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The FFTC Journal of Agricultural Policy
FFTC AGRICULTURAL POLICY PLATFORM

Management of Both Powdery Mildew Diseases and Pest-Insects on Vegetable Plants using A Single Microbial Agent

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Received June 17, 2024; Accepted November 25, 2024

ABSTRACT

The Ministry of Agriculture, Forestry and Fisheries Japan put out a policy as use of chemical pesticides decrease to 50% of values assessing risk of each pesticide to humans until 2050. Bio-pesticides are evaluated to have low risk and are expected to increase the use. A bio-insecticide confirmed to control powdery mildew diseases on vegetable plants in this study. The agent 'BotaniGard ES' includes a Beauveria bassiana strain as the effective ingredient. Several test cases controlling the disease with the agent was counted and the agent was legally registered to be used for the disease additive to pest insects in Japan. Expanding a range of target pests including diseases and insects is very desirable for increasing the value of a pesticide. The ingredient fungus in 'BotaniGard ES' presumed to induce local resistance to plant leaves. In farming conditions for tomato and strawberry in greenhouses, methods of using the agent were considered. Effects of the agent were enough to control both the disease and issued pest-insects when the agent was used once a week at low severity or density of the disease and pest insects. The agent intensified the effect of controlling chemical-resistant pest insects and do not affect predatory mites as enemies to pest mites. After more valuable information on the bio-agents including 'BotaniGard ES' and others will be accumulated, bioagents would be more useful.

Keywords: *Beauveria bassiana*-based bioagent, induced resistance, tomato, strawberry

INTRODUCTION

In Japan, 12 bacteria and fungi are legally registered and used as ingredients of biological pesticides to control crop diseases and pest insects by the country's Ministry of Agriculture Forestry and Fisheries. The biological agents are managed the same as chemical pesticides in legal registration and evaluation of those effects there. More than 10 billion yen (about US$ 60 million) and ten more years would be needed to develop a biological or chemical pesticide with strict assessment of safety to the environment and lives. Additionally, inconsistency in results and higher prices make biological agents difficult to compete with the chemical ones. Additive benefits are desired for the use of the bioagents, which are already commercialized and secured with safety. *Beauveria bassiana* is an entomopathogenic fungi and a few strains of the species suppressed plant diseases (Ownley *et al*., 2008, Quesda-Moraga *et al*., 2009, Raad *et al.*, 2019, Sinno *et al*., 2021). In our provisional tests with Japanese bio-insecticides, a *Beauveria bassiana*-based agent, 'BotaniGard ES' suppressed tomato powdery mildew diseases effectively. The active ingredient of 'BotaniGgard ES' is the spores of *B. bassiana*, an entomopathogenic fungus, strain GHA. The agent had been registered for control of sucking pests *viz*. aphids, thrips, whiteflies, and spider mites before our research. We attempted to control both the disease and pests-insects in fields with 'BotaniGard ES' supported by the Research Program on Development of Innovative Technology Grants (JPJ007097) from the Project of the NARO Bio-oriented Technology Research Advancement Institution (BRAIN) in 2017-2019 (29008B) and 2020-2022 (02028C) (Kubota 2023). Arista Life Science Ltd. as a provider of

'BotaniGard ES', National Agriculture and Food Research Organization and several institutes of prefectural governments took part in the research consortium.

CONTROL OF POWDERY MILDEW DISEASES ON VEGETABLE PLANTS WITH A BIOINSECTICIDE 'BOTANIGARD ES' (Kubota *et al.*, 2021)

We confirmed effects of 'BotaniGard ES' for control of powdery mildew diseases on plants of cucumber, tomato, eggplant, strawberry, and melon in greenhouses in several parts in Japan, during 2014 to 2018. Protection rates against the disease were calculated from percentages of infected area on leaves (Figure 1). The rates were 100-63% on cucumber plants, 99-88% on tomatoes, 78-66% on eggplants, 85-89% on strawberries, and 53-50% on melons. These experiments were conducted in imitation of productive farming and was authorized by the Food and Agricultural Materials Inspection Center, which officially operates the pesticide registration in Japan. Protection rates of 100-80% are generally judged as 'well effective', 80-60% as 'effective', 60-40% as 'weakly effective', under 40% as 'ineffective'. More than six results with protection rates of 100-40% are requested to register a pesticide against each disease, and two or more of the results on each of three (or more) plant families are needed for additive registration of the pesticide for use on the group of vegetable plants. Then, 'BotaniGard ES' was legally registered for control of powdery mildew diseases of the group of vegetable plants.

