UNIVERSITI PUTRA MALAYSIA

MASS PRODUCTION, FORMULATION AND DELIVERY OF METARHIZIUM ANISOPLIAE VAR. ANISOPLIAE FOR CONTROL OF SUNTERRANEAN TERMITE COPTOTERMES CURVIGNATHUS HOLMGREN (ISOPTERA: RHINOTERMITIDAE) IN OIL PALM ON PEAT

NYAM VEI TING

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By

NYAM VEI TING

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

July 2015

DEDICATION

Dedicated to my family and love ones who supported me throughout my research work

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

MASS PRODUCTION, FORMULATION AND DELIVERY OF *METARHIZIUM ANISOPLIAE* VAR. *ANISOPLIAE* FOR CONTROL OF SUBTERRANEAN TERMITE *COPTOTERMES CURVIGNATHUS* HOLMGREN (ISOPTERA: RHINOTERMITIDAE) IN OIL PALM ON PEAT

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March 2015

Chairman : Joseph Bong Choon Fah, PhD

Faculty : Agriculture and Food Sciences, (Bintulu)

The termite *Coptotermes curvignathus*is a very important pest of oil palm in peat. Conventional methods such as using spraying chemical pesticide and physical barrier do not provide long term control. An alternative method by using entomopathogenic fungus has great potential as a biopesticide against the termite. However, in order to effectively use entomopathogenic fungus to control the subterranean termite, the fungus quality in terms of the ability cause epizootic to termite, method of delivery and application in field are the main concern to researcher. In this study, liquid state fermentation was used to increase the fungus quality and to obtain large quantity in shorter time. Three different types of liquid mediums which were Jenkins medium, Leland medium and MPOB medium were used to mass a produce pathogenic local isolate of *Metarhizium anisopliae* var. *anisopliae*. Fungus in Jenkins medium yielded significantly highest number of submerged conidia with 2.36 x 10^9 submerged conidia/mL even in lowest growth rate on day 3 after inoculation. Fungus culture from Jenkins medium with concentration of 1:0 fungus culture to distilled water showed ability to cause 100% mortality in day 4 after treatment compared to MPOB medium in day 6 after treatment and Leland medium in day 9 after treatment. The fungus was then incorporated with 8 different combinations of bait matrix to formulate into a bait which can attract termite, reduce repellence, prolong active ingredient (fungus) shelf life, and sustain the ability to control termite colony. From the choice feeding test, bait matrix made of dextrin, PVP K90 and skim milk (DPS) impregnated with the fungus has the ability to attract termite compared to other bait matrix. Furthermore, DPS bait has ability to cause 100% mortality on 19 days after treatment. Termite took shorter time to finish the DPS bait with 11.2 day to consume all the baits, and consumed significantly larger quantity of DPS bait compared to the 7 other baits which consisted of different proportion of skim milk, dextrin, PVP K90 and kaolin. Shelf life of the baits were tested through bioassays. All baits were able

to maintain consistent termite mortality of more than 80% for over 6 months. A specially designed bait delivery system was adapted to local peat environment was developed to deliver the selected baits DPS and PS infested oil palm tree in the field. Over the 6 months trial period, bait designed delivery system was able to protect the bait from getting wet by rain water and the high water table in peat soil. Termites were found colonizing the PVP K90 plus skim milk (PS) bait chamber as early as one week after application. Termites collected from the vicinity of the bait chamber were found infected and killed by *M. anisopliae.* There were no *C. curvignathus* found in the previously infested palm trees at the end of the trial period. The formulated bait delivered in termite baiting system had shown effectiveness in the control of termite in oil palm plantation in peat soil area.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PRODUKSI BESAR-BESARAN, FORMULASI DAN CARA MENGHANTAR *METARHIZIUM ANISOPLIAE* VAR. *ANISOPLIAE* BAGI MENGWAL ANAI-ANAI BAWAH TANAH *COPTOTERMES CURVIGNATHUS* HOLMGREN (ISOPTERA: RHINOTERMITIDAE) PADA KELAPA SAWIT DI TANAH GAMBUT

