Fine particles of fungal active metabolites constructed from *Emericella* sp to control rice blast disease in China

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Abstract The biological control of rice blast in China was investigated by using the fine particles of active metabolites constructed from *Emericella* sp. The metabolite fine particles were inhibited *Magnaporthe oryzae* causing rice blast disease by using poisonous food method. Fine particles-EN inhibited the blast pathogen of 62 % in 12 days. *In vivo* biological activity of fine particles from active metabolites were proved to control blast disease of rice var. Co39 in tested tube and pot experiments compared with chemical fungicide, tebuconazole. Test tube experiment resulted that fine particles -EN reduced the blast disease incidence of 49 % which higher than tebuconazole of 33 %n when compared to the inoculated with *M. oryzae*. Pot experiment showed that tebuconazole treatment reduced the blast incidence of 63 % and fine particles-EN reduced the blast incidence of 44 % when compared to the inoculated control. The further research findings are being investigated for rice immunity to blast disease.

Keywords: rice blast, *Magnaporthe oryzae*, *Emericella* sp., fine particles

Introduction

The chemical pesticides in crop production has become deleterious effect to living organisms and agroecosystem over 100 years. Those toxic agrochemicals are residue in the soil, surrounding environment and agricultural products that harmful to human being and short life. Biological control strategy become one of the potential method to use for plant disease control instead of chemical fungicides.

Rice blast disease caused by *Magnaporthe oryzae*, is one of the most important rice disease. China is the world's top producer and consumer of rice, about 200 million tons a year. Rice blast is harmful disease that affects China's rice crop. Interspersing two varieties of rice have doubled yields in Yunnan and occurrence of rice blast decreased by 93 percent (Rice Agriculture in China, 2018).

Emericdella sp is reported as a biological control agent against Fusarium wilt of tomato (Sibounnavong, P., 2012). *Emericella rugulosa* is reported to produce five new prenylxanthones, ruguloxanthones A-C (1-3), 14-methoxytajixanthone (4), and tajixanthone ethanoate (5), a new bicyclo[3.3.1]nona-2,6-diene derivative, rugulosone (6), and seven known compounds, shamixanthone, tajixanthone, 14-methoxytajixanthone-25-acetate, tajixanthone hydrate, tajixanthone methanoate, isoemericellin, and ergosterol. , The structures of 1-6 were established using spectroscopic techniques. Compound 6 exhibited antimalarial and antimycobacterial activities, as well as cytotoxicity against three cancer cell lines (Moosophon *et al.*, 2009).

The construction of fine particles from natural products have been investigated to control plant pathogens and induce plant immunity. The objective of research finding was to investigate the fine particles derived from *Emericella* against *Magnaporthe oryzae* causing rice blast disease.

Materials and Methods

The isolate *Magnaporthe oryzae* is provided by Key Laboratory of Biotechnology, Zhejiang University, Hangzhou, P.R. China. It was cultured on completed medium (CM) which consisted of NaNO3 6 g., KCl 0.52 g, KH2PO4 1.52 g, MgSO4.7H_2O 0.52 g, D-glucose 10 g, peptone 2 g, yeast extract 1 g, casaminoacid 1 g and agae 15 g in 1 L, and adjusted pH to 6.5 and incubated at 27°C for 10 days. The morphology was observed under binocular compound microscope.

Pathogenicity tests were made in two methods by detached leaf and directly inoculated to seedling plants. The spore suspension of *Magnaporthe oryzae* at the concentration of $1x10^5$ spores/ml was inoculated by high pressure inoculation machine onto 20 days of rice seedlings var Co 39 or onto detached leaves. The inoculated seedlings and detached leaves were kept in 90% relative humidity in dark condition for 2 days in moist chambers.

