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A strategic approach to achieve healthy plant growth and decontaminated rhizome of *Curcuma alismatifolia* Gagenep. cultivation in modified substrate on raised-bed planting

K. Boontiang 1* , T. Siritrakulsak 1 and C. Nontaswatsri 2

*¹Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Kantharawichai 44150, Thailand. 2Department of Horticulture, Faculty of Agricultural Production, Maejo University, Sansai 50290, Thailand. *Email: kriangsuk.b@msu.ac.th*

Abstract

Curcuma alismatifolia Gagnep. is one of the most attractive tropical flowers exported from Thailand. However, distributing its rhizomes free of bacterial wilt is considered to be a critical quarantine issue. This study's strategic approach focused on cultivating *C. alismatifolia* in modified substrate on raised-bed planting without contamination by the wilt *Ralstonia solanacearum*. The experiment was conducted in a completely randomized design with 4 treatments (10 replications per treatment). Significant differences of the physical and chemical properties of appropriate substrates were observed to be correlated with mature green *Azolla* added in compost. This treatment promoted maximum growth and development, compared to the control. Moderate infection of *R. solanacearum* was detected in the rhizomes of plants grown with soil collected from the same location of a previous in-ground bedding crop (SSL) mixed in substrates. Healthy plants and decontaminated rhizomes were found in *C. alismatifolia* grown in modified substrates without SSL. Implementing the findings of this research has the potential to support sustainable production for *C. alismatifolia*.

Key words: *Curcuma*, bacterial wilt-free, modified substrates, raised-bed cultivation

Introduction

Curcuma alismatifolia Gagnep., also known as "Patumma" or "Siam Tulip," is a highly sought-after tropical flower exported from Thailand, its primary center of origin and diversity. It ranks second in market value after orchids and holds the 9th position in market share at the Aalsmeer Flower Auction in the Netherlands (Khumkratok *et al.*, 2012). Continuous introduction of new hybrid cultivars meets consumer demand. The primary use of *C. alismatifolia* is for rhizomes in landscape architecture and private gardening (70%), followed by cut flowers (20%) and potted plants (10%) (Prabhakaran Nair, 2013). However, global consumption faces quarantine challenges to ensure high-quality and healthy products. The bacterium causing wilt has been designated as a quarantine organism by the European Union Council Directive since 2000 and the Agricultural Bioterrorism Protection Act in the United States of America since 2002 (Miller, 2017).

Ralstonia solanacearum, formerly *Pseudomonas solanacearum*, has been reported as a wilt-causing soilborne disease that rapidly multiplies and blocks xylem vessels of host plants in the family Zingiberaceae (Narumol and Jirapa, 2007; Thekkan and Rajamma, 2020) or Solanaceae (Hao *et al.*, 2020). Infected plants turn brown from the tip of their leaves down through the root. Infected rhizomes of plants in the family Zingiberaceae are water-soaked, unable to shoot and eventually die. In regular production, a wide variety of *C. alismatifolia* cultivars has been grown in-ground bedding under rainfed conditions in the open field. Previous studies (Hayward and Pegg, 2013; Mitsuo *et al.*, 2014; Yuliar *et al.*, 2015) have reported that replanting rhizomes in the same location exacerbated the significant risk of contamination due to the pathogen, which survives free in

naturally infested soil without host plants for years and requires moisture for broad geographical distribution. The prevention of infectious diseases necessitates chemical treatments (Lin *et al.*, 2010) and soil solarization (Suseela *et al.*, 2019). Alternatively, most growers have tried to avoid spreading infection of bacterial wilt by integrated means of cultivating disease-free rhizomes and changing the cultivation location each year to interrupt the life cycle of *R. solanacearum*. However, crop rotation is ideally configured in a 3-year cycle to be effective.

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This study focused on cultivating *C. alismatifolia* in modified substrates on raised beds to prevent contamination by *R. solanacearum*. It aimed to evaluate substrate characteristics, plant growth, and rhizome yield for market standards.

