Brassica vegetable leaf residues as promising biofumigants for the control of root knot nematode, *Meloidogyne incognita* infecting cowpea

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Abstract: Under screenhouse conditions, two mashed leaves of plants belonging to the genus Brassica, cabbage (*Brassica oleracea* var. *Capitata* L.) and kohlrabi (*Brassica caulorapa* L.) were used as pre-and at sowing biofumigants at 20 g/pot for managing root knot nematode, *Meloidogyne incognita* on cowpea (*Vigna unguiculata* (L.) Walp) compared to metam potassium and metam sodium as chemical biofumigants as well as untreated check. It was found that, in general, there was a positive correlation between the time of the addition of the plant residue (10 and five days before sowing and at sowing time) and the percentage of final nematode population reduction i.e., the highest percentages of final nematode reduction (70.4% and 82.1%) occurred when *Brassica oleracea* and *B. caulorapa* residues were added, 10 days before sowing, respectively compared to those (61.9% and 61.1%) occurred after the respective residues were added at sowing time. Also, plant growth and yield (pods and seeds) criteria of cowpea plants behaved the same trend.

Keywords: Brassica plants, mashed leaves, cowpea, root knot nematode

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1 Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a dicotyledonous plant belonging to the Fabaceae family. It is the most important grain legume, providing a source of profitable revenue between 23% and 29% of the selling price in some countries (Langyintuo et al., 2000). It is also valued as the cheapest dietary and high quality vegetable protein of about 25%-43%. Root knot nematode is a major problem of cowpeas in the most crop growing regions of the world (Caveness, 1979). Losses on cowpeas are due to *Meloidogyne* species in some countries, which have been estimated between 10% and 89% including total crop losses in some cases (Adesiyan et al., 1992).

Fumigants have been used since 1950 against plant parasitic nematodes and especially used extensively for root knot nematode and soil borne pathogens control in the production of many fruit, vegetable and nursery crops (Noling and Becker, 1994). Some plant materials from several species within the family Brassicaceae are considered a promising alternative practice for controlling plant parasitic nematodes. Broccoli (Roubtsova et al., 2007) and cabbage (Youssef and Lashein, 2013; Kwerepe and Labuschagne, 2003) are proved to be effective against root knot nematode. As reviewed by Youssef (2015), biofumigation process was defined by many workers (Halberendt, 1996; Kirkegaard and Sarwar, 1998) as volatile compounds (e.g. thiocyanate, isothiocyanate) with pesticidal properties are released from certain plant materials, belonging to *Brassica* spp., or animal wastes when they are crushed or chopped.

Methyl bromide (MBr) is considered the main pesticide for reducing the populations of soilborne pests in tunnel production (Santos et al., 2008). It has been banned since 1992 in many countries because it is an

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atmosphere ozone depleting agent (Watson et al.,1992) and several alternatives can be applied for controlling soilborne pathogens and pests (Runia and Molendijk, 2007). Metam sodium (MS) and Metam potassium (MP) are soil fumigants used to control pathogens and pests affecting a wide range of the most economically important fruit and vegetable crops. MS at rates of 63-702 L ha^{-1} significantly reduces nematodes in soil before planting, and at all the growing season increases yield (Roberts et al., 1988). The use of synthetic nematicides is considered the most effective practical means of controlling plant-parasitic nematodes in cowpeas (Adesiyan et al., 1992). However, chemical control of root knot nematodes causes environmental pollution which refers to the high toxicity and persistence in the soil (Anastasiadis et al., 2008).

In view of this, the present investigation was carried out to assess the nematicidal activity of two Brassica plant species namely, cabbage (*Brassica oleracea*) and kohlrabi (*B. caulorapa*) in the form of mashed leaves as biofumigants compared to MS or MP as chemical fumigants for management of *Meloidogyne incognita* in cowpea.

2 Materials and Methods

Cowpea (*Vigna unguiculata* (L.) Walp) seeds were sown in 30-cm-diameter clay pots filled with 5 kg solarized sandy loam soil (1:1 w/w) in the growing season of Cowpea as summer crop. Three seeds were sown per pot at a depth of 2- cm, but the seedlings were thinned to one per pot, six days after emergence to ensure uniform plant vigor and size. The pots were watered regularly once a day. Treatments were applied as follows:

1-Mashed cabbage (*Brassica oleracea* var. *Capitata* L.) leaf was added at the rate of 20 g/pot.

2-Mashed kohlrabi (*Brassica caulorapa* L.) leaf was added at the rate of 20 g/pot.

3- MP [potassium methyl dithiocarbamate (Tamifume 69% SL) a. i, w/v] was added at the rate of 2.5 g/pot.

