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Identification of Postharvest Pathogens of *Amorphophallus muelleri* and Indoor Screening of Fungicides

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Abstract: Konjac (*Amorphophallus muelleri*), a genus of tuberous plants in the Araceae family, is one of high-value crops in Southwest China. This study aimed at identifying the main pathogens causing tuber rot during storage of *A. muelleri* and screening the effective fungicides, so as to prolong the storage period of *A. muelleri* and decrease the losses. Isolation and identification, as well as pathogenicity test and retro-inoculation experiments were made for the pathogen causing tuber rot during storage of *A. muelleri* in Kunming city, Yunnan province, China. The effective fungicides for the main pathogens were also screened in the laboratory. Six fungi were identified as the pathogens causing tuber rot of *A. muelleri*, which were *Fusarium solani* (Mart.) Sacc., *Fusarium oxysporum* Schlecht., *Botrytis cinerea* Pers., *Alternaria alternata* (Fr.) Keissl., *Rhizopus nigricans* Ehrenb., *Penicillium ulaiense* Hsieh, Su & Tzean. The main pathogens causing postharvest diseases of *A. muelleri* were *F. solani*, *F. oxysporum* and *B. cinerea*. The isolation frequencies of them were 33.9%, 10.5% and 19.4%, respectively. After artificial inoculation, the incidence of tubers infected by *F. solani*, *F. oxysporum* and *B. cinerea* was 100%, 83% and 95%, respectively. The results of chemical screening showed that, in potato dextrose agar (PDA) media plate, the compounds Fludioxonil (50% WP) and Boscalid (50% WG) were the most effective in controlling the three main pathogens, and the average effect reached more than 97%. The test of fungicidal antisepsis on tubers consisted of *A. muelleri* being dipped in the 9,000× diluted solution of Fludioxonil (50% WP) or in the 3,500× diluted solution of Boscalid (50% WG) for 3 min and stored at room temperature (25 °C) for 7 d and 15 d, respectively. The fungicidal effects of Fludioxonil against *F. solani*, *F. oxysporum* and *B. cinerea* for 7 d and 15 d were 88.6%/83.2%, 90.1%/84.7% and 93.0%/91.5%, respectively, whereas the fungicidal effects of Boscalid were 87.0%/85.3%, 89.0%/85.6% and 89.2%/89.1%, respectively. The results may provide useful information for the control of postharvest diseases of *A. muelleri*.

Key words: *A. muelleri*, postharvest disease, indoor screening of fungicides.

1. Introduction

Moyu with the byname of Juruo is the Chinese name, and *Amorphophallus konjac* and konjac are the common English name. It is a perrenial tuberous plant in the Araceae family [1-4]. *Amorphophallus muelleri* has a number of advantages, such as high disease resistance and high propagation coefficient as a species growing in tropical and sub-tropical areas [5-7]. Konjac tubers are rich in konjac glucomannan (KGM), a natural, neutral, hydrophilic polysaccharide [7-10].

Glucomannan is an excellent gelling agent used in food, pharmaceutical and chemical industry, and konjac is a specialty crop grown as a source of glucomannan for industrial use. It is an important cash crop and thus contributes to poverty alleviation in Southwest China. Its planting area is about 150 million mu (10 million ha) [11-15].

A. muelleri is an advantage cultivar with high

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disease resistance, high yield and high konjac glucomannan, and is suitable for planting at 500-1,700 m altitude, so there is a good prospect of popularization and application in tropical and sub-tropical areas. But, the konjac subterraneous tubers are susceptible to pathogen infection during postharvest and storage period because of their high moisture content. Infections may be caused by mechanical injuries of konjac tubers during field growth, harvest and transportation. Such injuries provide opportunities for the pathogenic microorganisms to infest the tubers [4]. The use of chemical fungicides is one of the main tactics being used for the management of plant diseases. Knowledge is scarce about the management of postharvest pathogens of *A. muelleri* by using chemical fungicides. The aim of the present work was to test the impact of different fungicides on growth inhibition and other biological parameters of postharvest pathogens of *A. muelleri*, which can provide the basic information for future management of postharvest pathogens of *A. muelleri* under field conditions.

