

## Allelopathic Activity of Itchgrass (*Rottboellia cochinchinensis*) and Its Phytotoxicity in Soil

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### Abstract

Farmers in Chaehom-Lampang area use itchgrass [*Rottboellia cochinchinensis* (Lour.) W.D. Clayton] as a mulching material to control the other weeds. The phytotoxicity of soil incorporated with itchgrass powder was investigated under an environment duplicating field conditions. The results showed that soil incorporating itchgrass powder had phytotoxic effects on the growth of *Bidens pilosa*, *Echinochloa crus-galli* and *Lactuca sativa*, the effect was intensified with increased concentrations of itchgrass powder. Allelopathic activity of itchgrass in soil exhibited a maximum effect at 0-15 days after incorporation and then decreased over time. Additionally, the allelopathic activity of itchgrass was lower in submerged condition than half-saturated conditions and in dry conditions allelopathic activity was the highest. These results suggested that the allelopathic activity is also influenced by timing and soil moisture condition. A better understanding of the allelopathic activity of itchgrass in soil will provide information crucial in enabling improvements in the basis of weed management.

**Keywords:** allelopathy, allelopathic activity, itchgrass, phytotoxic effects, soil moisture condition

### Introduction

Allelopathy, the metabolites are released into the environment by means of ecological processes by volatilization, leaching, decomposition of plant residues in soil, and root exudation (Rice, 1984; Jelenic, 1987). Many studies have suggested that most allelopathic activity occurs in soil. In Inderjit et al. (2001) study on allelopathic activity, the application of plant residues in the soil was closer to field conditions than the application of aqueous extracts. According to Kobayashi (2004) the phytotoxic effects of allelochemicals are affected by soil factors, usually leading to a greater decrease in their activity compared to non-soil conditions. Soil properties are the dominant factor determining the allelopathic activity of allelochemicals in soil (Tongma et al., 2001; Inderjit, 2002). Several

studies reported that, soil factors may increase or decrease allelopathic activity, such as the phytotoxic activity of dehydromatricaria ester released from *Solidago altissima* depends on its concentration in soil water, which is affected by soil characteristics through its adsorption and degradation activity in the soil (Ito et al., 1998; Kobayashi et al., 2004). Furthermore, the phenolic compound content in the soil released from *Parthenium* possible interactions with soil chemical properties reduces plant growth, either directly or indirectly by interfering with the soil chemical properties and nutrient availability (Batish et al., 2002; Tet-Vun and Ismail, 2006). Therefore, the factors affecting the phytotoxic effect of allelochemicals in soil are made more complex by soil factors, such as the properties and conditions of the soil.

The farmers in the Chaehom-Lampang area, in northern Thailand, have been cultivating itchgrass [*Rottboellia cochinchinensis* (Lour.) W.D. Clayton] and using it as a mulching material to control weeds in vegetable fields. Itchgrass seeds are sown, grown, and then spread as mulch before vegetable cultivation. Interestingly, the density of weed species was remarkably reduced in the fields that had been mulched with itchgrass. There have been reports that the allelopathy potential of itchgrass was observed in bioassays using filter paper applied with aqueous extracts on test plants (Casini et al., 1998; Meksawat and Pornprom, 2010). In addition, the report by Kobayashi et al. (2008) asserts that in research, under controlled conditions, soil incorporated with itchgrass shoot or root powder was found to inhibit the growth of radish seedlings and the allelopathic activity in the soil incorporated with the itchgrass powder decreased over time. However, there is limited information about the allelopathic activity of itchgrass in soil. A better understanding of the allelopathic activity of itchgrass in soil will provide a basis for improving weed control in vegetable fields. Therefore, the objectives of the present study were: 1) to investigate the phytotoxic effects of itchgrass in soil on the growth of test plant seedlings; 2) to determine the residual allelopathic activity of itchgrass in soil; and 3) to investigate the effects of soil moisture conditions on the allelopathic activity of itchgrass in soil.

