F. o. f sp. cumini* and *M. phaseolina*, wilt and dry root rot incidence and seed yield of cumin and clusterbean.

Treatments	Propagules (g ⁻¹ soil)				Disease incidence(%)		Seed Yield (kg ha ⁻¹)	
	<i>Fusarium</i> 1987 1989		<i>M. phaseolina</i> 1987 1988		Cumin	Guar	Cumin	Guar
Dry non-mulched	222	330	49	60	37.3	23	318	232
Wet non-mulched	162	255	31	51	29.7	19	371	277
Wet non-mulched+N+manure	149	239	25	50	23.7	15	420	326
Wet mulched+N+manure	56	98	8	27	12.6	0	557	398

In the moistened N plus manure solarized plots, a reduction in the formation of new persisting propagules was observed. Propagule counts remained significantly lower than in dry mulched soil even after the harvest of two successive crops of cumin. Loffler *et al.*, (1988) reported reduced formation and enhanced lysis of chlamydospores of *F. oxysporum* with urea-N application. Similarly, sclerotia of *M. phaseolina* germinate and the subsequent hypha is readily attacked by soil bacteria and actinomycetes whose population increase significantly in amended soils (Kovoor, 1954; Filho and Dhingra, 1980). Soil moisture alone affected the sensitivity of resting structures to a heat treatment, resulting in considerable reduction of *Fusarium* (31.6%) and *M. phaseolina* (42.6%) propagules at 30 cm in non-mulched soils. Incorporation of N plus manure further reduced the population of *Fusarium* (37.1%) and *M. phaseolina* (57.1%). These results suggest a novel approach for partial control of soil borne pathogen population in hot arid regions of India by merely one irrigation in the summer months even without polyethylene mulching in case the soil is amended with N plus manure. Reduction in the population of weeds in the standing crop could have been caused by thermal killing of weed seeds or germinating seedlings (Abdel-Rahim *et al.*, 1988).

Cultural Control – irrigated agriculture

Mustard oil-cake

Scarce information was available about the use of organic amendments in aridisols for the control of soil-borne plant pathogens. Laboratory experiments were, therefore, conducted to ascertain potentials of locally available crop residues/oil-cakes in influencing *M. phaseolina* and *Fusarium* population in soil.

Three levels of residues of pearl millet (PMR) and clusterbean (CBR) equivalent to 20, 40 and 60 kg N ha⁻¹ were incorporated in a known population of *M. phaseolina* infested soil amended with 40 kg N ha⁻¹ or without nitrogen. Considerable reduction was estimated in the population density of *M. phaseolina* in all the soil samples amended with nitrogen enriched residues. This reduction was significantly more in the N + PMR compared to N + CBR (Praveen-Kumar *et al.*, 1998). Among three levels, maximum reduction (96.3%) in *M. phaseolina* propagules was estimated in N + PMR equivalent to 40 kg N ha⁻¹ after 60 days. Reduction in pathogenic propagules was attributed to combined effects of soil moisture, volatiles and enhanced microbial activity including antagonism.

In a subsequent experiment, N + PMR, the best treatment was evaluated along with other organic sources like mustard and castor oil-cake (1%) to ascertain efficacy against *M. phaseolina* and *Fusarium*. A significant reduction in the population of *M. phaseolina* occurred in the cake and pearl millet residue amended soil (Table 12). In the MC alone and N+ PMR + MC amended soil, 100% reduction was achieved within a period of 30 and 45 days respectively (Sharma *et al.*, 1994). In N + PMR- amended soil, number of viable propagules of *M. phaseolina* were reduced by 94% in 45 days. Moist addition of urea (40 kg N ha⁻¹) decreased the C:N ratio and hastened the decomposition of crop residues. Low C: N ratio amendments have been found effective in reducing the population of *M. phaseolina* (Dhingra and Sinclair, 1974).

The population of *Fusarium* showed a dramatic rise (13 – 92 x 10³) after 15 days in all the cake-amended treatments. However, in MC-amended soil, a 100% reduction was achieved within 30 days (Table 12). After 60 days, the population declined in all the treatments. Contrary to the effect on *M. phaseolina*, the propagule population of *Fusarium* in the infested soil without amendments showed a gradual increase.

Table 12. Effect of soil amendments on population of *M. phaseolina* (M), *F.o. f. sp. cumini* (F) and the antagonistic actinomycetes population g⁻¹ soil after 45 and 60 days, respectively

Amendments ^a	<i>M. phaseolina</i>	<i>F. oxysporum</i> (x 10 ³)	Antagonistic actinomycetes	
			M (x 10 ⁶)	F (x 10 ⁶)
N + PMR	152	1.3	4.3	4.3
N + PMR + MC	0	8.3	23.3	10.6
N + PMR + CC	366	2.0	38.3	14.5
MC	0 ^b	0.0 ^b	27.3	11.0
CC	200	1.3	24.0	6.6
None	1132	1.7	2.3	0.3
Initial population	2753	0.3	0.9	0.3

^aN – Nitrogen (40 kg ha⁻¹) as urea; PMR – pearl millet; MC – mustard cake (1%); CC – castor cake (1%) and ^bsame as incubation for 30 days

Crucifer plant residues incorporation in the soil was shown to reduce the population of soil-borne pathogens (Ramirez-Villapudua and Munnecke, 1987). The effect was mainly attributed to the release of toxic volatiles such as mercaptan, methyl sulphide and isothiocyanate (Gamliel and Stapleton, 1993). Incorporation of N+PMR in MC and CC maintained higher populations of *M. phaseolina* and *F. oxysporum* till 30 days than when the cakes were added separately. This observation indicates that incorporation of nitrogen-enriched crop residues might have affected the release of toxic volatiles from cakes. The population of bacteria and actinomycetes increased considerably in amended soils. Over 90% of the total actinomycetes were antagonistic to *M. phaseolina*, with the highest numbers in N + PMR+ CC- amended soils (Table 12). However, populations of actinomycetes antagonistic to *Fusarium* were less as compared with *M. phaseolina*. Thus, apart from the toxic effects of cakes, increased population of antagonists might have also contributed in reducing the population of both the pathogens.

These results demonstrate the efficacy of mustard oil-cake in controlling *M. phaseolina* and *F.o. f. sp. cumini* within a period of 30 days. Increased populations of antagonistic actinomycetes, which were found to suppress *F. oxysporum* and *M. phaseolina* propagules (Mathur and Mathur, 1964; Lodha *et al.*, 1990a), further augmented the effect of amendments by inducing suppressiveness of sandy soils.

Natural Heating and Amendments

After ascertaining the efficiency of mustard oil-cake in simultaneous control of both the soil-borne pathogens, a field experiment was undertaken to evaluate the efficacy of cruciferous residues, mustard oil-cake (4 ton ha⁻¹) and cauliflower leaf residue (5 ton

ha⁻¹) combined with summer irrigation and/or solarization on the population of *M. phaseolina* at 0-15 and 16-30 cm soil depth (Lodha *et al.*, 1997). Two kg portions of *M. phaseolina* infested soil were mixed uniformly to 30 cm depth in 28 sub-plots (3 x 1 m). Eight sub-plots were amended with air-dried ground-up mustard oil -cake (4 ton ha⁻¹) and equal number with cauliflower residue (5 ton ha⁻¹). One irrigation was applied to field capacity in all sub-plots except four, which served as dry control. Four sub-plots of each amendment and merely irrigated plots were covered with polyethylene sheet. Soil temperatures were recorded at 7.5 and 22 cm depth. Polyethylene mulching was done for 15 days and then soil samples were collected from 0-15 and 16-30 cm depth to determine population of *M. phaseolina* and lytic bacteria (Greenberger *et al.*, 1987).

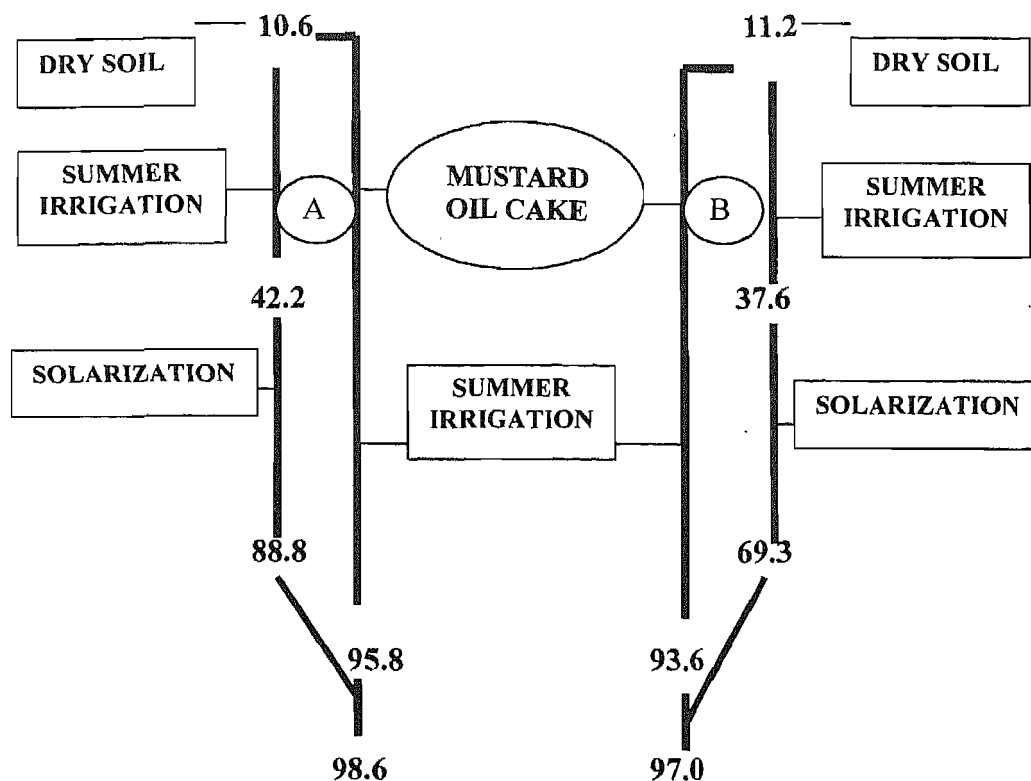


Fig 5. Per cent reduction in *M. phaseolina* propagules in mustard oil cake amended soil with summer irrigation /soil solarization at 0-15 (A) and 16-30 cm (B) soil depth

One summer irrigation (SI) of the dry plots (without amendment) initially reduced the soil temperature to 37°C but a gradual increase to 51°C (7.5 cm) and 46°C (22 cm) followed within 6-7 days. This caused 40% reduction in *M. phaseolina* counts at 0-30 cm depth. In non-amended irrigated solarized plots (SI+S), the temperature was 3-7°C higher

than in corresponding non-solarized plots. Amendment of soil with cruciferous residues augmented the efficiency of SI by eliminating a sizeable proportion of *M. phaseolina* propagules in non-solarized plots (Fig. 5). Soil solarization of amended and irrigated plots raised the maximal soil temperature to 4-6°C above that of the corresponding non-solarized plots. The combined effect of moisture, amendments and temperature was almost complete elimination of viable propagules of *M. phaseolina*, irrespective of soil depth. Cruciferous residues increased the population of lytic bacteria slightly in irrigated non-solarized soil but their density increased significantly in amended solarized soil, particularly at 0-15 cm depth.

The results of this study revealed that combining cruciferous residues with polyethylene mulching or natural heating of moistened soil improved the reduction in populations of *M. phaseolina* in hot arid climate. Soil temperatures under natural heating or solarization in the present study were higher than those observed in other parts of the world with a similar climate (Mihail and Alcorn, 1984; Abdel-Rahim *et al.*, 1988). The sharp decline in the viability of pathogenic propagules in the top soil layer could be attributed primarily to high soil temperatures (Sheikh and Ghaffar, 1984; Lodha and Solanki, 1992). This is also supported by the fact that temperatures at 22 cm remained relatively low (< 50°C) and mortality of the pathogen was also low at this depth.

In this study, pronounced reduction at the lower soil depth could be attributed to a combination of mechanisms. A weakening effect of sub-lethal soil temperature (47-50°C) may have facilitated the action of sulphur-containing toxic volatiles from cruciferous residues and microbial antagonism (Lewis and Pappas, 1970; Lifshitz *et al.*, 1983). After incorporation of cruciferous residues in moist soils, a greater release of toxic volatiles such as methyl sulphide, mercaptan and isothiocyanates at high temperatures was reported by Gamliel and Stapleton (1993). However, isothiocyanates were not detected at low temperatures (Lewis and Pappas, 1970; 1971) and these are mainly responsible for the inhibition or reduction of soil-borne pathogens (Angus *et al.*, 1994). A possible role of increased lytic bacterial density in reducing counts of *M. phaseolina* at lower soil depth in mustard cake amended soil can not be excluded as these bacteria are capable of lysing fungal mycelium of soil-borne pathogens (Mitchell and Alexander, 1963). Thus, combining amendments of cruciferous residues with one irrigation during hot summer days as a substitute for polyethylene mulching may be practical in the cultural control of *M. phaseolina* (Lodha and Mawar, 1999). This finding has a potential value and important implications for 'irrigated pockets of hot arid zone of India' as well as for many countries in the appropriate climatic conditions.

Concept of sub-lethal heating

Reduction in soil population densities of plant pathogens is achieved principally by fumigation, artificial heat treatment, solarization or use of organic amendments (Cook and Baker, 1983; Elad *et al.*, 1982; Katan, 1981; Gamliel, 2000). The death rate of a

population depends on both the dosages and exposure time, causing various degrees of reduction in viability (Bega and Smith, 1962; Pullman *et al.*, 1981). However, sub-lethal dosages, which eliminate only a part of the population, also may affect the surviving and possibly weakened propagules. Studies have shown that weakening of propagules of various pathogens following sub-lethal treatments may result in reduced viability and pathogenicity.

Exposing sclerotia of *Sclerotium rolfsii* to metham sodium caused reduced viability resulting from microbial activity in the damaged sclerotia (Elad *et al.*, 1980; Lifshitz *et al.*, 1983). Similarly, sub-lethal heating of conidia and chlamydo spores of *F. o. f. sp. niveum* at 38-42°C caused 0-33% reduction in propagules viability and resulted in a weakening effect on the surviving propagules (Freeman and Katan, 1988).

Any management strategy, if integrated after a requisite threshold of weakening is achieved, may require less energy, time and amount for improving the control (Fig. 6). Such information on the effect of prior weakening of *M. phaseolina* and *Fusarium* propagules on efficiency of *Brassica* amendments was not available. This information can be used to work out appropriate time of application of cost-effective concentrations of *Brassica* amendments to improve or augment control of *M. phaseolina* and *Fusarium*.

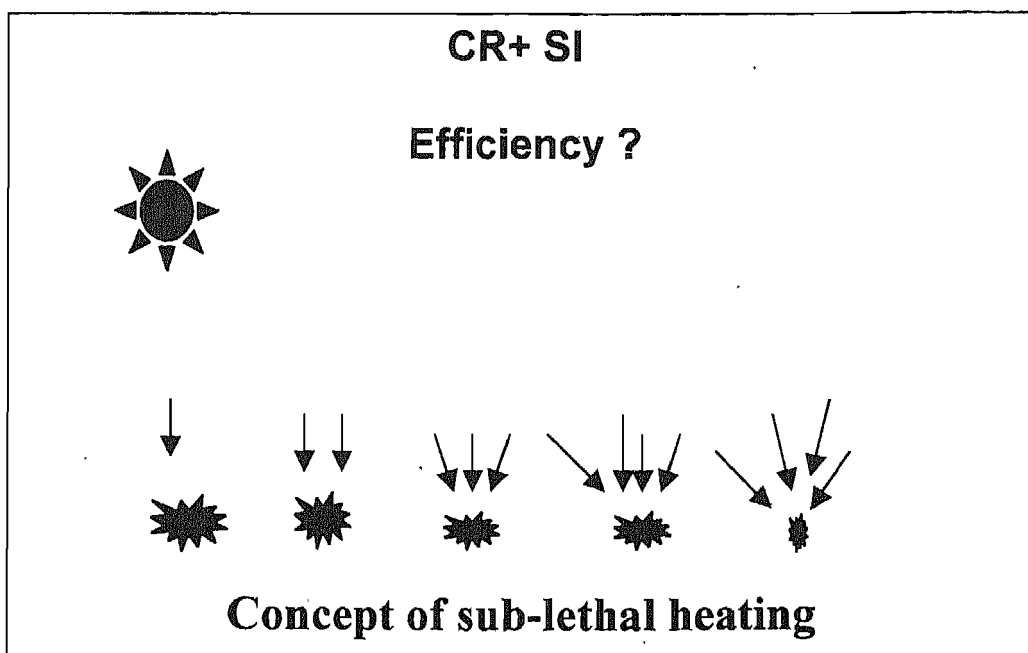


Fig. 6. Whether prolonged sub-lethal heating can increase the efficiency of cruciferous residues and summer irrigation (CR+SI) ?

