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Research Paper

Synergistic effect of bromadiolone and cholecalciferol (vitamin D_3) against house rat, *Rattus rattus*

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Abstract

Commensal rodents, especially house rat *(Rattus rattus)* causes extensive losses by feeding and contaminating the food products and also plays a role in spreading several diseases of health importance. House rats trapped from various commensal situations of Ludhiana city were fed for 5 days in no-choice on self made formulations of bromadiolone (Br) and cholecalciferol (Cc)/vitamin D_3 baits having different concentrations (less than their standard concentrations i.e <0.005% Br and <0.075% Cc) mixed in plain WSO- mix bait. Both male and female house rats were fed on four formulated baits viz. combination-I (0.0025% Br + 0.05% Cc), combination-II (0.001% Br + 0.05% Cc), combination-III (0.0025% Br + 0.01% Cc) and combination-IV (0.001% Br + 0.01% Cc). Out of these four tested formulated baits, combination-IV, having the lowest concentration of Br and Cc showed significantly higher rodenticidal potential in terms of 100% killing of rats within average time of 3.33±0.23 days in males and 4.00±0.40 days in female rats. Feeding of combination-IV resulted in delaying the blood clotting time by 55.00±1.90 sec. in males and 65.00±0.87 sec. in females as compared to the untreated rats due to the action of bromadiolone present in the formulated bait even at its concentration @ 0.001%. In this lowest tested formulation, the feeding of cholecalciferol @ 0.01% was able to increase the serum calcium level (mg/dL) significantly up to 3.66±0.93 in males and 2.94±0.92 in females in comparison to untreated rats. The study revealed that the combination-IV i.e formulation with least tested concentration of Br and Cc was found to have potent rodenticidal efficiency as well as cost effective bait for the control of house rats under commensal situations.

Keywords Anticoagulants, Commensal rodents, Hypercalcemia, Mortality, Rodenticide baits

Introduction

Rodents have played havoc with man's economy as they damage each and every food item in fields, godowns, storage houses, poultry farms and residential premises [1, 2]. The house rat, *Rattus rattus* is the most abundant, widely distributed and cosmopolitan commensal rodent [3]. House rat not only causes severe damage by consuming the stored food items, but also contaminate the food materials by urination and defecation, thus making it unfit for human consumption [4]. Due to its close proximity to human habitations, it is involved in spreading several diseases of public health importance, especially bubonic plague and act as reservoir of organisms that cause debilitating diseases in humans and livestock [5, 6]. The economic magnitude and health problems associated with rodent pests emphasize the need to develop techniques for their management. Integration of other methods like mechanical and environmental control, do form the technology package, but to a very limited extent.

The methods used for the management of rodent population such as trapping, habitat manipulation, use of repellents/attractants/pathogenic agents, that induce mortality or migration of rodents have never produced consistent results [7].

Chemical control by rodenticides is the most widely used and efficient method of all the available methods for the control of rodent pests both under agricultural and commensal situations [8, 9]. A number of highly toxic substances like strychnine, zinc phosphide, barium carbonate, red squill and bromethalin have been commonly used for the control of rodents [10]. However, rodenticides which are common in use for rodent control have their own drawbacks like poison aversion, bait shyness, lack of specificity and genetic resistance [11]. With the introduction of first and second generation anticoagulant rodenticides, the rodent control strategies have undergone a complete change. Nowadays anticoagulant rodenticides *viz.* bromadiolone, brodifacoum and warfarin are becoming more popular for rodent control. But these anticoagulants are the frequent cause of non-target animal poisoning and also potentially dangerous to all mammals, birds and humans [12]. Therefore, the chemical control of commensal rodents in human residential areas and animal dwellings requires the use of safe and less toxic rodenticides. Among various rodenticides, cholecalciferol (vitamin D_3) a sub-acute rodenticide which is being considered as more potent and relatively safe than anticoagulants. Use of cholecalciferol as a rodenticide in bait has been found to lower the risk of secondary poisoning, minimize the toxicity of non- target species and to overcome anticoagulant resistance in rats and mice [13]. No reports are available regarding the human poisoning from the use of cholecalciferol [10] and genetic resistance after its ingestion [14]. But cholecalciferol alone is also not suitable to control rat population, as it often shows the poor acceptance of bait. Another aspect of using vitamin D_3 as rodenticide is its higher cost, as its effective concentration is more expensive than that of effective concentration of most of the anticoagulants used [15].

