doi: 10.17221/82/2015-PPS

Synergism of Entomopathogenic Fungus, *Metarhizium anisopliae* Incorporated with Fipronil against Oil Palm Pest Subterranean Termite, *Coptotermes curvignathus*

Jing Ee YII[1], Choon Fah Joseph BONG[1], Jie Hung Patricia KING[1] and Jugah KADIR[2]

[1]Department of Crop Science, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Nyabau Road, Bintulu, Sarawak, Malaysia; [2]Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia

Abstract

Yii J.E., Bong C.F.J., King J.H.P., Kadir J. (2016): **Synergism of entomopathogenic fungus,** *Metarhizium anisopliae* **incorporated with fipronil against oil palm pest subterranean termite,** *Coptotermes curvignathus.* Plant Protect. Sci., 52: 35–44.

The compatibility and synergy in efficacy of the termiticide fipronil with a biocontrol agent *Metarhizium anisopliae,* alone or in combination, against the subterranean termite, *Coptotermes curvignathus* were investigated. Sublethal doses of fipronil were found relatively less detrimental to fungal growth of *M. anisopliae* local isolates in a compatibility test. The fungus–insecticide bait formulation showed the greatest synergistic effect that increased termite mortality as well as reduced the lethal time at a sublethal dose of 0.05 mg a.i./l fipronil with conidia concentrations of 10^7 conidia/g bait (χ^2 = 48.80) at LT_{50} value of 6.46 days, followed by 10^8 conidia/g bait (χ^2 = 5.09) at LT_{50} value of 4.89 days compared to the use of these control agents alone. The insecticidal stress caused by sublethal fipronil in the formulated bait may weaken the termites and reduce their defence mechanism, which facilitates fungus infection on termites. The observed synergism treatments show the potential for integrated fungus–insecticide control method and need to be further investigated on termite infested oil palm trees.

Keywords: compatibility; joint-action rhinotermitidae; termite baiting; toxicity

Termite damage has still been a critical problem worldwide, especially in agriculture in Indonesia and Malaysia, which are the two main palm oil producers in the world. Termite damage of oil palm caused by *Coptotermes curvignathus* (Holmgren) (Isoptera: Rhinotermitidae) in the long term will result in a significant loss in palm stand. Several methods have been developed against these natural calamities in the oil palm industry. However, the management of termites in most of the oil palm plantations and urban structure is still mainly based on chemical pesticide due to its fast action, yet low persistence of pesticide requires several rounds of prophylactic and blanket spraying to prevent re-infestation of termites. The intensive usage of the chemicals has jeopardised the environment and human health. Hence, more environmentally friendly alternative termite control methods should be studied in-depth and entomopathogenic fungi are believed to be one of the most appropriate agents for termite control (Rath 2000).

The use of entomopathogenic fungus biocontrol agents in pest control is a very promising method but it turns into a problematic issue when it comes to subterranean termites due to the their cryptic life habit. Nevertheless, the control effect of entomopathogenic fungi is unstable as they are easily influenced by abiotic and biotic factors such as soil

Supported by the Ministry of Higher Education (MOHE) Malaysia and by Research University Grant Scheme (RUGS) UPM Project No. 01-01-11-1128RU.

doi: 10.17221/82/2015-PPS

temperature, humidity, and antagonistic organisms or their interaction. It requires a long term combat against all these obstacles like termite defensive behaviours (Chouvenc & Su 2010, 2012; Chouvenc *et al.* 2009a,b, 2012b, 2013), competition for resources by other opportunistic fast growing fungi (Chouvenc *et al.* 2012a), formulation and so forth, in order to create successive disease outbreak in the termite population. Although plenty of difficulties and failures, it has still continued to attract a great deal of attention worldwide (Chouvenc *et al.* 2011). Hence, an enhancement of the infectivity and methods of application against termites by entomopathogenic fungi is essential by combining entomopathogenic fungi with sublethal dosages of pesticides as stressor to lower down immune response of the pests towards fungus infection (Hiromori & Nishigaki 2001).

Numerous studies have reported on the potential use of entomopathogenic fungi incorporated with agrochemicals in a whole range of pests. A study of Dayakar *et al.* (2000) has revealed that the virulence of the combination of *M. anisopliae* and *B. bassiana* with insecticides was 1.05–1.24 and 1.19–1.42 fold higher than single treatment. Spinosad was found to interact synergistically with *M. anisopliae*, causing higher mortality in exotic wireworms (Ericsson *et al.* 2007). Besides, combined toxicity of fipronil and *M. anisopliae* was proven to enhance the mortality of American cockroach (Wakil *et al.* 2012). Ramakrishnan *et al.* (1999) demonstrated that the susceptibility of termite *Reticulitermes falvipes* to *M. anisopliae* was improved in the presence of imidacloprid. However, there is still a lack of study focused on this integrated pest control method against termites.

Termite control is perhaps the most important pest management work of oil palm plantation on peat. The most common pesticide used by plantation owners is fipronil, a phenylpyrazole insecticide, which acts on the nervous system and is used for control of many soil and foliar insects through contact and stomach poison activity. Infested palms are treated with fipronil (5.0% w/w) at 5 and 10 ml/5 l water per palm immediately after monthly census (Lim & Silek 2001). Nevertheless, overuse of chemical pesticide not only raises toxicological as well as environmental problems, but also a problem in respect to other beneficial organisms such as insect pollinators of the oil palm. To alleviate these shortcomings, a combination of available control methods, such as entomopathogenic fungi and low dosages of pesticide, is one potential approach to increase the control efficiency and accelerate insect mortality for use in integrated pest management (IPM) strategies.