Integration of sub-lethal heating with *Brassica* amendments

Effect of different levels of sub-lethal heating, concentrations of *Brassica* amendments (mustard oil-cake and pod straw) and summer irrigation was studied on survival of *M. phaseolina* propagules (Lodha *et al.*, 1999 a; Lodha and Mawar, 2000b). The experiment was arranged in four sets (A-D) of 30 x 30 cm pits in order to study effect of four intensities of heat levels. *M. phaseolina* propagules were separately exposed to natural sub-lethal heating for 0 (A-April), 30 (B-May) and 60 (C-June) days under open field and for 30 days (D-May) under shaded conditions (Fig 7). Three sets (A-C), each comprised of 6 pits arranged in split-split plots design (1 m apart) were dug in 4 x 3 m plots in an open field. Pits for set D were dug under the dense canopy of trees 50 m away from other pits. On March 30, soil of each pit was thoroughly mixed with 9 kg of *M. phaseolina* infested soil and sub-samples were taken for the estimation of viable sclerotia of *M. phaseolina*.

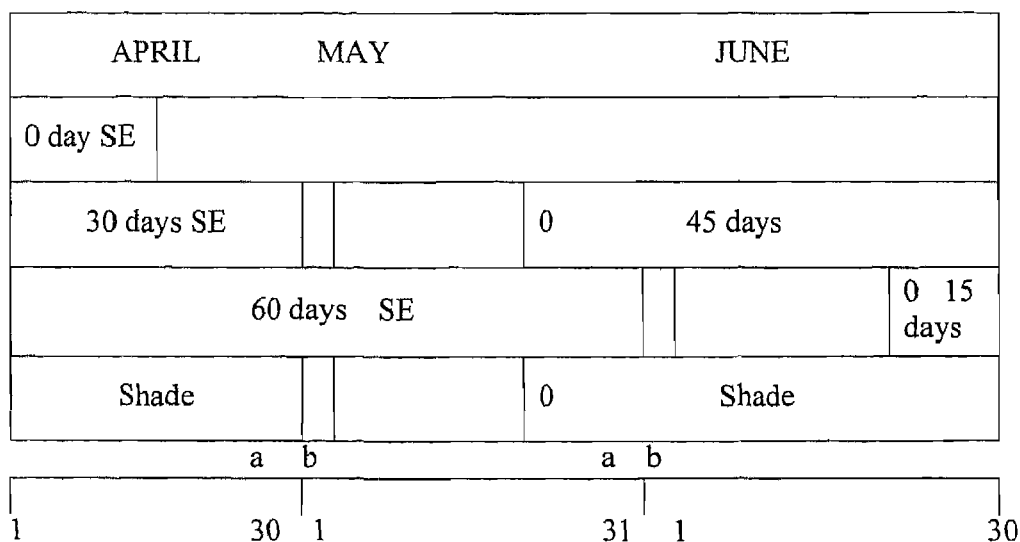


Fig. 7. Schematic plan of the experiment showing varying intensities of sub-lethal heating of *M. phaseolina* propagules in soil achieved by different days of summer exposure (SE). A-0 days, B- 30 days, C- 60 days and D- 30 days under shade.

The soil in pits of set A was separately amended with Indian Mustard (*Brassica juncea* (L.) Czern. and Coss) oil-cake (0.09 and 0.18% w/w) or mustard pod-straw (0.18 and 0.36 w/w) and then filled in randomly selected pits on March 31. Pits filled only with equal amount of *M. phaseolina* infested soil served as wet and dry non-amended controls.

On April 1, one irrigation was applied in plots having pits of set A except dry controls. Soil from pits of sets B and C were withdrawn on April 30 and May 31, respectively and amended separately with same doses of amendments. In set D also, soil was withdrawn on April 30 but only higher doses of mustard oil-cake (0.18% w/w) and pod straw (0.36% w/w) were separately mixed. Amended soil was refilled in the pits on the same day and then irrigated on May 1 (B and D) and on June 1 (C).

Soil temperatures were recorded at 15-cm depth in one pit of each treatment of all the sets from second day of irrigation. Soil samples were collected at depths of 0-15 and 15-30 cm on April 16 (A), May 16 (B and D), June 16 (C) and finally on July 1 from each pit of all the sets. Half of the each sample was used to determine soil moisture by gravimetric method, while the remaining soil was used for estimating the viable propagules of *M. phaseolina*. Population of *M. phaseolina* was estimated on selective medium (Meyer *et al.*, 1973).

During the experimental period (April – June), maximal air temperatures ranged from 34.5 – 47.2 °C; solar irradiations 18.01 – 24.81 MJm⁻²d⁻¹; available sunshine 8.1 – 11.8 hd⁻¹ and evaporation 7.5 – 19.2 mm water d⁻¹. These meteorological parameters remained higher in the first fortnight of June followed by those recorded under same period in May and April in that order. Soil temperatures at 15 cm depth were maximal at 4.00 PM throughout the experimental period. The maximum soil temperature of unshaded amended pits after irrigation reached 41- 45.1°C, however, under shade these remained 6.5 to 11°C lower than corresponding unshaded pits.

Effect of moderate sub-lethal heating (Shade)

Temperature of dry soil under shade did not exceed 38°C during experimental period. Only 9.6% reduction in viable propagules of *M. phaseolina* was estimated on July 1 (Lodha *et al.*, 2003). The soil temperature dropped to 27°C soon after one SI in the dry plots, but a gradual increase to 37°C followed within 9-10 days. In the samples collected on May 16 and July 1, there were reductions of 11.7 and 16.8%, respectively in the viable counts of *M. phaseolina* at 0-30 cm soil depth. Amendments with *Brassica* residues increased the temperature by 0.5-1°C over non-amended irrigated soil. Integration of mustard pod straw (0.36%) or oil-cake (0.18%) with summer irrigation caused 49 to 57% reductions in *M. phaseolina* propagules by May 16. A small improvement in this reduction was further estimated on July 1. Treatment contrasts of unshaded and shade conditions revealed that per cent reduction in *M. phaseolina* propagules was significantly higher under unshaded conditions. Among amendments, oil-cake appeared significantly better than mustard pod straw in reducing counts of *M. phaseolina* at both the depths.

Effect of sub-lethal heating (Unshaded conditions)

Maximum temperature of dry soil (DS) during the first fortnight of April reached 44 °C. This reduced viable propagules of *M. phaseolina* by 3.3% at 0 - 30 cm on April 16. Subsequent sub-lethal heating for the next 30 and 60 days reduced viability by 6.9 and 11.2% in the samples collected on May 16 and June 16, respectively. However, significant reductions were further evident in samples withdrawn on July 1. The reduction in *M. phaseolina* propagules, however, was significantly higher at 0-15 cm than that at 16-30 cm soil depth.

One summer irrigation (SI) of the non-amended dry pits initially brought down the soil temperature to 32 °C in April, but a gradual increase to 40.5 °C followed within 15 days. This reduced viable propagules of *M. phaseolina* by 31% at 0-30 cm soil depth. When SI was applied in sub-lethal heating level 30 and 60 days (May and June), after an initial drop to 34.5 and 37 °C, soil temperature reached 43 and 44.5 °C, respectively. Increased sub-lethal heating coupled with a small rise in temperature at sub-lethal heating level 2 (30 days) significantly improved (34%) the reduction in viable propagules of *M. phaseolina*. However, sub-lethal heating level 3 (60 days) did not improve this reduction. The magnitude of reduction increased in the final soil samples collected on July 1 from pits of 0, 30 and 60 days (Table 13) but significant increase was estimated only from sub-lethal heating level 1 (0 days) to 2 (30 days).

In amendment treatments, soil temperature remained 0.5-4.1 °C higher for the first 6-8 days than those recorded in corresponding non-amended pits of different sub-lethal heating levels. Summer irrigation augmented the efficiency of *Brassica* residues by eliminating a sizeable proportion of *M. phaseolina* propagules at both the soil depths. This reduction was significantly greater at higher concentration of both the amendments and in oil-cake compared to pod straw. In general, the level of reduction was significantly higher at 0-15 cm than that achieved at lower soil depth.

Analysis of variance showed that heat levels, amendments, depths, interaction of amendments x heat levels and amendment x depth were significant (Table 13). The other significant statistics across the levels of sub-lethal heating were the contrast of amendment versus the control and oil-cake versus pod straw. When the same concentration of amendments and one SI were applied at heat level 2 and 3, significant improvement (16.1-26.6%) in reduction of *M. phaseolina* propagules was estimated in the final soil samples compared to heat level 1, that was also significantly higher than that occurred on 16th of each month.

Amendment with *Brassica* residues retained comparatively more soil moisture than non-amended controls after 15 days of irrigation. Moisture retention was higher at 15-30 cm compared to 0-15 cm soil depth in April than in other two months. Among both

the amendments, mustard oil-cake retained greater soil moisture than pod-straw except under shade conditions where reverse was true.

During experimentation, often temperatures of dry soil during day time were quite close to the thermal death time-temperature (50 °C for 97 minutes) reported for *M. phaseolina* (Bega and Smith, 1962). In our earlier study, considerable reduction (94.7%) in counts of *M. phaseolina* was achieved by amendment of soil with 0.18% w/w mustard oil-cake and one summer irrigation merely by natural heat (Lodha *et al.*, 1997). In the

Table 13: Effect of sub-lethal heating, *Brassica* amendments and summer irrigation on the reduction of *M. phaseolina* population at different heat levels

Heat levels	<i>Brassica</i> amendments				
	Oil-cake		Pod straw		No amendment
	(0.09%)	(0.18%)	(0.18%)	(0.36%)	
1 (April)	67.8 (55.42)	71.6 (57.78)	60.4 (50.65)	67.9 (55.40)	33.9 (45.38)
2 (May)	81.8 (64.93)	86.7 (72.47)	80.7 (65.49)	82.8 (65.28)	36.2 (49.87)
3 (June)	94.2 (76.28)	96.1 (78.83)	89.4 (71.21)	94.5 (76.22)	41.3 (49.92)
Source of variation			d.f.	Mean square	
Heat levels			2	4633.30***	
Amendments			4	1276.23***	
Amend x heat levels			8	747.70***	
Amend vs. control			1	8760.60***	
Oil-cake vs. pod straw			1	460.46***	
Depth			1	584.24***	
Amend x depth			4	7.87 NS	
Heat levels x depth			2	4.37 NS	
Heat levels x amend x depth			8	16.16 NS	

present experiment, when sub-lethal heating for 60 days (April 1 - May 30) was given to *M. phaseolina* propagules, almost an equal reduction (94.2%) was achieved at reduced dose (0.09% w/w) of the same amendment. More than one mechanism might have operated concurrently or in a sequence in eliminating viable propagules of *M. phaseolina* from soil. Sub-lethal heating in dry soil for 90 days though reduced only 12.8% viable sclerotia of *M. phaseolina* but exerted a weakening effect on the surviving propagules. The effect was evident by significantly higher reduction in *M. phaseolina* propagules following sub-lethal heating for 60 days (38.9%) compared to 30 days (34.5%), though

the prevailing soil temperatures were comparable. The weakening effect depends on temperature level, exposure time and the environment into which the preheated propagules are introduced. However, a certain threshold of heating has to be reached to obtain a detectable weakening effect (Freeman and Katan, 1988). Significantly low levels of reduction in viable counts of *M. phaseolina* estimated in amended pits under shade compared to corresponding unshaded pits indicated that detectable weakening effect was not achieved at moderate heat level.

Increase in rate of decomposition was tenable by greater difference in temperature of amended soil in May and June (bright sunlight) compared to shade and merely irrigated pits. Increased temperature in amended compared to non-amended soil resulting from increased exothermic microbial activity may be an important factor for improving control of *M. phaseolina*, a heat - tolerant pathogen. Availability of less residual soil moisture after 15 days in pits irrigated in May or June compared to those irrigated in shade or April is a supporting evidence for this action. However, presence of more residual soil moisture in amended compared to non-amended pits probably encouraged microbial antagonism against remaining but weakened sclerotia particularly by bacteria at lower soil depth. In the final soil samples, population of bacteria and actinomycetes were invariably higher in amended compared to non-amended pits. The bio-toxic effects of volatile compounds from cruciferous residues and particularly those of allyl isothiocyanate (Mayton *et al.*, 1996) at high soil temperatures have been well demonstrated (Lewis and Papavizas, 1971; Gamliel and Stapleton, 1993). However, the quantity of pesticidal compounds produced during the decomposition may not be the only cause for this reduction. Perhaps, biological control of weakened propagules also is likely to play a role in the process. Increased colonization of heat treated sclerotia of *Sclerotium rolfsii* by bacteria has also been reported by Lifshitz *et al.* (1983).

Cumulative effects of these factors resulted in improved reduction of *M. phaseolina* propagules. Prior weakening of sub-lethal dosages of killing agents has been suggested as a tool for achieving integrated control through a synergistic effect (Baker and Cook, 1974). Combining heating and *Trichoderma harzianum*, both at sub-lethal doses resulted in improved control of *Sclerotium rolfsii* (Elad *et al.*, 1980) and *Rosellinia necatrix* (Sztejnberg *et al.*, 1987). Similarly, synergistic interaction between sub-lethal heating and *Talaromyces flavus* caused increased mortality of the microsclerotia of *Verticillium dahliae* (Tjamos and Fravel, 1995).

One major advantage of polyethylene mulching of the amended soil was that its surface was sealed to get maximum benefit from release of toxic volatiles (Ramirez-Villapudua and Munnecke, 1988). In the present concept of natural solar heating also, fast drying of top 1cm soil soon after irrigation leads to formation of a 2 mm crust. This

may act as a sealing material and prevents fast exchange of gases. Further, sub-lethal heating for long duration may substantiate this advantage where reduced dosages of amendments were found sufficient to kill already weakened propagules of *M. phaseolina*. This was evident with amendment of mustard oil-cake or pod residues where greater reduction in pathogenic propagules was achieved at low concentration (0.09% w/w) after 60 days of sub-lethal heating compared to that achieved at high concentration (0.18% w/w) after 0 and 30 days of sub-lethal heating. The results of our study demonstrate that the incorporation of *Brassica* residues combined with summer irrigation has a greater beneficial effect in reducing *M. phaseolina* viability during the crop-free period following harvest. More so, phytotoxicity symptoms were also not observed in succeeding clusterbean crop (July - October) in our field experiments.

After ascertaining efficiency of mustard oil-cake and pod straw, research efforts were made to improve efficiency of pod straw by combining other readily available sources of cruciferous residues including mustard oil-cake. Field experiments were conducted in crop-free period (April-June) of 1998 and 1999 with an objective to ascertain effect of cruciferous residues in controlling *Macrophomina* and *Fusarium* after prolonged summer exposure to their resting structures (Mawar and Lodha, 2000a). In 1998, four amendment combinations viz., mustard residues (MR – 2.5 ton ha⁻¹), MR + mustard cake (2.5 ton + 0.5 ton ha⁻¹), MR + *Eruca* (2.5 ton + 0.5 ton ha⁻¹), MR + cauliflower residue (2.5 ton + 0.5 ton ha⁻¹) along with non-amended dry and wet controls were arranged in different pits in two sets for May (A) and June (B). These were exposed to summer heat from April 1. Additional one set of infested soil was kept in laboratory at room temperature for 60 days and brought in the field only on June 1 (C), while another set of pits (D) were dug in shade and amendments and irrigation were given on June 1. In 1999, the experiment was repeated except that combination of *Eruca* and cauliflower with MR and treatment sets of May were deleted.

During the experimental period, temperature of dry soil ranged between 42-46°C and 40-43°C in May and June, respectively. One irrigation in dry soil initially brought down the soil temperature to 37°C but it reached 40-41°C within 15 days in May and June causing a reduction of 31 and 32.5% in viable propagules of *Fusarium*. Combining amendments with summer irrigation improved this reduction (Lodha and Mawar, 2000 b). Maximum reduction (86.8%) was achieved when a small dose of mustard oil-cake (0.5 ton ha⁻¹) was supplemented to mustard pod residues (2.5 ton ha⁻¹) and summer exposure was given for 60 days, compared to mustard pod residues alone (82.3%). This reduction was significantly higher (15.3-15.6%) than that recorded in the treatment where infested soil brought from laboratory was mixed with same amendments (Fig. 8).

