Research Article

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Infectivity and management of dry rot, eye rot and soft rot of ginger (*Zingiber officinale* Rosc.)

R. Suseela Bhai

ICAR-Indian Institute of Spices Research, Kozhikode-673 012, Kerala, India

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Abstract

Dry rot and eye rot of ginger are post-harvest infections noticed as caused by *Macrophomina phaseolina* and *Fusarium oxysporum,* respectively. To investigate whether these pathogens cause damage to the crop only during the cropping period and then remain latent, or are purely post-harvest in nature, an experiment was undertaken by artificially inoculating the pathogens and comparing them with soft rot caused by *Pythium myriotylum*. The results of the study indicate that *M. phaseolina* could infect ginger plants during the cropping period and cause rhizome infection, manifested as yellowing of the pseudostem and the pathogen reside inside the rhizome and develop latent infection as dry rot during storage. The pathogen could be re-isolated and proved Koch's postulates. However, none of the *Fusarium* challenged plants showed symptoms either manifested as yellowing or rotting of the pseudostem. In *Macrophomina*-challenged plants, the infection appeared only during the post-monsoon period that coincides with the yellowing of the leaves during maturity. This observation was supported by the occurrence of natural infection by *Macrophomina* in harvested fresh rhizomes during 2018 and manifested as scattered elongated streaks with black mycelia inside the rhizomes, which extended from the cut end to the tip. But *Pythium* inoculated plants succumbed to infection as rotting of the basal portion of the pseudostem and yellowing of the lower leaves. The intensity of infection varied from 0-63 per cent. *In vitro* testing of seven fungicides in four different concentrations showed that metalaxyl-Mz, copper oxychloride (COC), and mancozeb even at 500 ppm are not inhibitory to *M. phaseolina.* But carbendazim and carbendazim-mancozeb were highly effective, giving complete inhibition even at 50 ppm. In the case of *P. myriotylum*, metalaxyl-Mz, COC, and Bordeaux mixture showed >70 per cent inhibition at 500 ppm. Mancozeb alone at 500 ppm was not effective against *Pythium*. *In planta* evaluation was done with fungicides individually and in combinations along with a systemic insecticide, dimethoate. Most of these treatments resulted in reduction of *Macrophomina* infection, of which metalaxyl-Mz alone or in combination with carbendazim (0.2%) and dimethoate (0.05%) showed 100 per cent reduction in infection. Metalaxyl-Mz (0.125%) with dimethoate (0.05%) was highly effective in reducing the infection caused by *P. myriotylum*.

Keywords: Dimethoate, dry rot, eye rot, *Fusarium oxysporum,* ginger, *Macrophomina phaseolina, Pythium myriotylum*

Introduction

Ginger (*Zingiber officinale* Rosc.) is widely cultivated for the rhizomes which are used as a spice, flavoring agent in confectionaries as well as in medicine. The crop is affected by a number of diseases caused by fungal, bacterial and viral pathogens of which, soft rot caused by species of *Pythium*, bacterial wilt caused by *Ralstonia pseudosolanacearum* and yellows by *Fusarium* sp. cause heavy crop loss in many ginger growing regions (Dake and Edison, 1988; Sarma, 1994). Besides these, the stored rhizomes are also found affected by dry rot and eye rot symptoms caused by species of *Macrophomina* and *Fusarium* respectively, leading to rhizome spoilage (Fig. 1 and 2).

Dry rot disease is characterized by drying and shrinkage of stored rhizomes with charcoal black colouration inside (Sarma and Nambiar, 1974). The black colour is due to the presence of the fungus *M. phaseolina* (Fig. 1). Such rhizomes later become hollow and get converted to a black mass of fibres. The disease is also found in the fresh rhizomes at the time of harvest. Not much work has been carried

