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堅強芽孢桿菌可濕性粉劑在田間防治番茄南方根瘤線蟲的可行性評估

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摘　要

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本試驗主要目的為測試堅強芽孢桿菌 (*Bacillus firmus*) 在田間防治番茄南方根瘤線蟲 (*Meloidogyne incognita*) 病害的可行性評估。試驗以堅強芽孢桿菌 >1x10^9 CFU/g可濕性粉劑 (*B. firmus*, CGMCC No.1.2010 WP) 400、800、1200倍稀釋液，以500 g/L 氟派瑞水懸劑 (fluopyram SC) 稀釋4,000 倍為參考藥劑，並以無菌水作為對照組。田間試驗採逢機完全區集設計，分三場次進行。處理後60天調查田間土壤線蟲蟲口數，結果對照組介於33.6-42.8 nematodes/100 g soil，而堅強芽孢桿菌處理組與氟派瑞處理組，則分別介於20.8-30.8與10.3-22.4 nematodes/100 g soil之間，兩處理組的線蟲數皆與對照組具有顯著差異 ($p< 0.05$)。調查供試番茄植株根部根瘤線蟲的罹病度，結果顯示對照組的罹病度介於43.8-51.6%，而堅強芽孢桿菌處理組與氟派瑞處理組，則分別介於17.1-21.7%與11.2-18.8%之間，兩者的罹病度皆與對照組具有顯著差異 ($p< 0.05$)。進一步分析堅強芽孢桿菌處理組與氟派瑞處理組的根瘤線蟲病害防治率，分別介於58-61%與63.6-76.2%之間。根據以上結果顯示，堅強芽孢桿菌 (*B. firmus*, CGMCC No.1.2010 WP) 在田間具有防治番茄根瘤線蟲的潛力。

關鍵詞：南方根瘤線蟲、番茄、堅強芽孢桿菌、生物防治

緒　言

植物寄生性線蟲常造成田間作物根系受損，影響作物收穫品質與產量，甚至會造成植株死亡，其中以根瘤線蟲 (*Meloidogyne* spp.) 的危害最為嚴重[12]。根瘤線蟲是田間最常見的植物寄生性線蟲，尤其是排水良好的沙質土壤或介質栽培的環境，其主要傳播方式為水流、土壤或介質、種苗及繁殖體[11]。南方根瘤線蟲 (*M. incognita*) 是番茄 (*Solanum lycopersicum*) 的主要病害之一[16]，其寄主範圍廣泛，在土壤中的殘存能力強，因此短時間內難以以淹水、休耕、輪作等方式成功防治，如發生在長期栽培的果樹類作物，則更是一大隱憂[1]。已知農田中有超過61種雜草是根瘤線蟲的寄主[8]，加上農田土地利用率高，連作及周年栽植作物，常營造根瘤線蟲可大量繁殖的環境，而一般的罹病田很難完全清除根瘤線蟲的感染源[13]。隨著農業經營型態改變以及新興作物的引進，導致田間植物寄生性線蟲病害的發生更趨複雜。迄今為止，在田間防治植物寄生性線蟲病害，仍以殺線蟲劑為主要的選擇，主要有處理土壤燻蒸劑 (fumigant)，如1,3-二氯丙烯 (1,3-Dichloropropene，簡稱1,3-D)，或施用氨基甲酸鹽類殺線蟲劑，如歐殺滅 (oxamyl)，或施用新型殺線蟲劑，如氟派瑞 (fluopyram)[7]。但已上市的眾多殺線蟲劑多屬劇毒農藥，因此在考量作物線蟲病害的防治現況與環境保護兼顧的情況之下，採用綜合防治方法並著手研發具有殺死線蟲能力的微生物農藥，可降低化學藥劑的施用量與減低農藥殘毒的疑慮，更可達到良好的防治效果。本試驗主要在測試堅強芽孢桿菌 >1x10^9 CFU/g可濕性粉劑 (*Bacillus firmus*, CGMCC No.1.2010 WP) 的不同倍率稀釋液，於溫室栽培小番茄田間防治南方根瘤線蟲 (*M. incognita*) 的可行性。

材料與方法

供試植物材料與菌種

1. 供試藥劑：由利台化學工業股份有限公司取得堅強芽孢桿菌

>1x10^9 CFU/g可濕性粉劑 (*Bacillus firmus*, CGMCC No.1.2010 WP) 為供試藥劑。該藥劑為已經過檢測之微生物藥劑、淡褐色粉末物，並已有微生物藥劑原體審核通知書與註明有有效施用期限。參考藥劑使用500 g/L 氟派瑞水懸劑 (fluopyram SC, Bayer Crop Science LTD.)，作用機制代碼 FRAC 7、C2，每公頃推薦施用量為0.2 L，稀釋4,000 倍。依農業委員會藥物毒物試驗所植物保護資訊系統 (https://otserv2.tactri.gov.tw/PPM/menu.aspx) 推薦施用方法，植株定植後第3天澆灌稀釋藥液1次，每株澆灌40 mL，或每公頃澆灌800 L藥液，施用前應保持土壤濕潤。

2. 供試植株：由民間一般育苗場購買健康之番茄玉女 (*Solanum lycopersicum*) 幼苗為本試驗之供試植株。番茄幼苗定植適齡為4-5本葉，在預設株距上挖植穴，將幼苗置入並覆蓋土至子葉下方。
3. 試驗田區規劃：田間試驗規劃3場次，包括：
   (1) 田間試驗編號BF001，於雲林縣斗六市一處溫室小番茄田。整地時整成7畦，每畦畦寬100 cm，每畦可種2行，株距為45 cm。最旁邊左右2畦為緩衝區，以一般栽培管理方式管理。中間5畦為試驗區，試驗區前後有15株植株亦為緩衝區。每一畦再以每3.5 m劃分為11個試驗小區，則試驗田共有55個試驗小區，每一小區內可種植15-16株供試植株 (Fig. 1)。
   (2) 田間試驗編號BF002，於苗栗縣三義一處溫室小番茄田。整地時整成9畦，每畦畦寬100 cm，每畦可種2行，株距為45 cm。最旁邊左右2畦為緩衝區，以一般栽培管理方式管理。中間7畦為試驗區，試驗區前後有15株植株亦為緩衝區。每一畦再以每3.5 m劃分為7個試驗小區，則試驗田共有50個試驗小區，每一小區內可種植15-16株供試植株 (Fig. 2)。
   (3) 田間試驗編號BF003，於雲林縣崙背鄉一處溫室小番茄田。整地時整成4畦，每畦畦寬100 cm，每畦可種2行，株距為45 cm。最旁邊左右2畦為緩衝區，以一般栽培管

