Can Boric Acid-Based Bait Materials Be Used as Attractants for Red Ant, Dorylus orientalis Westwood?

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Article

Can Boric Acid-Based Bait Materials Be Used as Attractants for Red Ant, *Dorylus orientalis* Westwood?

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Simple summary: The use of attractants in insect traps can lead to significant improvements in trap catch. Infestations can be detected earlier, monitoring of population levels can be more accurate and alternative control measures can be developed. Such attractants would be of considerable use against infestations resulting from different species. Laboratory and field experiments carried out during the current study have proved that boric acid can be used as the active ingredient in baits as an alternative for different hazardous pesticides.

Abstract: Four groups (sugar, lipid, protein and waste based) of bait materials combined with boric acid were evaluated in laboratory and field conditions. When compared to all the sugar-based bait materials, a mixture of honey with boric acid registered to be the most effective in attracting the maximum number (83.00) of red ants followed by a mixture of molasses with boric acid (55.00). Likewise, among the oils tested, the highest number (47.33) of red ants registered was in the mixture of rice bran oil with boric acid followed by mustard oil with boric acid mixture (37.00). Similarly, from the protein-based bait materials, the maximum number of red ants (35.00) attraction was found in the mixture of gram flour with boric acid which was followed by dog biscuits with boric acid mixture (27.67). When all the waste-based bait materials were tested, sugarcane trash with boric acid mixture attracted most of the red ants (96.67) followed by banana peel with boric acid mixture (36.67). Based on the laboratory performance, the two best bait materials in each group were assessed against red ant, *Dorylus orientalis* in potato crops. Of the 8 bait materials tested, the use of rice bran oil with boric acid was found to be more attractive against red ants (1564.66, 1972.33 and 2636.33 during tuber formation, tuber maturity and harvesting stages, respectively) in potato crop when compared to other bait materials. Additionally, the attraction of *D. orientalis* was tested separately against rice bran oil, boric acid and rice bran oil in combination with boric acid using Y-tube olfactometer under laboratory conditions. Results indicated that rice bran oil in combination with boric acid had the highest attractiveness ratio of 86.66 per cent followed by boric acid and rice bran at 76.66 and 73.33 per cent, respectively. The outcome of the current investigation can be explored for the management of red ant menace in potato and other field crops, particularly grown under organic cultivation approach.

Keywords: red ant; *Dorylus orientalis*; boric acid; bait materials; attractant; potato; Y-tube olfactometer

1. Introduction

The red ant, *Dorylus orientalis* Westwood has been reported across India as a pest of numerous commercially significant crops. It has long been regarded as a significant pest of potato in both plains and hills [1]. Potatoes are an important source of income and a staple food in the Indian diet and are widely grown in North East India. However, potato production has declined sharply in recent years.

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There are several biotic and abiotic factors that cause yield loss in potatoes and amongst different biological constraints, pest problems are the main factor. As far as pests associated with potato crops are concerned, red ant causes considerable damage to underground potato tubers with numerous small holes (2-3 mm in diameter) appearing in the damaged tubers. The peak infestation occurs at harvest time, reducing tuber quality and market price, rendering them unfit for human consumption [2]. In severe cases, tuber infestation reaches 51.77-61.50 per cent [3], usually appears in December and is active until April. Studies on the biology and seasonal abundance of *D. orientalis* indicated that high temperatures followed by dry weather favor population buildup and damage severity of this obnoxious pest. Red ant populations have also been documented to be highest in non flooded potato growing areas as compared to the flood prone sites [4]. Apart from a few insecticidal trials in Sri Lanka [5] and Assam, India, practically very little work has been done so far to manage this significant soil dwelling pests [6–9]. The persistent use of common synthetic insecticides against *D. orientalis* on potatoes and other economically important crops initially managed the pest to an extent but posed long term threat to the ecosystem causing resurgence of other pest species like *Polyphagotarsoneus latus*, *Bemisia tabaci* and *Aphis gossypii* [10], in addition to harming the beneficial soil insect fauna. The long-term residual effects of synthetic insecticides are known to pose various hazards to human and animal health. The available literature on the bioecology and integrated management of *D. orientalis* is very limited and leaves room for further work. Moreover, the introduction of high yielding potato varieties without intensive monoculture and management strategies has contributed to the rapid colonization and spread of this pest. The dynamics of pest influxes and existing pest populations are also rapidly changing due to climate change.

