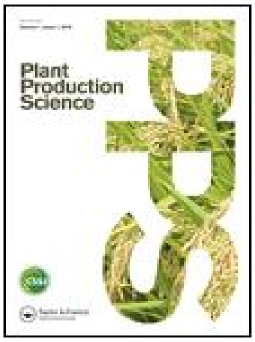

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Relationship between physical property of soil and growth of *Monochoria vaginalis* under paddy condition of organic farming—analysis using settled soil volume in water of superficial layer.

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ABSTRACT
Field experiments were conducted to analyze the relationship between the settled soil volume in water (SSVW) and the growth of *Monochoria vaginalis* (Burm. f.) Kunth under organic farming conditions. SSVW corresponds to the mud volume per dry matter weight. Soil was sampled from the superficial layer of the topsoil (<10 mm), which was of a finer texture than the rest of the topsoil. Without the application of rice bran, there was a negative correlation between SSVW and the number of individuals of *M. vaginalis*. This finding suggests that SSVW is useful as a physical indicator for the growth suppression of *M. vaginalis*. The application of rice bran dramatically reduced the number of *M. vaginalis*. The values of SSVW with rice bran were greater than those without rice bran. The analysis of SSVW indicates that the change in soil physical properties following the application of rice bran was one of the factors responsible for the suppression of *M. vaginalis* growth.

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Monochoria vaginalis; organic farming; paddy soil; rice bran; settled soil volumes in water (SSVW)

Introduction

Weed control via application of rice (*Oryza sativa*. L.) bran to paddy fields has recently received increased attention in Japan and has been adopted by farmers in many areas. Although this method is expected to be useful in organic agriculture, its use has been limited because its effectiveness varies for unknown reasons, particularly with respect to the control of *Monochoria vaginalis* (Burm. f.) Kunth (Shimamune & Suzuki, 2006). The growth suppression of *M. vaginalis* by rice bran varies greatly with soil properties, even when equal amounts of rice bran are applied. Developing a method for estimating the appropriate amount of rice bran to be applied would be useful for agriculture. However, identification of an appropriate soil property indicator correlated with the growth of *M. vaginalis* has received little attention. In our previous study, we reported that the addition of rice bran increased the electrical conductivity (EC) of the soil solution and that there was a negative correlation between the EC and germination of *M. vaginalis* (Nozoe et al., 2012). The increase in EC was suggested to be associated with amounts of toxic substances such as ferrous iron (Fe^{2+}) and organic acids that impaired the germination of *M. vaginalis*.

In the organic paddy field of the National Agricultural and Food Research Organization (NARO)/Agricultural Research Center (ARC) (Tsukubamirai City, Ibaraki, Japan), there were areas in which the growth of *M. vaginalis* was markedly different from that in other areas. This difference was not attributed to the accumulation of germination suppressors such as Fe^{2+} and organic acids, considering that rice bran had not been applied. Therefore, it could be assumed that other factors, such as physical properties of soil, were associated with this difference. During rice cultivation, a fine-grained layer with a smooth tactile texture gradually forms as the superficial layer of the topsoil. It is speculated that seeds of invasive weeds easily establish themselves in this layer, and the amount of light necessary for germination becomes insufficient, limiting the germination of weeds (Wang et al., 1996). Generally, the settled soil volume in water (SSVW) is used as an index expressing the ease of soil pulverization into small clods by tillage (Kitada et al., 1992). SSVW corresponds to the mud volume

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Table 1. Soil properties of topsoil.

Field name	Plot name†	Soil type	pH ‡ (H_2O)	Total N‡ (g kg^{-1})	Total C‡ (g kg^{-1})
A	(C)	Alluvial soil	6.5	2.5	28.8
	(R)		6.2	1.9	23.2
B	(C)	Andosol	6.4	3.0	39.3
	(R)		6.3	2.9	40.6

†C: Continuous irrigated rice, R: Rotation with irrigated rice and upland soybean.
‡Data anazyzed in April 2014.

Table 2. Cultivation System.