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Control treatment was done by spraying sterilize distilled water. The experiments were designed as a Completely Randomized Design (CRD) with four replications. Disease incidence was assigned a disease *index* (DI) *at* 5 days of post-inoculation using a 0 to 9 scales (*modified from* Xia *et al.*, 1993) where 0 =no infection observed;1 = small brown specks and small pinpoint infection < 1 mm and 9 = lesions with expanding open centers on > 90% of the leaf area evaluated.

Crude extract of natural product derived from *Emericella* sp was constructed to be fine-particles which followed the method of Dar and Soytong (2014) to yield fine particles-EN (FP-EN). The fine particles from natural products were kept in capped bottles after operated in electrospinning.

Fine particles were evaluated to control *M. oryzae* using poisonous food method. Treatments were non-treated control, fine particle-EN (FP-EN). FP-EN was mixed into media at the concentration of 100 ppm, autoclaved for 30 min. *M. oryzae* was cultured for 10 days under 25℃. The culture agar plug of 0.3 mm dia. was transferred to the middle poisonous plate, and incubated at 25℃. Data were collected as colony diameter and number of spores after 15 days. Experiment was designed by CRD with 4 replications. Data were subjected to analysis of variance.

Tube experimental test: Rice seedlings in big tube with 60 ml 1/5 Murashige & Skoog Medium (1/5 MS media) were prepared. The sterilized tubes were filled with 60 ml of sterilized 1/5 MS media, then transferred 3 rice seedlings into one tube and cultured for 14 days under 25 ℃. Experiment was designed by CRD with 4 replications. Treatments were T1 = non-inoculated control, T2 = inoculated control, T3 = FP-EN and T4 = Tebuconazole (chemical fungicide). Each treatment was sprayed 0.5 ml of 300 ppm FP-EN and 0.5 ml of Tebuconazole. Thereafter, spore suspension of the tested pathogen at concentration $1x10^5$ spores/ml was inoculated after 12 hours treatments. All treatments were maintained at 22℃ for 3 days. Data was gathered as chlorophyll, disease index, plant and root weights and subjected to analysis of variance and compared treatment mean using Duncan's Multiple Range Test (DMRT).

Pot experiment: Rice seeds were sterilized with 1% sodium hypochlorite for 20 min and sterilized water several times, then placed on tissue paper with sterilized water in petri dish at 37 ℃ for 3 days germination. The plastic pots (6 cm diameter) were prepared and put substrate: vermiculite at the ratio of 3:1, then planted 10 seeds per pot for 20 days before treatments. Experiment was designed by RCBD with 4 replications. Treatments were T1 = non-inoculated control, T2 = inoculated control, T3 = FP-EN and T4 = Tebuconazole at recommended rate. Each treatment was sprayed 2 ml at the rate of 1,000 ppm FP-EN and 2 ml Tebuconazole, then inoculated spore suspension of *M. oryzae* at concentration $1x10^5$spores/ml, 2ml/pot. All treatment were maintained at 22 ℃. Data was gathered as chlorophyll, disease index, plant fresh weight, plant high, plant dry weight and root fresh weight, root dry weight, root length. Data were subjected to analysis of variance.

Results

The isolate is offered from Key laboratotory of Biotechnology, Zhejiang University, Hangzhou, PR China. It was cultured on CM media for 15 days, incubated at 25 C. and observed under compound microscope. Cuture on CM medium appeared creamy white colony at first stage of growing and turned to yellowish brown. It showed septate mycelia, produced branched conidiophores and borne conidia. Conidia hyaline, 3-celled, 2 septa. The pathogenicity test in detached leaf method showed typical lesions after inoculation at averaged DI level 7 (lesions with expanding open centers on 51 to 75% of the leaf area evaluated) in rice. The pathogenicity test which directly inoculated the blast pathogen to seedling plants averaged DI level 6 (lesions with expanding open centers on 26 to 50% of the leaf area evaluated) in the same rice variety. The natural product *Emericella* sp was constructed to be fine particles yielded fine particle-EN(FP-EN).