Oleh

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Anai-anai *Coptotermes curvignathus* merupakan serangga pesrosak yang penting di ladang kelapa sawit pada tanah gambut. Pengawalan yang lazim digunakan seperti semburan racun bahan kimia dan membina halangan fizikal tidak memberi kawalan jangka masa panjang. Satu kawalan alternatif menggunakan kulat entomopatogenik mempunyai potensi besar dijadikan sebagai racun biologi untuk kawalan anai-anai. Namun, dalam usaha untuk menggunakan kulat entomopatogenik dengan lebih efektif dalam kawalan anai-anai, kualiti kulat dalam keupayaan penyebaran penyakit berjangkit dalam anai-anai, langkah penyampaian kulat kepada anai-anai dan aplikasinya di ladang merupakan tumpuan utama bagi para penyelidik. Dalam kajian ini, fermentasi dalam bentuk cecair digunakan untuk meningkatkan kualiti kulat dan untuk menghasilkan kuantiti kulat yang banyak dalam masa yang singkat. Tiga jenis medium cecair berlainan digunakan ialah medium Jenkins, medium Leland, dan medium MPOB bagi menghasilkan isolat tempatan *Metarhizium anisopliae* var. *anisopliae* secara besar-besaran. Kulat yang dikultur dalam mediums Jenkins menaghasilkan konidia terendam yang paling tinggi dengan 2.36×10^9 konidia terendam/mL walaupun dengan kadar pertumbuhan yang paling rendah pada 3 hari selepas inokulasi. Kultur kulat dari medium Jenkins dengan kepekatan nisbah 1:0 kultur kulat kepada air suling yang steril menunjukkan ada keupayaan menyebabkan 100% kematian pada hari ke 4 selepas diinokulasi berbanding dengan medium MPOB pada hari ke 6 selepas diinokulasi dan hari ke 9 selepas diinokulasi bagi medium Leland. Kulat kemudiannya dijadikan 8 jenis umpan dengan kombinasi matriks yang berbeza untuk dijadikan umpan yang boleh menarik perhatian anai-anai, mengurangkan pencegahan, memanjangkan jangka hayat bahan aktif (kulat), dan dapat mengekalkan mengawal koloni anai-anai. Dalam ujian pemilihan makanan, matriks umpan yang diperbuat daripada dextrin, PVP K90 dan susu skim (DPS)

direspi dengan kulat mempunyai keupayaan menarik anai-anai daripada matriks umpan lain. Tambahan lagi, umpan DPS mempunyai keupayaan menyebabkan 100% kematian pada hari ke 19 selepas dirawat. Anai-anai mengambil masa lebih pendek untuk menghabiskan umpan DPS dengan 11.2 hari untuk kesemua umpan dan ia juga memakan umpan DPS dengan kuantiti yang banyak berbanding dengan 7 jenis umpan lain yang terdiri daripada campuran antara susu skim, dextrin, PVP K90, dan kaolin. Jangkan hayat umpan telah diuji dengan bioesei. Semua umpan dapat mengekal lebih daripada 80% kadar kematian yang konsisten selama 6 bulan. Sistem penyampaian umpan yang direka khas sesuai untuk digunakan di persekitaran tanah gambut telah dibentuk bagi menyampaikan umpan pilihan DPS dan PS untuk mengawal pokok kelapa sawit yang diserang di ladang. Selama 6 bulan kajian ini dijalankan, sistem penyampaian umpan yang direka dapat melindungi umpan dari basah oleh air hujan dan parasa air yang tinggi di tanah gambut. Anai-anai ditemui menyerang kebuk umpan PVP K-90 dengan susu skim (PS) dalam satu minggu selepas aplikasi. Anai-anai yang dikutip daripada kawasan ruang umpan itu didapati dijangkit dan dibunuh oleh *M. anisopliae.* Tambahan lagi, tiada *C. curvignathus* dijumpa di pokok kelapa sawit yang diserang sebelum ini pada akhir tempoh kajian. Umpan yang dirumuskan dan sistem penyampaian telah menunjukkan keberkesanan dalam kawalan anai-anai di ladang kelapa sawit yang ditanam pada tanah gambut.

