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In vitro and *In planta* Effect of Newer Synthetic Compounds against Rice Root Knot Nematode *Meloidogyne graminicola* in Rice Nurseries

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Abstract: A Laboratory experiments were carried out to evaluate the effect of newer synthetic chemicals compounds in the control of rice root knot nematode (*Meloiodgyne graminicola*). Ten chemicals of two series namely coumarin series (5 chemicals) and coumarin carbamate series (5 chemicals) were evaluated at different concentrations (1000ppm, 500ppm, 250ppm and 125ppm) against J2 and eggs of M. graminicola. Approximately 100 eggs and 100 juveniles were dispensed into separate tissue culture cavity block containing the dilutions of chemicals except the control which contained only Acetone distilled water mixture. In vitro experiment revealed that all the compounds resulted in nematode mortality ranging from 20-87% and inhibition of egg hatching from 10 to 78%; the higher concentrations (1000 and 500 ppm) were being most effective. In Coumarin carbamate, Series III (4, 5 dimethyl -2-oxo-2H-benzopyran-7yl-phenyl carbamate) 70 % mortality was observed even at lowest concentration of this chemical i.e. 125 ppm. The lowest mortality was observed in acetone and water mixture (control) alone (5.3%). Maximum larval mortality and inhibition of egg hatching was recorded in one of the Coumarin carbamate series III i.e., 4,5 dimethyl-2-oxo-2H-benzopyran-7yl phenyl carbamate (96.33% and 11%) respectively. Coumarin carbamate derivates proved more nematicidal than Coumarin series in both larval mortality and egg hatching experiments. Based on larval mortality and egg hatching experiments only promising synthetic chemicals with 500 ppm concentration were selected for screen house study. Chemicals were used @ 70 ml/pot as soil drenching and untreated check was also maintained. Among synthetic chemicals maximum seedling weight (10 plants) and shoot length (2.76 g and 19.10 cm) was recorded with Coumarin carbamate III (4,5 dimethyl-2-oxo-2H-benzopyran-7yl phenyl carbamate) followed by Coumarin carbamate V (2.71 g and 18.53cm) and Coumarin carbamate IV (2.35 g and 17.48 cm). The minimum number of galls per 10 plants were recorded with Coumarin carbamate III (64.25) followed by Coumarin carbamate V (64.50) and Coumarin carbamate IV (70.50). A similar trend was also recorded in nematode population per root. The results of present investigation suggest suitable chemicals for grower having nematode problem in field to incorporate it in management strategies.
Keywords: Newer Synthetic compounds, *In vitro*, *In vivo*, Rice root Knot nematode, Rice nurseries

Introduction:

Root-knot nematode, *Meloidogyne*spp. is one of the most widespread and devastating nematode pests of agricultural crops (Sasser, 1989). The nematode has exceedingly wide host range and attacks almost all cereal, vegetable, pulse, fiber, fruit and beverage crops (Bridge *et al.*, 2005). Rice is the world's staple food and is cultivated in around 162 mha annually with annual global production of 464 mmt (FAOSTAT, 2013). Many root knot nematode species like *Meloidogyne incognita*, *Meloidogynegraminicola*, *Meloidogynetriticoryzae*, *Meloidogynejavanica*,

Meloidogyneoryzae and Meloidogynearenaria attacks the Rice crop both in nursery and main field conditions (Gaur and Pankaj, 2010). Among these species, *M. graminicola* is the most widely distributed and a potential damaging pest of upland, lowland, deep water rice and irrigated cultivation ((Prot*et al.,* 1994 &Netscher and Erlan, 1993). It causes a yield loss of 16-32% in rainfed and upland rice in India (Prasad *et al*., 2010). There are various methods available for the management of rice root-knot nematode including fallowing, flooding, deep ploughing, biological control and nematicide application. Despite concern about the adverse effects on the ecosystem by the chemical pesticides (Khan *et al*., 2012), but still chemical pesticides are the most effective means of management of nematodes in the rice ecosystem (Prasad *et al.*, 2010). In view of limited availability of rice cultivars resistant to *M. graminicola* (Dutta*et al*., 2012), chemicals are preferred by farmers, as they provide instant results, whereas other disease management practices only begin to have a recognizable impact over considerable time. Chemicals can function through contact and/or via systemic action, and are applied through different modes, such as seed, bare root-dipping (Jain and Bhatti, 1991) and nursery bed treatments (Jain and Gupta, 1990). A critical analysis of the available information reveals that most of the studies in this area have been carried out using phorate, carbosulfan and Carbofuran with multiple treatments, whereas other newer synthetic chemicals have seldom been tested against *M. graminicola* in rice. In the present study it has been attempted to target the newer synthetic chemicals specifically against *M. graminicola* both *in vitro* and *in planta* under rice nursery conditions.

