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COMPARARTIVE STUDIES BETWEEN ZINC PHOSPHIDE AND GOLDEN AHOWER CRUDE SEEDS PLANT EXTRACT AS A RODENTICIDE UNDER LABORATORY AND FIELD CONDITIONS

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ABSTRACT

The rodenticidal effect of golden shower crude seeds extract, *Cassia fistula* was studied comparatively with zinc phosphide under laboratory and field conditions of sharkia. Governorate. The laboratory results showed that in both non-choice and free - choice feeding tests a bait containing 1% either zinc phosphide or golden shower ethanolic extract gave 100% mortality for albino rat with shorter time to death for zinc phosphide treatment. Golden shower ethanolic extract bait was more palatable than zinc phosphide with 56.7 and 34.1% bait acceptance, respectively. Oral administration of sub-leathal dose (¼ LD_{50}) of each compound induced a noticeable dysfunction in aspartate aminotransferase (AST), alanine aminotransferase (ALT) enzymes and total protein as they increased in plasma at 24 and 48 hours after treatment indicating ahepatic damage. Aprolongation of the prothrombin time (PT) had occurred following administration of golden shower ethanolic extract indicating disorders in blood coagulation mechanism but no effect on (PT) was observed with zinc phosphide treatment. Under the field conditions, zinc phosphide caused 69.3% population reduction, While ethanol golden shower extract reduced 65.5%of black rat, *Rattus rattus rattus* population, while the water extract gave the lowest value.(9.7%).

INTRODUCTION

Egypt suffered from rodent problems in agricultural area at the beginning of the 1980's. The anticoagulant rodenticides were the major advance in rodent control. These compounds are generally effective against most rodent species, when used the surplus baiting, although long periods of feeding may be required in some cases. However, some species e.g. the Egyption spiny mouse, *Acomys cahirinus* and the house mouse, *Mus musculus* have a naturally low sensitivity to certain anticoagulants and their use would almost certainly lead to control failure (Gill, 1992). Zinc phosphide is the most commonly used acute rodenticide and has a relatively long history of use and it is becoming a standard to compare with newly developed rodenticides (Meehan, 1984).

The search for naturally occurring pesticides had resulted in a discovery of some plant derived compounds that are active against pest species, although their commercial viability is yet to be established. Golden shower, *Cassia fistula* is a commen plant consitituents with different biological properties (Satpathy 1983).

The present work aims to comparative studies between zinc phosphide and golden shower crude seeds plant extract as rodenticide under laboratory and field conditions.

MATERIALS AND METHODS

Tested compounds :-

Zinc phosphide :- Zn3 P2 (94) % was obtained from Kz pesticides company, Egypt.

Golden shower seeds extract :-

Golden shower, *Cassia fistula* seeds were purchased from a local market in Giza were cleaned and dried then150 grams of sieved powder were successive extracted with different solvents varied in their polarity i .e. hexane, ethanol, petroleum ether and water according to procedure of (Freedman *et al*, 1979). The golden shower extracts were weighted and frozen at- 20 C° as stoke till required.

Tested Animls

The adult individuals of albino rat, *Rattus norvegicus* were used for laboratory experiments. Animals were cuged individually with standard diet (65% crushed maize + 25% ground wheat + 5% sugar + 5% corn ail) and water supplied ad libitum. The unhealthy and pregnant animals were excluded. Animals were weighed and given a reference number for each one.

Laboratory Experiments.

Non choice feeding method:-

Agroup of 10 rats individually caged were used for each treatment. The first group was affered 50 gram of crushed maize containing 1% zinc phosphide for 24 h while the second group was offered 1% golden shower extracts fore 4 successive days.A nother group was offered plain crushed maize as check control. The consumed amount of bait was daily calculated

The treated bait was removed and the survivor animals were fed on the standard diet and observed for 28 days. During this period. Mortality was recorded.

Free choice feeding method.

The free choice feeding method according to palmateer (1974) was used to determine the poisoned bait acceptance by comparing its consumption with that of standard challenge diet.

