

臺灣產蟲生真菌 *Purpureocillium takamizusanense* 感染荔枝椿象 (*Tessaratoma papillosa*) 初報

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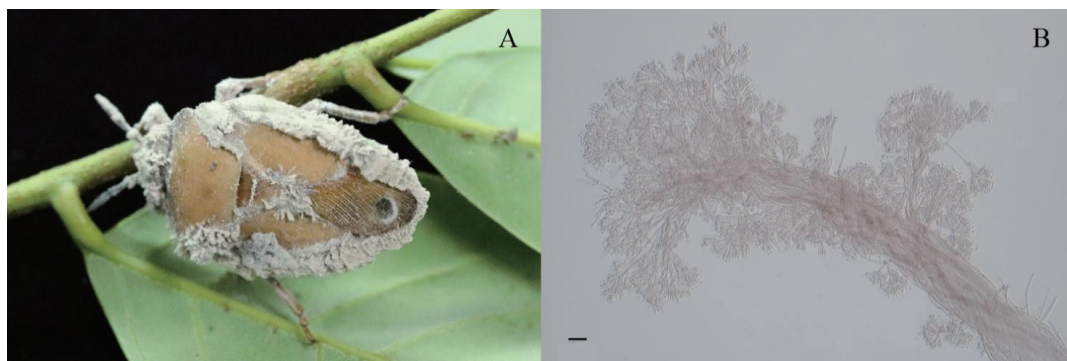
摘要

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本研究自臺中與南投地區龍眼樹上採集疑似受蟲生真菌感染之荔枝椿象 (*Tessaratoma papillosa* Drury)，其氣孔與體節間隙可見到菌絲團並佈滿粉紫色之分生孢子。該菌體經分離純化後，依其形態與分子生物學特性加以鑑定，配合內轉錄間隔區 (internal transcribed spacer)、增幅延長因子 (translational elongation factor 1 α) 及 β 微管蛋白 (β -tubulin) 的基因序列片段，將其鑑定為 *Purpureocillium takamizusanense* (Kobayasi) S. Ban, Azuma & Hirok. Sato。病原性測試結果，其分生孢子具感染荔枝椿象成蟲的能力，並於接種後14天開始造成蟲體死亡，至第28天致死率達85.2%，顯示該菌對防治荔枝椿象具有高度應用潛力。

