

# Development of a Novel Microbial Insecticide: Gottsu-A

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Gottsu-A is a new microbial insecticide containing spores of strain T1 of the entomopathogenic fungus *Paecilomyces tenuipes*, which was developed by Sumitomo Chemical Co., Ltd. to control chemical resistant whiteflies on greenhouse vegetables.

We have succeeded in developing a fermentation process and a subsequent formulation process for *P. tenuipes*, as well as developing a unique oil flowable formulation with high performance in the stable preservation of *P. tenuipes* spores.

Gottsu-A has a low toxicity to natural enemy insects and excellent suitability for integrated pest management (IPM) programs using other selective chemicals, physical control tools and beneficial insects.

In this report, the development of Gottsu-A is described focusing on biological information for *P. tenuipes*, insecticidal performance on whiteflies, formulation design, production process and toxicological studies.

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## Introduction

In greenhouse cultivation of vegetables centered on the warm areas of southwest Japan in recent years, damage by whiteflies has become a severe problem. The Q biotype of sweet potato whiteflies (*Bemisia tabaci*) has developed resistance to existing chemical pesticides. Damage to fruits and vegetables such as tomatoes, eggplants, sweet peppers, melons and cucumbers by them has come to the fore in addition to the conventional problems of greenhouse whiteflies (*Trialeurodes vaporariorum*) and the B biotype of sweet potato whiteflies.

It is known that the Q biotype of sweet potato whiteflies transmits the tomato yellow leaf curl virus (TYLCV) (Fig. 1) in the same way as the B biotype, and furthermore, it has become clear that they also propagate the cucurbit chlorotic yellow virus (CCYV).

In 2005, the Q biotype of sweet potato whiteflies was first found continuously in Kyushu Island, and since then it has extended to various locations in Japan. Since TYLCV, which is persistently transmitted by whiteflies, has spread to the southern Tohoku region, we need to plan for the control of these whiteflies in various areas.<sup>1), 2)</sup>

The situation is one where as a requirement for Integrated Pest Management (IPM) for controlling whiteflies, we need to use not only conventional chemicals, but also cultural, physical and biological control methods. However, we do not have the effective tools for controlling whiteflies that have the potential to be the backbone of IPM.

With concern for public opinion on environmentally friendly agriculture as a background, Sumitomo Chemical has actively moved forward in constructing an IPM model. The authors have focused on microbial pesticides, which are available for IPM, as the core agents for controlling whiteflies. We selected a new strain T1 of the entomopathogenic fungus *Paecilomyces tenuipes* as a promising candidate for development and have developed Sumitomo Chemical's first microbial insecti-

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**Fig. 1** Left) Larvae and adults of sweet potato whitefly, *Bemisia tabaci*  
Right) Typical tomato yellow leaf curl disease symptoms

cide derived from a filamentous fungus.

Three commercial microbial insecticides for whiteflies have been used mainly in areas advanced in IPM practices (such as Kochi and Miyazaki), but these have problems with performance such as insecticidal efficacy, phytotoxicity and storage stability. The development and research goal of the insecticide using *P. tenuipes* strain T1 is bringing to market a new microbial insecticide that performs well for these characteristics and is superior to other microbial insecticides.

In this paper, we will introduce the development history of a novel microbial insecticide used by *P. tenuipes* strain T1 “Gottsu-A”, covering its biological characteristics, evaluation of insecticidal efficacy, optimization of formulation ingredients, establishment of manufacturing method and safety assessment.

## History of Development

The T1 strain of *P. tenuipes*, which is the active ingredient in Gottsu-A, is a filamentous entomopathogenic fungus that was isolated from the soil in Japan in the first half of the 1990s. As a result of evaluations of the insecticidal properties of this strain that were a part of our selective research on beneficial microorganisms,

the authors have found it exhibits a superior insecticidal activity and spectrum. In 2001, we tried to apply global patents<sup>3)-5)</sup> for a new insecticidal agent with this strain (deposit number FERM BP-7861) and for the insecticidal method used by this strain, and continuously began developing a novel insecticide for controlling whiteflies, which are one of the serious pests in greenhouses.

In 2004, we began practical evaluations for a foliage applied insecticide for whiteflies through official tests (which were assigned by the Japan Plant Protection Association, development code: S-1276FL), evaluating its efficacy with the goal of registering it as an agricultural agent. As a result, it was proved to exhibit practical controlling effects on the sweet potato whitefly or greenhouse whitefly for tomatoes, eggplants, melons, cucumbers and strawberries. Through these insecticidal efficacy tests and also official safety assessments for humans and environmental effects required for microbial agricultural agents, we finally acquired domestic registration as an agricultural agent named “Gottsu-A” on June 11, 2008 from the Japanese Ministry of Agriculture, Forestry and Fisheries (Table 1).