The use of both elements in baiting technology may be able to act synergistically by weakening the termites and reducing their defense mechanism, whereby the entomopathogenic fungi can take advantage to kill the termites and spread secondarily, to cause an epizootic and rapid decline in termite colony provided that the pesticide does not affect the fungi, especially the viability, development, and virulence of the fungi (Moino & Alves 1998). The use of incompatible pesticide may cause inhibitory effects that often vary between fungal species and strains (Anderson *et al.* 1989). The variations of toxicity response (synergistic, additive, and antagonistic) of entomopathogenic fungi towards the chemical pesticide need to be evaluated to further determine the effect of the joint-action of both components. The present study evaluated the *in vitro* effects of a well-accepted termiticide fipronil from low dosages to commercially recommended dosage on growth and reproduction of three local isolates of the entomopathogenic fungus *M. anisopliae* var. *anisopliae*, and further tested the joint-action effect of the developed formulation on the termite *C. curvignathus*.

MATERIAL AND METHODS

Fungal culture. Three different local isolates of *M. anisopliae* which were potential biocontrol agents on controlling termite *C. curvignathus*, namely TA, LR2, and MG (Hoe *et al.* 2009), were obtained from the Entomology Research Laboratory of Universiti Putra Malaysia Bintulu Sarawak Campus. The cultures were maintained on Sabouraud dextrose agar with 1% yeast extract (both Merck, Kenilworth, USA) and 0.01% streptomycin sulphate (SDAY medium) in dark condition at 28 ± 1°C for 2 weeks before use.

Chemical pesticide. Technical grade fipronil was selected for the experiment. The active ingredient was dissolved in acetone for stock solution preparation. For compatibility test, different concentrations of fipronil (0.001, 0.01, 0.1, 1, 10, 100, 500 mg a.i./l) were prepared prior to adding into sterilised SDAY medium in the ratio of 1 : 9 to get the desired chemical suspension. The mixture was then shaken for 2 min to achieve a homogeneous distribution of the added compounds. The germination, vegetative growth, and conidial production of *M. anisopliae* isolates were

doi: 10.17221/82/2015-PPS

evaluated on chemical pesticide treated medium that ranged from 0.0001–50 mg a.i./l. Treatment without chemical pesticide and acetone treated medium served as control.

Compatibility test. Germination, vegetative growth, and conidiogenesis were evaluated based on three parameters to determine the fungitoxic effect of the pesticide fipronil on the fungus *M. anisopliae*.

Effect of pesticide on conidial germination. Two weeks old cultures were harvested gently using L-rod with sterile water containing 0.05% Tween 80. The mixture was vortexed for 15 min and filtered through double layers of cheesecloth. The conidial suspension of 10^7 conidia/l was prepared using a standard improved Neubauer haemocytometer (Assistent, Sondheim, Germany). Conidial suspension at 100 µl was spread on pesticide treated SDAY medium evenly and then incubated at 28 ± 1°C in darkness for 10 hours. Subsequently, five 1 cm^2 pieces were cut out of the agar and stained with lactophenol blue for assessment of spore germination. The percentage of germinated spores was counted on the basis of the number of conidia producing germ tubes that were at least as long as the swollen conidia per 100 counted under a microscope (400×). The experiment was repeated twice.

Effect of pesticide on vegetative growth and spore production. To assess the effect of pesticide on vegetative growth, a 5 mm mycelia disc was cored from the periphery of a 7-day-old culture and inoculated on the centre of the pesticide treated media. Diameter of the colony was measured daily with an average of two readings at right angles for 2 weeks. Four replicates were prepared for each concentration of pesticide-amended media and the Petri dishes were incubated in dark condition at 28 ± 1°C to allow maximum growth. After 2 weeks, the colonies were suspended in 0.05% Tween 80 and sterile water in order to quantify the conidial production of each isolate in the presence of pesticide. The conidial suspension was vortexed for 15 min and filtered using two layers of cheesecloth, before counting with the haemocytometer (Assistent) to determine the concentration.

Biological index for compatibility of pesticide. The toxicological effect of chemical compound on the entomopathogenic fungi growing on solid substrate was classified into compatible (BI > 66), moderately toxic (42 ≤ BI ≤ 66), and toxic (BI < 42) based on the biological index (BI) proposed by Alves *et al.* (2007) (as cited in Schumacher & Poehling 2012). The biological index was calculated using the formula:

$$BI = [47 \times VG + 43 \times SP + 10 \times GR]/100$$

where: VG – vegetative growth; SP – sporulation; GR – germination

The average percentage of VG, SP, and GR must first be corrected to the respective controls before subjected to the formula.