Materials and methods

Plant materials and management: Disease-free rhizomes of *C. alismatifolia* Gagnep. cv. 'Red Shadow' were selected. Plant materials had a rhizome size of 1.5-1.7 cm in diameter with 4 storage roots. All selected rhizomes were stored under ambient conditions at 15 ºC and relative humidity at 70 % for 4 months. Immediately after storage, the rhizomes were soaked in tap water for 3 days (with daily water changes). They were placed on a raised-bed frame filled with sand and rich husk ash at a ratio of 1:1 to stimulate germination following a described method (Boontiang *et al.*, 2021).

Germinating rhizomes were transplanted into 5×10 inches plastic bags (one rhizome in one plastic bag) filled with modified substrates. Plant materials were arranged on a raised bed with 30 \times 30 cm of plant space. 5 g NPK (15-15-15)/ plant was mixed into the substrate to promote optimal growth and development.

A ratio of 50 g KCL (0-0-60) mixed with 20 L of tap water was sprayed onto plants to support rhizome establishment. Combined fertilizers were applied tri-weekly after transplanting until the mid-period of the flowering stage. Irrigation with a mini-sprinkler system was used to supply water as necessary. All plant materials were managed on raised-bed planting in the open field from May 2022 to February 2023 at a research station in the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Thailand.

Raised-bed planting design: Raised-bed planting for *C. alismatifolia* cultivation was established. Diamond-shaped expanded galvanized steel-mesh sheet of 2 m width \times 6 m length was placed on concrete poles at 0.6 m height above the ground. Beds were precisely spaced with 0.5 m between them.

Experimental design: The experiment used a completely randomized design with 4 treatments and 10 replications (10 replicates per treatment). Treatments were arranged as follows: Treatment 1 was the plants were grown in soil collected from the same location of a previously grown in-ground bedding crop (SSL): rice husk: decomposed cattle manure at the ratio of 1: 1: 1 volume per volume (v/v) with 100 g NPK and 500 g calcium hydroxide $[Ca(OH)₂]$. This was non-treated (control) as used for regular cultivation. Treatment 2 was the plants were grown in SSL: rice husk: chaff charcoal: decomposed cattle manure: mature green *Azolla* (Pteridophyta, Polypodiopsida, Salviniales) at the ratio of 1: 1: 1: 1: 1 with 100 g NPK and 500 g Ca(OH)₂. Treatment 3 was the plants were grown in soil collected from a different location that had never been planted with plants in the family Zingiberaceae or Solanaceae (SDL): rice husk: chaff charcoal: decomposed cattle manure at the ratio of 1: 1: 1: 1 with 100 g NPK and 500 g Ca(OH)₂. Treatment 4 was the plants were grown in SDL: rice husk: chaff charcoal: decomposed cattle manure: mature green *Azolla* at the ratio of 1: 1: 1: 1: 1 with 100 g NPK and 500 g Ca(OH)2.

Compost was applied in treatments 2-4. The composting processes of raw materials involved watering and mixing at the start, then wrapping them in low-density polyethylene film. Composting substrates were turned upside down and rewrapped on day 30 after starting. The thermophile stage of the final composting temperature rose to 73 ºC until the end of composting on day 60 (*Fornes et al.*, 2012).

Data collection: Physical characteristics of modified substrates were analyzed on bulk density (kg/m3) and total water-holding capacity (ml. L-1) based on loosely packed material. Particle density (%v/v), total porosity (%v/v), air volume (%v/v) and water volume $(\frac{9}{v}v/v)$ were determined during the growth period (60 day after transplanting) through straightforward and efficient multicomponent-based methodology (Evans *et al.*, 1996).

Chemical properties of modified substrates were analyzed as total organic carbon (TOC), nitrogen (N), phosphorous (P), potassium (K), organic matter (OM), A carbon-to-nitrogen (C/N) ratio, cation exchange capacity (CEC), electrical conductivity (EC) and hydrogen ion concentration (pH). An elemental analyzer determined the capacity analysis of TOC and TN-burned materials at 1,020 ºC. OM and C/N ratio calculations were performed following a described method (Manuel *et al.* 2005). CEC, pH and EC were determined in a 1:5 (v: v) material: water suspension.

