A formulated container medium suppressive to Rhizoctonia damping-off of cabbage

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Abstract. Ten agricultural wastes were tested for their suitability as substrates for the growth of cabbage seedlings. Spent forest mushroom compost (SFMC) and spent golden mushroom compost (SGMC) were found to be more suitable than raw spent forest mushroom growth medium (RFM), raw spent golden mushroom growth medium (RGM), rice hull, carbonized rice hull, peanut husk, coconut fiber, bagasse meal or wasted cotton. The optimum composting period for SFMC and SGMC was 10 and 6 weeks, respectively. A new container medium (SSC-06) was formulated using SFMC, carbonized rice hull, shrimp and crab shell meal, blood waste, and lime. The SSC-06 medium was suitable for growth of cabbage seedlings and was suppressive to *Rhizoctonia solani* AG-4. The suppressive effect of 20-day-old SSC-06 medium on colonization of cabbage seeds by *R. solani* AG-4 was reduced after it was steamed in 100°C hot air for 15~30 min. However, the inhibitory effect was restored to the steamed SSC-06 medium by inoculation with *Trichoderma harzianum* isolate TH-05 at a concentration of 10⁵ cfu/g dry medium. After the medium was steamed for 5, 10, 15, 25 or 30 min, no fungal colonies were recovered, but the colony-forming units of the bacterial population were maintained at >10⁶/g dry medium. The potential for SSC-06 as a container medium for commercial nursery industries is discussed.

Keywords: Agricultural wastes; Biocontrol; Cabbage; Container medium; Rhizoctonia damping-off.

Abbreviations: BM, blood meal; CRH, carbonized rice hull; PDA, potato dextrose agar; RFM, raw spent forest mushroom growth medium; RGM, raw spent golden mushroom growth medium; RH, rice hull; RSP, rape seed pomace; SCSM, shrimp & crab shell meal; SFMC, spent forest mushroom compost; SGMC, spent golden mushroom compost.

Introduction

The use of synthetic media for container crops has become increasingly important to the greenhouse industry world-wide (Hoitink, 1980; Hoitink and Fahy, 1996; Huang, 1997; Trankner, 1992). Materials such as perlite, sand, vermiculite, expended styrofoam, peat, pine bark, and hardwood bark are commonly used in the synthetic growth substrate. Although container media are generally pathogen-free, infestation of these media by damping-off pathogens, such as Rhizoctonia solani Kühn, Pythium spp., and Fusarium oxysporum Schl. often occurs in the production of plugs for greenhouse vegetable and ornamental plant seedlings (Huang, 1997). During the late 1960s, observations made in nurseries indicated that the Phytophthora root rot of rhododendron was less severe on plants grown in media amended with composted tree bark than in those amended with peat (Hoitink and Fahy, 1986; Hoitink et al., 1991). Thus, peat media are considered conducive to damping-off pathogens, whereas composted hardwood bark media are considered suppressive. Currently, composted tree bark has largely replaced peat in container media for control of plant pathogens in the United States (Draft et al., 1979; Hoitink, 1980; Kuter et al., 1983).

A container medium, SSC-06, consisting of spent forest mushroom compost and other agricultural wastes has been developed for use as a growth substrate for cabbage seedlings. Results of preliminary studies showed that the SSC-06 medium also effectively controlled damping-off of cabbage caused by *R. solani* AG-4 (Huang, 1997). The objectives of this study were to develop the medium and to investigate the possible mechanism involved in the suppression of Rhizoctonia damping-off of cabbage grown in the SSC-06 medium.

Materials and Methods

Preparation of Container Media

Organic components used in container media were raw spent forest mushroom growth medium (RFM), spent forest mushroom compost (SFMC), raw spent golden mushroom growth medium (RGM), spent golden mushroom compost (SGMC), rice hull (RH), carbonized rice hull (CRH), peanut husk, coconut fiber, bagasse, wasted cotton and a commercial growth medium BVB No. 4 peat moss (Bas Van Burren, Maasland, the Netherlands). The term "RFM" refers to the spent mushroom growth medium removed from mushroom farms within a month after mush-