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Materials and Methods:

Ten chemicals of two series namely coumarin series (5 chemicals) and coumarincarbamate series (5 chemicals) represented below were synthesized by Kumari *et al.*, 2014 at CCS HAU were collected and These chemicals were tested against *M. graminicola* under *In vitro* and *In planta* conditions.

List of chemicals:

S. No.	Common name	Chemical name
	Coumarin series	
1	Coumarin I	4 hydroxy-2H-1-benzopyran-2-one (46)
2	Coumarin II	7 hydroxy-4, methyl, 2H-1-benzopyran-2-one (42)
3	Coumarin III	7 hydroxy-4,5 dimethyl, 2H-1-benzopyran-2-one (43)
4	Coumarin IV	7,8 dihydroxy-4, methyl, 2H-1-benzopyran-2-one (44)
5	Coumarin 4CR V	6 chloro - 7 hydroxy, 4 methyl, 2H-1-benzopyran-2-one (45)
	Coumarincarbamate series	
6	Coumarincarbamate-I	2-oxo-2H-benzopyran-4yl phenyl carbamate (51)
7	Coumarincarbamate- II	4 methyl-2-oxo-2H-benzopyran-7yl phenyl carbamate (47)
8	Coumarincarbamate- III	4,5 dimethyl -2-oxo-2H-benzopyran-7yl phenyl carbamate (48)
9	Coumarincarbamate- IV	4 methyl-2-oxo-2H-1-benzopyran-7,8 diylbis phenyl carbamate (49)
10	Coumarincarbamate-V	6 chloro- 4 methyl-2-oxo-2H-1-benzopyran-7yl phenyl carbamate (50)

Preparation of Dilutions:

Each compound was tested at four different concentrations, viz., 1000, 500, 250 and 125 ppm. The stock solutions of the compounds were prepared in acetone + distilled water (10: 90% by volume), and distilled water, as well as a mixture of water with acetone at concentrations equivalent to those in the treatment wells, were used as controls (Cheng *et al,* 2015). Then 1 mL of solution and 1 mL of rootknot nematodes J2 (containing average 100 J2 or 100 eggs) was added to each well of a 6-well tissue culture plate. Double concentrations of the compounds were mixed with equal amount of J_2 or Egg suspension containing ca 100 J_2 or eggof *M. graminicola* in sterile distilled water. The

assays were performed in 6 well tissue culture plates at 25±2º C for both larval mortality and egg hatching.

Obtaining eggs and J2 of *M.graminicola*:

M. graminicola populations were obtained from rice roots grown in culture pots. Eggs of M. *graminicola* were extracted using a modification of the Hussey-Barker method (Hussey & Barker 1973) wherein galled root pieces were placed in 0.1 per cent NaOCl solution and processed in a waring blender at 20 sec intervals for 3 min. The water suspension bearing the free eggs was passed through a bank of sieves – 100 mesh (pore size 150 µm) nested over 400 mesh (38 µm) and 500 mesh (26 µm) sieves. The contents of the sieves were washed thoroughly with running water to remove the chlorine. Finally the residues of 400 and 500 mesh sieves were collected in a beaker. The content of the beaker were examined under a microscope for the presence and density of eggs or from infected rice roots either by teasing galled roots with needles. The J2 were obtained by further pouring egg suspension over 4-ply facial tissue paper mounted on a piece of moulded wire net. The assembly was fixed in a Petri-plate and fresh water was added. The water was removed from the Petri-plate daily to collect the hatched J2 and replaced with fresh water. The assembly was maintained at 24 ± 2° C in a BOD incubator until the J2 hatched. Freshly hatched J2 were used for the experiments according to the treatments.

Mortality test of nematode larvae:

Eggs of *M. graminicola* were placed in distilled water and incubated at 28±2°C. After hatching, a suspension of juveniles in distilled water was prepared. One milliliter of juvenile suspension (100 J2/ml) and 1ml of chemical were transferred into different glass cavity block and kept at room temperature. Each treatment was replicated three times. The glass block containing 1 ml acetone water mixture served as control. Percentage mortality was calculated after 48 h of exposure,

the number of killed juveniles was counted under a low power stereomicroscope. Nematodes were considered dead if they did not move when probed with a fine needle (Abbasi*et al*., 2008).

Hatching Test:

Eggs of *M. graminicola* were collected by the method of Hussey and Barker (1973). A suspension of eggs in water was prepared. One milliliter of egg suspension (100 eggs/ml) and 1 ml of chemical was transferred in 6 well tissue culture plates and kept at room temperature. Each treatment was replicated three times. The 6 well tissue culture plates containing 1 ml egg suspension and 1ml acetone water mixture served as control. After ten days of exposure, the number of hatched eggs was counted under a low power stereomicroscope.