2. Materials and Methods

2.1 Isolation and Purification of Pathogens

During 2012-2013, the main pathogens causing tuber rot during storage of *A. muelleri* were investigated in Kunming city, Yunnan province, China, and the disease symptoms were recorded and photographed. Samples of diseased plants were collected at the junction of disease and healthy tissues in different diseased organs to isolate the pathogens on clean bench. The collected samples were cut into 5 mm × 5 mm pieces, sterilized with 0.1% mercuric chloride for 2-3 min and washed three times with sterile water, 3 min each time. The isolated tissues were transferred to potato dextrose agar (PDA) medium. A total 150 dishes of tissues were isolated and incubated at 28 °C. After 5 d, the number and morphology of isolated colonies were observed and recorded; the observed fungal colonies were respectively counted and coded. Single colonies were picked with a needle for purified culture, and preserved at 0-4 °C [16].

2.2 Pathogenicity Test

The purified strains were incubated on PDA medium for 6 d, prepared into fungus cake using a punch with diameter of 5 mm, stuck on stabbed and non-stabbed fresh, healthy tuber tissues of *A. muelleri*, which were pre-sterilized with 75% ethanol, and the inoculated tissues were moisturized with sterile water-soaked absorbent cotton. In addition, tuber inoculated with PDA medium and moisturized with sterile water-soaked absorbent cotton were used as control [4]. All the inoculated tubers were cultured in a greenhouse at 28 °C, and each treatment was repeated nine times. The inoculated tubers were observed every one day to record inoculation results. Furthermore, the isolated fungi were verified in accordance with Koch postulates to confirm whether these fungi were pathogens.

2.3 Pathogen Identification

The number and morphology of isolated colonies were observed and recorded; the observed fungal colonies were respectively counted and coded. Single colonies were picked with a needle for purified culture, and preserved at 0-4 °C.

Morphological identification of pathogens samples with typical symptoms was selected for free hand section. Diseased tissues were scraped or picked, prepared into sections and observed under a microscope to describe the morphological characteristics of pathogens. Fruiting bodies incubated on the plates were picked with a sterile inoculation needle, prepared into temporary sections, observed and photographed under a microscope. The size of 50 spores was measured. According to the morphological characteristics of arid culture and relevant data, pathogens were identified [17].

Molecular identification of pathogens by internal

transcribed spacer (ITS) sequencing was performed by BGI Co., Ltd.. Sequencing results were aligned on National Center of Biotechnology Information (NCBI) to identify the pathogen genus or species [4].

2.4 Experimental Germicides

2.4.1 Indoor Germicides

Various germicides, including Carbendazim (50% WP), Iprodione (50% SC), Fludioxonil (50% WP), Kresoxim-methyl (10% SC) and Boscalid (50% WG), were dissolved in appropriate amount of water and preserved in a refrigerator at 4 °C before use. List of pesticides used in the study and levels of dilution of each fungicide (unit: times) in this study are given in Table 1.

2.4.2 Inhibition Rates of Five Kinds of Fungicides

Various experimental germicides were successively diluted to a desired concentration; 1 mL of germicide was mixed evenly with 9 mL of medium in a Petri dish with diameter of 9.0 cm, and prepared into experimental PDA medium containing different gradient concentrations of germicides, with sterile water as a blank control. Each treatment was repeated four times. Pathogenic strains were inoculated to PDA plates and incubated at 28 °C for 48 h. Subsequently, fungus cake with diameter of 5 mm were collected at the edge of the colony using a punching bear, then transferred into experimental PDA medium containing germicides and blank control, and incubated at 28 °C. The diameter of colonies was measured with crisscross method, when colonies in control almost covered the Petri dish. Subsequently, the average diameter (mm) of every colony was calculated as Eq. (1) [18]:

$$\text{Average diameter (mm)} = (\text{long diameter} + \text{short diameter})/2 \quad (1)$$

Inhibitory percentage on mycelia growth was calculated as Eq. (2):

$$\text{Inhibition of mycelial growth (\%)} = [(Mc - Mt)/Mc] \times 100 \quad (2)$$

where, Mc = dimeter of mycelium in control and Mt = diameter of mycelium in treatment. The entire experiment was replicated three times on different dates.

