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Full Length Article

The Weeds Control of a Novel Bioorganic Fertilizer and its Effects on Agronomic Traits of Rice

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Abstract

Bio-organic fertilizer (BIO) is usually used as one of effective solutions to control weeds and reduce environmental pollution in agricultural ecosystems. A novel BIO for weed control and nutrient supply was manufactured and field trials at three rice fields were conducted for two years to evaluate its effect. The novel BIO was found to be effective in controlling grass and broad-leaved weeds in rice fields, with an average rate of more than 80% weed suppression. In addition, the BIO treatments significantly increased rice yield (16.3–29.8% relative to control) and yield components (e.g., number of spikes per square meter, plant height, number of kernels per spike). Results from these field experiments have implications for farmers and government agencies in regard for accept ability of bio-organic fertilizer for biological weed control in rice cultivation. © 2018 Friends Science Publishers

Keywords: Bioorganic fertilizer; Agricultural practices; Biological control; Weed

Introduction

During plant growth, weeds reduce crop yield through competition for moisture, nutrients, sunlight, and space, and negatively affect economic return for farmers. Crop yield reduction due to weeds varies from 5 to 50% (Ali *et al.*, 2013). Chemical herbicides are effective and have been widely adopted to control weeds. However, chemical herbicides affect agricultural biodiversity (Liu *et al.*, 2015). For example, pesticides have resulted in a loss of 42% of stream invertebrates in Europe and Australia (Beketov *et al.*, 2013). Furthermore, repeated use and overuse of pesticides can lead to weed resistance to chemical herbicides (Yu and Powles, 2014). There are currently 479 unique cases (species × site of action) of herbicide resistant weeds globally, numbering 252 species (147 dicots and 105 monocots) (http://weedscience.org/default.aspx/2017). Thus, to mitigate these problems from chemical pesticides, biological control seems as a promising long-term solution for weed control. At present, the biological control of weeds has been attempted through insects (Room *et al.*, 1981; Louda *et al.*, 1998; Cristofaro *et al.*, 2013; Hahn *et al.*, 2016), plant pathogens (Charudattan, 2001; Mishra *et al.*, 2013; Tehranchian *et al.*, 2014) and allelopathic products (Putnam and Duke, 1974; Lin *et al.*, 2004; Iqbal *et al.*, 2009; Mushtaq *et al*., 2010; Qasem, 2012; Kato-Noguchi *et al.*, 2013).

In addition, bio-organic fertilizer is considered to be a advanced biotechnology useful for developing and promoting organic agriculture and sustainable agriculture. Use of bio-fertilizers for biological control is to be a desirable technique for disease and pest control (Wei *et al.*, 2015). Two antagonistic fungi, *Penicillium* sp. and *Aspergillus* sp., were used as inocula to fortify organic fertilizer. Pot and field experiments showed that the two antagonistic fungi minimized the incidence of *Fusarium* wilt disease, maximized biomass production, and altered microbial community structure (Zhao *et al.*, 2011). Organic fertilizers supplemented with $CaCO_3$ increased soil pH and Ca^{2+} content in tobacco and decreased the *R. solanacearum* population by nearly 100 times (He *et al.*, 2014). This suggests that $CaCO_3$ could serve as a potential soil amendment for the control of bacterial wilt caused by *R. solanacearum*. The bio-organic fertilizer, inoculated *Bacillus amylolique- faciens* and *B. subtilis*, was effective in controlling *Fusarium* wilt disease in pepper (Wu *et al.*, 2015). A novel bio-organic fertilizer (BIO2) integrated the biocontrol agent *Bacillus subtilis* N11, and the application of the BIO2 significantly decreased the incidence rate of *Fusarium* wilt compared to the control (Zhang *et al.*, 2011).

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The application of biofertilizer in weed control is rarely reported (Sary *et al.,* 2009). Most ongoing research on biological weed control focuses on characteristics of the specific blems, potential control agents and only on small experimental scales. Thus, this study aimed to evaluate the effect of the novel bio-organic fertilizer on weed control and rice agronomic traits. Date from these large fields area were used originally in reports and suggested to farmers and government concerning the acceptability of bio-organic fertilizer for biological control of weeds in China rice fields.

Materials and Methods

Bio-organic Fertilizer (BIO) Manufacturing

The organic substrates in the BIO were composed of kitchen garbage, maize straw, wood-destroying, fungi dregs, rice straw, tobacco straw, plant ash, chicken and sheep manure (Table 1). The physical and chemical properties of the compost material measured are listed in Table 1. The combined process of ZF-5.5 fertilizer making and pile fermentation were used to produce composting manure at a temperature range of 40–80°C for 15 days. Man-made heating and cooling was used to control temperature on the first day.

