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Antifungal Activity of Water-Soluble Preparation Containing Hiba Oil on Plant-Pathogenic Fungi

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Hiba oil, extracted through distillation of the wood of *T. dolabrata* Sieb. *et* Zucc. *var* hondai Makino showed clear antifungal activity on five test plant-pathogenic fungi except for *Fusarium solani* and *Botryotinia fuckeliana.*, with a minimum inhibitory concentration(MIC) of 400 μ g/ml. A water-soluble preparation (aqua HB-10) which put containing hiba oil and polyglycerin lauric acid ester that was experimentally produced by improving the water solubility of hiba oil. also showed antifungal activity against four kinds of fungi, particularly for *Thanatephorus cucumeris.*

The preventive effect of aqua HB-10 on cucumber powdery mildew was also evaluated through pot and greenhouse tests. In the pot test, the diseased area observed as a result of a foliar spray application of aqua HB-10 at diluted 300 times (hiba oil concentration: 333 μ g/ml) was only 69.6% of that observed on controls only with water spray application.

In the greenhouse test, aqua HB-10 (diluted 300 times) applied twice at 7-day intervals clearly reduced the development of the cucumber powdery mildew compared with untreated control. No phototoxic symptoms were also observed on cucumber leaves after said applications.

Key words: water-soluble preparation, hiba oil, antifungal activity, plant-pathogenic fungi, cucumber powdery mildew.

1. INTRODUCTION

Agricultural chemicals is often used to control insects and pathogens that damage the crops of vegetables. However, conservation-oriented agriculture, for which the use of agrochemicals is restricted must now be established to reduce environmental impact.

Given this background, Okabe *et al* have examined how to control plant-pathogenic fungi by using hiba oil obtained through distillation of the wood of *T. dolabrata* Sieb. *et* Zucc. *var* hondai Makino [1] .

Hiba oil is commonly used as a raw material for cosmetics, shampoo, and preservatives due to its broad antibacterial activity. In addition to its antibacterial activity, hiba oil has reportedly shown insecticidal and acericidal effects [2] as well.

In this work, an attempt was made to develop effective control techniques utilizing hiba oil as a link to the management system being investigated to control the disease damage caused by pathogenic fungi. The approach to this development was made based on the following three standpoints: 1)Antifungal activity of hiba oil on plant-pathogenic fungi. 2) Production of water-soluble preparation containing hiba oil to improve the solubility of hiba oil in water,and 3) Control effect of the water–soluble hiba oil on cucumber powdery mildew which develops in most countries where cucumber is grown as a crop(as confirmed through pot test and green house tests).

2. EXPERIMENTAL METHOD

2.1 Production of Water-Soluble Preparation (Aqua HB-10) Containing Hiba Oil

Twenty grams of polyglycerin lauric acid ester (produced by Nikko Chemicals, Co, Ltd.) were added to 18 grams of ethyl alcohol dissolved in 10 grams of hiba oil obtained by distilling the wood of *T. dolabrata* Sieb. *et* Zucc. var *hondai*. Makino. and 2 grams of glycerin(produced by Sakamoto Yakuhin Kogyo Co.,Ltd.).Then 50 grams of ion-exchange water were added to this mixture, and stirred at 20 to 30℃ for one hour.

2.2 Antifungal Activity of Hiba Oil and Aqua HB-10 on Plant-Pathogenic Fungi

Each test fungus was cultivated at 24℃ for 14 days on slant agar containing potato extract (0.4%) and glucose(2%), and controlled to a pH of 6 in a test tube.

Then 200 mg of the preparation was dissolved in 10 ml of ethyl alcohol, with the solution diluted stepwise with ethyl alcohol. Next,0.2 ml of the diluted solution was added to 20 ml of melted agar (pH 6) containing potato extract(0.4%) and glucose (2%), then, the mixture was poured into a petri dish 90-mm in diameter and allowed to cool.

Approximately 10 ml of sterilized water containing 50-μ g/ml nontoxic wetting agent was introduced into the test tube and the spores were brought into a loopfull suspension by gently rubbing the spore layer with an inoculating hook. This suspension was then inoculated on the agar plate in a 5-cm streak. The fungi thus inoculated on the agar plate were cultured at 24℃ for 14 days, and MIC for them were determined by visual inspection.