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room harvesting and not subjected to thermophilic decomposition. The RFM was prepared from a mixture (pH 6) of forest mushroom (*Lentinus edodes* Singer) growth medium wastes and consisted of 75% (v/v) sawdust (mainly from *Liquidambar formosana* Hance), 20% (v/v) rice bran, 4% (v/v) wheat bran, and 1% (w/v) calcium carbonate. The RGM was prepared from a mixture (pH 6.5) of golden mushroom (*Flammuline velutipes* (Fr.) Sing.) growth medium wastes and consisted of 75% (v/v) sawdust [mainly from *Castanopsis carlesii* (Hemal.) Hay], 15% (v/v) rice bran, 5% (v/v) wheat bran, 3.5% (w/v) calcium phosphate, and 1.5% (w/v) calcium carbonate. The raw

(v/v) rice bran, 5% (v/v) wheat bran, 3.5% (w/v) calcium phosphate, and 1.5% (w/v) calcium carbonate. The raw spent mushroom growth medium was composted according to the following procedure: 3 kg NH,NO, and 2 kg triple superphosphate were added per cubic meter of RFM or RGM. The moisture was adjusted to 60~65% (w/w). The mixture was covered with a polyethylene sheet, incubated under field conditions, and turned weekly. Temperature in the compost ranged from 55~65°C. The heat in the compost dropped to near room temperature (30~35°C) after 10 wks of composting for SFMC or after 4 wks of composting for SGMC. After further incubation at 25°C for another 2 wks, SFMC and SGMC were air-dried and stored in polyethylene bags. Individual components or media were adjusted to $60 \sim 65\%$ (w/w) water content for one wk before use.

Preparation of Inoculum

A stock culture of *R. solani* AG-4 (isolate RST-04) isolated from Chinese kale (*Brassica alboglabra* Bailey) and maintained on potato dextrose agar (PDA) at 24°C was used in this study. The inoculum for infestation of the container medium was prepared by inoculation of a sterilized potato paste-perlite substrate made of 20 ml potato paste, 100 ml perlite No. 3, and 20 ml distilled water with a mycelial disk (5-mm-diameter) from 72-h PDA culture of *R. solani* (the modified method of Ko and Hora, 1971). After incubation for seven days at 24°C, the *R. solani* was mixed with the media in a polyethylene bag at a rate of 0.2% (v/v). Propagule density of *R. solani* in the container media was determined by the baiting technique described by Huang and Yang (1992).

Assays for Plant Growth and Disease Suppressiveness

RFM and RGM with composting periods ranging from 0 to 12 wks were used as container media. Pots (8-cm diam) were filled with *R. solani* infested or non-infested container media. Five cabbage seeds (*B. oleracea* L. var. *capitata* L. cv. EC-KELLY) were buried in the medium at a depth of 0.5 cm. Pots were kept in the greenhouse at 24°C, watered daily for 12 days and the number of healthy seed-lings was recorded. There were ten pots in each treatment. Seedlings showing damping-off symptoms were washed in water and plated on 2% (w/v) water agar plates containing 300 ppm streptomycin sulfate to isolate the casual agent. Fresh weight of healthy seedlings in each pot was measured at 30 days after seeding. Data on seedling emer-

gence and plant weight were analyzed statistically using SAS/STAT and SigmaPlot systems. The experiment was performed twice.

Screening Tests of Container Medium Amendments

Five SFMC-CRH medium combinations were prepared by mixing SFMC with CRH at rates of 9:1, 3:1, 1:1, 1:3 and 1:9 (v/v). SFMC-CRH, SFMC, CRH and BVB No. 4 peat moss media were each infested with 0.2% (v/v) R. solani. The cabbage seedling assay method (Huang and Yang, 1992) was used to determine the effects on seedling growth and disease suppression for each container medium. The medium composed of 3L SFMC and 1L CRH is designated as SSC-05. Among the eight media tested, SSC-05 was the best for the growth of cabbage seedlings, but it did not show suppressive effects to R. solani. Therefore, the SSC-05 medium was selected as a base substrate for formulation of a container medium suppressive to R. solani. The modified media were prepared by amending of SSC-05 with 0.2% (w/v) blood meal (BM), 0.5% (w/v) shrimp & crab shell meal (SCSM), and/or 0.4% (w/v) rape seed pomace (RSP). Each 5L of amended or non-amended SSC-05 medium was separately placed in a polyethylene bag, with or without the treatment of 0.3% (w/v) lime. Water content of amended and non-amended container media was brought to 55~60% (w/w) with sterilized distilled water and maintained for 10 days at 24°C. The cabbage seedling assay was used to evaluate effects of amended and nonamended SSC-05 on damping-off of cabbage by the technique of Huang and Yang (1992). In addition, pH values of the container media were measured at 0 and 20 days of incubation using a pH meter (Beckman Co., USA). The solutions were prepared by mixing each container medium with 0.01 M CaCl₂ at 1:2 ratio (v/v) for 30 min prior to measurement.

