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THESIS

SEED TRANSMISSION OF *Alternaria zinniae* PAPE, CAUSING LEAF SPOT IN ZINNIA

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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The infection biology of *Alternaria zinniae* were examined for conidia germination, number of germ tubes per conidium and appressoria formation. Conidia of *A. zinniae* germinated within 3 hr after inoculation and reached to maximum (100%) at 15 hr with an average of 5 germ tubes per conidium. Appressoria formed both on stomata and epidermal cell, but mostly the latter.

Disease severity (%) on the leaves of the inoculated zinnia plant at the age of 1 to 8 weeks were 2.8%, 4.7%, 10.3%, 18.8%, 17.4%, 16.4%, 23.5% and 31.3%, respectively. The blossoms were inoculated by *A. zinniae* at concentrations of 2.5 x 10^5, 2.5 x 10^6 and 2.5 x 10^7 conidia per ml. Disease severity on these plants were 18.0%, 16.1%, and 17.3% and the level of infected seeds were 99.6%, 75.3% , and 75.9%, respectively.

The infection of *A. zinniae* in zinnia seed is mostly in the seed coat. Transmission study of *A. zinniae* infected seed at 52% using seedling symptom test and sand method showed that seedling was infected at 59.8% and 56.0%, respectively.

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Student's signature Thesis Advisor's signature

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Wanida Seehachai

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INTRODUCTION

Zinnia (*Zinnia elegans* Jacq.) is one of the most popular summer and autumn flowering plants because of their large, colorful blooms and ability to withstand hot summer temperatures. Zinnia is the native flower of South America and Mexico and mostly uses as a bedding plant, pot plant and also cut flower (Stevens *et al*., 1993).

Seed health of various ornamental plants such as dianthus, globe amaranth, marigold, sunflower, celosia, hollyhock, periwinkle, snapdragon and zinnia has been studied by Chou and Wu, 1995; Wu *et al*., 2001; Li and Wu, 2002 and Wu and Wu, 2003. It is necessary to investigate the seed-borne pathogens of commonly grown flower crop.

Disease caused by *Alternaria* spp. is among the most important diseases of zinnia (Richardson, 1990). It causes leaf, stem and blossom spot of zinnia and other ornamentals especially in Compositae. High infections were shown on *Z. elegans* and *Z. haageana* (Baker, 1956; Mc Donald and Martens, 1963). Diseases often reduce the quantity and quality of zinnia flowers and sometimes cause significant plant mortality (Stevens *et al*., 1993). This disease is considered as an important seed borne disease of zinnia (Richardson, 1990). Agrios (1997), reported that Alternaria leaf spot occurs on the lower, senescent leaves, but the disease progresses upward and makes affected leaves turn yellowish and senescent and then, either dry up or fall off. Fungal pathogens can infect flowers at various stages of development either by penetration of one or more flower parts, or by systemic invasion through the apical meristem. The resultant symptoms range from flower spots, blotches, and blights (Ngugi and Scherm, 2006).

The genus *Alternaria* infects its host through the formation of appressoria and infection peg. Appressoria are usually globose, lobed or elongated structures produced at the hyphal apices. Some appressoria are intercalary, produced on one or both sides of growing hyphae. It penetrates the host by infection pegs produced from the attached appressoria. Appressoria formation has been reported to be affected by nutritional as well as other factors, such as surface hardness, topography and hydrophobicity (St Leger *et al*., 1989; Lee and Dean, 1994; Kuo and Hoch, 1996) and specific chemical signals (Podila *et al*., 1993; Gilbert *et al*., 1996). Suzuki *et al*. (2003) had been examined the infection behavior of *A. alternata*. There was no difference between the susceptible and resistant cultivars in term of the early infection behavior, such as conidia germination or appressorial formation. Saad and Hagedorn (1969), reported frequent stomata penetration during the first 24 hr after germ tube growth on the leaf surface whereas direct penetration occurred more frequently after 24 hr.

Many seed borne pathogens become active when seeds are sown, which may cause seed decay and/or pre-or post-emergence damping-off. This is resulted in a poor plant stand in the field (Agarwal and Sinclair, 1997). Severe infection of *A. zinniae* causes pre-emergence death, and superficial seed infection causes disease in plants after emergence (Gambogi *et al*, 1976). In 1980, Baker reported that *A. zinniae* carried with zinnia seed in form of vegetative structure (mycelium) in the inner part of seed. Gambogi *et al*. (1976) reported that an infection of *A. zinniae* was a deep-seated infection in zinnia seed. Shrestha *et al*.(2000), reported that *A. brassicae* was found predominantly in the seed coat and rarely in embryos of rapeseed (*Brassica campestris* L. var. *toria*) and mustard (*Brassica juncea*).

In these studies, infection biology of *A. zinniae*, disease severity at different growth stages and blossom infection in zinnia was investigated.

OBJECTIVES

The objectives of this study are:

1. To determine the infection of *A. zinniae* at different growth stages of zinnia plant

2. To determine relationship between blossom and seed infection of *A. zinniae*

3. To determine the localization of seed infection of *A. zinniae*

4. To evaluate the relationship of seed and seedling infection

LITERATURE REVIEW

Zinnia

Zinnia (*Zinnia elegans* Jacq.) is a one of 20 species of annual and perennial plants of family Asteraceae (Tusanee, 1996). Zinnias are popular garden flowers, cultivated worldwide (Stimart *et al*., 1987, Linderman and Ewart, 1990). It is one of the most popular summer and autumn flowering plants because of their large, colorful blooms and their ability to withstand hot summer temperatures. Recently, new hybrid zinnia cultivars have attracted attention as a cut flower crop. The majority of the zinnias are natives of Mexico, where they were cultivated at a very early date and also South America also (Paganun, 1995; Alice, 1968). Zinnias are mostly useful as a bedding plant, pot plant and also a cut flower (Stevens *et al.*, 1993).

Zinnia leaves are opposite and usually stalkless (sessile), with a shape ranging from linear to ovate, and pale to middle green in color. The flowers have a wide range of appearances, from a single row of petals to a dome shape, with the colors white, chartreuse, yellow, orange, red, purple, lilac, (Alfred, 1984; Eileen, 1995; John and Herbert,1968; Ken and Jill, 1995) and green such as Envy cultivar (Nuntiya, 1983).

Zinnia is also one of the most valuable annuals for summer gardens in Florida. All kinds can be grown even in poor soil if planted in a sunny location and given plenty of water and fertilizer, and bloom continuously for many weeks. Seed germination in a half week, and seedlings to bloom is about two months. Zinnias provide excellent cut flowers as well as color in the garden borders, and the small type do well as edgings (John and Herbert, 1978).

Based on a general survey by the Flower Seed Committee of the International Seed Testing Association (ISTA, FSC), the market for ornamental plants increasing with an improvement of living standards worldwide, including zinnia.

In Thailand, *A. zinniae* found to be seed borne in 2 Family, including Compositae such as *Calendula officinalis* Linn., *Cosmos bipinnatus* Cav., *Tagetes* sp., *Salvia splendens* Ker-Gawl. and Family Papaveraceae such as *Eschscholzia californica* Cham (Chauiprasit, 1981).

A. zinniae cause leaf and flower blight of zinnia and other ornamentals was found mainly in Compositae. It was more or less frequent in seeds of *Acacia farnesiana, Ageratum houstonianum, Bellis perennis, Calendula officinalis, Calliopsis bicolor, Callistephus chinenis, Centaurea cyanus, Chrysanthemum carinatum, Clarkia elegans, Cosmos bipinnatus, Gaillardia picta, Gentiana acaulis, Gerbera jamesonii, Helianthus annuus, Impatien* sp., *Lactuca sativa, Papaver nudicaule, Reseda odorata, Senecio cruentus, Tagetes erecta, Viola tricolor,* and very frequent and high infection percentages in *Zinnia elegans* and *Z. haageana* (Neergaard, 1945; Baker, 1956; Mc Donald and Martens, 1963; Andersen, 1974). Diseases often reduce the quantity and quality of zinnia flowers and sometimes cause significant plant mortality (Stevens *et al.*, 1993).

