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Laboratory evaluation of fipronil against *Periplaneta americana* & *Blattella germanica*

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***Background & objectives*: In cockroach control, bait formulations are extensively used. Fipronil is one of the broad spectrum insecticides from phenyl pyrazole family available in bait formulation. The compound has been tested under field conditions. However, information on its effectiveness on different surfaces and at various density levels is not known. Therefore, the efficacy of a new formulation of fipronil 0.03 per cent (Goliath gel) was tested under laboratory conditions.**

***Methods*: The bait formulation was tested against *Periplaneta americana* at the dosages of 0.025, 0.05, 0.1, 0.15 and 0.2 g/m^2, and *Blatella germanica* at 0.025, 0.05, 0.075, 0.1 and 0.125 g/m^2 on wood, cement, mud and thatch surface with three density levels of cockroaches *viz*., 5, 10 and 20 numbers/m^2. Mortality after 24 h of exposure was observed daily for five days.**

***Results*: Rate of mortality varied between 16.0 and 96.0 per cent in *P. americana* and 51.0 and 100.0 per cent in *B. germanica* on different surfaces tested. The mortality rate of *P. americana* increased line rarly from the dosage 0.025g to 0.15 g/m^2 and at 0.2 g/m^2, it was independent. The LD_{80} values of the gel against *P. americana* at high density were 0.208, 0.246, 0.361 and 0.466 g/m^2 on wood, cement, mud and thatch surfaces, whereas against *B. germanica* the values were 0.079, 0.081, 0.089 and 0.055 g/m^2 respectively, indicating a significantly ($P<0.001$) higher efficacy of Goliath gel against *B. germanica* than *P. americana*. The results of fitting logistic regression model to the observed percentage mortality with log dose and cockroach density as explanatory variables satisfactorily described the observations at all densities on each surface. The pattern of response to increasing dosages was similar for all the three density levels on each of the surfaces in *P. americana* and *B. germanica*.**

***Interpretation & conclusion*: The effectiveness assessed against *P. americana* and *B. germanica* using logistic regression model suggested that the gel when applied at the appropriate dosages on wood, cement, mud and thatch surfaces could cause >80 per cent mortality of these species in dwellings having these types of surfaces.**

Key words American cockroach - bait formulation - *Blatella germanica* - German cockroach - goliath gel - India - *Periplaneta americana* - phenyl pyrazole

Cockroaches are offensive pests visually and expel unpleasant smelling secretion that spoils the flavour of food and environment. Its obnoxious behaviour and movement while foraging makes it a potential carrier of various pathogenic organisms, as it contaminates food and water either by frequent regurgitation or defecation[1]. *Periplaneta americana* Linnaeus (Dictyoptera: Blattidae), the American cockroach infests kitchen, storeroom, drainage and latrine, while *Blatella germanica* (Linnaeus) (Dictyoptera: Blatellidae), the German cockroach inhabits peridomestic conditions, especially the garden having piles of garbage. Since control of cockroaches either by space spray or residual application of insecticides did not yield desired result, attention is being focused on the use of toxic food baits for suppression[2-4]. Baits offer the advantage of long term residual activity, safe application technology, fast action and reduced odour when compared with residual sprays. In addition, baits have also been reported to possess secondary poisoning effect through necrophagy[5] and coprophagy[6,7]. Besides chemicals, baits have some inert food substances, which attract cockroaches. A variety of active ingredients such as chlorinated hydrocarbons, carbamates, organophosphates, inorganic compounds including several new classes of insecticides have been incorporated in baits[8]. Fipronil, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulphinyl]-1-pyrazole-3-carbonitrile is one of the newly developed broad spectrum chemicals from the phenyl pyrazole family, when used as a bait formulation (Goliath gel) reported to cause mortality among certain species of insects at a low dosage[9]. It has moderate toxicity to mammals and in rat the acute oral lethal dose to kill 50 per cent population (LD_{50}) is 97 mg/kg[10]. Although, the efficacy of fipronil has been tested by Tilak *et al*[7], under field conditions, information on the influence of various dosages on density of cockroaches and effectiveness on various surface areas is not known. Therefore, we carried out this study to test Goliath gel (fipronil 0.03%) for its efficacy against adults and nymphs of both *P. americana* and *B. germanica*, under laboratory conditions.

Material & Methods

Adults and nymphs of *P. americana* were collected from a bakery in a small town, Villianur situated 10 km away from Pondicherry, south India, on its western side and *B. germanica* from a garbage dumping yard located behind the premises of Vector Control Research Centre, Pondicherry, and were maintained separately in 17 liter plastic buckets, having corrugated cardboard and covered with lid. This stock served for testing the efficacy of the gel. To facilitate ventilation, the central portion of the plastic lid (15 cm diameter) was replaced with brass mesh. Cockroaches were maintained in the laboratory at 28 ± 2°C, ambient humidity (65-75%) and a photoperiod of 8:16 (L: D) h, and were provided with food (dog biscuit, milk powder) and water.

