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Evaluation of single dose efficacy of difethialone- a second-generation anticoagulant for the control of rodents inhabiting arid ecosystem

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ABSTRACT

A study was conducted during 2002-03 to evaluate single dose efficacy of difethialone-a second generation anticoagulant rodenticide was evaluated in laboratory against Indian gerbil, *Tatera indica*, cutch rock rat, *Rattus cutchicus*, Indian desert gerbil, *Meriones hurrianae* and northern palm squirrel, *Funambulus pennanti*. All the test rodents were exposed to pearl millet-based difethialone (0.0025%) loose baits for one day under no-choice and choice condition. In toxicity trials under no-choice feeding, all the test rodents succumbed to the poison bait within 3–11 days after treatment. Minimum dose required to initiate mortality in *T. indica*, *R. cutchicus*, *M. hurrianae* and *F. pennanti* was 0.50, 0.94, 0.60 and 1.42 mg/kg respectively. Mortality of test rodents was reduced to 80, 80, 70 and 50% in *T. indica* (80%), *R. cutchicus* (80%), *M. hurrianae* (70%) and *F. pennanti* (50%) respectively when the poison bait was exposed under choice test. However, no significant difference was observed between the consumption of plain and poison bait indicating that anticoagulant was fairly well acceptable and palatable to all the test rodent species. Difethialone is an effective single dose anticoagulant rodenticide for managing rodents of arid ecosystem.

Key words: Rodents, Gerbils, Squirrels, Rodenticides, Anticoagulant, Difethialone, No choice, Choice tests

Rodents inflict considerable damage to field crops, vegetable and fruit crops both during pre- and post-harvest stages. Therefore, control of these small mammalian pests is necessary to optimize the production. Zinc phosphide, an acute rodenticide is being widely used to contain the menace of these pest rodents in crop fields, fruit orchards, grassland and afforestation sites. However, toxicity to non-targets, pre-baiting requirements, development of bait shyness and poison aversion are some of the drawbacks with zinc phosphide in achieving effective and long lasting control success under field conditions (Jain and Tripathi 2000). Therefore search for improved alternative rodenticides vis-à-vis screening of their bio-efficacy is considered to be of paramount importance. Evaluation of several second-generation anticoagulant rodenticides in recent years, viz bromadiolone, brodifacoum, flocoumafen and difethialone have helped in developing an effective and safe rodent management strategies world over. Presently bromadiolone is the only anticoagulant rodenticide of this generation registered by Government of India for public use. Thus there is a great need to screen other molecules of this nature against Indian rodents. Most of the second-generation anticoagulant rodenticides have been evaluated against important rodent pests of Indian agriculture (Saxena and Sahni 2000; Chaudhary *et al.* 2002; Prakash *et al.* 2003) but very little information is available pertaining to single dose toxicity and acceptability of difethialone against rodents inhabiting arid ecosystem. Difethialone belongs to family of hydroxy-4-benzo-thio-pyranones ($C_{31}H_{23}BrO_2S$) with chemical formula: 3-[(1RS, 3RS; 1RS, 3Rs) -3- (4'-bromobiphenyl-4-yi) -1,2,3,4- tetrahydro-1-naphthyl] -4-hydroxy-1-benzothi-in-2-one. The present study was therefore undertaken during 2002–03 to screen difethialone (0.0025%) baits against the important rodent pests of arid ecosystem in laboratory.