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This thesis is submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. Members of the
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DECLARATIONS

Declaration by graduate student

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Signature: _____ Date: 26.10.2015

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LIST OF ABBREVIATIONS

a.i	Active ingredient
mm	Milimetre
m	Micrometre
ha	Hectare
UV	Ultra violet
PDA	Potato Dextrose Agar
SDAY	Sabouraud Dextrose Agar with 1% yeast extract
°C	Degree Celsius
RH	Relative humidity
DNMRT	Duncan's New Multiple Range Test
mL	Millilitre
µL	Microlitre
h	Hour
CFU	Colony forming unit
sdH_2O	Sterile distilled water
%	Percentage
mg	Milligrams
mcg	Micrograms
CRD	Completely Randomized Design
ANOVA	Analysis of Variances
MPOB	Malaysia Palm Oil Board
LC	Lethal concentration
PVP K-90	Polyvinyl pyrrolidone K-90 series
g	Gram
cm	Centimetre
rpm	Round per minute
D	Day
USA	United State of America

CHAPTER 1

INTRODUCTION

Termite, the existence of which was fearsome to human, devours human goods that contain cellulose source especially in building manufactured with wood, agricultural assets, and others that contain cellulose materials. The existence of termites as a pest to human goods has cost extensive loss to individual, company or government. And yet, their biology notably their subterranean or cryptic habit makes it difficult to detect their presence in human goods (Zulkefli *et al.,* 2006). They are usually detected only after the damage appears in the surface and by that time it may be too late to revive or secure the infested goods.

In Malaysia, termite has been reported to attack the oil palm which is country's most important economic crop (Zulkefli *et al.,* 2006). Currently, spraying chemical pesticides is the only method used to control termite of oil palm in Malaysia. However, it does not provide long term control nor eliminate the termite colony in the field (Chan and Bong, 2008). Chemical treatment must be applied regularly to prevent the termite attack, and this is costly, and may cause environmental pollution.

Termite baiting system which is the most applicable method in termite control has evolved (Su, 2003; Lenz and Evans, 2002). However, it has not been applied or adapted in oil palm plantation, except for some minor trials on bait products provided by manufacturers. There is no report on the product effectiveness being published, except for termite control in building.

There are over two thousand species of termite and only a few species are considered pests to human (Krishna *et al*., 2013). *Coptotermes* sp. is one of the most aggressive species that destroy human properties. *Coptotermes curvignathus* is the most dominant species reported to infest oil palm in Malaysia and Indonesia (Lim and Silek, 2001, Cheng *et al.,* 2008, Chan *et al.,* 2011).

Various entomopathogenic fungus such as *Metarhizium anisopliae, Beauveria bassiana* and *Paecilomyces fumosoroseus* has been lab tested that can use for control termite (Chouvenc *et al.,* 2011), but only *Metarhizium anisopliae* has been develop into a product to use for control termite (Lenz, 2005). Nonetheless, there was no single report been publish on their efficacy in field. Sun *et al.* (2003) has stated that *Metarhizium anisopliae* was more effective compared with *Beauveria bassiana* for control *Coptotermes formosanus.* Local virulence isolate *Metarhizium anisopliae* var. *anisopliae* has ability to cause epizootic to termite (Hoe *et al.*, 2009).
Local isolate entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae* has the potential to use as biocontrol agent to control termite. However, how to produce a large quantity of fungus that is highly pathogenic, and how to deliver the fungus to termite are the main considerations to ensure effective use of fungus as biocontrol agent for termite in the field.

Therefore, the objective of this study were:

1. Mass production of a local isolate of entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae* in liquid state fermentation for control of *Coptotermes curvignathus*.
2. Develop a bait formulation for a local *Metarhizium anisopliae* var. *anisopliae* isolate to control *Coptotermes curvignathus* in oil palm on peat.
3. Design and develop a termite bait delivery system applicable in oil palm plantation in peat soil area.