4- MS [sodium methyl dithiocarbamate (Solasan 51% SL) a. i., w/v] was added at the rate of 2.5 mL/pot.

Each material was added at three times: 10 days and five days before sowing, and at sowing time. The treated soil was covered with a plastic sheet to hold back the fumes which were the result of leaves decomposition in soil. The sheet was removed at sowing time. After seven days of seed emergence, 1,000 freshly hatched juveniles of *Meloidogyne incognita*/pot were added. Each treatment was replicated five times and equal numbers of non-treated replicates served as control. Plants were uprooted at harvest stage; this was done 90 days after inoculation. The nematodes (Second stage juveniles) in the soil were extracted by sieving and decanting methods (Barker, 1985). The extracted nematodes were counted on Hawksly slide and identified under light microscope. In cowpea root samples, they were gently washed to clean them from soil, cut into small pieces and divided into two halves. The first half was incubated in distilled water in petri dishes according to Young (1954) to extract nematode second stage juveniles (J2). The second half stained by acid fuchsin lactophenol (Franklin and Goodey, 1949) was examined for counting galls and egg masses using binocular microscope (Mai et al., 1996). Plant growth and yield (pods and seeds) parameters were recorded. Carbon/nitrogen (C/N) ratio was recorded for each plant material in Table 1.

Table 1 C/N ratio of the tested plant materials.

Plant material	C/N
Cabbage (*Brassica oleracea* var. *Capitata*)	14.1/1
Kohlrabi (*Brassica caulorapa*)	11.4/1

3 Statistical analysis

Statistical analysis of designed experiment has been carried out according to analysis of variance (ANOVA) procedures. Treatment means were compared at 5% level of probability by Duncan's Multiple Range Test as reported by Snedecor and Cochran (1989). This was done by Computer Statistical Package (COSTAT) User Manual Version 3.03, Barkley Co.

4 Results

4.1 Effect on nematode population

Table 2 indicated that adding a rate of 20 g of mashed cabbage leaves (*Brassica oleracea* or *B. caulorapa*) significantly ($p\leq0.05$) reduced nematode criteria according to the time the cabbage residue added before and at sowing time. Average percentages nematode population reduction was calculated to compare among the different treatments. Thus, it was found that, in general, there was a positive correlation between the time of the addition of the plant residue and the percentage final nematode population reduction. I.e., the highest average percentages of nematode reduction (67.8% and 80.9%) occurred when *Brassica oleracea* and *B. caulorapa* residues were added, 10 days before sowing, respectively compared to those (58.5% and 59.7%) occurred after the respective residues were added at sowing time. On the other hand, effect of MP was higher than that occurred by MS, because for five days before sowing, the averages percentages nematode reduction (%) were 78.8% and 47.3%, respectively. Contrarily, they recorded 62.7% and 64.5% reductions, 10 days before sowing, respectively. However, these chemicals caused seed germination inhibition when added at sowing time.

Table 2 Effect of mashed leaf residues of cabbage and kohlrabi, Metam potassium and Metam sodium after different addition periods on root-knot nematode, *Meloidogyne incognita* infecting cowpea, *Vigna unguiculata*

Treatments	Biofumigation periods (days)	No. J_2 in soil/pot	Red. %	No. J_2 in roots/plant	Red. %	No. galls/plant	Red. %	No. egg-masses/plant	Red. %	Average percentages total nematode reduction
Cabbage (*Brassica oleracea* var. *Capitata*)	0 (at sowing)	240d	42.9	120f	77.2	32c	55.6	17c	59.5	58.5
Kohlrabi (*Brassica caulorapa*)		280c	33.3	90g	82.9	30c	58.3	15cd	64.3	59.7
Metam potassium		-	-	-	-	-	-	-	-	-
Metam sodium		-			-	-	-	-	-	-
Cabbage (*Brassica oleracea* var. *Capitata*)	5 (before sowing)	360b	14.3	300b	43.1	33c	54.2	27b	35.7	36.8
Kohlrabi (*Brassica caulorapa*)		120g	71.4	55i	89.6	22e	69.4	7e	83.3	78.4
Metam potassium		100g	76.2	90g	82.9	18f	75.0	8e	81.0	78.8
Metam sodium		280c	33.3	160e	69.6	39b	45.8	25b	40.5	47.3
Cabbage (*Brassica oleracea* var. *Capitata* L.)	10 (before sowing)	180f	57.1	100g	81.0	26d	63.9	13d	69.0	67.8
Kohlrabi (*Brassica caulorapa* L.)		100g	76.2	70h	86.7	20ef	72.2	7e	83.3	80.9
Metam potassium		210e	50.0	180d	65.8	23de	68.1	14d	66.7	62.7
Metam sodium		200fe	47.2	225c	57.3	18f	75.0	9e	78.6	64.5
Untreated check	-	420a	-	527a	-	72a	-	42a	-	-
LSD at 0.05	-	25.49	-	13.72	-	3.42		2. 34	-	-