Fipronil (0.03%) in the form of a pressurized gel bait (Goliath gel supplied by M/s. Aventis Crop Science India Ltd., Mumbai) was used in the study. The test was carried out in one square meter colony cage, made up of wooden slots. All four sides of the cage were covered with wire mesh, top was covered with a glass plate to view cockroaches and bottom was covered with plywood, where the gel was applied. Bottom plywood, the test surface was of detachable type and can be replaced either with mud or cement or thatch surface. Cockroaches were released with a male to female ratio of 1:1 and allowed to acclimatize for 2 to 2.5 h *i.e.*, until all of them aggregate within the harborages, which were kept in the cage for sheltering. Cockroaches were fed on dog biscuit and milk at the rate of 1g each and water 2 ml per cockroach. The cage was covered with a lint cloth to avoid light, as cockroaches prefer nocturnal condition.

Goliath gel was tested at five dosages *viz*., 0.025, 0.05, 0.1, 0.15 and 0.2 g/m^2 against *P. americana* adults and 0.025, 0.05, 0.075, 0.1 and 0.125 g/m^2 against *B. germanica*. *P. americana* were exposed to higher dosages as it weighs more than *B. germanica*. Prior to treatment, the amount of gel to be applied was measured using an electronic balance. Since cockroaches tend to aggregate[11] at corners of the cage, dosage of the gel was split into 5 equal parts and placed in 5 different spots, *i.e.*, one on each corner and the remaining in centre. Border of the treated surface was smeared with vaseline to prevent crawling of cockroach over the sides from treated area. Each dosage was tested at three density levels of cockroaches *viz*., 5 (low), 10 (medium) and 20 numbers (high) of cockroaches/m^2 respectively and on wood, cement, mud and thatch surfaces. Cockroaches were removed from the cage after one hour of exposure and held in

1000 ml plastic container, where food and water were also given. Mortality of the cockroaches was recorded daily for five days following the treatment.

The gel was also tested against nymphs (pre adult) of *P. americana* and *B. germanica* at 10 number/m^2 and at an application rate 0.1g/m^2, due to non-availability of nymph population of particular age group in sufficient quantities. To avoid bias if any, the experiment was blinded with coded dosages and decoded subsequently. Each set of the experiment was replicated five times. A control batch was also run concurrently, where the cockroaches of each species were released into the cage at different density level and food and water were given. The cockroaches were removed after 24 h and released in to 1000 ml plastic container and examined for mortality, if any. The cumulative average mortality was determined to assess the overall impact of the gel on the cockroaches exposed. The dosage at which more than 80 per cent mortality obtained (LD_{80}) was considered as an effective dosage in the suppression of cockroach density[7]. The entire study was completed within a span of 180 days.

The LD_{80} values and their 95 per cent confidence intervals (CI) were estimated by fitting a logistic regression model to the observed relationship of percentage mortality of adults with logarithmic concentration of the bait and level of cockroach density. Separate logistic models were fitted for each surface. The goodness of fit of the model was tested using Chi square test; $P < 0.05$ was considered as a significant departure, of the model from observations. In case of significant departure, a heterogeneity factor was used to calculate the 95 per cent confidence limits for LD_{80}. The slopes of the regression models for each density on a surface were compared with a common slope for all densities using Chi square test for parallelism[12]. Relative median potencies and their 95 per cent confidence intervals were calculated for each density level within each surface in which the slopes do not differ significantly.

Results

P. americana: The gel formulation caused mortality of *P. americana* at all the dosages tested with a maximum of 96.0 per cent mortality, at the application rate 0.2 g/m^2, on wooden surface with low density and a minimum of 16 per cent at the dosage 0.025 g/m^2, on thatched surface at the same density (Fig. 1). The rate of mortality increased almost linearly with dosage up to 0.15 g/m^2 and thereafter, it was independent of dosage. The mortality rate was >80 per cent at the application rate of 0.1, 0.15 and 0.2 g/m^2 with low density and at 0.15 and 0.2 g/m^2 with medium density on wood and cement surfaces (Fig. 1). At high density, motality was <80 per cent with all the dosages tested. Lowest mortality (<65%) was observed on thatch surface with all density levels and dosages (Fig.2). The LD_{80} of the gel against *P. americana* at the highest density was 0.208, 0.246, 0.361 and 0.466 g/m^2 on wood, cement and mud and thatch surfaces respectively (Fig.2).