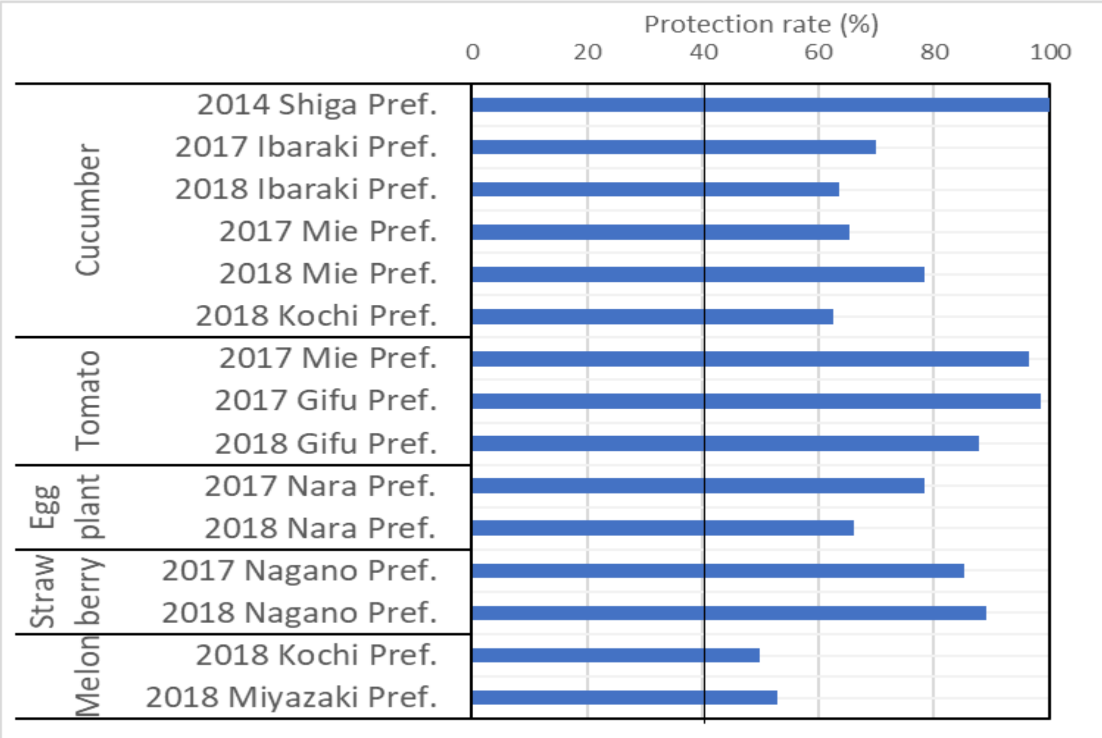

Figure 1. Protection rate from powdery mildew disease on vegetable plants.

'BotaniGard ES' was sprayed 3-5 times on plants for 6-11 days in each experiment and disease severity of leaves was investigated 6-13 days after the last spraying. Disease severity of each leaf was indexed with values as 0: no symptom, 1: powdery mildew covering 0-5% of leaf surface, 2: 5-25%, 3: 25-50% and 4: >50%. Then disease degrees and protection rates for each treatment were calculated as below.

Disease degree＝Σ((number of leaves with each index)×(index value))／((total number of leaves)×4)×100

Protection rate＝(1—(average of disease degrees) ／(average of disease degree of no treatment))×100

MECHANISM OF CONTROLING POWDERY MILDEW DISEASES WITH 'BOTANIGARD ES' (Iida *et al.,* 2023)