MATERIALS AND METHODS

Four species, viz Indian gerbil, *Tatera indica*, cutch rock rat, *Rattus cutchicus*, Indian desert gerbil, *Meriones hurrianae* and northern palm squirrel, *Funambulus pennanti* inhabiting different arid production systems, namely crop fields, rangelands, forestry areas and fruit orchards were used for the study. *M. hurrianae*, a diurnal rodent is essentially a native of sandy areas and is distributed only in arid tracts of Rajasthan, Gujarat, Punjab and Haryana states (Tripathi and Chaudhary 2004), whereas *T. indica* is widely distributed throughout the country. Both the gerbil species are considered as major pest to field crops in arid regions. The cutch rock rat *R. cutchicus* inhabits the rocky habitat and causes damage to forestry plantations. Similarly the squirrel, *F. pennanti*, an arboreal and diurnal rodent is a major vertebrate pest to fruit crops, nurseries and kitchen gardens (Jain and Tripathi 2000). All the 4 test species were live trapped with the help of Sherman traps from their respective habitats near Jodhpur

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(Latitude 26° 18'N, longitude 73°01'E). The live-trapped experimental animals were individually caged for 2 weeks for acclimatization in laboratory. During the period they were provided with whole grains of pearl millet (*Pennisetum typhoides*) as laboratory diet. Tap water was also available to the experimental rodents *ad libitum*. Ten healthy adult rodents of each species were taken for each set of experiments.

For understanding single dose toxicity of difethialone, the test rodents were exposed to poison baits under no-choice feeding trials. In this experiment the animals were offered difethialone (0.0025%) bait prepared in pearl millet grain only for one day. Consumption of poison baits (g/100g body wt), % mortality and days to death was worked out for each test species.

Acceptability of difethialone bait was studied under 4 experimental sets for each test species using 10 individuals in each trial. Herein the experimental animals were given a choice of difethialone (0.0025%) treated poisoned food and plain food (choice test). Same cereal grain (pearl millet) in which poison bait was prepared was used as plain bait in a separate container along with poison bait. These tests were also conducted for 1 day feeding period with 0.0025% concentration of anticoagulant to establish the single dose efficacy and palatability of difethialone.

Prior to exposure to difethialone treated baits under both the test conditions daily consumption of plain food by test rodents was measured for three days as pre-treatment consumption. On 4th day the test poison was given and its consumption was measured after 24 hr. With the help of data on consumption of poison bait, intake of active ingredient of rodenticide (mg/kg) was also calculated. During post treatment periods only plain food was given to the test animals and its consumption was recorded daily up to 15 days. Besides post treatment consumption, observations on signs of illness and other symptoms of anticoagulant poisoning were monitored regularly.

RESULTS AND DISCUSSION

Overall consumption of plain/poison bait, extent of mortality and days to death in no-choice and choice trials did not record any significant difference between the sexes. Therefore data for both the sexes were pooled and analyzed for drawing various inferences.

Toxicity

Mortality data under no-choice feeding trials showed that single day exposure of difethialone (0.0025%) yielded 100% kill of *T. indica, R. cutchicus, M. hurrianae* and *F. pennanti* (Table 1). It indicates that single day feeding of difethialone (0.0025%) was highly potent in knocking down the test animals. Minimum dose of test poison required to kill *T. indica, R. cutchicus, M. hurrianae* and *F. pennanti* by feeding was 0.50, 0.94, 0.60 and 1.42 mg/kg respectively whereas intake of ai to effect 100% mortality of test rodent species ranged from 1.04–2.3 mg/kg (Table 1). The mortality data indicated that though mortality in *T. indica* was initiated on day 4 but it recorded longest death period of 11 days for complete kill (Average 7.9 days) than the other 3 species. It may be due to relatively less poison intake (1.04 mg/kg) than others. Moreover, this gerbil was also been reported to take longer periods to die with flocoumafen (0.005%) in comparison to *M. hurrianae* and *F. pennanti* (Jain *et al.* 1992). In case of *R. cutchicus* the death period was shortest (3–7 days, average 5.0 days). *F. pennanti* and *M. hurrianae* too recorded a mean death period 6.10 and 5.9 days, respectively (Table 1). Perusal of data on ingestion of anticoagulant revealed that the cutch rock rat and squirrels ingested almost double the active ingredient of the poison as compared to the two gerbils (Table 1). This rodenticide was also reported to cause cent percent mortality in *Bandicota bengalensis, Mus musculus, Funambulus pennanti* and *Tatera indica* in one-day exposure (Sheikher and Sood 2000 and Chaudhary *et al.* 2002). Like difethialone (0.0025%), bromadiolone (0.005%), brodifacoum (0.005%) and flocoumafen (0.005%) too yielded 100% kill of *T. indica* with increased exposure period of 2–3 days (Rana and Tripathi 1999, Prakash *et al.* 2003), Similarly *F. pennanti* required 6 days exposure period for 100% mortality with brodifacoum (0.005%) and three days with bromadiolone (0.005%) (Mathur *et al.* 1992). However, flocoumafen (0.0025 and 0.005%) like difethialone (in the present trial) registered cent percent kill of *F. pennanti* in one-day exposure (Jain *et al.* 1992).