Testing under pot conditions (*In planta*):

On the basis of *in vitro*nematoxicity test, only promising compounds were selected along with their dilutions (500 ppm) for this experiment. Sandy-loam top soil (sand 60.8%, silt 34%, clay 5.2% and organic matter 6.8%) used for raising rice seedlings. Soil was dispensed into 1 kg pot in screen-house. The initial nematode population was 2J2 per g soil. The soil was drenched with 70 ml of the chemical with 500 ppm concentration by pouring the solution in each pot. The seeds of susceptible rice var. Pusa 1121 were sown @ 20 per pot. Unamended and without nematode pots served as positive control, while unamended and nematode inoculated pots served as negative controls. Four replicate pots per treatment and controls were arranged in a completely randomized design. The observations were taken after 35 days of germination of rice seedlings on seedling growth and nematode reproduction parameters. All the observations are subjected to ANOVA analysis through OPSTAT which is available in online at Hisar.

Results and Discussion:

Mortality test: It is evident from (Table 1 & 2) that the both Coumarin and Coumarincarbamate series are successful in larval mortality of *Meloidogynegraminicola*. All the compounds resulted in nematode mortality ranging from 20-96%; the higher concentrations (1000 and 500 ppm) were being most effective. Coumarincarbamatederivates proved more nematicidal than Coumarin series (Fig1 &2). Maximum larval mortality was recorded in one of the Coumarincarbamate series i.e., 4,5 dimethyl-2-oxo-2H-benzopyran-7yl phenyl carbamate (96.33%) which even exhibited 70% mortality even at the lowest concentration i.e., 125 ppm. The lowest mortality (21.67%) was observed in 7,8 dihydroxy-4, methyl, 2H-1-benzopyran-2-one even at highest concentration i.e. 1000 ppm. The lowest mortality was observed in acetone and water mixture (control) alone (5.33%). A similar type of results were found by Nabil and Reversat (2006) tested two pure isothiocyanates 2-phenylethyl (PEITC) and methylethylisothiocyanate (MITC) in vapour exposure tests for nematicidal activity against different stages of two nematodes. Two species were studied in vitro the cyst nematode *Heteroderasacchari* and the root knot nematode *M. gramminicola*. Suppressive effect on larval stages was comparable between MITC and PEITC. The MITC was more effective on eggs, cyst and galls than PEITC. The results indicate that the thicker the physical barrier, the higher the concentration required reaching 100% suppressive effect on the resistant nematode stage. Abbas *et al.*,(2015) study the effect of twenty chemicals currently available in market was evaluated against *M. incognita*. Juvenile's mortality of *M. incognita* was assessed under *in vitro* conditions. Four concentrations of each chemical were prepared viz., 2S, S, S/2, S/4 according to recommended dose of each chemical. Maximum mortality percentage was recorded in synthetic [Cartap (Thiocarbamate), Virtako (Thiamethoxam + chlorantraniliprole)] and bio [Cure (Abamectin), Azadirachtin (Aza)] chemicals. A similar experiment was conducted on Southern root-knot nematode (Meloidogyne incognita) test the emamectin benzoate for management of M. incognita in laboratory. Laboratory results showed that emamectin benzoate exhibited high toxicity to M. incognita, with LC50 and LC90 values 3.59 and 18.20 mg L-1, respectively by (Cheng *et al.*, 2015). Similar observations

were also found with using Isothiocyanates (ITCs), a series of new nematicides of the –N=C=S group, were evaluated for their efficacy against root-knot nematode *Meloidogynejavanica*. Of the compounds tested, AllylITC, AcITC,EtITC, BzTC, BzITC, 1-PEITC and 2-PEITC showed in vitro irreversible nematicidal activity against secondstagejuveniles of *M. javanica*, following exposure for 72 h at concentrations as low as 5 mg mL^{-1}. When exposed to AllylITC, AcITC and EtITC at lower concentrations, motile juveniles also became irreversibly immobile in 3 days, with a LC50 value at 2.76, 2.53 and 3.05 mg mL^{-1}respectively by HuaWu *et al.*, (2011).The results of present investigation suggest the suitable chemicals for grower having nematode problem in field to incorporate it in management strategies.

