

木黴菌對網室栽培萵苣菌核病之防治效果¹

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摘 要

本研究旨在探討利用木黴菌 (*Trichoderma harzianum* YM-1) 厚膜孢子製劑、枯草桿菌液與施用不同營養液對防治設施栽培萵苣菌核病之效益。萵苣種子分別在含木黴菌厚膜孢子 (1.5×10^7 cfu g⁻¹) 的泥炭土或滅菌之泥炭土中滲調 24 hr，以不滲調為對照，播種後灌注木黴菌懸浮液 (1.5×10^6 cfu⁻¹ ml⁻¹, 3 L m⁻²) 之田區，菌核病發病率分別為 10.7、10.5 及 14.7 %，產量分別為 2.8、2.2 與 2.2 kg m⁻²，而播種後不灌注木黴菌懸浮液之田區，菌核病發病率分別為 9.8、14.2 與 18.7 %，產量分別為 2.1、1.4 與 1.2 kg m⁻²，顯示種子以含木黴菌之介質滲調處理配合田區灌注木黴菌，可降低菌核病感染且產量較高，與對照組有顯著差異；未經木黴菌之介質滲調處理，且未灌注木黴菌之處理雖菌核病罹病度較對照組低，且達 5% 顯著水準，但產量則與對照無差異。施用不同堆肥對結球萵苣菌核病之防治效果顯示：不同肥料處理之田區若灌注木黴菌則發病率較低，其中尤以施用牛糞堆肥再灌注木黴菌之處理效果最佳，發病率僅 0.2%。結球萵苣由苗期至定植後分別灌注木黴菌厚膜孢子 (1×10^8 spore ml⁻¹, 3 L ml⁻²)、中興-100 植物營養液 500 倍與 1000 倍稀釋液及枯草桿菌 (1×10^8 cfu ml⁻¹, 3 L ml⁻²) 懸浮液及不處理 (對照) 之試驗結果顯示，菌核病之罹病度各處理間差異不顯著，產量則分別為 3.32、3.28、3.21、2.43 與 2.99 kg m⁻²，灌注木黴菌與中興-100 之處理產量明顯高於對照，但灌注枯草桿菌懸浮之處理則產量僅 2.43 kg m⁻²，較對照低且達 5% 顯著水準。

關鍵詞：木黴菌、枯草桿菌、菌核病、生物防治、種子滲調、厩肥、堆肥

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前 言

臺灣北部地區蔬菜栽培以塑膠布網室設施為主，複作指數相當高，常達 8-12 作，以致葉菜類受土壤傳播性病害危害之情形相當嚴重，尤其是菌核病、苗立枯病與萎凋病對葉菜類之危害特別嚴重。菌核病嗜低溫之環境（Adams and Ayers, 1979；Nelson 1998；Venette, 1998），每年秋末至次年春末造成北部地區葉菜類嚴重損失，尤其是萵苣、菠菜與甘藍。本病以菌核在土中長期存活，對不良環境與藥劑之耐受性極強，土壤消毒不但無法防治此病害（Agrios, 1988）甚至於會導致其他病害更嚴重（Altman, 1977；Altman and Campbell, 1977；Horsfall, 1979），而目前防治本病害所用之藥劑如撲滅寧、大克爛、貝芬替與貝芬依普同等藥劑因長期使用，病原菌之抗藥性逐漸浮現且抑制土壤中之拮抗菌（Adams et al., 1991），以致防治效果逐年下降，而且殘毒期長，在小葉菜類適用性低，因此，發展生物防治技術，利用木黴菌及其他拮抗微生物配合耕作管理策略，以減輕菌核病之危害已成為植病專家學者之研究主流（李與吳，1984；Huang, 1978；Huang, 1991；Huang et al., 1992；McLaren and Huang, 1996；Papavizas et al., 1984；Papavizas, 1985；Papavizas and Collins, 1990；Tu, 1980）。木黴菌是對菌核病最具抑制性的土棲微生物之一（李與吳，1984），因此，本研究探討利用木黴菌等生物製劑及施用不同堆肥防治萵苣菌核病之效益。

