
Study of the Host Range of Northern Corn Leaf Blight Disease and Effect of *Exserohilum turcicum* Toxin on Sweet Corn

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Abstract The isolate DI of *Exserohilum turcicum* (Pass.) Leonard & Suggs was used for studying the fungal toxin and the host range of Northern Corn Leaf Blight (NCLB) disease. This disease is an important disease of corn that causes significant economic loss in Thailand and worldwide. The isolate DI was selected for its highly virulence in previous studies. Twenty two different plant species belonging to 14 genera of the Poaceae family were used for the host range test by spraying with 10^5 spore/ml of isolate DI results showed that all tested plants showed symptoms of NCLB except sugarcane. There were three types of symptoms: in the rice and sorghum group, the symptoms appeared as single red-brown spots and clusters of dark red-brown spots; in the corn group, the symptoms were spherical red-brown, water soaked lesions that enlarged into oblong grayish-black lesions and finally the leaves became blighted and died; in the grass group, symptoms of the pathogen included small, single brownish to dark brown lesions. Toxin from the crude extract of the *E. turcicum* was prepared by culturing the fungus on PDB for 2 wk at room temperature on a rotary shaker. The mycelia mass and spores were extracted with 10% ethyl acetate followed by methanol and hexane. The extract was inoculated on the sweet corn variety Hibrix3. After 4 days, the symptoms of the toxin on the plant leaves were similar to the symptoms developed by natural and/or artificial inoculation of the fungal pathogen.

Keywords: Northern Corn Leaf Blight, *Exserohilum turcicum*, Crude Extract Toxin, Host range

Introduction

Exserohilum turcicum (Pass) Leonard & Suggs (syn. *Helminthosporium turcicum* Pass.) causing Northern Corn Leaf Blight (NCLB) is an important

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disease commonly found in corn and causes significant economic damage in Thailand and worldwide (Vincelli and Hershman, 2011). The disease becomes epidemic due to environmental factors: high humidity, frequent rain fall, and low temperature. The initial symptoms found are small blisters on the leaves and later change to greenish gray spots, which spread along the leaves and become brown elliptical-shaped lesion. The lesion size is about 2x10-15 cm and develops first on lower leaves and later causes severe damage to the upper leaves under favorable conditions. Disease symptoms can be found at the seedling stage up to the harvesting stage (Abebe and Singburadom, 2006). This disease is favored by high humidity which enables production of masses of conidia that appeared in the center of the wound which made the lesions turn to dark color and the whole leaf may become blight (Nakhon Sawan Field Crops Research Center Newsletter, 2008). Some research documents report severe outbreak of the disease, in certain areas, the disease could cause damage to corn as much as 40 to 68 percent and resulted in economic losses (Sittikul, 1996; Pataky *et al.*, 1998), especially sweet corn which showed more susceptible to the disease than field corn.

Disease dissemination is by wind and infected seed. The spread of inoculum can be by pathogen contaminating corncobs, corn ears, corn debris and/or alternate hosts. Following spore attachment and germination on the host surface, the emerging germ tube remarked from spore polar develops to hyphae and produce one or multiple appressoria. The infection peg develops from the appressorium then penetrates directly through the cuticle and epidermis of the leaf not often found through stomata (Martin, 2011, Knox-Davis, 1974). The infection period occurs within 6 - 18h after spore attachment to plant leaves (Lipps and Mills, 2002). In addition, the earliest detected disease symptom appears 3 d after infection. The spindle shaped lesions are light brown to dark brown, with a width of about 1.5-15.0 cm and are parallel to the midrib. The symptoms first occur in the lower leaves and spread to the upper leaves. In susceptible cultivars, many lesions are found and coalesce leading to severe blight and death of plants. The disease spreads very quickly during warm weather (24-30° C) and damp conditions (high relative humidity 80-100%). The NCLB fungus can survive between seasons by mycelium and chlamydospores in humus, leaves and corn seed (Abebe and Singburadom, 2006).

