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Abhishek Rana
Department of Entomology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India

YS Chandel
Department of Entomology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India

RS Chandel
Department of Entomology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India

Correspondence
Abhishek Rana
Department of Entomology, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India

Efficacy of soil sterilants and neem against plant parasitic nematodes, microarthropods and soil mycoflora in cabbage grown in pots under protected conditions

Abhishek Rana, YS Chandel and RS Chandel

Abstract
The effect of metham sodium, dazomet and neem cake was evaluated against plant parasitic and saprophytic nematodes, mites, collembolans and fungi in cabbage crop grown in pots under polyhouse conditions. The complete reduction was observed in the population of *Helicotylenchus* species with metham sodium and dazomet at 30 DAT and crop end. Neem cake was found to be least effective and it showed 57.55 percent reduction in the population of *Helicotylenchus* species at crop end. At crop end, the number of galls varied from 0.0 to 4.00 galls/ plant in metham sodium and dazomet treatments. Saprophytic nematodes were also adversely affected by metham sodium and dazomet showing reduction in their population by 70.54 (metham sodium @ 2 ml/ pot) to 86.48 (dazomet @ 3 g/ pot) percent, whereas in neem cake, decrease in the population of saprophytic nematodes was 62.85 percent. Microarthropod population in different treatments of metham sodium and dazomet was found to decline from 44.38 (dazomet @ 3g/ pot) to 82.00 (metham sodium 2 g/ pot) percent. Among fungi, only *Fusarium* species has been recorded. The maximum reduction in *Fusarium* population was recorded in higher dose of dazomet (3 g/ pot).

Keywords: *Helicotylenchus,* microarthropods, fungi, plant parasitic nematodes, soil sterilants

Introduction
Cabbage, *Brassica oleracea* L. var. *capitata* is an important vegetable crop grown throughout the country. Cultivation of a crop becomes profitable only when the effect of losses incurred could be made minimum. There are many soil pests which are responsible for these losses in cabbage crop. Soil fauna constitute 23% of the total diversity of living organisms (Decaens *et al.,* 2006) [8]. Among soil biota, nematodes, microarthropods and microbes are very important biotic components. Amongst nematodes, the plant parasitic nematodes remain a major challenge in cool season vegetable production. Cabbage crop is considered as a poor host crop for *Meloidogyne* sp (Goldi). The spiral nematode, *Helicotylenchus multicinctus* (Cobb) has been reported in Kenya, Uganda and other countries to be associated with cabbage (Bafokuzara, 1996) [3]. Maina (2011) [15] reported cabbage as a preferable host of *Helicotylenchus* sp in a survey conducted in selected agro-ecological zones of Kenya and revealed its frequency of occurrence of 82% in rhizosphere and 27% in roots. Saprophytic nematodes are organic matter decomposers and soil health regulators (Gaur *et al.,* 2003) [9]. Microarthropods are very important biotic components of soil ecosystem being actively involved in decomposition, nutrient cycling and soil health improvement (Begum *et al.,* 2014) [4]. *Folsomia hidakana* (Uchida and Tamura) (Collembola) has been observed to suppress the 'damping off' disease in cabbage caused by *Rhizoctonia solani* Kuhn (Shiraishi *et al.,* 2003) [17].
In the preliminary investigations, metham sodium and dazomet have been found to be very effective for the control of plant parasitic nematodes (Anonymous, 2015) [1] and soil borne diseases. Yan *et al.* (2014) [21] reported these soil sterilants are having a broad biocidal ability which also affect the non-target soil organisms. In present investigation, the effect of these soil sterilants and neem on plant parasitic and non-target nematodes, microarthropods and mycoflora was studied in cabbage grown in pots.

Material and Methods

The studies on the effect of dazomet, metham sodium and neem amendments on soil biota in cabbage crop were carried out at CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh (altitude 1290m amsl, between 320.11' North latitude and 760.23' East longitude).

