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# Poultry egg components as cereal bait additives for enhancing rodenticide based control success and trap index of house rat, Rattus rattus

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#### PEER REVIEW

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#### Comments

Present research is a very good study evaluating the potential of poultry egg albumin and egg shell (both crushed and powder form) in enhancing the acceptance of cereal bait by house rat. The acceptance was assessed based on bi-choice and no-choice experiments in laboratory cages and food scale consumption monitor. Authors have also tested the efficacy of these components after mixing in zinc phosphide bait, an acute rodenticide in poultry farms. Egg albumin has been found to have potential in increasing acceptance of cereal based bait. Details on Page S346

#### ABSTRACT

**Objective:** To compare the acceptance and efficacy of cereal bait containing different concentrations of poultry egg components in laboratory and poultry farms to control house rat, Rattus rattus (R. rattus).

Methods: Acceptance of cereal bait containing different concentrations (2%, 5% and 10%) of poultry egg components such as egg shell powder (ESP), egg albumin (EA) and crushed egg shell as bait additives were studied after exposing them to different groups of rats in bi-choice with bait without additive. Behaviour of rats towards cereal bait containing 2% concentration of different egg components was recorded in no-choice conditions through Food Scale Consumption Monitor. In poultry farm predominantly infested with R. rattus, acceptance and efficacy of 2% zinc phosphide bait containing 2% EA and ESP was evaluated. Trap success of single rat traps containing chapatti pieces smeared with 2% EA and 2% ESP was also evaluated in poultry farm.

**Results:** In bi-choice tests, significantly (P<0.05) higher preference was observed for baits containing 2% and 5% ESP and all the three concentrations of EA compared to plain bait by female rats and that of baits containing 5% and 10% EA by male rats. In no-choice test, non-significantly higher consumption, number of bouts made and time spent towards bait containing 2% EA was found by rats of both sexes. In poultry farm, acceptance and efficacy of 2% zinc phosphide bait containing 2% EA and ESP was significantly (P<0.05) more than 2% zinc phosphide bait without additive. No significant difference was, however, found in trap success of single rat traps containing chapatti pieces smeared with 2% concentration of EA and ESP placed in the poultry farm.

Conclusions: Present data support the use of 2% egg albumin and egg shell powder in cereal bait to enhance acceptance and efficacy of 2% zinc phosphide bait against R. rattus. This may further help in checking the spread of rodent borne diseases to animals and humans.

**KEYWORDS** Rattus rattus, Egg albumin, Egg shell, Bait enhancers, Rodenticide bait, Trapping

#### **1. Introduction**

Rodents have been identified as the most important mammalian pests at the global level. Damages caused by them lead to huge amount of crop losses and food shortages. Storage losses to rodents in India alone are 25%-30% costing

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at least \$5 billion annually<sup>[1]</sup>. The house rat, Rattus rattus (Linn.) (R. rattus) is one of the most common commensal rodent pest worldwide. It often damages, contaminates and spoils packed food and non-food materials in transit and storage besides being involved in spreading several diseases of public health importance<sup>[2]</sup>. It is the

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predominant pest species infesting poultry farms in India and posing a serious threat to poultry operations by feeding on poultry feed, contaminating it with their excrements, damaging eggs, attacking and killing chicks, causing structural damages to buildings, doors, windows, feed containers and transmitting several diseases<sup>[3]</sup>. The species acts as a wild reservoir horizontally transmitting infectious organisms to other rodent species and arthropod vectors living closer to anthropized environments, thus leading to inevitable exchange of pathogens between rodents, animals and humans<sup>[4]</sup>. Hence it is very important to control this species.

Rodenticide baiting is the most widely used method to control rodents throughout the world. Among rodenticides, acute poisons are more preferred and frequently applied, as people are anxious to see a rapid kill and get rid of damages caused by them<sup>[5]</sup>. However, rodent control with acute rodenticide baits has often been found ineffective in reducing rodent densities due to several factors. Poor poison bait acceptance, sub–lethal dosing and subsequent conditioned aversion, dietary preferences and neophobia can reduce the efficacy of rodenticide baiting<sup>[6]</sup>. Bait–shyness induced through conditioned taste aversion, can last more than a year, even when the rodenticides have been removed from the baits. Once shy, the rats prefer to remain hungry than eating an apprehensive food.

Use of poison baits is still the most reliable strategy for controlling field as well as commensal rodents, however, baiting techniques should be modified according to the psychological characteristics of the target species. Several researchers have noted the need for an additive that could be added to rodenticide baits for increasing their acceptance and efficacy<sup>[7-9]</sup>. Different workers have suggested the efficacy of different bait additives against different rodent species<sup>[10–14]</sup>. Since *R. rattus* is the predominant rodent pest species found in poultry farms and feeds on poultry eggs, the bait containing egg components may be more acceptable to it. Present study was hence conducted to compare the acceptance of bait containing different concentrations of poultry egg components by R. rattus in laboratory and to study their potential in increasing rodenticide based control success and trap index in poultry farms.

