



Bio-efficacy of denatonium benzoate added formulation of bromadiolone against commensal rodents



R.S. Tripathi*, Vipin Chaudhary

Central Arid Zone Research Institute, Jodhpur, India

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ABSTRACT

Present day rodent management technology mostly relies on use of rodenticides, which is unsafe for human beings and other non-targets. Denatonium benzoate (DB) is an intensely bitter and non-toxic substance which is detectable by humans at a concentration of 10 ppb. Denatonium benzoate added wax block formulation of bromadiolone (0.005%) (an anticoagulant rodenticide) was evaluated against commensal rodents (house rat, *Rattus rattus* and lesser bandicoot rat, *Bandicota bengalensis*), which are always found in the proximity of pets, domestic animals and human beings. Single day exposure to bromadiolone (0.005%) formulation with DB and without DB in no-choice tests yielded 100% mortality in both the test rodent species in 2–11 days. The amount of poison bait ingested by *R. rattus* and *B. bengalensis* for both the formulations did not differ significantly, ranging between 5.87–7.30 and 6.51–6.71 g/100 g body weight, respectively. In choice tests in the presence of alternate plain food (broken wheat grain), consumption of both the formulations of bromadiolone (0.005%) (with and without DB) was similar to plain food at both the exposure periods (1 and 2 days), indicating a good acceptability of test rodenticide. However, mortality with a single day exposure to bromadiolone (0.005%) formulation with DB was 70% and without DB was 60–80% in both test rodent species within 3–14 days. With a two days exposure period, mortality was 100% in both the species of test rodents in 3–13 days. Single day choice test between the formulations revealed that intake of baits of both formulations by both the test rodents was similar i.e., in the range of 8.16–8.94 g/100 g b wt (bromadiolone with DB) and 8.20–9.5 g/100 g b wt (bromadiolone without DB) indicating that incorporation of a bittering agent (DB) with bromadiolone did not alter the consumption of poison in both the test species. In field trials the mean control success as assessed by census baiting and trapping methods with the application of bromadiolone (0.005%) with DB ranged between 54.0% (houses and shops) to 68.4% (poultry farms) and was 55.6% (houses and shops) to 68.4% (poultry farms) in treatments with bromadiolone (0.005%) without DB. It can be concluded that denatonium benzoate could be a best additive in rodenticidal formulations as the acceptability, palatability and toxicity in the laboratory and also its performance in field in managing the pest rodents were not altered.

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1. Introduction

Rodents cause immense losses to standing crops in fields and food and other commodities in storage (Parshad, 1999). They are also regarded as vectors of several zoonotic diseases to man and his livestock (Singleton et al., 2003). Present day rodent management technology mostly relies on use of rodenticides, which are equally hazardous to a variety of non-target animals including human

beings. Safety of non-targets, while controlling the pest rodents has been a long standing matter of serious concern (Marsh, 1985). A number of methods and techniques, such as, warnings on product labels, warning colors, symbols, protective packing, selective/protective placement of baits, addition of aversive agents etc. are in vogue to make rodent management as selective as possible for safety of non-targets.

Addition of denatonium benzoate (DB) in rodenticidal baits may be an effective tool in reducing accidental ingestion of poison baits by human beings and other non-targets species because the compound (DB) is an intensely bitter and non-toxic substance. It is detectable by humans at a concentration of 10 ppb, discernibly

* Corresponding author.

E-mail address: dr_rstripathi@yahoo.co.in (R.S. Tripathi).

bitter at 50 ppb and unpleasantly bitter at 10 ppm (Payne, 1988). However its effectiveness may not be universal, as different animals may have different sensitivities to this chemical. This compound repels cats, dogs and birds, prevents cannibalism in pigs and prevents deer from nibbling trees (Payne, 1988). Keleinkauf et al. (1999) reported that it reduces the consumption of barley by wood mice (*Apodemus sylvaticus* (Linnaeus, 1758)), but had no effect on the amount of fly pupae eaten by common shrew (*Sorex araneus* Linnaeus, 1758) and barley eaten by bank voles (*Clethrionomys glareolus* (Schreber, 1780)).

Bromadiolone is a well-known single dose second generation anticoagulant rodenticide worldwide and is highly effective against commensal as well as field rodents (Malhi and Sheikher, 1985; Jain, 1985; Sridhara et al., 1988; Jain and Tripathi, 2000; Tripathi and Chaudhary, 2005). Its mode of action includes inhibition of formation of prothrombin necessary for coagulation of blood, thus causing fatal hemorrhages. Bromadiolone is the only second generation anticoagulant rodenticide registered by Central Insecticide Board and Registration Committee (Govt. of India) for managing commensal as well as field rodents in India (Tripathi and Jain, 1994; Parshad, 1999; Tripathi and Chaudhary, 2006). Commensal rodents always remain in the proximity of pets, domestic animals, human beings, their control through toxic rodenticides requires safe bait delivery systems to avoid hazards to non-targets.

