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Joint Action of Two Pesticides and an Oxidase Inhibitor on The Snail *Lymnaea* Acuminata

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ABSTRACT

Studies were conducted on synergism between an Organophosphate, Nuvan (dichlorvos) mixed with a carbamate, Sevin (carbaryl) in a 1:46 ratio against the snail Lymnaea acuminata. It was found that Sevin enhances the activity of Nuvan and thus the LC values (LC_{10}, LC_{50}, LC_{90}) obtained from this mixture were low as compared to Nuvan or Sevin alone. The acetylcholinesterase (AChE) inhibition was higher in case of Nuvan + Sevin (16.17%) as compared to Nuvan or Sevin (34.7% , 69.1%) alone. When Piperonyl Butoxide (PB), a mixed function oxidase inhibitor was given with Nuvan and Sevin in a 1:46:5 ratio, the LC value decrease still further. It appears that, in the tertiary mixture, while PB reduce the oxidative detoxification of Nuvan and Sevin the carbamate may also be preventing the hydrolysis of Nuvan may tertiary mixture lethal than of its components.

KEYWORD: *Nuvan, Oxidase inhibitor, Pesticide, Sevin, Snail (Lymnaea acuminata)*

INTRODUCTION

Several attempts have been made to control the population of snail *Lymnaea acuminata*, in order to reduce the incidence of fascioliasis in northern part of India. Different synergists like piperonyl butoxide (PB), Sulfoxide (SU), Dimethyl amino aniline (DAA) and MGK - 264 are found to increase the toxicity of various synthetic pesticides viz. carbamates, organophosphates and pyrethroids against the snail *L. acuminata*[3-8].

Sahay et al.[9] and sahay and Agarwal[10] have reported that even mixtures of two pesticides like a pyrethroid, Decis (deltamethrin) with the carbomate, sevin (carbaryl) and another pyrethroid, tenvalerate with the organophosphate phorate act synergistically when given to the snail *L. acuinata*. in a carbamate/organophosphate in case of snails, it has been also reported[11] that the activity of an organophosphate compound malathion is potentiated by a carbamate, sevin against *Musca domstica.*

Recently 12,8 found that tertiary mixtures of two pesticides and one synergist are very effective against the snail *L. acuminata*. As compared to binary mixtures of pesticides/synergists lower doses of pesticides were required in the tertiary mixture.

In the present study the toxicity of a binary mixture of the organophosphate Nuvan (dichlorvos, 0-0 Dimethyl, 2,2-dichlorovinyl phosphate) with a carbamate sevin carbaryl, 1-naphthalenyl methyl, in a 1:46 ratio and a tertiary mixture of Nuvan and sevin along with a mixed function oxidase inhibitor piperonyl butoxide (PB), 5-2 (2- butoxy ethoxy) ethoxy methyl 1-6- propyl-1, 3-benzodioxole, were given in a

1:46:5 ratio against the snail *Lymnaea acuminata*. Studies were also conducted on sublethal exposure of 40% of 24 h LC_{50} of sevin, Nuvan + sevin (1:46) and Nuvan + sevin + PB (1:46:5) on the in vivo inhibition of acetyl-cholinesterase (AChE) found in the nervous tissue of snail L. acuminate of acetyl-cholinesterase (AChE) found in the nervous tissue of snail *L. acuminata*.

EXPERIMENTAL METHODS

Adult *Lymnaea acuminata* (2.25 ± 0.3 cm) were collected from local ponds and pools. The animals were stored in glass aquaria containing dechlorinated tap water and kept for 72 h in order to acclimatize them to laboratory conditions. The aquaria were well aerated and covered with wire netting to prevent the animals from escaping. Dead animals were removed from the aquaria as soon as possible to prevent the contamination of water. The experiments were carried out within a temperature range of 26°C to 29° C.