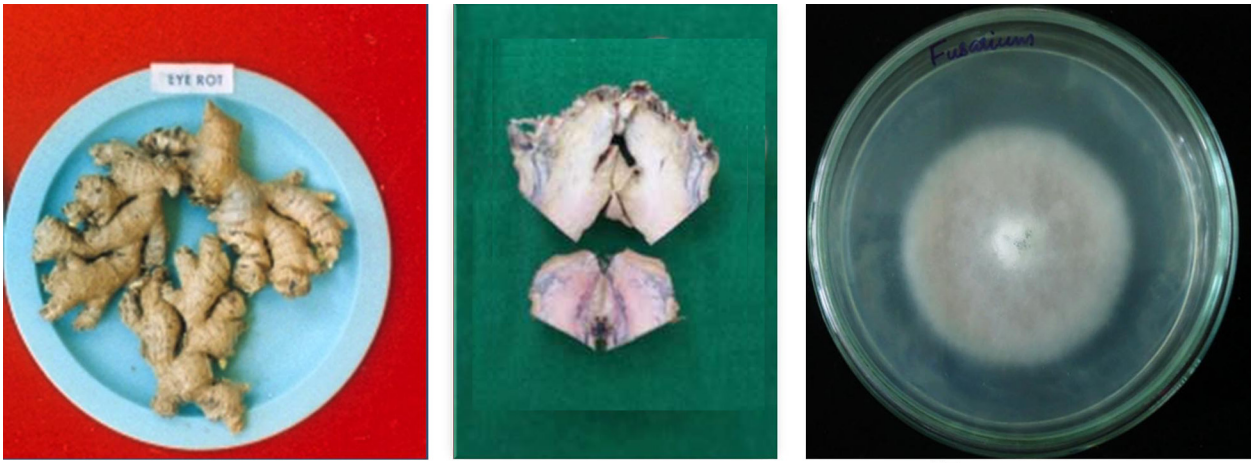
Corresponding Author: rsbhai@rediffmail.com

Dry rot infection ***M. phaseolina***

Fig. 1. Dry rot caused by *M. phaseolina*

out on the etiology and management of this disease. The initial symptom is manifested as small sunken spots at the cut end of rhizomes, which extends up to the tip through the fibrous tissues of ginger, making the tissue harder like roots. The LS of the root-like fibre structure shows black coloured thread which, on microscopic observation, will reveal the presence of the mycelium of *Macrophomina*. The infection in the tissues extends up to the tip of the buds, making the rhizome unusable. In later stages, the fungus grows from the cut end along the rhizomes as black growth extending along the rhizomes making the central core hollow (Bhai, 2018).

Eye rot occurs when the rhizomes put forth sprouts during storage, and the growing sprouts rot from the tip downwards (Fig. 2). The sprouts lose their viability and will not germinate further. *Fusarium oxysporum* was found to be associated with the infection. *F. oxysporum* has been reported to be associated with ginger yellows in Himachal Pradesh, India (Sharma and Dohroo, 1990; Dohroo, 2001). *F. solani* has been earlier reported from Chonbuk province, Korea as the causal agent of ginger yellows (Yang *et al.,* 1988). This disease occurs during the crop growth period in the field. Other *Fusarium* spp. reported in association with ginger are *F. moniliforme, F. graminearum* (Dohroo, 1987) and *F. equisetii* (Bhardwaj *et al.,* 1988). The most frequently isolated species is *F. solani* (Dohroo, 1987; Chauhan and Patel, 1990). Isolates of *F. oxysporum* f. sp. *zingiberi* differed in their aggressiveness (Dohroo and Sharma, 1992). The first report of ginger rhizome rot caused by



Eye rot infection ***F. oxysporum***

Fig. 2. Eye rot caused by *Fusarium oxysporum*

F. oxysporum was from China (Chi *et al.,* 2014). But, eye rot has not been reported from anywhere till date. Eye rot, caused by *F. oxysporum* is found to be a severe problem only during the storage of ginger (unpublished). However, not much work has been done on these two diseases *viz*., dry rot and eye rot Post-harvest seed treatment with a mixture of mancozeb and quinalphos is the recommended practice for seed rhizome storage to reduce the seed-borne infection during storage (Dohroo, 1995).