The results of fitting logistic regression model to the observed percentage mortality with log dose and cockroach density as explanatory variables are shown in Table I. The goodness of fit Chi square values suggested that the logistic regression model satisfactorily described the observations at all densities on each surface (P>0.05, Fig. 1). Further, the parallelism test showed that the slopes for different density levels is not significantly different and that a common slope fitted the data as well as three separate lines for three density levels. This suggested that the pattern of response to increasing dosages was similar for the three density levels on each of the surfaces. The estimated LD_{80} values for medium density did not differ significantly with that of low and high densities for wood, cement and mud surface (Table I, 95% confidence intervals overlap). However, the LD_{80} values for low density differed significantly (P<0.05) from that of high density on all the surfaces, except thatch (Table I). However, on thatch surface, the LD_{80} values were found to be independent of cockroach density.

At the low density the LD_{80} values did not vary significantly among wood, cement and mud surface (Fig. 3, Table I, 95% confidence intervals overlap). The difference in the potency of the bait at low density was eight times higher on wood compared with that of thatched surface. The corresponding figures for cement and mud surface were 7.7 and 4.7. At medium density the efficacy was 4.2, 3.6 and 2.4 times higher on wood, cement and mud respectively than on thatch. However, when the density was high, the efficacy did not differ significantly among wood, cement and mud

