In-vitro Efficacy of Different Bio-organic Pesticides Against Sooty Mold Disease (*Capnodium* sp.) in Tea (*Camellia sinensis*)

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Abstract

The study tried to diagnose and characterize the casual organism of tea foliar disease and to test the *in-vitro* efficacy of different bio-organic pesticides against *Capnodium sp*. The plant pathogenic fungi were collected in Maesai, Chiang Rai, Thailand and isolate into pure culture for *in-vitro* microbial bioassay. Bio-organic pesticides developed by the center was tested its antifungal activity against *Capnodium sp*. Such pesticides were herbal extract with surfactant, pure herbal extract, combination of microorganism (MMO1) and two bacterial stain (*Bacillus subtilis* and *Bacillus thuringiensis*). The use of *B. thuringiensis* gave a significant reduction of redial growth. The growth measured at 28.37 mm while the plates treated with other pesticides showed higher radial growth (47.33 mm to 50.80 mm). In addition, *B. thurigiensis* also exposed the highest zone of inhibition with a value of 18.37 mm. Zero of inhibition was observed when MMO 1 and *B. subtilis* was used while there was a slight zone of inhibition when herbal extract with surfactant and pure herbal extract (9.33 mm and 6.07 mm respectively) was used. Hence, in-vitro study indicates that harnessing *B. thurigiensis* can inhibit fungal growth of *Capnodium sp*.

Key words: Sooty mold, Bacillus subtilis, Bacillus thuringiensis, Tea, organic agriculture

Introduction

Sooty mold is a black coating on surface of plant composed of a weft of dark mycelial threads that live on insect honey dew. Sooty mold is a living plant surface habitat which will cover all infected area. Even though the fungi do not infect directly to plant but the growth of fungi might lower plant yield. Sooty mold usually found in orchid, mango, citrus, coffee and tea

Tea is one of the most common beverages in the world. It is a plant in Camellia sp. originally from China and India. It is commercially grown in more than 30 countries. In Thailand, there are a lot of tea plantations in northern part especially Chiang Mai and Chiang Rai because of the suitable conditions. The tea plantation in Maesai, Chiang Rai has found the spread of sooty mold disease in tea which effect tea quality and lower in yield.

Generally, sooty mold disease can be protected by using Carbaryl 85 WP for the killing of mouth-sucked insects. If the disease still occurs, it can be treated using fungicide e.g. Bennomil. However, the use of chemical agent such as pesticide or fungicide is strictly prohibited in organic agriculture production system. Microorganism and plant extract can be used as biological control in the system.

Bacillus subtilis and *Bacillus thuringiensis* were studied and reported that they could inhibit fungi. Therefore, this study will focus on testing of efficiency of five bio-organic pesticides which consist of *Bacillus subtilis*, *Bacillus thuringiensis*, herbal extract, herbal extract with surfactant and combination of microorganism (MMO1)

Figure 1 Sooty mold disease

Material and Method

Method

1. Collecting and Preparation of sample

Plant samples were collected from organic tea plantation in Maesai, Chiang Rai, Thailand. The sample is used to prepare fungi suspension in 50 ml distilled water.

2. Cultivation of *Bacillus subtilis* and *Bacillus thuringiensis*

Nutrient broth 500 ml has been prepared for cultivation of *Bacillus subtilis* and *Bacillus thuringiensis*. After inoculation, the suspension was shaken at 150 rpm for 24 hours.

3. Determination of in-vitro efficacy of bio-organic pesticide against Sooty mold

PDA was prepared in a cultivation plate, then drop Bacillus subtilis on one half of cultivation plate. A drop of fungi suspension was dropped on another half, then repeat for the others bio-organic pesticides.

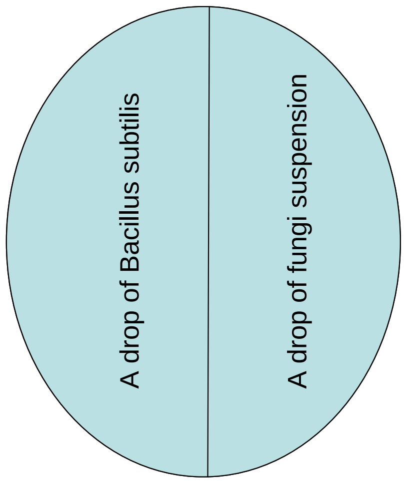

Figure 2 Determination of in-vitro efficacy of bio-organic pesticide against Sooty mold

Result and Discussion

Analysis of variance showed that there was an effect of the treatment used. Five organic bio pesticides were used in the study. Four of the treatments were comparable in terms of radial growth measurement and only one treatment gave significant inhibition of fungal growth. The used of T1-soap, T2-herbal extracts, T3-MMO and T4-Bacillus subtilis did not give significant results. The fungal growth ranged from

45.3 mm to 50.8 mm. The above result indicated that no inhibition of mycelial growth hence no active components present in the treatments. The use of *Bacillus thurigiensis* gave significant reduction in terms of radial growth. It means that the growth of mycelia would hampered by the invasion of bacteria. The mode of action could be attributing to the secretion of active components such as antifungal activity. The fungal radial growth measured at about 28.36 mm. the growth of *Capnodium* sp. was lower by 50% compared to the growth of the pathogen using the four treatments state above. Sooty mold, caused by *Capnodium* sp., is the result of a non plant pathogenic fungus that grows superficially as the thin black layer on leave or fruit, twigs and stems of various crop plants or trees. This fungus grows on the honeydew produced by insects with piercing –sucking mouthparts. The result of the study implied that the use of *B. thuringiensis* is effective in controlling sooty molds of tea.