Soft rot (rhizome rot) of ginger caused by species of *Pythium* is one of the most destructive diseases of ginger, worldwide. Crop loss of up to 90 per cent has been reported from major production areas. Almost 11 species of *Pythium* have been listed as causal organisms in different parts of the world (Dohroo, 1995), but the predominant species associated with the disease is *P. myriotylum,* followed by *P. aphanidermatum* (Kumar *et al*., 2008). Yang *et al.* (1988) isolated *P. zingiberum* from Chonbuk provinces, Korea, and the symptom appeared as collapsing of pseudostem. Symptoms may occur at any stage of crop growth during the peak monsoon period. In mature plants, the infection takes place through roots or *via* the collar region, the first aboveground symptoms being yellowing of lower leaves followed by the collapse of affected pseudostems even with a slight touch. On underground rhizomes, water-soaked lesions appear, rhizomes start decaying, and under suitable environmental conditions, the rhizome rots rapidly and is eventually destroyed. The present study aimed to find out whether the storage pathogens, *viz., M. phaseolina* from dry rot and *Fusarium oxysporum* from eye rot, cause any damage to the crop from the soil during the crop growth period and remain latent or are purely post-harvest in nature? It is also to find out a suitable strategy for the management of these pathogens in comparison with *Pythium* sp. causing soft rot.

Materials and methods

The experiment was conducted in two steps. The first step involved was the isolation of the pathogens and to study *in planta* infectivity, which was undertaken by challenge inoculation of the soil with the pathogens before planting seed rhizomes. The second step was to evaluate different doses of fungicides against the target pathogens *in vitro* and *in planta*.

Isolation of pathogen

The pure culture of the pathogens *viz*., *M. phaseolina* and *F. oxysporum* were isolated from dry rot and eye rot infected ginger rhizomes from storage. *Pythium myriotylum* was isolated from soft rot affected rhizomes from the field. All the infected samples were collected from ICAR-Indian Institute of Spices Research Experimental Farm, Peruvannamuzhi, Kerala, India. The organisms were isolated in potato dextrose agar (PDA) and maintained in PDA tubes for further use. It was sub-cultured at times for experiments.

Potting mixture preparation

The potting mixture, containing sand, soil and farmyard manure in the ratio of 1:1:1, was prepared and sterilized by formaldehyde fumigation (Rathaiah, 1987). Beds of 3 x 1 m size were prepared using the potting mixture and drenched with 4 per cent formaldehyde @ 20 litres per bed. The beds were covered with white polythene sheets of 100 microns and sealed the sides with clay soil to keep airtight. After fumigation for five days, the polythene sheets were removed, raked the soil thoroughly to remove excess fumes of formaldehyde and the mixture was filled in pots (size 30 cm diameter) @ 10 kg per pot.

Infectivity studies

For infectivity studies, the pathogens *viz*., *M. phaseolina*, *F. oxysporum* and *P. myriotylum*, were multiplied in PD broth for 10 days. The culture was macerated using a mixer grinder and incorporated into the pot filled as above @ 100 mL per pot, which was equivalent to 1 g mycelium per 100 mL (Thripathi and Grover, 1975). Ginger seeds, @ 25 g pot^{-1} were planted in each pot. The experiment was designed in CRD with three pots per replication. There were four treatments *viz., M. phaseolina* (dry rot), *F. oxysporum* (eye rot), *P. myriotylum* (soft rot) and the three combined. There was an absolute control without inoculation of any pathogen. The pots were kept under open conditions throughout the experimental period to mimic the natural environment.

Screening fungicides against the pathogens

Screening fungicides against *M. phaseolina, F. oxysporum* and *P. myriotylum* was done *in vitro.* Commercial formulations of six fungicides namely carbendazim, carbendazim+mancozeb, metalaxyl-Mz, mancozeb, copper oxychloride and Bordeaux mixture in four different concentrations (50, 100, 250 and 500 ppm) were tested against the pathogens by poison baiting method. The fungicides in different concentrations were prepared from stock solutions and incorporated into the lukewarm medium before pouring into plates. The pathogens were inoculated in the centre of Petri dish (90 mm) and incubated at 25± 1^{0}C. A control plate for each pathogen was kept without amending with fungicides. The linear growth of the pathogen was measured after 72 h, and the per cent inhibition was calculated by comparing with control using the formula I= C-T/Cx100 where, I is the per cent inhibition, C and T are the radial growth of the pathogen in the control and treatment respectively. The plates were incubated for >120 h to see whether there is any resurgence of the pathogens in the fungicides.

