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Effect of bio-fumigation on nematode population and nutrient status of soil in okra

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Abstract
To achieve the eco-friendly management practices inclusion of Brassicaceae crops in cropping systems is one such alternative in management of nematode in okra. With this background, a pot culture experiment was carried out in the net house to assess the effect of two bio-fumigants *viz.* Cabbage and Cauliflower leaf on plant parasitic nematode population infecting okra crop and its effect on soil nutrient status. Plant parasitic nematodes *viz.* Root knot nematode, *Meloidogyne incognita* (218.3J2/200cc soil), Lance nematode, *Hoplolaimus indicus* (56.6 /200cc soil), Spiral nematode, *Helicotylenchus dihystera* (42/200cc soil) and Stunt nematode, *Tylenchorhynchus mashoodi* (17.3/200cc soil) and initial nutrient status of soil was nitrogen (313kg/ha), phosphorus (21kg/ha) and potassium (237kg/ha). The bio-fumigation experiment was conducted by mixing chopped leaves of cabbage and cauliflower@ 2.5kg and 5.0kg/m2 each (44g and 88g/kg soil in 15cm diameter earthen pot) and covering the pots for 15 days with a thin polythene sheet along with a chemical check carbofuran@ 1.0kg a.i./ha and a untreated control were maintained for comparison. The experiment resulted in reduction of parasitic nematode population like root knot nematode (40-78%), lance nematode (40.8-80.1%), spiral nematode (49.1-79.7%) and stunt nematode (40.8-81.3%). Further, bio-fumigants enhanced the nutrient content of the soil; nitrogen (11.5-33.5%), phosphorus (1.1-30.9%) and potassium (11.5-43.6%) leading to better plant growth with encouragement of growth of saprophytes in soil (61.2-76.3%) resulting in reduced multiplication of post plant parasitic nematodes. Both cabbage and cauliflower were found to be at par with respect reduction of nematode population and enhancement of plant growth parameter. However, Cabbage leaf @ 5.0kg/m^2 exhibited the better performance in all above aspect as compared to other treatments.

Keywords: Bio-fumigation, root knot nematode, soil nutrient status, nematode population

Introduction
Okra **(***Abelmoschus esculentus* L. Moench), popularly known as ladies' finger, is a flowering plant in the malvaceae family. In India, okra is cultivated in 2,31,000 ha area with production of 63.50 lakh mt which is 70% of world's production. Pests and diseases including phyto-feeding nematodes are major production constraints. Among nematodes, the root knot nematode (*Meloidogyne* spp.) and Reniform nematode (*Rotylenchulus reniformis*) are the predominant nematode pests [1]. *Meloidogyne incognita* is the most prominent nematode species affecting okra in Odisha [2] with absolute frequency of 64.00% followed by reniform nematode, *Rotylenchulus reniformis.* Bio-fumigation, a classical biological approach emphasizing on the non-conventional method of nematode management by using cost effective sources of different bio-fumigants, to stabilize crop production in nematode infested soil is an increasingly desirable pursuit in the present time. Bio-fumigation refers to the suppression of soil-borne pests and pathogens by biocidal compounds (principally isothiocyanates-ITC) released in soil when glucosinolates (GLSs) in Brassica crop residues are hydrolysed [3]. It is a sustainable method of soil management that increases soil organic matter (SOM), moderates soil pH, suppresses weeds & soilborne pathogens through glucosinolates (GSLs) and increases water infiltration. Brassica crops are the most popular ones that are generally used for bio-fumigation due to the relatively high GSL contents. More than 200 GSLs have been identified from plants belonging to Brassicaceae family [4]. Three major groups of GSLs have been reported, namely aliphatic, aromatic and indole forms [5, 6], with sinigrin usually being the predominant GSL being identified from Brassicaceae plants [7].
So here we do this research work to know the effect of bio-fumigation on the nematode population infecting okra crop and soil nutrient status.

Materials and Methods

1. Collection of sick soil

Well pulverised sandy loam soil free from plant debris and gravels were collected from the sick experimental plots. The soil was spread on a clean polythene sheet, mixed thoroughly and three composite samples amounting to 200cc each were drawn for screening to estimate the initial nematode population and simultaneously to estimate the available N, P & K content of the soil.

