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Evaluation of Flocoumafen and difenacoum against lesser bandicoot rat, *Bandicota bengalensis* (gray) in rice

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Abstract
Rodenticide poison baiting is the most popular and preferred choice of rodent control in crop fields and urban dwellings as well. Second generation anticoagulants, flocoumafen and difenacoum were evaluated against lesser bandicoot, *Bandicota bengalensis* under choice and no-choice conditions in the laboratory. Both the rodenticides including bromadiolone has recorded 100% mortality in the test animal @0.005% concentration, but with varied time mortality periods of 3.8±0.24 and 4.6±0.25, respectively as against 5.5+0.56 in bromadiolone. In bi-choice laboratory tests, the mean daily intake of plain bait and poison bait was near similar with no significant difference. But, once the animal is ingested with anticoagulant poison bait during the 24 hr of test feeding period in no-choice condition, later its plain bait consumption was drastically reduced compared to its consumption before the test period. Flocoumafen has offered 71.3 and 62.8 per cent reduction in live burrows and tiller damage of *B. bengalensis* in rice, where as it was 61.9 and 51.7 per cent with difenacoum and 57.2 and 51.1 per cent with bromadiolone during first season field trials. Similar trend was noticed in the performance of new rodenticides during 2nd season also. Flocoumafen found highly effective and significantly superior in containing the live burrow counts and tiller damage caused by *B. bengalensis* in rice.

Keywords: Flocoumafen, difenacoum, bromadiolone, *Bandicota bengalensis*, rice

1. Introduction
Rodents create a great nuisance by causing extensive damage to field crops and structural damage in residential premises, besides transmitting several dreaded zoonotic diseases among humans and their animals. In India, among the pest rodent species, the lesser bandicoot, *Bandicota bengalensis* (Gray) is predominant and more challenging in rice and other crops. These rodents often inflict 10-15% tiller damage in rice, which accounts to a yield loss of 2-3 quintals of paddy per acre in rice growing deltas and even 100% crop loss is witnesses during rodent outbreak years, especially in Godavari delta of Andhra Pradesh (Srinivasa Rao and Nanda Kishore) [1]. Even though rodent trapping, burrow digging and burrow smoking are found effective in controlling the rodents, farmers and pest control operators rely mostly on rodenticides than these methods for their control, because rodenticides are cost effective and can cover larger area in a short period (Mohan Rao, 2003) [2]. Anticoagulants are the important group of rodenticides; they impair the blood clotting mechanism which results in haemorrhage and death. As per the provisions of Insecticide Act (1968) only four anticoagulants i.e. warfarin, coumachlor, coumatetralyl and bromadiolone were registered in India (Mohan Rao and Malla Reddy) [3]. Warfarin has been withdrawn from the market as most of the pest rodent species has developed resistance after its large scale use. Presently, bromadiolone is the only anticoagulant rodenticide available in India and it has been in use more than a decade, particularly in rodent endemic States like Andhra Pradesh. Chances of developing tolerance/ resistance by the lesser bandicoot, *B. bengalensis* against bromadiolone are quite fair, especially in areas of its long time use. Rice growing farmers of Godavari Delta of Andhra Pradesh often complaints in several forums that the bromadiolone is failing in containing the rodent damage in rice. Though this has no scientific evidence, there is a need for screening of other anticoagulants for their efficacy against predominant pest rodent species and make them available to the famers for field use, as the development of resistance against slow acting anticoagulant molecules by the target rodent species is a proven fact in several cases. Flocoumafen and Difenacoum are the 2nd generation anticoagulants and their efficacy against several other rodent species was reported since long back

(Hadler, 1975, John, 1976 and Parshad, 1988) [4-6], but not available for use in India. With this back ground, these two anticoagulants i.e. flocoumafen and difenacoum were tested for their efficacy against *B. bengalensis* in the laboratory and field as well.