Seed treatment and planting for disease management studies *in planta*

Rhizomes of required quantity were soaked in respective fungicide suspension for 30 min and air-dried. These were planted in the pots artificially infested with respective pathogens prepared as

Table 1. ***In vitro*** **evaluation of fungicides against** ***Pythium myriotylum*****,** ***Macrophomina phaseolina*** **and** ***Fusarium oxysporum***

Treatments	Concentration (ppm)			
	50	**100**	**250**	**500**
Pythium myriotylum (% inhibition)				
Metalaxyl - Mz	49.3	53.0	55.9	77.0
Mancozeb	0.0	0.0	0.0	0.0
Copper oxychloride	0.0	0.0	72.6	93.3
Carbendazim	0.0	0.0	4.8	34.4
Bordeaux mixture	10.4	11.1	64.8	82.6
Carbendazim + Mz	15.2	17.1	16.7	26.9
CV	8.9	6.1	4.6	2.8
SE	0.6	0.5	1.0	0.8
Macrophomina phaseolina (% inhibition)				
Metalaxyl - Mz	0.0	0.0	0.0	0.0
Mancozeb	0.0	0.0	0.0	0.0
Copper oxychloride	0.0	0.0	0.0	0.0
Carbendazim	100.0	10	100.0	100.0
Bordeaux mixture	0.0	62.6	71.8	74.1
Carbendazim + Mz	100.0	100.0	100.0	100.0
CV	0.0	2.4	2.5	2.9
SE	0.0	0.6	0.7	0.8
Fusarium oxysporum (% inhibition)				
Metalaxyl - Mz	10.5	9.6	12.3	20.0
Mancozeb	11.4	9.6	8.5	23.3
Copper oxychloride	0.0	0.0	0.0	0.0
Carbendazim	100.0	10	100.0	100.0
Bordeaux mixture	0.0	0.0	1.7	3.3
Carbendazim + Mz	100.0	100.0	100.0	100.0
CV	8.8	3.8	5.9	4.9
SE	1.9	0.8	1.3	1.7

Table 2. Effect of plant protection chemicals on infectivity by different ginger pathogens

Treatments details	% disease incidence		% disease reduction over control	
	P. myriotylum	*M. phaseolina*	*P. myriotylum*	*M. phaseolina*
T1 - Dimethoate 30% EC (0.05%)	50.4	2.2	0.0	89.2
T2 - Carbendazim 0.2%	63.2	2.7	0.0	86.8
T3 - Metalaxyl Mz (0.125%)	21.4	0.5	33.7	97.7
T4 - Carbendazim (0.2%) + Dimethoate (0.05%)	41.3	2.6	0.0	86.0
T5 - Metalaxyl Mz (0.125%) + Dimethoate (0.05%)	17.9	2.9	44.5	86.0
T6 - Metalaxyl Mz (0.125%) + Carbendazim (0.2%) + Dimethoate (0.05%)	27.1	0.0	16.0	100.0
T7 - Dimethoate (0.05%) + Metalaxyl Mz (0.125%)	37.0	2.5	0.0	88.0
T8 - Control	32.2	20.8	0.0	0.0
T9 - Absolute control	0.0	0.0	100.0	100.0
CD 0.05%	19.34	6.97		

above @ 25 g per pot (Table 2). These planted pots were drenched with the respective fungicidal suspensions @ 300 mL per pot at the time of planting the rhizomes and repeated the same treatments twice after 40 days interval. After harvest, the rhizomes from each treatment was stored and observed for storage infections of dry rot and eye rot.

Observation on germination was recorded based on the number of tillers produced. Disease incidence, based on the symptoms expressed either as yellowing or pseudostem infection, was recorded at fortnightly intervals by counting the number of tillers infected. After each observation, affected pseudostems were removed, and finally, percentage infection was calculated based on total infected and total pseudostem produced. Rhizome yield was recorded at the time of harvest.

Results and discussion

Planting rhizomes in artificially inoculated soil (sick soil) revealed the infection potential of the pathogens *viz*., *M. phaseolina, F. oxysporum* and *P. myriotylum*. Initially, no significant difference could be observed with respect to tiller production. However, there was enhanced tiller production in case of *Fusarium* inoculated plants (Fig. 3). Maximum disease incidence (40.2%) was observed with *Pythium* inoculated plants, followed by *Macrophomina* (20.8%) (Fig. 4). No symptoms of disease either manifested as yellowing or rotting of the pseudostem was noticed in case of *Fusarium* inoculated pots showing the non-infectiveness of *F. oxysporum* isolated from eye rot infection. Whereas in the case of *Macrophomina* treated plants, eight out of ten plants showed yellowing and in case of *Pythium* treated plants, all the plants