The fungal-strain GHA can survive on plants endo- or epiphytically, especially on wounds and presumed to interact with plants (Nishi *et al.* 2020). In leaves inoculated with powdery mildew pathogen after treatment with 'BotaniGard ES' a plant cell wall ingredient, callose was accumulated under penetration points by the pathogen and cell death after hypersensitive reaction was recognized (Figure 2). Then the penetration by the pathogen was suppressed. Analysis of plant hormones indicated an increase of salicylic acid, the signal of systemic acquired resistance, in leaves treated with 'BotaniGard ES' although induced resistance in this case was local, as only a-half side treated with 'BotaniGard ES' in a leaf was protected and another side without the agent got the disease. RNA-seq analysis of leaves inoculated with GHA strain and the pathogen also indicated induction of resistance via salicylic acid pathway. Additively, oil elements in 'Botanigard ES' would suppress spore germination of the pathogen and contribute to protect plants. Ownley *et al.* (2008) summarized plant defense against some diseases, although the mechanisms were not elucidated. Raad *et al.* (2019) showed expression of some genes related to disease resistance in Arabidopsis, but not activation of phytohormones, after inoculation of another strain of *B. bassiana*. We were the first to report detailed defense mechanisms against plant diseases by a *B. bassiana* agent. Explanation of the mechanism was described and printed as a leaflet which was dispersed around and opened on a website (in Japanese: https://www.setsunan.ac.jp/~pp/inobe).

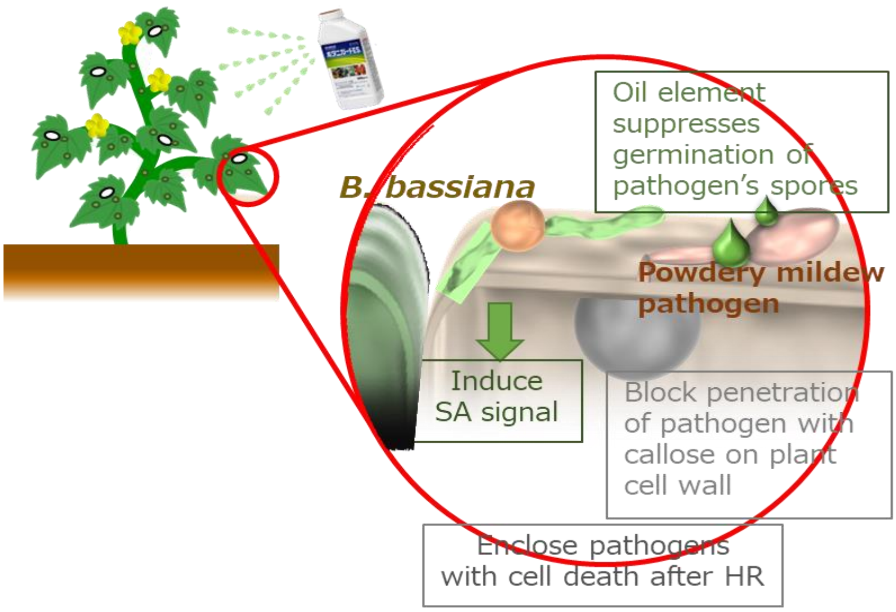

Figure 2. Mechanism of control of powdery mildew disease with 'BotaniGard ES'.

INFLUENCE OF CHEMICAL PESTICIDES ON 'BOTANIGARD ES'

In cases when the effect of bio-pesticides is not enough or non-target pests rise, chemicals would be used together with bio-pesticides in a field. Inhibitory effects of chemicals onto ingredient microbiota of bio-pesticides are suspected. Mixture of GHA spores with each of 94 chemical pesticides on sale in Japan are inoculated to cucumber leaves and then the survival of GHA strain was investigated. 25 fungi- and bactericides and 12 insecticides reduced colony forming units from GHA spores after 1 week of incubation. In mixtures of the agent 'BotaniGard ES', only 4 fungicides inhibited GHA survival. Components in the agent protect the spores from chemicals well. Mancozeb, captan, chlorothalonil, $KHCO_3$ are inhibitory chemical ingredients. A summary table of these results was printed as a leaflet which was dispersed around and opened on a website (in Japanese: https://www.setsunan.ac.jp/~pp/inobe).

CONTRIBUTION OF 'BOTANIGARD ES' IN TOMATO FARMING (Kawakami 2024)

Control of the powdery mildew disease and pest-insects on tomato plants was evaluated in Mie Prefecture. There is an insect target of whiteflies (Figure 3). Whiteflies disperse *Tomato yellow leaf curl virus* (TYLCV) and *Tomato chlorosis virus* (ToCV), and diseases caused by the viruses are serious in this area. Tomato plants are transplanted in greenhouses in August and the cultivation is finished in July in the next year. Powdery mildew and whiteflies concurrently damage the plants during March to June. As resistance to chemical insecticides of whiteflies occurred in this area, biocontrol agents are expected to control the resistant whiteflies (Sasaki *et al.* 2022).