2.4.3 Postharvest Efficacy Test

Select the same size of konjac tuber, and then were surface sterilized with 75% ethanol. The results of chemical screening showed that in PDA media plate, the compounds Fludioxonil (50% WP) and Boscalid (50% WG) were the most effective in controlling the main pathogens. The test of fungicidal antisepsis on tubers consisted of *A. muelleri* dipped in the 5,000×, 7,000×, 9,000× diluted solution of Fludioxonil (50% WP) or in the 1,500×, 2,500×, 3,500× diluted solution of Boscalid (50% WG) for 3 min, and then dry out. In addition, tubers inoculated with sterile water-soaked were used as control dip fruit after pathogen inoculation. Twenty konjac tubers of *A. muelleri* were sprayed with a 1.0×10^6 conidia/mL suspension from 7-day-old PDA cultures. Each concentration of a pesticide treatment and each treatment were repeated three times. Respectively, according to the following grading standards, and 7 d and 15 d observations of incidence, the survey results were analyzed to calculate the disease index and control effect. All the inoculated tubers were cultured in a greenhouse at 25 °C. The disease grades were recorded to calculate the disease index:

Grade 0—no lesion;

Grade 1—lesion area accounted for less than 5% of the entire organ area (tuber);

Grade 2—lesion area accounted for 6%-15% of the entire organ area;

Grade 3—lesion area accounted for 16%-25% of the entire organ area;

Grade 4—lesion area accounted for 26%-50% of the entire organ area;

Table 1 Levels of dilution of each fungicide in this study.

Fungicides	Level of dilution (times)		
Carbendazim (50% WP)	2,000	2,500	3,000
Iprodione (50% SC)	1,000	1,500	2,000
Fludioxonil (50% WP)	5,000	7,000	9,000
Kresoxim-methyl (10% SC)	2,000	3,000	5,000
Boscalid (50% WG)	1,500	2,500	3,500

Grade 5—lesion area accounted for more than 50% of the entire organ area.

2.4.4 Statistical Analysis

All experiments were carried out in triplicate, and the results were expressed as average of triplicate determinations. Radial growth and postharvest efficacy test data were analyzed by analysis of variance (ANOVA), and treatment means were compared by using Tukey's honestly significant difference (HSD) test for mean comparison at 5% level of significance. All statistical analysis was performed using SAS 8.01 [19].

3. Results and Discussion

3.1 Isolation and Purification of Pathogens

A. muelleri crop is generally harvested during December and January in most regions of China. The high humid conditions during this time favour dry rot and other types of rots of konjac tubers that are stored in heaps. This can lead to huge losses. The tubers were shrivelled, mummified and hard (Fig. 1). Six fungi identified as the pathogens causing tuber rot of *A. muelleri* were *F. solani* (A1), *F. oxysporum* (A 2), *B. cinerea* (A3), *A. alternate* (A4), *R. nigricans* (A5) and *P. ulaiense* (A6). The main pathogens causing postharvest diseases of *A. muelleri* were A1, A2 and A3. The isolation frequencies of them were 33.9%, 10.5% and 19.4%, respectively.

Fig. 1 Symptoms of tuber rot of *A. muelleri*.

3.2 Pathogenicity Test

After inoculation with different pathogenic strains A1, A2, A3, A4, A5 and A6 for 96 h, tubers showed symptoms similar to natural occurrence. The same pathogenic strains were isolated again from the diseased tuber. The incidence of tubers infected by A1, A2, A3, A4, A5 and A6 was 100%, 83.0%, 95.0%, 36.7%, 9.7% and 28.5%, respectively. The main pathogens causing postharvest diseases of *A. muelleri* were A1, A2 and A3.

