

Original Research Article

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Effect of Non-Polar Bioactive Molecules of *Tagetus* on Second Stage Larvae of *Meloidogyne javanica* in Lab Conditions

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ABSTRACT

An experiment was conducted under laboratory conditions in petri-plates, to study the effect of bioactive molecules extracted from roots and aerial parts of *Tagetus* plant on *Meloidogyne javanica* (second stage larvae) which forms root-knot in various crop plants and create severe loss. For its efficacy evaluation, the extracted compounds, initially the extracted fractions were converted into suitable E.C. (emulsified concentration) formulations and prepared formulations were tested against second stage larvae of *Meloidogyne javanica* under lab conditions. Solvent used for isolation were hexane(A), dichloromethane(B), ethyl acetate(C), acetone(D) and methanol(E), in which the first three solvents were non-polar in nature. The activity of EC formulations developed separately by isolated fractions A, B, C, D and E of *Tagetus* plant biocidal compounds varied according to compound doses and time of exposure. A great difference, in all respect, in the toxicity of all the developed formulations was observed. Results clearly indicates that the formulations developed from non-polar fractions exhibited severe impact on mortality and paralysis of second stage juveniles of *Meloidogyne javanica* in comparison to the formulations developed from polar fractions. But results indicate that the maximum mortality rate between the non-polar fractions were present in hexane soluble compounds. So, it is clear that maximum mortality rate was present in highest non-polar compound and followed by less non-polar compounds. The paper deals with the study of the effect of three different, non-polar compounds fractionated at different levels of polarity and their impact on juveniles.

Keywords

Allelochemicals,
Allelopathy, *Tagetus*,
Meloidogyne javanica,
Solvents,
Biopesticides

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Introduction

Chemical control of plant-parasitic nematode, essentially, involves the use of synthetic nematicides. As we know that nematodes are microscopic pathogens and create heavy loss in crop yield. Root-knot nematodes are sedentary endoparasites and most damaging agricultural pests, attacking a wide range of crops. The infection starts with root penetration of second stage juveniles (J_2) hatched in soil from egg encapsulated in egg masses laid by females on the infected roots.

Nematode infestation is considered as a prime constraints in realizing optimum yield potential of cultivated crops causing approximately 15-20% yield losses.

India produced 78.2 million tonnes of vegetables from 5.73 million hectare of land. Tomato is one of the important and popular vegetables grown in India and other parts of the world. It is a rich source of soluble sugars, several organic acids (Citric and Malic acids),

Mineral salts and large quantities of Vitamins A, B₂ and C. The vegetable is attacked by root-knot nematode. In India, reduction in the yield of tomatoes due to root-knot nematode has been reported 26.5 to 73.3%. The management of this nematode has been carried out by several workers (Akhtar and Mahmood, 1993; Das and Mishra, 2003; Dwivedi *et al.*, 2004; Gaur and Prasad, 1990).

So, it is the necessary to control these tiny, harmful worms in field conditions. However, apart from very high cost of chemical pesticides, increased concern for the environment has necessitated a reduction in amount of nematicide used for nematode control. Additionally, there has been an increase in the intensity of search for other efficient, ecologically sound and safe control methods.

Tagetes is an ornamental plant. It has several pesticidal properties in which nematicidal activity is well known in field conditions. We are using this plant as green technology which is safe for environment and easily available in our country. Total work is in the direction to extract particular potential molecules from plant which will be the pin-point for the mortality rate of second stage larvae of *Meloidogyne javanica* in field conditions. Allelochemicals refer mostly to the secondary metabolites released by intact live plant into their surrounding and are by product of primary metabolic process. Different classes of allelochemicals e.g. phenolic acids, coumarins, flavonoids, terpenoids, alkaloids and sulphides etc. have been identified. Allelopathy has direct (toxicity) or indirect (i.e. by supporting growth of biocarbon agents) effect on pests.

Materials and Methods

Plant of *Tagetes* has been grown in a trapping system, which was developed with the help of

Butchner funnel of 110 cm diameter and conical flask of 500ml. capacity. The sieve plate of buchner funnel has been cut and firstly muslin cloth was placed in it, then funnel was filled with the soil (loamy soil) of field capacity. The 2-3 seeds of *Tagetes* were grown in every trapping system. After the seedling growth when plants attained some height one plant was allowed to grow and other was thinned. After attaining the maturity (at the time of flowering) plant was taken out from the system and shade dried. After that the roots were cut into small pieces and powdered. Then it was processed for the recovery of biocidal compounds.

