

## Do organic silicon and imidacloprid synergistically induce toxicity to the new invasive mealybug *Phenacoccus solenopsis* Tinsley on *Portulaca grandiflora* plants?

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**Abstract:** In this study, the effects of organic silicon (Si) on the toxicity of imidacloprid to a new invasive mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae), infesting *Portulaca grandiflora* (Caryophyllales: Portulacaceae) plants were evaluated using spotting and dipping methods under laboratory and field conditions to improve management strategies for *solenopsis* mealybug. Results showed significant synergistic effects of 0.06% organic Si with imidacloprid solution against third-instar *P. solenopsis* nymphs and adult females. However, the percentage mortalities increased remarkably after *P. solenopsis* was exposed to 100, 50, and 25 mg a.i./L of imidacloprid combined with organic Si. For the third-instar nymphs, these values increased to 100%, 100%, and 92.5%, respectively; for the adult females, these values increased to 100%, 100%, and 82.4%, respectively. After 5 days, the percentage mortalities reached 84.7%, 56.1%, and 39.7% when the third-instar nymphs were exposed to 100, 50, and 25 mg a.i./L of imidacloprid solution, respectively. The percentage mortalities reached 57.5%, 38.9%, and 26.2% when adult females were exposed to the same imidacloprid solutions, respectively. Organic Si alone elicited toxicity to *P. solenopsis*. The percentage mortalities of the first-, second-, and third-instar nymphs and the adult females were 76.9%, 47.5%, 39.2%, and 18.9%, respectively. Field data showed that imidacloprid combined with organic Si caused sustainable control of *P. solenopsis*. The percentage mortalities of *P. solenopsis* reached maximum values (approximately 99.9%) after 21 days. Thus, the combined treatment with imidacloprid and organic Si is synergistically more effective than the individual use of these substances against third-instar *P. solenopsis* nymphs and adult females.

**Key words:** *Phenacoccus solenopsis*, imidacloprid, organic silicon, chemical control, synergistic effect

### 1. Introduction

The *solenopsis* mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) originated in the United States (Tinsley, 1898), but this species has now spread to different parts of the world including Asian countries such as Pakistan, India, Thailand, Australia, and China (Abbas et al., 2005; Hodgson et al., 2008; Wang et al., 2010). Like most of the scale insect species, *P. solenopsis* is an obligate phloem-feeding pest (Arif et al., 2009; Zhang et al., 2011; Huang F et al., 2012) and causes fruits, vegetables, and flower buds to drop off prematurely. In a bad infestation, their honeydew induces the development of sooty mold fungus (Saeed et al., 2007). This species has been recognized as an aggressively invasive species of agricultural and ornamental plants in China.

*P. solenopsis* in the fields is difficult to manage (Ram and Saini, 2010; Vennila et al., 2010; Huang J et al., 2012; Zhou et al., 2012) for the following reasons: 1) this species possesses a waxy coating that protects it from insecticides

and natural mortality factors, 2) *P. solenopsis* can also hide in soil cracks and crevices, 3) *P. solenopsis* exhibits a high reproductive rate and produces serious overlapping generations, and 4) the higher foraging tempo of ants provides additional effective protection for *P. solenopsis*. *P. solenopsis* cannot be controlled effectively; the losses in cotton yield in 2008 or 2009 were  $1.4 \times 10^6$  t in China,  $1.12 \times 10^6$  t in India, and  $0.48 \times 10^6$  t in Pakistan (Wang et al., 2010). Therefore, more efficient, energy-saving, and environmentally friendly chemical control methods are necessary to implement the emergency management of *P. solenopsis*.

Imidacloprid is a highly effective systemic neonicotinoid insecticide with low mammalian toxicity used to control sucking insects such as aphids, leafhoppers, planthoppers, thrips, and whiteflies, including resistant strains (Mullins, 1993; Elbert and Nauen, 2004; Feng et al., 2004; Ahern et al., 2005). Previous studies also found that imidacloprid was an effective insecticide against some

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species of mealybugs, such as *Planococcus citri* (Cloyd et al., 2012), *Planococcus ficus* (Mansour et al., 2010; Prabhaker et al., 2012), and *P. solenopsis* (Dhawan et al., 2009; Sahito et al., 2011; Huang J et al., 2012; Huang et al., 2013). However, less attention has been given to increasing the detention time of imidacloprid on leaves or stems and improving the permeability of imidacloprid into the body walls of mealybugs.