Result showed that FP-EN Na at the concentration of 100 ppm gave the inhibition of 78 % when comparted to the inoculated control, FP-EN treatment showed abnormal conidia leading to pathogenicity loss. It was observed that the conidia were deformed shape and being protoplast plugs inside the pathogen cells.

Rice seedings in tested tubes after treatment showed tebuconazole gave the lowest blast disease incidence of 3.7 % and highest disease reduction of 93% when comparted to inoculated with *M. oryzae*. FP-EN showed blast disease reduction of 73% when comparted to inoculated one.

The rice seedlings tested in pot experiment which inoculated with *M. oryzae* and treated with FP-EN compared to Tebuconazole (chemical fungicide). Result showed FP-EN reduced the blast incidence of 44n % and Tebuconazole reduced the blast incidence of 63 % when compared to the inoculated control.

The treated FP-EN and Tebuconazole in rice var Co39 were significantly higher chlorophyll content, plant height, fresh and dry weight of stems, root length, fresh and dry weight of roots than the inoculated with *M. oryzae* in 7 days after inoculation (Table 1).

Table 1. The effect of fine particles derived from Emericella sp. in comparison with to Tebuconazole to control blast incidence in pot experiment

Treatments	Index Chlorophyll content[1]	Disease Reduction (%)[1]	Plant			Root		
			Fresh Weight (g)[1]	Dry Weight (g)[1]	Height (cm)[1]	Fresh Weight (g)	Dry Weight (g)	Length (cm)[1]
inoculation	20.43*	-	2.22	0.60^{d}	34.37^{bc}	1.08^{b}	0.17^{b}	13.00^{ab}
FP-EN	25.42	44.44	2.71	0.79^{abcd}	38.30^{a}	1.31^{ab}	0.19^{ab}	13.38^{ab}
Tebuconazole	27.10	63.88	2.37	0.61^{cd}	36.00^{abc}	1.36^{ab}	0.21^{ab}	12.75^{b}

*$P < 0.01$

[1]/Disease reduction (%) = R1-R2/R1x100 where R1 is the disease incidence in the control rice plant and R2 is the disease incidence in treated rice plant.

Discussion

The research finding that *M. oryzae* which supported by by Key Laboratory of Biotechnology, Zhejiang University, Hangzhou, P.R. China was confirmed to be pathogenic to cause rice blast disease.

The natural product *E. nidulans* namely FP-EN inhibited the tested pathogen of 78 % which similar to the report of *Emericella rogulosa* inbihited some plant pathogenic fungi eg. E. rugulosa ER01 could inhibit the tested plant pathogen, *F. oxysporum* f. sp. lycopersici (Sibounnavong and Soytong, 2011). The treated FP-EN expressed abnormal spores of *M. oryzae* leading to lost of pathogenicity that also similar reports of Tann and Soytong (2017).

As result rice seedlings in testing tubes, tebuconazole gave the lower blast disease incidence than FP-EN when comparted to inoculated one. It is explained that the concentration of FP-EN may possible to test in various concentrations and tebuconazole treatment used as successfully recommendation rate. It was similar result which explained by Soytong (2014), who stated that bioproducts from *Chaetomium* sp. to control rice leaf spot caused by *Curvularia lunata*, but at a high application rate.

The pot experiment resulted that the treated with FP-EN and Tebuconazole decreased the blast incidence as compared to the inoculated control, and also higher chlorophyll content, plant height, fresh and dry weight of stems, root length, fresh and dry weight of roots than the inoculated with *M. oryzae*. These results confirmed successfully application either fine particles from natural product of *Emericella* sp or Tebuconazole chemical fungicide that reduce the disease incidence and increase plant strands. Similar results are reported that nanoparticles derived from *Chaetomium* sp reduced blast incidence, and increased plant growth parameters (Soytong, 1989). The future research findings are being evaluated induction immunity to blast disease of rice through phytoalexin production.

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