Toxicity effect of formulated bait against *C. curvignathus*. The potential fungal isolate was chosen to formulate into bait. Rubber wood sawdust was used as the substrate to develop three types of baits, i.e. fipronil bait, *Metarhizium* bait, and formulated bait that incorporated both fipronil and *Metarhizium* to determine the interaction in comparison with the application of each item individually. Sawdust was immersed in several low concentrations of fipronil working solution (0.001, 0.01, 0.05, 0.1 mg a.i./l), and air dried for 2 days. Dry harvested pure *M. anisopliae* conidia from broken maize were mixed into fipronil treated rubber wood sawdust and adjusted to three concentrations of 10^7, 10^8, and 10^9 conidia/g of formulated cellulose bait. A total of 0.01 g bait was applied to twenty pre-conditioned termites (18 workers and 2 soldiers) per replicate. During the bioassay, cadavers of dead termites were transferred into moist blotter to observe the mycosis of fungus. Data on termites mortality recorded daily over a period of two weeks and on mortality of 8 days post treatment (DPT) were corrected using Abbott's formula (Abbott 1925) before subjecting to analysis. The experiment was repeated thrice with five replications each.

Statistical analysis. All statistical analyses were performed using the SAS software (Version 9.0). The data on mortality percentage for each treatment were arcsine-square-root transformed prior to analysis using ANOVA in order to meet the normality assumption of the parametric statistical model. Treatment means were separated by Tukey's HSD test for laboratory fungi growth bioassay while percentage means of termite mortality were compared using Duncan's New Multiple Range Test (DNMRT) at $P \leq 0.05$. A factorial arrangement with randomised complete block design was performed to test the differences in the treatment combinations. The significant results indicated there was an interaction between *M. anisopliae* and fipronil and *vice versa*. Synergy between fungus and fipronil was analysed by comparing mortality rate induced by combinations of both agents (observed) with the sum of mortalities induced by each agent separately (expected). The expected mortality (M_E) was obtained using the formula:

doi: 10.17221/82/2015-PPS

$$M_E = M_{fip} + M_{ma} (1 - M_{fip}/100)$$

where: M_{fip} – observed mortality (%) caused by fipronil; M_{ma} – observed mortality (%) caused by *M. anisopliae*

Chi squared test (χ^2) was performed by calculating the χ^2 value using the formula:

$$\chi^2 = (M_{fm} - M_E)^2/M_E$$

where M_{fm} represents the observed mortality for the treatment combinations, and then compared to the table value for 1 df (> 3.84). If the calculated χ^2 value exceeds the tabulated value, it indicates a non-additive effect (either synergistic or antagonistic) of the two control agents. A significant interaction of the fungus–fipronil combination was determined through the difference of ($M_{fm} - M_E$), where positive = synergistic and negative = antagonistic. In contrast, if the tabulated value exceeds the calculated χ^2 value, it represents an additive effect at $P \leq 0.05$. LT_{50} and 95% confidence limits of each treatment were performed using the probit analysis of SPSS software. The treatments were considered as significantly different when there was no overlap in the 95% CL of lethal time values.

RESULTS

Conidia germination. All tested *M. anisopliae* local isolates showed a varying degree of inhibition of conidia germination percentage at different concentrations of fipronil amended media. Isolate TA achieved a high germination of 92.40% and only the germination at higher concentrations of fipronil (0.1–50 mg a.i./l) was significantly different from the control ($F = 11.24$, df = 8, $P < 0.0001$). For LR2 isolate, the germination of conidia was significantly affected by fipronil. The percentages of germination were comparatively lower (35.60–56.90%) in the presence of acetone and fipronil