Group of 10 rats caged individually were used for each compound and another one as a check control. Each rat was offered 50g of crushed maize containing either 1% zinc phosphide or Golden shower extract and 50g of standard challenge diet in small separate dishes. The position of the two dishes was altered daily to avoid feeding preference for a certain location. The consumed amount of the poisoned bait and standard diet was recorded daily for 4 successive days then the poisoned bait was removed and the survivor animals were fed on the standard diet. Dead animals were counted daily up to 28 days.

Bait acceptance was recorded as follows:-

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

Biochemical studies.

Effect of sub lethal dose ($^1/_4$ LD_{50}) of golden shower ethanolic extraction and zinc phosphide on some enzymes activity was studied as physiological response.

Animals were orally intubated with (43 mg /kg. b.w.) of golden extracted by ethanol (Mourad, 2007) or (7.18mg /kg.b .w) of zinc phosphide (Rezk, 2000). Blood samples were collected from each animal by retro- orbital sinus puncture in tri sodium citrate 3.8% as an anticoagulant and centrifuged at 3000 r.p.m. for 15 minutes. Plasma was collected and frozen until used.

Determination of A S T and A L T enzymes:

The activity of aspartate amino transferase (AST) and alanine amino transferase (ALT) was determined according to the method of Reitman and Frankel (1957) using commercial reagents of Boehring.

Determination of total protein:

Colorimetric determination of total protein in plasma of treated and untreated animals was conducted according to the method described by Gornall *et al.* (1968) using commercial reagents of Boehring.

Determination of blood coagulation Index:

Prothrombin time (PT) was determined in plasma of treated and untreated animals according to (Dacie and Lewis, 1984) using the fibrometer and commercial reagents obtained from Hoechst company.

Field performance:-

Field evaluation of crushed maize bait containing either 1% Zinc phosphide or 1% different extract of shower was carried out under the field conditions of Menia El-Kamh district, Sharkia Governorate. An infested area with the roof rat, *Rattus rattus* was chosen and divided into 5 plots, each of one feddan. One plot was chosen for each compound and one plot was left without treatment as check control. The population density of the rats was estimated pre-and post treatment using food consumption method according to Dubock (1984). Two Kilograms of the candiate bait were packed into plastic sacks each contained 50g and distributed in the chosen plot for 5 days.

The consumed amount of each tested bait was recorded. The percentage of population reduction was calculated as follows:-

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

RESULTS AND DISCUSSION

Laboratory studies:-

Non-choice feeding methods:-

Data in table (1) show that in non-choice feeding test a bait containing either 1% zinc phosphide or golden shower extracted by ethanol gave complete mortality with average bait consumption of 2.3 and 11.9 g/rat, respectively followed by 70 and 40% mortality with hexane and petroleum ether extracts with 10.3 and 7.1 g consumption, respectively.

Table (1): Effect of bait containing 1% zinc phoshide for 24 hour or 1% golden shower curde extractfor 4 days against albine rat using none choice feeding method.

Compound		Average bait consumption (g)	% Mortaltiy	Time to death	
				Rang	Mean
Zinc phosphide		2.3	100	7-19(h)	13
Golden shower extract b	Ethanol	11.9	100	5-9 (days)	5.8
	Hexane	10.3	70	5-9 (days)	8.6
	Petroleum ether	7.1	40	8-20 (days)	10.3
	Water	8.9	10	- (days)	13.0

The lowest mortality percentage was only 10% in case of golden shower extracted by water although a considerable amount of bait consumption 8.9g were up taken.

A considerable variation in the time to death was observed, whereas it ranged 7-19 hours with an average of 13 hours for zinc phosphide and the average time to death was 5.8, 8.6, 10.3 and 13 days for golden shower extracted by ethanol, hexane, petroleum ether and water, respectively. This mean that ethanol golden shower extract killed the animals in short time while the opposite was observed with golden shower extracted by water.

