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Evaluation of cellulose substrates treated with *Metarhizium anisopliae* (Metschnikoff) Sorokin as a biological control agent against the termite *Microcerotermes diversus* Silvestri (Isoptera: Termitidae)

Abstract - This article is the first report on the promising effect of an entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin to control populations of *Microcerotermes diversus* Silvestri. Biological control is an alternative to the long-term usage of chemical pesticides. *M. anisopliae*, the causal agent of green muscardine disease of insects, is an important fungus in biological control of insect pests. Bait systems can eliminate entire colonies of subterranean termites. Baiting reduces adverse environmental impacts caused by organochlorine and organophosphate pesticides in the control of termites and creates sustainable protection of buildings against their invasion. Treated-sawdust bait was applied by two methods: a) combination of treated sawdust and untreated filter paper, and b) combination of treated sawdust and untreated sawdust. When combinations of treated sawdust and untreated sawdust were used, LC_{50} and LC_{90} were 8.4×10^6 and 3.9×10^7 (spore/ml), respectively. With the use of improved bait formula and more virulent strains, we hope to achieve better control of termite colonies and enable pathogens to become a useful element in the Integrated Pest Management system.

Key words: termites, entomopathogenic fungus, biological control, sawdust, feeding.

INTRODUCTION

Microcerotermes diversus Silvestri (Isoptera: Termitidae) is an extremely destructive pest of wood and is considered to be the major termite species in Iran, Iraq and Oman. Methods for the control of termites, including chemical control, baiting system and wood protection, have hardly been investigated scientifically in Iran. Current management of subterranean termites in Iran mainly involves the application of a soil insecticide to reduce or isolate their foraging populations (Habibpour, 2006). In other parts of the world insecticidal baits have been shown to be an effective alternative to conventional soil insecticides for remedial termite control (Su, 1991). Bait systems can eliminate entire colonies of subterranean termites (Su & Scheffrahn, 1996). Biological control is recognized as a realistic alternative to chemical pesticides (Bayon *et al.*, 2000). The study of pathogens for termite control started as early as 1965 (Wang &

Powell, 2004). *Metarhizium anisopliae* (Metschnikoff) Sorokin, the causal agent of green muscardine disease of insects, is an important fungus in biological control of insect pests (Tajik Ghanbalani *et al*., 2009). Fungi exhibit qualities which can make them ideal for this application, including a slow-acting nature similar to that of successful chemicals, the ability to self replicate, and the ability of fungal spores to be spread by termite social behavior (Grace & Zoberi, 1992). Baiting reduces adverse environmental impacts caused by organochlorine and organophosphate pesticides in the control of termites and creates sustainable protection of buildings against their invasion (Su, 1991). Bait systems are composed of two parts: a) a slow-acting toxicant, and b) a nutritive substrate, such as sawdust, with absorbent additive materials (including sugars, nitrogenous compounds and pheromones). Foraging termites acquire slow-acting toxicants by feeding on the bait and then transfer it to other individuals in the colony through trophallaxis (Guadalupe & Morales-Ramos, 2001). This study was conducted to examine the effect of the fungus *M. anisopliae* on the termite *M. diversus*.

MATERIALS AND METHODS

Collection of termites: Termites were collected from wooden blocks of beech (*Fagus orientalis* Lipsky) ($3\times6\times20$ cm^3) placed in infected soil in Ahvaz (Iran). Worker termites were used for this experiment.

Fungal isolate: In this research *M. anisopliae* (DEMI 001) was used. The strain was originally isolated from *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) and stored at the Iranian Research Institute of Plant Protection.

Preparation of media: Fungi were cultured on Sabouraud Dextrose Agar with 1% yeast extract medium. Petri dishes were placed in an incubator (28 ± 1°C with 85 ± 5% R.H.) for two weeks.

Preparation of fungal suspension: To prepare the fungal suspension, 1 ml of polysorbate monoleate (Tween 80®) was added to 100 ml sterile distilled water and spores were harvested from the media surface with a shallow scalpel cut and placed into the solution. Spore suspension concentration was determined using a Haemocytometer.