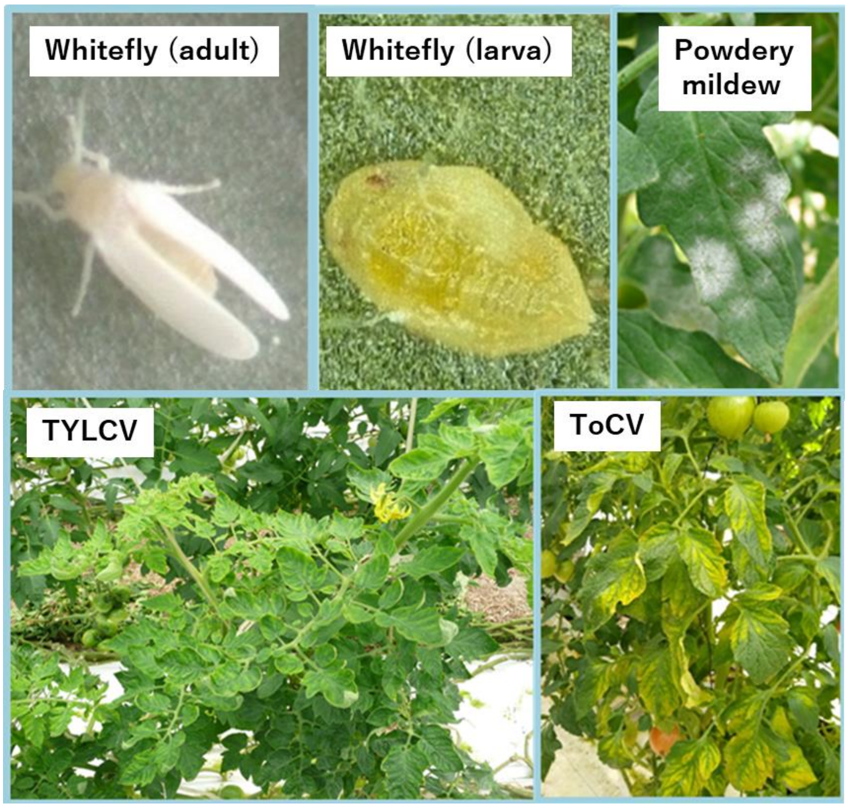

Figure 3. Targets of 'BotaniGard ES' on tomato plants.

In experiments conducted in Mie Prefectural Agricultural Research Institute, protection rates from powdery mildew disease were stably over 80% by weekly treatment with 'BotaniGard ES'. The effect of the bioagent was weak when treatment was started once every two weeks after the onset of disease. Mixtures of 'BotaniGard ES' and some chemical insecticides increased control effect on chemical-resistant whiteflies from that with each single chemical or bio insecticide (Figure 4). At cultivation in greenhouses in the institute, weekly treatment of 'BotaniGard ES' was enough to control powdery mildew and whitefly comparing to ordinal treatments with chemical pesticides once by two weeks (Figure 5, 6).

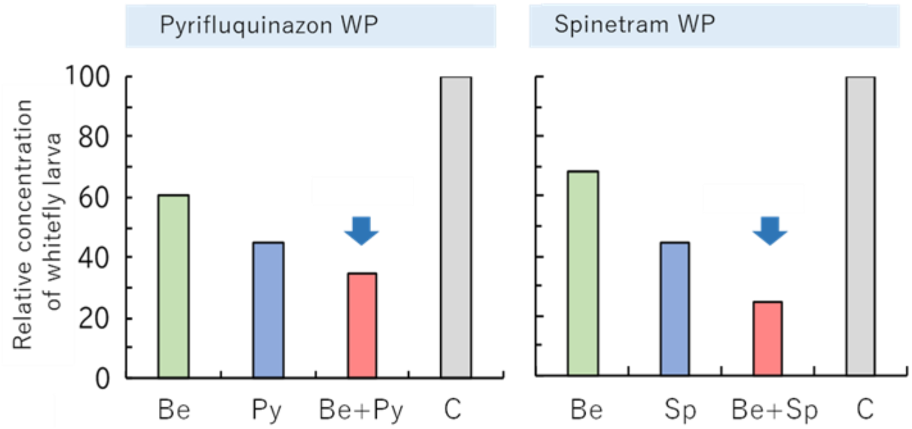

Figure 4. Relative concentration of whitefly larva on tomato plants sprayed with ‘BotaniGard ES’ and chemical insecticides. Be: ‘BotaniGard ES’, Py: pyrifluquinazon, Sp: spinetram, C: no treatment.