3.3 Pathogen Identification

According to the morphological analysis and ITS sequencing results, six fungi were identified as the pathogens causing tuber rot of *A. muelleri*.

A1 was plated on PDA and incubated at 28 °C. Colonies were woolly to cottony colour with cream to white aerial mycelium and a cream colour reverse on PDA media. Observed sporodochia were usually moist and blue-green colored. Macroconidia were moderately curved, stout, thick-walled, usually 3-5 septa, measuring 25.0-47.0 μm × 3.3-5.6 μm, and were borne on short conidiophores that soon formed sporodochia. Microconidia, formed on long monophialides, were one to three-celled, measuring 8.8-14.9 μm × 2.3-5.0 μm, and occurred in false heads. Chlamydoconidia occurred both singly and in pairs. The pathogen was identified as *F. solani* based upon colony and conidial morphology [20, 21]. The ITS region of rDNA was amplified using primers ITS4/ITS6, and then sequenced. Basic local alignment search tool (BLAST) analysis of the 536-bp segment showed 100% similarity with the sequence of *F. solani* (Mart.) Sacc. (Fig. 2a).

A2 was plated on PDA and incubated at 28 °C. Conidiophores were unbranched or branched short monophialides. Microconidia were abundant, generally single celled, oval to kidney shaped and produced only in false heads with 5.0-12.0 μm × 3.0-3.5 μm. Macroconidia were abundant, slightly sickle-shaped and thin-walled, with an attenuated

apical cell and a foot-shaped basal cell with 35.0-60.0 μm × 3.0-5.0 μm. Chlamydospores were single or in pairs and profusely distributed. These morphological features were typical of those described for *F. oxysporum* [22]. The ITS region of rDNA was amplified using primers ITS4/ITS6 and sequenced. BLAST analysis of the 518-bp segment showed 99% similarity with the sequence of *F. oxysporum* Schlecht. (Fig. 2b).

A3 was plated on PDA and incubated at 28 °C. Mycelium was initially whitish and turned gray with age. Black, irregular sclerotia formed at random in culture. Mycelium was initially whitish colour and turned gray with increasing age. Black conidiophores bore botryose heads of hyaline, ellipsoid, unicellular conidia, gray in mass, measuring 6.2-9.5 μm × 4.5-6.0 μm. Black, irregular sclerotia formed at random in culture. These morphological features were typical for *B. cinerea* [4]. The ITS region of rDNA was amplified using primers ITS4/ITS6 and sequenced. BLAST analysis of the 558-bp segment showed 99% similarity with the sequence of *Botryotinia fuckeliana* (perfect stage of *B. cinerea* Pers.) (Fig. 2c).

A4 was plated on PDA and incubated at 28 °C. Colonies of the fungus were deep green with white mycelium borders. Conidiophores were light brown with 2-4 septa. Conidia were obclavate, measuring 14.9-47.6 μm × 8.5-15.8 μm, with a short beak, and with 1-5 transverse septa and 0-3 longitudinal septa, light brown to olive-brown. Based on morphology, the pathogen was identified as *A. alternate* [23]. The ITS region of rDNA was amplified using primers ITS4/ITS6 and sequenced. BLAST analysis of the 529-bp segment showed 99% similarity with the sequence of *A. alternata* (Fr.) Keissl. (Fig. 2d).

A5 was plated on PDA and incubated at 28 °C. Fungal isolates developed copious, white, aerial mycelium that became dark gray after 2-3 d. Prostrate hyphal arcuate curved, in contact with the substrate to produce rhizoids at sporangiophore erect unbranched, light brown, sporangium spherical or oval, dark brown, diameter of 68.0-372.0 μm, spores of irregular shape, nearly spherical, oval, measuring 4.3-11.2 μm × 6.4-7.9 μm. Based on morphology, the pathogen was identified as *R. nigricans* Ehrenb. [24] (Fig. 2e).

A6 was plated PDA and incubated at 28 °C. Fungal isolates developed colonies green. Conidiophores of isolates were terverticillate, stipes were septate with rough walls and phialides were ampulliform. Conidia were smooth, borne in columns, and were spherical to subglobose, measuring 3.5-4.7 μm × 2.5-4.2 μm. Based on morphology, the pathogen was identified as *P. ulaiense* Hsieh, Su & Tzean [25] (Fig. 2f).