關鍵詞：荔枝椿象、蟲生真菌、*Purpureocillium takamizusanense*

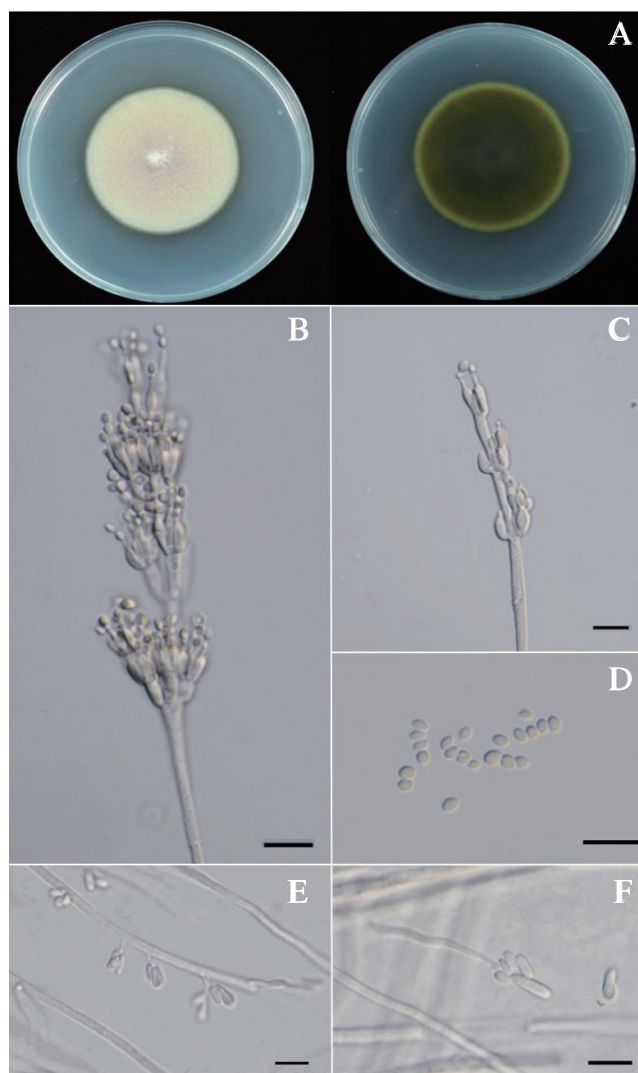
荔枝椿象 (*T. papillosa*) 以刺吸式口器危害荔枝、龍眼及臺灣欒樹等無患子科 (Sapindaceae) 植物，導致植株出現落花、落果及嫩枝、幼果枯萎⁽³⁾；此外，該蟲所噴出的臭液具腐蝕性，對農民於農事操作上亦造成威脅。目前對於荔枝椿象之防治方式以物理防治、化學藥劑防治及施放平腹小蜂 (*Anastatus* sp.) 防治為主⁽⁷⁾，而中國大陸亦有研究利用蟲生真菌 (*Beauveria bassiana* (Bals.-Criv.) Vuill.) 進行荔枝椿象之防治⁽⁶⁾。本研究於臺中市大坑與南投市等地區之龍眼樹枝幹與葉背處，採集到疑似受蟲生真菌感染死亡之荔枝椿象成蟲與若蟲，其蟲體呈現僵硬狀並環抱枝條，且可自死亡蟲體由觸角、足部、軀幹節間膜及氣孔等處觀察到菌絲 (圖一A) 與分生孢子束 (圖一B)，並佈滿淡紫色分生孢子。以單孢培養方式自蟲體分離蟲生真菌，培養於馬鈴薯葡萄糖瓊脂培養基 (potato dextrose agar, PDA) 平板上，於24°C 光照12小時恆溫生長箱培養，菌落生長速度約 3.1 mm/ day，初期產生白色菌落，氣生菌絲呈現粉絮狀，菌落上方覆蓋許多粉紫色之分生孢子，菌落背面呈現深褐色，邊緣則呈現淡黃色 (圖二A)。本菌經食品工業發展研究所鑑定，其菌株特性描述如下：菌絲薄壁，無色且平滑，寬度約2-4 μ m



圖一、遭蟲生真菌感染之荔枝椿象。(A) 自死亡蟲體之觸角、足部、軀幹節間膜及氣孔等處觀察到菌絲與分生孢子束；(B) 於光學顯微鏡下觀察到分生孢子束之形態 (標準尺: 20 μ m)。

Fig. 1. *Tessaratoma papillosa* (Drury) infected by entomopathogenic fungi. (A) Fungus mats and synnemata emerged from membranes between legs and antennal segments, the spiracles, and the abdomen lateral edges of dead bugs. (B) The morphology of synnemata. (Standard bar: 20 μ m).

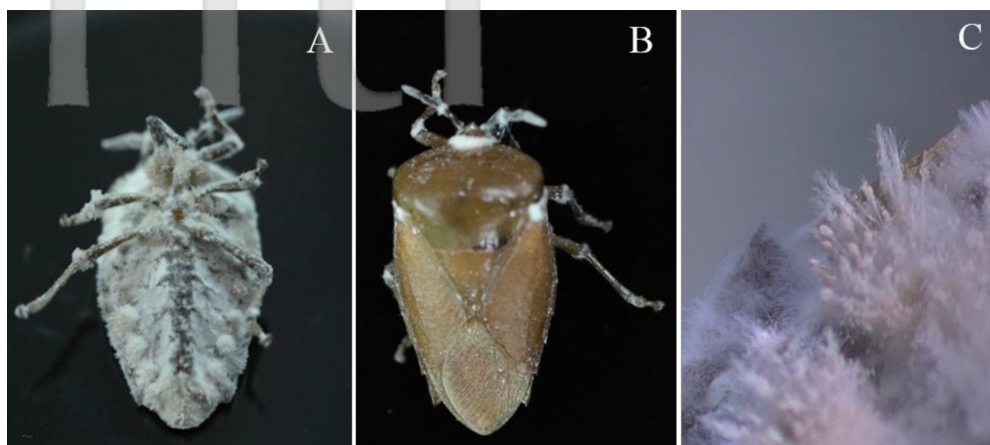
(3.0 μm)，分生孢子梗 (conidiophores) 從培養基表面菌絲生長，外壁粗糙呈現半透明至淺褐色，寬約2-4 μm (3.0 μm)，分生孢子梗頂部著生輪狀分枝 (verticillate branches)，每個分支點上常會形成2-6個瓶狀枝 (phialides) (圖二B)，瓶狀枝長約6-8 μm (7.1 μm)，寬約2-3 μm (2.5 μm)，基部膨大，具寬約1 μm 縮之短細



