Control of Subterranean Termite *Coptotermes Curvignathus* (Isoptera: Rhinotermitidae) by Entomopathogen *Metarhizium Anisopliae* Var. *Anisopliae* Cultured in Liquid State Fermentation

Vei-Ting Nyam, Choon-Fah J. Bong and Jie-Hung P. King

Department of Crop Science, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia, Bintulu Sarawak Campus, Malaysia

**Abstract:** Solid state fermentation has been used for mass production of *Metarhizium anisopliae* many years ago. However, solid state fermentation was time consuming, costly and unable to produce in large amount. We examined the suitability of liquid state fermentation to mass produce *M. anisopliae* and the effect of culture medium on fungal pathogenicity. Three different liquid medium selected for this study was Jenkins medium, Leland medium and MPOB medium. The fungus was cultured for 5 days to examine the growth rate. Yield of submerged conidia in different medium was assessed on day 3. Fungal virulence was bioassayed on termite *Coptotermes curvignathus*. The results indicated that Jenkins medium yielded significantly lower growth rate and dry weight (1.472 mg mL\(^{-1}\)), but the fungus exhibited highest virulence (100% mortality on day 4) among the 3 medium studied. MPOB medium had the highest growth rate and DW yield (1.961 mg mL\(^{-1}\)) but achieved 100% mortality only on day 6. The LC\(_{50}\) result showed Jenkins medium yielded the most virulent fungus culture compared to Leland medium and MPOB medium.

**Keywords:** Isoptera, Rhinotermitidae, *Coptotermes Curvignathus*, *Metarhizium Anisopliae*, Liquid State Fermentation, Termite

**Introduction**

The entomopathogen *Metarhizium anisopliae* has been widely used to control agricultural pests for many years. However, the lack of inexpensive manufacturing technique and the inability to maintain production quality and quantity for a long period have limited the entomopathogen to be used commercially (Jackson et al., 2004; Nelson et al., 1996). Most of the available fungal growth and pathogenicity research were conducted by using fungal surface culture and aerial conidia and very little work was published on using fungal submerged conidia. Robert and Sweeney (1982) reported that fungal submerged culture was less pathogenic compared to aerial conidia. However, Leland et al. (2005) and Jenkins and Prior (1993) found that alternation on liquid medium could produce pathogenic submerged conidia. Medium Carbon to Nitrogen ratio, carbohydrate sources, nitrogen sources, yeast extract and other nutrients have been reported to change fungal characteristic and pathogenicity (Mehta et al., 2012; Mustafa and Kaur, 2009; Jackson and Jaronski, 2009; Bharati et al., 2007; Rangel et al., 2006; Shah et al., 2005; Li and Holdom, 1995). The purpose of this study is to identify the suitable medium from three different sources derived from Moslim et al. (2009), Leland et al. (2005) and Jenkins and Prior (1993) to mass produce pathogenic submerged conidia for control of subterranean termite *Coptotermes curvignathus* and further use to formulate the biopesticide.

**Materials and Methods**

**Fungal Isolate**

Local isolate of *M. anisopliae* var. *anisopliae* (TA) originally isolated from peat soil in Bintulu Sarawak was obtained from Entomology Laboratory of Universiti Putra Malaysia Bintulu Sarawak campus (UPMKB). The culture was maintained on Sabouraud Dextrose Yeast Agar (SDYA) added with 0.01% streptomycin and incubated in total dark condition at room temperature (27±1°C).
Preparation of Liquid Medium for Fungal Mass Production

Three different liquid media were derived from Jenkins and Prior (1993) (Jenkins medium); Leland et al. (2005) (Leland medium); and Moslim et al. (2009) (MPOB medium). Jenkins medium consists of 1% yeast extract and 1% sucrose. Meanwhile, Leland medium consists of 0.4% yeast extract, 0.4% fructose and 0.5% lecithin and MPOB medium consists of 1% yeast extract, 1% dextrose and 1% peptone. The fungal growth rate, the yield of submerged conidia and the effect of medium on the fungal pathogenicity to C. curvignathus were determined by using these 3 medium.

