



No. 13-111842- -00009-0000

Fecha: 2014-02-27 16:16:48 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 457 PROCEDIPPH Folios: 68

DIRECCIÓN DE NUEVAS CREACIONES
Procedimiento acelerado de exámenes de patente (pph)

1	NÚMERO DE SOLICITUD ANTE LA SIC	13-111842
2	OFICINA DE PRIMERA PRESENTACIÓN (OPP)	ESTADOS UNIDOS DE AMERICA
3	NÚMERO(S) DE SOLICITUD O PUBLICACIÓN DE LA(S) SOLICITUD(ES) (OPP):	1. 13/288.864 2. 3.
4	☐ REPRESENTANTE LEGAL ☒ APODERADO	
APELLIDOS		NOMBRES
OLARTE GARCIA		CARLOS R.
IDENTIFICACIÓN		C.C.79782747 T.P.74295
DIRECCIÓN	CRA. 5 No. 34-03	No. TELÉFONO 601-7700
CIUDAD	BOGOTA	CORREO ELECTRÓNICO office@olartemoure.com
PAÍS	CO	No. RADICACIÓN O PROTOCOLO DE PODER GENERAL
5	CONDICIONES PARA ACCEDER A ESTE PROCESO:	
5.1.	DATOS DE PRIORIDAD ☒ SI ☐ NO	
(33) PAÍS DE ORIGEN		CÓDIGO PAÍS
1. US		(31) NÚMERO 61/410.279
2.		(32) FECHA (AAAA/MM/DD) 03/05/2013
3.		
5.2.	DATOS SOLICITUD INTERNACIONAL PCT ☒ SI ☐ NO	
Solicitud Internacional No. PCT/US2011/059197		Fecha 03 Noviembre 2011
Publicación Internacional No. WO2012/061622		Fecha 10 Mayo 2012
El solicitante presenta una copia de la(s) reivindicación(es) patentable(s) u otorgable(s) de la solicitud de la Oficina de Primera Presentación que profirió el examen de patentabilidad, junto con una traducción al castellano. ☒ SI ☐ NO		
REQUISITOS PARA EL RECONOCIMIENTO DE LOS RESULTADOS DE EXÁMENES DE PATENTABILIDAD EFECTUADOS A SOLICITUDES EXTRANJERAS		
Para que la Superintendencia de Industria y Comercio pueda reconocer los resultados de exámenes de patentabilidad producidos por otras Oficinas de Propiedad Industrial se debe cumplir con las siguientes condiciones:		
(a) Al momento de solicitar el examen de patentabilidad, si el solicitante pide que se reconozcan exámenes de patentabilidad de otras oficinas extranjeras, deberá aportar junto con el pago de la tasa correspondiente, los siguientes anexos:		
(i) Copia de las resoluciones, fallos o decisiones que han sido relevantes para la determinación de la patentabilidad de la(s) solicitud(es) en la Oficina que haya proferido el examen de patentabilidad que contienen reivindicaciones patentables u otorgables que son la base de la petición, y si es del caso, su traducción al castellano. ☒ SI ☐ NO		
(ii) Copia de todas las reivindicaciones determinadas como patentables u otorgables en la Oficina que profirió el examen de patentabilidad y, si es del caso, su traducción al castellano. ☒ SI ☐ NO		
(iii) Copia de los antecedentes, información o documentos citados por el examinador de la Oficina de Primera Presentación del examen de patentabilidad, en las resoluciones, fallos o decisiones correspondientes, incluyendo la literatura no patente. Los documentos de patente deberán presentarse siempre y cuando no estén disponibles para la Superintendencia de Industria y Comercio mediante el simple acceso a bases de datos gratuitas. ☒ SI ☐ NO		
(iv) Una tabla de correspondencias de las reivindicaciones que han sido determinadas como patentables u otorgables en la solicitud de la Oficina que haya proferido el examen de patentabilidad.		
7	FIRMA DEL SOLICITANTE, DEL APODERADO O DEL REPRESENTANTE LEGAL	
Junto a cada firma, indicar el nombre del firmante y su calidad (si tal calidad no es obvia al leer el petitorio)		
CARLOS R. OLARTE		

Claims Correlation Table for CO Patent App. No. 13-111842

Your Ref: P-CO-101.201/ba

MBI Ref: MOI-42018-CO (MBI-106)

2

Original US Application No. 13/288,864 claims as filed	Original CO claims as filed	US allowed claims
1. Un método para modular el crecimiento de una planta, caracterizado porque comprende tratar dicha planta con una cantidad de una composición que comprende uno o más derivados de antraquinona los cuales modulan el crecimiento de la planta efectivo para modular el crecimiento en la planta.	1. Un método para modular el crecimiento de una planta, caracterizado porque comprende tratar dicha planta con una cantidad de una composición que comprende uno o más derivados de antraquinona los cuales modulan el crecimiento de la planta efectivo para modular el crecimiento en la planta.	
2. El método de conformidad con la reivindicación 1, caracterizado porque la planta es un fruto, flores, plantas ornamentales, árboles, arbustos, plantas de bulbos, fibras, vegetales o vino.	2. El método de conformidad con la reivindicación 1, caracterizado porque la planta es un fruto, flores, plantas ornamentales, árboles, arbustos, plantas de bulbos, fibras, vegetales o vino.	
3. El método de conformidad con la reivindicación 1, caracterizado porque la planta es seleccionada del grupo que consiste de fresa, calabaza, pepino, tomate, rosas, pimienta, berenjena, uvas para vino, algodón, cebolla, ajo, trigo, soya, maíz y arroz.	3. El método de conformidad con la reivindicación 1, caracterizado porque la planta es seleccionada del grupo que consiste de fresa, calabaza, pepino, tomate, rosas, pimienta, berenjena, uvas para vino, algodón, cebolla, ajo, trigo, soya, maíz y arroz.	
4. El método de conformidad con la reivindicación 1, caracterizado porque además comprende transplantar la planta en el suelo.	4. El método de conformidad con la reivindicación 1, caracterizado porque además comprende transplantar la planta en el suelo.	

Claims Correlation Table for CO Patent App. No. 13-111842

Your Ref: P-CO-101.201/ba

MBI Ref: MOI-42018-CO (MBI-106)

3

Original US Application No. 13/288,864 claims as filed	Original CO claims as filed	US allowed claims
5. El método de conformidad con la reivindicación 4, caracterizado porque previo al trasplante de la planta en el suelo, una o más raíces del fruto, o vegetales, árboles o vino se tratan con la composición.	5. El método de conformidad con la reivindicación 4, caracterizado porque previo al trasplante de la planta en el suelo, una o más raíces del fruto, o vegetales, árboles o vino se tratan con la composición.	5. A method for modulating growth of a plant comprising treating one or more roots of said plant with a composition comprising one or more anthraquinone derivatives which modulate plant growth in an amount effective to modulate said growth in said plant prior to transplanting and transplanting said plant into soil, wherein said plant is a fruit, vegetable, tree, or vine plant.
6. El método de conformidad con la reivindicación 1, caracterizado porque la composición es aplicada a un fruto, vegetales, árboles o vino de la planta vía irrigación por goteo, inmersión de raíz o aplicación en surco.	6. El método de conformidad con la reivindicación 1, caracterizado porque la composición es aplicada a un fruto, vegetales, árboles o vino de la planta vía irrigación por goteo, inmersión de raíz o aplicación en surco.	
7. El método de conformidad con la reivindicación 1, caracterizado porque el derivado de antraquinona se deriva de una planta.	7. El método de conformidad con la reivindicación 1, caracterizado porque el derivado de antraquinona se deriva de una planta.	7. The method according to claim 5, wherein the anthraquinone derivative is derived from a plant.
8. El método de conformidad con la reivindicación 1, caracterizado porque el derivado de antraquinona se deriva de un miembro de una familia de plantas	8. El método de conformidad con la reivindicación 1, caracterizado porque el derivado de antraquinona se deriva de un miembro de una familia de plantas	8. The method according to claim 5, wherein the anthraquinone derivative is derived from a member of a plant family selected from the group consisting of

3

Claims Correlation Table for CO Patent App. No. 13-111842

Your Ref: P-CO-101.201/ba

MBI Ref: MOI-42018-CO (MBI-106)

4

Original US Application No. 13/288,864 claims as filed	Original CO claims as filed	US allowed claims
seleccionado del grupo que consiste de Polygonaceae, Rhamnaceae, Fabaceae, Asphodelaceae y Rubiaceae.	seleccionado del grupo que consiste de Polygonaceae, Rhamnaceae, Fabaceae, Asphodelaceae y Rubiaceae.	Polygonaceae, Rhamnaceae, Fabaceae, Asphodelaceae and Rubiaceae.
9. El método de conformidad con la reivindicación 1, caracterizado porque el derivado de antraquinona se deriva de una especie de Reynoutria.	9. El método de conformidad con la reivindicación 1, caracterizado porque el derivado de antraquinona se deriva de una especie de Reynoutria.	9. The method according to claim 5, wherein the anthraquinone derivative is derived from a Reynoutria species.
10. El método de conformidad con la reivindicación 1, caracterizado porque el derivado de antraquinona se deriva de Reynoutria sachalinensis.	10. El método de conformidad con la reivindicación 1, caracterizado porque el derivado de antraquinona se deriva de Reynoutria sachalinensis.	10. The method according to claim 5, wherein said anthraquinone derivative is derived from Reynoutria sachalinensis.
11. Un método para modular la iniciación de raíz y extensión en una planta, caracterizado porque comprende: (a) tratar una o más raíces de una planta con un extracto de planta en una cantidad efectiva para modular la extensión de la raíz cuando es transplantada en el suelo y (b) transplantar la planta tratada de (a) en el suelo.	11. Un método para modular la iniciación de raíz y extensión en una planta, caracterizado porque comprende: (a) tratar una o más raíces de una planta con un extracto de planta en una cantidad efectiva para modular la extensión de la raíz cuando es transplantada en el suelo y (b) transplantar la planta tratada de (a) en el suelo.	20. A method for modulating root initiation or extension of a plant comprising (a) treating one or more roots of said plant with a composition comprising one or more anthraquinone in an amount effective to modulate root extension when transplanted into soil in said plant and (b) transplanting the treated plant into soil, wherein said composition is a plant extract and said plant extract comprises said one or more anthraquinone derivatives.
12. El método de conformidad con la	12. El método de conformidad con la	21. The method according to claim 20,

4

Claims Correlation Table for CO Patent App. No. 13-111842

Your Ref: P-CO-101.201/ba

MBI Ref: MOI-42018-CO (MBI-106)

5

Original US Application No. 13/288,864 claims as filed	Original CO claims as filed	US allowed claims
reivindicación 11, caracterizado porque el extracto se deriva de la familia Polygonaceae.	reivindicación 11, caracterizado porque el extracto se deriva de la familia Polygonaceae.	wherein said extract is derived from the family Polygonaceae.
13. El método de conformidad con la reivindicación 11, caracterizado porque el extracto se deriva de Reynoutria sp. o Rheum sp.	13. El método de conformidad con la reivindicación 11, caracterizado porque el extracto se deriva de Reynoutria sp. o Rheum sp.	22. The method according to claim 20, wherein said extract is derived from Reynoutria sp. or Rheum sp.
14. El método de conformidad con la reivindicación 1, caracterizado porque el extracto se deriva de Reynoutria sachalinensis.	14. El método de conformidad con la reivindicación 1, caracterizado porque el extracto se deriva de Reynoutria sachalinensis.	23. The method according to claim 20, wherein said extract is derived from Reynoutria sachalinensis.
15. Un método para modular la germinación de una semilla de una planta, caracterizado porque comprende aplicar a la semilla una cantidad de (a) una composición que contiene uno o más derivados de antraquinona los cuales modulan el crecimiento en una planta y (b) opcionalmente un agente de revestimiento de semilla efectivo para modular la germinación de la semilla de la planta.	18. Un método para modular la germinación de una semilla de una planta, caracterizado porque comprende aplicar a la semilla una cantidad de (a) una composición que contiene uno o más derivados de antraquinona los cuales modulan el crecimiento en una planta y (b) opcionalmente un agente de revestimiento de semilla efectivo para modular la germinación de la semilla de la planta.	
16. Un método para modular la infección de enfermedad transmitida por el suelo sin Rhizoctonia en una planta, caracterizado porque comprende tratar una planta con	21. Un método para modular la infección de enfermedad transmitida por el suelo sin Rhizoctonia en una planta, caracterizado porque comprende tratar una planta con	

5

Claims Correlation Table for CO Patent App. No. 13-111842
Your Ref: P-CO-101.201/ba
MBI Ref: MOI-42018-CO (MBI-106)

6

Original US Application No. 13/288,864 claims as filed	Original CO claims as filed	US allowed claims
una cantidad de una composición que contiene uno o más derivados de antraquinona los cuales modulan el crecimiento en una planta efectivo para modular la enfermedad transmitida por el suelo en la planta.	una cantidad de una composición que contiene uno o más derivados de antraquinona los cuales modulan el crecimiento en una planta efectivo para modular la enfermedad transmitida por el suelo en la planta.	
17. El método de conformidad con la reivindicación 16, caracterizado porque la infección de enfermedad transmitida por el suelo en la planta resulta de la infección por un microorganismo patógeno seleccionado del grupo que consiste de Pythium, Phytophthora, Verticillium, Sclerotium, Colletotrichum y Fusarium.	22. El método de conformidad con la reivindicación 21, caracterizado porque la infección de enfermedad transmitida por el suelo en la planta resulta de la infección por un microorganismo patógeno seleccionado del grupo que consiste de Pythium, Phytophthora, Verticillium, Sclerotium, Colletotrichum y Fusarium.	
	15. El método de conformidad con la reivindicación 1 o 11, caracterizado porque el método además comprende tratar la planta con una segunda sustancia, en donde la segunda sustancia es un agente que promueve el crecimiento de la planta.	
	16. Uso de un extracto derivado de una planta, en donde el extracto contiene uno o más derivados de antraquinona los cuales modulan el crecimiento de una planta, en	

Claims Correlation Table for CO Patent App. No. 13-111842
Your Ref: P-CO-101.201/ba
MBI Ref: MOI-42018-CO (MBI-106)

7

Original US Application No. 13/288,864 claims as filed	Original CO claims as filed	US allowed claims
	la modulación del crecimiento de la planta.	
	17. Una composición para uso en la modulación del crecimiento en una planta, caracterizada porque comprende uno o más derivados de antraquinona los cuales modulan el crecimiento en una planta y opcionalmente una segunda sustancia, en donde la segunda sustancia es un agente que promueve el crecimiento de la planta.	
	19. Uso de un extracto derivado de una planta, en donde el extracto contiene uno o más derivados de antraquinona los cuales modulan la germinación de una semilla de una planta, en la modulación de la germinación de una semilla de la planta.	
	20. Una composición para uso en la modulación de la germinación de una semilla de una planta que comprende uno o más derivados de antraquinona los cuales modulan el crecimiento en una planta y opcionalmente una segunda sustancia, en donde la segunda sustancia es un agente de revestimiento de semilla.	