2. Materials and Methods

2.1 Collection, rearing and maintenance of test rodents

Live animals of *B. bengalensis* were collected from the rice fields of the research farm using multi-catch traps positioned in the trap barrier system. The field collected animals were transported to the laboratory and transferred individually into the laboratory rearing cages of 60 X 30 X 30 cm. During the one week of acclimatization period the animals were provided with broken rice and water *ad libitum* and their daily consumption rates were recorded. Sick and inactive animals found if any, were discarded and the remaining animals were sexed and weighed. Equal number of male and females weighing more or less uniform weight were selected for use in the experimentation.

2.2 Test Rodenticides: BASF, Mumbai has supplied the Blue colored bi-convex wax blocks of Flocoumafen 0.005% (Storm®) and green colored cube wax blocks of Difenacoum 0.005% (Sorexa®) weighing 8 g each, while Bromadiolone 0.005% RB (Roban®) and Bromadiolone CB 0.25% (Ratol®) were procured from the local market. Loose bait of bromadiolone 0.005% was prepared using broken rice: ground nut oil: bromadiolone 0.25%CB in 96:2:2 ratio on the day of experimentation.

2.3 No-choice test: In no-choice tests, no optional food was given to the test animal. The single caged rats were fed with 20 g of test rodenticide bait for 24h of test period. Later, they were provided with normal plain bait until the death. The mean poison and plain baits intake was recorded and corresponding percentage intakes were calculated. Test animals were observed daily for their mortality and time period required for mortality was recorded.

2.4 Choice test: In choice tests, animals were given option to feed either on plain bait or poison bait given 10 g each during the test period of 24h. Later, they were provided with normal plain bait until the death. The palatability of rodenticide is determined by comparing its consumption with that of challenge bait. The mean bait intake, mortality and time period required for mortality were recorded as in other tests. Typical haemorrhagic symptoms of anticoagulant poisoning were observed in autopsied animals.

The palatability of poison bait was arrived using the following formula

$$\text{Palatability} = \frac{\text{Total intake of poison bait}}{\text{Total intake of plain and poison bait}} \text{ X } 100$$

2.5 Field studies: Field experiments were conducted to study the efficacy against *B. bengalensis* in rice fields in kharif season during 2014-15 and 2015-16. Five villages (L.Koderu, B. Mogaturu, K. Chikkala, Kavitam and Maruteru) with 50-60 ha rice fields located at least 4-5 km apart with more or less uniform initial rodent incidence (live burrows) were selected for imposing the treatments. Each village was divided into 4 blocks and a unit of 4 ha rice field located at least 500 m apart were selected in each block on four corners of the village for sampling. Prior to the imposition of the treatment, rodent incidence was recorded by counting the live burrow counts (LBC) from the each selected unit of rice field. Tiller damage counts were also recorded using diagonal method from a randomly selected plot in each unit in the village as per the standard procedure (Mathur and Prakash, 1984) [7]. All the five treatments including control were randomized and replicated four times. Rodenticide treatment was given for the entire block @ 8 g of poison bait wax block per live burrow by pocketing once in a season in 60 days old crop, invariably before primordial initiation in the rice. In case of bromadiolone 0.005% loose bait weighing approximately 10 g bait packets are pocketed into the live burrows of *B. bengalensis*. Post treatment count of live burrows was recorded 12 days after the treatment, while the post treatment tiller damage was recorded at the time of harvest. The efficacy of the treatments was assessed as per cent reduction in live burrow counts and tiller damage over the control treatment as

Percent control success = 100 [1-(T2 X C1)/ T1 X C2)].

Where, T1 & T2 are the pre and post treatment live burrow counts and tiller damage in treatment plots, while C1 & C2 are the corresponding values in control plots. The data was statistically analyzed using ANOVA.

