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Research Article

The effects of bait shape and composition on acceptance and consumption of bait by *Microcerotermes diversus* (Isoptera: Termitidae) under laboratory and field conditions

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Abstract: *Microcerotermes diversus* Silvestri (Isoptera: Termitidae) is an extremely destructive structural wood pest in Khuzestan province, Iran. One of the effective methods for subterranean termites control is the use of baiting systems that have recently evolved as an environmentally safe tactic to protect structures. In this research, several factors for the preparation of bait were evaluated involving the bait composition. In the no-choice test of bait composition, termites had the highest levels of feeding on bagasse + molasses + yeast, bagasse + molasses, bagasse + yeast, and bagasse, respectively. In the further experiment, termites had the highest levels of feeding on bait containing bagasse + molasses + yeast compared to control. Termites showed the highest levels of feeding on the bait in the stems of the cane, raw bait, cooked bait and *pill-shaped bait*, respectively. In the field phase, significant differences were not observed among the treatments but termites had the highest levels of feeding on the bait in the stems of the cane, *pill-shaped bait*, cooked bait and raw bait, respectively. Both field and laboratory conditions, indicate that the target termite has shown a greater tendency to baits in the cane. Findings of this study suggests that the design of the bait and its shape can also affect the bait's attraction and its acceptance by the termite. Therefore, in addition to the ingredients of the bait, its shape, size, softness and texture quality also affect the amount of bait consumption.

Keywords: bagasse, cooked bait, molasses, pill-shaped bait, raw bait, yeast

Introduction

Microcerotermes diversus is an extremely destructive structural wood pest in Khuzestan province, Southwest Iran (Habibpour *et al.*, 2007; Habibpour *et al.*, 2010). This species was identified as the major pest of date palms *Phoenix dactylifera* L. in Iran, Iraq and Saudi Arabia (Habibpour, 2010; Cheraghi *et al.*, 2012). It has a broad scope of food foraging and also has the ability to create secondary nests in building walls and ceilings, as well as on trees (Habibpour, 2010; Cheraghi *et al.*, 2013). Eradication and control of this pest by conventional methods such as drilling and injection of insecticides into the ground is faced with problems and in some cases is not effective (Habibpour *et al.*, 2008). Current management of subterranean termites in Iran involves the application of soil insecticides. However, continuous use of chemical pesticides

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in the environment is a concern, especially in areas with a high ground-water table, as in the city of Ahvaz (Cheraghi *et al.*, 2013). Owing to these problems, one of the effective methods of control is the use of baiting systems that have recently evolved as a major technique (tactic) to protect structures and timber against termites (Dhang, 2011; Grace and Su 2001; Habibpour, 2006), and use of poisoned bait as an alternative control method is acceptable for the environment and effective for subterranean termites' control. In fact, baiting systems cause the elimination of colonies and reduce the population of subterranean termites (Habibpour *et al.*, 2011). A successful baiting system depends on understanding the foraging behavior of subterranean termites (Henderson and Wang, 2012). Little is known about how termites find food in nature, but many factors, such as the prevalence and attractiveness of the bait, can induce an effect on the foraging behavior to discover bait (Henderson *et al.*, 1998). In fact, a suitable bait will increase the attractiveness and reduce the the foraging time (Waller *et al.*, 1999; Paysen *et al.*, 2004; Swoboda, 2004; Sattar *et al.*, 2009; Jordan *et al.*, 2013). Termites use the odors and chemical signals in the bait to determine the position of food in the environment. One way to make the bait (cellulose and water as primary components in the termite bait) attractive and acceptable for the termites is the use of food stimulants (phagostimulants) such as nitrogenous compounds, carbohydrates, vegetable oils and pheromones (Cornelius, 2003; Rojas *et al.*, 2003; Castillo *et al.*, 2013). In addition to feeding stimulants that make the bait attractive for termite control, bait stations should be designed to encourage termites to maximize their consumption on bait matrix in order to expedite control in minimal time. Therefore, in addition to bait composition, its shape, size, softness and compactness also affect the amount of bait consumption (Evans and Gleeson, 2006). In this research, the effect of bait design in terms of shape and composition on bait consumption by the target termite was studied.