The causal agent

Classification of Genus *Alternaria* (Barnette, 1972)

Division	Eumycotina
Class	Deuteromycetes (Fungi Imperfecti)
Order	Moniliales
Family	Dematiaceae
Genus	*Alternaria*

A. zinniae produces dark-colored mycelium, and on older diseased tissue it produces short, simple erect conidiophores with a few conidia. Conidia are large, dark, long, obclavate or rostrate, brown to dark brown with both transverse and longitudinal septate (Rotem, 1994). Conidia are detached easily and can carry by air currents (Agrios, 1997). The length of conidiophores of *A. zinniae* varies from 35 to

200 µm, 3 – 12 septate, 10 – 28 x 19 – 105 µm in size. The length of beak varies from 16 – 108 µm and total length varies from 39 – 253 µm. (Ellis, 1976; Joly, 1967; Neergaard, 1945; Pape, 1942; Rao, 1971; Simmons, 1982).

A. zinniae Pape is seed borne and transmits from seed to seedling and disperses to blossom. On the zinnia seed, the growth of the fungus consists of conidia with long beaks in groups, sometimes single. Generally have no mycelium. Conidia mostly single, obclavate, pale to dark brown, 3 – 12 transverse septa, 0 – 9 longitudinal, few oblique, body 19.5 – 105 x 9 – 28.5 µm ; beak filiform, simple, 6 – 159 µm with 0 – 3 transverse septa. Secondary conidia may appear, but seldom (Mathur and Kongsdal, 2003).

Neergaard (1945) reported that the fungus grew over a temperature range; the optimum temperature for incubation was 27 – 28^{o} C. The fungus grows rather rapidly in culture and may cover 9 cm. on pethi dish of potato dextrose agar (PDA) within 7 – 8 days at optimum temperature. The optimum temperature of the organism is about 27^{o}C (81^{o}F). The rough mat of mycelium, about 2 mm. high, may have a narrow whitish margin but central growth rapidly assumes a dark grey color (Dimock and Osborn, 1943).

Survival and overseasoning

In most cases *Alternaria* pathogens overseason in debris and seed. Midsummer and early fall are normal times of outbreak. Conidia of *A. zinniae* can survive in the seed for 6 – 7 years (Khristova *et al*, 1964)**.** In many species conidia are preeminent in determining the survival of the fungus (Sussman, 1968). However, survival of *Alternaria* species, mycelium always and consistently survives longer and better than conidia (Rotem, 1994).

Symptoms and Diagnosis

Symptoms include large reddish brown or purple spots, at first round to oblong but becoming irregular in shape with gray or tan centers that may drop out leaving a hole. Severely affected leaves shown brown, dry, and become brittle. Seedlings can be killed when stems are attacked. Alternaria blight usually shows up towards the end of the cutting period for the zinnias. Warm and wet conditions with heavy rains favor disease development. This disease is considered of economically important seed borne disease in zinnia (Richardson, 1990). This is a seed-borne disease so treating seeds can help delay the onset of symptoms. Individual spots are at first circular but rapidly become irregular. The spots may vary from 2 to 10 millimeters or more in diameter, are reddish brown, and have grayish-white centers on the upper leaf surface. Differentiation of margin and center is lacking on the lower surface. As the spots increase in size and number, they coalesce and the affected leaves become brown and dry.

Blossoms may be severely affected. Brown spots, 1 or 2 millimeters in diameter, with grayish-white centers sometimes appear on the petal tissues of the ray flowers. Affected plants soon become darken and wither with unsightly blossoms (Dimock and Osborn, 1943). Numerous, small, reddish spots, sometimes with grayish-white centers, may be seen on stem internodes. Such spots usually are superficial. Spots develop at nodes, however, usually do not remain superficial. Instead, they grow or coalesce into larger lesions that frequently girdle the stem causing the upper portions of the affected stem to die back to the node. Dark brown to black cankers with sunken centers are also common at the base of the stem of diseased plants. Affected plants often wilt completely, even when the basal cankers do not encircle the stem. The outer tissues of affected roots may become dark gray, rot completely, and slough off, resulting in wilting and death of the plant. Damping-off of seedlings also may occur.

Development of disease

Plant pathogenic species of *Alternaria* overwinter as mycelium or spores in infected plant debris and in or on seeds. If the fungus is carried with the seed, it may

attack the seedling, usually after emergence, and cause damping-off or stem lesions and collar rot. Conidia are produced abundantly, especially during heavy dews and frequent rains, and are blown from infected debris or infected cultivated plants and weeds. As the spots increase in size and number, they coalesce and the affected leaves become brown and dry. Blossoms may be severely affected. Brown spots, 1 or 2 millimeters in diameter, with grayish-white centers sometimes appear on the petal tissues of the ray flowers.

From the previous microscopic examination by Dimock and Osborn (1943), cleared infected leaf tissue reveals the presence of light-colored intra- and intercellular hyphae within the lamina, and brown-walled conidiophores pushing through the stomata. Conidia of the fungus usually are visible on fresh specimens when atmospheric conditions favor sporulation. Such conidia always appear to be born singly on the conidiophores, but occasionally, on material which has been kept for a few days in a dry chamber, catenulation of conidia is evident. Chains of as many as 8 conidia have been seen. The conidia are large, averaging 170 – 210 by 20 – 24 microns, with a beak usually more than twice as long as the body of the conidia. The body of the spore has numerous longitudinal and transverse septa.

Inoculum location in/on seed

In 1980, Baker reported that *A. zinniae* carried with zinnia seed in form of vegetative structure (mycelium) in the inner part of seed. Gambogi *et al*. (1976) reported that an infection was a deep-seated infection.

Transmission

Seed transmission is a passage of inoculum from an infected or infested (contaminated) seed to germinating seed, seedling or plant (Neergaard, 1977; Maude, 1996; Agarwal and Sinclair, 1997). The frequency or rate of transmission, which is a measure of capability of a pathogen, can be expressed as percentage of infected seeds that result in infected plants in the field (Diekmann, 1993). Transmission in seeds is

probably the main mechanism for the introduction of *Alternaria* diseases into disease-free areas. For instance, in Ohio, infected seed was probably responsible for the introduction of *A. helianthi*, which appeared immediately in the first year of sunflower production (Herr and Lipps, 1982).

Many seed borne pathogens become active when seeds are sown, which may result in seed decay and/or pre-or post-emergence damping-off. This is results in a poor plant stand in the field (Agarwal and Sinclair, 1997). Severe infection of *A. zinniae* has caused pre-emergence death, and superficial seed infection has caused disease in plants after emergence (Gambogi *et al*, 1976). The transmission of *Alternaria* pathogens from infected seeds to seedlings are often affected through infection of cotyledons that emerge from soil with the remains of the contaminated seed coat. However, systemic transmission is also possible, as shown by *A. sesamicola* on sesame (Singh *et al*, 1983). Incubation period of approximately 24 hours was required for the maximum development of leaf infection (Dimock and Osborn, 1943).

Infection

The interaction of foliar fungal pathogens and plants begins with conidia attachment to host surfaces and continues with conidia germination, host recognition, formation of infection structures, and penetration. Active adhesion of fungal conidia and infection structures to plant surfaces is regarded as an important mechanism in early pathogenesis (Kuo and Hoch, 1996). In many foliar fungal pathogens, direct penetration of leaf surface is a common strategy (Knogge, 1998).

In the case of the genus *Alternaria*, appressoria are variously shaped. They appear as dilated hyphal tips in *A. cassiae* (van Dyke and Trigiano, 1987), near rectangular in *A. helianthi* (Allen *et al.*, 1983) and elongated in the Japanese pear pathotype of *A. alternata* (Takano *et al.*, 1997). In previous studies of *A. alternata* (van Dyke, unpublished data), entrance with or without appressoria of germ tubes through stomata and/or by direct penetration from appressoria has been observed.