buffer area							
buffer area	A (alternate)	buffer area	A	buffer area	buffer area		buffer area
	buffer area	B	buffer area	B	buffer area		
	C	buffer area	E	buffer area	buffer area		
	buffer area	C	buffer area	A	buffer area		
	B (alternate)	buffer area	D	buffer area	buffer area		
	buffer area	E	buffer area	D	buffer area		
	E	buffer area	B	buffer area	D (alternate)		
	buffer area	A	buffer area	C	buffer area		
	C (alternate)	buffer area	D	buffer area	A (alternate)		
	buffer area	E (alternate)	buffer area	B (alternate)	buffer area		
	E (alternate)	buffer area	C (alternate)	buffer area	D (alternate)		
buffer area							

圖一、田間試驗編號BF001的設計與藥劑處理分佈圖。

Fig. 1. Design and treatment distribution of field trial No. BF001. This experiment adopted a randomized complete block design (RCBD). Where "A" indicated control group treated with water; "B" indicated reference group treated with 500 g/L fluopyram SC 4,000-fold dilution; "C" indicated test group treated with *Bacillus firmus* CGMCC No.1.2010 WP 400-fold dilution; "D" indicated test group treated with *B. firmus* CGMCC No.1.2010 WP 800-fold dilution; "E" meant as test group treated with *B. firmus* CGMCC No.1.2010 WP 1,200-fold dilution.

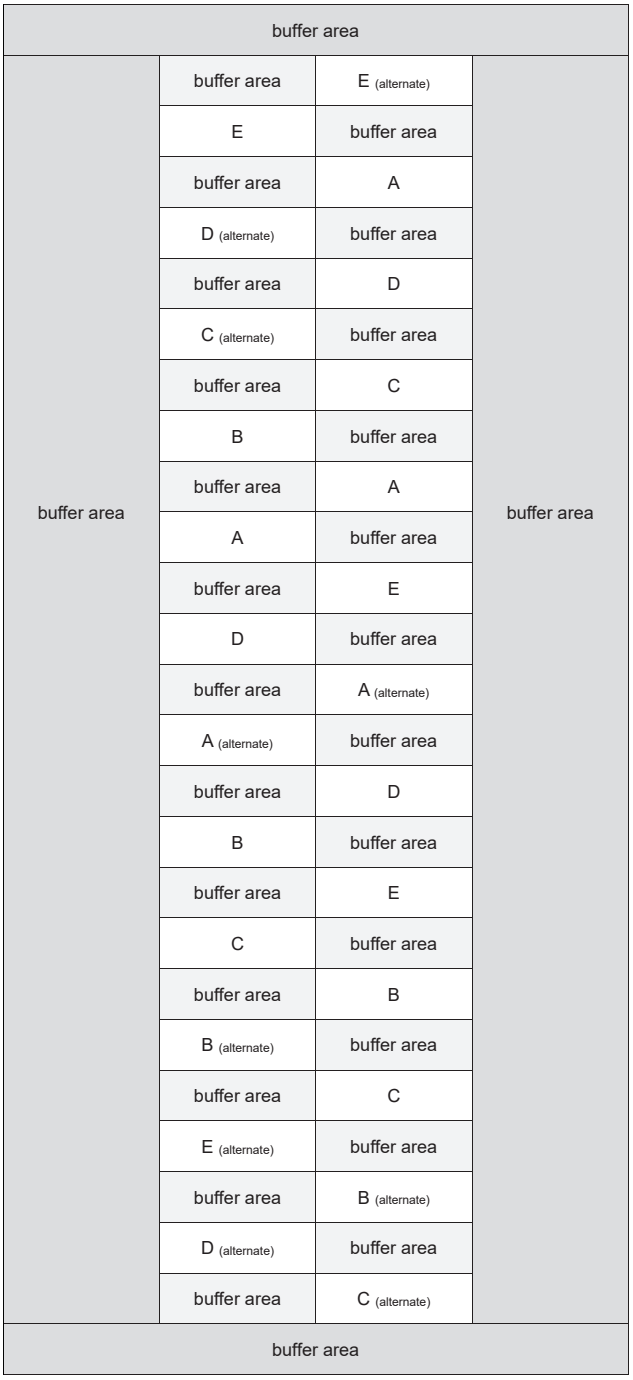
buffer area							
buffer area	D (alternate)	buffer area	C (alternate)	buffer area	D (alternate)	buffer area	E (alternate)
	buffer area	C	buffer area	D	buffer area	E	buffer area
	A	buffer area	D	buffer area	E (alternate)	buffer area	C
	buffer area	E	buffer area	B	buffer area	B	buffer area
	B (alternate)	buffer area	A	buffer area	B	buffer area	B (alternate)
	buffer area	A (alternate)	buffer area	D	buffer area	E	buffer area
	A (alternate)	buffer area	C	buffer area	A	buffer area	C (alternate)
buffer area							

圖二、田間試驗編號BF002的設計與藥劑處理分佈圖。

Fig. 2. Design and treatment distribution of field trial No. BF002. This experiment adopted a randomized complete block design (RCBD). Where "A" indicated control group treated with water; "B" indicated reference group treated with 500 g/L fluopyram SC 4,000-fold dilution; "C" indicated test group treated with *Bacillus firmus* CGMCC No.1.2010 WP 400-fold dilution; "D" indicated test group treated with *B. firmus* CGMCC No.1.2010 WP 800-fold dilution; "E" meant as test group treated with *B. firmus* CGMCC No.1.2010 WP 1,200-fold dilution.

