Effect of Saponin as Natural Product on Nematicidal Effect of Oxamyl and Cadusafos Nematicides against Root-Knot Nematode.

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ABSTRACT: The joint action between saponin 0.5 % and two nematicides namely Oxamyl and cadusafos at EC_{50}, EC_{25} and $EC_{12.5}$ was studied against second stage larvae of Root-Knot nematode under laboratory conditions. Apotentiation effect was recorded for ($EC_{12.5}$ of both tested nematicides + saponin 0.5 %) mixtures at all exposure periods (24, 48 and 72 hrs). Also the same trend was noticed with all tested mixtures of oxamyl with saponin (EC_{50} + 0.5 % saponin, EC_{25} + 0.5 % saponin and $EC_{12.5}$ + 0.5 % saponin) after 72 hrs in case of Oxamyl only. The physico-chemical properties of both tested nematicides at EC_{50}, EC_{25} and $EC_{12.5}$ and their mixtures with 0.5% saponin were studied. The obtained data showed an increased in conductivity values and decreased in pH and surface tension values of ($EC_{12.5}$ of both tested nematicides + 0.5 % saponin) mixtures and all tested mixtures of Oxamyl. These data indicated that the saponin 0.5 % change some physico-chemical properties of both tested nematicides at $EC_{12.5}$. These changes enhanced the nematicidal activity of both nematicides at $EC_{12.5}$.The results of this study should may be useful in establishing of field trials to enhance the effectiveness and to reduce the rate of field application.

Keywords: Root-knot nematode, nematicidal efficiency, saponin and joint action.

1.INTRODUCTION

Plant-parasitic Root-Knot nematodes are obligate sedentary endo parasites of many plant species. Their wide host range encompasses more than 3000 plant species (**Abad et al 2003**). Among the Root-Knot nematodes, *Meloidogyne javanica*, *M. incognita*, *M. arenaria* and *M. hapla*, are of major agronomic importance, being responsible for at least 90 % of all damage caused by these nematodes (**Castangnone serena, 2002**).

Nematode management can be maintained at level that does not cause economic loss. There are two broad categories for management practices: chemical and non chemical. The chemicals used earlier to control plant parasitic nematodes were usually fumigant and non-fumigant nematicides . These are not only expensive but also cause environmental pollution, phytotoxicity, contamination of ground water and adversely affect the land and its biotic environment. The dermites of hazardous chemicals have created interest in searching alternate methods for plant parasitic nematodes management (**Singh and Prasad, 2014**). Consequently, use of certain adjuvant agent with synthetic pesticides may provide a tool to improve their performance and increase pesticides bioactivity with decrease in their rates of application (**Betana et al, 2004**).

Saponins are secondary plant metabolites of glycosidic nature that occur in a wide range of plant species. The natural role of saponin in plants is through protection against attack by pathogens and pests. These molecules also have considerable commercial values and are processed as drugs and medicines, foaming agents (**Mohammed, 2010**). The objective of this study was to investigate the role of saponin as additive in decreasing the rate of applications for nematicides by evaluating their nematicidal effect separately and their mixtures with saponin. Also the impact of saponin on the physico-chemical properties of tested mixtures were measured.

2.MATERIALS AND METHODS

1) Nematicides used**:-**

a- Oxamyl (Vydate 24 % SL).FMC
b- Cadusafos (Rugby 20 % SC).FMC

2) Saponin: supplied by Riedel de Haen, Germany.

3) Bioassay**:**

The efficiency of tested nematicides (Oxamyl and Cadusfos) against second stage larvae of *Meloidogyne incognita* was evaluated as follows:-

Four serial concentrations were prepared using water. The suspension of newly hatched second stage larvae in water was prepared. Mean number of 2^{nd} stage larvae in suspension of 1 ml (100 larvae) was added to 1 ml of nematicide to reach the final tested concentration. Each treatment was replicated three times. The estimation of inhibition % was calculated according to effectiveness based on **Abbots formula** (**1925**) after 24, 48 and 72 hours. The obtained data was expressed as toxicity lines. The EC_{50}, EC_{90}, slope value were calculated. Saponin at 0.5 % was mixed with tested nematicides at EC_{50}, EC_{25} and $EC_{12.5,}$ these mixtures were evaluated against 2^{nd} stage larvae by the same bioassay method.