To achieve these goals, baits are the most effective means of managing pestiferous ants in both urban and agricultural environments. Earlier workers have also attempted food preference tests for other ant species. The Argentine ant, *Linepithema humile* especially prefers a 25 per cent honey solution and a 25 per cent sucrose solution [11–14]; red imported fire ant, *Solenopsis invicta* preferred molasses [15]; tropical fire ant, *Paratrechina* spp. showed attraction to sugar based baits [16]. The red wood ants, *Formica rufa* preferred sucrose to monosaccharides and was most attracted to solutions containing an amino acid source [17]. However, *Solenopsis geminata* was attracted to protein or oil-based attractants [18]. With these facts in the backdrop, a holistic and effective approach of managing this serious soil dwelling insect pest especially in the commercial cultivation of potatoes and other crops needs to be embraced to boost up the productivity of the crops under various cultivation systems. The present study, evaluating several boric acid-based bait materials as red ant attractants was conducted to explore information for the creation of eco-friendly, cost effective and safe management strategies.

2. Materials and Methods

Laboratory and field experiments on evaluation of some bait materials (sugar, lipid, protein and waste based) as attractant for *D. orientalis* was carried out during 2015-2017 at the Soil Arthropod Pests Laboratory, Department of Entomology, Assam Agricultural University (AAU), Jorhat (26.7509°N, 94.2037°E) and in the farmer's field of Charaibahi village, Jorhat, Assam.

2.1. Preparation of Sugar-Based Bait Materials

Sugar, honey, molasses and sucrose solution were used to test the food preferences of *D. orientalis*. In a beaker, 25 parts of boric acid was added in 50 parts of distilled water with continuous stirring. Then, 25 parts of sugar-based materials was added slowly while stirring to make a 100 ml stock solution from which 5 ml of aliquot were used for laboratory evaluation.

2.2. Preparation of Lipid-Based Bait Materials

50 parts of lipid test samples (oils of rice bran, coconut, soybean and mustard) were taken and then 50 parts of boric acid was added, stirred to make a 100 ml stock solution and then 5 ml of aliquot were used for laboratory evaluation.

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2.3. Preparation of Protein-Based Bait Materials

Baits were prepared by mixing protein-based bait materials (soybean powder, groundnut powder, dog biscuits and gram flour) individually (25 parts), of which 25 parts boric acid was added to 50 ml of distilled water, stirred to make a 100 ml stock solution and then 5 ml of aliquot was used for laboratory evaluation.

2.4. Preparation of Waste-Based Bait Materials

Four groups of different waste-based bait materials *viz*., fish waste, chicken waste, banana peel and sugarcane trash were used for testing. The samples were prepared by using 25 parts of boric acid in 50 ml of distilled water, to which 25 parts of waste based bait materials was added, stirred to make a 100 ml stock solution and 5 ml of aliquot was used for laboratory evaluation.

2.5. Laboratory Evaluation of Bait Materials

Red ants were dug up from a heavily infested potato field and carried to the laboratory for rearing. Large colonies were maintained in plastic containers (70 cm diameter) and 300 fully matured red ants of almost uniform sizes (6-7 mm) were released into four plastic containers (group wise based bait materials). The bait materials were prepared and loaded in urine pots (5 cm × 4 cm) as bait stations and kept randomly in the arenas and observation on red ants numbers attracted to the bait stations was recorded at 1, 3 and 5 days after treatment (DAT). This experimental set up was replicated three times. Based on attractiveness, two superior bait materials were selected from each group for further field evaluation.

