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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

氏名：

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学位論文題目：
Title of Dissertation　　Elucidation of Elicitors in *Sogatella furcifera* (Horváth) Causing the Japonica Rice Plant (*Oryza sativa* L.) to Induce the Ovicidal Substance, Benzyl Benzoate

学位論文要約：

Dissertation Summary

Some *Japonica* rice plant varieties show the resistance to the whitebacked planthopper (*S. furcifera*) and the varieties produce ovicidal compound, benzyl benzoate, to kill their eggs lying in the rice plant. We have tried to elucidate the mechanism for inducing the production of the benzyl benzoate by the Japonica rice varieties(Fig. 1).

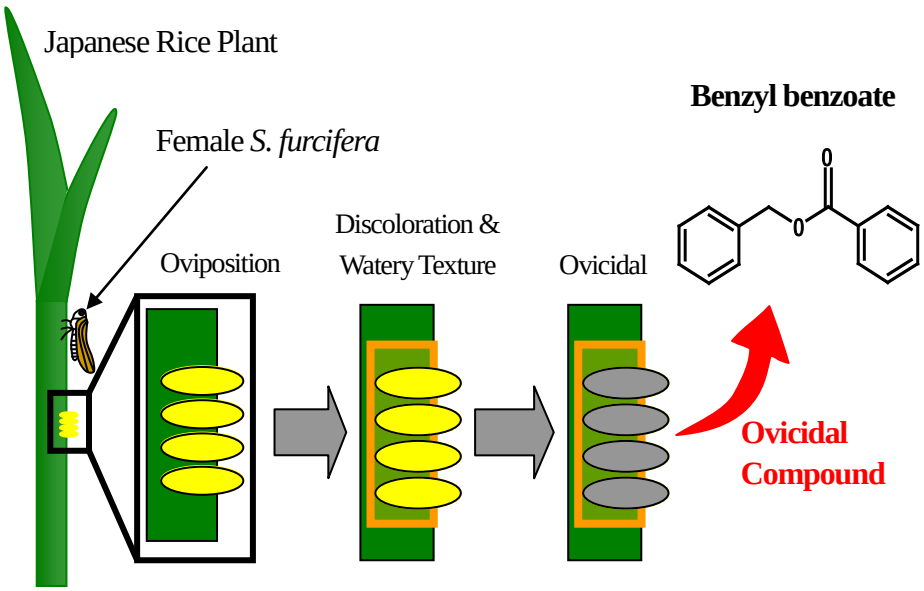

Fig. 1.　The induced mechanism of benzyl benzoate.

Even when the rice plant was given physical damages by needle or the damages with water, benzyl benzoate was not produced at all. On contrary, only when the plant was give the damages with the 80 % methanol extract of *S. furcifera* or homogenate of *S. furcifera*, benzyl benzoate was significantly produced (Fig. 2).