The soil used for filling the pots was collected from a polyhouse (having the history of nematode infestation) and mixed with FYM in ratio of 5:1 (soil: FYM) in a heap. From this heap, about one kg soil was drawn from different points of the heap for taking the pre-count of soil biota (nematodes, microarthropods and fungi). The soil was filled in pots, up to 3/ 4th of the total capacity which was approximately 16000 cm3 each. All the products viz. metham sodium (2 ml and 3 ml/ pot), dazomet (2 g and 3 g/ pot) and neem cake (40 g/ pot) were applied.

For the application of metham sodium, each dose was mixed in about 150 ml of water and poured onto the soil in the pots. In case of dazomet and metham sodium, each dose was mixed with the pot soil and the soil was saturated with water, followed by covering and tying of the pot tops with polythene sheet to make it airtight so that fumes of the chemical could not escape.

After 10 days, the polythene covers of all the pot were removed and pots were left undisturbed for five more days. After two weeks of removal of the covers, the seedlings of the crop were transplanted from the respective nurseries raised in plug trays. Neem cake was mixed in the pots and soil was kept moist for about three weeks before transplantation of the seedlings. All the pots were kept in a polyhouse and soil samples were taken before treatment, 30 DAT and at harvest from each experimental pot. Composite sample of 800 g from every pot was taken and brought to the laboratory in polythene bags for further processing.

Extraction of nematodes from soil

From the composite sample, 200 g soil was taken and processed by Cobb's Sieving and Decanting technique followed by Schindler's modifications (Southey, 1970) [18]. The volume of nematode suspension collected in a Petri plate after 24 hrs of washing was made up to 100 ml. An aliquot of one ml was drawn after gently agitating the suspension and nematodes were counted under stereoscopic microscope with the help of a nematode counting dish. Total population in the sample was determined by multiplying the count with 100.

Extraction of microarthropods from soil

The microarthropod population was assessed before soil treatment, 30 DAT and at crop harvest. The microarthropods were extracted from soil by 'Tullgren Funnel' method (Veeresh, 1988) [20]. Composite sample of 500 cc soil was placed in a container with a base made from gauze with a mesh designed to hold soil particles but permit the microarthropods to pass. The containers were then placed over funnels arranged in series. Thereafter, a conical flask (100 ml) was put under each funnel with about 100 ml of 70 percent alcohol. The assembly was lit up with bulbs (60 Watts) fixed over each funnel at a height of about 6 cm so as to keep each soil sample well exposed to light for 48 hrs (Plate 1). The population of microarthropods was counted under the microscope in Petri plates and preserved in vials in 70 percent alcohol. The percent change (increase or decrease) in the population of microarthropods was worked out using formula given by Henderson and Tilton (1955) [11] with slight modification.

$$\text{Percent change in the population} = \left\{\left(\frac{T_a \times C_b}{T_b \times C_a}\right) - 1\right\} \times 100$$

Where, T_a = Population after treatment
T_b = Population before treatment
C_b = Population in control before treatment
C_a = Population after treatment in control

Isolation of fungi from soil

The soil dilutions were prepared by using serial dilution method. Five test tubes were taken and marked as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Each tube was filled with 9 ml of double distilled water and 1 g of soil sample was added in the first test tube and shaken vigorously to mix the soil well in water. With the help of a sterilized pipette, one ml of this dilution was added to second test tube to obtain second dilution of 1:100. Likewise, the remaining dilutions *i.e.* 1:1000, 1:10000 and 1:100000 were prepared. The aliquot of final dilution (10^{-5}) was poured on the agar surface in the Petri plate @ 1 ml, and was spread uniformly with a sterilized bent glass rod under aseptic conditions under laminar air flow. These Petri plates were incubated at 25 °C for 48-72 hrs. After 2-3 days, colonies of different fungi were observed in the Petri plates. The number of colonies was counted, and multiplied with dilution factor to calculate the total number of colonies per g of soil sample as follows:

$$\text{Number of cfu per gram of original sample} = \frac{\text{Number of colonies x Dilution factor}}{\text{Volume of culture plate}}$$

Data recording and statistical analysis

The data collected on various parameters at different crop intervals were subjected to statistical analysis on the pattern of randomized block design (RBD) using CPCS software (Gomez and Gomez, 1984) [10].