2.6. Field Evaluation of Bait Materials

A field trial was conducted to determine the effectiveness of promising baits (2 superior baits from each group) in a highly endemic red ant field of potato crops. Nine different treatments including an untreated control were evaluated for their attractiveness to *D. orientalis* during 2016-17 (Table 5). The experiment was conducted in a randomized block design with three replicates. The bait material @ 25 ml/bait or 25 g/bait was loaded in a plastic container (8 cm × 7 cm). The bait stations were randomly placed at a depth of 15 cm and the red ant population was statistically analyzed by counting the number of red ants attracted to each bait station.

2.7. Y Tube-Olfactometer Bioassay

A series of single choice assays were carried out in a two arm Y-olfactometer to evaluate the attraction response of *D. orientalis* to different treatments. Adult ants, freshly collected from potato fields, were kept under laboratory conditions at ambient room temperature for two hours, following which, the active ants were selected for the olfactory experiments. Thirty adult ants were tested against three treatments, *viz*., rice bran oil, boric acid and rice bran oil in combination with boric acid with distilled water as control. The treatments were placed in the designated olfactometer arms @ 2 µl each on a filter paper. The adults were placed in the release chamber and were allowed 5 minutes to determine their choice. The olfactometer was cleaned using acetone after each treatment and the treatments were relocated after every five replicate to eliminate any source position effect. The data were analyzed using chi-square (χ^2) tests to determine significant differences of adult ants towards different treatments.

3. Results

3.1. Laboratory Performance of Different Bait Materials as Attractant for D. orientalis

Experimental results obtained from the laboratory evaluation of four sugar-based bait materials for different exposure periods against *D. orientalis* are presented in Table 1. Among the four sugar-based bait materials, the highest (25.00) mean number of red ant attraction was recorded in the

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mixture of boric acid with honey followed by molasses (18.33), sugar (7.33) and sucrose solution (5.00) as compared to untreated control at 1 DAT. At 3 DAT, the highest mean number of attraction (80.67) was recorded from the mixture of boric acid with honey followed by molasses (52.67) and sugar (13.33), respectively. The same trend of attraction of red ants was also seen at 5 DAT. However, sucrose solution and boric acid showed very low levels of attraction to red ants at various exposure days.

Analysis of the data shows that the attraction of *D. orientalis* on lipid-based bait materials at different exposure periods (Table 2). The highest (23.33 numbers) attraction was found in the mixture of boric acid with rice bran oil, mustard oil (19.00), soybean oil (5.00) and coconut oil (4.00) at 1 DAT. The mixture of rice bran oil with boric acid was found to be statistically superior to rest of the mixtures, showing the greatest attraction (42.67 and 47.33 numbers) of red ants at 3 and 5 DAT, respectively. For protein-based baits, gram flour with boric acid mixture was significantly superior to other treatments with mean number of red ants (20.67, 30.00 and 35.00) at 1, 3 and 5 DAT which was followed by mixture of dog biscuits with boric acid (13.00, 21.67 and 27.67) and groundnut powder with boric acid mixture (9.33, 14.00 and 18.67), respectively (Table 3). Data on evaluation of four waste-based bait materials reveals that the highest (22.67, 89.67 and 96.67) number of attraction was obtained from sugarcane trash with boric acid mixture followed by banana peel with boric acid mixture (17.00, 32.33 and 36.67) and fish waste with boric acid mixture (9.33, 12.33 and 15.00) at 1, 3 and 5 DAT, respectively (Table 4). The lowest numbers of red ants were recorded in chicken waste with boric acid mixture.

Table 1. Evaluation of sugar based bait materials against red ant, *D. orientalis* in laboratory conditions during 2015.

Treatments	Dosages	Exposure (in days)		
		1 DAT	3 DAT	5 DAT
T_1: Sugar + Boric Acid	5 ml	7.33	13.33	15.67
T_2: Honey + Boric Acid	5 ml	25.00	80.67	83.00
T_3: Molasses + Boric Acid	5 ml	18.33	52.67	55.00
T_4: Sucrose solution + Boric Acid	5 ml	5.00	9.00	11.67
T_5: Untreated control (water)	5 ml	0.00	0.00	0.00
S.Ed±	-	1.25	1.37	1.25
CD (5%)	-	2.67	2.92	2.67

*Data are mean of three replicates, DAT- Days After Treatment.

