

Effect of Capsaicin on Germination of *Colletotrichum capsici* Conidia

Wilawan Kraikruan¹, Somsiri Sangchote^{2*} and Sutevee Sukprakarn³

ABSTRACT

Different varieties of cultivated chilies (*Capsicum spp.*) produce different amounts of capsaicin, but the biological role of this compound has not yet been elucidated. High concentrations of capsaicin in some chili varieties are possibly associated with resistance to anthracnose, a fruit rot disease. Consequently, this paper examines the role of capsaicin in preventing conidial germination of the anthracnose-causing fungus *Colletotrichum capsici*. Conidia were germinated on potato-dextrose agar media supplemented with 40% methanol/aqueous capsaicin solution in final concentrations of 0, 25, 50, 100 and 200 mg/l. Each plate was inoculated with a solution containing approximately 400 conidia of *C. capsici*, then incubated at 25°C and 70% relative humidity under continuous light for 5, 6 and 7 hours.

Approximately 96% of conidia germinated on the capsaicin-free medium after six hours of incubation, but only 53% germinated on PDA containing 25 mg/l capsaicin. Subsequently, conidia germination increased to 91% after 7 hours on plates containing 25 mg/l capsaicin. Germination of *C. capsici* conidia was completely inhibited on the PDA with 100 and 200 mg/l of capsaicin.

Key words: pure capsaicin, anthracnose disease, conidia germination, acervulus formation

INTRODUCTION

Capsaicin, 8-methyl-N-vanillyl-6-nonenamide, which provides the pungency of chili peppers, is synthesized and accumulates in the placenta within the fruit (Iwai *et al.*, 1979; Suzuki *et al.*, 1980; Ishikawa *et al.*, 1998). It is slightly water-soluble but is preferably dissolved in diluted alcohol and is used as a medicine or food additive.

The pungency of chili is measured in Scoville Heat units (SHU) or mg/l capsaicin (Bosland and Votava, 1999). The relationship of the pungency level and capsaicin concentration revealed that the 'Thai bird chilies' and 'Leung

chili' varieties contained the highest capsaicin content followed by 'Cheefa', green peppers and sweet peppers (Yeuanongsawat, 2002).

Anthracnose of chili is caused by the fungus in the genus *Colletotrichum* and is one of the major constraints on chili production in tropical and subtropical areas (AVRDC, 2003). There are two species of the genus *Colletotrichum* that cause anthracnose disease on chili fruits, *C. gloeosporioides* (Penz.) Penz. & Sacc and *C. capsici* (Syd.) E.J. Butler & Bisby (Hadden and Black, 1987; Manandhar *et al.*, 1995). *Colletotrichum gloeosporioides* causes disease on both unripe (green) and ripe (red) chili fruits (Kim

¹ Kanchanaburi Horticultural Research Center, Department of Agriculture, Ministry of Agriculture and Cooperative, Kanchanaburi 71110, Thailand.

² Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

³ Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

* Corresponding Author, e-mail: agrsrs@ku.ac.th

et al., 1989; Park *et al.*, 1990), while the *C. capsici* infects immature fruits and remains quiescent, developing symptoms after fruit ripening (Higgins, 1926; Adikaram *et al.*, 1982). Since capsaicin produced in the chili fruit may affect the growth and development of the pathogen, the effects of capsaicin on conidial germination and placental acervuli formation of *Colletotrichum capsici* were investigated.

MATERIALS AND METHODS

The effect of purified capsaicin on conidial germination of *Colletotrichum capsici*

Inhibition of *C. capsici* conidial germination was studied using the poisoned food technique (Dhingra and Sinclair, 1994) on potato-dextrose agar (PDA) to which various concentrations of capsaicin had been added.

Pure capsaicin M2028® from Sigma-Aldrich (USA) was dissolved with 40% methanol to obtain stock solutions of 250, 500, 1,000 and 2,000 mg/l. One ml of each stock solution was mixed with 9 ml molten PDA to obtain media with 25, 50, 100 and 200 mg/l capsaicin. One milliliter of 40% methanol was also added to a fifth aliquot of PDA as a control treatment. The mixtures of each capsaicin concentration, as well as the control, were then mixed with the PDA in a rotary shaker, poured on petri dishes and left until the medium had cooled and solidified.

