

POTENTIATION EFFECT OF CERTAIN NATURAL COMPOUNDS TO CHLOROPHACINON TOXICITY AGAINST BLACK RAT *RATTUS RATTUS*

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Abstract

The potentiation effect of four natural compounds i.e. Oshar and Neem plant extracts, Vertimec and Spinosad biocides to Chlorophacinon anticoagulant toxicity was evaluated against black rat *Rattus rattus* under laboratory and field conditions. Results showed that when each compound was tested alone in both non-choice and free choice feeding methods, Oshar plant extract was the most effective one followed by Vertimec biocide and Neemix plant extract while Spinosad biocide did not exhibited noticeable effect. Concerning the palatability, the tested compounds could be arranged according to their acceptance in a descending order as follows : Spinosad > Oshar > Vertimec > Neemix. Regarding the potentiation effect, each tested compound was added at level 10 ml/ kg to chlorophacinon bait 0.005%. Oshar plant extract considerably enhanced the toxic effect of Chlorophacinon than Chlorophacinon alone followed by Vertimec and Neemix. On the other hand, Spinosad has no effect on the toxicity of Chlorophacinon. Also, the results which obtained from the field experiments were in complete harmony with the laboratory results as the combination of Chlorophacinon and Oshar gave the highest rate of population reduction against the black rat *Rattus rattus* followed by Vertimec while the mixture of Chlorophacinon with Neemix gave the lowest rate.

INTRODUCTION

Recently much effort has been put into screening and evaluation chemicals having different pharmacological and physiological modes of action to combat resistant and non-resistant rodents (Gabr, 1997). Some potentiators have been introduced to overcome the anticoagulant resistant rodents such as house mouse *Mus musculus*, roof rat *Rattus rattus*, Norway rat *Rattus norvegicus* and other rodent species. Also, these compounds have been employed as a proprietary compounds in combination with Warfarin to control rodent populations which developed resistance to Warfarin (Muktha *et al.*, 1978). However, very little information is available regarding the usage of additives, capable of enhancing the action of anticoagulant rodenticides and increase its toxicity. Therefore, the present study was conducted to evaluate the potentiation effect of some natural compounds, i. e. Oshar and Neem crude plant extracts, Vertimec and Spinosad biocide compounds on the toxicity of Chlorophacinon anticoagulant rodenticide against black rat *Rattus rattus*, one of the most common and most harmful rodent species in Egypt.

MATERIALS AND METHODS

1- Tested Compounds:

1-1- Chlorophacinon:

Master mix of Chlorophacinon anticoagulant rodenticide (0.5%) was supplied from KZ Company. It was mixed with crushed maize at 0.005% concentration as a feeding bait.

1-2- Oshar crude plant extract:

Oshar plant *Calotropis procera* was obtained from Aswan governorate, the plant leaves were dried and grounded. 150 g of powder were extracted with chloroform according to procedure of Freedman *et al.* (1979).

1-3- Neemix 4.5%:

Ready made crude plant extract of Neem *Azadirachta indica* was used. It was supplied by Agri Dyne Technologies INC.

1-4- Vertimec 1.8% E.C. (Abamectin):

Biocide compound produced by the soil microorganism, *Streptomyces avermitilis*, was supplied by Syngenta Company.

1-5- Spinosad 240 SC:

Biocide compound produced by the soil microorganism, *Saccharopolyspora spinosa*, was supplied by Dow Agro Sciences Company.

2- Tested Animals:

The black rats *Rattus rattus* were trapped from different locations of Giza and Kaliobyra governorates. Animals were transported to laboratory and caged individually for two weeks for acclimatization and fed on a free diet and water. The unhealthy and pregnant animals were excluded. Ten healthy adult animals were used for each test.

3- Non-choice feeding method:

Animals were divided into 5 groups (each of 10 rats). One group for each tested compound and another one as a control. Each animal was offered 50 g crushed maize mixed with one of the tested compounds at rate 10 ml / kg for 4 successive days. The consumed amount of bait was daily estimated. The treated bait was removed and the survivor animals were fed on untreated crushed maize and observed for 28 days. During this period, mortality was recorded.