Materials and Methods

We previously determined the best cellulose-based bait matrices and appropriate concentrations of additives and stimulants for attracting termites in previous experiments (Ekhtelat *et al.*, 2016 a). In the present study, several factors for the preparation of bait were evaluated: the composition of the bait (cellulose alone or with different ingredients and additives), and the shape and softness of the bait, which is in the form of a disk or block.

1- composition of bait (only cellulose or cellulose with different ingredients and additives).

2- Shape or amount of compression or softness of the bait in the form of disk-shaped or block shaped.

Composition of bait

A: Non-choice test

Based on the results of the previous experiments, we determined cane bagasse (Produced in the sugar cane development and Khuzestan subsidiary industries) to be the best cellulose-based bait matrix (Habibpour, 2006) with 4% molasses (Produced in the sugar cane development and Khuzestan subsidiary industries) and 1% yeast (Razavi instant yeast, *Saccharomyces cerevisiae*) as suitable concentrations of additives and stimulants for termites (Ekhtelat *et al*, 2016 a). Prior to testing, the sieved (with mesh No. 5) bagasse was placed in an oven at 100 °C for 24 hours. The experiment was then performed with 4 treatments: bagasse cellulose only (15g bagasse + 100ml distilled water); bagasse in combination with molasses (15g bagasse + 100 ml distilled water + 4g molasses); bagasse in combination with yeast (15g of bagasse + 100ml of distilled water + 1g of yeast); and bagasse in combination with molasses and yeast (15g of bagasse + 100ml distilled water + 4g molasses + 1g of yeast), with 5 replicates per treatment. With each composition, 30 grams were packed in 9-cm diameter plastic containers. Then, 200 workers and 1 soldier cast were added to the dishes and incubated at

28 ± 2 °C and a relative humidity of 90% (± 5%) and in the dark for 15 days. An additional 30 grams from each composition were heated to 100 °C for 24h to obtain the initial dry weight for each compound. After 15 days, the termites were separated from the cellulose substrates. The substrates were heated at 100 °C for 24h then weighed to obtain a secondary dry weight. The feeding rate was calculated from the weight loss associated with each cellulose substrate.

Data analysis

Normality test was performed using non-parametric Shapiro-Wilk tests at $P \leq 0.05$. Data were analyzed using one-way analysis of variance (One-way ANOVA). Means were separated by Tukey-Kramer honestly significant difference (HSD) test at $P \leq 0.05$ using SPSS software version 16.0 (SPSS, 2007).

B: Choice test

Based on the results of the non-choice test, the best compound in terms of the highest amount of feeding by termites was found to be the combination of bagasse, molasses, and yeast (BMY). Triple T-shaped containers were used in this test. These containers consisted of a central dish containing farm soil and wet vermiculite soil in the ratio of 2 to 1 and two dishes, one containing bagasse (control) and the other containing combination of bagasse, molasses, and yeast (BMY) (Fig. 1). Thirty g from each composition were poured into 9-cm Petri dishes and compressed. Then, 300 workers and 3 soldiers were added to the central dish and kept in the incubator at 28 ± 2 °C and 90 ± 5% relative humidity and dark conditions for 15 days. All experiments were conducted with four replicates. Determination of initial and secondary dry matter was performed according to the test of the previous section.

Data analysis

Normality test was performed using non-parametric Shapiro-Wilk tests at $P = 0.05$.
Data were analyzed using paired t-test (choice tests) at $P = 0.05$ using SPSS software version 16.0 (SPSS, 2007).

Effect of shape, amount of compression, and softness of the bait

The experiments were conducted in two phases of laboratory and field experiments: In the laboratory, the combination of bagasse, molasses and, yeast (BMY) was tested in four forms (four treatments): (A) Putting the bait in ordinary straw stalks (Fig. 2A); (B) putting raw bait into perforated 50 ml Falcon tubes (Fig. 2A); (C) putting baked bait into perforated Falcon tubes (Fig. 2A); and (D) putting the bait in the form of pills in a perforated aluminum foil (Fig. 2B).

