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DIMETHYL DISULFIDE- A POTENTIAL BIOPESTICIDE AGAINST ROOT-KNOT NEMATODE OF TOMATO (*Lycopersicon esculentum* L.)

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Abstract

Dimethyl disulfide (DMDS), the natural biopesticide extracted from *Allium* spp., was evaluated against root-knot nematode (*Meloidogyne incognita*) of tomato (*Lycopersicon esculentum* L.) in greenhouse pot culture. All concentrations of DMDS viz. 30 ml, 60 ml, and 80 ml and Aldicarb @ 2g per square meter of soil were effective against root-knot disease under both wet and dry conditions of soil. Nematode incidence was reduced drastically by higher dose of DMDS and Aldicarb @ 2g but did not accelerate vegetative growth of tomato plant especially when tomato seedlings were transplanted immediately after soil treatment. Low concentration of DMDS (30 ml per square meter of soil) was found appropriate for controlling root-knot nematode of tomato, accelerating saprophytic nematode population in soil and also enhancing vegetative growth of tomato plant under dry condition of soil.

Keywords: Dimethyl disulfide, Aldicarb, *Meloidogyne incognita*, tomato.

Introduction

Tomato (*Lycopersicon esculentum* L.) is the most important consumable and popular winter as well as summer vegetables throughout the world (Chowdhury, 1979). It suffers from several diseases caused by fungi, bacteria, nematodes, and viruses, which play major role in reducing yield of tomato. Among them, root-knot disease caused by *Meloidogyne incognita* is highly damaging and yield reducing factor for vegetable production including tomato all over the world (Mian, 1986; Netscher and Sikora, 1990; Kerry and Bourne, 2002 ; EPPO, 1994). The economic damage inflicted by these nematodes is enormous that estimates crop losses of 13% world wide while 24-38% for the sub-tropics and tropics (Sasser, 1979; Netscher and Sikora, 1990). Over the years cultural, chemical and biological methods have been developed to control the root knot nematodes.

Crop rotation is less effective against root-knot disease due to its wide host range (Brodie, 1993). Commercial cultivars of the crop are not sustainable to the resistance against root-knot nematode (Cook and Evans, 1987). This nematode has the ability to develop new physiological races that can overcome the

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resistance of the crop (Netscher, 1976). Root knot nematode control has traditionally been based on the use of chemical pesticides applied to soil or plants (Duponnis *et al.*, 2001) that posed significant effect on health hazard and environmental pollution. The development of biological control agents is considered as one of the alternatives in nematode control (Sikora, 1992). Limitation of mass production that associated with high cost has restricted large-scale application of biocontrol agent against nematodes (Gowen and Ahmed, 1990). Organic soil amendments can control nematodes by reducing nematode infection or by increasing microbial antagonistic activity (Watson, 1945; Linford *et al.*, 1938; Sitaramaiah and Singh, 1978). However, the use of various organic amendments in the soil has been greatly emphasized as an alternative easy, cheap and satisfactory method of nematode control (Hussain and Khan, 1988).

Different extracts and decomposition products of several indigenous medicinal plants and their parts such as fruits, seeds, leaves, stem and roots are known to have anthelmintic properties. Those plant materials have been reported to be toxic to many plant parasitic nematodes including root knot nematodes (Desai *et al.*, 1973; Hussain and Masood, 1975; Goswami and Vijayalakshmi, 1983 and 1986; Jain and Hasan, 1984). Dimethyl Disulfide (DMDS) is a natural biopesticide of *Allium* spp. Numerous wild *Allium* species are abundant natural source of DMDS. The aim of this study was to examine the effectiveness of DMDS against root knot nematode of tomato.

Materials and Method

Three experiments, two in wet soil and one in dry soil conditions, were carried out in the greenhouse of the Katholieke Universiteit Leuven, Belgium to evaluate the efficacy of Dimethyl Disulfide (DMDS) against root knot nematode (*Meloidogyne incognita*) of tomato. Root knot nematode culturing pot soil and infected tomato roots were used as source of inoculum. The tomato seedlings were raised by planting the tomato seeds cv. TREND in the tray with peat-soil medium. Irrigation was done once a day to grow the tomato seedlings. Three treatments with 3 doses of DMDS and one with Aldicarb were evaluated. The nematicide Aldicarb was used as standard for comparing the effectiveness of the test biopesticide DMDS against root-knot nematode.

