

## Study on Efficiency and Development of Anti-Anthrachnose Disease Products for Chili Peppers with Natural Substances, Nakhon Ratchasima Province, Thailand

Chetsada Promma<sup>a</sup>, Sutus Termsaithong<sup>b</sup>, Tarntip Rattana<sup>c</sup>, Waraporn Kosanlavit<sup>d</sup>

<sup>a</sup> Program of Environmental Management Technology, Graduated School, NRRU,

<sup>b,d</sup> Program of Environmental Science, Faculty of Science and Technology, NRRU

<sup>a</sup>aquatreatchemical@outlook.com., <sup>d</sup>waraporn.kslavit@gmail.com.

**Article History:** Received: 10 January 2021; Revised: 12 February 2021; Accepted: 27 March 2021; Published online: 20 April 2021

**Abstract:** The anthracnose outbreak occurs during the rainy season or a long raining period that facilitates the growth of fungi. Anthracnose can be commonly found during chili pepper growing, and it has caused considerable damage to farmers resulting in reduced production. Most farmers use chemicals to prevent anthracnose disease-causing toxic residues in the environment. The chemicals are also expensive and dangerous to the environment. For the study on the development of anti-anthrachnose disease products with natural substances for chili peppers, the researchers realized the importance of the purposes of 1) to study and develop natural substances and agricultural waste materials which are environmentally friendly products to prevent anthracnose disease in chili peppers and 2) to compare the efficiency between using chemicals substances that are commonly used at present. The study on efficiency and development of anti-anthrachnose disease products on chili peppers employed the Completely Randomized Design (CRD) to study the efficacy of substances used to prevent anthracnose disease. There are 4 treatments used in this study includes 1) Control, 2) Natural product Formula I (Bio-fermented water No. I), 3) Natural product Formula II (Bio-fermented water No. II), and 4) Chemical Product (Mancozeb chemical). Research operates by spray treatment products on chili every 7 days. After spraying treatments for 24 hours, spray *Colletotrichum* spp. to infect anthracnose in the period of three weeks. The study indicated that the natural product formula I and the natural product formula II found the efficiency of preventing the disease in chili peppers in significantly at 95 percent. The natural product formula I was highest anthracnose disease prevention, followed by the Mancozeb chemical, natural product formula II, and control, respectively.

**Index Terms:** Anti-Anthrachnose Disease, Natural Products, Bio-Fermented water

### 1. Introduction

Chili pepper is a plant in the genus *Capsicum* which is a member of the Solanaceae family [1]-[3]. The English names include chili peppers, chile, or chili. Chili is an economic crop that generates income and can be grown throughout Thailand. However, a problem with Chili peppers faced by many farmers is anthracnose, which is a common but very serious disease among pepper farmers. Anthracnose is a plant disease caused by fungi, namely *Colletotrichum* spp. *Colletotrichum gloeosporioides*, *Colletotrichum capsici*, and *Colletotrichum piperatum*. *C. gloeosporioides* attack big chili peppers and *C. capsici* attack small chili peppers (bird's eye chili), while *C. piperatum* attack large chili peppers (bell peppers) [4]-[6]. A common symptom found in big chili peppers such as spur chili peppers is that *C. gloeosporioides* cause a wet spot with a slight dent on the chili's surface or a wet circular or oval spot which then becomes bigger as the fungus produces spores, noticeable by a black spot [7]-[10]. For small chili peppers, *C. capsici* occurs during the rainy season causing a wet spot with a vague dent or without a dent. If the chili peppers get infected outside the rainy season, the shape of the dent will be unstable without a black wet spot. Anthracnose is sometimes called 'Dried shrimp disease' as it produces light orange slime shaped like a dried shrimp.

The anthracnose outbreak occurs during the rainy season or a long raining period that facilitates the growth of fungi. Anthracnose can be commonly found during chili pepper growing, and it has caused considerable damage to farmers resulting in reduced production. Most farmers use chemicals to prevent anthracnose disease-causing toxic residues in the environment. The chemicals are also expensive and dangerous to the environment.