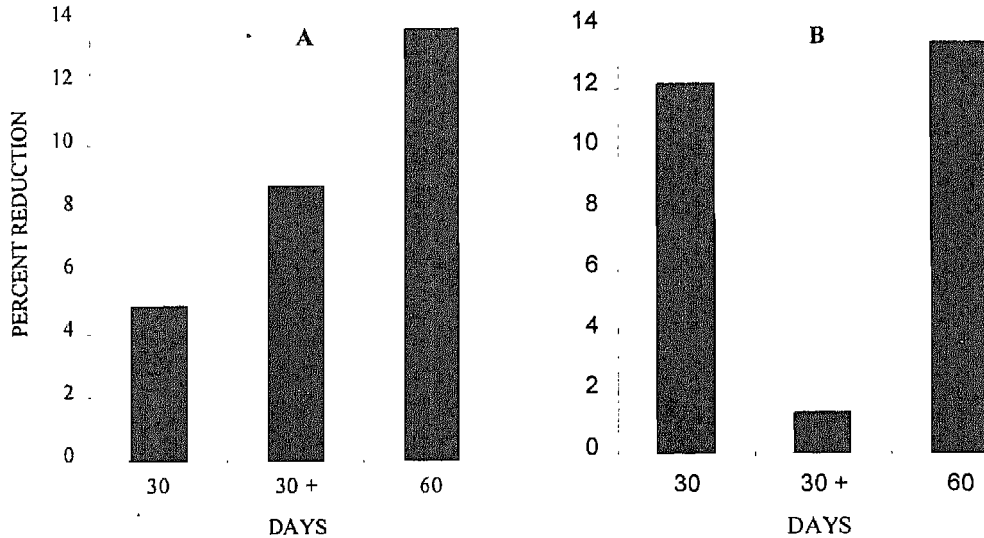


Fig. 8. Improvement in percent reduction in *M. phaseolina* (A) and *F. oxysporum* (B) propagules due to increased sub-lethal heating

These combinations of cruciferous amendments were also effective in reducing inoculum density of *M. phaseolina* below economic threshold level at 0-30 cm soil depth. Combining mustard pod straw with oil-cake caused 87-90% reduction in viable propagules of *Macrophomina* compared to that achieved by mustard residues alone (76-80%). Prolonged exposure to dry summer heat for 60 days significantly improved the reduction compared to zero and 30 days of exposure.

Comparatively higher reduction in *M. phaseolina* than *Fusarium* propagules achieved in this study may be a result of increased microbial antagonism against *M. phaseolina* due to soil moisture (Dhingra and Sinclair, 1975; Lodha, 1996). Improvement in reduction by supplementing a small dose of mustard oil-cake with pod residues could be due to presence of higher concentration of biotoxic volatiles and nitrogen in oil-cake. However, the suppressive activity of *E. sativa* with pod residues can be considered as intermediate probably due to its low glucosinolates content (18.6 M moles m⁻²) as reported by Lazzeri and Manici (2000).

Subsequently, field experiments were conducted in the year 2000 and 2001 with an objective to comparatively evaluate cruciferous residues and other effective weed residues of the region in controlling *Fusarium* after prolonged summer exposure (Israel

and Lodha, 2003b). In the year 2000, five amendments viz., *Euphorbia*, *Verbisina*, *Celosia*, onion and mustard, each at 0.18% w/w concentrations alongwith non-amended dry and wet controls were arranged in different pits in six sets (A-E). On April 1, *Fusarium* infested soil was filled in 9 kg capacity pits in field of sets A, B and E, while soil of set C was kept separately in laboratory at room temperature but filled in pits only on May 27. One lot each of 9 kg soil was filled in pits of set D and F. Pits of set A – D were dug in an open field while E and F were dug under the dense canopy of trees. On May 27, the soil in each pit of set A, B, C and E were amended individually with aforesaid residues and irrigation was applied on May 28 in all the plots except of set D and F. Polyethylene sheet was mulched in the plot having pits of set A. Soil temperatures were recorded at 14.00 and 16.00 h at 15 cm depth in one representative pit of each set. On June 30, the pouches containing *Fusarium* infested soil buried at 7 and 22 cm depth were retrieved from each pit and processed for estimating viable propagules of *Fusarium*. All the experimental details remained same in 2001, except that treatment of MR + *Verbisina* (MR 0.18% + V – 0.04%), MR + Onion (0.18% + 0.04%) and MR + MC (0.18% + 0.04%) were included, while *Euphorbia* and *Celosia* were omitted.

In general, a significant improvement in reduction of *Fusarium* propagules was achieved with the increase in duration and intensity of heat. Under shade conditions, 32-47% reduction in *Fusarium* propagules was estimated in all the amendments. Soil brought from laboratory and exposed to bright sunlight improved this reduction (75.7-86.5%). Further, a significant improvement was estimated in *Fusarium* infested soil continuously exposed to dry heat for 55 days, where reduction ranged from 76.6-88.3%. Elevation of soil temperature due to polyethylene mulching further augmented this reduction in the range of 80.2-95.5%. In the year 2001, combining a small dose of onion, *Verbisina* or mustard oil-cake with mustard residues further improved the reduction in *Fusarium* propagules at all the heat levels. These combinations also improved the reduction at the lower soil depth.

This study conclusively established that (i) combined application of mustard pod residues (2.5 t ha^{-1}) along with mustard oil-cake (0.5 t ha^{-1}) or *Verbisina* (0.5 t ha^{-1}) with one summer irrigation are highly effective in control of *Fusarium* propagules. (ii) Prolonged exposure to dry summer heat that exerted the weakening effect on pathogenic propagules enhanced the efficiency of amendments and summer irrigation.

Different Doses of Mustard Oil-Cake and Pod Straw

A field experiment was conducted to ascertain effectiveness of different doses of *Brassica* amendments in reducing viable propagules of *M. phaseolina* (Lodha and Sharma, 2002). In 7 cm^3 pits, ground up residues with concentration ranging from 0.022

to .27% (w/w) so as to give 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 ton ha⁻¹ doses of mustard oil-cake and pod straw, respectively were amended in *M. phaseolina* infested soil under shaded and unshaded conditions. Irrigation was given on June 1 and soil temperatures were recorded at 5 cm depth for 15 days. During June 1-15, 1996, temperature of dry soil ranged from 42-55°C and 29-40°C under unshaded and shaded conditions. In amended pits, soil temperature remained 0.5-3°C (unshaded) and 0.5-1.5°C (shaded) higher than non-amended pits. *Brassica* amendments significantly reduced *M. phaseolina* under both the environments. Of the amendments, mustard oil-cake was significantly better where complete reduction in viable propagules of *M. phaseolina* was achieved at 0.9% (2 ton ha⁻¹) compared to 0.22% (5 ton ha⁻¹) pod residues (Table 14). Under shade conditions, magnitude of reduction in *M. phaseolina* propagules was low but significant improvement in the reduction was estimated with increased concentration of amendments. Efficacy of mustard oil-cake even at low concentration (2 ton ha⁻¹) could be attributed to presence of 7-8% oil that release more quantity of volatiles at high temperature besides having 5% nitrogen. Organic amendments with high nitrogen have been shown to effectively control soil-borne pathogens (Rodriquez-Kabana *et al.*, 1990). The population of total aerobic cultivable bacteria and actinomycetes increased significantly with the increased concentration of mustard residues but in case of oil-cake after 0.09% significant decline was estimated in the population of both the types of microbes (Table 15).

A significant improvement in lytic bacterial density upto a certain level was estimated in all the amended pits compared to dry and irrigated non-amended pits. Possible role of increased lytic bacterial density in reducing *M. phaseolina* counts can not be excluded, as these bacteria are capable of lysing fungal mycelium of soil-borne pathogens (Mitchell and Alexander, 1963).

There was an increase in the population of mesophilic and thermophilic fungi with increasing concentration of amendments upto 0.09 and 0.18%, respectively. Enhanced survival of mesophilic and thermophilic fungi under natural heating process may induce soil suppressiveness. Heating cabbage-amended soil at 45°C has been found effective in controlling *Pythium* and *Sclerotium* population (Gamliel and Stapleton, 1993).

Table14. Effect of different *Brassica* amendments on survival of *M. phaseolina* propagules under unshaded and shaded conditions

Amendments*	Concentration (ha ⁻¹)	Reduction in <i>M. phaseolina</i> (%)		Dehydrogenase activity (P kat g ⁻¹ soil)	
		Unshaded	Shade	Unshaded	Shade
C + SI	0.5	88.8	27.6	15.9	15.8
	1.0	91.6	38.3	16.1	16.5
	1.5	97.2	39.1	18.3	17.3
	2.0	100.0	43.1	19.5	17.7
	2.5	100.0	47.9	16.7	19.2
	3.0	100.0	59.7	16.9	16.9
MR + SI	0.5	88.0	40.4	14.3	16.2
	1.0	94.4	45.6	16.5	17.7
	1.5	94.4	52.3	18.1	17.9
	2.0	97.2	52.4	19.8	18.5
	2.5	100.0	59.7	20.6	17.7
	3.0	100.0	63.8	17.8	17.6
SI	-	52.7	15.2	13.5	15.0
DS	-	12.3	8.8	10.8	13.3
LSD (<i>P</i> =0.05)	-	15.5	6.3	1.3	1.2

*MC – Mustard oil-cake, MR – Mustard residue, SI – Summer irrigation, DS – Dry soil

In our experiment, 44°C temperature was achieved by natural heating alone for more than 4 h a day for atleast 10 days after irrigation. As a result, effective doses of *Brassica* amendment for the control of *M. phaseolina* in hot arid region have been developed (Lodha and Sharma, 2002).

Table 15 . Effect of different concentrations of Brassica amendments on total bacteria actinomycetes, fungi and lytic bacterial density (CFU g⁻¹ soil)

Amendments*	Dose (ton ha ⁻¹)	Bacteria (x 10 ⁵)	Actinomycetes (x 10 ⁵)	Fungi		Lytic bacteria (x 10 ⁴ g ⁻¹)
				Meso-philic	Thermo-philic	
MC + SI	0.5	2.6	11.3	2.3	1.3	4.7
	1.0	4.1	11.6	3.0	1.3	4.7
	1.5	4.4	12.0	3.6	2.6	6.0
	2.0	4.8	13.7	5.0	3.7	6.5
	2.5	3.7	8.0	2.7	3.3	5.8
	3.0	2.1	5.3	2.7	1.0	5.9
MR + SI	0.5	2.5	12.0	3.0	1.3	6.1
	1.0	2.6	12.3	3.3	2.3	6.2
	1.5	3.1	13.0	3.6	2.7	6.2
	2.0	5.9	19.3	4.0	3.3	7.0
	2.5	9.6	24.3	3.6	3.3	6.6
	3.0	10.7	23.3	4.3	3.6	3.2
SI	-	2.4	8.0	2.3	1.0	2.8
DS	-	1.7	7.1	2.1	0.7	1.6
LSD (P=0.05)	-	0.9	5.3	1.9	1.5	1.6

* MC – Mustard oil-cake, MR – Mustard residue, SI – Summer irrigation, DS – Dry soil

Biological Control

Rationale

Studies on soil-borne plant pathogens led to development of many management strategies. One of the basic component of these strategies is the use of efficient bio-control agents to improve control of soil-borne plant diseases. While using bio-control agent in Integrated disease management (IDM), basic requirement is that it should be a resident isolate, relatively heat tolerant and able to survive in sandy soils of hot arid region. Several native bio-control agents were isolated from amended soils in these years.

Trichoderma harzianum

A native strain of *T. harzianum* was isolated from aridisols. The effectiveness of this strain was tested against major soil borne plant pathogens like *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. solani* etc. by dual culture method. Studies were conducted on multiplication of this strain on indigenous substrates like *Prosopis* pods. (Lodha *et al.*, 1999b).

Aspergillus versicolor

During the course of investigation, one native fungi isolated from the cruciferous residue amended soil was repeatedly found to parasitize *Fusarium oxysporum* f. sp. *cumini*. Antagonism of this fungus against *Fusarium* was confirmed by repeated dual culture methods. The fungus was identified as *Aspergillus versicolor* by Agharkar Research Institute, Pune. In a separate dual culture test, where discs of *Fusarium* were placed at the periphery, whitish mycelial growth was visible only during the initial stage, but at a later stage dense hyphal growth of *A. versicolor* overgrew and completely hyperparasitized Fusarial mycelial mat (Israel, 2002).

In *Fusarium* and *A. versicolor* infested soil, an initial population of 4.2×10^4 CFU g^{-1} soil of *Fusarium* drastically declined to 2.8×10^3 CFU g^{-1} soil after 15 days of incubation causing 93.3% reduction. In *Fusarium* and *T. harzianum* infested soil, after 15 days, 1.1×10^4 CFU g^{-1} soil of viable propagules of *Fusarium* causing 73.8% reduction were estimated under similar conditions. This declined further continued to the extent that after 30 days only 0.3×10^3 and 15 days 1.5×10^3 CFU g^{-1} soil of *Fusarium* were estimated in *A. versicolor* and *T. harzianum* infested soil, respectively (Israel, 2002).

In liquid culture tests, a cell-free filtrate even at a low concentration of 0.5 ml of both the biocontrol agents could inhibit mycelial growth of *Fusarium* (Israel, 2002). In general, percent reduction was considerably high in presence of filtrate of *A. versicolor* compared to that of *T. harzianum*. In a separate liquid test, effect of age of filtrate of *A. versicolor* and *T. harzianum* on mycelial growth of *Fusarium* was evaluated. There was a significant improvement in reduction of *Fusarium* in 8 day compared to that in 4-day-old cell free filtrate of both the biocontrol agents (Table 16). Further increase in age of filtrate did not improve reduction. The reduction was higher in *A. versicolor* compared to *T. harzianum* filtrate.

Table 16. Effect of age of metabolites on mycelial growth of *Fusarium*

Treatment	Days	Reduction (%) in <i>Fusarium</i> mycelial growth
<i>Aspergillus versicolor</i> (2-ml)	4	33.2
	8	54.3
	12	54.6
	16	54.9
<i>Trichoderma harzianum</i> (2-ml)	4	41.9
	8	44.4
	12	45.3
	16	46.2

Population changes of *A. versicolor* were followed for 120 days in a sandy soil maintained at different gradients of moisture. There was a sudden upsurge in the population of *A. versicolor* at all the moisture gradients after 30 days of incubation, with maximum being at 70% of MHC. At subsequent interval, populations fluctuated and showed a marginal increase and decrease at 90 and 120 days, respectively. In general, maximum survival and multiplication was estimated at 50% of MHC compared to any other moisture gradient.

Studies related to thermal resistance to *A. versicolor* showed that this bio-control agent was able to survive and multiply even at 65°C. There exist a positive correlation between increase in time interval and increase in population of *A. versicolor*. However, at 60°C, a sharp decline in viable propagules of *A. versicolor* occurred within 6 minutes of exposure, but subsequently a gradual increase was estimated both under moist and dry conditions. After 18 minutes of exposure, counts of *A. versicolor* were 2- and 3-fold higher than that of initial level. Similarly, population of *A. versicolor* declined after an exposure for 2 seconds at 62°C both under moist and dry conditions, but subsequently it increased again. Thus, after an exposure of 8 seconds, counts of *A. versicolor* were higher than that of initial level with more being under dry conditions. At 65°C also, where the test was run only under dry conditions, population of *A. versicolor* increased at 8 seconds exposure after an initial decline.

In a pot experiment, combined effects of biocontrol agents and *Verbisina* residues were ascertained. Studies revealed that amending soil with *A. versicolor* alone or in combination with *T. harzianum* (Fig.9) or *Verbisina* residues were significantly better in reducing *Fusarium* population compared to non-amended control. Maximum reduction in *Fusarium* population occurred where both the biocontrol agents (BCA's) were integrated with *Verbisina* residues. Of both the BCA's, reduction in *Fusarium* propagules was significantly better in *A. versicolor* amended soil. However, their combinations were significantly better than their lone application. A marked increase in root length over control was observed in the treatment with *A. versicolor* and *T. harzianum* and their integration. Further, integration of both the BCA's with *Verbisina* increased shoot length and weight. Synergistic effect of both the BCA's was clearly observed with the increase in shoot length and weight compared to that recorded in control.

These studies demonstrate that hyperparasitism of *A. versicolor* over *Fusarium* was comparable with that of *T. harzianum*. *A. versicolor* has also been reported as an antagonist against *M. phaseolina* (Bhattacharya *et al.*, 1985). Its potential in inhibiting germination of uredospores of *Puccinia helianthi* has also been demonstrated (Patil *et al.*, 2000). Its mutant is known to produce an antibiotic 'Mycoversilin' (Samanta *et al.*, 1983). Apart from release of antibiotic, various hydrocarbons, alcohols, ketones, ethers,

sulphur-containing compounds have also been identified as volatile metabolites from *A. versicolor* (Sunsseson *et al.*, 1995). Better survival and multiplication of *A. versicolor* at low soil moisture content and at high soil temperature are supportive evidence for its adaptation in dry sandy soils, where temperature often reaches in the ranges of 50-60°C during hot summer months.