Firstly, known quantity of the material was mechanically stirred in required solvent system for 2-3 hours at room temperature and then filtered. Filtrate was evaporated to minimum amount under vacuum at 50°C ± 1°C. The concentrated solution was transferred to a separating funnel and partitioned with some amount of ethyl acetate and hexane. The mixture was vigorously shaken for two hours and kept overnight to settle the different layers. By this allelocompounds got fractionated into two major groups (a) Polar (Which remained with H₂O layer) (b) Non-polar (Settle down with ethyl acetate layer).

Both were removed carefully and kept separately. Finally the ethylacetate layer was evaporated to dryness under vacuum at 50 ± 10°C. After that allelocompounds were recovered by using fractional crystallization technique. For the recovery of compounds, different solvents of different polarity were used. Before crystallization a number of information like numbers of compounds present in each fraction and their polarity etc. were being generated with the help of thin layer chromatography and then compounds were fractionally crystallized by selecting the suitable polarity of solvents.

Analysis of compounds

Thin layer chromatography method

TLC methods were developed to analyze and checking the purity of the isolated compounds of *Tagetes* root exudates present in each fraction.

After that bioefficacy of extracted compounds were evaluated against second stage of juveniles of *Meloidogyne javanica* in the laboratory and in pots on Tomato plants.

For bioefficacy evaluation initially the emulsified concentrations of the extracted fractions were converted into suitable concentrations.

EC formulations of different concentrations were tested against the second stage juveniles for which cultures were maintained in the laboratory of the Department of Botany, P.P.N. College, Kanpur on Chickpea and *Sisbanea* spp.

Preparation of EC Formulation

For bioassay study initially the isolated compounds were converted into suitable EC and EW formulations. Non-polar i.e. hexane, ethyl acetate and acetone soluble fractions were emulsified separately at 10% emulsifiable concentrate (EC) by taking the required quantity of products and adding them (10%) tween 80 as emulsifier and 80% xylene. ECs were obtained by constantly high speed agitating the mixture for two-three hours.

Preparation of Test Solution

Test solutions of different concentrations were prepared by taking the appropriate amount of EC diluting it in a definite volume of water.

Formula used for preparation of concentration

The amount for each experiment was decided on the basis of following formula:

$$\text{Amount of EC} = \frac{\text{ai required in Test solution} \times \text{Volume of solution}}{\% \text{ ai in formulation}}$$

Bioassay Test

Second stage juveniles (J_2) of root-knot nematode were allowed to hatch from egg masses obtained from culture maintained on Chick pea in Dept. of Botany, P.P.N. College, Kanpur in earthen pot.

Experiments for bioefficacy evaluation of extracted compounds were conducted on freshly hatched second stage juveniles in petriplates of 12 ml.

Capacity, 9 ml. distilled water was poured in each petriplate and with the help of micropipette desired quantity of formulations were added and mixed well.

One ml. of suspension of freshly hatched larvae were added to each petriplate and that make the total volume of water of 10ml. and plates were loosely capped by another petriplates and were kept for 24, 48 and 72 hours at room temperature ($30^{\circ} + 1^{\circ}\text{C}$).

All treatments were replicated thrice. A set of control was also placed containing only xylene and tween-80.

After specific time gap, the treated suspension was stirred and one ml. of suspension was taken out and transfer to another petri-plate containing water to reduce chemical toxicity much more below than its toxic level and to observe the revival of larvae if there was any.

Results and Discussion

Recovery and Purity of biocidal compounds

Approximately 2kg of *Tagetus* roots were processed for the recovery of biocidal compounds of different category viz. polar and non-polar category. A total of six different fractions named as A, B, C, D, E and F as per their clear-cut solubility in different polarity of solvents viz. hexane, dichloromethane, ethylacetate, acetone, methanol and water were recorded. TLC methods were developed to analyze the extracted fractions for their exact purity and total number of compounds present in each fraction. Silica gel of TLC grade was observed to be the appropriate coating materials for non-polar compounds. Highly non-polar compound hexane soluble compound resolved best in n-hexane while ethyl-acetate compounds resolved in 60: 40 n-hexane and ethyl-acetate mixture. All fractions were found more than 90% pure as a single compound as per TLC analysis.