Vegetative growth and physical development were monitored at week 10 post-transplanting, measuring plant height, number of new shoots per cluster, number of leaves, total leaf area (using a CI-203 Handheld Leaf Area Meter), days to inflorescence appearance, and days to true flower blooming. Inflorescence characteristics were measured as the length of peduncle, length of inflorescence, inflorescence diameter, number of fertile bracts, and number of coma bracts. Rhizomes were harvested after above-ground plant senescence, typically at week 20 posttransplanting. Rhizome yield was determined by assessing total new rhizomes per pot, rhizome diameter, number of storage roots per rhizome, length of storage root, and fresh weight of rhizome.

Infection testing of the wilt-causing bacterium, *R. solanacearum,* in the rhizomes used enzyme-linked immunosorbent assay (ELISA) following a described method (Umrao *et al.*, 2020). Contamination according to disease severity was rated on a scale of $(-)$ = no infection, $(+)$ = mild infection, $(+)$ = moderate infection and $(++)$ = severe infection. All determinations were replicated three times.

Statistical analyses: Data analysis utilized Statistix 8.1 software. Analysis of variance was conducted using the least significant difference test. Comparative means were determined using Duncan's New Multiple Range Test (DMRT).

Results

Physical characteristics and chemical properties of modified substrates: There were no significant differences in bulk density and particle density. Significant differences were observed in total porosity, air volume, water volume and water-holding capacity in compost conditions compared to the control. The composting process tended to cause an increase in total porosity, air volume, water volume and water-holding capacity than in the control. Total porosity and water-holding capacity were markedly higher and adequate for compost substrates (Table 1).

Table 1. The effect of treatments on some physical characteristics of modified substrates

Parameters	Bulk	Particle	Total	Air	Water	Water-
	density	density		porosity volume volume holding		
	(g/m^3)		(g/m^3) (% v/v) (% v/v) (% v/v) capacity			
						$(\% \text{ V/v})$
Control	1.18	2.57	90.34b	42.3c	34.2c	135.6b
Treatment 2	1.10	2.44	94.01a	46.5a	42.6a	141.7a
Treatment 3	1.13	2.50	93.23a	44.6b	39.1 _b	140.3a
Treatment 4	1.08	2.46	94.18a	46.3a	43.5a	142.0a
F-test	ns	ns	**	**	**	**

ns = non-significant difference, ** significant difference. Mean values in the same column with different letters were significantly different $(P \le 0.01)$

Large increases of nitrogen levels were observed in treatments 2 and 4 in which amounts of mature green *Azolla* were altered in the mixing ratios of compost substrates. Other essential nutrients (phosphorous and potassium) in the compost treatments were significantly higher compared to the control. The chemical properties in terms of total organic carbon, organic matter, and C/N ratio in compost treatments were lower than in the control. The proportion of CEC in the compost treatments was significantly higher compared to that in the control. However, the relationship between EC and pH was not significantly different (Table 2).

Growth and development of *C. alismatifolia***:** At week 10 posttransplanting, plants subjected to treatment 4 exhibited notable disparities in plant height (75.8 cm above substrate surface) and

Table 2. The effect of treatments on some chemical properties of modified substrates

Parameters	TOC.	N	P	K	OM	C/N	CEC	EC	pΗ
					$(\% dw)$ $(\% dw)$ (ppm) (ppm) $(\% dw)$ ratio		$(meq/100g)$ (mS/m)		
Control	35.0a	2.2 _b		12.5b 137.7b 82.3a		40.0a	47.6b		5.5
Treatment 2 32.4b		4.0a		21.0a 248.3a 73.4c		10.2 _b	55.8a	1.7	6.4
Treatment 3 31.8b		2.5 _b		20.2a 246.4a 76.1b		10.7 _b	56.9a	2.0	6.3
Treatment 4	32.0b	4.3a		21.5a 250.0a	73.6c	10.4 _b	55.6a	1.8	6.5
F-test	**	**	**	**	**	**	**	ns	ns