4

Claims Correlation Table for CO Patent App. No. 13-111842
Your Ref: P-CO-101.201/ba
MBI Ref: MOI-42018-CO (MBI-106)

8

Original US Application No. 13/288,864 claims as filed	Original CO claims as filed	US allowed claims
	23. Uso de un extracto derivado de una planta, en donde el extracto contiene uno o más derivados de antraquinona los cuales modulan la enfermedad transmitida por el suelo en una planta, en la modulación de la enfermedad transmitida por el suelo en la planta.	
	24. Una composición para uso en la modulación de enfermedad transmitida por el suelo sin Rhizoctonia en una planta, caracterizada porque comprende uno o más derivados de antraquinona los cuales modulan la enfermedad transmitida por el suelo en una planta y opcionalmente una segunda sustancia, en donde la segunda sustancia es un agente anti-fitopatogénico de la planta.	
		18. The method according to claim 5, wherein said composition is a plant extract and said plant extract comprises said anthraquinone derivatives.
		24. The method of claim 20, wherein the plant is a fruit, flower, ornamental, tree, shrub, bulb, vegetable, or vine plant.

9

Claims Correlation Table for CO Patent App. No. 13-111842
Your Ref: P-CO-101.201/ba
MBI Ref: MOI-42018-CO (MBI-106)

9

Original US Application No. 13/288,864 claims as filed	Original CO claims as filed	US allowed claims
		25. The method of claim 20, wherein the plant is a strawberry, squash, cucumber, tomato, rose, pepper, eggplant, grapevine, cotton, onion, garlic, wheat, soy, corn, or rice plant.

Notice of References Cited	Application/Control No. 13/288,864	Applicant(s)/Patent Under Reexamination SU ET AL.	
	Examiner ALI SOROUGH	Art Unit 1617	Page 1 of 1

U.S. PATENT DOCUMENTS					
*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS						
★		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS		
*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Inoue et al., Allelochemicals From Polygonum sachalinense FR. SCHM. (POLYGONACEAE), 1992, Journal of Chemical Ecology, vol. 18 no. 10, pp. 1833-1840.
	V	Kim et al., Anthraquinones Isolated from Cassia tora (Leguminosae) Seed Show an Antifungal Property against Phytopathogenic Fungi, 2004, Journal of Agricultural and Food Chemistry, vol. 52, pp. 6096-6100.
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Anthraquinones Isolated from *Cassia tora* (Leguminosae) Seed Show an Antifungal Property against Phytopathogenic Fungi

YOUNG-MI KIM, CHI-HOON LEE, HYO-GYUNG KIM, AND HOI-SEON LEE*

Research Center for Industrial Development of Biofood Materials and Faculty of Biotechnology,
College of Agricultural and Life Science, Chonbuk National University, Chonju 561-756, South Korea

The fungicidal activities of *Cassia tora* extracts and their active principles were determined against *Botrytis cinerea*, *Erysiphe graminis*, *Phytophthora infestans*, *Puccinia recondita*, *Pyricularia grisea*, and *Rhizoctonia solani* using a whole plant method in vivo and were compared with synthetic fungicides and three commercially available anthraquinones. The responses varied with the plant pathogen tested. At 1 g/L, the chloroform fraction of *C. tora* showed a strong fungicidal activity against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani*. Emodin, physcion, and rhein were isolated from the chloroform fraction using chromatographic techniques and showed strong and moderate fungicidal activities against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani*. Furthermore, aloe-emodin showed strong and moderate fungicidal activities against *B. cinerea* and *R. solani*, respectively, but did not inhibit the growth of *E. graminis*, *P. infestans*, *P. recondita*, and *Py. grisea*. Little or no activity was observed for anthraquinone and anthraquinone-2-carboxylic acid when tested at 1 g/L. Chlorothalonil and dichlofluanid as synthetic fungicides were active against *P. infestans* and *B. cinerea* at 0.05 g/L, respectively. Our results demonstrate the fungicidal actions of emodin, physcion, and rhein from *C. tora*.

KEYWORDS: *Botrytis cinerea*; *Cassia tora*; emodin; *Erysiphe graminis*; fungicidal activity; physcion; phytopathogenic fungi; *Phytophthora infestans*; *Puccinia recondita*; *Pyricularia grisea*; rhein; *Rhizoctonia solani*

INTRODUCTION

Plant diseases are estimated to cause yield reductions of almost 20% in the major food and cash crops worldwide (1–4). Synthetic fungicides have effectively controlled plant diseases for a number of years, but increasing concern over environmental effects of the currently used fungicides has highlighted the need for the development of alternative types of selective control or for methods of crop protection with the reduced use of conventional fungicides (2, 3). Research into plant natural products is now being intensified as it becomes evident that plant-derived fungicides still have an enormous potential to influence modern agrochemical research (3). Although it is difficult to define the ecological significance of most of the synthetic fungicides, there is good reason to suppose that the secondary metabolism of plants has evolved to protect them from attack by microbial pathogens (3).

Plant extracts or their constituents may provide an alternative to currently used synthetic fungicides to control phytopathogenic fungi, because they constitute a rich source of bioactive chemicals (4, 5). Because these are often active against a limited number of specific target species, are biodegradable to nontoxic products, and are potentially suitable for use in integrated

management programs, they could lead to the development of new classes of possibly safer disease control agents. Therefore, efforts have focused on secondary plant metabolites for potentially useful products as commercial fungicides or as lead compounds (6–8). The current authors already reported and confirmed that among 25 seed extracts of leguminous species, the extract of *Cassia tora* seeds exhibits potent fungicidal activities against *Pyricularia grisea*, *Botrytis cinerea*, *Phytophthora infestans*, and *Erysiphe graminis* (9). *C. tora* is not only important as a source of natural fungicides but is also considered important for its medicinal properties, such as an antiseptic, antidiarrheal, antioxidant, and antimutagen (10–12). However, relatively little work has been done on the management of fungi or their damage using the *Cassia* species despite their excellent biological actions (9–12). In the studies described herein, we assessed in vivo the fungicidal activities of *C. tora* seed-derived components, the synthetic fungicides chlorothalonil and dichlofluanid, and three commercially available anthraquinones against six phytopathogenic fungi.

MATERIALS AND METHODS

Chemicals. Anthraquinone, anthraquinone-2-carboxylic acid, and 1,2,4-trihydroxyanthraquinone were purchased from Sigma Chemical (St. Louis, MO), and aloe-emodin was supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing,

* To whom correspondence should be addressed. Tel: +82-63-270-2544. Fax: +82-63-270-2550. E-mail: hoiseon@chonbuk.ac.kr.

Table 1. Test Condition of Phytopathogenic Fungi in a Greenhouse

disease	plant/ stage	plant no./pot	pathogen	inoculation; inoculum dosage	holding period in humidity chamber	chamber temp ^a / period ^b
RCB	rice/2-leaf	3	<i>Py. grisea</i>	leaf spray; 1 × 10 ⁶ spore/mL	1 day	25 °C/5
RSB	rice/3-leaf	3	<i>R. solani</i>	pouring inoculum ^c on the soil; 10 mL/pot	7 days	28 °C/7
CGM	cucumber/1-leaf	1	<i>B. cinerea</i>	leaf spray; 1 × 10 ⁶ spore/mL	3 days	20 °C/3
TLB	tomato/2-leaf	2	<i>P. infestans</i>	leaf spray; 1 × 10 ⁵ zoosporangia/mL	4 days	18 °C/4
WLR	wheat/1-leaf	4	<i>P. recondita</i>	leaf spray; 1.5 mg of uredospores/pot	1 day	20 °C/10
BPM	barley/1-leaf	4	<i>E. graminis</i>	dusting ^d the conidia on barley plant	no need	20 °C/10

^a The chamber temperature was kept for treated and control plants. ^b Period (days) from inoculation of pathogen to evaluation of disease severity on host plants; includes the days that plants were needed in the humidity chamber. ^c Inoculum of *R. solani* was made by inoculating mycelial plugs in wheat bran medium at 25 °C for 7 days and macerated at the ratio of 500 g of medium incubated *R. solani* per 1 L of distilled water into the mixer. ^d The preparation of *E. graminis* conidia, known as an obligate parasite, was made by dusting the inoculation of conidia on 10 barley plants cultivated in the pot (φ 7.5 cm). The treated plants were dusted with *E. graminis* conidia formed on leaves of barley by the ratio of eight tested pots/maintained pot.

Peoples Republic of China). Chlorothalonil (C₈Cl₄N₂, MW 265.90) and dichlofluanid (C₉H₁₁Cl₂N₃O₂S₂, MW 333.24) were purchased from Dongbu HanNong Chemical Co., and all other chemicals were of reagent grade.

Extraction and Isolation. The seeds of *C. tora* were purchased from a local market in Chonju and identified by Prof. Sang-Hyun Lee (Forestry Department, Chonbuk National University, South Korea). *C. tora* seeds (6 kg) were ground in a blender, extracted twice with methanol (12 L) at room temperature for 2 days, and filtered. The combined filtrate was then concentrated in vacuo in a vacuum at 45 °C to yield 11.4% (684 g). Next, the combined filtrate (20 g) was partitioned into hexane (2.8 g), chloroform (4.6 g), ethyl acetate (2.4 g), butanol (2.5 g), and water soluble (7.7 g) portions. This step was repeated 34 times with the organic solvent. The organic solvent portions were concentrated to dryness by rotary evaporation at 45 °C, while the water portion was freeze-dried. Finally, hexane (96 g), chloroform (157 g), ethyl acetate (82 g), butanol (85 g), and water soluble (262 g) portions were obtained.

Because of its good fungicidal activity against *B. cinerea*, *E. graminis*, *P. infestans*, and *Rhizoctonia solani*, the chloroform (15 g) portion was chromatographed on a 95 cm × 6.5 cm (i.d.) silica gel column (Merck 70–230 mesh, 900 g) and successively eluted with a stepwise gradient of chloroform/methanol (0, 10, 20, 30, 40, and 50%). The active 30% fraction (7.1 g) showed strong fungicidal activities against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani*. This fraction was further chromatographed on a silica gel column and eluted with chloroform/methanol (30:1). The column fractions were analyzed by thin-layer chromatography (TLC) (silica gel 60 F₂₅₄, chloroform/methanol, 30:1), and fractions with similar TLC patterns were combined. One fraction (4.2 g) showed strong fungicidal activities against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani*. This bioactive fraction was then chromatographed over a Sephadex LH-20 column (Pharmacia, 800 mm × 49 mm) using chloroform/acetone/methanol (50:1:2). This operation was repeated three times. Again, an active fraction (2.4 g) exhibited strong fungicidal activities against the four test species. This fraction was chromatographed over a Polyclar AT column (Touzart and Matignon, 100 g) packed with chloroform/acetone (50:1, v/v) and eluted with an increasing ratio of methanol (1, 2, 5, 10, and 20%). The active fraction 2 (310 mg, compound I) showed strong fungicidal activities against *B. cinerea*, *P. infestans*, and *R. solani*. Active fraction 4, containing fungicidal activities against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani*, was finally purified successively on a Sephadex LH-20 column (Pharmacia) eluted with chloroform/methanol (6:4, v/v) and cellulose (Merck) eluted with chloroform/methanol (6:4, v/v). Finally, compounds II (203 mg) and III (215 mg) were isolated. Structural determination of the active isolates (compounds I–III) was made by a spectral analysis. ¹H NMR and ¹³C NMR spectra were recorded using a Bruker AM-500 spectrometer. The UV spectra were obtained using a Waters 490 spectrometer. IR spectra using a Biorad FT-80 spectrophotometer, and mass spectra using a JEOL JMS-DX 30 spectrometer.

In Vivo Fungicidal Activity. Six plant diseases evaluated in this study were rice blast (RCB), rice sheath blight (RSB), cucumber gray

mold (CGM), tomato late blight (TLB), wheat leaf rust (WLR), and barley powdery mildew (BPM) caused by *Py. grisea*, *R. solani*, *B. cinerea*, *P. infestans*, *Puccinia recondita*, and *E. graminis*, respectively (Table 1). Except for *P. recondita* and *E. graminis*, the others were routinely maintained on potato dextrose agar (PDA) slants and V-8 agar slants and kept for stock at 4 °C.

The fungicidal activities of test samples were determined by a whole plant method in a greenhouse, as previously described (7). The initial concentration of the test solution was 2 g/L, at which over 60% control value of the compounds (aloe-emodin, emodin, physcion, and rhein) showed fungicidal activity against all test fungi; further tests employed a dilution sequence of 1, 0.5, 0.25, 0.125, and 0.0625 g/L.