REFERENCES

Al Fazairy, A.A and F. A. Hassan. 1988. Infection of termites by *Spodoptera littoralis* Nuclear Polyhedrosis Virus. *International Journal of Tropical Insect Science* **9(1)**: 37-39.

Auld, B.A. 1992. Mass production, formulation, application of fungi as biocontrol agents. In *Biological Control of Locusts and Grasshoppers,* ed. C. J. Lomer and C. Prior: 239-244. C. A. B. International.

Basri, M.W., K. Norman; A.S. Idris, D. Ariffin, S. Shamala, M. Ramle, and S. A. A. Ramlah. 2003. Hand Book of Pests and Disease of Oil Palm, MPOB, Bangi: 113.

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. Agri. Sci.,* **20(2)**: 310-311.

Bignell, D.E. and P. Eggleton. 1998. Termites. In: Calow, P. (ed) *Encyclopedia of Ecology and Environmental Management.* Blackwell scientific, Oxford: 774-746.

Brey, P.T., J.P. Latge, and M.C. Prevost. 1986. Integumental penetration of pea aphid, *Acyrthosiphon pisum* by *Conidiobolus obscures* (Entomophthoraceae). *J. Invertebr. Pathol,* **48**: 34-41.

Bühler, V. 1998. Kolidon: Polyvinylpyrrolidone for the pharmaceutical industry 4th edition. BASF.

Carvalho, J., C. Gonçalves, A. M. Gil, and F. M. Gama. 2007. Production and characterization of a new dextrin based hydrogel. *European Polymer Journal,* **43**: 3050-3059.

Chan, S. P., C.F.J. Bong, and W.H. Lau. 2011. Damage pattern and nesting characteristic of *Coptotermes curvignathus* (Isoptera: Rhinotermitidae) in oil palm on peat. *American Journal of Applied Sciences* **8(5)**: 420-427.

Chan, S.P. and C.F.J. Bong. 2008. Susceptibility of planted forest species to *Coptotermes curvignathus* Holmgren. In *Proceedings of 2nd USM Penang International Postgraduate Convention 2008 and 4th Life Science Postgraduate Conference USM 2008.*

Cheng, S., L.G. Kirton and S. Gurmit. 2008. Termite attack on oil palm grown on peat soil: identification of pest species and factors contributing to the problem. The Planter 84(991);659-670

Chouvenc, T. and N. Y. Su. 2010. Apparent synergy among defense mechanisms in subterranean termite (Rhinotermitidae) against epizootic events: Limits and potential for biological control. *J. Econ. Entomol*, 103(4): 1327-1337.

Chouvenc, T., C.A. Efstathion, M.L. Elliott, and N.-Y. Su. 2013. Extended disease resistance emerging from the faecal nest of a subterranean termite. In *Proc. Roy. Soc. B Lond.* **280**:20131885. doi:10.1098/rspb.2013.1885.

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.

Chouvenc, T., N.Y. SU and J.K. Grace. 2011. Fifty years of attempted biological control of termite – Analysis of a failure. *Biological control* **59**: 69-82.

Chouvenc, T., N.Y. Su and M.I. Elliott. 2008. Antifungal activity of the termite alkaloid norharmane against the mycelial growth of *Metarhizium anisopliae* and *Apergillus nomius*. *Journal of Invertebrate Pathology* **99**: 345-347.

Cliquet, S. and M. A. Jackson, 1997. Comparison of Air-Drying Methods for Evaluating the Desiccation Tolerance of Liquid Culture-Produced Submerged conidia of *Peacilomyces fumosoroseous*. *World Journal of Microbiology and Biotechnology,* **13**: 299-303.

Cornelius, M.L. and J.K Grace. 1995. Laboratory evaluations of three ant species with the Formosan subterranean termite (Isoptera: Rhinotermitidae). *Sociobiology* **26**: 291-298.

Cornelius, M.L. and J.K Grace. 1996. Effect of two ant species (Hymenoptera: Formicidae) on the foraging and survival of the Formosan subterranean termite (Isoptera: Rhinotermitidae). *Environ. Entomol*. **25**:85-89.