Conidia of *C. capsici* were obtained from a 6-day-old culture of the Kampaengsaen isolate grown on PDA at 27°C, 70% relative humidity (RH), under 12 hours alternating fluorescent light and darkness conditions. Conidia were harvested by adding 5-10 ml of sterilized, distilled water and filtered through a sterilized, double layer of cheesecloth. The conidia concentration was adjusted to 4×10^5 conidia/ml using a hemacytometer. One micro liter of 4×10^5 conidia/ml solution (*i.e.* approximately 400 conidia/plate) was applied and spread on the culture media

supplemented with the various concentrations of capsaicin, and incubated at 27°C and 70% RH under continuous light. There were four replicates (agar plates) of each capsaicin concentration treatment and the control. The germination of conidia on various concentrations of capsaicin were observed and quantified after 5, 6 and 7 hours of incubation by counting the germinated conidia in five randomly selected objective fields at 100x magnification.

Comparison of the germinated conidia of *C. capsici* in various capsaicin concentrations at the different incubation periods were analyzed using the IRRI statistical program.

Investigation of acervuli formation on placenta of chili fruit infected with *Colletotrichum capsici*

Chili fruits (*Capicum annuum* var. leung) with anthracnose symptom were collected from the field. Twenty fruits infected with *Colletotrichum capsici* were transversely cut and fruit with lesions above the dissepiment were used for evaluation. The fruits were opened and then incubated in a moist-chamber at 27°C, 70% RH under alternate 12 hours light/dark for three days. The growth and development of fungus and acervulus formation were observed on the dissepiment and placenta. Three replicates of 20 fruits were studied.

RESULTS AND DISCUSSION

The effect of purified capsaicin on conidial germination of *Colletotrichum capsici*

The present study found that high concentrations of capsaicin inhibited conidial germination of *Colletotrichum capsici*. Germination rates of *C. capsici* conidia, as judged by the formation of germ tubes, varied with the capsaicin concentration of the medium and the incubation time (Table 1). In general, increased germination time in media with equivalent capsaicin concentrations increased conidial

germination, while increasing the capsaicin concentrations decreased conidial germination with varying incubation times. While this study tried to isolate the effects of capsaicin in a controlled experimental setting, it is likely that additional compounds in chili fruits also affect the growth of pathogens. To elucidate the role of capsaicin in a natural setting, fungal growth on media containing natural extracts from various chili varieties that differed in their capsaicin concentration could be compared. However, conidia on PDA with 100 and 200 mg/l of capsaicin had not developed germ tubes (Table 1, Figure 1, 2).

Capsaicin not only makes chilies pungent or hot, but also inhibits the growth of many microorganisms. Cichewicz and Thorpe (1996) reported that capsaicin inhibited the growth of

Bacillus cereus, *Bacillus subtilis*, *Clostridium sporogenes*, *Clostridium tetani* and *Streptococcus pyogenes*. In addition, concentrations that exceeded 10 $\mu\text{g/ml}$ of capsaicin inhibited the growth of the gastric pathogen, *Helicobacter pylori* (Jones *et al.*, 1997). Furthermore, high capsaicin concentration could retard the growth of *Escherichia coli* and *Pseudomonas solanacearum* and inhibit the growth of *Bacillus subtilis* (Torres *et al.*, 1991). Azad (1991) also reported that capsaicin content was high in anthracnose-resistant varieties, while the content was low in susceptible varieties. Moreover, Tenaya *et al.* (2001) found that the higher capsaicin content in red pepper (*C. annuum*) indicated resistance to anthracnose. Therefore, the capsaicin content in chili might be used as an indicator for the incidence of anthracnose disease.

Table 1 Conidial germination of *Colletotrichum capsici* grown on potato-dextrose agar with different concentrations of capsaicin.

Hours after inoculation	Conidia germination					Mean*
	0 (control)	25(mg/l)	50(mg/l)	100(mg/l)	200(mg/l)	
5	59.2	0.5	0.0	0.0	0.0	11.9 A
6	95.7	52.8	0.0	0.0	0.0	29.6 B
7	98.9	90.7	0.0	0.0	0.0	37.9 C
Mean*	84.6 a	48.0 b	0.0 c	0.0 c	0.0 c	

* Means with different letters are significantly different at $P < 0.05$ by multiple range testing (DMRT).