The Co-toxicity factor was calculated according to the equation of (**Mansour et al, 1966**)

$$\text{Co-toxicity factor} = \frac{\text{Observed mortality} - \text{Expected mortality}}{\text{Expected mortality}} \times 100$$

The factor was used to classify results into three categories, apositive factor ≥ 20 was considered potential , negative factor ≤ 20 means antagonism , intermediate value between - 20 and + 20 indicated additive.

4) Physico-chemical properties:

The physico-chemical properties of nematicides solution separately or blends with saponin were determined as the following: pH values and conductivity were measured by the pH, conductivity meter 1484-44,(Dobrat and Martijn 1995) Viscosity was determined by using (Brook field)viscometer according to ASTM D-2196 (2005)where cm poise is the unit of viscosity measurement and surface tension was determined according to ASTM1331(2001) by Du Nouy tensiometer where dyne / cm is the unit of surface tension measurement.

3.RESULTS

Data in table (1) indicated that both tested nematicides recorded a regration relationship between their concentrations and percentages of inhibition of 2nd stage larvae under laboratory conditions after three exposure periods.

Table 1: The nematicidal activity of Oxamyl and Cadusfos against second stage larvae of Root-Knot nematode under laboratory conditions.

Tested concentration ppm	Oxamyl			Tested concentration ppm	Cadusafos		
	% Inhibition after				% Inhibition after		
	Exposure period				Exposure period		
	24hrs	48hrs	72hrs		24hrs	48hrs	72hrs
1	35.2	42.7	23.3	2	11.3	15.6	17.6
2	50.7	61.5	55.6	4	25	28.3	31.3
4	66.5	77.8	84.4	8	44.5	44.5	48.1
8	78.8	89.3	97	16	65.4	61.7	65.2

On the other hand, in most cases the percentages of inhibition were increased by increasing exposure periods with all tested concentrations of both tested nematicides except inhibition percentage in case of Oxamyl at 1 and 2 ppm. In most cases the highest inhibition percentages were recorded after 72 hours with both tested nematicides.

Table 2: Toxicity data of Oxamyl and Cadusafos against second stage larvae of Root-Knot nematodes under laboratory conditions.

Pesticide	Exposure periods (hrs)	EC_{50} ppm	EC_{90} ppm	Lower limit	Upper limit	Slope
Cadusafos	24	9.5	51	4.07	5.36	1.783
	48	9.9	77.3	6.468	8.605	1.455
	72	8.6	65.8	5.42	6.69	1.467
Oxamyl	24	1.9	19.14	2.05	2.53	1.304
	48	1.31	8.6	1.17	1.62	1.586
	72	1.78	4.97	1.57	1.98	2.9

Data in table (2) showed that LCP lines of both tested nematicides against 2nd stage larvae under laboratory conditions after three exposure periods (24, 48 and 72 hrs). Generally Oxamyl was more effective than Cadusafos after three exposure periods. The EC_{50} values of Oxamyl were 1.9, 1.31 and 1.78 at 24, 48 and 72 hrs., whereas it was 9.5, 9.9 and 8.6 after the same exposure periods in case of cadusafos. Also the lowest EC_{50} value was recorded after 72 hours in case of Cadusafos. Whereas it was recorded after 48 hours in case of Oxamyl. On the other hand the sharpest slope value was recorded after 72 hours. In case of Oxamyl 2.9, whereas it was recorded after 24 hours in case of Cadusafos.

Data in table (3) indicated that the joint action between Oxamyl at ($EC_{12.5}$ + saponin 0.5 %) recorded potentiation effect after all periods also the same indication was noticed with all mixtures ($EC_{12.5}$, EC_{25}, EC_{50} of Oxamyl + 0.5 % saponin) after 72 hours.

Table 3: Effect of saponin on potential of Oxamyl against second stage larvae of Root-Knot nematodes under laboratory conditions.