2.3 Control Effects of Water-Soluble Preparation Containing Hiba Oil (Aqua HB-10) on Cucumber Powdery Mildew

Control effects of aqua HB-10 on cucumber powdery mildew were evaluated through foliar spray tests (i.e., pot and greenhouse tests).

For the pot test, aqua HB-10 diluted 300 times (hiba oil concentration: 333 μ g/ml) and hiba oil suspended in 1% polyoxyetylene sorbitan mono oleic acid ester (Tween80) by water diluted 3000 times (hiba oil concentration: 333 μ g/ml) were respectively sprayed on the leaf surfaces of 3-week-old potted cucumber (three plants for each treatment) one day after spraying isolated powdery mildew. The development of disease on each leaf was recorded seven days later. The following disease index (DI) was used 0 for no lesion; 1 for 1 to 5% of leaf surfaces covered by the fungus; 2 for 25%: 3 for 25 to 50%, and 4 for more than 50%. Disease severity (DS) was calculated with the following formula:

Disease severity = Σ (total of index values respectively multiplied by the number of corresponding leaves) /(4 × the total number of leaves)

Control (%) = [(DS on untreated leaf − DS on treated leaf)/ DS on untreated leaf] × 100

For field-testing, cucumber plants (cultivar: alufa10) were transplanted in soil in a field at Agricultural Food and

Environmental Sciences Research Center of Osaka Prefecture, Japan on August 21, 2003.

Aqua HB-10 diluted 300 times (250L/10a) was sprayed on the leaf surfaces 39, 46 and 53 days after the transplant, and 46, 53, and 60 days later, disease severity (DS) and control (%) were recorded in the same way as described above. Triflumizole emulsifiable concentrate diluted 2000 times was used as a positive control.

3 RESULTS AND DISCUSSION

3.2 The Antifungal Activity of Hiba Oil and HB-10 on Plant-Pathogenic Fungi

The antifungal activity of hiba oil on plant-pathogenic fungi was investigated using the agar dilution method. As listed in Table 1, hiba oil showed antifungal activity on five strains except for *Fusarium solani* and *Botryotinia fuckeliana*, with a minimum inhibitory concentration of 400 μ g/ml.

Table 1 also lists the antifungal activity of water-soluble hiba oil (aqua HB-10) against the same strains. This water-soluble preparation also showed antifungal activity on four plant-pathogenic fungi. It completely inhibited growth of *Thanatephorus cucumeris* even when diluted 1,600 times (hiba oil concentration: 62.5 μ g/ml). The antifungal activity against these plant-phathogenic fungi was thus enhanced by combining hiba oil and polyglycerin lauric acid ester. Although the reason for this enhancement was not clear at this stage of the research, T. Okabe *et al* have already reported on this finding, including the growth-inhibitory effect on plant-pathogenic fungi *Helicobasidum mompa* [3] and *Rosellinia necatrix* [4]. The antifungal activity of hiba oil against other plant- pathogenic fungi should also be investigated.

3.3 Pot Test and Greenhouse Test

In the pot test, foliar application of aqua HB-10 at diluted 300 times controlled *cucumber powdery mildew* caused by *Sphaerotheca fuliginea* (Fig.1) [5], and reduced the diseased area to 69.6% of that when only using water spray 7 days after treatment.

Table 1. Antifungal Activity of Aomori hiba oil and water-soluble preparation containg h on Plant-Pathogenic Fungi.

Plant-pathogenic fungi	MIC	
	Hiba oil[a]	aqua HB-10[b]
Pythium aphanidermatum IFO 32440	400	800 (125)[c]
Thanatephorus cucumeris IFO 30455	400	1600 (62.5)[c]
Fusarium solani IFO 9955	>400	200 (500)[c]
Botryotinia fuckeliana IFO 30915	>400	400 (250)[c]
Phomopsis obscurans MAFF 744018	400	N.T.[d]
Colletotrichum orbiculare MAFF 306518	400	N.T.[d]
Colletotrichum lagenarium	400	N.T.[d]

Potato dextrose agar medium, incubated at 24℃ for 15d.
a) Minimum inhibitory concentration (MIC:μg/ml) was determined by the agar dilution method.
b) diluted times
c) hiba oil concentration: μg/ml
d) N. T.:Not tested

Conversely, the control effect of hiba oil suspended in 1% polyoxyetylene sorbitan mono oleic acid ester (Tween80) by water against these pathogenic fungi was lower than that of aqua HB-10. This finding is believed to be due to differences treatment.

