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Germination Control Effect of Bees Wax Preparation Containing Hinokitiol on Garlic Sprout

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The aim of this research is to develop an effective technlogy of which hinokitiol was used for the control of garlic sprout, in order to avoid the economical damage. The evalution method was concretely established by introduction of hydroponics. The production method of wax preparation containing hinokitiol was attempted, to improve the stability of hinokitiol. As the sublimation of hinokitiol was suppressed by the its wax preparation, the stability of hinokitiol was improved. After the wax preparation containing hinokitiol was spread on the butt of garlic, the effect of hinokitiol on the sprout suppression was examined. The dose-dependent fashion was found between the suppression effect of hinokitiol and its garlic germination. The stability of hinokitiol was also investigated using its antibacterial and residual tests in the wax preparation. Hinokitiol in the wax preparation showed the residual rate of 75% for 14 days at 23℃.

1. INTRODUCTION

Vegetables with subterranean stem such as carrot,potato and garlic was often germinated at regular intervals after physiological dormancy during preservation. After their growth, the quality deterioration such as the decrease of the weight and the nutritive lose have been found. In garlic, the harvest was done from June to July. Germination and elongation of the root in garlic was suppressed until the middle of October. However, the germinations and elongations of garlic are opened again toward the end of October and its quality deterioration was promoted. To prevent the quality deterioration in garlic by its

germination and root elongation, germination inhibitors have often been used. However, the improvement of the alternative technology are required at present, as carcinogens have frequently been point out in various germination inhibitors. The establishment of conservation oriented agriculture, which are restricted for use of agricultural chemicals, are also needed.

From the above-mentioned background, Okabe *et al* have examined the development of the control of garlic using hinokitiol [1-4], wood-extracted component which is used as food preservative.

Hinokitiol has been isolated from *Thujopsis dolabrata* var. *hondai* Makino [5] and it has widely been used as cosmetics, toothpaste [6,7] and preservatives [8-10] on account of its broad antibacterial activity [11,12] . In addition to the antibacterial activity, hinokitiol has already been reported to show the germination inhibitory action [2-4] .

In this work, as the link in the chain of manegement system which control the economic damge by garlic germination, the development of effective techniques using hinokitiol is attemped. The concrete measure is the following three points, namely, 1) the establishment of estimation method by introduction of hydroponics, 2) execution of wax preparation containing hinokitiol for the purpose of the improvement of its stability and 3) germination inhibitory activity test using wax preparation containing hinokitiol. The stability of hinokitiol in wax preparation was also estimated by hinokitiol - residual test using antibacterial activity test and spectrophotometer.

2. EXPERIMENTAL METHOD

2.1 Production Method of Bees Wax Preparation Containg Hinokitiol

One hundred gram of bees wax was added to 899g of soybean oil dissolving 1g of hinokitiol and then heated to 60～70℃. Further, the mixture was stirred at 60～70℃ for 2 hours and cooled to produce wax preparation containing hinokitiol.

2.2 Control Effect Test of Wax Preparation Containg Hinokitiol on Garlic Sprout

Garlic used for the germination control effect test were harvested in Aomori Prefecture Tokiwa-mura in July 2003 and dried for 2 weeks at 40℃. The germination control effect on garlic sprout was investigated by introduction of hydroponics.

Two grams of wax preparation containing 100ppm, 1000ppm and 10000ppm of hinokitiol were spread on the butt of garlic respectively, then incubated at 23℃ for 7days. After treatment, the butt of garlic (7samples/group) were soaked in water tank equipped with temperature controller and the moter filter made from the apple charcoal as a quality of the material (bath size: 495×295×150mm, SIBATA Co, Ltd). After 1, 3, and 5 days at 25℃, the length of the bud and the root of garlic were investigated. Each values represented the mean±SD (n=7).

2.3 Hinokitiol-Residual Test in Wax Preparation

Calibration curves was obtained by the following method: One hundred mg of hinokitiol was dissolved in 100ml of tetrahydrofurane (THF) and then 0.2ml, 0.5ml, and 1.0ml of this solution were each diluted in 20ml of THF to concentration of 10, 25 and 50 μg/ml, respectively. The absorbance of each standard solution of hinokitiol were measured at 320nm by

spectrophotometer (Shimazu Uvmini-1240 ultraviolet visibility spectrophotmeter), using THF as a blank. The caribration curve was obtained by plotting the absorbance values (ABS) versus the concentration of hinokitiol(μg/ml).

Next, One g of wax preparation contaning 1000μ g/ml of hinokitiol was spread on 5.5cm Petri dish, then incubated at 23℃. Five hundreds mg of each wax preparation were removed from these dishes at 0, 14, 20, 28 and 35 days after incubated at 23℃ to dissolved in 20ml of THF. The concentration of hinokitiol in the wax was calculated by previously prepared calibration of hinokitiol. The experiments were repeated three times. Each values represented the mean (n=3).

Fig. 1 Germination Control Test on Garlic Sprout by Introduuction of Hydroponics.

2.4 Antibacterial Effect Test of Wax Preparaton Containig Hinokitiol on *Aspergillus* species

The lasting antibacterial effect was investigated according to the following method: Five hundreds mg of the powder of *Aspergillus* species (Akita konno shoten Co) was suspended in 10ml of sterile-distilled water and 2ml of this suspensions was pipeted onto potato dextrose ager (PDA). Then, the filter paper (5mm diameter), which applied wax preparation (concentration: 13.1-20.5mg/cm^2), was placed in the center of PDA inoculated with fungus and the culture were incubated at 35℃ for 30 days.

The inhibition halo surrounding filter paper lager than 5mm in the size were expressed as inhibition of growth.

