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RESEARCH

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Efficacy of combined formulation of bromadiolone and cholecalciferol in reducing rodent population and damage in agricultural crop fields

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Abstract

Background Field rodents cause significant damage to standing crops in agroecosystems at vulnerable stages. Of all the methods available, chemical rodent control is the most practical and economically feasible. Laboratory studies demonstrate the potential of synergistic bait formulations containing bromadiolone and cholecalciferol. This study is the first multi-site multi-crop trial to assess the efficacy of cereal bait formulations containing lower than standard doses of bromadiolone (0.0025 and 0.001%) and cholecalciferol (0.02 and 0.04%) compared to presently recommended bait formulations of zinc phosphide (2.0%) and bromadiolone (0.005%) to protect wheat, rice and sugarcane crop fields against rodent attack.

Results Rodent control success was highest (74.21–88.80%) in fields treated with a combination bait formulation containing bromadiolone (0.0025%) and cholecalciferol (0.04%), which led to a significant reduction in crop damage (from 6.82 to 26.56% cut tillers/canes and 251.75–1448.00 kg/ha yield loss (in reference block) to 1.18–6.18% cut tillers/canes and 46.67–745.00 kg/ha yield loss (in treated blocks).

Conclusions This study therefore found that cereal bait formulation containing bromadiolone (0.0025%) and cholecalciferol (0.04%) can be effectively used to manage rodent population in agricultural crop fields and it is suggested that consideration be given to registering this combination rodenticide formulation to improve global food security.

Keywords Bromadiolone, Cholecalciferol, Crop fields, Zinc phosphide, Rodents

Background

Agriculture in India is the largest enterprise in the private sector, employing about 60% of the workforce and contributing significantly to the country's GDP. Rodents are the main culprits of damage to standing crops in all the agroecosytems. This damage is more pronounced in developing countries like India. Rodents destroy rice and wheat crops at all the crop stages viz. tillering, panicle initiation, flowering, dough and ripening stages (Gogoi & Borah, 2013; Sarwar, 2015; Singla & Babbar, 2010), but the greatest damage was observed at the ripening stage. Rodent damage to rice and wheat crops is estimated at 5–15% (Chellappan, 2021). Rodents also cause damage to sugarcane, India's main cash crop. India is the world's second largest producer of sugarcane. Rodents feed on lower stem internodes and damage roots through burrowing behaviour (Singla & Babbar, 2012; Singla & Parshad, 2010). Additionally, bacterial and fungal pathogens can also infect rodent damaged sugarcane stems causing secondary damage. *Bandicota bengalensis* is the most common rodent in

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sugarcane fields, causing considerable damage (Pervez et al., 2019; Rao, 2003).

There are various strategies to manage rodents in crop fields, such as trapping, burrow fumigation, habitat management, biological control and the use of chemical sterilizing agents, but chemical control of rodent pests (using rodenticide baits) is the most practical and economically viable option (Smith & Mayer, 2015; D'Silva & Krishna, 2019). In India, the recommended rodenticide baits are zinc phosphide (2%, acute rodenticide) and bromadiolone (0.005%, anticoagulant). Acute rodenticide causes death within 24 h of ingestion. But their regular use can cause bait-shyness in rodent populations surviving after acute poisoning (Horak et al., 2018). Bromadiolone is effective against bait-shy rodent populations, but reduced efficacy of bromadiolone has been reported, possibly due to the development of resistance (Garg et al., 2017). Furthermore, it tends to bioaccumulate, posing a secondary poisoning threat to predatory and scavenger reptiles, birds and mammals (Lettoof et al., 2010). In addition, rodents share their habitat with other organisms, so, there is a risk of poisoning non-target species (Elmeros et al., 2019). Considering the limitations of traditional rodenticides, the sub-acute rodenticide cholecalciferol has been used in many countries.

Given the above limitations, the efficacy of combination bait formulation of bromadiolone and cholecalciferol has been evaluated in the laboratory against major rodent species and resulted in significant mortality at much lower doses (Kocher & Kaur, 2013; Singla & Kaur, 2015). The efficacy of three rodenticides viz. acute rodenticide, first generation anticoagulant rodenticide and combination rodenticide (containing an acute toxicant and an anticoagulant) was also compared (Witmer et al., 2017). Combination rodenticide group had the highest effective rate of 93%, followed by the acute rodenticide (50%) treatment group. Similar results were recorded when squirrel control tests were performed on a combination bait, containing diphacinone and cholecalciferol (Witmer & Samra, 2018). Klemann et al. (2023) reported more than 90% control success of *Rattus norvegicus* in fields treated with this combination bait.

