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Culture Conditions Effect on Ferment Liquid Activity of *Paecilomyces griseoviride* Strain U-2 against Aphids

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Abstract Based on the result of biological assays, the current study determined the suitable conditions of *Paecilomyces griseoviride* strain U-2 to produce active substance for aphid control. The conditions were: Potato Dextrose Agar (PDA) media with initial PH 6.0 added in 0.18% $MgCl_2$, 0.3% citrate sodium and 0.4% beef extract; the strain U-2 of *Paecilomyces griseoviride* was cultured for 6 days on the PDA media in culture flasks and shaken in 26-28 ℃, at 200 rpm.

Key words: *Paecilomyces griseoviride,* activity against aphid, culture condition

Gray-green mould, *Paecilomyces griseoviride* strain U-2 was isolated by the author from cotton aphid *Aphid gossypii* Glover, that died due to disease infection. Biological assay proved that the ferment liquid of *P. griseoviride* had an active substance for aphid control [2]. In order to develop environmentally safe pesticides against aphids on crops, forests, fruits, vegetables, *etc.* this study explored the conditions for producing the active substance of *P. griseoviride*. A rod-shaped crystal substance (element A), dissolvable in grease and active against aphids, was isolated from the ferment liquid of *P. griseoviride* with methods of active carbon adsorption, methanol elusion, HPLC isolation and extraction. Another water dissolvable component existed, which was proofed by repeated extraction with acetic ether. However, no effective method was found to quantify the active component. We used the method of biological assay, based on the effect on aphid control, to select and optimize the conditions for strain U-2 of *P. griseoviride* producing the active component against aphids.

1 MATERIAL AND METHOD

1.1 Material

1.1.1 **Strain** *P. griseoviride* strain U-2 (isolated from dead cotton aphid, *Aphid gossypii* Glover, and stored by author).

1.1.2 **Aphids for the experiment** Aphids were fed on young vegetable plants cultured in pots in a greenhouse.

1.2 Method

1.2.1 Culture with flask-shaking

P. griseoviride strain U-2 was cultured using the following steps: we poured 100ml liquid culture media into a 500 ml triangle culture flask; covered and bound 6 layers of gauze on the mouth of the flask; sterilized the flask with the culture liquid; inoculated 2ml of U-2 conidium suspending liquid ($5X10^8$ conidia ml^{-1}) into the sterilized culture liquid in the flask; cultured with the method of flask shaking in 26±1° C, at 200 rpm for 6 days; made up water loss caused by evaporation to 100ml; centrifuged the culture liquid of U-2 at 3000rpm for 20 min; diluted the upper part of U-2 liquid to 50 times; and carried out biological assays of strain U-2.

1.2.2 Activity assay for aphid control

The dipping method was used in this experiment. Each time 100-300 aphids were tested, with three replications. The corrected rate of aphid reduction in 48h presented the activity of strainU-2 against aphids.

2 RESULTS AND DISCUSSION

2.1 Effect of the culture medium types on activity against aphids

Various liquid media were poured into 500 ml triangle culture flasks separately, 100 ml for each. Six layers of gauze were covered and bound on the mouth of flask. Two ml of conidial suspension ($5X10^8$ conidia ml^{-1}) of strain U-2 were inoculated into the flask. Then the flasks were sterilized. The conidiophores of strain U-2 were cultured with the method of flask shaking in 26±1° C, at 200 rpm for 6 days. The culture liquid was

centrifuged at 3000rpm for 20 min. The upper part liquid of U-2 conidiophores was diluted to 50 times. The results (table 1) indicated that among the 7 kinds of culture media, PDA was the best, in which *P. griseoviride* strain U-2 could produce the most active component against aphid. The bran medium took the second place, following PDA.

