Research Article

# Development and Evaluation of a Maize Cob Based Bait Formulation against Subterranean Termites (*Odontotermes* spp.)

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**Abstract** | Subterranean termites are devastating pests of many agricultural and horticultural crops, forest plantations and wooden infrastructures. Farmers rely primarily on direct applications of liquid insecticides which usually get off the target site resulting in unsatisfactory and short-term termite eradication along with environmental contaminations. This situation necessitates looking for more target-oriented and ecologically safer strategies such as baiting insecticides with some cellulose attractant. In this study, 5% technical grade fipronil was formulated as small beads (3.5 mm) made by a matrix composed of 65% maize cob powder (as source of natural cellulose for termite attraction), 18% starch, 6% sodium alginate, 4% sodium silicate, and 2% urea. Termites infested monitoring stations installed in a sugarcane field were treated with the formulated bait beads and were inspected for termite activity at 15 and 30 days post-treatment. Results of initial *in-vitro* trials indicated that formulated beads remained intact and did not disintegrate by consecutive episodes of wetting and drying and were able to attract more than 50% termite individuals exposed. This formulation suppressed the termites in field as no termite foraging was recorded inside the bait-treated monitoring stations for about a month post-treatment, while the termites fed on all beads in the control monitoring stations. Overall study findings validate the effectiveness of fipronil and formulated beads for their potential incorporation in the management of subterranean termites in agricultural crops. However, further studies are needed to get more empirical data regarding the formulation's effectiveness and to determine the insecticide (fipronil) release kinetics from the formulated beads under laboratory and field conditions.

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## Introduction

With more than 3,000 described species, termites (Insecta: Isoptera) constitute an important eusocial fauna in the tropical and subtropical ecosystems. These invertebrates feed upon decaying plant material including damp and dry wood, grasses and leaf litter. They are key components of plant decomposition and nutrients cycling and render many ecosystem services in terrestrial biomes (Abe *et al*., 2000; Schuurman, 2005; Majeed, 2012; Constantino, 2021).

Apart from their great ecological significance, many termite species (about 10% of known termite species) are economically important pests of agricultural and urban sectors (Mitchell, 2002; Govorushko, 2019). Subterranean termite species particularly are very damaging agricultural pests and cause substantial injury to wooden infrastructures, agricultural and horticultural crops and forest plantations all over the world including Pakistan (Rouland-Lefèvre, 2011; Manzoor *et al*., 2011; Rust and Su, 2012; Paul *et al*., 2018; Hussain *et al*., 2021).

In Pakistan, around 53 species of termites have been recorded in various biogeographical areas (Manzoor *et al*., 2009; Manzoor and Mir, 2010). In Punjab province, most damaging and economically important subterranean termite species belong to genera *Microtermes* and *Odontotermes* such as *M. mycophagus*, *M. obesi*, *O. obesus* and *O. guptai*. These species infest many agricultural crops including sugarcane, maize, sorghum, cotton, sesame, gram, wheat etc. and agro-forestry and tree plantations (Ahmed *et al*., 2008; Manzoor *et al*., 2011; Hussain *et al*., 2021).

Farmers rely primarily on the direct and extensive applications of synthetic insecticides such as imidacloprid, chlorpyrifos, bifenthrin, deltamethrin, lambda-cyhalothrin, hexaflumuron, indoxacarb and fipronil *etc*. to combat subterranean termite infestations (Su and Scheffrahn, 1996; 1998; Ahmed and Farhan, 2006; Iqbal and Saeed, 2013). These insecticides are mostly applied as liquid formulations and often get off the target site through leaching, evaporation and rapid decomposition by weather extremes resulting in unsatisfactory and short-term termite eradication and also pose ecological consequences such environmental contaminations and human health hazards (Fernández-Pérez, 2007; Huang *et al*., 2018; Meftaul *et al*., 2020).

This situation necessitates looking for more target-oriented and ecologically safer strategies such as slow-release formulations in which a non-repellent toxicant is baited with some pest attractant material and is formulated in such a way that it can persist and remains effective against pests for longer period of time than the direct application of toxicant itself (Grace and Su, 2001; Davies *et al*., 2021). Termite baits are being effectively used to eliminate the pest termites for last few decades and usually toxicant-laced cellulosic foodstuff such as cardboard or wood are often employed in commercial termite baiting systems (Grace and Su, 2001; Tracy, 2003; Glenn *et al*., 2008; Zhang *et al*., 2009; Hamm *et al*., 2013; Su *et al*., 2016; Davies *et al*., 2021).