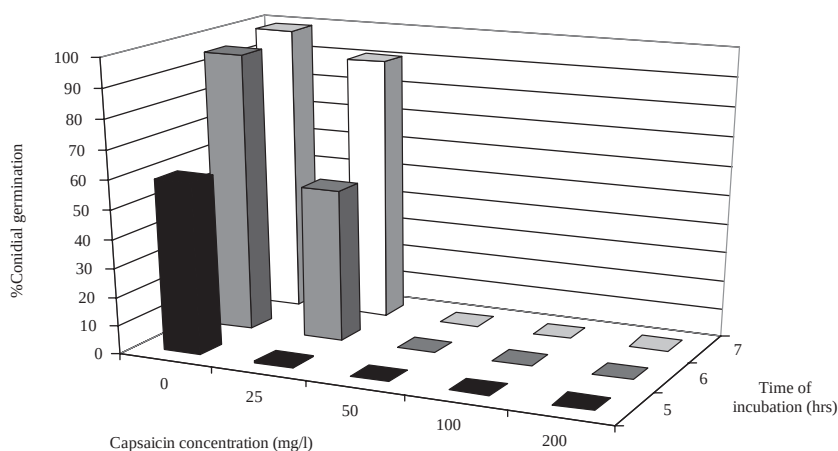


Figure 1 Germinated conidia of *Colletotrichum capsici* on potato-dextrose agar media with different capsaicin concentrations after 5, 6 and 7 hours of incubation.

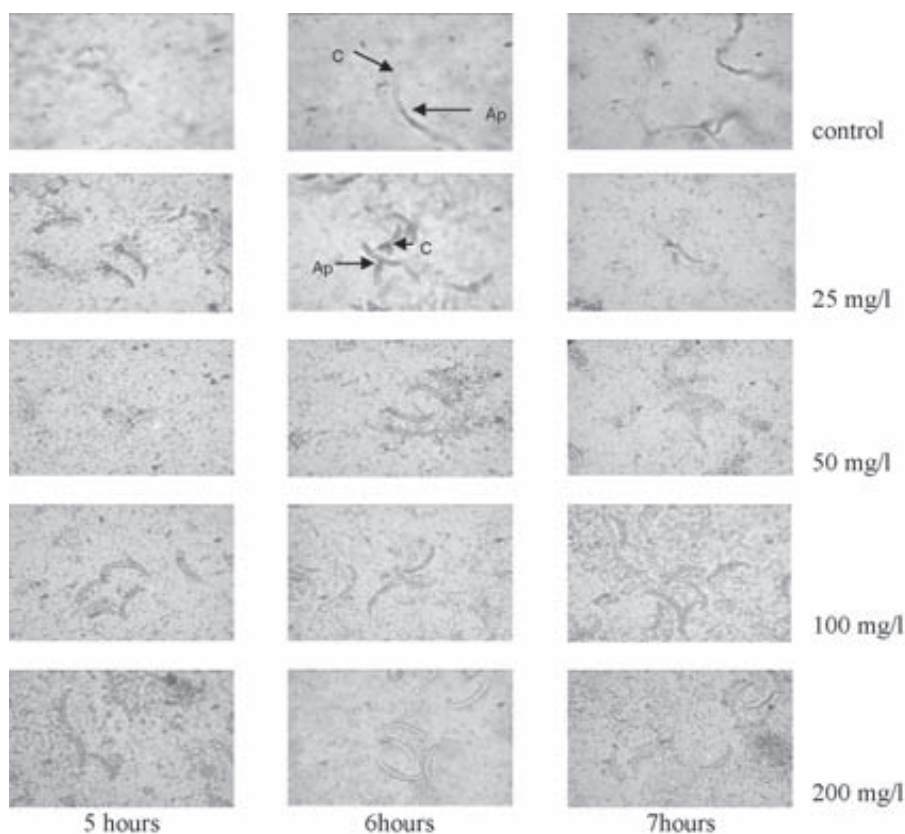


Figure 2 Conidial germination of *Colletotrichum capsici* (Ap= appresorium, C= conidia) on potato-dextrose agar media with 0, 25, 50, 100 and 200 mg/l of capsaicin at 5, 6 and 7 hours after incubation (100X).