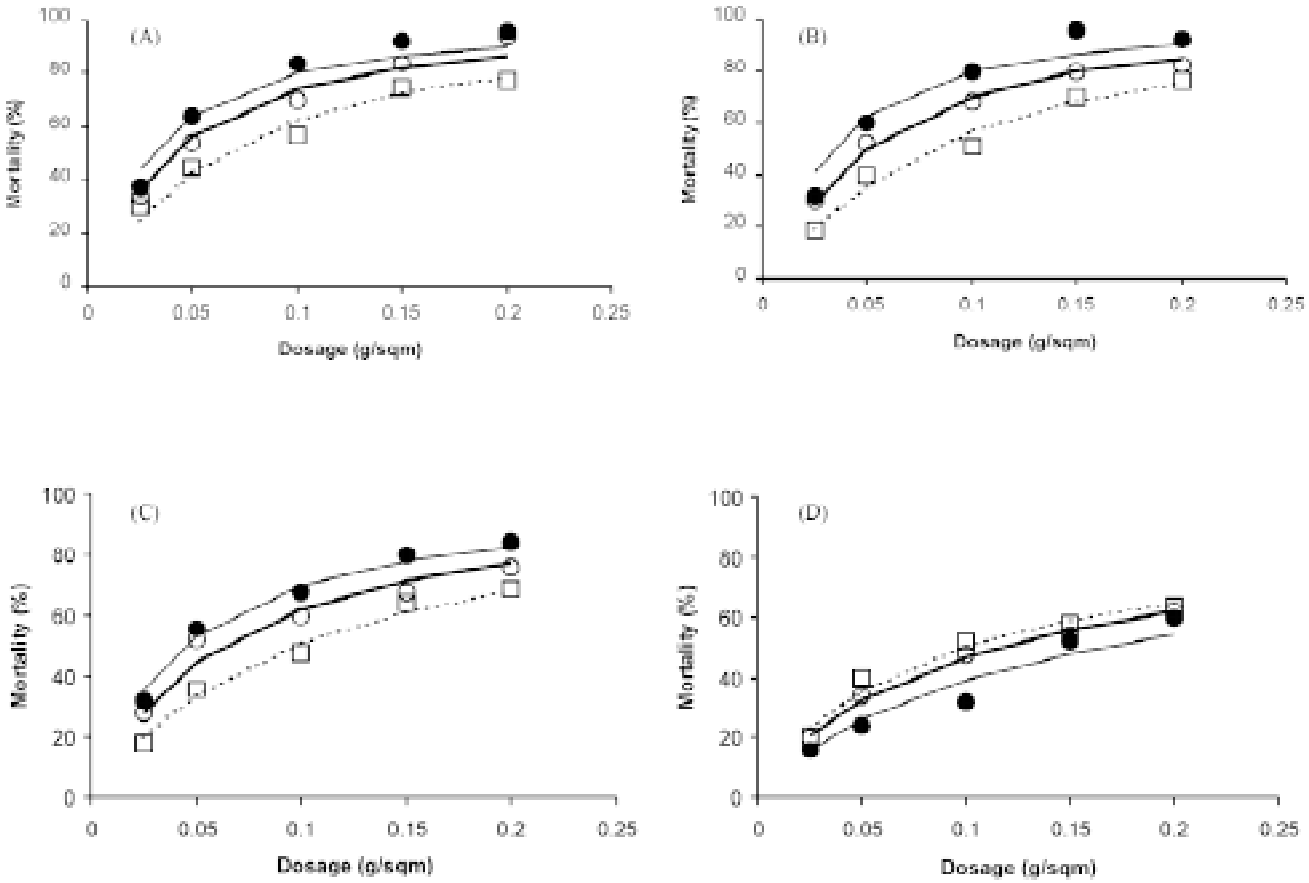

Fig. 1. Observed (closed circle: low density; open circle: medium density and open square: high density) and fitted (thin line: low density; solid line: medium density; dotted line: high density) logistic regression of percentage mortality of *P. americana* due to different dosages of Goliath gel on various surfaces; (**A**) wood, (**B**) cement, (**C**) mud and (**D**) thatch.

and the effectiveness of the gel was 2.2, 1.9 and 1.3 times higher on wood, cement, mud when compared with thatch at this density.

Mortality, in *P. americana* was found after 24 h of exposure to the gel in all the sets of experiments irrespective of surfaces and dosages. The mortality rate observed was 61.2, 12.7 and 2.8 per cent after 24, 48 and 72 h of exposure to the gel respectively.

B. germanica: In *B. germanica*, the Goliath gel caused cent per cent mortality at the application rate 0.1 as well as 0.125 g/m^2 on thatch at low density, and lowest mortality of 51.0 per cent at 0.025 g/m^2 on cement surface at high density (Fig. 3). Mortality was >80 per cent at the application rate of 0.05, 0.075, 0.1 and 0.125 g/m^2 on wood, cement, mud and thatch respectively at low density level and similar trend was also observed on thatch at medium as well as high density levels at these dosages. Mortality observed was >80 per cent in medium density, at the dosages of 0.075, 0.1 and 0.125 g/m^2 on wood and mud and similar effect was seen only with the last two higher dosages on cement. At high density, >80 per cent mortality was observed at the dosages of 0.075, 0.1 and 0.125 g/m^2 on wood and mud and only with the last two higher dosages on cement. The mortality rate increased with dosages and decreased with density on wood, cement, mud and thatch surfaces (Fig. 3). The LD_{80} values for *B. germanica* were 0.079, 0.081, 0.089 and 0.055 g/m^2 respectively at low density, showing the significantly (P<0.05) higher efficacy of Goliath gel against *B. germanica* than *P. americana*.

The results of fitting logistic regression model to the observed logit of the percentage mortality for each surface in *B. germanica* are given in Table II. As observed in *P. americana*, the logistic regression model satisfactorily described the observed mortality at all densities on each surface (Fig. 2). The pattern of