Saad and Hagedorn (1969) reported frequent stomata penetration during the first 24 hr after germ tube growth on the leaf surface whereas direct penetration occurred more frequently after 24 hr.

Generally, plant pathogenic fungi may use a combination of enzymatic activity and mechanical pressure to penetrate their hosts and several *Alternaria* spp. have been shown to produce enzymes such as cutinases and pectinases (Kolattukudy, 1985; Rotem, 1994). Many species of *Alternaria* also produce host-specific and non-host-specific toxin (Rotem, 1994) which precondition or chemically degrade host tissues, aiding in penetration (Allen *et al.*, 1983; Van Dyke and Trigiano, 1987).

Yield losses

Crop losses, as related to disease intensity, present a considerable range of aspects, of which quantitative losses of the harvested product in terms of weight, number of units or volume are usually the most simple to record but not always economically the most importance. Qualitative losses may, for instance, spoiling of cut flowers by leaf spot diseases (different species of *Alternaria* in carnation, *Senecio cruentus, Zinnia* and other). Seed of *Z. elegans,* produced in California, is rarely and if so only weakly infected by *A. zinniae*, whereas seed produced in Italy is so often heavily infected that about 40 percent of the seed lots inspected for export from Denmark are rejected in spite of a very lenient tolerance (Neergaard, 1965).

Control

Diseases often reduce the quantity and quality of zinnia flowers and sometime cause significant plant mortality. Alternaria blight usually can be controlled with sound cultural management and chemicals. It may be advisable to treat seed with a fungicide dust such as captan or thiram for direct seeding in the field. If possible, sow the seed on a slightly raised bed with drip irrigation. Avoid overhead watering, and keep plants adequately spaced for good air circulation. Watering the plants early in the day permits good foliage drying before nightfall. Closely monitor plants for signs

of diseases or insect activity. And rotate planting beds in the field annually to avoid buildup of soil borne fungal pathogens (Stevens *et al*, 1993).

MATERIALS AND METHODS

1. Seed-borne fungi of zinnia

Five samples of zinnia seed were obtained from seed company and used in this study (Table 1). Four hundred seeds of each sample were randomly selected and tested by blotter method (Neergaard, 1973). The tested seeds were incubated at 25^{o}C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness for 7 days before identification and recording.

Table 1 Samples of zinnia seeds used in this study

Name of samples	Scientific name
ZNUD	*Zinnia elegans* Jacq.
ZNClassic	*Zinnia angustifolia* Kunth
ZN#3	*Zinnia elegans* Jacq.
ZN#4	*Zinnia elegans* Jacq.
ZN#7	*Zinnia elegans* Jacq.

2. Pathogenicity Test

The seeds of zinnia were randomly selected and plated onto the moist blotter paper. The tested seeds were incubated at 25^{o}C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness. Seven days after incubation, the seed was observed for the appearance of *A. zinniae.*

Single conidia of *A. zinniae* were transferred and cultured on potato dextrose agar (PDA) and incubated at 25^{o}C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness for 7 days. Afterwards, a piece of each colony of *A. zinniae* was sub-cultured on carrot agar (CA) and incubated at 25^{o}C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness for 14 days. A conidial suspension was prepared with sterile distilled water and adjusted to 10^{6} conidia per ml. The

suspension of *A. zinniae* was sprayed to five healthy plants of each sample (*ZNUD, ZN#3* and *ZN#7*). After inoculation, inoculated plants were kept under moist conditions at 25^{o}C for 12 hr. Seven days after incubation, inoculated plants were checked for disease severity that meant to the pathogenic potential of *A. zinniae.*

3. Conidia germination and appressoria formation of *A. zinniae*

Plant material

Zinnia plants (*Z. elegans* Jacq.) were obtained from germinated zinnia seeds which were incubated in the moist chamber for 7 days at 25^{o}C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness. The seedlings were transferred and planted in plastic pot contained soils (1 seedling per pot). The seedlings were maintained in the nursery until use.

Inoculum preparation

The single conidia were obtained from infected seeds which were incubated on moistened blotter paper for 7 days and transferred to carrot agar (CA). The culture was incubated at 25^{o}C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness for 10 days. The conidia were collected and suspended in distilled water to the desired concentrations.

3.1 On agar plate

Thirty microlitre of conidia suspension of *A. zinniae* (10^6 conidia per ml) were dropped onto 1% water agar and spread. The plates were incubated at 25^{o}C under near ultraviolet (NUV) light for 1 to 6 hr. A total of 200 conidia were randomly selected from each incubation time to determine the conidial germination under compound microscope.

3.2 On leaf surfaces by using compound microscope

The conidial suspension (10^6 conidia per ml) of *A. zinniae* were mixed with 0.05% glucose and sprayed to the leaves of 6 weeks old zinnia plants. Inoculated leaves were incubated for 1, 3, 6, 9, 12, 15, 18, 21 and 24 hr in a moist plastic bag at 25°C. For each time of incubation, leaves were detached and painted with enamel on abaxial side of leaf and let it dried. The enamel sheath were peeled off to remove the epidermal layer of leaf and mounted on the glass slide with lactophenol. A total of 200 conidia were randomly checked from each incubation time to examine the conidia germination, number of germ tube per conidium, appressoria formation and its localization under compound microscope.

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3.3 On leaf surfaces by using scanning electron microscope (SEM)

The conidial suspension of *A. zinniae* (10^7 conidia per ml) were sprayed to the leaves of 6 weeks old zinnia plants. Inoculated leaves were incubated in moist plastic bag for 18, 21 and 24 hr at 25°C. For each time of incubation, leaves were detached and cut into pieces of 3x5 mm. These samples were fixed in 1% osmium tetra oxide for an hour. Dehydration was done in alcohol series (10, 20, 35, 50, 75, 90, 95% ethanol) for 10 min per each step and 3 times (10 min x 3) for absolute alcohol. They were critical-point dried in liquid carbon dioxide (liquid CO_2), mounted on aluminum stubs, and sputter-coated with gold (Balzers SCD 040). Observation of germinated conidia and appressorial formation were made under JOEL JSM-5410LV scanning electron microscope.

4 Infection of *A. zinniae* and disease severity at different growth stages of zinnia plant

Plant material

Zinnia plants (*Z. elegans* Jacq.) were obtained from germinated zinnia seeds which were incubated in the moist chamber for 7 days at 25°C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness. The seedlings were transferred

and planted in plastic pot contained soils (1 seedling per pot). The seedlings were maintained in nursery until use.

Inoculum preparation

The single conidia were obtained from infected seeds which were incubated on moistened blotter paper for 7 days and transferred to carrot agar (CA). The culture was incubated at 25^{o}C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness for 10 days. The conidia were collected and suspended in distilled water to the desired concentrations.

One to eight weeks old of zinnia plants were used for this experiment. These zinnia plants were used for inoculation and observed for their susceptibility. Conidial suspension of *A. zinniae* (10^6 conidia per ml) was prepared and sprayed to the plants. The control treatment was also done by using distilled water instead of the conidial suspension. After inoculation, inoculated plants were incubated in moist condition for 12 hr and then, transferred to greenhouse. One week after inoculation, three leaves of each plant were observed and assessed for infected leaf area.

$$\text{Infected leaf area (\%)} = \frac{\text{infected leaf area}}{\text{total leaf area}} x100$$

5 Relasionship between disease severity on the blossom of zinnia and infection of *A. zinniae* on zinnia seed

Plant material

Zinnia plants (*Z. elegans* Jacq.) were obtained from germinated zinnia seeds which were incubated in the moist chamber for 7 days at 25^{o}C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness. The seedlings were transferred and planted in plastic pot contained soils (1 seedling per pot). The seedlings were maintained in the nursery until use.