Note: -Each value is an average of five replicates. Different letter (s) of each column are significantly different according to Duncan's Multiple Range test at 0.05 level.
-= inhibited seed germination

4.2 Effect on plant growth

As shown in Table 3, all plant growth criteria and number of nodules increased by using the tested treatments. The obtained results indicated that there was a positive correlation between the percentage plant growth vigor increase and the time of treatment addition. I.e., the highest percentages plant growth vigor increase were 86.3% and 168.5% when *Brassica oleracea* and *B. caulorapa* plant residues were added, 10 days after sowing, respectively compared to the percentages increase (8.7% and 26.8%) when the respective plant residues were added at sowing time. The same trend occurred for MP and MS as they achieved the highest percentages plant growth vigor (83.2% and77.3%) when they were added, 10 days after the sowing time compared to those (43.9% and 28.3%), occurred when the two respective chemicals were added, five days before sowing time. However, these chemicals caused seed germination inhibition when added at sowing time.

4.3 Effect on yields

As shown in Table 4, all plant yield criteria increased as influenced by the different treatments. It is worthy to note that there was a positive correlation between the percentage plant yield vigor (pods and seeds) increase and the time of plant residue addition. I. e., the highest percentages of yield vigor index increase were 66.0% and 203.8%, when *Brassica oleracea* and *B. caulorapa* plant residues were added, 10 days before sowing compared to 7.5% and 86.9% when plant residues were added at sowing time. The same trend was noticed for the two chemical fumigants, MP and MS, as they caused the highest percentages yield vigor index increases (179.2% and 47.2%), 10 days before sowing compared to 66.0% and 30.1%, respectively, occurred when these chemicals were added, five days before the sowing time. However, these chemicals caused seed germination inhibition when added at sowing time.

Table 3 Plant growth of cowpea as affected by mashed leaf residues of cabbage and kohlrabi, Metam potassium and Metam sodium at different addition periods on root-knot nematode, *Meloidogyne incognita* infecting cowpea, *Vigna unguiculata*

Treatments	Biofumigation periods (days)	Plant height (cm)	Fresh weight of shoots (g)	Dry weight of shoots (g)	Fresh weight of roots (g)	No. of nodules	Plant growth vigor index	%Plant vigor index increase
Cabbage (*Brassica oleracea* var. *Capitata* L.)	0 (at sowing)	67g	53.3g	14.9f	19.5d	20de	34.9	8.7
Kohlrabi (*Brassica caulorapa* L.)		72f	71.5f	17.9de	20.1d	22cd	40.7	26.8
Metam potassium		-	-	-	-	-	-	-
Metam sodium		-	-	-	-	-	-	-
Cabbage (*Brassica oleracea* var. *Capitata* L.)	5 (before sowing)	75 ef	51.7g	17.0ef	20.7d	23cd	37.5	16.8
Kohlrabi (*Brassica caulorapa* L.)		84 ab	119.1c	20.0c	24.2ab	20de	53.5	66.7
Metam potassium		80bcd	87.8d	18.2cde	23.1bc	22cd	46.2	43.9
Metam sodium		76def	78.1e	15.4f	12.4e	24cd	41.2	28.3
Cabbage (*Brassica oleracea* var. *Capitata* L.)	10 (before sowing)	79cde	79.2e	19.2cd	25.7a	17e	59.8	86.3
Kohlrabi (*Brassica caulorapa* L.)		88a	151.7a	29.2a	26.1a	48a	86.2	168.5
Metam potassium		82bc	134.8b	25.9b	21.5cd	30b	58.8	83.2
Metam sodium		78cde	74.3f	20.1c	8.9f	25c	56.9	77.3
Untreated check	-	58b	43.0h	13.0g	23.4bc	23cd	32.1	-
LSD at 0.05	-	4.19	3.35	1.93	1.95	3.52		

Note: -Each value is an average of five replicates. Different letter(s) of each column are significantly different according to Duncan's Multiple Range test at 0.05 level.
Plant growth vigor index= average plant growth criteria
-= inhibited seed germination

Table 4 Yield of cowpea as affected by mashed leaf residues of cabbage and kohlrabi, Metam potassium and Metam sodium at different addition periods on root-knot nematode, Meloi*dogyne incognita* infecting *Vigna unguiculata*