To prepare test solutions at the concentration of 2 g/L, 100 mg of the test sample was dissolved in 0.5 mL of dimethyl sulfoxide and then diluted with 49.5 mL of water containing Tween 20 (250 μg/mL). Fifty milliliters of each test sample solution was sprayed onto two pots on a turntable at the same time. The treated plants were kept in a greenhouse for 1 day, before being inoculated by each pathogen. The controls were sprayed with the Tween 20 solution. All tests were replicated three times.

In a test with RCB caused by *Py. grisea*, rice plants at the second leaf stage (three plants/pot) were sprayed with each test solution. The treated plants were inoculated with a suspension of conidia in distilled water (1 × 10⁶ spores/mL) and kept in a chamber (25 °C) for 24 h under 100% relative humidity (RH). The treated and control plants were then held in a lighted chamber (26 ± 2 °C; 85% RH; photoperiod, 12 h; light intensity, 3000 lux) for 5 days and rated for disease severity. The severity was assessed on a 0–5 scale where 0 = no disease, 1 = disease affecting <1% area of second leaf, 2 = 1–10%, 3 = 10–25%, 4 = 25–60%, and 5 = >60% of leaf area affected. For RSB caused by *R. solani*, each test solution was sprayed onto rice plants at the third leaf stage (three plants/pot). The plants were inoculated by injecting the inoculum at the base of the plants. The inoculum of *R. solani* was made by inoculating mycelial plugs in wheat bran medium at 25 °C for 7 days and macerated at the ratio of 500 g of medium incubated *R. solani* per 1 L of distilled water into the mixer. The treated and control plants were held in a lighted chamber (28 °C; photoperiod, 12 h; light intensity, 3000 lux) for 5 days. With CGM caused by *B. cinerea*, the cucumber plants at the first leaf stage (one plants/pot) were sprayed with each test solution. The cucumber was inoculated with conidia (1 × 10⁶ spores/mL) of *B. cinerea* incubated on PDA medium at 20 °C for 15 days by leaf spray and then placed in a chamber (20 °C) for 4–5 days. For TLB caused by *P. infestans*, each test solution was sprayed onto tomato plants at the second leaf stage (two plants/pot). The plants were inoculated with a suspension of 1 × 10⁵ zoosporangia/mL made from a 14 day culture of V-8 juice agar medium at 20 °C. They were kept in a chamber (18 °C) for 4 days, and disease ratings were made. For WLR caused by *P. recondita*, wheat plants at the first leaf stage (four plants/pot) were sprayed with each test solution. The plants were sprayed with a suspension (60 mg/100 mL of 250 ppm Tween 20) of uredospores collected from the second leaf of wheat and then placed in a moist chamber. One day after inoculation, the plants were held in a growth chamber (20 °C and 70% RH). The

Table 2. Antifungal Activities of *C. tora* Seed-Derived Materials against Phytopathogenic Fungi^a

material	concn (g/L)	control values ^b (%)					
		RCB	RSB	CGM	TLB	WLR	BPM
methanol extract	2	0	100	100	100	0	100
	1	0	91	82	75	0	87
hexane fraction	2	0	0	71	0	0	0
	1	0	0	45	0	0	0
chloroform fraction	2	0	100	100	100	0	100
	1	0	95	86	85	0	93
ethyl acetate fraction	2	0	0	0	68	0	85
	1	0	0	0	45	0	62
LSD (0.05)			4.5	8.5	9.6		8.8

^a Activity is preventive value (%), 100% complete killing, 0% zero killing. ^b RCB, caused by *Py. grisea* on rice; RSB, caused by *R. solani* on rice; CGM, caused by *B. cinerea* on cucumber; TLB, caused by *P. infestans* on tomato; WLR, caused by *P. recondita* on wheat; and BPM, caused by *E. graminis* on barley.

fungicidal activities of the test samples were tested 10 days after inoculation. For BPM caused by *E. graminis*, barley plants with a fully expended first leaf (four plants/pot; ϕ 7.5 cm) were sprayed with a suspension of the test material. The treated plants were dusted with *E. graminis* conidia formed on leaves of barley. The control effect of test samples on each disease was evaluated with a control value (CV) calculated by the formula $CV (\%) = [(A - B)/A] \times 100$, where A and B represent the disease area on the untreated and treated plants, respectively. The LC₅₀ values were calculated by probit analysis.

Statistical Analysis. Analysis of variance was performed using the PROC GLM procedure (SAS Institute, Cary, NC). If P was less than 0.01, the means were separated with the least significant difference (LSD) test at the $P = 0.05$ level.

RESULTS AND DISCUSSION

During the initial experiments, we observed that a methanolic extract of *C. tora* seeds possessed significant fungicidal activities (100%) against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani* at the concentration of 2 g/L. However, this methanolic fraction showed no fungicidal activity against *Py. grisea* and *P. recondite*. Further solvent fractionation showed strong fungicidal activities in the chloroform fraction against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani* with 86, 93, 85, and 95% control values, respectively, at the concentration of 1 g/L (Table 2). Furthermore, the ethyl acetate fraction had fungicidal activities against *P. infestans* and *E. graminis* with 45 and 62% control values, and the hexane fraction had a fungicidal activity against *B. cinerea* with 45% control value at 1 g/L. However, little fungicidal activity was observed with the butanol and water fractions (not shown).

Because of the strong activity of the chloroform fraction, purification of the biologically active compounds was achieved with silica gel column, Sephadex LH-20 column, Polyclar AT column, Sephadex LH-20 column, and cellulose chromatography, and the isolates were bioassayed. Three active isolates showed potent fungicidal activities against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani* (Table 3). The structural determination of the isolates was made by spectroscopic methods including MS and NMR and by direct comparison with authentic reference compounds, and they were characterized as the anthraquinones rhein (compound I), emodin (compound II), and physcion (compound III) (Figure 1). These compounds were identified on the basis of the following evidence. Rhein: orange needles from CHCl₃, mp 328–330 °C. IR (KBr): $\nu_{\max}$ 3600 (OH), 2200, 1689, 1625, 1605, 1484, 1265, 1187, 1094, 752 cm⁻¹. UV (MeOH) $\lambda_{\max}$ nm (log ϵ): 230 (4.6), 260 (4.2), 432 (4.0). ¹H NMR [(CD₃)₂SO]: δ 2.07 (1H, d, $J = 1.7$ Hz, H-1),

Table 3. Antifungal Activities of Components Isolated from *C. tora* Seeds, Three Commercially Available Anthraquinones, and Synthetic Fungicides against Phytopathogenic Fungi^a

material	concn (g/L)	control values ^b (%)					
		RCB	RSB	CGM	TLB	WLR	BPM
emodin	1	0	100	100	81	0	100
	0.5	0	100	85	65	0	100
	0.25	0	76	65	32	0	94
	0.125	0	54	43	0	0	80
	0.0625	0	23	18	0	0	60
	LC ₅₀ (g/L)		0.102	0.163	0.385		0.046
	1	0	100	92	75	0	100
physcion	0.5	0	90	71	48	0	90
	0.25	0	52	49	12	0	79
	0.125	0	28	25	0	0	65
	0.0625	0	0	0	0	0	49
	LC ₅₀ (g/L)		0.248	0.263	0.518		0.073
	1	0	83	78	100	0	0
	0.5	0	65	51	100	0	0
rhein	0.25	0	35	34	90	0	0
	0.125	0	23	14	81	0	0
	0.0625	0	0	0	60	0	0
	LC ₅₀ (g/L)		0.375	0.478	0.047		
	1	0	100	91	0	0	0
	0.5	0	86	74	0	0	0
	0.25	0	67	47	0	0	0
aloe-emodin	0.125	0	36	20	0	0	0
	0.0625	0	11	0	0	0	0
	LC ₅₀ (g/L)		0.177	0.275			
	0.05	0	0	0	94	0	0
	0.05	0	0	91	0	0	0
	LSD (0.05)		9.1	10.7	8.4		7.5

^a Activity is preventive value (%), 100% complete killing, 0% zero killing. ^b RCB, caused by *Py. grisea* on rice; RSB, caused by *R. solani* on rice; CGM, caused by *B. cinerea* on cucumber; TLB, caused by *P. infestans* on tomato; WLR, caused by *P. recondita* on wheat; and BPM, caused by *E. graminis* on barley. ^c Commercial name.

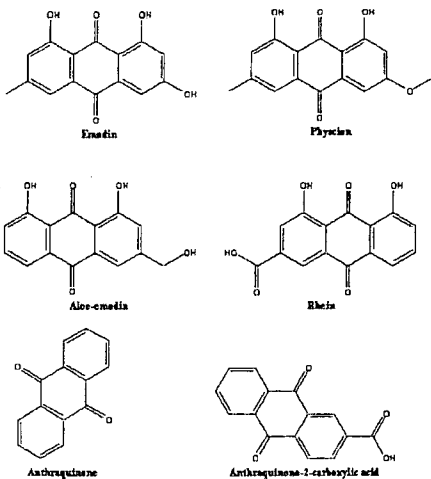


Figure 1. Structure of anthraquinones isolated from *C. tora* seed.

7.37 (1H, d, $J = 8.2$ Hz, H-3), 7.70 (1H, m, H-2), 8.09 (1H, t, $J = 3.4$ Hz, H-10), 11.87 (1H, s, H-7). HREIMS m/z 284.2205 (C₁₅H₈O₆). ¹³C NMR [(CD₃)₂SO]: δ 191.3, 181.0, 165.4, 161.4, 161.1, 138.2, 137.6, 133.8, 133.2, 124.6, 124.1, 119.4, 118.8, 118.7, 116.2. Emodin: orange needles from EtOH, mp 260–263 °C. IR (KBr): $\nu_{\max}$ 3425 (O-H), 1677, 1627 (C=O) cm⁻¹. UV (MeOH) $\lambda_{\max}$ nm (log ϵ): 248 (4.11), 262 (4.12), 285 (4.18), 432 (3.92). ¹H NMR [(CD₃)₂CO]: δ 2.46 (3H, s, Ar-CH₃), 6.65 (1H, d, $J = 2.5$ Hz, H-2), 7.12 (1H, br s, H-7), 7.24 (1H, d, $J = 2.5$ Hz, H-4), 7.55 (1H, br s, H-5), 12.05 (1H, s, OH), 12.18 (1H, s, OH). HREIMS m/z 270.0517 (C₁₅H₁₀O₅). ¹³C NMR

$[(\text{CD}_3)_2\text{CO}]$: δ 191.9, 182.2, 167.0, 165.9, 161.9, 149.3, 136.6, 134.0, 124.8, 121.2, 114.4, 110.0, 109.7, 108.6, 21.8. Physcion: orange needles from EtOH, mp 207–209 °C. IR (KBr): ν_{max} 3400 (OH), 1630 (C=O) cm^{-1} . UV (MeOH) λ_{max} nm (log ϵ): 223, 254, 265, 287, 434. ^1H NMR $[(\text{CD}_3)_2\text{CO}]$: δ 2.46 (3H, s, Ar-CH₃), 3.94 (3H, s, Ar-CH₃), 6.68 (1H, d, J = 2.5 Hz, H-2), 7.08 (1H, br s, H-7), 7.36 (1H, d, J = 2.5 Hz, H-4), 7.63 (1H, br s, H-5), 12.12 (1H, s, OH), 12.31 (1H, s, OH). HREIMS m/z 284.0708 (C₁₆H₁₂O₅). ^{13}C NMR $[(\text{CD}_3)_2\text{CO}]$: δ 187.0, 166.8, 159.6, 158.5, 142.5, 141.8, 140.7, 123.6, 123.1, 119.8, 118.9, 108.0, 104.7, 56.0, 21.2. The spectroscopic analyses of emodin, rhein, and physcion were found to be the same as those for emodin from *Cassia obtusifolia* (13) and rhein and physcion from *Rheum emodi* (14).

The fungicidal activities of emodin, rhein, and physcion isolated from *C. tora* seeds against six phytopathogenic fungi when treated with various concentrations were determined in vivo (Table 3). Emodin and physcion showed strong to moderate activities against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani* at 1, 0.5, and 0.25 g/L, except for WLR caused by *P. recondita* and RCB caused by *Py. grisea*. Rhein exhibited strong fungicidal activities against *B. cinerea*, *P. infestans*, and *R. solani* at 1, 0.5, and 0.25 g/L, except for WLR and BPM caused by *E. graminis* and *P. recondita* and RCB caused by *Py. grisea*. Emodin has an apparent LC₅₀ value of approximately 0.102, 0.163, 0.385, and 0.046 g/L against *R. solani*, *B. cinerea*, *P. infestans*, and *E. graminis*, respectively, and physcion has an LC₅₀ value of 0.248, 0.263, 0.518, and 0.073 g/L against *R. solani*, *B. cinerea*, *P. infestans*, and *E. graminis*, respectively (Table 3). Furthermore, the LC₅₀ value of rhein is 0.375, 0.478, and 0.047 g/L against *R. solani*, *B. cinerea*, and *P. infestans*, respectively. This study is the first to report the fungicidal functions of these components isolated from *C. tora* seeds against *B. cinerea*, *E. graminis*, *P. infestans*, *P. recondita*, *P. grisea*, and *R. solani*. The *Cassia* species are well-known traditional Chinese medicinal plants. The seeds of the plant have been widely used for the treatment of red and tearing eyes, headache, and dizziness (15). They are also used as an essential ingredient in medicine as antiseptic, antidiarrheal, antioxidant, antimicrobial, and antimutagen medicines (10–12, 16). Among the constituents of *C. tora*, anthraquinones constitute a major group of secondary metabolites (16). Anthraquinones (aloe-emodin, chrysophanol, physcion, and rhein) from *R. emodi* have been reported to exhibit antifungal activities against *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes*, and *Aspergillus fumigatus* (14). Furthermore, anthraquinones (anthraquinone, anthraflavine, anthrurufin, and quinizarin) exhibited a strong inhibition against *Clostridium perfringens* and *Staphylococcus aureus* and strongly inhibited the mouse liver microsomal conversion of aflatoxin B₁ into aflatoxin B₁-8,9-epoxide (16).

