Antinematicidal Efficacy Of Root Exudates Of Some Crotalaria Species On Meloidogyne Incognita (Root-Knot Nematode) (Kofoid And White) Chitwood Isolated From Infected Lycopersicum Esculentum L.(Tomato) Plant

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ABSTRACT: The antinematicidal efficacies of exudates of four common weeds: Crotalaria breviflora, Crotalaria juncea, Crotalaria retusa, and Crotalaria spectabilis were carried out against Meloidogyne incognita. The young actively growing seedling of the common weeds were uprooted and taken to the laboratory for analyses. The root exudates of test plants were prepared by growing the young actively growing seedlings in test tubes wrapped with black carbon paper for five days under lighted florescent bulbs. Root exudates of Crotalaria breviflora, Crotalaria juncea, Crotalaria retusa and Crotalaria spectabilis exhibited nematicidal properties against the Meloidogyne incognita. The effects varied with concentrations of the exudates ($P<0.05$) using analysis of variance (ANOVA). The effects also differed among test plants with Crotalaria retusa topping in terms of reduction in nematode population. This was followed by C.breviflora, C.juncea and C.spectabilis respectively. The results thus confirmed that all the test plants are potentially viable trap weeds, and can be used for the control of Meloidogyne incognita and should be employed as such.

Keywords: Antinematicidal efficacy, exudates, kofoid and white, tomato, root-knot

1. INTRODUCTION

Root-knot disease of tomato caused by Meloidogyne incognita is common throughout Nigeria. It is destructive in the savannah regions including Plateau State. Severe losses in tomato yields occur in fields infested with root-knot nematodes [36]. Several control strategies such as host plant resistance, rotation with non-hosts, sanitation and avoidance, destruction of residual crop root, and judicious use of nematicides have been reported to effectively control root-knot nematodes [5]. However, the use of resistant varieties remains the most viable option, particularly for small –scale farmers with limited resources [33] Even with these control strategies root-knot nematodes are often cited as major limiting factor of crop production. Despite their relative importance in the biology and growth and yield of crops, root-knot nematodes have not been fully addressed in Nigeria. Taking into account the world-wide distribution of root-knot nematodes, it is necessary to find out the most effective and feasible control measure. The use of chemical for nematode control on large scale is an expensive and impracticable operation [3]. Record has it that the relatively few users of synthetic pesticides on crops are faced with several problems such as their high cost which make them unaffordable.

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Non-availability of synthetic pesticides when needed by the farmers and adulteration and sale of expired products also compound the problem [28]. Cases of side effects such as phytotoxicity, accidental poisoning of the farmers leading to ill-health and residual effect such as soil toxicity and water pollution have been reported too [25]. Due to the phase out of methyl bromide and constraints of use of other fumigant nematicides, such as the length of the period following fumigant application when crops cannot be planted, growers developed an increase interest in non-chemical control options [10], [17]. The use of chemicals (nematicides) which are the most effective method of controlling nematodes is, however, not economical, because these chemicals are very expensive particularly on large scale farming and most farmers cannot afford them. They are currenly being reappraised with respect to the environmental hazards and human health [32]. Indiscreminate use of synthetic pesticides for controlling nematodes is likely to give rise to phytoxicity, environmental pollution and nematode resistance. Unsafe use of pesticides may result in poisoning of humans and is a problem especially in developing countries [38], [7]. There is a need to develop naturally occurring nematicides, which may be less toxic to man and animals but as effective against nematodes of various crops as synthetic ones. Toxicity of root extracts of different plants against nematodes has been reported by many researchers [22]. Dropkin [8] gave some control methods to include sanitation, crop rotation, fallow rotation, cultural practices (early planting, drying, steam sterilization, solar pasteurization etc) but he equally stated that certain factors such as expenses and type of crop may limit their application in some cases. Taylor and Sasser [31] stated that the major problem with crop rotation is that the rotation resistant or immune crop may not always be profitable as susceptible crops and also if weed are not adequately controlled, the success of crop rotation is jeopardized. Crop rotation is also land demanding. There are reports that