3. Results and Discussion

Under no-choice conditions, the mean weigh of the test animal varied between 157.5 to 176.5 g and the mean plain bait intake was 6.81 to 8.21 grams per 100 g body weight. More than half of the bait (20.0) placed under no-choice was eaten by the test animals in all the cases before the poison baiting. 20 g of poison bait was given per each animal and allowed 24 hr of feeding, the consumption varied from 5.20 to 6.30 g per 100 g body weight among the treatments. There was no significant difference between percent consumption of plain bait before treatment and poison bait during treatment; further the palatability indices were ranged between 40.4 to 48.8 per cent, indicating that the test animals doesn't showed any food aversion towards the anticoagulant poison bait. Research workers worked on anticoagulant molecules *viz*., bromadiolone (Saravanan and Knakasabai, 1998) [8], flocoumafen (Lund, 1988) [9] and difenacoum (Rowe *et al.*, 1981) [10] also recorded that the test animals ingested poison baits in lethal quantities without any aversion. There was a significant reduction in consumption of plain bait by the intoxicated animals after the test period; the consumption was only 16.3 to 32.8 per cent as against 59.6 per cent in control. All the anticoagulant molecules @ 0.005% caused 100 per cent mortality in the test animals, but with varied time period required for mortality. It was, 3.8 days in Flocoumafen, 4.6 days in Difenacoum, 6.2 days in Bromadiolone CB and 6.9 days in Bromadiolone RB (Table 1). It was understood that the time period required for mortality is influenced by the amount of poison bait ingestion, time period exposure to the poison bait and toxicity of poison bait. In the present case, lowest mean mortality of 3.8 days in flocoumafen certainly due to its higher toxicity to the test animal as other parameters were mere constant. Similarly, Parshad, (1988) [6] also has recorded the 100% mortality of the test animals but in other species when fed with flocoumafen @0.005% in under bi-choice and no-choice laboratory tests. Another new anticoagulant, difenacoum @ 0.005% also recorded the lower mean mortality period over the bromadiolone 0.005%.

Under choice condition also, flocoumafen and difenacoum performed well even though all the three anticoagulant molecules recorded 100% mortality in *B. bengalensis*. The mean daily plain bait consumption was varied between 2.50 to 2.77 g per 100g of body weight, where as it was 1.88 to 2.18 g in anticoagulant baits (Table 2). There was no significant difference between the per cent consumption of poison bait and plain bait offered simultaneously, expect in the case with bromadiolone 0.005% RB wax block. This shows that none of the anticoagulant showed issue of un-palatability. But, Hadler *et al*., (1975) [4] has recorded some evidence of un-palatability with difenacoum against warfarin resistant ship rats, though it has recorded the 100% mortality in the test population. Under bi-choice condition also all the test animals were died when fed with any of the anticoagulant but with varied mean mortality time periods. The lowest time required for mortality was in flocoumafen (4.1d) followed by difenacoum (4.9d). Further, it was observed that the mortality time period were higher in bi-choice tests compared to no-choice tests for all the anticoagulants tested, this is quiet expected as the poison bait consumption was relatively low in choice condition due to availability of the alternate food, which might have resulted in delayed mortality.

The mortality time period required for individual *B. bengalensis* rat with different test anticoagulants was presented in the graph (fig 1). The mortality period repose to the Flocoumafen and Difenacoum was found more or less uniform with a range between 3-5 days. But, in case of bromadiolone it was uneven and ranged between 3 to 11 days. Animals with huge peaks of mortality periods is a sign for existence or development of bromadiolone tolerant individuals, which is quite possible as this rodenticide has been in use since 12-15 years very long time in the study area.

Field studies were conducted during *kharif* 2014-15 in rice crop; the pre treatment live burrow counts were ranged from 17.3 to 20.8/ha, while the tiller damage was 9.5 to 12.0 per cent which are non-significant. Studies revealed that the new rodenticide, flocoumafen 0.005% @ 8g/live burrow achieved 71.3 and 62.8 per cent control success in reducing the live rodent burrows and tiller damage, respectively, which is significantly superior over the other treatments. The other new rodenticide difenacoum @ 8g/ burrow has achieved 61.9 and 51.7 per cent control success with respect to LBC/ha and tiller damage (Table 3). Flocoumafen performed significantly superior in containing the rodent burrows and their tiller damage caused by *B. bengalensis* in rice fields. Parshad (1988) [6] achieved 65% rodent control with one time application of 0.005% flocoumafen bait @ 1kg/ha against *B. bengalensis* and *Mus* spp. in sugarcane and wheat and much more significant control if applied second time also with 10-day interval. Lund *et al.,* (1988) [9] also showed in his reports that flocoumafen is highly potent even in controlling the bromadiolone resistant mouse populations.