Preparation of Conidia for Liquid Medium Mass Production

Conidia of M. anisopliae var. anisopliae were harvested from 14 days old culture in SDYA by using sterile water containing 0.05% Tween 80. The conidia suspension was further adjusted to 1×10^6 conidia mL^{-1}. Sterile liquid medium at 100 mL per was inoculated with 1 mL conidia suspension and incubated on rotary shaker with 180 rpm at room temperature for 5 days.

Fungal Growth Rate on 3 Different Medium

Fungal growth rate was determined daily until day 5. Growth rate were collected daily by filtering the culture in liquid medium into pre-weighted filter paper (Whatman no. 1) through water filter vacuum pump to discard the liquid medium. The fungal culture on filter paper was dried at 60°C for 1 week. One day prior to weighing, the fungus was transferred into a desiccator. It was weighed using an analytical balance (Satrious) until a constant weight was obtained. Each treatment contained 4 replicates with 4 times repetition.

Submerged Conidia Yield on 3 Different Medium

The inoculated mediums were incubated as before for 3 days. Prior to harvesting, each medium was vortexed for 20 min to separate the submerged conidia from the phialides. The culture medium was then filtered through 2 layers of cheese cloth (pore size ≈ 75 µm) and concentration of submerged conidia in the filtrate was determined using a haemacytometer (Petroff-Hausner). The morphology of submerged conidia was confirmed by using description from Jenkins and Prior (1993) and Leland et al. (2005) as references. Each treatment had 4 replicates and repeated 4 times.

Viability Assays

After 24 h incubation, all submerged conidia treatments recorded more than 90% of submerged conidia were viable There was no varied significantly (p<0.05) in the Colony Forming Unit (CFU) for all 3 different submerged treatment.

Virulence of Fungal Mycelia with Submerged Conidia and Submerged Conidia Alone from Different Medium

Fungal submerged conidia and mycelia were prepared from 3 days old liquid culture. Submerged conidia were prepared as before and the filtrate was adjusted to 7 different concentrations. For fungal mycelia with submerged conidia, the culture was pipetted into a 15 mL sterile falcon tube and diluted to 7 different concentrations and the respective dry weights are shown in Table 1.

Bioassay for Virulence

C. curvignathus was collected from an infested oil palm plantation in Bintulu. Termite cultures were maintained on humid black PVC tank with rubber wood as food source. A day before treatment, termites were transferred into a Petri dish which contained a moist filter paper (food sources) for conditioning (80% relative humidity). For the virulence of submerged conidia alone, 1 µL of freshly harvested submerged conidia was topically inoculated on to the termite’s thorax and termite mortality was recorded daily until 100% mortality. In the bioassay of mycelia with submerged conidia, 1 µL of culture was topically inoculated on to the termite’s thorax and the termite mortality was recorded daily until 100% mortality. Each treatment contained 8 replicates at 10 termites per rep with 2 soldiers and 8 workers per replicate. Bioassays were repeated four times.

Statistical Analysis

All data were analyzed by SAS software version 9.0 and treatment means were separated by Duncan New Multiple Range Test.

Table 1. The dry weight of mycelia with submerged conidia in percentage from different medium

| Treatment (Percent fungal culture) | Jenkins medium (J) | MPOB medium (M) | Leland medium (L) |
|-----------------------------------|--------------------|-----------------|-------------------|
| 100%                              | 29.44              | 36.40           | 39.22             |
| 50%                               | 14.72              | 18.20           | 19.61             |
| 10%                               | 2.94               | 3.64            | 3.92              |
| 4%                                | 1.18               | 1.46            | 1.57              |
| 2%                                | 0.59               | 0.73            | 0.78              |
| 1.3%                              | 0.38               | 0.47            | 0.51              |
| 1%                                | 0.29               | 0.36            | 0.39              |

*Dry weights were calculated based on growth rate at Day 3 from Fig. 1
Results

Growth Rate

Fungal growth was significantly (p<0.05) affected by the use of different liquid culture medium (Fig. 1). Fungus cultivated in MPOB medium produced the highest growth compared to Leland medium and Jenkins medium. Medium for fungus liquid state fermentation had significant effect on the fungal growth. During day 1 and day 2 fungal cultures in Jenkins medium showed significantly lower dry yield compared to Leland medium, but no significant difference was observed when compared to MPOB medium.