## Biological Characteristics

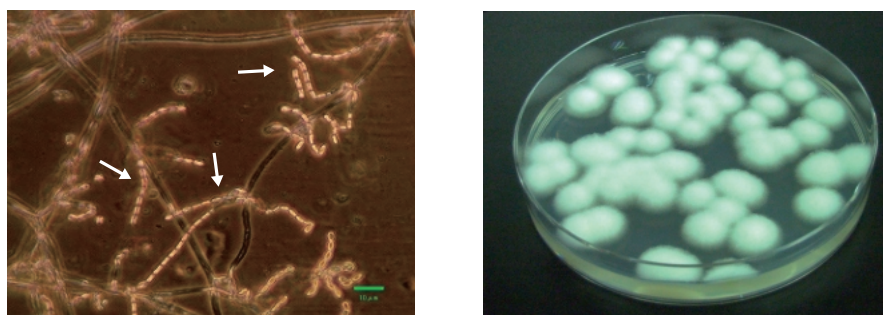
*P. tenuipes* is a filamentous entomopathogenic fungus (mold) that is widely distributed everywhere in the world. Its sexual generation has been known in the countries of eastern Asia as “caterpillar fungus” (“Hana-sanagi-take”) for a long time. It has been used as a stimulating tonic and cough suppressant.

The optimum range of temperature and pH for growth of *P. tenuipes* is 22 to 28°C and 4 to 7, and it may be grown in a medium that contains a carbon source, nitrogen source and inorganic salts (e.g. potato dextrose medium and malt extract medium). These characteristics are similar to other filamentous fungi growths. Downy fungal threads are formed on an agar medium, and their spores form vigorously under light conditions (Fig. 2).

**Table 1** Domestic registration of *Paecilomyces tenuipes* formulation, Gottsu-A

| Target crops             | Target pests | Dilution rate  | Application  |                     |                                 |
|--------------------------|--------------|----------------|--------------|---------------------|---------------------------------|
|                          |              |                | Method       | Volume (liters/10a) | Timing                          |
| Vegetables in greenhouse | Whiteflies   | fx 500 to 1000 | Foliar spray | 100 to 300          | At the first sign of whiteflies |

Application frequency is not limited to any given season.



**Fig. 2** Left) Spore formation of *P. tenuipes* strain T1 (arrows indicate the spores, bar indicates the length of 10µm) Right) Colony formation of *P. tenuipes* on PDA medium

The mode of action of *P. tenuipes* for infection of the host insect is also similar to other filamentous entomopathogenic fungi. The spores of the fungus germinate after the detection of skin constituents when they come into contact with the body surface of an insect. After they penetrate the cuticle by physical power and enzymes (such as protease, chitinase and lipase), blastospores are formed and grow in body fluids under suitable nutritional conditions. Host insects are killed by multiple accidents of body fluid circulation and other physiological balances, when these blastospores spread inside the body.<sup>6)</sup> The fungus appears outside the body of the host and spores form on it, then they disperse in air or soil. These spores possibly live in the soil waiting for contact with a host insect in the next generation.

## Evaluation of Insecticidal Efficacy

### 1. Insecticidal spectrum

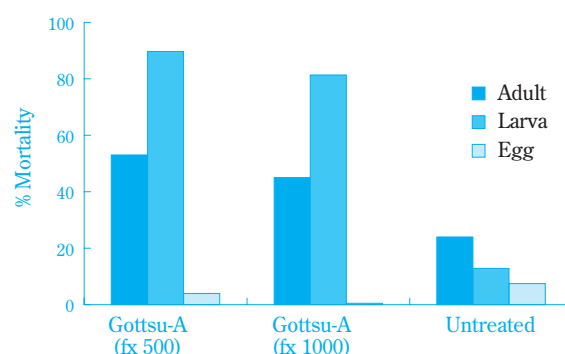
Gottsu-A has a high level of insecticidal activity on whiteflies (sweet potato whitefly and greenhouse whitefly), and also a certain level of activity to another hemipteran pests, such as aphids. The population inhibiting effects of Gottsu-A on the cotton aphid (*Aphis gossypii*), green peach aphid (*Myzus persicae*) and potato aphid (*Macrosiphum euphorbiae*) are superi-

or to other commercial microbial insecticides (*Verticillium lecanii* wettable powder) (Table 2). We expect that Gottsu-A can be applied as a beneficial and practical control agent for aphids.

Furthermore, it has been made clear that *P. tenuipes* strain T1 is infectious for the diamondback moth (*Plutella xylostella*) and corn earworm (*Helicoverpa armigera*).