Considering the effectiveness of two combination baits (based on bromadiolone (Br) and cholecalciferol (Ch)) in our laboratory trials, and the lack of data on the field effectiveness of combined rodenticide baits in India, the current study was conducted for the first time to compare the potential of already recommended rodenticide baits i.e. zinc phosphide (2%) and bromadiolone (0.005%) with two combination baits containing lower concentrations of bromadiolone (0.0025 and 0.001%) and cholecalciferol (0.02 and 0.04%) in managing rodent population in wheat, rice and sugarcane crop fields located in three different agro-climatic zones of Punjab in India.

Methods

Study location

This study was carried out in wheat, rice and sugarcane crop fields located in villages of five districts under three different agro-climatic zones of Punjab, India viz. the Sub-mountain undulating zone (District Hoshiarpur), the Central Plain Zone (Districts Kapurthala, Jalandhar and Ludhiana) and the Western Plain zone (District Fazilka) (Fig. 1) from February, 2021 to September, 2021. For the wheat and rice crops, three locations were selected, each consisting of five blocks (I–V) (total 12.0 ha), while for sugarcane crop, six blocks (I to VI) were selected (total 7.2 ha). Each block also had three replicated fields of 0.4 ha. Rodent infestation in different crop fields was determined based on typical rodent burrows (Singla & Babbar, 2010).

Treatment and data collection

Pre-census bait consumption (g/100 g) was recorded for all the fields by placing 400 g of plain WSO bait (loose mixture of cracked Wheat grains, powdered Sugar and edible refined Oil in the ratio 96:2:2) at 40 bait points on pieces of paper, and the remaining bait was collected and weighed on the third day.

After the census, wheat and rice crop fields in blocks I–IV were treated with zinc phosphide (2%) bait (T1), bromadiolone (0.005%) bait (T2), combination bait I (0.0025% Br + 0.02% Ch) (T3) and combination bait II (0.0025% Br + 0.04% Ch) (T4), respectively. Fields of block V were considered as untreated references. Treatment baits were placed on pieces of paper 400 g/0.4 ha @ 40 bait points along the bunds, near burrow holes and other rodent activity sites.

Given the high rate of rodent infestation in sugarcane crop, a second rodenticide treatment after 15 days is recommended to manage surviving rodent populations (Mahal & Kaur, 2021). In this study, sugarcane crop fields in blocks I-V were treated with zinc phosphide bait (2%) followed by bromadiolone (0.005%) baiting after 15 days (T1), combination bait I once (T2), combination bait I twice at an interval of 15 days (T3), combination bait II once (T4) and combination bait II twice with an interval of 15 days (T5), respectively. Fields in block VI were considered as untreated references.

