

# 綠殭菌與木黴菌之劑型配方研究

簡年佑、謝建元

E-mail: 9318481@mail.dyu.edu.tw

## 摘要

本文乃針對綠殭菌(*Nomuraea rileyi*)及木黴菌(*Trichoderma virens*)之劑型配方加以研究探討，主要克服微生物製劑在保存期間菌體活性的穩定度，以及減緩紫外線對孢子之殺傷力。在綠殭菌劑型方面，將回收之分生孢子以35℃、1hr加熱處理後，懸浮於含有1%葡萄糖之沙拉油中，此劑型配方在冷藏條件(8±1℃)下具有最佳保存效果，保存3~6個月之發芽率，僅由63%降為46%，其對三齡期甜菜夜蛾幼蟲(*Spodoptera exigua*)致死率仍有63.3%；而無製劑保存之孢子粉，發芽率於保存5個月已降至4%。若將新鮮孢子油劑添加0.5%氧化鋅，經30Lux紫外光照射30min後，孢子活性仍有74%，其對三齡期甜菜夜蛾幼蟲致死率達80%，而無劑型之孢子粉活性已趨於零。在木黴菌劑型方面，最佳保存配方是將分生孢子經35℃加熱1hr後，懸浮於含有1%蔗糖之沙拉油中，在冷藏條件下保存6個月，發芽率仍可維持51%，而無劑型之孢子粉發芽率僅剩23%。此外，在孢子油劑中添加0.5%氧化鋅，經30Lux紫外光照射120min，其孢子發芽率仍達41%，而無劑型之孢子粉發芽率已趨於零。

關鍵詞：綠殭菌；木黴菌；劑型配方

## 目錄

封面內頁 簽名頁 授權書.....	iii	中文摘要.....	v	英文摘要.....	v
要.....	vi	誌謝.....	viii	目錄.....	ix
錄.....	x	表目錄.....	ix	第一章 前言.....	1
文獻回顧.....	3	2.1劑型配方之種類及其重要性.....	3	2.2研發微生物製劑所應具備之條件.....	4
2.3影響製劑穩定性之因素.....	6	2.3.1填料.....	7	2.3.2儲藏溫度.....	9
2.3.3含水量.....	9	2.3.4孢子儲存濃度.....	10	2.3.5包裝條件.....	11
2.3.6界面活性劑.....	11	2.3.7抗氧化劑.....	12	2.3.8添加促進發芽的營養物.....	12
2.3.9紫外線保護劑.....	12	2.4環境因素對微生物劑型之影響.....	14	2.4.1物理因素.....	15
2.4.2化學因素.....	17	2.4.3生物因素.....	18	第三章 材料與方法.....	20
3.1試驗菌種.....	20	3.2實驗設備.....	20	3.3試藥.....	21
3.3.1綠殭菌之培養基.....	21	3.3.2木黴菌之培養基.....	21	3.3.3界面活性劑.....	22
3.3.4惰性填充物.....	22	3.3.5紫外線保護劑.....	22	3.3.6其他添加物.....	23
3.4固態培養分生孢子.....	24	3.4.1培養綠殭菌分生孢子.....	24	3.4.2培養木黴菌分生孢子.....	25
3.5平板製作.....	26	3.6發芽率之檢測.....	26	3.7孢子之回收.....	26
3.8維持孢子活性之劑型配方.....	27	3.8.1油基劑.....	27	3.8.1.1不同溶劑油及孢子保存濃度對活性之影響.....	27
3.8.1.2不同加熱溫度及添加物對孢子活性之影響.....	28	3.8.1.3不同水含量對孢子活性之影響.....	28	3.8.1.4添加抗氧化劑對孢子活性之影響.....	29
3.8.1.5界面活性劑之選擇.....	29	3.8.2可濕性粉劑.....	30	3.8.2.1測定劑型在水中的懸浮率.....	31
3.8.2.2研磨對於孢子活性之影響.....	31	3.9添加營養源對孢子發芽之影響.....	31	3.10最佳保存製劑之生物檢定.....	31
3.11抗紫外線之劑型配方.....	33	3.11.1紫外光照射強度(Lux)之測量.....	33	3.11.2不同紫外光照射強度對孢子及油劑活性之影響.....	33
3.11.3不同保護劑對於孢子抗紫外線之影響.....	33	3.11.4添加沙拉油對孢子抗紫外線之影響.....	34	3.12最適保護劑配方之生物檢定.....	34
3.13統計分析.....	35	第四章 結果與討論.....	36	4.1綠殭菌.....	36
4.1.1綠殭菌油劑.....	36	4.1.1.1綠殭菌孢子儲存濃度與油劑種類對活性之影響.....	39	4.1.1.2不同加熱溫度及添加物對綠殭菌油劑活性之影響.....	47
4.1.1.3不同水含量對綠殭菌孢子活性之影響.....	48	4.1.1.4添加抗氧化劑對綠殭菌油劑活性之影響.....	52	4.1.1.5界面活性劑之選擇.....	57
4.1.2綠殭菌可濕性粉劑.....	57	4.1.2.1測定劑型在水中之懸浮率.....	57	4.1.2.2研磨對於綠殭菌孢子活性之影響.....	59
4.1.2.3添加營養源對綠殭菌孢子發芽之影響.....	59	4.1.3最佳保存製劑之生物檢定.....	61	4.1.4抗紫外線之劑型配方.....	64
4.1.4.1不同紫外光照射強度對綠殭菌孢子及油劑活性之影響.....	64	4.1.4.2不同保護劑對於綠殭菌孢子抗紫外線之影響.....	64	4.1.4.3添加沙拉油對綠殭菌孢子抗紫外線之影響.....	64
4.1.4.4最適抗紫外線配方之生.....	73	4.1.5.1.....	73	4.1.5.2.....	73
4.1.5.3.....	73	4.1.5.4.....	73	4.1.5.5.....	73