4- Free choice feeding method:

Free choice feeding test is important to determine the acceptability of each tested compound by comparing its consumption with that of challenge diet (65% crushed maize + 25% ground wheat + 5% sugar + 5% corn oil) according to

Palmateer (1974). One of each tested compound and challenge diet were offered to each rat (50 g of each) in small separate dishes. Their position was daily altered to avoid feeding preference for a certain location. The consumed amount of bait and diet was recorded daily for 4 successive days. The same previous proceeding was followed. Bait acceptance was recorded as follows:

$$\text{Acceptance \%} = \frac{\text{Consumed amount of treated bait}}{\text{Consumed amount of treated bait} + \text{challenge diet}} \times 100$$

5- Potentiation effect:

The potentiation effect of Oshar, Neemix, Vertimec and Spinosad to the toxicity of Chlorophacinon anticoagulant was tested against black rat *Rattus rattus*. Animals were divided into five groups. One of them was fed only on Chlorophacinon bait 0.005%. The rest four groups were fed on mixture of Chlorophacinon bait + one of the four examined compounds at rate of 10 ml/ kg for 4 successive days using free choice method. Animals were observed for 28 days. Acceptance and mortality rates as well as time to death were determined.

6- Field Evaluation:

Chlorophacinon anticoagulant alone and in combination with one of each tested compounds were evaluated under the field conditions of Qaha district, Kaliobya governorate. An area infested with the black rat *Rattus rattus* was chosen and divided into 5 plots (each of one feddan) represented the number of tested compounds and another plot was left without treatment as a check control. The population density of rodent was estimated pre and post treatment according to Dubock (1984). Two kg of each tested compound were divided into plastic sacks (200 g of each) and distributed in every plot and exposed for two weeks. The consumed amount of each tested compound was recorded. The percentage of population reduction was calculated as follows :

$$\text{Population reduction \%} = \frac{\text{Pre treatment consumed} - \text{Post treatment consumed}}{\text{Pre treatment consumed}} \times 100$$

RESULTS AND DISCUSSION

1- Laboratory Studies :

The toxic effect of 4 natural compounds i. e. Oshar and Neem plant extracts, Vertimec and Spinosad biocides was studied against black rat *Rattus rattus* using non and free choice methods.

1-1- Non-choice feeding test:

Data in Table (1) showed that Oshar plant extract gave complete mortality 100% with average bait consumption 10.5 g followed by 60 and 20% mortality for Vertimec biocide and Neemix plant extract with 12.6 and 3.4 g bait consumption, respectively. The lowest mortality percentage was only 10% in case of Spinosad biocide although a considerable amount of bait consumption 14.2 g were uptaken. A considerable variation in the average time required to death was observed, whereas it was 5.8, 10, 12.5 and 11 days for Oshar, Neemix, Vertimec and Spinosad, respectively. This mean that Oshar plant extract killed the animals in short time, while the opposite was observed with Vertimec biocide.

1-2- Free choice feeding test:

The efficacy of the tested compounds in free choice test was shown in Table (2). Data indicate that Oshar plant extract induced 80% mortality followed by 40% for Vertimec biocide, while both Neemix plant extract and Spinosad biocide fail to achieve any mortality percentage. Regarding the mean of time required to death, results revealed that it was 8.6 days for Oshar extract and 11.7 days for Vertimec biocide. On the other hand, the palatability of rats to baits which treated with the four tested compounds, a wide variation was observed as it ranged between 7.2% for Neemix and 31.4% for Spinosad. The tested compounds could be arranged according to their acceptance in a descending order as follows: Spinosad > Oshar > Vertimec > Neemix.