After initiation of 8 h, 10 mL kg^{-1} of a suspension of 10^{10} CFU *Bacillus subtilis* L5 was added to the compost. After 24 h, the compost was moved out and piled fermentation began. After 15 days, the compost turned taupe gray, exhibited threadiness and had a slightly sour fragrance. The finished bio-organic fertilizer contained L5 more than 1×10^9 CFU g^{-1} DW of L5.This compost contained 53.4% organic matter, 2.0% N, 3.7% P_2O_5 and 1.1% K_2O.

Field Sites

The experiments were conducted from January 1, 2014 to October 30, 2015 at three main arable area of China: Xiangying, Yueyang, Hunan province (113°55′32″N, 28°39′55″E); Yachen, Haikou, Hainan province (109°10′42″N, 18°21′45″E); and Lanleng, Haerbing, Heilongjiang province (126°12′5″N, 45°13′18″E). The fields have been cultivated with till farming practices for decades. The soils are a loam at Xiangying, an arenosol at Yachen and a black soil at Lanleng. Mean annual precipitation and temperature at the planting season in the last three years were 1392.62 mm and 17°C at Xiangying, 1347.5 mm and 21.4°C at Yachen, 481 mm and 4.4°C at Lanleng. The rice varieties were Yuzhengxian at Xiangying, Long-xianyou 130 at Yachen and Wuyoudao4 at Lanleng.

Bioorganic Fertilizer Field Trials

Experimental concentration gradient: BIO treatments included four rates, 750 kg/ha (BIO-50), 1500 kg/ha (BIO-100), 2250 kg/ha (BIO-150) and 3000 kg/ha (BIO-200). Each concentration, handing weed (HW) and an untreated control (CK) were each replicated four times. No fertilizer and herbicides application in handing weed and an untreated control were done.

Experimental Plot Arrangement

The experimental fields were tilled by tractors to build rows. Each site included 32 plots (5 m×6 m). The height and width of the plot ridges were about 15–30 cm and about 15 cm, respectively. Around 750 rice plants were planted in each plot, with 20 cm interplant spacing in rows. Gutterways (50 cm in width) were built between two plots. The same experiment was replicated in both 2014 and 2015.

Application Time

After two days of rice transplanting, the BIO was evenly sprinkled by hand. Water layer was hold about seven days after BIO application. Chemical herbicides were not used in experimental fields. Field management was organized according to local the traditional methods.

Field survey

The changes of BIO in fields were photographed day by day for 7 days after nursery transplanting. The changes in soil electrical conductivity (EC, Spectrum EC 110, USA) were consistently measured day by day for15 days after BIO application. Weeds in fields were investigated at 20 and 40 days after BIO application. Five points (1 m^2) were chosen randomly in every plot. The species, number of grass and broad-leaf weeds were record separately. The aboveground fresh biomass of weeds was measured 40 days after BIO application. Plant height, spike length (SL), number of spikes per square meter (SPm^2), thousand kernel weights (TKW), numbers of kernels per spike and yield were measured in each plot after harvesting. For plant height, five plants were randomly selected in each plot and measured from the ground to the tip of the longest leaf at maturation stage of rice. For spike length, spikes were randomly selected and measured from the bottom to the tip. SP m^2, TKW and KPS were measured randomly five times from each plot. For yield, plant grains were weighed at five 1 m^2 acreage points per plot (calculated by standard water percentage 13%). Total yield was estimated at 20 times the average of five points.

Data Analyses

Control effect (%)=(CK-PT)/CK×100 CK: Blank control plots weeds number or fresh weight, PT: BIO and handing plots weeds plants number or fresh weight.

All data were analyzed using SPSS version 17.0 and variance (ANOVA). Least significant differences (LSD at 5%) were used to compare the treatments means.