## 2. Materials and methods

## 2.1. Collection and maintenance of animals

Adult *R. rattus* of both sexes were trapped using single and multi rat catch traps from poultry farms in and around Ludhiana, India and kept individually in laboratory cages (each of size, 36 cm×23 cm×23 cm) for acclimatization (10– 15 d) before experimentation. Food and water were provided *ad libitum*. Food consisted of preferred cereal based bait containing loose mixture of cracked wheat, powdered sugar and groundnut oil (WSO bait) at a ratio of 96:2:2. Rats were used and maintained as per the guidelines of Institutional Animal Ethics Committee.

## 2.2. Bi-choice experiments

During experimentation, mature and healthy rats of both sexes were weighed and divided into four groups (I to IV) of 10 rats each (five of each sex). In the first experiment, rats of Groups I, II and III were exposed to WSO bait containing 2%, 5% and 10% egg shell powder (ESP) as additive, respectively in bi-choice with WSO bait without additive for 5 d. There was no significant difference in average body weight of rats in treated and untreated groups. Bait was kept in bowls and water was provided ad libitum. The position of bowls was changed daily to avoid any difference in consumption due to side preference. In the second experiment, rats of Groups I, II and III were exposed to WSO bait containing 2%, 5% and 10% poultry egg albumin (EA) as additive, respectively, whereas in the experiment third, similar sets of rats were exposed to WSO bait containing 2%, 5% and 10% crushed egg shells (CES) as bait additive, respectively. Rats of Group IV kept as untreated control were fed on WSO bait without additive in all the experiments. Before and after the treatment, all the rats were fed on WSO bait only.

Bait consumption was recorded after every 24 h and every time bait was replaced to the original 30 g. Before weighing, the bait of all the treated and untreated rats was cleared of fecal pellets and dried if needed. Mean daily consumption of food (g/100 g body weight) was calculated separately for each group of rats during pre-treatment, treatment and posttreatment periods. The percent acceptance of WSO bait with additive over WSO bait without additive during treatment period was determined as per the formula given below:

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Percent \ acceptance = \frac{Consumption \ of WSO \ bait \ with \ additive \ during \ treatment}{Total \ bait \ consumed \ during \ treatment} \times 100
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#### 2.3. No-choice experiments

Four rats of each sex were exposed to WSO baits containing 2% concentration of ESP, EA and CES in no-choice test under Food Scale Consumption Monitor (FSCM) (Columbus Instruments, USA). Each rat was kept in a cage provided with a feeding bowl and a feeding sensor below. Each rat was exposed to a particular type of bait formulation for 4 d. Rats were kept without food overnight prior to experimentation. Behaviour of rats in the form of consumption of bait (g/kg body weight), number of bouts made and time spent (second) was recorded for 2 h a day by means of the software of FSCM loaded in the computer.

## 2.4. Poison baiting experiment

Three blocks (I, II and III) of poultry farm, with each block further consisting of four replicated sheds were selected at village Ghutani Kalan, District Ludhiana (India). One block (IV) consisting of four sheds selected at the Campus, Guru Angad Dev Veterinary and Animal Science University, Ludhiana was kept as untreated control. All the sheds were predominantly infested with *R. rattus*. Before treatment, precensus bait consumption (g/100 g bait) was recorded from all blocks by keeping WSO bait to record level of rodent activity before treatment. After pre-census, sheds of blocks I, II and III were treated with 2% zinc phosphide bait (prepared in WSO bait without any additive), 2% zinc phosphide bait containing 2% EA and 2% zinc phosphide bait containing 2% ESP, respectively. Treatment bait was kept 500 g/shed in two rows of 10 bait points each. The treatment bait was exposed to rats for 2 d after which the remaining bait was collected and weighed to record the consumption (g/100 g bait). After 1 week of treatment, post census bait consumption of WSO bait was recorded from all the poultry blocks to record level of rodent activity after treatment. Reduction in level of rodent activity (%) in treated blocks with respect to untreated block was determined as per the formula given below:

Percent reduction in rodent activity=100 × 
$$\left[1 - \frac{t_2 x r_1}{t_1 x r_2}\right]$$

Where,  $t_1$  and  $t_2$  represent pre- and post-treatment bait consumptions, respectively in treated sheds and  $r_1$  and  $r_2$  represent pre- and post-treatment bait consumptions, respectively in untreated reference sheds.