certain plant parts and extracts possess nematicidal properties [14], [28]. Applying the plant parts or extracts to nematode infested soil affects or reduces their population [15], [25]. The Nigerian rural farmers use several plant materials for protecting their crops [4] and also recent studies have confirmed that some botanicals have nematostatic, nematicidal, fungistatic or fungicidal activity [12], [9], [27]. Usage of botanical crop protestants attracts lower capital investments as there is little or no additional training needed to prepare and apply them [23]. Identification of plants with nematicidal or nematostatic properties facilitates safer, cheaper, practical and profitable control of nematodes through plant residues or biological control by cultivation of such plants with nematicidal properties on heavily infected agricultural soils to bring the population of root-knot nematodes down to safe levels. Generally, in pest control, the method used must be of economic value, that is the increase in monetary value of the crop must be more than enough to offset the cost of control measures. According to Taylor and Sasser [31] the expected benefits should exceed the expenses by a ratio of three to one (3:1).

AIMS AND OBJECTIVES

To proffer a control measure for Meloidogyne incognita (root-knot nematode) with the use of nematicidal plant root exudates.

2.0 MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

The seed of common weeds; Crotalaria breviflora, Crotalaria juncea, Crotalaria retusa, and Crotalaria spectabilis were collected and grown on prepared land. The young actively growing seedlings were uprooted put in polyethene bags and brought to the Laboratory for analyses. The common weeds were earlier identified botanically.

2.2 Preparation of root Exudate of test Plants

The test plants with nematicidal properties against the Meloidogyne incognita were subjected to exudates preparation. Young and actively growing test plants of similar sizes were inserted into test-tubes wrapped with black carbon papers and properly seal with masking tape containing water. The set-up containing the plants was held in upright position in a test-tube rack. Cotton wool was used to support the seedlings in the test-tube in an up right position under constant illumination of fluorescent light for 5days. The water in the test-tube continued to reduce due to transpiration and was carefully replaced by removing the cotton wool used to hold the plants in position and some water added and a new cotton wool placed back on the test-tube mouth, to hold the plant in position. The plants grew under this normal condition and in the process or within the period produced exudates that were released into the test-tubes. After this, the set up was dismantled and the plants were removed and the water in the test-tubes containing the exudates was used to test for nematicidal effects on Meloidogyne incognita.

2.3 The Nematicidal Effect of root Exudates on Meloidogyne incognita

The preparation of root exudates was carried out concurrently to ensure that fresh exudates and freshly hatched nematode juveniles from galls containing females egg masses was used always. Five (5) ml of each of the root exudates were obtained within 5days. The galls that contained egg masses were teased off, introduced into Petri dishes containing the exudates to hatch into juveniles. Twenty (20) of them in each Petri dish were arranged in triplicates and kept on the Laboratory bench for varying time intervals of 6h, 12h and 24h. The number of nematodes dead or immobilized for each time interval was recorded. Another gall that contained females egg masses was teased off and introduced into distilled water without root exudates and was kept in separate Petri dish to serve as control. The average of the replicates was computed to determine the number of live/dead nematodes for each plant root exudates at the various time intervals.

3.0 RESULTS

3.1 Effect of root Exudates for Nematicidal Properties against Meloidogyne incognita

The results of nematicidal effects of root-exudates on Meloidogyne incognita revealed that death of root-knot nematode juveniles were recorded with all the root exudates extracted for 5-days. This result also revealed that the roots of the test plant exude nematicidal substances in their natural habitat. The mean individual effect of concentration of exudates on life/dead Meloidogyne incognita per/mls are as shown below. Distilled water that was used as control showed slow mortality rate after various time interval of exposure. The statistical analysis of variance (ANOVA) showed that there was significance difference ($P< 0.05$) in the effect of various plant exudates on Meloidogyne incognita mortality at various concentrations compared to the control. The means were compared by using least significant difference test at 5% level of significance (Table 1).

Table 1: *Effects of Root Exudates Concentrations on Meloidogyne incognita*

		Time of exposure of juveniles to extracts					
		6H		12H		24H	
	Test Plants	Alive	Dead	Alive	Dead	Alive	Dead
1.	Crotalaria breviflora	13.67	7.67	8.00	18.00	0.00	18.67
2.	Crotalaria juncea	14.00	7.33	11.67	18.33	0.00	18.67
3.	Crotalaria retusa	13.33	7.67	12.00	18.33	0.00	19.67
4.	Crotalaria spectabilis	13.67	6.67	12.00	18.00	0.00	18.33
	Distilled water	14.00	2.00	11.00	3.00	9.00	5.00
	LSD P<0.05	0.533	0.000	0.006	0.000	0.000	0.000

Each value is a mean of three replicates and pairs of means that differ by more than their LSD are significantly different at 5% level of significance.