To further examine the fungicidal activity of anthraquinones, three additional structurally related anthraquinones, aloe-emodin, anthraquinone, and anthraquinone-2-carboxylic acid, were determined against the six phytopathogenic fungi (Figure 1 and Table 3). Aloe-emodin revealed strong to moderate fungicidal activities against *B. cinerea* and *R. solani* at 1, 0.5, and 0.25 g/L but did not inhibit the growth of *E. graminis*, *P. infestans*, *P. recondita*, and *Py. grisea*. Aloe-emodin has an apparent LC₅₀ value of 0.177 and 0.275 g/L against *R. solani* and *B. cinerea*, respectively. Little or no activity was observed for anthraquinone and anthraquinone-2-carboxylic acid when treated at 1 g/L (not shown). In the current study, these results indicated that the aromatic hydroxyls are probably important moieties for anti-

fungal actions, given that the four compounds (aloe-emodin, emodin, physcion, and rhein) have at least two such groups whereas anthraquinone and anthraquinone-2-carboxylic acid lack them. Because of the fungicidal activities of rhein, emodin, and physcion against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani*, the compounds were compared with the synthetic fungicides chlorothalonil and dichlofluanid (Table 3). A potent fungicidal activity was observed with chlorothalonil against *P. infestans* at 0.05 g/L and dichlofluanid against *B. cinerea* at 0.05 g/L, whereas no fungicidal activity was produced for chlorothalonil against *B. cinerea*, *P. grisea*, *R. solani*, and *P. recondita* or for dichlofluanid against *P. grisea*, *P. infestans*, *P. recondita*, and *R. solani*. In this study, although the fungicidal activity of rhein, emodin, and physcion is less than that of the synthetic fungicides used, rhein, emodin, and physcion may be useful as leads developing new types of fungicides for controlling *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani* on crops.

Certain plant extracts and phytochemicals act in many ways on various types of disease-causing agents and may be applied to the crop in the same way as other agricultural chemicals. They are being considered as potential alternatives for synthetic fungicides (17, 18) or lead compounds for new classes of synthetic fungicides such as podoblastin produced by *Podophyllum peltatum* (19, 20).

LITERATURE CITED

- (1) Agrios, G. N. Significance of plant diseases. *Plant Pathology*, 4th ed.; Academic Press: San Diego, 1997; pp 25–37.
- (2) Lee, S. E.; Kim, J. E.; Lee, H. S. Insecticide resistance in increasing interest. *Agric. Chem. Biotechnol.* 2001, 44, 105–112.
- (3) Benner, J. P. Pesticidal compounds from higher plants. *Pestic. Sci.* 1993, 39, 95–102.
- (4) Swain, T. Secondary compounds as protective agents. *Annu. Rev. Plant Physiol.* 1977, 28, 479–501.
- (5) Wink, M. Production and application of phytochemicals from an agricultural perspective. In *Phytochemistry and Agriculture*; van Beck, T. A., Breteler, H., Eds.; Proceeding of the Phytochemical Society Europe; Clarendon Press: Oxford, 1993; Vol. 34, pp 171–213.
- (6) Balandrin, M.; Klocke, J.; Wurtele, E. S.; Bollinger, W. H. Natural plant chemicals: Sources of industrial and medicinal materials. *Science* 1985, 228, 1154–1160.
- (7) Cho, J. H.; Kim, J. C.; Kim, M. K.; Lee, H. S. Fungicidal activities of 67 herb-derived oils against six phytopathogenic fungi. *Agric. Chem. Biotechnol.* 2002, 45, 202–207.
- (8) Kim, M. K.; Choi, G. J.; Lee, H. S. Fungicidal property of *Curcuma longa* L. rhizome-derived curcumin against phytopathogenic fungi in greenhouse. *J. Agric. Food Chem.* 2003, 51, 1578–1581.
- (9) Lee, H. S.; Kim, B. S.; Kim, H. T.; Cho, K. Y.; Ahn, Y. J. Fungicidal activities of leguminous seed extracts toward phytopathogenic fungi. *J. Pestic. Sci.* 1998, 2, 21–27.
- (10) Choi, J. S.; Lee, H. J.; Park, K. Y.; Ha, J. O.; Kang, S. S. *In vitro* antimutagenic effects of anthraquinone aglycones and naphthopyrone glycosides from *Cassia tora*. *Planta Med.* 1997, 63, 11–14.
- (11) Yen, G. C.; Chen, H. W.; Duh, P. D. Extraction and identification of an antioxidative component from Jue Ming Zi (*Cassia tora* L.). *J. Agric. Food Chem.* 1998, 46, 820–824.
- (12) Yen, G. C.; Chung, D. Y. Antioxidant effects of extracts from *Cassia tora* L. prepared under different degrees of roasting on the oxidative damage to biomolecules. *J. Agric. Food Chem.* 1999, 47, 1326–1332.
- (13) Yang, Y. C.; Lim, M. Y.; Lee, H. S. Emodin isolated from *Cassia obtusifolia* (Leguminosae) seed shows larvicidal activity against three mosquito species. *J. Agric. Food Chem.* 2003, 51, 7629–7631.

- (14) Agarwal, S. K.; Singh, S.; Verma, S.; Kumar, S. Antifungal activity of anthraquinone derivatives from *Rheum emodi*. *J. Ethnopharmacol.* **2000**, *72*, 43–46.
- (15) Guo, H.; Chang, Z.; Yang, R.; Guo, D.; Zheng, J. Anthraquinones from hairy root cultures of *Cassia obtusifolia*. *Phytochemistry* **1998**, *49*, 1623–1625.
- (16) Lee, H. S. Inhibitory effects of quinizarin isolated from *Cassia tora* seeds against human intestinal bacteria and aflatoxin B₁ biotransformation. *J. Microbiol. Biotechnol.* **2003**, *13*, 529–536.
- (17) Hedin, P. A. New concepts and trends in pesticide chemistry. *J. Agric. Food Chem.* **1982**, *30*, 201–215.
- (18) Hostettman, K.; Potterat, O. Strategy for the isolation and analysis of antifungal, molluscicidal, and larvicidal agents from tropical plants. In *Phytochemicals for Pest Control*; Hedin, P. A., Hollingworth, R. M., Masler, E. P., Miyamoto, J., Thompson, D. G., Eds.; ACS Symposium Series No. 387; American Chemical Society: Washington, DC, 1997; pp 14–26.
- (19) Miyakado, M. The search for new insecticidal and fungicidal compounds from plants. *J. Pestic. Sci.* **1986**, *11*, 483–492.
- (20) Seifert, K.; Unger, W. Insecticidal and fungicidal compounds from *Isatis tinctoria* L. *J. Naturforsch.* **1994**, *44C*, 44–48.

Received for review April 18, 2004. Revised manuscript received June 26, 2004. Accepted June 28, 2004. This work was supported by a grant from BioGreen 21 Program, Rural Development Administration, South Korea.

JF049379P

ALLELOCHEMICALS FROM *Polygonum sachalinense* FR. SCHM. (POLYGONACEAE)

M. INOUE,¹ H. NISHIMURA,^{2,*} H.-H. LI,¹ and J. MIZUTANI¹

¹Research Development Corporation of Japan (JRDC)
Mizutani Plant Ecochemicals Project
Eniwa 061-13, Japan

²Department of Bioscience and Technology
Hokkaido Tokai University
Sapporo 005, Japan

(Received February 18, 1992; accepted June 10, 1992)

Abstract—The root exudates from *Polygonum sachalinense* in a recirculating system significantly inhibited lettuce seedling growth. The rhizomes and roots of *P. sachalinense* were extracted with 80% acetone. Bioassay of the neutral-acidic fraction on the TLC agar plate showed the inhibitory activity corresponded to the two yellow pigment bands. Two orange needles were isolated and identified as anthraquinone compounds: emodin and physcion. Both compounds exhibited inhibitory activities against the seedling growth of several testing plant species. Glucosides were isolated from *P. sachalinense* and were identified as emodin-1-*O*- β -D-glucoside and physcion-1-*O*- β -D-glucoside, respectively. On plant growth bioassay, these glucosides showed no phytotoxic activity against lettuce seedlings. The concentrations of emodin, physcion, and their glucosides from rhizome with roots, aerial parts, fallen leaves, and soil were determined. The rhizome with roots and fallen leaves contained emodin and physcion at relatively high concentrations. Emodin also occurs in the soil of this plant community with effective concentrations in the fall. The results indicate that these anthraquinones are responsible for the observed interference and are potent allelopathic substances.

Key Words—*Polygonum sachalinense*, allelopathy, allelochemicals, anthraquinone, emodin, emodin-1-*O*-D-glucoside, physcion, physcion-1-*O*-D-glucoside, root exudates.

INTRODUCTION

Chemical interactions among plants have long been recognized. Many scientists have shown that certain plants produce secondary compounds that have the

*To whom correspondence should be addressed.

ability to inhibit growth and germination of other plants (Rice, 1984; Harborne, 1988). The genus *Polygonum* has about 300 species and is distributed all over the world. AlSaadawi and coworkers demonstrated that *Polygonum aviculare* was allelopathic, and several phenolic compounds and long-chain fatty acids appeared to be responsible for this activity (AlSaadawi and Rice, 1982a,b; AlSaadawi et al., 1983).

P. sachalinense (Sachaline giant knotweed), or "Ohitadori" in Japanese, is a perennial grass found in Honshu and Hokkaido. Its rhizome has been used as an herbal medicine for laxative and diuretic purposes. This grass forms colonies along roadsides and river banks in gravelly soils. Judging from its ability to rapidly colonize, we hypothesize that allelopathy contributes to its aggressiveness.

This paper deals with the isolation and identification of allelochemicals in *P. sachalinense* and the evaluation of these allelochemicals in the ecosystem.

METHODS AND MATERIALS

Root Exudate Recirculating Systems. The evaluation of the effect of the root exudates as allelochemicals was carried out according to the root exudate recirculating system of Stevens and Tang (1985). Lettuce seedlings were planted in the acceptor pot, while *P. sachalinense* was grown in the donor pot. Nutrient solution (Tadano and Tanaka, 1976) from the donor pot was lifted through 8-mm-diameter glass tubing to the acceptor pot using an air pump. Nutrient levels were maintained, and pH was kept 5.5 with H_2SO_4 or NaOH. Recirculating solution was not changed during the experiment except for replenishing water and nutrient solution. After 10–14 days, the growing condition of lettuce (*Lactuca sativa* L.) seedlings was compared with that of the control growing under the same conditions without the donor plant. The experiment was carried out in a greenhouse.

Plant Materials and Extraction. Rhizomes with roots, aerial parts, and fallen leaves of *P. sachalinense* were collected at several wild places near the campus of Hokkaido Tokai University. Excavated rhizomes were rinsed with tap water and brushed lightly to remove soil, and air-dried. The rhizome (5.1 kg) and aerial part (3.8 kg) were sliced and extracted in separate containers with 80% acetone for about two months at room temperature. The extracts were filtered and concentrated under reduced pressure. In another experiment, 100 g of fallen leaves were extracted with 300 ml of ether for 24 hr, and the residues were removed by filtration.

Fractionation. The acetone extract of rhizome with roots was divided into ether-soluble and water-soluble fractions. The ether-soluble fraction was further divided into neutral, acidic, and basic fractions in an usual manner. The water-

soluble fraction was evaporated to dryness for bioassay; it did not show any significant activity against the growth of lettuce seedlings.

TLC Agar Plate Bioassay. Thin-layer chromatography was carried out on a 20 × 7-cm silica gel glass plate (Merck, Kieselgel 60 F254) 0.25 mm thick using hexane-CHCl₃-MeOH (4:10:1 v/v/v) as developing solvents. The chromatogram was covered with a 0.5% agar layer (2 mm), and the seeds of green amaranth (*Amaranthus viridis* L.), timothy grass (*Phleum pratense* L.), crabgrass (*Digitaria adscendens* Henr.), and Chinese cabbage (*Brassica campestris* L.) were sown in a row perpendicular to the bands on the chromatogram. The seeds were incubated in a moisture saturated growth chamber at 24–26°C with 14 hr light and 10 hr dark. After four to seven days, seed germination and plant growth were examined.

Seedling Growth Bioassay. In a 25-mm-high × 40-mm-ID glass Petri dish 10 germinated seeds were placed on 0.5% agar gel with a known concentration of the test sample. Lettuce, green amaranth, and timothy grass were used for bioassay. The assay was done in duplicate. Incubation was for seven days in a growth chamber at 24–26°C with a 14-hr light and 10-hr dark cycle. The average length of hypocotyl or leaf sheath and the longest roots of the testing plants were measured and compared with that of the control growing on 0.5% agar gel only.

Isolation and Identification of Allelochemicals. The inhibitory substances against seedling growth were fractionated by SiO₂ column chromatography using a hexane-CHCl₃-MeOH gradient as an eluent, and the desired products were recrystallized from hexane-CHCl₃ to give two orange-needle crystals. The isolated compounds were identified from IR, UV, mass, and NMR spectra.

Emodin (1): IR (KBr) $\nu_{\max}$ 3400 (H)(OH), 1630 (C=O) cm⁻¹; UV (MeOH) $\lambda_{\max}$ 221, 253, 265, 289, 437 nm; [¹H]NMR [(CD₃)₂CO] δ 2.46 (3H, s, Ar-CH₃), 6.65 (1H, d, J = 2.5 Hz, H-2), 7.12 (1H, br s, H-7), 7.24 (1H, d, J = 2.5 Hz, H-4), 7.55 (1H, br s, H-5), 12.05 (1H, s, OH), 12.18 (1H, s, OH); HR-EI-MS m/z 270.0515 (C₁₅H₁₀O₅).