Table 1: Physical and chemical properties of the compost material

Compost Material	Organic Carbon (%)	Total Nitrogen (%)	C/N	Total phosphorus (%)	Potassium (K) (%)
Kitchen garbage	45.07±0.78	2.89±0.55	15.67	0.46	1.83±0.83
Maize straw	53.87±0.07	0.74±0.19	72.48	0	3.03±1.35
Wood-destroying fungi dregs	51.62±0.14	0.91±0.01	56.7	0.85	0.85±0.63
Rice straw	49.56±0.01	1.74±0.30	28.52	0	2.94±1.62
Tobacco straw	50.73±0.16	1.55±0.22	32.73	0	2.85±1.04
Plant ash	14.29±0.13	0.41±0.32	34.85	1.11	5.76±1.39
Chicken manure	39.15±0.12	3.63±0.27	10.78	3.88	3.01±0.94
Sheep manure	48.14±0.24	2.59±0.41	18.59	0.40	1.68±1.01

Table 2: Weed number control effects in 20 days after different BIO application gradients at Hunan

Treatment	*Echinochloa crus-galli*				*Monochoria vaginalis*				*Total weeds*			
	2014		2015		2014		2015		2014		2015	
	N p/m^2	EF %	N p/m^2	EF %	N p/m^2	EF %	N p/m^2	EF %	N p/m^2	EF %	N p/m^2	EF %
BIO-50	19.75	75.3c	21.5	73.8c	9.75	78.9b	12	77.3b	41.75	75.3c	42.25	75.0c
BIO-100	16	80.0b	15.75	80.6b	8.75	81.2b	10.5	80.5b	33.25	80.3b	33	80.7b
BIO-150	10.5	86.8a	10.75	86.8a	5.75	87.0a	6.75	87.5a	21.75	87.1a	22.25	86.9a
BIO-200	8.25	90.0a	8	90.2a	4.25	90.9a	5.25	90.5a	16.5	90.3a	16.5	90.4a
HW	25	69.4d	28	66.0d	14.5	68.6c	17.75	66.2c	54.5	67.9d	59.5	65.2d
CK	81.25		82.5		46.75		53.5		169.25		171	

N: number (plants/m^2); EF: control effect (%). Data analysis is based on the average of four repetitions. Means with the same letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$)

Results

Field Survey

The bio-organic fertilizer began to decay as soon as it was applied. After 3 days, decay layers formed on the surface of the water, which was papescent, dark and was 3–4 cm thick (Fig. 1). The decay layers existed about 40 days after irrigation.

Soil EC exhibited a great change after BIO use. In Hainan (Fig. 2), for example, as the BIO degraded, soil EC began to rise. After two days, the EC reached its maximum. The BIO200, BIO150, BIO100 and BIO50 EC average reached 3008, 1502, 1389, 1331 us/cm respectively, far beyond the blank control EC (591 us/cm). Then, after three days the EC began to decrease and about 15 days later, it was stable. The BIO200, BIO150, BIO100 and BIO50 had an EC with averages of 1363, 1041, 966, 903 us/cm, respectively, still beyond the blank control EC (638 us/cm).

Effects on Weeds Control

The most common weeds in the treatment plots were grassy (*Echinochloa crus-galli*, *Cyperus iria*, *Leptochloa chinensis*, *Eleocharis yokoscensis*, *Scirpus planiculmis*) and broad-leaved weeds (*Lindernia procumbens*, *Ammannia baccifera*, *Monochoria vaginalis*, *Potamogeton distinctus*, *Sagittaria pygmaea*).

In 2014 and 2015, application of the bio-organic fertilizer had a positive control effect on grassy and broad-leaved weeds (Fig. 3). Twenty (20) days after fertilizing, the plant number control effect was 78% under treatment BIO-50 (750 kg/ha BIO). Under treatments BIO-100 (1500 kg/ha BIO), BIO-150 (2250 kg/ha BIO) and BIO-200 (3000 kg/ha BIO), the plant number control effect were all above 80%. With incremental BIO dosage, the weed plant number control effect increased, but did not result in a significant difference (Table 2). After 40 days, the plant number control effect was about 75% by the treatment BIO-50, and above 80% for treatments BIO-100, BIO-150 and BIO-200 (Table 3). After 40 days, the fresh weight control effect was 76% for treatment BIO-50, and above 80% for the treatments BIO-100, BIO-150 and BIO-200. With incremental BIO dosage, the fresh weight control effect increased, but not significantly (Table 4). The other sites had similar results with regard to the weed control effect. BIO had a positive effect in controlling grassy and broad-leaved weeds in rice fields. For the 1500 kg/ha and above treatment, more than 80% of weeds were eliminated at 20 and 40 days after BIO application.