2. Processing of soil samples for nematode extraction

200cc of soil was taken and processed by Cobb sieving [8] and modified Baermann's funnel technique based on the principles of shifting and gravitation. Collection from sieves were compounded and poured over a moist double layered tissue paper supported on an aluminium wire gauge. When the excess water got drained, the tissue paper assembly was kept over a petri dish filled with water touching the bottom of aluminium wire gauge. The whole system was kept undisturbed for 24hrs for movement of the nematode through the tissue paper into the water in the petri dish. The nematode suspension so obtained was examined under a binocular stereoscopic microscope for preliminary observation.

3. Identification and estimation of nematode population of soil

The nematode in the suspension were killed by emerging the bottle in boiling water for about 3-4 min with constant stirring. An equal volume of double strength formalin (8% v/v) was added reducing the final volume to 4%. Then the suspension was taken in a 7×7 square counting dish, species were identified and their numbers were counted under a stereoscopic microscope.

Estimation of available nutrient contents of soil

Nitrogen

It was determined by alkaline permanganate method [9]. Twenty gram of soil samples were taken in one litre flask and to it added 100 ml of 0.32% KMnO4 and 2.5% NaOH each. The flask was immediately connected to distillation assembly and heated. The distilled ammonia was collected in 0.1N H2SO4 using methyl red indicator. The excess of sulphur acid was titrated against 0.1N NaOH. Results have been expressed as N in kg/ha.

Phosphorus

Available phosphorus was determined by using Olsen's extractant i.e. 0.5N sodium bicarbonate solution of pH 8.5 [10]. Standard solution was prepared by dissolving 0.2195 g of pure dry KH2PO4 in one litre of distilled water. This solution contained 50 microgram (0.05 mg) per millilitre. This was prepared as a stock standard solution of phosphate, 100 ml of this solution was diluted to 1000 ml with distilled water. This solution contained 5 microgram (0.005 mg) of P per millilitre. Then 1, 2, 3, 4, 6 and 10ml of this solution were taken in separate 25 ml volumetric flasks and 5 ml of the extractant solution and 5ml of molybdate reagent were added and diluted with distilled water to about 20 ml. After the addition of 1 ml of SnCl2 solution, it was diluted to 25 ml mark and then contents were shaken vigorously. After two minutes the transmittance per cent of the solution was read on spectrophotometer at 660mμ. The transmittance per cent was plotted against microgram of P and standard curve was prepared accordingly. One gram of soil sample was taken with 20 ml of 0.5 N NaHCO3 of pH 8.5 as an extractant [10] together with 0.5 g of Darco G-60 (free from phosphorus). The contents were shaken for 30 minutes in 100ml conical flask and then filtered through Whatman filter paper No.40. Five ml of the colourless filtrate was taken in 25ml of volumetric flask for determination and then 5ml of ammonium molybdate hydrochloric acid solution was added. The contents were diluted to about 22ml, now 1ml of working solution of stannous chloride was added to each flask to develop blue colour. The contents of flasks were shaken well and diluted to the mark. Colour intensity was measured in spectrophotometer within 10 minutes after setting the instrument to 100 reading of transmittance with blank prepared. The amount of phosphorus was calculated as P in kg/ha.

Potassium

One gram of soil was shaken with 100 ml of neutral normal ammonium acetate solution as an extractant in 200 ml conical flask for 30 minutes and then filtered through Whatman number 40 filter paper. The amount of potassium present in extract was determined by flame photometer [11]. The results were calculated as K in kg/ha.

Preparation of pot and addition of bio-fumigants

Earthen pots of 15 cm diameter were cleaned and surface sterilized in 1% formaldehyde solution and made air dry. Pots were then filled with the collected soil. The leaves of cabbage and cauliflower were chopped and incorporated in different dosages in the pot soil as per the treatment requirements. The soil of pots was moistened through sprinkling and were covered with polythene sheet making air tight. The nematicide carbofuran was also added to the soil as a chemical check along with an untreated control treatment. After covering the pots, a waiting period of 15 days was given for proper decomposition of bio-fumigants. After 15 days, the polythene sheet was removed, composite samples from each pot were drawn and post bio-fumigation nematode population and soil nutrients were estimated as per the methods described earlier.