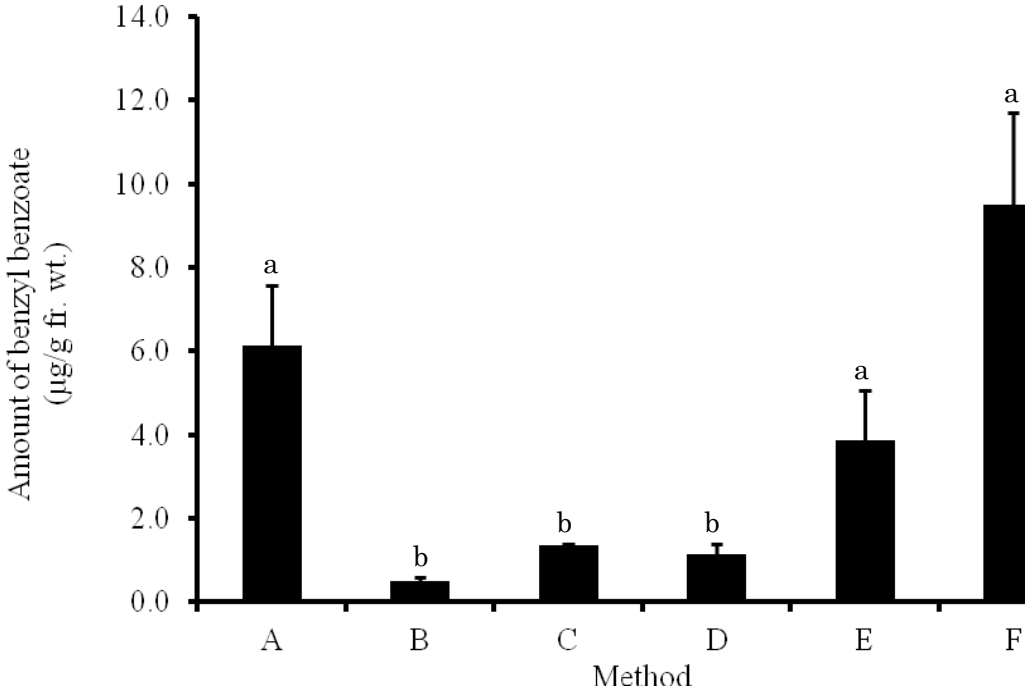

Fig. 2. The amount of benzyl benzoate induced by several treatments. A, the ovipositional site created by *S. furcifera*; B, the intact rice plant; C, only physical damage; D, physical damage with water; E, physical damage with *S. furcifera* homogenate (20 female equivalent); F, physical damage with the 80% methanol extract of *S. furcifera* (20 female equivalent). Each value represents the mean ± SE (n = 3). Values with different letters are significantly different at $p < 0.05$ according to a Student's *t*-test.

The produce amount of benzyl benzoate of the extract was higher than that of the homogenate and the extract of female only induced the produce of benzyl benzoate. Judging from these results, we concluded that benzyl benzoate was induced by some elicitor being in female of *S. furcifera*. The females of *S. furcifera* were extracted with 80 % MeOH/H_2O. When 20 females equivalent extract was

applied to rice plant (ver. Natsuhikari) after the plant was hurt by 50 of needles, the benzyl benzoate was produced with the formation of watery lesions. This result clearly showed that this phenomenon was induced by elicitor(s) in the females of *S. furcifera* (Fig. 3).

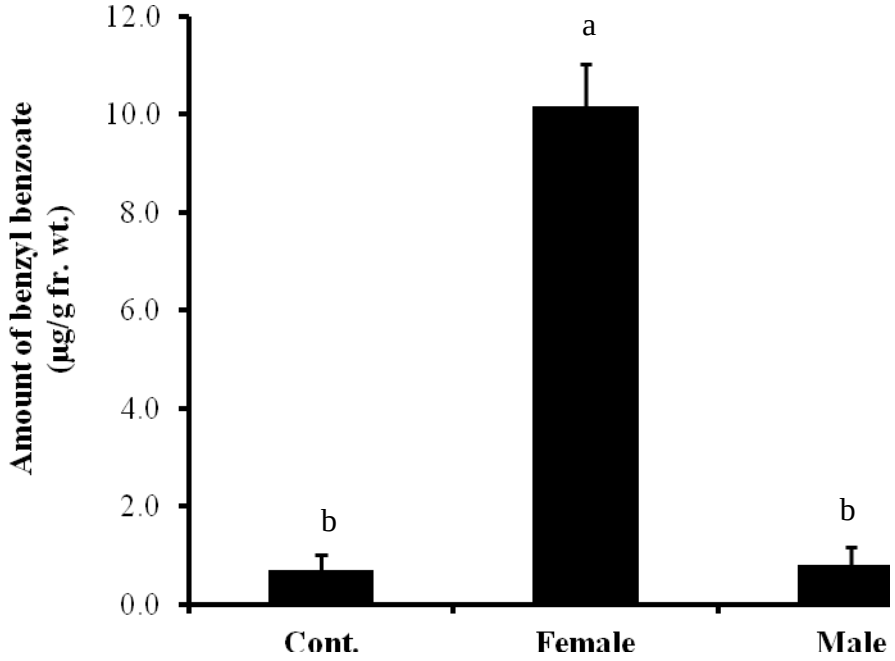

Fig. 3. The amounts of benzyl benzoate induced by methanol extracts of *S. furcifera* females and males. Each value represents the mean ± SE (n = 3). Values with different letters are significantly different at $p < 0.05$ according to a Student's t-test.