Overcoming the poison resistance and poison shyness is critical to the success of rodent control operation, so there is a need to formulate such compounds which have efficient rodenticidal potential, low or no resistance, good susceptibility, safe against non-target species and cost effective. To achieve this success some workers have studied the effect on mortality of rodents by preparing attractive bait formulations having combination of anticoagulant rodenticides with hypercalcaemia causing agent like cholecalciferol [16, 17]. So as to overcome the inefficacy of anticoagulants caused by their resistance and compensate the higher cost of cholecalciferol, the present study was carried out to standardize the best combination of bromadiolone and cholecalciferol (vitamin D_3) to be used as a formulated bait against house rats (*R. rattus*).

Materials and Methods

House rats were trapped live from poultry farms, grocery shops, store houses and godowns of Ludhiana city (Latitude 30°56'N, Longitude 75°52'E) with single and multicatch rat traps. The mature house rats, both male and female (having body weight >100 g) were maintained individually in laboratory cages. These rats were acclimatized for 15 days by providing them plain WSO-mix bait, prepared by mixing of cracked wheat, sugar powder and groundnut oil in the ratio of 96:2:2 and water *ad libitum* before starting the experiment. Food and water were replenished daily. Metallic trays were kept under each cage to collect the faeces and spilled food and were cleaned daily. Approval of Institutional Animal Ethics committee, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana was obtained for the usage of animals. Two chemicals used as rodenticide during the present study were bromadiolone (Roban) and cholecalciferol (vitamin D_3). Both these chemicals were purchased from standard sources i.e. bromadiolone from Pest Control (India) Pvt. Ltd. and cholecalciferol from MP Biochemicals, Inc.

Adult and healthy rats were categorized into two main groups, GroupI and Group II. Group I was further divided into three feeding trials viz; trial 1, 2 and 3 (having 3 male and 3 female rats /trial). The house rats of trial 1 and 2 were fed on standard baits of bromadiolone (0.005% Br) and cholecalciferol (0.075% Cc) in no-choice, respectively (bait formulations mentioned in table 1) and rats of trial 3 were kept as control (fed on untreated /plain WSO-mix bait). Group II was divided further into four feeding trials (having 3 male and 3 female rats /trial), and rats were fed in no-choice on combination I (0.0025%Br + 0.05%Cc), combination II (0.001%Br +0.05%Cc), combination III (0.0025%Br + 0.01% Cc) and combination IV (0.001% Br + 0.01% Cc), as given in table 1. With each combination, a set of untreated rats was also kept. Rats of each set were fed on their respective treated baits for five consecutive days and consumption of treated / untreated baits was recorded. Consumption of plain

bait before treatment and after treatment (if rats survived) for five days was also recorded. Different observations recorded were; daily treated bait consumption (g), acceptability/palatability of baits (%), mortality rate (%). The following formulae were used for calculation of different parameters like:

$$\begin{pmatrix} Average\ daily\ bait\ consumption \\ (g\ per 100\ g\ body\ weight) \end{pmatrix} = \frac{Average\ daily\ consumption\ of\ bait\ by\ rats\ (g)}{Average\ body\ weight\ of\ rats\ (g)} \times 100$$

$$(Acceptance\ of\ treated\ bait) = \frac{Consumption\ of\ treated\ bait\ during\ treatment\ period}{Consumption\ of plain\ bait\ during\ pretreatment\ period} \times 100$$

$$(Molarity\ \%) = \frac{Number\ of\ rats\ died}{Total\ number\ of\ rats\ in\ feeding\ trial} \times 100$$

The action of bromadiolone was detected in the form of delay in blood clotting time (sec) which was recorded by Sabraze's capillary tube method by taking blood from the tail vein of mildly anaesthetized treated rats of group I (except trial 2) and II at 0 hour and 48 hours after treatment. Delay in blood clotting time was calculated as per the formula:

$$Delay\ in\ clotting\ time\ (sec) = Blood\ clotting\ time\ after\ treatment\ (48\ Hours) - Blood\ clotting\ time\ before\ treatment\ (0\ Hours)$$

Cholecalciferol causes increase in serum calcium level (mg/dL) which was estimated by Cresolphthalein complex method [18] from the treated rats of group I (except trial 3) and group II at 0 and 48 hours after treatment and rise in serum calcium level was determined by the following formula:

$$Rice\ in\ serum\ calcium\ level\ (mg\ per\ dL) = Serum\ calcium\ level\ after\ treatment\ (48\ Hours) - Serum\ calcium\ level\ before\ treatment\ (0\ Hours)$$

Significance of difference between two mean values within the same group were determined by using 'paired t-test' and significance of difference among mean values of different treatments were determined by using critical difference, CD [19].

