Screening Brassicaceous Plants as Biofumigants for Management of *Rhizoctonia solani* AG1-IA

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Abstract

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Brassicaceae plants rich in glucosinolates have been used as biofumigants for management of *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) and other soilborne pathogens. Efficacy of brassica plant tissue has mainly been attributed to toxic isothiocyanates released upon hydrolysis of glucosinolates. Management of *R. solani* AG 1-IA, the causal agent of sheath blight in rice (*Oryza sativa*), using biofumigation, is promising but needs more validation. Biofumigation activity of nine Brassicaceae plants and two other related species were evaluated in vitro with soils from Texas, Arkansas, or Mississippi. All plants evaluated significantly suppressed the mycelium growth of *R. solani* AG 1-IA. Mustard (*Brassica juncea*) cultivars ('Brand 199', 'Ruby Streak', 'Florida Broadleaf', and 'Green Wave') consistently provided the greatest (>90%) mycelial inhibition, while sunn hemp (*Crotalaria juncea*) and Chinese cabbage (*B. rapa*) had the least suppressive effect. *B. juncea*

'Red Giant' and 'Sheali Hong', turnip (*B. rapa*), kale (*B. oleracea*), and arugula (*Eruca sativa*) showed intermediate efficacy or were inconsistent. Effects of soil pasteurization and plant tissue amendment rates were examined with *B. juncea* Brand 199 and Texas soil. Inhibition of mycelial growth became greater with increasing plant amendment rates up to 3.2% (wt/wt) in the soil. Soil pasteurization almost completely suppressed the release of allyl isothiocyanate (AITC). The nonpasteurized soil amended with 0.5% (wt/wt) of the plant material released 96% more AITC than the soil amended with 0.25% (wt/wt) of the plant material. The highest levels of AITC release were observed at 12 and 24 h after soil amendment, with 0.25 and 0.5% (wt/wt) of the plant material, respectively. Antifungal effects of *B. juncea* are attributed to dose-dependent production of volatile AITC and could be used for managing rice sheath blight caused by *R. solani* AG 1-IA.

Modern intensive crop farming systems with short crop rotations tend to result in an increase in inoculum densities of soilborne pathogens (Hassan and Yousif 2010). In rice (*Oryza sativa* L.) production, accumulation of soilborne inoculum of *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (A. B. Frank) Donk) AG1-IA, the causal agent of sheath blight, can lead to severe disease outbreaks and consequent yield losses. Sheath blight is one of the major rice diseases in the United States, causing varying degrees of grain yield and quality losses every year. Conventional management of sheath blight is dependent on cultural practices and synthetic fungicides. However, heavy dependence on the use of synthetic fungicides causes adverse effects on human health and the environment. Interest in using environment-friendly management practices for sustainable rice production has increased in recent years.

Biological treatments such as use of plant organic residue can be an alternative to synthetic pesticides for management of soilborne pathogens (Lazzeri et al. 2003; Pascual et al. 2004). Glucosinolate hydrolysis products have inspired investigators to explore the use of Brassicaceae plant residue to control different agricultural pests (Mazzola et al. 2007). Brassicaceae species can be incorporated into soil as a biofumigant and have shown positive effects on combating soilborne diseases in various field crops (Angus et al. 1994; Brown and Morra 1996; Kirkegaard et al. 2000; Larkin and Griffin 2007; Matthiessen and Kirkegaard 2006; Smolinska and Horbowicz 1999; Yulianti et al. 2006).

Soil amendment with *Brassica* plants increases organic matter, improves soil structure, and boosts beneficial microbial communities as well as their biocidal effects on soilborne pathogens (Larkin and

Griffin 2007). Different cultivars of *Brassica* spp. have different efficacy due to varying concentrations and chemical types of the glucosinolates in their plant tissue (Kirkegaard and Sarwar 1998; Kirkegaard et al. 1996). The degree of fungal suppression by *Brassica* plant materials was determined by the concentration and type of biocidal volatile gases released (Kirkegaard et al. 1996).

Previous in vitro bioassays have shown inconsistent effects of *Brassica* plant materials on growth suppression of several plant-pathogenic fungi (Charron and Sams 1999; Lazzeri and Manici 2001; Sarwar et al. 1998; Smith and Kirkegaard 2002; Yulianti et al. 2006), especially when advanced to field evaluations. Such inconsistencies between in vitro bioassays and field efficacy may be due to the fact that the methods used for screening plant materials as biofumigants exclude the role of soil properties and soil microbes. Gimsing et al. (2006) reported that microbial enzymes myrosinases in soil influence the *Brassica* biofumigation process, emphasizing the importance of soil properties and microbial activities.