Shurleff (1980) studied the host range of *E. turcicum* and found that corn, sorghum, barley, oat, rice, millet, Sudan grass, Johnson grass and tobacco seedlings are alternate hosts of this pathogen. In addition, he stated that the fungus strain from Johnson grass and isolates from sorghum and Sudan grass will not infect the corn. In the same year, Frederiksen and Franklin (1980) reported that *E. turcicum* was often found in sorghum, barley, wheat, oat,

sugarcane, rice and corn. Bashan *et al.* (1996) reported that *E. turcicum* produces E.t toxin which is a phytotoxic peptide and can increase the number of appressoria and the ramification of germinating conidia both on host leaves and on artificial media. They claimed that this toxin plays an important role in infection of corn in northern corn leaf blight. Robeson and Strobel (1982) reported that the toxin produced by *E. turcicum* (syn. *Drechslera turcica*), monocerin, isolated from Johnson grass (*Sorghum halepense*) could inhibit seedling growth of the Johnson grass, and cucumber to a lesser extent. It was also active against tissues of tomato and Canada thistle. Chauhan *et al.* (2008) studied the toxicity of the compounds in the culture filtrates of *E. turcicum*, which inhibited shoot and root growth, callus growth and reduced the chlorophyll content and cell viability of corn. Results showed that no germination occurred in non-autoclaved extracts at 100% (undiluted) and 50% concentrations, whereas germination was normal at all the concentrations of autoclaved toxic compounds. Zhao and Dong (2000) reported that 18 compounds were used to test their inactive reaction to HT-toxin produced by *E. turcicum*. The results indicated that mancozeb; one out of 18 compounds tested could inactivate the activity of HT toxin.

The aim of this research was to study the fungal toxin and to examine the host range of *E. turcicum* Pass., causal pathogen of NCLB disease, in alternate cultivated hosts and in weeds in the epidemic region. The results obtained will be useful for extension personnel to extend the knowledge to the farmers and enable them to control this disease effectively.

Material and methods

A study on host range of E. turcicum on some plants in the Poaceae family soil and host plants preparation procedures

Soil preparation was conducted by mixing loam soil with coconut husk powder and dried cow dung at the ratio of 2:1:1. The mixtures were sterilized and used to fill the pots (20 cm). Rice, corn, sorghum and grass seeds were separately sown in the mixed soil. The potted plants were watered regularly.

Spore suspension preparation (inoculum)

The *E. turcicum* isolate DI was grown on V-8 agar for 7 d. Spore suspensions were prepared by scraping spores from the agar surface with an L-shaped glass rod and filtered through two layers of cheese cloth. The number of

spores was determined using a haemocytometer and the concentration was adjusted to 10^5 spore/ml.

Inoculation of E. turcicum on some plants in Poaceae family

Two-week-old seedlings of the corn group e.g. sweet corn variety Hibrix3, field corn, popcorn, waxy corn, Tuxpeno corn, Indian fancy corn, big white corn and Tak-Ngai corn; the rice and sorghum group; rice (*Oryza sativa*)-Tanjung Native I, sorghum (*Sorghum bicolor* (L.) Moench), white sorghum, red sorghum and sweet sorghum and the grass group; atratum grass (*Paspalum atratum*), plicatulum grass (*Paspalum plicatulum*), ruzi grass (*Brachiaria ruziziensis*), guinea grass (*Panicum maximum*), napier grass (*Pennisetum purpureum*), bamboo grass (*Pogonatherum panieum*), umbrella plant / reed (*Cyperus involucratus* Rottb.), millet (*Coixlacryma-jobi*), *Digitaria adscendens*, paragrass (*Brachiaria mutica*), *Echinochloa* spp. and sugarcane were sprayed with 10^5 spore/ml of *E. turcicum* isolate DI. They were kept in a moist chamber for 3 d in the greenhouse. Observation of the symptoms on the inoculated plants were made, details of the symptoms were recorded and photographs taken.

Effects of toxin from a crude extract of E. turcicum on disease symptoms development in sweet corn

The fungal isolate DI, previously tested as a virulent strain, had been grown on PDA for 8 d before transferring 5 discs (0.5 cm diameter) to each flask containing potato dextrose broth (PDB), and incubated on a rotary shaker at room temperature for 2 wk. The cultures were then filtered through Whatman paper No.1 via vacuum pump. The cultures were mixed with 10% ethyl acetate and were shaken thoroughly. The cultures stood for 30 min to separate toxin from suspension. The suspension was separated into two parts; the top part was taken to be extracted, using a rotary evaporator at 55°C with a pressure of 200 lb and speed of 50 rpm, repeated three times. The crude extracts were then dissolved with deionized water and mixed with methanol at the ratio of 9: 1 and then mixed with hexane at 2: 1. The extracts stood for a period of time to let the solution separate. Once again the top part was mixed with hexane (1: 1). The crude extracts (toxin) were then tested for their effect by dropping the fungal toxin on the leaves of sweet corn variety Hibrix3 (2-wk-old), then incubated in a moist chamber. The results of *E. turcicum* toxin on the leaves were recorded at 3 d after application.