Treatments

T1: Cabbage leaves @ 2.5 kg/m^2 (44g/pot of 15cm diameter).
T2: Cabbage leaves @ 5 kg/m^2 (88g/pot of 15cm diameter).
T3: Cauliflower @ 2.5 kg/m^2 (44g/pot of 15cm diameter).
T4: Cauliflower @ 5 kg/m^2 (88g/pot of 15cm diameter).
T5: Carbofuran @ 1.0 kg a.i/ha (56mg/pot of 15cm diameter).
T6: Untreated control.

Results

Effect of bio-fumigation on initial soil nematode population

Bio-fumigation with leaves of two crucifer's *viz*. Cabbage & Cauliflower @ 2.5kg and 5.0kg/m2 (44g and 88g/pot) each along with standard nematicide Carbofuran reduced the initial population of nematode of the experimental soil (Table-1). There was no clear cut difference between the leaves of cabbage and cauliflower at lower dose in respect of reduction of root knot nematode population since T1-cabbage leaf@ 2.5kg/m2 (44g/pot) & T3-cauliflower leaf@ 5.0kg/m2 (88g/pot) were at par (Table-1). However, T2 i.e. cabbage leaf @ 5.0kg/m2 (88g/pot) was significantly different from both the lower doses (T1 & T3) as well as the higher dose of cauliflower (T4) in reduction of population of root knot

nematode (Table-1). However, both the leaves exhibited similar trend in both lower and higher doses in population reduction of lance nematode *Hoplolaimus indicus*, spiral nematode *Helicotylenchus dihystera* and stunt nematode *Tylenchorhynchus mashoodi.* Among all the treatments the Carbofuran (T5) was distinctly superior over others in respect of nematode population reduction (Table-1). Regarding saprophytes, their population rather enhanced from the initial level irrespective of type of bio-fumigation as well as their different doses. But their population reduced under carbofuran application as well as in untreated control pot (T6) (Table-1).

Effect of bio-fumigation on soil nutrient status

Bio-fumigation by use of chopped leaves of crucifers i.e. cabbage and cauliflower revealed variation in the nutrient contents of the soil. The observed variations are given below.

Nitrogen

All the bio-fumigation treatments enhanced nitrogen content of soil. Statistical analysis of the data indicated that T1 & T3 i.e. bio-fumigants at lower doses and T2 & T4 i.e. bio-fumigants at higher doses are statistically at par in respect of addition of nitrogen to the soil (Table-2). But T2 & T4 are found to be significantly different from T1 & T3. However, T2-cabbage leaves@ 5.0kg/m^2 (88g/pot) was significantly superior over rest other three treatments i.e. T1, T3 & T4. (Table-2).

Phosphorus

Treatment means exhibited significantly different result in respect of addition of phosphorus content of test soil following bio-fumigation. Both cabbage & cauliflower leaves at higher doses (T2 & T4) enhanced the phosphorus content to the tune of 30.95% and 19.05% respectively applied @5.0kg/m2 (88g/pot) (Table-2). However, their lower doses are insignificant in enhancement of the phosphorus content of soil.

Potassium

Enhancement of potassium content of test soil following bio-fumigation was in the range of 11.5% (T3) to 43.67% (T2) (Table-2). Higher doses of both the leaves added more potassium as compared to the lower doses. However, maximum enhancement of potassium content was observed from T2-cabbage leaves@5.0kg/m2 (88g/pot) which was significantly superior over T3 but at par with T1 & T4 (Table-2).

Table 1: Effect of Bio-Fumigation on Initial Soil Nematode Population

Treatment	*Meloidogyne incognit*a			*Hoplolaimus indicus*			*Helicotylenchus dihystera*			*Helicotylenchus dihystera*			Saprophytes		
	BBF	ABF	% Decrease	BBF	ABF	% Decrease	BBF	ABF	% Decrease	BBF	ABF	% Decrease	BBF	ABF	% Change
T_1	218.33	131	40	56.66	33.5	40.8	42.00	21.25	49.13	17.33	10.25	40.89	80.66	132	64.89
T_2		109	50		27	52.48		16.25	61.28		8.5	50.98		120.5	76.35
T_3		129.75	40.5		32.25	43		20.25	52.09		10	43.27		118.75	61.27
T_4		120	45		28.25	50.3		17	59.53		8.75	48.93		109	70
T_5		48	78		11.25	80.1		8.5	79.77		3.25	81.34		9	87.79
T_6		202.25	7.3		53	6.54		39.5	6		16.5	5		76.5	5.12
SE(m) (+/-)		3.87			1.50			1.07			1.11			2.77	
CD (0.05)		8.13			3.16			2.25			2.35			5.83	