Hydrolysis of glucosinolates in soil depends not only on the concentration of glucosinolates but also soil factors, extent of plant tissue disruption, soil temperature, and water content (Gabler et al. 2006; Hanschen et al. 2015; Morra and Kirkegaard 2002). Concentrations of glucosinolate hydrolysis products can decline in soil due to their volatility (Dungan et al. 2003), sorption to soil particles (Borek et al. 1995; Gimsing et al. 2009), and chemical or biodegradation (Borek et al. 1995; Dungan et al. 2003; Gimsing et al. 2009). Microbiota have been identified as a major factor for biodegradation and consequent isothiocyanate reduction in soils (Dungan et al. 2003).

Biodegradation, which is also known to occur in pesticides, increases with higher pH value in soils amended with *Brassica* plant tissue (Matthiessen and Kirkegaard 2006; Warton et al. 2003). In soils amended with *Brassica* plant tissue, degradation was shown to be both chemically but mostly biologically mediated, because autoclaving soil severely reduced degradation of glucosinolate hydrolysis products (Hanschen et al. 2015).

The objective of this study was to screen brassicaceous plant species and other related plant species for mycelial growth inhibition of

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the rice sheath blight pathogen *R. solani* AG1-IA. The in vitro screening method used in this study simulated soil amendment in field conditions and allowed us to evaluate the antifungal activity of plants tissue as influenced by soil properties.

Materials and Methods

Soil collection and preparation. Soils used in this study were collected from rice fields in three major rice-producing states in the United States: Texas (Texas A&M AgriLife Research and Extension Center, Beaumont), Arkansas (Rice Research and Extension Center, Stuttgart), and Mississippi (Delta Research and Extension Center, Stoneville). The soils were sieved using a 2-mm² sieve to establish a homogenous soil sample. Soil properties, including texture, pH, nutrients, and organic content, were analyzed at the Soil, Water and Forage Testing Laboratory, Texas A&M AgriLife Extension Service, College Station.

In vitro screening. Nine *Brassica* spp. and two nonbrassicaceous plant species were tested. The *Brassica* spp. comprised six mustard (*Brassica juncea* (L.) Czern.) cultivars ('Brand 199', 'Ruby Streak', 'Florida Broadleaf', 'Green Wave', 'Red Giant', and 'Sheali Hong'), one turnip (*B. rapa* L. 'Southern Green'), one Chinese cabbage (*B. rapa* 'Napa'), and one kale (*B. oleracea* L. 'Starbor'). The two nonbrassicaceous plant species were arugula (*Eruca sativa* (L.) Mill 'Nemat') and sunn hemp (*Crotalaria juncea* L.). The plants were grown in the greenhouse with temperatures ranging from 14 (night) to 36°C (day). At flowering, the aboveground biomass of the plants were harvested and stored at -20°C until used for lab assays.

Frozen plant materials were thawed and macerated with a blender. A 3-g sample of the macerated plant tissue sample was confined to the lid of an upside-down petri dish (54 mm in diameter) containing one-fifth strength potato dextrose agar (PDA) medium. An agar disc (8 mm in diameter) of *R. solani* AG 1-IA (TX-RS-1, an isolate collected from a commercial rice field in Texas) was placed on the center of PDA without physical contact with the plant sample attached on the petri dish lid. The petri dishes were incubated at room temperature (25°C) for 72 h; then, radial mycelial growth was measured. Relative mycelial growth inhibition of each treatment was calculated by comparing it with the nontreated control mycelium. Each treatment consisted of 12 petri dishes per experiment arranged in a completely randomized design. The experiment was independently conducted three times.

Mixtures of plant tissue and soil were evaluated using the in vitro assay method with the experimental protocol and design described above. A macerated plant tissue sample for each plant species tested was mixed at a 1:1 ratio with each of the soils collected from Texas, Arkansas, or Mississippi.