Here we developed and evaluated a maize cob based bait formulation of fipronil against subterranean termites (*Odontotermes* spp.) under the laboratory and field conditions. Fipronil is a non-repellent phenylpyrazole insecticide acting as GABA-gated chloride channel blocker (Grant *et al*., 1998), and has been widely used as an active ingredient in different baits against many insect pests including termites (Collins and Callcott, 1998; Shelton and Grace, 2003; Huang *et al*., 2006; Mao *et al*., 2011; Iqbal and Evans, 2018; Peters *et al*., 2019).

## Materials and Methods

This research work was carried out in the Laboratory of Entomology, College of Agriculture, University of Sargodha and in a sugarcane (*Saccharum officinarum* L.) field situated in surroundings of College of Agriculture (72°41'35" E; 32°07'54" N).

*Procurement of experimental material*

Fipronil ((5-amino-1-[2,6-dichloro-4-(trifluoromethyl) -phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazole-3-carbonitrile) is an effective synthetic termiticide and belongs to phenylpyrazole group of insecticides. Technical grade fipronil (purity: 95%) was procured from Ruina International Co., Beijing, China through Ali Akbar Group Pvt. Ltd, Pakistan. Sodium silicate and sodium alginate (97.9%; Sigma-Aldrich, MO, USA) were imported and provided by Chemical House Pvt. Ltd., Lahore, Pakistan. Starch and Urea (99.9%; Merck-Schuchardt, Germany) were purchased from local scientific store. Cobs of a full season white grain variety (Gohar-19) of maize (*Zea mays* L.) were acquired from a local farmer. PVC pipes and corrugated cardboard sheets for termite monitoring stations were procured locally from the market.

*Preparation of bait matrix*

Three kilogram maize cobs were cut into small pieces and were air-dried under sunlight for three days. These dried cobs were further crushed into fine powder through a heavy duty electric blender and then were sieved to get fine powder. Maize cob based bead formulation was prepared following a slightly modified previously described method (Singh *et al*., 2009). In brief, a 500 mL glass beaker was filled with 100

**Figure 1:** *Preparation steps of maize cob based fipronil bait formulation. A) mixing of ingredients, B) final form of bait matrix spread in glass Petri-plates, C) blowing of bait matrix through the straw, D) finally formulated beads, E) bead size measurement.*

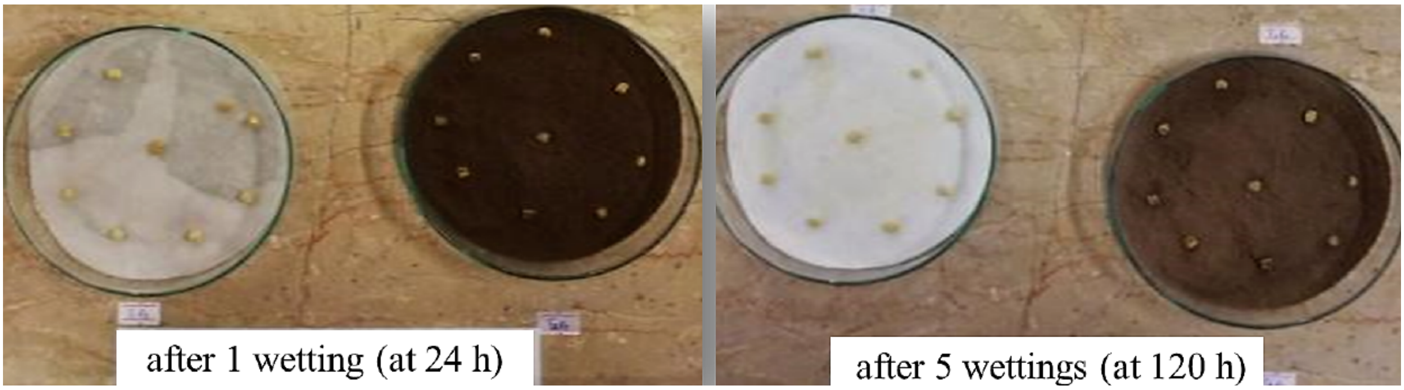


**Figure 2:** *Stability test of formulated bait beads. Wetting and drying of beads were done at 24 h intervals for five consecutive days.*