Treatment	Exposure periods					
	24hr		48hr		72hr	
	% I	Co. toxicity factor	% I	Co. toxicity factor	% I	Co toxicity factor
Oxamyl $EC_{12.5}$ + saponin 0.5 %	18	+ 44 potential	16.3	+ 30.4 potential	29.6	+ 136.8 potential
Oxamyl EC_{25} + saponin0.5 %	24.2	- 3.2 additive	18.5	- 26 anta-gonism	41	+ 64 potential
Oxamyl EC_{50} + saponin 0.5 %	18	- 64 antagonism	19.6	- 60.8 antagonism	63	+ 26 potential

% I: percentage of inhibition

On contrast the mixtures of Oxamyl at EC_{25} + saponin 0.5 % and Oxamyl at EC_{50} + saponin showed antagonistic effect after 24 and 48 hours from treatment except oxamyl EC_{25} +saponin0.5% at 24hr.that recorded additive effect.

Table 4: Effect of saponin 0.5 % on the potential of Cadusafos against second stage larvae Root-Knot nematodes under laboratory conditions.

Treatment	Exposure periods					
	24hrs		48hrs		72hrs	
	% I	Co.toxicity factor	% I	Co.toxicity factor	% I	Co.toxicity factor
Cadusafos $EC_{12.5}$ + saponin 0.5 %	16.3	+ 30.4 potential	28	+ 124 potential	32.3	+ 158.4 potential
Cadusafos EC_{25}+ saponin 0.5 %	16.3	- 34.8 antagonism	18.5	- 26 antagonism	19.6	- 21.6 antagonism
Cadusafos EC_{50} + saponin 0.5 %	6	- 88 antagonism	11	- 78 antagonism	84	+ 68 potential

% I: percentage of inhibition

According to data in table (4) the joint action of the saponin 0.5 % and Cadusafos at $EC_{12.5}$ produced different levels of potentiation after the three exposure periods. On the other hand the potentiation effect increased by increasing the exposure periods. Whereas joint action of the other mixtures produced antagonistic effect after all exposure periods except Cadusafos at EC_{50} and saponin 0.5 % after 72 hours.

Data concerning the effect of saponin 0.5 % on the physico-chemical properties of Oxamyl are tabulated in table (5), the results indicated that surface tension and pH values of Oxamyl + saponin mixtures decreased on comparison with Oxamyl only. The highest decrease was recorded at $EC_{12.5}$ (Oxamyl + 0.5 % saponin). On the other hand no considerable changes were found between viscosity of Oxamyl alone and its mixtures whereas the highest viscosity values was recorded in case of mixture ($EC_{12.5}$ Oxamyl + 0.5 % saponin). The mixtures of saponin 0.5 % + Oxamyl EC_{25} and $EC_{12.5}$ showed high conductivity values 800 and 900 μ mhos respectively.

Table 5: Effect of saponin 0.5 % on the physico-chemical properties of Oxamyl at EC_{50}, $EC_{25}$and $EC_{12.5}$.

Treatment	Surface tension dyne/cm	Viscosity cm poise	pH	Conductivity µ mhos
EC_{50} of Oxamyl	78.37	7.1	9.02	0.100
EC_{25} of Oxamyl	81	7.4	9.40	0.100
$EC_{12.5}$ of Oxamyl	83.81	7.13	9.57	400
EC_{50} of Oxamyl + 0.5 % saponin	75.95	7.05	8.80	100
EC_{25} of Oxamyl + 0.5 % saponin	71.5	7.07	8.62	800
$EC_{12.5}$ of Oxamyl + 0.5 % saponin	69.44	7.51	8.57	900
Saponin	77.1	7.5	7.91	990
Water	72	10	7.2	390

EC_{25} and $EC_{12.5}$ alone or in mixtures with saponin 0.5 % were studied in table (6).

Table 6: Effect of saponin 0.5 % on the physico-chemical-properties of Cadusafos at EC_{50}, $EC_{25}$and $EC_{12.5}$.

Treatment	Surface tension dyne/cm	Viscosity cm poise	pH	Conductivity µ mhos
EC_{50} of Cadusafos	77.142857	22.25894	9	0.100
EC_{25} of Cadusafos	77.142857	20.032644	9	0.100
$EC_{12.5}$ of Cadusafos	85.263158	8.034095	9.04	400
EC_{50} of Cadusafos + 0.5 % saponin	70.434783	4.2999637	8.37	100
EC_{25} of Cadusafos + 0.5 % saponin	67.5	4.2304437	8.35	1000
$EC_{12.5}$ of Cadusafos + 0.5 % saponin	70.434783	7.5021158	7.98	1020
Saponin	77.142857	7.5553137	7.91	990
Water	72	10	7.2	390

