

# Associative effect of biofumigation and biocontrol agents in management of root knot nematode *Meloidogyne hapla* in Gerbera

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## Abstract

Protected cultivation is an emerging technology in Nilgiris for raising cut flower crops. Due to controlled environmental condition and continuous growing of crops, the root-knot nematode *Meloidogyne hapla* has emerged as a major problem in gerbera cultivation causing enormous yield loss. The damage progressively increases if proper sanitation control measures are not followed during the polyhouse cultivation. In the absence of Methyl Bromide, it is necessary to use other options. Biofumigation was evaluated to control soil borne nematode parasites with the aim to develop bio-pesticides, which could be effective against root knot nematodes without deteriorating soil environment. Two field trials were conducted to study the effect of combined use of biofumigants and bioagents on the nematode population. Mustard crop was ploughed *in situ* before flowering and covered with a plastic mulch for one month in polyhouses. The biocontrol agents *viz.*, *Pseudomonas fluorescens* and *Trichoderma viride* were tried either alone or in combination with biofumigation. The stem length, flower yield and nematode population in soil were recorded. The study conducted at two different locations indicated that biofumigation with mustard followed by soil application of *P. fluorescens* @1.25 kg/ha at the time of planting significantly suppressed the population of *M. hapla* in soil by 67.5 to 67.8% and enhanced the flower yield of gerbera by 41 to 44%.

**Key words:** Gerbera, *Meloidogyne hapla*, biofumigation, mustard, biocontrol agents

## Introduction

Over the last years, in the search for a more environmental friendly pest and disease control, efforts have been directed towards a better understanding of the biological effects of natural products and the transformation products thereof. Plant extracts and crop residues have also been considered to have a potential role in sustainable agriculture, controlling pests and diseases, while minimizing dependence on artificial inputs such as pesticides (Halbrendt and LaMondia, 2004 and Kratochvil *et al.*, 2004). Biofumigation is the agronomic practice of using volatile chemicals (allelochemicals), released from decomposing *Brassica* tissues, to suppress soil-borne pests and pathogens. The most common volatiles produced during the breakdown of Brassicas are isothiocyanates (ITCs). ITCs are related to the active ingredient in the commercial fumigant metham sodium and dazomet and are highly toxic to pests and pathogens (Donkin *et al.*, 1995 and Jing and Halbrendt, 1994). They are released following tissue damage, when myrosinase enzymes, at neutral pH, hydrolyse (in presence of water) glucosinolates (GCs). GCs are sulfur-containing chemicals (thioglucosides) that are produced as secondary metabolites by Brassicas and most researchers believe their evolutionary role is to provide resistance against pests and pathogens. Isothiocyanates have often been regarded as the most toxic among the various types of glucosinolate derived products, and they have been found to have nematicidal, fungicidal, insecticidal and herbicidal properties. The biocidal activity of various isothiocyanates (ITCs) released by *Brassica* tissues is well-known (Brown and Morra, 1996) and the potential of Brassicas to suppress a range of soil-borne pests and diseases is supported by considerable empirical field evidence (Matthiessen and Kirkegaard, 2006). Biofumigation with brassicaes has been

found quite effective when used under plastic mulch in protected cultivation. Hence, this study was aimed to study the associative effect of the biofumigation potential of Brassicas in combination with biocontrol agents against root knot nematodes in gerbera which is being grown in polyhouses under protected condition in Nilgiris.

## Materials and methods

Trials were conducted in polyhouses cultivating gerberas at two different locations in Nilgiris with a history of severe infestations by root knot nematodes. Mustard (*Brassica juncea*) seeds were broadcasted at the rate of 10g/m<sup>2</sup> and just before flowering the plants were chopped and incorporated into soil at the rate of 5 kg fresh biomass m<sup>-2</sup>. After incorporation the plot was covered with linear low density polyethylene sheets and left as such for one month. At the time of planting the polyethylene sheet was removed and the soil was thoroughly mixed. The other treatments were applied as soil application just before planting. Thirty day-old side suckers of gerbera were planted at a spacing of 30 × 20 cm, accommodating 16 plants/ m<sup>2</sup>. Foliar sprays of pesticides were made according to recommended schedules in all the beds to control mites, thrips, leaf miner and foliar diseases. Talc based formulations of local strains of *Pseudomonas fluorescens* and *Trichoderma viride* were used in this study as follows