Dry soil experiment

The nematode culturing pot soil with infected tomato roots was taken in a tray and kept in the greenhouse at 23±2°C for drying. After one week the dry soil was mixed with the field soil at the rate of 50% of each. The mixed soil was treated with DMDS at the concentration of 3, 6, and 8 ml per 10 liter of soils (30 ml, 60

ml and 80 ml per square meter of soil). Ten liter of soil was taken in each container and the required amount of DMDS with 20 ml of water was added to the soil. The DMDS was properly mixed with the soil by shaking the containers and kept closed for one week. After one week the container was opened and the soil was poured into the plastic pots. The nematicide Aldicarb (2 g/10 liter of soil) was used with mixed soil at the time of seedling transplanting. Pots for control treatment contained only mixed soil. Twenty five days old tomato (cv. TREND) seedlings were transplanted into the pots and each pot received one seedling. The experiment was laid out in a CRD design with 8 replications. The pots were placed on a greenhouse bench at 23±2° C. The tomato seedlings were allowed to grow for nine weeks with necessary irrigation and intercultural operation. After nine weeks all the tomato plants were uprooted carefully from the pots minimizing the loss of the roots. The roots were washed with tap water and data on fresh shoot height, shoot weight; root length and root weight were recorded. The gall indexing was done on the basis of 0-10 scoring scale (Zeck, 1971), where 0 represented free from gall and 10 severely galled root system. The nematode population per 33 g (then converted to 100 ml) soils were recorded by extraction of nematodes from soil of each pot of each treatment.

Wet soil experiment

Wet soil was prepared for setting up experiment to find out the effect of DMDS on root knot nematode and growth of tomato plant. The nematode culturing pots with tomato plant were taken and the plants were removed. The pot soil with nematode infected roots was mixed with field soil at the rate of 50% of each. The mixed soil was treated with DMDS at the concentration of 3, 6, and 8 ml per liter of soil as described previously. Two different experiments were conducted in wet soil condition. In the first experiment, the soil was poured into the plastic pots immediately after treatment with DMDS. The nematicide Aldicarb (2g/10 liter of soil) was mixed with soil at the time of seedling transplanting. Five pots were poured with mixed soil as a control treatment. In the second experiment, the soil was poured into the plastic pots five days after treatment with DMDS. The nematicide Aldicarb was used (2 g/10 liter of soil) at the time of seedling transplanting. Pots under control treatment contained only mixed soil. In both the cases experimental design, seedling transplanting, intercultural operation, data collection etc. were done as before. The number of weeds per pot was also recorded after 25 days of seedling transplanting.

Estimation of nematode population

The nematodes were isolated from dry and wet soil using Baermann Tray technique to determine the initial nematode density, the number of nematodes at

the time of seedling transplanting and nematode density in soil at the end of growing of tomato plant. Each pot soil was transferred into a tray and mixed thoroughly and 33 g was taken for extraction of nematodes. This method consists in a plastic tray upon which a plastic mesh supported a piece of tissue paper. The soil was spread thinly on the tissue and the water was added to plastic tray so that the materials were just submerged in the tissue. The trays were kept for 24 hours to allow the nematodes for coming down into the water. After 24 hours the plastic mesh with soil was removed gently and the nematode suspension was transferred to a beaker. The beaker was kept undisturbed for one hour to settle down the nematodes. After one hour the nematode suspension in the beaker was made 100 ml to pour out supernatant. This 100 ml suspension was used for counting the nematode population.

All statistical analyses were performed using SPSS Statistical Package. Analysis of variance (ANOVA), Duncan's multiple range tests was used for comparison of means of data parameters.

Results and Discussion

Wet soil experiment

Effect of DMDS and Aldicarb on root-knot nematode of tomato was tested in wet soil condition when tomato seedlings were transplanted immediately after soil treatment. All the concentrations of DMDS and Aldicarb significantly reduced the gall index produced by root-knot nematode in tomato under wet soil condition. The highest gall index value 6.6 was observed in control treatment while the lowest gall index value 1.0 was recorded from the highest concentration of DMDS (80 ml per square meter of soil) treated pots (Table 1). Maximum reduction in gall index value over control was observed in DMDS @ 80 ml per square meter of soil followed by DMDS @ 60 ml per square meter of soil and DMDS @30 ml per square meter of soil and Aldicarb. The similar results were also observed in reducing plant parasitic nematode populations in soil where the highest number 1614 per 100 ml soil of plant parasitic nematode was recorded in control (Table 1). The initial populations of plant parasitic nematodes were reduced after the soil treated with DMDS and this reduction trend was continued up to the harvest of tomato plant i.e. after 55 days growing of tomato plants. The initial population of saprophytic nematode was also reduced after treatment with DMDS but during growing stage the population of saprophytic nematode was increased while in case of Aldicarb the population of saprophytic nematode was drastically reduced at the time of crop harvest (Table 1).