Effect of Aging of Medium

The SSC-06 medium was prepared by evenly mixing 10 liters of SSC-05 medium with 0.2% (w/v) BM, 0.5% (w/v) SCSM and 0.3% (w/v) lime in a polyethylene bag. The SSC-06 and BVB No. 4 media (500-ml samples) were adjusted to 60~65% (w/w) water content in 1 liter beakers. They were covered with aluminium foil and incubated at $24~28^{\circ}$ C for 0, 10, 20, 30, 40, 50 and 60 days. The samples from various aging treatments were inoculated with 0.2% (v/v) *R. solani*, incubated for 0 and 5 days, and tested for seed colonization and damping-off of cabbage by the method described previously.

Microbial Population in the Container Medium

The 20-day-old, SSC-06 medium was steamed at 100°C for 0, 5, 10, 15, 20 and 30 min. Six random samples from each medium were mixed, air-dried, and sieved through a 10-mesh (2-mm) screen. Microbial populations were estimated by the serial dilution plate technique, using nutrient agar for bacteria and peptone-dextrose-rose bengal agar for fungi (Huang and Kuhlman, 1991).

Inoculation of Trichoderma harzianum

In another experiment, the 20-day-old, SSC-06 medium steamed at 100°C for 15 min was artificially infested with 0.2% (v/v) R. solani and inoculated with Trichoderma harzianum Rifai isolate TH-05 which was originally obtained from SSC-06 medium. A spore suspension of T. harzianum TH-05 was prepared by adding 20 ml sterile distilled water to the culture grown on malt extract agar (Difco), culturing for 2 wks in a 9-cm-dia. petri dish, and gently scraping the colony to release spores. The suspension was filtered through double-layer cheesecloth and centrifuged (10,000 g for 10 min), and the pellet was resuspended in sterile distilled water. Spore concentrations were determined with a hemacytometer (Cambridge Instruments Inc., N.Y., USA). The level of T. harzianum TH-05 was 2×10^5 colony-forming units (cfu)/g dry medium. Steamed and non-steamed media without microbial infestation were used as controls. The effect of T. harzianum TH-05 on Rhizoctonia damping-off of cabbage was measured by previously described procedures.

Results

Effect of Agricultural Wastes on the Growth of Cabbage Seedlings

Of the ten agricultural wastes tested, SFMC and SGMC were the most effective substrates in promoting the growth of cabbage seedlings (Figure 1). SFMC was significantly better than the commercial container medium, BVB No. 4 peat moss. Growth of cabbage seedlings was markedly (p<0.001) affected by the composting period for SFMC and SGMC: the longer the composting period, the stronger the growth vigor (Figure 2). The optimum composting period for SFMC and SGMC was ³10 and ³6 wks, respectively.

Effect of Formulated Container Media on Rhizoctonia Damping-off

Incidence of Rhizoctonia damping-off of cabbage was high (>94.7%) in all ten container media (Table 1). However, the media of SFMC mixed with 10 to 50% (v/v) of CRH were suitable for growth of cabbage seedlings (Table 1). The SSC-05 medium made of 75% SFMC and 25% CRH (v/v) was selected for use as a key component for formulation of container media suppressive to R. solani AG-4. Amendment of SSC-05 medium with 0.3% (w/v) lime significantly reduced incidence of Rhizoctonia dampingoff of cabbage (Table 2). Amendment of SSC-05 with BM and SCSM or SCSM and RSP, significantly increased the suppressiveness to Rhizoctonia damping-off of cabbage, especially when the media were treated with lime. However, the treatment of SSC-05 medium mixed with BM, SCSM, RSP and lime showed phytotoxic effects resulting in poor seed germination, root discoloration, and growth retardation in cabbage (Table 2). Liming caused a drastic increase of pH in all container media initially but the values decreased with time of incubation (Table 3). For



