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RELATIVE SUSCEPTIBILITY OF *RATTUS RATTUS* POPULATIONS TO WARFARIN ANTICOAGULANT

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Abstract

Variations in the susceptibility were found in different *Rattus rattus* populations reared either in areas exposed to the extensive anticoagulant treatment for long time before or in areas its back history ensured that the anticoagulants were not applied for at least three years at Menofia, Kafr El-Sheikh and Benisuef Governorates. Therefore, series of field and laboratory trials were done. Results showed that rat populations in treated areas exhibited noticeable tolerance to warfarin when applied in the field as compared with those in areas free from any anticoagulant treatments. Warfarin baits induced 57.5 & 90.0; 81.5 & 95.8 and 87.2 & 93.4% reduction in rat populations in fields treated before or not at the three governorates.Data of laboratory trials supported those obtained from field experiments, whereas warfarin toxic effect was considerably lower against animals populations collected from agricultural lands treated before than those from untreated ones at the three governorates, i.e. LD50 values were 90.5,88.0 and 79.4 mg a.i./kg b.wt. for animals from the first areas in comparison to 79.4, 82.5 and 75.0 mg/kg b.wt. for animals from untreated areas, respectively.

A parallel trend was measured against warfarin administration as bleeding time (B.T.) and prothrombin time (P.T.) did not considerably alter before and after warfarin treatment in the case of animals exposed for anticoagulant treatment before, while a noticeable prolongation in their values was observed after warfarin treatment in case of animals obtained from unreated areas.

INTRODUCTION

Rodents are the most important vertebrate pests in the wold. Food loss due to rodent has been assessed as 33 million metric ton per year of stored grains (mjoe, 1969). Egypt suffered from rodent problem in agricultural areas at the beginning of the 1980's. Anticoagulant rodenticides have been used for the purpose of rodent control allover the world. These compounds have two main advantages over the older acute poisons; firstly they do not induce bait shyness, secondly safe in the use.

All the anticoagulant rodenticides are of two chemical groups, i.e. hydroxycoumarine and indanedione derivatives. All anticoagulant rodenticides differ in their chemical structure but exert the same mode of action, i.e. preventing the normal mechanisms that controls blood clotting rendering blood incapable of coagulation and causing death which generally results from internal haemorrhage. The toxicity symptoms take some days to develop, therefore, bait shyness does not occur.

Different species of rodents vary in their natural level of resistance to anticoagulant rodenticides. Accordingly, it is important to distinguish clearly between species that evolve a new and abnormal level of resistance and those possessed initially a high level of natural resistance.

In Egypt, anticoagulant rodenticides have long been used on a large scale to control rodents. However, there are very little, if any, studies carried out to determine the susceptibility level of the Egyptian commensal rodents after this long and extensive use of anticoagulants (Kandil et al., 1994; Gabr, 1997).

Previous studies made by Harmful Animal Department, plant protection Research Institute, A.R.C., Egypt revealed that the roof rat *Rattus rattus* has the upper hand among rodent fauna of most agricultural lands (Hussein, 1991; Ibrahim, 1995; Mikhail, 1995; Youssef, 1996; Mourad, 1997; Gabr, 1997).

The present trials were conducted to detect the relative susceptibility of *R.rattus* populations infested areas treated before with anticoagulant in comparison to those in area free from any previous anticoagulants treatment.

MATERIALS AND METHODS

Rodenticides

Warfarin: active ingredient (98%),water soluble, was obtained from KZ Pesticides Company, Egypt. Chemical name: 3- (α-acetonylbenzyl)-4-hydroxy coumarine.

1. Field Experiment

The field trials were conduted in orchards at Quesna district (Menofia Governorate), Sakha area (Kafr El-Sheikh Governorate) in Lower Egypt and El-Wasta district (Beni-Suef Governorate) in Middle Egypt. In each district, two orchards were assigned, the first its back history showed that it was treated with anticoagulant rodenticides for a long time, while the other free from anticoagulant treatment at least three years before. In orchards of each district, the population density of rats was estimated before and after application using crushed maize consumption (Hussein, 1991). Saturation baiting method was used where 20 bait stations were distributed in both orchard of each district and 250 grams warfarin bait (0.025% on wheat) per station were exposed to rats. The consumed warfarin bait was recorded and replaced at 3-day intervals until the consumption was stopped. Reduction in *R.rattus* population in both orchards was estimated from the following equation.

$$\text{Population reduction} = \frac{A - B}{A} \times 100$$

Where :
A = consumed maize bait before treatment
B = consumed maize bait after treatment

Efficacy of warfarin bait and its consumed amount per feddan were estimated in orchards previously treated with anticoagulant and compared with those free from anticoagulant treatment.