Field name	Plot name	Area (m^2)	Year		
			2012	2013	2014
A	(C)	420	Paddy rice†	Paddy rice	Paddy rice†
	(R)	420	Paddy rice†	Upland soybean	Paddy rice†
B	(C)	913	Paddy rice†	Paddy rice	Paddy rice†
	(R)	664	Paddy rice†	Upland soybean	Paddy rice†

†Soil, *M. vaginalis* and/or rice were sampled for the report.

per dry matter weight. When continuously irrigated rice cultivation is converted to upland crop cultivation, SSVW tend to decrease with increasing numbers of upland crops.

In the present study, field experiments were first conducted to investigate whether SSVW is useful as a physical indicator for the evaluation of the growth of *M. vaginalis*. Second, we investigated whether soil physical properties affect the suppression of *M. vaginalis* following the application of rice bran, using SSVW.

Materials and methods

Rice cultivation

Procedure

Organic rice cultivation is continuous at the NARO/ARC in the Yawara experimental paddy fields, Tsukubamirai City, Japan (36°00′N, 140°01′E). Soil properties of the fields are shown in Table 1. Two cropping systems have been implemented in these fields since 2008, one being continuous cultivation of irrigated paddy rice and the other being crop rotation of irrigated paddy rice with upland soybean (Table 2). Samples of soil and of *M. vaginalis* were collected from the paddy fields in 2012 and 2014. Rice straw had been incorporated into the soil after the harvest of the previous year. In the succeeding year, the fields were puddled during the early or middle part of May after the introduction of water. Japonica rice (cv. Koshihikari) was transplanted on June 8, 2012 and June 3, 2014 at a plant density of 18.5 (18 cm × 30 cm) hills m^{-2}. As a basal dressing, a commercial organic fertilizer that consisted mainly of fish and rapeseed meal was applied to the topsoil 4 weeks before transplanting. The amounts of nitrogen (N), phosphorus (as P_2O_5), and potassium (as K_2O) were 30 kg ha^{-1} each. Topdressing of the same fertilizer was applied during late July at 20 kg ha^{-1} of each nutrient. One or two days before transplanting, the field was puddled again for weeding. Intra-row rice bran application to the soil surface and mechanical weeding in the spaces between rows was performed to reduce weeds. Both methods were performed twice during cultivation, with the first application of rice bran (600 kg ha^{-1}) occurring at transplanting, the first mechanical weeding and second application of rice bran (600 kg ha^{-1}) occurring on day 10 after transplanting, and the second mechanical weeding occurring on day 20 after transplanting.

Sample collection for analysis I

(i) This analysis was performed in 2012 and 2014. (ii) The sampling site was located in the area adjoining the border of the experimental fields. At this site, rice was not cultivated and neither application of rice bran nor mechanical weeding was performed. (iii) On July 5, 2012 (day 27 after transplanting), and July 1, 2014 (day 28 after transplanting), soil samples were collected from the superficial layer for analysis of SSVW. (iv) On July 25, 2012 (day 47 after transplanting), and July 22, 2014 (day 49 after transplanting), plant material of *M. vaginalis* was sampled and the number of individuals per unit area was calculated. (v) The sampling location of *M. vaginalis* corresponded with that of soil. Thus, the data for *M. vaginalis* were paired with those for SSVW.

Sample collection for analysis II

(i) This analysis was performed in 2014. (ii) At the sampling site, rice cultivation and weeding, both mechanically and by rice bran application, were performed. (iii) Soil samples of the superficial layer were periodically collected during the 29-day period after transplanting. The soil was collected from the intra-row space. The soil in the spaces between planting rows was not sampled because the weeding machine agitated the weeds in this part. After the collection of soil, SSVW was analyzed. (iv) On July 22 (day 49 after transplanting), the plant material of *M. vaginalis* was sampled in the intra-row space and the number of individuals per unit area was calculated. (v) The data for *M. vaginalis* were not paired with those for SSVW because the sampling locations of *M. vaginalis* and soil were different in each plot. (vi) On September 22 (day 111 after transplanting), rice samples were collected, and brown rice yield per unit area was measured.