Plate 28: *A live Meloidogyne incognita juvenile treated with test plant root exudates (x 40).*

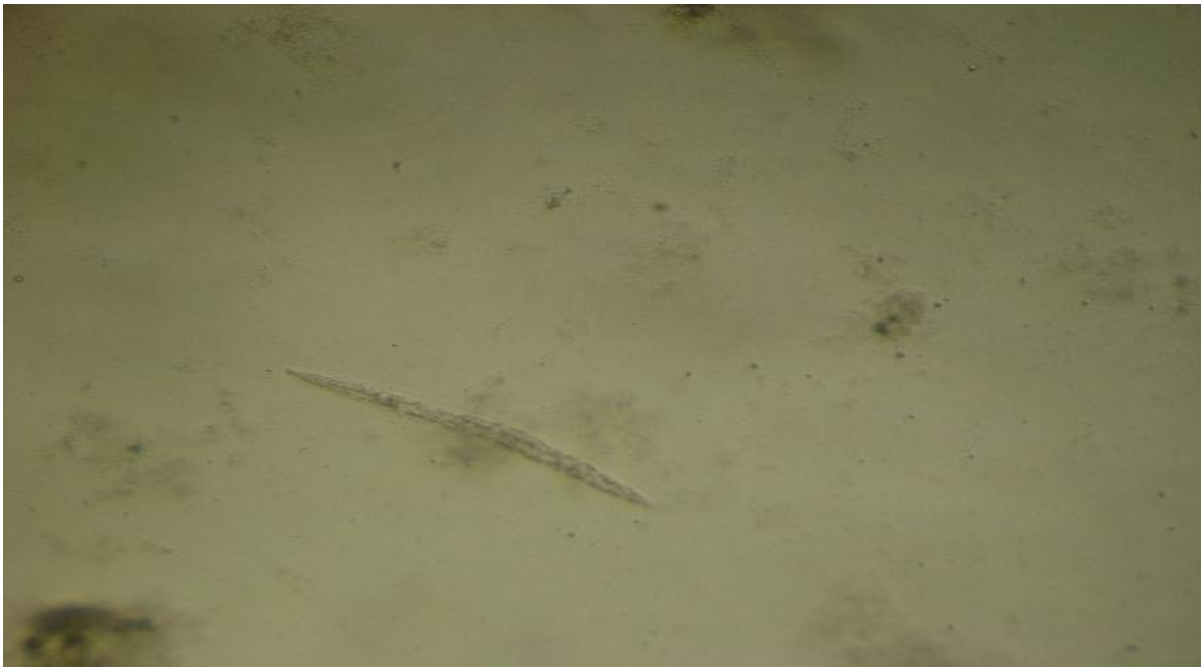

Plate 29: *A dead Meloidogyne incognita treated with test plant root exudates (x 40).*

4.0 DISCUSSION

The result of this research work on the screening of some common weeds for nematicidal effects against Meloidogyne incognita indicated that the four plants screened for nematicidal properties root exudates, showed positive nematicidal activity against the Meloidogyne incognita. The results of nematicidal test of root exudates of the test plants on Meloidogyne incognita revealed that death was recorded with all the root exudates extracted for same number of days. This indicates that the root of the test plant exudes nematicidal substances in their natural habitat and can be used in crop rotation or fallowing with susceptible crops to