mL of warm distilled water and 5 g fipronil, 6 g sodium alginate, 4 g sodium silicate were added in it and were mixed thoroughly with the help of a stirrer. Then, 2 g urea, 18 g starch and 350 mL semi-hot water was further added and stirred for two to three min. In the third step, 65 g grinded maize cob was added in it and the matrix was thoroughly mixed for 5 min. Composition of bait matrix ingredients is detailed in Table 1.

**Table 1:** *List of ingredients used for the preparation of maize-cob based matrix of fipronil bait formulation.*

| Bait ingredients | Composition (%) |
|---|---|
| Technical grade fipronil (96% pure) | 5 |
| Maize-cob powder | 65 |
| Sodium alginate | 6 |
| Sodium silicate | 4 |
| Urea | 2 |
| Starch | 18 |

*All these ingredients were mixed in a sequence in 450 mL semi-hot distilled water to prepare the stock bait matrix.*

*Preparation of formulation beads*

Prepared bait matrix was formulated in the form of small beads (Figure 1). For this purpose, bait matrix was first lined as a 1.5 mm layer in a Petri-plate and was allowed to be dried for thirty min. Then, small quantity of bait matrix was collected from the Petri-plate by using a plastic straw and was blown on another Petri-plate set on an orbital shaker running at a speed of 270 rpm. The purpose of this shaking was to make the beads round. The size of bead was measured with the help of a screw gauge. The average bead size was 3.5 mm.

*Stability test of formulation beads*

This preliminary test was conducted in order to determine the integrity of prepared formulation beads that whether they get distorted or not after the irrigation/rain episode under the field conditions. It was checked in two ways. One was evaluation on a Whatman No. 1 filter paper disc and second was on sieved agricultural soil to simulate field conditions (Figure 2). Three replications were set for each method. For this test, nine beads were arranged in each Petri-plate (150 mm Ø) and plates were moistened regularly at 24 h time intervals for 5 consecutive days. Observations on texture and integrity of the bait beads were recorded on daily basis.