Experiment: After preliminary trials, concentrations of 1.1×10^5, 2.7×10^6, 3.7×10^7 and 3.5×10^8 (spore/ml) were selected for testing. Treated sawdust bait was applied by two methods: a) combination of treated sawdust and untreated filter paper, and b) combination of treated sawdust and untreated sawdust. a) In this test 2 g of sugarcane molasses and 2 g of agar mixed in 100 ml of fungus spore suspension were used. This combination was placed in shaker for 30 minutes. Then 25 g of beech sawdust had been added and mixed well. At this stage the bait was ready for testing. Also pieces of filter paper (Whatman® cat No 1001 42) were used. The filter paper with a diameter of 42 mm cut into two halves and these pieces were used in this experiment. Four grams of

bait were placed inside a plastic Petri dish together with a piece of filter paper in other side of the dish. The filter paper was moistened with sterile distilled water. b) In this method the bait was prepared as in the previous test and the same sawdust was prepared and sterile distilled water had been used instead of spore suspension. Four grams of bait were placed inside a Petri dish (9 cm diameter, 1 cm height) after weighing and 4 g of untreated sawdust was placed in the other side. One hundred worker termites were added into every Petri dish. Sterile distillated water was applied instead of spore suspension in control treatment. Four replications were carried out for every treatment. Petri dishes were incubated in 28 ± 1°C with 85 ± 5% R.H. in the dark. The rate of mortality had been registered for 14 days. Analysis of variance performed by SAS (9.1). Graphs were drawn by software Excel 2007. Comparison of means calculated by LSD test at the 0.05 level.

RESULTS

The rate of LC_{50} and LC_{90} in both methods is summarized in Tab. 1. When combinations of treated sawdust and untreated sawdust was used in one Petri dish, LC_{50} and LC90 were 8.4×10^6 and 3.9×10^7 (spore/ml), respectively (df=14, F=23 and p<0.0001). Also when combination of treated sawdust and untreated filter paper was used in one Petri dish, LC_{50} and LC_{90} were 4.8×10^6 and 3.3×10^7 (spore/ml), respectively (df=14, F=13 and p<0.0001) (Tab. 1).

The rate of LT_{50} and LT_{90} in both methods is summarized in Tab. 2. In both techniques the rate of LT_{50} and LT_{90} were decreased with increasing concentration of spore suspension in bait. The lowest level of LT_{50} LT_{90} related to the concentration of 3.5×10^8 (spore/ml) in both methods that over 90% of the population were killed in less than a week. When filter paper was applied instead of sawdust in untreated section, the rate of LT_{50} and LT_{90} were low (Tab. 2).

The mean comparisons of termites mortality with the two methods is reported in Fig. 1. The highest mortality was observed with concentrations of 3.7×10^7 and 3.5×10^8

Baits	**LC_{50} (Spore/ml) (95% Fiducial limits)**	**LC_{90} (Spore/ml) (95% Fiducial limits)**	**F**	**Estimate±SE**
SS*	8.4×10^6 (6.2×10^6-1.0×10^7)	3.9×10^7 (1.1×10^7-9.8×10^7)	23	1.92±1.09
SF**	4.8×10^6 (2.8×10^6-8.2×10^6)	3.3×10^7 (1.8×10^7-9.0×10^7)	13	1.52±0.21

Tab. 1 - The rate of LC_{50} and LC_{90} in both methods. * Treated sawdust and untreated sawdust. ** Treated sawdust and untreated filter paper.

Concentration (Spore/ml)	Baits	LT_{50} (day) (95% Fiducial limits)	LT_{90} (day) (95% Fiducial limits)
1.1×10^5	SS*	671 (213-7262)	10695 (1659-519581)
	SF**	344 (142-1874)	3759 (876-61282)
2.7×10^6	SS*	96 (55-263)	666 (247-4104)
	SF**	24.75 (22.24-28.26)	73.22 (59-96.10)
3.7×10^7	SS*	5.02 (4.66-5.37)	13.39 (12.15-15.03)
	SF**	3.84 (3.46-4.22)	10.23 (9.21-11.61)
3.5×10^8	SS*	2.85 (2.63-3.07)	6.14 (5.66-6.74)
	SF**	2.42 (2.33-2.5)	4.68 (4.51-4.88)

Tab. 2 - The rate of LT_{50} and LT_{90} in both methods.
* Treated sawdust and untreated sawdust . ** Treated sawdust and untreated filter paper.