Inoculum preparation

The single conidia were obtained from infected seeds which were incubated on moistened blotter paper for 7 days and transferred to carrot agar (CA). The culture was incubated at 25^{o}C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness for 10 days. The conidia were collected and suspended in distilled water to the desired concentrations.

Eight weeks old of zinnia plants were used for this experiment. The conidial suspension at concentrations of 2.5 x 10^5, 2.5 x 10^6 and 2.5 x 10^7 conidia per ml was sprayed to the blossom of zinnia plants (using 10 replication / treatment). The control treatment was also done by using distilled water instead of the conidial suspension. After inoculation, inoculated plants were incubated in moist condition for 12 hr and then, transferred to greenhouse. One week after inoculation, disease severity of each blossom was calculated as following;

$$\text{disease severity (\%)} = \frac{\text{number of infected petal}}{\text{total of the petal}} x\,100$$

The infected blossoms were cut and dried after disease assessment and separated from the seeds. Seeds of each blossom were placed on 3 layers of moistened blotters in petri dishes and incubated at 25^{o}C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness. After 7 days of incubation, each seed was examined for growth of *A. zinniae* under a steromicroscope.

6 Localization of *A. zinniae* on/in zinnia seed

Four hundred seed samples of zinnia grown in the north- eastern of Thailand were collected. The infected seeds were tested using the component plating method as described by Neergaard and Mathur (1985). The seeds were surface sterilized for 3 minute with 1% sodium hypochlorite solution (NaOCl) and then washed thoroughly with sterile water. Individual infected seeds were dissected into embryo and seed coat,

and each part was then placed on 3 layers of moistened blotters. The dishes were incubated at 25^{o}C under 12h alternating cycles of near ultraviolet (NUV) light and darkness using 100 seeds/ replication (4 replications). Each component was examined under a stereomicroscope for growth of *A. zinniae* after 7 days of incubation.

7 Transmission of *A. zinniae* from infected seed to seedling

Transmission of *A. zinniae* from infected seed to seedling was studied under controlled environment in growth chamber using blotter method, test tube agar, and seedling symptom test (sand method). The infected seed was obtained from the seed sample of the same lot. The infected seed sample was tested by blotter method on the incidence of infected seed before preceded to transmission study. The infected seedling was monitored for appearance of symptom and different methods were compared.

7.2 Comparison of testing methods

7.2.1 Blotter method

The infected seeds were incubated on moistened blotter at 25^{o}C under 12 hr alternating cycle of near ultraviolet (NUV) light and darkness for 7 days.

7.2.2 Test tube agar

The infected seeds were surface sterilized with 1% sodium hypochlorite (NaOCl) for 3 min and then grown in test tube on 5 ml of 1% water agar. The tubes were incubated at 25^{o}C under 12 hr alternating cycle of near ultraviolet (NUV) light and darkness for 14 days. Infected seedlings were counted and confirmed by sterilized with 1% sodium hypochlorite (NaOCl) for 3 min and then plating on PDA. Growth of *A. zinniae* were checked after 7 days of incubation under 12 hr alternating cycle of near ultraviolet (NUV) light and darkness at 25^{o}C.

7.2.3 Sand method

The infected seeds were surface sterilized with 1% sodium hypochlorite (NaOCl) for 3 min and sown in the sterile sand tray. The disease was monitored after 14 days by washing to remove sand from the tray and examining under a stereomicroscope. The number of infected seedlings was examined.

7.3 Transmission of infected seed to seedling infection

Seed sample of approximately 100 grams were collected from the field tested by blotter method on the incidence of *A. zinniae* and used for this experiment. The samples were checking for appearance of *A. zinniae* by using blotter method and seedling symptom test using method selected from previous experiment.

7.3.1 Blotter method

The infected seeds were incubated on moistened blotter at 25^{o}C under 12 hr alternating cycle of near ultraviolet (NUV) light and darkness for 7 days.

7.3.2 Seedling symptom test

The infected seeds were surface sterilized with 1% sodium hypochlorite (NaOCl) for 3 min and sown in the test tube contained 5 ml of 1% of water agar. The disease was monitored after 14 days. The number of infected seedlings was examined.

RESULTS AND DISCUSSION

Results

1. Seed-borne fungi of zinnia

Eleven fungi were existed on zinnia seeds of 5 samples. These included common field and storage fungi (Table 2). From these results, *A. zinniae* existed mostly (52%) on the zinnia seeds of *ZNUD*. Hence, zinnia seeds of *ZNUD* were used for further studies.

Table 2 Fungi detected on zinnia seed samples

Sample	Percentage of infected seeds[1]										
	Aa [2]	Az	As	Ce	Cl	Cls	Cor	F	Fm	H	P
ZNUD	6.75	52	-	-	20.25	1.5	-	0.75	4	-	-
ZNClassic	37.25	5.5	2.25	-	42.5	-	-	53.5	-	-	9.75
ZN#3	84.5	11.5	-	1	13.5	6.5	-	-	3.25	-	-
ZN#4	2.75	29.75	-	13.25	37.5	1	-	-	9.5	-	-
ZN#7	16	2.5	-	3.25	21.25	0.75	1.25	6	3.25	1.25	-

[1] 400 seeds of each sample were examined by means of blotter tests. Seeds were incubated at 25°C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness for 7 days before examination.

[2] Aa: *Alternaria alternata*; Az: *A. zinniae*; As: *Aspergillus* sp.; Ce: *Curvularia eragrostidis*; Cl: *C. lunata*; Cls: *Cladosporium sphaerospermum*; Cor: *Corynespora* sp.; F: *Fusarium* sp.; Fm: *Fusarium moniliforme*; H: *Helminthosporium* sp.; P: *Penicillium* sp.

2. Pathogenicity test

A. zinniae infected the leaves of zinnia and caused reddish brown spots with approximately 35.4%, 32% and 25.6% on the zinnia plants of *ZN#3, ZN#7* and *ZNUD*, respectively (Table 3). There was no significantly different between diseases severity of the samples. Hence, *A. zinniae* had a potential to infected and caused leaf spot on these samples and *ZN#3* was selected and used for the next experiment.

Table 3 Means of diseases severity (%) after inoculated with *A. zinniae* and incubated at 25^{o}C for 7 days

Sample	Mean of diseases severity (%)
ZN#3	35.4b
ZN#7	32.0b
ZNUD	25.6b
Control	0a
F-test	**

Mean followed by different letters in the columns are significantly different by Duncan's multiple range test ($p \leq 0.05$)

Figure 1 Diseases severity (%) of zinnia plants after inoculated with *A. zinniae* and incubated at 25^{o}C for 7 days, *ZN#3* (A), *ZN#7* (B) and *ZNUD* (C)

3. Conidia germination and appressoria formation of *A. zinniae*

Conidia of *A. zinniae* germinated within 3 hr after incubating on 1% water agar (Figure 3) and reached a maximum (95.9%) at 6 hr (data not shown) and 100% at 15 hr on zinnia leaves surface (Figure 2 and Table 4). An average of 5 germ tubes per conidium was observed on zinnia leaves at 15 hr after inoculation (Figure 4). Germ tubes from germinating conidia produced an appressoria at their tip. At 21 hr after inoculation, appressoria formation was about 52.1% (Table 4). These appressoria was formed directly on epidermal cell and stomata with approximately 77.2% and 11.6%, respectively (Table 5). Sometimes the appressoria were mostly produced close to the conidia, but sometimes produced at the tip of elongated germ tube. The results from SEM were showed that germinated conidia formed a terminal globose appressorium at the end of germ tube and mostly appear on the epidermal cell (Figure 5). Within 60 hr after inoculation, brownish black lesions were observed on the infected leaves of zinnia.