Table 1 Effect of the culture media type on the activity against aphid

No.of media	Name of media	Activity against aphid (%)
1	PDA	65.8
2	Ca	22.9
3	Bran	57.5
4	Soybean powder cake steep	46.8
5	Maize powder	27.8
6	Turnip juice	20.1
7	Bean sprout	35.8

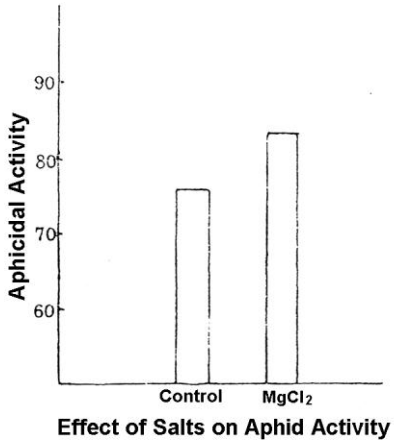
2.2 Saccharide adding effects on the activity of aphid control

Only the types of sugars in PDA were changed; the other ingredients were kept the same as described in 2.1. The results (table 2) showed that different kinds of sugars in potato culture medium affected aphid control activity. Among the different sugars, sucrose was the best, which led to the highest activity.

Table 2 Effect of adding saccharide on activity against aphid

Saccharid added	Activity against aphid (%)	Ratio of activity against aphid*
Glucose	65.8	1.000
Lactose	55.3	0.840
Galactose	51.3	0.780
Sucrose	76.6	1.164
Maltose	66.5	1.011

*The ratio was defined as activity against aphids of each tested saccharide / activity against aphids on potato-glucose medium.



Effect of Salts on Aphid Activity

2.3 Effect of salts on aphid activity

In our research we noticed that adding some salt to the medium had certain effects on *P. griseoviride* strain U-2 producing component in the ferment liquid, which had activity against aphid. More than 20 kinds of salts (single or mixed) were assayed repeatedly. It was found that adding 0.18% $MgCl_2$ and 0.3% citrate sodium in PDA had significant enhancing activity against aphids (Fig.1).

2.4 Beef extracted cream and bran steep effects on activity against aphids

Adding certain amounts of beef extracted cream into PDA containing 0.18% $MgCl_2$ and 0.3% citrate sodium could enhance the activity of the ferment liquid against aphids. The results in table 3 show that adding 4g beef extracted cream or 10g bran resulted in the highest activity against aphids. However, when adding beef extracted cream and bran steep simultaneously in culture medium, the activity of the component was not obviously improved compared with only adding 0.4% beef extracted cream, even though the proportion of two ingredients were adjusted several times [sic.].

Table 3 Beef extracted cream and bran steep effects on the activity against aphids

Added ingredient	Dose (g/L)	Activity against aphid (%)	Ratio of activity against aphid*
Beef extracted cream	2	87.9	1.069
	4	89.4	1.088
	8	86.2	1.049
	10	81.9	0.996
Bran steep	5	86.2	1.049

	10	88.7	1.079
	15	85.4	1.039
	20	85.5	1.040
CK	0	82.2	1.00

* The ratio represented the activity in ingredient adding group / the activity in control (ck).

2.5 PH of culture media effects on the activity against aphid

The PDA with 0.18% $MgCl_2$, 0.3% citrate sodium and 0.4% beef extracted cream was selected to adjust the PH to different values. Every 500ml triangle culture flask with 80ml adjusted PH liquid media was sterilized. *P. griseoviride* strain U-2 was inoculated with the same quantity of spore suspension and was cultured with the shaking method in 26±1° C, at 200 rpm for 6 days. The assay results (table 4) indicated that the culture medium with initial PH 6 – 6.5 could produce the most active component in the ferment liquid by *P. griseoviride* strain U-2. When the initial PH value was more than 8.0, no activity occurred.