Seed density and bulk density of soil

During April, a sample of topsoil was collected from a depth of approximately 10 cm before the introduction of water. The moist sample was air-dried, mixed well, and passed through a 2-mm mesh sieve. Bulk density on an air-dried basis was determined from a portion of the sample (Heuscher et al., 2005). Seeds were recovered from the soil sample by a flotation method using potassium carbonate (K_2CO_3) as described (Buhler & Maxwell, 1993; Ishikawa-Goto & Tsuyuzaki, 2004; Kobayashi & Watanabe, 2010). After 100 g of the soil sample was weighed in a 500-mL beaker, 200 mL of K_2CO_3 solution was added to the beaker. The specific gravity of the solution was adjusted to 1.5 g mL^{-1}. The mixture was stirred and allowed to stand for >30 min. After the floating seeds of weeds were collected, the number of *M. vaginalis* was counted. The seed density in the soil sample was expressed as seed number divided by soil weight on an air-dried basis.

Analytical method of SSVW

After the introduction of water, soil samples from the superficial layer (<10 mm in depth) were collected at three places selected at random. Soil in this layer was of a smooth tactile texture compared with the rest of the topsoil. After a portion of the sample was dried at 105 °C for 24 h, the moisture content of the sample was measured (Gardner, 1986). A volume of moist soil corresponding to 10 g of oven-dried soil was placed in a measuring cylinder, which was filled with 50-mL distilled water. The mixture was shaken thoroughly and allowed to stand for 18–24 h. The soil volume in the cylinder, divided by the soil weight on an oven-dried basis, was expressed as SSVW.

Number of M. vaginalis

In the field without rice cultivation (analysis I), three quadrats of 50 × 50 cm were placed randomly in every plot, and all weeds in the quadrats were collected. In the field with rice cultivation (analysis II), all weeds were sampled from the intra-row space. The sampling area was 30 cm in width and its length (50 cm–3 m) was determined according to the amount of weeds.

After collection, the number of *M. vaginalis* in the weed sample was counted in the laboratory. The number of *M. vaginalis* was expressed as the mean of the numbers in the three sites.

Brown rice yield

In each plot, plant samples were collected from three or four sites. In each site, all plant materials within 2 m in length of the row were sampled. After air drying for 2 or 3 weeks, brown rice yield adjusted to 14% moisture content was determined.

Statistical analysis

Statistical data analyses were performed with SPSS Statistics version 20 (IBM Japan, Tokyo, Japan).

Results and Discussion

Analysis I: relationship between SSVW and M. vaginalis without application of rice bran

SSVW as a physical indicator

A distinct layer of soil of a smooth and fine texture is formed on the surface of the topsoil during cultivation (Kurihara & Kikuchi, 1988). The formation of the layer appears to be useful for weeding because seeds of weeds are easily buried under the layer. However, the relationship between the formation of a smooth and fine textured layer of soil and the burial of seeds has not been elucidated. Considering that there is no suitable physical indicator for the evaluation of the degree of "smoothness" of the soil, predicting the growth of *M. vaginalis* using this property is difficult.

As stated in the Materials and Methods, rice bran was not applied to the area adjacent to the borders of the experimental fields. Although the growth of *M. vaginalis* in the border area differed among years and cropping systems used, a negative and significant correlation between the number of *M. vaginalis* and SSVW was observed (Figure 1L). We also show an inverse relationship between the number of *M. vaginalis* and the reciprocal of SSVW in the superficial layer that corresponded to the bulk density of the mud (Figure 1R). In this study, the reciprocal of SSVW was expressed as mud bulk density [(SSVW)$^{-1}$], as will be