## Materials and Methods

### Soil Collection

Phytotoxic effects of itchgrass in soil was investigated using soil samples from the farmer's field at Chaehom-Lampang, Northern Thailand (CH-LP soil; sand=43.63%, silt=35.00%, clay=21.38%, field capacity=37.57%, and permanent wilting point=9.52%) and from the experiment field at the Department of Agronomy, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom (KPS-NP soil; sand=52.64%, silt=6.94%, clay=40.42%, field capacity=21.45%, and permanent wilting point=7.47%). The CH-LP soil were collected from infested and none infested itchgrass plant areas, and the KPS-NP soil was not infested by itchgrass plant and herbicide free prior to collection. The soils, at a

depth ~ 10 cm from the surface, were air-dried and passed through a 2 mm mesh sieve, then collected in plastic bag and stored at room temperature until used.

### Plant Materials

Itchgrass powder was derived from itchgrass plants, grown in a uniform environmental and agronomical conditions at the farmer's field in the CH-LP area. They were harvested at maturation stage then separated as shoot and root parts. These plant portions were individually cut into 1-2 cm sections, dried at 40°C for one week, and ground into a powder with an electrical grinder to pass through a 0.5 mm screen mesh. The itchgrass powder was stored in plastic bottles at -30°C until required.

### Effects of Soil Infested by Itchgrass on Test Plants Seedling Growth

Phytotoxic effects of itchgrass powder were investigated by means of the allelochemicals released into the soil. The experiment design was laid out as a completely randomized design (CRD) with four replications. The soil samples and itchgrass powder were prepared as described above before used. The experiment was a modified methodology reported by Kobayashi et al. (2008), the shoot and root powder of itchgrass was incorporated with the KPS-NP soil at the following itchgrass powder to soil sample ratios 0.1 g of itchgrass powder per 1 g of soil to replicate the CH-LP soil that was infested by itchgrass plant.

Bioassay tests were performed according to the method of Meksawat and Pornprom (2010) with some modification. Twenty five germinated seeds (the radical ~ 2 mm) of *Bidens pilosa*, *Echinochloa crus-galli* and *Lactuca sativa* were planted on top of fifteen grams soil samples in the petri dishes, distilled-water was added to reach the maximum water holding capacity (MWHC) of the soil (Tongma et al., 2001) and the samples were placed in the incubator at 25°C for five days then a bioassay test was performed. The growth measurement of the three test plant seedling species was determined as the shoot and root lengths at 5 days after incubated.

All the data were subjected to analysis of variance (ANOVA) including the calculation of the means. Treatment means were compared with least

significant difference test (LSD) and indicated a significant difference between control and treatment. The statistical analyses were evaluated with the application of the R-program.

#### **Residual of Allelopathic Activity of Soil Incorporated with Itchgrass Powder**

The residual phytotoxic effect of soil incorporated with itchgrass powder was determined using the bioassay test and soil property analysis. Treatments were arranged as a split-split plot in CRD with four replications. The main plots were the periods; i.e. 0, 15 and 30 days after incorporated (DAI), the sub-plots were the distinct parts of itchgrass powder; i.e. shoot and root powders, and the sub-sub plots were concentrations of itchgrass powder; i.e. 0, 0.01 and 0.1 g g<sup>-1</sup> (soil). The soil incorporated with itchgrass powder was moistened one time per week with distilled-water to MWHC as half-saturated soil condition and incubated at 25 °C for 0, 15 and 30 DAI before use. The germinated three species of test plant seedling having the radical ~ 2 mm were measured in the bioassay test described above.

Measurement of the electrical conductivity (ECe) and pH values of the soil samples were taken using a pH/conductivity meter (CyberScan PC 10) for all samples, adding 35 mL distilled water to 7 g of soil samples for each treatment, shaking for 15 min and keeping at room temperature for 1 h. The organic matter was determined by the percentage of organic matter (%OM), this method was conducted according to the Walkley-Black method (Nelson and Sommers, 1982). All data were subjected to statistical analysis using R-program as described above.

#### **Effects of Soil Moisture Condition on Allelopathic Activity**

Three levels of soil moisture conditions dry, half-saturated and submerged were investigated for the residual allelopathic activity of soil incorporated with itchgrass powder. Itchgrass powder and KPS-NP soil was prepared as described above. The experimental design was split-split plot in CRD with four replications. Three periods consisting of 0, 15 and 30 DAI were applied to the main plots. The sub plots with itchgrass powder at the ratio 0.1 g g<sup>-1</sup> (soil); i.e. shoot powder and root powder consisted

of three levels of soil moisture conditions dry, half-saturated and submerged soils. The soil samples were adjusted to the three levels of soil moisture condition using distilled-water (dry soil; not watered, half-saturated soil; watered one time/week and submerged soil; flooded) and were placed in the greenhouse (25-27°C) when the experiments were initiated. The soil samples were air-dried and soil moisture content adjusted to MWHC before measurement in the bioassay test. Three species of test plant seedling were used, as described above. The measurement of percent OM, ECe and pH was conducted as described above. All the data were analyzed using R-program.