Results and discussion

A study on host range of E. turcicum on some plants in Poaceae family

Seven to ten days after inoculation with *E. turcicum* isolate DI, disease symptoms appeared on various host plants except sugarcane. Each plant species showed different disease symptoms, the lesion forms which indicate the biodiversity of the collected isolates as shown in three characteristics i.e. in the rice and sorghum group e.g. Taijung Native I, white sorghum, red sorghum and sweet sorghum symptoms were expressed as single reddish brown spots and clusters of dark reddish brown spots (Fig. 1), whereas in the corn group, the symptoms were spherical reddish brown water soaked lesions that enlarged into oblong grayish-black lesions and finally, the leaves became blighted and died. Symptoms found in sweet corn variety Hibrix3, field corn, popcorn, waxy corn, Tuxpeno, Indian fancy corn, big-white corn, sticky corn, Tak-Ngai corn and Tian Lueng corn are shown in Fig. 2. In the grass group, the symptoms were small, single brownish to dark brown lesions. Such the symptoms were found in Atratum grass (*Paspalum atratum*), Plicatulum grass (*Paspalum plicatulum*), Ruzi grass (*Brachiaria ruziziensis*), Guinea grass (*Panicum maximum*), Napier grass (*Pannisetum purpureum*), bamboo grass (*Pogonatherum panieum*), umbrella plant/reed (*Cyperus involucratus* Rottb.), millet (*Coixlacryma-jobi*), *Digitaria adscendens*, paragrass (*Brachiaria mutica*) and *Echinochloa* spp. (Fig. 3) but not found in sugarcane.

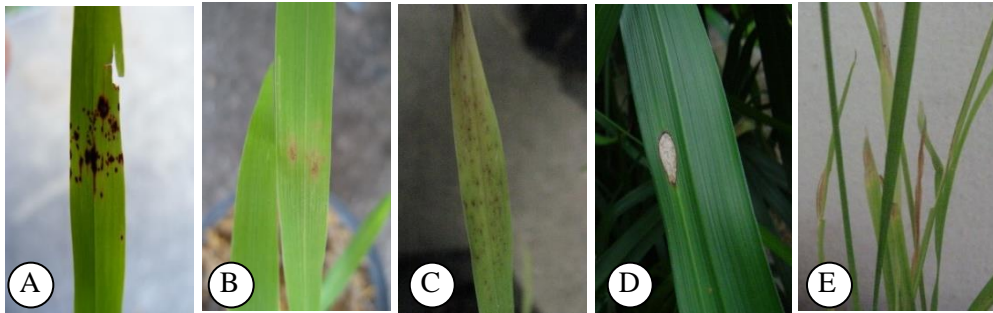


Figure 1. Reddish brown symptoms induced by *E. turcicum* found on (A), sweet sorghum (B), white sorghum (C), umbrella plant / reed (D) and rice-Taijung Native I (E).