Effect on juveniles

The activity of EC formulations developed separately by isolated fractions like A, B and C of *Tagetus* root exudate biocidal

compounds varied according to compound doses and the time of exposures obtained observations are revealed in table 1-3. A great difference, in all respect, in the toxicity of all the developed formulations was observed. Results clearly indicated that formulations developed from non-polar fractions exhibit severe impact on the mortality rate and paralysis of second stage juveniles of *Meloidogyne javanica*. But in between three of non-polar compounds maximum juveniles mortality rate at different exposure times was achieved by the formulations developed from highly non-polar fraction i.e. hexane (A, Table 1) followed by ethylacetate and acetone (B, C Table 2 and 3). Emulsion concentrate formulation developed from less non-polar fraction was found practically least effective at the same concentration and at maximum time of exposure (72 h).

Results indicate that the polarity of the extracted compounds of *Tagetus* root exudate remained as the key factor in imparting the toxicity against second stage juveniles of *Meloidogyne javanica*.

Results also indicate that as the polarity of solvent changes from non-polar to polar even every high concentration is not much effective in comparison of non-polar compounds.

Table.1 Effect of hexane soluble compounds

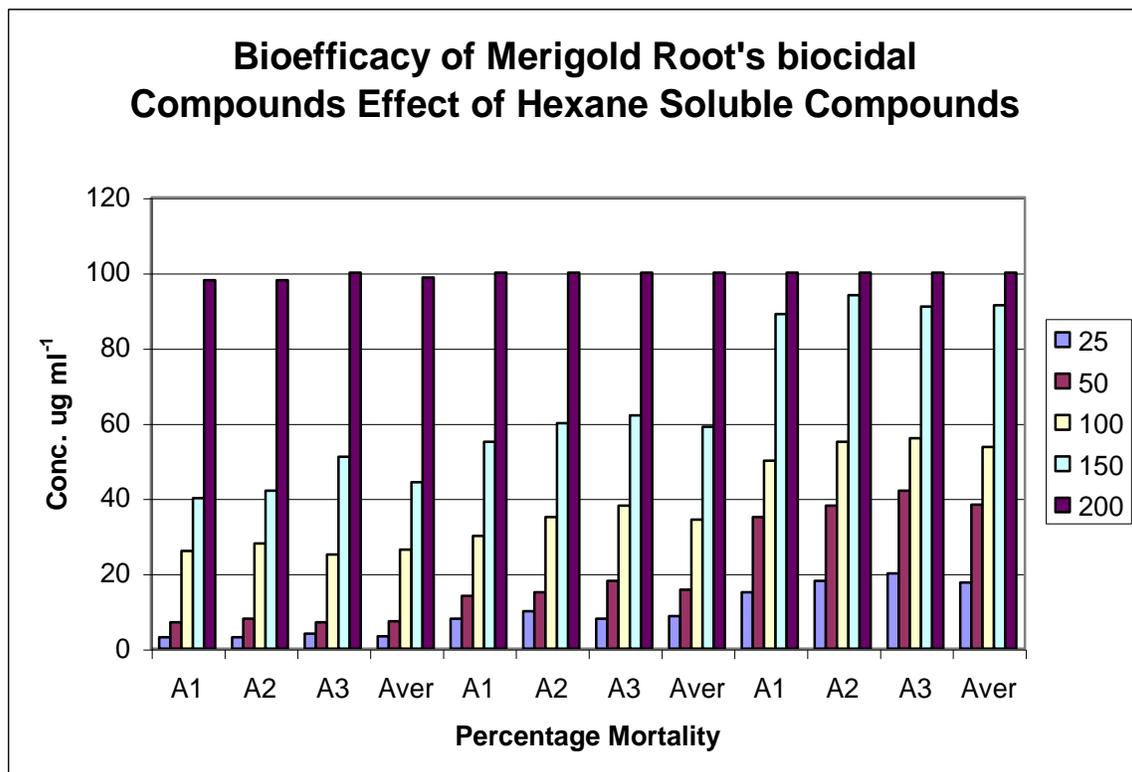
Conc. $\mu\text{g ml}^{-1}$	Percentage mortality after 24 hours.				Percentage mortality after 48 hours.				After 72 hours.			
	A ₁	A ₂	A ₃	Aver	A ₁	A ₂	A ₃	Aver	A ₁	A ₂	A ₃	Aver
25	3	3	4	3.3	8	10	8	8.7	15	18	20	17.6
50	7	8	7	7.3	14	15	18	15.7	35	38	42	38.3
100	26	28	25	26.3	30	35	38	34.3	50	55	56	53.6
150	40	42	51	44.3	55	60	62	59.0	89	94	91	91.3
200	98	98	100	98.7	100	100	100	100.0	100	100	100	100.0