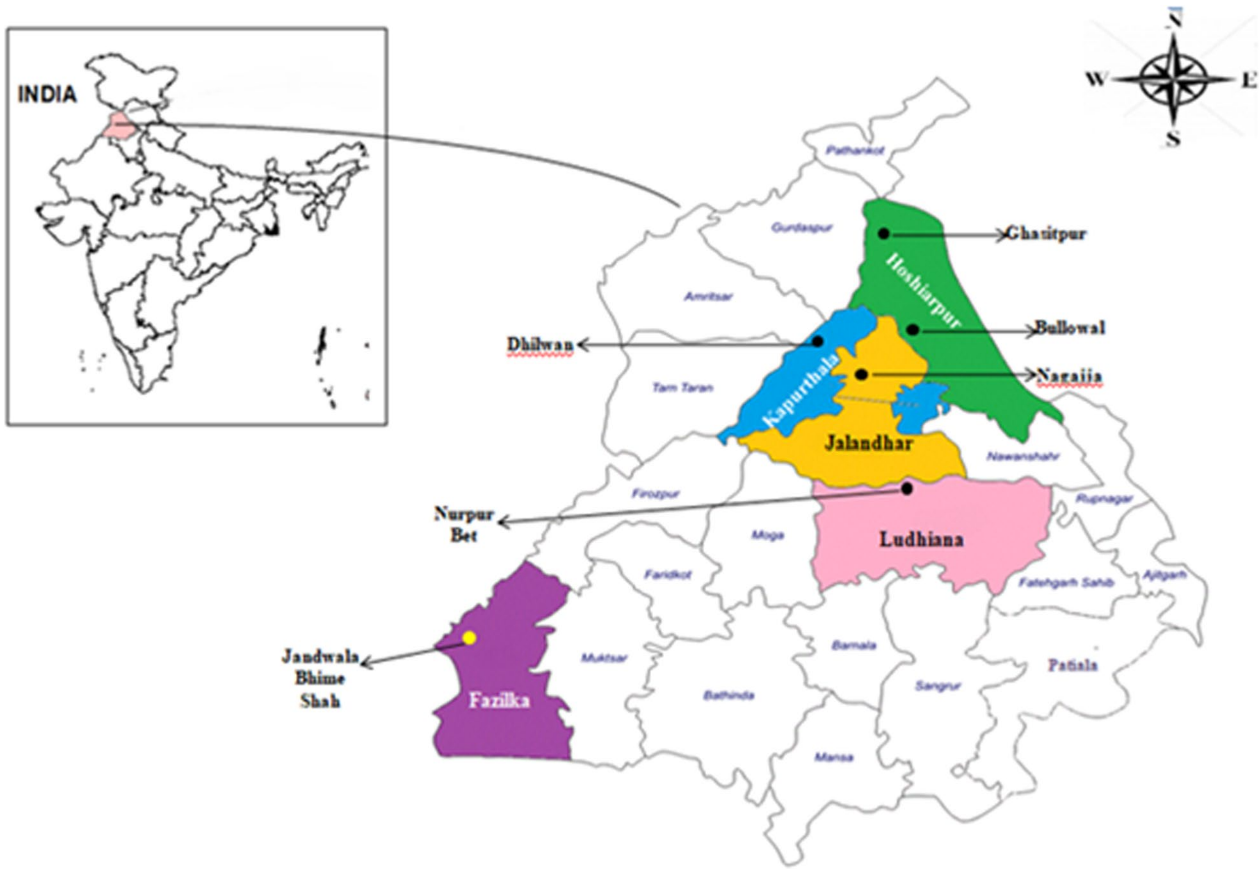

Fig. 1 Map of Punjab State of India showing study areas

In different crops, treatments were carried out according to the schedule recommended for each crop (Mahal & Kaur, 2021; Kumar & Kaur 2022). In wheat crop, the treatments were carried out in the last week of February 2021 and in rice crop, the treatments were carried out in the third week of August 2021 before the milky grain stage. In the sugarcane crop, treatments were carried out in the months of July–August 2021 (after transplantation of rice in the surrounding fields).

Post-census bait consumption (g/100 g) was recorded according to the method used in the pre-census, 15 days after all treatments. To document the treatment effects, rodent control success was determined by the following formulae:

$$\text{Control success(\%)with respect to same field} = \frac{\text{Pre treatment census bait consumption} - \text{Post treatment census bait consumption}}{\text{Pre treatment census bait consumption}} \times 100$$

$$\text{Control success (\%) with respect to reference field} = 1 - \frac{\text{T2} \times \text{R1}}{\text{T1} \times \text{R2}} \times 100$$

where R1 = Pre-treatment census consumption in the reference field; R2 = Post-treatment census in reference field; T1 = Pre-treatment census consumption in the treated field; T2 = Post-treatment census consumption in the treated field.

Pre-harvest rodent damage (%) and yield loss (kg/ha) were determined in different crops by the following formulae:

$$\text{Cut tillers in wheat and rice crops}(\%) = \frac{\text{Number of cut tillers per square metre}}{\text{Total tillers per square metre}} \times 100$$

$$\text{Cut canes in sugarcane crop}(\%) = \frac{\text{Number of cut canes per square metre}}{\text{Total canes per square metre}} \times 1$$

Mean yield loss per cane damaged by rodents = 0.4 kg (Singla & Prashad, 2010).

$$\text{Yield loss in sugarcane crop}(\text{kg/ha}) = \text{Number of cut canes per square metre} \times 0.4\text{kg} \times 4047$$

$$\text{Yield Loss in wheat and rice crops}(\text{kg/ha}) = \frac{\text{Number of cut tiller} \times \text{yield per tiller}(\text{g}) \times 2.5 \times 4,047}{10,00}$$

Table 1 Comparison of mean percent rodent control success, rodent damage and yield loss in wheat crop fields of three locations treated with different rodenticide baits