Table 1. Effect of DMDS and Aldicarb on root knot nematode under wet soil condition when tomato seedlings were transplanted immediately after soil treatment.

Treatment (per square meter soil)	Gall index (0-10 scale)	Gall index reduction over control (0-10 scale)	Plant parasitic nematode per 100 ml soil		Saprophytic nematode per 100 ml soil	
			At seedling transplanting	At harvest	At seedling transplanting	At harvest
DMDS 30 ml	1.2 b	5.4	540	180 b	750	1188 b
DMDS 60 ml	1.2 b	5.4	360	150 b	510	882 bc
DMDS 80 ml	1.0 b	5.6	300	114 b	390	618 cd
Aldicarb 2g	1.2 b	5.4	975*	150 b	1500*	348 d
Control	6.6 a	-	975*	1614 a	1500*	2004 a

Means in the same column followed by a common letter do not differ significantly at P≤0.05 (DMRT, n= 5) * Initial population in the soil

Efficacy of DMDS and Aldicarb on the incidence of root-knot disease was evaluated in tomato under wet soil condition by transplanting tomato seedlings after 5 days of soil treatment. Pre-sowing soil treatment with DMDS at the rate of 30 ml, 60 ml, and 80 ml per square meter of soil and nematicide Aldicarb at 2g remarkably reduced the root-knot incidence in tomato (Table 2). The highest gall index value 6.0 was observed in the control treatment and the lowest value 1.0 was recorded in DMDS at the rate of 80 ml per square meter of soil treated pots. The highest reduction of root knot disease incidence over control was observed when DMDS was applied at the rate of 80 ml per square meter of soil. Due to soil treatment with DMDS the population of plant parasitic nematode was reduced in all treatments over control (Table 2). The highest number of plant parasitic nematode was recorded in control treatment and lower number was in the DMDS and Aldicarb treated soil. In case of saprophytic nematode population, the highest number was observed in DMDS (30 ml) treated pots and it was 6270 per 100 ml soil in the control treatment (Table 2). Lower numbers of saprophytic nematodes were recorded with higher concentration of DMDS and Aldicarb.

In case of weed management, all the concentrations of DMDS significantly reduced the incidence of weeds in the pot soil of tomato (Table 2). The highest number of weeds per pot was observed in control treatment followed by Aldicarb. The lower number of weeds was recorded from the pot soil treated with DMDS over Aldicarb treatment.

Table 2. Effect of DMDS and nematicide in wet soil on root knot disease incidence, nematode development and weeds management in tomato when seedlings were transplanted five days after soil treatment.

Treatment (per square meter soil)	Gall index (0-10 scale)	Gall index reduction over control (0-10 scale)	Nematode per 100 ml soil after 45 days of plant age		Number of Weeds per pot
			Plant parasitic nematode	Saprophytic nematode	
DMDS 30 ml	1.6 b	4.4	552 b	7152 a	3.2 c
DMDS 60 ml	1.4 b	4.6	180 b	3654 b	2.2 c
DMDS 80 ml	1.0 b	5.0	210 b	4272 b	0.6 c
Aldicarb 2g	1.2 b	4.8	348 b	2916 b	12.4 b
Control	6.0 a	-	1434 a	6270 a	15.6 a

Means in the same column followed by a common letter do not differ significantly at $P \leq 0.05$ (DMRT, n= 5)

Higher concentrations of DMDS gave reduced growth of tomato plant while lower concentration of DMDS accelerated the vegetative growth of tomato plant (Figure 1). Maximum shoot height and shoot weight were recorded with lower dose of DMDS. Both shoot height and weight were lower in higher dose of DMDS. Similarly, root length and weight were also enhanced by the lower dose of DMDS (Fig. 1).