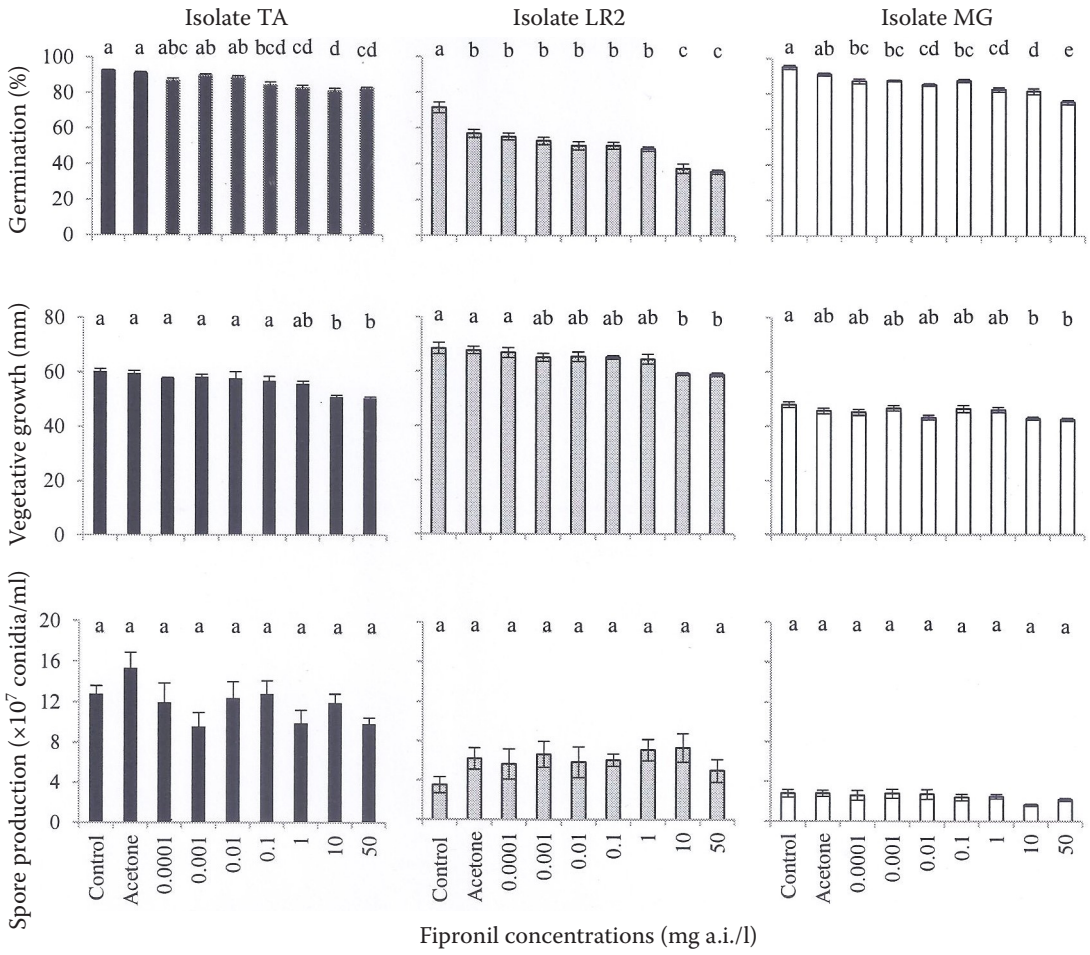

Figure 1. Germination (means ± SE, $n = 10$), colony diameter (means ± SE, $n = 4$), and spore production (means ± SE, $n = 10$) by *Metarhizium anisopliae* isolates TA, LR2, and MG on different concentrations of fipronil. Bar with different letters is significantly different ($P \leq 0.05$) (Tukey's test)

doi: 10.17221/82/2015-PPS

Table 1. Biological index (BI) values of different concentrations of the pesticide with three isolates of *Metarhizium anisopliae* (TA, LR2, and MG)

Fipronil concentration (mg a.i./l)	TA	LR2	MG
Acetone	107.81	130.43	97.44
0.0001	94.75	122.75	93.57
0.001	87.26	133.39	97.89
0.01	96.16	123.48	93.49
0.1	96.37	125.71	91.95
1	85.65	137.75	92.24
10	88.50	135.13	75.99
50	81.22	106.16	83.30

Classification: compatible (BI > 66), moderately toxic (42 ≤ BI ≤ 66), and toxic (BI < 42)

compared with the control (71.40%) (F = 25.53, df = 8, P < 0.0001). However, significantly fewer conidia of isolate MG germinated ranging from 75.00 to 87.20% on media amended with ≥ 0.0001 mg a.i./l fipronil compared with the control (95.00%) (F = 25.87, df = 8, P < 0.0001) (Figure 1).

Vegetative and spore production. The vegetative growth of three local isolates showed the same tendency, whereby only the highest concentrations of fipronil (10 and 50 mg a.i./l) had the most adverse effect on fungal growth. Conversely, other lower concentrations of fipronil were statistically at par according to each isolate. In terms of spore production, it was not affected by the presence of fipronil, judging from the result which showed no significant difference between control and fipronil (Figure 1).

Biological index for compatibility of pesticide. From the biological index, all tested concentrations of fipronil could be used along with the entomopathogenic fungi *M. anisopliae* isolates TA, LR2, and MG as all the combinations were classified as compatible according to the biological index formula. The value decreased with increasing concentrations of fipronil (Table 1).

Toxicity effect of formulated bait. The results showed that termite mortality was significantly affected by fipronil concentration (F = 71.38, df = 4, P < 0.0001), spore concentration (F = 280.98, df = 3, P < 0.0001), and the interaction of pesticide and fungi (F = 13.51, df = 12, P < 0.0001). Mixing of 10^9 conidia/g with either 0.1, 0.05, 0.01, or 0.001 mg a.i./l of fipronil showed no significant difference with the treatment of 10^9 conidia/g alone, while the combination treatments of 10^8 and 10^7 conidia/g with fipronil 0.1 and 0.05 mg a.i./l caused higher termite mortalities compared to treatments at 10^8 and 10^7 conidia/g alone, respectively. Moreover, mortalities of fipronil + spore combination at 0.1 + 10^8 and 0.05 + 10^8 were similar to the highest mortality treatment of 10^9 spores (F = 60.94, df = 18, P < 0.0001) (Table 2). The result of the chi-squared test also showed synergistic interaction between fipronil and *M. anisopliae* in both concentrations of 0.05 + 10^8 and 0.05 + 10^7 combinations, wherein the greatest synergistic effect occurred when 10^7 conidia/g bait acted synergistically with 0.05 mg a.i./l fipronil (χ^2 = 48.80). However, a combination of 10^7 spores of fungus and 0.001 mg a.i./l fipronil caused an antagonistic effect. The overall interaction of *M. anisopliae* and fipronil in combination treatments is additive in killing the termite colony (Table 3).