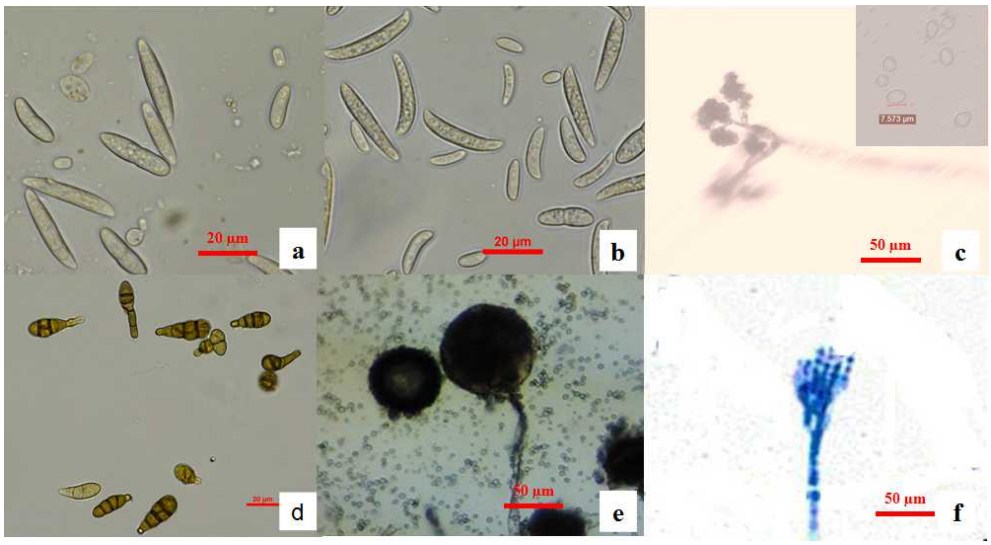

Fig. 2 Morphological characteristics of six fungi causing tuber rot of *A. muelleri*.
a: *F. solani*; b: *F. oxysporum*; c: *B. cinerea*; d: *A. alternata*; e: *R. nigricans*; f: *P. ulaiense*.

3.4 Effect of Different Fungicides

3.4.1 Radial Growth of Three Pathogens

The effect of five fungicides on radial growth of three pathogens (*F. solani*, *F. oxysporum* and *B. cinerea*) was studied to screen out which fungicides are highly effective against this fungus. The average radial growth of fungus was significantly affected by different fungicides. The results of chemical screening showed that in PDA media plate, the compounds Fludioxonil (50% WP) and Boscalid (50% WG) were the most effective in controlling the three main pathogens, and the average effect reached more than 97%. The different levels of dilution of Iprodione (50% SC), Carbendazim (50% WP) and Kresoxim-methyl (10% SC) had different antifungal effects. The antifungal effect of Iprodione (50% SC) was apparent (*F. solani*, *F. oxysporum* and *B. cinerea* with 93.2%, 97.8% and 98.9%, respectively), when the level of dilution was more than 2,000 times. The inhibition rate of Kresoxim-methyl (10% SC) was less than 65% (*F. solani* and *F. oxysporum* with 59.2% and 63.1%, respectively), when the dilution level was more than 5,000 times (Table 2).

3.4.2 Postharvest Efficacy Test

The test of fungicides antisepsis of tubers showed that *A. muelleri* were dipped in the 9,000× diluted solution of Fludioxonil (50% WP) or in the 3,500× diluted solution of Boscalid (50% WG) for 3 min and stored at room temperature (25 °C) for 7 d and 15 d, respectively. The control effects of Fludioxonil against *F. solani*, *F. oxysporum* and *B. cinerea* were 88.6%/83.2%, 90.1%/84.7% and 93.0%/91.5%, respectively, and the control effects of Boscalid were 87.0%/85.3%, 89.0%/85.6% and 89.2%/89.1%, respectively.