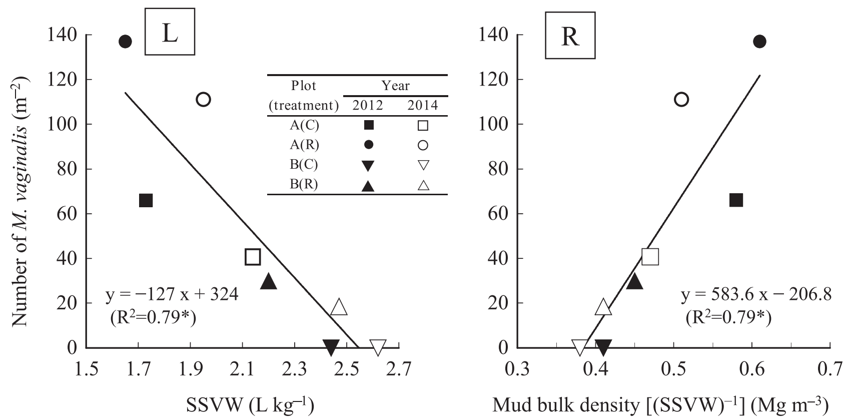

Figure 1. Relationship between the number of *M. vaginalis* and settled soil volume in water (SSVW) (L) and mud bulk density [(SSVW) $^{-1}$] (R). Regression lines are shown in the figures. Each year, the soil sample was collected in late June, and the plant material of *M. vaginalis* was sampled in late July. *Note.* * indicates significant correlation at the 5% level.

Table 3. Bulk density of topsoil and mud bulk density [(SSVW)$^{-1}$] of superficial layer.

		Bulk density† (Mg m^{-3})					Mud bulk density‡ [(SSVW§)$^{-1}$] (Mg m^{-3})				
				Av.#	±	S.D.¶			Av.#	±	S.D.¶
Field name	Plot name	2012	2014		(n=4)		2012	2014		(n=4)	
A	(C)	0.97	0.98	0.97**	±	0.01	0.58	0.47	0.54**	±	0.06
	(R)	0.97	0.96				0.61	0.51			
B	(C)	0.81	0.88	0.85	±	0.04	0.41	0.38	0.41	±	0.03
	(R)	0.83	0.88				0.45	0.41			

† air-dried basis.
‡each year, the soil sample was collected in late June.
§settled soil volume in water.
average.
¶standard deviation.
**the value in the field A is significantly greater than that in the field B at 1% level (Student's *t* test).

discussed below. Hosokawa et al. (2012) reported that the percentage of clay or silt + clay in the layer was greater than that in the soil below it. Generally, there is a negative correlation between bulk density and the percentages of clay or silt (Jones, 1983). Although the particle size distribution of the layer was not analyzed in this study, the bulk density of topsoil was measured (Table 3). Based on Figure 1R, we also show the mud bulk density [(SSVW)$^{-1}$] in Table 3. Both the bulk density of the topsoil and mud bulk density [(SSVW)$^{-1}$] of the superficial layer in field B were significantly greater than those in field A, suggesting that the bulk density of the topsoil affected the SSVW of the superficial layer.

The seed of *M. vaginalis* requires light for its germination and that germination percentage decreases with increasing soil depth (Wang et al., 1996). Thus, one of the factors suppressing the germination of *M. vaginalis* in deep soil may have been insufficient light. If we shake a mixture of soil and water, soil particle with higher density such as sand will sink faster. Generally, soil with higher bulk density tends to consist of soil particles with higher density (Jones, 1983). Thus, if the mixtures of the seed of *M. vaginalis* and muds with different bulk densities [(SSVR)$^{-1}$] are agitated, the mud with higher bulk density [(SSVW)$^{-1}$] will settle faster. As a result, the seed will be placed in relatively shallower soil. In this study, there was a significant positive correlation between the number of *M. vaginalis* and the mud bulk density [(SSVW)$^{-1}$] (Figure 1(R)). After soil agitation by such operations as puddling, transplanting, and the activity of soil animals, a greater mud bulk density [(SSVW)$^{-1}$] might thus have placed the seed in shallower soil. Thus, the germination percentage might have been higher and the number of *M. vaginalis* greater.