Physcion (2): IR (KBr); $\nu_{\max}$ 3400 (OH), 1630 (C=O) cm⁻¹; UV (MeOH) $\lambda_{\max}$ 223, 254, 265, 287, 434 nm; [¹H]NMR (CDCl₃) δ 2.45 (3H, s, Ar-CH₃), 3.94 (3H, s, Ar-OCH₃), 6.68 (1H, d, J = 2.5 Hz, H-2), 7.08 (1H, br s, H-7), 7.36 (1H, d, J = 2.5 Hz, H-4), 7.63 (1H, br s, H-5), 12.12 (1H, s, OH), 12.31 (1H, s, OH); HR-EI-MS m/z 284.0704 (C₁₆H₁₂O₅).

Isolation and Identification of Anthraquinone Glycoside. The rhizomes with roots and the aerial part of *P. sachalinense* were collected in June 1991. Immediately after cutting, they were extracted in EtOH by refluxing for 5 hr and the EtOH solution concentrated to one third of the original volume. The aqueous concentrate was extracted with ether to remove free anthraquinones and other ether-soluble constituents. Ba(OH)₂ solution (5%) was added to precipitate the organic acids. After filtration, the filtrate was adjusted to pH 3 and was extracted

with *n*-BuOH. The *n*-BuOH layer was separated and evaporated in vacuo to give the crude glycoside fraction, which was further fractionated by SiO₂ column chromatography using a hexane—CHCl₃—MeOH as an eluent to obtain anthraquinone glycosides. Rechromatography and further recrystallization from MeOH gave two glycosides as orange-needle crystals. Identification was based on the comparison of the melting point, *R_f* values, and spectrometric data with those in Kang and Woo (1982) and Kato and Morita (1987).

Emodin-1-*O*-β-D-glucoside (**3**): mp 193°C; IR (KBr) $\nu_{\max}$ 3400 (OH), 1630 (free C=O), 1600 (chelated C=O) cm⁻¹; UV (EtOH) $\lambda_{\max}$ 221, 253, 265, 286, 421 nm; [¹H]NMR (CD₃OD) δ 2.40 (3H, s, Ar-CH₃), 4.96 (1H, d, *J* = 7.5 Hz, anomeric H), 7.04 (1H, br s, H-7), 7.10 (1H, d, *J* = 2.5 Hz, H-2), 7.32 (1H, d, *J* = 2.5 Hz, H-4), 7.48 (1H, br s, H-5); FAB-MS (DMSO) *m/z* (NaCl addition) 455 (M + Na)⁺, 478 (M + 2Na)⁺.

Physcion-1-*O*-β-D-glucoside (**4**): mp 240°C; IR (KBr); $\nu_{\max}$ 3400 (OH), 1640 (free C=O), 1600 (chelated C=O) cm⁻¹; UV (MeOH) $\lambda_{\max}$ 226, 273, 417 nm; [¹H]NMR (CD₃OD) δ 2.46 (3H, s, Ar-CH₃), 3.97 (3H, s, Ar-OCH₃), 4.96 (1H, d, *J* = 7.5 Hz, anomeric H), 7.11 (1H, br s, H-7), 7.30 (1H, d, *J* = 2.5 Hz, H-2), 7.57 (1H, d, *J* = 2.5 Hz, H-4), 7.60 (1H, br s, H-5).

Quantitation of Anthraquinones and Anthraquinone Glycosides. Plant samples were collected at several wild places near the campus of Hokkaido Tokai University. Fresh aerial parts, rhizome with roots, and fallen leaves were extracted with 80% acetone. After concentration, the aqueous residues were extracted with ether. The ether-soluble fractions were quantitatively analyzed by HPLC (JASCO System-800) and compared with standard emodin and physcion (purchased from Funakoshi Co. Ltd.). The analytical conditions are as follows: column—YMC-Pack ODS-AM (30 cm × 10 mm ID); solvent—MeOH/H₂O (80:20, v/v); flow rate—4 ml/min, wavelength of detection—245 nm.

Soil samples were collected in November 1990 from the center of a *P. sachalinense* community near the campus of Hokkaido Tokai University at the depth of about 10 cm. Litter was removed as completely as possible by sieving and the soil was dried at room temperature overnight. A 500-g soil sample was stirred in 2 liters of acetone-1 N HCl (2:1, v/v) for 1 hr at 50°C and further treated with ultrasonic energy for 1 min. After leaving overnight at room temperature, it was filtered. The filtrate was evaporated to remove acetone and extracted three times with ether. The ether extract was evaporated in vacuo and fractionated by SiO₂ column chromatography. The contents of emodin and physcion were determined by HPLC. The amounts of anthraquinone glycosides were determined by weighing the isolated crystals of emodin-1-*O*-β-D-glucoside and physcion-1-*O*-β-D-glucoside.

Spectrometers. IR spectra were recorded on a JASCO IR-810 as KBr disk for crystals. UV spectra were recorded on a Hitachi U-3210 spectrophotometer. NMR spectra were measured on a Bruker AM-400 (400 MHz) spectrometer

using TMS as an internal standard. Mass spectra were recorded using JEOL JMS-DX303HF GC-MS spectrometer.

RESULTS AND DISCUSSION

The root exudates from *P. sachalinense* showed an inhibition on lettuce seedling growth using the recirculating system. It is well known that organic compounds are exuded from healthy undamaged plant roots (Rovira, 1969), and we expect that allelochemicals or their precursors are present in the plant tissue and also released from the rhizome and roots.

From the results of the TLC agar plate bioassay, growth inhibitory activity was shown in the two bands of yellow pigments against green amaranth and timothy grass on the chromatogram of the neutral-acid fraction (Figure 1). Two corresponding orange needles were isolated and identified as anthraquinone compounds: emodin (1) and physcion (2) (Figure 2).

Emodin and physcion exhibited inhibitory activities against the seedling growth of several test species (Figure 3). Emodin inhibited root and hypocotyl or leaf sheath growth over 100 ppm (3.7×10^{-4} M). It severely inhibited the growth of lettuce seedlings at 50 ppm (1.85×10^{-4} M). Physcion was less active and inhibited root and hypocotyl growth at 200 ppm (7.0×10^{-4} M).

The presence of anthraquinone glucosides was expected since they are common for transportation and storage and are less toxic in the plant. As a result, two glucosides were isolated from both the rhizome and aerial part of *P.*

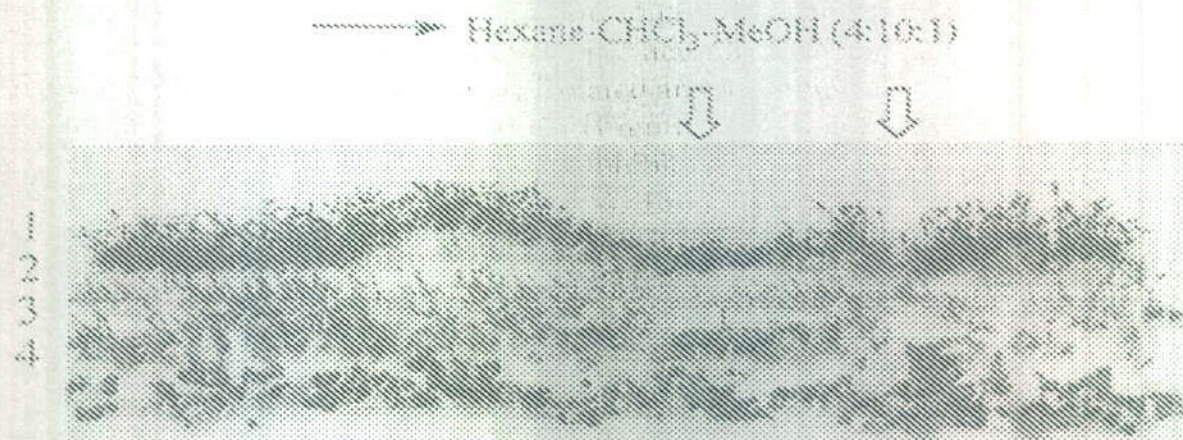


FIG. 1. Bioassay of neutral and acid substances from *Polygonum sachalinense* by TLC agar plate method (5 days). Arrows point to areas of inhibited plant activity. (1) green amaranth, (2) timothy grass, (3) crab grass, (4) Chinese cabbage.

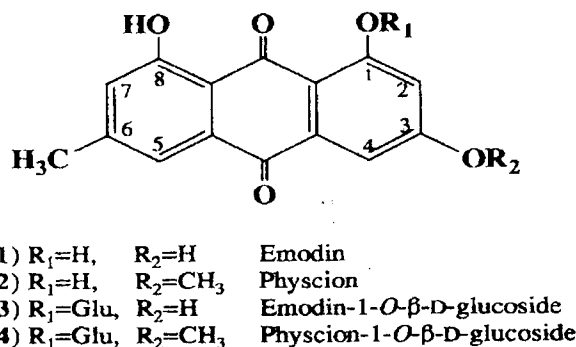


FIG. 2. Chemical structures of allelochemical candidates from *Polygonum sachalinense*.

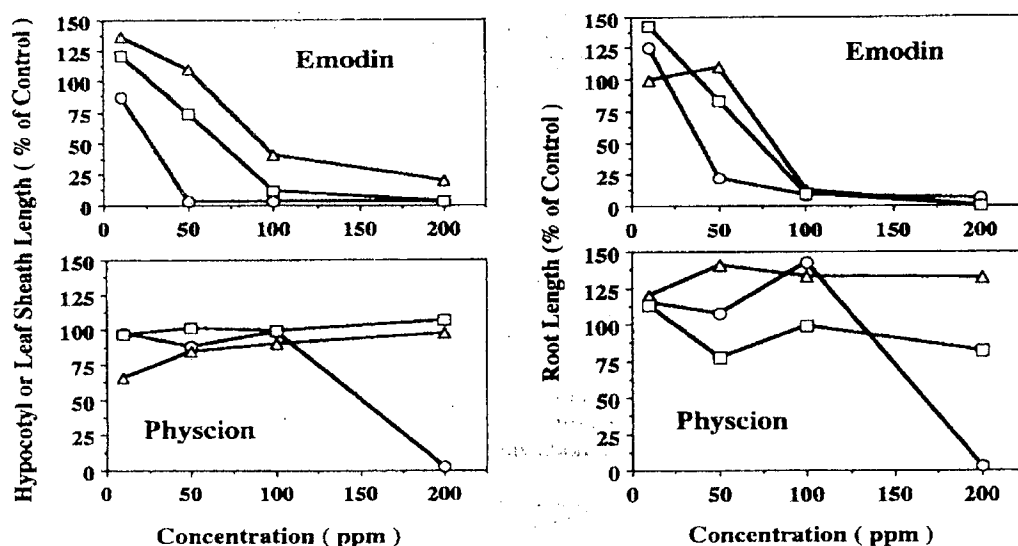


FIG. 3. Growth inhibitory activities of emodin and physcion against plant seedlings. ○: lettuce, △: green amaranth, □: timothy grass.

sachalinense and identified as emodin-1-*O*- β -D-glucoside (3) and physcion-1-*O*- β -D-glucoside (4) (Figure 2). In plant growth bioassay, both emodin and physcion glucosides showed no phytotoxicity at 200 ppm against lettuce seedlings.

The concentrations of emodin and physcion, and their glucosides in the rhizome, aerial part, fallen leaves, and soil were quantitatively determined. Contents of emodin and physcion in the fresh rhizome were ca. 158 and 32 mg/kg fresh weight, and in the aerial part, ca. 72 and 22 mg/kg fresh weight, respectively. Large amounts of anthraquinones were still detected in dry fallen leaves more than four months after defoliation: emodin, 213 mg/kg dry weight; physcion, 180 mg/kg dry weight. In addition, contents of emodin and physcion in the soil were 55 and 30 mg/kg dry weight, respectively; these concentrations

are enough to inhibit plant seedlings. These facts indicate that the anthraquinones are very stable in the ecosystem.

Allelopathic substances are released from plants in four ways (Tukey, 1969): (1) Litter of leaves and stems decomposes by physical or biological processes and the substance is released. (2) The active substance is released to the soil from the root directly by exudation or through the decay of dead roots. (3) Volatile material is vaporized from plants and acts on other plants through the air. (4) Rain and fog drips transfer toxic compounds from leaves to the soil.

We consider that the first two items above are associated with the allelopathy of *P. sachalinense*. It has anthraquinone glucosides in the plant tissue. We propose that either these glucosides or the aglycons are released from the rhizome. The glucosides are then decomposed to active forms—emodin and physcion—and subsequently exhibit plant growth inhibitory activities against other plant species. To confirm this hypothesis, isolating glucosides and aglycons from the root exudate solution is currently under investigation. Furthermore, anthraquinones exuded from the fallen leaves of *P. sachalinense* accumulate on the soil surface and affects early seedling growth of nearby plants of the community.

There are reports about antimicrobial activities of emodin and physcion (Podojil et al., 1978; Anke et al., 1980; Kitanaka and Takido, 1986). Some antimicrobial activity by emodin and physcion may directly or indirectly affect the growth of other plants. Rice (1964, 1984) and AlSaadawi et al. (1983) pointed out that some weeds produce allelopathic chemicals that inhibited soil bacteria and changed the soil environment to their own advantage. While these anthraquinone compounds are potent allelopathic substances, the question of the extent of their impact against the flora in *P. sachalinense* is yet to be fully evaluated.

Acknowledgments—We wish to thank Mr. A. Sato (Hokkaido Tokai University) for NMR and mass spectral measurements.