Reduction in level of rodent activity (%) was also recorded with respect to same shed of poultry farm which was determined as per the formula given below:

| Pre-census bait consumption – Post-census bait consumption |       |
|--|-------|
| Pre-census bait consumption                                | -×100 |

## 2.5. Trapping experiments

Six poultry sheds were selected at the poultry farm located in the Campus of Guru Angad Dev Veterinary and Animal Science University, Ludhiana. All the sheds were predominantly infested with R. rattus. Rodent trapping was carried out in all the sheds by placing eight single rat traps in each shed. Each trap was baited with a piece of chapatti. The effect of 2% EA and 2% ESP in enhancing rodent trapping was studied by smearing these components on the chapatti pieces which were then used for baiting the traps. For the first time, trapping was carried out from all the sheds by alternatively placing traps containing chapatti pieces without additive and those containing chapatti pieces smeared with 2% ESP. For the second time, trapping was carried out from all the sheds by alternatively placing traps containing chapatti pieces without additive and those containing chapatti pieces smeared with 2% EA. For the third time, trapping was carried out from all the sheds by alternatively placing traps containing chapatti pieces smeared with 2% ESP and 2% EA. Every time, trapping was carried out for 1 week and the number of rats trapped of each sex was recorded. From the data on number of rats trapped of each sex, trap index in the form of number of rats trapped per 100 trap nights per day was calculated.

#### 2.6. Statistical analysis

All values were expressed as mean±SEM. The data on food consumption for two sexes, three bait additives, three concentrations of each bait additive, 5 d of treatment and five replicated rats was collected using factorial experiments in completely randomized design. Analysis was done using general linear model in SAS 9.3. All pair wise treatment comparisons were made using Tukeys' HSD test at 5% level of significance.

#### **3. Results**

#### 3.1. Bi-choice experiments

The data on consumption of WSO bait containing different bait additives are presented in Tables 1–3. There was no significant difference observed in mean daily consumption (g/100 g body weight) of WSO bait during pre– and post– treatment periods among all the treated and untreated groups of rats of both sexes in all the experiments.

## Table 1

Acceptance of bait containing ESP by R. rattus.

|        |       |       |                    | Mean            | daily bait c    | onsumption g/10        | 00 g bw)        | Percent    |
|--------|-------|-------|--------------------|-----------------|-----------------|------------------------|-----------------|------------|
| Sex    | Group | Conc. | Body wt (g)        | Pre-            | During          | ; treatment            | Post-           | acceptance |
|        | (n=5) | (%)   |                    | treatment       | WSO bait        | WSO+additive           | treatment       | (%)        |
| Female | Ι     | 2.0   | 130.00±6.32        | 6.25±0.44       | 2.03±0.17       | $4.02 \pm 0.21^{b}$    | $5.70 \pm 0.20$ | 67.21±4.88 |
|        | II    | 5.0   | 130.00±9.38        | $6.52 \pm 0.62$ | $2.70 \pm 0.16$ | $4.06 \pm 0.26^{b}$    | $5.49 \pm 0.16$ | 58.04±5.09 |
|        | III   | 10.0  | 130.00±6.93        | 7.18±0.68       | 3.87±0.35       | $2.77 \pm 0.20^{ab}$   | 5.27±0.26       | 49.18±5.59 |
|        | IV    | 0.0   | $130.00 \pm 8.48$  | 6.61±0.37       | $6.78 \pm 0.52$ | -                      | 5.81±0.24       | -          |
| Male   | Ι     | 2.0   | $135.00 \pm 10.77$ | 4.73±0.19       | 4.36±0.30       | 2.83±0.20 <sup>a</sup> | $5.07 \pm 0.10$ | 35.19±4.29 |
|        | II    | 5.0   | 133.00±6.26        | 5.04±0.26       | 3.44±0.17       | 3.03±0.27 <sup>a</sup> | 4.94±0.03       | 41.20±4.39 |
|        | III   | 10.0  | $135.00 \pm 10.20$ | 5.37±0.43       | 5.01±0.64       | 3.93±0.27 <sup>a</sup> | 6.41±0.25       | 47.32±6.34 |
|        | IV    | 0.0   | 141.00±6.39        | $5.76 \pm 0.40$ | $5.85 \pm 0.27$ | -                      | 4.13±0.08       | -          |

Values are mean $\pm$ SE, bw: body weight. Values with different superscripts (a–b) in a row for during treatment bait consumption differ significantly at *P*<0.05.

#### 3.1.1. Acceptance of ESP as bait additive

During treatment period, significantly (P<0.05) higher mean daily consumption (g/100 g body weight) of WS0 bait containing 2% and 5% ESP from that of WS0 bait alone was observed in female rats. There was no significant difference in consumption between WS0 bait without additive and that containing 10% ESP by female rats (Table 1). There was no significant difference in mean daily consumption and percent acceptance of WS0 bait containing egg shell powder among the three treated groups I, II and III. The percent acceptance was found to be 67.21%, 58.04% and 49.18%, respectively in rats of Groups I, II and III.