Since denatonium benzoate is known to be extremely aversive against a variety of mammals including human beings, the present investigation was attempted to determine the bio-efficacy of a wax block formulation of bromadiolone (0.005%) containing denatonium benzoate (10 ppm) versus wax block formulation of bromadiolone (0.005%) without denatonium benzoate against two commensal rodents, viz., the house rat, *Rattus rattus* (Linnaeus, 1758) and the lesser bandicoot rat, *Bandicota bengalensis* Gray, 1835 in laboratory and field conditions.

2. Materials and methods

2.1. Laboratory studies

2.1.1. Test animals

Two rodent species viz., *R. rattus*, an important pest of godowns and residential premises and *B. bengalensis*, an important pest of field crops as well as storage, were used for this investigation. Test rodents were live trapped using Sherman traps from residential areas, stores, grain markets and the railway station area of Jodhpur city (Lat. 26°14'N Long. 72°59'E). Before experimentation, it was ascertained that trapped rodents had no previous experience of feeding on any anticoagulant baits, as these areas were never treated with any rodenticides for control of rodents. All trapped animals were weighed, sexed and lodged individually in wire mesh cages (152.4 × 76.2 × 76.2 cms) for two weeks for acclimatization in the laboratory before initiating the experiments. They were provided with broken wheat as food and tap water *ad libitum*. Ten healthy animals (5 males and 5 females) were selected for each set of experiments and the injured, sick animals and pregnant females were discarded as per the guidelines of the European Plant Protection Organization (Mathys, 1975). Three days before experimentation, the rodents were again weighed and their food consumption (pre-treatment) was recorded daily.

2.1.2. Test rodenticide

Ready to use wax block formulations of bromadiolone (0.005%) containing 10 ppm denatonium benzoate (DB) and another ready to use wax block formulations of bromadiolone (0.005%) without DB were evaluated in the study.

2.1.3. Feeding trials

Two types of feeding trials (no-choice and choice) were employed for the study. In no-choice feeding trials, both the test species were exposed separately to both the wax block formulations of bromadiolone (0.005%) (i.e. with and without DB) in separate cages for one day. No food was given during the one-day treatment period. However, in choice trials two sets of experiments were laid. In the first experiment an alternate plain food (broken wheat) was also offered along with test poison baits in separate feeding bowls. For this experiment two sets were maintained separately for bromadiolone with DB and without DB for each test species. The choice tests were run separately for two exposure periods (1 and 2 days). In the second choice experiment both the formulations of bromadiolone (0.005%) (i.e., with and without DB) were offered together to each of the test species for one day exposure period only. For both types of choice experiments, weighed quantities of both the formulations of bromadiolone (0.005%) (with and without DB) and plain food were given to experimental rodents along with tap water *ad libitum*. Consumption of bait for pre-treatment, treatment and post-treatment periods was measured daily and observation on symptoms of poisoning (i.e. bleeding from nose, ears, wounds (if any) etc.) and days to death was recorded. The formulation of bromadiolone (0.005%) without DB was taken as control for comparing the bio-efficacy of bromadiolone (0.005%) with DB.

2.2. Field trials

Field trials for both the formulations of bromadiolone (0.005%) were conducted separately in three commensal rodent habitats viz., (i) poultry farms, (ii) ware houses/godowns and (iii) houses/shops in the Jodhpur city. In each habitat three sites having uniform pest infestation, as revealed from pre-treatment census and counts of infested locations, were selected. The methods for assessing rodent population levels directly or indirectly involve monitoring relative activity before and after treatment. The efficacy of test rodenticidal bait (bromadiolone with DB versus bromadiolone without DB) was thus evaluated using two census methods viz., census baiting and trapping (Kaukeinen, 1979; Saxena et al., 1990). Pre- and post-treatment populations/infestations were assessed by laying plain baits and Sherman traps for three consecutive nights. On average, 50 g of plain (broken wheat mixed with oil) bait/bait station was laid in 10–15 bait stations/site. The bait stations were placed 10 m apart at places where signs of infestation were seen. The bait was replenished after every 24 h. Thirty Sherman traps (23 × 8 × 9 cms) baited with peanut butter were laid at intervals of 10 m preferably near walls where tracks of rat movement were visible (Advani, 1992). Traps were checked after 24 h and trapped rodents were weighed and again released to the same site. The traps were again laid after baiting with peanut butter. On the basis of pre-treatment assessment of population/infestation poison baits (bromadiolone (0.005%) with and without DB) @ 50 g/bait station were laid for two consecutive nights on the runways of rodents in all the study habitats. Monitoring of sites after poisoning was initiated immediately after completion of treatment period and continued upto 15 days. Thereafter, post-treatment census was initiated for three consecutive nights. The consumption of plain bait and trapping data (pre- and post-treatment period) and poison bait (treatment period) were recorded daily during the experiment.

2.3. Statistical analysis

The absolute consumption values of plain/poison baits by each test animal in laboratory were converted to relative values (g/100 g of body weight) for comparison. However the ingestion of active

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