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Germ Metabolites and Plant Extracts as Aphicide, their Formulation and Tests for Stability.

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Abstract: From studying aphicidal activity in bioassays, we developed a compound from germ metabolites and plant extracts, abbreviated as "G-P aphicide". The G-P aphicide is composed of culture concentrates of *Paecilomyces griseiviride*, datura (*Datura stramenium*) extracts, and Chinese prickly ash (*Zanthoxylum bungeanum*) extracts in the ratio of 5:1:0.2. The aphicidal activity of 1:400 diluted G-P aphicide against *Lipaphis erysimi* was 97.9% 48 hours after spraying. The G-P aphicide lost 7.17% and 10.8% of its aphicidal activity after six and 12 months' storage at room temperature, respectively.

Keywords: Germ metabolites and plant extracts compound aphicide, G-P aphicide, stability
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Aphids are among the important harmful insects to agriculture, forestry, and horticulture, causing widespread, severe damage themselves, and spread several viruses as well [1]. In order to meet the demand for organic foods, and fruits or vegetables free of insectice residues, the development of new, environmentally friendly bioaphicides is a research area of significant importance. We started our research in this area in 1993 and isolated a *Paecilomyces griseiviride* strain U-2 with high aphicidal activity. The ecological and physiological conditions for its growth, development and production of active aphicidal compounds were carefully studied [3]. Forty-five plants selected from 118 plant species belonging to 45 families showed aphicidal activity. Among these 45 plants, 12 had significant aphicidal activity.

The addition of certain plant extracts at a specific level to the concentrates of *P. griseiviride* strain U-2 culture supernatant could markedly increase the aphicidal activity of the concentrates. Thus, we developed the germ metabolites and plant extracts compound aphicide with ideal aphicidal activity, which we abbreviated as G-P aphicide. The results of our research work are briefly reported as follows.

1. Material and Method

1.1 Preparation of concentrates of culture supernatant. Potato dextrose broth with a pH value of 6.0 supplemented with 0.18% $MgCl_2$, 0.3% sodium citrate, and 0.4% beef extract was inoculated with the spore suspension of *P. griseiviride* strain U-2. The inoculated broth was cultured on a rotary incubator rotating at 200 rpm at 26-28 °C for 6 days. After removing

mycelia by filtration or centrifugation, the culture supernatant was concentrated 5 times under reduced pressure.

1.2 Plant extracts tested. The plant extracts with aphicidal activity used in these experiments were prepared from plants selected from 118 plant species (Table 1). Solvent extraction followed the procedures reported by Zhang[2].

1.3 Aphid species used. The test species *Lipaphis erysimi* was fed and reproduced on radish seedlings in a greenhouse.

1.4 Bioassay method. Aphicidal activities of the test preparations were expressed as corrected reduction in aphid density or 50% lethal concentration (LC_{50}) 48 hours after spraying. About 160-250 aphids were used in each treatment, and each treatment was replicated three times.

2. Results and Conclusions

2.1 Effects of single plant extract additions on aphicidal activity of germ metabolites. Equal amounts of plant extracts were added to the culture supernatant concentrated 5 times (G), and 1: 400 diluted solutions were used for bioassay. Results (Table 2) showed that the addition of extracts of rosebay, datura, wartwort, black falsehellebore, and gingko significantly increased the aphicidal activity of the germ metabolites (G). The addition of rosebay extracts gave the best results, followed by the addition of datura extracts. The aphicidal effects of adding different preparations of datura extracts to G were also tested. The results showed that the addition of extracts prepared by 60 °C water extraction (twice) achieved better aphicidal results. The addition of prickly ash extracts to G slightly but not significantly increased the aphicidal activity, and the extracts prepared by different methods showed similar results. The addition of sweet wormwood extracts and rhubarb extracts only very slightly increased the aphicidal activity of G or may even decrease its aphicidal activity. Thus we inferred that there might be some interactions between the active ingredients in the plant extracts and those in the germ metabolites, because the extracts of sweet wormwood and rhubarb had showed good aphicidal activity alone.

Table 1. Plant extracts tested

Serial #	Plants	Parts used	Extracting method	Plant tissue Wt (g) /raw extracts (mL)
1	Black falsehellebore	Whole plant	Extract with 60 ºC water	1(fresh)/3
2	Stemona root	Tuber	Extract with 60 ºC water	1(dried)/5
3-1	Sweet wormwood	Whole plant	Water Decoction (reflux)	1(dried)/5
3-2	Sweet wormwood	Whole plant	Extract with ethanol	1(dried)/5
4-1	Datura	Stem, leaf, fruit	Extract with 60 ºC water	1(dried)/5
4-2	Datura	Stem, leaf, fruit	Water Decoction (reflux)	1(dried)/5
5	Rosebay	Leaf, bark	Water Decoction (reflux)	1(fresh)/2
6	Wartwort	Whole plant	Ground & extract with Water	1(fresh)/2
7-1	Chinese prickly ash	Leaf, pericarp	Extract with 60 ºC water	1(dried)/5
7-2	Chinese prickly ash	Leaf, pericarp	Eextract with ethanol	1(dried)/5
8	Rhubarb	Root	Water Decoction (reflux)	1(dried)/5
9	Gingko	Pericarp	Extract with Water	1(fresh)/3