The Zone of Inhibition (ZOI) is a microbial bioassay method to determine the effect of the different treatments against the growth of phytopathogenic fungi, the *Capnodium* sp. Zero ZOI of fungal growth was observed when MMO 1 and *Bacillus subtillis* was used as treatment. The use of herbal extract and herbal extract with surfactant showed a little effect because the range of ZOI was observed at a ranged of 6.06 mm to 9.33 mm. It means that there was a slight effect of the two treatments used. It was observed that *B. thuringiensis* was consistent in inhibiting both radial growth and gave higher ZOI. The ZOI measured as high as 18.36 mm which is higher than the accepted standard ZOI. Hence, the use of *B. thuringiensis* was effective in inhibiting fungal growth and at the same time it exhibited the highest ZOI against fungal growth.

Table 1 Zone of inhibition (mm)

Treatment	**Zone of inhibition (mm)**
Herbal extract with surfactant	9.33
Herbal extract	6.07
MMO 1	0
Bacillus subtilis	0
Bacillus thuringiensis	18.37

Table 2 Final Radial Growth Measurement (mm)

Treatment	Final Radial Growth Measurement (mm)
Herbal extract with surfactant	50.80
Herbal extract	45.30
MMO 1	47.77
Bacillus subtilis	47.33
Bacillus thuringiensis	28.37

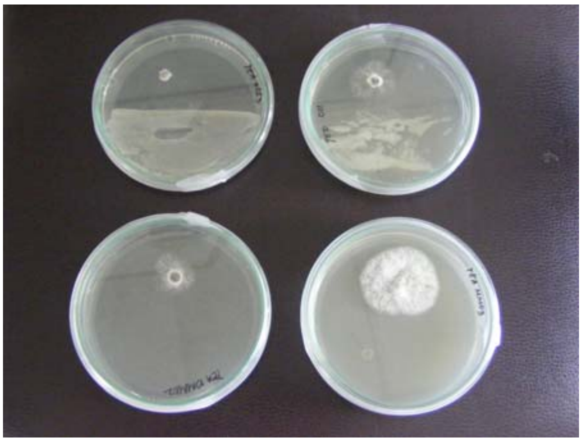

Figure 3 Zone of inhibition

According to De la Vega (1996), *B. thuringiensis* is a gram positive, rod-shaped, spore forming bacterium which often has antifungal and insecticidal properties. During sporulation *in B. thuringiensis*, some strain produce one or more inclusions or parasporal bodies with in a sporangium. The parasporal body is often toxic to specific insect an fungal groups and may different insecticidal crystal proteins (δ-endotoxin) can be found in different *B. thuringiensis* subspecies and strains. Bacterial chitinases degrade chitin primarily for use as carbon and energy sources (Barboza et al,1999) Chitinase-producing microorganisms have been reported as biocontrol agents for different type of fungi. Driss et al. (2005) observed that the culture supernatant of *B. thuringiensis* strain BUPM 225 has high antifungal activity against *Aspergillus niger*. The chitinolytic bacterium Bacillus cereus 28-9 produces a chitinase (ChiCW) that is found to be effective against the fungal pathogen Botrytis elliptica (Huang et al, 2005)

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The genus *Bacillus* is a collection of diverse bacteria that run the gamut from important producers of antibiotics; industrial metabolizers of solvents, alcohols, enzymes and vitamins; biopesticide agents (*Bacillus thuringiensis*); instigators of food poisoning (*Bacillus cereus*); and frank pathogens (*Bacillus anthracis).* Pictured is *Bacillus thuringiensis* with the parasporal body next to the forming endospore. The parasporal body (or parasporal crystal) acts as a biopesticide against the caterpillar stage of over 100 species of moths.

Bacillus thuringiensis subspecies *kurstaki* was isolated on nutrient broth yeast agar (8 g of nutrient broth, 3 g of yeast extract, 15 g of agar per liter of water) from Greenlight Dipel Dust, a commercial bioinsecticide. Isolated colonies were then used to inoculate 24-hour nutrient broth yeast broths for subsequent fixation and observation.

Bacillus thuringiensis is widely used as a Lepidopteron-specific bioinsecticide. During sporulation, the bacterium produces a toxin crystal otherwise known as the parasporal body. Once ingested by the tomato hornworm or other susceptible leaf-eating caterpillars, the parasporal body is subjected to alkaline conditions in the hindgut resulting in fragmentation into protoxin (250 kD) crystals. The protoxin is then activated by gut proteases to a 68 kD active toxin. Active toxin units aggregate to form hexagonal-shaped pores within the plasma membrane of the midgut leading to loss of osmotic balance and finally cell lysis leading to caterpillar death. The crystal and endospores are incorporated into a commercial powder for application to plants to control various leaf-eating caterpillars while having no deleterious effects on humans or pets. Use of the biopesticide overcomes the insecticide resistance and toxicity problems associated with traditional chemical pesticides. Since *B. thuringiensis* biopesticides have a specific target host, they do not kill beneficial insects such as honeybees unlike most insecticides. Additionally, the *Bacillus thuringiensis* toxin gene has been incorporated into corn and other crops in order to confer the toxin's protective effects against caterpillar attack (2).