REFERENCES

- AL SAADAWI, I.S., and RICE, E.L. 1982a. Allelopathic effects of *Polygonum aviculare* L. I. Vegetational patterning. *J. Chem. Ecol.* 8: 993–1009.
- AL SAADAWI, I.S., and RICE, E.L. 1982b. Allelopathic effects of *Polygonum aviculare* L. II. Isolation, characterization, and biological activities of phytotoxin. *J. Chem. Ecol.* 8:1011–1023.
- AL SAADAWI, I.S., RICE, E.L., and KARNS, T.K.B. 1983. Allelopathic effects of *Polygonum aviculare* L. III. Isolation, characterization, and biological activities of phytotoxin other than phenol. *J. Chem. Ecol.* 9:761–774.
- ANKE, H., KOLTHOUM, I., and LAATSCH, H. 1980. Metabolic products of microorganisms. 192. The anthraquinones of the *Aspergillus glaucus* group. II. Biological activity. *Arch. Microbiol.* 126:231–236.

- HARBORNE, J.B. 1988. Introduction to Ecological Biochemistry. Academic Press, New York.
- KANG, S.S., and WOO, W.S. 1982. Anthraquinones from the leaves of *Polygonum sachalinense*. *Kor. J. Pharmacol.* 13:7-9.
- KATO, T., and MORITA, Y. 1987. Anthraquinone components in *Rumex acetosa* L. *Syoyakugaku Zasshi* 41:67-74.
- KITANAKA, S., and TAKIDO, M. 1986. Studies on the constituents in the roots of *Cassia ofusifolia* L. and the antimicrobial activities of constituents of the roots and the seed. *Yakugaku Zasshi* 106:302-306.
- PODOJIL, M., SEDMERA, P., VOKOUN, J., BETINA, V., BARÁTHOVÁ, H., DURACKOVÁ, Z., HORÁKOVÁ, K., NEMEC, P. 1978. *Eurotium (Aspergillus) repens* metabolites and their biological activity. *Folia Microbiol.* 23:438-443.
- RICE, E.L. 1964. Inhibition of nitrogen-fixing and nitrifying bacteria by seed plant. *Ecology* 45:824-837.
- RICE, E.L. 1984. Allelopathy, 2nd ed. Academic Press, New York.
- ROVIRA, A.D. 1969. Plant root exudates. *Bot. Rev.* 35:35-59.
- STEVENS, G.A., JR., and TANG, C.S. 1985. Inhibition of seedling growth of crop species by recirculation root exudates of *Bidens pilosa* L. *J. Chem. Ecol.* 11:1411-1425.
- TADANO, T., and TANAKA, A. 1976. Differences for crop species of ammonium and nitrogen suitability. *Nippon Dojo-Hiryogaku Zasshi* 47:321-328.
- TUKEY, H.B. 1969. Implications of allelopathy in agricultural plant science. *Bot. Rev.* 35:1-16.



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

117607 7590 12/23/2013
Marrone Bio Innovations c/o Yuko Sonoka
2121 Second Street, Ste. B-107
Davis, CA 95618

EXAMINER

SOROUGH, ALI

ART UNIT PAPER NUMBER

1617

DATE MAILED: 12/23/2013

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/288,864	11/03/2011	Hai Su	MOI-42018-US	5883

TITLE OF INVENTION: COMPOSITIONS CONTAINING ANTHRAQUINONE DERIVATIVES AS GROWTH PROMOTERS AND ANTIFUNGAL AGENTS

APPL. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$890	\$300	\$0	\$1190	03/24/2014

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: **Mail** Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or **Fax** (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

117607 7590 12/23/2013
Marrone Bio Innovations c/o Yuko Soncoka
2121 Second Street, Ste. B-107
Davis, CA 95618

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/288,864	11/03/2011	Hai Su	MOI-42018-US	5883

TITLE OF INVENTION: COMPOSITIONS CONTAINING ANTHRAQUINONE DERIVATIVES AS GROWTH PROMOTERS AND ANTIFUNGAL AGENTS

APPL. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$890	\$300	\$0	\$1190	03/24/2014

EXAMINER	ART UNIT	CLASS-SUBCLASS
SOROUSII, ALI	1617	504-189000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363). ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached. ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. Use of a Customer Number is required.	2. For printing on the patent front page, list (1) The names of up to 3 registered patent attorneys or agents OR, alternatively, (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. 1. _____ 2. _____ 3. _____
---	---

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE _____ (B) RESIDENCE: (CITY and STATE OR COUNTRY) _____

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted: ☐ Issue Fee ☐ Publication Fee (No small entity discount permitted) ☐ Advance Order - # of Copies _____	4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above) ☐ A check is enclosed. ☐ Payment by credit card. Form PTO-2038 is attached. ☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).
--	--

5. Change in Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29

☐ Applicant asserting small entity status. See 37 CFR 1.27

☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____	Date _____
Typed or printed name _____	Registration No. _____



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/288,864	11/03/2011	Hai Su	MOI-42018-US	5883
11/607 7590 12/23/2013 Marrone Bio Innovations c/o Yuko Soncoka 2121 Second Street, Ste. B-107 Davis, CA 95618			EXAMINER SOROUSH, ALI	
			ART UNIT 1617	PAPER NUMBER

DATE MAILED: 12/23/2013

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 0 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 0 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND THIS OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

**Notices of Allowance and Fee(s) Due mailed between October 1, 2013 and
December 31, 2013**

(Addendum to PTOL-85)

If the "Notice of Allowance and Fee(s) Due" has a mailing date on or after October 1, 2013 and before January 1, 2014, the following information is applicable to this application.

If the issue fee is being timely paid on or after January 1, 2014, the amount due is the issue fee and publication fee in effect January 1, 2014. On January 1, 2014, the issue fees set forth in 37 CFR 1.18 decrease significantly and the publication fee set forth in 37 CFR 1.18(d)(1) decreases to \$0.

If an issue fee or publication fee has been previously paid in this application, applicant is not entitled to a refund of the difference between the amount paid and the amount in effect on January 1, 2014.

Notice of Allowability	Application No. 13/288,864	Applicant(s) SU ET AL.	
	Examiner ALI SOROUGH	Art Unit 1617	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--
All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

- 1. ☒ This communication is responsive to the response filed on 08/26/2013.
☐ A declaration(s)/affidavit(s) under 37 CFR 1.130(b) was/were filed on _____.
- 2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
- 3. ☒ The allowed claim(s) is/are 5-10, 18 and 20-25. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
- 4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:
a) ☐ All b) ☐ Some *c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

- 5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
- 6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

- Attachment(s)**
- | | |
|---|--|
| 1. ☐ Notice of References Cited (PTO-892) | 5. ☐ Examiner's Amendment/Comment |
| 2. ☐ Information Disclosure Statements (PTO/SB/08),
Paper No./Mail Date _____ | 6. ☒ Examiner's Statement of Reasons for Allowance |
| 3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material | 7. ☐ Other _____ |
| 4. ☐ Interview Summary (PTO-413),
Paper No./Mail Date _____ | |

/ALI SOROUGH/
Primary Examiner, Art Unit 1617

Application/Control Number: 13/288,864
Art Unit: 1617

Page 2

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Acknowledgement of Receipt

Applicant's response filed on 08/26/2013 to the Office Action mailed on 04/25/2013 is acknowledged.

Claim Status

Claims 5-10, 18, and 20-25 are pending.

Claims 1-4, 12-17 and 19 are cancelled and claim 11 was previously cancelled.

Claims 5-10, 18, and 20 are currently amended.

Claims 21-25 are newly added.

Claims 5-10, 18, and 20-25 have been examined.

Claims 5-10, 18, and 20-25 are allowed.

Priority

Priority to application 61/410279 filed on 11/04/2010 is acknowledged.

Application/Control Number: 13/288,864
Art Unit: 1617

Page 3

Withdrawn Claim Rejections - 35 USC § 102

Response to Applicant's Arguments

The rejection of claims 6-10 and 18 under 35 U.S.C. 102(b) as being anticipated by Inoue et al. (Allelochemicals From Polygonum sachalinense FR. SCHM. (POLYGONACEAE), Published 1992) is withdrawn in view of the amendments to the claims.

The rejection of claims 1-4, 12-15, and 19 under 35 U.S.C. 102(b) as being anticipated by Inoue et al. (Allelochemicals From Polygonum sachalinense FR. SCHM. (POLYGONACEAE), Published 1992) is moot since the claims are cancelled.

The rejection of claims 16 and 17 under 35 U.S.C. 102(b) as being anticipated by Kim et al. (Anthraquinones Isolated from Cassia tora (Leguminosae) Seed Show an Antifungal Property against Phytopathogenic Fungi, Published 2004) is moot since the claims are cancelled.

REASONS FOR ALLOWANCE

The following is an examiner's statement of reasons for allowance: the prior art is free of any teaching or suggestion of modulating plant growth and/or root initiation or extension by application to the root of a plant a composition comprising anthraquinone. Therefore, claims 5-10, 18, and 20-25 are allowed.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Application/Control Number: 13/288,864
Art Unit: 1617

Page 4

Conclusion

Claims 5-10, 18, and 20-25 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ALI SOROUGH whose telephone number is (571)272-9925. The examiner can normally be reached on M-F (9am-6pm).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Fereydoun G. Sajjadi can be reached on (571)272-3311. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ALI SOROUGH/
Primary Examiner, Art Unit 1617

December 13, 2013

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
)
Hai Su et al.) Atty. Dkt. **MOI-42018US**
)
Appl. No.:13/288,864)
) Examiner: Ali Soroush
)
Filed: November 3, 2011) Group Art Unit: 1617

For: **COMPOSITIONS CONTAINING ANTHRAQUINONE DERIVATIVES AS
GROWTH PROMOTERS AND ANTIFUNGAL AGENTS**

RESPONSE TO OFFICE ACTION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the Office Action of April 25, 2013, applicants submit the following amendments and response.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

A **Memorandum of Interview** is presented on page 5 of this response.

Remarks begin on page 6 of this paper.

Amendments to the Claims:

Listing of Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

CLAIM AMENDMENTS

1. -4. (Canceled)

5. (Currently amended)

A method for modulating growth of a plant comprising treating one or more roots of said plant with a composition comprising one or more anthraquinone derivatives which modulate plant growth in an amount effective to modulate said growth in said plant prior to transplanting and transplanting said plant into soil, wherein said plant is a fruit, vegetable, tree, or vine plant. ~~The method according to claim 4, wherein prior to transplanting said plant into soil, the one or more roots of said fruit, or vegetable, tree or vine are treated with said composition.~~

6. (Currently amended) The method according to claim [[1]] 5, wherein the composition is applied to a said one or more roots of said fruit, vegetable, tree or vine of said plant via drip irrigation, root dip or in-furrow application.

7. (Currently amended) The method according to claim [[1]] 5, wherein the anthraquinone derivative is derived from a plant.

8. (Currently amended) The method according to ~~claims 1~~ claim 5, wherein the anthraquinone derivative is derived from a member of a plant family selected from the group consisting of Polygonaceae, Rhamnaceae, Fabaceae, Asphodelaceae and Rubiaceae.

9. (Currently amended) The method according to ~~claims 1~~ claim 5, wherein the

anthraquinone derivative is derived from a *Reynoutria* species.

10. (Currently amended) The method according to claim [[1]] 5, wherein said anthraquinone derivative is derived from *Reynoutria sachalinensis*.

11-17. (Canceled)

18. (Currently amended) The method according to claim [[1]] 5, wherein said composition is a plant extract and said plant extract comprises said anthraquinone derivatives.

19. (Canceled)

20. (Currently amended) A method for modulating root initiation or extension of a plant comprising (a) treating one or more roots of said plant with a composition comprising one or more anthraquinone in an amount effective to modulate root extension when transplanted into soil in said plant and (b) transplanting the treated plant into soil, wherein said composition is a plant extract and said plant extract comprises said one or more anthraquinone derivatives. The method according to claim 19, wherein said root initiation and extension in said plant is modulated by (a) treating one or more roots of a plant with a plant extract in an amount effective to modulate root extension when transplanted into soil and (b) transplanting the treated plant of (a) into soil.

21. (New) The method according to claim 20, wherein said extract is derived from the family *Polygonaceae*.

22. (New) The method according to claim 20, wherein said extract is derived from *Reynoutria* sp. or *Rheum* sp.

23. (New) The method according to claim 20, wherein said extract is derived from *Reynoutria sachalinensis*.

24. (New) The method of claim 20, wherein the plant is a fruit, flower, ornamental, tree, shrub, bulb, vegetable, or vine plant.

25. (New) The method of claim 20, wherein the plant is a strawberry, squash, cucumber, tomato, rose, pepper, eggplant, grapevine, cotton, onion, garlic, wheat, soy, corn, or rice plant.

MEMORANDUM OF INTERVIEW

On July 18, Examiner Soroush kindly granted the undersigned a telephonic interview.

During the interview, the undersigned presented suggestions with regard to the subject matter not found allowable, in particular, replacing the term “modulating” with promoting in claims 1 and 15. Examiner Soroush expressed 112 concerns in view of those amendments.

REMARKS

CLAIM AMENDMENTS

Claim 5 has been amended to incorporate all of the limitations of the base claim and intervening claims, i.e., the claim combines the limitations of former claims 1, 4, and 5. Claim 20 has been amended to incorporate all of the limitations of the base claim and intervening claims, i.e., the claim combines the limitations of former claims 1, 18, 19 and 20. New claims 21-23 are equivalent to former claims 12-14, but dependent on claim 20. New claims 24 and 25 are equivalent to former claims 2-3, but dependent on claim 20.

ALLOWABLE SUBJECT MATTER: Claim 5 and 20

On page 5 of the Action, the Office objected to claims 5 and 20 as being dependent on rejected base claims, but found these claims otherwise allowable. The claims have been appropriately amended. All remaining claims depend directly or indirectly on claims 5 or 20.

ANTICIPATION REJECTIONS

On page 3, claims 1-4, 6-10, 12-15, 18 and 19 are rejected under 35 U.S.C. 102(b) as being anticipated by Inoue et al. (Allelochemicals From *Polygonum sachalinense* FR. SCHM. (POLYGONACEAE), Published 1992).