In male rats, no significant difference was observed between mean daily consumption (g/100 g body weight) of WSO bait containing all the concentrations of ESP and that without ESP (Table 1). Also there was no significant difference in mean daily consumption and percent acceptance of WSO bait containing ESP among the three treated Groups I, II and III. The percent acceptance was found to be 35.19%, 41.20% and 47.32%, respectively in rats of Groups I, II and III.

Percent acceptance of WSO bait containing ESP was

generally low in male rats as compared to female rats. This difference in acceptance of bait between male and female rats was found to be statistically significant (P<0.05) when rats were exposed to WSO bait containing 2% and 5% ESP in choice with WSO bait without additive (Table 1).

## 3.1.2. Acceptance of EA as bait additive

Significantly (P<0.05) higher mean daily consumption (g/100 g body weight) of WSO bait containing 2%, 5% and 10% EA from that of WSO without additive was observed in female rats (Table 2). There was no significant difference in mean daily consumption and percent acceptance of WSO bait containing EA among the three treated groups I, II and III. The percent acceptance was found to be 67.45%, 69.13% and 65%, respectively in rats of Groups I, II and III. Table 2

Acceptance of bait containing poultry EA by R. rattus.

|        |       |       |                    | Mean             | Percent          |                      |                 |                         |
|--------|-------|-------|--------------------|------------------|------------------|----------------------|-----------------|-------------------------|
| Sex    | Group | Conc. | Body wt (g)        | Pre-             | During           | ; treatment          | Post-           | acceptance              |
| JUA    | (n=5) | (%)   | body wit (g)       | treatment        | WSO bait         | WSO+additive         | treatment       | (%)                     |
| Female | Ι     | 2.0   | 131.00±0.89        | 11.05±0.43       | 6.39±0.79        | $10.04 \pm 0.32^{b}$ | 8.92±0.46       | 67.45±3.83 <sup>a</sup> |
|        | Π     | 5.0   | 136.00±14.31       | 12.77±1.03       | 7.25±0.45        | $13.04 \pm 0.57^{b}$ | 11.21±0.21      | 69.13±4.55 <sup>a</sup> |
|        | III   | 10.0  | $134.00 \pm 14.58$ | $12.95 \pm 0.24$ | $8.09 \pm 0.58$  | $12.71 \pm 0.52^{b}$ | 8.72±0.38       | $65.00 \pm 3.86^{a}$    |
|        | IV    | 0.0   | 127.00±6.87        | $11.15 \pm 0.17$ | $10.52 \pm 0.52$ | -                    | 9.39±0.37       | -                       |
| Male   | Ι     | 2.0   | 130.00±5.65        | $12.70 \pm 0.81$ | 8.17±0.60        | $9.98 \pm 0.40^{ab}$ | 10.61±0.75      | 57.12±4.36 <sup>a</sup> |
|        | Π     | 5.0   | 138.00±17.06       | $9.58 \pm 0.40$  | $5.79 \pm 0.32$  | $10.97 \pm 0.05^{b}$ | $9.01 \pm 0.14$ | $70.63 \pm 3.81^{b}$    |
|        | III   | 10.0  | 132.00±3.34        | $10.49 \pm 0.49$ | $4.69 \pm 0.42$  | $11.57 \pm 0.48^{b}$ | $7.88 \pm 0.11$ | $76.22 \pm 4.11^{b}$    |
|        | IV    | 0.0   | 123.00±3.89        | 10.33±0.46       | 9.94±0.41        | -                    | 8.79±0.34       | -                       |

Values are mean $\pm$ SE, bw: body weight. Values with different superscripts (a–b) in a row for during treatment bait consumption and in a column for percent acceptance by male and female rats separately differ significantly at *P*<0.05.

In male rats, significantly (P<0.05) higher mean daily consumption (g/100 g body weight) of WSO bait containing 5% and 10% EA was found from that of WSO bait alone (Table 2). There was no significant difference between the consumption of WSO bait without additive and that containing 2% EA. Also there was no significant difference in mean daily consumption of WSO bait containing EA among the three treated Groups I, II and III. Percent acceptance of WSO bait with additive over WSO bait alone was, however, found to be significantly (P<0.05) high in male rats of Groups II (70.63%) and III (76.22%) as compared to rats of Group I (57.12%).

Percent acceptance of WSO bait containing EA by male rats as compared to female rats was generally low at 2% EA and high at 5% and 10% EA. This difference in acceptance of bait between male and female rats was, however, found to be statistically non-significant at all the concentrations tested (Table 2).