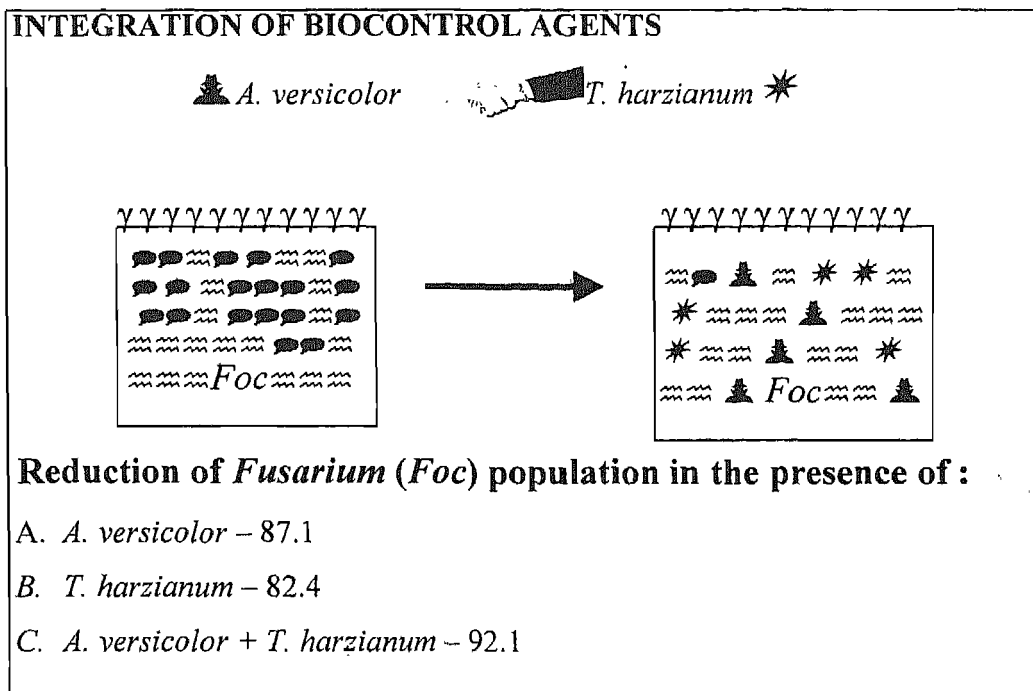


Fig 9. The effect of integration of *Aspergillus versicolor* and *T. harzianum* on survival of *Fusarium (Foc)* propagules in soil

Bacillus firmus

Cruciferous residue amended soil was screened for the presence of antagonists against *M. phaseolina*. A soil bacterium exhibiting clear inhibition zone was isolated and brought into pure culture. Dual inoculation test was carried out to confirm antagonism of bacteria against *M. phaseolina*. Observations on the interaction of antagonistic bacterium and *M. phaseolina* on Czapeck's Dox agar indicated development of a clear inhibition zone after 5-6 days of inoculation (Lodha *et al.*, 2000). The growth of *M. phaseolina* continued to remain restricted but developed a scarlet pigmentation after 4 days. To confirm that antagonism is antibiosis in nature, several 3-mm discs were cut from

bacteria free inhibition zone and placed at four equi-distant points on Czapeck's Dox agar. *M. phaseolina* discs were placed in the center of each petri dish. Observations taken after four days indicated that bacterial free disc from scarlet pigmentation also inhibited the growth of *M. phaseolina* though the area of inhibition zone was significantly reduced compared to that recorded in presence of bacterium.

The bacterium was identified as *Bacillus firmus* by IMTC, Chandigarh, after analyzing morphological, physiological and biochemical characteristics. This gram +ve bacterium was able to grow at a temperature range of 22-45°C.

To study the pattern of inhibition in presence of different substrates, three solid media PDA, Czapeck's Dox agar and nutrient agar were used and well method was adopted. The pattern and zone of *M. phaseolina* inhibition varied when both the microbes were tested on media having different composition. Maximum inhibition was recorded on nutrient agar to the extent that at the periphery of *M. phaseolina* colony sclerotial formation was checked (Mawar, 2001).

Separate dual culture tests were performed to ascertain the activity of antagonistic bacterium against prevalent soil fungi like *Aspergillus terreus*, *A. niger*, *Paceilomyces fusiformis*, *A. fumigatus*, *A. regulosus*, *A. awamosi* and cumin wilt pathogen *Fusarium oxysporum* f.sp. *cumini* and a bio-control agent *T. harzianum*. *B. firmus* failed to inhibit growth of all the soil fungi including *T. harzianum*. Scarlet pigmentation was also not observed.

In a separate experiment, growth of *M. phaseolina* in presence of antagonistic bacterium was significantly higher than that recorded with its cell free filtrate. When *B. firmus* or its filtrate were incorporated in Czapeck's Dox broth, maximum activity of *B. firmus* on mycelial growth of *M. phaseolina* was recorded when both were simultaneously inoculated. However, the activity of *B. firmus* reduced with increased time interval of its inoculation. It produced scarlet pigmentation in liquid medium also.

In a pot experiment, effect of *B. firmus* seed treatment on growth parameters and nodulation of clusterbean seedlings was studied. The experiment had four treatments 1. Field soil (without *M. phaseolina*), 2. *M. phaseolina* infested soil + *B. firmus* coated seeds, 3. Field soil + *B. firmus* coated seeds and 4. *M. phaseolina* infested soil. After 25 days of sowing, observations on plant height, fresh weight and number of nodules were recorded. Studies revealed that height, fresh and dry weights of seedlings were significantly higher in the treatments where *B. firmus* coated seeds were sown in the field soil (without *M. phaseolina*) compared to treatments where uncoated seeds were sown (Table 17). Presence of *M. phaseolina* along with *B. firmus* though affected all the growth parameters studied but reduction was not significant compared to lone presence of *B. firmus*.

Nine different residues : mustard pod straw, mustard oil-cake, cauliflower, radish, eruca, pearl millet, *Euphorbia hirta*, *Aerva persica*, *Celosia argentea* and three composts of *E. hirta*, *A. persica* and *C. argentea*, which were found effective in the control of *M. phaseolina* were evaluated as growth substrates for the multiplication of bacterial antagonist. Each substrate was amended @ 1% in separate soil lots and population of bacterium was estimated after 20 days of incubation.

Amendment of soil with all the cruciferous residues except mustard oil-cake significantly enhanced the multiplication of *B. firmus* within 20 days. Maximum multiplication was recorded in residues of radish amended soil. However, it was significantly equal to that recorded in mustard and cauliflower residue amended soil. Among other residues, pearl millet supported maximum multiplication of *B. firmus* while *Euphorbia* residues inhibited its multiplication. Other weed residues also did not support multiplication.

Table 17. Effect of *B. firmus* and *M. phaseolina* on growth parameters of clusterbean seedlings

Treatments	Fresh weight (mg)	Dry weight (mg)	Shoot height (cm)	Nodules
Field soil	385.8	129.0	9	0.5
<i>M. phaseolina</i> ^a + <i>B. firmus</i>	571.8	137.0	10	0.8
<i>B. firmus</i> ^b	613.0	157.0	12	4.3
<i>M. phaseolina</i>	366.0	119.9	9	0.5
LSD (0.05)	184.8	23.9	2	-

^a Soil was infested with *M. phaseolina* sclerotia

^b Seeds were treated with 10 ml solution of *B. firmus* and carboxy methyl chloride

Characteristic feature of growing well on a wide range of temperature corroborates well with faster multiplication of *B. firmus* in heated soil. Broadbent *et al.*, (1971) also reported that the percentage of *Bacillus* isolates antagonistic to plant pathogens increased with the temperature of soil treatments up to 60°C for 10 minutes, and then declined. Nutrients from decomposing residue and certain compounds such as ethylene, ammonia, acetone, ethanol, methanol, formaldehyde, acetaldehyde were reported to stimulate germination of micro-organisms (Harman *et al.*, 1980). Many of these and related compounds have been isolated and characterized from heated cruciferous residues (Gamliel and Stapleton, 1993). Release of some antibiotic due to interaction of both the microorganism is known as antibiosis. Analysis of mutants of *B. cereus* shows a significant quantitative relationship between disease suppressiveness and the production of two antibiotic, Zwittermicin A and Kanosamine (Silo-Suh *et al.*, 1994; Milner *et al.*, 1996).

Bioformulations

T. harzianum – Maru Sena 1.

Studies revealed that population of *T. harzianum* increased gradually in composts prepared from residues of *P. juliflora*, *Calotropis* and weeds compared to FYM and non-amended control (Bareja and Lodha, 2002). Maximum survival was estimated in *P. juliflora* compost. In subsequent experiment, better survival of *T. harzianum* was estimated in mixture of *P. juliflora* compost and talc compared with that in only talc. After achieving this success, in the year 2002, 300 packets (100 g each) were prepared of this bioformulation and named as **Maru Sena 1**. These packets were sold to farmers in *Kharif* season through Agricultural Technology Information Centre (ATIC).

A. versicolor – Maru Sena 2.

Among composts, maximum population and survival rate of *A. versicolor* was estimated in sterilized neem compost. Accordingly, a bioformulation of *A. versicolor* in a mixture of neem compost and talc was prepared and distributed to only selected farmers as **Maru Sena 2**. Use of *A. versicolor* has an additional advantage in hot arid region due to its thermal resistance and ability to survive under low soil moisture conditions. Information gathered through farmer's fields gave encouraging results where 10-15% reduction in wilt incidence was recorded in year 2001-02.

B. firmus - Maru Sena 3.

Bioformulation of *B. firmus* was prepared in locally available lignite and by maintaining adequate soil moisture as **Maru Sena 3**.

Integration of Cultural and Bio-Control Agent

After working out potential value of cruciferous residues, sub-lethal heating on survival of both the pathogens and antagonistic effect of bacterium against *Macrophomina*, next step was to study effects of these strategies in an integrated manner on both the diseases. A field experiment was, therefore, conducted (1998-2000) to study combined effects of *Brassica* amendments and summer irrigation on survival of *M. phaseolina* and *Fusarium* in soil, and dry root rot intensity on clusterbean (July-Oct) and wilt of cumin (Nov-Mar) in the same field.

Two *Brassica* amendments, mustard oil-cake and pod straw (2.5 ton ha⁻¹) were amended in pathogenic infested field either in the end of May with one summer irrigation (MC+SI, MR+SI) or in July (MC+NS, MR+NS). In case of dry root rot of clusterbean, antagonistic bacterium was also integrated as seed treatment (MR+SI+ST). Summer irrigation, seed treatment and normal sowing (SI, ST, NS) were kept as corresponding controls.

Results of field study have shown that both the amendments combined with one summer irrigation were significantly superior in reducing incidence of dry root rot and wilt on clusterbean and cumin, respectively (Lodha *et al.*, 2001; Mawar and Lodha, 2002). The soil temperature of amended soil after one summer irrigation in June ranged from 38-44°C at 15 cm depth. These temperatures were 0.5-5°C higher than those recorded in unamended soil for the same period and 6-16°C higher when amendments were incorporated in July. Merely one summer irrigation in unamended plots reduced dry root rot severity significantly by 34-39% compared with the unamended control without irrigation (Table 18).

Bacillus coated seeds also reduced mortality, which was significantly lower than SI alone. A single irrigation in summer further augmented the efficiency of cruciferous residues to control dry root rot in both the years. Of the residues, mustard oil-cake was 38% more effective than mustard residues in reducing disease severity. However, planting of *Bacillus* coated seeds in combination with MR+SI was significantly better than MR+NS.

Table 18. Effect of cruciferous residues as soil amendment on population of *M. phaseolina* and clusterbean mortality due to dry root rot on clusterbean

Treatment ^a	Mortality (%)		<i>M. phaseolina</i> population ^b (sclerotia g ⁻¹ soil)	
	1998	1999	1998	1999
MC + SI	4.7	5.1	180	120
MC + NS	9.8	8.7	220	168
MR + SI	7.2	6.5	206	148
MR + NS	10.3	10.7	248	188
MR + SI + ST	6.8	5.4	200	136
MR + NS + ST	7.2	10.6	240	170
SI	11.3	13.9	284	220
ST	7.9	11.6	352	388
Control (unamended)	18.8	21.3	385	432

^aMC, Mustard oil-cake (2.5 ton ha⁻¹); MR, Mustard residues (2.5 ton ha⁻¹); NS, amendment at the time of clusterbean sowing in July; SI, Summer irrigation in June; ST, Seed coating with *Bacillus* spp.

^bInitial population was 340 sclerotia g⁻¹ soil.

Maximum wilt incidence (26.8%) was recorded in the unamended control plots (Table 19). A single summer irrigation, or soil amendment with residues either combined with SI or applied in July at the time of clusterbean planting significantly reduced wilt incidence. Amending the soil with MR or MC in summer or with mustard oil-cake in July significantly reduced wilt incidence as compared with SI alone. Of the residues, mustard oil-cake was significantly more effective than mustard pod straw with a 34% greater reduction in wilt incidence, but differences relating to time of application were not significant (Table 19).

In an earlier study, it was found that amending the soil with urea-N and farmyard manure improved the effectiveness of summer irrigation in controlling *M. phaseolina* and *Fusarium oxysporum* (Lodha, 1995b). Substituting *Brassica* residues for these amendments in the present study further reduced the pathogenic agents. This reduction may be a cumulative effect of bio-toxic volatile compounds released during the decomposition of the residues at prevalent high soil temperatures (38-42 °C) and subsequent microbial antagonism. Amendment of the soil with mustard oil-cake caused a significant reduction in populations of *M. phaseolina* and *Fusarium* (Sharma *et al.*, 1994; Lodha *et al.*, 1997). A lower dose of this amendment in the present field experiment was almost equally effective; probably because the prolonged exposure of infested soil to dry heat exerted a weakening effect on pathogenic propagules (Lodha and Mawar, 2000b).

Table 19. Efficacy of cruciferous residues as soil amendment on population of *F. o. f. sp. cumini* and wilt incidence on cumin

Treatment ^a	Wilt incidence		<i>Fusarium</i> population ^b (x 10 ⁻³ cfu g ⁻¹ soil)	
	1999	2000	1999	2000
MC + SI	5.2	7.2	8.5	4.2
MC + NS	12.1	10.2	11.2	8.9
MR + SI	8.6	7.8	10.4	4.9
MR + NS	11.4	12.7	12.5	9.3
SI	17.8	15.2	13.2	13.8
Control (non-amended)	23.3	26.8	18.6	21.4

^aMC - Mustard oil-cake (2.5 ton ha⁻¹); MR, Mustard residues (2.5 ton ha⁻¹); NS - amendment at the time of clusterbean sowing in July; SI, Summer irrigation in June.

^b Initial population was 17.5 X 10⁻³ cfu g⁻¹ soil

Statistically significant control of dry root rot merely by coating clusterbean seeds with the *Bacillus* sp. is also of practical value in low-input sustainable agriculture (LISA)

(Lodha *et al.*, 2000). Greater control of dry root rot with this *Bacillus* in the 1998 crop season was probably due to adequate soil moisture enhancing bacterial antagonism (Dhingra and Sinclair, 1975; Lodha, 1996). The bacterial activity decreased with decreasing soil moisture (Griffin and Quail, 1968). There was no significant improvement in disease control when *Brassica* residues were combined with the *Bacillus* coating of the seeds. This was most likely due to concurrent operation of two different management strategies. However, coating will help in establishing the *Bacillus* sp. in amended soil for inducing suppressiveness.

Subsequently a field experiment was conducted to study combined effects of residues, summer irrigation and soil solarization on survival of *Fusarium* in soil and wilt of cumin in field (Lodha *et al.*, 2002a). Three promising weed residues viz. *Euphorbia hirta*, *Celosia argentic*a and *Aerva persicae*, residues of mustard and oil-cake were utilized for the study. Experimental plots (4x3m) were arranged in completely randomized block design with nine treatments.

Soil temperature in irrigated (SI) plots ranged from 50.9-51.7 °C, 42.9-50.6°C and 39.3-42.1°C at 5, 15 and 30 cm depth, respectively in both the years of study. In the mustard residue amended (MR+SI) plots, an increase by 0-2.5 °C in soil temperature over non-amended plots (SI) was recorded. Polyethylene mulching (SS+SI) elevated the soil temperature by 2.5-13.1 °C compared to non-solarized plots. Further, temperatures in solarized amended (MR+SS+SI) plots remained higher by 0.5-5.5 and 0-4.1 °C over corresponding non-amended plots (SS+SI).

***M. phaseolina* population**

A reduction to the tune of 9.2% in viable *M. phaseolina* propagules was recorded merely by dry heating, which improved to 28.3% when one summer irrigation was given. Polyethylene mulching augmented this reduction in the range of 73.5-100 %. However, at 30 cm soil depth only 72% viable propagules of *M. phaseolina* could be eliminated. Combining mustard residues with polyethylene mulching completely eliminated viable propagules of the pathogen at 5 and 15 cm depth and also significantly improved reduction at 30 cm depth.

In non-mulched treatments, combining mustard residues with one summer irrigation caused 68-92% reduction in *M. phaseolina* propagules at various studied depths. Addition of a small dose of weed residues or mustard oil-cake augmented the level of reduction. In case of MR+MC+SI, at 15 and 30 cm depth, the magnitude of reduction in *M. phaseolina* propagules was significantly greater than that achieved with polyethylene mulching.

Dry root rot incidence

All the treatment combinations having polyethylene mulching, amendments, summer irrigation and coating seeds with *B. firmus* were significantly better in reducing dry root rot incidence on clusterbean compared to control (Table 20).