Table 1: Preparation of standard baits and combined baits having bromadiolone and cholecalciferol at their different concentrations

Baits	Prepared by mixing of		
	WSO-mix bait (Kg)	WSO-mix bait (Kg)	WSO-mix bait (Kg)
Standard bait of Br (0.005%)	1	20	---
Stanmdard bait of Cc (0.075%)	1	---	750
Combination-I (0.0025% Br+ 0.05% Cc)	1	10	500
Combination-II (0.001% Br + 0.05% Cc)	1	4	500
Combination-III (0.0025% Br + 0.01% Cc)	1	10	100
Combination-IV (0.001% Br + 0.01% Cc)	1	4	100

Br represents bromadiolone and Cc represents cholecalciferol

Results and discussion

a) Acceptance and efficacy of standard bait of bromadiolone (0.005%)

Feeding of male house rats on standard bait of bromadiolone (0.005%) in no-choice for 5 days under laboratory conditions, resulted in 81.50 ± 0.97% and 95.80 ± 3.19% acceptance of this bait over plain bait consumed during pre-treatment period (n=5 days) respectively by male and female house rats (Table 2). This acceptance in the form of average daily consumption (g/100g bw) of treated bait was non-significantly different from that of plain bait during pre-treatment period, indicating good acceptability of standard bait of bromadiolone. Also, this bait was found to be effective as it resulted in 100% mortality of both male and female house rats. In case of males, rats started dying on the 3rd day of treatment and all the rats died till 6th day after consuming the 0.005% bromadiolone bait and in females all the rats died on 4th day of treatment (Table 2). Anticoagulant rodenticidal potential of bromadiolone has chronic toxicity and rodents are capable of withstanding these rodenticides generally for 48 hours after ingestion and with increased intake of their dose, delayed mortality occurs [20]. Standard bait of bromadiolone (0.005%) has been found to cause mortality of commensal rodents upto 100% by different workers with in a period of 2-15 days [21, 22]. The house rats fed on plain bait (control) showed non-significant difference in average daily consumption (Table 2).

b) Acceptance/palatability of standard bait of cholecalciferol (0.075%)

Feeding of standard bait of cholecalciferol (0.075%) by male house rats for 5 days resulted in significantly less average daily consumption (g/100g bw) i.e. 3.66 ± 0.88 as compared to that of plain bait during pre-treatment period (8.91 ± 0.83), which resulted in 40.10 ± 1.55 % acceptance of cholecalciferol treated bait over plain. Similarly, a significantly less average daily consumption (g/100g bw) of 0.075% cholecalciferol bait i.e. 2.70 ± 0.61 was found in female rats as compared to that of plain bait during pre-treatment period (8.06 ± 1.38). This resulted in 35.30 ± 0.64% acceptance of cholecalciferol treated bait over plain bait by females. Though the acceptance of 0.075% cholecalciferol by male and female house rats was significantly less, but whatever amount of cholecalciferol was ingested by them was able to cause their 100% mortality. Delayed mortality of house rats was observed, as male and female rats started dying respectively on 4th and 7th day and all died till the 10th and 14th day after feeding of treated bait (Table 2). Rodents generally take a lethal dose of cholecalciferol (sub-acute poison) during first 24 hours like acute rodenticides and repeated feeding may occur, causing delayed mortality of rats like anticoagulants [7]. Different formulations of cholecalciferol i.e. cakes, pellets and baits have also been used for effective killing of rodents and delayed effects have been reported [15, 22]. However, untreated group of rats (both male and female) showed non-significant difference in the average daily consumption (Table 2).

c) Acceptance and efficacy of formulated baits having different concentration of bromadiolone and cholecalciferol