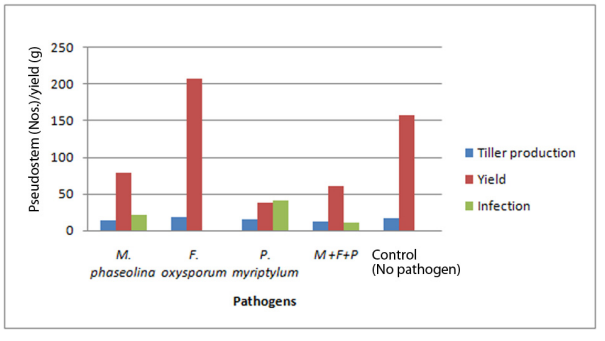


Fig. 3. Influence of pathogens on pseudostem production, yield and infection in ginger

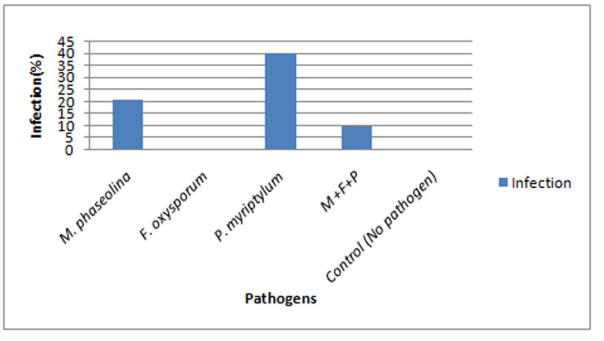


Fig. 4. Infectivity of pathogens on ginger

Fig. 5. Symptoms due to different pathogens

showed infection as rotting of the basal portion of the pseudostem and yellowing of the lower leaves (Fig. 5). The intensity of infection varied from 0-63 per cent in both cases (Fig**.** 3). When all the pathogens were inoculated together, there was suppression in disease incidence, which was also reflected in the yield (Fig. 1). This observation may be due to the protection offered by *F. oxysporum* to some extent, where there are reports of non-pathogenic *Fusarium* species that can act as biological control agents (Campanile *et al*., 2008). The harvested rhizomes after storage showed *Macrophomina* infection as well as *Pythium* infection in the respective treatments, whereas, no infection of eye rot occurred in *Fusarium* inoculated rhizomes. *Macrophomina* induced yellowing was noticed only during the post-monsoon period that coincided with the yellowing of the leaves during maturity (Fig. 6). Thus, the present infectivity study clearly revealed that *M. phaseolina* is infecting ginger plants during the crop growth period and cause rhizome infection and is manifested as yellowing of the pseudostem and the pathogen reside in the rhizome and develop as dry rot during storage (Fig. 7). Under challenge inoculated conditions, an incidence of 21 per cent was obtained due to this pathogen. More than 60 per cent of the rhizomes harvested showed *Macrophomina* infection manifested as small sunken spots at the cut end of rhizomes which extends up to the tip through the fibrous tissues of ginger making the tissue harder like roots. Isolation from infected