### 2. Host stage

Gottsu-A at a conventional dilution rate (500 and 1000 times dilutions; following “fx 500 and 1000”) has high activity against larvae of sweet potato whiteflies



**Fig. 3** Insecticidal activity of Gottsu-A on adult, larva and egg of *B. tabaci* by direct spray at 25°C and 99%RH

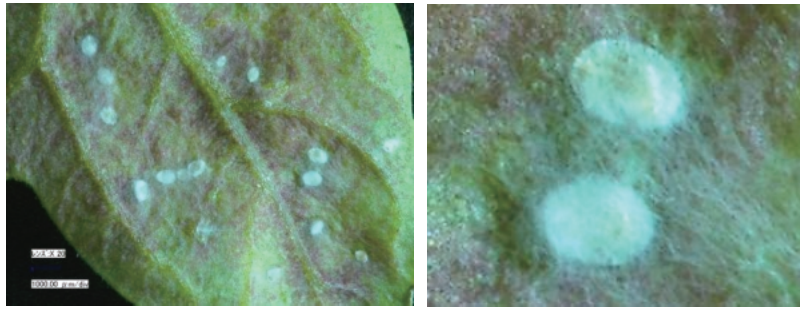
**Table 2** Insecticidal activity of *P. tenuipes* FL (Gottsu-A) and *Verticillium lecanii* WP on aphids

| Aphids            |                               | Corrected population density index* (DAT6 at 25°C and 90%RH) |                                |
|-------------------|-------------------------------|--|--------------------------------|
|                   |                               | <i>P. tenuipes</i> FL (fx 500)                               | <i>V. lecanii</i> WP (fx 1000) |
| Cotton aphid      | <i>Aphis gossypii</i>         | 15   | 113                            |
| Green peach aphid | <i>Myzus persicae</i>         | 58   | 90                             |
| Potato aphid      | <i>Macrosiphum euphorbiae</i> | 35   | 97                             |

\* Corrected population density index =  $((A \times b) / (B \times a)) \times 100$

A : number of living insects in the untreated sample before application  
a : number of living insects in the untreated sample after application

B : number of living insects in the treated sample before application  
b : number of living insects in the treated sample after application



**Fig. 4** Sweet potato whitefly, *B. tabaci* larvae infected by *P. tenuipes* strain T1

under 25°C and 99% RH conditions. Gottsu-A has no activity against eggs, but has a middle range of activity against adults when they contact these dilutions directly (Fig. 3).

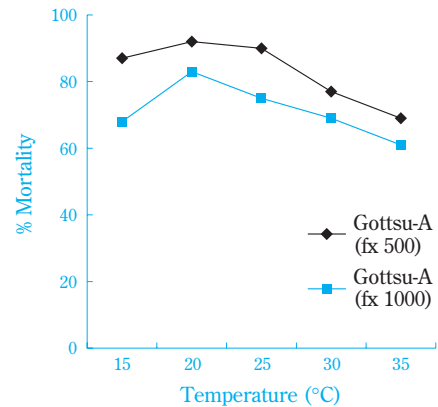
As the mode of action of Gottsu-A as described in the section on biological characteristics, larvae are killed by spores contacting the body surface and by the fungus growing continuously inside the body. Larvae infected and killed by Gottsu-A usually fall from the leaves of the host plant. When the infected larvae maintained under suitable conditions for the fungus growth stay on leaves, we can see dead larvae covered with a white fungus colony around four to seven days after first contact with Gottsu-A (Fig. 4).

### 3. Temperature and humidity requirements

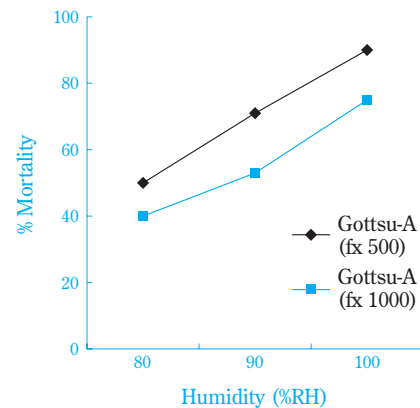
The expression of insecticidal activity of Gottsu-A is dependent on *P. tenuipes* infection and growth inside the host body. Since insecticidal activity was expected to be greatly affected by external environmental conditions, such as temperature and humidity, we determined the optimal temperature and humidity conditions for the activity.

When we applied Gottsu-A at fx 500 and fx 1000 to the 2nd instar larvae of sweet potato whiteflies under 15 to 35°C (99% RH), the range of maximum activity was exhibited at 20 to 25°C (Fig. 5). These results substantially tuned with the optimal temperature range for growth of the fungus. It is clear that the best temperature range for effective insecticidal activity of Gottsu-A is 15 to 28°C.