Submerged Conidia Yield

The yield of submerged conidia was significantly affected by different medium. Compared to Leland medium and MPOB medium, Jenkins medium had significantly higher (p<0.0001) total yield of submerged conidia while there was no difference in yield for both MPOB medium (1.87×10^9 submerged conidia mL^−1) and Leland medium (1.74×10^9 submerged conidia mL^−1) (Table 2).

Viability Assays

After 48 h incubation, all submerged conidia treatments recorded more than 90% of submerged conidia were viable There was no varied significantly (p<0.05) in the Colony Forming Unit (CFU) for all 3 different submerged treatment.

Virulence of Fungal Submerged Conidia and Mycelia with Submerged Conidia from Different Medium on C. Curvignathus

In high concentration of submerged conidia (1×10^9 submerged conidia mL^−1), there was no 100% mortality over 10 days of assessment (Table 3). Conversely, fungal mycelia with submerged conidia did cause 100% mortality over 10 days of assessment (Table 4). In addition, the 100% of fungal mycelium with submerged conidia (no dilution) from Jenkins medium caused 100% of termite mortality on day 4 post inoculation and 100% mortality on day 7 post inoculation for 50% concentration (1:1 ddH2O dilution of fungal culture with submerged conidia in medium). Probit regression analysis of the mortality data of C. curvignathus on day 14 for submerged conidia of M. anisopliae var. anisopliae on different liquid medium (Table 5), showed that submerged conidia from Jenkins medium had lower LC50 compared to Leland medium and MPOB medium. It means that submerged conidia from Jenkins medium were significantly more virulent compared to the other two medium.

Table 3. Pathogenicity of M. anisopliae submerged conidia from different medium on C. curvignathus

| Submerged conidia mL^−1 | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 |
|-------------------------|----|----|----|----|----|----|----|----|----|-----|
| Jenkins medium          | 5.0±0.58a | 7.5±0.29k | 10.0±1.00n | 12.5±0.58l | 15.0±0.58h | 17.5±0.29k | 20.0±1.15l | 22.5±0.29k | 25.0±0.29m | 27.5±0.29p |
| MPOB medium             | 7.5±0.58a | 10.0±1.00n | 12.5±0.58l | 15.0±0.58h | 17.5±0.29k | 20.0±1.15l | 22.5±0.29k | 25.0±0.29m | 27.5±0.29p | 30.0±1.00k |
| Leland medium           | 10.0±1.00n | 12.5±0.58l | 15.0±0.58h | 17.5±0.29k | 20.0±1.15l | 22.5±0.29k | 25.0±0.29m | 27.5±0.29p | 30.0±1.00k | 32.5±0.29n |

| Mean values presented as means ±standard errors |

\(^1\)Means values presented as means ± standard errors
Table 4. Pathogenicity of *M. anisopliae* mycelia with submerged conidia from different medium on *C. curvignathus*

| Treatment | Daily mortality rate in mean percentage (% mortality) |
|-----------|-----------------------------------------------------|
| Concentration (%) | M | J | L | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 |
| 0% | 42.7±0.17a | 25.3±0.12g | 16.0±1.15j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j |
| 0.5% | 38.7±0.17b | 43.3±0.12j | 32.0±0.10n | 32.0±0.10n | 32.0±0.10n | 32.0±0.10n | 32.0±0.10n | 32.0±0.10n | 32.0±0.10n | 32.0±0.10n | 32.0±0.10n | 32.0±0.10n | 32.0±0.10n |
| 1% | 32.7±0.15d | 21.3±0.47h | 20.0±0.58h | 20.0±0.58h | 20.0±0.58h | 20.0±0.58h | 20.0±0.58h | 20.0±0.58h | 20.0±0.58h | 20.0±0.58h | 20.0±0.58h | 20.0±0.58h | 20.0±0.58h |
| 1.5% | 29.3±0.10g | 18.0±1.00i | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j | 16.0±1.00j |
| 2% | 25.3±0.10f | 14.0±0.06i | 12.0±0.06i | 12.0±0.06i | 12.0±0.06i | 12.0±0.06i | 12.0±0.06i | 12.0±0.06i | 12.0±0.06i | 12.0±0.06i | 12.0±0.06i | 12.0±0.06i | 12.0±0.06i |
| 4% | 23.3±0.10l | 8.0±0.06i | 7.0±0.06i | 7.0±0.06i | 7.0±0.06i | 7.0±0.06i | 7.0±0.06i | 7.0±0.06i | 7.0±0.06i | 7.0±0.06i | 7.0±0.06i | 7.0±0.06i | 7.0±0.06i |
| 50% | 18.0±1.00i | 4.0±0.06i | 3.0±0.06i | 3.0±0.06i | 3.0±0.06i | 3.0±0.06i | 3.0±0.06i | 3.0±0.06i | 3.0±0.06i | 3.0±0.06i | 3.0±0.06i | 3.0±0.06i | 3.0±0.06i |