The postharvest diseases of non-pollution, comprehensive prevention and cure methods are particularly important for konjac. The test selected agents are toxic fungicide, and Fludioxonil, Boscalid were available for post-harvest tuber disinfection flooding, which has been widely used in Boscalid fruit preservative field [26, 27]. Postharvest diseases by

Table 2 Effect of different fungicides on radial growth of A1, A2 and A3.

Fungicides	Level of dilution (times)	*F. solani*		*F. oxysporum*		*B. cinerea*	
		Average diameter (mm)	Inhibition rate (%)	Average diameter (mm)	Inhibition rate (%)	Average diameter after 48h (mm)	Inhibition rate after 48h (%)
Carbendazim (50% WP)	2,000	20.5	72.7^{aA}	21.3	72.7^{aA}	10.5	82.8^{aA}
	2,500	25.1	66.5^{bB}	25.3	67.6^{bB}	14.6	76.1^{cC}
	3,000	28.6	61.9^{cC}	29.3	62.4^{cC}	17.2	71.8^{cC}
Iprodione (50% SC)	1,000	1.0	98.7^{bB}	0.6	99.2^{bB}	0.0	100.0^{aA}
	1,500	3.9	94.8^{bB}	1.3	98.3^{bB}	0.5	99.2^{bB}
	2,000	5.1	93.2^{bB}	1.7	97.8^{bB}	0.7	98.9^{bB}
Fludioxonil (50% WP)	5,000	0.0	100.0^{aA}	0.0	100.0^{aA}	0.0	100.0^{aA}
	7,000	0.0	100.0^{aA}	0.0	100.0^{aA}	0.0	100.0^{aA}
	9,000	1.6	97.7^{bB}	1.3	98.3^{bB}	0.0	100.0^{aA}
Kresoximmeth-yl (10% SC)	2,000	23.8	68.3^{bC}	23.5	69.9^{cC}	8.3	86.4^{cC}
	3,000	29.1	61.2^{cC}	28.6	63.3^{cC}	11.2	81.6^{cC}
	5,000	30.6	59.2^{cC}	28.8	63.1^{cC}	15.0	75.4^{cC}
Boscalid (50% WG)	1,500	0.0	100.0^{aA}	0.0	100.0^{aA}	0.0	100.0^{aA}
	2,500	0.0	100.0^{aA}	0.0	100.0^{aA}	0.0	100.0^{aA}
	3,500	0.0	100.0^{aA}	0.0	100.0^{aA}	0.0	100.0^{aA}
Control check	-	75	-	78	-	61	-

Different lowercase letters represent significant difference at 0.05 level; different uppercase letters represent extremely significant difference at 0.01 level.

inhibiting fungicides in combination with other anticorrosion methods pathogens will effectively extend the konjac tuber storage time. In this paper, konjac postharvest diseases and pathogens were identified. Major pathogens were fungicide screening for postharvest diseases. Konjac major causative pathogen characteristics and fungicides, and other control techniques in the warehouse during storage problem have not been involved, but also need for further study. The findings of the current studies will provide basic information for *A. muelleri* postharvest disease management in field conditions.

4. Conclusions

Progressive increase of production and application of chemicals fungicides for agriculture as well as for plant protection has converted the problem of environmental pollution into national and international issue. Therefore, more efforts are being directed using selective chemicals, as well as assessment and usage of their minimum concentration required for disease management. Initially, according to the morphological analysis and ITS sequencing results, six fungi were identified as the pathogens causing tuber rot of *A. muelleri*. The main pathogens causing postharvest diseases of *A. muelleri* were *F. solani*, *F. oxysporum* and *B. cinerea*.

Screening bioassays were carried out to determine the most suitable fungicides for the management of *A. muelleri* postharvest disease on the basis of their effectiveness as well as economics. All the chemicals used in this study effectively reduced the radial mycelial growth of six pathogens. The concentrations of different fungicides were selected on the base of reduction in radial growth. Indoor toxicity test results indicated that five germicides used in this study all exhibited varying degrees of inhibitory effect on mycelial growth of three main pathogens. In PDA media plate, the compounds Fludioxonil (50% WP) and Boscalid (50% WG) were the most effective in controlling the three main pathogens, and the average effect reached more than 97%, while inhibitory effects of other germicides were different, which may be related to the different control characteristics or molecular structures of various germicides.