Acceptability and palatability

In general intake of poison bait was reduced in choice in all test species, but *R. cutchicus* (from 8.12 to 3.25 g) and *F.*

Table 1 Bait consumption and mortality pattern in different rodent species feeding on difethialone (0.0025%) mixed in pearl millet in no-choice trials

Rodent species	Feeding period	Mean body weight Mean ± SE	Poison consumption g/100g body weight Mean ± SE	Anticoagulant (ai) ingested (mg/kg) Mean ± SE	Mortality	Days to death Mean ± SE	Days to death Range
Meriones hurrianae	1	105.70 ± 3.57	4.64 ± 0.03	1.17 ± 0.09	10/10	5.90 ± 0.57	4 -7
Tatera indica	1	112.30 ± 6.79	4.50 ± 0.53	1.04 ± 0.16	10/10	7.90 ± 0.77	4-11
Rattus cutchicus	1	54.8 ± 2.18	8.12 ± 1.08	2.03 ± 0.26	10/10	5.00 ± 0.42	3 -7
Funambulus pennanti	1	106 ± 3.18	7.99 ± 0.40	2.01 ± 0.10	10/10	6.10 ± 0.62	3-9

Table 2 Bait acceptability and mortality in different rodent species given choice between plain and difethialone (0.0025%) treated pearl millet bait

Rodent species	Feeding period	Mean daily bait intake (g/100 g body weight) Mean ± SE		Anticoagulant consumed (mg/kg) Mean ± SE	Mortality	Days to death	
		Poison (1)	Plain (2)			Mean ± SE	Range
Meriones hurrianae	One	3.87 ± 0.47	4.07 ± 0.71	0.96 ± 0.14	7/10	7.86 ± 0.92	5–11
Tatera indica	One	3.06 + 0.27	4.60 + 0.62	0.77 + 0.81	8/10	7.40 + 1.34	6–11
Rattus cutchicus	One	3.25 ± 0.21	4.80 ± 0.41	0.81 ± 0.31	8/10	6.80 + 0.71	4–7
Funambulus pennanti	One	3.07 ± 0.27	6.38 ± 0.84	0.76 ± 0.11	5/10	6.00 ± 0.89	4–9

*Non-significant at P >0.01 ('t' test)

pennanti (7.99 to 3.07 g) recorded maximum reduction (Table 1and 2). Such a variation was quite obvious due to availability of an alternate un-poisoned food in choice tests. However, relative consumption of plain and poison bait in choice feeding (Table 2) did not record any significant variation by any of the test species. Irrespective of the test species this intake was also at par ranging between 3.06 and 3.87-g/100 g body wt (poison bait) and from 4.07 to 6.38 g/100 g body wt (plain bait). It clearly reflects that the pearl millet based difethialone bait (0.0025%) is fairly well acceptable and palatable to all the test rodents of arid ecosystem. Similar trends have been observed by various workers with other second-generation anticoagulants, viz bromadiolone, brodifacoum and flocoumafen also (Saravanan and Kanaksabai 1998, Jain and Khare 2004).