Means values presented as means ± standard errors. All values of LC50 and 95% confidence limits are in submerged conidia/mL.

Table 5. Probit regression analysis of the mortality data of *C. curvignathus* on day 14 for submerged conidia of *M. anisopliae* var. *anisopliae* on different liquid medium

| Liquid medium | Regression equation | Chi-square heterogeneity (calculated) | LC50 | 95% Confidence limits of LC50 |
|---------------|---------------------|--------------------------------------|------|-----------------------------|
| Jenkins medium | Y=0.297X+3.936 | 5.050 | 3.807×10^7 | 3.360×10^7 | 5.050 | 1.786×10^4 |
| MPOB medium   | Y=0.270X+3.846 | 1.672 | 1.904×10^7 | 2.227×10^7 | 1.672 | 8.227×10^6 |
| Leland medium | Y=0.283X+3.009 | 1.932 | 1.099×10^7 | 3.018×10^6 | 1.932 | 5.681×10^5 |

All values of LC50 and 95% confidence limits are in submerged conidia/mL.

Similarly, the probit regression analysis of the mortality data of *C. curvignathus* on day 2 for mycelia with submerged conidia of *M. anisopliae* var. *anisopliae* on different liquid medium (Table 6), showed fungal culture from Jenkins medium required lower quantity of mycelia with submerged conidia to cause mortality of termites.

**Discussion**

On day 1 and day 2, fungal spores were adapting in the new environment and thus the fungal growth rate was lower. However, on day 3 the germinated fungal spores started utilizing the nutrient in the liquid medium and growth rate increased rapidly. During the growth phase, the fungus utilized the nutrient in the medium and caused...
decrease of the nutrient. On day 4 there was a gradual decrease in growth as a result of the lower nutrient availability. Fungal growth in Jenkins and Leland medium entered into stationary phase in day 4 while that of MPOB medium which had higher nutrient contents continued on the growth phase till day 5 after which the fungus entered stationary phase (Fig. 1). *Metarhizium anisopliae* growth was greatly dependent on the sources and amount of carbon and nitrogen (Mustafa and Kaur, 2009; Shah et al., 2005). In Jenkins medium, sucrose was a carbon source and yeast extract was a nitrogen source. In Leland medium carbon source was derived from fructose and nitrogen from yeast extract and lecithin. MPOB medium which has longer growth phase in the fermentation process consists of high carbon sources with 2% of dextrose and high nitrogen sources with 1% of yeast extract and 1% of peptone. Although fungal culture in Jenkins medium had significantly lower growth rate compared to Leland medium and MPOB medium on day 3, it had higher submerged conidia yield. In favourable condition with lots of nutrient, the fungus grew vegetatively until most of the nutrients were depleted. Then, in unfavourable condition with high competition for nutrients, the fungus induced spores for reproduction. In Jenkins medium which has lower nutrients, the fungus achieved maximum growth at day 3 producing corresponding highest weight yield of submerged conidia, after which the lag phase set in. For Leland medium and MPOB medium which had higher nutrient contents than Jenkins medium, the fungus continued its growth phase after day 3 with mycelia yield dominating the growth medium. The adaptation and characteristic of fungus culture from different medium had impact on the fungus pathogenicity and nutrition had effect on fungal conidia production and conidia quality (Shah et al., 2005). Hence, liquid fermentation with Jenkins medium can produce *M. anisopliae* var. *anisopliae* cultures pathogenic to termite *C. curvignathus*. Fungal cultures from the liquid state fermentation have previously been reported to have lower pathogenicity compared to aerial conidia produced from solid state fermentation (Roberts and Sweeney, 1982). However, mass production of fungal cultures from liquid fermentation was much easier compared to solid state or two stage fermentation. Furthermore, the former was cheaper and the resources to mass produce were more readily available compared to solid state fermentation. In addition some research showed that the alteration of medium can enhance the pathogenicity of submerged form of fungal culture (Leland et al., 2005).