圖二、*Purpureocillium takamizusanense* 之形態。(A) 於PDA平板上生長15天之菌落正面與反面之形態；(B) 分生孢子梗上輪狀著生分枝，其上具有2-6個輪生之瓶狀枝；(C) 瓶狀枝基部膨大具有約1 μm 之縮；(D) 分生孢子為梭狀或檸檬狀；(E) 由菌絲長出之瓶狀枝呈現圓柱狀，孢子於上聚集黏性頭狀成團；(F) 菌絲上長出較長之細頸狀瓶狀枝，並產生橢圓形孢子。(標準尺:10 μm)

Fig. 2. Morphology of *Purpureocillium takamizusanense*. (A) The upperside and bottom of a 15-day culture on PDA. (B) Conidiophores consist of verticillate branches with whorls of two to six phialides. (C) Phialides have a swollen basal portion with narrowing abruptly into a short neck approximately 1 μm wide. (D) Conidia in limoniform or fusiform. (E) Atypical phialides in cylindrical with slimy head. (F) Unusual phialides in lageniform with oval conidia. (Standard bar: 10 μm)

頸狀 (圖二C)。分生孢子串生，形狀為梭狀 (fusiform) 或檸檬狀 (limoniform)，單細胞無隔，外壁平滑，半透明，孢子大小約為2-3x1.5-2.2 μm (2.6x1.9 μm)，聚集時呈現淺紫色 (圖二D)。菌落邊緣具類似Acremonium之產孢構造，瓶狀枝多由菌絲上單獨長出，呈現細頸瓶狀 (lageniform) 至圓柱狀 (cylindrical)，長度變化大；分生孢子聚集成黏性頭狀成團 (slimy head)，形狀為近球形 (subglobose)、橄欖形 (oliverly) 或橢圓形 (ellipsoidal)，單細胞，外壁圓滑，透明，約為2.5-6x2-3.5 μm (4.1x 2.6 μm) (圖二 E、F)。進一步透過分子生物學特性鑑定，以V9G/ LR1引子對增幅內轉錄間隔區 (internal transcribed spacer, ITS) rDNA 序列^(11, 12)，全長約700 bp (Accession No. MK592777)，與*Isaria takamizusanensis* (LC008201) 具有99.1%之相似度；另以EF1-983F/ EF1-2281R引子對增幅延長因子 (translational elongation factor 1 α , EF-1 α) 部分序列⁽¹³⁾，全長約572 bp (Accession No. LC469050)，與*I. takamizusanensis* (LC008333) 具有99.1%之相似度；另以Bt2a/ Bt2b引子對增幅 β 微管蛋白 (β -tubulin) 部分序列⁽⁵⁾，總全長約311 bp (Accession No. LC469051) 與*I. takamizusanensis* (GU980010) 具有96.15%相似度，綜合形態與分子生物學特性，將其鑑定為*I. takamizusanensis*。而*I. takamizusanensis*於2010年透過形態、ITS rDNA及EF-1 α 鑑定，並在one fungus one name之概念下由*I. takamizusanensis*更為新學名*Purpureocillium takamizusanense*⁽¹⁾。於室內接種試驗中，以0.01%之tween 80將培養於PDA平板之分生孢子洗下，並配製成 2×10^7 spores/ ml之孢子懸浮液，以噴瓶噴灑3-4 ml之孢子懸浮液至荔枝椿象成蟲表面，將其培養於具龍眼植株之養蟲籠 (L x W x H = 63 cm x 63 cm x 124 cm) 內，每重複15隻，共3重複，並以噴施0.01% tween 80作為對照組，每7天觀察一次蟲體死亡情形。於接種後14天蟲體開始死亡，第21天死亡率達52.0%，第28天之死亡率可達85.2%，死亡之蟲體以環抱龍眼枝條或攀附於葉背之形態死亡。蟲體經過75%酒精表面消毒並保溼，3-5天後可見蟲體觸角、足部、軀幹之節間膜及氣孔等處產生白色絨毛狀菌絲 (圖三A、B)，並且產生分生孢子束 (圖三C)，將菌絲再次培養於PDA平板上，可產生佈滿淡紫色孢子之菌落形態，與接種之菌株具有相同菌落形態，可見*P. takamizusanense* 可感染荔枝椿象成蟲並造成其死亡，為對荔枝椿象具防治潛力之蟲生真菌。