Figure 1. Effect of different agriculture wastes: raw spent forest mushroom growth medium (RFM), spent forest mushroom compost (SFMC), rice hull (RH), carbonized RH, raw spent golden mushroom growth medium (RGM), spent golden mushroom compost (SGMC), peanut husk, coconut fiber, bagasse meal and wasted cotton and the commercial product BVB No. 4 peat moss on cabbage seedlings (cv. EC-Kelly) grown for 30 days in the greenhouse. BVB No. 4 is from Maasland Corporation, the Netherlands. a-g, Means of treatments followed by the same letter are not significantly different (p<0.05) according to Duncan's multiple range test.



Figure 2. Effect of spent forest mushroom compost (SFMC), spent golden mushroom compost (SGMC), and BVB No. 4 peat moss with different incubation periods on cabbage seedlings grown for 30 days in the greenhouse.

Container medium	Fresh wt. (g/5 seedlings)	Disease incidence (%) 95.0 a	
SFMC	17.6 b ¹		
CRH	9.6 d	100.0 a	
BVB No. 4 peat moss	15.4 c	94.7 a	
SFMC:CRH=9:1(v/v)	19.6 a	100.0 a	
SFMC:CRH=3:1(v/v) or SSC-05	19.3 a	100.0 a	
SFMC:CRH=1:1(v/v)	20.3 a	100.0 a	
SFMC:CRH=1:3(v/v)	14.6 c	100.0 a	
SFMC:CRH=1:9(v/v)	10.5 d	100.0 a	

Table 1. Effect of spent forest mushroom compost (SFMC) amended with different ratios of carbonized rice hull (CRH) on growth of cabbage seedlings and incidence of damping-off caused by *Rhizoctonia solani* AG-4.

¹Values within a column followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test.

Table 2. Effect of several formulated container media with or without lime on damping-off of cabbage caused by *Rhizoctonia solani*AG-4.

Container medium ¹	Disease incidence (%)			
	Lime (0.3%, w/v)	Without lime		
SFMC	80 ab ²	87 ab		
CRH	92 a	97 a		
BVB No.4 peat moss	67 bc	85 ab		
SSC-05	54 c	86 ab		
SSC-05+BM	53 c	79 bc		
SSC-05+SCSM	54 c	64 cd		
SSC-05+RSP	70 bc	84 ab		
SSC-05+BM+SCSM	28 d	59 d		
SSC-05+BM+RSP	50 cd	73 bcd		
SSC-05+SCSM+RSP	38 d	76 bcd		
SSC-05+BM+SCSM+RSP	Injury*	61 d		

¹SSC-05 medium consisted of 3 liters spent forest mushroom compost (SFMC) and 1 liter carbonized rice hull (CRH). BM=blood meal, amended at 0.2% (w/v); SCSM=shrimp and crab shell meal, amended at 0.5% (w/v); RSP=rape seed pomace, amended at 0.4% (w/v).

²Values within a column followed by the same letter are not significantly different (p<0.05) according to Duncan's multiple range test.

Table 3. pH values of 11 formulated container media with or without liming.

Container medium ¹	Lime (0	Lime (0.3%, w/v)		Without lime	
	0 day ²	20 days	0 day	20 days	
SFMC	8.78	7.09	5.94	5.88	
CRH	10.34	8.05	7.61	7.50	
BVB No.4 peat moss	8.18	7.06	5.80	5.75	
SSC-05	8.74	7.03	6.33	6.21	
SSC-05+BM	8.68	6.71	6.28	5.68	
SSC-05+SCSM	8.73	6.67	6.58	5.98	
SSC-05+RSP	8.78	6.77	6.22	5.83	
SSC-05+BM+SCSM	8.71	6.70	6.51	5.76	
SSC-05+BM+RSP	8.75	6.54	6.20	5.41	
SSC-05+SCSM+RSP	8.38	6.74	6.51	5.66	
SSC-05+BM+SCSM+RSP	8.69	6.50	6.68	6.02	

¹Refer to Table 2.

²pH values were measured at 0 and 20 days after container media were prepared.

example, liming of SSC-05 resulted in an increase of pH from 6.33 to 8.74 initially but the value dropped to 7.03 after incubation for 20 days. Similar effects of liming on changes of pH values were observed for other container media (Table 3).