### **Results and Discussion**

#### **Effects of Soil Infested by Itchgrass on Test Plants Seedling Growth**

Allelopathic activity of itchgrass under field condition was observed from phytotoxic effects on test plants seedling growth in soil. The infested CH-LP soil and the itchgrass powder incorporated with KPS-NP soil significantly inhibited the growth of *Bidens pilosa*, *Echinochloa crus-galli* and *Lactuca sativa* when compared to none infested CH-LP and KPS-NP soils. Infested CH-LP soil and KPS-NP soil incorporated with itchgrass powder had significant inhibitory effects on the growth of shoot and root lengths of all test plant species. Itchgrass powder from each part displayed uniform inhibitory effects on the growth of the test plants (Table 1). These results indicate that, the KPS-NP soil incorporated with itchgrass powder resulted in an inhibitory effect on test plants seedling growth and the effect was similar to soil previously planted with itchgrass, for more than 5 years, under field conditions (infested CH-LP soil).

Phytotoxic effects of soil incorporated with itchgrass powder were induced by the plant materials released into the soil. Our finding was consistent with previous reports (Kobayashi et al., 2008, Meksawat and Pornprom, 2010). Thus, the phytotoxicity of soil incorporated with itchgrass powder was investigated replicating field conditions. Itchgrass powder had a phytotoxic effect on many test plants, indicating the possibility that itchgrass used as mulch may release allelochemicals into the soil. The residual

**Table 1** Phytotoxicity of soil infested by itchgrass plant on the growth of test plant seedlings.

Treatment	<i>Bidens pilosa</i>		<i>Echinochloa crus-galli</i>		<i>Lactuca sativa</i>	
	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
	(----- cm -----)					
None infested KPS-NP soil	1.83 a <sup>1/</sup>	2.83 a	3.48 a	5.50 a	1.40 a	4.98 a
None infested CH-LP soil	1.78 a	2.88 a	3.50 a	5.40 a	1.45 a	4.97 a
Infested CH-LP soil	0.69 b	1.71 b	2.03 b	3.03 b	1.10 b	2.83 b
Shoot powder+None infested KPS-NP soil	0.59 b	1.58 b	1.85 b	2.68 b	0.59 c	0.92 c
Root powder+None infested KPS-NP soil	0.52 b	1.47 b	1.88 b	2.63 b	0.54 c	0.83 c
<i>F-test</i>	**	**	**	**	**	**
LSD <sub>0.01</sub>	0.330	0.338	0.689	1.210	0.131	0.597

<sup>1/</sup>All data are the means and standard errors of four replications and the treatment means were compared with LSD test (\*\**P* < 0.01).

allelopathic activity of itchgrass was reported by Kobayashi et al. (2008) to remain for only 14 day after incorporating the itchgrass powder, but we believed that the allelopathic activity of itchgrass may remain in soil for more than 14 days after mixing, because the of farmer's practice in CH-LP area who use the itchgrass plant as mulching materials for about 1-2 months for weed control. Therefore, the residual allelopathic activity from itchgrass for a longer term should be determined in the next experiment.

#### Residual Allelopathic Activity of Soil Incorporated with Itchgrass Powder

The residual allelopathic activity in soil was determined by the decreasing phytotoxic effects of soil incorporated with itchgrass powder in upland fields (half-saturated condition) on three test plants growth. The results showed that the shoot and root lengths of *Bidens pilosa* and *Echinochloa crus-galli* were generally significantly sensitive to the phytotoxic effects at 0-30 DAI, except for the shoot and root growths of *Lactuca sativa* which increased after 30 DAI. Based on the continuous periods, itchgrass root powder showed greater inhibitory effects on the growth of the test plants seedling than shoot powder through bioassay determination. In addition, it was also observed that itchgrass powder at the highest concentration had greater phytotoxic effects on the test plants growth (Table 2).