Effects on Agronomic Traits

In 2014, for the number of spikes per square meter, BIO-50 treatments in Hunan, Heilongjiang and Hainan had statistically significant results beyond the blank control, roughly 32.5, 41.1 and 17.2%, respectively (Table 5). BIO-100 treatment in Hunan, Heilongjiang and Hainan had significant beyond the blank control at 35.8, 41.9 and 19.9%, respectively; BIO-150 treatment in Hunan, Heilongjiang and Hainan had significance beyond blank control at about 38.1, 46.4 and 22.4%, respectively; BIO-200 treatment in Hunan, Heilongjiang and Hainan had resulted in significance beyond the blank control at roughly 39.3, 49.3 and 23.5%, respectively. For plant height, BIO-50 treatments in Hunan, Heilongjiang and Hainan had

Fig. 1: Decay layers formed 3 days after application of BIO on rice field in Hainan

a: Feb.27, 2014 (First day); b: Feb.28,2014 (Second day): c: Mar.1,2014 (Third day)

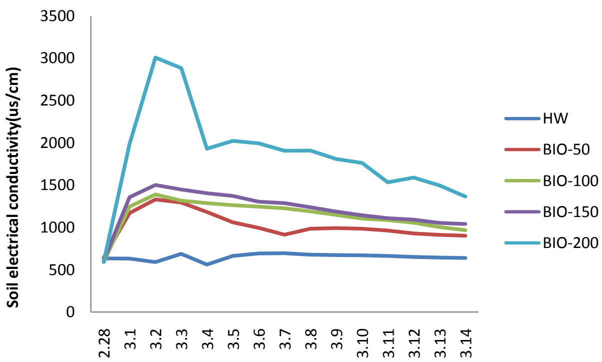

Fig. 2: The change of soil EC after used BIO 15 days

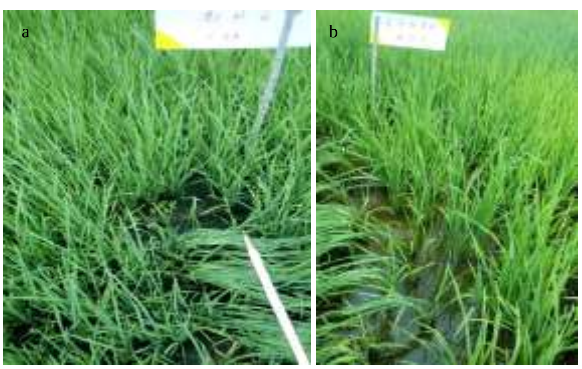

Fig. 3: Effect of weed control by BIO after 40 days in Hunan

a. untreated control (CK); b. rate 1500 kg/ha (BIO-100)

significant effect beyond to blank control at about 27.4, 26.9 and 13.7%, respectively; BIO-100 treatments in Hunan, Heilongjiang and Hainan were significant beyond the blank control at about 41, 26.6 and 21.7%, respectively; BIO-150 treatments in Hunan, Heilongjiang and Hainan were significant beyond the blank control at about 45, 27.2 and 27.4%, respectively; BIO-200 treatments in Hunan, Heilongjiang and Hainan were significant beyond the blank control at about 57.3, 28.7 and 30.8%, respectively. For number of KPS, BIO-50 treatments in Hunan, Heilongjiang and Hainan were significant beyond the blank control at about 17, 16.8 and 84.3%, respectively; BIO-100 treatments in Hunan, Heilongjiang and Hainan were significant beyond the blank control at roughly 20.4, 26.9 and 89.2%, respectively; BIO-150 treatments in Hunan, Heilongjiang and Hainan had significant beyond blank control at about 23.7, 39.9 and 100.7%, respectively; BIO-200 treatments in Hunan, Heilongjiang and Hainan were significant beyond blank control at about 28.3, 44.7 and 110%, respectively.

For yield, BIO-50 treatments in Hunan, Heilongjiang and Hainan were significant beyond blank control at about 24.5, 100 and 13.3%; BIO-100 treatments in Hunan, Heilongjiang and Hainan were significant beyond blank control at about 31.7, 115.3 and 15.8%, respectively; BIO-150 treatments in Hunan, Heilongjiang and Hainan were significant beyond blank control about 37.9, 138.3 and 18.6%; BIO-200 treatments in Hunan, Heilongjiang and Hainan were significant beyond blank control about 42.5, 150.7 and 20.2%, respectively. Plant heights, numbers of SPm^2, numbers of KPS and PGY also increase when BIO treatments were compared the blank control. However, with incremental BIO dosage, agronomic traits increased, but not significantly.