#### 3.1.3. Acceptance of CES as bait additive

No significant difference in mean daily consumption (g/100 g body weight) between WSO bait containing 2%, 5% and 10% CES and WSO bait without additive was observed in both male and female rats (Table 3). Also there was no significant difference in mean daily consumption and percent acceptance of WSO bait containing CES among the three treated groups I, II and III of male and female rats. The percent acceptance was found to be 55.34%, 54.25% and 53.62%, respectively by female rats and 62.39%, 55.35% and

## 58.94%, respectively by male rats of Groups I, II and III. Table 3

Acceptance of bait containing CES by R. rattus.

|        | Group<br>(n=5) | (%)  | Body wt (g)        | Mean             | Percent               |                  |                  |            |
|--------|----------------|------|--------------------|------------------|-----------------------|------------------|------------------|------------|
| Sex    |                |      |                    | Pre-             | Pre- During treatment |                  | Post-            | acceptance |
|        |                |      |                    | treatment        | WSO bait              | WSO+additive     | treatment        | (%)        |
| Female | Ι              | 2.0  | 143.00±2.68        | 12.85±0.37       | $7.00 \pm 0.42$       | 8.67±0.47        | 11.54±0.29       | 55.34±6.10 |
|        | Π              | 5.0  | 144.00±2.19        | 14.66±0.51       | 8.28±0.65             | 9.67±0.62        | $11.78 \pm 0.18$ | 54.25±4.16 |
|        | Ш              | 10.0 | 142.00±1.79        | $12.88 \pm 0.42$ | 6.61±0.49             | 7.13±0.52        | 10.99±0.36       | 53.62±3.62 |
|        | IV             | 0.0  | $146.00 \pm 10.80$ | $14.20 \pm 0.74$ | $15.02 \pm 0.40$      | -                | $11.09 \pm 0.18$ | -          |
| Male   | Ι              | 2.0  | 149.00±5.72        | 11.17±0.63       | $7.62 \pm 0.78$       | 9.92±0.30        | $10.28 \pm 0.24$ | 62.39±4.69 |
|        | Π              | 5.0  | 149.00±5.72        | 13.89±0.33       | 8.18±0.64             | $8.98 \pm 0.48$  | $13.84 \pm 0.42$ | 55.35±5.07 |
|        | Ш              | 10.0 | 146.00±7.79        | 13.12±0.37       | 7.08±0.39             | $10.46 \pm 0.75$ | 11.82±0.39       | 58.94±3.31 |
|        | IV             | 0.0  | $144.00 \pm 4.56$  | 13.85±0.11       | $12.92 \pm 0.26$      | -                | $12.36 \pm 0.42$ | -          |

Values are mean±SE, bw: body weight.

Percent acceptance of WSO bait containing CES by male rats as compared to female rats was generally high at all the three concentrations of CES tested. This difference in acceptance of bait between male and female rats was, however, not found to differ significantly at any of the concentrations tested (Table 3).

In female rats, the overall percent acceptance among WSO baits containing 2% ESP, EA and CES was not found to differ significantly (Tables 1–3). However, the percent acceptance of WSO bait containing 5% EA was significantly (P<0.05) high from WSO bait containing 5% CES and non–significantly high from WSO bait containing 5% ESP. The percent acceptance of WSO bait containing 10% EA was significantly (P<0.05) high from WSO bait containing 10% ESP and non–significantly high from WSO bait containing 10% CES. There was no significant difference in percent acceptance of WSO bait containing 10% CES. There was no significant difference in percent acceptance of WSO bait containing ESP and CES at all the concentrations tested (Table 3).

In male rats, the overall percent acceptance of WSO bait containing EA and CES was found to be significantly (P<0.05) high from that of WSO bait containing ESP at all the concentrations tested (Tables 1–3). At 5% and 10% concentrations, percent acceptance of WSO bait containing EA was comparatively more than WSO bait containing similar concentrations of CES. Statistically, there was no significant difference in percent acceptance of WSO bait containing EA and CES.

## 3.2. No-choice experiments

No significant difference was observed in average consumption (g/kg body weight) of bait for 2 h by female rats among WSO bait without additive and WSO baits containing 2% concentration each of ESP, EA and CES in no-choice feeding tests conducted under FSCM (Table 4). The average consumption of WSO bait with all the three types of additives was non-significantly high than WSO bait without additive. Among the three treated baits, the average consumption of bait containing 2% EA was non-significantly higher than other two bait additives. No significant difference in food consumption for 2 h a day was observed among the four replicated rats when exposed to both treated and untreated baits. The total number of bouts made and total time spent (seconds) by female rats in 2 h towards WSO bait without additive and those with three bait additives were also not found to differ significantly (Table 4). The number of bouts made and time spent by female rats were the highest towards WSO bait containing 2% EA.