理方式管理。中間2畦為試驗區，試驗區前後有15株植株亦為緩衝區。每一畦再以每3.5 m劃分為25個試驗小區，則試驗田共有50個試驗小區，每一小區內可種植15-16株供試植株 (Fig. 3)。

4. 田間試驗設計：試驗依逢機完全區集設計 (randomized complete block design, RCBD)，有5個試驗藥劑濃度處理，包括 (A) 為對照組，表澆水處理。(B) 為參考藥劑組，表500 g/L氟派瑞水懸劑，稀釋4,000倍處理。(C) 為CGMCC No.1.2010 WP 400倍稀釋液處理組。(D) 為CGMCC No.1.2010 WP 800倍稀釋液處理組。(E) 為CGMCC No.1.2010 WP 1200倍稀釋液處理組。處理間的試驗小區設計為緩衝區，用以避免處理間的藥效試驗互相干擾。另為避免藥劑處理前的田間土壤線蟲蟲口數有落差，導致發病不一致。每一藥劑處理事先規劃有5個重複，再依田間土壤測得之線蟲蟲口數，每一藥劑處理各選擇其中蟲口數大於25 nematodes/100 g soil 的3個試驗小區為重複數。番茄定植後第3天澆灌各式稀釋藥液1次，每星期一次，連續4次。每株澆灌250 mL，施用前先檢視田間土壤是否濕潤，並適時給水。參考藥劑組500 g/L氟派瑞水懸劑則依推薦用量與方法，於植株定植後第3天澆灌稀釋藥液1次，每株澆灌40 mL，施用前先檢視田間土壤是否濕潤，並適時給水。
5. 田間線蟲數的調查：試驗田區規劃之後，先於田區採集土樣調查田間土壤線蟲數，由每一藥劑處理的每一重複試驗小區採集3個點，則每一藥劑處理會有9個點。首先以採土器去除深度0-5 cm表土，採集深5-15 cm田間土壤，每採土點至少採集100 g土樣。於實驗室，利用改良式柏門氏漏斗分離法 (Modified Baermann Funnel Method)，取每份樣本100 g，置於60孔目網篩上 (已鋪2張衛生紙)，再將衛生紙摺好，避免底泥樣本漏出。靜置24-36 hr後收集指形管中的線蟲懸浮液，將分離所得之線蟲樣本，倒入直徑5 cm的玻璃鏡檢皿中，以解剖顯微鏡 (Leica KL 300 LED, Japan) 進行初步鏡檢，再以挑針挑取線蟲置於光學顯微鏡 (BX53, Olympus, Japan) 下觀察，記錄線蟲重要分類依據之構造特徵，並利用數位相機 (UC-90, Olympus, Japan) 拍照記錄不同線蟲之重要特徵 (口腔、食道及生殖系統等)，由其蟲體之特徵判定科別、屬別，並計算其數量。無法立即觀察之樣本則置於4℃冰箱中暫時保存，後續要觀察時再取出回溫觀察。本次試驗標的線蟲為南方根瘤線蟲，於田間採取根瘤線蟲感染之根部挑出母蟲後，切取母蟲陰門切片，依據Willmott 等人 (1977) 的描述，確認模紋形態特徵為南方根瘤線蟲所特有[21]。以改良式柏門式漏斗分離出之根瘤線蟲二齡幼蟲，則參考Hu等人 (2011) 描述的PCR方法確認為南方根瘤線蟲[9]。
6. 藥效試驗之病害調查：供試植株定植後分別每星期施用供試藥劑1次，連續4次，並於試驗開始後60天，再由每一藥劑處理的每一重複試驗小區採集3個地點，則每一藥劑處理會有9個地點，調查土壤線蟲族群密度。三場次試驗時間分別為，



圖三、田間試驗編號BF003的設計與藥劑處理分佈圖。

Fig. 3. Design and treatment distribution of field trial No. BF003. This experiment adopted a randomized complete block design (RCBD). Where "A" indicated control group treated with water; "B" indicated reference group treated with 500 g/L fluopyram SC 4,000-fold dilution; "C" indicated test group treated with ***Bacillus firmus*** CGMCC No.1.2010 WP 400-fold dilution; "D" indicated test group treated with ***B. firmus*** CGMCC No.1.2010 WP 800-fold dilution; "E" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 1,200-fold dilution.

(1) 田間試驗編號BF001，供試植株於2021年9月3日定植，定植後分別於9月6日、9月13日、9月20日與9月27日各施用供試藥劑1次。(2) 田間試驗編號BF002，供試植株於2021年10月11日定植，定植後分別於10月14日、10月21日、10月28日與11月4日各施用供試藥劑1次。(3) 田間試驗編號BF003，供試植株於2021年8月10日定植，定植後分別於8月13日、8月20日、8月27日與9月3日各施用供試藥劑1次。

另一處理，則挖取所有供試植株根系，調查根瘤線蟲的結瘤率。依根瘤發病級別 (root-knot index) 區分為0-5級：0級表根系健康無根瘤；1級表有極少數的根瘤產生，根瘤數量佔全根系的1%-20%；2級表有少量的根瘤產生，根瘤數量佔全根系的21%-40%；3級表有中量的根瘤產生，根瘤數量佔全根系的41%-60%；4級表有多量的根瘤產生，根瘤數量佔全根系的61%-80%；5級表有極多根瘤產生，根瘤數量佔全根系的81%-100%。所得資料，再換算罹病度 (%, disease severity, DS) 與防治率 (%, control rate, CR)。

(1) 罹病度 (%)＝Σ(發病株數x相對應級別數)／((試驗總株數 x 最高發病級數)) x 100 (%)

(2) 防治率 (%)＝(對照組罹病度-處理組罹病度)／對照組罹病度 x 100 (%)

7. 資料統計分析：試驗所得資料，比較不同處理之蟲口數、發病度與防治率，係利用統計分析軟體進行變方分析 (analysis of variance, ANOVA) 及最小顯著差異性 (least significant difference, LSD, p= 0.05) 測驗。