材料與方法

一、木黴菌製劑與種子滲調處理對圓葉萵苣菌核病之防治效果

萵苣種子分別在含分離自楊梅之木黴菌（*T. harzianum* YM-1， 1.5×10^7 cfu g⁻¹）之泥炭土，或滅菌之泥炭土中滲調 24 hr，不作滲調為對照，播種後土壤灌注木黴菌厚膜孢子懸浮液（ 1.5×10^6 cfu⁻¹ ml⁻¹，3 L m⁻²），不灌注為對照，每小區為 3×2.5 m²，採裂區設計（split plot design）每處理四重複。於採收前 4 天調查菌核病發生株率，調查時周圍之 25 cm 寬度作為保護行捨棄調查，中間之（2.5×2）m² 畫成 50×50 cm 之 20 小區隨機調查 5 小區。

二、木黴菌厚膜孢子與不同堆肥處理對結球萵苣菌核病之防治效果

於第二次整地前先分別施牛糞堆肥、豬糞堆肥、雞糞堆肥、豆粕堆肥、碗豆-穀殼

堆肥，及化學肥料（對照）為主區，施肥後以中耕機整地耙勻，而後再分別灌注木黴菌厚膜孢子懸浮液（ 1.5×10^8 spore ml⁻¹，3 L ml⁻²），灌注 48 hr 後定植結球萵苣，於定植後 14 與 28 天再分別灌注一次，及不灌注（對照）為副區，採裂區設計，每處理四重複。株距 35 cm 行距 30 cm 每畦 5 行，種植方式採交叉排列每小區 50 株，於採收前 4 天調查菌核病罹病程度，每小區周圍之 18 株為保護行捨棄調查，中間之 32 株全數調查，罹病程度依地上部表現之症狀分成 5 級。0：無病徵；1：最下位葉呈萎凋（少於 3 片葉）；2：萎凋葉片數達總外葉數之 1/3；3：萎凋葉片數超過總外葉數 1/2 但未腐爛；4：全株 3/4 外葉萎凋且開始腐爛但結球仍乾淨；5：全株外葉腐爛枯死且結球污穢。

$$\text{罹病度} = \Sigma (\text{指數} \times \text{該指數罹病株數}) \times (5 \times \text{總調查株數})^{-1} \times 100\%$$

三、木黴菌厚膜孢子及枯草桿懸浮菌液與植物營養液處理對結球萵苣菌核病之防治效果

萵苣穴盤苗定植前 15、10 及 5 天每穴盤苗分別灌注 500 ml 木黴菌孢子懸浮液（ 1×10^8 spore ml⁻¹）之 500 倍稀釋液、中興-100（CH-100）之 500、1000 倍稀釋液，以及枯草桿菌（*Bacillus subtilis* 百泰生技公司， 1×10^8 cfu ml⁻¹）500 倍稀釋液，及澆水（對照）為副區。以各處理再分別於定植當日、定植後 14 與 28 天每平方公尺再各灌注 3 公升一次，及澆水（對照）為主區，採裂區設計，每處理四重複，種植方式與罹病度調查與前項處理相同。

結果與討論

一、木黴菌製劑與種子滲調處理對防治圓葉萵苣菌核病之效益

萵苣種子在含 1.5×10^7 cfu g⁻¹ 之木黴菌的泥炭土，或滅菌之泥炭土中滲調，及不作滲調處理之對照田區，播種後灌注木黴菌懸浮液之處理，菌核病發病率分別為 10.7、10.5 及 14.7%（表 1），產量分別為 2.8、2.2 與 2.2 kg m⁻²，而播種後不灌注木黴菌懸浮液之田區，菌核病發病率分別為 9.8、14.2 與 18.7%（對照），產量分別為 2.1、1.4 與 1.2 kg m⁻²（對照），顯示含木黴菌之介質滲調處理的種子，播種後不論田區是否灌注木黴菌，均可降低菌核病感。以對立枯絲核菌之感染而言，發芽及胚軸發育較快之苜蓿品系（variety）抗病性較強（Fower et al., 1999），而 Harman 等（1989）