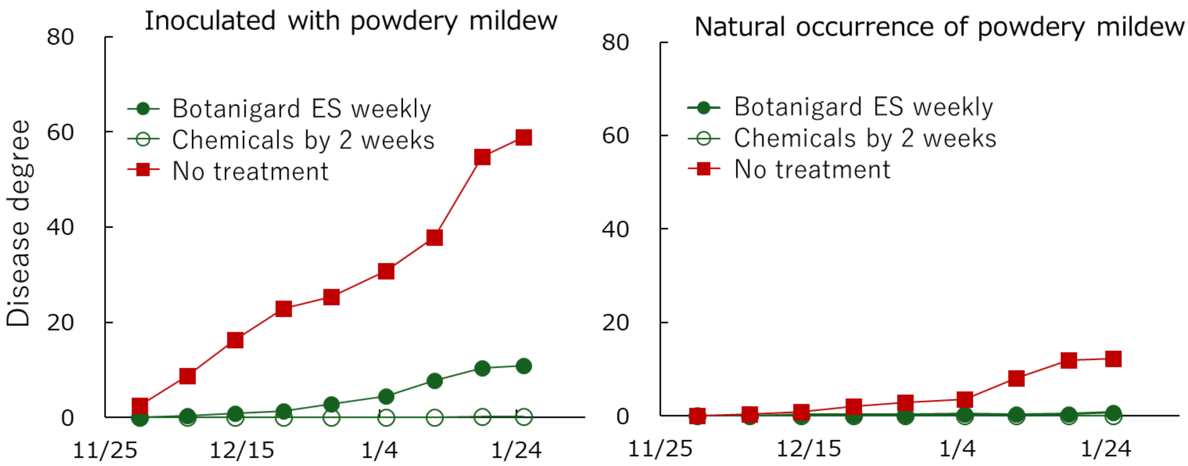

Figure 5. Disease severity of powdery mildew on tomato plants sprayed with ‘BotaniGard ES’ weekly or chemicals by 2 weeks.

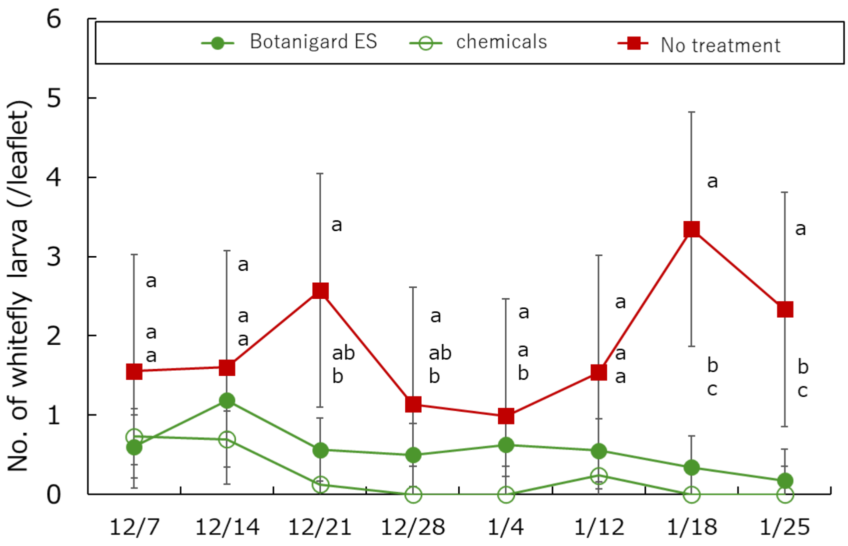

Figure 6. Number of whitefly larva on tomato plants sprayed with ‘BotaniGard ES’ weekly or chemicals once by 2 weeks.

At cultivation in farmers greenhouses, ‘BotaniGard ES’ was treated additively to ordinally chemical treatments because production and farmer’s income had to be secured. On treatments adding ‘BotaniGard ES’ whiteflies grew at lower level than on ordinal chemical treatments and control effect of the bioagent on chemical resistant whiteflies were suggested (Figure 7). Plants infected with TYLCV did not increase in 2021-22 in tuned with whiteflies. ToCV would be dispersed and infected latently in autumn, then symptoms would appear in spring. Powdery mildew was suppressed in both additive ‘BotaniGard ES’ and ordinal chemical treatments.