It has been reported that >90% of seedlings emerged from puddled paddy soil ≤2 mm in depth (Koarai & Shibayama, 2001). Based on the seed density (Figure 2L) and the bulk density (Table 3), the seed number of the soil volume 1 m^2 × 2 mm in depth was estimated (Figure 2R). There appeared to be no relationship between the estimated numbers of seeds and the numbers of *M. vaginalis* in the fields, with the estimated number of seeds being considerably greater than the number of *M. vaginalis*.

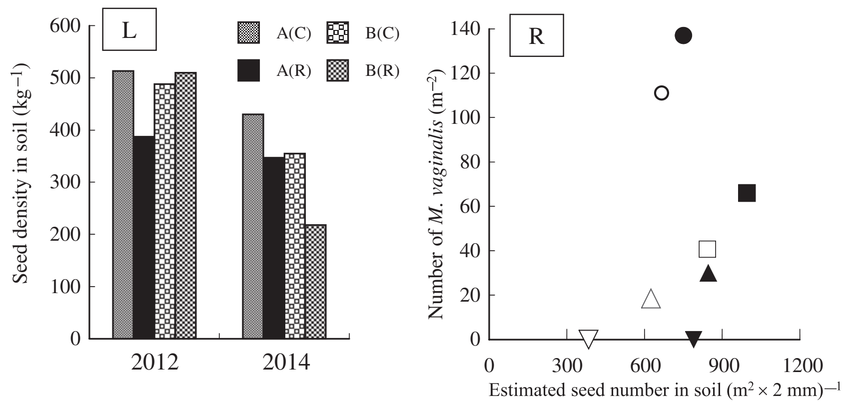

Figure 2. Relationship between seed number in soil and number of *M. vaginalis* in the field. (L): seed density; (R): relationship between number of plants in the field and the estimated seed number in superficial layer. Symbols in (R) are the same as those in Figure 1.

These findings indicate that seed density was not a factor restricting the number of *M. vaginalis* in the fields. Thus, seed density did not affect the relationship between SSVW and the number of *M. vaginalis* in the fields (Figure 1L). Effects of seed density on germination have been described (Inouye, 1980; Forcella, 1992). Although the seedling number increased with seed density, it reached a plateau when the density exceeded a certain level. This response by seeds may be an adaptation to avoid unfavorable competition. However, the mechanism underlying density-dependent germination has not been fully elucidated, particularly in paddy soil. Grable and Danielson (1965) suggested carbon dioxide (CO_2) as a germination suppressor. Further study of the mechanism is needed.

In the present study, we showed that SSVW was useful as a physical indicator of soil to predict the growth of *M. vaginalis*, provided that seed density did not restrict the number of *M. vaginalis*.

Crop rotation

Takahashi et al. (1999) stated that the conversion of continuously irrigated field into upland field reduces SSVW. They expressed SSVW as sediment volume in the report. They speculated that this change was associated with the stabilization of soil microstructure, with an increase in crystallization of Fe(III) oxides which played a roll of "cementing reagent." The crystalline Fe(III) oxides combined soil particles, and the complex of particles and crystalline Fe(III) oxides was formed. When the soil microstructure was stabilized, SSVW decreased because the volume/weight of the complex was smaller than that of original particle. If the history of upland cultivation in crop-rotated plots affects SSVW, the SSVW in the plots should be lower than that in continuous paddy plots. Based on the data shown in Figure 1L, the averages of SSVW (L kg^{-1}) in 2012 and 2014 were calculated and expressed as follows (average ± standard deviation, n = 2). The values of A(C), A(R), B(C), and B(R) were (1.94 ± 0.29), (1.80 ± 0.21), (2.53 ± 0.13), and (2.34 ± 0.19), respectively. There were no significant differences between either A(C) and A(R) or B(C) and B(R).