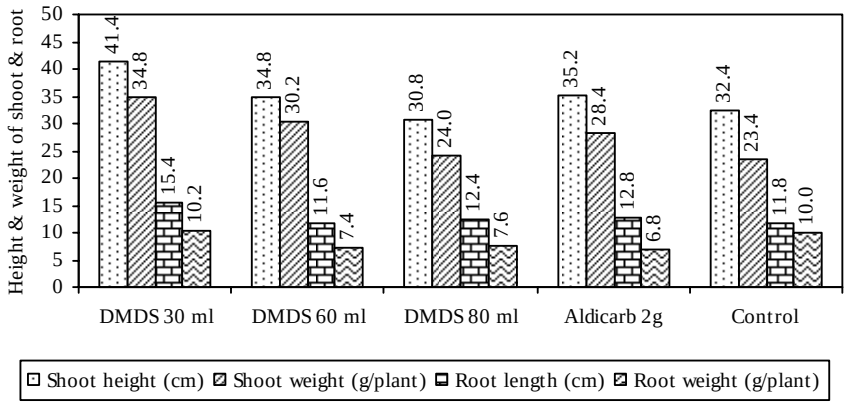

Fig. 1. Effectiveness of DMDS and Aldicarb on shoot and root growth of tomato plant when seedlings were transplanted immediately after soil treatment under wet condition.

Efficacy of DMDS and Aldicarb were evaluated on the growth of tomato plant when seedlings were transplanted 5 days after soil treatment under wet soil condition. Plant growth such as shoot height, shoot weight and root length, root weight were accelerated due to soil treatment with DMDS over control (Fig. 2).

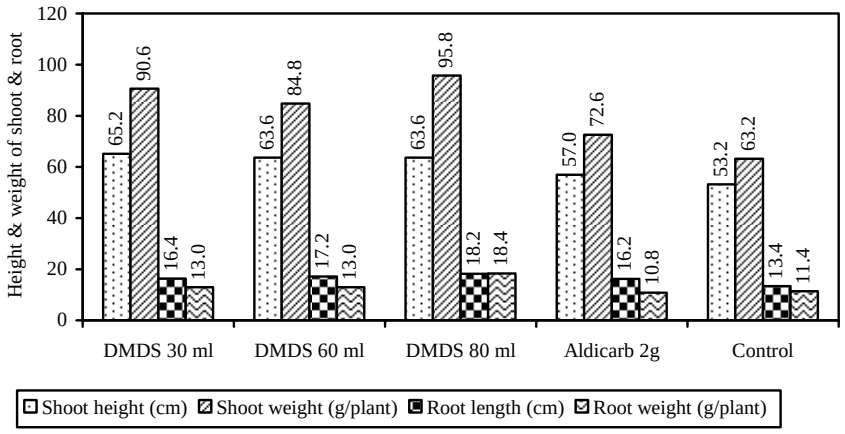

Fig 2. Effect of DMDS and Aldicarb on shoot and root growth of tomato plant when seedlings were transplanted five days after soil treatment under wet condition.

Dry soil experiment

Role of DMDS and Aldicarb on root knot of tomato was investigated by seedlings transplanting immediately after soil treatment under dry condition. Application of DMDS at 30 ml, 60 ml, and 80 ml per square meter of soil and nematicide Aldicarb remarkably reduced the galling incidence of root-knot in tomato (Table 3). The highest gall index value 5.2 was observed in control pot while the lowest gall index value 1.2 was recorded from DMDS treated plots. All the concentration of DMDS and Aldicarb gave statistically similar results in reducing root knot disease of tomato.

Soil treated with DMDS and Aldicarb significantly reduced the development of plant parasitic nematode in the soil (Table 3). The highest number of plant parasitic nematode was recorded in control treatment and lower numbers were in DMDS and Aldicarb treated soil. The initial population of plant parasitic nematode was decreased after DMDS treatment and the decreasing trend was continued until crop harvest. In case of saprophytic nematodes, the initial population was also decreased after DMDS treatment but this number increased rapidly during growing of plant. Besides, the initial population of saprophytic nematode was remarkably reduced during growing of tomato plant in case of Aldicarb treatment (Table 3).

Table 3. Effect of DMDS and Aldicarb on root knot nematode under dry soil condition when tomato seedlings were transplanted immediately after soil treatment.