For bioassay, we used the rice plants from a climate chamber. The development of rice plant, however, was not good condition and benzyl benzoate was not induced. From this results, we used the rice plants to grow of the vinyl house. It was difficult to control the temperature, day/night time and humidity from this vinyl house. The different conditions made the different development stage of rice plants. Especially, leaf stage of rice plant was not same. This different leaf stage of rice plants occur different induce rate of benzyl benzoate. So, we tested the experiment to get small standard deviation of leaf stages of rice plants. Leaf stage in rice leaf stage 8 and 9, benzyl benzoate was not induced in

the rice extract that injected the extraction of *S. furcifera*. This is still insufficient of the growth of the rice, because it could not have been thought to induce benzyl benzoate. In contrast, 10 and 11 leaf stage of rice that was injected extracts of *S. furcifera* had been induced benzyl benzoate. The rice plant of 11 leaf stage was also induced the benzyl benzoate at the control that injected the water. So, after all bioassay used the rice plant of the 10 leaf stage (Fig. 4).

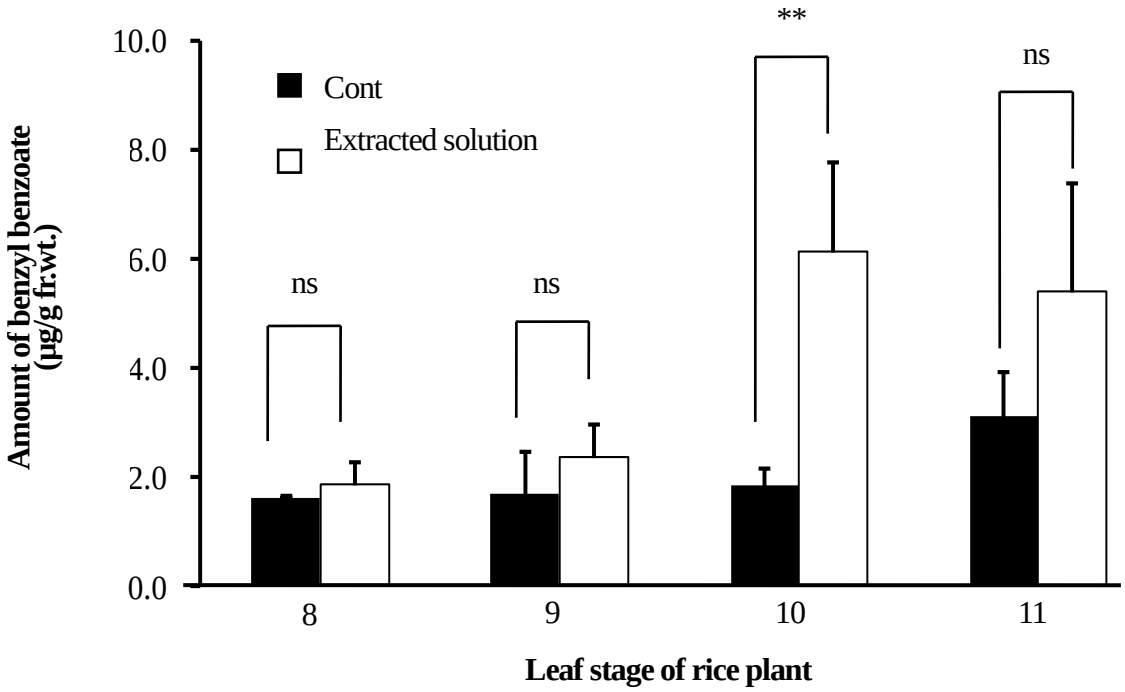

Fig. 4. Rice plant responses to the methanol extract of *S. furcifera* females at several leaf stages. Each value represents the mean ± SE ($n = 6$). ** Indicates statistically significant values ($p < 0.01$) compared with the values for the Cont. group (Student's *t*-test). Not significant (ns).