Effects of Aging of Medium

The effect of SSC-06 medium on the incidence of damping-off of cabbage seedlings (Figure 3) and colonization of cabbage seeds by *R. solani* AG-4 (Figure 4) could be detected in the medium incubated at 24~28°C for 10 days or longer. The suppressive effect of SSC-06 lasted for more than 60 days. Moreover, SSC-06 medium was more effective than BVB No. 4 in preventing colonization of cabbage seeds by *R. solani* (Figure 4). Also, SSC-06 inoculated with *R. solani* AG-4 and incubated for 5 days was more suppressive to seed colonization by the pathogen than the medium treated with the pathogen and used for planting immediately (Figure 4).

Microbial Population in the Container Medium

The suppressive effect of 20-day-old SSC-06 medium on colonization of cabbage seeds by *R. solani* AG-4 was significantly greater than that of BVB No. 4 (Figure 5).



Figure 3. Effect of incubation periods of SSC-06 medium on incidence of damping-off of cabbage caused by *Rhizoctonia solani* AG-4.





Figure 4. Effect of incubation periods of SSC-06 and BVB No. 4 media on colonization of cabbage seeds by *Rhizoctonia solani* AG-4. Cabbage seeds were sown after the media were infested with the pathogen and incubated at 24~28°C for 0 and 5 days.

Figure 5. Colonization of cabbage seeds by *Rhizoctonia solani* AG-4 when planted in SSC-06 and BVB No. 4 media treated with different steaming periods at 100°C and incubated for 0 and 5 days prior to planting.



Figure 6. Population densities of bacteria and fungi in SSC-06 medium steamed at 100°C for 0, 5, 10, 15, 20, 25 and 30 min.

However, the inhibitory effect was partially nullified after it was steamed at 100°C for 15~30 min and used for planting immediately (Figure 5). SSC-06 medium steamed for 15~30 min, inoculated with *R. solani*, and incubated for 5 days prior to planting remained suppressive to the pathogen (Figure 5).

No fungal colonies were observed in the SSC-06 medium after steaming at 100°C for 5~30 min, but the colony-forming units of bacterial population were maintained at >10⁶ cfu/g dry medium (Figure 6). Addition of *T. harzianum* TH-05 to the steamed SSC-06 medium at 0-day immediately inhibited cabbage seed colonization by *R. solani* AG-4 (Figure 7) and the level of inhibition was equivalent to that observed in 20-day-old, non-steamed SSC-06 medium. Application of *T. harzianum* at a concentration of 10⁵ cfu/g dry medium to SSC-06 was more effective than the untreated, steamed SSC-06 in the suppression of *R. solani* (Figure 7).

Discussion

Composts made of agricultural and industrial wastes have been widely used as soil amendments (Sun and Huang, 1985a,b; Voland and Epstein, 1994) or as amendments to growing media (Chung and Hoitink, 1990; Hoitink, 1980; Nelson and Hoitink, 1982; Nelson and Hoitink, 1983) for production of horticultural crops. In our study, RFM and GRM were used as basic substrates for making SFMC and SGMC, respectively. SFMC and SGMC have been used as container media for growing cabbage seedlings in greenhouse nurseries in Taiwan (Huang, 1997). However, SFMC and SGMC were ineffective in the suppression of damping-off of cabbage caused by *R*.



Figure 7. Inhibition of *Rhizoctonia solani* AG-4 colonization of cabbage seed at 0 day after SSC-06 medium was steamed at 100°C for 15 min and inoculated with spores of *Trichoderma harzianum* (TH-05).

solani. The formulated container medium SSC-06 with SFMC as a major component, is a potential medium for cabbage because it improves seedling growth and suppresses Rhizoctonia damping-off.