Free Choice feeding method

The efficacy of 1% Zinc phosphide and different extracts of golden shower plant when tested with free choice method was illustrated in Table (2). Data indicate that both zinc phosphide and golden shower ethanol extract induced complete mortality followed by 60, 30 and 20% for golden shower extracted with hexane, petroleum ether and water, respectively. Regarding the mean of time required to death, results revealed that it was 17 hours for zinc phosphide and 6.4, 7.8, 9.5 and 16.0 days for golden shower extracted with ethanol, hexane, petroleum ether and water, consecutively. On there other hand golden shower ethanol extract bait was more palatable to albino rat than zinc phoshoide. Awide variation was observed for the palatability of rats to baits which treated with the four extracts. The tested extracts could be arranged according to their acceptance in a descending order as follows golden shower extracted by ethanol, >hexane> water> petroleum ether.

Table (2): Effect of bait containing 1% zinc phoshide for 24 hour or 1% golden shower curde extract for 4days against albino rat using free choice feeding method.

Compound		% Acceptance	% Mortality	Time to death	
				Rang	Mean
Zinc phosphide		34.1	100	6-24 (hours)	17 (hours)
Golden shower extract by	Ethanol	56.7	100	5-10 (days)	6.4 (days)
	Hexane	48.0	60	6-16 (days)	7.8 (days)
	Petroleum ether	29.0	30	7-19 (days)	9.5(days)
	Water	33.0	20	10- 23 (days)	16.0 (days)

Reviewing the aforementioned results, it is obvious that both zinc phosphide and golden shower ethanol extract proved to be effective.

The toxic effect of golden shower plant when extracted by different solvents differed according to variation in bioactive compound types. Asran (1994), Khidr(2001) Gabr *et al* (2004) and Mourad (2007).

Biochemical Response:-

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are important and critical in biological processes. They have role in amino acid metabolism and biosynthesis and they are considered as specific indicators of liver damage.

Data in Table (3) show that administration of ¼ LD_{50} of zinc phosphide or ethanolic golden shower extract resulted in significant rise of transaminases (AST and ALT) activity and total protein indicating a hepatic disorder and /or damage. AST, ALT and total protein level in plasma was significantly elevated to 37.3, 48.4 and 26.3% than control value when measured at 24 hours and raised to 51.8, 59.1 and 44.1% at 48 hours post-zinc phosphide treatment, respectively.

Table (3): Effect of subleathal dose (1/4 LD_{50}) of zinc phoshide and golden shower ethanolic extract on AST, ALT enzymes, Total protein and prothrombin Time(P.T) in plasma **of albino rat.**

Tested Compound	Biochemic al Parameter	Control	Period after treament					
			24 h		48h		72h	
		Mean ± S.E	Mean ± S.E	% diff	Mean ± S.E	% diff	Mean ± S.E	% diff
Zinc phosphide	AST (U/L)	19.3 ± 0.3	26.5± 0.2	**37.3	29.33±0.72	**51.8	23.6±0.46	**22.3
	ALT (U/L)	9.3 ± 1.33	13.8 ± 1.3	* 48.4	17.8±0.67	**59.1	12.7±0.43	*36.6
	Total protein(g/dl)	6.8 ± 0.1	8.6 ± 0.4	* 26.3	9.8±0.3	**44.1	7.5±0.7	10.1
	P.T time (second)	11.4 ± 1.3	12.3±1.4	7.9	13.2±1.3	12.2	12.1±1.5	6.1
Golden shower ethanolic extract	AST (U/L)	19.33±0.3	28.3±0.86	*16.6	31.2±0.43	**61.7	22.3±0.25	**15.5
	ALT (U/L)	9.3 ± 1.33	11.6±0.23	24.3	13.6±0.42	**16.2	10.7±0.32	15.1
	Total protein(g/dl)	6.81 ± 0.1	8.41±0.6	*23.5	9.43±0.5	**38.5	7.3±0.4	7.2
	P.T time (second)	11.4 ±1.3	15.5±1.6	*35.9	18.6±2.3	*63.2	14.4±1.3	26.3

* Significant.

** Highly significant.

Similar results were obtained when these parameters were measured in the plasma of animals treated with golden shower extract. An Elevation of prothrombin time (PT) had occurred by golden shower extract treatment. The P.T. value was raised from 11.4 seconds of control to reach 15.5 and 18.6 seconds at 24 and 48 hours post- treatment, respectively. However, Zinc phosphide treatment had no significant effect on prothrombin time. Anoticeable decreasing in all values level of AST, ALT, total protein and prothrombin time was recorded at 72 hours post-treatment of Zinc phosphide and golden shower extract but it was over than control.

