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Biological metabolites from *Chaetomium* spp to inhibit *Drechslera oryzae* causing leaf spot of rice

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The antagonistic fungi, *Chaetomium cupreum, Chaetomium brasilense*, *Chaetomium cochliodes*, *Chaetomium globosum* and *Chaetomium elatum*, were isolated from soil in Thailand, effectively controlled the *Drechslera oryzae* causing leaf spot of rice (*Oryza sativa).* The bioactivities test demonstrated the antagonistic activity of *Ch. cupreum*, *Ch. brasilense*, *Ch. cochliodes*, *Ch. globosum* and *Ch. elatum* to inhibit the conidial production of *D. oryzae*. To elucidate the control mechanism involved in the inhibition of *D. oryzae*, crude extracts of *Ch. cupreum*, *Ch. brasilense*, *Ch. cochliodes*, *Ch. globosum* and *Ch. elatum* were confirmed for antifungal activity againt of *D. oryzae*. All tested crude extracts of *Ch. cupreum*, *Ch. brasilense*, *Ch. cochliodes*, *Ch. globosum* and *Ch. elatum* were significantly inhibited conidia production of *D. oryzae*. It is indicated that crude extracts from hexane, EtOAc and MeOH from *Ch. cupreum* inhibited *D. oryzae* at the ED_{50} of 0.58, 14.92 and 8.77 µg/ml, respectively. Crude extracts from hexane, EtOAc and MeOH from *Ch. brasilense* inhibited *D. oryzae* at the ED_{50} of 45.60, 31.38 and 145.10 µg/ml, respectively. Crude extracts from hexane, EtOAc and MeOH from *Ch. cochliodes* inhibited *D. oryzae* at the ED_{50} of 37.26, 2.37 and 27.91 µg/ml, respectively. Crude extracts from hexane, EtOAc and MeOH from *Ch. globosum* inhibited *D. oryzae* at the ED_{50} of 63.01, 49.74 and 10.15 µg/ml, respectively. Crude extracts from hexane, EtOAc and MeOH from *Ch. elatum* inhibited *D. oryzae* at the ED_{50} of 166.98, 20.24 and 29.44 µg/ml, respectively. This research finding is the first report using *Chaetomium brasilense*, *Chaetomium cochliodes* and *Chaetomium elatum* to inhibit *D. oryzae* causing leaf spot of rice

Keys word: *Chaetomium cupreum, Chaetomium brasilense*, *Chaetomium cochliodes*, *Chaetomium globosum, Chaetomium elatum*, *Drechslera oryzae*, fungal metabolites

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Introduction

Rice **(***Oryza sativa***)** is one of the world's major food crops. Particularly popular in Asia where rice is the daily diet than in other regions of the world production, consumption and trade, most of it concentrated in Asia (www.thaifta.com/thaifta/Portals/0/File/ascn_rice1.doc) *Drechslera oryzae* (Breda de Haan) Subram. & Jain was revised the name from *Helminthosporium oryzae* Breda de Haan)Alcorn, 1988). The fungus causes leaf spot disease of rice seedlings (*Oryza sativa*), the fungus will spread to the internal field and hard and can cause death of seedlings to 10-58% . The output reduced up to 45% in severe infections and infections in 12% of the average level in 1942, has reported losses of 50-90% yield and also causes the death of the population. 2 million peoples with the disease in India, which yielded 14-41 % loss in high-yielding varieties. Under the right environment and the state of Florida in the United States are reported to lose up to 16-40% of the total rice production (IRRI, 1983). At present, biological control of plant pathogens has been widely used and interested to investigate tne new biological control agents. Because farmers are usually applied chemical fungicidesto eradicate the disease prevention which polluted to the environment and harmful to living things. It is also another reason for the current climate as well. *Chaetomium* spp. belong to the Ascomycota, and have been reported as antagonists against several plant pathogens (Soytong *et al*., 2001; Dhingra *et al*., 2003; Aggarwal *et al*., 2004; Park *et al*., 2005). Many species of *Chaetomium* with the potential to be biological control agents suppress the growth of bacteria and fungi through competition (for substrate and nutrients), mycoparasitism, antibiosis, or various combinations of these (Zhang and Yang, 2007). *Chaetomium globosum* and *Ch. cupreum* in particular have been extensively studied and successfully used to control root rot disease of citrus, black pepper and strawberry, and have been shown to reduce damping off disease of sugar beet (Soytong *et al*., 2001; Tomilova and Shternshis, 2006). The plant disease control mechanism may involve in antibiosis, with the antagonistic fungus releasing antibiotic substances (Soytong *et al.,* 2001; Kanokmedhakul *et al*., 2002, 2006; Park *et al*., 2005).