During 2015, plant heights, SL, numbers of spikes per square meter, TKW, KPS and yield also showed significant increases when compared to the blank control (Table 6). However, with incremental BIO dosage, these agronomic attributes increased, but not significantly. Above results showed that yield and yield components of rice also had significant differences between BIO treatments plots and the blank control plots.

Discussion

The bio-organic fertilizer began to decay as it was fertilized. After 3 days, decay layers formed on the surface of the water, which was papescent, dark and 3–4 cm thick. Sunlight cannot penetrate through the decay layer. Weed seed did not germinate, because of lack of sunlight (Wang *et al.*, 2016). Soil EC was above 1000 µs/cm in soil at a depth of 3–4 cm two days after the application of BIO. An increase in EC had a negative effect on plant growth (Griffin and Hollis, 2013; Mamat *et al.*, 2016). When the papescent decay layer formed, transplanting rice seedlings of roots growing down firmly in the soil beyond 5 cm depths, and did not damage by the application of BIO. The germination weeds of roots rotted in the decay layer soil. However, the weeding principle is not completely understood. One or two reasons may be find in later work, such as allelopathic matter or secondary metabolite.

Many weed management techniques have been directed at total weed eradication, however, those are not realistic possibilities in most arable fields, pastures and rangelands, and only a few of them have reached the market as commercial products (Liebman *et al.*, 2001). Many limitations to bio-herbicides have been suggested, with low pathogen virulence and fastidious environmental conditions identified as the key restraints to overcome (Ghorbani *et al.*, 2005). Regarding this control technology, its unique

Table 3: Weed number control effects in 40 days after different BIO application gradients at Hunan

Treatment	*Echinochloa crus-galli*				*Monochoria vaginalis*				*Total weeds*			
	2014		*2015*		*2014*		*2015*		*2014*		*2015*	
	N p/m²	*EF %*	*N p/m²*	*EF %*	*N p/m²*	*EF %*	*N p/m²*	*EF %*	*N p/m²*	*EF %*	*N p/m²*	*EF %*
BIO-50	21	74.1b	21.25	75.0b	11	75.8c	12.5	74.3c	44.5	73.8c	43.25	75.1c
BIO-100	16	80.2b	16	81.4b	9.25	79.8b	9.5	79.7b	34	80.0b	32.5	81.1b
BIO-150	11	86.4a	11.25	86.9a	6.25	86.1a	7.25	84.7a	24	85.9a	24.25	86.0a
BIO-200	9	89.2a	8.25	90.3a	4.25	90.6a	5	89.4a	17.25	89.9a	17	90.1a
HW	26	68.6c	27.75	67.4c	16.25	63.4d	17.25	63.2d	57.75	66.0d	59	65.7d
CK	82.5		86		45.5		47.25		169.75		173	

N: number (plants/m^2); EF: control effect (%). Data analysis is based on the average of four repetitions. Means with the same letter are not significantly different according to Fisher's protected LSD test (α= 0.05)

Table 4: Weed fresh weight control effects in 40 days after different BIO application gradients at Hunan

Treatment	*Echinochloa crus-galli*				*Monochoria vaginalis*				*Total weeds*			
	2014		*2015*		*2014*		*2015*		*2014*		*2015*	
	WT g	*EF %*	*WT g*	*EF %*	*WT g*	*EF %*	*WT G*	*EF %*	*WT g*	*EF %*	*WT g*	*EF %*
BIO-50	68.84	74.3 c	79.65	74.9d	28.91	75.7c	30.41	75.9d	125.36	74.1c	142.18	75.4d
BIO-100	52.99	80.0b	60.67	81.2c	23.90	80.2b	24.67	80.0c	94.24	80.6b	107.81	81.1c
BIO-150	35.56	86.69a	43.18	86.5b	16.53	85.9a	19.4	84.3b	66.32	86.3a	81.62	85.8b
BIO-200	26.76	90.31a	31.47	90.1a	10.60	91.0a	12.54	89.9a	46.12	90.6a	56.27	90.2a
HW	84.03	69.48d	106.37	66.5e	43.90	62.0d	46.12	62.6e	162.61	66.6d	198.51	65.3e
CK	272.35		321.01		119.84		124.27		485.38		575.32	

WT: Weed freash weight (g); EF: control effect (%). Data analysis is based on the average of four repetitions. Means with the same letter are not significantly different according to Fisher's protected LSD test (α= 0.05)

Table 5: Effect of weed control treatments on yield and agronomic traits of rice 2014