For the study on efficiency and development of anti-anthrachnose disease products with natural and chemical substances for chili peppers, the researchers realized the importance of using natural substances and agricultural waste materials to prevent the anthracnose disease in chili peppers and would like to compare with chemical substances that are commonly used at present. The researchers hope that this study will help to obtain environmentally friendly products.

### 2. Material and Method

#### A. The studies areas

The research was conducted in a specific cultivation area in Nai Muang Sub-District, Muang District, Nakhon Ratchasima Province, and in the laboratory of the Environmental Science Department, Faculty of Science and

Technology, Nakhon Ratchasima Rajabhat University.

## **B. The studies samples**

The population and sample group included chili peppers. Chili pepper type was Chili spur pepper (*Capsicum annuum* Linn. Var. *acuminatum* Fingerh).

## **C. Chemical and Research Instruments**

The substances used in the production are divided into 2 groups: natural products and chemical products. Chemical and substances were used in this research include:

1. Natural product Formula I: Bio-fermented water A (betel nutshell and banana tree)
2. Natural product Formula II: Bio-fermented water B (channeled apple snail and banana tree)
3. Chemical product: Mancozeb
4. The anthracnose pathogens (*Colletotrichum* spp.)

## **D. Research Methodology**

The study on efficiency and development of anti-anthracnose disease products on chili peppers employed the Completely Randomized Design (CRD) to study the efficacy and production cost of substances used to prevent anthracnose disease and to provide anthracnose disease prevention guidelines for farmers.

The substances used in the production are divided into 2 groups: natural and chemical. The products tested for efficiency by the researchers consist of 3 products and 1 control product, namely: 1) Control, 2) Natural product Formula I (Bio-fermented water No. I), 3) Natural product Formula II (Bio-fermented water No. II), and 4) Chemical Product (Mancozeb chemical).

Cultivation of anthracnose pathogens, *C. gloeosporioides*, and *C. capsici* was separated from chili with anthracnose. The infected area was removed from the normal area, sanitized, placed on Potato Dextrose Agar (PDA) and incubated for 7 days at room temperature. Then, *C. gloeosporioides* and *C. capsici* were cultivated on the PDA petri dish for 7 days to produce spores. The spores were diluted to have a concentration of  $10^5$  conidia/ml.

Natural product Formula I: bio-fermented water A (betel nutshell and banana tree) was produced by mixing 5 kg of a cracked betel nutshell, 5 kg of chopped banana tree, 5 liters of molasses, and Microbial Activators PD2 solution (dissolve 2 bags of Microbial Activators PD2 powder in 5-liter water and leave it for 5 minutes) in a 20-liter tub with the lid slightly open for ventilation for 60 days. Then, filter the water. To use the natural product Formula I: bio-fermented water A, use 2 tablespoons per 20 liters of water and spray it on the chili every 7 days starting 2 weeks after the trees produce chili.

Natural product Formula II: bio-fermented water B (channeled apple snail and banana tree) was produced by mixing 5 kg of banana tree, 5 kg of chopped channeled apple snail, 5 liters of molasses, and Microbial Activators PD2 solution (dissolve 2 bags of Microbial Activators PD2 powder in 5-liter water and leave it for 5 minutes) in a 20-liter tub with the lid slightly open for ventilation for 60 days. Then, filter the water. To use the natural product Formula II: bio-fermented water B, use 2 tablespoons per 20 liters of water and spray it on the chili every 7 days starting 2 weeks after the trees produce chili.

Chemical product (Mancozeb chemical) is an ethylenebis chemical group. To use, dissolve about 70 grams of Mancozeb in 20 liters of water and spray it on the chili every 7 days starting 2 weeks after the trees produce chili.