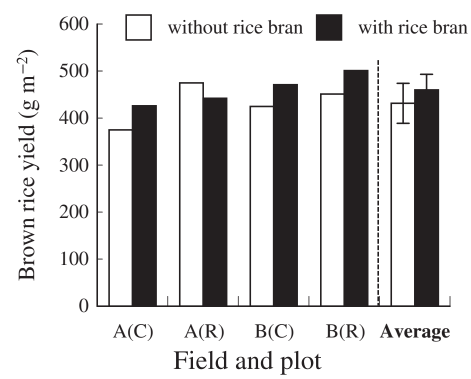

Figure 3. Effects of application of rice bran on brown rice yield (2014). Average; the means of four plots: A(C), A(R), B(C), and B(R). The error bars indicate standard deviations (n = 4).

To summarize, there was a strong correlation between the number of *M. vaginalis* and SSVW, provided that seed density did not restrict the number of *M. vaginalis* in the fields. Thus, SSVW was a useful physical indicator by which the growth of *M. vaginalis* could be evaluated. The effects of crop rotation on the SSVW were not determined in this analysis.

Analysis II: effect of application of rice bran on SSVW and M. vaginalis

This analysis was performed in the fields where rice was cultivated, and rice bran was applied to the soil surface. In the plots of A(C), B(C), and B(R), brown rice yields with rice bran were greater than those without rice bran (Figure 3). The result in the plot of A(R) was opposite. In this plot, the lower yield with the application of rice bran was attributed to yield reduction by lodging.

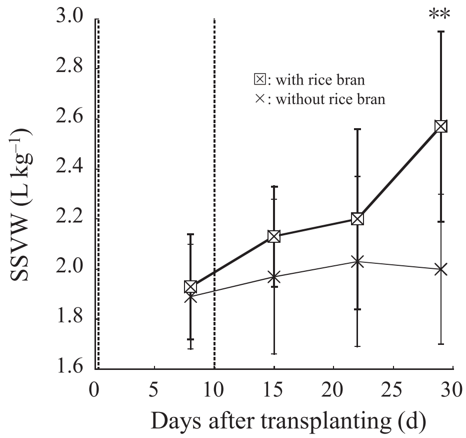

Figure 4. Effects of application of rice bran on changes in settled soil volume in water (SSVW) of the superficial layer (2014). Broken lines both at transplanting and on day 10 after transplanting indicate the application of rice bran. Each data point represents the mean of four plots: A(C), A(R), B(C), and B(R). The error bars indicate standard deviations. ** the SSVW with rice bran is significantly greater than that without rice bran at 1% level (Student's *t* test).

SSVW with the application of rice bran increased throughout the period of 29 days after transplanting (Figure 4). The difference in SSVW between the plots with and without rice bran increased over this period. Generally, the application of organic materials to paddy soil improves soil physical properties such as aggregation, compaction, and retention of water (Larson & Clapp, 1984). However, these properties do not change over a short period. Tubificid worms (*Tubifex tubifex*) are responsible for the production of the fine-textured layer of soil (Kurihara & Kikuchi, 1988). The tubificid worms ingest mud, absorb nourishment, and excrete the soil on the surface of topsoil. The formation of a smooth textured layer is attributed to the accumulation of excreted soil as soil particles in this layer are relatively light and fine. Simpsons et al. (1993) reported that the density of soil oligochaete including tubificid worms increased with soil carbon content. They suggested that the organic matter in soil was a food source for oligochaete. Presumably, in the present study, the use of rice bran by tubificid worms in the soil was associated with the increase in SSVW. Further investigation is required to identify the mechanism behind the increase in SSVW.

The application of rice bran dramatically reduced the number of *M. vaginalis* (Figure 5L). The values of SSVW with rice bran were greater than those without rice bran (Figure 5R), and all values of SSVW with rice bran were 2.4 L kg^{-1}, a value at which the growth of *M. vaginalis* was markedly suppressed (Figure 1L). These findings suggest that the change in soil physical properties with the application of rice bran was one of the factors responsible for the suppression of *M. vaginalis* growth (Figure 5L). Although many factors, some unknown, could be associated with the decrease in the number of the *M. vaginalis* with the application of rice bran, these factors have not been identified. In the previous report, we suggested that the addition of rice bran to flooded soil suppressed the germination of *M. vaginalis* (Nozoe et al., 2012) and also that the EC of the soil solution is useful as a chemical indicator of the suppression of *M. vaginalis* growth. Generally, the amounts of substances such as Fe^{2+} (Nozoe et al., 2008) and aromatic carboxylic acids (Tanaka et al., 1990) increase in flooded soil, and these substances sometimes suppress the growth of paddy rice. We have reported that Fe^{2+} could suppress the growth of *Echinochloa* weeds (Nozoe et al., 2010). The tolerance of paddy weeds to Fe^{2+} was different among species, with that of *M. vaginalis* being relatively strong (Nozoe et al., 2009). The value of EC was presumably associated with the total amounts of germination suppressors. Here we suggest that SSVW is useful as an additional indicator to evaluate the suppression of *M. vaginalis* by application of rice bran.