The physico-chemical properties of Cadusafos EC_{50}, Data indicated that, the surface tension and pH values were decreased in mixtures compared with Cadusafos alone. The highest decrease was recorded at EC_{25} Cadusafos + 0.5 % saponin in case of surface tension and viscosity, whereas it was found with $EC_{12.5}$ + 0.5 % saponin in case of pH. On the other hand no considerable changes in conductivity values were found between EC_{50} and EC_{25} in case of Cadusafos only whereas the highest change in this properties was found between $EC_{12.5}$ and its mixture; the descending order on conductivity was 400 and 1020 µ mhos.

4.DISCUSSION

The obtained data showed that, saponin 0.5 % caused potentiation in effectiveness of both tested nematicides after all exposure periods when it was mixed with $EC_{12.5}$ of both nematicides. Also the same indication was noticed with all tested mixtures after 72 hrs from treatment in case of Oxamyl only. This may be due to saponin ability to form complex with cell membrane chlesterol leading in consequence to pore formation and cell permeabilization (Guthier et al 2009) as well as surface activity responsible for foaming properties which alternated the physico-chemical properties of the tested nematicides and their mixtures table (5) and (6). Generally the surface tension and pH values of $EC_{12.5}$ + 0.5 % saponin mixture was decreased on comparison with the same properties of both tested nematicides at $EC_{12.5}$. Also conductivity of the same mixture was increased upon conductivity of both nematicides only at the same concentration.

Green and **Hale** (**2005**), reported that, the reduction in PH values of formulated solutions led to more attraction between the pesticide particles and treated surface **El-Attal et al** (**1984**) reported that, increase of electric conductivity of insecticide spray solution would led to, deionization of insecticide and increase its deposits and penetration to the treated surface, then cause an increase in insecticidal efficiency. Also **Ryckaert et al** (**2007**) concluded that, the reduction of surface tension of spray solution cause a good wettability, spreading and depositing of particles of that solution on treated surface. From another point of view the obtained data could be discussed depending on the biological effect of saponin that possessed several forms of toxicity against harmful pests (antifeedency, disturbance of mout, growth regulation, etc) the insecticide activity of saponines comes from their interaction with alimentary cholesterol causing disturbance of synthesis of molting hormone (**Chaleb 2012**). According to (**Ibrahim et al, 2014**), no gall formation was recorded on roots of egg plant that was infected by 330 2^{nd} stage larvae of Root-Knot nematodes when treated with 5000 and 10000 ppm from saponin of *Portulacea Oleracea* and *Lantana Camara*, the respective percentage of inhibition in root galling formation was 98.9 and 100 %.

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الملخص العربى

تأثير الصابونين كمنتج طبيعى على الكفاءة النيماتودية للاوكساميل والكاديوسافوس على نيماتودا تعقد الجذور

هالة سعد ابراهيم

المعمل المركزى للمبيدات- مركز البحوث الزراعية

تم دراسة التأثير المشترك ما بين الصابونين بتركيز ٠.٥ % ومبيدا نيماتودا هما الاوكساميل والكاديوسافوس بتركيز النصف والربع والثمن مميت على الطور اليرقى الثانى لنيماتودا تعقد الجذور تحت ظروف المعمل. أظهر مخلوط الصابونين بتركيز ٠.٥ % وكلا من ثمن التركيز المميت للاوكساميل والكاديوسافوس زيادة فى فاعلية المبيدين خلال فترات التعريض ٢٤ و ٤٨ و ٧٢ ساعة وقد تكرر نفس التأثير مع كل الخلائط المختبرة لمبيد الاوكساميل بعد ٧٢ ساعة ومن ناحية اخرى فقد درست الخواص الفيزوكيمائية لخلائط الصابونين مع المبيدين تحت الاختبار وأظهرت النتائج ان هناك زيادة فى درجة التوصيل الكهربى ونقص فى قيمة ال pH والتوتر السطحى للمخلوط (٠.٥ % صابونين + $EC_{12.5}$) مما يوضح سبب زيادة الفاعلية مع هذا المخلوط . حيث ان هذة الصفات الكيمائية تؤدى الى زيادة تبلل السطوح المعاملة وسهولة اختراق المبيد لها.