Di Pietro *et al*. (1992) reported that *Ch. globosum* can produce chetomin, which effectively inhibited *Pythium ultimum* causing damping-off of sugar beet. *Ch. globosum* strain KMITL 0802 has been shown to produce chaetoglobosin – C, which inhibits some pathogens (Kanokmedhakul *et al*., 2002). Park *et al.* (2005) also reported that *Ch. globosum* F0142 can produce chaetoviridin A to control rice blast, wheat leaf rust and tomato late blight. Soytong (1992) and Soytong *et al*. (2001) showed that a specific isolate of *Ch. cupreum* produced secondary metabolites that significantly suppressed tomato

wilt caused by *F. oxysporum* f. sp. *lycopersici* in the tomato fields in Thailand, and later found that this isolate of *Ch. cupreum* produced rotiorinols A-C and rotiorin, which exhibited antifungal activity against *Candida albicans* (Kanokmedhakul *et al*., 2006). *Chaetomium cochlioides*- strains VTh01 and CTh05 have been shown to exhibit antimicrobial activity against a *Phytophothora* sp. that causes root rot, the anthracnose fungus *Colletotrichum gloeosporioides* , and *F. oxysporum* f. sp. *lycopersici*.

Materials and methods

Isolation

Disease sample were collected from leaf from rice fields in Bankok provinces in Thailand. The pathogen was isolated by transferring surface sterilized plant tissue to a broken rice agar (BRA) medium. Pure culture of *Drechslera oryzae* were identified by morphological characteristic under a binocular compound microscope, maintained on BRA slant and deposited at the Biocontrol Research Unit and Mycology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Lardkrabang, Bangkok, Thailand.

Antagonism test

Effective antagonists were tested, *Chaetomium cupreum, Chaetomium brasilense, Chaetomium cochliodes, Chaetomium globosum* and *Chaetomium elatum* which offered from Assoc. Prof. Dr. Kasem Soy tong, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bankok, Thailand. These isolates were tested to determine their ability to antagonize *Drechslera oryzae*. The test was conducted using the methods of Soytong (1992), Sibounavong *et al*. (2009) and Charoenporn *et al*. (2010). The antagonistic fungi and pathogen were separately cultured on PDA at room temperature (30 to 32 ºC) for seven day. A 0.5 cm diameter sterilized cork borer was used to remove agar plugs from the actively growing edge of cultures of the pathogenic and antagonistic fungi and transferred onto 9 cm diameter PDA plates, an agar plug of the pathogen was placed on one side of the plate which opposited an agar plug of an antagonistic fungus. PDA plates were transferred with a single plug of an antagonistic fungus or of the pathogen acted as the controls. The bi-culture plates were incubated at room temperature (30 to 32 ºC) for 30 days. The experiment was performed using a completely randomized design (CRD) with four replications. Data were collected regarding tp colony diameter (cm) and the number of conidia produced by the pathogen.

A haemcytometer was used to count the number of conidia. Percentage inhibition of pathogen colony growth and of conidia production were calculated using the following formula:

$$\% \text{ inhibition} = \frac{A - B}{A} \times 100$$

Where, A is the colony diameter or number of conidia produced by the pathogen on the control plate and B is the colony diameter or number of conidia produced by the pathogen when inoculated opposite an antagonistic fungus. Analysis of variance was statistically analyzed and treatment mean were compared using Duncan's Multiple Range Test (DMRT) at p = 0.5 and 0.01.

Crude extract method

Crude extracts from each antagonistic fungus were followed the method used by Kanokmedhakul *et al*. (2006), Moosophon *et al*. (2009) and Thohinung *et al*. (2010). The fungi were cultivated in potapo dextrose broth at room temperature (30 to 32 ºC) for 30 days. The dried fungal biomass of each antagonistic fungus was ground and sequentially extracted with hexane, ethyl acetate and methanol. The solvents were then evaporated in vacuo to yield crude hexane, crude ethyl acetate (EtOAc) and crude methanol (MeOH) extracts, respectively.