Cornelius, M.L. and J.K Grace. 1997. Effect of termite soildiers on the foraging behaviour of *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in the presence of predatory ants. *Sociobiology* **29**:247-253.

Dawn, H.G., A.S. Krik, O. Carl, and B. Paul. 2001. Drywood Termites. The University of Arizona.

Delate, K.M., K.J. Grace and C.H.M. Tome. 1995. Potential use of pathogenic fungi baits to control the Formosan subterranean termite (Isopt., Rhinotermitidae). *Journal of Applied Entomology* **119**: 429-433.

Driver, F., R.J Milner, and J.H.W. Trueman. 2000. A Taxonomic Revision of *Metarhizium* Based on a Phylogenetic Analysis of rDNA Sequence Data. *Mycol. Res* **104(2**): 134-150.

Easwaramoorthy, S. 2003. Entomopathogenic Fungi. In *Biopesticides & Bioagents in Intergrated Pest management of Agricultural Crop.* Ed. Srivastava, R.P.: 341-379. International Book Distributing Co.

Eggleton, P. 2000. Global Patterns of Termite Diverdsity. In *Termites: Evolution, Sociality, Symbioses, Ecology,* ed.Abe:, T.,Bignell, D. E. & Higashi, M. (eds.). Kluwer Acedemic Publishers: 25-52.

Fishel, F.M. 2010. Pesticide formulations. University of Florida, Agronomy Department.

Forschler, B.T. and T.M. Jenkins. 2001. Subterranean termites in the urban landscape: Understanding their social structure is the key to successfully implementing population management using bait technology. *Urban Ecosystem* **4**: 231-251. Kluwer Academic Publisher.

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

Gay, F.J. 1969. Species Introduce by man. In *Biology of Termite volume 1*, ed. Krishna, K. and Weesner, F.M. 459-494. Academic Press, New York and London.

Ghadimi, H. and P. Pecora. (1963) Frees amino acids of different kinds of milk. *American Journal of Clinical Nutrition* **13**: 75-81.

Goettel, M.S. and D.W. Robert. 1992. Mass Production, Formulation and Field Application of Entomopathogenic Fungi. In *Biological Control of Locusts and Grasshoppers,* ed. C. J. Lomer and C. Prior: 230 - 238. C. A. B. International.

Grace, J.K. 1997 Biological control strategies for suppression of termites. *Journal of Agricultural Entomology* **14(3**): 281-289.

Grace, J.K. and N.Y. Su. 2001 Evidence supporting the use of termite baiting systems for long-term structural protection (Isoptera). *Sociobiology* **37(2)**: 301-310.

Greathead, D.J. 1992. Natural enemies of tropical locusts and grasshoppers: their impact and potential as biological control agents. In *Biological Control of Locusts and Grasshoppers,* ed. C. J. Lomer and C. Prior: 105 - 121. C. A. B. International.

Gunner, H.B., Kane, J. and Duan, H. 1994. Biological Control of Termites. PCT Patent Application #WO94/04034.

Haifig, I., F. F. Marchetti and A. M. Costa-Leonardo. 2010. Nutrients affecting food choice by the pest subterranean termite *Coptotermes gestroi* (Isoptera: Rhinotermitidae). *International Jounral of Pest Management* **5(4):** 371-375.

Harris, W.V. 1961. Termite: Their recognition and control. Longman Group Ltd.

Hickin, N.E. 1971. Termites. A world problem. Hutchinson and Co Ltd, London: 13-23.

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

Homathevi, R. and T. Noel. 2003. Isoptera (Termite): Adapted From Inventory and Collection. Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, *Malayan Nature Journal*.

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. *Mycological Research*: 1-9.

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.

Jackson, M.A., M.R. Mcguire, L.A. Lacey, and S.P. Wraight. 1997. Liquid culture production of desiccation tolerant blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus. Mycological research* **101(1)**: 35-41.