2.1 Toxicity estimation

After acclimatization 10 snails were transferred to experimental glass aquaria containing 3 L dechlorinated tap water. The animals were exposed to 6 different concentrations viz., 0.02, 0.05, 0.09, 0.2, 0.5 and 0.7 mg/ l of Nuvan (Hindustan, Ciba - Geigy Ltd.) and Sevin (Union Carbide India Ltd.) mixed in a 1:46 ratio and 6 different concentration viz., 0.001, 0.005, 0.008, 0.01, 0.03 and 0.05 mg/1 of a mixture of Nuvan, Sevin and PB (McLaughlin Gormley King Co. U.S.A.) in a 1:46:5 ratio, six aquaria were set up for each concentration. This ration was determined after a series of pilot experiments. Doses given are the final concentration of active ingredients in the aquarium water. Control groups were kept along side in a similar manner without any treatment. The animals were exposed for a period of 96 h and mortality counts were recorded every 24 h. Dead animals were removed from the aquaria every day in ordr to prevent contamination of water. Probit-log analysis[13] was carried out in order to calculate the LC values for different exposure period using a computer programme 'POLO'[14]. This programme also calculates the Upper (UCL) and lower confidence Limits (LCL) of LC values, heterogeneity factor of the sample and fiducial probability limits at 90, 95 and 99%. Using the data obtained from probit analysis further statistical analysis was carried out in order to assess the expected mortality (E.M.) of the mixtures assuming that the ingredients do not influence each other's activity and comparing in with the mortality actually observed (O.M.) after exposing the snails to the binary and tertiary mixtures. This analysis was carried out with the help of another computer programme 'MIX'[15].

ACETYLCHOLINESTERASE (AChE) ESTIMATION

Using the data obtained from the toxicity experiments, (Table -1) snails were treated from 24 h with 40% LC_{50} of Nuvan + Sevin and Nuvan + Sevin + PB mixture. The concentration of sovin were based on the 24 h LC_{50} reported earlier by sahay. According the experimental snails were exposed for 24 h to 9.45 mg/1 of Sevin; 0.058 mg/1 of Nuvan+Sevin to a 1:46 ratio and 0.036 mg/1 of ratio. The control snails did not receive any treatment. After 24 h the snails were removed from the aquaria and washed with fresh water. AChE activity was measured according to the method of Ellman et.al.[16] as modified for snails by Singh et. al.[17]The 250 milligram of nervous tissue around the buccal mass was homogenized in 5 ml of 0.1 M phosphate buffer, pH 8, for 5 min in an ice bath and centrifuged at 1000 g for 30 min in a refrigerated centrifuge maintained at -4°C. Supernatants were used as enzyme source. Enzyme activity at 25°C was measured in a 10 mm path-length cuvette using an incubation mixture consisting of 2.9 ml of 0.1 M phosphate buffer pH 8.0, 0.1 ml of enzyme-containing supernatant, 0.1 ml of chromomeric agent DTNB (5:5 dithiobis-2-nitroben-zoate), and 0.02 ml of freshly prepared acetyl-thio-choline iodide solution in distilled water. The change in optical density at 412 mμ was monitored for 3 min. Enzyme activity has been ex-pressed as μm "SH" hydrolyzed $min^{-1}mg^{-1}$ protein. Protein estimation was carried out by the method of Lowry et al. [18]. Each experiment was replicated at least six times and values have been expressed as means ± SE.

Students's 't' test was applied to determine significant difference ($P<0.05$) between treated and control animals.

TABLE-1: Table showing Probit-log analysis of mortality data following treatment of *Lymnaea acuminata* with Nuvan + Sevin (1:45) and Nuvan + Sevin + PB (1:46:5) for 24, 48, 72 and 86 h exposure.

Exposure period	Pesticides	LC_{10} (mg/1)	LC_{50} (mg/1)	Limits LC_{50} (mg/1)	LCT	LC_{90} (mg/1)	Hetero-geneity	(g)
24 h	Nuv+Sev Nuv+Sev+PB	0.041 0.003	0.145 0.009	0.171 0.011	0.123 0.008	0.510 0.032	0.47 0.92	0.027 0.061
48 h	Nuv+Sev Nuv+Sev+PB	0.018 0.001	0.089 0.006	0.109 0.005	0.449 0.036	0.449 0.036	0.31 0.61	0.043 0.054
72 h	Nuv+Sev Nuv+Sev+PB	0.011 0.00006	0.00 64 0.005	0.041 0.003	0.257 0.027	0.26 0.49	0.26 0.49	0.049 0.056
96 h	Nuv+Sev Nuv+Sev+PB	0.009 0.00 03	0.039 0.002	0.048 0.003	0.030 0.002	0.179 0.016	0.10 0.47	0.054 0.061

Abbreviation : UCL = Upper Confidence Limits, UCL = Lower Confidence Limites, Nuv= Nuvan, PB=Piperonyl butoxide, Sev = Sevin, 9 = Fiducial limites.