Table 2. Effects of single plant extracts addition on aphicidal activity of germ metabolites

Group	Dilution factor	Aphicidal activity (%)
G	250	87.2
	400	62.8
	800	54.5
G+1	400	81.8
G+2	400	58.2
G+3-1	400	60.4
G+3-2	400	63.1
G+4-1	400	86.4
G+4-2	400	85.1
G+5	400	88.0
G+6	400	81.2
G+7-1	400	66.2
G+7-2	400	68.1
G+8	400	56.2
G+9	400	80.2

2.2 Effects of different germ metabolites to plant extracs ratios on their aphicidal activities. Based on the results in section 2.1, the datura extracts (60 ºC water) was chosen as the study material to mix with the G at different ratios. Results in Table 3 showed that the mixed aphicide had the highest aphicidal activity when the concentrated culture supernatant to datura extracts ratio was 5 to 1 (the aphicidal activity of 1: 400 diluted solution was 90.5% 48 hours after application).

Table 3. Effects of mixing ratios on the aphicidal activity

Ratio	Dilution factor	Aphicidal activity (%)
20:1	400	71.2
10:1	400	81.3
5:1	400	90.5
2.5:1	400	88.6
1:1	400	86.4
1:1.25	400	73.9
1:5	400	67.2

2.3 Effects of addition of compound plant extracts on aphicidal activity. Based on the ratio determined in section 2.2, the effects of adding additional plant extracts on the aphicidal activities of the mixture were observed. Results of repeated experiments showed (Table 4) that addition of wartwort, or prickly ash, or gingko extracts at appropriate ratios could further improve the aphicidal activity of the mixtures. The mixture of G with datura extracts and wartwort extracts (5:1:0.5) showed the best aphicidal effects (1: 400 diluted solution with an aphicidal effect of 98.8% 48 hours after application). The mixture with addition of prickly ash extracts showed the second highest aphicidal effects. The aphicidal effects of the prickly ash extracts prepared by different methods gave similar results. The optimum mixing ratio of G to datura extracts and prickly ash extracts was 5:1:0.2.

Table 4. Effects of compound plant extracts on aphicidal activity

2nd plant extracts added	Adding ratios	Aphicidal activity (%)
Wartwort(6)	5:1:1	93.1
	5:1:0.5	98.8
	5:1:0.2	92.5
	5:1:0	89.8
Chinese prickly ash (7-1)	5:1:1	87.2
	5:1:0.5	94.4
	5:1:0.2	97.9
	5:1:0	89.8
Chinese prickly ash (7-2)	5:1:1	88.9
	5:1:0.5	96.0
	5:1:0.2	98.1
	5:1:0	89.8
Gingko (9)	5:1:1	94.6
	5:1:0.5	93.4
	5:1:0.2	90.1
	5:1:0	89.8

Since the cost of solvent extraction is higher, its technical procedures are more complicated, and organic solvents are inflammable, we decided to use the 60 ºC water extracting method to prepare prickly ash extracts. Based on the results of our studies and considering other factors such as lowering production costs, simplifying technical procedures, and the readiness of raw materials, we formulated our germ metabolites and plant extract compound aphicide as follows: 5 times concentrated culture supernatant (G): datura extracts (extracted with 60 ºC water): prickly ash extracts (extracted with 60 ºC water) in the ratio of 5:1:0.2, which was abbreviated as G-P aphicide.

2.4 Tests on the stability of G-P aphicide. The G-P aphicide was prepared in our laboratory according to the procedures and ratio described in section 2.3. After storing at room temperature for a certain period of time, its aphicidal activity was tested with *Lipaphis erysimi*. We tried our best to maintain identical or similar experimental conditions. The results in Table 5

Table 5. Effects of storage on aphicidal activity of G-P aphicide

Storage time (Month)	LC_{50} (dilution factor)	Aphicidal activity loss (%)
CK (0)	2009	
2	1968	2.04
6	1865	7.17
12	1792	10.80

indicated that the G-P aphicide lost 2.04% of its aphicidal activity after 2 month's storage at room temperature; and it lost 7.17% and 10.80% after 6 months and one year storage. The losses in aphicidal activity for over one year of storage need to be further studied.

References

1) Editorial committee for the illustrated dictionary of major pests on crops and forests in Shandong. *The Illustrated dictionary of pests on major crops in Shandong*. Jinan: Shandong People's Press. 1977, 52-64.

2) Zhang, Jijie. *Chemistry of Traditional Chinese Medicine (second edition)*. Beijing: People's Public Health Press, 8-10.

3) Dai, Meixue and Zu, Aimin. 1995. Studies on the growth conditions of *Paecilomyces griseiviride* strain U-2 and its contagious ability on aphids. *Journal of Microbiology*. 1997, 17(3): 37-40.