*Termite attraction test for formulated beads*

In this experiment, potential attraction of 15 work


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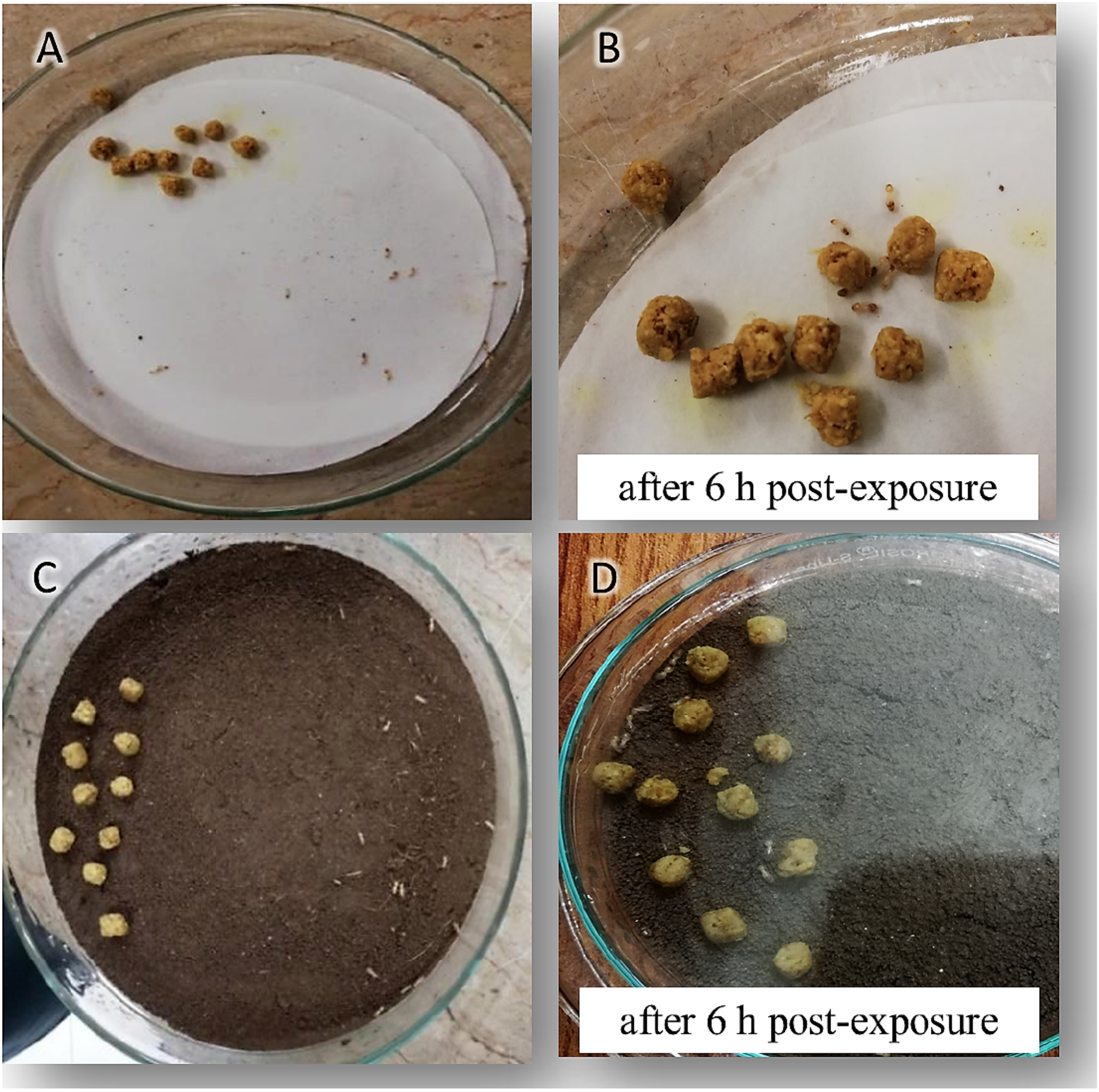


**Figure 3:** *Test for assessing the termite attraction towards formulated bait beads on Whatman No. 1 filter paper disc (A and B) and sieved agricultural field soil (C and D).*

er individuals of subterranean termite (*Odontotermes* spp.; Termitidae) was evaluated by placing 10 formulation beads per Petri-plate (150 mm Ø). One of the Petri-plates was having moist Whatman No. 1 filter paper disc and the second had moistened sieved agricultural field soil as test media (Figure 3). The variation in media was kept in order to observe if termites feed on the filter paper or get attracted and feed on the maize cob based formulation beads.

*Preparation of termite monitoring stations*

Eleven PVC pipes, each with 3 inches diameter and 1.5 feet length, were taken and twenty holes (8 mm Ø) were made randomly on all sides of each pipe with a drill machine (Figure 4). Then, a moistened corrugated cardboard roll was inserted into each pipe. One side of each pipe was wrapped up with a plastic sheet in order to hold the cardboard roll intact inside the pipe.

*Field assessment of bait formulation of fipronil against subterranean termite colonies*

Alive and active 11 termite mounds were searched and selected in a sugarcane field and it was ensured that all mounds had active termite colonies and activ

**Figure 4:** *Preparation and installation of PVC-made termite monitoring stations and placement of formulated beads inside termite infested monitoring stations.*

ities. Each monitoring stations were installed 6 inches away from the mound, after digging up the soil with a metallic soil corer (90 mm Ø). These termite-monitoring stations were inspected regularly for the infestation of subterranean termites at every third day and cardboards were changed on every sixth day. After observing the activity of termites, 10 highly infested monitoring stations were selected for bait application and evaluation. Five stations were treated with fipronil-loaded formulation beads while five were exposed to beads without fipronil as control. Within each termite-infested monitoring station, 15 pre-moistened bait beads (weighing approximately 0.5 g) were placed (Figure 4) and observations regarding the termites' activity inside each station were observed at 15 and 30 days post-treatment. For ensuring termite infestation, the cardboards present inside the monitoring stations were pulled out and the termite individuals were counted on them and the condition of formulation beads were also inspected carefully.