- T1- Biofumigation with mustard
- T2- *P. fluorescens* (SA)- 2.5 kg/ha
- T3- *T. viride* (SA)-2.5 kg/ha
- T4- Biofumigation+ *P. fluorescens* (SA)- 1.25 kg/ha
- T5- Biofumigation+ *T. viride* (SA)-1.25 kg/ha
- T6- *P. fluorescens* + *T. viride* (SA)-2.5 kg/ha
- T7- Cartap hydrochloride-10 kg/ac

T8- Carbofuran 3G-12 kg/ac

T9- Untreated control

The overall mean initial population level of *M. hapla* before treating the beds was estimated (five random soil samples/bed). Soil samples (100 g each) from the beds designated for each treatment and the control were processed and active nematode juveniles were extracted by combining Cobb's sieving and decanting method with the Baermann technique. Juveniles of root-knot nematodes per mL of aqueous suspension were counted with a microscope. The number of the nematodes per 100 g soil from each replicate was pooled and the mean value for each treatment was calculated. The stem length and flower yield/plant/month were measured in the gerbera crop in treated and untreated beds. Root-gall index (on a 1-5 scale, according to Heald *et al.*, 1989) and nematode population levels in root (Byrd *et al.*, 1983) and soil (Trudgill *et al.*, 1972) were recorded in each bed. The experiment was conducted in a randomized block design and the treatments were replicated thrice. Data collected in the two polyhouses were subjected to analysis of variance and significance assessed by means of a modified Duncan's multiple range test (Steel and Torrie, 1980).

## Results and discussion

The root-knot nematode, *M. hapla* is a serious limiting factor for the production of gerbera in commercial polyhouses in Nilgiris district. Trials were conducted in polyhouses cultivating gerberas at two different locations in Nilgiris with a history of severe infestations by root knot nematode, *M. hapla*. As an ecofriendly approach biofumigation was evaluated along with biocontrol agents in polyhouses for the management of root knot nematodes. At both the locations, significant reduction in nematode population in soil was observed in mustard biofumigated plots. In the polyhouse at Kanneri Mandanai village the population in biofumigated plots was reduced by 58.94 to 64.67% compared to untreated control plots (Table 1). Similar results were also obtained in the polyhouse located in Kappathorai village where there was 49.02 to 52.14% reduction in root knot nematode population in the biofumigated plots (Table 2). However there was a gradual increase in the population of root knot nematodes after a period of three months. Application of biocontrol agents *viz.*, *P. fluorescens* and *T. viride* in the biofumigated plots had an additive and prolonged effect in maintaining the nematode population at low levels. Among the two bioagents tested *P. fluorescens* was found more effective than *T. viride* at both the locations. There was 41 to 44% increase in flower

yield in plots biofumigated with mustard followed by application of *P. fluorescens* @ 1.25 kg/ha. The root knot nematode population reduced by 67.5 to 67.8% and the root gall index was 2 in these plots compared to 5 in untreated plots. Cartaphydrochloride was also effective against root knot nematodes. When applied at the rate of 10 kg/ac the population of nematodes in soil was reduced by 54.03% three months after planting.

Biofumigation is a sustainable strategy to manage soil-borne pathogens, nematodes, insects, and weeds. Brassicaceous cover crops have several characteristics that make them particularly suitable for plant-parasitic nematode control. Glucosinolate-containing brassicaceous cover crops have long been studied in the laboratory for nematode suppression (Chitwood, 2002). Upon enzymatic hydrolysis of glucosinolates in brassicaceous tissue, toxic byproducts are released and have been shown to suppress weed seed germination (Brown and Morra, 1996; Vaughn and Boydston, 1997; Weil and Kremen, 2007), fungal pathogens (Smolinska *et al.*, 2003) and nematodes (Akhtar and Mahmood, 1994; Mojtahedi *et al.*, 1991; Zasada and Ferris, 2003, 2004). Gardiner *et al.* (1999) found higher concentrations of glucosinolate-degradation products from roots than shoot, despite accounting for only 25% of the plant biomass. They proposed that the prolonged release of hydrolysis products from the roots could contribute to the potential effectiveness of rapeseed (*Brassica napus* cv., Humus and Dwarf Essex) as a soil fumigant. A lot of investigations have been made about root-knot nematodes *Meloidogyne* spp and show that the use of different plant materials can be efficient for controlling these pests (Gardner and Caswell-Chen, 1994). Fresh tissues (2.5 % w/w) of *B. napus*, *B. rapa*, *B. juncea*, *B. Carinata*, *Raphanus sativus* and *Crambe abyssinica* incorporated in soil infested with *M. Incognita* significantly suppressed nematode multiplication on the roots, compared to the untreated control (Djian-Caporilano *et al.*, 2005).