The test of fungicides antisepsis of tubers showed that *A. muelleri* were dipped in the 9,000× diluted solution of Fludioxonil (50% WP) or in the 3,500× diluted solution of Boscalid (50% WG) for 3 min and stored at room temperature (25 °C) for 7 d and 15 d, respectively. The control effects of Fludioxonil against *F. solani*, *F. oxysporum* and *B. cinerea* were 88.6%/83.2%, 90.1%/84.7% and 93.0%/91.5%, respectively, and the control effects of Boscalid were 87.0%/85.3%, 89.0%/85.6% and 89.2%/89.1%, respectively, showing no significant differences, which were both extremely significantly higher than control efficacies of other experimental germicides. These suggest that Fludioxonil (50% WP) and Boscalid (50% WG) are appropriate germicides for controlling postharvest disease of *A. muelleri*.

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References

[1] Impaprasert, R., Borompichaichartkul, C., Srzednicki, G., Zhao, J. R., and Yu, L. 2013. "Improving Production of Purified Konjac Glucomannan from *Amorphophallus muelleri* by Multistage Drying." *Acta Horticulturae* 1011: 155-62.

[2] Impaprasert, R., Borompichaichartkul, C., and Srzednicki, G. 2014. "A New Drying Approach to Enhance Quality of Konjac Glucomannan Extracted from *Amorphophallus muelleri*." *Drying Technology* 32 (7): 851-60.

[3] Liu, E. X., Mou, F. G., Pan, Y., Teng, J. X., Jiang, Q. L., Zhao, Q. H., and Yang, C. Z. 2011. "Study on Biological Characteristics of *Amorphoph mlus* Blume Flower and Cross Compatibility." *Chinese Agricultural Science Bulletin* 27 (31): 126-31. (in Chinese)

[4] Yu, L., Zhao, J. R., Xu, S. G., Su, Y., Gao, D., and

Srzednicki, G. 2014. "First Report on Gray Mold of *Amorphophallus muelleri* Caused by *Botrytis cinerea* in China." *Plant Disease* 98 (5): 692.

[5] Zhao, J. R., Zhang, D., Zhao, J. P., and Srzednicki, G., Kanlayanarat, S., and Borompichaichartkul, C. 2010. "The Morphological and Growth Characteristics of *Amorphophallus muelleri* Blume—A Commercially Important Konjac Species." *Acta Horticulturae* 875: 501-8.

[6] Zhao, J. R., Yu, L., Srzednicki, G., and Borompichaichartkul, C. 2013. "Effects of Different Concentrations of Gibberellin on Flower-Bud Differentiation of *Amorphophallus muelleri*." In *Proceedings of International Symposium on Agri-Foods for Health and Wealth,* 93-9.

[7] Zhao, J. R., Donghua, Z., Srzednicki, G., Kanlayanarat, S., and Borompichaichartkul, C. 2008. "A Simplified Method of Purifying Konjac Glucomannan." *Acta Horticulturae* 837: 375-80.

[8] Zhao, J. R., Zhang, D. H., Srzednicki, G., Kanlayanarat, S., and Borompichaichartkul, C. 2009. "Asexual Reproduction of *Amorphophallus bulbifer* by Low-Cost Artificial-Induction Technique." *Acta Horticulturae* 837: 351-8.

[9] Zhao, J. R., Zhang, D., Srzednicki, G., Kanlayanarat, S., and Borompichaichartkul, C. 2010. "Development of a Low-Cost and Two-Stage Technique for Production of Low Sulphur Purified Konjac Flour." *International Food Research Journal* 17 (4): 1113-24.

[10] Zhao, J. R. 2011. *Integrated Production of Purified Konjac Flour.* Bangkok: KMUTT.