## Results and Discussion

*Stability test of formulated maize cob based beads*

Whatman No. 1 filter paper discs were used in the 1$^{st}$ treatment and sieved soil collected from agricultural field was used in the 2$^{nd}$ treatment. Water was applied to simulate field irrigation in each Petri-plate and then was allowed to dry. There was no evidence of disintegration of beads after five simulated field irrigation in five consecutive days. The formulated beads were stable after irrigated five times with the interval of 24 h and no beads were found broken or disintegrated till the end of experiment (Figure 2).

*Termite attraction test*

Termites (*Odontotermes* spp.) were collected from the adjacent sugarcane field and were placed in the dark for 24 h, then randomly collected active and healthy termite workers were exposed to formulated beads in Petri-plates. First Petri-plate had moistened Whatman No. 1 filter paper discs and the second Petri-plate had moistened sieved agricultural field soil with 10 beads placed in each treatment. After 6 h, almost 50% of the released termite individuals moved towards the formulated beads. Some termite individuals were attached on the beads and fed on it. In other treatment, some termites fed on the edges of the filter paper discs, while about 50% individuals were attracted towards the formulated maize cob based beads (Figure 3).

*Field evaluation of fipronil bait bead formulation against foraging subterranean termites*

Maximum termites' feeding and foraging activities were observed inside the control termite infested monitoring stations (Figure 5). Whole stations were filled up with the excavating soil when viewed from top or down side of the station. Cardboard sheets were completely infested by the termites throughout the height of station with a lot of foraging termite individuals found in the entire cardboard material. Moreover, when installed monitoring stations were removed out, we found some intact portions of termite fungal garden (termitarium) along with the eggs and immatures of the termites (Figure 5) ensuring that infesting termite species was *Odontotermes* spp.

On the other hand, when termite monitoring stations,


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**Figure 5:** *Observations made on non-fipronil bait treated (control) termite monitoring stations at the end of 4th week post-treatment. Formulation beads not found and station was filled with soil (station upper view A), foraging termite individuals and some part of their fungal garden along with colony immatures were observed (station basal view B-D), corrugated cardboards were filled with soil and termites were found feeding on cardboard sheets (E-G).*


**Figure 6:** *Observations made on fipronil bait treated termite monitoring stations at the end of 4th week post-treatment. Formulated bait beads were exploited by the termites (station upper view A), no foraging termites were found at base (B and C) and in corrugated cardboard sheets (D-F).*

treated with fipronil-containing formulation beads, were dug out, no activity or population of termites was observed inside or at bottom of the monitoring sheet as shown in Figure 6. Formulation beads seemed to be semi-exploited by the termite individuals. Cardboard sheets were thoroughly inspected but there were no live termite foragers inside the cardboard sheets.

Subterranean termites pose a serious threat to agricultural and urban sectors all over the world. In semi-arid subtropical regions such as Sargodha and other rain-fed areas of Pakistan, subterranean termites is emerging as a challenging pest attacking a number of agricultural crops including wheat, gram, maize, sorghum, sugarcane and sesame *etc*. (Manzo-or *et al*., 2011; Buczkowski and Bertelsmeier, 2017; Hussain *et al*., 2021). Currently available termiticides being used by the indigenous farmers are mostly in liquid form (Hu, 2011; Akbar *et al*., 2019) and exhibit unsatisfactory and short-term control of termite infestations most probably due to off-target movement (Wiltz, 2012; Rashid *et al*., 2018) and quick degradation of these applied insecticides (Huang *et al*., 2018). More target-oriented and persistent formulations such as controlled-release termiticide baits present a promising strategy to alleviate this problem. Many such bait formulations have been demonstrated effective against various insect pests including subterranean termites and other soil inhabiting pests such as cockroaches, ants, wasps, fleas and turf insects (Collins and Callcot, 1998; Shelton and Grace, 2003; Overmyer *et al*., 2005; Zhang *et al*., 2009; Durier and Rivault, 2000; Mao *et al*., 2011; Rashid *et al*., 2012; Hamm *et al*., 2013; Su *et al*., 2016; Davies *et al*., 2021).