Jenkins, N. E. and C. Prior, 1993. Growth and Formation of True Conidia by *Metarhizium flavoviridae* in a simple liquid medium. *Mycol Res.* **97(12)**: 1489-1494.

Jenkins, N.E., G. Heviefo, J. Langewald, A.J. Cherry, and C.J. Lomer. 1998. Development of Mass Production of Technology for Aerial Conidia for use as Mycopesticides. *Biocontrol News and Information.* **19**: 21N-31N.

Kambhampati, S. and P. Eggleton. 2000. Phylogenetics and Taxanomy. In *Termites: Evolution, Sosiality, Symbiosis, Ecology,* ed.Abe:, T.,Bignell, D. E. & Higashi, M. (eds.). Kluwer Acedemic Publishers: 1-23.

Khan, K.I., R.H. Jafri, and M. Ahmad. 2008. Discovery and pathogenicity of *Pseudomonas fluorescens* against various species of termite. *Punjab University Journal of Zoology* **23(1-2)**: 47-57.

Khoo, K.C., P.A.C. Ooi and C.T. Ho. 1991. Crop pests and their management in Malaysia.1st Edn., Tropical Press, Kaula Lumpur, Malaysia, ISBN-10:9677300695: 242.

Koehler, P.G. and C.L. Tucker. 2003. Subterranean Termites. University of Florida, Entomology and Nematology Department.

Krishna, K. and F.M. Weesner. 1969. Biology of termite, volume 1. Academic Press, New York and London.

Krishna, K., D.A. Grimaldi, V. Krishna, and M.S. Engel. 2013. Treatise on the Isoptera of world: References and index. *Bulletin of the American Museum of Natural History.*

Leland, J. E., D. E. Mullins, L. J. Vaughan, and H. L. Warren, 2005. Effect 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 science and Technology,* **15(4)**: 393-409.

Lenz, M. 2005. Biological control in termite management: The potential of nematodes and fungal pathogens. In *Proceeding of the Fifth International Conference on Urban Pests,* ed. C.Y. Lee and W. H. Robinson: 47-52.

Lenz, M. and T. A. Evans. 2002. Termite bait technology: perspectives from Australia. In *Proceeding of the 5th International Conferences on Urban Pests.* Charleston, South Carolina, US. 7-10 July 2002. Pocahantas Press: Blacksburg Va.

Li, D. P. and D. G. Holdom. 1995. Effects of nutrients on colony formation, growth, and sporulation of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). *Journal of Invertebrate Pathology* **65**: 253-260.

Lim, K.H. and B. Silek. 2001. Termite infestation on Oil Palm Planted on deep peat in Sarawak: Tradewinds experience. Proceeding of the 2001 PIPOC International Palm Oil Congress Agricultural Conferences, Aug. 20-22, Kuala Lumpur Malaysia: 365-368.

Lo, N., Tokuda, G., Watanabe, H., Rose, H., Slaytor, M., Maekawa, K., Bandi, C. and Noda, H. 2000. Evidence from multiple gene sequences indicates that termites evolved from wood-feeding cockroaches. *Current Biology* **10**: 801-804.

Logan, J.W.M. and R.H. Cowie and T.G. Wood. 1990. Termite (Isoptera) control in agriculture and forestry by non-chemical methods: a review. *Bulletin. of Entomological. Research*. **80**:309-330.

Lucas-Clements, R. H. 1981. Use of skimmed milk in human foods in developing countries. *Journal of the Society of Diary Technology*, **34**: 67-70.

Mehta, J., N. Kaushal, P. Sen, D. R. Sharma, M. S. Dhillon, and B. L. Mathuriya. 2012. Impact of carbon and nitrogen sources on the *Verticillium lecanii* and *Metarhizium anisopliae* – entomopathogenic fungi. *European Journal of Experimental Biology,* 2012, **2(4)**: 1278-1283.

Mendonca, A. F. 1992. Mass Production, Formulation and Application of *Metarhizium anisopliae* for Control of Sugarcane Froghopper, *Mahanarva posticata* in Brazil. In *Biological Control of Locusts and Grasshoppers,* ed. C. J. Lomer and C. Prior: 239-244. C. A. B. International.