Results and Discussion

Effect on nematode fauna

The data on *Helicotylenchus* sp, saprophytic nematodes and number of galls per plant were recorded before treatment, 30 DAT and at the crop end (Table 1). *Helicotylenchus* species caused small necrotic lesions on cabbage roots, and under severe infestation, die-back symptoms were evident on roots. The population of *Helicotylenchus* species before treatment ranged from 30.0 to 180.0 J2/ 200 cc soil in the pots of metham sodium and dazomet treatments. No population of *Helicotylenchus* species was recorded at 30 DAT and crop end in any of the treatments of metham sodium and dazomet which is showing higher efficacy of these products. At 30 DAT the population (21.33 individuals/ 200 cc soil) was recorded from the pots where neem cake treatment was given as against 26.33 individuals/ 200 cc soil in untreated control. The population of nematodes in neem treatment as well as in control increased when recorded at crop end. Population reduction of 57.55 percent was found in neem treatment at crop end Fig 1. Cabbage variety was observed to be poor host for *M. incognita* in the present studies; therefore the number of galls recorded was very low at different stages of crop growth. At 30 DAT, no nematode galls were recorded in any of the treatment of either metham sodium or dazomet. It was only in neem treatment where 1.67 galls/ plant were recorded which were significantly less compared to the control where 7.33 galls/ plant were recorded. Whereas, at crop end number of nematodes galls was ranged from 0.0 to 4.0 galls/ plant in different treatments of metham sodium and dazomet but the number was very low as compared to control where number

of galls was increased in comparison to data taken at 30 DAT. There are several reports to support that metham sodium and dazomet lead to lesser number of galls and increase the yield of different crops (Candido *et al*., 2011; Kaskavalci 2007) [6, 14].

Table 1: Effect of different treatments on *Helicotylenchus* species

Treatments		*Helicotylenchus* population (per 200 cc soil)			Number of galls/ plant		Saprophytic nematode population (per 200 cc soil)		
		Before treatment	30 DAT	At crop end	30 DAT	At crop end	Before treatment	30 DAT	70 DAT
T_1	Metham sodium %(2 ml/ pot)	30.10	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	1.65 (1.48)	738.30	153.33 (11.38)	621.67 (24.62)
T_2	Metham sodium (3 ml/ pot)	160.60	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	356.17	41.00 (6.36)	238.50 (15.36)
T_3	Dazomet (2 g/ pot)	180.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	4.00 (2.10)	196.67	108.33 (10.08)	89.67 (9.32)
T_4	Dazomet (3 g/ pot)	62.17	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	1.00 (1.33)	140.55	99.33 (9.97)	54.33 (7.41)
T_5	Neem cake (40 g/ pot)	80.00	21.33 (4.16)	92.67 (9.39)	1.67 (1.48)	0.67 (1.24)	225.56	346.67 (17.57)	239.50 (15.44)
T_6	Untreated control	35.00	26.33 (5.22)	95.50 (9.78)	7.33 (2.73)	9.33 (3.19)	195.87	443.00 (20.28)	559.83 (22.83)
CD(P=0.05)		(NS)	2.08	2.38	0.99	0.96	(NS)	(NS)	8.01

Values in the parentheses are $\sqrt{n+1}$ transformed values

The data on saprophytic nematodes were also recorded in experimental pots Table 1. Before treatment, the population of saprophytic nematodes was ranged from 140.55 to 738.30 in different doses of methem sodium and dazomet. The gross population of saprophytic nematodes was reduced at 30 DAT in various treatments of metham sodium and dazomet, but increased slightly in neem treated plots *i.e*. from 225.56 (before treatment) to 346.67 (30 DAT). However, at crop maturity, the order of efficacy was dazomet (3 g/ pot) > dazomet (2 g/ pot)> metham sodium (3 ml/ pot) > neem cake (40 g/ pot) and metham sodium (2 ml/ pot) Fig 1. Saprophytic nematodes showed more susceptibility to neem cake than metham sodium (2 ml/ pot). Jahan *et al*. (2007) [12] has reported antibacterial properties of neem against different bacteria. Since saprophytic nematodes feed on bacteria in the soil therefore, reduction in bacterial population in soil can possibly lead to reduction in the population of saprophytic nematodes.