#### Table 4

Behaviour of male and female rats recorded under FSCM.

| Bait       | Average bait const | Number of bouts made |           | Total time spent (seconds)   |              |                            |
|------------|--------------------|----------------------|-----------|------------------------------|--------------|----------------------------|
| ban        | Female             | Male                 | Female    | Male                         | Female       | Male                       |
| WSO bait   | 1.96±0.49          | 7.28±1.82            | 4.85±1.48 | 12.55±4.35"                  | 175.10±78.38 | 689.45±350.08 <sup>a</sup> |
| WSO+2% ESP | 4.01±1.00          | 2.61±0.65            | 4.50±1.43 | $4.10 \pm 1.22^{b}$          | 156.50±63.39 | $172.45 \pm 77.12^{\rm b}$ |
| WSO+2% EA  | 6.33±1.58          | 6.04±1.51            | 6.20±3.16 | $4.40\pm0.56^{\mathrm{b}}$   | 181.50±92.21 | 163.35±42.76 <sup>b</sup>  |
| WSO+2% CES | 6.01±1.50          | 6.22±1.55            | 2.45±0.51 | $2.80{\pm}0.19^{\mathrm{b}}$ | 69.10±18.88  | 99.05±14.30 <sup>b</sup>   |

Values are mean±SE, bw: body weight. Values with different superscript (a–b) in a column for number of bouts made and total time spent by male rats differ significantly at P<0.05. Body weight of female and male rats are (135.00±8.10) g and (185.00±9.84) g, respectively.

In male rats also, no significant difference was observed in average consumption (g/kg body weight) of bait for 2 h among WSO bait without additive and those containing 2% concentration each of ESP, EA and CES in no-choice feeding tests conducted under FSCM (Table 4). Among the three baits with additives, the average consumption of WSO bait containing 2% CES and EA was non-significantly high than bait containing 2% ESP. No significant difference in food consumption for 2 h a day was observed among the four replicated rats. The total number of bouts made by male rats in 2 h period towards WSO bait without additive was significantly (P < 0.05) more than those made towards baits containing three types of additives (Table 4). No significant difference was, however, found in the number of bouts and total time spent among the WSO baits containing 2% ESP, EA and CES. The total time spent (seconds) in bouting in 2 h period a day by male rats was also significantly (P < 0.05) more in WSO without any additive than in baits containing three additives (Table 4). No significant difference was, however, found in the time spent among the WSO baits containing 2% ESP, EA and CES. The time spent for bouting in the baits containing 2% ESP and EA was non-significantly more than that spent in bait containing 2% CES.

In overall, no significant difference was found in average food consumption (g/kg body weight) for 2 h a day between male and female rats (Table 4) in respect of WSO bait without additive as well as of WSO bait containing 2% ESP, EA and CES. The number of bouts made towards WSO bait without additive was, however, significantly (P<0.05) more by male rats compared to female rats (Table 4). However, there was no significant difference in number of bouts made towards WSO bait containing three bait additives between male and female rats. The total time spent in bouting towards WSO bait without additive was also significantly (P < 0.05) more by male rats compared to female rats (Table 4) and there was no significant difference in time spent in bouting towards WSO bait without additive male rats (Table 4) and there was no significant difference in time spent in bouting towards WSO bait containing three bait additives between male and female rats compared to female rats (Table 4) and there was no significant difference in time spent in bouting towards WSO bait containing three bait additives between male and female rats.

#### 3.3. Poison baiting experiment

Pre-census bait consumption (g/100 g bait) recorded before

treatment from all four blocks of poultry farm at village Ghutani Kalan, District Ludhiana by keeping plain WSO bait revealed 29.3% to 55.8% consumption (Table 5). No significant difference was found in pre-census bait consumption among the four treated and untreated blocks indicating almost similar level of rodent activity in all the blocks. The consumption of treatment bait was found to be 26.95%, 68.20% and 68.65% in blocks I, II and III, respectively (Table 5). The consumption of 2% zinc phosphide bait containing 2% egg albumin and 2% egg shell powder was (P<0.05) significantly higher than that of 2% zinc phosphide bait alone. Record of post census bait consumption after 1 week of treatment from all the blocks revealed 29.29% to 33.52% reduction in rodent activity in the three treated blocks with respect to untreated block and 19.23% to 42.69% with respect to same shed. There was no significant difference in reduction in rodent activity between the three treated blocks despite of significantly higher consumption of 2% zinc phosphide bait containing bait additives.