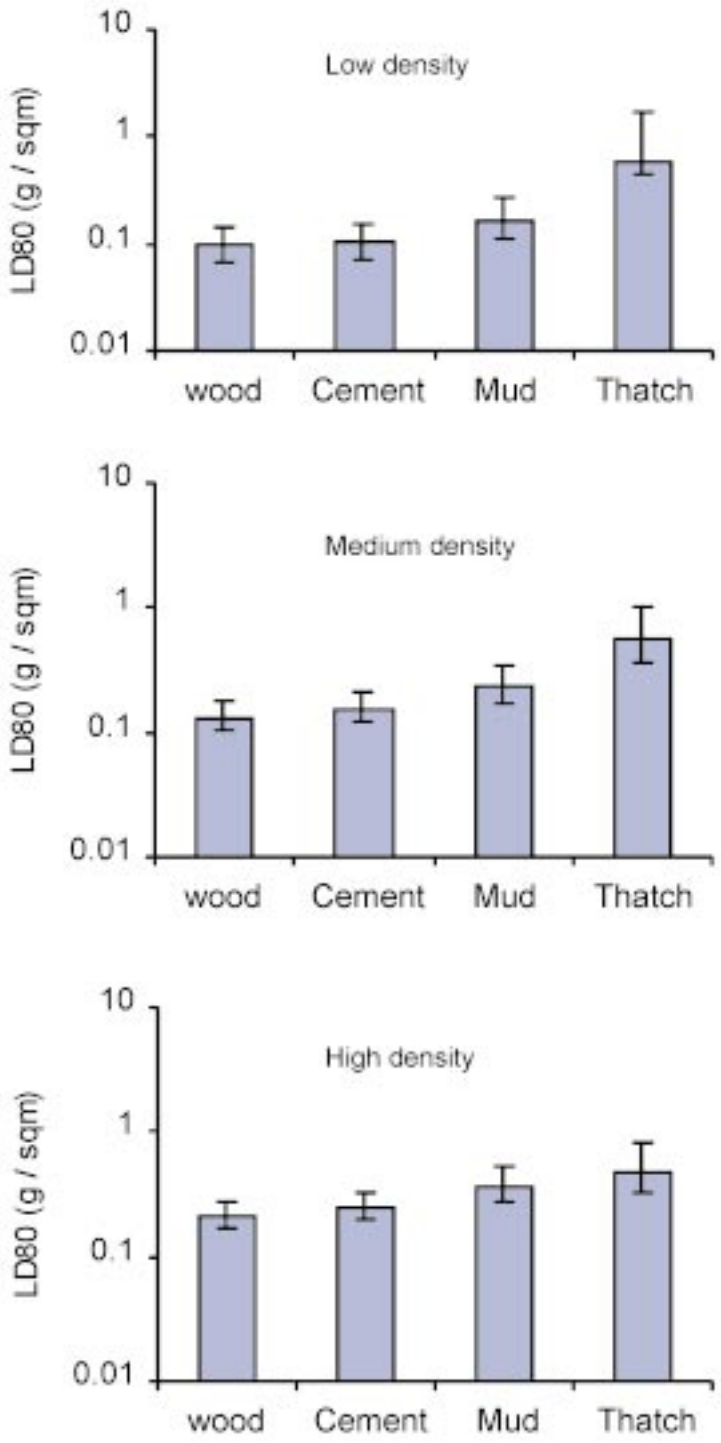

Fig. 2. Lethal dosage of Goliath gel required to kill 80 per cent (LD_{80}) of the population of *P. americana* in relation to different density (low, medium and high) on various surfaces *viz*., wood, cement, mud and thatch.

Table I. Results of fitting logistic regression model to the observed percentage mortality with log dose and density (low, medium and high) of *Periplaneta americana* as explanatory variables

Surface	Density (per m^2)	Regression equation	χ^2	*P*	LD_{80} (95% CI)
Wood	5	4.08 + 1.17 ln dose			0.099 (0.068-0.147)[a]
	10	3.75 + 1.17 ln dose	8.488	0.669	0.132 (0.102-0.177)[a,b]
	20	3.22 + 1.17 ln dose			0.208 (0.168-0.273)[b]
Cement	5	4.21 + 1.24 ln dose			0.102 (0.072-0.148)[a]
	10	3.71 + 1.24 ln dose	5.815	0.885	0.154 (0.121-0.205)[a,b]
	20	3.12 + 1.24 ln dose			0.246 (0.199-0.323)[b]
Mud	5	3.25 + 1.04 ln dose			0.167 (0.114-0.262)[a]
	10	2.91 + 1.04 ln dose	3.073	0.99	0.233 (0.173-0.342)[a,b]
	20	2.45 + 1.04 ln dose			0.361 (0.272-0.532)[b]
Thatch	5	1.59 + 0.88 ln dose			0.578 (0.435-1.713)[a]
	10	1.91 + 0.88 ln dose	2.54	0.99	0.553 (0.360-1.031)[a]
	20	2.06 + 0.88 ln dose			0.466 (0.352-0.789)[a]

Same superscript [a,] within a surface indicates no significant difference in LD_{80} values and different superscripts[a,b] indicate significant difference in LD_{80} values