Effect of brassicaceous plant amendment rate on mycelium growth. Texas soil (10 g) was placed in an aluminum weighing dish (57 mm in diameter). The soil sample was amended with *B. juncea* Brand 199 at 0.2, 0.4, 0.8, 1.6, or 3.2% plant tissue (wt/wt), and a nonamended control was included. The aluminum dishes containing *B. juncea*-amended soil were placed in a double zipper Ziploc bag (14.9 by 19.6 cm; S. C Johnson and Sons, Racine, WI). Three petri dishes of one-fifth strength PDA, each of which contained a PDA agar disc (8 mm in diameter) of isolate TX-RS-1 of *R. solani*, were placed in the bag. Petri dish lids were taken off before the bag was zipped and sealed. The bag containing three *R. solani* culture plates and *B. juncea*-amended soil was incubated at 25°C. After 48 h of incubation, radial mycelial growth of *R. solani* was measured. Relative mycelium growth inhibition was calculated. The experiment was independently conducted three times.

Effect of soil pasteurization and *B. juncea* Brand 199 amendment rate on allyl isothiocyanate release. The soil from Texas was pasteurized by heating in a dry oven at 60°C for 18 h. The soil that received no pasteurization treatment served as the natural soil control. A 1-gal. Ziplock bag was filled with 250 g of natural or pasteurized soil and thoroughly mixed with macerated *B. juncea* Brand 199 at a rate of 0.25 or 0.5% (wt/wt). The experiment included five treatments: natural soil with no amendment (nontreated control), natural soil + *B. juncea* amended at a rate of 0.25 or 0.5% (wt/wt), and pasteurized soil + *B. juncea* amended a rate of 0.25 or 0.5% (wt/wt).

Each treated soil sample (250 g) was placed into four 0.946-liter mason jars assigned in a complete randomization design. Each jar was closed with an air-tight-seal lid containing a rubber septum in the center and incubated at room temperature. Vapor samples (1 ml) were continually extracted from the head space of each jar by piercing through a rubber septum with a 3.8-cm-long 18-gauge needle (Hamilton Co., Reno, NV) at 12, 24, 36, 48, and 72 h after incubation (Mazzola et al. 2007). After extraction of gases at each time point, the jar lids were briefly removed to release the remaining allyl isothiocyanate (AITC), then tightly closed again.

Gas chromatography-mass spectrometry analysis was carried out using a modified protocol of Mazzola et al. (2007). The vapor samples (0.5 ml) were manually injected into an Agilent 7890 gas chromatograph (GC) coupled with an Agilent 5975 inert mass spectrometer (MS) (Agilent Technologies, Santa Clara, CA) under a splitless injection mode. The GC was equipped with an HP-5MSI column at 30 m by 0.25 mm internal diameter and 0.25-μm film thickness. Helium used as a carrier gas flowed at 2.0 ml/min, with an inlet pressure of 120 kPa. The inlet and MS transfer line temperatures were set at 125 and 100°C, respectively. The oven temperature programmed for AITC separation was held at 60°C for 3 min, increased from 60 to 180°C at a rate of 9°C/min, from 180 to 280°C at a rate of 20°C/min, and finally was held at 280°C for 2 min, requiring a total run time of 32.3 min. The MS was operated in scan and selected ion monitoring modes for quantification of AITC. Mass spectrometry source temperature was maintained at 230°C, and the mass spectral scanning range was 20 to 450 atomic mass units. Ions of 41, 45, 72, and 99 m/z were monitored at retention time 4.0 min for AITC. The standard calibration curve was prepared with a commercial AITC solution (Sigma-Aldrich, St. Louis) as reference to determine the concentrations of AITC. Enhanced Chemstation software (version E.02.01; Agilent Technologies) was used to acquire and process chromatographic peak areas.

Data analysis. Data collected from the repetitions of each experiment were combined and subjected to analysis of variance (ANOVA) using SAS (version 9.2; SAS Institute Inc., Cary, NC). Bartlett's test of homogeneity of error variance was carried out prior

Table 1. Properties of soils from Texas, Arkansas, and Mississippi used in this study

Soil properties	Texas	Arkansas	Mississippi
Type classification	League clay	Dewitt silt loam	Sharkey clay
Sand (%)	3	18	3
Silt (%)	32	70	32
Clay (%)	64	12	65
pH	6.3	5.8	7.9
Conductivity (μmho/cm)	254	139	598
Nitrate-N (ppm)	0	27	40
Phosphorus (ppm)	10	36	49
Potassium (ppm)	168	115	368
Calcium (ppm)	3,953	827	6,745
Magnesium (ppm)	637	125	1356
Sulfur (ppm)	16	10	18
Sodium (ppm)	107	68	62
Total C (ppm)	10,350	7,238	12,158
Organic Matter (%)	1.82	1.2	2.24
Total N (ppm)	1,032	952	1,462
Ammonium-N (ppm)	12.9	6	2.3