	Number of spikes per square meter			Plant Height (cm)			Number of Kernels per spike			Yield (kg/ha)		
	Hunan	Heilongjiang	Hainan	Hunan	Heilongjiang	Hainan	Hunan	Heilongjiang	Hainan	Hunan	Heilongjiang	Hainan
BIO /50	229.99b	213.94b	254.87b	102.66c	74.53a	110.09c	144.42c	94.78c	110.25b	5575.65c	4458.3b	5970.15b
BIO /100	235.53a	215.29ab	260.72a	113.57b	74.36a	117.86b	148.6bc	102.95b	113.18b	5894.7b	4791.15b	6098.85b
BIO/150	239.58a	222.19a	266.51a	116.81b	74.68a	123.38a	152.7ab	113.52a	120.05a	6175.05ab	5302.35a	6250.35a
BIO/200	241.68a	226.54a	268.52a	126.71a	75.57a	126.64a	158.35a	117.41a	125.64a	6377.7a	5578.05a	6333.45a
Handing	222.04c	170.01c	231.48c	99.03c	63.55b	105.43d	129.82d	84.68d	66.04c	4656.45d	2474.1c	5580.6c
CK	173.46d	151.72d	217.39d	80.55d	58.72c	96.82e	123.4e	81.12e	59.82d	4476.75e	2225.4d	5268.3d

Means with the same letter are not significantly different according to Fisher's protected LSD test (α= 0.05)

Table 6: Effect of weed control treatments on yield and agronomic traits of rice 2015

	Number of spikes per square meter			Plant height (cm)			Number of kernels per spike			Yield (kg/ha)		
	Hunan	Heilongjiang	Hainan	Hunan	Heilongjiang	Hainan	Hunan	Heilongjiang	Hainan	Hunan	Heilongjiang	Hainan
BIO /50	230.28b	215.74a	258.62a	106.03b	74.5a	111.35c	142.85c	93.9c	108.03c	5350.65c	4148.1c	5781.15c
BIO /100	234.93ab	217.54a	261.32a	110.42b	74.51a	118.12b	145.25bc	103.2b	112.14c	5780.4b	4968.9b	6002.55b
BIO/150	238.68a	222.64a	265.97a	117.67a	74.29a	122.78ab	150.43ab	114.65a	119.09b	6124.65a	5289.3a	6214.8a
BIO/200	243.18a	229.69a	268.07a	123.6a	75.63a	127.64a	157.65a	119.96a	127.58a	6293.25a	5635.05a	6307.2a
Handing	221.14c	168.97b	240.03b	97c	61.73b	104.64d	135.74d	85.03d	67.53d	4695.15d	2532.3d	5369.1d
CK	185.16d	152.77c	220.99c	82.28d	58.52c	95.42e	126.25e	81.47d	60.22e	4428.6e	2282.55e	4969.2e

Means with the same letter are not significantly different according to Fisher's protected LSD test (α= 0.05)

characteristic is that bio-organic fertilizer can be applied directly to fields. Using this approach, many organisms are introduced into an environment in much the same way the herbicides are applied. Most of these organisms with pathogen virulence were wiped out by high temperature fermentation. Those organisms meet the demands of the bio-herbicide (inundative) biological control organisms, such as being safe, providing easy cultivation on a large scale, being easy to produce, store and highly virulent against the target (Charudatan *et al.*, 1985). Those organisms meet the demands may be one of reason for that BIO can be applied directly to fields. From conventional viewpoints, biological weed control methods are more dependent on specific environmental conditions than on chemical methods (Charudattan, 2001). Under this new way, other environmental conditions were not as except continuous watering seven days after fertilizing in the whole rice growth stage. The novel bio-organic fertilizer may have two above characteristics; therefore, it could be used to control weeds in rice fields.

However, this novel bio-organic fertilizer also has some disadvantages. Firstly, application time should be very accurate. In 2012–2013, our exploratory research showed that when the timing of the application was too early or late, the effect of weed control obviously decreased (date not shown). Secondly, the BIO may not suit for cultivation by direct seeding. From 2012 to 2015, we investigated the weed control effect after application of BIO in direct seeding rice. We found that the effect of weed control is about 30–35% in direct seeding rice field (date not shown). In the early stage, water layer should hold about seven days after BIO application in order to degrade the BIO organisms, but the rice seed cannot germinate after long time water soaking in the direct-planting rice field. This conflict may be the reason BIO has not failed in the direct-planting rice field.

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