Feeding of male and female house rats on different formulated baits having bromadiolone and cholecalciferol mixed in different concentrations (less than their standard one) in the form of combination I, II, III and IV resulted in significantly lower acceptance of these baits over the plain baits and the % acceptance was found to range from 35.10±2.23 to 71.30±0.72. However only one set of female rats which were fed on combination - III (having 0.0025% bromadiolone and 0.001% cholecalciferol) showed maximum % acceptance i.e. 86.80±1.88 which was statistically non-significant as compared to the combination of plain bait during pre- treatment period (Table 3). However, whatever more or less quantity of these formulated baits was accepted by the house rats was able to cause their 100% mortality in all the feeding trials. All the male and female rats died within 3-5 days of feeding of these formulated baits, indicating their good rodenticidal efficiency (Table 3) in comparison to the standard bait of bromadiolone and much better than that of standard bait of cholecalciferol (Table 2). Even the combination IV (having the lowest concentration of bromadiolone and cholecalciferol) was able to result in 100% mortality of both male and female rats within 3-5 days (Table 3). Literature reveals that feeding of *R. rattus* on formulated bait having calciferol and warfarin resulted in complete kill at lower dosages of calciferol in the combination as compared to either calciferol or warfarin alone [16]. Also, 100% killing of rats and mice was found after feeding them in the bait having coumatetralyl (0.04%) and cholecalciferol (0.025%) mixed in their lower concentratrion [24].

Table 2: Acceptance and efficacy of standard baits of bromadiolone (0.005%) and cholecalciferol (0.075%) against *Rattus rattus* in no-choice feeding trial

Feeding Trial	Sex	Body weight (g)	Average daily consumption of bait (g /100g body weight), n= 5 days			Acceptance of treated bait over plain bait (%)	Percent Mortality (Range in days)
			Pre-treatment	During treatment	T value at 5% level		
Standard bromadiolone bait (0.005%)	Male	168.30±1.59	5.12±0.50	4.07±0.24^{NS}	2.26	81.50±0.97	100 (3 – 6)
	Female	126.60±1.47	5.51±0.038	5.30±0.75^{NS}	0.97	95.80±3.19	100 (4)
Standard cholecalciferol bait (0.075%)	Male	121.10±0.85	8.91±0.83	3.66±0.88^{S}	7.50	40.10±1.55	100 (4-10)
	Female	128.30±1.10	8.06±1.38	2.70±0.61^{S}	3.87	35.30±0.64	100 (7 – 14)
Control (Untreated)	Male	138.30±2.24	7.03±0.66	6.50±0.97^{NS}	1.37	-	Nil
	Female	125.00±0.73	6.90±0.48	5.98±0.50^{NS}	1.40	-	Nil

All values are Mean±S.E.
T value at 5% level is 2.92
NS represents the non-significant difference and S represents significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.

Table 3: Acceptance and efficacy of different combinations of bromadiolone and cholecalciferol mixed in in bait against *Rattus rattus* in no-choice feeding trial.

Feeding Trial	Sex	Group	Body weight (g)	Average daily consumption of bait (g /100g body weight), n= 5 days			Acceptance of treated bait over plain bait (%)	Percent Mortality (Range in days)
				Pre-treatment	During treatment	T value at 5% level		
Combination-I (0.0025% Br + 0.05% Cc)	Male	Treated	141.66±2.91	11.81±0.70	5.61±1.20^{S}	4.49	47.90±3.65	100 (3-5)
		Untreated	118.33±1.32	10.36±0.32	10.10±0.24^{NS}	1.05	-	Nil
	Female	Treated	140.00±0.91	11.62±0.66	6.15±0.11^{S}	7.04	55.80±1.73	100 (3-5)
		Untreated	116.60±0.57	6.40±0.17	8.75±0.18^{NS}	2.28	-	Nil
Combination-II (0.001% Br + 0.05% Cc)	Male	Treated	140.0±2.10	10.63±0.42	5.56±0.32^{S}	14.9	52.20±0.29	100 (3-4)
		Untreated	135.0±1.86	10.90±0.40	9.45±0.34^{NS}	2.04	-	Nil
	Female	Treated	121.0±1.09	12.20±0.68	8.63±1.06^{S}	5.61	71.30±0.72	100 (3-4)
		Untreated	148.3±0.77	9.56±0.68	9.64±0.59^{NS}	0.88	-	Nil
Combination-III (0.0025% Br + 0.01% Cc)	Male	Treated	156.6±0.13	5.04±0.13	3.03±0.61^{S}	4.80	59.20±2.53	100 (3-4)
		Untreated	125.0±0.73	9.04±0.28	7.25±0.40^{NS}	2.08	-	Nil
	Female	Treated	151.6±1.67	6.15±0.11	5.30±0.34^{NS}	1.93	86.80±1.88	100 (3-4)
		Untreated	141.6±1.58	5.24±0.67	5.88±0.49^{NS}	2.84	-	Nil
Combination-IV (0.001% Br + 0.01% Cc)	Male	Treated	146.6±2.03	9.75±0.55	3.30±0.68^{S}	5.91	35.10±2.23	100 (3-4)
		Untreated	135.0±1.86	9.99±0.52	7.53±0.30^{NS}	1.64	-	Nil
	Female	Treated	140.3±1.21	9.05±0.95	4.04±0.63^{S}	4.43	47.90±2.28	100 (3-5)
		Untreated	135.0±1.05	8.86±0.60	9.05±0.50^{NS}	1.10	-	Nil