The study on efficiency and development of anti-anthracnose disease products on chili peppers employed CRD by using 4 treatments, each of which was done in triplicate as follows:

T1R1	T2R1	T3R1	T4R1
T2R2	T3R2	T4R2	T1R2
T3R3	T4R3	T1R3	T2R3

The chili trees are 1 meter away from one another. T represents 4 treatments consisting of T1 Control, T2 Natural product Formula I, T3 Natural product Formula II, and T4 Chemical product. Also, R represents 3 replications: R1 First replication, R2 Second replication, and R3 Third replication.

## E. Experiment Methods

Grow chili in a pot of 15cm height and 20cm diameter. When the chili is 2-3 months old or bears the fruit, spray 30ml of disease prevention product per one chili every 7 days. After spraying for 24 hours, scratch the chili to create a wound and spray *C. gloeosporioides* and *C. capsici* to infect anthracnose at the concentration of  $1 \times 10^5$  conidia/ml on all the 12 chili trees, 20ml each. When the chili trees bear the fruit, spray the anthracnose disease prevention product on them. After 24 hours, spray the pathogens on the chili trees. Count the number of chili peppers that do not have anthracnose 3 times on the 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> days after spraying. Count the total number of chili peppers, the number of chili peppers with anthracnose, and the number of those without.

## F. Data Analysis

Calculate anthracnose disease prevention using the percentage. Analyze and compare the differences of anthracnose disease prevention and compare the total differences using the CRD statistics

## 3. Results

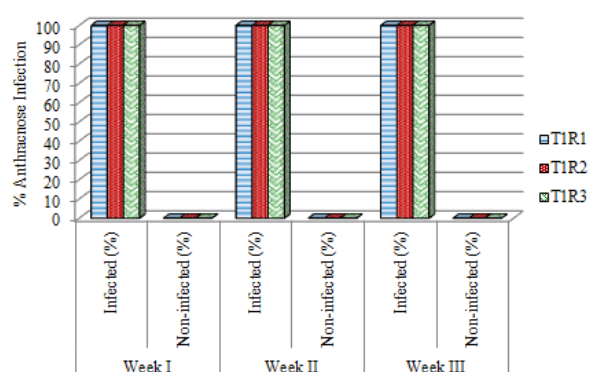
The study on efficiency and development of anti-anthracnose disease products with natural and chemical substances for chili peppers were found the results as follow:

### The efficiency of natural products and chemical product to prevent anthracnose disease

**Table I** Percentage of anthracnose infection of chili pepper

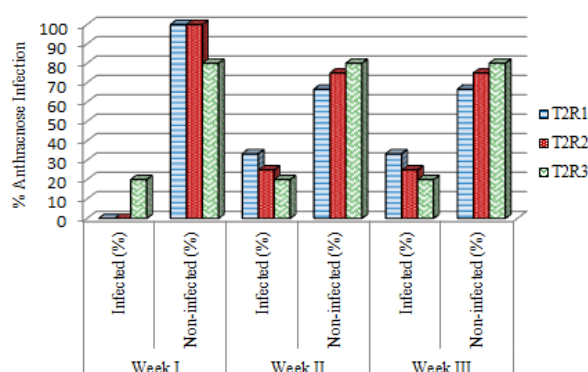
Treatments Rep.	Week I		Week II		Week III	
	Infected (%)	Non-infected (%)	Infected (%)	Non-infected (%)	Infected (%)	Non-infected (%)
Control						
T1R1	100.00	0.00	100.00	0.00	100.00	0.00
T1R2	100.00	0.00	100.00	0.00	100.00	0.00
T1R3	100.00	0.00	100.00	0.00	100.00	0.00
Natural product Formula I						
T2R1	0.00	100.00	33.33	66.67	33.33	66.67
T2R2	0.00	100.00	25.00	75.00	25.00	75.00
T2R3	20.00	80.00	20.00	80.00	20.00	80.00
Natural product Formula II						
T3R1	77.78	22.22	77.78	22.22	77.78	22.22
T3R2	25.00	75.00	25.00	75.00	25.00	75.00
T3R3	20.00	80.00	20.00	80.00	40.00	60.00
Chemical product						
T4R1	0.00	100.00	33.33	66.67	33.33	66.67
T4R2	0.00	100.00	33.33	66.67	33.33	66.67
T4R3	20.00	80.00	20.00	80.00	20.00	80.00

The results of the efficiency of natural products and chemical product to prevent anthracnose disease from Table I found the natural product formula I, the natural product formula II, and chemical product (Mancozeb) have the efficiency to prevent anthracnose disease when compare with control. There show the different results following Fig.1-Fig.4.