結　果

1. 試驗處理前田間土壤線蟲數調查：依田間試驗設計與藥劑處理分佈圖 (Fig.1, 2, 3)，於試驗前分別由每一藥劑處理採集田間土樣，於實驗室利用改良式柏門氏漏斗分離法分析其田間土壤線蟲蟲口數。

(1) 田間試驗編號BF001：結果顯示對照組、參考藥劑組、CGMCC No.1.2010 WP 400倍、800倍與1200倍稀釋液處理組的田間土壤線蟲蟲口數分別為29.7、30.2、32.9、29.6與33.7 nematodes/100 g soil。經統計分析，試驗前各藥劑處理土樣的線蟲數無差異 (p>0.05) (Fig. 4)。

(2) 田間試驗編號BF002：結果顯示對照組、參考藥劑組、CGMCC No.1.2010 WP 400倍、800倍與1200倍稀釋液處理組的田間土壤線蟲蟲口數分別為42.1、49.4、47.4、46.7與42.8 nematodes/100 g soil。經統計分析，試驗前各藥劑處理土樣的線蟲數無差異 (p>0.05) (Fig. 5)。

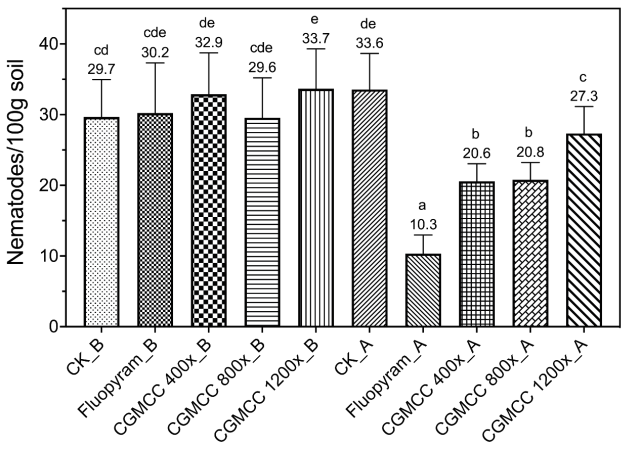
(3) 田間試驗編號BF003：結果顯示對照組、參考藥劑組、CGMCC No.1.2010 WP 400倍、800倍與1200倍稀釋液處理組的田間土壤線蟲蟲口數分別為31.8、33.4、39.6、39.7與42.8 nematodes/100 g soil。經統計分析，試驗前各藥劑處理土樣的線蟲數無差異 (p>0.05) (Fig. 6)。

2. 試驗處理後田間土壤線蟲數調查：供試植株定植後每週施用供試藥劑1次，連續4次，並於試驗開始後60天分析其田間土壤線蟲蟲口數。

(1) 田間試驗編號BF001：結果顯示對照組、參考藥劑組、CGMCC No.1.2010 WP 400倍、800倍與1200倍稀釋液處理組的田間土壤線蟲蟲口數分別為33.6、10.3、20.6、20.8與27.3 nematodes/100 g soil。經統計分析，試驗後各藥劑處理土樣的線蟲數與對照組具顯著差異 (p< 0.05) (Fig. 4)。進一步分析對照組的藥劑處理前、後土壤線蟲蟲口數分別為29.7與33.6 nematodes/100 g soil，兩者不具顯著差異 (p> 0.05)。參考藥劑組的藥劑處理前、後土壤線蟲蟲口數分別為30.2與10.3 nematodes/100 g soil，兩者具顯著差異 (p< 0.05)。CGMCC No.1.2010 WP 400倍稀釋液處理組的藥劑處理前、後土壤線蟲蟲口數分別為32.9與20.6 nematodes/100 g soil，兩者具顯著差異 (p< 0.05)。CGMCC No.1.2010 WP 800倍稀釋液處理組的藥劑處理前、後土壤線蟲蟲口數分別為29.6與20.8 nematodes/100 g soil，兩者具顯著差異 (p< 0.05)。CGMCC No.1.2010 WP 1200倍稀釋液處理組的藥劑處理前、後土壤線蟲蟲口數分別為33.7與27.3 nematodes/100 g soil，兩者具顯著差異 (p< 0.05) (Fig. 4)。

(2) 田間試驗編號BF002：結果顯示對照組、參考藥劑組、CGMCC No.1.2010 WP 400倍、800倍與1200倍稀釋液處理組的田間土壤線蟲蟲口數分別為42.8、22.4、32.3、30.8與35.3 nematodes/100 g soil。經統計分析，試驗後各藥劑處理土樣的線蟲數與對照組具顯著差異 (p< 0.05) (Fig. 5)。進一步分析對照組的藥劑處理前、後土壤線蟲蟲口數分別為42.1與42.8 nematodes/100 g soil，兩者不具顯著差異 (p> 0.05)。參考藥劑組的藥劑處理前、後土壤線蟲蟲口數分別為49.4與22.4 nematodes/100 g soil，兩者具顯著差異 (p< 0.05)。CGMCC No.1.2010 WP 400倍稀釋液處理組的藥劑處理前、後土壤線蟲蟲口數分別為47.4與32.3 nematodes/100 g soil，兩者具顯著差異 (p< 0.05)。CGMCC No.1.2010 WP 800倍稀釋液處理組的藥劑處理前、後土壤線蟲蟲口數分別為46.7與30.8 nematodes/100 g soil，兩者具顯著差異 (p< 0.05)。CGMCC No.1.2010 WP 1200倍稀釋液處理組的藥劑處理前、後土壤線蟲蟲口數分別為42.8與35.3 nematodes/100 g soil，兩者具顯著差異 (p< 0.05) (Fig. 5)。

(3) 田間試驗編號BF003：結果顯示對照組、參考藥劑組、CGMCC No.1.2010 WP 400倍、800倍與1200倍稀釋液處理組的田間土壤線蟲蟲口數分別為37、12.6、19、30.8與37.4 nematodes/100 g soil。經統計分析，試驗後參考藥劑組、CGMCC No.1.2010 WP 400倍與800倍稀釋液處理組的線蟲數與對照組具顯著差異 (p< 0.05) (Fig. 6)。