Hatching Test: Based on larval mortality only certain compounds were taken in egg hatching experiment. It is evident from (Table 3 & 4) that the both Coumarin and Coumarincarbamate series are successful in inhibiting the egg hatching of *Meloidogynegraminicola*. Coumarincarbamatederivates proved more efficient in inhibiting the egg hatching than Coumarin series. All the compounds resulted in nematode hatching ranging from 10-80%; the higher concentrations (1000 and 500 ppm) were being most effective in inhibiting the egg hatching (Fig 3 &4).Minimumegg hatching was recorded in one of the coumarincarbamate series i.e., 4,5 dimethyl-2-oxo-2H-benzopyran-7yl-phenyl carbamate (11.00 %). The highest egg hatching (94.75%) was observed in acetone and water mixture (control). As the concentration of the chemical decreases the egg hatching increases. All the chemicals showed a good inhibition (below 50%) of egg hatching at 500 ppm. A similar type of observations was done by Abbas *et al.*,(2015) study the effect of twenty chemicals currently available in market was evaluated against *M. incognita*. Egg hatching inhibition test of *M. incognita* was assessed under *in vitro* conditions. Four concentrations of each chemical were prepared viz., 2S, S, S/2, S/4 according to recommended dose of each chemical. Data on hatching inhibition was recorded after 2, 4 and 6 days. Maximum hatching inhibition was recorded in synthetic [Cartap (Thiocarbamate), Virtako (Thiamethoxam + chlorantraniliprole)] and bio [Cure (Abamectin), Azadirachtin (Aza)] chemicals. The Efficacy of lac based chemicals on egg hatching of root-knot nematode (*Meloidogyne incognita*) under in vitro

condition. Two lac based chemicals viz. 9-hydroxy Δ2 –nonenoic acid and its methyl ester when evaluated against juveniles (J2) of *M. incognita* at different concentrations [T1 (1000ppm), T2 (500ppm), T3 (250ppm), T4 (125ppm) and T5 (62.5ppm)] showed marked suppression over control. There was a significant difference among the concentrations tested. Highest inhibition of egg hatching (100%) was recorded in T1 with both the chemicals, while other concentrations showed decrease in percentage inhibition of egg hatch with time was reported by (Srivasta*et al.*, 2013)

Testing under pot conditions (*In planta*):

An experiment was conduct to study the effect of synthetic chemical compounds on *Meloidogynegraminicola* as soil drenching on plant growth characters and reproduction of *M. graminicola* on rice. For this study seven chemicals with their concentrations (which showed 50% mortality and suppression of hatching) were selected *viz.*,Coumarin I,Coumarin III, Coumarincarbamate I, Coumarincarbamate II, Coumarincarbamate- III, IV and V were used @ 70 ml/pot as soil drenching and untreated check were also maintained. Observations on plant growth characters (10 seedling weight, shoot length) and nematode reproduction (number of galls and nematode population per root were recorded and presented in Table (5) showed that all the synthetic chemicals applied significantly increased the seedling weight and shoot length as compared to untreated check (nematode alone). Among synthetic chemicals maximum seedling weight and shoot length (2.76 g and 19.10 cm) was recorded withCoumarincarbamate III followed by Coumarincarbamate V (2.71 g and 18.53cm) and Coumarincarbamate IV (2.35 g and 17.48 cm). However, maximum seedling weight and shoot length was observed in sterilized soil (3.10 g and 21.53 cm) while minimum (1.56 g and 13.49 cm) was recorded with untreated check (nematode alone).Results showed that the numbers of galls produced by *M. graminicola* on rice seedlings were reduced significantly as compared to untreated check (118.25) when synthetic chemical compounds applied as soil drenching. The minimum number of galls per 10 plants were recorded with Coumarincarbamate III (64.25) followed by Coumarincarbamate V (64.50) and Coumarincarbamate IV (70.50). A similar trend was also recorded in nematode population per root. A parallel

type of work was carried out by Hua Wu *et al.*, (2011) using 1.0 ml AllylITC and 1.1 ml AcITC per kg of soil in the pot for control of *M. javanica*, similarly to or better than metham sodium at its recommended dose. Similar results were obtained in the field experiments using 1.0 kg AllylITC or 1.0 kg AcITC ha^{-1}. Based on the results of this study, AllylITC and AcITC have potential to be used as new bio-fumigant nematicides. In greenhouse tests and field trials, emamectin benzoate provided control efficacy against *M. incognita* and resulted in increased tomato yields. Compared with the untreated control, there was a 36.5%to 81.3%yield increase obtained from all treatments and the highest yield was received from the highest rate of emamectinbenzoate.(Cheng *et al.*, 2015). A Field trials evaluating AgNP were conducted on a bermudagrass(Cynodon dactylon3C. transvaalensis) putting green infestedwith *M. graminis*. Biweekly application of 90.4 mg/m2 of AgNP improved turfgrass quality in one year and reduced gall formationin the roots in two years without phytotoxicity. The AgNP application did not significantly reduce the number of *M. graminis* J2in plots during the growing season (Cromwell *et al.*, 2013).