之研究亦顯示，棉花、玉米、胡瓜與碗豆等作物經滲調處理再播種，對猝倒病菌及立枯絲核菌等之感染也表現逃避之情形，但本試驗的結果顯示，萵苣種子以含木黴菌之介質滲調處理可降低菌核病感染，但用滅菌介質之單純滲調處理則無法避免菌核病之危害，係因病原菌的感染型態不同所致。Lifshitz 等（1985）之報告顯示，生物防治製劑對萌前感染（preemergence infection）之病原菌防治效果較佳，而菌核病為萌後感染，故利用木黴菌防治萵苣菌核病，則萵苣種子應提前進行滲調處理，才能逃避菌核病之危害。

表 1. 圓葉萵苣種子利用木黴菌滲調處理與灌注木黴菌厚膜孢子對菌核病感染與產量之影響

Table 1. Efficacy of control of leaf lettuce sclerotinia drop by seed priming and drenching of chlamydospore suspension of *T. harzianum* YM-1

種子處理 Seed treatments	發病率 Disease severity		產量 Yield	
	灌注木黴菌 Drench <i>Trichoderma</i>	不灌注木黴菌 Non-drench check	灌注木黴菌 Drench <i>Trichoderma</i>	不灌注木黴菌 Non-drench check
	-----%-----		-----kg m ⁻² -----	
木黴菌泥炭土滲調 Priming with <i>Trichoderma</i> enriched peat moss	10.7 a* A [#]	9.8 a A	2.8 a A	2.1 a B
滅菌泥炭土滲調 Priming with autoclaved peat moss	10.5 a A	14.2 b B	2.2 b A	1.4 b B
不作滲調對照 Non-priming check	14.7 b A	18.7 c B	2.2 b A	1.2 c B

*：同一欄之數據後的小寫英文字母相同，為鄧肯氏多變域分析 5%水準無顯著差異。

*：Means in the same column with same lowercase letters are not significantly different ($\alpha=0.05$) by Duncan's multiple range test.

#：同一列之數據後的大寫英文字母相同，為鄧肯氏多變域分析 5%水準無顯著差異。

#：Means in the same row with same uppercase letters are not significantly different ($\alpha=0.05$) by Duncan's multiple range test.

[（發病率（Disease incidence）L S D=1.2；產量（Yield）L S D=0.18]

在產量方面，木黴菌不論是在種子滲調或是播種後灌注處理均較高，尤其是種子

以含木黴菌介質滲調，播種後再灌注木黴菌之處理最高，與 Chang 等人之報告一致（Chang et al., 1986；Inbar et al., 1994；Ousley et al., 1993；Paulitz et al., 1986），主要是因木黴菌會誘發植物產生抗病物質（Howell et al., 2000），或寄生於病原菌以抑制病原菌（Chang et al., 1986；Inbar et al., 1994）外，同時具有根圈微生物的特性會促進植物生長（Ahmad and Baker, 1987a；Ahmad and Baker, 1987b；Ristaino and Papavizas, 1985；Windham et al., 1986），因此種子以木黴菌滲調處理，除了抑制病害之外亦可改善作物生長（Harman et al., 1989）。

二、木黴菌厚膜孢子與不同堆肥處理對結球萵苣菌核病防治之效果

專家指出禽畜糞便堆肥（厩肥，cattle manure）可用於增殖木黴菌或導引木黴菌至土壤中加強抑制立枯絲核菌之感染（Gorodecki and Hadar, 1990；Kok et al., 1996；Volland and Epstein, 1994），本試驗中田區施不同堆肥再配合灌注木黴菌以防治結球萵苣菌核病之結果顯示，灌注木黴菌之區域發病率大致較低，在木黴菌灌注處理區以施用牛糞堆肥之處理發病率僅 0.2%，較其他處理為佳（表 2），雞糞堆肥處理區之抑病效果較差，是因木黴菌在雞糞堆肥中不但存活率差，甚至於降低（葉等，2002）。施用牛糞堆肥或碗豆-穀殼堆肥等兩處理，若不灌注木黴菌，則菌核病發生率與對照處理相同，可能係因牛糞堆肥或碗豆-穀殼堆肥含有菌核病喜好的有機物質，而提高菌核病之初始感染源(primary inoculum)的感染潛勢（inoculum potential, Adams and Ayers, 1979；Dow et al., 1988）。