From the result, submerged conidia alone were less pathogenic to termite. However, the submerged conidia when applied en masse with liquid medium were more virulent to the termite. This may be due to the isolation process that detached the submerged conidia from the fungal phialides which may affect viability of the submerged conidia. This explains why the submerged conidia without removed from the fungal submerged culture were more virulent to termite. Hoe et al. (2009) reported that *M. anisopliae* var. *anisopliae* at a high concentration of 1×108 conidia mL⁻¹ (aerial conidia) caused 100% mortality in day 3; while in the present study it took 4 days to achieve similar mortality result. However, the time period required to produce that quantity of aerial conidia were much longer compared to liquid state fermentation. In recent years, entomopathogen such as *M. anisopliae* has been reported as not effective or not practical to use for biocontrol due to the defense mechanisms in allogrooming fungal spores by termites nest mate and cellular encapsulation by termite’s own immune system (Chouvenc et al., 2009; Yanagawa et al., 2008). In most or all research on fungus as a biocontrol agent of termites, fungal aerial spores produced in agar plate or from solid state fermentation was the sole infective unit to apply to termite. However, the aerial spores were usually harvested from surface culture with use of surfactant such as Tween 20, Tween 80, Triton-X or SDS and there was a report that surfactant will reduce spore viability and germination (Mishra et al., 2013). The pathogenicity of aerial spores depended on the active spores; however, topical application using a specific spore concentration did not indicate that all the spores used were viable. Spore reproduction from both sexual and asexual cycle in fungi can either be capable to germinate immediately or stay in dormancy until a favorable condition for germination (Garraway and Evans, 1984). Thus, the inactive, unviable or dormant spores may lead to the termite itself or the nest mates to remove the spores from the body. Consequently, further research is required to evaluate the application and practical use of entomopathogen *M. anisopliae* to control termite. The present study which compares the effectiveness of fungal submerged conidia and submerged culture with both submerged conidia and mycelia may help enhance the effectiveness of the entomopathogenic fungus, although the mechanism of infection in this case remain unknown.

**Conclusion**

Mass production of fungus on liquid state fermentation was much easier and faster compared to solid state fermentation. From the result has showed even though the fungus cultured in Jenkins medium does not has higher growth rate but it has the higher submerged conidia yield and higher pathogenicity compared to fungus cultured in MPOB medium and Leland medium. Thus, Jenkins medium can produce much batter fungus culture to apply as entomopathogenic fungus.

**Acknowledgement**

This research was supported by Fundamental Research Grant Scheme (FRGS) 07-04-10-9111FR of the Ministry of Higher Education, Malaysia.
Author's Contributions

All authors equally contributed in this work.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Bharati, T., J. K. Kulkarni, P.U. Krishnaraj and A.R. Alagawadi, 2007. Effect of different carbon sources on the biomass of Metarhizium anisopliae (Ma2). Karnataka J. Agric. Sci., 20: 310-311.

Chouvenc, T., N.Y. Su and A. Robert, 2009. Cellular encapsulation in the eastern subterranean termite, Reticulitermes flavipes (Isoptera), against infection by the entomopathogenic fungus Metarhizium anisopliae. J. Invertebr. Pathol., 101: 234-241. DOI: 10.1016/j.jip.2009.05.008

Garraway, M.O. and R.C. Evans, 1984. Spore dormancy, activation and germination. Fungal Nutrition Physiology, A Wiley-Interscience Publication, pp: 212-230.