The active 80% MeOH/H_2O extract was chromatographed on ODS open column and separated into 6 fractions; 100% H_2O, 20% MeOH/H_2O, 60% MeOH/H_2O, 80% MeOH/H_2O, 100% MeOH,

100% EtOH. Of these 6 fractions, 100% MeOH and 100% EtOH fractions evidently induced benzyl benzoate at 43.95 μg/g of fresh rice plant (frp) and 31.04 μg/g frp, respectively. The active 100% MeOH fraction was further separated by reverse phased HPLC into fractions from A to E (Fig. 5).

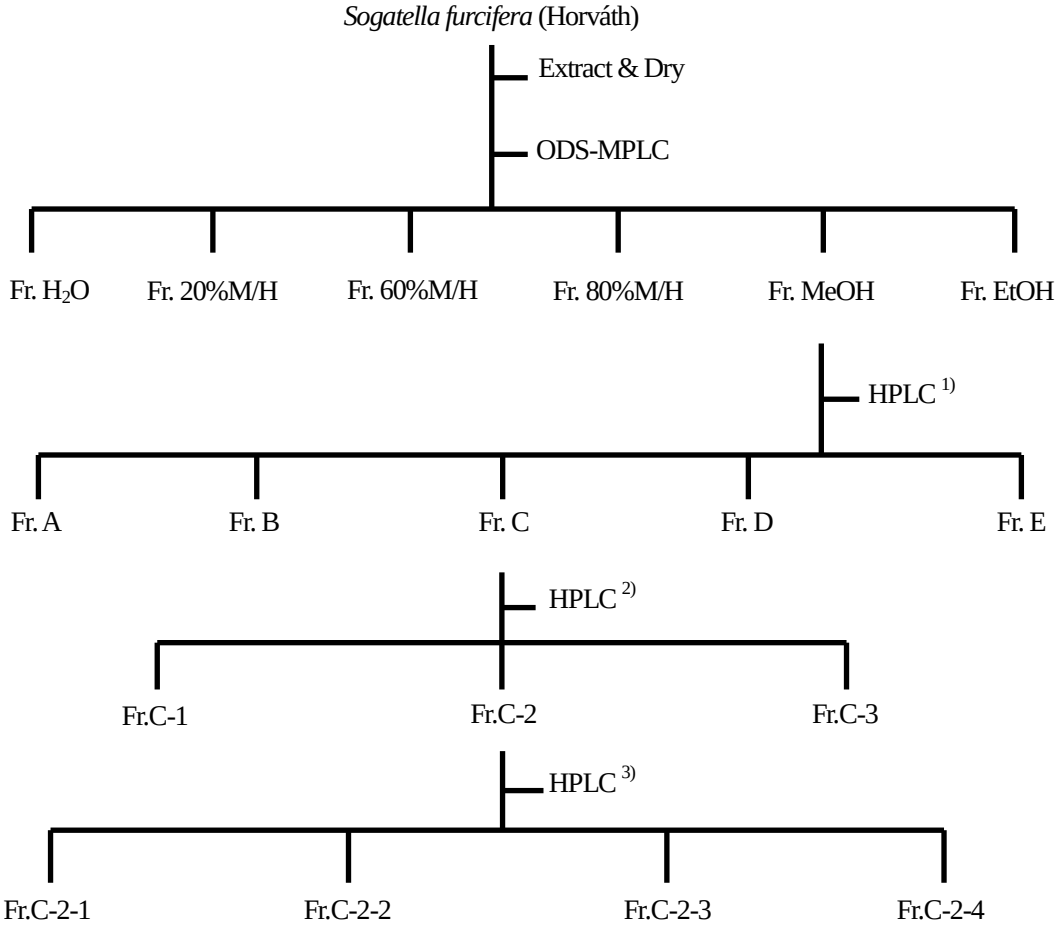

Fig. 5. A fractionation scheme for the extraction and isolation of *S. furcifera*.