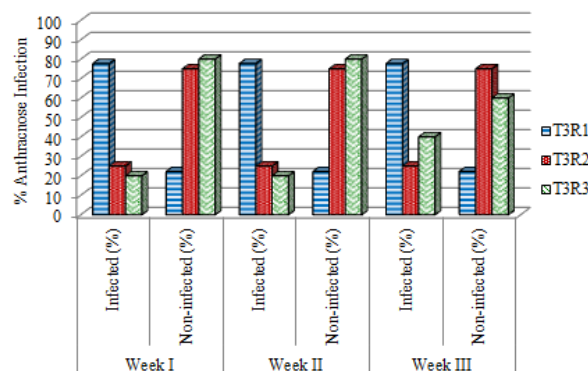
**Fig. 1** Percentage of anthracnose infection and non-infection of chili pepper where control treatment using for prevention

The percentage of control treatment using found 100% of chili pepper infected anthracnose disease in a week I, II, and III (Fig. 1). There was mean the chili pepper cannot prevent anthracnose disease by themselves.



**Fig. 2** Percentage of anthracnose infection and non-infection of chili pepper where natural product formula I treatment using for prevention

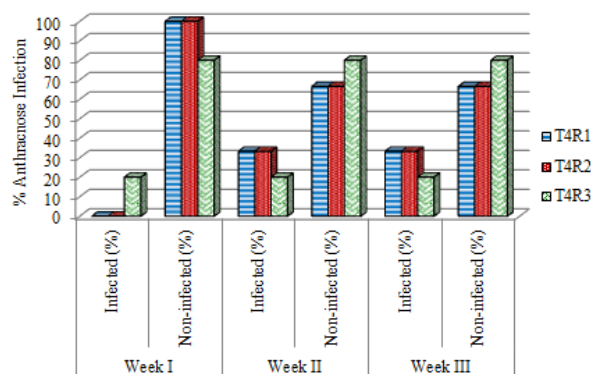
Natural product formula I was bio-fermented water A where betel nutshell and banana tree (two main ingredients) were mixed show the percentage of efficiency for anthracnose prevention 80-100% in a week I, 66.67-80% in week II and III. Moreover, the results show that the efficiency of anthracnose prevention were decreasing from week I to week II, but constancy from week II to week III as shown in Fig. 2.



**Fig. 3** Percentage of anthracnose infection and non-infection of chili pepper where natural product formula II treatment using for prevention

Natural product formula II was bio-fermented water B where channeled apple snail and banana tree (two main ingredients) were mixed show the percentage of efficiency for anthracnose prevention 22.22-80% in a week I, 22.22-80% in week II, and 22.22-75% in week III, whereas there found the chili pepper were anthracnose infection 20-

33.33% approximately. Moreover, the results show that the efficiency of anthracnose prevention was decreasing from week I to week III, respectively. There means an increase of anthracnose infection in chili pepper as shown in Fig. 3.

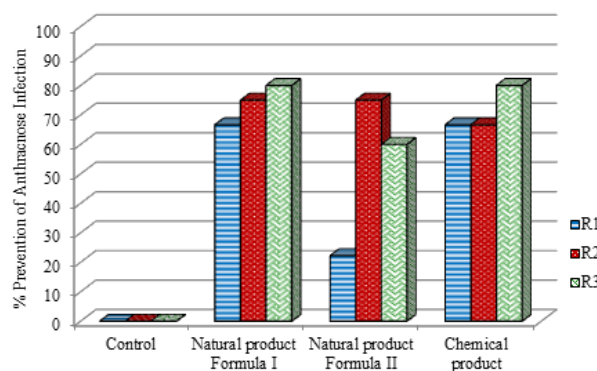


**Fig. 4** Percentage of anthracnose infection and non-infection of chili pepper where chemical product treatment using for prevention

The chemical product was Macozeb show the percentage of efficiency for anthracnose prevention 80-100% in a week I, 66.67-80% in week II and week III, whereas there found the chili pepper was anthracnose infection 20-33.33% approximately. Moreover, the results show that the efficiency of anthracnose prevention was decreasing from week I to week II, but constancy from week II to week III as shown in Fig. 4.