表 2. 灌注木黴菌厚膜孢子及施用不同有機質肥料對結球萵苣菌核病之防治效果

Table 2. Efficacy of controlling of head lettuce sclerotinia drop by soil treatment with various compost followed by drenching chlamydospore suspension of *T. harzianum* YM-1

小區肥料處理 MFertilizer treatment	罹病度 Disease severity	
	灌注木黴菌 Drench <i>Trichoderma</i>	不灌注木黴菌 Non-drench check
	----- % -----	
牛糞堆肥 Composted cattle feces	0.2 a* A [#]	7.2 c B
豬糞堆肥 Composted pig's feces	1.5 ab A	3.3 a B
雞糞堆肥 Composted chicken feces	4.5 c A	4.5 ab A
豆粕堆肥 Composted soybean waste	3.1 bc A	4.3 ab A
碗豆-穀殼堆肥 Composted pea waste + rice hull	2.4 b A	7.5 c B
化學肥料-對照 Chemical fertilizer (ck)	1.1 a A	7.0 c B

*：同一欄之數據後的小寫英文字母相同，為鄧肯氏多變域分析 5%水準無顯著差異。

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#：Means in the same row with same uppercase letters are not significantly different ($\alpha=0.05$) by Duncan's multiple range test.

[發病率 (Disease incidence) L S D=1.2]

三、木黴菌厚膜孢子及枯草桿菌懸浮菌液與植物營養液處理對結球萵苣菌核病之防治效果

已有報告顯示枯草桿菌可防治水稻白葉枯病（林等，2003），且會促進水稻秧苗之生長及抑制 *Sclerotium rolfsii* 造成之水稻秧苗立枯病（林等，2008），及抑制長豇豆鐮孢菌萎凋病（Ho, 2007），也是發展生物防治製劑的項目之一。但本研究之結果顯示，對結球萵苣菌核病而言仍以木黴菌之抑病效果較佳（表 3），其次為 CH-100 植物營養液稀釋 500 倍之處理，自苗期即開始灌注與定植後才開始處理間無顯著差異。CH-100 植物營養液稀釋 1,000 倍及枯草桿菌之處理則均無法抑制菌核病。

有些報告顯示 CH-100 等植物營養液，可促進作物根系生長，及誘發土壤中之拮抗微生物繁殖，改善作物之產量（黃，1993；Huang, 1991；Huang et al., 1992），本試驗中結球萵苣若由苗期至定植後分別各灌注三次木黴菌孢子懸浮液及 CH-100 植物營養液 500 倍與 1000 倍稀釋液處理之結果，產量分別為 3.32、3.28 與 3.21 kg m⁻² 較對照之 2.99 kg m⁻² 提高（表 3），處理間差異達顯著水準，但灌注枯草桿菌懸浮液之處理產量 2.43 kg m⁻²，較對照處理低，處理間差異亦達顯著水準。若苗期不處理至定植後才開始灌注，則灌注木黴菌孢子懸浮液及 CH-100 植物營養液 500 倍稀釋液之處理產量分別為 3.03 及 3.17 kg m⁻² 與對照處理間差異達顯著水準；CH-100 植物營養液之 1000 倍稀釋液、枯草桿菌懸浮液與對照處理之產量分別為 2.94、2.89 與 2.81 kg m⁻²，處理間差異不顯著，顯示 CH-100 植物營養液之 1000 倍稀釋液須自苗期即開始灌注才有實質效益，而灌注枯草桿菌處理對產量似有不良影響，可能係因枯草桿菌對植物之胚軸與幼根之發育初期會造成遲滯現象（Mahaffee and Backman, 1993），若苗期不灌注枯草桿菌懸浮液，則苗期根系發育不受影響，對產量影響就較小。