The bioassay had the activities of each fraction. Most active C fraction was separated from C-1 to C-3 and each fraction was evaluated by the bioassay. The Highest active compound of C-2 fraction was further divided 4 fractions by HPLC. The Fr-C-2-3 and the Fr-C-2-4 showed the active about bioassay, compounds were submitted to analyses by using NMR and GC- and LC-MS in order to

elucidate the structure. As a result, the structure is confirmed as 1,2-dilinoleoyl-*sn*-glycero-3-phospholcholine(Fig. 6), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine, 1-palmitoyl-2-oleoyl-*X*-glycero-3-phosphoethanolamine, and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine, respectively.

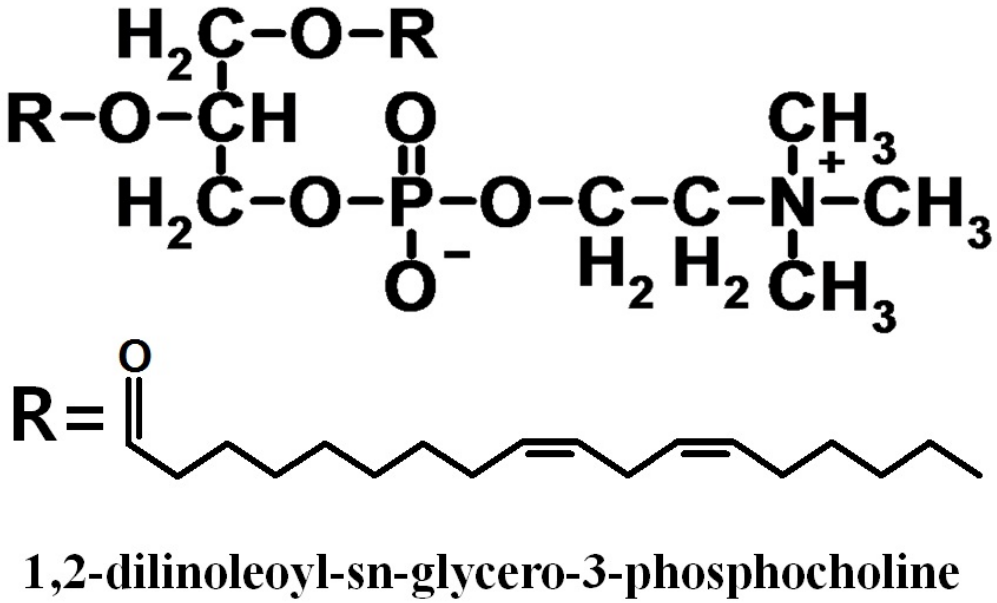

Fig. 6. The structure of compound of Fr-C-2-3.

The other active fraction of frs. C-1, C-3, and E in the 100% methanol fr. separated by using a HPLC and these compounds showed an activity to make an induce production of benzyl benzoate. These 3-compounds elucidated structures as 1,2-dilinoleoyl-*sn*-glycero-3-phosphoethanolamine, 1-stearoyl-2-linoleoyl-*X*-glycero-3-phosphoethanolamine, and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine by using NMR, LC-MS, and GC-MS.

When the active 80% MeOH extract of *S. furucifera* was separated on a reverse-phase ODS-MPLC, the elicitor(s) was eluted in 100% MeOH and 100% EtOH frs. Bioassay-guidanced fractionations lead to isolate two active compounds from the 100% EtOH fr. and the structures of each compound were determined as 1,2-dioleoyl-3-palmitoyl-*X*-glycerol, and triolein respectively (Table 1).