Calculated LT_{50} values and 95% confidence limits (Table 4) for different treatments against termites showed the same results as in Table 2, whereby there were no significant differences in LT_{50} between 10^9 spores alone with other fipronil incorporated

Table 2. Toxicity of fipronil (mg a.i./l) and *Metarhizium anisopliae* (conidia/g) alone and in combination treatments against termite *Coptotermes curvignathus* at 8 days post treatment (n = 300)

Treatment	Mortality (%) ± SE*
M. anisopliae (10^9)	100.00 ± 0.00^{a}
M. anisopliae (10^8)	48.29 ± 8.76^{c}
M. anisopliae (10^7)	17.65 ± 4.66^{de}
Fipronil (0.1)	72.60 ± 5.23^{b}
Fipronil (0.05)	23.63 ± 5.89^{d}
Fipronil (0.01)	3.51 ± 1.42^{f}
Fipronil (0.001)	9.11 ± 3.17^{ef}
0.1 + 10^9	98.29 ± 1.19^{a}
0.1 + 10^8	91.78 ± 3.47^{a}
0.1 + 10^7	71.23 ± 4.75^{b}
0.05 + 10^9	98.63 ± 1.37^{a}
0.05 + 10^8	94.86 ± 2.40^{a}
0.05 + 10^7	79.45 ± 6.56^{b}
0.01 + 10^9	99.66 ± 0.34^{a}
0.01 + 10^8	49.50 ± 8.61^{c}
0.01 + 10^7	11.48 ± 3.76^{def}
0.001 + 10^9	98.97 ± 0.74^{a}
0.001 + 10^8	51.03 ± 7.96^{c}
0.001 + 10^7	13.35 ± 3.58^{de}

*means with the same letters are not significantly different at P ≤ 0.05 by using Duncan's New Multiple Range Test

doi: 10.17221/82/2015-PPS

Table 3. Synergy bioassay of a different combination of fipronil and *Metarhizium anisopliae* in termite mortality at 8 days post treatment

Treatment		Mortality (%)					
Fungi (conidia/g)	Fipronil (mg a.i./l)	fungi	Fipronil	expected (M_E)	observed (M_{fm})	χ^2	effect
10^7	0.001	17.47	8.56	24.53	13.36	5.09	antagonistic
10^7	0.01	17.47	2.05	19.17	11.30	3.23	additive
10^7	0.05	17.47	23.63	36.97	79.45	48.80	synergistic
10^7	0.1	17.47	72.60	77.39	71.23	0.49	additive
10^8	0.001	48.29	8.56	52.72	51.02	0.05	additive
10^8	0.01	48.29	2.05	49.35	49.32	0.00	additive
10^8	0.05	48.29	23.63	60.51	94.86	19.50	synergistic
10^8	0.1	48.29	72.60	85.83	91.78	0.41	additive
10^9	0.001	100.00	8.56	100.00	98.97	0.01	additive
10^9	0.01	100.00	2.05	100.00	99.66	0.00	additive
10^9	0.05	100.00	23.63	100.00	98.63	0.02	additive
10^9	0.1	100.00	72.60	100.00	98.28	0.03	additive

χ^2 comparison that exceeds 3.84 with df = 1 and α = 0.05 is considered a synergistic or antagonistic interaction

10^9 treatments. In addition to the synergistic and additive effect of 0.1 and 0.05 mg a.i./l fipronil with 10^7 and 10^8 conidia/g *M. anisopliae* spores, respectively, the combinations showed the shortest lethal time for causing 50% mortality in termite colony in comparison to sole fungus treatments of 10^7 and

Table 4. Calculated LT_{50} values for the formulated bait of *Metarhizium anisopliae* (conidia/g) and its combination with fipronil (mg a.i./l)

Treatment	LT_{50}	95% CL*	Slope ± SE	χ^2	df
Fipronil (0.001)	21.82	16.78–40.19	0.09 ± 0.03	0.47	12
Fipronil (0.01)	16.51	14.42–20.96	0.18 ± 0.04	1.62	12
Fipronil (0.05)	10.52	9.81–11.36	0.26 ± 0.03	0.29	12
Fipronil (0.1)	5.89	5.22–6.52	0.30 ± 0.03	6.56	12
M. anisopliae (10^7)	13.83	12.28–16.49	0.15 ± 0.03	0.32	12
M. anisopliae (10^7) + Fipronil (0.1)	6.14	5.53–6.73	0.33 ± 0.03	1.96	12
M. anisopliae (10^7) + Fipronil (0.05)	6.46	5.97–6.94	0.47 ± 0.05	0.68	12
M. anisopliae (10^7) + Fipronil (0.01)	11.52	10.71–12.57	0.25 ± 0.03	3.88	12
M. anisopliae (10^7) + Fipronil (0.001)	13.71	12.15–16.36	0.15 ± 0.03	0.72	12
M. anisopliae (10^8)	8.62	7.89–9.38	0.24 ± 0.03	4.62	12
M. anisopliae (10^8) + Fipronil (0.1)	5.22	4.72–5.71	0.47 ± 0.05	4.97	12
M. anisopliae (10^8) + Fipronil (0.05)	4.89	4.44–5.33	0.57 ± 0.07	4.89	12
M. anisopliae (10^8) + Fipronil (0.01)	7.99	7.38–8.60	0.31 ± 0.03	0.82	12
M. anisopliae (10^8) + Fipronil (0.001)	8.12	7.50–8.75	0.30 ± 0.03	2.63	12
M. anisopliae (10^9)	3.94	3.54–4.32	0.77 ± 0.10	2.08	12
M. anisopliae (10^9) + Fipronil (0.1)	3.67	3.24–4.09	0.67 ± 0.09	3.46	12
M. anisopliae (10^9) + Fipronil (0.05)	4.08	3.66–4.49	0.68 ± 0.09	1.86	12
M. anisopliae (10^9) + Fipronil (0.01)	4.02	3.54–4.47	0.55 ± 0.07	0.88	12
M. anisopliae (10^9) + Fipronil (0.001)	4.13	3.70–4.55	0.63± 0.08	0.47	12

*significant on LT_{50} if there was no overlap of 95% CL

doi: 10.17221/82/2015-PPS

10^8 spores. This implied that the mortality among termite population was accelerated by the addition of fipronil. Besides, there was no significant difference in LT_{50} between 0.05 mg a.i./l of fipronil and 0.1 mg a.i./l of fipronil when both were incorporated with fungus spores whereas in fact there was a significant difference in termite mortality when each level of fipronil was applied individually (Table 4).