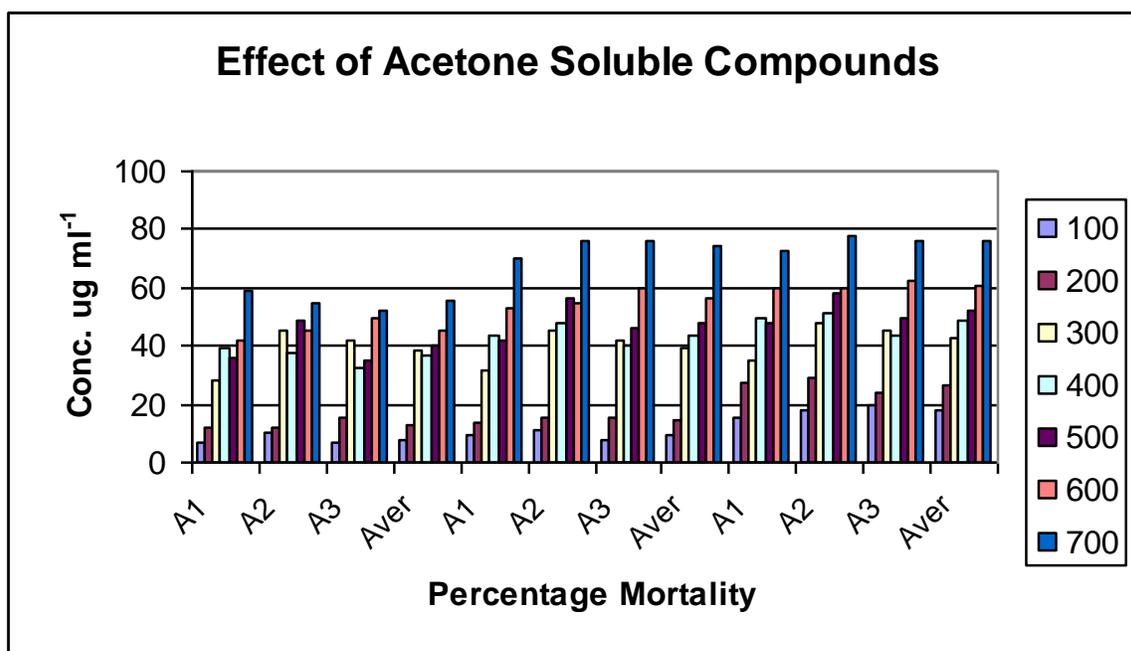
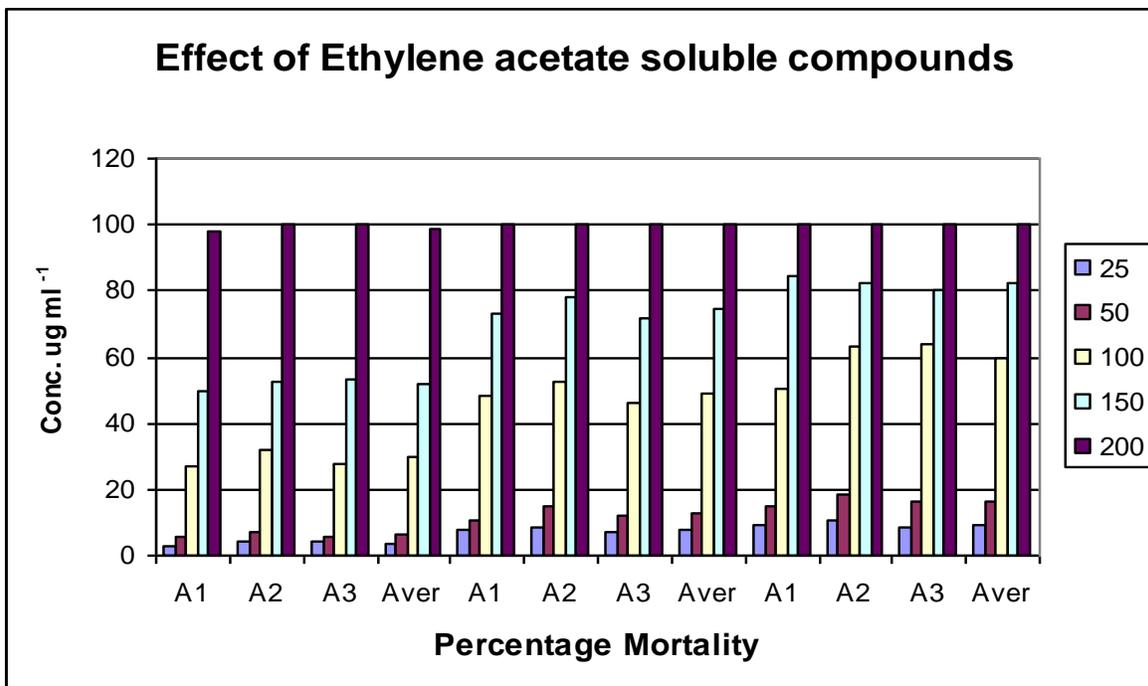
Table.2 Effect of ethyl acetate soluble compounds