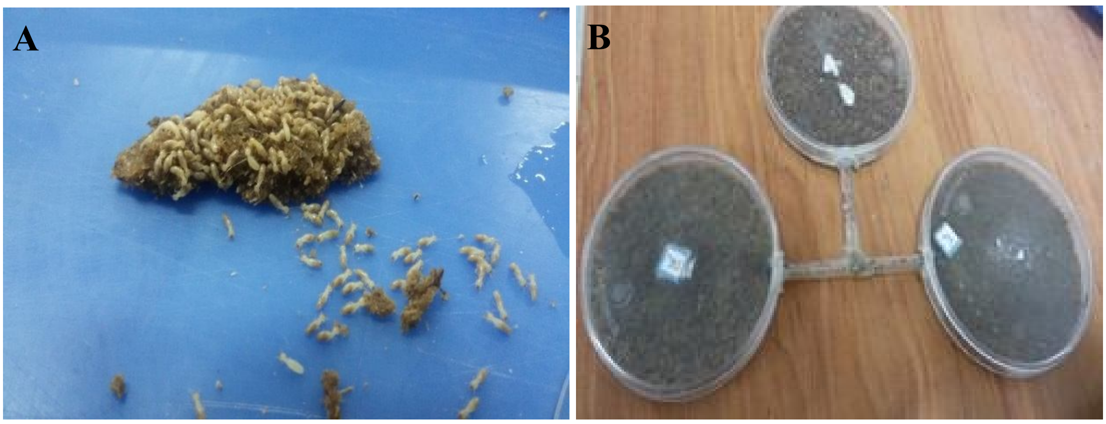

Figure 1 (A) Triple T-shaped containers for determination of bait composition attractiveness in choice laboratory tests, (B) attracted termites *Microcerotermes diversus* to the bait.

Figure 2 (A-C) Bait shapes in laboratory tests for *Microcerotermes diversus*.

For putting the bait in ordinary straw stalks, the bait was placed at a certain level in 12cm straw stalks and heated at 100 °C for 24 hours to allow the bait to dry completely. Then the straw containing the bait was weighed to obtain the initial dry weight. After determining the initial weight, the straws were packed in 3 bundles and about 5cm of the bundles of straws was covered with aluminum foil. For putting the raw bait into the perforated Falcon tubes 20g of bait was placed in perforated 50ml Falcon tubes (four columns with five holes) and compressed. For putting the baked bait into the perforated 50ml Falcon tubes a quantity of bait was heated at 100 °C for one hour after which it was cooled, Then 20g of the dried bait was compressed into the tubes.

For determination of the initial dry weight of the bait in the Falcon tube, 20g from each composition was placed in pre-weighed aluminum foil and placed in an oven at 100 °C for 24 hours to obtain initial dry weight of each composition.

To create the bait in the form of pills in a perforated aluminum foil, 20g of bait was shaped into the form of a pill (1 cm thick and 7 cm in) which was then heated in an oven for 24 hours at 100 °C until completely dried. The pills were weighed to obtain their initial dry weight. The pills were then placed in perforated aluminum foil to be located in the laboratory phase and in the field.

In the laboratory, plastic containers with a height of 14 cm and a diameter of 20 cm were used for testing. PVC pipe cut in the lower part in the shape of sinuate, was placed in the center of each container. Then, 1.5Kg of farm soil and wet vermiculite mixture were placed in the bins, around the PVC pipe. All four treatments were placed in soil. A total of 1000 worker and 7 soldier termites were released into the PVC pipe (Fig. 2C). The experiment was repeated in its entirety four times.

For the field experimentation, land infested with colonies of the target termite was selected based on surveys. Before applying the treatments, traps of beech blocks (dimensions 20 × 4 × 2.5cm) were placed in the soil in order to determine the activity of the termites. When the wooden blocks were infested by the target termites, they were slowly removed from the soil and replaced with each of four treatments (all four treatments were simultaneously in place) (Fig. 3). The experiment was performed in four replicates for each treatment over the course of a month. After a month, the baits were transferred to the lab and the number of termites attracted to the baits determined (Fig. 4). The amount of bait consumption was determined by oven drying the baits as described earlier.

Data analysis

Normality test was performed using non-parametric Shapiro-Wilk tests at $P \leq 0.05$. Data were analyzed using one-way analysis of variance (One-way ANOVA). Means were separated by Tukey-Kramer Honestly Significant Difference (HSD) test at $P \leq 0.05$ using SPSS software version 16.0 (SPSS, 2007).