RESULTS

Table 1 shows the LC_{10}. LC_{50} and LC_{90} values for 24, 48, 72 and 96 h of a mixture of Nuvan + Sevin and Nuvan + Sevind + PB in a ration of 1:46 and 1:46:5 respectively. This table brings out that the LC values of the binary mixture, e.g. the LC_{50} for Nuvan+Sevin were 0.145, 0.052 and 0.039 mg/1 as copared to 0.009, 0.006, 0.004 and 0.002 mg/1 of Nuvan+Sevind + PB for 24, 48, 72 and 96 h respectively.

Calculation of expected mortality (E.M.) for 24, 48, 72 and 96 h caused by exposure to Nuvan and Sevin and observed mortalities caused by the binary mixture of Nuvan+Sevind (1:46) at hoses ranging from 0.02, to 0.07 mg/1 have been given in Table 2. The calculation for E.M. are based on the assumption that the two chemicals do not affect each other activity. It is clear from the their expectd mortalities e.g. after an exposure of 0.7mg/1 for 24 h, all the test animals died against an expectation of only 85% death. Likewise, against an expectation of 57.9% mortality after 96 h of exposure from a dose of 0.09 mg/1, 76% animals actually died during the experiment.

TABLE-2: Table showing expected percent mortality of *Lymnaea acuminata* from Nuvan and Sevin and the actual observed observed percent mortal when a Nuvan + Sevind mixture was given in a nation of 1 : 46

Dos (mg/1)	24 h		48 h		72 h		96 h	
	E.M.	O.M.	E.M.	O.M.	E.M.	O.M.	E.M.	O.M.
0.62	1.99 %	3.3 %	R.10 %	13 %	14.9 %	23%	20.8 %	30 %
0.65	10.4 %	13 %	23.1 %	27 %	35.1 %	47%	41.9 %	57 %
0.09	22.9 %	30 %	38.1 %	53 %	51.9 %	67%	57.9 %	76 %
0.2	48.2 %	63 %	61.6 %	77 %	74.1 %	87%	77.9 %	90 %
0.5	77.5 %	83 %	84.3 %	90 %	91%	92.2 %	92.8 %	100 %
0.7	85.4 %	100 %						

Abbereviations : E.M. = Expected percent mortality from Nuvan and Sevin.

O.M. = Observed percent mortality from Nuvan + Sevin.

Table 3 given the expected mortalities (E.M.) and observed mortalities (O.M.) of the six doses of the tertiary mixture of Nuvan+Sevin+PB ranging from 0.001 mg/1 to .05 mg/1, when the snails were exposed fro 24, 48, 72 and 96 h. It can be seen that while no mortality was expected from 0.03 mg/1, of the mixture 83% of the animals actually died after 24 in exposure. Likewise, against an expectation of 0.03% deaths after 24 h from a dose of 0.05 mg/1, all the animals died during this period. Similarly a dose of 0.005 mg/1 was expected to cause only 0.01% mortality after 96 h of exposure, but 57% of the animals actually died during this period. A similar trend i seen at all the doses and all exposure period. The effect of the tertiary mixture was also time and dose dependent.

TABLE -3: Table showing the synergistic effect of PB on a mixture of Nuvan + Sevin. It shows the expected percent mortality of *Lymnaea acuminata* from Nuvam + Sevin (1:46) and the actu7al observed percent mortality when Nuvan and Sevin was given with PB in a ratio of 1 : 46 : 5.

Dos (mg/1)	24 h		48 h		72 h		96 h	
	E.M.	O.M.	E.M.	O.M.	E.M.	O.M.	E.M.	O.M.
0.001	Nil	3.3 %	Nil	17 %	Nil	23 %	Nil	37 %
0.005	Nil	17 %	Nil	30 %	0.01 %	43 %	0.01 %	57 %
0.008	Nil	40 %	0.006 %	53 %	0.04 %	63 %	0.04 %	77 %
0.01	Nil	63	0.02	77	0.07	83	0.08	87

		%	%	%	%	%	%	%
0.03	Nil	83 %	0.37	90 %	1.09 %	93 %	1.37 %	100 $
0.06	0.03 %	100 %						

Abbereviations : E.M. = Expected percent mortality from Nuvan and Sevin.