Table 2. Evaluation of lipid based bait materials against red ant, *D. orientalis* in laboratory conditions during 2015.

Treatments	Dosages	Exposure (in days)		
		1 DAT	3 DAT	5 DAT
T_1: Rice bran oil + Boric Acid	5 ml	23.33	42.67	47.33
T_2: Coconut oil + Boric Acid	5 ml	4.00	6.67	10.33
T_3: Soybean oil + Boric Acid	5 ml	5.00	10.00	17.00
T_4: Mustard oil + Boric Acid	5 ml	19.00	33.33	37.00
T_5: Untreated control (water)	5 ml	0.00	0.00	0.00
S.Ed±	-	0.84	1.74	1.49
CD (5%)	-	1.80	3.72	3.19

*Data are mean of three replicates, DAT- Days After Treatment.

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Table 3. Evaluation of protein based bait materials against Red ant, *D. orientalis* in laboratory conditions during 2015.

Treatments	Dosages	Exposure (in days)		
		1 DAT	3 DAT	5 DAT
T1: Soybean powder + Boric Acid	5 g	6.67	11.00	15.33
T2: Ground nut powder + Boric Acid	5 g	9.33	14.00	18.67
T3: Dog biscuits + Boric Acid	5 g	13.00	21.67	27.67
T4: Gram flour + Boric Acid	5 g	20.67	30.00	35.00
T5: Untreated control (water)	5 ml	0.00	0.00	0.00
S.Ed±	-	1.10	1.23	1.21
CD (5%)	-	2.34	2.63	2.59

*Data are mean of three replicates, DAT- Days After Treatment.

Table 4. Evaluation of waste based bait materials against Red ant, *D. orientalis* in laboratory conditions during 2015.

Treatments	Dosages	Exposure (in days)		
		1 DAT	3 DAT	5 DAT
T1: Fish waste + Boric Acid	5 g	9.33	12.33	15.00
T2: Chicken waste + Boric Acid	5 g	5.00	9.00	12.33
T3: Banana peel + Boric Acid	5 g	17.00	32.33	36.67
T4: Sugarcane trash + Boric Acid	5 g	22.67	89.67	96.67
T5: Untreated control (water)	5 ml	0.00	0.00	0.00
S.Ed±	-	1.35	1.46	0.97
CD (5%)	-	2.89	3.13	2.07

*Data are mean of three replicates, DAT- Days After Treatment.

3.2. Field Evaluation of Selected Bait Materials as Attractant for D. orientalis

To determine the efficacy of superior bait materials (2 from each group), a field trial was conducted in chronically affected red ant endemic potato fields during 2016-17. Experimental results indicated that all treatments were significantly superior as compared to untreated control (Table 5). Among the 8 treatments, use of boric acid with rice bran oil attracts maximum numbers of *D. orientalis* (1564.66, 1972.33 and 2636.33) on potato crop as compared to other bait materials which was followed by mixture of boric acid with honey (347.66, 488.66 and 675.66 numbers) at tuber formation, tuber maturity and harvesting stages, respectively. However, boric acid treated with molasses attracted 326.33, 501.33 and 575.66 numbers of *D. orientalis* at the aforementioned stages, respectively.

Table 5. Evaluation of bait materials against Red ant, *D. orientalis* under field conditions in potato crop during 2016-17.

Treatments	Dosages	Mean number of attraction of red ant, *D. orientalis*		
		Tuber Formation Stage	Tuber Maturity Stage	Harvesting Stage
T1: Honey + Boric Acid	25 ml	347.66	488.66	675.66
T2: Molasses + Boric Acid	25 ml	326.33	501.33	575.66
T3: Rice bran oil + Boric Acid	25 ml	1564.66	1972.33	2636.33
T4: Mustard oil + Boric Acid	25 ml	14.00	39.00	50.66
T5: Dog biscuits + Boric Acid	25 ml	86.33	101.33	134.33
T6: Gram flour + Boric Acid	25 ml	55.66	73.66	126.33
T7: Banana peel + Boric Acid	25 g	134.33	196.66	272.00
T8: Sugarcane trash + Boric Acid	25 g	234.00	283.66	379.00
T9: Untreated control (water)	25 ml	2.00	1.66	2.33

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S.Ed±	-	3.07	4.19	3.61
CD (5%)	-	6.58	9.00	7.75

*Data are mean of three replicates.