Results

The effects of bait composition on bait acceptance and consumption

A: laboratory no-choice trial of bait composition

Based on the results obtained in the no-choice test, significant differences were observed among the treatments ($F = 73.238$, $df = 3$, $P = 0.001$). Termites had the highest levels of feeding on bagasse plus molasses and yeast (BMY), bagasse plus molasses (BM), bagasse plus yeast (BY), and bagasse (B), respectively. The mean amount of feeding on the bait containing bagasse, molasses and yeast, expressed as weight loss of the baits (1090 ± 16.43mg) was significantly higher than other baits (Fig. 5).

B: Laboratory choice trial of bait composition

Based on the results obtained in the choice test, significant differences were observed among the two treatments ($t = 49.842$, $P < 0.0001$, $df = 3$). The mean comparison of bait containing bagasse + molasses + yeast (BMY) with control (only bagasse) through feeding in choice test showed that termites have the highest levels of feeding on bait containing bagasse + molasses + yeast (BMY) (Fig. 6).

The effects of bait shape on bait acceptance and consumption

Based on the results, under laboratory conditions, significant differences were observed among the treatments at $P = 0.05$. Termites had the highest levels of feeding on the bait in the stems of the cane, raw bait, cooked bait and *pill-shaped bait* respectively ($F = 191.984$, $P < 0.0001$, $df = 3$) (Table 1) (Fig. 3A-B). In the field phase, significant differences were not observed among the treatments but termites had the highest levels of nutrition on the bait in the stems of the cane, pill-shaped bait, cooked bait and raw bait, respectively ($F = 1.163$, $df = 3$, $P = 0.364$) (Table 2) (Fig. 4).

Figure 3 Bait shapes in the field phase of laboratory tests *Microcerotermes diversus*.

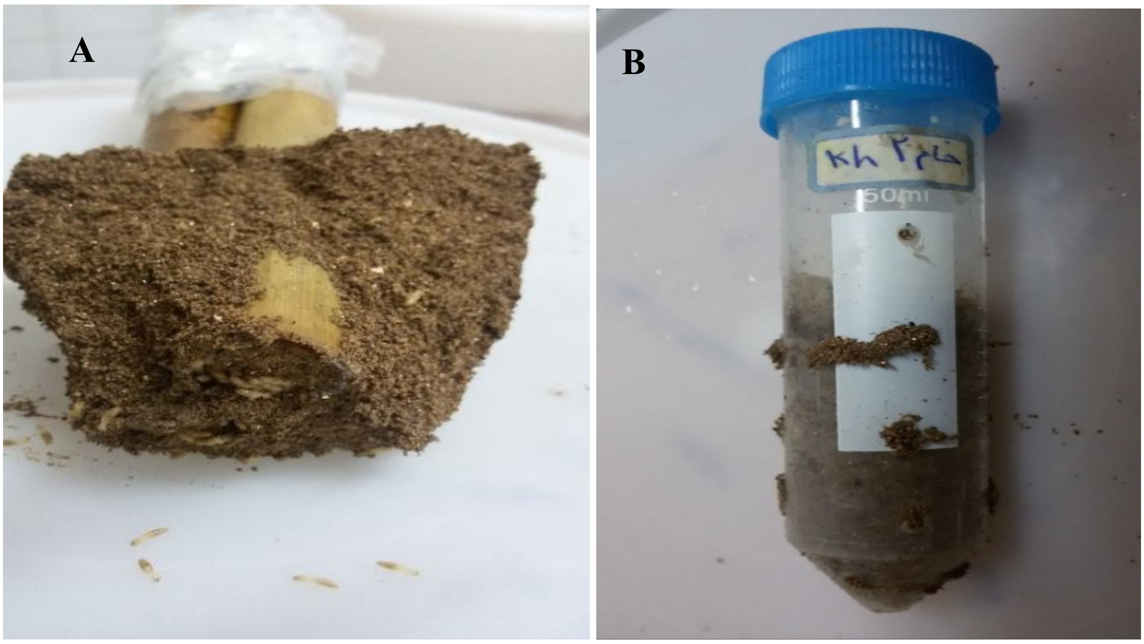

Figure 4 (A-B) Attracted termites *Microcerotermes diversus* to various forms of bait under laboratory conditions.