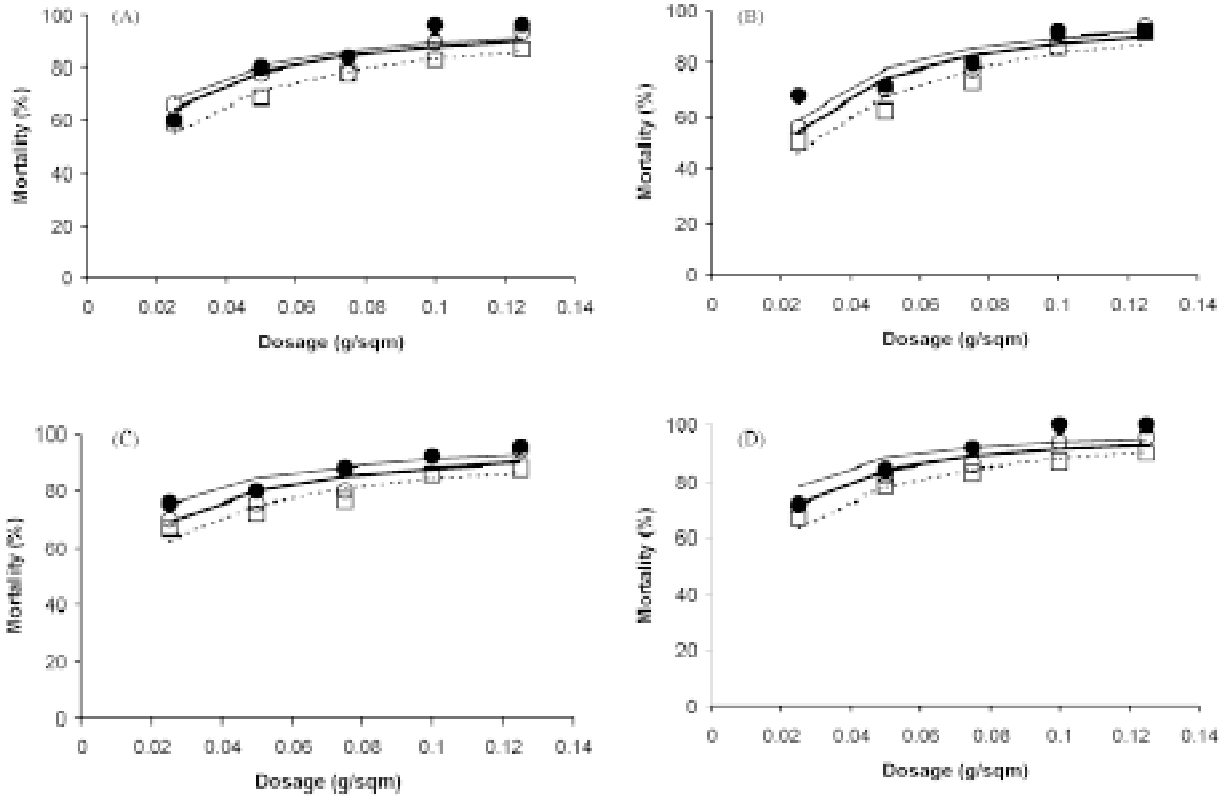

Fig. 3. Observed (closed circle: low density; open circle: medium density and open square: high density) and fitted (thin line: low density; solid line: medium density; dotted line: high density) logistic regression of percentage mortality of *B. germanica* due to different dosages of Goliath gel on various surfaces; (**A**) wood, (**B**) cement, (**C**) mud and (**D**) thatch.