物檢定	74	4.2木黴菌	77	4.2.1木黴菌油劑	77	4.2.1.1木黴菌孢子儲存濃度與油劑種類對活性之影響	80
						4.2.1.2不同加熱溫度及添加物對木黴菌油劑活性之影響	80
						4.2.1.3不同水含量對木黴菌孢子活性之影響	84
						4.2.1.4添加抗氧化劑對木黴菌油劑活性之影響	84
						4.2.2木黴菌可濕性粉劑	91
						4.2.2.1測定劑型在水中之懸浮率	91
						4.2.2.2研磨對於木黴菌孢子活性之影響	91
						4.2.3添加營養源對木黴菌孢子發芽之影響	94
						4.2.4抗紫外線之劑型配方	96
						4.2.4.1不同紫外光照射強度對木黴菌孢子及油劑活性之影響	96
						4.2.4.2不同保護劑對於木黴菌孢子抗紫外線之影響	96
						4.2.4.3添加沙拉油對於木黴菌孢子抗紫外線之影響	99
						4.2.4.4添加沙拉油對於木黴菌孢子抗紫外線之影響	99
五章 結論						109 參考文獻	112
藥的劑型形態						120 附錄二碳、氮、氫元素分析表	121
						122 附錄四玉米澱粉之成分表	123
方						124 附錄六論文口試問題	125
影響						圖4.1儲存溫度對綠殭菌孢子活性之影響	41
						圖4.2室溫下綠殭菌孢子儲存濃度與油劑種類對活性之影響	40
						圖4.3冷藏下綠殭菌孢子儲存濃度與油劑種類對活性之影響	42
						圖4.4室溫下加熱溫度及添加物對綠殭菌油劑活性之影響	45
						圖4.5冷藏下加熱溫度及添加物對綠殭菌油劑活性之影響	45
						圖4.6室溫下不同水含量對綠殭菌孢子活性之影響	46
						圖4.7冷藏下不同水含量對綠殭菌孢子活性之影響	49
						圖4.8添加抗氧化劑TBHQ對綠殭菌油劑活性之影響	53
						圖4.9研磨對於綠殭菌孢子活性之影響	50
						圖4.10添加營養源對室溫最佳劑型之影響	60
						圖4.11添加營養源對冷藏最佳劑型之影響	62
						圖4.12紫外光照射強度對綠殭菌孢子及油劑活性之影響	63
						圖4.13添加0.1%紫外線保護劑對綠殭菌孢子活性之影響	66
						圖4.14添加0.5%紫外線保護劑對綠殭菌孢子活性之影響	69
						圖4.15添加0.1%紫外線保護劑對綠殭菌孢子油劑活性之影響	70
						圖4.16添加0.5%紫外線保護劑對綠殭菌孢子油劑活性之影響	71
						圖4.17在30Lux紫外光下添加沙拉油及0.5%氧化鋅對綠殭菌油劑活性之影響	72
						圖4.18在30Lux紫外光下添加沙拉油及0.5%氧化鋅對綠殭菌油劑活性之影響	75
						圖4.19儲存溫度對木黴菌孢子活性之影響	81
						圖4.20室溫下木黴菌孢子儲存濃度與油劑種類對活性之影響	82
						圖4.21冷藏下木黴菌孢子儲存濃度與油劑種類對活性之影響	83
						圖4.22室溫下加熱溫度及添加物對木黴菌油劑活性之影響	83
						圖4.23冷藏下加熱溫度及添加物對木黴菌油劑活性之影響	85
						圖4.24室溫下不同水含量對木黴菌孢子活性之影響	86
						圖4.25冷藏下不同水含量對木黴菌孢子活性之影響	89
						圖4.26添加抗氧化劑TBHQ對木黴菌油劑活性之影響	90
						圖4.27研磨對於木黴菌孢子活性之影響	92
						圖4.28添加營養源對室溫最佳劑型之影響	95
						圖4.29添加營養源對冷藏最佳劑型之影響	97
						圖4.30紫外光照射強度對木黴菌孢子及油劑活性之影響	98
						圖4.31添加0.1%紫外線保護劑對木黴菌孢子活性之影響	100
						圖4.32添加0.5%紫外線保護劑對木黴菌孢子活性之影響	101
						圖4.33添加0.1%紫外線保護劑對木黴菌孢子油劑活性之影響	102
						圖4.34添加0.5%紫外線保護劑對木黴菌孢子油劑活性之影響	103
						圖4.35在30Lux紫外光下添加沙拉油及0.5%氧化鋅對木黴菌油劑活性之影響	104
						圖4.36在300Lux紫外光下添加沙拉油及0.5%氧化鋅對木黴菌油劑活性之影響	107
						表目錄 表4.1不同界面活性劑對綠殭菌孢子油劑懸浮率之影響	55
						表4.2不同HLB值對綠殭菌孢子油劑懸浮率之影響	56
						表4.3不同界面活性劑對綠殭菌可濕性粉劑懸浮率之影響	58
						表4.4綠殭菌製劑配方之生物檢定	65
						表4.5綠殭菌抗紫外線配方之生物檢定	65
						表4.6不同界面活性劑對木黴菌可濕性粉劑懸浮率之影響	78