In addition to soil fumigation, implementation of an integrated approach not only benefits the flower production but also provide safety to the environment. Rhizosphere bacteria, mainly fluorescent pseudomonads have been reported to be antagonistic to nematodes. In the present study, introduction of naturally occurring biocontrol agent *P. fluorescens* in the biofumigated plots significantly reduced nematode population and enhanced gerbera flower production. According to Nagesh and Parvatha Reddy (2005) protection of plants in polyhouses against nematode infection needs to begin before planting, with a soil sterilant such

Table 1. Management of root knot disease in Gerbera by biofumigation and biocontrol agents (Kanneri Mandanai)

Treatments	Initial soil nematode population/ 100g	Nematode population/ 100g (after biofumigation)	Stem length (cm)	Flower yield/ plant/ month	Soil nematode population/ 100g (90 DAP)	Root gall index
Biofumigation with mustard	353.66	136.50	21.68	6.0	308.33	4
<i>P. fluorescens</i> (SA)- 2.5 kg/ha	360.02	389.27	23.36	7.6	282.66	3
<i>T. viride</i> (SA)-2.5 kg/ha	349.26	365.24	22.73	5.3	327.33	4
Biofumigation + <i>P. fluorescens</i> (SA)- 1.25 kg/ha	354.00	149.08	28.51	8.3	138.66	2
Biofumigation + <i>T. viride</i> (SA)-1.25 kg/ha	362.31	158.66	23.72	6.0	275.33	3
<i>P. fluorescens</i> + <i>T. viride</i> (SA)-2.5 kg/ha	345.24	396.84	27.75	6.6	258.00	3
Cartaph hydro chloride-10 kg/ac	363.47	410.35	26.90	7.0	196.00	2
Carbofuran-12 kg/ac	360.85	395.80	26.63	7.3	164.00	2
Untreated control	356.60	386.43	17.50	4.3	426.33	5
CD ( $P=0.05$ )	NS	12.3	1.12	0.94	11.51	-

Table 2. Management of root knot disease in Gerbera by biofumigation and biocontrol agents (Kapathorai)

Treatments	Initial soil nematode population/100g	Nematode population/100g (after biofumigation)	Stem length (cm)	Flower yield/plant/month	Soil nematode population/100g (90 DAP)	Root gall index
Biofumigation with mustard	364.40	196.50	29.42	7.00	320.35	4
<i>P. fluorescens</i> (SA)- 2.5 kg/ha	359.04	396.85	30.99	7.33	258.21	3
<i>T. viride</i> (SA)-2.5 kg/ha	366.94	410.31	29.13	6.00	324.37	3
Biofumigation + <i>P. fluorescens</i> (SA)- 1.25 kg/ha	361.20	209.34	33.77	8.33	125.98	2
Biofumigation + <i>T. viride</i> (SA)-1.25 kg/ha	358.40	208.85	30.53	5.66	198.80	3
<i>P. fluorescens</i> + <i>T. viride</i> (SA)-2.5 kg/ha	351.60	410.52	31.63	7.66	245.60	3
Cartap hydro chloride-10 kg/ac	344.50	386.00	28.30	6.33	228.65	3
Carbofuran-12 kg/ac	356.50	402.79	30.10	7.00	147.84	2
Untreated control	365.39	410.62	24.80	4.66	390.65	5
CD ( $P=0.05$ )	NS	10.5	1.30	1.02	12.62	-

as dazomet, followed by establishment of antagonistic potential in the substratum soil/media using fungi like *P. lilacinus* and *P. chlamydosporia*. Based on the results obtained in the experiments conducted in polyhouses at two different locations in Nilgiris it could be concluded that soil biofumigation with mustard fresh tissues for a period of one month followed by introduction of *P. fluorescens* as pre plant treatment significantly reduces the incidence of *M.hapla* in Gerberas grown in polyhouses. These preliminary studies have revealed the biofumigant potential of mustard. To achieve a higher degree of use of biofumigation further research has to be taken up to identify appropriate species of Brassica having high amount of GLS-s in their tissue, identify the biocidal compounds responsible for suppression and demonstrate a correlation between concentrations of these in the tissues and the degree of nematode suppression and work out details of incorporating biobumigation as an efficient tool in sustainable and integrated nematode management systems.

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