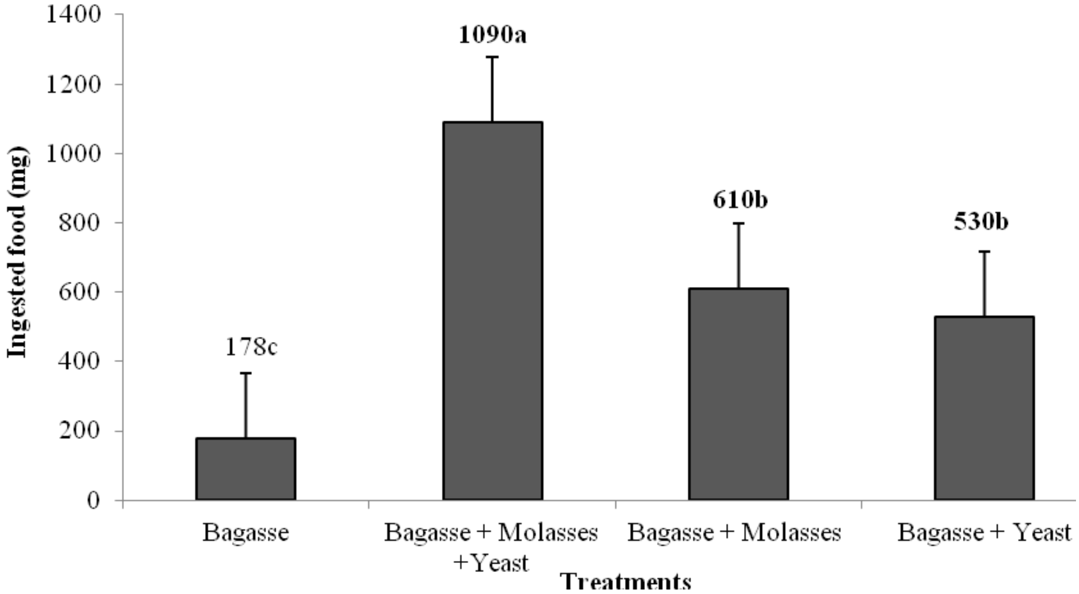

Figure 5 Feeding of *Microcerotermes diversus* on different combinations of bait composition in no-choice trial in laboratory.

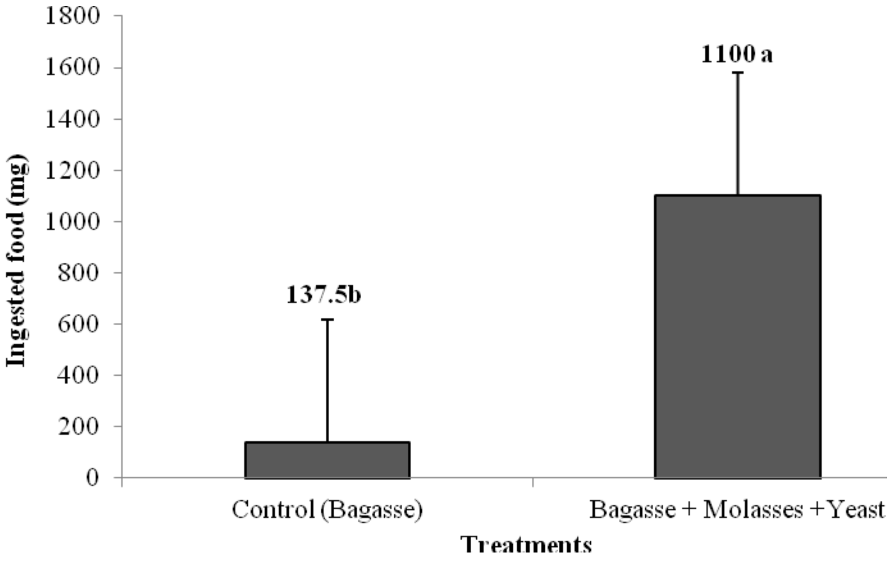

Figure 6 Comparison of bait containing bagasse, molasses and yeast with control through feeding (mg) of *Microcerotermes diversus* in choice trial in laboratory.

Table 1 Weight loss of the baits and the number of attracted termites *Microcerotermes diversus* to various forms of bait under laboratory conditions 20 days after exposure.