O.M. = Observed percent mortality from Nuvan + Sevin + PB.

TABLE -4: Activity of Acetyl-cholinesterase in the nervous tissue of the snail after injection of 40% of 24 h of Sevin, Nuvan + Sevin (1:46) and Nuvan + Sevin + PB (1:46:0)Acetyl-cholinesterase activity mol "SH" hydrolyzed mg^{-1} protein min^{-1}

40 % of 24 h LC_{50}	Amount Nuvan (mg/1)	Actually injected Sevin (mg/1)	PB (mg/1)
Control	None	None	None
$Nuvan^{-1}$	0.0084	None	None
Sevin	None	9.452	None
Nuvn + Sevin (1:46)	0.00124	0.05704	None
Nuvan + Sevin + PB (1 : 46 : 5)	0.00007	0.00322	0.0035

Exposure of snails to 40% of 24 h LC_{50} of Sevin : Nuvan + Sevin (1:46) and Nuvan + Sevin + PB (1 : 46 : 5) induced a significant ($P<0.05$) dose dependent inhibition of AChE activity in the nervous tisse of snail Lymnaea acuminate (Table 4). This table shows that 24 h exposure to Nuvan to Sevin caused a reduction in the activity of AChE present in the nervous tissue of Lymnaea acuminata. Reduction caused by Nuvan was mor than the reduction caused by Sevin. Treatment with 40% of LC_{50}. of Nuvan+Sevin caused 84% reduction in the activity of AChE. Highest reduction in the activity of this enzyme was caused by the tertiary mixture of Nuvan + Sevind + PB.

DISCUSSION

It has been reported[8] that Nuvan is the most potent organophosphates against the snails Lymnaea acuminata. The 24, 48, 72 and 96 h LC_{50} of this compound is 0.021, 0.014, 0.009 and 0.007 mg/1 respectively. The carbomate Sevin too is a powerful

The major route of detoxification of the carbamate Sevin is generally believed to be the oxidation of the compound while major pathway for the detoxification of Nuvan is the hydrolysis of ester bond[19]. Tripath ann Agarwal[8], however, have reported that addition of piperonyl butoxide, a mixed function oxidase inhibitor has a synergistic effect on Nuva as well. It seems that as in other organaphosphates, oxidative degradation any also be an important rout for the detoxification of Nuvan as well[19]. Because of this a concentration of only 0.00007 mg/1 of Nuvan and 0.00322 mg/1 of Sevind together with PB can caused greater inhibition of AChE (9.55 of control) as compared to 0.00124 mg/1 of Nuvan and 0.05704 mg/1 of Sevin in Nuvan + Sevin mixture (16.17 of control) (Table 4).

It is generally believed that both carbomates and organophosphates are lethal nerve poisons bcaused they cause inhibition of AChE leading to the accumulation of ACh at the synapses. It the present investigation it was found that the extent of AChE leading to the accumulation of ACh at the synapses. It the present investigation it was found that the extent of AChE inhibition however, was not the same even when doses of comparable toxicity i.e. 40% 24 h LC_{50}. were given. This shows that AChE inhibition alone may not be responsible for the death of the snails. It has been domenstrated that sevin, in addition to being a AChE inhibitor, also inhibits other esterases e.g. alkalike phosphateses and trypsin[5,12]. Since alkaline phosphatase plays a critical role in the synthesis of certain enzymes, formation of sheell and other secretary activities in gastropods, its inhibition may produce a variety of metabolic disturbances in Lymnaea acuminata[21]. Morever, in the tertiary mixture PB would be preventing the detoxification of both Nuvan and Sevin while sevind owing to its esterase inhibiting character may also be preventing the hydrolysis of Nuvan.

This might be the reason for the greater toxicity of the tertiary mixture. The above study suggest that tertiary mixtures of pesticides and synergists would can be applied for the control to harmful pests. This would beneficiation as low doses of pesticides would be released in the environment.

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