We have reported that EC increased with the decrease in soil Eh (oxidation–reduction potential) (Nozoe et al., 2012), and Nakai and Toritsuka (2009) have reported that the decrease in soil Eh with rice bran was completed over a 4-day period of cultivation. Presumably, the suppression of *M. vaginalis* with the increase in EC began immediately after transplanting. In contrast, the effect of rice bran application on SSVW gradually increased during the 29-day cultivation period (Figure 4). These findings suggest that the chemical factor expressed as an increase in EC was responsible for the initial suppression of *M. vaginalis*. However, the physical factor expressed as the increase in SSVW gradually replaced the chemical factor as the suppressor. Namely, the relationship between chemical and physical factors is not competitive but complementary. Further studies will be necessary to elucidate the relationship between the chemical and physical effects.

Based on the results obtained in the present study, SSVW is a useful indicator of the growth of *M. vaginalis*. In addition, the results suggest that the change in physical properties of soil with the application of rice bran is a factor responsible for the suppression of *M. vaginalis* growth. Under the current conditions of organic farming, farmers wishing to determine the amount of rice bran to apply use no formal, scientifically determined method. The results of the present study could provide useful information to farmers. For example, farmers could determine the appropriate application period and the amount of rice bran to apply on the basis of scientific indicators such as SSVW of the superficial layer of topsoil.

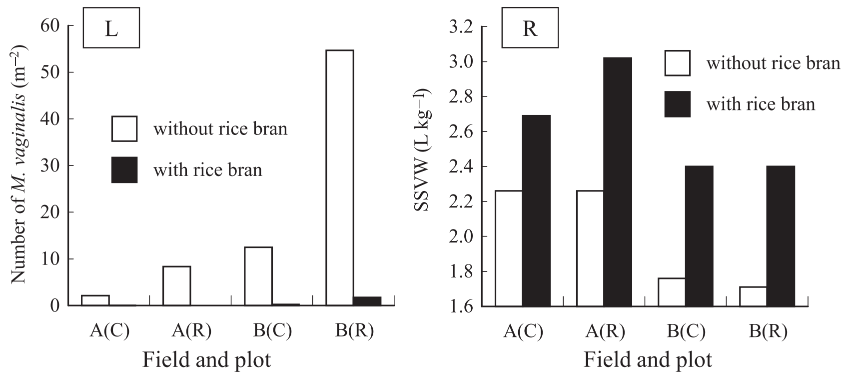

Figure 5. Effects of application of rice bran on number of *M. vaginalis* in field (L) and settled soil volume in water (SSVW;R) (2014). The soil sample was collected on July 2 (day 29 after transplanting). Plant material of *M. vaginalis* was sampled on July 22 (day 49 after transplanting).

Conclusion

The physical properties of the soil superficial layer affected the growth of *M. vaginalis*. There was a negative correlation between the number of *M. vaginalis* in the field and SSVW. This finding indicates that SSVW is a useful indicator for the evaluation of the relationship between the physical property of soil and growth of *M. vaginalis*. SSVW with the application of rice bran was greater than that without rice bran throughout a 29-day period after transplanting. The application of rice bran reduced the number of *M. vaginalis*. The analysis using SSVW indicated that the change in soil physical properties with the application of rice bran was one of the factors responsible for the suppression of *M. vaginalis* growth.

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