In laboratory tests, it was recorded that formulated bead baits remained intact and did not disintegrate even after several wetting and drying episodes demonstrating the binding properties of polymers sodium silicate, sodium alginate and starch. All these polymers are used in most of slow- or controlled-release pesticide formulation matrices (Fernandez-Perez, 2007; Glenn *et al*., 2008; Singh *et al*., 2009; Osbrink *et al*., 2011; Chen *et al*., 2017; Ashitha and Mathew, 2020). Similarly, other additives such as urea are known to influence the release dynamics and physico-chemical properties of such pesticide formulations (Wang and

Wu, 2003; Cao *et al*., 2005).
It was observed that formulated bait beads attracted more than 50% termite workers in 6 h exposure. This finding corroborates the fact that maize cob constitute a cost-effective source of natural cellulose with dual advantage that it can keep the pesticidal compounds for longer time and can attract termites more efficiently than other natural cellulose sources (Lenz and Evans, 2002; Varvel and Wilhelm, 2008; Wang and Henderson, 2012).

Current investigations report significant suppression of termites' feeding and foraging activities inside the monitoring stations treated with fipronil baited beads. This might be possible due to the elimination of complete or partial disruption of foraging individuals approaching baited stations and nearby subterranean termite colonies. It was further verified by the presence of foraging ants observed in the termite stations treated with fipronil-loaded beads at 1st and 2nd week inspection as ants are efficient predators of subterranean termites (Tuma *et al*., 2020). Many previous studies have concluded that entire colony of subterranean termites can be eliminated by termite baits and controlled-release formulations (Grace *et al*., 1996; Tsunoda *et al*., 1998; Peters and Fitzgerald, 1999; Prabhakaran, 2001; Fernández-Pérez, 2007; Evans, 2010; Neoh *et al*., 2011; Eger *et al*., 2012; Webb, 2017).

Our results are also in line with those of Iqbal and Evans (2018) who showed under laboratory and field conditions that 5-7% fipronil treated toilet paper baits were more effective than imidacloprid as bait ingredient and eliminated all five colonies as compared to control and imidacloprid treatments. Moreover, our research findings are in line with Huang *et al*. (2006) revealing that fipronil bait was very efficient in eliminating *O. formosanus* colonies in the field. Similarly, findings of this study corroborate the results of Sarmad *et al*. (2020), where they demonstrated that macrocosm soils, treated with 5% technical grade fipronil formulated as maize cob based pellets, were toxic against subterranean termite *O. obesus* worker individuals till 15–21 days post-treatment and caused 70–85% termite mortality in 48 h bioassays.

## Conclusions and Recommendations

Results of these laboratory and field trials indicate that formulated fipronil beads remained intact and did not disintegrate by consecutive episodes of wetting and drying of five days and were able to attract more than 50% termite individuals exposed in 90 mm diameter Petri-plates. Moreover, these beads seem suppressing the foraging activities of subterranean termite workers in the field for few weeks post-treatment. These study results overall corroborate the effectiveness of fipronil and formulated beads to be further tested and potential incorporation in environment-friendly and target-specific management of subterranean termites in agricultural crops. However, further more detailed investigations are needed to get more empirical data regarding this formulation and to determine the insecticide release kinetics (Cao *et al*., 2005) and potential biological and physico-chemical and environmental factors influencing the efficiency (Glenn *et al*., 2008) of these formulated beads under laboratory and field conditions.

## Novelty Statement

This study describes an attempt to develop and evaluate a 5% technical grade fipronil against subterranean termites (*Odontotermes* spp.) under field conditions. Results demonstrated a considerable suppression of active termite colonies in the sugarcane field by the application of formulated beads of fipronil bait.

## Authors' Contribution

**Muhammad Zeeshan Majeed and Muhammad Asam Riaz:** Conceived the idea and designed the experimental protocols.
**Hassan Raza:** Conducted the experiments and recorded data.
**Muhammad Irfan Majeed:** Provided the technical support during research work.
**Hassan Raza and Muhammad Zeeshan Majeed:** Prepared the manuscript.
**Muhammad Zeeshan Majeed and Muhammad Asam Riaz:** Provided financial assistance and technically proofread the manuscript.

*Conflict of Interest*
The authors declare no conflict of interest regarding the publication of this research work.

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