Location	Treatments	Control success (%)		Cut tillers (%)	Yield loss (kg/ha)	Yield loss saved (kg/ha)
		w.r.t. reference field	w.r.t. same field			
Nurpur Bet Distt Ludhiana (n = 3 each)	T1	79.15 ± 1.67^{B}	79.32 ± 1.66^{AB}	3.25 ± 1.01^{B}	129.17 ± 44.32^{B}	139.42 ± 55.24^{A}
	T2	71.16 ± 4.24^{A}	71.38 ± 4.20^{A}	3.45 ± 1.07^{B}	124.78 ± 33.25^{B}	143.80 ± 86.57^{A}
	T3	77.09 ± 2.98^{B}	77.28 ± 2.96^{A}	2.93 ± 0.27^{AB}	103.20 ± 18.23^{B}	167.98 ± 60.24^{A}
	T4	90.42 ± 0.29^{C}	90.50 ± 0.29^{B}	1.04 ± 0.43^{A}	38.45 ± 17.90^{A}	230.14 ± 64.14^{B}
	Reference	–	–	6.69 ± 1.87^{C}	268.59 ± 53.86^{C}	–
Jandwala Bhime Shah Distt Fazilka (n = 3 each)	T1	60.16 ± 0.35^{A}	63.33 ± 0.21^{A}	2.97 ± 0.63^{B}	111.56 ± 28.42^{B}	132.16 ± 34.12^{A}
	T2	69.42 ± 1.16^{B}	71.72 ± 1.09^{B}	2.58 ± 0.35^{B}	108.26 ± 21.44^{B}	135.91 ± 30.04^{A}
	T3	75.72 ± 2.77^{B}	77.52 ± 2.57^{B}	2.84 ± 0.15^{B}	100.60 ± 9.78^{B}	143.58 ± 30.18^{A}
	T4	88.60 ± 0.60^{C}	89.28 ± 0.63^{C}	1.28 ± 0.43^{A}	51.13 ± 13.39^{A}	191.35 ± 35.29^{B}
	Reference	–	–	6.52 ± 0.89^{C}	244.17 ± 24.59^{C}	–
Ghasitpur Distt Hoshiarpur (n = 3 each)	T1	62.45 ± 3.79^{A}	64.59 ± 3.60^{A}	2.74 ± 0.31^{B}	92.74 ± 16.10^{B}	149.74 ± 31.80^{A}
	T2	66.52 ± 1.25^{AB}	68.42 ± 1.18^{AB}	1.82 ± 0.80^{AB}	70.15 ± 31.60^{B}	172.33 ± 30.03^{B}
	T3	70.74 ± 6.89^{AB}	72.40 ± 6.50^{AB}	2.99 ± 0.51^{B}	103.70 ± 26.85^{B}	138.79 ± 22.35^{A}
	T4	85.92 ± 3.77^{C}	86.72 ± 3.56^{B}	1.24 ± 0.64^{A}	50.42 ± 26.77^{A}	192.06 ± 35.39^{B}
	Reference	–	–	7.52 ± 0.51^{C}	242.48 ± 24.69^{C}	–
Mean (n = 9 each)	T1	67.30 ± 3.25^{A}	68.37 ± 3.00^{A}	3.00 ± 0.36^{B}	110.49 ± 9.96^{B}	140.58 ± 21.02^{A}
	T2	69.08 ± 1.50^{A}	69.88 ± 1.55^{A}	2.62 ± 0.46^{B}	101.06 ± 16.19	150.68 ± 29.30^{A}
	T3	74.65 ± 2.54^{A}	75.18 ± 2.54^{A}	2.92 ± 0.17^{B}	102.50 ± 0.96^{B}	149.25 ± 23.42^{A}
	T4	88.34 ± 1.30^{B}	88.57 ± 1.30^{B}	1.18 ± 0.24^{A}	46.67 ± 4.12^{A}	205.08 ± 23.89^{B}
	Reference	–	–	6.82 ± 0.47^{C}	251.75 ± 8.44^{C}	–

Values are Mean ± SE, n = number of fields in each block, w.r.t. = with respect to, ZnP = Zinc phosphide, Br = Bromadiolone, Ch = Cholecalciferol, T1 = ZnP (2%), T2 = Br (0.005%), T3 = Br (0.0025%) + Ch (0.02%) and T4 = Br (0.0025%) + Ch (0.04%). Values with different superscripts (A–C) in a column differ significantly at $P \leq 0.05$

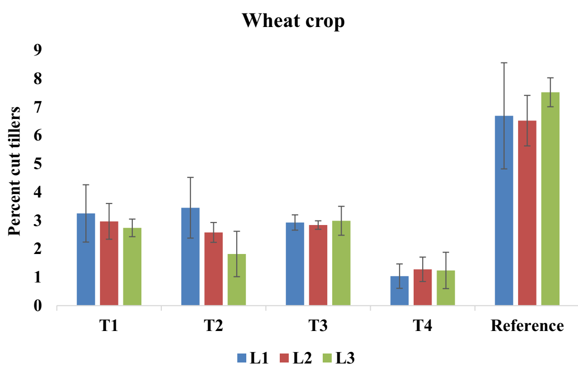

Fig. 2 Comparison of percent cut tillers in wheat crop fields treated with different rodenticide treatments at three different locations. ZnP = Zinc phosphide, Br = Bromadiolone, Ch = Cholecalciferol, T1 = ZnP (2%) followed by Br (0.005%), T2 = Br (0.0025%) + Ch (0.02%) once, T3 = Br (0.0025%) + Ch (0.02%) twice, T4 = Br (0.0025%) + Ch (0.04%). Location 1 = Village Nurpur Bet, District Ludhiana, Location 2 = Village Jandwala Bhime Shah, District Fazilka and Location 3 = Village Ghasitpur, District Hoshiarpur. Bars with superscripts (A–B) differ significantly at $P \leq 0.05$