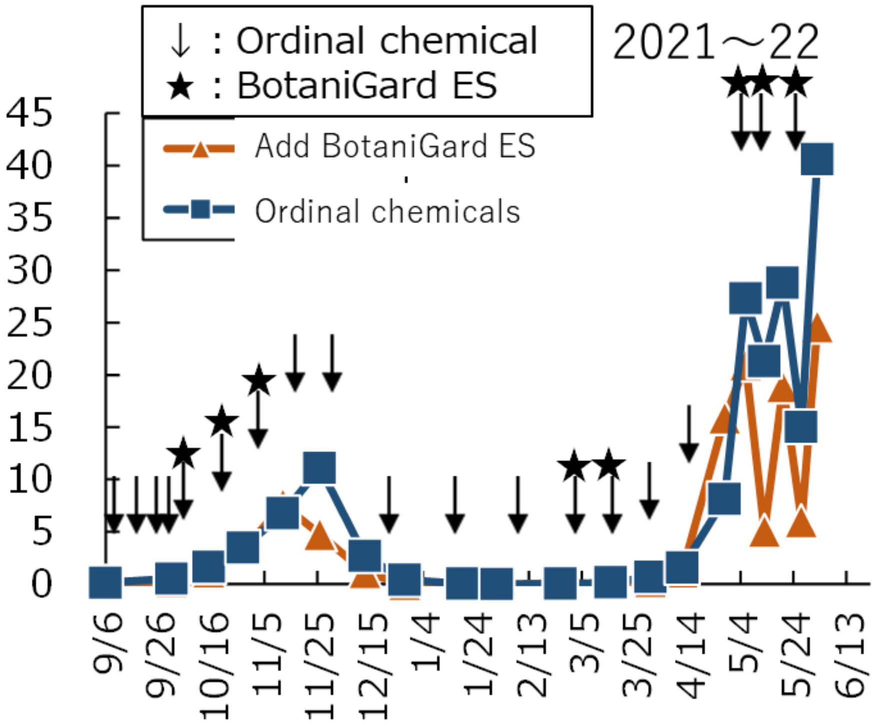

Figure 7. Number of whiteflies captured on yellow adhesive sheets in a tomato greenhouse sprayed with ordinal chemicals and additive ‘BotaniGard ES’.

CONTRIBUTION OF ‘BOTANIGARD ES’ IN STRAWBERRY FARMING

In Nara Prefecture, control of powdery mildew and pest-insects was attempted on strawberry plants. The main insect target was herbivorous pest mites, although predatory mites as natural enemies against pest mites are introduced in the area (Figure 8).

Figure 8. Target of ‘Botanigard ES’ on strawberry plants.

On strawberry plants ‘BotaniGard ES’ could control powdery mildew well in usage at low severity of the disease, as occurrence at <20% leaflets (Figure 9). Fruits of a cultivar Kotoka grown frequently in Nara Prefecture are weak to powdery mildew and effect of ‘Botanigards ES’ on the fruits was unclear. At cultivation in greenhouses in Nara Prefecture Agricultural Research and Development Center, treatments with only ‘BotaniGards ES’ could not control enough powdery mildew on fruits in winter from January. Low temperatures would make the effect of the bio-agent unsuitable, additively. ‘BotaniGard ES’ controlled pest mites compared to a spiracle-blocking insecticide. In this area, two predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus*, to control pest mites. ‘BotaniGard ES’ did not have an impact on the predators. A predatory mite, *Amblyseius cucumeris*, also got no impact from the fungus as reported by Zimmermann (2007).