**Table II** Percentage of non-infected chili pepper to anthracnose disease

Replication	Control	Natural product Formula I	Natural product Formula II	Chemical Product	
R1	0.00	66.66	22.22	66.66	
R2	0.00	75.00	75.00	66.66	
R3	0.00	80.00	60.00	80.00	
Total: T <sub>1</sub>	0.00	221.66	175.22	213.32	GT = 610.20
Size (Rep.): n <sub>1</sub>	3	3	3	3	N = 12
Mean	0.00	73.87	58.41	71.11	GM = 50.85



**Fig. 5** Comparisons of natural products and chemical product for anthracnose infection prevention (%) in chili pepper

Table II and Fig. 5 show the trend of overall 4 treatments includes 1) Control, 2) Natural product Formula I: Bio-fermented water A (betel nutshell and banana tree), 3) Natural product Formula II: Bio-fermented water B (channeled apple snail and banana tree), and 4) Chemical products (Mancozeb). The results show that the average

percentage of anthracnose disease prevention of Natural product Formula I was highest (73.87 %), follow by Chemical product (Mancozeb) (71.11 %), Natural product Formula II (58.41 %), and control (0.00%), respectively.

#### **Cost-Benefit of natural products and chemical product**

Cost-Benefit of Natural product Formula I: Bio-fermented water A (betel nutshell and banana tree), Natural product Formula II: Bio-fermented water B (channeled apple snail and banana tree), and Chemical product (Mancozeb) in anthracnose disease prevention were calculated from product cost and dose (volume; ml) using per chili pepper tree.

Natural product formula I: the cost of bio-fermented water A, volume 30 ml is 0.0009 baht, in the trial using natural product formula I 3 times/chili pepper tree, which is 0.0027 baht. Normally, the farmer can grow about 3,200 chili pepper tree/1 rai, which is equal to 8.64 baht/rai of natural product formula I (140 liters/rai). The average percentage of anthracnose disease prevention of Natural product Formula I was highest (73.87 %).

Natural product formula II: the cost of bio-fermented water A, volume 30 ml is 0.0008 baht, in the trial using natural product formula I 3 times/chili pepper tree, which is 0.0024 baht. Normally, the farmer can grow about 3,200 chili pepper tree/1 rai, which is equal to 7.68 baht/rai of natural product formula II (140 liters/rai). The average percentage of anthracnose disease prevention of Natural product Formula I was 58.41 %.

Chemical product (Mancozeb): the cost of Mancozeb, volume 30 ml is 0.045 baht, in the trial using natural product formula I 3 times/chili pepper tree, which is 0.135 baht. Normally, the farmer can grow about 3,200 chili pepper tree/1 rai, which is equal to 432 baht/rai of Mancozeb (140 liters/rai). The average percentage of anthracnose disease prevention of chemical products (Mancozeb) was 71.11 %.