Organic silicon (Si) is found in organic and inorganic structures. The unique composition and molecular structure of organic Si allows it to exhibit organic and inorganic characteristics. In agriculture, organic Si exhibits low surface tension and surface energy (Knoche, 1994; Deng et al., 2002). Organic Si added to insecticides and herbicides can increase the effectiveness of pest control (Liu et al., 2010). Ferreira et al. (2011) determined that Si combined with soybean cultivar IAC-19 can significantly decrease silverleaf whitefly populations. However, the synergistic effect of organic Si on the toxicity of imidacloprid to solenopsis mealybugs remains unknown.

This study aimed to provide evidence of the effectiveness of imidacloprid combined with organic Si against different developmental stages of *P. solenopsis* on *Portulaca grandiflora* (Caryophyllales: Portulacaceae) plants by using topical application and dipping methods under laboratory and field conditions. We assumed that organic Si added to imidacloprid solution could significantly increase the percentage mortality of adult females and larval *P. solenopsis*. The results of these experiments may provide a new and promising option for controlling *P. solenopsis* by combining imidacloprid with suitable organic Si.

## 2. Materials and methods

### 2.1. Insect and insecticide

*P. solenopsis* was collected from infested *Torenia fournieri* (Scrophulariales: Scrophulariaceae) in the suburbs of Hangzhou, Zhejiang Province, China, and was reared on potted *P. grandiflora* (Caryophyllales: Portulacaceae). Laboratory culture was maintained in an insectary at  $25 \pm 1$  °C with a photoperiod of 14 L:10 D and 60%–70% relative humidity (RH). The developmental period of *P. solenopsis* was divided into 4 stages (first-, second-, and third-instar nymph and adult females), as previously described (Zhu et al., 2011; Huang J et al., 2012).

Imidacloprid 10% Wp (commercial name: Yashijing; molecular formula:  $C_9H_{10}ClN_5O_2$ ) and organic Si (commercial name: Tianda) were supplied by Nanjing Baofeng Pesticides Factory (Nanjing, China) and Shandong Tianda Bio-Pharmaceutics Co., Ltd. (Shangdong, China), respectively. The concentration of organic Si added to imidacloprid was 0.06%, as described by Huang and Zhang (2013). Imidacloprid was diluted to 25, 50, and

100 mg a.i./L based on the commercially recommended concentrations.

### 2.2. Laboratory experiment

*P. grandiflora* stems (5 cm to 7 cm in length) were chosen, and their bases were moisturized using a humid pledget. A total of 15 *P. solenopsis* at the same stage were selected randomly from the colonies and carefully transferred to their respective *P. grandiflora* stems using a writing brush. Each stem was placed in a culture dish (9 cm in diameter) and prepared for the pesticide treatments. Four stems were randomly assigned to each of the 4 treatments: imidacloprid with organic Si, organic Si only, imidacloprid only, and control treatment (purified water). The experiment was repeated 4 times with 16 replicates per treatment.

The mixture or the control treatment (approximately 100  $\mu$ L) was sucked up using a mechanical adjustable pipette (20–200  $\mu$ L; Dragon Medical Co., Shanghai, China), and 200  $\mu$ L of the treatment or the control treatment was topically applied to the body of the first- and second-instar nymphs in each replicate. A total of approximately 10  $\mu$ L was applied to each nymph body. The third-instar nymphs and the adult females were treated using the dipping method. Each stem was dipped carefully in a 100-mL beaker containing the respective treatments (100 mL) for 15 s. After the pesticide treatment was performed, each covered culture dish was maintained in a growth cabinet at  $27 \pm 1$  °C with a photoperiod of 14 L:10 D and 60%–70% RH. The number of *P. solenopsis* survivors was counted after 1, 3, and 5 days. Death was determined based on the coordinated muscle response of mealybugs to gentle prodding with an insect needle.

### 2.3. Field experiment

The experiment was conducted under field conditions in the gardens of the Flower Research and Development Center, Zhejiang Academy of Agricultural Sciences, to evaluate the effects of imidacloprid with organic Si against *P. solenopsis*. In this procedure, the imidacloprid concentration was 50 mg a.i./L, as used in a previous study (unpublished data). Three potted *P. grandiflora* infested with *P. solenopsis* were randomly assigned to each of the 4 treatments as described in the Section 2.2. The experiment was repeated 4 times with 12 replicates per treatment. The spraying method was used to treat *P. grandiflora* plants, and approximately 150 mL of the treatment was administered to each plant. The number of *P. solenopsis* from each plant was counted before the test, and the numbers of *P. solenopsis* survivors were determined after 1, 3, 9, 13, and 21 days.