Further studies on the surfactant effect of antifungal activity of hiba oil are needed.

In a-field (greenhouse) test conducted to evaluate the effect of aqua HB-10 on cucumber powdery mildew, aqua HB-10 was found to effectively improve cucumber plants under moderate disease severity of cucumber powdery mildew slightly less than the effect of triflumizole emulsifiable concentrate, which was used as a positive control. However, HB-10 (diluted 300 times) applied (2 sprays) at 7-day intervals clearly reduced the development of cucumber powdery mildew compared with the untreated control (Table 2), showing an effect of 71.6%. No phytotoxicity was observed on the tested plants.

Conversely, the surfactant components other than hiba oil (SA-20) did not show a preventive effect against cucumber powdery mildew. This finding suggests that hiba oil serves as an active antifungal component of this preparation.

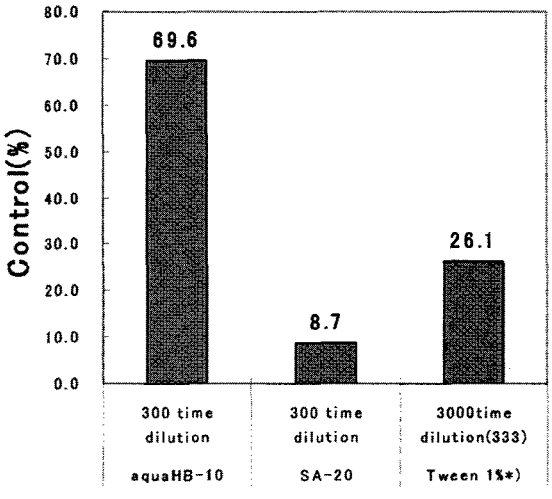

Fig. 1 Control effect of hiba oil on cucumber powdery mildew (in pot test)

Table 2 Efficacy of aqua-HB-10 on *Cucumber Powdery Mildew* (Greenhouse test)

Treatment	dilution time	leaves number of treatments	Disease severity (%)	Control (%)	Phytotoxity
aqua HB-10	300	100	12.5	18.3	non
		150	13	71.6	non
		150	52.7	44.6	non
SA-20	300	100	12.3	19.6	non
		150	29	36.5	non
		150	61.7	35.1	non
Triflumizole emulsifiable concentrate	2000	90	6.4	58.2	non
		135	5.2	88.6	non
		135	29.8	68.7	non
control	-	90	15.3	-	non
		135	45.7	-	non
		135	95.2	-	non

Experiment size: 5 plants/group, 2 groups (100-150 leaves treatments).

However, the conditions of cucumber plants suffering from serious cucumber powdery mildew were not improved even when HB-10 was sprayed three times in each application.

These findings have showed that spray application of HB-10 at 7-day intervals have a preventive effect on cucumber powdery mildew of less than moderate diseases severity.

4. CONCLUSION

1) Hiba oil showed antifungal activity on five Fungus strains, with a minimum inhibitory concentration of 400 μ g//ml.

2) Polyglycerin lauric acid ester was added to ethyl alcohol dissolved in hiba oil, followed by the addition of glycerin, and then stirred at 20 to 30℃ for one hour to produce a water-soluble preparation [aqua HB-10] . This water-soluble preparation showed strong antifungal activity particularly on *Thanatephorus cucumeris* and completely inhibited the growth of this fungus even when diluted 1,600 times (hiba oil concentration: 62.5 μ g/ml).

In pot test, an application of aqua HB-10 containing hiba oil diluted 300 times reduced the extent of powdery mildew by 69.6% compared to controls using only water spray (Fig. 1).

In the greenhouse test, aqua HB-10 applied at 7-day intervals clearly reduced the development of cucumber powdery mildew compared with the untreated control (Fig. 1).

Further studies should also be conducted on applying hiba oil to other crops, such as melon and tomato. The effect of hiba oil on other cucumber pathogens is now under investigation.

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