#### **4. Conclusion**

The study on efficiency and development of anti-anthracnose disease products on chili peppers employed the Completely Randomized Design (CRD) to study the efficacy of substances used to prevent anthracnose disease. There are 4 treatments used in this study includes 1) Control, 2) Natural product Formula I (Bio-fermented water No. I), 3) Natural product Formula II (Bio-fermented water No. II), and 4) Chemical Product (Mancozeb chemical). Research operates by spray treatment products on chili every 7 days. After spraying treatments for 24 hours, spray *Colletotrichum* spp. to infect anthracnose in the period of three weeks. The results of the study indicated that the natural product formula I and the natural product formula II found the efficiency of preventing the disease in chili peppers in significantly at 95 percent significant. The natural product formula I was highest anthracnose disease prevention, followed by the Mancozeb chemical, natural product formula II, and control, respectively. These results were due to the reason of different main ingredients of each formula treatment. Two main ingredients of natural product Formula I (Bio-fermented water A) were betel nutshell and banana tree which were astringent plants and anti-mold properties. Natural product Formula II (Bio-fermented water B) has two main ingredients were channeled apple snail and banana tree, which channeled apple snail consist of *Bacillus* sp., *Lactobacillus* sp., and *Streptococcus* sp. There were anti-mold properties.

Cost-Benefit of Natural product Formula I: Bio-fermented water A (betel nutshell and banana tree), Natural product Formula II: Bio-fermented water B (channeled apple snail and banana tree), and Chemical product (Mancozeb) in anthracnose disease prevention were calculated from product cost and dose (volume; ml) using per chili pepper tree.

Natural product formula I: 8.64 baht/rai (73.87% Efficiency)

Natural product formula II: 7.68 baht/rai (58.41% Efficiency)

Chemical product (Mancozeb): 432 baht/rai (71.11% Efficiency)

Therefore, Natural product formula I (Bio-fermented water A: betel nutshell and banana tree) show the highest cost-benefit for the anti-anthracnose disease. This research realized the importance of using natural substances and agricultural waste materials to prevent anthracnose disease in chili peppers and environmentally friendly products. The natural products of this research can be alternative methods and products for the farmer in anthracnose disease control in chili pepper.

#### **References**

1. V.V. Shah, N.D. Shah, and P.V. Patrekar. "Medicinal Plants from Solanaceae Family," Research J. Pharm. and Tech. vol. 6, no. 2, pp. 143-151, February 2013.

2. J.J Tewksbury, and G.P Nabhan. "Directed deterrence by capsaicin in chilies," *Nature*. Vol. 412, no. 6845, pp. 403–404, 2001.
- A. Rodríguez-Burruezo, C. González-Mas, and F. Nuez. "Carotenoid composition and vitamin a value in ají (*Capsicum baccatum* L.) and rocoto (*C. Pubescens* R. & P.), 2 pepper species from the Andean region," *Journal of Food Science*. vol. 75, no.8, pp. S446–53, 2010.
3. P.F. Cannon, P.D. Bridge, and E. Monte. "Linking the past, present, and future of *Colletotrichum* systematics. In: D Prusky, S. Freeman, M. Dickman, eds. *Colletotrichum: Host Specificity, Pathology, and Host-Pathogen Interaction*," St Paul, MN, USA: APS Press, pp.1– 20 2000.
4. S. Freeman, T. Katan, and E. Shabi. "Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits," *Plant Disease* vol. 82, pp. 596– 605, 1998.
5. J.C. Guerber, B. Liu, J.C. Correll, and P.R. Johnston. "Characterization of diversity in *Colletotrichum acutatum* sensu lato by sequence analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility," *Mycologia* vol. 95, pp.872– 95, 2003.
6. B.B. Higgins. "Anthracnose of pepper (*Capsicum annum* L.)," *Phytopathology* vol. 16, pp. 333-343, 1926.
7. N.K.B. Adikaram, A.E. Brown and T.R. Swinburne. "Phytoalexin involvement in latent infection of *Capsicum annum* L. fruit caused by *Glomerella cingulate* (Stonem.)," *Physio.Plant. Pathol.* Vol. 12, pp.161-170, 1982.
8. S. Freeman, and R.J. Rodriguez. "A Rapid, Reliable Bioassay for Pathogenicity of *Colletotrichum Magna* on Cucurbits and Its Use in Screening for Nonpathogenic Mutants," *Plant Dis.* vol.76, pp. 901-905, 1992.
9. R.T. McMillan. "Enhancement of anthracnose control on mango by combining copper with Nui-film-17," *Pro. Fla. State Hort. Soc.* vol. 85, pp. 268-270, 1972