Conc. $\mu\text{g ml}^{-1}$	Percentage mortality after 24 hours.				Percentage mortality after 48 hours.				After 72 hours.			
	A ₁	A ₂	A ₃	Aver	A ₁	A ₂	A ₃	Aver	A ₁	A ₂	A ₃	Aver
25	3.0	4.2	4.0	3.7	7.5	8.2	7.1	7.6	9.0	10.4	8.8	9.4
50	6.0	7.2	6.0	6.4	11.0	14.8	12.4	12.7	15.0	18.2	16.0	16.4
100	27.2	32.0	28.0	29.6	48.0	52.4	46.3	48.9	50.2	63.0	64.0	59.6
150	50.0	52.5	53.5	52.0	73.0	78.2	72.0	74.4	84.3	82.3	80.2	82.3
200	98	100	100	98.7	100	100	100	100.0	100	100	100	100.0

Table.3 Effect of acetone soluble compounds

Conc. $\mu\text{g ml}^{-1}$	Percentage mortality after 24 hours.				Percentage mortality after 48 hours.				After 72 hours.			
	A ₁	A ₂	A ₃	Aver	A ₁	A ₂	A ₃	Aver	A ₁	A ₂	A ₃	Aver
100	7	10	7	8.0	9	11	8	9.3	15	18	20	17.7
200	12	12	15	13.0	14	15	15	14.7	27	29	24	26.7
300	28	45	42	38.3	32	45	42	39.6	35	48	45	42.7
400	39.0	38	32.2	36.4	44	48	40	44	50	51	44	48.3
500	36.2	49	34.8	40.0	42	56	46	48.0	48	58	50	52.0
600	42.0	45	50	45.6	53	55	60	56	60	60	62	60.6
700	59	55	52	55.3	70	76	76	74	73	78	76	75.7





The results suggest that the biocidal compounds extracted from the *Tagetes* root especially the non-polar group of compounds possess molecules with tremendous nematocidal activity, which can be utilized effectively as a new control strategy with

minimal or nil environmental impact for effective control and management of *Meloidogyne* spp. and possibly other nematodes in agricultural and other important commercial crops. In all directions emulsion concentrate formulation developed from

highest non-polar compounds was found most effective at the same concentrations in field conditions.

After lab experiments, all the results indicate that the polarity of extracted compounds of *Tagetus* root exudates remained as the key factor in imparting the toxicity against second stage of juveniles of *Meloidogyne javanica* in lab conditions. Therefore, the formulations developed especially from non-polar fraction hexane was found detrimental to the juveniles and Root Galls.

The results suggested that the biocidal compounds extracted from the *Tagetus* root, especially, the non-polar group of compounds possess molecules with tremendous nematicidal activity, which can be utilized effectively as a new control strategy with minimal or nil environmental impact for effective control and management of *Meloidogyne* spp. and possibly other nematodes in agricultural and other important commercial crops. It will also curtail the budget of chemical pesticides.

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