2. Laboratory experiments

By the termination of the field experiment, individuals of *R.rattus* collected from the two tested orchards of each district were directly transported to be used in the laboratory trials, i.e. LD50, bleeding and prothrombin time. Animals were caged individually for at least 3 weeks for acclimatization and given a daily fresh supply of water and standard diet.

2.1. Acute oral LD50 determination

Series of different dosages of warfarin anticoagulant calculated on the basis of mg active ingredient/kg body weight (b.wt.) were prepared. Mortality and time to death were recorded up to 28 days post-treatment. LD50 was derived by moving average method using special tables given by weil (1952) and simplified calculation according to Horn (1956).

2.1. Bleeding and prothrombin time measurement

Tested animals (20 animals) from each district were administered orally 1.0 mg warfarin/kg b.wt. Blood samples were taken before and after treatment with 24 hours. Bleeding time (B.T.) was measured using the method of Duke (1910). Prothrombin time (B.T.) was measured using the method of Duke (1910). Prothrombin time (B.T.) was measured according to Dacie and Lewis (1984).

RESULTS AND DISCUSSION

1. Field Studies

According to Agric. Credit Bank and Egyptian German Project of Rodent Control, Menofia Governorate applied the highest amount of anticoagulant either belonged to first or second generation as compared with consumed ones in the other governorates.

1.2. Efficacy of warfarin anticoagulant against *R.rattus* populations

Data in Table 1 showed that using of warfarin bait induced 90.0, 95.8 and 93.4% population reductions when applied in orchards untreated before with anticoagulants at Menofia, Kafr El-Sheikh and Beni-Suef Governorates, respectively. On the other hand, warfarin efficacy against *R.rattus* was noticeable less when applied in orchards previously treated. Corresponding values were 57.5, 81.5 and 87.2% for the three governorates. This means that rats on areas treated before exhibited a considerable tolerance than those on areas free from anticoagulant treatment in all cases and this tolerance relatively varied depending on the tested governorate. Variations in rat susceptibility at both treated and untreated areas with anticoagulants before were obvious at Menofia Governorate than those of the other two tested governorates. In the same time, the relative susceptibility of *R.rattus* population considerably varied between the two tested populations of each Governorate when measured by the total consumed poisoned bait. Amount noticeably increased from 592 to 725g, 540 to 895 g and 1670 to 1950 g when it was applied on areas treated before and free from anticoagulants treatment at the three governorates, respectively. These findings are in harmony with (El-Zemaity et al., 1991; Gill, 1992; Mikhail, 1995; Gabr 1997).

Table 1. Efficacy of warfarin rodenticide under field conditions at different governorates.

Governorate	Efficacy %			Consumed rodenticide		
	Untreated	Treated	Ratio	Untreated	Treated	Ratio
Menofia	90.0	57.5	6.4	592	725	1.2
Kafr El-Sheikh	95.8	81.5	8.5	540	895	1.6
Beni-Suef	93.4	87.2	9.23	1670	1950	1.2

2. Laboratory Studies

2.1. Warfarin LD50 determination

Data in Table 2 showed the same trend of field results, whereas rats collected from treated areas of Menofia Governorate were more tolerant than those from the untreated areas (LD50 90.5, 79.4 mg/kg b.wt.). The variation in response of the two populations at Kafr El-Sheikh and Beni-Suef Governorates to warfarin was less (88.0, 82.5 and 79.4,75.0 mg/kg b.wt., respectively). It is to be noted that the variation among strains should be taken into account when testing anticoagulant compounds. Our findings are in accordance with those obtained by Greaves and Ayres (1976), Mikhail (1995), Gabr (1997) and Hussein (1997).

Table 2. LD50 values of warfarin to *Rattus rattus* collected from different governorates.

Governorate	Treated orchards				Untreated orchards			
	Dose mg/kg	Animal dead/total	Mortality %	LD_{50}	Dose mg/kg	Animal dead/total	Mortality %	LD_{50}
Menofia	21.5	1/5	20.0		21.5	0/5	0.0	
	46.4	0/5	0.0	90.5	46.4	1/5	20.0	79.4
	100.0	2/5	40.0		100.0	3/5	60.0	
	215.0	5/5	100.0		215.0	5/5	100.0	
Kafr	21.5	2/5	40.0		21.5	2/5	40.0	
	46.4	1/5	20.0	88.0	46.4	0/5	0.0	82.5
El-Sheikh	100.0	2/5	40.0		100.0	4/5	80.0	
	215.0	5/5	100.0		215.0	5/5	100.0	
	21.5	0/5	0.0		21.5	1/5	20.0	
	46.4	2/5	40.0	79.4	46.4	1/5	20.0	
Beni-Suef	100.0	2/5	40.0		100.0	3/5	60.0	75.0
	215.0	5/5	100.0		215.0	5/5	100.0	