### 2.4. Statistical analysis

Data were analyzed by Shapiro–Wilk and Levene tests to determine the normal distribution and homogeneity of variances, respectively. If the data were normally

distributed and exhibited similar variances, then a paired t-test was performed to compare the percentage mortality of *P. solenopsis* exposed to different treatments (imidacloprid only and a combination of imidacloprid and organic Si, for 1 and 3 days). The mortality data of *P. solenopsis* were corrected via blank control before the process of analysis. The mortality data for different *P. solenopsis* developmental stages and treatments after 5 days were statistically examined by 2-way ANOVA and Duncan multiple range tests. The mortality data of *P. solenopsis* in different treatments at different sampling dates in the field, or kinetic changes in *P. solenopsis* mortality in different treatments, were analyzed by repeated measures ANOVA and Dunnett tests. All statistical analyses were conducted in SPSS 14.0 (SPSS Inc., Chicago, IL, USA).

### 3. Results

#### 3.1. Laboratory experiment

The mortality of *P. solenopsis* at all developmental stages increased rapidly with the combined treatment after 1 day and 3 days (Figure 1). Additionally, the mortality of *P. solenopsis* with imidacloprid was only significantly lower than mortality with the combined treatment, with the exception of first ( $t = 1.00$ ;  $P < 0.05$ ) and second ( $t = 1.73$ ;  $P < 0.05$ ) instars exposed to 100 mg a.i./L of imidacloprid after 3 days (Figure 1a).

Mealybug developmental stage, treatment, and their interaction significantly affected the mortality data of *P. solenopsis* after 5 days (Table 1; Figure 1). Mortality decreased significantly when the third-instar nymphs ( $84.68 \pm 3.37\%$ ) and the adult females ( $57.50 \pm 10.14\%$ ) were exposed to 100 mg a.i./L of imidacloprid only (Figure 1a;  $F_{6,36} = 11.24$ ;  $P < 0.001$ ); the synergistic toxicity of the 2 compounds was greater than the sum of the effects of the 2 individual treatments for the adult females at 100 mg a.i./L ( $100\% > 57.5\% + 18.9\%$ ). The percent mortalities of the mealybugs treated with the combined treatment were significantly higher than those treated with imidacloprid only when *P. solenopsis* was exposed to 50 mg a.i./L of imidacloprid, with the exception of first-instar nymphs (Figure 1b;  $F_{6,36} = 30.52$ ;  $P < 0.001$ ). The percentage mortality also decreased to 38.90% when the adult females were exposed to 50 mg a.i./L of imidacloprid only. Synergistic toxicity of the 2 compounds occurred for the third-instar nymphs ( $100\% > 56.1\% + 39.2\%$ ) and adult females ( $100\% > 38.9\% + 18.9\%$ ). The effect of the combined treatment of 25 mg a.i./L against *P. solenopsis* was more effective than imidacloprid only, although the percent mortalities of the third-instar nymphs and the adult females decreased when they were exposed to the combined treatment (Figure 1c;  $F_{6,36} = 11.15$ ;  $P < 0.001$ ); synergistic toxicity of the 2 compounds occurred for the third-instar nymphs ( $92.5\% > 39.7\% + 39.2\%$ ) and adult females ( $82.4\% > 26.2\% + 18.9\%$ ).