The suppressiveness of container media amended with composted hardwood bark to Rhizoctonia damping-off varies with age of the compost and the presence of microbial agents (Kuter et al., 1988; Nelson and Hoitink, 1982; Nelson and Hoitink, 1983). The production of container media that were consistently suppressive to R. solani required not only the addition of antagonists, but also the introduction of the antagonist into an environment that favoured antagonistic activity (Chung and Hoitink, 1990; Craft and Nelson, 1996; Mihuta-Grimm and Rowe, 1986; Tunlid et al., 1989). Our study demonstrated that SSC-06 medium inhibited colonization of cabbage seeds and seedlings by R. solani AG-4 and reduced the severity of damping-off. The inhibitory effect of the SSC-06 medium on R. solani is associated with microbial activity because: (1) the inhibitory effect was partially nullified by heat steaming of the medium; (2) the inhibition was restored to the steamed medium after inoculation with T. harzianum TH-05; and (3) the SSC-06 medium required an incubation period of 5 days for inhibiting R. solani AG-4. Trichoderma harzianum is well known for its ability to control R. solani (Papavizas, 1985). The low incidence of damping-off of cabbage in SSC-06 medium inoculated with T. harzianum suggests that the growth substrate is conducive for rapid proliferation of the biocontrol agent, T. harzianum.

The breakdown of organic wastes in a formulated culture medium may have direct harmful effects on soilborne pathogens (Huang and Huang, 1993). Lee et al. (1997) reported that the addition of blood meal to HECO medium (Product Par Heveco Ltd, Tabusintac, New Brunswick Canada) rapidly increased the pH value and concentration of NH_4^+ -N, which peaked 10 days after amendment. They also found that *R. solani* colonization plunged as NH_4^+ -N was released at high a concentration from microbial degradation of the blood meal. Our study showed that the steam-heat treated SSC-06 medium remained suppressive to *R. solani* (Figure 5). This result suggests that SSC-06 medium may also contain chemical substances that are harmful to the growth and survival of *R. solani*. Further studies on factors affecting survival of pathogens and non-pathogens in the SSC-06 medium are warranted.

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研製具有抑制甘藍立枯病的栽培介質

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評估廢棄香菇培養基質、香菇太空包堆肥、廢棄金針菇培養基質、金針菇堆肥、稻殼、炭化稻殼、 花生殼、椰殼纖維、蔗渣及廢棉等十種農業廢棄物對甘藍幼苗生育的影響,發現腐熟的香菇太空包堆肥與 金針菇堆肥兩者最適合作為培育甘藍幼苗的基質。香菇太空包堆肥與金針菇堆肥堆積時間的長短可顯著 (P<0.001)影響甘藍幼苗的生長。試驗結果顯示香菇太空包堆肥與金針菇堆肥分別堆積 10 與 6 星期以 上,才使可甘藍幼苗生育良好。將腐熟香菇太空包堆肥與炭化稻殼按三比一體積均匀拌合,再分別添加 0.5% (W/V) 蝦蟹殼粉、0.2% (W/V) 牛血粉與 0.3% (W/V) 石灰等,即可製成 SSC-06 介質。SSC-06 介 質除具有抑制甘藍種苗立枯病(Rhizoctonia solani AG-4)發生的效果外,還可培育生長強壯的甘藍幼苗。 剛配置的 SSC-06 介質不具有抑制甘藍立枯病發生的效果,惟將其堆積 10 至 50 天後,即呈現顯著 (P< 0.001)的抑病功效。在不同堆積程度的 SSC-06 介質中,分別接種 R. solani AG-4 後,再利用甘藍種子 誘釣法檢測 R. solani AG-4 纏據種子的百分率,發現堆積 10~60 天的 SSC-06 介質呈現抑制 R. solani AG-4 纏據種子的功效;至於不堆積和堆積的 BVB No.4 介質均不具有抑制 R. solani AG-4 的效應。堆積 過 20 天的 SSC-06 介質,經 100°C 熱蒸氣處理 15~30 分鐘後,隨即喪失部分抑制 R. solani AG-4 的功 效。利用 Peptone-dextrose-rose bengal agar (PDRA) 與 Nutrient agar (NA) 分別檢測不同程度熱處理的 SSC-06 介質之真菌與細菌相,發現 SSC-06 介質經過 100℃ 熱蒸氣處理 5~30 分鐘後, PDRA 平板已檢測不 出任何真菌的菌落;然而,在 NA 平板卻還可分離到 10⁶ cfu/g medium 的細菌。若在處理過熱蒸氣 (100°C) 15 分鐘的 SSC-06 介質中,接種 Trichoderma harizanum TH-05 (10⁵ cfu/g dry medium),發現該 介質可立即顯現抑制 R. solani AG-4 的功效。

關鍵詞:栽培介質;農業廢棄物;甘藍立枯病;生物防治。