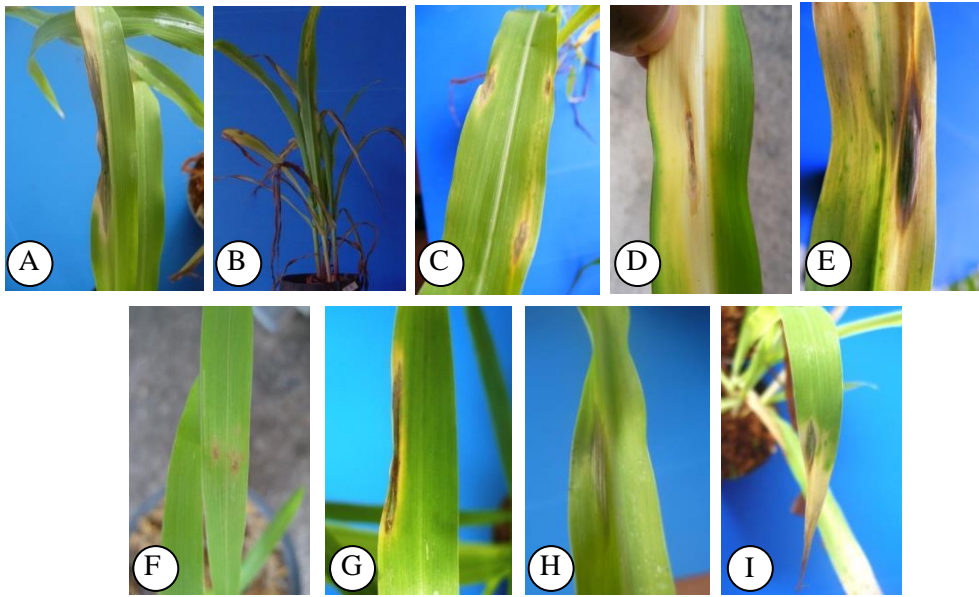


Figure 2. Typical lesion and blight symptoms of northern corn leaf blight on the corn group: Hibrix3 (A), field corn (B), Indian fancy (C), Tak-Ngai (D), Big white (E), waxy (F), Tuxpeno (G), popcorn (H) and Tian Lueng corn (I).

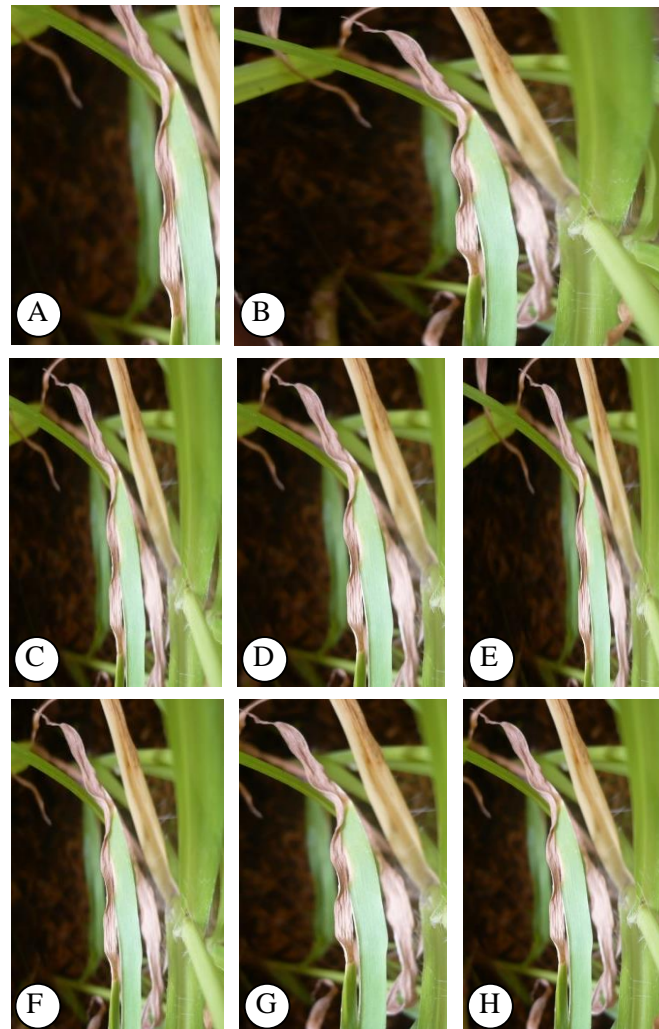


Figure 3. Blight and brownish or dark brown lesions in the grass group: bamboo grass (A), napier grass (B), atratum grass (C), plicatum grass (D), ruzi grass (E), guinea grass (F), *Eleusineindica* (G) and millet (H).

Effects of toxin from a crude extract of Exserohilum turcicum on disease symptom development in sweet corn

After treating the 2-wk-old susceptible sweet corn plants, 'Hibrix3', with the toxin of *E. turcicum* isolate DI, the results showed that the leaves of sweet corn revealed brownish black lesions with a yellow rim around the lesions. The lesions have a long oval shape along the length of the leaf. The lesion induced by the toxin was the same as the one caused by the *E. turcicum* infection. Moreover, the expanded chlorotic area varied by the concentration of the toxin used; a high dose of the toxin caused symptoms throughout the whole leaf (Fig. 4).