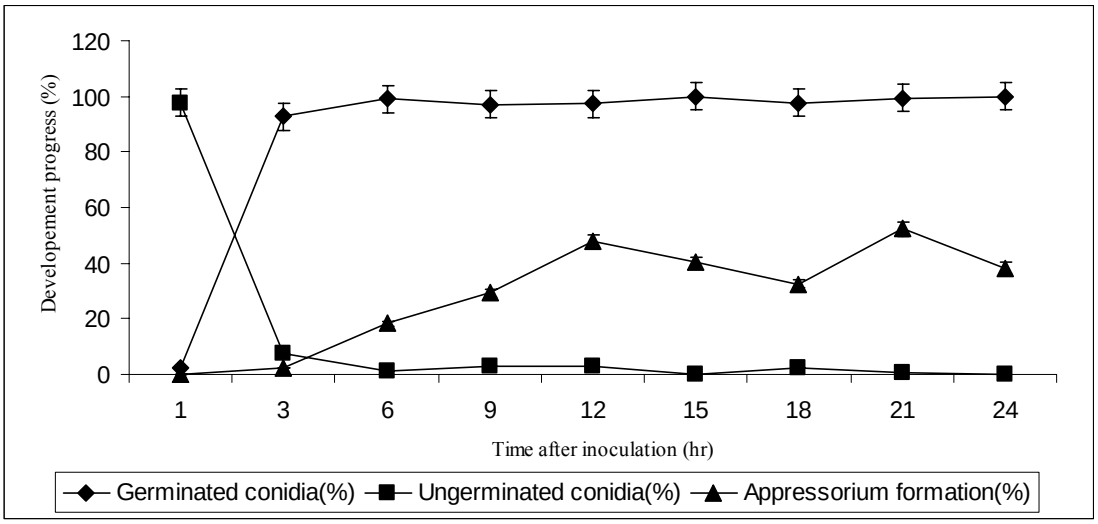

Figure 2 Development progress of *A. zinniae*, expressed by the percentage of ungerminated conidia (square), conidial germination (rhombus) and appressorium formation (triangle), after inoculation on zinnia leaves surfaces

Table 4 Germinated conidia and appressoria formation (%) and numbers of germ tube per germinated conidia on zinnia leaf after inoculation, and incubated at 25°C for 1, 3, 6, 9, 12, 15, 18, 21, and 24 hr

Incubation time (hr)	*Germinated conidia (%)*	*Germ tube per germinated conidia*	*Appressoria (%)*
1	2.3c	0.9e	0.0f
3	92.7b	3.3bcd	2.2f
6	99.0a	3.3cd	18.4e
9	97.2a	4.0b	29.3d
12	97.5a	3.3bcd	46.6ab
15	100.0a	3.8bc	40.8bc
18	97.6a	5.0a	32.6cd
21	99.5a	3.9bc	52.1a
24	100.0a	3.1d	39.1bcd
F-test	**	**	**
C.V. (%)	2.1%	11.1%	23.4%

Mean followed by different letters in the columns are significantly different by Duncan's multiple range test ($p \leq 0.05$)

Table 5 Localization of appressoria formation of *A. zinniae* on the leaf of zinnia after inoculated and incubated at 25°C for 1, 3, 6, 9, 12, 15, 18, 21 and 24 hr

Incubation time (hr)	Appressoria (%)	Location of appressorium on leaf (%)	
		Stomata	Epidermal cell
1	0.0f	0.0d	0.0d
3	2.2f	4.2cd	95.8a
6	18.4e	28.4a	71.6c
9	29.3d	16.2b	83.9ab
12	46.6ab	9.7bc	88.4ab
15	40.8bc	10.8bc	89.3ab
18	32.6cd	10.6bc	90.3ab
21	52.1a	12.0bc	88ab
24	39.1cd	12.3bc	87.8ab
F-test	**	**	**
C.V. (%)	23.4%	46.7%	6.9%

Mean followed by different letters in the columns are significantly different by Duncan's multiple range test ($p \leq 0.05$)

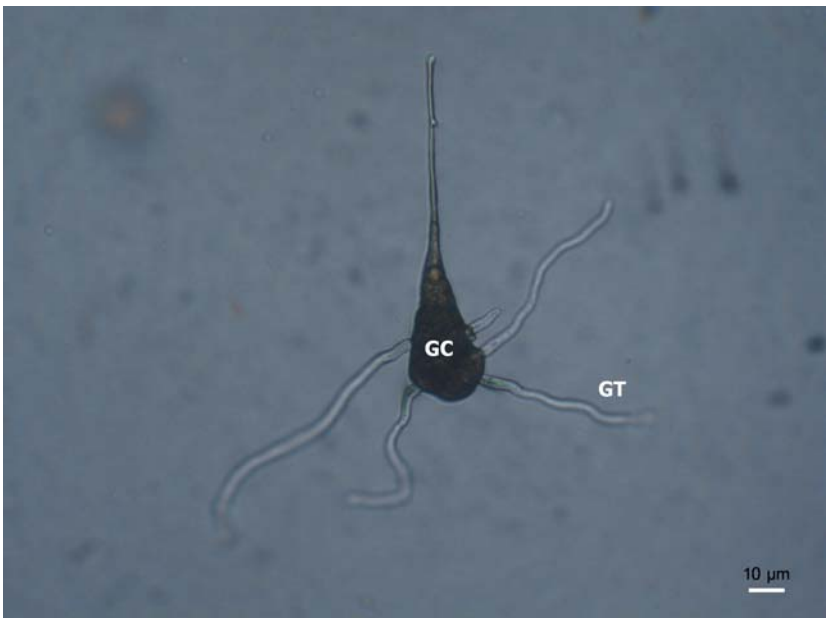

Figure 3 A germinated conidia (40X) with five germ tubes after incubated on 1% water agar for 6 hr; germinated conidia (GC), germ tube (GT)

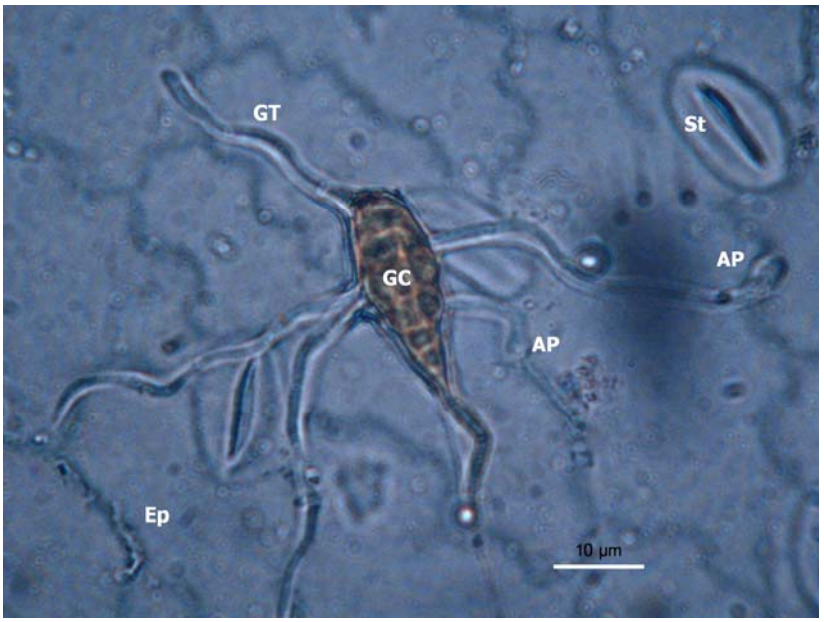

Figure 4 Germinated conidia with five germ tubes and produced an appressorium at their end after inoculation on zinnia leaf at 25^{o}C for 6 hr; germinated conidia (GC), germ tube (G), appressorium (AP), Epidermal cell (Ep), Stomata (St)