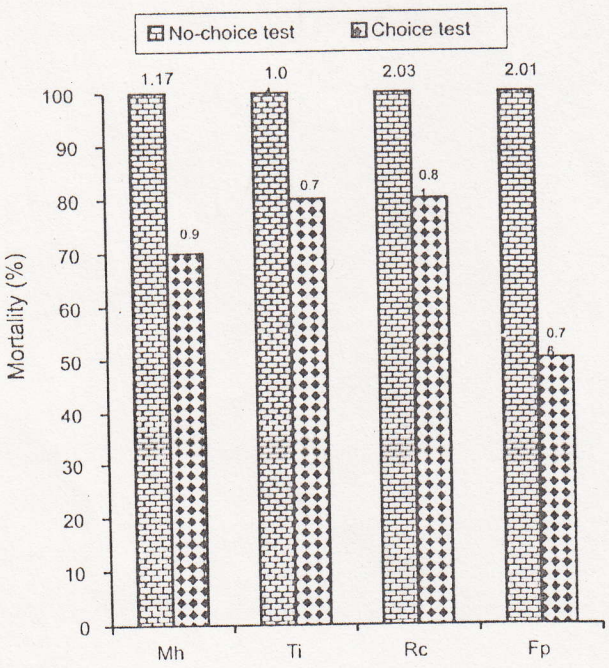

Fig 1 Mortality rates under no-choice and choice condition after one day exposure to difethialone (0.0025%) (Figures at the top of bars are of mg/kg ai of poison ingested)

Mortality patterns

Reduced intake of poison bait in choice trials led to decreased ingestion of active ingredient of rodenticide (0.76–1.79 mg/kg), which was clearly reflected in reduced mortality data in all the test species in comparison to no-choice test. Single day feeding of difethialone (0.0025%) resulted in least mortality in *F. pennanti* (50%), whereas *M. hurrianae, T. indica* and *R. cutchicus* registered 70, 80 and 80% kill, respectively. Contrary to this Saxena *et al.* (1992) have reported 100% mortality of *M. hurrianae, F. pennanti* and *M. musculus* in choice test after single day exposure of difethialone, which might be due to ten times higher dosage (0.025%) evaluated by these authors. Mean days to death in choice test was between 6 and 7.86 days (Table 2) which was comparable with that of no-choice tests (Table 1). Maximum mortality was observed between 7-9 day (*T. indica* and *M. hurrianae*) and between 6-8 day in (*R. cutchicus and F. pennanti*). Flocoumafen (0.500%) was also reported to cause reduction in mortality (60%) of these rodents under choice test in single day exposure, as compared to 100% in no-choice test (Jain *et al.* 1992). Duration of death of desert rodents with difethialone baiting in the present study was also similar to that reported by Jain *et al.* (1992) with other anticoagulants of the same generation. Sheikher and Sood (2000), Saxena and Sahni (2001) found difethialone (0.0025%) quite efficacious in containing a variety of field rodents. Chaudhary and Tripathi (2003) have reported that difethialone (0.0025%) was effective in managing the zinc phosphide induced bait shy rodents also.

The first generation anticoagulant rodenticides like warfarin, fumarin, coumatetralyl, chlorophacinone etc. have also been found effective against the rodents of arid region but they required very long exposure periods of 7-14 days (Kumar and Kushwaha 1998, Saxena and Sahni 2001). However, the second generation anticoagulant rodenticides required relatively shorter feeding period of 1-4 days (Saravanan and Kanaksabai 1998, Prakash *et al.* 2003).

Thus the present findings indicated that difethialone (0.0025%) is an effective single dose anticoagulant rodenticide having an edge not only over the first generation multi-dose anticoagulant rodenticides, requiring 7-14 days feeding but over the second-generation anticoagulant rodenticides also which yield similar results at relatively higher concentration

(0.005%) (Jain *et al.* 1992) for managing the rodents of arid agro-ecosystem.

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