As the persistence of the chemical decreases the soil microflora rejuvenates as a consequence of which there can be possible increase in the population of other saprophytes. In the present studies, well rotten FYM was added to the plots which certainly enrich the soil microflora. Thus increase in population of soil microflora ultimately contributes to higher population of saprophytic nematodes in the experimental plots at crop maturity.

Effect on Microarthropods

Among microarthropods, collembolans and mites were observed in low numbers in experimental plots (Table 2). Before the application of various treatments, the population of microarthropods was ranged from 4.67 to 9.0 individuals/ 500 cc soil. At 30 DAT, the population in different treatments was suppressed and was revived in some of the treatments when data was recorded at the crop end. At the crop end, population was lowest in pots treated with metham sodium (3 ml/ pot) and dazomet (2 g/ pot) treatments in comparison to other treatments. Microarthropod population in different treatments of metham sodium and dazomet was found to decline from 44.38 (dazomet @ 3g/ pot) to 82.00 (metham sodium 2 g/ pot) percent, while reduction with neem cake was recorded to be 49.56 percent. In metham sodium treatment (2 ml/ pot) the population was found to be increased by 4.0 percent at crop end. The average population of microarthropds at crop end was found to be very low. The low population of microarthropods may be because of the sensitivity of the organisms to high soil temperature coupled with low organic matter. The population is reported to be higher in forest soils where organic matter is high and disturbances are very less. Vats and Narula (1990) [19] recorded the population of soil arthropods ranging from 17 to 23,253/ m^2 in cereal fields, and from 509 to 1,39,436/ m^2 in forest stand. Axelsen and Kristensen (2000) [2] observed up to 1,20,000 and 90,000 individuals/ m^2 of collembolans and mites, respectively, from organically grown plots. Culik *et al*. (2002) [7] studied the biodiversity of collembolan in tropical agricultural environments of Brazil and found total collembolan densities to be greater with no tillage versus conventional tillage.

Table 2: Effect of different treatments on microarthropods population

Treatments		Microarthropod population (per 500 cc soil) in the rhizosphere of plants		
		Before treatment	30 DAT	70 DAT
T_1	Metham sodium (2 ml/ pot)	4.67	1.67 (1.58)	9.83 (3.15)
T_2	Metham sodium (3 ml/ pot)	7.33	4.33 (2.29)	2.67 (1.81)
T_3	Dazomet (2 g/ pot)	9.00	6.33 (2.67)	5.50 (2.54)
T_4	Dazomet (3 g/ pot)	5.33	1.33 (1.47)	6.00 (2.55)
T_5	Neem cake (40 g/ pot)	7.67	10.00 (3.31)	7.83 (2.90)
T_6	Untreated control	6.67	11.00 (3.45)	13.50 (3.73)
CD (P=0.05)		(NS)	0.51	(NS)

Values in the parentheses are $\sqrt{n+1}$ transformed values

Effect on Fungi

Only *Fusarium* species (Link) was recorded in the experimental pots kept in polyhouse Table 3. Before treatment, the population of *Fusarium* species was ranged from 16.00 x 10^5 to 27.41 x 10^5 in different treatments of metham sodium and dazomet. After 30 DAT, population of *Fusarium* sp was found to decline variably in all the treatments except neem treatment compared to pre-treatment population. Population of the fungus in different treatments of metham sodium and dazomet was found to be between 4.33 x 10^5 and 19.67 x 10^5 whereas, in neem cake, population was increased from 17.33 x 10^5 to 22.67 x 105 cfu/ g soil. At the crop end, population of the fungus increased in all the treatments where slight decline was observed in dazomet (2 g/ pot) and neem treatment. Percent reduction in population of this fungi over control was maximum in dazomet treatment (3 g/ pot) Fig 1. Fungi population was revived in almost all treatments which indicated its tolerance towards different treatments. This tolerance of *Fusarium* sp can be attributed to two reasons. Firstly, *Fusarium* forms chlamydospores which are resistant to unfavorable situations and have the ability to perpetuate in the soil (Bloomberg, 1976; Nelson *et al.*, 1983) [5, 16]. Secondly, most of the species belonging to genus *Fusarium* are saprophytic and the residual population has the potential to multiply at faster rate when the effect of chemical is over.