3.3. Response of D. orientalis to Different Treatments

Overall, when tested against a control treatment of distilled water, *D. orientalis* had more than 70 per cent attractiveness towards all the three treatments. *D. orientalis* showed highest level of attraction towards the combination treatment of boric acid and rice bran at 86.66 per cent, this was followed by boric acid at 76.66 per cent. The lowest attraction was recorded towards rice bran oil at 73.33 per cent (Figure 1).

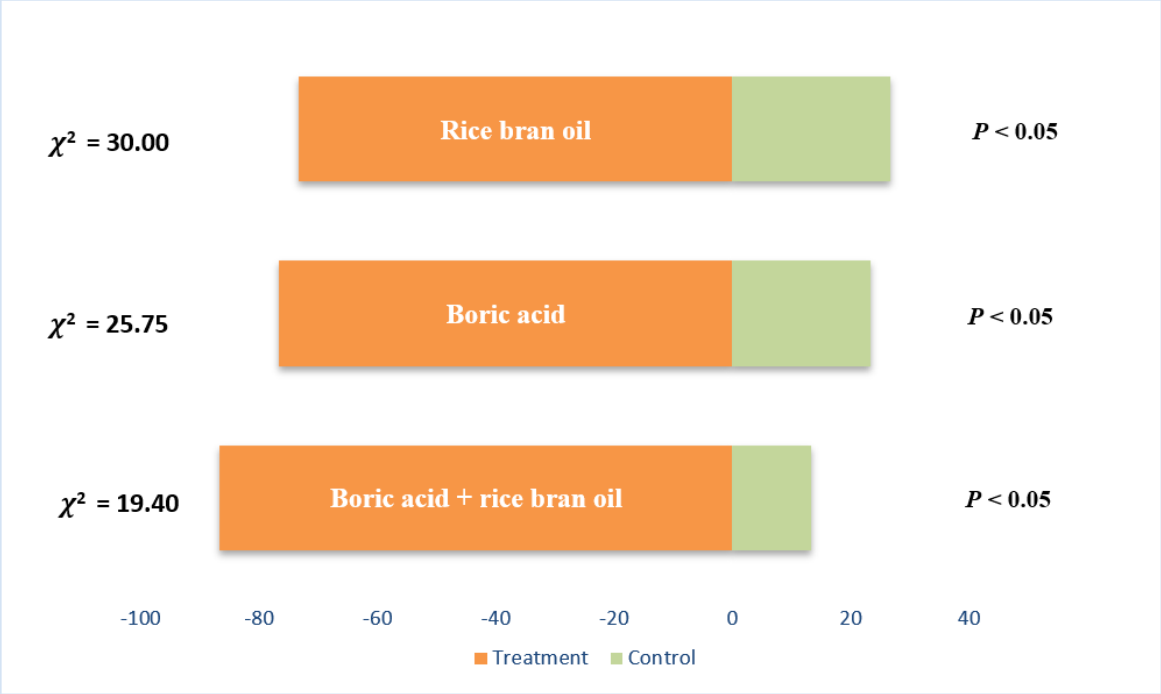

Figure 1. Per cent response by *D. orientalis* adults.