(spore/ml). The rate of mortality was enhanced by increasing the concentration of spore suspension in the bait. There was no significant difference between concentrations of 3.7×10^7 and 3.5×10^8 (spore/ml). The lowest level of mortality (less than 10%) was observed with concentration of 1.1×10^5 (spore/ml). The level of mortality with concentration of 2.7×10^6 (spore/ml) was less than 10% in combination of treated sawdust and untreated sawdust and was less than 30% in combination of treated sawdust and untreated filter paper (Fig. 1).

The mean comparisons of feeding in combination of treated sawdust and untreated filter paper method is reported in Fig. 2. The highest level of feeding was achieved from

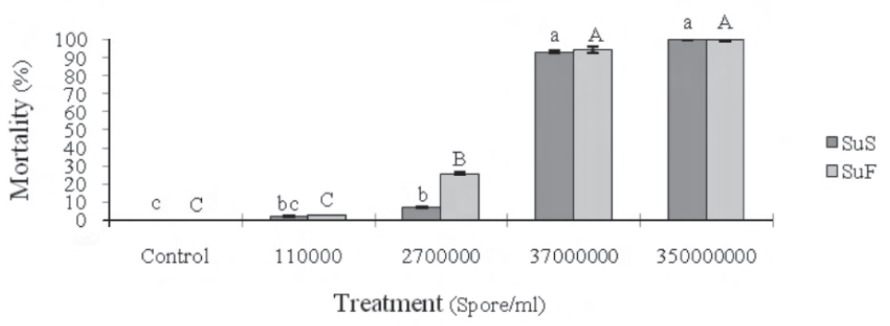

Fig. 1 - Comparison of mean mortality in both methods. The same letter above bars indicates absence of significant differences (LSD test, p = 0.05). * SuS: Treated sawdust and untreated sawdust. ** SuF: Treated sawdust and untreated filter paper.

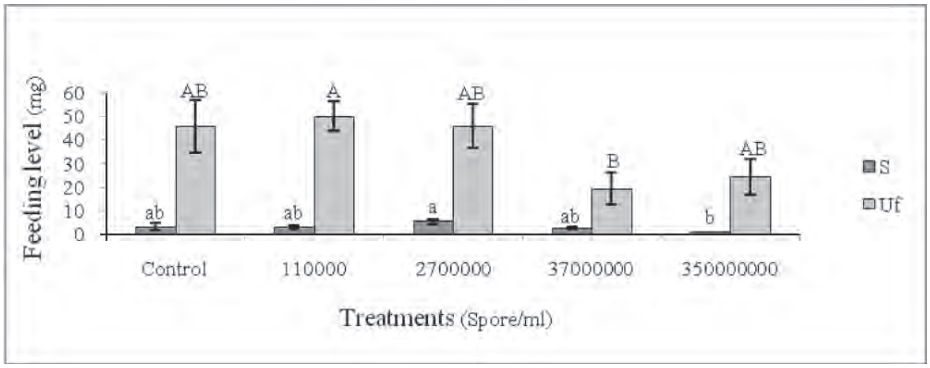

Fig. 2 - Comparison of mean feeding in combination of treated sawdust and untreated filter paper method. The same letter above bars indicates absence of significant differences (LSD test, p = 0.05). * S: Treated sawdust. ** Uf: Untreated filter paper.

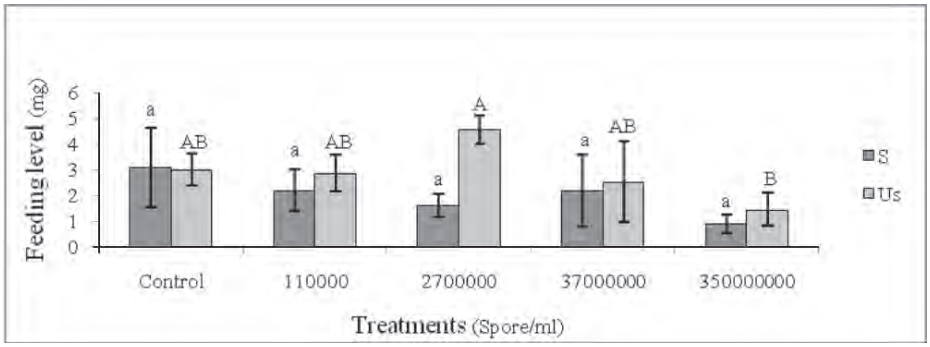

Fig. 3 - Comparison of mean feeding for combination of treated sawdust and untreated sawdust method. The same letter above bars indicates absence of significant differences (LSD test, p = 0.05). * S: Treated sawdust. ** Us: Untreated sawdust