During the 2nd season also flocoumafen and difenacoum recorded significantly superior performance over the bromadiolone in reducing the live burrow counts of *B. bengalensis* with 68.5 and 66.5 per cent control success, respectively in rice fields. The per cent reduction in tiller damage due to these two rodenticides was 60.5 and 50.7 per cent as against 43.5 to 47.4 in bromadiolone. Hadler *et al.*, (1975) [4] worked out the optimal field dose of Difenacoum as 0.005% for Warfarin resistant ship rats and mice and at this dose Rowe *et al.* (1981) [10] recorded a control success of 60.4 to 100 per cent in pen studies and 70.2 to 100 per cent in field trials against mice. John (1976) [5] also reported that Difenacoum is highly effective against wild rodents that are resistant to conventional anticoagulants.

Table 1: Effect of different rodenticides against *B. bengalensis* under laboratory conditions (No-choice tests)

Treatments	Mean animal weight (g) + SE	Mean Bait consumption(g) per 100g body weight per day						% Mortality	Mean Days to mortality + SE
		Plain bait before treatment		Poison bait during treatment		Plain bait after treatment period			
		Plain Bait	% consumption	Poison bait	% consumption	Bait consumed	% consumed		
Flocoumafen	176.5+2.63	7.50 + 1.6	58.6+4.1	6.23 + 0.18	54.9+2.09^{NS}	0.97+ 0.27	16.3+1.15**	100 (10)	3.8+0.24
Difenacoum	172.5+2.96	8.21 + 1.2	62.2+3.2	6.30 + 0.42	57.5+3.03^{NS}	1.12+ 0.45	22.0+1.33**	100 (10)	4.6+0.25
Bromadiolone 0.005% RB	169.9+2.90	7.20 + 0.6	56.8+4.8	5.20 + 0.36	44.2+2.90^{NS}	1.12+ 0.15	32.8+1.54**	100 (10)	6.9+0.66
Bromadiolone 0.005% CB	157.5+5.90	6.81 + 1.1	53.6+7.4	5.81 + 0.27	45.2+1.45^{NS}	1.22+ 0.42	29.0+1.60**	100 (10)	6.2+0.47
Control (Broken Rice)	166.8+5.09	7.92 + 0.8	59.6+6.8	-	-	7.21+ 0.42	59.6+2.18^{NS}	0 (10)	-

NS- Non significant, * T-test significant at 0.01 CD

Table 2: Effect of different rodenticides against *B. bengalensis* under laboratory conditions (Choice tests)

Treatments	Mean animal weight (g) + SE	Mean Bait consumption (g) per 100g body weight per day				Palatability (%)	% Mortality	Mean Days to mortality + SE
		Bait consumption during treatment period						
		Plain Bait	% Consumption	Poison bait	% Consumption			
Flocoumafen	170.5+4.01	2.50 + 0.26	42.0+3.9	2.18 + 0.16	37.0+2.9 ^{NS}	48.8+ 3.3	100 (10)	4.1+0.18
Difenacoum	162.3+5.40	2.77 + 0.27	45.0+4.6	2.11 + 0.23	34.0+3.50^{NS}	43.8+ 3.2	100 (10)	4.9+0.35
Bromadiolone 0.005% RB	165.1+2.03	2.62 + 0.20	43.0+3.2	1.88 + 0.20	31.0+3.3*	40.4+ 2.6	100 (10)	9.2+0.36
Bromadiolone 0.005% CB	168.9+3.10	2.50 + 0.21	42.0+3.4	2.06 + 0.10	35.0+2.5^{NS}	45.7+ 2.6	100 (10)	8.6+0.29
Control (Broken Rice)	171.9+ 3.21	-	-	-	-	-	0 (10)	-