Table 2. Analysis of variance for the effect of plant sample and soil type on the growth of *Rhizoctonia solani* mycelium

Source	DF	MS	F value ^a
Plant sample	10	27,655	297***
Soil type	3	1,343	14***
Plant sample × soil type	30	843	9***

^a Asterisks (***) = significant at *P* = 0.0001

to combining the data from repetitions of the experiment. Multiple comparisons of significant independent variables were conducted with Fisher's protected least significant difference test at $P = 0.05$. When interaction was significant, the effect of each factor was partitioned by running the respective ANOVA.

Results

Soil collection and preparation. Soils from Texas and Mississippi were clay soil type whereas the soil from Arkansas was silt loam with higher percentages of sand and silt (Table 1). Soil from

Mississippi had higher levels of pH, conductivity, organic matter, total C, total N, potassium, calcium, and magnesium than soil from Texas and Arkansas.

In vitro screening. Plant species, soil type, and their interaction were all highly significant ($P \leq 0.01$) in affecting the mycelial growth of *R. solani* (Table 2). Soil amendments with each of the 11 plants tested resulted in a significant degree of *R. solani* mycelia growth inhibition (Table 3; Fig. 1). All the six mustard (*B. juncea*) cultivars (Brand 199, Ruby Streak, Florida Broadleaf, Green Wave, Sheali Hong, and Red Giant) consistently inhibited mycelium growth

Table 3. Analysis of variance for effect of plant sample when mixed with Texas, Arkansas, or Mississippi soil or no soil on the growth of *Rhizoctonia solani* mycelium^a

Source	DF	Texas soil		Arkansas soil		Mississippi soil		No soil	
		MS	F value	MS	F value	MS	F value	MS	F value
Plant sample	10	6,689	180.46***	7,014	71.72***	6,954	58.43***	9,527	80.77***

^a Asterisks (***) = significant at $P = 0.0001$.

Fig. 1. Effect of plant samples on the inhibition of *Rhizoctonia solani* mycelium. **A**, Amendment to Texas soil. **B**, Amendment to Arkansas soil. **C**, Amendment to Mississippi soil. **D**, No soil used. Treatment abbreviations are as follows: BjB199 = *Brassica juncea* Brand 199, BjRuby = *B. juncea* Ruby Streak, BjFLBroad = *B. juncea* Florida Broadleaf, BjC.wave = *B. juncea* Green Wave, BjSheali = *B. juncea* Sheali Hong, BjR.Giant = *B. juncea* Red Giant, Br.napa = *B. rapa* Napa, Bo.Kale = *B. oleracea* Kale Starbor, Br.Turnip = *B. rapa* Southern Green, Cj.Sunnhemp = *Crotalaria juncea* sunn hemp, and Ev.Nemat = *Eruca sativa* Nemat. Bars with the same letter are not significantly different according to Fisher's protected least significant difference at $P = 0.05$.

of *R. solani* in three different soil types tested. Brand 199, Ruby Streak, Florida Broadleaf, and Green Wave (except a mixture with Mississippi soil) were the most effective, inhibiting >90% mycelial growth. In the non-brassicaceous species tested, arugula (*E. sativa*) Nemat achieved over 60% of *R. solani* mycelia inhibition when amended into the soils from Texas or Arkansas, which was significantly higher than kale (*B. oleracea*), turnip (*B. rapa*), and sunn hemp (*C. juncea*).

Soil type significantly affected the efficacy of plant species on inhibiting mycelial growth (Fig. 1). Except for Chinese Napa, kale, and sunn hemp, the degrees of mycelial growth suppression by the plant samples amended into the soils from Texas or Arkansas were greater than from Mississippi soil. There were no significant differences in the efficacy of mycelial growth inhibition between the soils from Texas and Arkansas.

Effect of brassicaceous plant amendment rate on mycelium growth. Plant amendment rate, soil use, and their interaction were significant ($P \leq 0.01$) in affecting the mycelial growth of *R. solani* (Table 4). Amendment rate of *B. juncea* Brand 199 accounted for 87% of the variation in *R. solani* mycelium inhibition. Mycelia

Table 4. Analysis of variance for the effect of soil medium and *Brassica juncea* application rate on the growth of *Rhizoctonia solani* mycelium

Source ^a	DF	MS	F value ^b
Soil medium	1	1,156.2	16.58***
Rate	5	9,095	130.42***
Medium × Rate	5	197	2.83**

^a Medium refers to whether *Brassica juncea* plant tissue was mixed with soil or no soil.