Treatment (per square meter soil)	Gall index (0-10 scale)	Gall index reduced over control (0-10 scale)	Plant parasitic nematode per 100 ml soil		Saprophytic nematode per 100 ml soil	
			At seedling transplanting	At harvest	At seedling transplanting	At harvest
DMDS 30 ml	1.2 b	4.0	450	270 b	540	1302 ab
DMDS 60 ml	1.4 b	3.8	330	234 b	390	1152 ab
DMDS 80 ml	1.2 b	4.0	210	180 b	270	930 bc
Aldicarb 2g	1.8 b	3.4	720*	246 b	810*	624 c
Control	5.2 a	-	720*	1152 a	810*	1494 a

Means in the same column followed by a common letter do not differ significantly at $P \leq 0.05$ (DMRT, n= 5) * Indicate the initial population into the soil

All the concentrations of DMDS and Aldicarb increased the growth of tomato plant compared to the control (Fig. 3). The highest shoot height and weight were recorded when the soil was treated with DMDS at 30 ml and 60 ml per meter square of soil. The lowest shoot highest and weight was in the control treatment. Root length was maximum in case of medium dose of DMDS (60 ml). But in case of root weight there was no significant difference among the treatments.

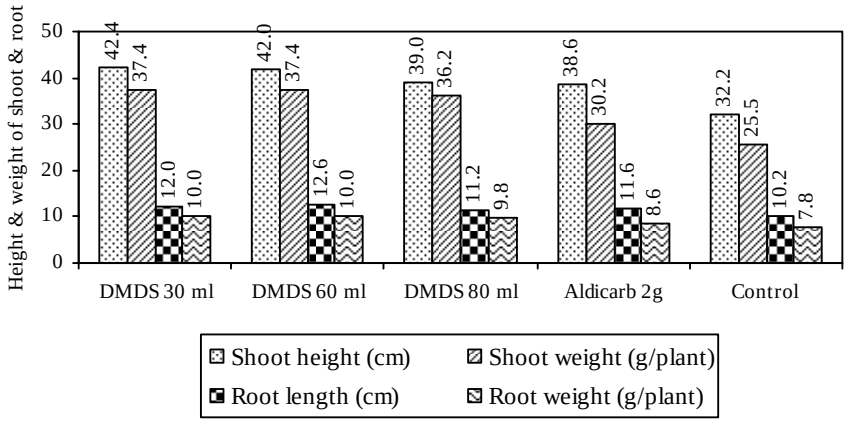

Fig. 3. Effectiveness of DMDS and Aldicarb on shoot and root growth of tomato when seedlings were transplanted immediately after soil treatment under dry soil condition.

Higher concentration of DMDS (80 ml and 60 ml per square meter of soil) showed toxicity on the growth of tomato plant, which reduced the growth of tomato plant especially when the tomato seedlings were transplanted immediately after soil treatment whereas low concentration of DMDS at 30 ml per square meter of soil has no toxicity on the growth of tomato plant. This toxicity of DMDS differed between two soil conditions; in wet soil the toxicity was higher and longer than that in the dry soil. This could be due to higher absorption of DMDS by wet soil than dry soil. Stephan *et al.*(1989) supported these results who found that pre-plant application of Furfural at 4000 ppm increased plant growth, while the same concentration applied post planting caused plant toxicity and significantly decreased the dry shoot and root weight of tomato compared to control. Application of DMDS, especially lower doses significantly increased tomato plant growth, saprophytic nematode population and reduced root-knot disease incidence as well as plant parasitic nematode development in the soil. Nath and Mukherjee (2000) supported these results who found that the aqueous extracts of tubers of medicinal yam, *Dioscorea floribunda* inhibited egg hatching of *Meloidogyne incognita* at 0.5 to 20% concentrations, respectively both *in vitro* and *in vivo*. They observed that the concentrations of the aqueous extracts at 20, 10, and 5% killed 100% juveniles within 2.5, 3, and 4 hr, respectively, and improved the plant growth of tomato compared to control. Stephan *et al.*(1989) however found that 3 alkaloids of *Haplophyllum tuberculatum* was effective against *M. javanica* and killed greater number of eggs and larvae than controls and the higher concentrations being more effective. Al-Saba *et al.* (2001) reported that the aqueous plant extract of *Datura stramonium* was more effective on the second stage juveniles (larvae) on *Meloidogyne javanica* in tomato plants and mortality of second stage juveniles increased with increasing the extract concentration. Many other investigators also achieved appreciable control of root knot nematode and increased plant growth by treating nematode infested soil with aqueous plant and seed extract (Gupta and Sharma, 1993; Nath and Mukherjee, 2000) and chemical extracts of plant and its parts such as leaves, stem and buds (Kumari *et al.*, 1986; Al-Obaedi *et al.*, 1987).

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