T1: Cabbage leaves @ 2.5 kg/m^2 (44gm/pot of 15cm diameter)
T2: Cabbage leaves @ 5 kg/m^2 (88gm/pot of 15cm diameter)
T3: Cauliflower @ 2.5 kg/m^2 (44gm/pot of 15cm diameter)
T4: Cauliflower @ 5 kg/m^2 (88gm/pot of 15cm diameter)
T5: Carbofuran @ 1.0 kg a.i/ha (56mg/pot of 15cm diameter)
T6: Untreated control
BBF: Before bio-fumigation population
ABF: After bio-fumigation population

Table 2: Effect of bio-fumigation on soil nutrient status

Nitrogen (kg/ha)				Phosphorus (kg/ha)			Potassium (kg/ha)		
Treatment	BBF	ABF	% Change	BBF	ABF	% Change	BBF	ABF	%Change
T_1	313	359.25	14.78	21	22	4.76	237	276.75	16.77
T_2		418	33.55		27.5	30.95		340.5	43.67
T_3		349	11.50		21.25	1.19		264.25	11.50
T_4		401.75	28.35		25	19.05		319.5	34.81
T_5		313			21			237	
T_6		313			21			237	
SEM (+/-)		15.931			1.425			15.218	
CD (0.05)		33.775			3.022			32.263	

T_1: Cabbage leaves @ 2.5 kg/m^2 (44g/pot of 15cm diameter)
T_2: Cabbage leaves @ 5 kg/m^2 (88g/pot of 15cm diameter)
T_3: Cauliflower @ 2.5 kg/m^2 (44g/pot of 15cm diameter)
T_4: Cauliflower @ 5 kg/m^2 (88/pot of 15cm diameter)
T_5: Carbofuran @ 1.0 kg a.i/ha (56mg/pot of 15cm diameter)
T_6: Untreated control
BBF: Before bio-fumigation population
ABF: After bio-fumigation population

Discussion

The bio-fumigation experiment revealed that both cabbage and cauliflower leaves in both the doses i.e. 2.5kg & 5.0kg/m^2 (44g & 88g/kg soil in 15cm diameter pot) are capable of reducing the nematode population of the soil within 15 days of application. This might be due to production of hematotoxic glucosinolates degradation products, *viz*. isothiocyanates, thiocyanates, nitriles or oxazolidinediones which are lethal to phytonematodes [6, 12, 13]. This principle of bio-fumigation has earlier been reported [14, 15, 16, 17]. Glucosinolates degradation products are formed as a result of the hydrolyzation of sulphur containing secondary metabolites by the enzyme myrosinase (stored separately in plant cells) to yield nitriles, epi-thio nitriles and thiocyanates following the bio-fumigation by brassica plants [18]. Youssef [19] reported that crushed cabbage leaves incorporated into the soil at different rates 10 days before transplanting of tomato reduced the nematode population significantly. Higher the rate of residue, higher was the percentage of nematode reduction. The data of present experiment also indicated that higher the dose, higher is the nematode mortality. However, the cabbage leaf@ 5.0kg/m^2 (88g/pot) was found to be significantly superior over other treatments in overall nematode control. Anita [20] has reported that the bio-fumigation with sulphur containing cruciferous vegetables (Cabbage, Cauliflower, Radish & Chinese cabbage) waste@ 1kg/5kg soil reduced the root knot nematode, *Meloidogyne hapla*, population significantly infecting celery crops. The population reduction of *Meloidogyne incognita*, to the tune of 50% & 45% by incorporation of cabbage & cauliflower leaves@ 5.0kg/m2 (88g/ kg soil in 15cm diameter pot) obtained in the present investigation is in agreement with the above findings. Bio-fumigation by use of chopped leaves of cabbage and cauliflower enhanced the nitrogen, phosphorus & potassium content of the soil to different extent. Higher the doses of bio-fumigant, higher is the percent increase in N, P & K content. However, the cabbage leaves@ 88g/pot was observed to add more N, P & K to the soil.

Conclusion

Basically in case of higher dose of bio-fumigants, nematode population reduction percentage was higher as compared to lower doses. Besides reduction in nematode population, the bio-fumigant crops also added different nutrients like nitrogen, phosphorus & potassium to the soil in some amount.

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