According to the obtained data from bioassay, it is highly probable that allelochemicals are contained in both itchgrass shoot and root powders, and can residue in soil. Similarly, Kobayashi et al. (2008) also observed the allelopathic activity of

itchgrass under field situations. Thus, it is suggested that the inhibition of test plants growth in the soil incorporated with itchgrass powder was principally caused by the phytotoxic substance release from the powder.

Changing the main soil properties was investigated after incorporation of with itchgrass powder at different periods. The investigation of soil properties is shown in Table 3. The ECe value significantly decreased after incorporated with itchgrass powder. Conversely, pH value and percent OM were not significantly changed at the different periods. Interestingly, the soil incorporated with itchgrass powder at the concentration of 0.1 g g<sup>-1</sup> (soil) showed a higher ECe value, pH value and percent OM than 0.01 and 0 g g<sup>-1</sup> (soil), respectively. Remarkably, the highest ECe value and percent OM showed a significant inhibitory effect on the growth of the three test plants. These results are similar to Kobayashi et al. (2008), who reported the high electrical conductivity values in the growth media of non-soil conditions inhibited plant growth. This can be used to assess the allelopathic activity in soil.

The allelopathic activity of itchgrass is due to the direct release of a possible toxic substance from the plant parts. Our results suggest that the maximum periods for residues in the soil are 15-30 DAI depended on the growth susceptible of the test plant species. The residual allelochemicals, under field condition, will decrease over time and it may be affected by the concentration, type of compound and soil properties (Belz et al., 2009, Cecchi et al., 2004). However, our results suggest that the highest concentration showed significantly different results

**Table 2** Residual phytotoxic effects of soil incorporated with itchgrass powder.

Treatment	<i>Bidens pilosa</i>		<i>Echinochloa crus-galli</i>		<i>Lactuca sativa</i>	
	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
(----- cm -----)						
Time (factor A)						
0 DAI <sup>1/</sup>	1.79	2.85	2.33	4.05	0.67 b	3.00 b
15 DAI	1.58	3.06	2.26	3.82	0.75 b	3.05 b
30 DAI	1.60	3.61	2.21	4.46	0.94 a	4.30 a
<i>F-test</i>	ns <sup>2/</sup>	ns	ns	ns	**	**
LSD <sub>0.01</sub>	0.671	1.959	0.632	1.081	0.164	0.759
Itchgrass part (factor B)						
Shoot powder	1.79 a	3.22	2.39	4.40	0.85 a	3.74 a
Root powder	1.52 b	3.12	2.14	3.81	0.73 b	3.17 b
<i>F-test</i>	*	ns	ns	ns	*	**
LSD <sub>0.01</sub>	-	1.130	0.957	3.047	-	0.725
LSD <sub>0.05</sub>	0.431	-	-	-	0.244	-
Concentration (factor C)						
0 g.g <sup>-1</sup> (soil)	2.15 a	3.99 a	2.80 a	5.40 a	0.97 a	4.80 a
0.01 g.g <sup>-1</sup> (soil)	1.62 b	3.27 b	2.43 b	4.26 b	0.79 b	3.70 b
0.1 g.g <sup>-1</sup> (soil)	1.19 c	2.26 c	1.57 c	2.66 c	0.60 c	1.85 c
<i>F-test</i>	**	**	**	**	**	**
LSD <sub>0.01</sub>	0.283	0.448	0.312	0.880	0.159	0.760
A x B	ns	*	ns	ns	ns	*
A x C	**	**	**	**	*	**
B x C	ns	ns	ns	ns	ns	ns
A x B x C	ns	ns	ns	ns	ns	ns

<sup>1/</sup>DAI = Days after incorporated.<sup>2/</sup>All data are the means and standard errors of four replications and the treatment means were compared with LSD test (ns = not significant, \**P* < 0.05 and \*\**P* < 0.01).**Table 3** Changing of ECe, pH and %OM of soil incorporated with itchgrass powder.