Treatments	Ingested food (g)	The number of attracted termites
The bait in the stems of the cane	2.250 ± 0.070 a	725W
Raw bait	1.162 ± 0.065 b	443W + 2S
Cooked bait	1.072 ± 0.033 b	100W + 2S
Pill-shaped bait	0.427 ± 0.039 c	237W + 1S

The means followed by the same letters are not significantly different (HSD test, $P \leq 0.05$).
W: Worker, S: Soldiers.

Table2 Amount of feeding and the number of attracted termites *Microcerotermes diversus* to various forms of bait under field conditions.

Treatments	Ingested food (g)	The number of attracted termites
The bait in the stems of the cane	5.26 ± 2.38 a	2631W + 7S
Raw bait	1.30 ± 0.60 a	12W
Cooked bait	1.74 ± 0.46 a	34W
Pill-shaped bait	2.94 ± 2.15 a	1297W + 13S

The means followed by the same letters are not significantly different (HSD test, $P \leq 0.05$).
W: Worker, S: Soldiers.

Discussion

Based on the results obtained in the non-choice test of the bait composition, the termites had the highest amount of bait consumption on bagasse plus molasses and yeast, followed by bagasse plus molasses, bagasse plus yeast, and bagasse (only), respectively. The mean consumption of the bait containing bagasse, molasses and yeast (1090.43mg) was significantly higher than on other baits.

Termites also prefer foods that decompose by fermentation and have a pH of less than 5 and preferably less than 4.5, Because these conditions are favorable for intestinal microorganisms of termites, including protozoa and bacteria that decompose cellulose, (Rojas *et al.,* 2003), yeast was used in this study to induce fermentation of the molasses and bagasse.

The results of our study are consistent with the results obtained by other researchers, including those of Waller *et al.* (1999), who showed in field experiments that *Reticulitermes flavipes* Kollar and *Reticulitermes virginicus* Banks select and occupy baits containing sucrose and yeast more than baits without these materials. Subada (2004) also showed that *Reticulitermes* spp. preferentially consume paper baits containing fructose, galactose, glucose, or sucrose.

Haifig *et al*. (2008) examined the food preferences of *Coptotermes gestroi* Wasmann and *Heterotermes tenuis* Hagen for papers treated with sucrose, yeast, and urea, and observed that the *C. gestroi* preferred papers treated with sucrose and yeast, while *H. tenuis* chose the paper treated with 0.015 urea, thus they concluded that the food preferences of the two termites were different for some of the same foods and suggested using sugar and yeast in the bait to control *C. gestroi* and urea to control *H. tenuis*.

Henderson and Wang (2012) studied the consumption efficiency of two commercially used termite bait materials: southern yellow pine wood and cardboard, and one potential bait material, maize *Zea mays* cob, against *Coptotermes formosanus* Shiraki under laboratory conditions. They observed that in no-choice tests, the consumption of wood and cob was similar and also significantly more than cardboard, while in the two-choice test, the consumption was cob > wood, wood > cardboard, cob = cardboard. In a three-choice test, no significant difference was detected in consumption.

Studies by Castillo *et al.* (2013) showed that termites fed baits containing supplementary materials more than baits without these materials. The results obtained from studies of Rasib and Ashraf (2016) also showed that bait systems can be used effectively to reduce subterranean termite population's area wide and changes in the presentation of materials in the bait station can efficiently affect bait matrix consumption. Evidently, much more work on food and foraging preferences is required to design more efficacious baiting protocols.

The results of the choice test confirmed this subject, so that the termites and the amount of consumption of the bait consisting of bagasse, molasses and yeast was significantly higher than the control bait (bagasse only).

The results obtained from the effect of designing the bait's shape in the laboratory phase demonstrated a significant difference between treatments. Termites had the highest amount of bait consumption in the stems of the cane, raw bait, cooked bait and pill-shaped bait. There was no significant difference between treatments in the field tests. However, the termites had the highest amount of consumption of the bait in the cane stalks, followed by pill-shaped, cooked bait, and raw bait respectively. These results, under both field and laboratory conditions indicated that the target termites showed a greater tendency to baits in the cane. The reason for this choice can be that, according to previous studies, canes themselves have an attraction for termites (Ekhtelat *et al*., 2016 b). On the other hand, the tube-like shape of the cane can play the role of a food searching tunnel for the termite, and in fact, the termite will spend less energy on soil transfer and tunneling and concealing itself. Also an increase in compression of the bait leads to more feeding of termites.

