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Control of the Root-knot Nematode, *Meloidogyne incognita*, on Okra (*Abelmoschus esculentum*) by a Neem Tree (*Azadirachta indica*) Product and Urea

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멀구슬나무(Neem Tree) 부산물과 요소(Urea)를 이용한 오크라의 뿌리혹선충 억제

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ABSTRACT

A neem based product, Suneem-G (granules) alone as well as in combination with urea was tested against root-knot nematode, *Meloidogyne incognita,* on okra (*Abelmoschus esculentum*) in clay pots of 20-cm-diameter, containing 2 kg sterilized soil, inoculated with 2,000 freshly hatched second stage juveniles (J_2) of *M. incognita.* Application of different dosages of Suneem-G with or without urea significantly reduced nematode population and incidence of galling on okra roots and increased vegetative plant growth compared with the untreated control. Suneem-G with urea was the most effective and its effect increased with the increase of dosage. Suneem-G 6 g+ urea 25 g per pot gave maximum plant growth and root gall reduction, as plants shoot and root lengths were increased by 84 and 58% and root galling was reduced by 58%, respectively. The lowest dosage of Suneem-G alone, 2 g per pot increased shoot and root lengths of plants by 25.7 and 17%, respectively, and reduced root galling by 24%. The lowest effect was recorded in the pots treated with 25 g urea alone; shoot and root lengths were increased and root galling was decreased by 14% each.

Key words : Control, *Meloidogyne incognita*, neem product, okra, Suneem-G, urea

INTRODUCTION

The root-knot nematode, *Meloidogyne incognita*, is one of the most common and important plant parasites in tropical and subtropical regions of the world (Sasser *et al.* 1983). *M. incognita* and other root-knot nematodes cause root galls in many crops impeding normal uptake of water and nutrients. Root-knot nematodes typically have wide host ranges, causing problems in many annual and perennial crops, including okra among the seriously affected. According to Jain *et al.* (1994), *M. incongnita* is responsible for yield losses of up to 30% under field conditions. Control of root-knot nematodes relies mainly on chemical pesticides, but recently several nematicides have been withdrawn from market because of health and environmental problems associated with their production and use (Thomason 1987). As a result of this, and increasing public concern over the use of pesticides in food production, there has been increased interest in the development of other effective, and environment friendly methods of nematode control.

Nowadays plant byproducts are receiving greater attention as prophylactic and therapeutic applications against pests and pathogens. The neem tree (*Azadirachta indica* A. Juss.) is considered to be one of the most promising trees of the 21st century, since it possesses great potential in the fields of pest management, environment protection and medicine. Neem is native to Southeast Asia and growing in many countries throughout the world (Schmutterer 1990, Ascher 1993). The neem tree can tolerate heat up to 50°C and poor, shallow, and even saline soils (Ascher 1993), but it is sensitive to injury at

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temperature around 0°C, which limit its distribution in temperate regions of the world (Jacobson, 1989; Ascher, 1993). Mishra and Prasad (1973) reported that neem cake is the most toxic to *M. incognita* juveniles on the basis of LC_{50} value of aqueous extract of neem, mahuwa, karanj and mustard cakes. Neem products including leaf, kernel, seed powder, seed extracts, oil cakes and oil have been reported to control several agricultural pests including many plant-parasitic nematodes (Lvibijaro 1983, Schmutterer 1990, Akhtar and Mahmood 1994).

In the past, many workers had reported that applications of nitrogenous materials (ammonium-nitrogen) as nematode controlling strategy (Heald and Burton 1968, Rodriguez-Kabana 1986, Oka and Pivonia 2001). Nematicidal efficacy of addition of urea to soil, which readily converted to ammonia by urease present in soil, was dosage dependent (Ridriguez-Kabana and King 1980, Huebner *et al.* 1983). Ridriguez-Kabana (1986) pointed out that nitrogen fertilizers releasing ammonium N in the soil are very effective in suppressing nematode populations and recommended that the rate required for effective suppression of nematode population is generally in excess of 150 kg N y ha^{-1}. Present study was aimed to evaluate the efficacy of a neem product i.e Suneem-G alone or in combination with urea for the control of *M. incognita* on okra.

MATERIALS AND METHODS

Pot culture study was conducted under glasshouse conditions in clay pots of 20-cm-diameter, filled with 2 kg sterilized soil mixture of clay, sand and compost in the ratio of 2 : 1 : 1 to study the effect of a neem product, Suneem-G (Sunida Exports Ltd., Mumbai, India) against *M. incognita.* Soils were treated with Suneem-G at 2, 4, 6 g alone or in combination with 25 g urea per pot. Neem product was diluted with 100 ml water and thoroughly mixed with potted soil. After 24 hrs of treatments, 3 seeds of okra cv. "Pusa Swami" were sown in all pots and were thinned to one per pot after germination. Control treatments with 25 g urea per pot and Carbofuran 3G (a.i.) at 1.2 g per pot and untreated pots were maintained for comparison. Each pot was inoculated with freshly hatched second stage juveniles (J_2) of *M. incognita* at the rate of one nematode per gram of soil after 15 days of sowing.