To test the effects of humidity on activity, we applied conventional dilutions of Gottsu-A to the 2nd instar larvae at 80 to 100% RH (25°C). The insecticidal activity improved as the humidity increased, and it was clear that excessive moisture was the optimal condition (Fig. 6). Generally, microbial insecticides with fungus require higher humidity for insecticidal activities. The



**Fig. 5** Insecticidal activity of Gottsu-A on *B. tabaci* larvae at different temperature at 99%RH



**Fig. 6** Insecticidal activity of Gottsu-A on *B. tabaci* larvae at different relative humidities at 25°C

optimal range of humidity for Gottsu-A arise from the requirement for moisture for the fungal infection steps, namely attachment of spores to the body, germination on the body surface and penetration, and this is similar to characteristics for other insecticides.

For the maximization of the insecticidal activity by Gottsu-A, it is necessary that the high humidity conditions be maintained for 8 hours or more just after spraying of dilutions. Since the humidity generally



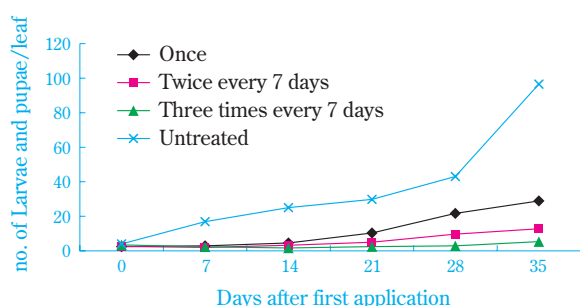
maximizes during the night in greenhouses, we recommend that Gottsu-A is sprayed in the evening.

#### 4. Field trials for control of whiteflies

##### (1) Number of applications and intervals

Gottsu-A is mainly active against the larvae and pupae of whiteflies by means of infection by a fungus. Since it requires several days for the host to die, the speed of death by microbial insecticide is more delayed than with chemical insecticides that are active against the insect nervous system directly. Additionally adult whiteflies continually invade greenhouses and increase the populations during early occurrence of the flies. In this season, a single application of microbial insecticide is not sufficiently effective for controlling the whitefly populations. From such a background, we tried to determine the optimal number of applications and intervals (days) for controlling whiteflies.

As a result of one to three applications of Gottsu-A at fx 500 on greenhouse tomatoes, a clear inhibitory effect on increases in the number of whitefly larvae and pupae was seen with all of the treated groups when compared to the untreated group. It was apparent that the population inhibiting effect was greatest when



**Fig. 7** Control efficacy of Gottsu-A on *B. tabaci* at different application frequencies for tomatoes

there were three applications (Fig. 7). Moreover, a period of approximately 7 days was shown to be optimal by examination of the application interval (days) that provided the greatest control effect with multiple applications.

##### (2) Practical evaluation

The results of field trials by an official research organization on the control effects of Gottsu-A (at fx 500 and fx 1000) on whiteflies for various crops are as follows.

The control effect of Gottsu-A for sweet potato whiteflies on tomatoes was the same or higher than the compared commercial microbial, *Beauveria bassiana* FL at fx 500 (Table 3). Additionally a high control effect was found for sweet potato whiteflies on melons, and it showed an effect superior to the compared commercial microbial, *Verticillium lecanii* WP at fx 1000 (Table 4). Moreover, a sufficient effect was shown on greenhouse whiteflies on strawberries and a control effect higher than or equal to the compared commercial microbial, *Paecilomyces fumosoroseus* WP at fx 1000 was found (Table 5). For these evaluation results of field trials, we confirm that Gottsu-A has practical control effects on whiteflies for various crops, and its efficacy is equal to or higher than these other commercial microbial insecticides.

No phytotoxicities, such as problems with growth, deformation and discoloration on leaves or fruits from Gottsu-A, were found on these crops. And, when Gottsu-A was applied at fx 250 –that is a double the concentration of conventional dilutions– phytotoxicities were not found on the stems, leaves or fruit after treatment of the stems and leaves of the seedlings or the growing plants of the main crops cultivated in greenhouses.

**Table 3** Control efficacy of Gottsu-A on *B. tabaci* for tomatoes

| Research Institute of Japan Plant Protection Association (Kochi) (2004) |               |  |              |              |              |
|---|---------------|--|--------------|--------------|--------------|
| Insecticide   | Dilution rate | Corrected population density index <sup>1)</sup> |              |              |              |
|   |               | DAT <sup>2)</sup> 7                              | DAT14        | DAT21        | DAT28        |
| Gottsu-A  | fx 500        | 35.0   | 21.2         | 4.5          | 9.0          |
| Gottsu-A  | fx 1000       | 63.6   | 22.9         | 15.7         | 31.5         |
| <i>B. bassiana</i> FL   | fx 500        | 39.7   | 18.6         | 9.9          | 36.6         |
| Untreated   |               | 100<br>(56.9) <sup>3)</sup>                      | 100<br>(201) | 100<br>(610) | 100<br>(327) |

1) Refer to Table 2 for the corrected population density index.