*Purpureocillium*屬之蟲生真菌於國外已有相關研究指出，其對害蟲具防治潛力，如半翅目*Edessa rufomarginata* (De Geer)、*Aphis gossypii* (Glover)及鞘翅目*Tribolium confusum* (DuVal) 等^(2, 4, 8)，亦具防治根瘤線蟲 (*Meloidogyne incognita* (Kofoid & White)) 之效果⁽¹⁰⁾，可見該屬蟲生真菌具有廣泛之寄主範圍與害蟲防治能力。於中國大陸地區Meng氏等人 (2017) 自荔枝椿象上分離到該屬蟲生真菌*P. lilacinum* (昔學名為*Paecilomyces lilacinus* (Thom) Samson) 並證實其對荔枝椿象具致病性⁽⁹⁾。然目前國內外仍未有應用*P. takamizusanense*蟲生真菌防治荔枝椿象之相關研究，未來將持續評估其對荔枝椿象之



圖三、接種後死亡之荔枝椿象蟲體。(A)(B) 由蟲體之觸角、足部、軀幹節間膜及氣孔等處觀察到菌絲與分生孢子束；(C) 灰白色的分生孢子束具有淡紫色之頂端。

Fig. 3. The inoculated dead bugs. (A)(B) The fungus mats and synnemata emerged from the intersegmental membranes of antennae, legs and body, and the spiracles of dead bugs. (C) Synnemata arising from the bugs with grey stipe and greyish purple swollen tips.

田間防治效果。

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ABSTRACT

Lo, P. H.^{1*}, Yu, Y. C.¹, and Pai, K. F.¹. 2019. First report of *Purpureocillium takamizusanense* as an entomopathogenic fungus infecting *Tessaratoma papillosa* (Drury) in Taiwan. J. Plant Med. 61(2_3): 27-30.

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In this study, entomopathogenic fungi were isolated from dead Lychee stink bug (*Tessaratoma papillosa*) in Taichung and Nantou of Taiwan. White mats with pinkish purple conidia emerged from the intersegmental membranes of antennae, legs and body, and the spiracles of dead bugs. The fungi were identified as *Purpureocillium takamizusanense* (Kobayasi) S. Ban, Azuma & Hirok. Sato based on morphology and molecular characters, sequencing intergenic transcribed spacer (ITS) rDNA, transcribed elongation factor 1 α (EF- α), and β -tubulin genes. Moreover, pathogenicity test showed that it killed adult bugs 14 days post-inoculation and had 85.2% mortality rate after 28 days of inoculation. Therefore, it shows high potential on biological control of Lychee stink bug in Taiwan.

Keywords: *Tessaratoma papillosa* (Drury), entomopathogenic fungi, *Purpureocillium takamizusanense*