Pure culture of *M. incognita* was maintained on tomato plants (*Lycopersicon esculentum* Mill) cv. Pusa Ruby in a green-house. Eggs of *M. incognita* were extracted from nematode infected tomato roots with sodium hypochlorite solution (Hussey and Barker 1973). J_2 of *M. incognita* were hatched from eggs spread on a 30 μm sieve, collected daily, and stored at 10°C. J_2 aged less than 3 days were used in pot experiments. They were counted in 2 ml aliquots of water suspension to prepare inocula. J_2 of *M. incognita* were added to soil around the roots in each pot by removing topsoil to a depth of 2-3 cm and pipetting nematode suspension on the exposed surface. Each treatment was replicated five times and pots were arranged in complete randomized blocks design on benches in the greenhouse maintained at 23-26°C. The experiments were terminated 60 days after inoculation. Plants were carefully uprooted from pots and the roots were washed gently with tap water to free them from adhering soil particles. Root galls and eggmasses per plant root system were counted and index of 0-5 was assigned (0 = no galls, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = > 100 galls per root system) (Taylor and Sasser 1978). The numbers of developing juvenile stages and mature females of *M. incognita* were also recorded in roots after fixation and staining of the roots with 0.1% cotton blue-lactophenol and clearing in lactophenol (Franklin and Goody 1949). Soil populations of *M. incognita* J_2 were estimated by extracting nematodes from 200 g soil samples from each pot by centrifugal-flotation techniques (Caveness and Jensen 1955). Plant parameters, shoot and root lengths, and fresh and dry weights of shoots and roots were recorded. Plants were dried at 70°C in an oven for 12 h before recording the dry weight of shoots and roots. All data obtained were subjected to statistical analysis.

RESULTS AND DISCUSSION

The data presented (Table 1) revealed that all the plant growth characteristics were significantly enhanced by all treatments compared with the untreated plants. However, among the various treatments, Suneem-G (granule) with urea was the most effective in increasing plant growth and reducing the development of root galls, eggmass formation and nematode population (Table 1, 2). Galling on okra roots was significantly reduced by the application of different treatments compared to the untreated control. Final population densities of *M. incognita* were significantly decreased in all treatments. A significant difference in mean length and weight of shoots and roots between treated and untreated (control) plants was also observed in all treatments. Similarly, fewer root galls and eggmasses were found on roots of treated plants than those inoculated with *M. incognita* alone (control). The developing

Table 1. Effect of different dosages of Suneem-G separately as well as in combination with urea on growth parameters of okra plants inoculated with 2,000 J_2 of *Meloidogyne incognita*.

Treatments	Dosages/pot (gram)	Shoot length (cm)	Root length (cm)	Shoot weight (gram)		Root weight (gram)	
				Fresh	Dry	Fresh	Dry
Seneem-G	2	32.2 g	22.8 fg	20.8 f	9.5 c	8.6 f	2.7 fe
Suneem-G	4	36.3 f	23.3 f	22.5 e	9.9 d	9.2 e	3.0 e
Suneem-G	6	42.1 d	26.2 d	27.5 c	10.8 c	11.8 c	3.8 d
Suneem-G+Urea	2+25	39.8 e	25.1 e	25.4 d	11.0 c	11.1 d	3.6 d
Suneem-G+Urea	4+25	44.0 c	28.2 c	28.2 c	11.9 b	13.4 b	4.1 c
Suneem-G+Urea	6+25	47.3 b	30.8 a	30.4 b	12.3 b	14.3 a	5.1 b
Urea	25	29.1 h	22.2 g	20.6 f	9.4 de	8.1 f	2.5 f
Carbofuran	1.2	50.1 a	29.2 b	32.1 a	14.5 a	14.6 a	6.3 a
Control	–	25.6 i	19.5 h	18.5 g	8.8 e	6.3 g	2.1 g

Means with the different letters in a column are significantly different from each other at P=0.05 by DMRT.