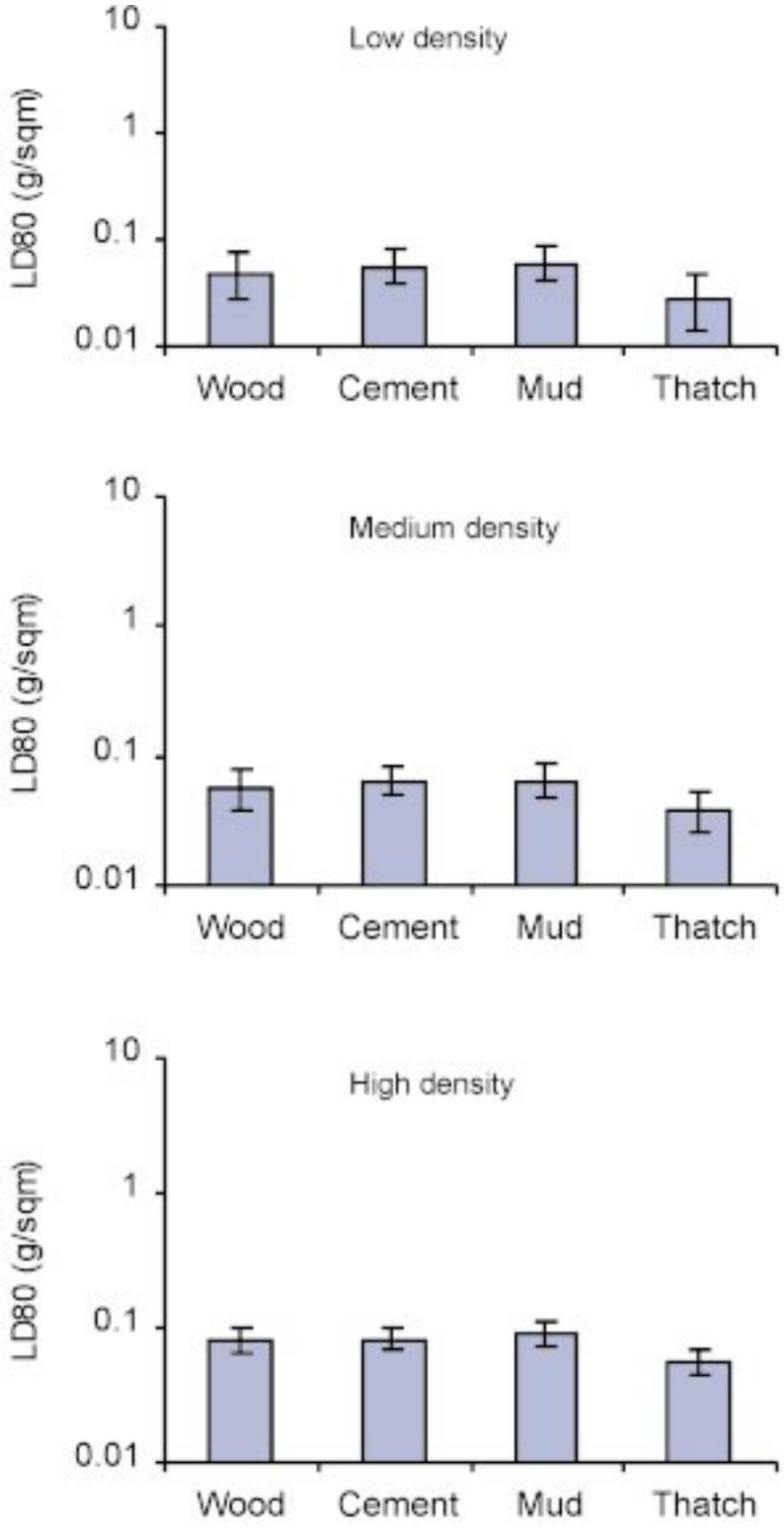

Fig. 4. Lethal dosage of Goliath gel required to kill 80 per cent (LD_{80}) of the population of *B. germanica* in relation to different density (low, medium and high) on various surfaces *viz*., wood, cement, mud and thatch.

Table II. Results of fitting logistic regression model to the observed percentage mortality with log dose and density (low, medium and high) of *B. germanica* as explanatory variables

Surface	Density (per m^2)	Regression equation	χ^2	*P*	LD_{80} (95% CI)
Wood	5	4.53 + 1.03 ln dose			0.047 (0.028-0.076)[a]
	10	4.39 + 1.03 ln dose	6.31	0.85	0.055 (0.039-0.077)[a]
	20	4.02 + 1.03 ln dose			0.079 (0.064-0.102)[a]
Cement	5	5.14 +1.29 ln dose			0.052 (0.038-0.080)[a]
	10	4.95 + 1.29 ln dose	11.53	0.4	0.063 (0.050-0.083)[a]
	20	4.64 + 1.29 ln dose			0.081 (0.069-0.100)[a]
Mud	5	4.28 + 0.86 ln dose			0.058 (0.017-0.063)[a]
	10	3.96 + 0.86 ln dose	6.96	0.802	0.064 (0.032-0.074)[a]
	20	3.67 + 0.86 ln dose			0.089 (0.054-0.094)[a]
Thatch	5	5.19 + 1.06 ln dose			0.027 (0.014-0.047)[a]
	10	4.84 + 1.06 ln dose	6.44	0.842	0.038 (0.026-0.054)[a]
	20	4.46 + 1.06 ln dose			0.055 (0.044-0.069)[a]

Same superscript [a] within a surface indicates no significant difference ($P = 0.05$) in LD_{80} values

response to increasing dosages is similar for the three density levels on each surface (Parallelism test, $P>0.05$, Table II). The LD_{80} values at low density did not differ significantly from that of medium and high density on all the surfaces tested. At low density, the LD_{80} value did not differ significantly among wood, cement, thatch and mud. The same trend was also seen with regard to medium and high density (Fig. 4) (95% confidence intervals overlap on all surfaces and density levels). The efficacy of the bait on wood, cement and mud when compared with that of thatch was 1.7, 2.0 and 2.1 times lower at low density. The corresponding figures at medium and high density were 1.4, 1.7 and 1.7 times; and 1.4, 1.5 and 1.6 times respectively. The mortality was 72.5, 15.3 and 1.5 per cent after 24, 48, and 72 h of exposure to the gel.

Nymphs: The gel was effective in causing mortality of nymphs of *P. americana* and *B. germanica* at the application rate of 0.1 g/m^2 on all the four surfaces tested. The mortality of *P. americana* nymphs was more when the bait was applied on wooden surface (86.0%) followed by cement (84.0%), mud (80.0%) and thatch (54.0%). As has been observed in the adult population, the mortality was maximum on wooden surface and minimum on thatched surface. The rate of mortality varied significantly ($P<0.05$) with the types of surfaces tested.

Cent per cent mortality in the nymphs of *B. germanica* was seen on thatched surface. The corresponding figures for wood, cement and mud surfaces were 98.0, 94.0 and 96.0 per cent respectively. Though the mortality rate was high on thatched surface, it did not vary significantly with the types of surfaces. Logistic regression analysis showed that nymph population of *B. germanica* was more vulnerable to the gel than that of *P. americana*.