^b Asterisks: ** or *** = significant at $P = 0.01$ or 0.0001 , respectively.

Table 5. Analysis of variance for the effect of *Brassica juncea* application rate when mixed with soil or without soil on the growth of *Rhizoctonia solani* mycelium^a

Source	DF	Soil		No soil	
		MS	F value	MS	F value
Rate	5	3,148	45.35***	5,382	53.06***

^a Asterisks (***) = significant at $P = 0.0001$.

Fig. 2. Effect of *Brassica juncea* plant tissue amendment rate on *Rhizoctonia solani* mycelium growth when mixed with Texas soil or without soil.

inhibition increased with an increase in the amendment rate regardless of whether the plant tissue was amended with or without soil (Table 5; Fig. 2). Plant tissue without soil achieved higher levels of mycelium inhibition of *R. solani* than plant tissue amended with soil.

Effect of soil pasteurization and *B. juncea* Brand 199 amendment rate on AITC release. Soil pasteurization, *B. juncea* plant amendment rate, time of sampling, and their interactions were highly significant ($P \leq 0.01$) in affecting the amount of AITC emitted from plant tissue of *B. juncea* Brand 1999 (Table 6). Plant tissue amendment in natural (nonpasteurized) soil resulted in a significantly higher AITC release than plant tissue amendment in pasteurized soil (Table 7; Figs. 3 and 4). In natural soil, emission of AITC reached higher levels from the soil amended with 0.5 than 0.25% (wt/wt) *B. juncea* (Fig. 4). The soil amended with 0.5% (wt/wt) *B. juncea* released 96% more AITC than the soil amended with 0.25% (wt/wt) *B. juncea*. The highest AITC levels were recorded from the amendment treatment of 0.5% (wt/wt) *B. juncea* at 24 h (3.5 $\mu\text{g/ml}$) and 0.25% *B. juncea* at 12 h (0.215 $\mu\text{g/ml}$). In pasteurized soil, AITC amounts increased from 12 to 24 h and then leveled off from 36 to 72 h regardless of the amendment rate of *B. juncea* evaluated (Fig. 4).

Discussion

Effective mycelium inhibition by *B. juncea* plant species tested in this study can be attributed to high levels of glucosinolates in their plant tissue, as previously reported by Mazzola and Zhao (2010). Variation in efficacy of Brassicaceae plants for inhibiting *R. solani* mycelium growth in our in vitro assay is associated with glucosinolate content. Different *Brassica* spp. or cultivars have different concentrations and types of the glucosinolates (Kirkegaard and Sarwar 1998; Kirkegaard et al. 1996). *B. juncea* evaluated in this study outperformed other species in antifungal activity because they contained higher levels of allyl glucosinolate type, resulting in the release of higher concentrations of AITC upon hydrolysis (Mayton et al. 1996). The efficacy of mycelium growth inhibition by sunn hemp (*C. juncea*) and arugula (*E. sativa*) could be attributed to a different chemistry (Lazzeri et al. 2003; Sharma et al. 2003).

Soil property is another important factor that determines the efficacy of biofumigants because it directly affects hydrolysis of glucosinolates and subsequent release of AITC from brassicaceous plant tissue (Brown and Morra 1996; Tsao et al. 2000). Our in vitro study treatments involved mixing *B. juncea* plant tissue with soil, thus mimicking field conditions in which soil microbes are present.

Table 6. Analysis of variance for the effect of soil pasteurization treatment, *Brassica juncea* application rate, and sampling time emission of allyl isothiocyanates

Source	DF	MS	F value ^a
Soil pasteurization treatment	1	24.5	33.0***
Application rate	1	25.8	34.7***
Sampling time	4	10.9	3.6**
Soil × rate	1	21	28.2***
Soil × time	4	8.7	2.9**
Rate × time	4	11.4	3.8**
Soil × rate × time	4	10.1	3.3**

^a Asterisks: ** or *** = significant at $P = 0.01$ or 0.0001 , respectively.

Table 7. Analysis of variance for the effect of *Brassica juncea* application rate and sampling time emission of allyl isothiocyanates in natural or pasteurized soil^a

Source	DF	Natural soil		Pasteurized soil	
		MS	F value	MS	F value
Application rate	1	46.82	31.56***	0.12	24.09***
Sampling time	4	4.89	3.3*	0.02	3.98*
Rate × time	4	5.36	3.62**	0.009	0.12*

^a Asterisks: *, **, or *** = significant at $P < 0.05$, $P = 0.01$, or $P = 0.0001$, respectively.