## 參考文獻

- 王樹成 (1996) 真菌殺蟲劑劑型的研究現狀。安徽農業大學學報, 23 (3): 375-380。
- 李農昌、王樹成、唐燕平、李增智 (1996) 白殭菌油劑劑型的研究。安徽農業大學學報, 23 (3):329-335。
- 林明申 (2003) 二階段發酵時不同生長條件對綠殭菌產孢之影響。私立大葉大學食品工程學系碩士論文, 彰化。
- 林姿儀、柯伶怡、謝建元、羅朝村 (2002) 以農業廢棄物進行木黴菌固態發酵之探討。第七屆生化工學研討會論文集(二), pp.1099-1104。
- 唐立正 (1997) 本地產綠殭菌感染玉米穗夜蛾之研究。國立中興大學昆蟲學系博士論文, 台中。
- 高穗生、黃莉欣 (1992) 甜菜夜蛾核多角體病毒紫外線保護劑之效果評估。中華昆蟲, 12:31-40。
- 黃建忠、梁宗琦、劉愛英 (1992) 粉被蟲草無性型對蘇雲桿菌抗紫外線的保護效應。西南農業大學學報, 5(2):63-67。
- 劉惠盈 (1999) 黑殭菌及蠟蚧輪枝菌之劑型配方研究。朝陽科技大學應用化學研究所碩士論文, 台中。
- 殷鳳鳴 (1983) 白殭菌孢子粉儲存試驗。廣東林業科技通訊, (3/4):13-16。
- 殷鳳鳴 (1986) 用不同包裝材料儲存白殭菌孢子粉試驗。廣東林業科技通訊, 3:35-36。
- 湯堅、黃長春、丁珊、王成樹、李增智 (1996) 安徽農業大學學報, 23(3):351-354。
- 蔡俊良 (1997) 生物型農藥製劑配方之研究 水分散性粒劑。中國文化大學造紙印刷研究所造紙組碩士論文, 臺北。
- 謝建元 (2000) 黑殭菌之研發與應用。2000年台灣生物農藥生物科技研討會論文集, pp.38-50。
- 謝建元、洪文凱、高穗生、王順成、曾耀銘 (1998) 本土黑殭菌以固態和液態發酵生產黑殭菌素之探討。中國農業化學會誌, 36(4):371-379。
- 薛一祥 (2001) 綠殭菌發酵最適化條件之探討。大葉大學食品工程學系碩士論文, 彰化。
- Anderson, T. E., Hajek, A. E., Roberts, D. W., Preisler, H. K. and Robertson, J. L. (1989) Colorado potato beetle (Coleoptera : Chrysomelidae):Effect of combinations of Beauveria bassiana withinsecticides, J. Econ. Entomol., 82: 83-89.
- Ball, B. V., Pye, B. J., Carreck, N. L., Moore, D. and Bateman, R. P. (1994) Laboratory testing of a mycopesticide

on non-target organisms: the effects of an oil formulation of *Metarhizium flavoviride* applied to *Apis mellifera*, *Biocontrol Sci. Technol.*, 4: 289-296.