Table 1. The list of structures in each fraction that exhibited an activity

Fractions	IUPAC name	M.F.	M.W. (g/mol)	Equivalent (μg/20)
C-1	1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine	$C_{41}H_{74}NO_8P$	740.0	22.40
C-2-3	1,2-dilinoleoyl-sn-glycero-3-phosphocholine	$C_{44}H_{80}NO_8P$	782.1	40.80
C-2-4	1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine	$C_{37}H_{74}NO_8P$	692.0	3.32
C-2-4	1-palmitoyl-2-oleoyl-x-glycero-3-phosphoethanolamine	$C_{39}H_{76}NO_8P$	718.0	7.96
C-2-4	1,2-dioleoyl-sn-glycero-3-phosphoethanolamine	$C_{41}H_{78}NO_8P$	744.0	5.12
C-3	1-stearoyl-2-linoleoyl-x-glycero-3-phosphoethanolamine	$C_{41}H_{78}NO_8P$	744.0	5.68
E	1,2-dioleoyl-sn-glycero-3-phosphocholine	$C_{44}H_{84}NO_8P$	786.1	4.40
EtOH	1,2-dioleoyl-3palmitoyl-x-glycerol	$C_{55}H_{102}O_6$	859.4	4.04
EtOH	Triolein	$C_{57}H_{104}O_6$	885.4	18.80

From the results, the substance to induce benzyl benzoate is species of the phospholipids and glycerol. We had experiments to combine of all substance by a bioassay. As results, the substance to sum had same activity with the MeOH extraction of *S. furcifera* (Fig. 7).

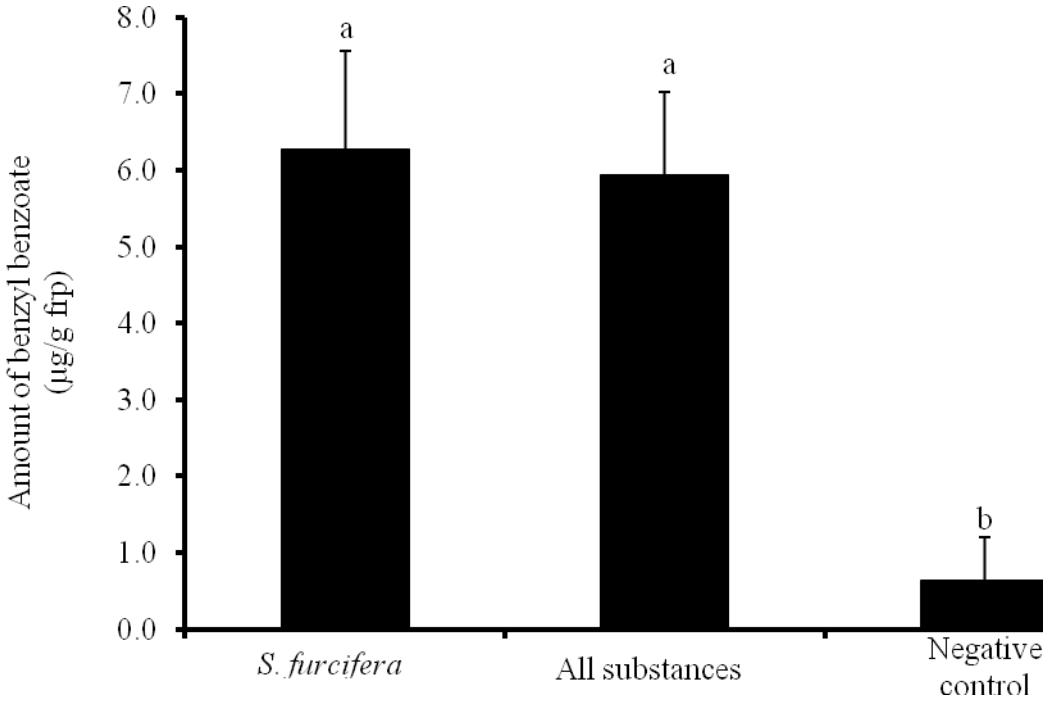

Fig. 7. The amount of benzyl benzoate induced by the *S. furcifera* extract and all substances. Each value represents the means ± SE (n = 9), and the values with different letters are significantly different at $p < 0.05$ according to a Student's t-test.

We find elicitors to induce with *S. furcifera*. The elicitors are in the female of *S. furcifera* and the structures of elicitors are species of phospholipid and glycerol. This results are made us why Japanese rice plants killed only the eggs of *S. furcifera*? We will compare the eggs of *S. furcifera* with the eggs of *N. lugens* and *N. cincticeps*.