Conclusion: In mortality test, Coumarincarbamate derivates proved more nematicidal than Coumarin series. Maximum larval mortality (96.3%) was recorded in 4,5 dimethyl-2-oxo-2H-benzopyran-7yl phenyl carbamate at 1000 ppm. The lowest mortality (21.7%) was in 7,8 dihydroxy-4, methyl, 2H-1-benzopyran-2-one even at its highest concentration (1000 ppm). Its 1000 and 500 ppm concentrations were more effective than lower concentrations (250 and 125 ppm).Hatching test revealed that the maximum inhibition of egg hatching(11.0 %) was recorded in 4,5 dimethyl-2-oxo-2H-benzopyran-7yl phenyl carbamate at 1000 ppm. Its 1000 and 500 ppm concentrations were more effective than lower concentrations (250 and 125 ppm) in inhibiting the egg hatching. All the chemicals showed > 50% inhibition of egg hatching at 500 ppm. Under pot conditions, all the chemicals, increased the plant growth parameters of rice, reduced galls and nematode population significantly over untreated check. Maximum increase in plant growth parameters and, reduction in

number of galls and nematode population were found in4,5 dimethyl-2-oxo-2H-benzopyran-7yl phenyl carbamate.

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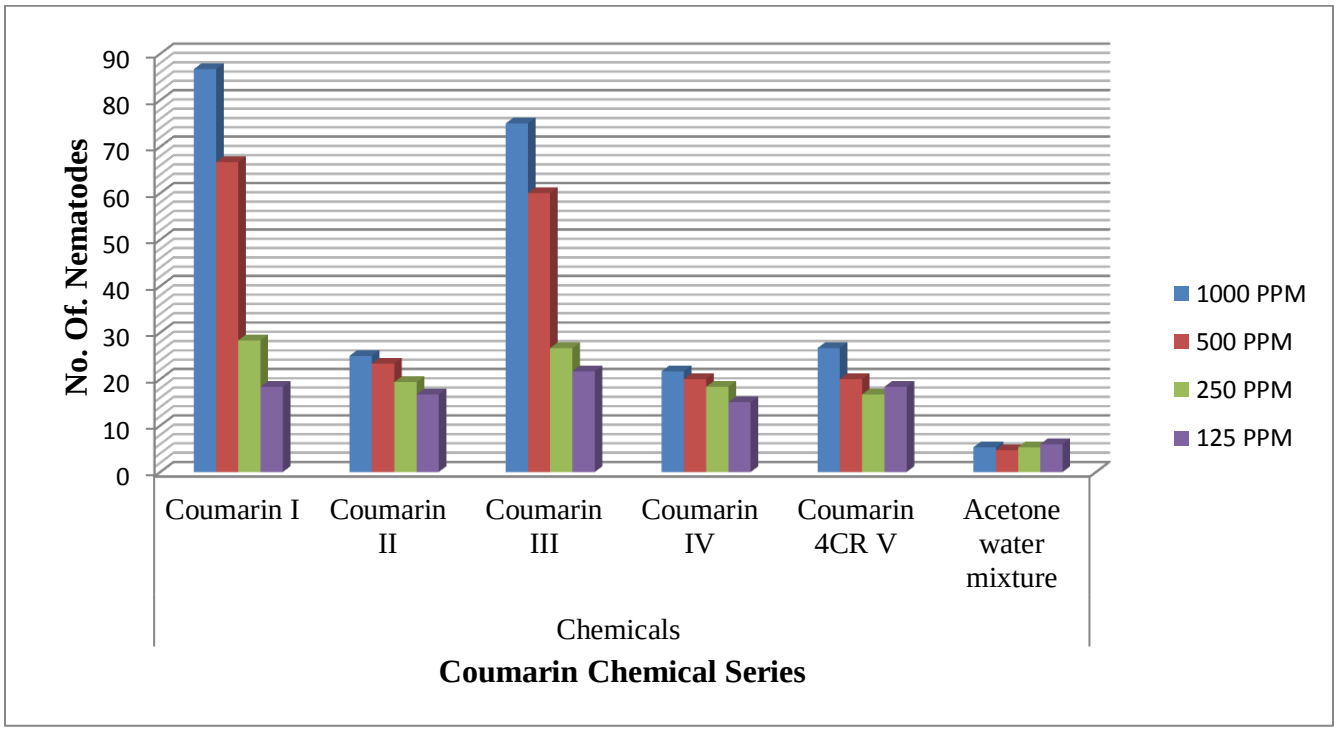

Fig. 1.Effect of Coumarin chemical derivatives on larval mortality of *M. graminicola*.