Table 20. Effect of cruciferous and weed residues, soil solarization and summer irrigation on incidence of dry root rot on clusterbean

Amendments ^a	% dry root incidence	
	2000	2001
MR + SS + SI	2.3	5.1
SS + SI	2.8	6.1
SI	9.5	15.7
MR + SI	5.6	9.8
MR + MC + SI	4.3	7.4
MR + Weed + SI	4.5	8.3
MR + Weed + SI + <i>B. firmus</i>	4.9	8.0
<i>B. firmus</i> seed coating	6.9	11.6
Non-amended control	14.0	27.5

^a MR –Mustard residues (2.5 t ha⁻¹); MC-Mustard oil-cake (0.5 t ha⁻¹);
SS – Soil solarization; SI-Summer irrigation.

Summer irrigation alone reduced incidence of dry root rot by 39% compared to control. Maximum reduction, however, was achieved in amended and non-amended solarized plots but the level of reduction was significantly equal in both the treatments. In non-solarized amended plots maximum reduction was achieved in the MR+MC+SI treatment, which was significantly equal to the treatments having soil solarization in both the years of study. Combining weed residues with those of mustard though improved reduction in dry root rot incidence over mustard residues alone but was not significantly better. Coating seeds with *Bacillus* also, considerably reduced dry root rot incidence compared to control and the level of reduction was significantly equal to all the non-solarized amended treatments in both the years of study.

Fusarium population

Treatments having amendments, soil solarization and summer irrigation alone or in combination were significantly superior than control in eliminating *Fusarium* propagules. One summer irrigation in dry heated soil caused 32.5 % reduction in viability of *Fusarium* propagules. Soil amendment with mustard residue (MR+SI) eliminated 78 % of the viable *Fusarium* propagules. However, amendment of soil with weed residue in combination with mustard (MR+W+SI) could not improve this reduction. Addition of a

small dose of mustard oil-cake with mustard residue (MR+MC+SI), on the other hand, significantly improved this reduction.

Wilt incidence

All the treatment combinations having amendments, soil solarization, summer irrigation, and *Trichoderma harzianum* were significantly superior than non-amended control in reducing wilt incidence on cumin crop (Table 21).

Table 21. Effect of cruciferous and weed residues, soil solarization and summer irrigation on wilt incidence on cumin

Amendments ^a	% wilt incidence	
	2001	2002
MR + SS + SI	4.0	4.5
SS + SI	4.1	12.5
SI	11.9	27.4
MR + SI	6.0	16.7
MR + MC + SI	5.1	15.0
MR + Weed + SI	7.3	18.7
<i>Trichoderma harzianum</i>	7.1	21.8
MR + Weed + SI + <i>T. harzianum</i>	7.4	16.1
Non-amended control	16.0	38.2

^a MR –Mustard residues (2.5 t ha⁻¹); MC-Mustard oil-cake (0.5 t ha⁻¹); SS – Soil solarization; SI-Summer irrigation.

Summer irrigation alone had 7.4 % less wilt incidence than control (27.1%). Mustard residue alone or in combination with mustard oil-cake or weed residue further improved control of wilt on cumin crop. Application of bio-control agent, *T. harzianum* resulted in 7.1-21.8 %wilt incidence. Polyethylene mulching of amended or non-amended soil had lowest wilt incidence (4.0-12.5 %) during both the years of study.

The ability of mustard pod residues to control pathogens and diseases, demonstrated in this study, is important in resource-deficient farming since this source of *Brassica* residue is cheaper and more readily available than oil-cake (Mawar *et al.*, 2001). Wheat-mustard-cumin rotation in the winter seasons ensures that *Brassica* residues are available in March.

Weed Suppression

During experimentation on use of *Brassica* amendments it was observed that weed population and composition had significant variations in different treatments. Complete reduction of weeds like *Gisokia pharnacioides* L., *Heliotropium subulatum* Hochst., and *Eragrotis ciliaris* (L.) R.Br. in mustard pod straw and *Corchorus tridens* L., *H. subulatum* Hochst. and *E. ciliaris* L. in mustard oil-cake amended plots with one summer irrigation was observed (Saxena and Lodha, 2003). These amendments also reduced the weed population significantly in clusterbean during the rainy season. *Brassica* amendments with summer irrigation at high soil temperatures (39-44 °C) resulted in significant reduction to the extent of 42.6 and 10.9%, respectively, in the population of summer and rainy season weeds in all the amended plots compared to their application at the time of normal sowing in July at moderate soil temperatures (23-35 °C). The reduction in summer weeds was significantly equal with the amendment of mustard oil-cake and pod straw. Among *Brassica* amendments, oil-cake gave better results in terms on weed suppression compared to pod straw, irrespective of the time of application. Of the 16 weed species recorded during rainy season, certain species like *Pulicaria crispa* Cass., *Cenchrus biflorus* Roxb. and *Cyperus rotundus* L. were completely eradicated whereas *C. tridens*, *C. biflorus*, *H. subulatum*, *E. ciliaris*, *Boerhaavia diffusa* L., *Digera muricata* (L.) Mart., *Crotolaria burhia* Buch.-Ham., *Euphorbia hirta* L. and *Prosopis juliflora* (Sw.) DC. were suppressed.

Mustard oil-cake with one irrigation in summer increased the seed yield by 2.7 and 61.4% over incorporation of oil-cake in rainy season and non-amended control. The corresponding values for mustard pod straw were 6.6 and 32.4%, respectively. Mustard oil-cake was significantly superior than mustard pod straw irrespective of time of application. Mustard pod straw gave 32.4% (MS+SI) and 24.2% (MS+NS) higher seed yield over non amended control. Considerable improvement (20.3%) in seed yield in SI treatment was also recorded.

Although the major benefit of combining *Brassica* residues with summer irrigation was the reduction in the population of *M. phaseolina*, *Fusarium*, dry root rot incidence on clusterbean and wilt incidence on cumin, but additional advantage accrued was the partial weed suppression.

Biochemical aspects

Cruciferous residue as soil amendment have been reported to reduce the population density of many soil borne pathogens (Angus *et al.* 1994, Mohjtahedi *et al.* 1993). In hot arid regions, cruciferous residues along with one summer irrigation results in 70-80 % reduction in population of *Fusarium* (Lodha and Mawar 2000b). This is likely

to effect growth, water relations and metabolism of cumin plants under both healthy and diseased conditions.

Field experiment was conducted for two consecutive years with four treatments viz., Mustard oil-cake amendment (MC, 0.11 % or 2.5 ton ha⁻¹) in May + Summer irrigation (to bring 45 cm soil depth to FC ;SI), (MC + SI); Mustard residue amendment (MR, 0.11%) in May + SI, (MR + SI); SI only and control. Data on wilt incidence were recorded one day prior to sampling for shoot and root growth, plant water status and metabolite content.

Symptoms of wilt due to *Fusarium* were not visible on cumin plants up to pre-flowering stage. Amendment of soil with *Brassica* residues, in general, improved the plant growth and their effect was more conspicuous on root compared to shoot growth. The differences in plant water potential of cumin grown under different soil amendments were not significant but relative water content (RWC) of leaves varied significantly at pre-flowering stage. RWC was least in plants raised in treatments SI and MR+SI while MC+SI resulted in higher RWC, which was statistically equal to control.

At post-flowering stage and onwards cumin plants succumbed to wilt in all the treatments. Maximum plant mortality due to wilt was recorded in non-amended control (25.0 %) followed by the treatment where only summer irrigation (SI) was given (16.5%) and then in MR + SI (8.2 %). Mortality was lowest in the treatment MC+SI (6.2 %). In addition to reducing *Fusarium* propagules and wilt incidence in field, significant changes in water relation and metabolism of cumin plants were recorded in amended plots resulting in higher seed yield compared to non-amended plots (Table 22).

Table 22. Effect of cruciferous residues and summer irrigation on water relations of cumin plants under healthy and diseased conditions at post-flowering stage.

Treatments ^a	Plant Water Potential (-bars)			Leaf Relative Water Content (%)		
	Healthy	Diseased	Mean	Healthy	Diseased	Mean
MC+SI	19.0	23.6	21.3	71.3	72.7	72.0
MR+SI	16.3	23.6	19.9	69.1	63.1	66.0
SI	20.0	22.0	21.0	73.5	69.7	71.6
Non amended control	16.3	26.7	21.5	67.4	54.0	60.7
Mean	17.9	23.9	-	70.3	64.8	-

^a MC : Mustard oil-cake (2.5 t ha⁻¹); MR : Mustard residues (2.5 t ha⁻¹) and SI : summer irrigation at the field capacity (10.4 % w/w).

Differences among healthy plants in different amended plots were not significant in terms of plant water potential at post-flowering stage, but RWC of cumin leaves was invariably higher in plants from amended compared to non-amended plots (Table 22). Plants in MR + SI treatment recorded maximum change in the content of free amino acid and starch in shoot. Total soluble carbohydrates content in shoot, however, was maximum in MC+SI.

The percent drop in plant water potential due to fungal infection was maximum in non-amended plots followed by those amended with mustard residue, oil-cake and only summer irrigation in that order. Relative water content of leaves also decreased due to infection, maximum drop being in control plants followed by SI and MR + SI. Decrease in water status of diseased plants in terms of water potential and RWC is probably due to disturbance in water conductance and increased petiolar resistance due to plugging of vessels (Hall and Mc Hardy 1981). Less plant water deficit coupled with high RWC in diseased plants from amended plots (Table 22) clearly indicated the favourable effect of amendments on plant water status. Increase in available soil moisture in amended soil, as one of the probable reason for such a response cannot be ruled out. In summer irrigated plots, more moisture availability due to reduced weed population (Saxena and Lodha 2003) may also account for the better water status of the standing cumin crop to some extent.

Amendments did not significantly influence the major fractions of leaf carbohydrate (total soluble carbohydrates and starch) except reducing sugars, which were higher in amended compared to non-amended control (Fig 10).

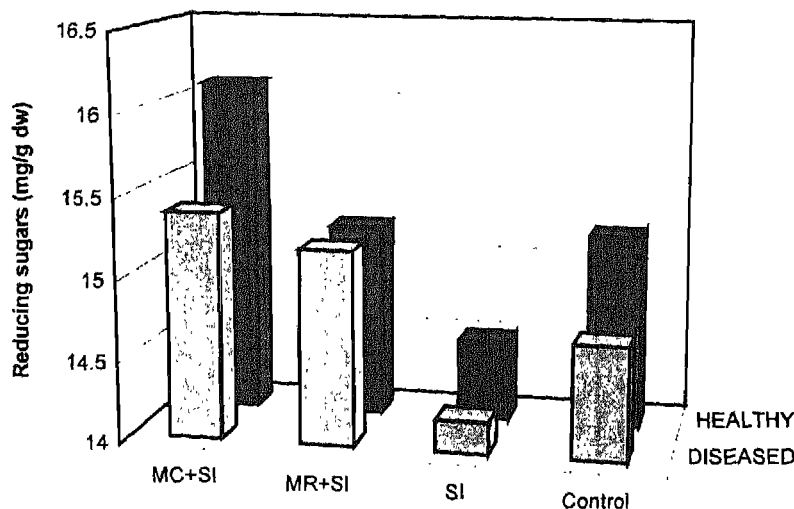


Fig 10. Effect of amendments on content of reducing sugars in shoots of healthy and diseased cumin plants

This might have resulted in decreased fungal infection in amended as *Fusarium* wilt occurs primarily at low sugar levels (Horsfall and Dimond 1957). The role of total free amino acid level in the pathogen infection or its expression is ruled out in the present study contrary to the increased susceptibility of trees to fungal wilt at high level of amino acids (Bell and Marshall 1981). In roots, however, fungal infection significantly decreased the content of total soluble carbohydrate.

Amendment associated increase in the content of different metabolites in shoot and root may be due to direct positive effect on growth or indirectly due to better plant water status and nutrient availability in soil system. A significant decrease in the content of total soluble carbohydrates in diseased roots (Fig 11) may be due to adverse effect on CO₂ exchanges (Orcutt and Nilsen 2000) besides preferential translocation of photosynthates to the growing shoot apex (i.e. floral buds during post-flowering stage).

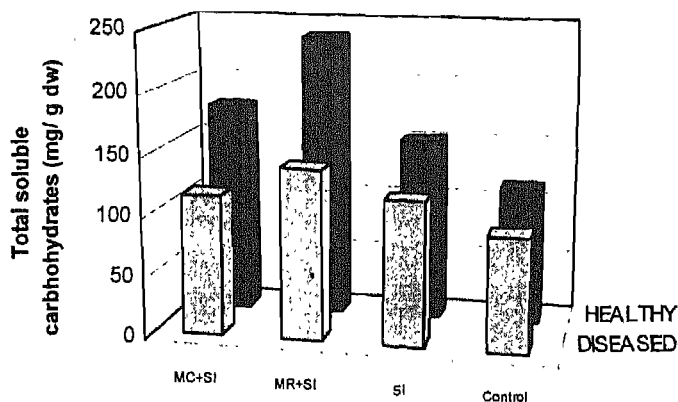


Fig 11. Effect of amendments on content of total soluble carbohydrates in roots of healthy and diseased cumin plants

The contents of N, P, K, Fe and Mn in shoot also decreased after infection. This may be due to general impairment of mineral uptake, transport and utilization as reported for other diseases (Rengal, 2001). This makes plants more vulnerable to fungal infection as low levels of Mn deleteriously effects phenol synthesis (Rengal *et al.* 1994). Like wise, low levels of Fe and poor growth of diseased plants could be explained through the adverse effect on chlorophyll synthesis and electron transport chain.

Effect of saline irrigation water

Amendment of soil with cruciferous residues particularly those of mustard, have been found to reduce soil population densities of *M. phaseolina* and *F. oxysporum* f.sp. *cumini* in soil (Lodha *et al.*, 1997; Lodha and Mawar, 2000b). Since use of cruciferous residues for pathogen control requires irrigation in aridisols, the objective of present investigation was to study influence of saline irrigation water on survival of these pathogens in mustard residue amended soil (Mawar and Lodha, 2003).

Twenty five ml of the saline water each of the five EC levels (2.24, 4.44, 6.60, 8.88 and 10.60 dS), was added separately in 200 g of non-amended infested soil while water of only three levels of EC, 2.24, 6.60 and 10.60 dSm⁻¹ was separately added in amended (with 1% mustard residue) infested soil. Polyethylene bags moistened with equal amount of distilled water served as amended and non-amended controls. Population changes of *M. phaseolina* and *Fusarium* were followed for a period of 120 days at monthly interval. The loss of moisture was replenished by adding 2 ml of respective saline or distilled water at weekly interval. In general, there was a gradual increase in EC with the addition of saline or distilled water in amended and non-amended soil (Fig. 12).

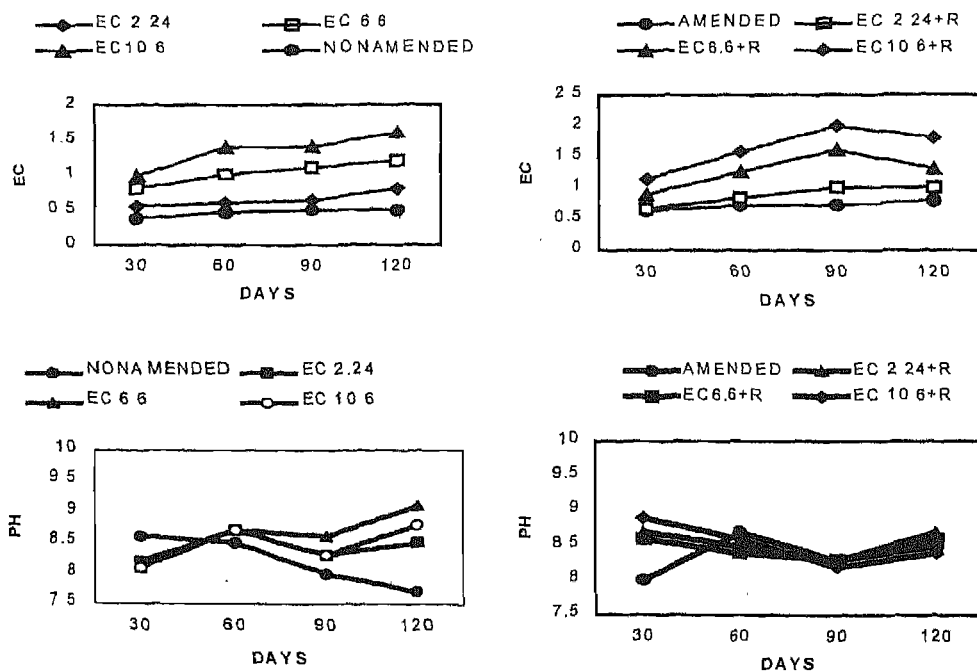


Fig 12. Effect of saline irrigation on development of electrical conductivity (EC) and pH in amended and non amended soil (R- 1 %mustard residue).