Our results agree with those obtained by Abdel-Halim *et al* (1995), Sebaii (1996), Bhakta *et al* (1999), El-Deeb *et al* (2002), Gabr *et al* (2004), Rezk (2006), Hussien *et al* (2007)and Mourad (2007).

Field Performance.

The efficiency of 1% zince phosphide and the four golden shower extracts bait was test against the black rat *Rattus rattus rattus* under the field conditions of Sharkia Governorate. Results in Table (4) indicate that zinc phosphide was the most effective one whereas it achieved 69.5% rat population reduction followed by golden shower ethanol extract 65.5% while golden shower extracted by hexane and petroleum ether coused 40.5 and 16.4% rat population reduction, rspectively .The lowest effective was for extract by water as it gave only 9.7% population reduction.

Table (4): Field Performance of zinc phoshide and golden shower plant extract against the black rat *Rattus rattus rattus* at Sharkia Governorate.

Compound		Bait Consumption (g)/Feddan			% Population reduction
		Pre-treatment	Treatment	Post-treatment	
Zinc phosphide		469	658	144	69.3
Golden shower extract by	Ethanol	470	852	155	65.5
	Hexane	580	837	345	40.5
	Petroleum ether	730	902	610	16.4
	Water	720	985	650	9.7

The average of bait consumption was 658 g / feddan for zinc phosphide while it was 852, 837 and 902 g / feddan for golden shower extracts with ethanol, Hexane and petroleum ether while the highest amount was 985 g in case of extract by water.

From the obtained results, it is cleared that golden shower ethanol extract proved to be promising compound that com be effectively used as a rodenticide in comparison with zinc phosphide.thise agree with EL Deeb *et al* (1991), Ibrahim (2001) and Hussien *et al*(2007)..

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دراسات مقارنة بين مبيد فوسفيد الزنك و مستخلص بذور الخيار شنبر كمبيد قوارض تحت الظروف المعملية و الحقلية

حسن الديب، ابراهيم قطب ابراهيم ، فاطمة كامل خضر و عبد المقصود عبد المقصود ابوهاشم
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تم مقارنة تأثير مبيد فوسفيد الزنك و مستخلص بذور الخيار شنبر كمبيد قوارض تحت الظروف المعملية و الحقلية لمحافظة الشرقية.

أظهرت النتائج المعملية فى كلا من التغذية الإختيارية و اللإختيارية إن التغذية على طعم يحتوى على ١% لكلا المركبين أدى الى حدوث نسبة موت ١٠٠%للحيوانات المختبره مع قصر الفترة الزمنية اللازمة لحدوث الموت وذلك عند المعاملة بفوسفيد الزنك وكان طعم المستخلص الايثانولى لبذور الخيار شنبر أكثر أستثاغة للفأرالألبينو من طعم فوسفيد الزنك حيث بلغت النسبه المئويه للأستساغة ٥٦,٧ ،٣٤,١% على التوالى .

وقد أدت المعاملة بجرعة تحت مميته (LD_{50} ¼) عن طريق الفم لكلا المركبين الى حدوث نشاط الأنزيمات الناقله للأمين (ALT ، AST) و كذلك البروتين الكلى حيث أرتفعت فى بلازما الدم بعد مرور ٢٤ ، ٤٨ ساعة من المعاملة وهذا يدل على حدوث خلل أو تلف فى أنسجة الكبد ومن جهه أخرى أدت المعاملة بمستخلص بذور الخيار شنبر الى حدوث أطالة فى زمن البروثرمبين (PT)مما يدل على حدوث خلل فى آلية تجلط الدم . وتحت الظروف الحقلية بمحافظة الشرقية حقق فوسفيد الزنك و مستخلص بذور الخيار شنبر الأثانولى نسبه خفض فى تعداد الفأر المتسلق (٦٩,٣% ، ٦٥,٥%) على التوالى فى حين أن مستخلص بذور الخيار شنبربا لماء أعطى أقل قيمة(٧و٩%) .

قام بتحكيم البحث

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