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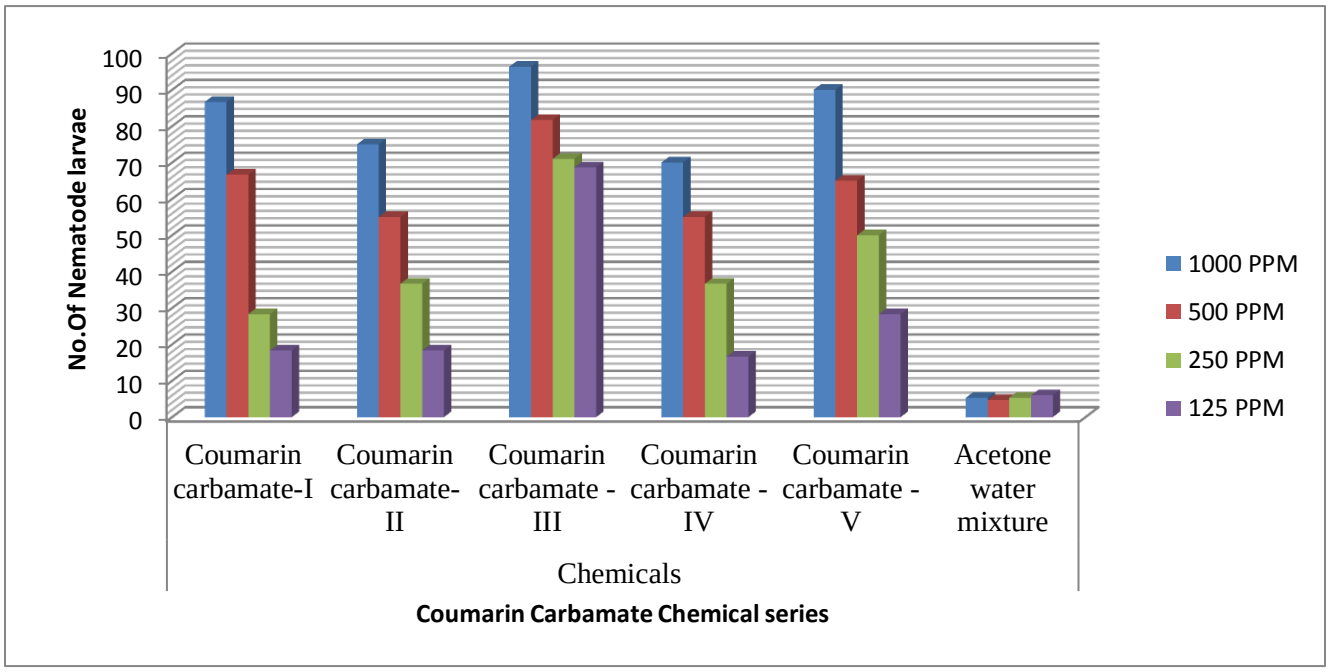

Fig. 2.Effect of Coumarin Carbamate chemical derivatives on larval mortality of *M. graminicola*.