combination treated sawdust in concentration of 2.7×10^6 (spore/ml) that didn't exhibit significant different with control treatment and concentrations of 1.1×10^5 and 3.7×10^7 (spore/ml). But there was significant different with concentration of 3.5×10^8 (spore/ml) and were placed on a higher level. The highest feeding activity occurred in concentration of 1.1×10^5 (spore/ml) from filter paper (Fig. 2).

The mean comparisons of feeding for combination of treated sawdust and untreated sawdust method is reported in Fig. 3. No significant different was detected from treated part. Furthermore, there were no significant different between rate of feeding from

untreated sawdust in different concentrations except concentration of 3.5×10^8 (spore/ml) which was lower than the other. Overall rate of feeding was less in treated part than in the untreated part in all concentrations (Fig. 3)

DISCUSSION AND CONCLUSIONS

When combination of untreated sawdust was applied as untreated part, LC_{50} and LC_{90} exhibited higher level in comparison of untreated filter paper. Probably this is because of the higher attractiveness of a sawdust bait for termite than the filter paper. Comparison between LC_{50} and LC_{90} prepared acceptable information about performance of bait in different concentrations. The rate of LC_{50} and LC_{90} decreased with increasing concentration and due to lower level of these two parameter when untreated filter paper used instead of untreated sawdust. Mean comparisons of mortality revealed the importance of choosing the appropriate concentration in both methods. Collectively no significant different observed between rates of mortality in every concentration in two methods. Figs. 2 and 3 offer considerable information regarding the amount of eaten untreated and treated parts. According to Fig. 3 it can be expressed that termites feed less from treated baits. However, it should not be assumed that fungi are repellent for termites in high concentration and that the termites avoided the bait containing fungi. It should be noted that population of termites did decreas regarding to the rate of LC_{50} and LC_{90} in both methods in less than a week. Probably the reason of lower feeding rate in high concentrations with comparison of control and lower concentrations is related to this case. Wang & Powell (2004) declared that *M. anisopliae* was not repellent for *Reticulitermes flavipes* Kollar (Iso.: Rhinotermitidae) and *Coptotermes formosanus* Shiraki (Iso.: Rhinotermitidae) in cellulose powder bait with effective concentrations. They suggested use of attractive baits as a suitable alternative for increasing performance of fungi against these two implanted termites. Habibpour *et al*. (2006) in research about laboratory evaluation of chemical additives as feeding stimulants for *M. diversus* represented that additive nitrogenous compounds such as Lesitin may increase efficacy of toxicant baits against termites in field condition. Using of such cases may increase efficacy of entomopathogenic fungi against of termites. Habibipour *et al*. (2008) applied borax-treated bait against of *M. diversus* and exploded that level of mortality and rapid of mortality of *M. diversus* depend on toxin concentration that was according to the present study. Collectively this research investigated possibility of using entomopathogenic fungus *M. anisopliae* in form of bait against *M. diversus* and trying to raise awareness of performance of this fungus. According to this findings fungus in combination of sawdust bait revealed efficient alternative *in vitro* and therefore it can be a candidate for optimizing performance and field trials. It may take a long treatment period and many treatment sites to eliminate field colonies using *M. anisopliae*. Most field studies failed to eliminate termite colonies by using fungal pathogens. These failed experiences had prevented the fungus from becoming a stand-alone termite treatment measure. Developing a palatable formulation with appropriate concentration is the key to improve its efficacy. With the study of improved bait formula and virulent

strains, we hope to achieve better control of termite colonies and enable pathogens to become a useful element in the Integrated Pest Management system.

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