Using same bioassay method with *S. furcifera*, we checked the activities with three insects. This bioassay result showed the other insects, *N. lugens* and *N. cincticeps* induced ovicidal compound (benzyl benzoate). This result showed that the induced benzyl benzoate with *S. furcifera* is not specific phenomenon to have only *S. furcifera*. The different resistance with three insects showed different mortality (Fig. 8).

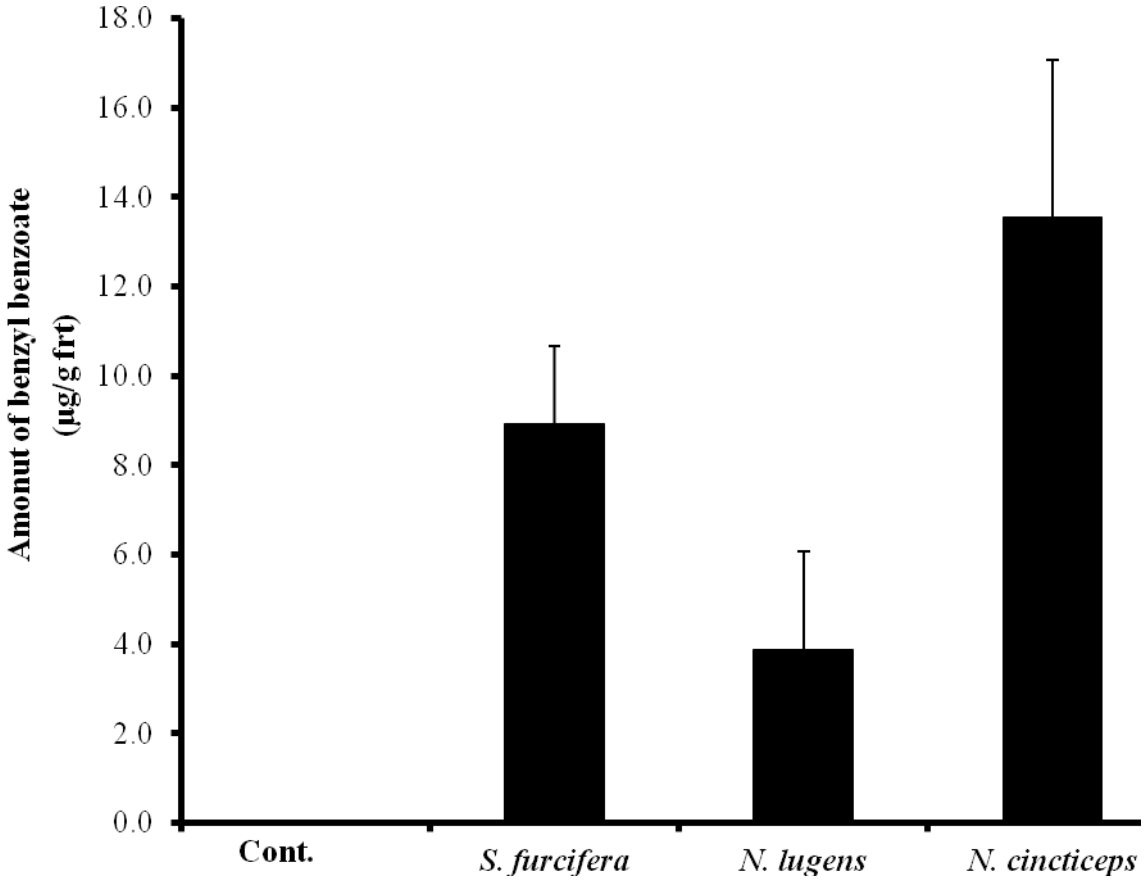

Fig. 8. The activities of *S. furcifera*, *N. lugens*, and *N. cincticeps* eggs. Each value represents the mean ± SE ($n = 3$).

Pure water with 1% triton X100 dissolved the benzyl benzoate because pure water cannot dissolve this compound. As a result, this solution killed more 100 times the number of *S. furcifera* eggs then the eggs from the other two insects with benzyl benzoate (Fig. 9). This result showed that each insect had different degrees of resistance to benzyl benzoate.