Crude extract bioassay

The crude extracts were assayed for inhibition of *Drechslera oryzae*. The experiment was conducted by using a factorial experiment in CRD with four replications. Factor A represented the different solvents: A1 = crude hexane, A2 = crude ethyl acetate and A3 = crude methanol. Factor B represented the different concentrations: B1 = 0 µg/ml (control), B2 = 50 µg/ml, B3 = 100 µg/ml and B4 = 500 µg/ml. Each crude extract was dissolved in 2% dimethyl sulfoxide and added to BRA before autoclaving at 121 ºC (15 psi) for 30 min. To perform the assay, a sterilized 3 mm diameter cork borer was used to remove agar plugs from the actively growing edge of the pathogen culture. An agar plug was transferred to the center of 5 cm diameter Petri dishes of PDA containing crude extract at each concentration and incubated at room temperature (30 to 32 ºC) until the pathogen on the control plates.. Data were collected regarding to the number of conidia produced by the pathogen and used to calculate the percentage of conidia inhibition. The effective dose (ED_{50}) was calculated using Probit analysis.

Results

Antagonism test

The result showed in bi-culture antagonistic tests that *Ch. cochliodes* inhibited mycelia growth and production of conidia by *Drechslera oryzae* of 38.60 and 91.49% inhibition, respectively followed by *Ch. globosum*, *Ch. brasilense*, *Ch. cupreum* and *Ch. elatum* which inhibited mycelial growth by 36.80, 35.83, 35.69 and 32.4%, respectively and inhibited conidial production by 91.49, 87.94, 75.13, 87.25 and 26.43 respectively (Table 1).

Table 1. Mycelial and conidia inhibition of *Drechslera oryzae* at 30 days

Antagonistic fungi	Mycelial inhibition (%)[1]	Conidia inhition (%)[1]
Ch. cochliodes	38.60	91.49
Ch. globosum	36.80	87.94
Ch. brasilense	35.83	75.13
Ch. cupreum	35.69	87.25
Ch. elatum	32.49	26.43

[1]Average of four replications

Crude extract bioassay

Crude hexane from *Ch. cupreum* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 4.37 x 10^6, 3.75 x 10^6, 3.12 x 10^6 and 2.50 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 10.62 x 10^6 spore/ml. Crude ethyl acetate (EtOAc) from *Ch. cupreum* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 5.62 x 10^6, 5.00 x 10^6, 5.00 x 10^6 and 3.12 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 11.25 x 10^6 spore/ml. Crude methanol (MeOH) from *Ch. cupreum* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 5.00 x 10^6, 3.75 x 10^6, 2.50 x 10^6 and 2.50 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 8.75 x 10^6 spore/ml.crude hexane from *Ch. brasilense* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 11.25b x 10^6, 5.62 x 10^6, 4.37 x 10^6 and 2.50 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 13.75 x 10^6 spore/ml. Crude ethyl acetate (EtOAc) from *Ch. brasilense* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 14.75 x 10^6, 10.00 x 10^6, 8.12 x 10^6 and 2.50 x 10^6 spore/ml, respectively when

compared to the control (0 µg/ml) of 14.75 x 10^6 spore/ml. Crude methanol (MeOH) from *Ch. brasilense* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 9.12 x 10^6, 7.50 x 10^6, 5.00 x 10^6 and 2.50 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 10.00 x 10^6 spore/ml. Crude hexane from *Ch. cochliodes* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 12.50 x 10^6, 15.62 x 10^6, 7.50 x 10^6 and 3.12 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 22.50 x 10^6 spore/ml. Crude ethyl acetate (EtOAc) from *Ch. cochliodes* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 16.87 x 10^6, 10.62 x 10^6, 7.50 x 10^6 and 3.12 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 25.00 x 10^6 spore/ml. Crude methanol (MeOH) from *Ch. cochliodes* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 6.87 x 10^6, 6.87 x 10^6, 5.62 x 10^6 and 3.75 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 15.00 x 10^6 spore/ml. Crude hexane from *Ch. globosum* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 11.62 x 10^6, 7.50 x 10^6, 5.62 x 10^6 and 3.75 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 22.37 x 10^6 spore/ml. Crude ethyl acetate (EtOAc) from *Ch. globosum* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 9.00 x 10^6, 6.25 x 10^6, 4.37 x 10^6 and 2.87 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 11.87 x 10^6 spore/ml. Crude methanol (MeOH) from *Ch. globosum* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 8.25 x 10^6, 6.25 x 10^6, 5.25 x 10^6 and 4.00 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 11.87 x 10^6 spore/ml. Crude hexane from *Ch. elatum* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 33.75 x 10^6, 33.12 x 10^6, 30.62 x 10^6 and 22.62 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 54.37 x 10^6 spore/ml. Crude ethyl acetate (EtOAc) from *Ch. elatum* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 26.25 x 10^6, 18.12 x 10^6, 10.00 x 10^6 and 6.87 x 10^6 spore/ml, respectively when compared to the control (0 µg/ml) of 39.37 x 10^6 spore/ml. Crude methanol (MeOH) from *Ch. elatum* at the concentration of 10, 50, 100 and 500 µg/ml gave significantly different in spore production of *Drechslera oryzae* were 31.87 x 10^6, 26.25 x 10^6, 20.62 x 10^6 and 16.25 x 10^6 spore/ml, respectively when compared to the

control (0 µg/ml) of 58.12 x 10^6 spore/ml, respectively when compared to the control (Table 2).