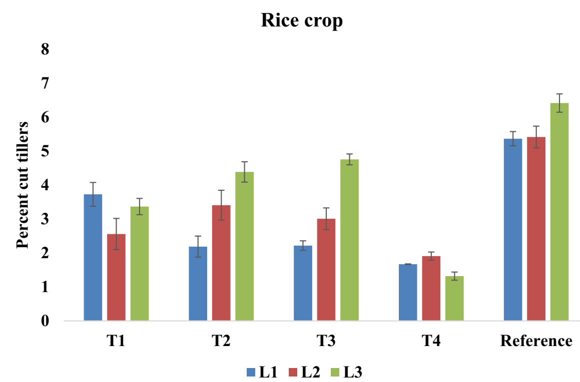

Fig. 3 Comparison of percent cut tillers in rice crop fields treated with different rodenticide treatments at three different locations. ZnP = Zinc phosphide, Br = Bromadiolone, Ch = Cholecalciferol, T1 = ZnP (2%) followed by Br (0.005%), T2 = Br (0.0025%) + Ch (0.02%) once, T3 = Br (0.0025%) + Ch (0.02%) twice, T4 = Br (0.0025%) + Ch (0.04%). Location 1 = Village Nurpur Bet, District Ludhiana, Location 2 = Village Jandwala Bhime Shah, District Fazilka and Location 3 = Village Bullowal, District Hoshiarpur. Bars with superscripts (A–C) differ significantly at $P \leq 0.05$

$$\text{Saved in yield loss(kg/ha)} \\ = \text{Yield loss in reference field} \\ - \text{yield loss in treated field}$$

Statistical analysis

Values were determined as Mean ± SE. Used one way ANOVA to determine the significance ($P \leq 0.05$) for differences between treated and untreated blocks for control successes, crop damage and yield loss. The statistical software used was SAS version 9.3.

All the fields selected for assessing the comparative potential of rodenticides were found infested by rodent pests, based on the number of rodent burrows. *Bandicota bengalensis* was the predominant species in all the fields followed by *Tatera indica*, *Millardia meltada* and *Mus booduga*.

Efficacy of rodenticide treatments in wheat crop

Percent rodent control success with respect to reference field and same field in wheat crop treated with combination bait II (Br (0.0025%) + Ch (0.04%), T4) was significantly ($P \leq 0.05$) high as compared to that observed with other three treatments at all the three locations viz village Nurpur Bet, district Ludhiana, village Jandwala Bhime Shah, district Fazilka and village Ghasitpur, district Hoshiarpur. In the fields treated with T4, the mean control success w.r.t. the reference field and same field was 88.34 ± 1.30% and 88.57 ± 1.30%, respectively. This was also supported by the lowest rodent damage (1.18 ± 0.24% cut tillers) and yield loss (46.67 ± 4.12 kg/ha) in fields with T4 treatment as compared to the reference fields (6.82 ± 0.47% cut tillers and 251.75 ± 8.44 kg/ha yield loss, respectively) (Table 1) at all the locations (Fig. 2). The mean protection in the yield loss (205.08 ± 23.89 kgha) was also significantly ($P \leq 0.05$) high in fields treated with combination bait II (T4).

Efficacy of rodenticide treatments in rice crop

Percent rodent control success with respect to reference field and same field in rice crop treated with combination bait II (Br (0.0025%) + Ch (0.04%), T4) was also significantly ($P \leq 0.05$) high as compared to that achieved with other three treatments at all the three locations viz. village Nurpur Bet, district Ludhiana, village Jandwala Bhime Shah, district Fazilka and village Bullowal, district Hoshiarpur. In the fields treated with T4, the mean control success w.r.t. the reference field and the same field was 74.21 ± 1.70 and 73.50 ± 1.74%, respectively. This was also supported by the lowest mean rodent damage (1.78 ± 0.9% cut tillers and 367.10 ± 25.51 kg/ha yield loss) in fields with T4 treatment as compared to the reference fields (5.74 ± 0.22% cut tillers and 1040.75 ± 83.84 kg/ha yield loss) (Table 2) at all the locations (Fig. 3). The mean protection in yield loss (673.66 ± 86.17 kg/ha) was also significantly ($P \leq 0.05$) high in fields treated with combination bait II (T4).

Table 2 Comparison of mean percent rodent control success, rodent damage and yield loss in rice crop fields of three locations treated with different rodenticide baits