Treatment	ECe (dS m <sup>-1</sup> )	pH (value)	Organic matter (%)
Time (factor A)			
0 DAI <sup>1/</sup>	1.22 a <sup>2/</sup>	6.48	3.32
15 DAI	0.85 b	6.22	3.40
30 DAI	0.69 b	6.01	3.22
<i>F-test</i>	**	ns	ns
LSD <sub>0.01</sub>	0.465	0.787	1.021
Itchgrass part (factor B)			
Shoot powder	1.09 a	6.25 a	3.25 b
Root powder	0.75 b	6.22 b	3.37 a
<i>F-test</i>	**	**	**
LSD <sub>0.01</sub>	0.057	2.85E-15	0.003
Concentration (factor C)			
0 g.g <sup>-1</sup> (soil)	0.41 c	6.26 b	1.30 c
0.01 g.g <sup>-1</sup> (soil)	0.64 b	6.16 c	2.22 b
0.1 g.g <sup>-1</sup> (soil)	1.70 a	6.30 a	6.42 a
<i>F-test</i>	**	**	**
LSD <sub>0.01</sub>	0.043	2.23E-15	0.003
A x B	**	**	**
A x C	**	**	**
B x C	**	**	**
A x B x C	**	**	**

<sup>1/</sup>DAI = Days after incorporated<sup>2/</sup>All data are the means and standard errors of four replications and the treatment means were compared with LSD test (ns = not significant and \*\**P* < 0.01).

when compared to lower level treatments. Therefore, we decided to use the ratio 0.1 g g<sup>-1</sup> (soil) to observe the effect of soil moisture conditions on allelopathic activity of itchgrass as under field conditions.

### Effects of Soil Moisture Conditions on Allelopathic Activity

Soil moistures were observed for allelopathic activity of itchgrass under field condition, replicating the plantation fields included in this study. Three soil condition experiments were included in this study; they were dry, half-saturated and submerged soil conditions. The result of the soil moisture conditions affect to the residual allelopathic activity in soil was determined by measuring the decrease of phytotoxic effects in the soil incorporated with itchgrass powder.

The effect from the different soil moisture conditions dry, half-saturated and submerged conditions were investigated on three test plants growth. The results showed that the phytotoxic of soil incorporated with itchgrass powder under dry condition strongly inhibited the growth of the three test plant species and was greater than half-saturated and submerged conditions. The residue of phytotoxic effects of soil incorporated with itchgrass powder under the three soil conditions had a more significant inhibitory effect on the growth of the three test plants at periods of 0-15 DAI. Phytotoxic activity decreased after 30 DAI. In addition, the itchgrass root powder had a significantly greater inhibition effect than the shoot powder (Table 4).

In addition, the E<sub>Ce</sub> value and percent OM of dry condition showed a higher significance than half-saturated and submerged conditions (Table 5). The several studies have reported that allelopathic activity is affected by soil moisture conditions (Tongma et al., 2001). In addition, soil moisture condition is related to the decomposition rate of plant materials in the soil (Thongjoo et al., 2005). These results suggest that dry condition may have a low decomposition rate of plant materials compared with other moisture conditions, and depend on the soil microbial ecology factor (Inderjit, 2005). Therefore, the allelochemicals residue can remain inside the plant materials under dry conditions longer than other, this is the reason that the dry condition

showed a stronger inhibition than the other moisture conditions.

These results indicate that allelochemicals may be contained inside the plant residue and slowly released into the soil, until plant materials is degraded by soil microorganisms or leached by soil water. These are related to the disappearance of allelochemicals in soil, even though allelopathic activity is also influenced by timing and soil moisture condition, however further investigations should be performed to determine the influence of soil characteristics, soil microorganisms and the seasonal variations on the phytotoxic activity in soil under natural field conditions.

Residual phytotoxic activity from itchgrass powder in soil is determined by its concentration and the soil moisture condition. The results obtained in this study demonstrated that the greatest phytotoxic effect of itchgrass in soil was found at 0-15 DAI after which activity decreased. The inhibition of test plants growth in the soil incorporated with itchgrass powder was principally caused by itchgrass powder releasing allelochemicals into the soil; similar to the results obtained in the studies of radish (Kobayashi et al., 2008). From the results, it is evident that itchgrass released allelochemicals into the soil causing growth inhibition. Therefore, further investigations are needed to identify the chemical structure of allelochemicals and characterize the soil sorption-desorption of the allelochemicals in soil.