18. Barnes, S. E. and Moore, D. (1997) The effect of fatty, organic or phenolic acids on the germination of conidia of *Metarhizium flavoviride*, *Mycol. Res.*, 101: 662-666.

19. Bateman, R. P., Carey, D. M. and Prior, C. (1993) The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities, *Ann. Appl. Biol.*, 122: 145-152.

20. Batta Y. A. (2003) Production and testing of novel formulations of the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina : Hyphomycetes), *Crop Protection.*, 22:415-422.

21. Bull, D. L., Ridgway, R. L., House V. S. and Pryor, N. W. (1976) Improved formulations of the *Heliothis nuclear polyhedrosis virus*, *J. Econ. Entomol.*, 69: 731-736.

22. Couch, T. L. and Ignoffo, C. M. (1981) Formulation of insect pathogens, in " Microbial Control of Pests and Plant Disease 1979~1980 " Academic press, London., pp. 621-634.

23. Douro-Kpindou, O. K., Godonou, I., Houssou, A., Lomer, C. J. and Shah, P. A. (1995) Control of *Zonocerus variegatus* by ultra-low volume application of an oil formulation of *Metarhizium flavoviride* conidia, *Biocontrol Sci. Technol.*, 5:131-139.

24. Daoust, R. A., Ward, M. G. and Roberts, D. W. (1983) Effect of formulation on the viability of *Metarhizium anisopliae* conidia, *J. Invertebr. Pathol.*, 41:151-160.

25. Fargues, J., Reisinger, O., Robert, PH. and Aubart, C. (1983) Biodegradation of entomopathogenic hyphomycete: influence of clay coating on *Beauveria bassiana* blastospore in soil, *J. Invertebr. Pathol.*, 41:131-142.

26. Glare, T. R. (1987) Effect of host species and light conditions on production of conidia by an isolate of *Nomuraea rileyi*, *J. Invertebr. Pathol.*, 50: 67-69.

27. Hidalgo, E., Moore, D. and Lepatourel, G. (1998) The effect of different formulations of *Beauveria bassiana* on *Sitophilus zeamais* in stored maize, *J. stored Prod. Res.*, 34(2/3):171-179.

28. Homer, D. W. (1988) *Trichoderma* as a biocontrol agent, CRC Press, Boca Raton., pp. 211.

29. Hunt, M. D. (1994) Effect of sunscreens, irradiance and resting periods on the germination of *Metarhizium flavoviride* conidia, *Entomophaga.*, 39(3/4): 2509-2512.

30. Im, D. J., Aguda, R. M. and Rombach, M. C. (1988) Effect of nutrients and pH on the growth and sporulation of four entomogenous hyphomycetes fungi, *Korean J. Appl. Entomol.*, 27:41-46.

31. Inglis, G. D., Goettel, M. S. and Johnson, D. L. (1995) Influence of ultraviolet light protectants on persistence of the entomopathogenic fungus, *Beauveria bassiana*, *Biological Control.*, 5:581-590.

32. Ignoffo, C. M., Garcia, C. and Hostetter, D. L. (1976) Effects of temperature on growth and sporulation for the entomopathogenic fungus *Nomuraea rileyi*, *Environ. Entomol.*, 5: 935-936.

33. Ignoffo, C. M., Garcia, C. and Kroha, M. J. (1982) Susceptibility of larvae of *Trichoplusia ni* and *Anticarsia gemmatialis* to intrahemocoelic injections of conidia and blastospores of *Nomuraea rileyi*, *J. Invertebr. Pathol.*, 39: 198-202.

34. Ignoffo, C. M., Garcia, C., Hostetter, D. L. and Pienll, R. E. (1975) Sensitivity of the entomopathogenic fungus *Nomuraea rileyi* to chemical pesticides used on soybeans, *Environ. Entomol.*, 4: 765-768.

35. Jaques, R. P. (1971) Tests on protectants for foliar deposits of a polyhedrosis virus, *J. Invertebr. Pathol.*, 17: 9-16.