表 3. 木黴菌及中興-100 植物營養液處理對結球高苣菌核病防治效益

Table 3. Effect of chlamydo spores of *Trichoderma* and CH-100 nutrient suspension on sclerotinia drop of head lettuce

定植後土壤處理 Soil treatments	罹病度 Disease severity		產量 Yield	
	苗灌注 Drench	苗不處理 None	苗灌注 Drench	苗不處理 None
	----- % -----		----- kg m ⁻² -----	
木黴菌厚膜孢子 Trichoderma	9.7a * A [#]	10.3a A	3.32a A	3.03a A
中興-100 500 倍 CH-100 500 ×	10.9ab A	11.1ab A	3.28a A	3.17a A
中興-100 1000 倍 CH-100 1000×	12.1b A	12.4b A	3.21a A	2.94b B
枯草桿菌 Bacillus	11.3ab A	13.9c B	2.43c B	2.89b A
對照不處理 Check	12.9b A	13.1bc A	2.99b A	2.81b B
平均(Average)	11.4 A	12.2 A	30.5 A	29.7 A

*：同一欄之數據後的小寫英文字母相同，為鄧肯氏多變域分析 5%水準無顯著差異。

*：Means in the same column with same lowercase letters are not significantly different ($\alpha=0.05$) by Duncan's multiple range test.

#：同一列之數據後的大寫英文字母相同，為鄧肯氏多變域分析 5%水準無顯著差異。

#：Means in the same row with same uppercase letters are not significantly different ($\alpha=0.05$) by Duncan's multiple range test.

[發病率 (Disease incidence) L S D=0.78；產量 (Yield) L S D=1.2]

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Efficacy of *Trichoderma harzianum* as biocontrol agents for management of Lettuce Sclerotinia drop in plastic nethouse¹

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Abstract

The purpose of this study was to evaluate the effectiveness of chlamydo-spores of *Trichoderma* on management of lettuce sclerotinia drop. In the experiment, lettuce seeds were conditioned respectively with peat containing 1.5×10^7 spores g^{-1} of *T. harzianum* YM-1, disinfected peat, or non-conditioned check, followed by drenching field with chlamydo-spore suspension (1.5×10^6 spores ml^{-1} , 3 L m^{-2}). Disease severities of sclerotinia drop of lettuce were 10.7, 10.5, and 14.7%, respectively, yields were 2.8, 2.2 and 2.2 kg m^{-2} , respectively. On the other hand, in the non-drenched field, disease severity of sclerotinia drop of lettuce was 9.8, 14.2, and 18.7%, respectively, and yields were 2.1, 1.4 and 1.2 kg m^{-2} , respectively. It showed that seed priming and field drench with *Trichoderma* could reduce the damage of lettuce by sclerotinia drop disease, and we suggest that priming substrate have to contain *Trichoderma*. Disease severity of sclerotinia drop of head lettuce was lower in the field drenched with *Trichoderma* chlamydo-spore, especially that previously treated with certain composted cattle feces. The disease severity of lettuce drop was 0.2%, the difference was significantly against 7.0% of check. In the experiments that drenching with *Trichoderma* chlamydo-spore, CH-100 500x, CH-100 1000x, or *Bacillus* during nursery stage or after transplant. The disease severities of lettuce drop in the fields drenched with *Trichoderma* chlamydo-spore and CH-100 500x, were significantly different from that of other treatments. On the other hand, the difference were insignificantly among CH-100 1000x, *Bacillus* and check. The yields were 3.32, 3.28, 3.21, 2.43 and 2.99 kg m^{-2} respectively for that drenched full stage. The yields of lettuce in the field treated with *Trichoderma* chlamydo-spore and CH-100 were higher than the check, but that treated with *Bacillus* was even lower than the check.

Key words: *Trichoderma*, *Bacillus*, sclerotinia drop, biocontrol, seed priming, CH-100 stable manure, compost

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