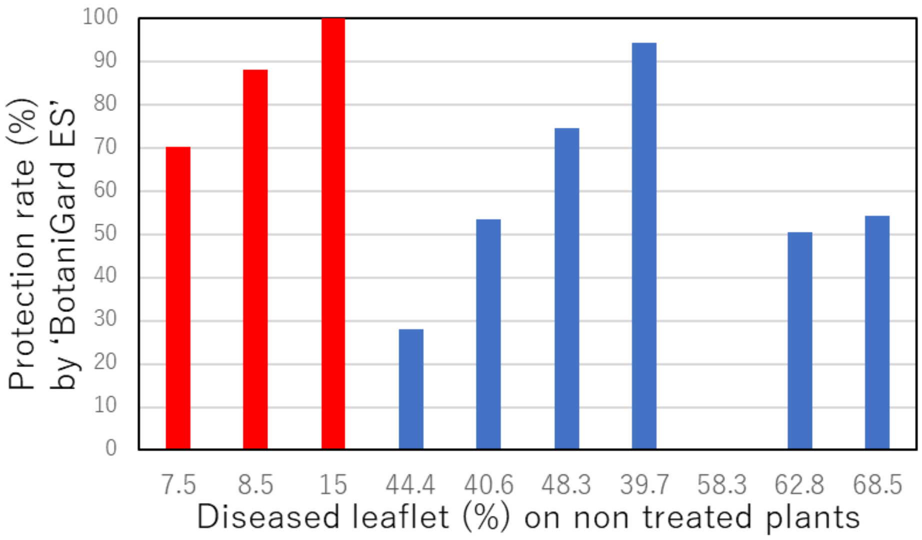

Figure 9. Protection rates by 'BotaniGard ES' in different severity of powdery mildew disease.

Strawberry plants are generally transplanted in September for harvest cultivation in greenhouses and new seedlings are isolated in May to June and then grown until transplanted. In summer season, anthracnose is the most serious disease and never miss control of the disease with chemical fungicides. As most of the fungicides can simultaneously control powdery mildew, especially protection tools from powdery mildew disease are not needed in this season. Expected periods for control of both powdery mildew and insects with 'BotaniGard ES' are March and April in spring and October in autumn. In autumn, early occurrence or increase of powdery mildew and pest mites would be avoided with 'BotaniGard ES'.

CONCLUSION

In this study, a bioagent 'BotaniGard ES' was proved to be able to control both pest-insects and diseases and techniques getting better control effect of it were considered. Bio-agents are registered as low risk to the environment and human and expected to be used increasingly. Though, environmental conditions and suitable techniques would be needed to get enough effect. More information on bio-agents including 'BotaniGard ES' and others is needed to suggest treatment techniques avoiding failures in pest control, and then improve the value of the agents.

In Japan, the standards for evaluating the safety and effectiveness of biopesticides and chemical pesticides are completely the same, so the number of registered bio-agents is few. In the market, it is difficult to promote bio-agents due to price competition and ease of use. The government has begun to hold events to introduce IPM (integrated pest management) technology, but it seems that policy support, including subsidies and revision of pesticide registration standards, is necessary for the wider adoption of bio-agents. In addition, it is hoped that research institutes present scientific evidence suggesting techniques for achieving sufficient effects with bio-agents, as in this study. Then, public and private supervisory organizations can explain this to farmers, so that they will accept the bio-agents.

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ACKNOWLEDGMENT

This work was supported by the Research Program on Development of Innovative Technology Grants (JPJ007097) from the Project of the NARO Bio-oriented Technology Research Advancement Institution (BRAIN) in 2017-2019 (29008B) and 2020-2022 (02028C). I am incredibly grateful to members of the project, Dr. Yuichiro Iida (Setsunan Univ.), Dr. Satoshi Yamanaka and Ms. Minori Sekiguchi (Arysta LifeScience Co. Ltd.), Mr. Taku Kawakami, Ms. Kaori Nakajima, Mr. Minoru Nishino, Ms. Chiharu Tanaka, Ms. Ayano Sasaki, Ms. Tsubasa Murata and Mr. Katsutoshi Kuroda (Mie Pref. Agric. Res. Inst.), Dr. Shunsuke Asano, Dr. Yoshihiko Hirayama, Mr. Takeo Imura, Mr. Masahiro Katsuma and Mr. Sota Fujimori (Nara Pref. Agric. Res. Develop. Center), Mr. Takayuki Sekine, Dr. Yasuyuki Komagata and Mr. Shogo Kakui (Miyagi Pref. Agric. Hortic. Res. Center), Dr. Hirotsugu Tsueda, Mr. Akiyoshi Miyazaki, Mr. Yuki Ito and Mr. Katsuyuki Kamiya (Gifu Pref. Agric. Technol. Center), Dr. Fumihiro Terami and Ms. Yumiko Higashi (NARO), Mr. Masashi Fujinaga and Ms. Naho Yamagishi (Nagano Veg. Ornam. Crop Experiment Station).

AUTHORS' CONTRIBUTIONS

Masaharu Kubota took up remarkable results from plenty of data on the project and wrote this manuscript.

ACOMPETING INTERESTS

The author declares no conflicts of interest.