Table 3: Effect of different treatments on *Fusarium* sp

Treatments		*Fusarium* sp (cfu/ g soil)		
		Before treatment	**30 DAT**	**70 DAT**
T_1	Metham sodium (2ml/ pot)	21.57 x 10^5	12.67 x 10^5 (13.84)	51.33 x 10^5 (15.29)
T_2	Metham sodium (3ml/ pot)	16.00 x 10^5	5.67 x 10^5 (13.23)	22.00 x 10^5 (14.44)
T_3	Dazomet (2 g/ pot)	25.50 x 10^5	19.67 x 10^5 (9.84)	18.67 x 10^5 (13.93)
T_4	Dazomet (3 g/ pot)	27.41 x 10^5	4.33 x 10^5 (12.89)	12.67 x 10^5 (9.43)
T_5	Neem cake (40 g/ pot)	17.33 x 10^5	22.67 x 10^5 (9.93)	8.50 x 10^5 (13.36)
T_6	Untreated control	12.00 x 10^5	34.33 x 10^5 (14.94)	39.17 x 10^5 (15.15)
CD (P=0.05)		(NS)	(NS)	(NS)

Values in the parentheses are $\sqrt{n+1}$ transformed values

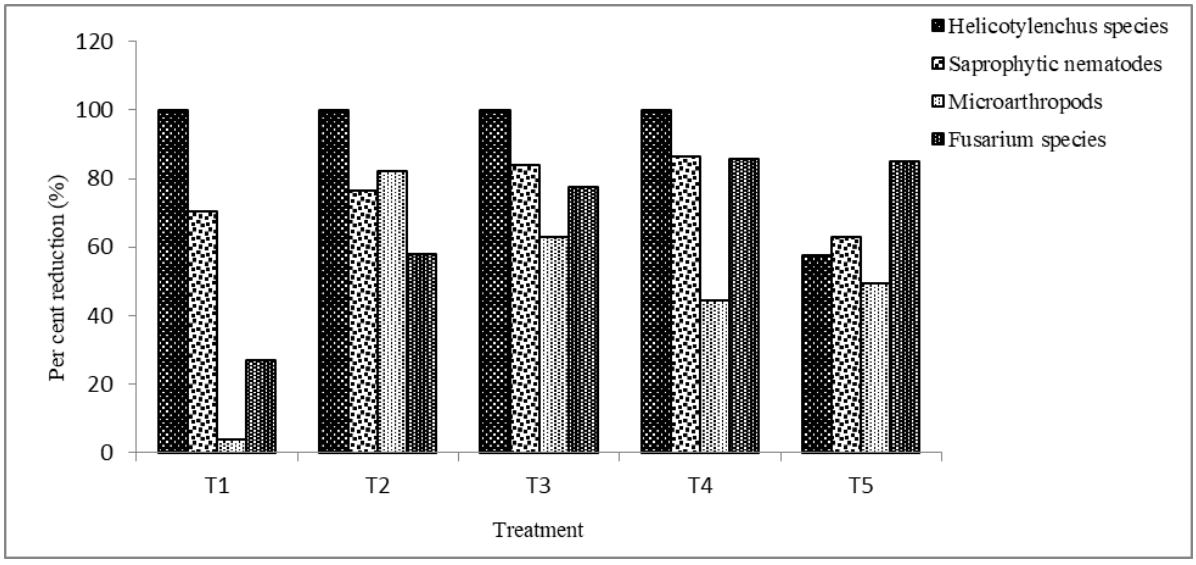

Fig 1: Percent reduction in soil biota at crop end

Conclusion

From the results as obtained above, it is inferred that metham sodium and dazomet at the evaluated doses are toxic to soil biota *i.e.* nematodes (plant parasitic and saprophytic), microarthropods and fungi variably. There is considerable reduction in the population during the initial weeks after the application of these chemicals and with time, population in most of the cases was found increased when data recorded at crop end. Fungi population was revived in almost all treatments which indicated its tolerance towards different treatments. It is likely that with time, biota may revive to normal levels which are at par with the pre count of respective organisms.

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