2) DAT : Days after treatment

3) Numbers in parentheses indicate number of eggs, larvae and pupae on 10 leaves Applied three times every seven days (June 29, July 6 and 13, 2004)

**Table 4** Control efficacy of Gottsu-A on *B. tabaci* for melons

Research Institute of Japan Plant Protection Association (Kochi) (2004)

| Insecticide          | Dilution rate | Corrected population density index <sup>1)</sup> |              |               |               |
|----------------------|---------------|--|--------------|---------------|---------------|
|                      |               | DAT <sup>2)</sup> 7                              | DAT14        | DAT21         | DAT28         |
| Gottsu-A             | fx 500        | 28.6   | 9.7          | 4.3           | 2.0           |
| Gottsu-A             | fx 1000       | 32.8   | 22.7         | 8.7           | 5.1           |
| <i>V. lecanii</i> WP | fx 1000       | 44.3   | 40.6         | 21.5          | 11.8          |
| Untreated            |               | 100<br>(12.7) <sup>3)</sup>                      | 100<br>(7.5) | 100<br>(19.5) | 100<br>(61.8) |

1) Refer to Table 2 for the corrected population density index.

2) DAT : Days after treatment

3) Numbers in parentheses indicate number of eggs, larvae and pupae on leaf disk (15mm in diameter)

Applied three times every seven days (May 13, 20 and 27, 2004)

**Table 5** Control efficacy of Gottsu-A on *Trialeurodes vaporariorum* for strawberries

Nagano Nanshin Agricultural Experiment Station (2004)

| Insecticide               | Dilution rate | Corrected population density index <sup>1)</sup> |              |                       |               |
|---------------------------|---------------|--|--------------|-----------------------|---------------|
|                           |               | Adults   |              | Eggs + Larvae + Pupae |               |
|                           |               | DAT <sup>2)</sup> 14                             | DAT21        | DAT14                 | DAT21         |
| Gottsu-A                  | fx 500        | 12.6   | 17.2         | 9.7                   | 8.6           |
| Gottsu-A                  | fx 1000       | 7.5  | 15.8         | 20.1                  | 12.5          |
| <i>P. fumosoroseus</i> WP | fx 1000       | 55.3   | 44.5         | 19.9                  | 32.7          |
| Untreated                 |               | 100<br>(12.0) <sup>3)</sup>                      | 100<br>(2.6) | 100<br>(19.4)         | 100<br>(33.1) |

1) Refer to Table 2 for the corrected population density index.

2) DAT : Days after treatment

3) Numbers in parentheses indicate number of insects on leaf

Applied twice every seven days (May 24 and 31, 2004)

## Optimization of Formulation Ingredients

We were focusing on the performance of insecticidal activity, storage stability and safety for crops, which were superior to the compared commercial microbial insecticides for selection of the formulation ingredients for Gottsu-A.

The content of the active ingredient, spores of *P. tenuipes* strain T1, was established as  $5 \times 10^8$ /ml or greater (0.3 to 3% by weight) to guarantee insecticidal efficacy as per the product specifications.

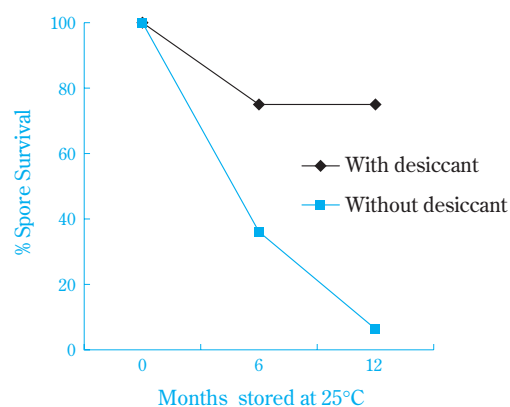
In terms of the formulation for showing the maximum insecticidal effect for a filamentous fungus insecticide, it was clear that an oil flowable formulation was more suitable. The conventional dilution of this formulation would make it easier for the spores to contact the insect bodies and cause infection because of the physicochemical effects of the oil. Furthermore, because it is important to suppress to a low degree of the amount of moisture in the liquid to increase the storage stability of the product, we

decided that the formulation of Gottsu-A should be composed of an oil component, which would be a non-aqueous liquid.

Since phytotoxicity was sometimes found with commercial oil flowable (FL) formulations, we tried to select oil, which reduced the risk of damage to crops. And we selected an emulsifier, which would maintain high survival for the spores and provide high of emulsifiability performance with the selected oil.