#### 3.2. Field experiment

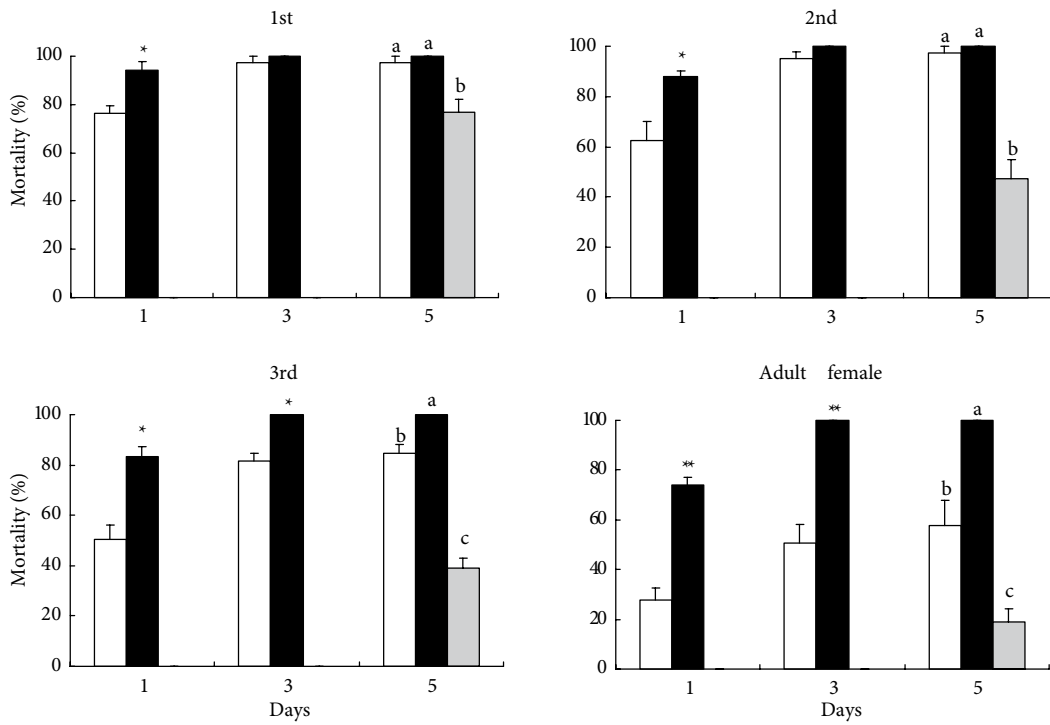
The different mortalities of *P. solenopsis* among the combined pesticide, imidacloprid only, and organic Si only treatments were compared at different sampling dates (Table 2; Figure 2). Adding organic Si to imidacloprid solution caused a significant increase in the mortality of *P. solenopsis* (Table 2;  $F_{8,45} = 11.27$ ;  $P < 0.001$ ). However, the synergistic toxicity of the 2 compounds occurred 13 days ( $99.8\% > 75.6\% + 22.3\%$ ) and 21 days ( $99.9\% > 74.7\% + 16.1\%$ ) later.

### 4. Discussion

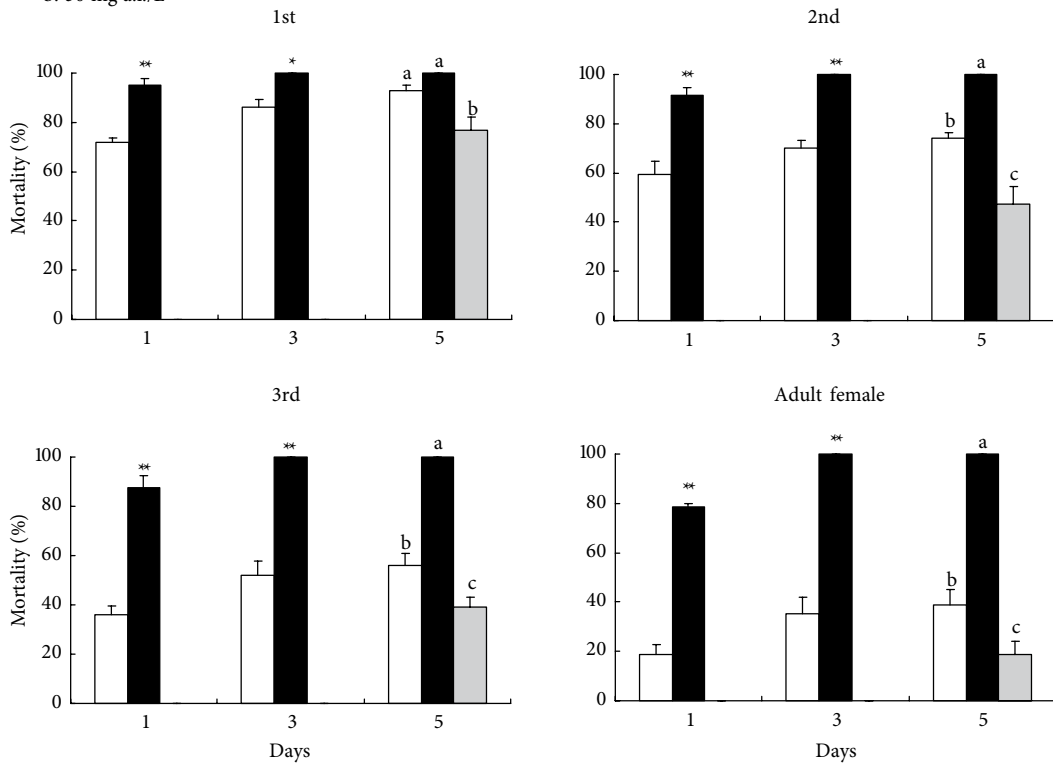
Numerous pesticides such as profenofos (Bhosle et al., 2009; Dhawan et al., 2009), imidacloprid, and neem oil (Sahito et al., 2011; Huang J et al., 2012; Huang et al., 2013) have been approved against *P. solenopsis*. Using the combined treatment of imidacloprid and organic Si, Huang and Zhang (2013) found that the mortality of *P. solenopsis* on *Torenia fournieri* plants increased to a maximum of 100% after 15 days. Likewise, we found that the same treatment caused a significant increase in the mortality of *P. solenopsis*, particularly the third-instar nymphs and the adult females, after 5 days under laboratory conditions. The combined treatment elicited constant and efficient control of *P. solenopsis*, but the percentage mortalities of *P. solenopsis* treated only with imidacloprid fluctuated as days passed under field conditions. Two factors may explain this result. First, organic Si could increase the adhesion of imidacloprid solution on the body of *P. solenopsis* (direct effect) or plants (indirect effect) and improve the toxicity of imidacloprid. Second, the treatment with organic Si only could also elicit toxicity to *P. solenopsis*. However, the toxicity of the combined treatment was greater than the sum of the effects of the 2 individual treatments on the third-instar nymphs and the adult females. We also found that *P. solenopsis* treated with imidacloprid only exhibited high mortality. This result may be the basis of administering large volumes of imidacloprid solution to small mealybugs each time. The combined treatments did not elicit toxicity to *P. grandiflora* plants. Therefore, different concentrations of imidacloprid and organic Si can be applied without eliciting toxic effects on pest-infested plants.