Fig. 6. Pot experiment showing symptoms of pathogens

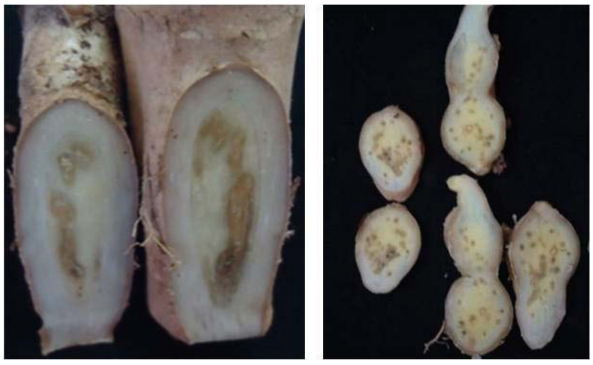


Fig. 7. Dry rot symptoms on cut rhizomes caused by *Macrophomina phaseolina*

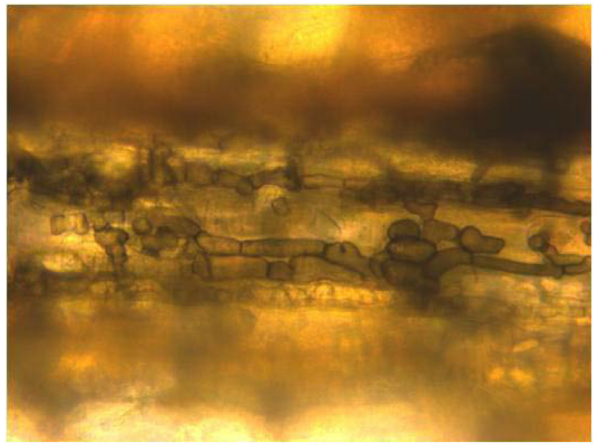


Fig. 8. Rhizome LS showing *Macrophomina* growth (20X)

rhizomes showed the presence of the pathogen (Figs. 8 & 9). This observation is supported by the fact that *M. phaseolina* is a heat and drought favouring pathogen (Smith and Wyllie, 1999) and it produces large numbers of microsclerotia inside the rhizomes under relatively low water potentials and relatively high temperatures. Under favourable conditions, hyphae germinate from these microsclerotia. Germination of the microsclerotia occurs throughout the growing season when the temperature is between 28 and 35 ^{0}C (Wyllie, 1988). Microsclerotia germinate on the root surface, germ tubes form appressoria that penetrate the host epidermal cell walls by mechanical pressure and enzymatic digestion or through natural openings (Bowers and Russin, 1999). Studies on the *in vitro* effect of different concentration of fungicides against *Macrophomina* and *Fusarium* showed that metalaxyl-Mz, mancozeb or copper oxychloride alone has no inhibitory effect on these pathogens even at 500 ppm whereas carbendazim alone or its combination product (mancozeb 63% + carbendazim 12%) were highly inhibitory even at 50 ppm. BM showed 83 per cent inhibition at 500 ppm concentration, when compared to carbendazim or its combination product (Table 1). Dwivedi and Dubey (1987) tested three different concentrations (50-1000 µg g^{-1}) of fungicides *viz*., carbendazim (Bavistin), pentachloronitrobenzene (PCNB) and Dithane M45 to reduce the population of *M. phaseolina* and found Bavistin 250 µg g^{-1} as the most effective and the same effect was obtained with PCNB and Dithane M45 at 1000 µg g^{-1}. *Pythium* was inhibited by copper oxychloride, Bordeaux mixture and metalaxyl-Mz in the descending order, where 500 ppm gave 75-90 per cent inhibition (Table 1). In cotton, reasonable control of *M. phaseolina* was obtained by seed treatment, and pre-sowing soil drenching with quintozene and carbendazim (Chauhan *et al.,* 1990). Charcoal rot of black gram, caused by *M. phaseolina*, is a key disease in India which is managed through bio-control agents like *Trichoderma viride* and *Pseudomonas fluorescens*. Also, the fungicide carbendazim has been used as a seed treatment chemical at recommended doses. Carbendazim @ 0.1 per cent was highly effective in the management of root rot disease by controlling mycelial growth (Athira, 2017). The results obtained in our study corroborates this observation.

For *in planta* studies, only those chemicals which were found highly inhibitory under *in vitro* conditions were used. Since *Fusarium* was found non-infective, it was not included in the experiment. In the case of *Macrophomina*, the infection was found less than 5 per cent in all the treatments with a disease reduction of 98-100 per cent except in control where >20 per cent disease incidence was observed. The results of the *in planta* trial showed that application of metalaxyl-mancozeb @ 0.125 per cent alone or along with dimethoate was highly effective in controlling soft rot of ginger caused by *Pythium* sp. Jayasekhar *et al*. (2000) recorded the effect of metalaxyl-Mz at 1 per cent for reducing the rhizome rot incidence (4.23%) with the highest disease reduction of 88.85 per cent. In our study, metalaxyl - Mz with dimethoate showed around

Fig. 9. Stages of dry rot infection caused by *M. phaseolina*

44 per cent reduction in disease incidence when compared to 33.66 per cent with metalaxyl-Mz alone (Table 2, Fig.10). It has reported previously that fungicides of different chemical groups may be alternated or combined in a control program to limit development of resistant populations of *Pythium* spp. (Allen *et al*., 2004). Here dimethoate was used to prevent the secondary infection of ginger rhizome by *Mimigrella coeruleifrons* which aggravate the disease (Koya, 1990).