[11] Adamiec, J., Borompichaichartkul, C., Srzednicki, G., Panket, W., Piriyapunsakul, S., and Zhao, J. R. 2012. "Microencapsulation of Kaffir Lime Oil and Its Functional Properties." *Drying Technology* 30 (9): 914-20.

[12] Keithley, J., and Swanson, B. 2005. "Glucomannan and Obesity: A Critical Review." *Alternative Therapies in Health and Medicine* 11 (6): 30-4.

[13] Mekkerdchoo, O., Holford, P., Srzednicki, G., Prakitchaiwattana, C., Borompichaichartkul, C., Wattananon, S., and Zhao, J. R. 2010. "Determination of Relationships and Genetic Variation among *Amorphophallus* sp. from Northern Part of Thailand." In *Proceedings of International Conference on Agricultural and Agro-Industry, Food, Health and Trade.*

[14] Pérols, C., Piffaut, B., Scher, J., Ramet, J. P., and Poncelet, D. 1997. "The Potential of Enzyme Entrapment in Konjac Cold-Melting Ge Beads." *Enzyme and Microbial Technology* 20 (1): 57-60.

[15] Zhang, Y., Xie, B., and Xin, G. 2005. "Advance in Applications of Konjac Glucomannan and Its Derivatives." *Carbohydrate Polymer* 60: 27-31.

[16] Fang, Z. D. 1998. *Research Methods of Plant Disease.* Beijing: China Agriculture Press. (in Chinese)

[17] Zhao, S. Y., Li, X. H., Chen, L., and Zhang, J. S. 2005. "Studies on the Varieties of Pathogenic Fungi and Their Ways of Post-Harvest Rot Disease Infectin on Peach." *Food Science* 26 (10): 253-5. (in Chinese)

[18] Ali, S., Huang, Z., and Ren, S. X. 2009. "Media Composition Influences on Growth, Enzyme Activity and Virulence of the Entomopathogen Hyphomycetes *Isaria fumosoroseus*." *Entomologia Experimentalis et Applicata* 131 (1): 30-8.

[19] SAS Institute. 2000. *SAS User's Guide: Statistics.* Cary, North Carolina, USA: SAS Institute.

[20] Leslie, J. F., and Summerell, B. A. 2006. *The Fusarium Laboratory Manual.* Ames, IA: Blackwell.

[21] Pérez, B. A., Castelar, I. I., Argentina, Murillo, F., Divo de Sesar, M., and Wright, E. R. 2007. "Occurrence of *Fusarium solani* on Blueberry in Argentina." *Plant Disease* 91 (8): 1053.

[22] Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium Species: An Illustrated Manual for Identification.* Pennsylvania, PA, USA: Pennsylvania State University Press.

[23] Zhou, Z., Li, Y. L., Yuan, C. Y., and Duan, P. L. 2014. "First Report of *Alternaria alternata* Causing a Leaf Spot on Philodendron 'Con-Go' in China." *Plant Disease* 98 (11): 1588.

[24] Araceli, T., Verónica, F., Diana, C., and Javier, B. G. 2001. "An Isolate of *Rhizopus nigricans* Capable of Tolerating and Removing Pentachlorophenol." *World Journal of Microbiology and Biotechnology* 17 (2): 201-5.

[25] Holmes, G. J., Eckert, J. W., and Pitt, J. I. 1993. "A New Postharvest Disease of Citrus in California Caused by *Penicillium ulaiense.*" *Plant Disease* 77 (5): 537.

[26] Sallato, B. V., Torres, R., Zoffoli, J. P., and Latorre, B. A. 2007. "Effect of Boscalid on Postharvest Decay of Strawberry Caused by *Botrytis cinerea* and *Rhizopus stolonifer*." *Spanish Journal of Agricultural Research* 5 (1): 67-78.

[27] Xiao, C. L., and Boal, R. J. 2009. "Preharvest Application of a Boscalid and Pyraclostrobin Mixture to Control Postharvest Gray Mold and Blue Mold in Apples." *Plant Disease* 93 (2): 185-9.