TOC: Total organic carbon; N: Nitrogen; P: Phosphorous; K: Potassium; OM: Organic matter; C/N: A carbon-to-nitrogen ratio; CEC: Cation exchange capacity; EC: Electrical conductivity and pH: Hydrogen ion concentration. ns = non-significant difference, ** significant difference. Mean values in the same column with different letters were significantly different ($P \le 0.01$)

shoot count (4.5 plants/cluster) compared to other treatments. However, there were no significant differences in leaf number, total leaf area, days to inflorescence appearance, or days to flower blooming (Table 3). Regarding inflorescence characteristics, treatment 4 plants displayed the longest peduncle (60.0 cm), largest inflorescence size (16.8 cm), and maximum inflorescence diameter (7.8 cm). The number of fertile bracts significantly differed from other treatments, whereas coma bract count did not (Table 4).

Table 3. The effect of modified substrates on growth and development of *C. alismatifolia* cultivation on raised-bed planting

Parameters	Plant height (cm)	No. of new shoots (plant/cluster)	No. of leaf (leaf)	Total area of leaves $\rm (cm^3)$	Day to inflorescence appearance (day)	Day to flower blooming (day)
Control	65.4c	2.8 _b	4.8	7.3	56	
Treatment 2	72.2 _b	3.2 _b	5.3	8.5	54	74
Treatment 3	72.6 _h	3.0 _b	5.2	8.7	55	73
Treatment 4	75.8a	4.5a	5.3	9.2	55	75
F-test	**	**	ns	ns	ns	ns

ns = non-significant difference, ** significant difference. Mean values in the same column with different letters were significantly different (*P ≤* 0.01)

Table 4. The effect of modified substrates on inflorescence qualities of *C. alismatifolia* cultivation on raised-bed planting

Parameters	Length of peduncle (cm)	Length of inflorescence cm	Inflorescence diameter (cm)	No. of fertile bracts (bract)	No. of coma bracts (bract)
Control	52.3c	14.0 _b	5.4 _b	6.3 _b	6.4
Treatment 2	55.2 _b	14.5b	6.3 _b	6.5 _b	6.7
Treatment 3	55.6b	14.7b	6.2 _b	6.4 _b	6.3
Treatment 4	60.0a	16.8a	7.8a	8.5a	6.8
F-test	**	**	**	**	ns

ns = non-significant difference, ** significant difference. Mean values in the same column with different letters were significantly different (*P ≤* 0.01)

Rhizome yield and infection testing of wilt-causing bacterium: *C. alismatifolia* grown in treatment 4 showed significant higher in the number of new rhizomes, number of storage roots and length of storage roots, while the fresh weight of the rhizomes of plants grown in treatment 2 was not significantly different as compared to treatment 3. The comparison of critical contamination testing of *R. solanacearum* infections in the rhizomes showed moderate infection in *C. alismatifolia* grown in the control and in treatment 2. However, no bacterial wilt-infected plants were observed in treatments 3 and 4 (Table 5).

Table 5. The effect of modified substrates on rhizome yield and scales of infection of *C.alismatifolia* cultivation on raised-bed planting

Parameters	No. of new	Diameter	No. of		Length of Fresh weight	Scales of
	rhizomes/pot of rhizome		storage root/ storage root of rhizome/ infection			
	(rhizome)	cm	rhizome cm^3)	(cm)	pot(g)	
Control	2.9 _b	2.1	2.6c	10.3 _b	326.4c	$^{++}$
Treatment 2	3.1 _b	2.2	3.6b	10.4 _b	329.1b	$^{++}$
Treatment 3	3.3 _b	2.2	3.4 _b	11.5ab	330.3b	
Treatment 4	4.5a	2.3	4.7a	13.1a	334.2a	
F-test	**	ns	**	**	**	