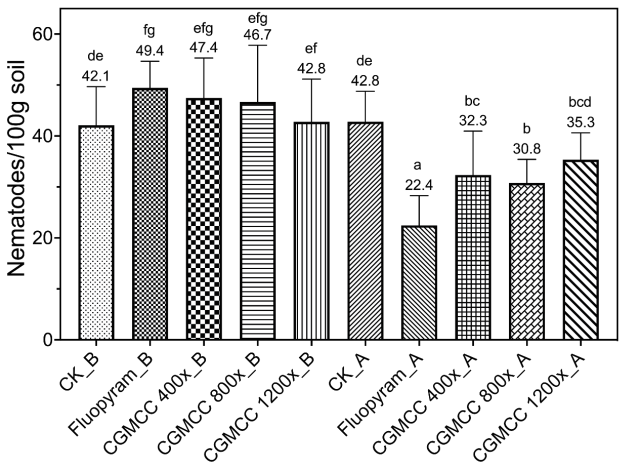
圖四、田間試驗田 (BF001) 土壤根瘤線蟲數調查。

Fig. 4. Soil nematode number surveys of root-knot nematode in field trial No. BF001. Where "CK" meant as control group treated with water; "Fluopyram" meant as reference group treated with 500 g/L fluopyram SC 4,000-fold dilution; "CGMCC 400x" meant as test group treated with ***Bacillus firmus*** CGMCC No.1.2010 WP 400-fold dilution; "CGMCC 800x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 800-fold dilution; "CGMCC 1200x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 1,200-fold dilution; "B" meant as before treatment; and "A" meant as after treatment. Values followed by the same letter were not significantly different at p> 0.05 according to LSD test, and err bars were SD.

進一步分析對照組的藥劑處理前、後土壤線蟲蟲口數分別為31.8與37 nematodes/100 g soil，兩者不具顯著差異 (p> 0.05)。參考藥劑組的藥劑處理前、後土壤線蟲蟲口數分別為33.4與12.6 nematodes/100 g soil，兩者具顯著差異 (p< 0.05)。CGMCC No.1.2010 WP 400倍稀釋液處理組的藥劑處理前、後土壤線蟲蟲口數分別為39.6與19 nematodes/100 g soil，兩者具顯著差異 (p< 0.05)。CGMCC No.1.2010 WP 800倍稀釋液處理組的藥劑處理前、後土壤線蟲蟲口數分別為39.7與30.8 nematodes/100 g soil，兩者具顯著差異 (p< 0.05)。CGMCC No.1.2010 WP 1200倍稀釋液處理組的藥劑處理前、後土壤線蟲蟲口數分別為42.8與37.4 nematodes/100 g soil，兩者不具顯著差異 (p> 0.05) (Fig. 6)。

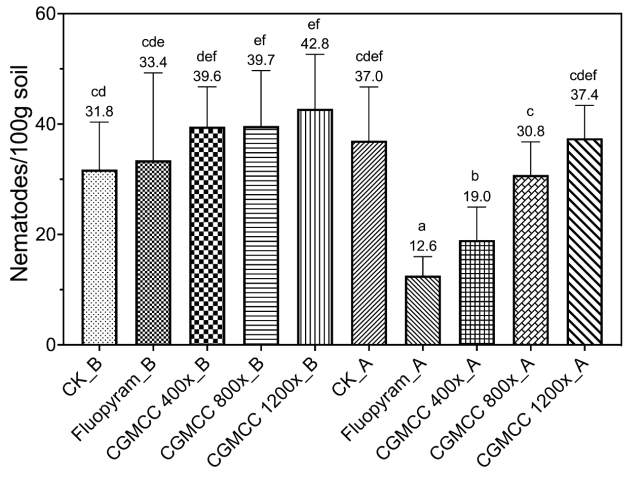
3. 田間防治試驗之病害調查：供試植株定植後連續施藥4次，並於試驗開始後60天分析田間供試植株根部的根瘤指數與罹病度 (%)。

(1) 田間試驗編號BF001：結果顯示對照組、參考藥劑組、CGMCC No.1.2010 WP 400倍、800倍與1200倍稀釋液處理組的供試植株根部罹病度分別為43.8%、12.1%、16.7%、17.1%與21.9%。經統計分析，試驗後各藥劑處理的罹病度與對照組具顯著差異 (p< 0.05) (Fig. 7)。進一步分析不同藥劑濃度對根瘤線蟲的病害防治率，結果顯示參考藥劑組、CGMCC No.1.2010 WP 400倍、800倍與1200倍稀



圖五、田間試驗田 (BF002) 土壤根瘤線蟲數調查。

Fig. 5. Soil nematode number surveys of root-knot nematode in field trial No. BF002. Where "CK" meant as control group treated with water; "Fluopyram" meant as reference group treated with 500 g/L fluopyram SC 4,000-fold dilution; "CGMCC 400x" meant as test group treated with ***Bacillus firmus*** CGMCC No.1.2010 WP 400-fold dilution; "CGMCC 800x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 800-fold dilution; "CGMCC 1200x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 1,200-fold dilution; "B" meant as before treatment; and "A" meant as after treatment. Values followed by the same letter were not significantly different at p> 0.05 according to LSD test, and err bars were SD.