Table 2. Effect of crude extracts from antagonistic fungi against conidia production of *Drechslera oryzae*

Crude extracts	**Number of conidia (x10^6) of *Drechslera oryzae* in each concentration (µg/ml)**				
	0	10	50	100	500
Ch. cupreum					
Hexane	10.62a	4.37de	3.75ef	3.12fg	2.50g
EtOAc	11.25a	5.62c	5.00cd	5.00cd	3.12fg
MeOH	8.75b	5.00cd	3.75ef	2.50g	2.50g
Ch. brasilense					
Hexane	13.75a	11.25b	5.62f	4.37f	2.50g
EtOAc	14.75a	10.00bc	8.12de	2.50g	2.50g
MeOH	10.00bc	9.12cd	7.50e	5.00f	2.50g
Ch. cochliodes					
Hexane	22.50a	12.50bcd	15.62bc	7.50def	3.12f
EtOAc	25.00a	16.87b	10.62cde	7.50def	3.12f
MeOH	15.00bc	6.87def	6.87def	5.62ef	3.75f
Ch. globosum					
Hexane	22.37a	11.62b	7.50cde	5.62defg	3.75fg
EtOAc	11.87b	9.00b	6.25def	4.37fg	2.87g
MeOH	11.87b	8.25cd	6.25def	5.25efg	4.00fg
Ch. elatum					
Hexane	54.37a	33.75c	33.12c	22.62efg	30.62cd
EtOAc	39.37b	26.25def	18.12gh	10.00i	6.87i
MeOH	58.12a	31.87cd	26.25def	20.62fgh	16.25h

[1]Average of four replications. Means with the same common letters in each column were not significantly different according to Duncan's multiple rage test at p = 0.01.

It revealed that crude extract at 500 µg/ml from crude hexane of *Ch. cupreum* gave significantly better inhibited spore production of *Drechslera oryzae* as 76.30% better than crude ethyl acetate (EtOAc) and crude methanol (MeOH) which were 72.23 and 71.40%. Crude extract at 500 µg/ml from crude ethyl acetate (EtOAc) of *Ch. brasilense* gave significantly better inhibited spore production of *Drechslera oryzae* as 83.03% better than crude hexane and crude methanol (MeOH) which were 81.80 and 74.93%. Crude extract at 500 µg/ml from crude hexane of *Ch. cochliodes* gave significantly better inhibited spore production of *Drechslera oryzae* as 86.11% better than crude ethyl acetate (EtOAc) and crude methanol (MeOH) which were 85.84 and 74.92%. Crude extract at 500 µg/ml from crude hexane of *Ch. globosum* gave significantly better inhibited spore production of *Drechslera oryzae* as 83.19% better than

crude ethyl acetate (EtOAc) and crude methanol (MeOH) which were 75.77 and 66.49%. Crude extract at 500 µg/ml from crude ethyl acetate (EtOAc) of *Ch. elatum* gave significantly better inhibited spore production of *Drechslera oryzae* as 82.26% better than crude hexane and crude methanol (MeOH) which were 58.27 and 71.95%, respectively. It is indicated that crude hexane, crude ethyl acetate (EtOAc) and crude methanol (MeOH) extracted from *Ch. cupreum* inhibited the conidial production of *Drechslera oryzae* with the ED_{50} of 0.58, 8.77 and 14.92 µg/ml respectively. The crude hexane, crude ethyl acetate (EtOAc) and crude methanol (MeOH) extracted from *Ch. brasilense* inhibited the conidial production of *Drechslera oryzae* with the ED_{50} of 45.60, 31.38 and 145.10 µg/ml respectively. The crude hexane, crude ethyl acetate (EtOAc) and crude methanol (MeOH) extracted from *Ch. cochliodes* inhibited the conidial production of *Drechslera oryzae* with the ED_{50} of 37.26, 27.91 and 2.37 µg/ml respectively. The crude hexane, crude ethyl acetate (EtOAc) and crude methanol (MeOH) extracted from *Ch. globosum* inhibited the conidial production of *Drechslera oryzae* with the ED_{50} of 10.15, 49.74 and 63.01 µg/ml respectively. Moreover, the crude hexane, crude ethyl acetate (EtOAc) and crude methanol (MeOH) extracted from *Ch. elatum* inhibited the conidial production of *Drechslera oryzae* with the ED_{50} of 166.98, 29.44 and 20.24 µg/ml, respectively (Table 2). The blue highlight should be deleted.