As a result of carrying out the selections above, we found an optimal combination of the oil component and emulsifier for the *P. tenuipes* strain T1, and therefore, we established a qualitative and quantitative formulation of Gottsu-A with ideal performance of insecticidal activity, storage stability and safety for crops.

Furthermore, we found that it is possible to maintain high survival for the spores at room temperatures by putting desiccant within a bag inside the bottle for suppressing the moisture content of the product (Fig. 8). Finally, we succeeded in making a product with a longer quality guarantee period



**Fig. 8** Effects of desiccant with bag on spore survival of *P. tenuipes* in Gottsu-A

(guaranteed for two years after manufacture at room temperatures) than conventional microbial insecticides.

## Establishing the Manufacturing Method

### 1. Production process

The manufacture of Gottsu-A comprises the fermentation process of *P. tenuipes* and the formulation process (Fig. 9). We succeeded in establishing an original manufacturing process for both of them.

The production of an active ingredient comprises the pre-culturing process of *P. tenuipes* in a liquid medium and next the main culturing process in a solid medium to promote the formation of spores. Optimization of the

spore production technique in the main culturing process was the most important item in establishing the manufacturing of Gottsu-A.

It was necessary to select the culturing trays, optimize the volume for the solid culture, establish a technique for environmental conditioning and control the bacterial contamination in order to produce a spore low-cost.

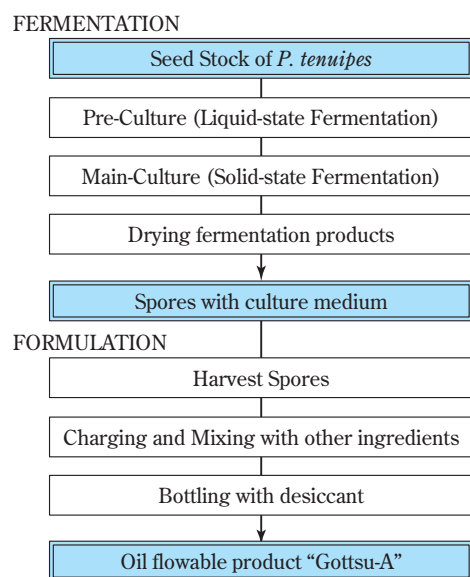
Since we found that *P. tenuipes* constantly required a high illumination for spore production, we have selected the light transmissive trays. To maintain an appropriate temperature and moisture content in the solid medium for growth and spore production of *P. tenuipes*, we strictly adjusted the temperature and humidity in the culturing space and decided to use trays with moderate ventilation for culturing. In addition, we established methods for sterilization operations and components of the medium for controlling bacterial contamination. After the main culturing, the entire culture product was dried to efficiently recover the spores on the solid medium.

In the formulation process, the spores were first harvested from the dried culture product. The prescribed amounts of spores were charged and mixed with substrate (oil), surfactants, thickening agents and other additives. The uniform mixture was filled into bottles containing a desiccant as the product for sale.

Parts of the fermentation process and formulation process were established in joint development with Idemitsu Kosan Co., Ltd.

### 2. Quality control

We have checked the quality and quantity of products in the fermentation process by observing bacterial contamination and determining the number of spores in the culture medium. Since commercial microbial (filamentous fungus) insecticide needs to guarantee a standard spore concentration (live spores/ml), it was necessary to minimize the mortality of spores in the product during the storage period under room temperature. We clarified that moisture content of product was ascertained to be important as the factor with the greatest impact on spore survival in the formulation, so compliance with standards for the moisture content was clearly important. The values for the standards for this moisture content were determined through storage stability tests at room temperatures. In addition, we established an analytical method through careful examinations into



**Fig. 9** Fermentation and formulation process for Gottsu-A

increasing precision in measurements of spore concentration.

We finally established original quality control methods for Gottsu-A with standard values for each analytical item, the appearance of the final product, spore concentration, moisture content and physical properties (viscosity, specific gravity and emulsifiability).

## Safety Assessment

The safety evaluation items for microbial insecticide registration requirements are mainly infectiousness and pathogenesis for humans and animals, organisms including crops, microorganisms and beneficial insects. Most of the fungi used in microbial insecticides are distributed universally throughout the natural world, and the hosts for those fungi are extremely limited. Therefore experience shows that microbial insecticides have less toxicity for humans, animals and other organisms without target insects.

The safety performance of Gottsu-A was toxicologically evaluated in “Safety Tests for Humans” and “Tests of Effects on Environmental Organisms” described in the guidelines for microbial agricultural agents.<sup>7), 8)</sup> We also toxicologically evaluated the effects of the formulated products on beneficial insects existed on target crops of Gottsu-A.