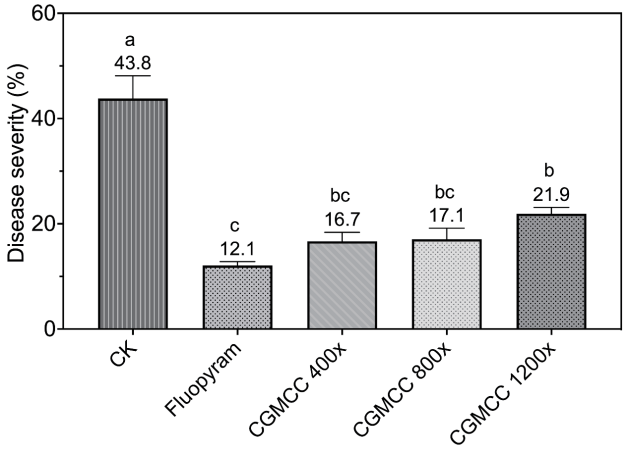
圖六、田間試驗田 (BF003) 土壤根瘤線蟲數調查。

Fig. 6. Soil nematode number surveys of root-knot nematode in field trial No. BF003. Where "CK" meant as control group treated with water; "Fluopyram" meant as reference group treated with 500 g/L fluopyram SC 4,000-fold dilution; "CGMCC 400x" meant as test group treated with ***Bacillus firmus*** CGMCC No.1.2010 WP 400-fold dilution; "CGMCC 800x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 800-fold dilution; "CGMCC 1200x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 1,200-fold dilution; "B" meant as before treatment; and "A" meant as after treatment. Values followed by the same letter were not significantly different at p> 0.05 according to LSD test, and err bars were SD.

釋液處理組的病害防治率分別為72.5%、61.9%、61%與50%。經統計分析，CGMCC No.1.2010 WP 400倍與800倍稀釋液處理組的病害防治率，不具顯著差異 (p> 0.05) (Fig. 8)。

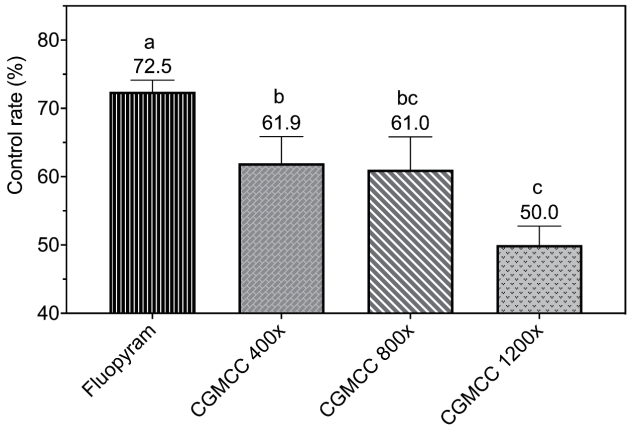
(2) 田間試驗編號BF002：結果顯示對照組、參考藥劑組、CGMCC No.1.2010 WP 400倍、800倍與1200倍稀釋液處理組的供試植株根部罹病度分別為51.6%、18.8%、22.2%、21.7%與28.3%。經統計分析，試驗後各藥劑處理的罹病度與對照組具顯著差異 (p< 0.05) (Fig. 9)。進一步分析不同藥劑濃度對根瘤線蟲的病害防治率，結果顯示參考藥劑組、CGMCC No.1.2010 WP 400倍、800倍與1200倍稀釋液處理組的病害防治率分別為63.6%、56.9%、58%與45.2%。經統計分析，CGMCC No.1.2010 WP 400倍與800倍稀釋液處理組的病害防治率，不具顯著差異 (p> 0.05) (Fig. 10)。

(3) 田間試驗編號BF003：結果顯示對照組、參考藥劑組、CGMCC No.1.2010 WP 400倍、800倍與1200倍稀釋液處理組的供試植株根部罹病度分別為47%、11.2%、17.3%、19.7%與29.8%。經統計分析，試驗後各藥劑處理的罹病度與對照組具顯著差異 (p< 0.05) (Fig. 11)。進一步分析不同藥劑濃度對根瘤線蟲的病害防治率，結果顯示參考藥劑組、CGMCC No.1.2010 WP 400倍、800倍與1200倍稀釋液處理組的病害防治率分別為76.2%、63.2%、58.1%與36.5%。經統計分析，CGMCC No.1.2010 WP 400倍與800



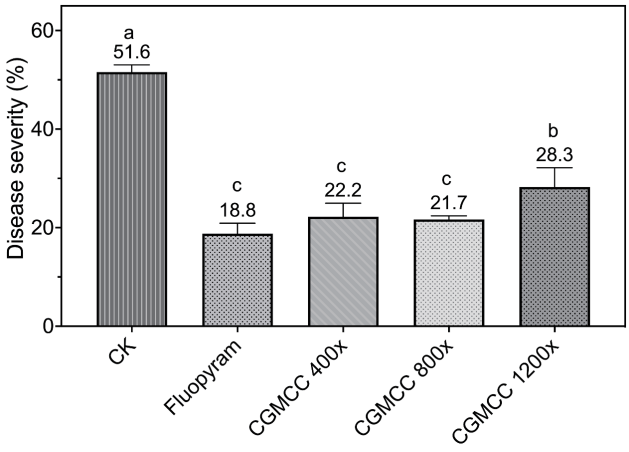
圖七、田間試驗田 (BF001) 根瘤線蟲發病度調查。

Fig. 7. Disease severity surveys of root-knot nematode in field trial No. BF001. Where "CK" meant as control group treated with water; "Fluopyram" meant as reference group treated with 500 g/L fluopyram SC 4,000-fold dilution; "CGMCC 400x" meant as test group treated with ***Bacillus firmus*** CGMCC No.1.2010 WP 400-fold dilution; "CGMCC 800x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 800-fold dilution; and "CGMCC 1200x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 1,200-fold dilution. Values followed by the same letter were not significantly different at p> 0.05 according to LSD test, and err bars were SD.