DISCUSSION

The current study focused on the concentration response of the pure chemical pesticide on biocontrol agents since the additives in an agrochemical pesticide formulation are known to cause effect on entomopathogenic fungi (Anderson & Roberts 1983). Three local *M. anisopliae* isolates (TA, MG, and LR2) which have great potential to be developed as biopesticide and able to cause pathogenic effect against termite *C. curvignathus* (Hoe *et al.* 2009) were chosen for evaluation in the test of compatibility among fipronil and *M. anisopliae.* Luan *et al.* (2012) revealed that the *Metarhizium* spp. populations were temporarily heterogeneous based on the analysis of 51 isolates sampled on diversified occasions. Therefore, the evaluation of the three isolates against fipronil was crucial as different species, and isolates within a species, can display varying characteristics in host specificity, infection levels, germination rates, temperature optima (Sierotzki *et al.* 2000; Pell *et al.* 2001; Shaw *et al.* 2002) and also have different susceptibilities (Schumacher & Poehling 2012) to counteract the existence of different toxicant.

The latest biological index proposed by Alves *et al.* (2007) (as cited in Schumacher & Poehling 2012) includes three critical parameters to determine compatibility: germination, vegetative growth, and sporulation after realising the importance of germination which reflected on the viability of the fungus at the beginning of the infection process. Asi *et al.* (2010) also revealed conidia germination was comparatively more sensitive to pesticides than vegetative growth of the fungi which was also shown in the present study. Schumacher & Poehling (2012) tested five concentrations of fipronil (0.32, 1.6, 8, 40, and 200 ppm) on two *M. anisopliae* strains and all the tested concentrations were compatible with both strains. Generally, the results were in accordance with the present study which also indicated compatibility of the three local isolates in the biological index. However, isolates LR2 and MG showed a significant reduction in spore germination even only in the medium of residual solvent acetone without pesticide treatment, while the vegetative growth and spore yield were not much different from control and some were even greater than those obtained in control treatment. These conflicting data were common in *in vitro* experiments due to the physiological mechanism of pesticide resistance of fungus whereby the fungus was presumably making a reproductive effort by increasing spore production in a toxic medium and used the metabolised chemical substances as secondary nutrients to prolong its survival (Moino & Alves 1998). However, using the current biological index imposes a limitation and may disguise the actual toxic effect of a pesticide on pathogen (Silva *et al.* 2013), whereby the germination parameter only consisted of 10% compared to 90% of the formula is attributed to vegetative growth (47%) and sporulation (43%), respectively.

Apparently, *in vitro* studies are important to evaluate the development of disease and the deleterious way to growth of fungi in the worst condition by direct exposing the fungus to maximum toxicity of the chemicals in synthetic environment which could not occur under field conditions (Neves *et al.* 2001). Compatible fungus and pesticide may help avoid undesirable side effects under field conditions (Alves *et al.* 1998) and facilitate the selection of proper products for IPM practices. In the present study, isolate TA did not cause a significant reduction in the spore production, despite a slight effect on spore viability and average diameter of the colony in the presence of fipronil compared to the control. With respect to the good performance of TA in all aspects, TA isolate was chosen for further investigation on synergy bioassay.

A simple bait formulation incorporating the conidia of isolate TA and the fipronil treated rubber wood sawdust was used in the current study to investigate the interaction of the combinations. The combination of both agents in termite baiting has the advantages of not contaminating the soil with chemicals as the chemical concentration is low, difficult to be leached out after formulating, and more target specified. Among all tested treatments, treatment of 10^9 conidia/g bait obtained the highest mortalities either in combination with fipronil or as a sole treatment at 8 DPT. A relatively high dosage of fungus is necessary to increase the risk of infection in most tested species (Chouvenc *et al.* 2008,

doi: 10.17221/82/2015-PPS

2009c). Meanwhile, the presence of a massive amount of fungus triggered avoidance behaviour (Milner *et al.* 1998; Mburu *et al.* 2009) in the miniature termite colonies, whereby bait repellence occurred in the treatments with 10^9 conidia/g bait, slight repellence at 10^8 conidia/g bait, and non-repellence at 10^7 conidia/g bait. However, the high toxicity of 10^9 conidia/g *Metarhizium* bait still manages to infect the exposed termites when they foraged or buried the bait area to prevent further spreading among all individual termites. It can be effective for curative control of termite but the LT_{50} was relatively shorter than other treatments, whereby the termites might fail to carry the disease back to their nest.