control root–knot nematodes. This study provides suitable alternative and a cheaper and safer means of controlling root-knot nematodes especially where there is sufficient land for fallowing. Interestingly, the weeds used in this investigation are legumes which have the added advantage of enriching the soil and promoting crop growth, vigour and yield through nitrogen fixation. This study was designed to examine all potential nematicidal plants that may suppress Meloidogyne incognita. It was focused on treatment with exudates of plants with nematicidal properties, identified as economically feasible practices by Plateau State vegetable grower for their farm operation. It is obviously feasible as these weeds are readily available and grow well on the Plateau. The Meloidogyne incognita were found in all studied areas where tomatoes are grown in Plateau state. Infected tomato plants developed root-knot galls two or three times as large in diameter as compared to healthy roots, indicating high population densities of the pathogens and thus the envisaged heavy loss of crops especially vegetables on the Plateau and its attendant economic loss and catalyst to poverty as the economy of Plateau State is mainly agro based. The revelation that root exudates of the four test plants: Crotalaria breviflora, Crotalaria, Crotalaria juncea, Crotalaria retusa and Crotalaria spectabilis have nematicidal properties on Meloidogyne incognita suggest that they contain biologically active ingredient that can kill or inhibit growth of the nematodes. Several studies demonstrated that Crotalaria sp suppressed Meloidogyne spp, better than chemical nematicides, because it continued to suppress the nematodes population development after a host was planted [11], [30]. The results was thus, exciting as it suggest a remedy to heavy economic expenditure usually incurred during chemical control as well as guarantee safety and higher yield to the farmers. The mortality of the juveniles might be attributed to the nematicidal properties contained in the exudates. These nematicidal properties might have penetrated nematodes body and inhibited metabolic reactions such as those of respiratory enzymes, acetyl cholinesterase enzyme, Hydrolysis of acetyl choline by acetyl cholinesterase and esterases that function in various metabolic systems [2]. The current studies on root-knot nematodes in one of the middle belt states equally gives information on the menance of the organisms in this part of Nigeria. Since, most of the information on root-knot nematode population and crop damage in Nigeria has been reported from the Southern (Ogbuji [18] Western (Ogunfowora [19] and [20] and for Northern parts (Okatachi [21] Chindo and Khan [6] of the country while the middle belt states particularly Plateau has very limited information [37]. The root-knot nematodes are particularly very destructive on vegetable including tomato, okra, potato, spinach etc, which form the basis for search for the test nematodes from these vegetables. The protection of these important crops from root-knot nematodes attack would suggests more food on the table of people within the middle belt region of Nigeria and health promotion through increased availability of vegetables, the principal sources of minerals and vitamins for body health. There has been so many reports on the damage cause by root-knot nematodes but with little suggestion on safe and cheaper control measures, Some of these reports are by; Nastcher[16] reported that Meloidogyne spp have been found to be a major problem with vegetable growers in Senegal. Jones and Jones [13] report that the genus Meloidogyne is the most widely distributed nematode genus in Africa. It causes galls in roots and the infected plants grow poorly and can wilt on hot days. In tropical countries, including Nigeria, farmers often suffer heavy field losses of their crops especially vegetables due to attack by these nematodes [35]. The Jos, Plateau has a conducive climate for dry and rainy season farming and crops cultivated include vegetables especially in dry season. This continuous cropping system (dry and rainy seasons) favours a very high build up of the nematode in the soil and consequently bring about reduce productivity. Hence, a good and cheap control measure will go a long way to increase yield since nematodes can reduce yield up to 10-60% [6]. The result of this study has revealed that root exudates of the four plants: Crotalaria breviflora, C. juncea, C. retusa, and C. spectabilis have nematicidal properties on Meloidogyne incognita suggesting that they contain biologically active ingredients that can kill or inhibit the growth of these nematodes. Since these non-crop plants grow wildly as weed, they can compete and outgrow some persistent weeds that host these nematodes besides killing them directly. Several studies have demonstrated that Crotalaria suppressed Meloidogyne spp better than chemical nematicides, because it continued to suppress the nematode population development after a host was planted [11], [2], [30]. The result of this study to control Meloidogyne incognita with root exudates and showed little but significant death after some time, which can be attributed to lack of food. This agrees with the facts that the distribution of nematodes is influenced by availability of host plants. It has been reported that Meloidogyne naasi has two population peaks in the soil; they were few in winter when the plants were scanty and increased in spring when the plants become many [1].

5.0 CONCLUSION

It can be concluded from the present study that certain plant exudates of the same test plants are a source of cheap and effective nematicides of Meloidogyne incognita. The root exudates of Crotalaria species were found to have nematicidal properties. This finding is important from the point of view of controlling Meloidogyne incognita infecting tomato without the use nematicides in view of the environmental pollution likely to be caused. The future looks bright for identifying new classes of pesticides from natural plants to replace the synthetic harmful and expensive chemicals used in the control of these nematodes at present.

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