#### Table 5

Bait acceptance and level of rodent activity in different blocks of poultry farm.

|                 |         | Bait consumption (g/100 g bait) |                          | Reduction in rodent activity (%) |                      |  |
|-----------------|---------|---------------------------------|--------------------------|----------------------------------|----------------------|--|
| Block Treatment |         | D                               | m                        | With respect to                  | With respect to      |  |
|                 |         | Pre-census                      | Treatment                | untreated block                  | same block           |  |
| I               | ZnP     | 29.30±11.89                     | 26.95±13.99 <sup>a</sup> | 33.52±3.46                       | 24.07±3.95           |  |
| Π               | ZnP+EA  | 55.60±7.74                      | 68.20±8.86 <sup>b</sup>  | 28.13±8.64                       | 19.23±9.71           |  |
| III             | ZnP+ESP | 55.80±13.42                     | 68.65±13.95 <sup>b</sup> | 44.41±6.81                       | 42.69±9.58           |  |
| IV              | Control | 33.95±3.80                      | -                        | -                                | Increase in activity |  |

Values are mean±SE, ZnP=2% zinc phosphide, EA=2% egg albumin, ESP=2% egg shell powder. Values with different superscripts (a,b) in a column for treatment bait consumption differ significantly at P<0.05.

## 3.4. Trapping experiments

Rodent trapping carried out with wooden single rat traps from all the sheds by alternatively placing traps containing chapatti pieces without additive and those containing chapatti pieces smeared with 2% ESP revealed total trap index of *R. rattus* to be 11.60 (6.84 for male and 4.76 for female rats) in traps baited with chapatti piece smeared with ESP and 13.38 (6.84 for male and 6.54 for female rats) in traps baited with chapatti piece without additive (Table 6). No significant difference was found in total trap index as well as that of male and female rats separately between the traps baited with chapatti pieces with and without ESP. Also no significant difference was found in the trap index of traps baited with chapatti pieces smeared with and without ESP between the two sexes.

Trapping carried out by alternatively placing traps containing chapatti pieces without additive and those containing chapatti pieces smeared with 2% EA revealed total trap index of *R. rattus* to be 5.35 (3.27 for male and 2.08 for female rats) in traps baited with chapatti piece smeared with EA and 7.13 (2.08 for male and 5.05 for female rats) in traps baited with chapatti piece without additive (Table 6). No significant difference was found in total trap index as well as that of male and female rats separately between the traps baited with chapatti pieces with and without EA. Also no significant difference was found in the trap index of traps baited with chapatti pieces smeared with and without EA between the two sexes.

Trapping carried out by alternatively placing traps containing chapatti pieces smeared with 2% ESP and 2% EA revealed total trap index of *R. rattus* to be 13.69 (6.25 for male and 7.44 for female rats) in traps baited with chapatti piece smeared with ESP and 12.79 (8.03 for male and 4.76 for female rats) in traps baited with chapatti piece smeared with EA (Table 6). No significant difference was found in total trap index as well as that of male and female rats separately between the traps baited with chapatti pieces smeared with ESP and EA. Also no significant difference was found in the trap index of traps baited with chapatti pieces smeared with ESP and EA. Also no significant difference was found in the trap index of traps baited with chapatti pieces smeared with ESP and EA between the two sexes.

In overall, the results of poultry trapping experiments revealed no significant effect of poultry egg components *i.e.* ESP and EA in trapping of rats by single rat traps. This may be due to the reason that rats might not have come to know about the additive smeared on chapatti piece before they pulled it from the string of the trap and got trapped. There was no significant difference in trap index between traps baited with chapatti piece smeared with and without additive and also between the traps baited with chapatti smeared with ESP and EA.

#### Table 6

Comparison of trap index of rats trapped by smearing chapatti pieces with different additives.

|            | Additive used on | Trap index (    | Trap index (no. of rats trapped/100 traps/day |            |  |  |  |  |
|------------|------------------|-----------------|---|------------|--|--|--|--|
| Experiment | chapatti         | Male            | Female  | Total      |  |  |  |  |
| Ι          | ESP              | 6.84±2.04       | 4.76±1.87                                     | 11.60±3.69 |  |  |  |  |
|            | Untreated        | 6.84±1.22       | 6.54±2.87                                     | 13.38±3.61 |  |  |  |  |
| II         | EA               | 3.27±1.06       | 2.08±0.78                                     | 5.35±1.68  |  |  |  |  |
|            | Untreated        | $2.08 \pm 1.29$ | 5.05±1.42                                     | 7.13±2.59  |  |  |  |  |
| III        | ESP              | 6.25±2.01       | 7.44±2.61                                     | 13.69±4.12 |  |  |  |  |
|            | EA               | 8.03±2.22       | 4.76±1.55                                     | 12.79±2.03 |  |  |  |  |

Values are mean ±SE.