Most of the treatments in the study generally increased the mortalities of termites when in combination of both components than those caused by individual agents, but synergistic interaction was not apparent. Only 0.05 mg a.i./l fipronil was interacting synergistically with 10^7 and 10^8 conidia/g bait by increasing the mortality and lowering the LT_{50} values as compared to the spore treatment alone. Synthetic insecticides can act as physiological stressors and/or behavioural modifiers (Inglis *et al.* 2001) among insects at sublethal doses, resulting in fungal conidia bypassing the primary defense mechanism (grooming behaviour) and penetrating the termite cuticle successfully. The mechanism of synergistic effect could be achieved by weakening the immune system of the insects through insecticidal stress, when the phenoloxidase activity of insect hemolymph decreased with time after *M. anisopliae* and pesticides treatment, thus making it more vulnerable to the fungus attack (Hiromori & Nishigaki 2000) and increasing the killing speed. Beyond the synergistic interactions detected in this study, an antagonistic interaction was obtained in combination of the lowest doses of fungus and fipronil. This might be due to inability of both agents with low toxicant and conidia load to overwhelm the termite's defense mechanism which becomes less susceptible for disease attack. However, further studies on factors or mechanisms for enhanced efficacy of *M. anisopliae* in combination with fipronil against termites are yet to be unravelled.

In conclusion, incorporating the chemical pesticide fipronil with the fungus *M. anisopliae* was compatible. The inclusion of a low concentration of fipronil and the fungus in the bait was proven to obtain synergistic effect against termite *C. curvignathus* by increasing termite mortalities and the lethal time in laboratory bioassay was shortened, too. Sublethal chemical pesticide may be able to break the termite defense mechanisms and the entomopathogen may induce an epizootic within the colony. This suggests that baiting technology with fungus–insecticide mixtures can serve as an alternative termite control method to reduce termite populations without the use of conventional pesticides. Further studies should be done in field to determine whether the combined treatments of fipronil and *M. anisopliae* using formulated bait are practical and effective against termite colonies with developed field delivery systems. It might provide valuable data useful for the development of effective termite IPM strategies for long-term sustainable and economical control of insect pests in the agro-ecosystem.

References

Abbott W.S. (1925): A method for computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265–267.

Alves S.B., Moino Jr. A., Almeida J.E.M. (1998): Produtos fitossanitários e entomopatógenos. In: Alves S.B. (ed.): Controle microbiano de insetos. São Paulo, Fealq: 269–289.

Anderson T.E., Roberts D.W. (1983): Compatibility of *Beauveria bassiana* isolates with insecticide formulations used in Colorado potato beetle (Coleoptera: Chrysomelidae) control. Journal of Economic Entomology, 76: 1437–1441.

Anderson T.E., Hajek A.E., Roberts D.W., Preisler H.K., Robertson J.L. (1989): Colorado potato beetle (Coleoptera: Chrysomelidae): Effects of combinations of *Beauveria bassiana* with insecticides. Journal of Economic Entomology, 82: 83–89.

Asi M.R., Bashir M.H., Afzal M., Ashfaq M., Sahi S.T. (2010): Compatibility of entomopathogenic fungi, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* with selective insecticides. Pakistan Journal of Botany, 42: 4207–4214.

Chouvenc T., Su N.Y. (2010): Apparent synergy among defense mechanisms in subterranean termites (Rhinotermitidae) against epizootic events – The limits and potential for biological control. Journal of Economic Entomology, 103: 1327–1337.

Chouvenc T., Su N.Y. (2012): When subterranean termites challenge the rules of fungal epizootics. PLoS ONE, 7: e34484.

Chouvenc T., Su N.Y., Elliott M.L. (2008): Interaction between the subterranean termite *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) and the entomopathogenic fungus *Metarhizium anisopliae* in Foraging Arenas. Journal of Economc Entomology, 101: 885–893.

doi: 10.17221/82/2015-PPS

Chouvenc T., Efstathion C.A., Elliott M.L., Su N.Y. (2012a): Resource competition between two fungal parasites in subterranean termites. Naturwissenschaften, 99: 949–958.

Chouvenc T., Efstathion C.A., Elliott M.L., Su N.Y. (2013): Extended disease resistance emerging from the faecal nest of a subterranean termite. Proceedings of the Royal Society B, 280: 20131885.

Chouvenc T., Robert A., Sémon E., Bordereau C. (2012b): Burial behaviour by dealates of the termite *Pseudacanthotermes spiniger* (Termitidae, Macrotermitinae) induced by chemical signals from termite corpses. Insectes Sociaux, 59: 119–125.

Chouvenc T., Su N.Y., Grace J.K. (2011): Fifty years of attempted biological control of termites – Analysis of a failure. Biological Control, 59: 69–82.

Chouvenc T., Su N.Y., Robert A. (2009a): Cellular encapsulation in the eastern subterranean termite, *Reticulitermes flavipes* (Isoptera), against infection by the entomopathogenic fungus *Metarhizium anisopliae*. Journal of Invertebrate Pathology, 101: 234–241.

Chouvenc T., Su N.Y., Robert A. (2009b): Inhibition of *Metarhizium anisopliae* in the alimentary tract of the eastern subterranean termite *Reticulitermes flavipes*. Journal of Invertebrate Pathology, 101: 130–136.

Chouvenc T., Su N.Y., Robert A. (2009c): Susceptibility of seven termite species (Isoptera) to the entomopathogenic fungus *Metarhizium anisopliae*. Sociobiology, 54: 723–748.

Dayakar S., Kanaujia K.R., Rathore R.R.S. (2000): Compatibility of entomogenous fungi with commonly used insecticides for the management of *Spodoptera litura* (Fab.). In: Ignacimuthu S., Sen A. (eds): Microbials in Insect Pest Management. Oxford and IBH Publishing Co. Delhi, Kolkata: 47–52.