### 1. Safety for humans

In toxicological studies on single-dose tests (oral, dermal, intratracheal and intravenous routes) of the active ingredient *P. tenuipes* spores or the formulated product of Gottsu-A, a mild irritation to the skin was found, but no remarkable toxicity nor infectiousness, pathogenesis or live persistence of this fungus in the body were found. Gottsu-A caused minimal irritation to

the eyes and had skin sensitizing potential (Table 6). Whoever handles Gottsu-A should follow the cautions for toxicological information cited on the product labels.

### 2. Effect on environmental organisms

In safety evaluation tests of Gottsu-A for aquatic organisms, it was clarified that *P. tenuipes* spores were not harmful on common carp (*Cyprinus carpio*) and *Daphnia magna* in cases where Gottsu-A was applied under the practical conditions. Additionally, in safety evaluation tests for crops, it was confirmed that the spores were not harmful on the vegetation of main crops belonging to seven families with 10 species when Gottsu-A was applied as foliar spray on seedling plants or the soil treatment at seeding. Since no phytotoxicity in double the concentration of conventional dilutions of the formulated product on crops was observed, it was concluded that Gottsu-A had less effect on crops.

Effects were not found on natural enemy insects, such as parasitic Hymenoptera (*Encarsia formosa*, *Aphidius colemani*, *Neochrysocharis formosa* “MIDORI-HIME”) and *Eretmocerus eremicus*), predatory Hemiptera (*Orius strigicollis* “ORISTAR® A”), predatory Coleoptera (*Harmonia axyridis*) and predatory mites (*Phytoseiulus persimilis*, *Amblyseius cucumeris*, *A. californicus* and *A. swirskii*) with the formulated product or the *P. tenuipes* spores (Table 7). Those results led to the conclusion that we can use Gottsu-A in combination with natural enemies for pursuing the concept of systematic pest control.

In tests of the effects of the *P. tenuipes* spores on honeybees (*Apis mellifera*), it was observed that the mortality of adults depended on the spore concentration. However, it was not found that there were any effects of the formulated product (at fx 500) on honeybees. In addition, the formulated product did not show

**Table 6** Toxicological information for Gottsu-A on animals

| Study              | Administration route  | Animal         | Period  | LD <sub>50</sub> or NOEC (cfu/animal)* |
|--------------------|-----------------------|----------------|---------|--|
| Acute toxicity     | Oral                  | Rat (♂ ♀)      | 21 days | > 1 × 10 <sup>8</sup>                  |
|                    | Dermal                | Rabbit (♂ ♀)   | 14 days | > 2.1 × 10 <sup>10</sup>               |
|                    | Intratracheal         | Rat (♂ ♀)      | 21 days | > 1 × 10 <sup>8</sup>                  |
|                    | Intravenous           | Rat (♂ ♀)      | 21 days | > 1 × 10 <sup>7</sup>                  |
| Eye irritation     | Eye                   | Rabbit (♂)     | 7 days  | Minimally irritating                   |
| Skin irritation    | Dermal                | Rabbit (♂ ♀)   | 14 days | Mild irritant                          |
| Skin sensitisation | Intradermal injection | Guinea pig (♂) | —       | Positive                               |

\* cfu : colony forming unit



**Table 7** Toxicological information for Gottsu-A on beneficial insects (natural enemies)

| Insects                        | (stage)          | Application          |                      | Evaluation Item <sup>3)</sup> | Result        |
|--------------------------------|------------------|----------------------|----------------------|-------------------------------|---------------|
|                                |                  | Method <sup>1)</sup> | Sample <sup>2)</sup> |                               |               |
| PARASITIC WASPS                |                  |                      |                      |                               |               |
| <i>Encarsia formosa</i>        | (adult ♀, pupa)  | BS/BC/D              | S/F                  | M/Pa/E/B                      | Not effective |
| <i>Aphidius colemani</i>       | (adult ♀, pupa)  | BS                   | F                    | M/Pa/E/B                      | Not effective |
| <i>Neochrysocharis formosa</i> | (adult)          | BS                   | F                    | M/Pa/B                        | Not effective |
| <i>Eretmocerus eremicus</i>    | (adult ♀, pupa)  | BS                   | F                    | M/Pa/E/B                      | Not effective |
| PREDATORS                      |                  |                      |                      |                               |               |
| <i>Orius strigicollis</i>      | (adult ♀, larva) | BS/BC/D              | S/F                  | M/R/E/B                       | Not effective |
| <i>Harmonia axyridis</i>       | (adult, larva)   | BC                   | S/F                  | M/Pu/B                        | Not effective |
| PREDATORY MITES                |                  |                      |                      |                               |               |
| <i>Phytoseiulus persimilis</i> | (adult)          | BS                   | F                    | M/FC/B                        | Not effective |
| <i>Amblyseius cucumeris</i>    | (adult)          | BS                   | F                    | M/FC/B                        | Not effective |
| <i>Amblyseius californicus</i> | (adult)          | BS                   | F                    | M/FC/B                        | Not effective |
| <i>Amblyseius swirskii</i>     | (adult)          | BS                   | F                    | M/FC/B                        | Not effective |

1) BS : body spray, BC : body contact, D : dipping

2) S : spore, F : formulation

3) M : mortality, Pu : pupation, E : emergence, R : reproductivity, Pa : parasitism, FC : food consumption, B : behavior

any effects such as infective and foliar residual toxicities on honeybee a colony.