Milner, R.J. and Staples, J.A. 1996. Biological control of termites results and experiences within a CSIRO project in Australia. *Biocontrol Science and Technology* **6:** 3- 9.

Milner, R.J., Staples, J.A., Lenz, M., Lutton, G.G., McRae, C.F. and Watson, J.A.L. 1997 Insect pest control. US Patent #5,595,746.

Mishra, S., P. Kumar, and A. Malik. 2013. Evaluation of *Beauveria bassiana* spore compatibility with surfactants. *World Academy of Science, Engineering and Technology,* **73:** 115-119.

Moslim, 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). *Journal of Oil Palm Research* **21**: 37-49.

Murray, H. H. 1961. Industrial applications of Kaolin. In *Proceedings of the Tenth National Conference on Clay and Clay Minerals,* ed. A. Swineford: 291-298.

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 Journal of Agricultural research,* **3(10)**: 922-930.

Nelson, T. L., A. Low, and T. R. Glare. 1996. Large Scale Production of New Zealand Strain of *Beauveria* and *Metarhizium. Proc. 49th N.Z. Plant Protection Conf. 1996:* 257-261.

Pawar, V.M., P.S. Borikar and U.T. Thombre. 2005. Microbial pesticide. In *Crop protection: management strategies,* ed. Parsad, D.:3 -26. Daya Publishing House Delhi.

Pearce, M. J. 1987. Seals, tombs, mummies and tunnelling in the drywood termite *Cryptotermes* (Isoptera: Kalotermitidae). *Sociobiology* **13**:217-226.

Peng, G.X., Z.K. Wang, Y.P. Yin, D.Y. Zeng, and Y.X. Xia. 2008. Field Trials of *Materhizium anisopliae* var. acridum (Ascomycota: Hypocreales) Against Oriental Migratory Locusts, *Locusta migratoria manilensis* (Meyen) in Northern China. *Journal of Crop Protection* **27**: 1244-1250.

Prior, C. 1992. Discovery and Characterization of Fungal for Locust and Grasshopper Control. In *Biological Control of Locusts and Grasshoppers,* ed. C. J. Lomer and C. Prior: 159-180. C. A. B. International.

Ramle, M., M.W. Basri, K. Norman, S. Mukesh, and S.A.A. Ramlah. 1999. Impact of *Metarhizium anisopliae* (Deuteromycotina: Hyphomyycetes) Applied by Wet and Dry Inoculums on Oil Palm Rhinoceros Beetles, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). *Journal of Oil Palm Research* vol. **11 (2)**: 25-40.

Ramle, M., M.W. Basri, K. Norman, S.A.A Ramlah, and N.H. Hisham. 2006. Research into the Commercialization of *Metarhizium anisopliae* (Hyphomycetes) for Biocontrol of Rhinoceros Beetle, *Oryctes rhinoceros* (Scarabaeidae), in Oil Palm. *Journal of Oil Palm Research (special issue – April 2006)*: 37-49.

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. *Journal of Invertebrate Pathology*, **93**: 127-134.

Rath, A.C. 2000. The use of entomopathogenic fungi for control of termites. *Biocontrol Science and Technology* **10**: 563-581.

Robert, D. W. and A. W. Sweeney. 1982. Production of fungi imperfecti with vector control potential. In *Invertebrate Pathology and Microbial Control. Proceedings 3rd International Colloquium on Invertebrate Pathology, University of Sussex,* 6- 10 September 1982: 409-413.

Robert, D.W., 1981. Toxins of entompathogenic fungi. *Microbial control of pests and Plant Disease.*: 441-464.

Sajap, A. S., L.C. Lee, D. Ouimette and A.M. Jaafar. 2005. Field evaluation of noviflumuron for controlling Asian subterranean termite, *Coptotermes gestroi* (Isoptera: Rhinitermitidae). Proceeding of the Fifth International Conferences on Urban Pests: 238-241.