3. RESULTS AND DISCUSSION

3.1 Control Effect of Wax Preparation Containing Hinokitiol on Garlic Sprout

After wax preparations containing various concentrations of hinokitiol were spread on the butt of garlic, its control effect was estimated by hydroponics (Fig. 1). The results are summarized in Fig. 2. Garlic sprout was not completely found in groups treated at concentration of 10000ppm of hinokitiol for 3 days after treatment and sprout with 23mm was confirmed for 7days. Garlic sprout was not found in groups treated at concentration of 1000ppm of hinokitiol, for 1 day after treatment, and sprouts with 1.3 and 15.8mm were found at 3 and 7 days after treatment, respectively. Garlic sprout was found in groups treated at concentration of 100ppm of hinokitiol at 1day after treatment and the elongation of roots with 3.6 and 51.0mm, respectively, was found. The elongation of roots with 3.8mm was found in control group which was not treated with hinokitiol was seen at 1 day after treatment and their root elongations with 20.6 and 102.9mm was found at 3 and 7 days after treatment.

As mentioned above, wax preparation containing hinokitiol showed strong repressive effect on garlic germination in a dose dependent fashion at concentration range from 100 to 10000ppm of this compound. On the other hand, suppression effect of hinokitiol on root elongation in garlic was not

confirmed even at higt concentration of 10000ppm (data not shown).

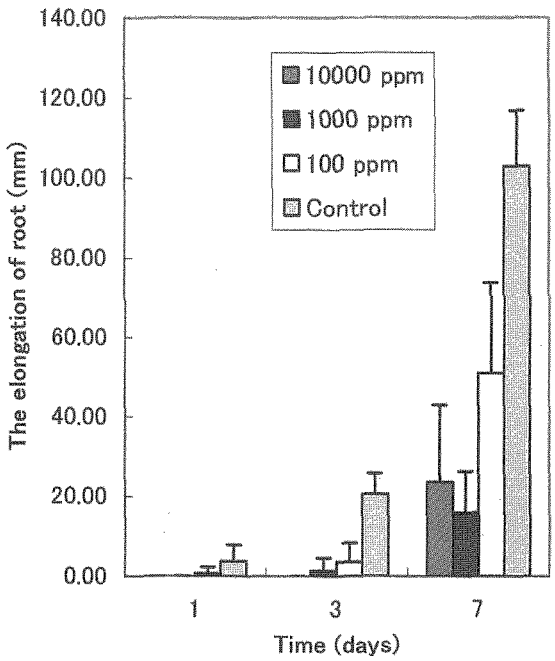

Fig. 2 Germination Control Effect of Wax Preparation Containig Hinokitiol on Garlic Sprout

3.2 Antibacterial Activity of Bees Wax Containing Hinokitiol

The stability of hinokitiol in bees wax containing this compound was investigated by antibacterial activity test on *Aspergillus* species. As shown in Table 1, bees wax containing hinokitiol exhibited lasting antibacterial effect at 35℃ for 7 days.

Table 1. Antibacterial Effect of Wax Preparation Hinokitiol on *Aspergillus* Species

Time (days)	Concentration (mg/cm^2)	Growth[2)]
0	15.1	−
0	14.1	−
1	16.8	−
1	13.1	−
2	15.5	−
2	10.0	−
7	17.6	−
7	20.4	−
14	14.7	+
14	15.9	+
30	20.3	+
30	14.8	+

1) − Inhibition + Growth

3.3 Stablity of Hinokitiol in Wax Preparation

Wax preparation containing hinokitiol inhibited the growth of *Aspergillus* species for 7 days. In order to verify the lasting antibacterial effect in wax containing hinokitiol, its residue was examined using spectrophotometer. As shown in Fig. 3, hinokitiol in wax preparation was decreased with the passage of time, but its residual rate for 7 days showed high level of 75%. From these results, it was found that sublimation of hinokitiol was suppressed in wax preparation and it was stabilized. Above-mentioned lasting antibacterial effect is considered to be due to the stability of hinokitiol.

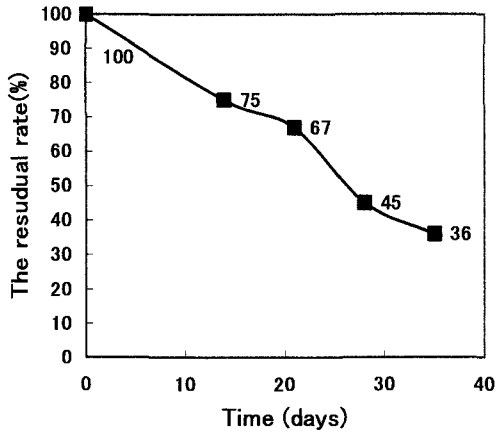

Fig. 3 Residual Rate of Hinokitiol in Wax Preparation

4. CONCLUSION

1) In estimation of suppressive effect of hinokitiol on garlic germination, system about sprout and growth by hydroponics was adopted. By the introduction of hydroponics in garlic for 3 to 7 days, rapid and simple estimation in its sprout was realized.

2) After addition of 10% bees wax to soybean oil dissolving hinokitiol and heating at 60～70℃, wax preparation containing this compound was produced. Hinokitiol content was measured by spectrophotometer.

3) In germination inhibition test by spread of wax preparation containing hinokitiol on butt of garlic, its effect on inhibition showed dose dependent fashion and correlation. On the other hand, suppressive effect of hinokitiol on root elongation was not found even at its high concentration of 10000ppm.

4) Bees wax containing hinokitiol showed lasting antibacterial activity against *Aspergillus* species at 35℃ for 7 days. Hinokitiol content in wax at 23℃ was decreased with the passage of time, but its residual rate at 5 days showed rather high level of 75%. From these results, it was inferred that the sublimation of hinokitiol was suppressed, so that this compound showed lasting antibacterial activity.

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