All values are Mean ±S.E ; T value at 5% level is 2.92
Br represents bromadiolone and Cc represents cholecalciferol.
NS represents the non-significant difference and S represents significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.

Table 4: Comparative rodenticidal potential of standard baits of bromadiolone and cholecalciferol and their different combinations against *Rattus rattus* in no-choice feeding trial.

Feeding trial	Active ingredient of rodenticide ingested (mg/kg bw) (n= 5 days)		Average time for 100% mortality (days)		Delay in blood clotting time(sec)*		Rise in serum calcium level (mg/dL)*		Cost of rodenticides (Rs/kg of WSO) Female
	Male	Female	Male	Female	Male	Male	Female	Male	
Standard bait of Br (0.005%)	153.00±4.49	245.90±3.69	4.33±0.59	4.00±0.00	46.66±1.82	45.00±3.39	ND	ND	24.00
Standard bait of Cc (0.075%)	122.30±3.80	98.02±3.28	7.33±0.92	9.66±0.80	ND	ND	4.37±0.72	3.21±0.21	1240.00
Combination-I	180.31±5.06	222.43±2.45^{S}	3.66±0.49	3.66±0.49	16.66.08	56.66±0.62	3.40±0.38	5.86±1.53	840.00
Combination-II	117.0±1.50	203.23±3.16^{S}	3.66±0.24	3.66±0.24	85.00±1.53	65.00±0.87	3.95±0.55	6.17±0.16	832.00
Combination-III	55.00±2.67^{S}	96.20±1.46^{S}	3.66±0.24	3.33±0.25	55.00±4.15	70.00±0.84	3.21±0.16	5.96±0.95	177.00
Combination-IV	26.90±2.34^{S}	46.88±1.35^{S}	3.33±0.25	4.00±0.40	55.00±1.90	65.00±0.87	3.66±0.93	2.94±0.92	170.00
CD at 5% level	90.11	81.40	NS	NS	NS	NS	NS	NS	--

*refers to difference of the values between 48 hours and 0 hour of feeding
NS represents the non-significant difference
ND represents not detected, as in bromadiolone treated rats there will be no rise in serum calcium level and in cholecalciferol treated rats there will be no delay in blood clotting time
Combination-I (0.0025%bromadiolone and 0.05% cholecalciferol), Combination-II (0.001% bromadiolone and 0.05% cholecalciferol), Combination-III (0.0025% bromadiolone and 0.01% cholecalciferol), Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol

b) Comparative of rodenticidal potential of standard baits of bromadiolone and cholecalciferol and their formulations

When standard bait of bromadiolone (0.005%) alone was fed, its total active ingredient ingested (mg/kg body weight) was found to be 153.00±4.49 and 245.90±3.69 by male and female house rats, respectively which led to 100% mortality within an average of 4.33±0.59 days in males and 4 days in females. The killing of house rats was due to excessive flow of blood or as a result of delay in blood clotting time i.e. 46.66±1.82 sec in males and 45.00±3.39 sec in females ,as compared to their normal clotting time (Table 4). On the other hand the mortality of rats was delayed when fed on the standard cholecalciferol bait (0.005%) in comparison to standard bromadiolone feeding and average time for 100% killing of males was found to be 7.33±0.92 days and 9.66±0.80 days in females after ingestion of its active ingredient 122.30±3.80 and 98.02±3.28 (mg/kg bw) respectively. The ingestion of cholecalciferol resulted in rise of serum calcium level (mg/dL) i.e. 4.37±0.72 in males and 3.21±0.21 in females as compared to the normal rats (Table 4). Out of the four tested combinations, combination III and IV showed significantly less ingestion of active ingredient both by male and female house rats as compared to that of during the feeding of their standard baits. Feeding of four different formulations resulted in their good rodenticidal efficacy as 100% mortality was also observed in all the experiment trials and there was non- significant difference in the average time taken for 100% killing of both male and female house rats. Delay in blood clotting time (sec) and rise in serum calcium level (mg/dL) of rats fed on different formulations were statistically similar to that of their standard baits.