4. Discussion

The present study indicates that in case of laboratory evaluation of bait materials, sugarcane trash and boric acid mixture attracted more red ants than other bait materials. However, in field evaluation, it was found that the use of boric acid with rice bran oil was far superior to other bait materials in attracting the maximum numbers of *D. orientalis* in potato crops. This may be because rice bran oil has a high smoke point (232°C) and is less likely to break down in sunlight. As a carrier material, boric acid destroys the ant's stomach, inhibits its nervous system and also damages its exoskeleton. In field, foraging worker ants are attracted to bait containing boric acid and carry pieces of the bait back to the nest. Later, the boric acid bait is transferred between the mouth of the insects in the nest, effectively killing other ants and even the queen ant. Laboratory evaluations have shown that sugarcane trash and boric acid mixture attracts the highest number of red ants. The cause may be due to improper mixing of both sugarcane trash and boric acid.

Boric acid has been used to control ants and cockroaches since late 1800's [19–21]. It is slow acting and non-repellent, so long term use is recommended [22,23]. Boric acid baits have been shown to be effective against Pharaoh ants [24], Argentine ants [25] and Florida carpenter ants [26] both in the laboratory as well as field conditions. Similar results were obtained using liquid boric acid baits to control pharaoh ants [23]. The boric acid in sucrose solutions also relaxes the water regulatory mechanism, causing the ants to consume more bait to compensate for water loss [27]. Although little

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is known about the mode of physiological action of boric acid in insects, borate ions have shown to form strong complexes with sugar alcohols and other organic functional groups [28]. Boron may be involved in the disruption of intercellular adhesion as saturated solutions of boric acid can be used to dissociate cells [29]. Boric acid acts as a stomach poison and may be toxic to the insect's nervous system. In addition to being toxic to the stomach, most borate salts are also abrasive to the exoskeleton of insects. *Monomorium chinensis* and *Anoplolepis gracilipes* are regular feeders seeking carbohydrates, lipids and proteins as food sources [30]. Ants are ideal pests for bait control because of their highly social habits, such as chemically recruiting food sources using odour trails and sharing food among nest mates (trophallaxis). If the bait is attractive to foraging ants, it is quickly collected, carried back to the nest to be distributed throughout the entire colony. A novel boric acid gel baits was developed for household ant species *viz*., the Asian needle ant, *M. chinensis* and the Yellow crazy ant, *A. gracilipes* and found to be a potential alternative control strategy for household ant species [31]. The results obtained from the Y-tube olfactometer bioassays show that the three treatments had a positive influence on the olfactory behavior of *D. orientalis.* This further establishes the superiority of rice bran oil and boric acid as potential baits. In case of chemical cues, variations in behavioral response of insects have been associated with the composition and concentration of chemical compounds [32]. The percentage response of *D. orientalis* to the treatments in the current study indicate a similar pattern. The combination treatment of boric acid and rice bran oil had higher attraction than the individual treatments. More often than not, synergistic interactions between the different components of an odor mixture enhance the attraction of insects to their hosts [33].

5. Conclusion

The use of insecticides is an integral part of pest control. Impacts on non-target organisms and public opposition to the widespread use of insecticides are the main concerns of these programmes. Targeted investigation of bait stations has therefore been proposed as an alternative treatment in areas where broadcast insecticides are unacceptable. A potential search for bait development should begin with laboratory preference testing. Extensive field testing must then be performed due to the large variability in food preferences and gathering between laboratory and field populations of the same ant species. Baits have become one of the most popular agents used by pest management experts to control household pests and termites. One advantage of using bait formulations is that they are used in low concentrations and can be placed only where and when they are needed. They are easy to use and require only a small amount of toxicant. Ant baits are designed for specific purposes using traditional control methods and have been found to be cost effective. Baits are very effective tools for eradication of various ant species. Effective management and eradication of these species of ant requires not only taking the right appropriate tools available but also using them efficiently *i.e*, using the right type of bait at the suitable time of the day and season. Control and eradication are important factors in evaluating the baits effectiveness for controlling different species of ants. But in many control programmes, it is very difficult to separate the efficacy of the bait from the success of the control programme itself. The bait must be effective against a particular ant species that may not be controlled or eradicated due to unsuccessful control programmes themselves such as lack of monitoring or tracking. Considering the effectives and immense potential of baits, the pesticide industry has expanded testing and screening programmes for extended labeling of bait formulations against diverse groups of insect pests.

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