Location	Treatments	Control success (%)		Cut tillers (%)	Yield loss (kg/ha)	Yield loss saved (kg/ha)
		w.r.t. reference field	w.r.t. same field			
Nurpur Bet Distt Ludhiana (n = 3 each)	T1	58.06 ± 10.62^{A}	59.29 ± 19.85^{A}	3.73 ± 0.35^{A}	971.275 ± 61.29^{B}	347.47 ± 105.05^{A}
	T2	68.95 ± 3.16^{A}	69.85 ± 5.32^{A}	2.19 ± 0.31^{B}	440.76 ± 59.57^{A}	813.78 ± 59.71^{B}
	T3	49.49 ± 4.96^{A}	51.11 ± 5.32^{A}	2.22 ± 0.14^{B}	496.10 ± 128.99^{A}	758.48 ± 74.78^{B}
	T4	86.63 ± 3.74^{A}	87.34 ± 6.28^{A}	1.67 ± 0.01^{B}	325.59 ± 25.43^{A}	928.95 ± 25.42^{B}
	Reference	–	–	5.37 ± 0.21^{C}	1254.00 ± 81.33^{B}	–
Jandwala Bhime Shah Distt Fazilka (n = 3 each)	T1	71.02 ± 3.11^{B}	69.17 ± 3.32^{B}	2.56 ± 0.46^{B}	1136.51 ± 240.29^{AB}	1150.69 ± 240.29^{B}
	T2	67.48 ± 2.34^{B}	65.41 ± 2.49^{B}	3.41 ± 0.44^{B}	1520.56 ± 246.33^{B}	766.67 ± 246.31^{A}
	T3	53.95 ± 0.43^{A}	53.98 ± 0.43^{A}	3.01 ± 0.32^{B}	1327.00 ± 203.86^{AB}	959.80 ± 203.54^{A}
	T4	72.57 ± 3.93^{B}	70.82 ± 4.18^{B}	1.91 ± 0.12^{A}	833.35 ± 91.09^{A}	1453.92 ± 91.75^{B}
	Reference	–	–	5.42 ± 0.32^{C}	2287.22 ± 348.68^{C}	–
Bullowal Distt Hoshiarpur (n = 3 each)	T1	70.06 ± 1.41^{B}	68.48 ± 1.48^{AB}	3.37 ± 0.24^{B}	1049.50 ± 160.59^{B}	1407.59 ± 160.70^{B}
	T2	70.41 ± 2.94^{B}	68.85 ± 3.10^{AB}	4.39 ± 0.30^{B}	1784.00 ± 60.21^{BC}	673.09 ± 60.22^{A}
	T3	57.89 ± 7.14^{A}	55.67 ± 7.52^{A}	4.76 ± 0.16^{B}	1396.20 ± 45.375^{B}	1060.93 ± 45.37^{B}
	T4	77.58 ± 2.38^{B}	76.40 ± 2.51^{B}	1.32 ± 0.12^{A}	451.89 ± 71.35^{A}	2007.25 ± 71.33^{C}
	Reference	–	–	6.42 ± 0.27^{C}	2566.44 ± 269.39^{C}	–
Mean(n = 9 each)	T1	66.38 ± 3.83^{B}	65.65 ± 3.54^{B}	3.22 ± 0.50^{B}	626.83 ± 51.60^{B}	413.92 ± 68.36^{A}
	T2	68.95 ± 1.48^{B}	68.04 ± 1.60^{B}	3.30 ± 0.37^{B}	678.27 ± 105.40^{B}	362.49 ± 82.36^{A}
	T3	53.78 ± 2.80^{A}	53.54 ± 2.70^{A}	3.53 ± 0.37^{B}	671.91 ± 89.21^{B}	368.84 ± 74.53^{A}
	T4	74.21 ± 1.70^{B}	73.50 ± 1.74^{B}	1.78 ± 0.09^{A}	367.10 ± 25.51^{A}	673.66 ± 86.17^{B}
	Reference	–	–	5.74 ± 0.22^{C}	1040.75 ± 83.44^{C}	–

Values are Mean ± SE, n = number of fields in each block, w.r.t. = with respect to, ZnP = Zinc phosphide, Br = Bromadiolone, Ch = Cholecalciferol,

T1 = ZnP (2%), T2 = Br (0.005%), T3 = Br (0.0025%) + Ch (0.02%) and T4 = Br (0.0025%) + Ch (0.04%)

Values with different superscripts (A–C) in a column differ significantly at $P \leq 0.05$

Efficacy of rodenticide treatments in sugarcane crop

Percent rodent control success with respect to reference field and same field in sugarcane crop treated with combination bait II (Br (0.0025%0 + Ch (0.04%) twice, T5) at three locations i.e., village Nagajja, district Jalandhar, village Bullowal, district Hoshiarpur and village Dhilwan, district Kapurthala was significantly ($P \leq 0.05$) high as compared to that observed with other treatments. In fields treated with T5, percent control success w.r.t. the reference field and the same field was 88.80 ± 1.34% and 86.11 ± 1.48%, respectively. This was also supported by the lowest mean rodent damage (6.18 ± 1.50% cut canes and 7.45 ± 1.92 q/ha yield loss) in fields with T5 treatment as compared to the reference fields (26.56 ± 2.35% cut canes and 29.91 ± 2.14 q/ha yield loss) (Table 3) at all the locations (Fig. 4). The protection in mean yield loss (22.32 ± 1.85 q/ha) was also significantly ($P \leq 0.05$) high in fields treated with combination bait II (T5).