Table 2. Effect of different dosages of Suneem-G separately as well as in combination with urea on development of root galls, eggmasses and nematode population on okra roots inoculated with 2,000 J_2 of *Meloidogyne incognita*

Treaments	Dosages/pot (gram)	Females/ g root	J_2/200 g soil	Root-knot index	Eggmass index
Suneem-G	2	24.4 c	234 c	3.8 c	3.6 c
Suneem-G	4	19.4 d	178 d	3.6 c	3.2 cd
Suneem-G	6	14.5 e	98 e	3.0 d	2.8 de
Suneem-G +Urea	2+25	16.8 de	170 d	3.6 c	3.0 de
Suneem-G +Urea	4+25	11.6 f	92 ef	2.6 de	2.6 e
Suneem-G +Urea	6+25	8.6 g	61 ef	2.0 f	2.0 f
Urea	25	27.5 b	295 b	4.4 b	4.4 b
Carbofuran	1.2	6.6 g	51 f	2.2 ef	2.0 f
Control	–	37.1 a	358 a	5.0 a	5.0 a

Means with the different letters in a column are significantly different from each other at P=0.05 by DMRT.

stages of juveniles and females of *M. incognita* in okra roots were significantly different in pots receiving different dosages of Suneem-G. With the increase in level of dosages, there was a corresponding decrease in development of root galls and production of eggmasses. Suneem-G 6 g+urea 25 g gave maximum plant growth and root gall reduction. This was significantly superior next to the standard control by Carbofuran. Thus, results obtained from this experiment clearly indicated that Suneem-G suppressed *M. incognita* and enhanced plant growth.

Reduction in gall development and in nematode populations due to Suneem-G treatments may be attributed to unfavorable conditions causing poor penetration and retardation in biological activities of *M. incognita* such as mobility, feeding and reproduction. Several chemicals commonly occurring in neem e.g. nimbdin, thionemone, azadirachtin, nimbin, nimbidic acid, kaemferol and quercetin, have been attributed for the antagonistic nature of neem (Alam and Jairajpuri 1990). Khan *et al.* (1975) reported high nematode mortality and inhibition of juvenile hatch by nimbidin and thionemone. Similar results were also obtained by Siddiqui (1986) with azadirachtin, nimbidic acid, nimbin, kaemferol, and quercetin. Azadirachtin, nimbidic acid and nimbin, when used as root-dip treatment, significantly inhibited the penetration of root-knot juveniles and subsequent root galling on tomato and eggplant (Siddiqui 1986). Azadirachtin, a neem triterpene, acts as delaying the rapid transformation of ammonium nitrogen into nitrate nitrogen. This ensures slow and continuous availability of nitrogen during the plant growth. Rodriguez-Kabana (1986) concluded that nitrogenous fertilizers that release ammonium N in the soil are the most effective in suppressing nematode population. Urea is a good nematicide when applied at level in excess of 300 mg N/kg soil (Huebner *et al.* 1983, Rodriguez-Kabana and King 1980).

The efficacies of treatments were increased with the increased dosages that may be due to differences in concentration of neem products in soil. Suneem-G was non-phytotoxic in all the dosages used and also did not show any adverse effect on seed germination. The tested neem products may be used as biopesticides to control the root-knot nematodes. Application of natural inhibitors, such as Suneem-G, provides an economical option for controlling plant parasitic nematodes without disturbing agroecosystems. However, further researches on this neem product under field conditions are needed.

적 요

고구마뿌리혹선충(*Meloidogyne incognita*)에 대하여 멀구슬나무(*Azadirachta indica*) 부산물인 Suneem-G와 요소의

억제효과를 연구하였다. 폿트 당 살균토양을 2 kg씩 넣고 *M. incognita* 2령기 유충 2,000마리를 접종한 후 오크라를 심고 Suneem-G를 처리하였다. Suneem-G의 처리량에 따라 유충 밀도 및 혹 형성은 유의성 있게 차이가 있었고, Suneem-G와 요소를 동시에 처리하였을 때 그 효과는 상승하였으며, 선충 밀도가 낮을수록 오크라 식물체의 생장이 좋았다. 요소와 Suneem-G를 함께 처리한 조합에서 가장 높은 효과를 보였으며, 처리량의 증가에 따라 억제 효과도 증가하였다. 폿트 당 Suneem-G 6 g과 요소 25 g 조합의 처리에서 최대의 식물생육과 뿌리혹의 최대감소를 보였는데, 식물체 엽육과 뿌리의 길이가 84%와 58%나 높았으며, 뿌리혹은 58%나 감소하였다. Suneem-G의 최저 처리인 폿트 당 2 g 처리에서 식물체의 엽육과 뿌리의 길이는 25.7과 17%까지 증가하였으며, 뿌리혹은 24% 감소하였다. 그러나, 요소 25 g 처리에서 가장 낮은 효과를 나타내었으며, 식물의 엽육과 뿌리의 길이는 증가하였으며 뿌리혹은 14% 감소하였다.

검색어 : 고구마뿌리혹선충, 멀구슬나무 부산물, 오크라, Suneem-G, 요소

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