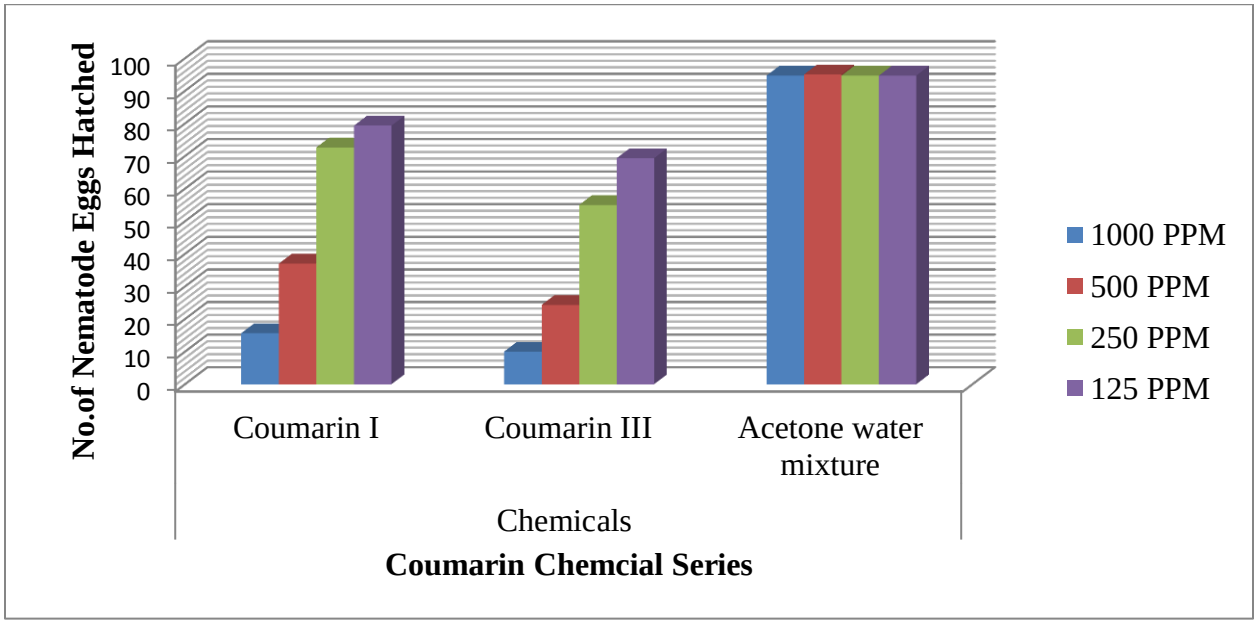

Fig 3: Effect of Coumarin Chemcial derivatives on egg hatching of *M. graminicola* under *In vitro* conditions

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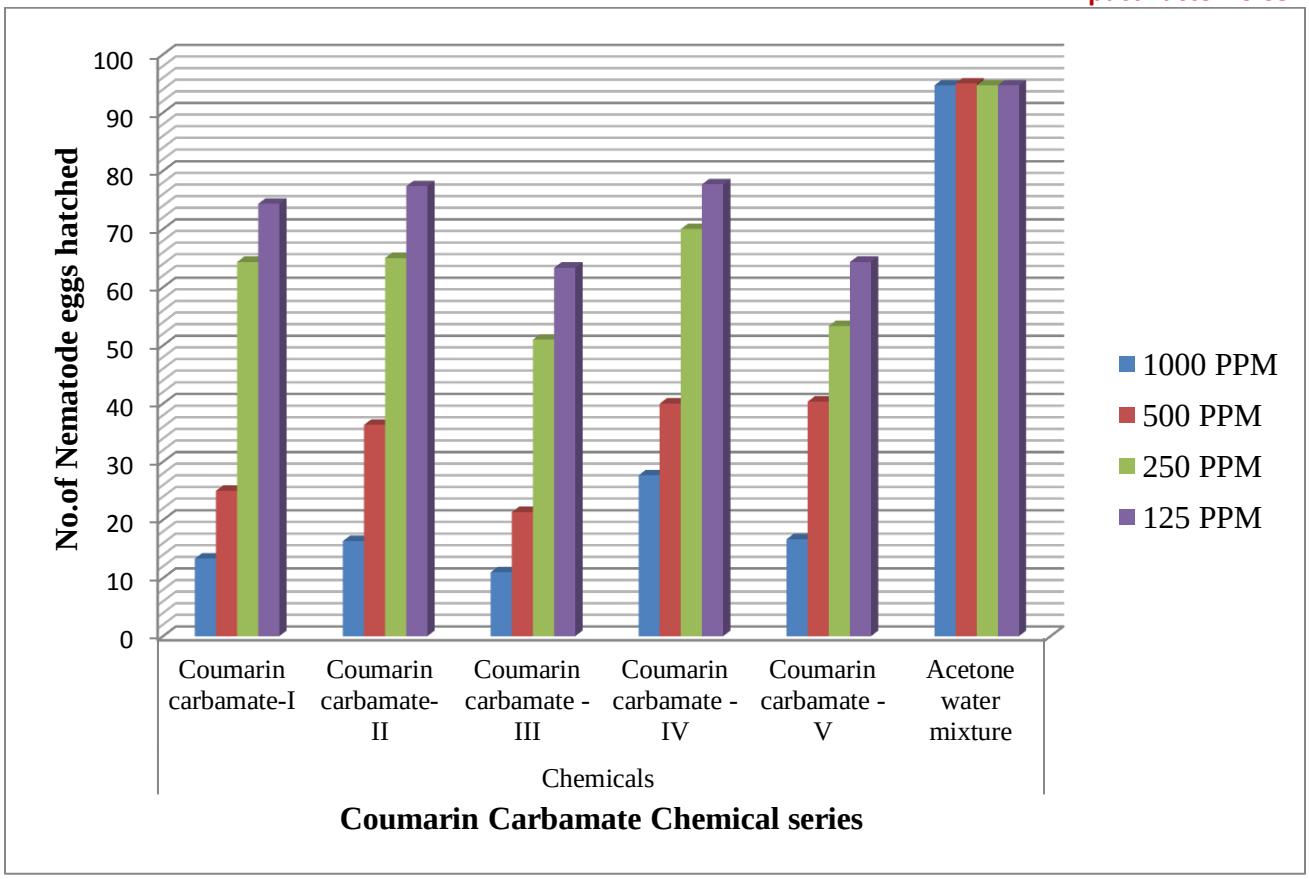

Fig4: Effect of Coumarin Carbamate Chemcial derivatives on egg hatching of *M. graminicola* under *In vitro* conditions

Table 1: Effect of Coumarin derivatives on larval mortality of *M. graminicola*.