On page 4, claims 16 and 17 are rejected under 35 U.S.C. 102(b) as being anticipated by Kim et al. (Anthraquinones Isolated from *Cassia tora* (Leguminosae) Seed Show an Antifungal Property against Phytopathogenic Fungi, Published 2004).

The rejections are moot in view of the amendments to the claims. All claims are now directly or indirectly dependent on subject matter which has been found allowable.

Applicants submit that in view of the above, the claims should now be in condition for allowance and the issuance of an early notice of allowance is respectfully requested.

The Commissioner is authorized to charge any fee deficiencies and overpayments to deposit account number 50-3135.

Respectfully submitted,
 /Joyce v. Natzmer/
 Joyce von Natzmer
 Reg. No. 48,120
Agris & von Natzmer LLP
 Customer No. 46002
 Direct Telephone: (301) 657-1282

Monday, August 26, 2013



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/288,864	11/03/2011	Hai Su	MOI-42018-US	5883
25538 7590 04/25/2013 CHERYL H AGRIS PHD PO BOX 8495 PELHAM, NY 10803			EXAMINER SOROUSH, ALI	
			ART UNIT 1617	PAPER NUMBER
			NOTIFICATION DATE 04/25/2013	DELIVERY MODE ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

c.agris.patlaw@pobox.com
clhrasa@aol.com
realtargetscompany@yahoo.com

49

Office Action Summary

Application No.
13/288,864

Applicant(s)
SU ET AL.

Examiner
ALI SOROUGH

Art Unit
1617

AIA (First Inventor to File)
Status
No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 10 January 2013.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.
- 4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 5) ☒ Claim(s) 1-10 and 12-20 is/are pending in the application.
5a) Of the above claim(s) ____ is/are withdrawn from consideration.
- 6) ☐ Claim(s) ____ is/are allowed.
- 7) ☒ Claim(s) 1-4, 6-10 and 12-19 is/are rejected.
- 8) ☒ Claim(s) 5 and 20 is/are objected to.
- 9) ☐ Claim(s) ____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

- 10) ☐ The specification is objected to by the Examiner.
- 11) ☐ The drawing(s) filed on ____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some * c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. ____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Interim copies:

- a) ☐ All b) ☐ Some c) ☐ None of the: Interim copies of the priority documents have been received.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☒ Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date 11122012, 03212013
- 3) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. ____
- 4) ☐ Other: ____

Application/Control Number: 13/288,864
Art Unit: 1617

Page 2

DETAILED ACTION

Acknowledgement of Receipt

Applicant's response filed on 01/10/2013 to the Office Action mailed on 01/10/2013 is acknowledged.

Claim Status

Claims 1-10 and 12-20 are pending.

Claim 11 is cancelled.

Claims 1-10 and 12-20 have been examined.

Claims 1-4, 7-10, 12-19 are rejected.

Claims 5 and 20 are objected to.

Election/Restrictions

Applicant's election without traverse of Group I (claims 1-10 and 16-17) in the reply filed on 01/10/2013 is acknowledged. In view of Applicant's amendments to claims 12-15 the claims are rejoined with claims 1-10 and 16-17. Further, new claims 18-20 are dependent upon claim 1 directly or indirectly and therefore are joined to Group I. For the foregoing reasons, claims 1-10 and 12-20 are hereby examined.

Priority

Priority to application 61/410279 filed on 11/04/2010 is acknowledged.

Application/Control Number: 13/288,864
 Art Unit: 1617

Information Disclosure Statement

The information disclosure statements (IDSs) submitted on 11/12/2012 and 03/21/2013 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statements have been considered by the examiner.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-4, 6-10, 12-15, 18 and 19 are rejected under 35 U.S.C. 102(b) as being anticipated by Inoue et al. (Allelochemicals From Polygonum sachalinense FR. SCHM. (POLYGONACEAE), Published 1992).

The claims are directed to a method of modulating growth of plant comprising treating the plant with a composition comprising one or more anthraquinone derivatives. The claims are further directed to the plant being a vegetable. The claims are further directed to the plant being transplanted into soil. The claims are further directed to the plant growth is modulated by modulating germination of a seed by applying to the composition to said seed. The claims are further directed to the plant growth is modulated by modulating root initiation and extension. The claims are further directed to the composition is a plant extract comprising anthraquinone derivatives, wherein the plant is from the member Polygonaceae such as Reynoutria (Polygonum) sachalinense.

Application/Control Number: 13/288,864

Page 4

Art Unit: 1617

Inoue et al. teach lettuce (vegetable plant) seedlings were planted in the acceptor pot (transplanting plant into soil) and nutrient solution from *P. sachalinense* is lifted through tubing into the acceptor pot (in-furrow application) (page 1834, paragraph 4). Emodin and physcion exhibited inhibitory (modulated) activity against lettuce seedling growth (page 1837, paragraph 4). Emodin and physcion are anthraquinone extracted from *P. sachalinense* (abstract). Therefore, the instant claims are anticipated by the prior art.

Claims 16 and 17 are rejected under 35 U.S.C. 102(b) as being anticipated by Kim et al. (Anthraquinones Isolated from *Cassia tora* (Leguminosae) Seed Show an Antifungal Property against Phytopathogenic Fungi, Published 2004).

The claims are directed to a method for modulating soil borne infection in a plant comprising treating a plant with an amount of a composition containing one or more anthraquinone. The claims are further directed to the soil borne disease infection by a pathogenic microorganism such as *Phytophthora*.

Kim et al. teach applying a solution of emodin or physcion to tomato plant for treating infection of *Phytophthora infestans* (page 6097, Table 1, column 2, paragraph 4 and page 6098, Table 3). Therefore the instant claims are rendered obvious by the teachings of the prior art.

Application/Control Number: 13/288,864

Page 5

Art Unit: 1617

Claim Objections

Claims 5 and 20 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. The prior art is free of any teaching or suggestion wherein a composition comprising anthraquinone is applied to the root of a plant.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ALI SOROUSH whose telephone number is (571)272-9925. The examiner can normally be reached on M-F (9am-6pm).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Fereydoun G. Sajjadi can be reached on (571)272-3311. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

C16

Application/Control Number: 13/288,864

Page 6

Art Unit: 1617

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ALI SOROUGH/
Primary Examiner, Art Unit 1617

April 19, 2013

DOCKET NO. MOI-42018-US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Su et al.

Serial No.: 13/288,864

Group Art Unit: 1616

Filed: 03-NOV-2011

Examiner: A. Soroush

For: COMPOSITIONS CONTAINING ANTHRAQUINONE DERIVATIVES AS
GROWTH PROMOTERS AND ANTIFUNGAL AGENTS

Confirmation No. 5883

RESPONSE TO RESTRICTION REQUIREMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is in response to the Restriction Requirement dated November 9, 2012.

CLAIM AMENDMENTS

1. (original) A method for modulating growth of a plant comprising treating said plant with an amount of a composition comprising one or more anthraquinone derivatives which modulate plant growth effective to modulate said growth in said plant.
2. (original) The method according to claim 1, wherein said plant is a fruit, flower, ornamental plant, tree, shrub, bulb plant or vegetable or vine.
3. (original) The method according to claim 1, wherein said plant is selected from the group consisting of strawberry, squash, cucumber, tomato, rose, pepper eggplant, grapevine, cotton, onion, garlic, wheat, soy, corn and rice.
4. (original) The method according to claim 1, further comprising transplanting said plant into soil.
5. (original) The method according to claim 4, wherein prior to transplanting said plant into soil, one or more roots of said fruit, or vegetable, tree or vine are treated with said composition.
6. (original) The method according to claim 1, wherein the composition is applied to a fruit, vegetable, tree or vine of said plant via drip irrigation, root dip or in-furrow application.
7. (original) The method according to claim 1, wherein the anthraquinone derivative is derived from a plant.
8. (original) The method according to claims 1, wherein the anthraquinone derivative is derived from a member of a plant family selected from the group consisting of Polygonaceae, Rhamnaceae, Fabaceae, Asphodelaceae and Rubiaceae.

9. (original) The method according to claims 1, wherein the anthraquinone derivative is derived from a *Reynoutria* species.

10. (original) The method according to claim 1, wherein said anthraquinone derivative is derived from *Reynoutria sachalinensis*.

Claim 11 (canceled)

12. (withdrawn-currently amended) The method according to claim 11, wherein said extract is derived from the family *Polygonaceae*.

13. (withdrawn-currently amended) The method according to claim 11, wherein said extract is derived from *Reynoutria* sp. or *Rheum* sp.

14. (withdrawn-currently amended) The method according to claim 11, wherein said extract is derived from *Reynoutria sachalinensis*.

15. (withdrawn-currently amended) ~~A method~~The method according to claim 1 wherein the growth of the plant is modulated by modulating germination of a seed of said plant and wherein the for modulating germination of a the seed of a the plant is modulated by comprising applying to said seed an amount of (a) a composition containing one or more anthraquinone derivatives which modulate growth in a plant and (b) optionally a seed coating agent effective to modulate said germination of said seed of said plant.

16. (original) A method for modulating non-*Rhizoctonia* soil borne disease infection in a plant comprising treating a plant with an amount of a composition containing one or more anthraquinone derivatives which modulate growth in a plant effective to modulate said soil borne disease in said plant.

17. (original) The method according to claim 16, wherein said soil borne disease infection in said plant results from infection by a pathogenic microorganism selected

from the group consisting of *Pythium*, *Phytophthora*, *Verticillium*, *Sclerotium*,
| *Colletotrichum* and *Fusarium*.

18. (new) The method according to claim 1, wherein said composition is a plant extract and said plant extract comprises said anthraquinone derivatives.

19. (new) The method according to claim 18, wherein said plant growth in said plant is modulated by modulating root initiation and extension in said plant.

20. (new) The method according to claim 19, wherein said root initiation and extension in said plant is modulated by (a) treating one or more roots of a plant with a plant extract in an amount effective to modulate root extension when transplanted into soil and (b) transplanting the treated plant of (a) into soil.

REMARKS

Before addressing the Restriction Requirement, Applicants wish to note that claim 15 has been amended to more distinctly claim the subject matter of the invention and to have claim 15 depend from claim 1. Claim 11 has been canceled without prejudice. However, the substance of claim 11 is presented in new claims 18-20. Furthermore, claims 12-14 have been amended to depend from claim 18. Amended claim 15 and new claims 18-20 are supported by the specification (see, for example, page 2, lines 21-23 and page 3, line 9). No new matter has been added.

The following Restriction Requirement has been issued:

- I. Claims 1-10 and 16-17, drawn to a method of modulating plant growth and/or soil borne disease with application of anthraquinone derivative, classified in class 544, subclass 156.
- II. Claims 11-14, drawn to a method of modulating root initiation with application of a plant extract to the plant root, classified in class 424, subclass 725.
- III. Claim 15, drawn to a method of modulating seed germination with application of anthraquinone derivative to the seed, classified in class 544, subclass 155.

The Restriction Requirement specifically asserts:

Inventions I and II are directed to related processes. The related inventions are distinct if: (1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.050(j). In the instant case, the inventions as claimed different modes of operation since Group I requires the application of anthraquinone to the plant whereas the invention of Group II requires the application of a plant extract to the seed of the plant. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants

Inventions I and III are directed to related processes. The related inventions are distinct if: (1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the

inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.050(j). In the instant case, the inventions as claimed have different mode of operation, since the Invention of Group I requires the application to be to the plant and the Invention of Group III requires the application to be to the seed of the plant. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants.

Inventions II and III are directed to related processes. The related inventions are distinct if: (1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.050(j). In the instant case, the inventions as claimed different modes of operation since Group III requires the application of anthraquinone to the plant seed whereas the invention of Group II requires the application of a plant extract to the seed of the plant. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants.

Applicants, in response, elect Group I with traverse. However, in view of the cancellation of claim 11 and the amendment of claim 15, Applicants do respectfully request reconsideration of the Restriction Requirement and rejoinder of the withdrawn claims for the reasons set forth below.

First, with respect to the claims of Groups I and II, Applicants note that different modes of operation are not required. Specifically, claim 11 has been canceled. New claim 18 recites that the composition may be a plant extract and that the plant extract may comprise anthraquinone derivatives. Claims 12-14 now depend from claim 18.

Second with respect to the claims of Groups I and III, Applicant asserts that different modes of operation are indeed not required. To more distinctly claim the subject matter of the invention, claim 15 has been amended to depend from claim 1. Specifically claim 15 recites that the growth of the plant is modulated by modulating seed germination, a specific embodiment of the subject matter of claim 1 and that seed germination is modulated by treating the plant.

Similarly, with respect to the claims of Groups II and III, it is also Applicants' position that different modes of operation are not involved. As noted above, claim 11 has been canceled and new claim 18 recites that the composition may be a plant extract. Claims 12-14 now depend from claim 18. Furthermore, the claims of Groups II and III involving modulating the growth of a plant.

In summary, in view of the above arguments, the cancellation of claim 11, the amendment of claim 15 and the addition of claims 18-20, Applicants respectfully request reconsideration of the Restriction Requirement. Early action to that end is respectfully requested. The Examiner is invited to contact the undersigned if there are any questions regarding this Restriction Requirement or application at (914) 712-0093.

Respectfully submitted,

/Cheryl H. Agris/

Date: January 10, 2013

Cheryl H. Agris, Reg. No. 34,086
P.O. Box 8495
Pelham, N.Y. 10803
(914) 712-0093
Customer No. 25538



CHERYL H AGRIS PHD
PO BOX 8495
PELIHAM, NY 10803

Paper No.

Application No.:	13/288,864	Date Mailed:	2012-12-17
			
First Named Inventor:	Su, Hai,	Examiner:	SOROUGH, ALI
Attorney Docket No.:	MOI-42018-US	Art Unit:	1617
Confirmation No.:	5883	Filing Date:	2011-11-03

Please find attached an Office communication concerning this application or proceeding.