### Conclusions

Soil incorporating itchgrass powder had phytotoxic effects on the growth of *Bidens pilosa*, *Echinochloa crus-galli* and *Lactuca sativa*, the effectiveness was intensified with increased concentrations of itchgrass powder. Allelopathic activity of itchgrass in soil showed a maximum at 0-15 days after incorporation and then decreased over time. The allelopathic activity of itchgrass was lower in submerged condition than half-saturated conditions and in dry conditions allelopathic activity was the highest. These results suggested that both timing and soil moisture condition were an influence on the allelopathic activity of itchgrass in soil.

**Table 4** Effect of soil moisture conditions on allelopathic activity of soil incorporated with itchgrass powder.

Treatment	<i>Bidens pilosa</i>		<i>Echinochloa crus-galli</i>		<i>Lactuca sativa</i>	
	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
(----- cm -----)						
Time (factor A)						
0 DAI <sup>1/</sup>	1.23 b <sup>2/</sup>	2.90	1.44 b	3.24 b	0.63 b	2.97 c
15 DAI	1.38 b	2.94	1.48 b	3.24 b	0.68 b	3.27 b
30 DAI	1.75 a	3.31	1.82 a	4.27 a	0.76 a	3.95 a
<i>F-test</i>	**	ns	*	**	*	**
LSD <sub>0.01</sub>	0.424	1.311	-	0.558	-	0.287
Itchgrass part (factor B)	-	-	0.329	-	0.101	-
Shoot powder						
Root powder	1.50	3.29 a	1.91 a	3.62	0.78 a	3.94 a
<i>F-test</i>	1.41	2.81 b	1.25 b	3.55	0.60 b	2.84 b
LSD <sub>0.01</sub>	ns	*	**	ns	**	**
LSD <sub>0.05</sub>	0.321	-	0.522	0.819	0.260	0.326
Concentration (factor C)	-	1.012	-	-	-	-
0 g.g <sup>-1</sup> (soil)						
0.01 g.g <sup>-1</sup> (soil)	0.89 c	1.68 b	1.08 b	1.69 b	0.43 c	1.73 c
0.1 g.g <sup>-1</sup> (soil)	1.62 b	3.60 a	1.74 a	4.34 a	0.73 b	3.97 b
<i>F-test</i>	1.85 a	3.89 a	1.90 a	4.72 a	0.91 a	4.48 a
LSD <sub>0.01</sub>	**	**	**	**	**	**
A x B	0.256	0.754	0.491	0.909	0.192	0.419
A x C	ns	ns	**	**	*	**
B x C	**	ns	ns	ns	**	**
A x B x C	ns	ns	**	**	ns	**

<sup>1/</sup>DAI = Days after incorporated.<sup>2/</sup>All data are the means and standard errors of four replications and the treatment means were compared with LSD test (ns = not significant, \**P* < 0.05 and \*\**P* < 0.01).**Table 5** Measurement of ECE, pH and %OM under different soil moisture conditions.

Treatment	ECE (dS m <sup>-1</sup> )	pH (value)	Organic matter (%)
Time (factor A)			
0 DAI <sup>1/</sup>	3.80 a <sup>2/</sup>	6.46	6.43
15 DAI	3.41 b	6.29	6.41
30 DAI	3.33 b	5.94	6.20
<i>F-test</i>	**	ns	ns
LSD <sub>0.01</sub>	0.487	0.787	1.023
Itchgrass part (factor B)			
Shoot powder	4.24 a	6.09 b	6.37 a
Root powder	2.78 b	6.37 a	6.32 b
<i>F-test</i>	**	**	**
LSD <sub>0.01</sub>	0.003	2.51E-15	0.002
Concentration (factor C)			
0 g.g <sup>-1</sup> (soil)	5.94 a	6.10 c	6.53 a
0.01 g.g <sup>-1</sup> (soil)	1.70 c	6.30 a	6.42 b
0.1 g.g <sup>-1</sup> (soil)	2.90 b	6.29 b	6.09 c
<i>F-test</i>	**	**	**
LSD <sub>0.01</sub>	0.003	2.06E-15	0.001
A x B	**	**	**
A x C	**	**	**
B x C	**	**	**
A x B x C	**	**	**

<sup>1/</sup>DAI = Days after incorporated<sup>2/</sup>All data are the means and standard errors of four replications and the treatment means were compared with LSD test (ns = not significant and \*\**P* < 0.01).

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