36. Jaques, R. P. (1972) The inactivation of foliar deposits of viruses of *Trichoplusia ni* (Lepidoptera : Noctuidae) and *Pieris rapae* (Lepidoptera : Pieridae) and tests on protectant additives. *Can. Ent.*, 104: 1985-1994.

37. Keller, S. (1991) The use of blastospore of *Beauveria brongniartii* to control *Melolontha melolontha* in Switzerland, in Proceedings of an International Conference. Biopesticides, Theory and Practice, Ceske Budejovice, Czechoslovakia.

38. Lingg, A. J. and Donaldson, M. D. (1981) Biotic and abiotic factors affecting stability of *Beauveria bassiana*, *J. Invertebr. Pathol.*, 38:191-200.

39. Matewele, P. O. B. (1986) The biology of the fungi *Tolyposcladium cylindrosporum* and *Culicinomyces clavispurus* in mosquitoes, PhD thesis, University of Southampton.

40. Moore, D. and Caudwell, R. W. (1997) Formulation of entomopathogens for the control of locusts and grasshoppers, *Mem. Entomol. Soc. Can.*, 171:49-67.

41. Moore, D., Bridge, P. D., Higgins, P. M., Bateman, R. P. and Prior, C. (1993) Ultra-violet radiation damage to *Metarhizium flavoviride* conidia and the protection given by vegetable and mineral oils and chemical sunscreens, *Annals of Applied Biology.*, 122: 605-616.

42. Moore, D., Douro-Kpindou, O. K., Jenkins, N. E. and Lomer, C. J. (1995) Long term storage of *Metarhizium flavoviride* conidia in oil formulations for the control of locusts and grasshoppers, *Biocontrol Sci. and Technol.*, 5:193-199.

43. Pinnock, D. E. (1977) Effect of water content to the storage of *Beauveria bassiana*, *J. Invertebr. Pathol.*, 23:341-346.

44. Prior, C., Jollands, P. and Le-Patourel, G. (1988) Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina : hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera : Curculionidae), *J. Invertebr. Pathol.*, 52: 66-72.

45. Shapiro, M., Agin, P. P. and Bell, R. A. (1983) Ultraviolet protectants of the gypsy moth (Lepidoptera : Lymantriidae) nucleopolyhedrosis virus, *Environ Entomol.*, 12: 982-985.

46. Stathers, T. E., Moore, D. and Prior, C. (1993) The effect of different temperatures on the viability of *Metarhizium flavoviride* conidia stored in vegetable and mineral oils, *J. Invertebr. Pathol.*, 62:111-115.

47. Tang, L. C. and Hou, R. F. (2001) Effects of environmental factors on virulence of the entomopathogenic fungus, *Nomuraea rileyi*, against the cynr earworm, *Helicoverpa argimera*, *J. Appl. Ent.*, 125: 243-248.

48. Terror, G. E. and Kramer, J. P. (1977) Effect of ultraviolet radiation on the microsporidian *Octospora muscaedomesticae* with reference to protectants provided by the host *Phormia regina*, *J. Invertebr. Pathol.*, 30: 348-353.

49. Vimala Devi, P. S. (1994) Conidia production of the entomopathogenic fungus *Nomuraea rileyi* and its evaluation for control of *Spodoptera litura* (Fab) on *Ricinus communis*, *J. Invertebr. Pathol.*, 63: 145-150.

50. Vimala Devi, P. S. and Prasad, Y. G. (1996) Compatibility of oils and antifeedants plant origin with the entomopathogenic fungus *Nomuraea rileyi*, *J. Invertebr. Pathol.*, 68: 91-93.

51. Walstad, J. D., Anderson, R. F. and Stambaugh, W. J. (1970) Effects of environmental conditions on two species of muscardine fungus (*Beauveria bassiana* and *Metarhizium anisopliae*), *J. Invertebr. Pathol.*, 16:221-226.

52. Ward, M. G. (1984) Formulation of biological insecticides surfactant and diluent selection. *Advances in Pesticide Formulation Technology.*, pp.177-184.

53. Witt, D. J. and Stairs, G. R. (1975) The effect of ultraviolet irradiation on a Baculovirus infecting *Galleria mellonella*, *J. Invertebr. Pathol.*, 26:321-327.

54. Zimmermann, G. (1982) Effect of high temperatures and artificial sunlight on the viability of conidia of *Metarhizium anisopliae*, *J. Invert. Pathol.*, 40: 39-40.

55. Zimmermann, G. (1993) The entomopathogenic fungus *Metarhizium anisopliae* and its potential as a biocontrol agent, *Pestic. Sci.*, 37: 375-379.