Commissioner for Patents

Notice of Non-Compliant Amendment (37 CFR 1.121)	Application No. 13/288,864	Applicant(s) SU ET AL.
		Art Unit 1617

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

The amendment document filed on 10 December, 2012 is considered non-compliant because it has failed to meet the requirements of 37 CFR 1.121 or 1.4. In order for the amendment document to be compliant, correction of the following item(s) is required.

THE FOLLOWING MARKED (X) ITEM(S) CAUSE THE AMENDMENT DOCUMENT TO BE NON-COMPLIANT:

- ☒ 1. Amendments to the specification:
 - ☐ A. Amended paragraph(s) do not include markings.
 - ☐ B. New paragraph(s) should not be underlined.
 - ☒ C. Other Response was not signed.
- ☐ 2. Abstract:
 - ☐ A. Not presented on a separate sheet. 37 CFR 1.72.
 - ☐ B. Other _____.
- ☐ 3. Amendments to the drawings:
 - ☐ A. The drawings are not properly identified in the top margin as "Replacement Sheet," "New Sheet," or "Annotated Sheet" as required by 37 CFR 1.121(d).
 - ☐ B. The practice of submitting proposed drawing correction has been eliminated. Replacement drawings showing amended figures, without markings, in compliance with 37 CFR 1.84 are required.
 - ☐ C. Other _____.
- ☐ 4. Amendments to the claims:
 - ☐ A. A complete listing of all of the claims is not present.
 - ☐ B. The listing of claims does not include the text of all pending claims (including withdrawn claims)
 - ☐ C. Each claim has not been provided with the proper status identifier, and as such, the individual status of each claim cannot be identified. Note: the status of every claim must be indicated after its claim number by using one of the following status identifiers: (Original), (Currently amended), (Canceled), (Previously presented), (New), (Not entered), (Withdrawn) and (Withdrawn-currently amended).
 - ☐ D. The claims of this amendment paper have not been presented in ascending numerical order.
 - ☐ E. Other: _____.
- ☐ 5. Other (e.g., the amendment is unsigned or not signed in accordance with 37 CFR 1.4): For further explanation of the amendment format required by 37 CFR 1.121, see MPEP § 714.

TIME PERIODS FOR FILING A REPLY TO THIS NOTICE:

- 1. Applicant is given **no new time period if the non-compliant amendment is an** after-final amendment or an amendment filed after allowance, or a drawing submission (only) If applicant wishes to resubmit the non-compliant after-final amendment with corrections, the **entire corrected amendment** must be resubmitted.
- 2. Applicant is given **one month**, or thirty (30) days, whichever is longer, from the mail date of this notice to supply the correction, if the non-compliant amendment is one of the following: a preliminary amendment, a non-final amendment (including a submission for a request for continued examination (RCE) under 37 CFR 1.114), a supplemental amendment filed within a suspension period under 37 CFR 1.103(a) or (c), and an amendment filed in response to a Quayle action. If any of above boxes 1 to 4 are checked, the correction required is only the corrected section of the non-compliant amendment in compliance with 37 CFR 1.121.

Extensions of time are available under 37 CFR 1.136(a) only if the non-compliant amendment is a non-final amendment or an amendment filed in response to a *Quayle* action.

Failure to timely respond to this notice will result in:

- Abandonment** of the application if the non-compliant amendment is a non-final amendment or an amendment filed in response to a *Quayle* action; or
- Non-entry** of the amendment if the non-compliant amendment is a preliminary amendment or supplemental amendment.

Legal Instruments Examiner (LIE), if applicable /DORIAN EVANS/ Telephone No: (571)272-1572

DOCKET NO. MOI-42018-US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Su et al.

Serial No.: 13288864

Group Art Unit: 1616

Filed: 03-NOV-2011

Examiner: A. Soroush

For: COMPOSITIONS CONTAINING ANTHRAQUINONE DERIVATIVES AS
GROWTH PROMOTERS AND ANTIFUNGAL AGENTS

Confirmation No. 5883

RESPONSE TO RESTRICTION REQUIREMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is in response to the Restriction Requirement dated November 9, 2012.

CLAIM AMENDMENTS

1. (original) A method for modulating growth of a plant comprising treating said plant with an amount of a composition comprising one or more anthraquinone derivatives which modulate plant growth effective to modulate said growth in said plant.
2. (original) The method according to claim 1, wherein said plant is a fruit, flower, ornamental plant, tree, shrub, bulb plant or vegetable or vine.
3. (original) The method according to claim 1, wherein said plant is selected from the group consisting of strawberry, squash, cucumber, tomato, rose, pepper eggplant, grapevine, cotton, onion, garlic, wheat, soy, corn and rice.
4. (original) The method according to claim 1, further comprising transplanting said plant into soil.
5. (original) The method according to claim 4, wherein prior to transplanting said plant into soil, one or more roots of said fruit, or vegetable, tree or vine are treated with said composition.
6. (original) The method according to claim 1, wherein the composition is applied to a fruit, vegetable, tree or vine of said plant via drip irrigation, root dip or in-furrow application.
7. (original) The method according to claim 1, wherein the anthraquinone derivative is derived from a plant.
8. (original) The method according to claims 1, wherein the anthraquinone derivative is derived from a member of a plant family selected from the group consisting of Polygonaceae, Rhamnaceae, Fabaceae, Asphodelaceae and Rubiaceae.

9. (original) The method according to claims 1, wherein the anthraquinone derivative is derived from a *Reynoutria* species.

10. (original) The method according to claim 1, wherein said anthraquinone derivative is derived from *Reynoutria sachalinensis*.

Claim 11 (canceled)

12. (withdrawn-currently amended) The method according to claim 1, wherein said extract is derived from the family *Polygonaceae*.

13. (withdrawn-currently amended) The method according to claim 1, wherein said extract is derived from *Reynoutria* sp. or *Rheum* sp.

14. (withdrawn-currently amended) The method according to claim 1, wherein said extract is derived from *Reynoutria sachalinensis*.

15. (withdrawn-currently amended) ~~A method~~ The method according to claim 1 wherein the growth of the plant is modulated by modulating germination of a seed of said plant and wherein the for-modulating-germination of a-the seed of a-the plant is modulated by comprising-applying to said seed an amount of (a) a composition containing one or more anthraquinone derivatives which modulate growth in a plant and (b) optionally a seed coating agent effective to modulate said germination of said seed of said plant.

16. (original) A method for modulating non-*Rhizoctonia* soil borne disease infection in a plant comprising treating a plant with an amount of a composition containing one or more anthraquinone derivatives which modulate growth in a plant effective to modulate said soil borne disease in said plant.

17. (original) The method according to claim 16, wherein said soil borne disease infection in said plant results from infection by a pathogenic microorganism selected

from the group consisting of *Pythium*, *Phytophthora*, *Verticillium*, *Sclerotium*,
Colletotrichum and *Fusarium*.

18. (new) The method according to claim 1, wherein said composition is a plant extract and said plant extract comprises said anthraquinone derivatives.

19. (new) The method according to claim 18, wherein said plant growth in said plant is modulated by modulating root initiation and extension in said plant.

20. (new) The method according to claim 19, wherein said root initiation and extension in said plant is modulated by (a) treating one or more roots of a plant with a plant extract in an amount effective to modulate root extension when transplanted into soil and (b) transplanting the treated plant of (a) into soil.

REMARKS

Before addressing the Restriction Requirement, Applicants wish to note that claim 15 has been amended to more distinctly claim the subject matter of the invention and to have claim 15 depend from claim 1. Claim 11 has been canceled without prejudice. However, the substance of claim 11 is presented in new claims 18-20. Furthermore, claims 12-14 have been amended to depend from claim 18. Amended claim 15 and new claims 18-20 are supported by the specification (see, for example, page 2, lines 21-23 and page 3, line 9). No new matter has been added.

The following Restriction Requirement has been issued:

- I. Claims 1-10 and 16-17, drawn to a method of modulating plant growth and/or soil borne disease with application of anthraquinone derivative, classified in class 544, subclass 156.
- II. Claims 11-14, drawn to a method of modulating root initiation with application of a plant extract to the plant root, classified in class 424, subclass 725.
- III. Claim 15, drawn to a method of modulating seed germination with application of anthraquinone derivative to the seed, classified in class 544, subclass 155.

The Restriction Requirement specifically asserts:

Inventions I and II are directed to related processes. The related inventions are distinct if: (1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.050(j). In the instant case, the inventions as claimed different modes of operation since Group I requires the application of anthraquinone to the plant whereas the invention of Group II requires the application of a plant extract to the seed of the plant. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants.

Inventions I and III are directed to related processes. The related inventions are distinct if: (1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the

inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.050(j). In the instant case, the inventions as claimed have different mode of operation, since the Invention of Group I requires the application to be to the plant and the Invention of Group III requires the application to be to the seed of the plant. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants.

Inventions II and III are directed to related processes. The related inventions are distinct if: (1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.050(j). In the instant case, the inventions as claimed different modes of operation since Group III requires the application of anthraquinone to the plant seed whereas the invention of Group II requires the application of a plant extract to the seed of the plant. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants.

Applicants, in response, elect Group I with traverse. However, in view of the cancellation of claim 11 and the amendment of claim 15, Applicants do respectfully request reconsideration of the Restriction Requirement and rejoinder of the withdrawn claims for the reasons set forth below.

First, with respect to the claims of Groups I and II, Applicants note that different modes of operation are not required. Specifically, claim 11 has been canceled. New claim 18 recites that the composition may be a plant extract and that the plant extract may comprise anthraquinone derivatives. Claims 12-14 now depend from claim 18.

Second with respect to the claims of Groups I and III, Applicant asserts that different modes of operation are indeed not required. To more distinctly claim the subject matter of the invention, claim 15 has been amended to depend from claim 1. Specifically claim 15 recites that the growth of the plant is modulated by modulating seed germination, a specific embodiment of the subject matter of claim 1 and that seed germination is modulated by treating the plant.

Similarly, with respect to the claims of Groups II and III, it is also Applicants' position that different modes of operation are not involved. As noted above, claim 11 has been canceled and new claim 18 recites that the composition may be a plant extract. Claims 12-14 now depend from claim 18. Furthermore, the claims of Groups II and III involving modulating the growth of a plant.

In summary, in view of the above arguments, the cancellation of claim 11, the amendment of claim 15 and the addition of claims 18-20, Applicants respectfully request reconsideration of the Restriction Requirement. Early action to that end is respectfully requested. The Examiner is invited to contact the undersigned if there are any questions regarding this Restriction Requirement or application at (914) 712-0093.



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/288,864	11/03/2011	Hai Su	MOI-42018-US	5883
25538 7590 11/09/2012 CHERYL H AGRIS PHD PO BOX 8495 PELHAM, NY 10803			EXAMINER SOROUSH, ALI	
			ART UNIT 1617	PAPER NUMBER
			NOTIFICATION DATE 11/09/2012	DELIVERY MODE ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

c.agris.patlaw@pobox.com
clhrasa@aol.com
realtargetscompany@yahoo.com

Office Action Summary	Application No.	Applicant(s)	
	13/288,864	SU ET AL.	
	Examiner	Art Unit	
	ALI SOROUGH	1617	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on 03 November 2011.

2a) ☐ This action is **FINAL**. 2b) ☐ This action is non-final.

3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5) ☒ Claim(s) 1-17 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☐ Claim(s) _____ is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☒ Claim(s) 1-17 are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10) ☐ The specification is objected to by the Examiner.

11) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some * c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. _____.

3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____.

4) ☐ Other: _____.

Application/Control Number: 13/288,864
Art Unit: 1617

Page 2

DETAILED ACTION

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

- I. Claims 1-10 and 16-17, drawn to a method of modulating plant growth and/or soil borne disease with application of anthraquinone derivative, classified in class 544, subclass 156.
- II. Claims 11-14, drawn to a method of modulating root initiation with application of a plant extract to the plant root, classified in class 424, subclass 725.
- III. Claim 15, drawn to a method of modulating seed germination with application of anthraquinone derivative to the seed, classified in class 544, subclass 155.

The inventions are distinct, each from the other because of the following reasons:

Inventions I and II are directed to related processes. The related inventions are distinct if:

(1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants.

See MPEP § 806.05(j). In the instant case, the inventions as claimed different modes of operation since Group I requires the application of anthraquinone to the plant whereas the invention of Group II requires the application of a plant extract to the seed of the plant. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants.

Inventions I and III are directed to related processes. The related inventions are distinct if: (1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.05(j). In the instant case, the inventions as claimed have different mode of operation, since the Invention of Group I requires the application to be to the plant and the Invention of Group III requires the application to be to the seed of the plant. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants.

Inventions II and III are directed to related processes. The related inventions are distinct if: (1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.05(j). In the instant case, the inventions as claimed different modes of operation since Group III requires the application of anthraquinone to the plant seed whereas the invention of Group II requires the application of a plant extract to the seed of the plant. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants.

Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and/or examination burden if restriction were not required because at least the following reason(s) apply:

- *the inventions have acquired a separate status in the art in view of their different classification*
- *the inventions have acquired a separate status in the art due to their recognized divergent subject matter*
- *the inventions require a different field of search (e.g., searching different classes /subclasses or electronic resources, or employing different search strategies or search queries).*

Applicant is advised that the reply to this requirement to be complete must include

(i) an election of a invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected invention.**

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the

Application/Control Number: 13/288,864

Page 5

Art Unit: 1617

application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ALI SOROUGH whose telephone number is (571)272-9925. The examiner can normally be reached on M-F (9am-6pm).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Fereydoun G. Sajjadi can be reached on (571)272-3311. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ALI SOROUGH/
Examiner, Art Unit 1617

November 5, 2012