In our study, the composition of all the baits was the same, but the shape and design of the baits were different, which suggests that the design of the bait and its shape can also affect attraction to bait and its acceptance by the termite. Therefore, in addition to the ingredients of the bait, its shape, size, softness, and compression (texture) also affect the amount of bait consumption. Evans and Gleeson (2006) examined the effect of bait design on its consumption by termites of family Rhinotermitidae.

The results showed that the most effective baits were large, soft, and paper plus wood baits. Another advantage of using bait containing straw is that, because of termites hiding in the straw, they do not transfer too much soil and block only the entrance of the cane with soil. But in the other three forms, the termites transfer the soil into the bait, which causes an error in the amount of bait secondary weight, especially in the raw and cooked baits that are placed in the falcon tubes and the soil is mixed with bait.

Acda (2004) used bamboo stalk to collect termites of *Coptotermes* spp. because of the darkness and humidity of these hollow stems. By placing these canes in places that were infested with the termite, the termites quickly migrated to these stems (one side of these stems was closed). By filling the stems with termites, they were transferred to the laboratory, then separated with small impact on the insect. In fact, this is a quick collection method without injuring termites or transferring a lot of soil to the bait. Therefore, the use of hollow cane such as ordinary cane and even bamboo cane for placing toxic poisons is recommended attracting more termites and controlling them in the field.

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اثرات شکل و ترکیب طعمه بر پذیرش و میزان مصرف طعمه توسط موریانه *Microcerotermes diversus* (Isoptera: Termitidae) در شرایط آزمایشگاهی و صحرایی

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چکیده: موریانه *Microcerotermes diversus* Silvestri به‌عنوان مخرب ترین گونه موجود در استان خوزستان خسارت اقتصادی شدیدی را به تولیدات چوبی در ساختمان‌ها وارد می‌کند. از سیستم‌های طعمه‌گذاری اخیراً به‌عنوان یک تاکتیک مهم برای حفاظت درازمدت ساختمان‌ها و الوار در برابر آسیب موریانه‌ها استفاده می‌شود. در این آزمایش چندین فاکتور برای ساخت طعمه مورد ارزیابی قرار گرفت که شامل نحوه ترکیب طعمه بود در آزمون غیرانتخابی نحوه ترکیب طعمه، موریانه‌ها به‌ترتیب بیش‌ترین میزان تغذیه را از باگاس حاوی ملاس و مخمر، باگاس حاوی ملاس، باگاس حاوی مخمر و باگاس داشتند. در آزمون انتخابی نیز، میزان تغذیه از طعمه شامل باگاس، ملاس و مخمر خیلی بیش‌تر از طعمه شاهد (فقط باگاس) بود و اختلاف معنی‌داری بین دو تیمار مشاهده شد. موریانه‌ها به‌ترتیب بیش‌ترین میزان تغذیه را از طعمه در ساقه‌های نی، طعمه خام، طعمه پخته و طعمه به شکل قرص داشتند. در فاز صحرایی (مزرعه) اختلاف معنی‌داری بین تیمارها مشاهده نشد. اما موریانه‌ها به‌ترتیب بیش‌ترین میزان تغذیه را از طعمه در ساقه‌های نی، طعمه به شکل قرص، طعمه پخته و طعمه خام داشتند. هر دو حالت مزرعه و آزمایشگاه نشان داد که موریانه هدف، تمایل بیش‌تری به طعمه‌های موجود در نی نشان داده است. هم‌چنین این تحقیق نشان می‌دهد که طراحی طعمه و شکل آن نیز می‌تواند بر میزان پذیرش و جلب طعمه توسط موریانه تأثیر داشته باشد. بنابراین علاوه بر مواد تشکیل‌دهنده طعمه، شکل، اندازه، نرمی و فشردگی آن نیز بر میزان مصرف طعمه تأثیر می‌گذارد.

واژگان کلیدی: باگاس، طعمه پخته، ملاس، طعمه به شکل قرص، طعمه خام، مخمر