In surveys of the effects of the *P. tenuipes* spores on silkworms (*Bombyx mori*), the No-Observable-Effect Level (NOEL) was estimated to be around  $1.0 \times 10^5$ /ml (equivalent to 1/10 times the maximum conventional concentration for use). Because the possibility of direct exposure of mulberry leaf to the dilutions of Gottsu-A is low in the field, it seems not to cause a problem for practical use of Gottsu-A if the cautions for toxicological information regarding silkworms cited on labels are complied with.

In soil incorporation test with the *P. tenuipes* spores (corresponding to an amount 10 times that of the maximum application per unit surface area), no effects were found on abundance of soil microorganisms (bacteria, actinomycetes and fungi).

## Conclusion

With the background of Sumitomo Chemical Company's (SCC) well-thought-of concern for environmentally friendly agriculture, we have promoted work on IPM from the standpoint of agricultural production. As a part of this action, we have put effort into developing new resources that lead to the development of original SCC IPM technology for various crops. For example, proposals for systematic pest control models by a combination of selective insecticide, PLEO<sup>®</sup> FLOWABLE (Pyridaly)<sup>9)</sup> and natural enemies of SCC, and IPM pro-

grams utilizing agricultural chemicals, physical control materials and functional fertilizers are some of the results. Within this trend, we tried to develop novel microbial insecticides and Gottsu-A is one such product we have succeeded to commercialize in this way.

For overcoming the present problems of performance of commercial microbial insecticides, such as insufficient efficacy, phytotoxicity and low storage stability, we established an original manufacturing and formulation process for Gottsu-A. Eventually we could accomplish commercialization of Gottsu-A as a high-performance microbial insecticide.

The entry of Gottsu-A as a novel insecticide is a timely solution being proposed in a situation where the number of farmers who cannot sufficiently control whiteflies on greenhouse crops is increasing. Finally, it is possible to implement an IPM system centered on SCC's products by using Gottsu-A. For example, Gottsu-A is mainly used as the key control agent and sufficient effects for controlling whiteflies can be expected with yellow sticky traps and a starch formulation ("NENCHAKUKUN" Liquid) in the autumn culture of tomatoes. For controlling other pests, such as leaf miners and worms, selective chemical insecticides and physical control materials are effective in this system (Fig. 10).

We would like to promote and spread quickly IPM programs utilizing Gottsu-A mainly in areas with greenhouse cultivation where farmers cannot sufficiently control whiteflies.

| MAJOR PESTS   | SEP                                  | OCT                                      | NOV                      | DEC | JAN | FEB | MAR           | APR                 | MAY | JUNE |
|---|--------------------------------------|--|--------------------------|-----|-----|-----|---------------|---------------------|-----|------|
|   | Plantation                           | ← Harvest (Bumblebees for pollination) → |                          |     |     |     |               |                     |     |      |
| Whiteflies<br>• <i>Bemisia tabaci</i> (Q-Biotype)<br>• <i>Trialeurodes vaporariorum</i> |                                      | Yellow Sticky Trap                       |                          |     |     |     |               | Gottsu-A            |     |      |
|   |                                      | Gottsu-A                                 |                          |     |     |     |               | Gottsu-A            |     |      |
|   |                                      | Liquid Starch                            |                          |     |     |     | Liquid Starch |                     |     |      |
| Leafminer flies<br>• <i>Liriomyza spp.</i>  | Clothianidin G<br>Nitenpyram G       | Parasitic Wasp                           |                          |     |     |     |               |                     |     |      |
| Lepidopterans<br>• <i>Spodoptera litura</i><br>• <i>Helicoverpa armigera</i>            |                                      | <i>B.t. aizawai</i>                      |                          |     |     |     |               |                     |     |      |
|   | Insect Repellent<br>Fluorescent Lamp |  | Insect Proof Net (0.4mm) |     |     |     |               |                     |     |      |
|   | Pyridalyl                            | <i>B.t. kurstaki</i>                     |                          |     |     |     |               |                     |     |      |
| Aphids<br>Erineum mites   |                                      | Selective Chemicals                      |                          |     |     |     |               | Selective Chemicals |     |      |

**Fig. 10** IPM program utilizing Gottsu-A with other insecticide, beneficial insect and tools to control major pests in greenhouse tomato

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