Table 3. Bioassay of extracts against *Drechslera oryzae*

Antagonistic fungi	Crude extract	Conidial inhibition (%)	ED_{50} µg/ml
	Hexane	76.30a	0.58
Ch. cupreum	Ethyl acetate	72.23ab	8.77
	Mehanol	71.40ab	14.92
	Hexane	81.80a	45.60
Ch. brasilense	Ethyl acetate	83.03a	31.38
	Mehanol	74.93ab	145.10
	Hexane	86.11a	37.26
Ch. cochliodes	Ethyl acetate	85.84a	27.91
	Mehanol	74.92ab	2.37
	Hexane	83.19a	10.15
Ch. globosum	Ethyl acetate	75.77ab	49.74
	Mehanol	66.49bc	63.01
	Hexane	58.27de	166.98
Ch. elatum	Ethyl acetate	82.26a	29.44
	Mehanol	71.95bc	20.24

[1]Average of four replications. Means with the same common letters in each column were not significantly different according to Duncan's multiple rage test at p = 0.01.

Discussion

The Bi-culture antagonistic test fungi *Ch. cupreum, Ch. brasilense*, *Ch. cochliodes, Ch. globosum* and *Ch. elatum* inhibited the conidial production of *D. oryzae* causing rice leaf spot as 91.49, 87.94, 75.13, 87.25 and 26.43%. Similar result was in accordance with the study from Sibounnavong (2012) who reported that *Ch. brasilense* CB01, *Ch. cupreum* CC03 and *Ch. elatum* ChE01 were tested for abilities to inhibit the pathogen conidia of *Fusarium oxysporum* f.sp. *lycopersici* NKSC02 in bi-culture plates were 77.50, 86.51, 77.72%.

The antagonistic fungi *Ch. cupreum, Ch. brasilense, Ch. cochliodes, Ch. globosum* and *Ch. elatum* inhibit the conidial production of *D. oryzae* causing rice leaf spot as 85.84, 83.19, 83.03, 82.26 and 76.30% respectively. Similar result was in accordance with the study from Sibounnavong (2012) who reported that *Ch. brasilense* CB01 and *Ch. cupreum* CC03 inhibit the conidial production of *F. oxysporum* f.sp. *lycopersici* NKSC02 between 63-77%. All tested crude extracts of *Ch. cupreum, Ch. brasilense*, *Ch. cochliodes*, *Ch. globosum* and *Ch. elatum* were significantly inhibited conidia production of *Drechslera oryzae*. This result was similar to the report of Sibounnavong (2012) who stared that crude hexane, crude ethyl acetate and crude methanol from *Ch. brasilense* CB01 and *Ch. cupreum* CC03 inhibited *F. oxysporum* f.sp. *lycopersici* NKSC02 with the ED_{50} of 9.13, 18.10 and 1.63 µg/ml, wile in this study, those crude extracts inhibited the conidial production of different isolate of *D. oryzae* with the ED_{50} of 0.58, 31.38, 2.37, 10.15 and 20.24 µg/ml which were lower than those from previous report. Similar result were also report by Soytong *et al*. (2005) reported that crude ethyl acetate extract of *Ch. globosum* CG at 1000 µg/ml inhibited conidial production of this pathogen.

It concluded that *Ch. cupreum* can be produced some metabolites to inhibit *D. oryzae* which Kanokmedhakul *et al*. (2006) found antifungal azaphilones from *Ch. cupreum* CC3003 effectively inhibited some human pathogens. Moreover, in this study *Ch. brasilense* proved to produce antifungal metabolites against *D. oryzae* causing rice leaf spot which it is similar to the work of Khumkomkhet *et al*. (2009) who reported *Ch. brasilense* CB01 produced found four new desidones, mollicelins K-N which exhibited antimalarial activity against *Plasmodium falcipurum* and mollicellin K exhibited antmycobacterial activity against *Mycobacterium tuberculosis* and natifungal activity against *Candida albicans* and some cancer cell line.

This research finding is the first report using *Chaetomium brasilense*, *Chaetomium cochliodes* and *Chaetomium elatum* to inhibit *D. oryzae* causing leaf spot of rice.

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