Discussion

Based on the results of this study, a synergistic cereal bait formulation containing bromadiolone (0.0025%) and cholecalciferol (0.04%) had a significantly higher potential to manage rodent populations in all three major crops. Few field studies have been conducted to test the effectiveness of combination rodenticide baits on rodents. In Europe, cholecalciferol has been added to baits containing the first generation anticoagulant, coumatetralyl

Table 3 Comparison of mean percent rodent control success, rodent damage and yield loss in sugarcane crop fields of three locations treated with different rodenticide baits

Location	Treatments	Control success (%)		Cut canes (%)	Yield loss (q/ha)	Yield loss saved (q/ha)
		w.r.t. reference field	w.r.t. same field			
Nagajja Distt Jalandhar						
(n = 3 each)	T1	91.19 ± 1.89^{C}	77.94 ± 7.36^{BC}	9.59 ± 2.12^{B}	13.23 ± 2.39^{A}	14.17 ± 2.50^{B}
	T2	32.88 ± 7.86^{A}	68.82 ± 3.87^{B}	14.57 ± 1.84^{BC}	18.87 ± 2.48^{B}	8.50 ± 2.50^{A}
	T3	82.35 ± 3.34^{B}	72.33 ± 2.18^{B}	10.92 ± 1.45^{B}	14.18 ± 1.65^{A}	13.22 ± 1.65^{B}
	T4	77.86 ± 1.75^{B}	37.22 ± 7.35^{A}	14.04 ± 1.58^{BC}	19.82 ± 1.65^{B}	7.55 ± 1.64^{A}
	T5	66.67 ± 4.15^{B}	88.98 ± 2.36^{C}	4.95 ± 2.66^{A}	7.55 ± 3.98^{A}	19.83 ± 3.99^{C}
	Reference	–	–	19.73 ± 1.71^{C}	27.37 ± 2.49^{C}	–
Bullowal Distt Hoshiarpur (n = 3 each)	T1	90.33 ± 1.04^{B}	73.23 ± 1.62^{BC}	7.82 ± 1.64^{A}	9.45 ± 2.48^{A}	17.94 ± 1.79^{C}
	T2	75.00 ± 0.75^{B}	47.95 ± 2.13^{A}	16.74 ± 1.55^{BC}	22.65 ± 3.28^{C}	4.72 ± 4.11^{A}
	T3	79.91 ± 1.22^{B}	74.98 ± 1.54^{BC}	13.19 ± 1.99^{B}	13.22 ± 0.95^{AB}	14.16 ± 4.32^{B}
	T4	51.63 ± 4.18^{A}	66.64 ± 0.99^{B}	12.84 ± 1.70^{B}	13.22 ± 0.95^{AB}	14.16 ± 3.27^{B}
	T5	81.24 ± 1.10^{B}	87.62 ± 13.90^{D}	5.94 ± 2.91^{A}	5.66 ± 2.82^{A}	22.66 ± 4.32^{C}
	Reference	–	–	19.01 ± 3.79^{C}	27.38 ± 4.11^{C}	–
Dhilwan Distt Kapurthala						
(n = 3 each)	T1	84.46 ± 1.68^{B}	69.84 ± 2.80^{B}	9.91 ± 3.17^{A}	12.35 ± 4.12^{A}	22.56 ± 4.12^{AB}
	T2	70.45 ± 1.20^{AB}	56.28 ± 2.80^{A}	20.17 ± 2.89^{B}	23.60 ± 4.12^{B}	11.32 ± 4.13^{A}
	T3	74.36 ± 2.38^{B}	69.72 ± 3.05^{B}	13.67 ± 2.05^{A}	16.05 ± 2.50^{B}	18.88 ± 2.48^{A}
	T4	63.57 ± 2.38^{A}	65.24 ± 1.41^{AB}	16.49 ± 3.20^{A}	19.82 ± 3.28^{B}	15.35 ± 3.28^{A}
	T5	74.26 ± 2.59^{B}	81.72 ± 1.98^{C}	8.05 ± 4.13^{A}	9.45 ± 5.00^{A}	25.50 ± 4.89^{AB}
	Reference	–	–	31.62 ± 2.48^{C}	34.95 ± 3.78^{C}	–
Mean(n = 9 each)	T1	74.47 ± 1.93^{B}	60.16 ± 2.26^{A}	9.11 ± 1.24^{A}	11.61 ± 1.62^{A}	18.20 ± 1.99^{B}
	T2	60.38 ± 2.90^{AB}	57.68 ± 3.40^{A}	17.16 ± 1.35^{B}	21.68 ± 1.80^{B}	8.17 ± 1.89^{A}
	T3	77.79 ± 1.40^{B}	72.35 ± 1.40^{B}	12.49 ± 0.96^{A}	14.48 ± 0.98^{A}	15.39 ± 1.27^{A}
	T4	59.44 ± 7.05^{A}	56.37 ± 5.26^{A}	14.58 ± 1.28^{A}	17.63 ± 1.51^{B}	12.27 ± 1.56^{A}
	T5	88.80 ± 1.34^{C}	86.11 ± 1.48^{C}	6.18 ± 1.50^{A}	7.45 ± 1.92^{A}	22.32 ± 1.85^{C}
	Reference	–	–	26.56 ± 2.35^{C}	29.91 ± 2.14^{C}	–