Sajap, A. S., S. Amit and J. Welker. 2000. Evaluation of Hexaflumuron for Controlling the Subterrranean Termite *Coptotermes curvignathus* (Isoptera: Rhinotermitidae) in Malaysia. *J. Econ. Entomol.* **93**: 429-433.

Sandoval-Coronado, C. F., H. A. Luna-Olvera, K. Arevalo-Nino, M. A. Jackson, T. J. Poprawski, and L. J. Galan-Wong. 2001. Drying and Formulation of Balstospores of *Peacilimyces fumosoroseus* (Hyphomycetes) produced in two different liquid media. *World Journal of Microbiology and Biotechnology* **17**: 423-428.

Shah, F. A., S. W. Cheng, and T. M. Butt. 2005. Nutrition influences growth and virulence of the insect-pathogenic fungus *Metarhizium anisopliae. FEMS Microbiology Letters,* **251**: 259-266.

Stansly, P.A., N.Y. Su and J.M. Conner. 2001. Management of subterranean termite *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in a citrus orchard with hexaflumuton bait. *Crop protection,* **20**: 199-206.

Su, N.Y. 1994. Field evaluation of hexaflumuron bait for population suppression of subterranean termites (Isoptera: Rhinotermitdae). *J. Econ. Entomol,* **87(2)**: 389-397.

Su, N.-Y. 2003. Overview of the global distribution and control of the Formosan subterranean termite. *Sociobiology* **41**: 7-16.

Su, N.Y. 2005. Response of the Formosan subterranean termites (Isoptera: Rhinotermitidae) to baits or nonrepellent termiticides in extended foraging arenas. *Journal of Economic Entomology* **98(6)**: 2142-2152.

Su, N.Y., P. M. Ban and R.H. Scheffrahn. 2002. Control of subterranean termite populations at San Critóbal and El Morro, San Juan National Historic Site. *Journal of Cultural Heritage*, **3**: 217-225.

Sun, J.Z., J.R., Fuxa, and G. Henderson. 2003. Effects of Virulence, Sporulation, and Temperature on *Metarhizium anisopliae* and *Beauveria bassiana* Laboratory Transmission in *Coptotermes formosanus. Journal of Invertebrate Pathology* **84**: 38-46.

Thapa, R.S. 1981. Termites of Sabah. Sabah Forest Record Number 12. Sabah Forest Department. Sandakan.

Tho, Y. P. 1992. Termites of Peninsular Malaysia. Malayan Forest Record No. 36. *Forest Research Institute Malaysia.*

Tulloch, M. 1976. The Genus *Metarhizium. Trans. Br. Mycol. Soc.* **66(3)**: 407-411.

Watanabe, T. 2002. Soil and Seed Fungi, Morphologies of Cultured Fungi and Key to Species. 2nd edition: 306.

Wood, T.G. and W.A. Sands. 1978. The role of termites in ecosystems. In Production ecology of ants and termites (M.V. Brian, Ed.): 245-292. Cambridge University.

Yanagawa, A., F. Yokoharu, and S. Shimizu, 2008. Defense mechanism of termite, *Coptotermes formosanus. Journal of Invertebrate Pathology* **97**: 165-170.

Zulkefli, M., K. Norman, M.W. Basri, A. Zamani, and N.J. Khairul. 2006. Rubstake – Rubber Wood Stake for Detecting Subterranean Termites in Peat Soil. MPOB Information Series: June 2006.

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LIST OF PUBLICATIONS

Nyam, V. T. and C. F. J. Bong. 2010. Mass production of local isolates of *Metarhizium anisopliae* for control of subterranean termite *Coptotermes curvignathus.* 15th Biological Sciences Graduate Congress (BSGC), 15-17th December 2010, Institute of Biological Sciences, University of Malaya.

Nyam, V. T., C. F. J. Bong and P. J. H. King. 2015. Control of subterranean termite *Coptotermes curvignathus* (Isoptera: Rhinotermitidae) by entomopathogen *Metarhizium anisopliae* var. *anisopliae* cultured in liquid state fermentation. *American Journal of Agricultural and Biological Sciences* **10(1):** 35-40 **(**published)