Dilutions	Chemicals						
	Coumarin I	Coumarin II	Coumarin III	Coumarin IV	Coumarin 4CR V	Acetone water mixture	Means Of Dilutions
1000 PPM	86.67	25.00	75.00	21.67	26.67	5.33	**40.06**
500 PPM	66.67	23.33	60.00	20.00	20.00	4.67	**32.44**
250 PPM	28.33	19.33	26.67	18.33	16.67	5.33	**19.11**
125 PPM	18.33	16.67	21.67	15.00	18.33	6.00	**16.00**
Means Of Chemicals	**50.00**	**21.08**	**45.83**	**18.75**	**20.42**	**5.33**	

C.D. at 5 % level
Chemicals: 4.08; Dilutions: 3.33; Chemicals X Dilutions: 8.16

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Table 2: Effect of Coumarincarbamate derivatives on larval mortality of *M. graminicola*.

Dilutions	Chemicals						
	Coumarincarbamate-I	Coumarincarbamate- II	Coumarincarbamate - III	Coumarincarbamate - IV	Coumarincarbamate -V	Acetone water mixture	Means Of Dilutions
1000 PPM	86.67	75.00	96.33	70.00	90.00	5.33	**70.56**
500 PPM	66.67	55.00	81.67	55.00	65.00	4.67	**55.22**
250 PPM	28.33	36.67	71.00	36.67	50.00	5.33	**39.11**
125 PPM	18.33	18.33	68.67	16.67	28.33	6.00	**26.61**
Means Of Chemicals	**50.00**	**46.25**	**79.42**	**44.58**	**58.33**	**5.33**	

C.D. at 5 % level
Chemicals: 3.74; Dilutions: 3.06; Chemiclas X Dilutions: 7.48

Table 3: Effect of Coumarin derivatives on hatching of *M. graminicola*.

Dilutions	Chemicals			
	Coumarin I	Coumarin III	Acetone water mixture	Means Of Dilutions
1000 PPM	15.67	10.00	94.67	**40.11**
500 PPM	37.00	24.33	95.00	**52.11**
250 PPM	72.67	55.00	94.67	**74.11**
125 PPM	79.33	69.33	94.67	**81.11**
Means Of Chemicals	**51.17**	**39.67**	**94.75**	

C.D. at 5 % level
Chemicals: 2.20; Dilutions:2.54; Chemicals X Dilutions: 4.40

Table 4: Effect of CoumarinCarbamate derivatives on suppression of hatching of *M. graminicola*.

Dilutions	Chemicals						
	Coumarincarbamate-I	Coumarincarbamate- II	Coumarincarbamate - III	Coumarincarbamate - IV	Coumarincarbamate -V	Acetone water mixture	Means Of Dilutions
1000 PPM	13.33	16.33	11.00	27.67	16.67	94.67	**29.94**
500 PPM	25.00	36.33	21.33	40.00	40.33	95.00	**43.00**

250 PPM	64.33	65.00	51.00	70.00	53.33	94.67	**66.39**
125 PPM	74.33	77.33	63.33	77.67	64.33	94.67	**75.28**
Means Of Chemicals	**44.25**	**48.75**	**36.67**	**53.83**	**43.67**	**94.75**	

C.D. at 5 % level
Chemicals: 3.08; Dilutions: 2.51; Chemiclas X Dilutions: 6.15

Table 5: Effect of Some newer synthetic chemicals on rice plant growth and nematode population of *M. graminicola*.

Treatments	**10 seedling weight (g)**	**Shoot height (cm)**	**Galls per 10 plants**	**Nematode population per root**
Coumarincarbamate-I	2.16	17.17	82.75	1,357.50 (36.86)
Coumarincarbamate- II	2.14	17.28	81.50	1,338.50 (36.60)
Coumarincarbamate- III	2.76	19.10	64.50	1,282.50 (35.83)
Coumarincarbamate- IV	2.35	17.48	70.50	1,318.75 (36.33)
Coumarincarbamate-V	2.71	18.53	64.25	1,255.00 (35.44)
Coumarin I	2.15	17.17	76.75	1,362.00 (36.92)
Coumarin III	2.15	17.31	75.75	1,377.00 (37.12)
Control (nematode alone)	1.56	13.49	118.25	2,682.25 (51.80)
Sterilize soil alone	3.10	21.53	0.00	0.10 (1.05)
C.D. at 5%	**0.13**	**0.32**	**2.87**	**0.33**
SE(m)	**0.04**	**0.11**	**0.99**	**0.11**

*figure in Parenthesis are √n+1 transformed values