This increase was greater in amended than non-amended soils. No significant variation could be measured in the soil pH recorded after 120 days in all the amendment treatments and at EC 2.24 and 8.88 dSm⁻¹ in non-amended soils. However, considerable increase in soil pH was measured in the treatments where saline water of EC 4.44, 6.60 and 10.6 dSm⁻¹ was frequently added in non-amended soils.

There was a gradual decline in the viable population of *M. phaseolina* by the addition of saline or distilled water in amended and non-amended soil. More than 80% reduction in pathogenic propagules in non-amended soil maintained at 100% of MHC confirmed the significance of soil moisture alone in reducing *Macrophomina* population due to enhanced microbial antagonism (Dhingra and Sinclair, 1975; Lodha, 1996). However, the rate of decline was significantly greater in amended compared to non-amended soil. In the final soil samples analysed after 120 days, reduction to the tune of 81.8 and 91.7% was estimated in non-amended and amended soil, respectively (Table 23).

Table 23. Effect of soil salinity on *Macrophomina* (sclerotia g⁻¹ soil) and *Fusarium* population (x 10⁻³ g⁻¹ soil) in mustard pod residue amended and non-amended soil

Electrical conductivity (dSm ⁻¹)	<i>M. phaseolina</i>		<i>F. oxysporum</i>	
	30 days	120 days	30 days	120days
Non-amended 2.24	486	256	44	28
4.44	520	266	34	28
6.60	660	206	48	25
8.88	640	352	48	25
10.60	500	360	37	38
Amended 2.24	246	80	24	13
6.60	226	106	22	7
10.60	260	140	10	16
Control(non-amended)	560	246	48	34
Control(amended)	332	112	27	13

Initial population 1352 sclerotia g⁻¹ soil (*M. phaseolina*) and 76.2 x 10⁻³ g⁻¹ soil (*Fusarium*)

In all the amended treatments, a sharp decline in *M. phaseolina* was estimated at first sampling date. This decline was significantly higher than that at the same EC levels in non-amended treatments. In the final soil samples, reduction in *M. phaseolina* propagules was 59% in amended compared to only 50% in non-amended treatments over first sampling date. Maximum reduction (94%) was estimated at EC 2.24 dSm⁻¹ with the

amendment of mustard residues. This reduction was significantly greater than that recorded with the increased EC levels (6.60 and 10.60 dSm⁻¹) and control.

In the final soil samples analysed after 120 days, 83% propagules of *Fusarium* were eradicated in amended compared to a reduction of 55% in non-amended control. At first sampling date, a sharp decline in viable propagules of *Fusarium* was observed at all the EC levels in amended treatments. This decline was significantly higher than that recorded at same EC levels in non-amended treatments and amended control. The reduction was greater at 6.60 dSm⁻¹ to the extent that almost 91 % of viable *Fusarium* propagules were eliminated in the final soil sample (Table 23). On the contrary, population of *Fusarium* propagules fluctuated at EC level 10.6 dSm⁻¹ at subsequent intervals. Thus at this EC level, population of *Fusarium* in the final soil samples was significantly higher than lower EC levels and amended control.

Our findings demonstrate that saline irrigation water up to a certain level of electrical conductivity did not alter the potential of mustard residues in reducing the soil population densities of *Macrophomina* and *Fusarium*. Increased electrical conductivity in amended soil could be a result of release of SO₄⁻ ions during decomposition of mustard residues at low temperatures, which are converted into bio-toxic volatiles at high temperatures. Better survival of fungal propagules at high level of EC (10.60 dSm⁻¹) observed in the present study explains in part why wilt of cumin is more severe in the fields irrigated with saline water in arid region as the host is experiencing two concurrent stresses.

High level of salinity nullified the impact of residue amendment in pathogen control. Such situation may warrant supplemental use of other ameliorative measures like gypsum in aridisols (Joshi and Dhir, 1990). Adverse effects of salinity can also be mitigated by amendments with farm yard manure and urea-N (Gupta *et al.*, 1995).

Cultural Control- rainfed agriculture

Moisture Conservation Techniques

Soil water stress to host plants has been considered as the most important predisposing factor for the development of *Macrophomina* induced diseases (Ghaffar and Erwin, 1969; Sheikh and Ghaffar, 1979). Numbers of practices are recommended to conserve soil moisture in water scarce region (Unger and Stewart, 1983). This study was carried out to determine effect of moisture conservation practices on available soil moisture and role of resident microbial population on survival of *M. phaseolina* population, dry root rot intensity and seed yield of clusterbean (Lodha, 1996). The experiment was conducted during 1984 and 1985 *Kharif* seasons.

The quantity of rainfall was 221 mm in 1984 and 142 mm in 1985. The post-sowing period in 1984 did not favour the development of dry rot due to evenly distributed rainfall. In 1985, a 30-days dry spell followed sowing, after that 60 mm of rainfall was received within 3 days. The plants suffered from mild moisture stress during the seedling growth and from severe moisture stress at 50 days after planting till harvest. This situation favoured the development of dry root rot. All the moisture conservation techniques (MCTs) i.e. farmyard manure @ 10 ton ha⁻¹, low plant population (1.6 lakh ha⁻¹) and mulching with a layer of pearl millet stover (3.5 ton ha⁻¹) alone or in combination effectively retained soil moisture compared to control plants (Lodha, 1996). There was a continuous and varied depletion of soil moisture in all the treatments during absence of rain events. The increase in soil moisture at 45 DAP favoured the microbial population but reduced the counts of *M. phaseolina* population. At subsequent intervals *M. phaseolina* population tended to increase in different treatments with a progressive decline in soil moisture and bacterial counts. There was a significant positive correlation between soil moisture and total bacteria in the treatment LPP + FYM ($r = 0.67$), LPP + mulch ($r = 0.51$) and LPP + FYM + mulch ($r = 0.84$) plots. On the other hand, soil moisture was negatively correlated with *M. phaseolina* in control ($r = -0.52$), mulch ($r = -0.74$), FYM ($r = -0.45$), LPP + FYM ($r = -0.61$), LPP + Mulch ($r = -0.61$) and LPP + FYM + Mulch ($r = -0.58$) plots. Total bacteria also were negatively correlated with *M. phaseolina* in mulch ($r = -0.58$), LPP ($r = -0.52$) and FYM + LPP ($r = -0.56$) plots. In path coefficient analyses, soil moisture had the highest negative effect on *M. phaseolina* in control (-0.75), mulch + LPP (-1.05) plots. In LPP and Mulch + FYM amended plots, total bacteria had the high negative effects (-0.65 and -0.62, respectively) on *M. phaseolina*. Total actinomycetes had highest negative effects on *M. phaseolina* in FYM (-0.77) and FYM + LPP (-0.75) plots.

Dry root rot mortality was significantly lower in the treatments having MCTs in 1985. However, in 1984 only FYM and Mulch + FYM + LPP had significant reductions in mortality over the control. These results demonstrated that mulching with a layer of pearl millet stover (3.5 ton ha⁻¹), farmyard manure (10 ton ha⁻¹) and low plant density (1.6 lakh ha⁻¹) alone or in combination, effectively retained available soil moisture of varying levels during different stages of crop growth. Conserved soil moisture affected increases in native bacterial population with corresponding decrease in total fungi including *M. phaseolina* (Fig 13). In the presence of adequate soil moisture, antagonistic role of some soil bacteria in reducing the sclerotial population is well documented (Dhingra and Sinclair, 1975). The bacterial activity decreased as soil moisture decreases (Griffin and Quail, 1968). In the present studies, although bacterial population did increase at some sampling intervals (e.g. in LPP, FYM + LPP), but in the absence of adequate soil moisture necessary for their antagonistic activity, population of *M. phaseolina* was not

reduced. These results suggested that soil moisture probably modified the behaviour of competitors rather than directly influencing the *M. phaseolina* propagules. Highly significant positive correlations of soil moisture with total bacteria, the negative correlations of both with *M. phaseolina* population and the highest negative indirect effects of soil moisture via total bacteria on *M. phaseolina* in many of the promising treatments support this view.

Correlations between *M. phaseolina* population and mortality were significant ($r = 0.66$ to 0.69) at 60 to 90 DAP. The multiple regression equations worked out for these stages were:

$$\hat{Y} = - 8.85 + 0.36x_1 + 0.46x_2 \quad (R^2 = 0.47) \quad - 60 \text{ DAS}$$

$$\hat{Y} = - 0.12.03 + 1.17x_1 + 0.30x_2 \quad (R^2 0.63) \quad - 75 \text{ DAS}$$

$$\hat{Y} = 9.93 + 0.43X_1 + 0.37x_2 \quad (R^2 0.49) \quad - 90 \text{ DAS}$$

Where \hat{Y} = plant mortality, x_1 = soil moisture and x_2 = *M. phaseolina* population.

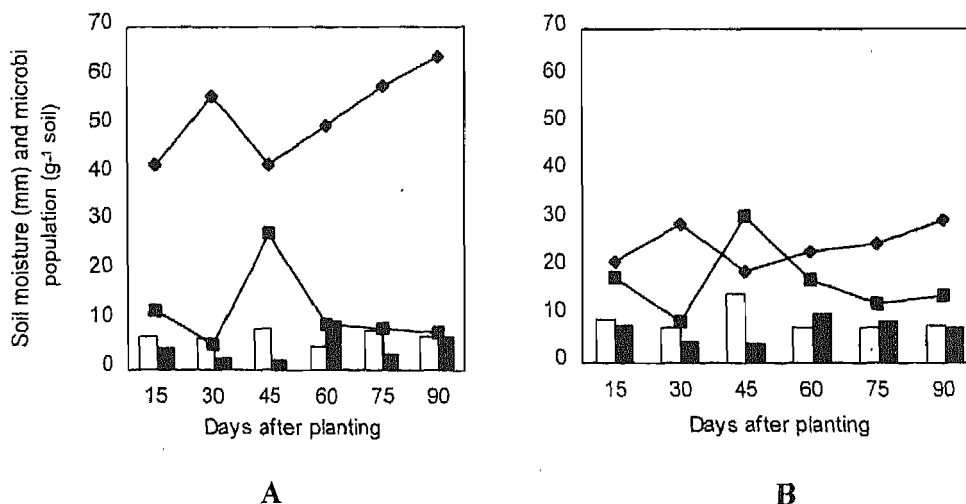


Fig 13. Effect of moisture conservation techniques on soil moisture (■), actinomycetes (□), bacteria (▣) and *M. phaseolina* population (◆). A – control and B- mulch + FYM + Low plant population

Enhanced soil moisture and a decrease in *M. phaseolina* density significantly reduced the dry root rot mortality (72%) and increased the seed yield by 58% (Lodha, 1996). This study established that in the presence of a crop (i) soil moisture and bacterial population are the principal factors governing the population of *M. phaseolina* and (ii) use of moisture conservation practices reduced the population of pathogen in soil and dry root rot mortality of clusterbean in field.

Compost

Inactivation of M. phaseolina from residues

The incorporation of crop residues changes the soil characteristics in many ways including enrichment of nutrients, and improvement in microbial activity, water holding capacity, soil aeration and permeability. However, low moisture content, small microbial populations and high temperature prevailing in arid regions prolong the process of decomposition of crop residues when incorporated in a nutrient deficient sandy soil. Another disadvantage of direct incorporation is that crop residues may carry plant pathogens (Hoitink and Fahy, 1986). Composting is a method to inactivate pathogens and decompose crop residues more rapidly. Most pathogens are inactivated during the heating phase of composting (Bollen *et al.*, 1989), but information on *Macrophomina phaseolina* is available

Concerned with the risk of spreading *M. phaseolina* by amending compost in the soil, we analysed several samples from on-farm composts and detected 60-80 sclerotia g⁻¹ of compost. *M. phaseolina* is a heat tolerant pathogen since sclerotia could withstand a temperature range of 60-65°C (Bega and Smith, 1962; Mihail and Alcorn, 1984). Efforts are therefore required to eliminate or bring down the sclerotial population of *M. phaseolina* from composts before their incorporation in soil.

Amendment of soil with composts prepared from organic wastes have been used with various levels of success for suppression of several soil-borne plant pathogens (Hoitink and Fahy, 1986; Ben-Yephet and Nelson, 1999), but information on *M. phaseolina* is not available. The present investigation deals with (i) the survival of *M. phaseolina* during composting and (ii) the disease suppressive characteristics of compost with respect to *M. phaseolina* (Lodha *et al.*, 2002b).

Fully dried residues of pearl millet [*Pennisetum glaucum* (L.) R.Br], clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.], cauliflower [*Brassica oleracea* var. *capitata* L.] and a mixture of off-season weeds (*Boerhavia diffusa* L., *Cenchrus biflorus* Roxb., *Cyanodon dactylon* (L.) Pers., *Heliotropium subulatum* Hocht., *Tephrosia purpurea* L) were used for the preparation of composts. The process of composting was initiated under partially anaerobic conditions in separate pits (1.7 m³) according to the principles of the Indore type (Howard and Ward, 1931). A 5 cm cowdung layer completely saturated with water was spread over the base of all the pits. In each pit, a 30 cm layer was filled with 40 kg of residues enriched with 400 g gypsum (1%) and 800 g urea (2%). Each layer was provided with 60% moisture (W/W) and then covered with a 10 cm thick layer containing a mixture of 68 kg cowdung and 100 kg field soil (Fig. 14). Four such layers of residues with soil-dung mixture were piled in each pit. The soil having a native population of *M. phaseolina* (135 sclerotia g⁻¹ soil), as estimated on selective medium (Meyer *et al.*, 1973),

was used in this mixture to enhance multiplication and activity of resident antagonists that can survive peak heating (Lodha and Solanki, 1992) and to absorb part of the liberated ammonia until being used by the microflora. Compost pits were finally covered with a 5 cm layer of weeds. Loss of moisture was replenished by adding 20 L of water every 10-15 days. C:N ratio of all the residues, cowdung, soil and each compost mixture was estimated separately. Carbon was estimated by oxidizing it with chromic acid in presence of H₂SO₄. The excess chromic acid was back titrated with ferrous ammonium sulphate. For N estimation, samples were digested with H₂SO₄ and distilled using Kjeltac Auto System II (Tecator). Once the composting process was over, matured composts were uniformly mixed separately and three sub-samples (500 g) were collected randomly from each compost to estimate C:N ratio. *M. phaseolina* and microbial populations including antagonists were also estimated by standard procedures.

In clusterbean and pearl millet residue amended compost pits, several samples (20 g) each containing 2 g pieces (0.5-1.5 cm) of *M. phaseolina* infected roots and 18 g uninfected (apparently healthy) residues of clusterbean, enriched with 2 or 4% urea-N, were separately placed in small nylon pouches (120 µm pore). These were tied with long nylon strings and buried at 30 and 60 cm depth. Twenty four samples, corresponding to 3 replications for each depths (2), each urea-N concentrations (2) and each date (2), were collected. Temperatures at both depths were recorded every day at 14 and 16 h for 6 weeks, because maximum temperature at 30 cm and 60 cm are attained at these two periods of the day. Pouches were retrieved after 2 and 4 months with a minimum disturbance from pits. These samples were air-dried and passed through a 2 mm sieve before estimating viable propagules of *M. phaseolina*. After 4 months, all the composting material of each pit was given three turnings at an interval of 15 days.

Composts retrieved from pits in May were separately spread over soil surface as a 10-12 cm thick layer in an open field during hot summer days of June and were moistened once at 10% (W/W) with water. Temperatures at 5 cm depth were recorded every day at 14 h. After seven days of exposure, three sub-samples (50 g) were collected randomly from each compost for estimating the density of *M. phaseolina* propagules.