From the previous results it could be concluded that Oshar plant extract proved to be the most effective one against rats followed by Vertimec biocide. Ibrahim (2001) found that Oshar leaves extract was the most effective one followed by Datura while Black pepper seed extract exhibited the lowest toxic effect against albino rat. Also, Gabr *et al.* (2004) recorded that in both non and free choice feeding tests, Oshar plant extract was most effective against albino rat. Keshta (2003) stated that Vertimec palatability was 36.1 and 35.6% for male and female albino rats, respectively. Also, he mentioned that Vertimec gave 80 and 30% mortality in the non and free choice feeding test with time to death of 9 and 16 days, respectively. Ogugo *et al.* (1997) noticed that Neem products caused 7 to 20% mortality for two rodent species in Kenya and reduce the rat body weight with 12.6%. They also concluded that Neem products reduce the consumption of maize grain by rats as a result of their repellent effect. Abd El-Maksoud (2005) indicated that the palatability of Neem plant extract was more than 30% for both albino rats and mice.

1-3 - Potentiation Effect:

The potentiation effect of the four tested compounds to Chlorophacinon anticoagulant was evaluated against black rats. Data in Table (3) revealed that when 0.005% Chlorophacinon bait was used alone, only 60% of animals were killed with time to death 8.6 days. When Oshar plant extract was added to Chlorophacinon bait at 10 ml/kg, all the examined animals were killed and the time required to death reduced to 6.5 days while acceptance rate was 33.8%. Also, the combination of Vertimec biocide with Chlorophacinon raised the mortality to 80% and the time required to death to 9.7 days while its acceptance rate was 28.4%. On the other side, Spinosad has no effect on Chlorophacinon toxicity with 33.5% acceptance and required time to death of 8.2 days. On the contrary, Neemix took an adverse way as it caused only 30% mortality with 7.8 days time to death and reduced the acceptance to 14.7% comparing with 34.2% for Chlorophacinon alone.

Generally, it noticed that Oshar plant extract and Vertimec biocide raised the toxic effect of Chlorophacinon anticoagulant while Spinosad and Neemix plant extract had no and opposite effect, respectively.

2- Field Performance:

The potentiation effect of the same tested compounds when combined with Chlorophacinon was tested against the black rat *Rattus rattus* under the field conditions of Kaliobya governorate. Results in Table (4) showed that the mixture of Oshar plant extract with Chlorophacinon was the most effective one as it caused 91.2% rat population reduction followed by 87.4 % for Vertimec in comparison with 72.8% for Chlorophacinon when applied alone. The combination of Spinosad with Chlorophacinon caused 71.0% population reduction while that of Neemix reduced these ratio to 44.3%. Regarding the consumed amount per feddan, it was observed that the highest amount combination (1505 g) was of Chlorophacinon bait when applied alone followed by 1470, 1450 and 1285 g for Spinosad, Oshar and Vertimec, respectively, while the lowest amount (320g) was recorded in case of Neemix - Chlorophacinon combination.

Discussing the aforementioned results, it could be noticed that the field performance was in harmony with laboratory results as the combination of Oshar plant extract with Chlorophacinon bait considerably enhanced the efficiency of Chlorophacinon anticoagulant followed by Vertimec biocide. These results may be lead to overcome the tolerance of rodent to anticoagulants and may help in the future in critical situation. Similar observations were reported by Greaves *et al.* (1974) and Muktha *et al.* (1978). They found that mixture of lower dosages of calciferol in combination with Warfarin bait resulted in not only a complete kill but reduced the death time considerably as compared to either toxicant alone. Also, Muktha (1979) and Gabr (1997) reported that the increased mortality of roof rat when L-histidine was combined with low dosages of Warfarin could alternatively be due to the simultaneous

action of the two compounds on the blood vascular system. Kandil *et al.* (1991) mentioned that sulphaquinoxaline enhanced the efficiency of the anticoagulant when it was given daily for 4 to 7 days before treatment with the anticoagulant.

Table 1. Effect of certain natural compounds against black rat *Rattus rattus* using non-choice feeding test.