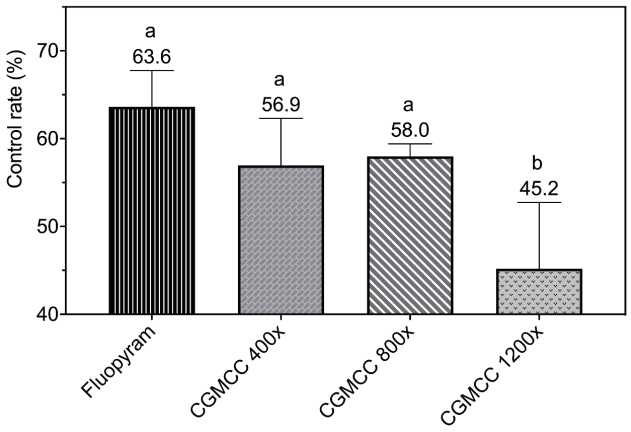
圖八、田間試驗田 (BF001) 根瘤線蟲病害防治率調查。

Fig. 8. Disease control surveys of root-knot nematode in field trial No. BF001. Where "Fluopyram" meant as reference group treated with 500 g/L fluopyram SC 4,000-fold dilution; "CGMCC 400x" meant as test group treated with ***Bacillus firmus*** CGMCC No.1.2010 WP 400-fold dilution; "CGMCC 800x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 800-fold dilution; and "CGMCC 1200x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 1,200-fold dilution. Values followed by the same letter were not significantly different at p> 0.05 according to LSD test, and err bars were SD.



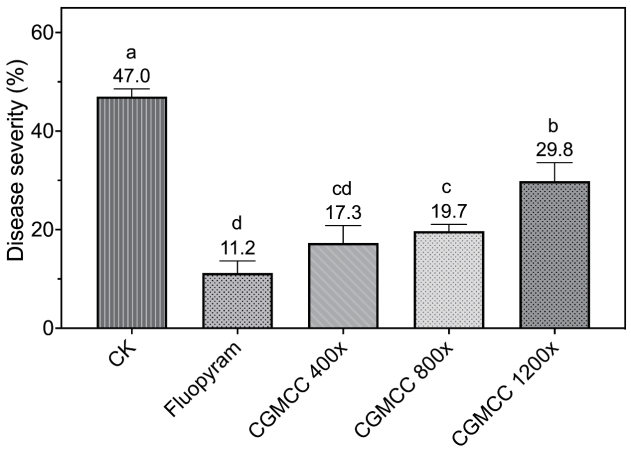
圖九、田間試驗田 (BF002) 根瘤線蟲發病度調查。

Fig. 9. Disease severity surveys of root-knot nematode in field trial No. BF002. Where "CK" meant as control group treated with water; "Fluopyram" meant as reference group treated with 500 g/L fluopyram SC 4,000-fold dilution; "CGMCC 400x" meant as test group treated with ***Bacillus firmus*** CGMCC No.1.2010 WP 400-fold dilution; "CGMCC 800x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 800-fold dilution; and "CGMCC 1200x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 1,200-fold dilution. Values followed by the same letter were not significantly different at p> 0.05 according to LSD test, and err bars were SD.



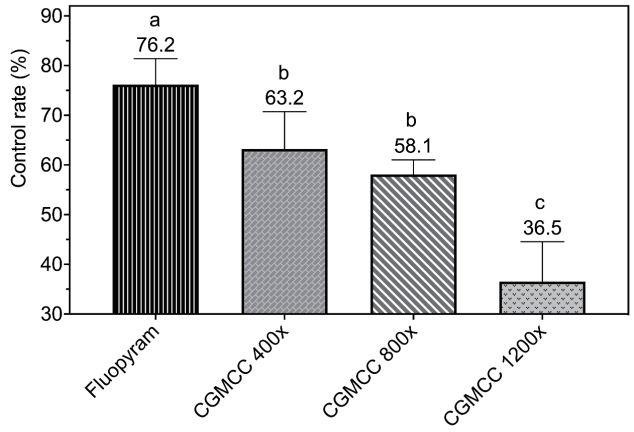
圖十、田間試驗田 (BF002) 根瘤線蟲病害防治率調查。

Fig. 10. Disease control surveys of root-knot nematode in field trial No. BF002. Where "Fluopyram" meant as reference group treated with 500 g/L fluopyram SC 4,000-fold dilution; "CGMCC 400x" meant as test group treated with ***Bacillus firmus*** CGMCC No.1.2010 WP 400-fold dilution; "CGMCC 800x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 800-fold dilution; and "CGMCC 1200x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 1,200-fold dilution. Values followed by the same letter were not significantly different at p> 0.05 according to LSD test, and err bars were SD.



圖十一、田間試驗田 (BF003) 根瘤線蟲發病度調查。

Fig. 11. Disease severity surveys of root-knot nematode in field trial No. BF003. Where "CK" meant as control group treated with water; "Fluopyram" meant as reference group treated with 500 g/L fluopyram SC 4,000-fold dilution; "CGMCC 400x" meant as test group treated with ***Bacillus firmus*** CGMCC No.1.2010 WP 400-fold dilution; "CGMCC 800x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 800-fold dilution; and "CGMCC 1200x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 1,200-fold dilution. Values followed by the same letter were not significantly different at p> 0.05 according to LSD test, and err bars were SD.



圖十二、田間試驗田 (BF003) 根瘤線蟲病害防治率調查。

Fig. 12. Disease control surveys of root-knot nematode in field trial No. BF003. Where "Fluopyram" meant as reference group treated with 500 g/L fluopyram SC 4,000-fold dilution; "CGMCC 400x" meant as test group treated with ***Bacillus firmus*** CGMCC No.1.2010 WP 400-fold dilution; "CGMCC 800x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 800-fold dilution; and "CGMCC 1200x" meant as test group treated with ***B. firmus*** CGMCC No.1.2010 WP 1,200-fold dilution. Values followed by the same letter were not significantly different at p> 0.05 according to LSD test, and err bars were SD.