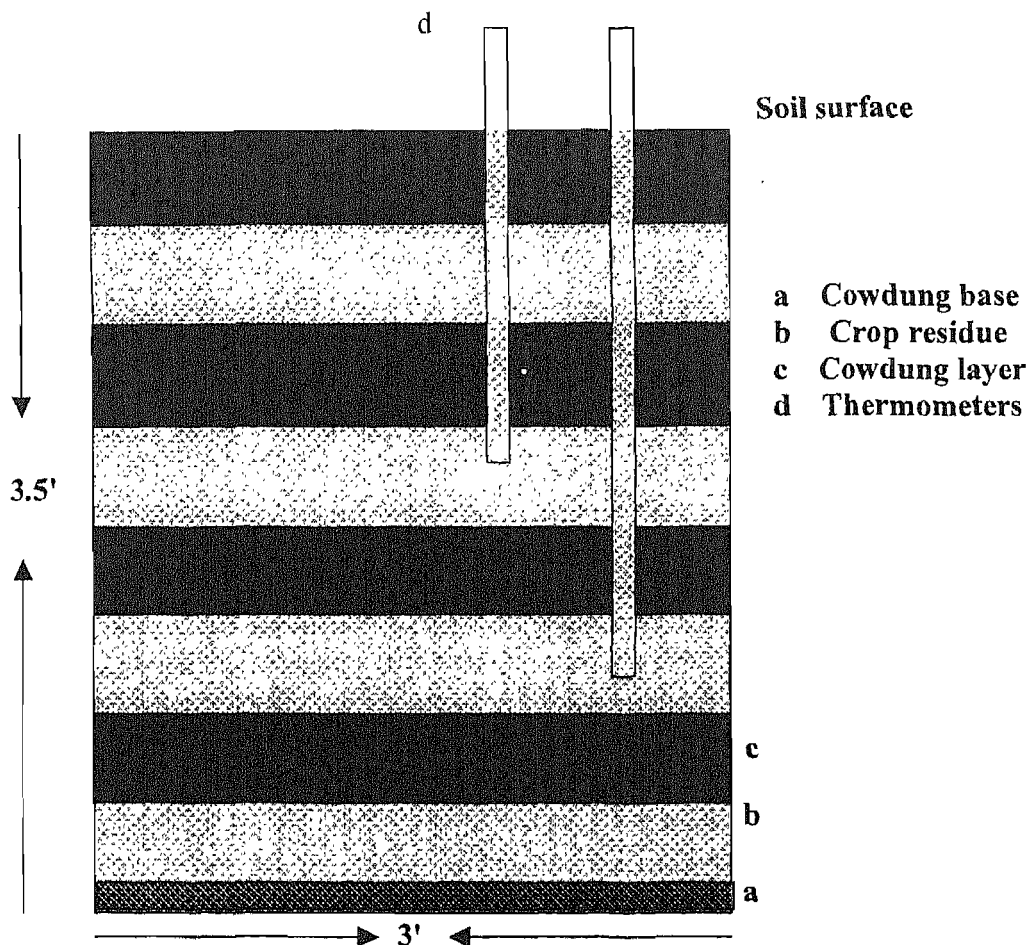


Fig 14 . A cross-section of compost pit

Effect on dry root rot mortality and legume seed yield

Suppressive effect of composts on dry root rot was studied in a fixed layout using completely randomized block design with five replications during 1994 and 1995. The soil of the experimental field was naturally infested with sclerotia of *M. phaseolina* (640 g⁻¹soil) as determined on selective media (Meyer *et al.*, 1973). After summer exposure 4-week-old composts (after retrieval from pits) of pearl millet, clusterbean, weeds and cauliflower residues (each 4 t ha⁻¹) were incorporated to a depth of 0-30 cm by a spade in separate plots (4x4 m). Plots without any amendment served as control. Clusterbean seeds (cv. HG 75, 8 rows plot⁻¹) were sown on 25th (1994) and 22nd July (1995) and harvested on 5th Nov (1994) and 28th Oct (1995).

Three soil samples from same depth were collected with a 2.5 cm diameter tubular probe before amendment and 15 days after harvest from each plot. For each date,

these were bulked to form one sample for each of the 5 replication and processed for biological assays. Data on plant mortality due to dry root rot were recorded 12-15 days before harvest in third and fourth row of each plot. Seed yield of clusterbean was also recorded.

Population densities of *M. phaseolina*, total fungi, bacteria, actinomycetes and *Nitrosomonas* were determined following standard procedures. Lytic bacterial density was estimated following Greenberger *et al.*, (1987). Antagonistic actinomycetes were also estimated (Ghaffer *et al.*, 1969).

The process of composting was completed in all the pits in a period of 6 months. Maximum temperatures observed were 48-51°C at 30 cm and 60-62°C at 60 cm depth after 9-13 days and 14-18 days, respectively, after filling the compost pits. The heat phase lasted 3 to 4 weeks. A reduction of 28 to 35% of the initial volume was observed in compost pits with maximum being in the cauliflower pit. In general, considerable decrease in C:N ratio was estimated in all the composts. Density of aerobic cultivable bacteria was maximal in cauliflower compost but that of actinomycetes, fungi, lytic bacterial and antagonistic actinomycetes were significantly higher in clusterbean compost. In the final composts, viable sclerotia of *M. phaseolina* were significantly lower in cauliflower compost compared to other composts.

Density of viable sclerotia of *M. phaseolina* was significantly lower in the partially decomposed clusterbean residue samples retrieved from 60 cm depth compared to those from 30 cm depth in both the pits. The counts of *M. phaseolina* were significantly lower in the residues enriched with 4% compared to 2% urea-N. Viable propagules of *M. phaseolina* reduced drastically in all the samples retrieved after 4 months with similar trend for all the treatments except that differences were not significant in the samples enriched with urea-N at 30 cm depth from pearl millet pit.

During a 7 day period of natural heating, maximum air temperature ranged from 39-47°C. Moistening the composts with water initially brought down the maximal soil temperature to 37°C but a gradual increase of 53°C at 5 cm depth followed within 2-3 days. As a result, 53-61% reduction in counts of *M. phaseolina* was estimated in different composts.

During both years of field experiments, disease severity due to dry root rot was significantly reduced and seed yield of clusterbean was significantly enhanced by soil amendment with the different composts (Table 24). Among composts, the lowest disease severity was recorded with pearl millet compost except in 1995 where disease suppression was not significantly different from that obtained in the presence of weed compost. However, seed yield was the highest in plots amended with cauliflower compost. The increase in seed yield in amended treatments over control was more

conspicuous in a normal rainfall year 1994 (28.1-52.3%) than in a low rainfall year such as 1995 (28.9-38.4%).

Table 24. Effect of different compost amendment on dry root rot mortality induced by *M. phaseolina* and seed yield of clusterbean

Compost amended soil ^a	Mortality (%)		Seed yield (kg ha ⁻¹)	
	1994	1995	1994	1995
Pearl millet	2.0	4.4	508.3	296.8
Clusterbean	3.0	6.3	572.7	299.5
Weeds	4.2	6.8	585.7	311.4
Cauliflower leaves	2.2	6.0	604.1	318.7
Control	5.4	16.0	396.5	230.2
LSD (P<0.05)	1.2	5.5	54.5	45.5

^aSoil was amended with 4 t compost per hectare

Amendment of soil with compost, in general, increased the population of antagonistic actinomycetes, lytic bacteria and *Nitrosomonas* and decreased the population of *M. phaseolina* in soil (Table 25). Among composts, increase in antagonistic actinomycetes was significantly higher in cauliflower compost amended soil that also had maximum reduction of *M. phaseolina* density. In both years, the population of *Nitrosomonas* spp. was also significantly higher in cauliflower compost followed by pearl millet compost amended soil compared to the other two composts.

Table 25. Densities of *M. phaseolina*, associated antagonists and *Nitrosomonas* in compost amended soil.

Compost amendment	<i>M. phaseolina</i> ^b (cfu g ⁻¹ soil ^a)		Antagonistic actinomycetes log cfu g ⁻¹		Lytic bacteria log cfu g ⁻¹		<i>Nitrosomonas</i> log cfu g ⁻¹	
	1994	1995	1994	1995	1994	1995	1994	1995
Pearl millet	313	510	11.40	11.46	12.47	12.67	10.33	10.51
Clusterbean	266	394	11.55	11.28	12.64	12.87	10.15	10.19
Weeds	386	477	11.45	11.51	12.54	12.75	9.83	9.94
Cauliflower leaves	253	381	11.90	12.20	12.69	12.85	10.42	11.04
Control	426	768	11.28	11.16	12.36	12.38	9.84	9.78
LSD (P<0.05)	76	43	0.19	0.13	0.12	0.07	0.23	0.18

^aPopulations estimated 15 days after the harvest of clusterbean crop.

^bInitial population of *M. phaseolina* was 640 propagules g⁻¹ soil.

Release of *M. phaseolina* inoculum from infected plant residues was significantly reduced by organic composting. Many factors may be involved in reducing population of *M. phaseolina* during composting (i) heat generated in the first phase (Bollen, 1985), (ii) toxicity of conversion products formed during or after the self-heating process (Berestetsky and Kravchenko, 1984), and (iii) microbial antagonism in presence of moisture (Dhingra and Sinclair, 1975). Amendment of soil with composts showing a reduced density of *M. phaseolina* lead to a significant decrease of dry root rot severity and a significant increase of seed yield of clusterbean both in normal and low rainfall conditions.

In the present study low survival of *M. phaseolina* propagules, particularly at 60 cm could be attributed to elevated temperatures during the heat phase that reached values reported to be lethal (60°C for 3 seconds) for sclerotia of *M. phaseolina* (Bega and Smith, 1962). This hypothesis is supported by the survival of a higher number of sclerotia in the samples kept at 30 cm depth where maximum temperatures (48-51°C) were lower than at 60 cm depth. However, presence of viable sclerotia could still be retrieved in the samples from pits after 2 months. This observation could be ascribed to the heat tolerant nature of *M. phaseolina* propagules, which could withstand even a temperature range of 58-63 °C achieved under polyethylene mulching in hot arid conditions (Lodha, 1989; Lodha and Solanki, 1992; Mihail and Alcorn, 1984). Further reduction in viable sclerotia of *M. phaseolina* after the heat phase could be a result of combined effects of fungitoxic compounds and microbial antagonism in the presence of moisture and nitrogen. Several workers have demonstrated production of fungitoxic volatile compound during decomposition of crop residues (Berestetsky and Kravchenko, 1984; Spring *et al.*, 1980). Release of such volatiles from decomposing pearl millet residues was shown to reduce density of viable propagules of *M. phaseolina* in a previous study (Sharma *et al.*, 1994). The release of volatiles, particularly isothiocyanates from cruciferous residues was shown to be maximal, during the second and third week of decomposition (Lewis and Papavizas, 1970, 1971; Gamliel and Stapleton, 1993).

The higher reduction in viable propagules of *M. phaseolina* in the decomposing tissues enriched with 4% urea-N than from 2% urea-N recorded in the present study could result from a higher concentration of nitrogen. In our experiment, high populations of antagonists estimated in final compost samples might have also accelerated antagonism particularly activity of lytic bacteria in the presence of moisture and nitrogen.

Survival of *M. phaseolina* propagules in matured composts could be due to static state of residues in pits where lethal temperatures was not reached at all the sites. However, our effort to expose moistened composts to prevailing high temperatures was found highly effective in further reducing the viable propagules of *M. phaseolina*. Sub-

lethal temperatures (48-53 °C) exerted a weakening effect on remaining sclerotia of *M. phaseolina* which might have accelerated microbial antagonism.

Organic amendment may increase, decrease or not affect diseases caused by soil-borne pathogens (Hoitink and Fahy, 1986). In the present study, disease suppression due to addition of composts into the soil could possibly be ascribed to the following factors: (i) composts support high levels of total microbial population including antagonists and (ii) composts improves the moisture holding of the soil which in turn could reduce the pathogenic propagules and disease.

Increased densities of bacteria and actinomycetes along with antagonists by amendment of compost in the field study could be ascribed to its blending with field soil in each layer of residues during composting. Their population multiplied rapidly from initial $1.6 \times 10^5 \text{ g}^{-1}$ soil to $1.2 \times 10^6 \text{ g}^{-1}$ compost at high temperature attained during heat phase, more so, in the presence of nutrients like urea-N. *Bacillus* spp. that can withstand peak heating may induce biological control in composts (Bareja *et al.*, 2003). One *Bacillus* strain was found to inhibit growth of *M. phaseolina* in separate *in vitro* tests.

Our results showed greater potentials of cauliflower compost amendment to reduce *M. phaseolina* density compared to other composts, in which increased antagonist density brought proportionate reduction in dry root rot incidence. Beneficial effects of incorporating cruciferous residues on growth and yield of watermelon and wheat due to disease suppression and increase in microbes beneficial to plant growth has been well documented (Keinath, 1996; Kirkegaard *et al.*, 1994). Increased seed yield in all the compost-amended treatments may be a cumulative effect of reduced disease incidence, more retention of soil moisture, availability of nutrients and qualitative and quantitative improvements in microbiological properties. Improvement in the population of *Nitrosomonas* in amended soil in our study is an indication of mineralization of N from compost (Hadas *et al.* 1996). *Nitrosomonas* oxidizes ammonium to nitrite, which in turn is converted into nitrate by *Nitrobacter*. Increased microbial populations particularly those of antagonistic actinomycetes and lytic bacteria against *M. phaseolina* in compost amended soil probably lead to long-term beneficial effects. The high microbial activity and biomass caused by the “general soil microflora” in compost amended soil prevents germination of pathogenic propagules and infection of the host, presumably through microbiostasis (Hoitink and Boehm, 1999).

Our study demonstrated that after sanitation of crop residues from *M. phaseolina*, amendment of compost in nutrient deficient sandy soil reduced dry root severity, improved microbial properties of soil and seed yield. In resource deficient farming of arid region, the beneficial effect of compost as an integral part of low-input sustainable agriculture can also be a practical way of managing soil-borne pathogens.

In a subsequent study, efficacy of composts prepared from non-farm wastes was ascertained against dry root rot pathogen, nitrogen fixation and seed yield of cowpea and clusterbean (Lodha and Burman, 2000). Fully dried residues of pearl millet, a mixture of on- and off- season weeds {*Berhavia diffusa* L., *Cenchrus biflorus* Roxb., *Cynodon dactylon* (L) Pers, *Heliotropium subulatum* Hochst, *Coelosia argenticia* L and *Polycarpea crispa* Cass.} and green and fallen dried leaves and twigs of neem (*Azadirachta indica*) were used for the preparation of composts in December, 1995.

Composts prepared from on-farm wastes were incorporated @ 4 tones/ha to a depth of 30 cm in the first week of July in 1996 and 1997. Plots without any amendment served as control. 'HG75' clusterbean and 'Durgapura' cowpea seeds were sown in *Kharif* season of 1996 and 1997. Population densities of *M. phaseolina*, antagonistic bacteria and actinomycetes were estimated by standard procedures. Nitrogenase activity of roots were estimated by acetylene reduction assay activity. Data on dry root rot mortality and seed yield were recorded.

Amendment of soil with pearl millet and weed composts were significantly superior in reducing plant mortality due to dry root rot and enhancing seed yield of cluster bean and cowpea in both the years compared to non-amended plots (Table 26). Invariably, seed yield also was maximum in pearl millet compost amended plots in both the years and crops. Activity of nitrogenase was maximum when both the crops were grown in pearl millet amended plots (Table 26). The amendment with weed compost was also significantly equal to pearl millet compost in reducing dry root rot disease. In both the years, mortality due to dry root rot was less severe on clusterbean than cowpea under identical growing conditions. Higher susceptibility of cowpea than clusterbean to *M. phaseolina* in spite of similar conditions of moisture stress has been well documented (Lodha and Singh, 1984; Burman and Lodha, 2000).

Higher reduction in disease incidence in soil amended with pearl millet compost may be attributed to presence of some fungistatic or fungitoxic compounds. Residues of pearl millet were also found highly effective in bringing down propagules of *M. phaseolina* (Sharma *et al.* 1994). Use of weed compost as soil amendment has a potential advantage in nutrient deficient sandy soil as it was prepared purely from on- and off-season weeds. Many weeds are also host of *M. phaseolina* though composting inactivate *M. phaseolina* propagules during the heat phase to a greater extent. However, precaution should be taken to use only those weeds which are tolerant to *M. phaseolina* for preparing composts.

Table 26. Effect of different compost on dry root rot intensity, seed yield and nitrogenase activity of clusterbean and cowpea

Composts	Clusterbean			Cowpea		
	Mortality (%)	Seed Yield (kg ha ⁻¹)	Nitrogenase (n moles C ₂ H ₄ l ⁻¹ p ⁻¹)	Mortality (%)	Seed Yield (kg ha ⁻¹)	Nitrogenase (n moles C ₂ H ₄ h ⁻¹ p ⁻¹)
Weed	5.1	583	5920	12.1	423	5978
Neem	6.1	557	5353	14.8	370	4684
Pearl millet	5.2	659	6493	10.3	480	6751
Control	9.8	463	5261	20.0	339	6486

There was a considerable reduction in *M. phaseolina* propagules in amended plots, which was greater in clusterbean than cowpea plots. There was a significant increase in the population of antagonistic actinomycetes in the plots amended with pearl millet and weed compost after two years of study compared to that estimated in non-amended plots (Table 27). Enhanced population of actinomycetes in compost amended soils would induce suppressiveness because in fairly dry soils also their presence reduced counts of *M. phaseolina* (Lodha *et al.* 1990a). Lytic bacterial density was also significantly higher in all the amended compared to non-amended plots except when clusterbean was sown in neem compost amended plots (Table 27). In general, population of both the group of antagonists were maximum in plots amended with weed compost. Activity of nitrogenase was maximum in crops grown in amended soil, but number of nodules did not corroborated with nitrogenase activity. However, amendment with neem compost was not found to benefit these legumes with respect to nitrogenase activity. It may be due to release of some toxic compounds affecting the ability of rhizobium to form effective nodules.