Table 4 PH of culture media effects on the activity against aphids

PH of culture media	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
Activity against aphid (%)	52.3	74.2	81.6	89.4	88.9	62.8	26.2	—

2.6 Ventilation effects on the activity against aphid

The optimal medium selected previously was used for this experiment. PH was adjusted to 6.0. Different amount of media were poured into 500ml triangle flasks respectively. Six layers of gauze were covered and bound on the mouth of each flask. Strain U-2 was cultured with the method of flask shaking in 26±1° C, at 200 rpm for 6 days, making up water loss caused by evaporation. After centrifugation the upper part liquid with 50 times dilution was assayed. The results (table 5) showed that 80-100ml medium in one 500ml triangle flask could gain the highest activity against aphids. Over 120ml in one flask, the more medium was poured into a flask, the lower the activity of component produced by U-2. The 30ml medium in one flask dried on the fourth day of culture due to evaporation. It did not show activity against aphids when water was added to the dry medium and centrifuged on the sixth day.

Table 5. The amount of liquid medium effects on the activity against aphids

Content (ml/500ml)	Activity against aphid (%)
30	—

50	83.9
80	89.3
100	89.2
120	85.2
150	78.3
180	68.8
200	57.6

When the medium remained at 100ml per flask and we changed shaking speed, the results (table 6) revealed that the activity of ferment liquid against aphids was raised while shaking frequency was increased to the range of 30-200 rpm. As the shaking frequency was raised to 230, rpm the activity appeared to decline.

Table 6. Effect of shaking frequency on activity against aphids

Shaking frequency (rpm)	Activity against aphids (%)
30	47.2
50	56.2
100	65.2
120	78.6
150	80.6
200	89.1
230	87.8
250	87.6

2.7 Culture temperature effects on the activity against aphids

Potato - sucrose medium (PH 6.0) consisting of 0.18% $MgCl_2$, 0.3% citrate sodium and 0.4% beef extracted cream was used for this experiment. Each 500ml triangle culture flask contained 100ml the medium. Strain U-2 was cultured in the medium in different temperatures at 200 rpm shaking speed for 6 days. After the water component had almost evaporated, the suspension of centrifuged liquid was diluted by 50 times and used for the biological test. The results (table 7) indicated that the ferment liquid of *P. griseoviride* strain U-2 was most active when the culture temperature was 26—28° C. It did not show activity against aphids because the fungal hyphae were suffocated during growth when the culture temperature was raised to 40° C.

Table 7. Effect of culture temperature on aphid control activity

culture temperature (° C)	activity against aphid (%)
18	19.6
20	53.5
22	74.6
24	82.5
26	89.6
28	89.4
30	81.5
32	62.6
34	50.6
36	22.6
38	9.4
40	—

2.8 Culture days effect on the activity against aphid

P. griseoviride strain U-2 was cultured under the same conditions described in **2.7**. Under 26—28° C culture temperature, the flasks with U-2 in the medium were removed one by one at different culture time-spans and water was added to compensate for evaporation. The suspension of the centrifuged culture liquid was stored in a refrigerator. Eventually the dilution of the suspension was made and tested at the same. It was found that (table 8) the ferment liquid of *P. griseoviride* strain U-2 was inactive after 1-2 days culture. It began to be active after 3 days cultivation. The activity reached a peak when the culture was up to the 6th day. The activity was reduced significantly if the U-2 was cultured continuously until the 8th day.

Table 8. Effect of culture time span on the activity against aphids

Culture time span (Day)	activity against aphids (%)
1	—
2	—
3	15.2
4	54.4
5	76.6
6	89.8
7	89.4
8	72.2
9	65.5
10	59.5

3 Brief summary

Based upon results obtained in this study, it was determined that aphid control activity of the ferment liquid was affected by the medium of sugar types, ingredients, PH, ventilation, culture temperature and duration. The suitable conditions for *Paecilomyces griseoviride* strain U-2 producing active component against aphid were: initial PH 6.0, adding 0.18% $MgCl_2$, 0.3% citrate sodium and 0.4% beef extracted cream in PDA, 80-100ml medium per 500ml triangle culture flask, in 26—28° C temperature, at 200 rpm shaking speed for 6 days cultivation.