Compound	Average bait consumption (g)	% Mortality	Time to death (day)	
			Range	Mean
Oshar	10.5	100.0	5 - 10	5.8
Neemix	3.4	20.0	8 - 12	10.0
Vertimec	12.6	60.0	9 - 15	12.5
Spinosad	14.2	10.0	—	11.0

Table 2. Effect of certain natural compounds against black rat *Rattus rattus* using free choice feeding test.

Compound	% Acceptance	% Mortality	Time to death (day)	
			Range	Mean
Oshar	29.6	80.0	6 - 11	8.6
Neemix	7.2	0.0	—	—
Vertimec	27.3	40.0	9 - 17	11.7
Spinosad	31.4	0.0	—	—

Table 3. Potentiation effect of certain natural compounds to 0.005% Chlorophacinon anticoagulant against black rat *Rattus rattus*.

Treatment	% Acceptance	% Mortality	Time to death (day)	
			Range	Mean
Chlorophacinon alone	34.2	60	6 - 10	8.6
Chlorophacinon + Oshar	33.8	100	5 - 12	6.5
Chlorophacinon + Neemix	14.7	30	6 - 14	7.8
Chlorophacinon + Vertimec	28.4	80	7 - 15	9.7
Chlorophacinon + Spinosad	33.5	60	6 - 11	8.2

*All the potentiator compounds were added to Chlorophacinon bait at the rate of 10 ml/kg.

Table 4. Field evaluation of combination of 0.005% Chlorophacinon anticoagulant with certain natural compounds against black rat *Rattus rattus*.

Treatment	Bait consumption (g) / Feddan			% population reduction
	Pre - treatment	Treatment	Post - treatment	
Chlorophacinon alone	1880	1505	510	72.8
Chlorophacinon + Oshar	1250	1450	110	91.2
Chlorophacinon + Neemix	1470	320	818	44.3
Chlorophacinon + Vertimec	1635	1285	206	87.4
Chlorophacinon + Spinosad	1140	1470	330	71.0

*All the potentiator compounds were added to Chlorophacinon bait at the rate of 10 ml/kg.

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التأثير التنشيطي لبعض المركبات الطبيعية علي سمية مبيد الكلوروفاسينون
ضد الفأر المتسلق *Rattus Rattus*

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معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقي - جيزة - مصر

تم تقييم التأثير التنشيطي لأربعة من المركبات الطبيعية هي أثنين مستخلص نباتي (مستخلص نبات العشار ومستخلص نبات النيم) وأثنين مبيد حيوي (فيرتيميك وسباينوساد) لمبيد القوارض المضاد للتجلط كلوروفاسينون ضد الفأر المتسلق *Rattus Rattus* تحت الظروف المعملية والحقلية . عند إختبار كل من هذه المركبات علي حدة أوضحت النتائج أنه في كل من اختباري التغذية الإختياري واللاختياريه فأن مستخلص نبات العشار كان الأعلى تأثيراً يليه الفيرتيميك ثم مستخلص نبات النيم بينما كان المبيد الحيوي سباينوساد كان أقلهم تأثيراً . بالنسبة لإختبار نسبة الإستساعة فيمكن ترتيب المركبات علي حسب نسبة الإستساعة تنازلياً كالآتي : سباينوساد < العشار < فيرتيميك < النيم .

وبالنسبة لإختبار التأثير التنشيطي فعند إضافة كل مركب علي حدة بنسبة ١٠ مللي / كجم علي طعم الكلوروفاسينون ٠,٠٠٥ % فأن مستخلص نبات العشار رفع التأثير السام للكلوروفاسينون عنه في حالة إستخدام الكلوروفاسينون فقط يليه الفيرتيميك ثم النيمكس بينما المبيد الحيوي سباينوساد لم يكن له أي تأثير علي زيادة سمية الكلوروفاسينون . وكانت النتائج الحقلية متوافقة مع النتائج المعملية حيث أعطي خليط الكلوروفاسينون مع العشار أعلي نسبة خفض في تعداد القوارض يليه خليط الكلوروفاسينون مع الفيرتيميك بينما أعطي خليط الكلوروفاسينون مع النيمكس أقل نسبة خفض في التعداد.