Table 27. Effect of compost on the population of antagonistic actinomycetes, lytic bacteria and *M. phaseolina* in clusterbean and cowpea plots^a

Composts	Antagonistic actinomycetes (x 10 ⁵ g ⁻¹ soil)		Lytic bacteria (x 10 ⁵ cfu ml ⁻¹) ^b		<i>M. phaseolina</i> (g ⁻¹ soil)	
	Cluster-bean	Cowpea	Cluster-bean	Cowpea	Cluster-bean	Cowpea
Weed	8.6	9.5	33.1	31.6	205	230
Neem	6.5	6.6	22.6	24.1	256	287
Pearl millet	8.5	8.1	32.6	31.6	240	253
Control	6.1	6.3	20.3	18.1	306	362

^a After the harvest of crops in November 1997

^b Colony forming units

Disease suppression due to addition of compost into the soil could possibly be explained due to following factors 1. composts improve the soil physical structure, thus aeration of the roots improves, 2. composts support higher levels of total microbial population including antagonists, *M. phaseolina* propagules might also be prevented to infect the roots because of the presence of these antagonists, 3. Composts improves the moisture holding capacity of the soil, which in turn could reduce the pathogenic propagules and disease and 4. biological factors in compost amended soil also contributed to increased nitrogenase activity resulting in vigorous plants that may resist the attack of pathogen.

Increased seed yield may therefore be a cumulative effect of reduced disease incidence, more retention of soil moisture, availability of nutrients besides qualitative and quantitative improvement in microbiological properties.

Weed Residues

During cultivation under rainfed conditions, a number of weeds also compete with principal crops for soil moisture and nutrients. Under moisture stress, few of these weeds may be infected with *M. phaseolina* thus help in increasing its inoculum density. However, it is expected that many weeds may also be resistant to *Macrophomina* infection. These can be used as soil amendment in rainfed agriculture. Therefore, an experiment was initiated to study efficacy of weed residues against *M. phaseolina*. Of the eleven weeds screened to confirm the association of *M. phaseolina*, roots of *E. hirta*, *C. depressus*, *H. subulatum* and *A. persica* were found completely free but *A. hispidissima*, *V. divaricata* and *C. argentic* roots had 10% infection. Other four weed species recorded higher infection.

Five promising weeds alongwith one highly susceptible *Polycarpea cormbosa* were selected for further studies on the basis of resistance or susceptibility to *M. phaseolina*. Population changes of *M. phaseolina* were followed at an interval of 30 days in sandy soil separately amended with ground-up residues (1%) of these weeds for 90 days under laboratory incubation (Mawar and Lodha, 2000b).

A significant reduction in the population of *M. phaseolina* was estimated in all the weed residues except *Polycarpea* amended compared to non-amended soil. In *Celosia* and *Euphorbia* amended soil, 94 and 80% reduction in *M. phaseolina* propagules occurred within 30 days and reached 100 per cent in 90 days (Fig 15). In *Aerva*, *Heliotropium* and *Corchorus*, 87-94% reduction in pathogenic counts was estimated in the same period. A dramatic increase (44-61%) in the population of antagonistic actinomycetes was observed in *Corchorus* and *Euphorbia* amended soil (Table 28).

In subsequent field experiment, efficacy of weed residues viz., *Euphorbia*, *Aerva*, *Celosia* and *Polycarpea* was ascertained against dry root rot of clusterbean during *Kharif* season of 1998 and 1999. Partially decomposed residues were separately incorporated @ 0.5 t ha⁻¹ to a depth of 30 cm before seeds were sown. Data on soil moisture, dry root rot incidence and *M. phaseolina* population were recorded. Populations of antagonists were also estimated.

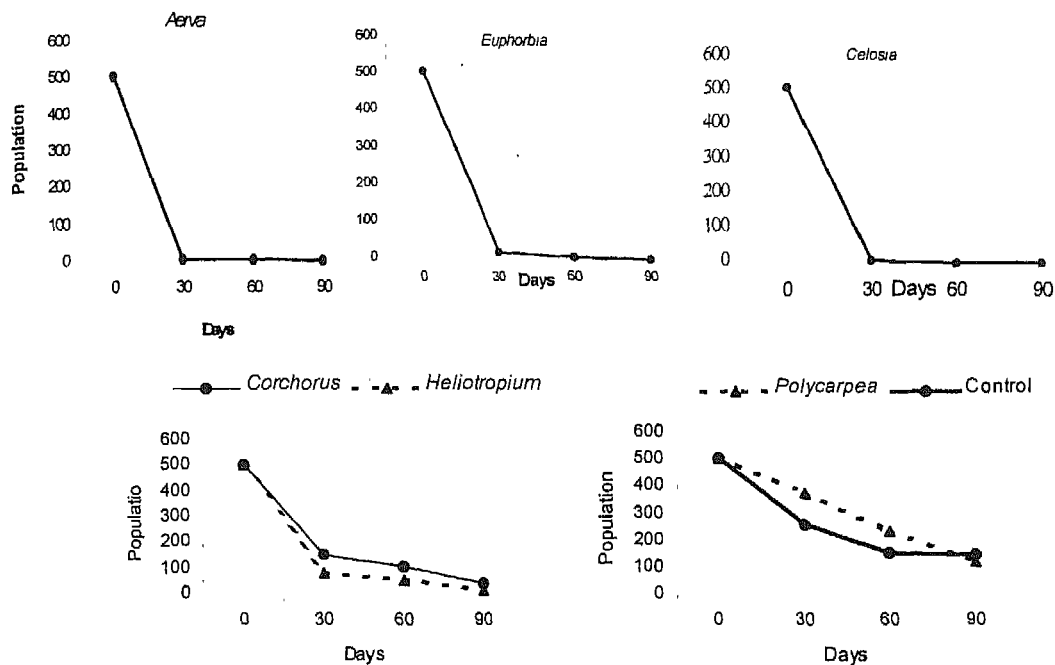


Fig 15. Population changes of *Macrophomina phaseolina* in weed residue amended and non-amended soil at 30 days interval

Clusterbean plants remained disease free till harvest in *Aerva* residue amended plots in 1998, *Euphorbia* and *Celosia* amendments were significantly superior than non-amended control in reducing dry root rot incidence. However, maximum mortality due to dry root rot was recorded in *Polycarpea* amended plots (Table 29). In 1999 also same trend was observed. Interestingly, *Aerva* and *Celosia* amendments restricted development of dry root rot on clusterbean plants for a period of 21 days even after initiation of the disease. In *Polycarpea* amended plots, there was a steep increase in disease intensity after 14 days. This finding is of great significance in delaying losses due to dry root rot in the years of low rainfall or terminal drought.

Another beneficial consequence of amending these on-farm weeds was attained by way of improving soil moisture retention and overall microbial properties of sandy soils particularly antagonistic actinomycetes. This has induced suppressiveness in soil against *M. phaseolina*. Increased inoculum build-up and dry root rot incidence in *Polycarpea* amended plots indicate that this and similar other weeds contribute in multiplication and survival of *M. phaseolina* sclerotia in soil. Farmers will have to make little efforts to uproot *Polycarpea* from their fields before ploughing down efficient bio-toxic weeds like *Aerva*, *Celosia* and *Euphorbia* to get a better harvest.

Table 28. Effect of weed residues on total numbers of bacteria, fungi, actinomycetes and the antagonistic actinomycetes population ^a (g⁻¹ soil) after 90 days of incubation

Treatments	Bacteria (x10 ⁵)	Fungi (x10 ³)	Actinomycetes (x10 ⁵)	
			Total	Antagonistic
<i>Aerva</i>	21	145	102	15
<i>Celosia</i>	14	135	123	33
<i>Corchorus</i>	22	122	81	36
<i>Euphorbia</i>	16	213	54	33
<i>Heliotropium</i>	12	95	79	12
<i>Polycarpea</i>	21	142	113	27
Control (non amended)	8	39	52	16

Occurrence under concurrent heat and moisture stresses, ubiquitous nature and other associated factors are the principle reasons why more than 500 species, are susceptible to *M. phaseolina*. Besides commercially valuable plants many on- and off-season weeds are susceptible hosts of this pathogen (Singh *et al.*, 1990). Since sclerotia are formed during pathogenesis and released in the soil after disintegration of host tissues (Cook *et al.*, 1973), infected weeds also contribute in increasing inoculum density of *M. phaseolina* in soil. These can be important sites where pathogen populations can increase and serve as inoculum reservoirs for adjacent cultivated crops (Mihail *et al.*, 1987). Much of the weed flora of arid region can be termed as on-farm wastes that can be utilized as a readily available cheaper source of organic amendments. But susceptibility to *M. phaseolina* restricts their *in toto* use. Screening done in the present study helped in selecting field resistant weeds only. A sharp reduction in the counts of *M. phaseolina* within 30 days of *Aerva* and *Celosia* residue incorporation in soil could be attributed to

Table 29. Efficacy of weed residues as soil amendment on plant mortality due to dry root rot on clusterbean

Treatments ^a	Mortality (%)		<i>M. phaseolina</i> population (g ⁻¹ soil) ^b	C:N ratio
	1998	1999	1999	1999
<i>Aerva</i>	0.5	13.00	223	13.3
<i>Celosia</i>	1.86	17.80	167	8.5
<i>Euphorbia</i>	1.43	13.10	213	17.5
<i>Polycarpea</i>	5.80	24.59	353	12.3
Control	4.33	25.33	310	11.4

^a@0.5 ton ha⁻¹; ^b After 15 days of harvesting in 1999.
Initial population was 504 sclerotia g⁻¹ soil in July 1998.

toxic nature of plant products and release of certain bio-toxic volatiles during decomposition. Further, complete eradication of *M. phaseolina* propagules in *Celosia* or *Euphorbia* amended soil within 90 days suggest the possibility of release of different types of volatiles during different stages of decomposition in the presence of soil moisture (Gamliel, 2000). Anti-microbial activity of various plant part extracts of *Aerva* against *M. phaseolina* and virus has been well documented (Gehlot and Bohra, 1998; Verma and Srivastava, 1985). *Euphorbia* is considered as a natural source of certain hydrocarbons and many phyto-chemicals (Sekar and Francis, 1998), and breakdown products during decomposition may be bio-toxic in nature. Relatively less decline in *Corchorus* and *Heliotropium* residue amended soil might be due to presence of low quantity of volatiles compared to those released by *Aerva*, *Celosia* and *Euphorbia*. Reduction of 70% of viable propagules of *M. phaseolina* in the non-amended moistened soil confirmed the significance of soil moisture alone in reducing population of *M. phaseolina* (Lodha, 1996). However, survival of almost equal counts of *M. phaseolina* in *Polycarpea* amended and non-amended soil can be attributed to release of such breakdown products during decomposition of *Polycarpea* residues that favoured survival of *M. phaseolina*.

Studies have shown that amendment of soil with cruciferous residues and summer irrigation (irrigated) or with composts from on-farm weeds (rainfed) will extend profitable cultivation of cumin and legumes for atleast two seasons. Thus, one needs to amend these residues only once in three years.

Conclusion

In arid regions of India, occurrence of soil-borne plant pathogens like *Macrophomina phaseolina* causing dry root rot in many legume and oilseed crops under rainfed conditions and *Fusarium oxysporum* f. sp. *cumini* causing wilt on cumin in winter season has become a serious limiting factors for the profitable production of these crops. The objective of integrating basic and applied research findings in developing management strategies against these soil-borne pathogens of hot arid region has been achieved to a reasonable extent in the work presented here.

We were conscious of the fact that agriculture in arid region ranges from resource deficient (rainfed) to resource affluent (irrigated) cropping systems. If rainfed agriculture is the main stay, lush green fields of wheat, chilies, cumin and yellow flower laden mustard are also a common site in the irrigated pockets. Under such varied milieu, management of these pathogens by any single method may not be adopted or result in economic gains. Therefore, a host of technologies suited to specific conditions of farming is a circumstantial requirement of the region.

Since inoculum density of these pathogens in the soil is directly proportional to disease intensity in the field, major efforts were made to bring down propagule density below the economic threshold. Thus, influence of various physical, cultural and biological management strategies on survival of *M. phaseolina* and *F. oxysporum* were studied under laboratory and field conditions. Combining indigenous resources of the hot arid region like intense and ample solar irradiations, high temperature during crop-free summer periods, resident biocontrol agents and on-farm wastes (cruciferous and weed residues), a host of technologies were developed specific to rainfed and irrigated agriculture.

In any cropping system, seed treatment with effective seed dressers like carbendazim (0.2%) has been found as a prerequisite requirement to eliminate transmission of disease in soil particularly in newer areas and to check seedling infection. Similarly, rotation with less susceptible crops like pearl millet or moth bean against *M. phaseolina* and non-hosts like wheat, mustard or Isabgol against *F. oxysporum* will restrict the increase of pathogenic propagules in the soil. Cultivation of field tolerant strains is another prerequisite for minimizing losses.

Studies have shown that in rainfed agriculture, mulching the soil with pearl millet stover (3.5 ton ha⁻¹), low plant density (1.6 against 2 lakh ha⁻¹), and soil amendment with farmyard manure (10 ton ha⁻¹) singly or in combination effectively conserved the soil moisture and reduced the population of *M. phaseolina* and dry root rot incidence by 55-72% in the field, thereby significantly increasing the seed yield of clusterbean. There exists ample scope for the use of composts prepared from on-farm wastes that has long-

term effects for control of *M. phaseolina* and other soil-borne pathogens besides improving population of *Nitrosomonas* and resident antagonists like actinomycetes and lytic bacteria, soil fertility and moisture holding capacity of sandy soils. The highest disease suppression and yield promotions were recorded in soil amendment with pearl millet and cauliflower leaf residue composts (4 ton ha⁻¹), respectively. Further, amendment with composts in general was beneficial in terms of nodulation and nitrogenase activity in legumes. Our effort to inactivate pathogenic propagules of *Macrophomina* during and after composting reduced the chances of spreading this pathogen in the soil is first of its kind from India. Incorporation of on-farm weeds like *Aerva*, *Euphorbia* and *Celosia* in soil before sowing of rainfed crops reduces dry root rot incidence besides inducing soil suppressiveness.

In irrigated agriculture, soil solarization (polyethylene mulching) elevated the soil temperature in the ranges found to be lethal for propagules of *Macrophomina*, *Fusarium oxysporum*, *F. solani* and *Cylindrocarpon lichenicola*. The increase in temperature due to polyethylene mulching in our experiments was maximum compared to those recorded from other parts of the world. This resulted in pronounced reduction in disease incidence and increased seed yield of economically valuable crops. Further, partial control of *Fusarium* and *Macrophomina* was also achieved just by one summer irrigation during May-June. Amendment of soil with *Brassicac*s (Mustard pod straw or oil-cake) augmented the efficiency of summer irrigation in reducing sizeable proportion of viable pathogenic propagules of *Fusarium* and *Macrophomina*, a technology developed as a substitute for expensive polyethylene mulching. This finding has a potential value and important implications for irrigated pockets of hot arid zone of India as well as for many countries in the appropriate climatic conditions. Control was further improved when pathogenic propagules were first continuously exposed to dry summer heat for 60 days and then the soil was amended with *Brassica* residues. Thus, after prolonged exposure of *Macrophomina* and *Fusarium* infested fields to dry summer heat, amendment of soil with a combination of mustard pod straw (2.5 ton ha⁻¹) and oil-cake (0.5 ton ha⁻¹) and one summer irrigation considerably reduced the soil population densities of both the pathogens and disease intensity on clusterbean during rainy season and on cumin in subsequent winter season in the same field. Generally, farmers are adopting a wheat-mustard-cumin rotation. Mustard residues are, thus available in March-April. Our technology requires application of these residues in May end or early June during intense solar irradiations and high temperatures in crop-free period. Moreso, sufficient time is available for the release of toxic volatiles before succeeding crop is grown in rainy season. Amendment of soil with these residues at this time allows adequate heating of soil so as to get maximum benefit of weakening effect on chlamydo spores/sclerotia of these pathogens. Once these volatiles are released under prevailing temperatures,

decomposed material will also serve as an amendment to enrich nutrient deficient sandy soils. Besides, disease control, partial weed suppression was also observed in *Brassica* residue amended soil.

One additional benefit accrued due to *Brassica* amendment was in the form of enhanced population and activity of certain bio-control agents like *Bacillus firmus*, *Aspergillus versicolor*, *Trichoderma harzianum* and antagonistic actinomycetes inducing soil suppressiveness. Survival and shelf life of *T. harzianum* was improved and a bioformulation was developed as Maru Sena 1, while bioformulation of *A. versicolor* in a mixture of neem compost and talc was prepared and termed as Maru Sena 2. *B. firmus* found specifically antagonistic to *M. phaseolina* is the first report from the world. Integration of *B. firmus* seed treatment with *Brassica* amendments and summer irrigation improved the control of dry root rot of clusterbean. Bioformulation of this bacterium has been developed as Maru Sena 3. Studies on yet another bio-control agent *Penicillium oxalicum* are in progress.

Technological interventions for management of these diseases have produced encouraging results. Demonstrations for seed treatment with *B. firmus* resulted in significant increase in seed yield of legumes.

Thus, efforts made during last two decades led to the development of eco-friendly, viable and cost effective alternatives to manage soil-borne plant diseases in hot arid region. In the resource deficient farming community of the region, the beneficial effects of these technologies formed an integral part of low externally input sustainable agriculture (LEISA).

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