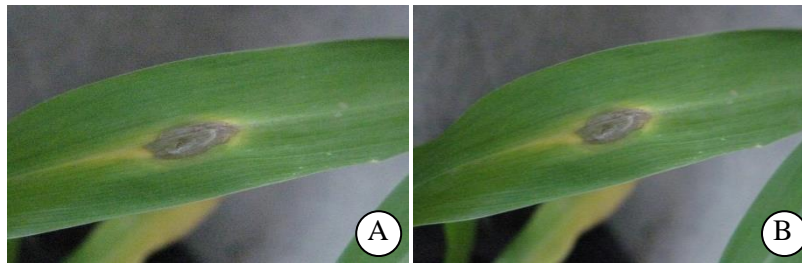


Figure 4. Lesion on sweet corn variety Hibrix3 expressed after applied the *E. turcicum* toxin: 4 d (A) and 10 d after application (B).

Conclusion

A study of the host range of *Exserohilum turcicum*, using isolate DI sprayed on the plant leaves as a spore suspension (10^5 spore/ml) showed that all isolates of *E. turcicum* produced three distinct symptoms including: (1) in the corn group; early lesions are gray-black and enlarged along the length of the leaves as brownish-gray, elliptical-shaped lesions. Severe symptoms included coalescence of lesions as brown stripes, blight and death (2) in the rice and sorghum group symptoms included single small red-brown spots or dense clustering of reddish-brown lesions, or dark red stripes throughout an entire leaf and (3) in the grasses group symptoms were brown or grayish-black lesions surrounded by a yellow area. Our results agree with the work of Shurleff (1980), and Frederiksen and Franklin (1980) whose reports characterized the infection of these plants by the NCLB pathogen. However, no symptoms were detected on the sugarcane, though Haripur *et al.* (2007) reported that in India NCLB the disease was found in sugarcane which was grown close to corn field. This difference might be due to the specificity of the Indian strain of *E. turcicum* to

sugarcane. In addition, Shurleff (1980) stated that the pathogen that infected Johnson grass which was isolated from sorghum and Sudan grass had lost the ability to infect corn plants. Kucharek and Raid (2000) reported that *E. turcicum* - sorghum and *E. turcicum* - Sudan grass strains had lost the capability to infect corn plants. Nevertheless, *E. turcicum*- corn strains showed low pathogenicity in Sudan grass.

It has been shown that the lesions on the leaves typical of NCLB result from chlorosis due to the inhibition of chlorophyll formation and necrosis of tissues from the toxin produced by the pathogen (Bashan and Levy, 1992). There are many papers supporting this concept as follows: Robeson and Strobel (1982) stated that monocerin, a toxin produced by *E. turcicum* (syn. *Drechslera turcica*), isolated from Johnson grass (*Sorghum halepense*) could inhibit seedling growth of the Johnson grass and, to a lesser extent cucumber. The toxin also has activity against tissues of tomato and Canada thistle. Bashan *et al.* (1996) reported that *E. turcicum* produces E.t toxin, which is a phytotoxic peptide, could increase the number of appressoria and the ramification of germinating conidia both on host leaves and on artificial media. They claimed that this toxin plays an important role in infection by *E. turcicum*. Chauhan *et al.* (2008) studied the toxicity of the compounds in the culture filtrates of *E. turcicum*, which caused inhibition of shoots, roots and callus growth, and reduction of chlorophyll content and cell viability of corn. They concluded that there was no germination of fungal conidia in non-autoclaved extracts at 100% (undiluted) and 50% concentrations, whereas germination was normal at all the concentrations of autoclaved toxic compounds; indicating the heat labile character of the toxin. To control the disease is to stop production of the fungal toxin by using fungicides or the resistant varieties. Zhao and Dong (2000) tested 18 compounds on their inactivation reaction to HT-toxin produced by *E. turcicum*. The results indicated that mancozeb, one out of 18 compounds could inactivate the activity of HT toxin. The effect of toxins from the crude extracts of *E. turcicum* on sweet corn leaves was also tested in the current study. The results showed that brown lesions were found at 4 d after toxin application. The symptoms found were oval shaped, brownish-black in the middle and having yellow rim around the lesions. The results indicated that the lesions developed on the host plants due to the effect of toxin which agrees with the report of Hailmi *et al.* (2011) who found that with the bioherbicide from the fungus *E. monoceras* used for controlling *Echinochloa crus-galli*, the symptoms on the weed resulted from the crude extract from the fungus.

Our study of toxin from *E. turcicum* indicates that the disease symptoms developed on the host plants were induced by the toxin of the pathogen. Results from studying the host range of NCLB disease in this project could be used as

database in controlling NCLB disease by avoiding planting the corn close to the alternate hosts and also eradicating these hosts in the non-corn growing period in order to cut the disease cycle.

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