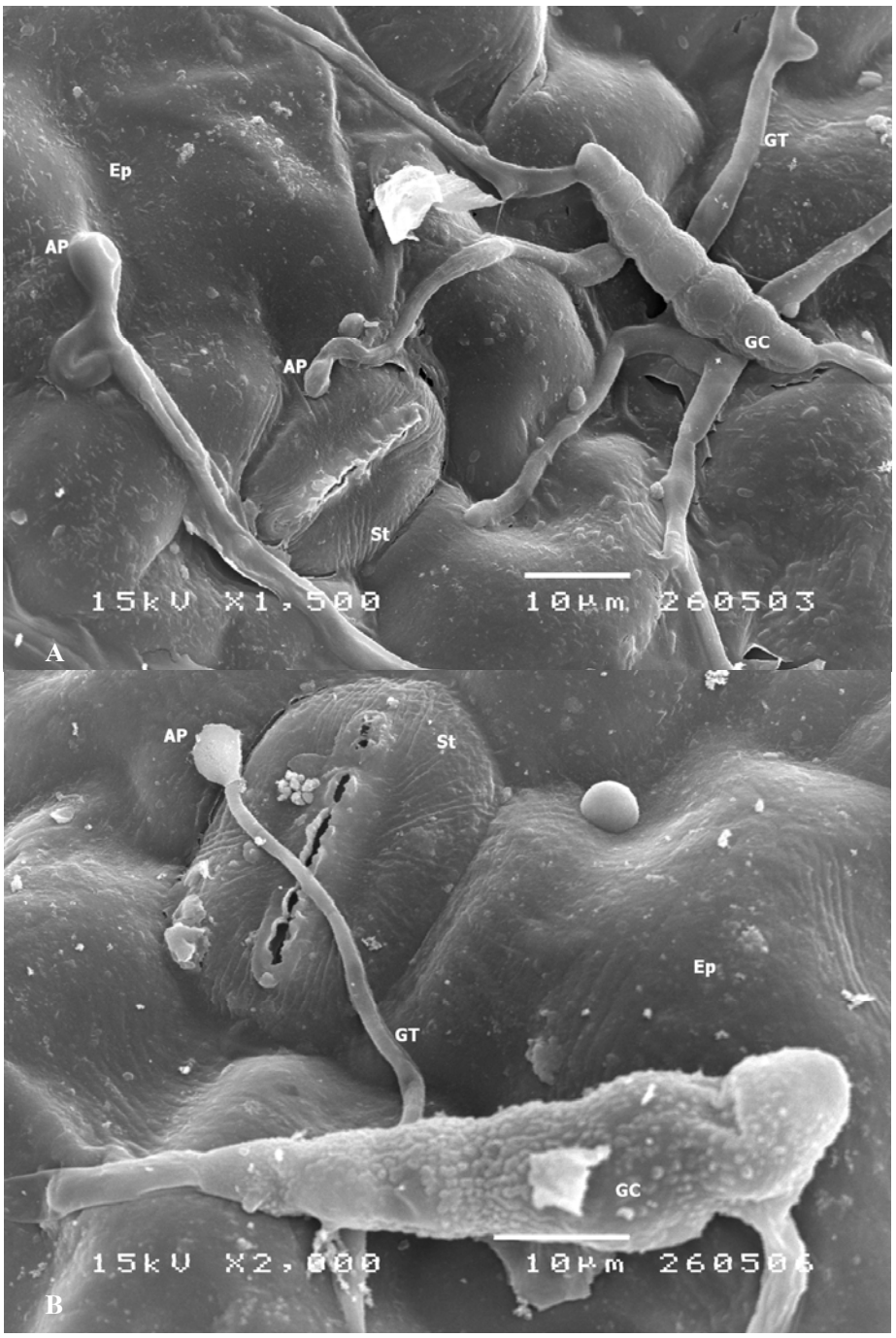

Figure 5 Scanning electron microscope of a germinated conidia (GC) of *A. zinniae* on the leaf surface of *Z. elegans*. (A) Numerous germ tubes (GT) extend from conidia and formed an appressorium (AP) at their tip. Germ tube (upper left) was extended from other conidia and formed an appressorium on the epidermal cell (Ep). (B) A germinated conidia (GC) with two germ tubes, one passed over stomata (St) and formed a terminal globose appressorium (AP) at the end.

4. Infection of *A. zinniae* and disease severity at different growth stages of zinnia plant

In the experiment field, the different of infected levels on three leaves of inoculated plant was observed at eight stages. The zinnias were infected by *A. zinniae* and produced reddish brown spot on the leaves and blossoms in the age of 6, 7 and 8 weeks, but rarely from 1 to 3 weeks. The results of incidence and severity of the spot symptoms were increased according to the development stages of plant from 1 to 8 weeks. The mean of infected plants was 93.75%. Means of severity of infected leaves from 1 to 8 weeks were 2.8%, 4.7%, 10.3%, 18.8%, 17.4%, 16.4%, 23.5% and 31.3%, respectively. Therefore, the most susceptible period of zinnia was 8 weeks old (Figure 6).

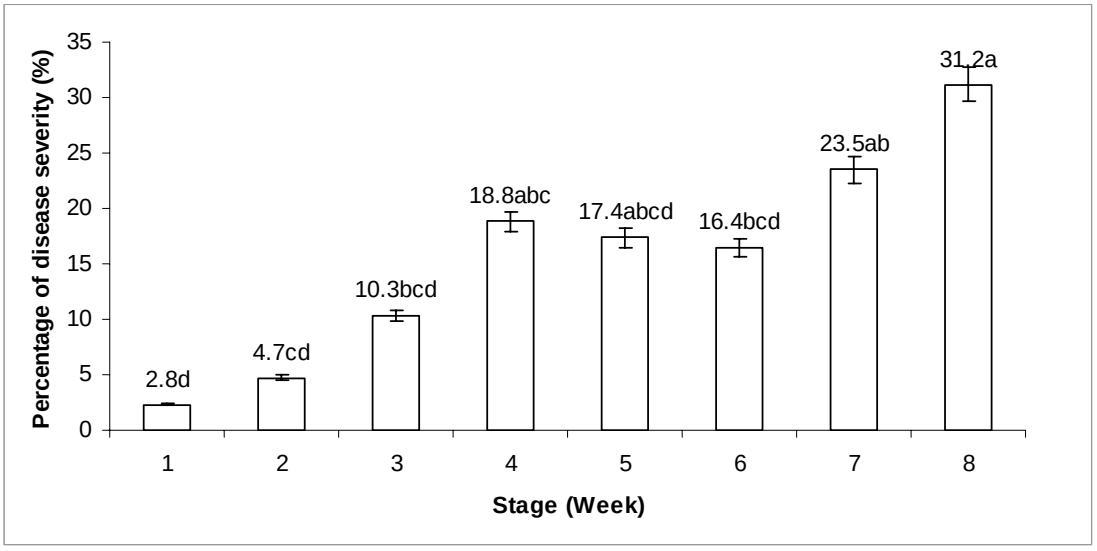

Figure 6 Percentage of disease severity of *A. zinniae* at different stages (week) of zinnia plant. Mean followed by different letters in the bar are significantly different by Duncan's multiple range test ($p \leq 0.05$)

5. Relationship between disease severity on the blossom of zinnia and infection of *A. zinniae* on zinnia seed

This experiment was conducted to determine percentage of disease severity and percentage of seed infection. The blossoms which were inoculated with *A. zinniae* at concentration of 2.5 x 10^7, 2.5 x 10^6, 2.5 x 10^5 conidia per ml and distilled water. After 7 days, inoculated plants were produced reddish brown spot on the petals. Disease severity were 18.0%, 16.1%, 17.3% and 0.2% on the blossom and percentage of infected seeds were 99.6%, 75.3%, 75.9% and 4.2%, respectively (Table 6, Figure 7 and Figure 8).