Mycelial growth inhibition resulting from different *B. juncea* amendments seem to be consistent in the soils from Texas and Arkansas in contrast to Mississippi. These differences might be associated with the differences in soil properties such as pH, organic matter, and total N (Borek et al. 1995; Dungan et al. 2003; Gimsing et al. 2009).

Most soil properties, except soil texture, were similar in the soils from Texas and Arkansas and were dissimilar from Mississippi soil. These findings indicate that soil texture does not affect the efficacy of *B. juncea* amendment but the other soil properties contribute to biofumigation efficacy. Soil types did not affect the degradation of glucosinolate breakdown products; however, stability was affected by pH (Hanschen et al. 2012). Gimsing et al. (2009) reports that there was no difference in degradation of glucosinolate hydrolysis products in two soils (pH values 6.9 and 7.2) but degradation significantly increased in soils when pH changed (pH 6.1 and 7.6). More detailed research is needed to understand the impacts of pH, organic matter, total N, and other soil properties on the release of AITC and microbial activities.

Biofumigation is dose dependent. As the amount of *B. juncea* plant tissue amended into soil increases, AITC emission increases and, consequently, antifungal activity becomes stronger. Disease suppression is achieved in response to chemistry-based modes of

action, depending upon the efficiency at which glucosinolate is converted into isothiocyanate (Mazzola and Zhao 2010). Our in vitro assay shows a relationship among antifungal activity, AITC emission, and the amount of glucosinolate in *B. juncea* plant tissue. This supports a major role of glucosinolates in the biofumigation process, indicating that total glucosinolate contents in biofumigants are a primary factor in in vitro screening and biofumigation efficacy.

Release of AITC appeared to be very rapid within 12 to 24 h after treatment and then decreased to undetectable levels after 72 h regardless of the amount of *B. juncea* tissue used. In a previous relevant study by (Mazzola and Zhao 2010), AITC emission from *B. juncea*-amended soil was completed within 72 h postamendment, even at an application rate to soil up to 1.0% (wt/wt). Morra and Kirkegaard (2002) reported that maximum AITC release in the field occurred at 2 h after *Brassica* tissue incorporation.

The lower AITC emission observed in the pasteurized soils compared with the natural soils is attributed to a reduction of microbial or enzymatic activities by soil pasteurization. Autoclaving not only inactivates microorganisms that might be able to degrade glucosinolates but also degrades myrosinase in the soil (Hanschen et al. 2015). Previous studies reported the importance of the enzyme myrosinase in the biofumigation process. Soil sterilization resulted in a significant reduction of myrosinase activity in the soil (Gimsing, et al. 2006). The rapid dissipation of glucosinolates in brassicaceous plant materials amended in the natural soil is attributed mainly to myrosinase activity (Omirou et al. 2013). Glucosinolate hydrolysis products in soils amended with *Brassica* plant tissue declined rapidly due to volatilization of AITC, hydrolysis products binding to soil matrix, or degradation by soil microbiota (Hanschen et al. 2015).

The reduction of AITC emission in pasteurized soils observed in this study suggests that activities of soil microbes play an important role in glucosinolate hydrolysis. Degradation of pure AITC glucosinolate in autoclaved soil revealed that nonenzymatic degradation of glucosinolates occurred but was less than in native soil (Hanschen et al. 2015).

Both biotic (soil microbes) and abiotic (soil properties) factors need to be considered for screening for antifungal efficacy of brassicaceous biofumigants and implementing biofumigation practices to manage soilborne disease in the field. This study demonstrates antifungal property of *B. juncea* plants, especially Brand 199, as a biofumigant candidate to manage rice sheath blight caused by *R. solani* AG1-1A. Incorporation of *B. juncea* plant tissue may also improve soil microbial activities and soil abiotic properties suppressive to sheath blight.

Fig. 3. Cumulative allyl isothiocyanates emitted from *Brassica juncea* plant tissue amended in natural and pasteurized soils.

Fig. 4. Time-course emission of allyl isothiocyanate from *Brassica juncea* plant tissue amended in natural and pasteurized soils. *B. juncea* amendment at A, 0.25% (wt/wt) and B, 0.5% (wt/wt).

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