Discussion

Control of cockroaches relies mainly on the use of synthetic chemicals. Most of the chemicals, when used in the form of either aerosol or spray, did not prevent cockroach infestation in and around human habitations due to increase in the incidence of insecticide resistance[2,3,13]. The use of baits in cockroach abatement programme is now gaining popularity, due to non repellent action, preference even in the presence of food materials, easy application techniques and specific and fast action on target species[14,15]. Hence

baits incorporated with active ingredients, *i.e*., chemicals having moderate mammalian toxicity and safe application techniques are being developed for controlling cockroaches. Performance of such baits in apartments and commercial kitchens infested with cockroaches has been demonstrated. However, the efficacy of baits varies depending on the type of dwelling surfaces and target species[14,16]. Fipronil (0.03%) when tested in the form of bait, caused mortality in both *P. americana* and *B. germanica*. The logistic regression model made in this investigation showed that the efficacy of Goliath gel was significantly higher against *B. germanica* than *P. americana*. It is assumed that the size of the species influences the efficacy of gel, as *B. germanica* weighs less than that of *P. americana*.

Though the effectiveness of gel showed a linear relationship initially, it was independant of the dosages thereafter. An inverse relationship was observed between cockroach density and mortality on all surfaces, in both the species except on thatch, where it was reverse with *P. americana*. The estimated LD_{80} value for *P. americana* on thatch surface was based on logistic regression model. Model predictions were in agreement with the observations. However, the observed mortality was found to be below the estimated LD_{80} value at all the tested dosages. The estimated LD_{80} value suggested that for thatch surface the dosages were 5.6, 4.2 and 2.2 times higher in low, medum and high density than that of wood surface. The estimated risk of mortality of *P. americana* was lower on thatch, when compared to that of other surfaces, suggesting that, it was not effective on thatch surface. Perhaps, *P. americana*, the nocturnal species concealed among the leaflets as soon as released and seldom came out on to the treated surface; hence the rate of kill was 16 per cent at low density. When cockroach density was increased, the chance of exposure to the gel also increased, as they shared the same surface area and thereby the mortality increased to 64 per cent at high density. In contrast, the gel was more effective, on thatch surface against *B. germanica*, causing >80 per cent mortality and unlike in *P. americana* the rate of mortality decreased with the increase in the density on this surface. It is obvious that the thatch mimics the natural habitat of *B. germanica*. The size of this species would have facilitated to crawl actively among the leaflets.

Lee[17] carried out a study to determine the effect of fipronil and 2 hydramethylnon as bait formulations against *B. germanica*. The mortality rate observed was 92.0 per cent by both fipronil and chlorpyrifos, which is one of the well known and frequently used baits in cockroach control. This finding corroborates with the present finding, where fipronil also caused 100 per cent mortality in *B. germanica*.

Buczkowshi and Schal[10] observed that the German cockroaches fed on fipronil bait, produced liquid excretions, which were toxic to conspecifics. They also reported that nymphs were attracted to the excretions and preferentially contacted oral region of dying females and imbibed the liquid exudates. They concluded that ingestion of fipronil-induced regurgitate constitutes an important mechanism, through which insecticides were disseminated within cockroach populations. This novel behaviour would appreciably increase the efficacy of the gel. Bait shyness has also been reported in cockroaches[8]. In an another study Lee and Soo[15] have reported glucose aversion towards certain baits among German cockroaches, however, in the present study the species tested showed an inclination towards the Goliath gel. This is evident from the observation that the cockroaches moved towards the bait and consumed, even in the presence of other food materials.

Though both the species infest human habitations, *P. americana* prefers wooden structures rather than cracks and crevices in cement and mud surfaces, whereas *B. germanica* which is common in thatched dwellings with mud wall, also invades buildings with wood or cement surfaces[2]. Since the gel is effective in causing mortality of cockroaches, when tested on wood, cement, mud and thatch sufaces under laboratory conditions, their menace can be reduced in such habitats in nature, using the gel. Further the logistic regression model also supported that the gel when applied at the appropriate dosages on various surfaces tested could suppress the infestation to a desirable level *i.e*., >80 per cent. Besides the Goliath gel appears to be palatable and relatively non-repellent and its application technique is simple and easy. Even in an inaccessible infested surfaces, the gel can be treated with syringe like applicator, which is an added advantage.

Hence for the control of these species of cockroaches, fipronil (0.03%) formulation in the form

a bait can be advocated. However, potency of the bait against cockroaches under field conditions needs to be explored.

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