Table 6 Disease severity and seed infection (%) after inoculated with different concentrations of *A. zinniae* and incubated for 7 days of zinnia seeds on moistened blotter paper at 25°C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness

Treatment (conidia/ml)	Disease severity (%)	Seed infection (%)
2.5 x 10^7	18.0a	99.6a
2.5 x 10^6	16.1a	75.3b
2.5 x 10^5	17.3a	75.9b
Distilled water	0.2b	4.3c
F-test	**	**
C.V. (%)	53.8%	21.3%

Mean followed by different letters in the columns are significantly different by Duncan's multiple range test ($p \leq 0.05$)

Figure 7 Disease severity on the blossom of zinnia after inoculated with different concentrations of *A. zinniae*. A: 2.5 x 10^7 conidia/ml, B: 2.5 x 10^6 conidia/ml, C: 2.5 x 10^5 conidia/ml, D: uninoculated

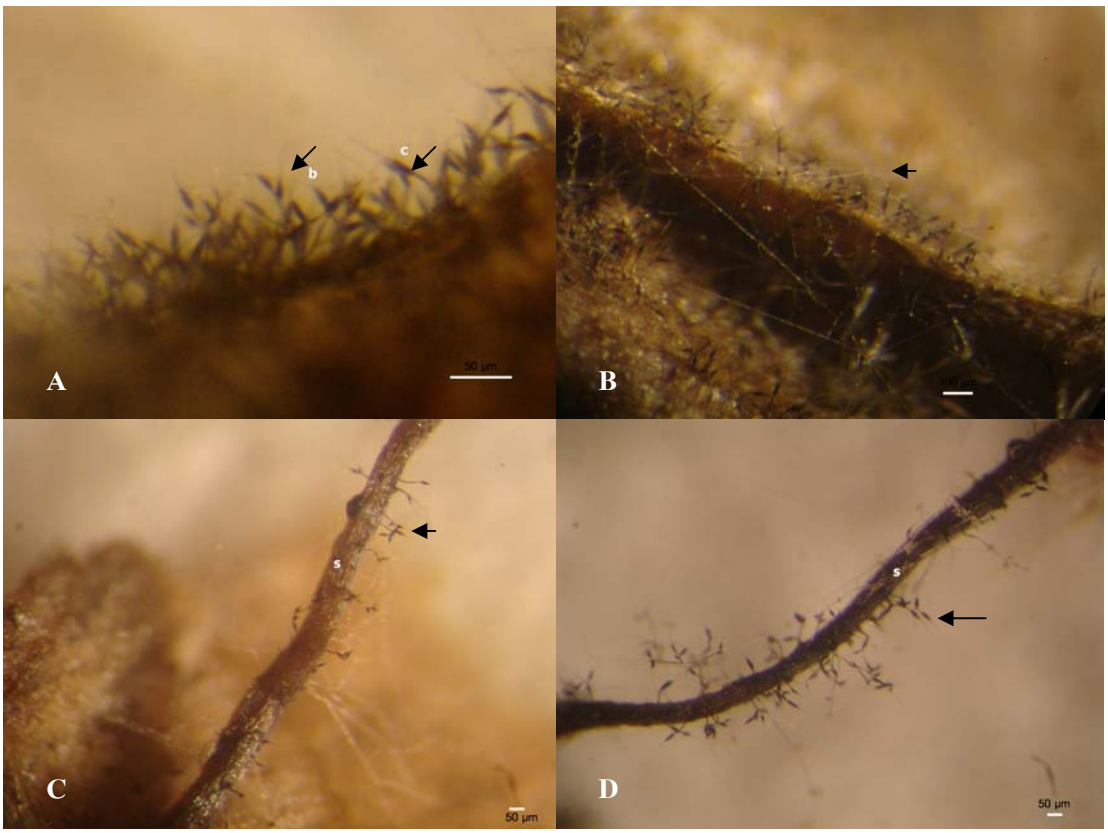

Figure 8 Sporulation (arrow) of *A. zinniae* on infected zinnia seeds. A and B, sporulation of the fungus on seed coat, conidia (c), beak (b) (bar = 50 µm and 100 µm respectively). C and D, sporulation of the fungus on the stigma (s) of zinnia seed (bar = 50 µm)

6. Localization of *A. zinniae* on/in zinnia seed

The components including embryo and seed coat were separated completely and incubated for 7 days. These components were examined under stereomicroscope. The infection of *A. zinniae* was found in both components. The infection of *A. zinniae* in zinnia seed is mainly located in the seed coat and rarely in the embryo but not significant. The range of infected seed coat varied between 2 – 12% and 0 – 8 % for infected embryo (Table 7). From the result, *A. zinniae* was mostly contaminated in seed coat and can infect deeply into the embryo (Figure 9).

Table 7 Percent infection of *A. zinniae* on two components of zinnia seed by using blotter method after incubating at 25 C under 12 h alternating cycles of near ultraviolet (NUV) light and darkness for 7 days

Component	Infection (%)
Seed coat	7
Embryo	4.5
F-test	**
CV (%)	12.30

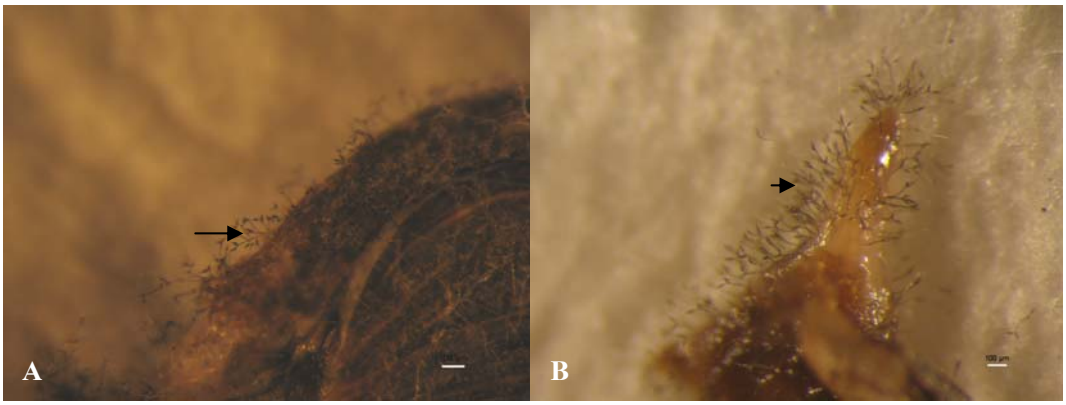

Figure 9 Sporulation of *A. zinniae* (arrow) on seed coat (A) and embryo (B) of zinnia seed after incubating at 25 C under 12 h alternating cycles of near ultraviolet (NUV) light and darkness for 7 days. (bar = 100 µm).

7. Transmission of *A. zinniae* from infected seed to seedling

a. Comparison of testing method

Three methods, which were used in this experiment, were the most common used in the seed health testing laboratories. These methods are simple and easy to practice. The experiment was conducted by using blotter method, test tube agar method, and seedling symptom test to study the transmission of *A. zinniae* from infected seed to seedling. Infection seed of *A. zinniae* using seedling symptom test

and test tube agar showed infection at 56.0% and 59.8% of infected seedling while blotter method obtained at 52.0% (Table 8).

Blotter method, this method is good for checking the incidence of *A. zinniae* from infected seed to seedling of zinnia. In this method, the seedlings were observed directly under stereomicroscope.

Test tube agar method, germination and seedling development was good and the disease progress was monitored easily. The symptom and conidia were observed under stereomicroscope for all parts of seedling. Mostly of infected seedling were died and the causal pathogen was confirmed directly under stereomicroscope.

Sand method, germination and seedling development was good. The disease progress was monitored easily at an upper part of seedling, but complicated and difficult for monitoring disease progress on rootlets of seedling. From these results, test tube agar method was the best for studying seed transmission of *A. zinniae*.