As the soft rot disease is aggravated by the presence of insect larvae of *Mimigrella* sp., seed treatment with a systemic insecticide is found useful in reducing the disease incidence. Effect of metalaxyl and copper fungicides against species of

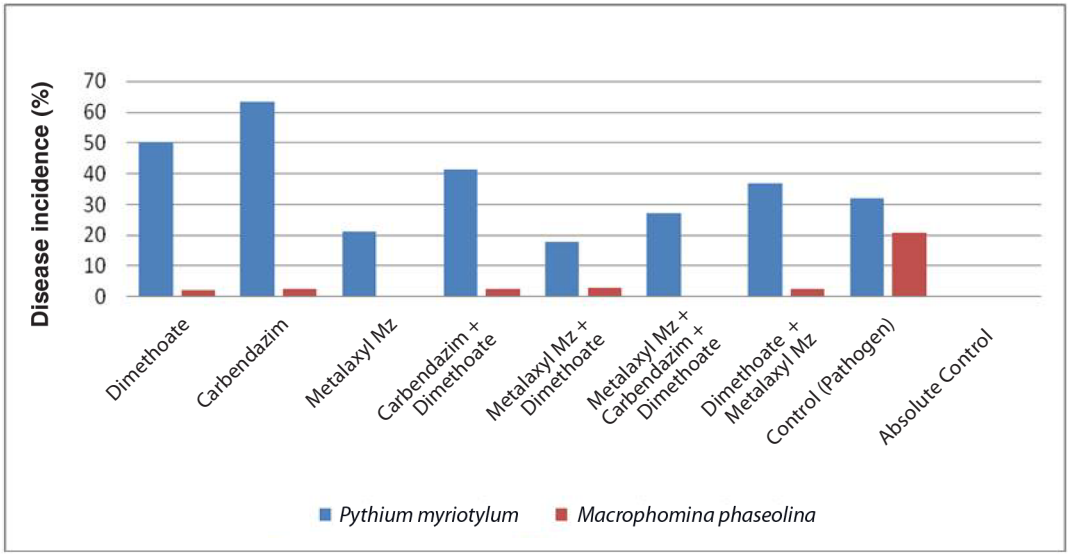


Fig. 10. Effect of different chemicals in the management of soft rot and dry rot of ginger

Pythium has been reported for the control of *Pythium* blight of snap beans (Locke *et al.,* 1983). The study was also supported by the work of Mathur *et al*. (2002), where it was reported that soil solarization followed by fungicide application could effectively minimize soft rot occurrence caused by *P. myriotylum*. They reported that seed treatments with Ridomil Mz (metalaxyl + mancozeb) at 6.25 g L^{-1} in addition to soil drench with Thimet and Ridomil Mz at 10 L in 3 × 1 M plot at 60 days after sowing gave the best control of *P. myriotylum* in an experimental field in southern Rajasthan, India.

Dry rot caused by *M. phaseolina* and soft rot caused by species of *Pythium* are two major diseases spoiling the crop during the crop season and at post-harvest storage. Although *Macrophomina* infection is found mainly during storage, the current study clearly suggests that the infection by *Macrophomina* can occur in the standing crop and latently carried through seed rhizomes resulting in dry rot. *F. oxysporum* strain causing eye rot is not infective to the crop in the field. It is purely post harvest in nature. Carbendazim and its combination (mancozeb 63% + carbendazim 12%) even at 50 ppm were highly inhibitory to *M. phaseolina in vitro.* However soil application of metalaxyl - Mz, carbendazim or its combination can take care of the soil population, thereby protecting the plants from *Macrophomina*. In case of soft rot infection occurring during the heavy monsoon period, seed treatment and soil drenching with metalaxyl - Mz (0.125%) along with dimethoate (0.05%) is highly effective in managing the disease.

Conclusion

The infectivity study clearly proved that *M. phaseolina* infects ginger plants during crop growth period causing rhizome infection and is manifested as yellowing of the pseudo stem and the pathogen reside in the rhizome and develop as dry rot during storage. The infection can be managed by the soil application of carbendazim alone or its combination product (mancozeb 63% + carbendazim 12%) even at 50 ppm. Further research is warranted on the effect of new generation fungicides and on biological control agents for combating *Macrophomina* infection.

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