倍稀釋液處理組的病害防治率，不具顯著差異 (p> 0.05) (Fig. 12)。

討　論

根瘤線蟲 (*Meloidogyne* spp.) 為對作物危害性最為嚴重的植物寄生性線蟲，主要乃是因為其寄主範圍廣泛，世界各地皆有危害紀錄報導，以及繁殖力強等優勢[11]。番茄植株根部遭受根瘤線蟲危害後，首先根尖會萎縮，被害部組織分化腫大呈圓形至橢圓形小腫瘤狀物，常多數連在一起，使根部呈現不規則腫狀瘤，後期根系腐敗。在地上部則出現生長不良的病徵，繼之葉片開始黃化、萎凋，但植株不會馬上死亡。接著植株葉片數會減少，出現小葉與結果不良、果實畸形等徵狀。土壤燻蒸劑1,3-二氯丙烯 (1,3-Dichloropropene) 可有效在田間防治番茄南方根瘤線蟲的危害，包括病徵的減緩與根部結瘤數的降低[7]。而非土壤燻蒸殺線蟲劑歐殺滅 (oxamyl) 或氟派瑞 (fluopyram)，則可能因溫度、土壤濕度與線蟲族群接種源潛勢的影響，其對田間線蟲病害的防治效果則不如土壤燻蒸劑有效[2]。近年來在植物寄生性線蟲的防治上，都是以減少對化學殺線蟲劑的依賴，可行的替代方案包括使用帶有抗性基因 (R-基因) 的作物[18]，或利用微生物及化學藥劑誘導作物的抗病性，以及增加田間土壤對線蟲的拮抗作用[6]。透過有益微生物的添加可以

增加田間土壤對線蟲的拮抗作用，以減緩有害生物在土壤中的傳播[19]，其中已獲准上市的微生物殺線蟲劑包括有*Bacillus firmus*、*Purpureocillium lilacinum* (=*Paecilomyces lilacinus*)，以及 *Pasteuria nishizawae*[3, 6]。

本研究利用堅強芽孢桿菌 >1x10^9 CFU/g可濕性粉劑 (*Bacillus firmus*, CGMCC No.1.2010 WP) 為供試藥劑，測試其對番茄根瘤線蟲 (*Meloidogyne incognita*) 的防治效果。結果顯示 CGMCC No.1.2010 WP在三場次的田間試驗結果400倍與800倍可明顯降低土壤中的線蟲蟲口數 (Fig. 4, 5, 6)。在棉花腎形線蟲 (*Rotylenchulus reniformis*) 的防治上，利用*B. firmus* (1.4×10^7 CFU/seed) 與 *P. lilacinus* (0.3% vol/vol of water) 的複合施用，可以在溫室與田間試驗中減少腎形線蟲的幼蟲、母蟲與卵數量，並可增加腐生性線蟲的族群數[3]。而在本研究的三場次田間試驗結果，試驗後各藥劑處理的罹病度與對照組具顯著差異 ($p<0.05$) (Fig. 7, 9, 11)，進而達到病害防治的效果 (Fig. 8, 10, 12)。文獻報導指出*Bacillus firmus* 菌株可纏聚在玉米、棉花與大豆根系上[6, 14]，而有益微生物細菌菌株在植株根系的纏聚能力，則可保護根系不受根瘤線蟲的感染，並透過對線蟲表皮的影響，降低線蟲的活動力與存活力，以及利用對卵殼的分解能力，進而可抑制二齡幼蟲的孵化率，以降低土壤中線蟲蟲口數[15, 20, 22]。另外，*Bacillus* species 具有誘導性抗病的能力 (ISR, induced systemic resistance)[4]，*B. firmus* 菌株可在大豆植株上誘發系統性抗性，減少大豆包囊線蟲 (*Heterodera glycines*) 的感染，在棉花則可減少根瘤線蟲 (*Meloidogyne* spp.) 的感染[17]。進一步利用綠色螢光蛋白 (GFP, green florescent protein) 轉殖技術，則可發現*B. firmus*菌株可纏聚在番茄與胡瓜的根系上，並可纏聚在根瘤線蟲的卵上，且具有分解卵的能力。配合RT-qPCR技術，則可發現*B. firmus*菌株在番茄植株上可誘導對根瘤線蟲的抗病性，但是在胡瓜植株上則無[6]。絲氨酸蛋白酶 (serine proteases) 是防治植物寄生性線蟲的關鍵因子之一[10]，已知*B. firmus* DS-1菌株具有可產生絲氨酸蛋白酶Sep 1的能力，可以分解多種角質層與腸道相關蛋白[5]。

綜合上述，*Bacillus firmus*菌株可以在作物根系上纏聚，並具備多種可殺死線蟲的能力，又可以就近影響內寄生性線蟲的活動力與卵塊的孵化，因此在防治根瘤線蟲上具有在生物防治上的效果[23]。

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ABSTRACT

Su, J. F., Wu, C. Y., Chien, L. Y., Lin, T. C., and Yen, J. H. 2022. *Bacillus firmus*, CGMCC No.1.2010 WP, a potential biocontrol agent against root-knot nematode of tomato in field. J. Plant Med. 64(4): 159-168.

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This study evaluated the application feasibility of a strain of *Bacillus firmus*, CGMCC No.1.2010 WP, to possess biocontrol activity against root-knot nematode on tomato in greenhouse. Three field trials were conducted in randomized complete block design (RCBD). The *B. firmus* >1x10^9 CFU/g wettable powder 400-fold, 800-fold, 1200-fold dilutions were used as test group. The reference group was 500 g/L fluopyram SC of 4,000-fold dilution, and sterile water was used as control. Soil nematode numbers of root-knot nematode in the control groups were between 33.6 and 42.8 nematodes/100 g soil 60 days after the experimental treatment, and the numbers in *B. firmus* groups and fluopyram groups were 20.8-30.8 and 10.3-22.4 nematodes/100 g soil, respectively. Those numbers of nematodes of both treated groups were significantly different from the control group ($p<0.05$). The disease severities in the control groups were between 43.8% and 51.6%, and the disease severities in *B. firmus* groups and fluopyram groups were 17.1-21.7% and 11.2-18.8%, respectively. The disease severities of both treated groups were also significantly different from the control group ($p<0.05$). The disease control rates in the *B. firmus* groups and fluopyram groups were 58-61% and 63.6-76.2%, respectively. According to the above described results, the *B. firmus* CGMCC No.1.2010 WP had the potential to control root-knot nematode on

tomato in the fields.

Keywords: *Meloidogyne incognita*, root-knot nematode, *Bacillus firmus*, *Solanum lycopersicum*, tomato, biological control