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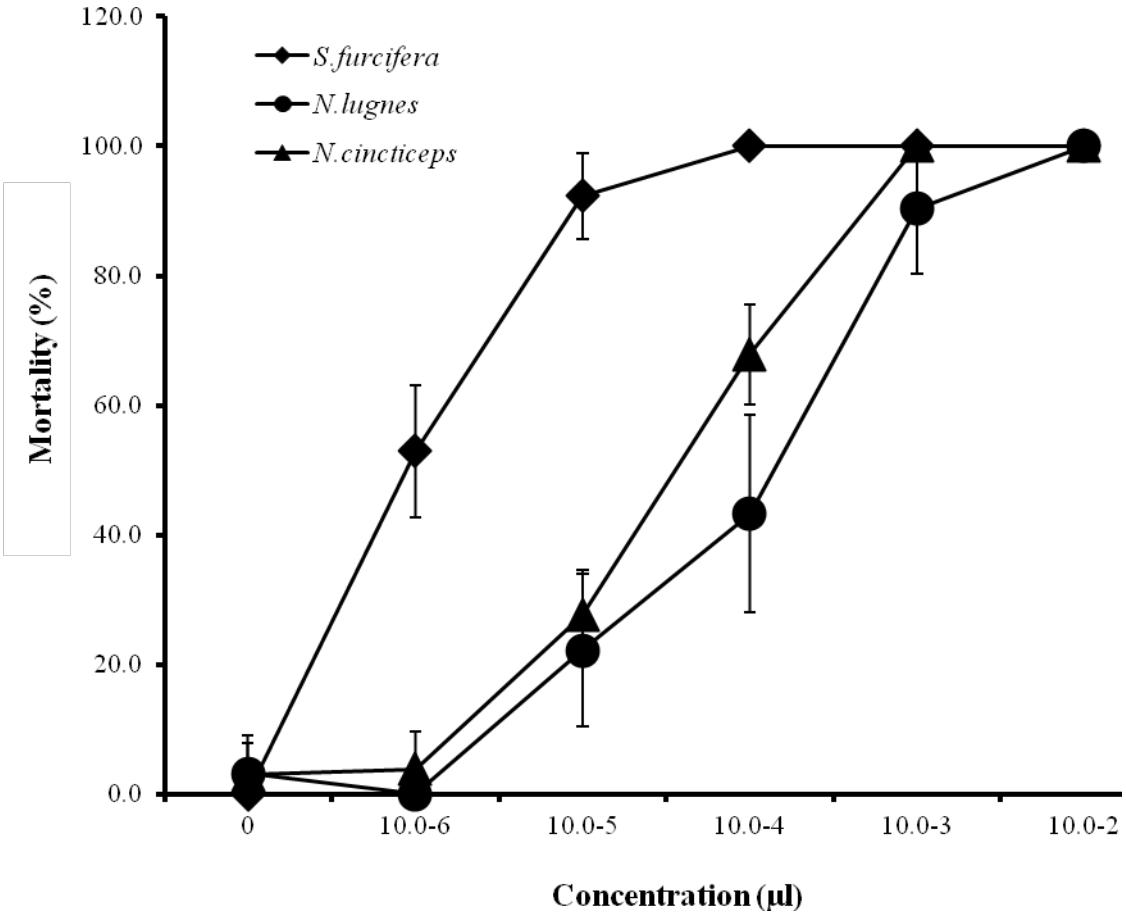

Fig. 9. The mortality of three insects when treated with different amounts of benzyl benzoate. Each value represents the mean ± SE (n = 3).

N. lugens and *N. cincticeps* also have elicitors. However *S.furucifera*'s eggs are 100 times as weak as the others against BB. This is reason why *Japonica* rice plant varieties show a resistance against only *S. furucifera* and kill only the eggs of *S. furucifera*.

(注)　要約の文量は，学位論文の文量の約１０分の１として下さい。図表や写真を含めても構いません。
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