Acervulus formation on placenta of chili fruit infected by *Colletotrichum capsici*

After *C. capsici* conidia were allowed to incubate on chili fruits for 3 days, acervuli formed on the dissepiment and placenta of 41.7% of the fruits. Approximately 58.3% of the acervuli formed only on the dissepiment and not on the placenta (Figures 3, 4). Sangchote (1984) reported that there are two pathways for spore transmission in a *Colletotrichum capsici* invasion. Usually, the mycelium develops along the dissepiment and spreads to the remnant of the placenta, then enters through the opening of the seed testa. Since capsaicin is produced and accumulated in the placenta (Iwai *et al.*, 1979; Suzuki *et al.*, 1980; Ishikawa *et al.*, 1998), a large amount of this compound should be present in these tissues. In

this investigation, 58.3% of infected chili fruits had no lesions on the placenta. However, the other 41.7% did have infected placentas. The results also indicated that the placenta might have had an inhibitory effect on the fungus and capsaicin may have been the primary inhibitor found in the placenta, which correlates with other observations from this study that the pathogen could only be found on either the dissepiment or both the placenta and the dissepiment.

CONCLUSIONS

This study on the effect of pure capsaicin on *Colletotrichum capsici* conidial germination indicated that at capsaicin concentrations above 50 mg/l, conidial germination was totally inhibited,

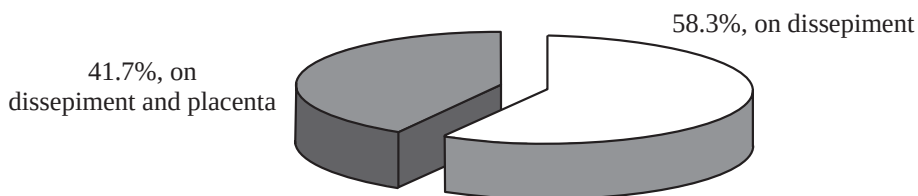


Figure 3 Acervuli formation on the dissepiment and placenta of *Capsicum annuum* var. leung infected by *Colletotrichum capsici* after three days of incubation.

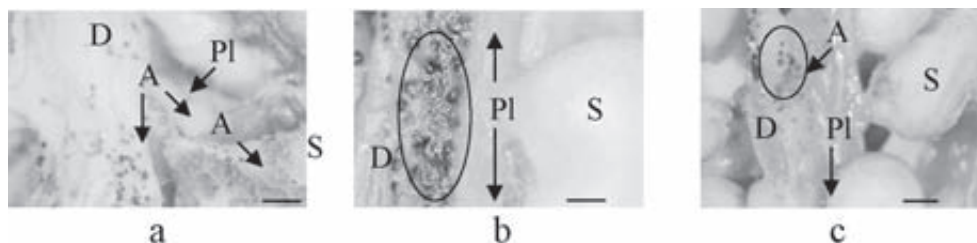


Figure 4 Acervulus formation lesions of chili fruits (*Capsicum annuum* var. leung) infected by *Colletotrichum capsici* 3 days after incubation (all bar = 1mm). a. Acervulus formation on the dissepiment, placenta and seed (D = dissepiment, Pl = placenta, S= seed, A = acervulus) b and c) Acervulus formation on the dissepiment, but not on placenta.

whereas a concentration of 25 mg/l had little overall effect on the conidial germination after seven hours of incubation.

The investigation of the acervuli formation of *Colletotrichum capsici* on the placenta of the chili fruit (*Capsicum annuum* var. leung) infected with *Colletotrichum capsici* showed that 58.3% of placenta were not infected. Capsaicin found in the placenta was possibly the main compound that provided anthracnose resistance.

ACKNOWLEDGEMENTS

Thanks are due to the Department of Agriculture, Ministry of Agriculture and Cooperative for financial support. Thanks are also due to Ms. Srueangson Neumjeang for assistance in the laboratory, Dr. Srimeak Chowpongpan and Dr. David Lohman for revision of the final draft version.

LITERATURE CITED

- Adikaram, N.K.B., A.E. Brown and T.R. Swinburne. 1982. Phytoalexin involvement in the latent infection of *Capsicum annuum* L. fruit by *Glomerella cingulata* (Stonem.). **Physiol. Plant Pathology** 21: 161-170.
- AVRDC. 2003. Evaluation of phenotypic and molecular criteria for the identification of *Colletotrichum* species causing pepper anthracnose in Taiwan. **AVRDC Progress Report 2003**: 92-93.
- Azad, P. 1991. Fate and role of chemical constituents of chilli fruits during infection with *Colletotrichum capsici*. **Indian Phytopath.** 44: 129-131.
- Bosland, P.W. and E. Votava. 1999. **Peppers: Vegetable and Spice Capsicums**. CABI Publishing. New York. 204 p
- Cichewicz, R.H. and P.A. Thorpe. 1996. The antimicrobial properties of chili peppers (*Capsicum* species) and their uses in Mayan