## 4. Discussion

Present study indicates sex specific differences in acceptance of cereal based bait containing different poultry egg components as bait additives in bi-choice and nochoice feeding tests by *R. rattus*. During present studies, in no-choice feeding tests, individual variation in response of rats towards difference baits was observed. Similar individual variations in rats of different species have also been reported earlier<sup>[15,16]</sup>.

Results of bi-choice experiments revealed significantly (P<0.05) higher consumption of WSO bait containing all the three concentrations (2%, 5% and 10%) of EA and 2% and 5% ESP by female rats and that of bait containing 5% and 10% EA by male rats. In no-choice experiments, the consumption, number of bouts made and the time spent in WSO bait containing 2% EA by both male and female rats was non-significantly higher than that containing 2% ESP and CES. In

poultry farm, significantly higher consumption of 2% zinc phosphide bait containing 2% EA and 2% ESP leading to upto 42.69% reduction in rodent activity was observed compared to up to 33.52% reduction in rodent activity with only 2% zinc phosphide treatment.

The literature also reveals the use of cereals in wholesome or cracked form mixed with additives such as vegetable oil, egg shell, egg yolk, minced meat, sugar and flavours<sup>[11]</sup>. Such food items are intermittently preferred because of the energy they provide chiefly due to their carbohydrate and protein contents. Khan et al. reported preference for poison bait containing egg shells by rodents in rice crop fields<sup>[10]</sup>. Pervez *et al.* found the highest potential of egg mixed brodifacoum bait in enhancing acceptance by field rodents in wheat crop<sup>[12]</sup>. The addition of egg shell did not show a significant increase in relative consumption for the cereal base by Bandicota bengalensis<sup>[17]</sup> and Hystrix indica<sup>[18]</sup>. Shafi et al. also observed Bandicota bengalensis showing higher preference toward minced meat bait than egg shell and egg yolk mixed bait<sup>[13]</sup>. However, Pervez et al. found an additive effect of 2% egg shell in enhancement of bait preference<sup>[11,19]</sup>. These contradictory results may primarily be attributed to the nature of bait material used and due to different species tested.

Based on present studies, it is suggested that the use of 2% EA and ESP in cereal bait may enhance bait intake and this combination may yield higher control of *R. rattus* if used in 2% zinc phosphide bait. This may further help in checking the spread of rodent borne diseases to animals and humans.

## **Conflict of interest statement**

We declare that we have no conflict of interest.

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## Comments

#### Background

The house rat, *R. rattus* is one of the most important commensal rodent pest worldwide. It is the predominant pest species infesting poultry farms in India as well as in other countries. Use of poison baits specially the acute rodenticides is the most reliable strategy for controlling field as well as commensal rodents. However, rodent control with acute rodenticides has often been found ineffective in reducing rodent densities due to poor poison bait acceptance, sub-lethal dosing and subsequent conditioned aversion. The acceptance and efficacy of such rodenticide baits can be improved by using certain bait additives.

## Research frontiers

*R. rattus* poses a serious threat to poultry operations by feeding on poultry feed, damaging eggs and killing chicks. Hence, rodenticide bait containing poultry egg components may be more acceptable to it. Present research compares the acceptance of cereal bait containing different concentrations of poultry egg components such as egg shell and egg albumin in laboratory as well as in the poultry farms.

## Related reports

Present study indicates sex specific and individual differences in acceptance of cereal bait containing different poultry egg components as bait additives by *R. rattus.* Similar differences have also been reported earlier. Earlier workers have evaluated the acceptance of different bait additives such as peanut butter, egg shell, minced meat *etc.* Some workers have found them good additives and some not. These contradictory results may be due to different species tested and different bait base used.

## Innovations and breakthroughs

Authors have studied the behaviour of rats in the form of food consumed, number of bouts made and time spent towards the food source containing different bait additives. In present studies, egg albumin as bait additive has been evaluated for the first time. The results reveal this component to be more acceptable by house rats.

## Applications

Results of present study can be directly applied in poultry farms for control of *R. rattus* populations. Use of these additives will increase the acceptance and hence the efficacy of acute poison such as zinc phosphide. Controlling rats in poultry farms will not only reduce the damage caused by them but will also prevent the transmission of rodent borne diseases.

#### Peer review

Present research is a very good study evaluating the potential of poultry egg albumin and egg shell (both crushed and powder form) in enhancing the acceptance of cereal bait by house rat. The acceptance was assessed based on bi-choice and no-choice experiments in laboratory cages and food scale consumption monitor. Authors have also tested the efficacy of these components after mixing in zinc phosphide bait, an acute rodenticide in poultry farms. Egg albumin has been found to have potential in increasing acceptance of cereal based bait.

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