Treatments	Biofumigation periods (days)	No. of pods/plant	Weight of Pods/plant (g)	No. of seeds/pod	Dry weight of seeds/pod (g)	Plant yield vigor index	%Plant yield vigor index increase
Cabbage (*Brassica oleracea* var. *Capitata*)	0 (at sowing)	10bcd	9.6e	7a	6.02d	5.7	7.5
Kohlrabi (*Brassica caulorapa*)		13b	12.4d	7a	6.93d	9.9	86.8
Metam potassium		-	-	-	-	-	-
Metam sodium		-	-	-	-	-	-
Cabbage (*Brassica oleracea* var. *Capitata*)	5 (before sowing)	6ef	5.8g	7a	7.14cd	6.5	22.6
Kohlrabi (*Brassica caulorapa*)		18a	17.4c	8a	8.32c	12.9	143.4
Metam potassium		9cde	9.6e	8a	8.40c	8.8	66.0
Metam sodium		8def	6.3fg	7a	6.23d	6.9	30.1
Cabbage (*Brassica oleracea* var. *Capitata*)	10 (before sowing)	12bc	9.0e	7a	7.28cd	8.8	66.0
Kohlrabi (*Brassica caulorapa*)		20a	23.6a	8a	12.8a	16.1	203.8
Metam potassium		20a	21.7b	8a	9.60b	14.8	179.2
Metam sodium		9cde	8.0ef	7a	7.07cd	7.8	47.2
Untreated check	-	5f	5.6g	6a	4.56e	5.3	-
LSD at 0.05	-	3.16	1.80	3.29	1.22		

Note: Each value is an average of five replicates. Different letter(s) of each column are significantly different according to Duncan's Multiple Range test at 0.05 levels.
Plant yield vigor index= average plant yield criteria
-= inhibited seed germination

5 Discussion

In the present study, mashed leaves of cabbage and kohlrabi when treated and compared to MP and MS as chemical fumigants were effective in reducing population density of root knot nematode, *M. incognita* infecting cowpea plant. It is clearly noticed that the highest average percentage nematode population reduction occurred when cabbage and kohlrabi residues were added, 10 days before sowing than those when added at sowing time. This may be due to the slow decomposition of these plant residues in soil which contain glucosinolate compounds as toxic products (Chew, 1988; Brown et al., 1991; Brown and Morra, 1996). These compounds are produced when plant or animal waste cells are damaged by crushing or chopping and interact with enzyme called myrosinase producing D-glucose, isothiocyanate (biofumigant) and nitrite in the presence of water (Anonymous, 2011) which are lethal against nematodes. The present results agree with those obtained by Anita (2012) who reported that ethanol extracts of cabbage, cauliflower, radish and Chinese cabbage leaves reduced population of *M. hapla* and improved celery plant growth criteria of which, radish leaf residue was the most effective resulting in 60.6% reduction in nematode population in soil and 41.9% increase in celery green leaves and stalk yield. The same trend was noticed with El-Sherbiny and Awd Allah (2014) who reported that air-dried powdered of some plants of which cauliflower (belonging to crucifer plants) when added as pre-planting reduced *M. incognita* on tomato plants and improved plant growth criteria. Roubtsova et al. (2007) stated that biofumigants to be effective against nematodes require uniform distribution through the soil profile as the target nematodes exists. Also, when the C/N ratio of the amendment is less than 20:1, more effect on nematodes is evident (Stirling, 1991). On this basis, kohlrabi (*Brassica caulorapa*) residue used in this study, has a C/N ratio which equals to 11.4:1, so more effect of its toxic biofumigants on the nematode population than cabbage (*Brassica oleracea*) residue which has C/N equals to14.6/1 could be expected which corresponds to the previous study (Youssef and Lashein, 2013). Other factors including a very thorough disruption of the plant tissue before mixing with soil, sufficient soil moisture (Morra and Kirkegaard, 2002) and soil temperature (Ploeg and Stapleton, 2001; López-Pérez et al., 2005) at the time of tissue incorporation were shown to greatly enhance the suppressive activity of biofumigation against pests. El- Ghonaimy et al. (2014) reported that the soil fumigated with MS and MP significantly reduced the population criteria of *M. incognita* which agree with the present results. Also the present results agree with those obtained by Roberts et al. (1988), Runia and Molendijk (2007), Santos et al. (2008) and Asif et al. (2017).

6 Conclusions

From the obtained results, it can be concluded that , mashed leaves of cabbage and kohlrabi belonging to Family Brassicaceae when treated and compared to MP and MS as chemical fumigants were effective in reducing population density of root knot nematode *M. incognita* infecting cowpea plant and improved plant growth criteria. The highest percentage of nematode population reduction occurred when cabbage and kohlrabi residues were added to soil, 10 days before sowing than those when added at sowing time.

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