- medicine. **J. Ethnopharmacology** 52: 61-70.
- Collins, M., L.M. Wasmund and P.W. Bosland. 1995. Improved method for quantifying capsaicinoids in *Capsicum* using high performance liquid chromatography. **HortScience** 30: 137-139.
- Dhingra, O.D. and J.B. Sinclair. 1994. **Basic Plant Pathology Methods**. 2nd ed. CRC Lewis. Florida. 434 p.
- Grewal, A.S. and R.K. Grover. 1974. Change in amino acids, carbohydrates and capsaicin content of red fruits of chilies infected with *Colletotrichum piperatum*. **Indian Phytopath.** 27: 603-604.
- Higgins, B.B. 1926. Anthracnose of pepper (*Capsicum annuum* L.) **Phytopathology** 16: 333-347.
- Ishikawa, K., T. Janos, S. Sakamoto and O. Nunomura. 1998. The contents of capsaicinoids and their phenolic intermediates in the various tissues of the plants of *Capsicum annuum* L. **Capsicum and Eggplant News**. 17: 22-25.
- Iwai, K., T. Suzuki and H. Fujiwake. 1979. Formation and accumulation of pungent of hot pepper fruits, capsaicin and its analogues, in *Capsicum annuum* var. *annuum* cv. Karayatsubusa at different growth stages after flowering. **Agric. Biol. Chem.** 43(12): 2493-2498.
- Jeyalakshmi, C., K. Seethalaman and E.G. Ebenezer. 1999. Qualitative losses of chili fruits due to infection by *Colletotrichum capsici* (Syd) Butler and Bisby. **Capsicum and Eggplant News**. 18: 80-82.
- Jones, N.L., S. Shabib and P.M. Sherman. 1997. Capsaicin as an inhibitor of the growth of the gastric pathogen *Helicobacter pylori*. **FEMS Microbiol. Letter** 146: 223-227.
- Kim, B.S., H.K. Park and W.S. Lee. 1989. Resistance to anthracnose (*Colletotrichum* spp.) in pepper. pp. 184-188. In **Proc. International Symposium on Integrated Management Practices: Tomato and Pepper Production in the Tropics**. AVRDC, Tainan, Taiwan.
- Manandhar, J.B., G.L. Hartman and T.C. Wang. 1995. Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. **Plant Disease** 79: 380-383.
- Park, H.K., B.S. Kim and W.S. Lee. 1990. Inheritance of resistance to anthracnose (*Colletotrichum* spp.) in pepper (*Capsicum annuum* L.) II. Genetic analysis of resistance to *Colletotrichum dematium*. **J. Korean Soc. Hortic. Sci.** 31: 207-212.
- Sangchote, S. and P. Juangbhanich. 1984. Seed transmission of *Colletotrichum capsici* on pepper (*Capsicum* spp.). **The Kasetsart J. (Nat. Sci.)** 17(1): 7-13.
- Suzuki, T., H. Fujiwake and K. Iwai. 1980. Intracellular localization of capsaicin and its analogues, capsaicinoids, in *Capsicum* fruit 1. Microscopic investigation of the structure of the placenta of *Capsicum annuum* var. *annuum* cv. Karayatsubusa. **Plant Cell Physiol.** 21:839-853.
- Szallasi, A. 1995. Autoradiographic visualization and pharmacological characterization of capsaicin receptors in several species including man. **Acta Physiologica Scandinavica Supplementum** 629: 1-65.
- Tenaya, I.M.N., R. Setiamihardja and S. Natasasmita. 2001. Correlation of capsaicin content, fructose, and peroxidase activity with anthracnose disease in chili pepper x red pepper (Abstract). **Zuriat** 12(2): 73-83.
- Torres, J.M., A.G. Chavez and E.R. Chavez. 1999. Antimicrobial properties of alkaloids present in flavoring plants traditionally used in Mesoamerica: affinin and capsaicin. **J. Ethnopharmacology** 64(3): 241-248.
- Yeuanyongsawat, S. 2002. Academic article: March, 2002. <http://pcog.pharmacy.psu.ac.th/thi/Article/2545/03-45/chili.html>