Values are Mean ± SE, n = number of fields in each block, w.r.t. = with respect to, ZnP = Zinc phosphide, Br = Bromadiolone, Ch = Cholecalciferol, T1 = ZnP (2%) followed by Br (0.005%), T2 = Br (0.0025%) + Ch (0.02%) once, T3 = Br (0.0025%) + Ch (0.02%) twice, T4 = Br (0.0025%) + Ch (0.04%) once and T5 = Br (0.0025%) + Ch (0.04%) twice. Values with different superscripts (A–D) in a column differ significantly at $P \leq 0.05$

(registered as Racumin® plus) to overcome anticoagulant resistance and reduce its cost in rats and mice (Eason et al., 2008; Pospischil & Schnorbach, 1994; Tobin et al., 1993). Similar results were also recorded when a combination of diphacinone and cholecalciferol was tested in California to control voles (Baldwin et al., 2016). A recent field study in New Zealand showed the effectiveness of diphacinone (0.005%) and cholecalciferol (0.06%) based baits in possums and rodents. Combination bait treatment gave 94% and 80% success in controlling possums and house rats, respectively. Prior to the field trials, laboratory tests were also carried out, which showed a mortality rate of 87% for possums and 86% for house rats. This new bait combination was approved by the New Zealand Environmental Protection Agency in 2018 and eventually registered as a commercial product with the Ministry of Primary Industries in 2019 (Eason et al., 2020). Combination bait treatment have been shown to be effective in controlling anticoagulated rats (Endepolis et al., 2017; Witmer et al., 2014).

Combination bait treatments also reduced time to death compared to bromadiolone and cholecalciferol alone (Kocher & Kaur, 2013; Singla & Kaur, 2015), which may reduce threats to non-target organisms. The use of bromadiolone (0.005%) and cholecalciferol (0.075%) in

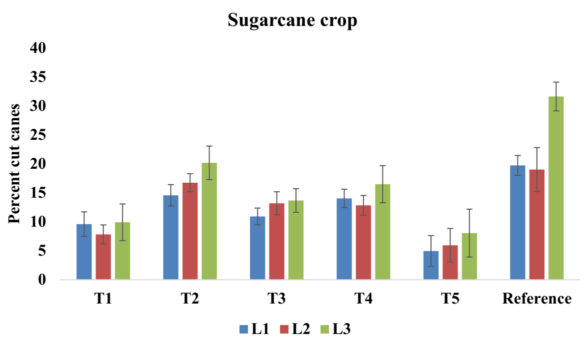

Fig. 4 Comparison of percent cut canes in sugarcane crop fields treated with different rodenticide treatments at three different locations. T1 = Zinc phosphide (2%) followed by Bromadiolone (0.005%), T2 = Bromadiolone (0.0025%) + Cholecalciferol (0.02%) once, T3 = Bromadiolone (0.0025%) + Cholecalciferol (0.02%) twice, T4 = Bromadiolone (0.0025%) + Cholecalciferol (0.04%) once and T5 = Bromadiolone (0.0025%) + Cholecalciferol (0.04%) twice. Location 1 = Village Nagajja, District Jalandhar, Location 2 = Village Bullowal, District Hoshiarpur and Location 3 = Village Dhilwan, District Kapurthala. Bars with superscripts (A–C) differ significantly at $P \leq 0.05$

combination baits at lower than recommended concentrations of active ingredients have several advantages, such as reduced risk of secondary poisoning, increased consumption of bait and reduced costs (Kocher & Kaur, 2013; Singla & Kaur, 2015).

Conclusions

From the present study, it is evident that the combination II of bromadiolone and cholecalciferol (Br (0.0025%) + Ch (0.04%) can be a promising alternative to traditional rodenticides (zinc phosphide and bromadiolone as solo treatments). Field trials confirmed that the synergistic mode of action of an anticoagulant with hypercalcemic cholecalciferol was more lethal to rodents even at lower concentrations. It can therefore be concluded that this combination of bromadiolone and cholecalciferol has the potential to manage rodent populations in different crops and registration of this rodenticidal combination should therefore be considered for use against field rodents worldwide.

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Author contributions
DS carried out the field studies, wrote draft of manuscript. NS managed and coordinated the field studies, analysed data and finalized the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data and materials used and/or analyzed during the current study are available in this manuscript.

Declarations

Ethics approval and consent to participate
Not required for field study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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