Ericsson J.D., Kabaluk J.T., Goettel M.S., Myers J.H. (2007): Spinosad interacts synergistically with the insect pathogen *Metarhizium anisopliae* against the exotic wireworms *Agriotes lineatus* and *Agriotes obscures* (Coleoptera: Elateridae). Journal of Economic Entomology, 100: 31–38.

Hiromori H., Nishigaki J. (2001): Factor analysis of synergistic effect between the entomopathogenic fungus *Metarhizium anisopliae* and synthetic insecticides. Applied Entomology and Zoology, 36: 231–236.

Hoe P.K., Bong C.F.J., Jugah K., Rajan A. (2009): Evaluation of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycete) isolates and their effects on subterranean termite *Coptotermes curvignathus* (Isoptera: Rhinotermitidae). American Journal of Agricultural and Biological Sciences, 4: 289–297.

Inglis G.D., Goettel M.S., Butt T.M., Strasser H. (2001): Use of hyphomycetous fungi for managing insect pests. In: Butt T.M., Jackson C., Magan N. (eds): Fungi as Biocontrol Agents: Progress, Problems and Potential. Wallingford, CAB International: 23–69.

Lim K.H., Silek B. (2001): Termite infestations on oil palms planted on deep peat in Sarawak: Tradewinds experience. In: Proceedings 2001 PIPOC International Palm Oil Congress, Agriculture Conference, Kuala Lumpur 2001 – Cutting-edge Technology for Sustained Competitiveness. MPOB, Kuala Lumpur: 355–368.

Luan F., Zhang S., Wang B., Huang B., Li Z. (2013): Genetic diversity of the fungal pathogen *Metarhizium* spp., causing epizootics in Chinese burrower bugs in the Jingting Mountains, eastern China. Molecular Biology Reports, 40: 515–523.

Mburu D.M., Ochla L., Maniania N.K., Njagi P.G.N., Gitonga L.M., Ndung'u M.W., Wanjoya A.K., Hassanali A. (2009): Relationship between virulence and repellency of entomopathogenic isolates of *Metarhizium anisopliae* and *Beauveria bassiana* to the termite *Macrotermes michaelseni*. Journal of Insect Physiology, 55: 774–780.

Milner R.J., Staples J.A., Lutton G.G. (1998): The selection of an isolate of the hyphomycete fungus *Metarhizium anisopliae*, for control of termites in Australia. Biological Control, 11: 240–247.

Moino Jr. A., Alves S.B. (1998): Efeito de imicacloprid e fipronil sobre *Beauveria bassiana* (Bals.) Vuill. e *Metarhizium anisopliae* (Metsch.) Sorok. e no comportamento de limpeza de *Heterotermes tenuis* (Hagen). Anais da Sociedade Entomológica do Brasil, 27: 611–620.

Neves P.M.O.J., Hirose E., Tchujo P.T., Moino Jr. A. (2001): Compatibility of entomopathogenic fungi with neonicotinoid insecticides. Neotropical Entomology, 30: 263–268.

Pell J.K., Eilenberg J., Hajek A.E., Steinkraus D.C. (2001): Biology, ecology and pest management potential of Entomophthorales. In: Butt T.M., Jackson C., Magan N. (eds): Fungi as Biocontrol Agents: Progress, Problems and Potential. Wallingford, CAB International: 71–153.

Ramakrishnan R., Suiter D.R., Nakatsu C.H., Humber R.A., Bennett G.W. (1999): Imidacloprid-enchanced *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) susceptibility to the entomopathogen *Metarhizium anisopliae*. Journal of Economic Entomology, 92: 1125–1132.

Rath A.C. (2000): The use of entomopathogenic fungi for termite control. Biocontrol Science and Technology, 10: 563–581.

Schumacher V., Poehling H.M. (2012): *In vitro* effect of pesticides on the germination, vegetative growth and conidial production of two strains of *Metarhizium anisopliae*. Fungal Biology, 116: 121–132.

Shaw K.E., Davidson G., Clark S.J., Ball B.V., Pell J.K., Chandler D., Sunderland K.D. (2002): Laboratory bioassays to

doi: 10.17221/82/2015-PPS

assess the pathogenicity of mitosporic fungi to *Varroa destructor* (Acari: Mesostigmata), an ectoparasitic mite of the honeybee, *Apis mellifera*. Biological Control, 24: 266–276.

Sierotzki H., Camastral F., Shah P.A., Aebi M., Tuor U. (2000): Biological characteristics of selected *Erynia neoaphidis* isolates. Mycological Research, 104: 213–219.

Silva R.A.D., Quintela E.D., Mascarin G.M., Barrigossi J.A.F., Lião L.M. (2013): Compatibility of conventional agrochemicals used in rice crops with the entomopathogenic fungus *Metarhizium anisopliae*. Scientia Agricola, 70: 152–160.

Wakil W., Yasin M., Qayyuan M.A., Asim M. (2012): Combined toxicity of *Metarhizium anisopliae* with sublethal doses of chlorpyrifos, fipronil and chlorantraniliprole against *Periplaneta americana* (Dictyoptera: Blattidae). Pakistan Entomologist, 34: 59–63.

Received: 2015–08–12
Accepted after corrections: 2015–09–14

Corresponding author:

Dr Choon Fah Joseph Bong, Universiti Putra Malaysia, Bintulu Sarawak Campus, Faculty of Agriculture and Food Sciences, Department of Crop Science, Nyabau Road, 97000 Bintulu, Sarawak, Malaysia;
E-mail: josephbcf@upm.edu.my