Although organic Si and imidacloprid could elicit a significant synergistic toxicity to the third-instar nymphs and the adult females of *P. solenopsis*, the earliest instars remain the critical periods for chemical pest control. The main reason is that the earliest instar nymphs are not covered with powdery wax (Vennila et al., 2010; Huang J et al., 2012), thereby increasing the effectiveness of insecticides. This control period is difficult to target because the earliest instar nymphs develop and move very fast. Therefore, the population dynamics of *P. solenopsis* should be monitored to implement chemical pest control effectively. Natural enemies are

a: 100 mg a.i./L



b: 50 mg a.i./L



**Figure 1.** Percent mortalities of *Phenacoccus solenopsis* (first-, second-, and third-instar nymphs and adult females) exposed to 100 mg a.i./L (a), 50 mg a.i./L (b), and 25 mg a.i./L (c) of imidacloprid combined with or without organic Si and exposed to organic Si only, respectively, after 1, 3, and 5 days (average  $\pm$  SE). \* or \*\* on a bar indicates a significant difference at 0.05 or 0.01, respectively (paired t-test). The bars labeled with the same letter are not significantly different ( $P < 0.05$ , Duncan test).

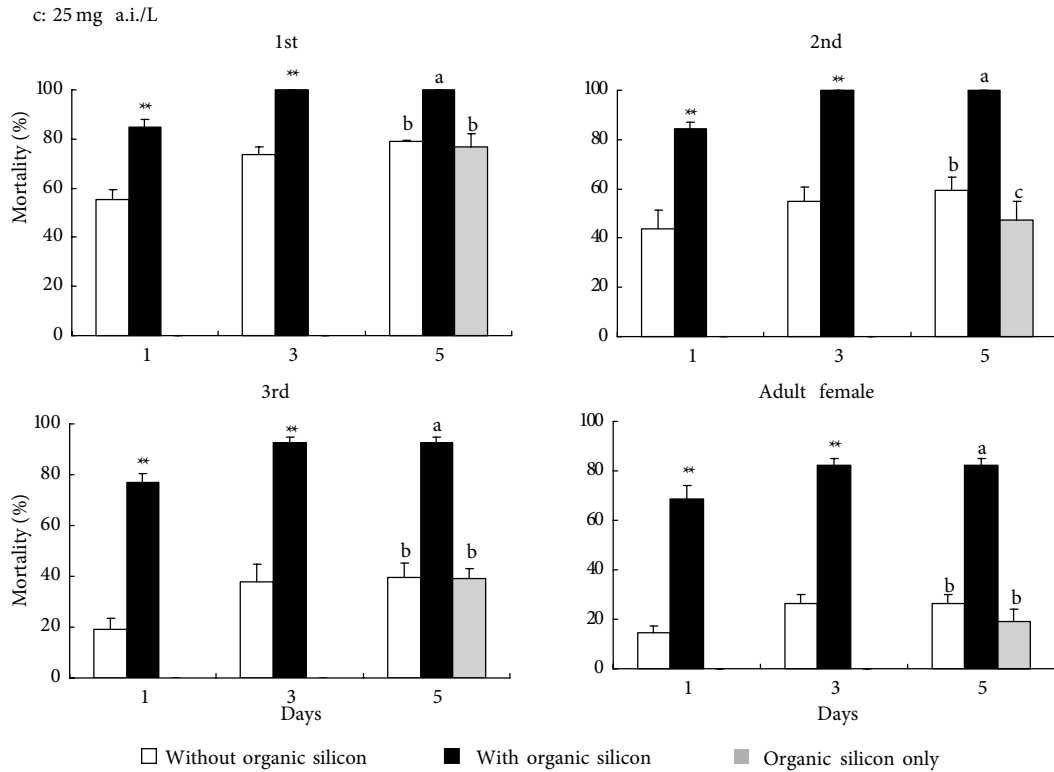


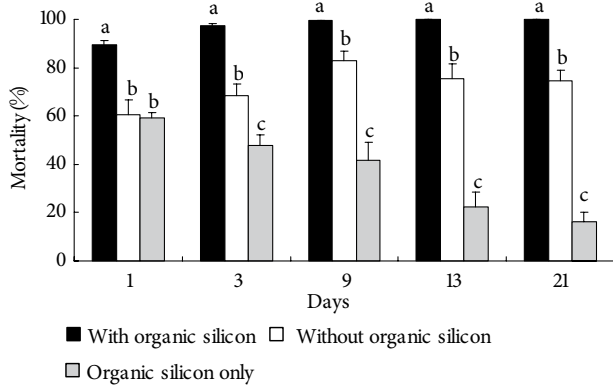
Figure 1. (continued).

Table 1. Results of 2-way ANOVA on the effects of *P. solenopsis* developmental stages, treatments, and their interaction on the mortality data of *P. solenopsis* after 5 days.

Concentrations	Factors	df (numerator, denominator)	Mean squares	F	P
100 mg a.i./L	Stage	3, 36	711.24	11.24	<0.001
	Treatment	2, 36	1973.49	31.18	<0.001
	Stage × treatment	6, 36	711.24	11.24	<0.001
50 mg a.i./L	Stage	3, 36	1071.76	30.52	<0.001
	Treatment	2, 36	9573.82	272.64	<0.001
	Stage × treatment	6, 36	1071.76	30.52	<0.001
25 mg a.i./L	Stage	3, 36	1894.04	42.03	<0.001
	Treatment	2, 36	14599.13	323.95	<0.001
	Stage × treatment	6, 36	502.33	11.15	<0.001

Table 2. Results of repeated-measures ANOVA on the effects of treatments, infestation time, and their interaction on the mortality data of *P. solenopsis* in the field.

Source	df (numerator, denominator)	Mean squares	F	P
Treatment	2, 45	18146.13	179.17	<0.001
Time	4, 45	244.08	3.70	0.014
Treatment × time	8, 45	732.67	11.27	<0.001



**Figure 2.** Percent mortalities of *Phenacoccus solenopsis* treated with 50 mg a.i./L of imidacloprid only, 50 mg a.i./L imidacloprid and 0.06% organic Si combined, and 0.06% organic Si only after 1, 3, 9, 13, and 21 days, respectively (average  $\pm$  SE). The bars labeled with the same letter are not significantly different ( $P < 0.05$ , Duncan test).

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important in a sustainable pest management system. Since *P. solenopsis* invaded China in 2008, some natural enemies such as *Cryptolaemus montrouzieri* Mulsant, *Lemnia biplagiata* (Swartz), *Menochilus sexmaculatus* (Fabricius), *Nephus quadrimaculatus* (Kamiya), *Acerophagus coccois* Smith, *Prochiloneurus nagasakiensis* (Ishii), and *Aenasius bambawalei* Hayat (Chen et al., 2011) have been found in the fields. Therefore, the impact of the combination of imidacloprid and organic Si on natural enemies should be assessed in future studies.

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