Mean values in the same column with different letters were significantly different ($P \le 0.01$). $1(-)$ = no infection, (+) = mild infection, (++) = moderate infection and (+++) = sever infection

Discussion

The high quality and refinement of the substrate are crucial for ornamental plant cultivation. Professional floriculturists and nurseries use peat-based substrates in their culture media (Jeong et al., 2016). As a finite resource and expensive substrate, peat presents challenges for sustainable and economically viable horticulture. Thai and other developing country farmers' entrepreneurial competencies in SMEs are cost-burdened by this critical situation. To produce disease-free rhizomes from bacterial wilt-causing *R. solanacearum*, SDL soil, local materials, and mature green *Azolla* were added to compost for *C. alismatifolia* cultivation on raised beds. They also improved the physical and chemical characteristics of substrate. These properties allowed sufficient available air and water for plant growth and development. Improved air and water volumes were observed in treatments including mature green *Azolla* mixed into the compost substrates. Particle size was expected to decrease as composting proceeded due to fragmentation and decomposition of larger particles (Gola *et al.*, 2018). This approach also enhanced the porosity of substrates, which were uniform in texture, held sufficient moisture and were well drained. Total organic carbon decreased in the composting processes mainly due to microbial respiration.

Composting, an aerobic bio-oxidative process, converts organic matter into nutrient-rich, sanitized substrates through the activity of microorganisms. This transformation results in a sustainable alternative to conventional substrates, contributing to soil health and plant growth. Mature green *Azolla*, a fast-growing water plant, serves as a valuable bio-fertilizer when cultivated alone or with other crops, enriching soil nitrogen and reducing nitrogen requirements (Roy *et al.*, 2016). Microbial activity during composting alters both the physical and chemical properties of substrates, benefiting plant growth (Raviv, 2005). Previous studies confirm that mature green *Azolla* enhances the growth of ornamental plants (Torkashvand *et al.*, 2014; Kaushal and Kumari, 2020). Treatment 1, with a higher C/N ratio attributed to rice husk without composting, exhibited noticeable differences. A lower C/N ratio indicates better nutrient availability for plants (Anandyawati *et al.*, 2023). Maintaining suitable levels of electrical conductivity (EC) and pH is crucial for optimal plant development.

The utilization of raised-bed planting technology has been consistently recognized for its positive impact on economic efficiency and environmental sustainability (Xiangbei *et al.*, 2022). In this study, agricultural waste, including locally sourced materials with *Azolla* incorporated into the composting process, was employed to enrich the soil for cultivating *C. alismatifolia* on raised beds. Notably, the addition of *Azolla* significantly improved the physical and chemical properties of the compost substrates, particularly in terms of their rapid decomposition capacity. The highest rhizome yield was closely linked to the optimal physical and chemical characteristics of the modified substrates, particularly those modified with *Azolla*. Composting mature green *Azolla* initiates a thermophilic phase, ensuring ample nitrogen sources in the final product to enhance plant growth and rhizome yield. However, there were no significant differences in the growth and yield of plants in treatments 2 and 3, possibly due to an observed epidermal change characterized by conspicuous browning edges on leaves, particularly in plants grown in SSL soil. This physical anomaly was detected earlier than visually expected in the *C. alismatifolia* cv. 'Red Shadow', despite its designation as a highly resistant cultivar (Thano and Akarapisan, 2018). These findings suggest that strains of *R. solanacearum* may have contaminated the soil from the same location as a previous crop, as reported in earlier studies (Akarapisan *et al.*, 2021).

The study suggests that decontaminated soil from non-host plant locations controls *R. solanacearum*-induced bacterial wilt. Sanitised soil with mature green *Azolla* in compost substrates is the best way to grow *C. alismatifolia*, saving farmers money. This method eliminates crop rotations to combat R. solanacearum, promoting sustainable production of healthy plants and rhizomes that meet market standards. Future research should examine raised-bed planting's cost savings for farmers.

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