NS- Non significant, *'t' significant at 0.05 CD

Table 3: Efficacy of Flocoumafen and Difenacoum against *B. bengalensis* in rice

Treatments	2014-15						2015-16					
	LBC/ha			Percent tiller damage			LBC/ha			Percent tiller damage		
	PTC	ATC	% control success	PTC	ATC	% control success	PTC	ATC	% control success	PTC	ATC	% control success
Flocoumafen 0.005%	20.0^{a}	6.0^{a}	71.3^{a}	12.0^{a}	5.8^{a}	62.8^{a}	16.3^{a}	5.3^{a}	68.5^{a}	11.5^{a}	5.0^{a}	60.5
Difenacoum 0.005%	17.3^{a}	6.5^{ab}	61.9^{b}	10.5^{a}	6.3^{a}	51.7^{b}	17.5^{a}	6.3^{a}	66.5^{a}	12.0^{a}	6.5^{a}	50.7
Bromodiolone 0.005% loose bait	20.5^{a}	9.0^{bc}	57.2^{bc}	10.3^{a}	6.5^{a}	51.1^{b}	19.3^{a}	9.8^{b}	54.7^{b}	10.8^{a}	6.3^{a}	47.4
Bromodiolone 0.005% RB cake	18.8^{a}	9.3^{c}	50.6^{c}	9.5^{a}	6.8^{a}	45.1^{b}	19.3^{a}	11.0^{b}	47.0^{b}	14.0^{a}	8.8^{a}	43.5
Control	20.8^{a}	21.8^{d}	-	9.8^{a}	13.0^{b}	-	19.5^{a}	21.8^{c}	-	12.8^{a}	14.3^{b}	-
F-test	NS	Sig	Sig.	NS	Sig	Sig	NS	Sig	Sig	NS	Sig	NS
CD (P=0.05)		2.7	8.1		2.5	7.9		3.4	15.6		4.2	
CV		16.6	7.8		21.3	9.4		14.4	16.5		33.3	
PTC- Pre treatment count, ATC- After treatment count												

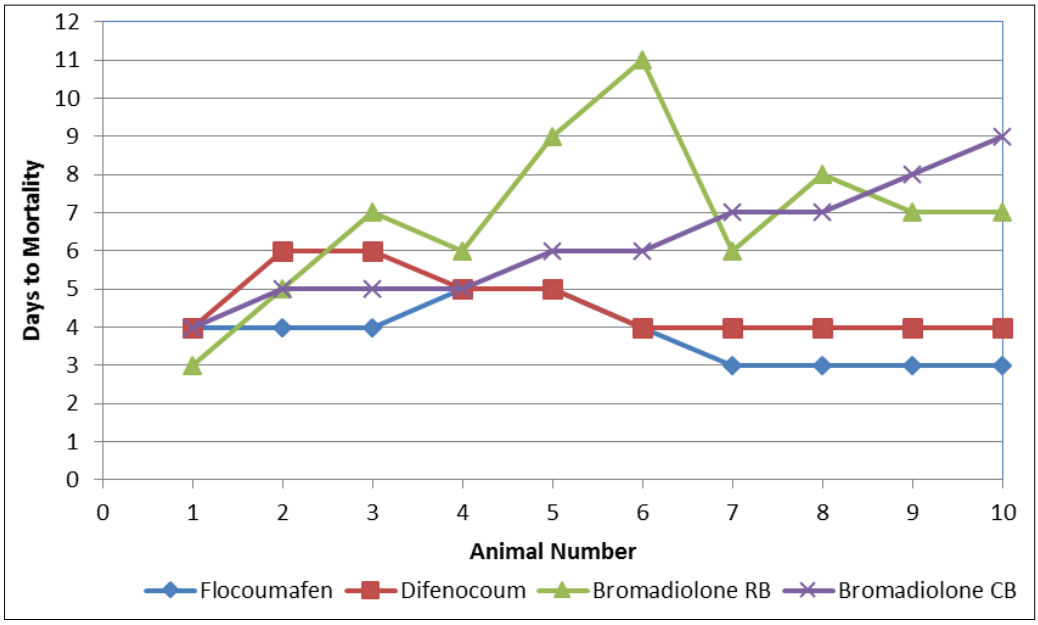

Fig 1: Time mortality response in *B. bengalensis* against anticoagulant rodenticides

4. Conclusion

Anticoagulants, flocoumafen and difenacoum showed superior performance against *B. bengalensis* in the laboratory and field as well. Bromadiolone has been introduced long back and is use for more than a decade; in such scenario these two rodenticides can be found as alternatives to the presently using bromodiolone in the country.

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