2.2. Haematological effects induced by warfarin treatment

Effect of warfarin treatment (1mg/kg b.wt.) on bleeding time (B.T.) and prothrombin time (P.T.) of the two tested *R.rattus* populations of each governorate was complied in Table 3. It is obvious that haematological effect of warfarin treatment was noticed in the case of animals collected from the free anticoagulant treatment orchards of all tested governorates as compared with those collected from treated orchards. B.T. and P.T. values increased after treatment in case of animals from treated orchards of Menofia, Kafr El-Sheikh and Beni-Suef Governorates with 0.07, 0.57, 0.43, 0.85 and 0.47, 0.96 times than pre-treatment, respectively, while the haematological effect of warfarin treatment was clear in case of animals from untreated orchards, i.e. B.T. and P.T. values increased after treatment with 0.67 & 1.49; 1.25 & 4.1 and 3.0 & 2.2 times than before treatment for the same tested governorates. Many investigators agree with our data (Mikhail et al., 1971; Mourad et al. 1982; Kansouh et al., 1990), they reported that *Rattus rattus* was the dominant rodent species followed by *R.norvegicus* in most governorates in Egypt.

Table 3. Bleeding and prothrombin time before and after 1 mg warfarin/kg b.w.t. of *Rattus rattus* in different governorates.

Governorate		Bleeding time (B.T.) min.			Prothrombin time (P.T.) sec.		
		Before treatment	After treatment		Before treatment	After treatment	
Menofia	Treated area	3.21	3.45	(0.07)*	16.4	25.9	(0.57)*
	Untreated area	2.55	4.25	(0.67)*	6.1	15.2	(1.49)*
Kafr El-Sheikh	Treated area	3.50	5.00	(0.43)*	17.2	31.9	(0.85)*
	Untreated area	1.55	3.50	(1.25)*	4.7	23.9	(4.10)*
Beni-Suef	Treated area	3.10	4.55	(1.47)*	9.6	18.8	(0.96)*
	Untreated area	1.10	4.42	(3.00)*	6.2	19.9	(2.20)*

* Ratio of prolongation relative to values before treatment.

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حساسية الفأر المتسلق لمبيد الوارفارين

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معهد بحوث وقاية النباتات ، مركز البحوث الزراعية ، الدقى ، مصر.

أجريت هذه الدراسة فى منطقة قويسنا (محافظة المنوفية)،وسخا (محافظة كفر الشيخ)، والواسطى (محافظة بنى سويف) لدراسة حساسية الفأر المتسلق المتحصل عليه من منطقتين فى كل محافظة : الأولى عوملت بالمبيدات المسيلة للدم لفترة طويلة والثانية لم يتم استخدام المبيدات فيها منذ ثلاث سنوات على الأقل .. وكانت النتائج كالتالى:

١. التجارب الحقلية :

الفئران المتحصل عليها من مناطق معاملة بالمبيدات من قبل أظهرت مقاومة لمبيد الوارفارين بالمقارنة بالفئران المتحصل عليها من مناطق لم يتم فيها استخدام المبيدات، حيث أدى استخدام طعم الوارفارين (٠.٠٢٥ ٪) إلى خفض تعداد الفئران فى المناطق المعاملة من قبل بالمبيدات المسيلة للدم فى محافظات المنوفية وكفر الشيخ وبنى سويف إلى ٥٧,٥، ٨١,٥ ، ٢،٨٧ بالمقارنة بـــ ٩٠,٠ ، ٩٥,٨ ، ٩٣,٤ فى المناطق التى لم يتم استخدام المبيدات فيها من قبل فى المحافظات الثلاث على التوالى.

٢. التجارب العملية :

أيدت النتائج المتحصل عليها مَن التجارب المعملية النتائج الحقلية حيث التأثير السام للوارفارين كان أقل ضد الفئران المتحصل عليها من الأراضى الزراعية المعاملة سابقا بالمبيدات عن غير المعاملة من قبل فى كل المحافظات الثلاث. وكانت الجرعة المميتة للنصف ٩٠,٥ ، ٨٨,٠ ، ٧٩,٤ ملليجرام/كيلو جرام من وزن الفأر لفئران المنطقة الأولى بالمقارنة بـــ ٧٩,٤، ٨٢,٥ ، ٧٥,٠ ملليجرام/كيلو جرام من وزن الجسم للمنطقة الثانية. أيضا قياس زمن النزف وزمن البروثروجين أوضح أن المناطق المعاملةسابقا بالمبيدات أكثر مقاومة من المناطق التى لم يتم فيها استخدام المبيدات من قبل. وعموما يتضح من النتائج أن درجات التحمل للمبيدات المسيلة للدم تختلف باختلاف المناطق المجموع منها الفئران، وكذلك باختلاف المحافظات حيث أظهرت الفئران التى صيدت من محافظة المنوفية تحملا أكثر لمركب الوارفارين عن تلك التى صيدت من محافظتى بنى سويف وكفر الشيخ.