Even the combination IV (the formulation having the least concentration) i.e. 0.001% bromadiolone and 0.01% cholecalciferol mixed in bait showed efficient rodenticidal potential in terms of 100% killing of male and female rats with an average feeding of 3.33±0.25 days (Table 4), this is because that synergism increase the rodenticidal activity and such combined preparations have been known to eradicate the anticoagulant resistant rats [17].Literature also indicated that calciferol or warfarin baits given individually to *R. rattus* produced partial mortality, however in combination produced a complete killing. Excess of calcium due to cholecalciferol and the unavailability of vitamin K due to the action of bromadiolone might explain the added effectiveness of cholecalciferol, indicating that cholecalciferol and bromadiolone act on different loci in the same process of blood physiology [16]. There is an important feature of calciferols (vitamin D_2 and vitamin D_3) toxicology that they are synergistic with anticoagulant toxicants, it means that mixture of anticoagulant and calciferols in same bait are more toxic than sum of toxicities of anticoagulant and the calciferol in the bait. Thus, a massive hypercalcaemia effect can be achieved by substantially lower calciferol content in bait and vice-versa, more pronounced anticoagulant/hemorrhagic effects are observed if the calciferol is present. This synergism is mostly used in calciferol low concentration baits, because effective concentration of calciferols is more expensive than that of the most anticoagulants. Such type of synergistic calciferol based baits having less concentration of anticoagulant are considered generally safer to birds and other non-target species than anticoagulants or acute toxicants [25].

When standard baits of bromadiolone and cholecalciferol were prepared, the cost of rodenticide for preparation of their 1 kg bait was Rs 24/- and Rs 1240/-, respectively. Cost of rodenticide used for preparation of 1 kg of combination-I, II, III and IV bait was Rs 840, Rs 832, Rs 177 and Rs 170, respectively (Table 4). Bromadiolone bait was though cheaper as it cost only Rs 24/kg bait, but it is very toxic and has been found to have primary and secondary toxic effects in non-target species [26, 27]. Out of all the other feeding trials of formulated baits, the combination-IV (0.001% bromadiolone and 0.01% cholecalciferol) was found to be cost effective i.e. Rs 170/kg (Table 4), as less amount of the costly chemical i.e. cholecalciferol was needed to prepare it, while the efficiency and rodenticidal potential equivalent or more than that of standard baits of bromadiolone and cholecalciferol and other combination baits was achieved (Tables 4) while spending less money (Rs 170/kg of bait). As the concentration of bromadiolone in combination-IV is reduced, its lethal effects to other non-target species will also be reduced. Such type of combination could benefit in terms of reduction of active ingredients whilst retaining the efficiency and minimizing the primary and secondary hazards [25].

Conclusion

Feeding of formulated baits having bromadiolone (anticoagulant) and cholecalciferol/Vitamin D_3 (sub-acute rodenticide) mixed in the concentration less than their standard one were capable to show the comparative results in the form of 100% killing of house rats. Out of the tested four formulations, combination with least concentration (having 0.001% bromadiolone + 0.01% cholecalciferol) was

found to be most effective and economic bait against house rats for its safe usage especially under commensal situations.

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References

1.Jackson W. B., Current management strategies for commensal rodents. In : Genoway H H (ed) Current Mammology. 495-512. Plenum Press, New York and London **(1987)**.

2. Prakash I. and Ghosh P. K., Rodents in Indian Agriculture. 707. Jodhpur Scientific Publishers **(1992)**.

3. Parshad V. R. Rodent Control in India. Integ. Pest Mgmt. Rev., 4, 97-126 **(1999)**.

4. Parshad V. R., Saini M. S., Ahmad N. and Malhi C. S., Food contamination by commensal rodents in Ludhiana : A Preliminary report. Pestology, 18, 30-32 **(1994)**.