Hoe, P.K., J. Bong, K. Jugah and A. Rajan, 2009. Evaluation of Metarhizium anisopliae var. Anisopliae (Deuteromycotina: Hyphomycete) Isolates and their Effects on Subterranean Termite Coptotermes curvignathus (Isoptera: Rhinotermitidae). Am. J. Agric. Biological Sci., 4: 289-297. DOI: 10.3844/ajabssp.2009.289.297

Jackson, M.A. and S.T. Jaronski, 2009. Production of microsclerotia of the fungal entomopathogen Metarhizium anisopliae and their potential for use as a biocontrol agent for soil-inhabiting insect. Mycol. Res., 113: 842-850. DOI: 10.1016/j.myeres.2009.03.004

Jackson, M.A., A.R. Payne and D.A. Odelson, 2004. Liquid-culture production of blastospores of the bioinsecticidal fungus Paecilomyces fumosoroseus using portable fermentation equipment. J. Ind. Microbiol Biotechnol., 31: 149-154. DOI: 10.1007/s10295-004-0127-8

Jenkins, N.E. and C. Prior, 1993. Growth and formation of true Conidia by Metarhizium flavoviridae in a simple liquid medium. Mycol. Res., 97: 1489-1494. DOI: 10.1016/S0953-7562(09)80223-2

Leland, J.E., D.E. Mullins, L.J. Vaughan and H.L. Warren, 2005. Effects of media composition on submerged culture spores of the entomopathogenic fungus, Metarhizium anisopliae var. acridum Part 2: Effects of media osmolality on cell wall characteristics, carbohydrate concentrations, drying stability and pathogenicity. Biocontrol Sci. Technol., 15: 393-409. DOI: 10.1080/09583150400016910

Li, D.P. and D.G. Holdom, 1995. Effects of nutrients on colony formation, growth and sporulation of Metarhizium anisopliae (Deuteromycotina: Hyphomycetes). J. Invertebrate Pathol., 65: 253-260. DOI: 10.1006/jipa.1995.1039

Mehta, J., N. Kaushal, P. Sen, D.R. Sharma and M.S. Dhillon et al., 2012. Impact of carbon and nitrogen sources on the Verticillium lecanii and Metarhizium anisopliae-entomopathogenic fungi. Eur. J. Exper. Biology, 2: 1278-1283.

Mishra, S., P. Kumar and A. Malik, 2013. Evaluation of Beauveria bassiana spore compatibility with surfactants. World Academy Sci. Eng. Technol., 73: 115-119.

Moslin, K., N. Kamarudin and M.B. Wahid, 2009. Pathogenicity of granule formulations of Metarhizium anisopliae against the larvae of the oil palm rhinoceros beetle, Oryctes rhinoceros (L.). J. Oil Palm Res., 21: 602-612.

Mustafa, K. and G. Kaur, 2009. Effects of carbon and nitrogen sources and ratio on the germination, growth and sporulation characteristic of Metarhizium anisopliae and Beauveria bassiana isolates. African J. Agric. Res., 3: 922-930.

Nelson, T.L., A. Low and T.R. Glare, 1996. Large Scale Production of New Zealand Strain of Beauveria and Metarhizium. New Zealand Plant Protection Society (Inc.), pp: 257-261.

Rangel, D.E.N., A.J. Anderson and D.W. Robert, 2006. Growth of Metarhizium anisopliae on non-preferred carbon sources yields conidia with increased UV-B tolerance. J. Invertebrate Pathol., 93: 127-134. DOI: 10.1016/j.jip.2006.05.011

Robert, D.W. and A.W. Sweeney, 1982. Production of Fungi Imperfecti with Vector Control Potential. In Invertebrate Pathology and Microbial Control. Proceedings of the 3rd International Colloquium on Invertebrate Pathology, University of Sussex, pp: 409-413.

Shah, F.A., S.W. Cheng and T.M. Butt, 2005. Nutrition influences growth and virulence of the insect-pathogenic fungus Metarhizium anisopliae. FEMS Microbiol. Lett., 251: 259-266. DOI: 10.1016/j.femsle.2005.08.010

Yanagawa, A., F. Yokohari and S. Shimizu, 2008. Defense mechanism of the termite, Coptotermes formosanus Shiraki, to entomopathogenic fungi. J. Invertebrate Pathol., 97: 165-170. DOI: 10.1016/j.jip.2007.09.005