5. Weber W. J., Diseases Transmitted by Rats and Mice. 182. Thomson pub. Fresno, California, USA **(1982)**.

6. Sullivan L., Commensal rodents. http://cals.arizona.edu/yavapai **(2007)**.

7. Buckle A. P. and Muller F., Rodenticides. In: Muller F (ed) Agrochemicals: Composition, Production, Toxicology and Application. 667-686. Wiley-VCH, Weinheim **(2000)**.

8. Prakash I. and Mathur R. P., Management of Rodent Pests. 1-133, ICAR Publications **(1987)**.

9. Chopra G., Kaur P. and Guraya S. S., Predominant rodent species in India. In : R Chand and Co. (ed) Rodents : Ecology, Biology and Control. 3-19, New Delhi **(1996)**.

10. Fishel F. M., Pesticide toxicity profile : Miscellaneous rodenticides. http://edis.ifas.ufl.edu PI-78 **(2005).**

11. Buckle A. P., Prescott C. V. and Ward K. J., Resistance to the first and second generation anticoagulant rodenticides - A new perspective. Proc. 16th Vetebr. Pest Conf., 138-42, **(1994)**.

12. Koehler P. G. and Kern W. H., Rat and mouse control. UF/IFAS EDIS Document ENY-224 http://edis.ifas.uff.edu/DHO44 **(2005)**.

13. Eason C. T., Wickstorm M., Henderson R., Milne L. and Arthur D., Non-target and secondary poisoning risks associated with cholecalciferol. NZ. Plant Prot., 53, 299-304, **(2000)**.

14. Saini M. S. and Parshad V. R., Control of Rattus rattus with cholecalciferol: Laboratory manual of freshly prepared and ready to use bait formulations. Intl. Biodet. Biodr., 30, 87-96, **(1992)**.

15. Gould E. M. and Holmes S. J., The effect of dextromethorphan in preventing cholecalciferol-induced poison shyness and sickness-induced anorexia in the laboratory Norway rat. Pest Mgmt. Sci., 64(2), 197-202, **(2008)**.

16. Mukhta Bai K., Krishnakumari M. K. and Majumder S. K., Toxicity of Calciferol, Warfarin and their Combination to Rattus norvegicus (albino) and R.rattus. Pestic. Sci,. 9, 44-50, **(1978)**.

17. Zatsepin V. G., Kadirov A. F. and Klement-eva S A., Resistance of rats to first generation anticoagulants and ways of overcoming it using synergists. Russ. Agri. Sci,. 9, 39-42, **(2006)**.

18. Henry R. J. and Dryer R. L., Standard methods of clinical chemistry 205. Acord Press, New York **(1963)**.

19. Singh S., Bansal M. L., Singh T. P. and Kumar R., Statistical Methods for Research workers. Kalyani Publishers, New Delhi **(2004)**.

20. Revathi K. and Yogananda M., Effect of bromadiolone on haematology, liver and kidney in Mus musculus. J. Env. Biol., **27**(1), 135-40 **(2006)**.

21. Kocher D. K. and Parshad V. R., Acceptance and Efficacy of baits of three rodenticides against Rattus rattus alexandrinus. Pestology, 27, 18-20, **(2003)**.

22. Chaudhary V. and Tripathi R. S., Field Efficacy of Second Generation Avticoagulant Rodenticides in Managing Rodents Pests in Poultry Farms. Pestology ,33 (10), 41-45, **(2009)**.

23. Eason C., Baigent D., Wilson L., Hix S., Macmorran D., Ross J., Miller A. and Ogilvie S., Toxicity of cholecalciferol to rats in a multi-species bait. Sci. of Conser., 12, 1-9, **(2002)**.

24. Pospischil R. and Schnorbach H. J., Racumin Plus, A New Promising Rodenticide against rats and mice. Proc 16th Vertebr. Pest Conf. Pp 180-87 University of Nebraska, Lincoln **(1994)**.

25. Anonymous (1998) Rodenticides. http://en.wikipedia.org/wiki/rodenticide.

26. Brar R. S. and Sandhu H. S., Text Book of Veterinary Toxicology 181-200. Kalyani Publishers, Ludhiana, India **(2000)**.

27. Rao K. S., Encyclopedia of Toxicology (second edition). Wexler P (ed) Pp 338-340 National lib of medicine Bethesda MD, USA **(2005)**.