Table 8 Transmission study of *A. zinniae* from infected seed to seedling using blotter, test tube agar and sand methods

method	Infection (%)
Test tube agar	59.8a
Sand	56.0b
Blotter	52.0c
F-test	**
C.V.	2.8%

Means followed by different letters in the column are significantly different using Duncan's Multiple Range Test ($p \leq 0.05$)

b. Infected seed and seedling infection

In this experiment, seed samples were used for checking infection of *A. zinniae* in both blotter method and seedling symptom test. Infection seed of *A. zinniae* using those methods obtained at 52% and 51%, respectively. Transmission of *A. zinniae* from an infected seed can cause seed rot, spots, blight and damping off on seedlings under the favorable conditions especially at high humidity similar to Mc Donald and Martens (1963) and Krisana (1983). The result indicated that the percent of transmission for infected seed to seedling were 59.8 and 56.0% by test tube agar and sand method respectively (Table 8).

Based on previous results and comparing advantages and disadvantages among methods, test tube agar was used for studying of seed transmission of *A. zinniae*. Infected seeds with highest percentage of appearance of *A. zinniae* from previous result were used for this experiment (50 seeds per replication for 4 replications). The disease progress was examined after incubating at 25^{o}C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness for 14 days.

Symptom on cotyledons, stems and roots were observed. There was no significantly different on the occurrence of symptom on cotyledons, roots and stems of seedling (Table 9). The symptom as brownish spots on cotyledons, stems and roots was observed at 45.72%, 48.93% and 30.95%, respectively. Death of seedling was observed at 85.78%. From the previous experiment, seed infection was found at 52% of *A. zinniae* and its potential to caused damaged on seedling of zinnia with approximately 73.2%.

Table 9 Infection and symptom occurrence of *A. zinniae* from infected seeds to seedling by using test tube agar method and incubated at 25°C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness for 7 days

Seedling	Part of seedling	Occurrence of symptom (%)	Mean of infection
Infected seedling			
	Roots	30.95	8.75b±2.06*
	Stem	48.93	8.5b±1.73
	Cotyledon	45.72	9b± 1.83
	Seed coat	70[1]	19a± 2.71
Healthy seedling		0.00	0.00c
Death seedling		85.78	7.25b±1.89
C.V. (%)			21.62

Means followed by different letters in the column are significantly different using Duncan's Multiple Range Test ($p \leq 0.05$)

* Mean ± standard deviation

[1] Occurrence of *A. zinniae* (%)

Discussion

Among 8 seed-borne fungi isolated from 5 samples, some well known pathogens of various crops, e.g. *Alternaria recommend, A. zinniae, Curvularia eragrostidis, C. lunata, Corynespora* sp., *Fusarium* sp., *Fusarium moniliforme* and *Helminthosporium* sp.. For instance, *A. recommend* has diverse hosts and was isolated from seeds of Balsam, Celosia, Cosmos, Hollyhock, Japan pink, Rainbow pink, Periwinkle, Pot marigold, Snapdragon, Yellow cosmos and zinnia. *A. zinniae* also isolated on the seed of Yellow cosmos (Chou and Wu, 1995). This study reported that commercial zinnia seeds were infected and contaminated with a variety of seed-borne fungi. Although some seed-borne fungi isolated from 5 samples of zinnia seeds were not pathogenic to zinnia crop, the potential to be pathogenic to other crops should not be ignored.

A. zinniae readily infected the leaves of zinnia and caused reddish brown

spots on the zinnia plants of *ZN#3, ZN#7* and *ZNUD*. There were no differences observed among the samples used in this study. Hence, *A. zinniae* had a potential to infected and caused leaf spot on any samples which were tested in this study.

The symptoms of Alternaria leaf spot have been described by various authors (Andersen, 1974; Baker, 1956; Baker, 1980; Ellis, 1976; Joly, 1967; Mc Donald and Martens, 1963; Neergaard, 1945; Pape, 1942; Rao, 1971 and Simmons, 1982), but the biology of infection of zinnia leaf by *A. zinniae* has not been documented except in previous studied of Dimock and Osborn (1943), in the development of *A. zinniae* on zinnia leaf. However, this is the first study of the infection process of *A. zinniae* on zinnia leaves. The investigation of the artificial inoculation of zinnia with *A. zinniae* showed that conidia of *A. zinniae* germinated within 3 hr after incubating on 1% water agar and reached a maximum (95.9%) at 6 hr without inducer and 100% at 15 hr on zinnia leaves surface after spraying with 0.05% glucose, it produced high germination rates on the leaves of zinnia. Similarly to the previous studied of Suzuki *et al*. (2003) showed that most *A. recommend* Japanese pear pathotype conidia (approximately 95%) germinated within 3 hr after inoculation. An average of 5 germ tubes per conidium was observed at 15 hr after inoculation. Similar to Van Dyke and Trigiano (1987), were observed an average of 6 germ tubes of *A. cassiae* on sickle pod seedling parts collected 18 hr after inoculation in the field. Germ tubes from germinating conidia produced an appressoria at their tip. These appressoria formed directly on epidermal cell and stomata with approximately 11.6% and 77.2%, respectively. Sometimes the appressoria were produced at the tip of elongated germ tube, but mostly produced close to the conidia. Van Dyke and Trigiano (1987) reported that appressoria of *A. cassiae* formed directly on epidermal cells or over stomata of sickle pod with about equal frequency and sometimes germ tubes grew towards stomata; whereas other germ tubes passed near stomata but without appressoria formation.

Localization of appressoria mostly appear on epidermal cell that similar to the appressoria formation and leaf penetration by *A. cirsinoxia* that occurred predominantly at adjoining anticlinal cell walls of the epidermis of Canada thistle and rarely via stomata (Green *et al*., 2001). According to Allen *et al*. (1983), van Dyke

and Trigiano (1987) and Aveling *et al*. (1994) were reported that *Alternaria* species penetrate the host epidermis directly whereas other fungi utilize mainly stomata for host penetration (Saad and Hagedorn, 1969).

According to Green *et al.* (2001), reported the infection process of *A. cirsinoxia* on Canada thistle (*Cirsium arvense*) in the controlled environment and in the field. In the controlled environment, germination of conidia began at 2 hr and appressoria formation at 4 hr after inoculation. Approximately 75% of appressoria formed at the anticlinal wall junctions of the epidermis. Leaf penetration occurred between 6 and 24 hr, most commonly in between adjoining anticlinal walls. Penetration through stomata was rare.

The zinnias were infected by *A. zinniae* and produced reddish brown spot on the leaves and blossoms in the age of 6, 7 and 8 weeks, but rarely from 1 to 3 weeks. The results of incidence and severity of the spot symptoms were increased according to the development stages of plant from 1 to 8 weeks. According to Allen *et al.* (1983), reported that older sunflower leaves were more susceptible to infection by *A. helianthi* than younger leaves at both optimal (25^{o}C) and suboptimal temperatures (22^{o}C). Agrios (1997) reported that Alternaria leaf spot occurs on the lower, senescent leaves, but the disease progresses upward and makes affected leaves turn yellowish and senescent and then, either dry up or fall off. Chupp and Sherf (1960) also reported that the Alternaria symptoms often occur on the older leaves.

For most flower-infecting fungi, infection of petals is more common than infection of sepals (Ngugi and Scherm, 2006). The petals are easier to breach as their cell walls usually are not lignified (Bowes, 1996). This experiment was limited to testing the inoculum concentrations of *A. zinniae* that were successfully infection on the blossom of zinnia even in the lower concentration (2.5 x 10^5 conidia per ml).

However, this concentration was commonly used such as the previous studied of Hildebrand *et al.* (2001) were studied in the relationship of inoculum concentration of *Botrytis cinerea* and wetness duration for disease severity index on flower of

blueberry and reported that at the concentration of 10^5 or 10^6 conidia/ml at 20°C and incubated over 96 hr wet period, lesions spread from the corolla to the peduncle, but when the inoculum concentration was 10^3 or 10^4 conidia/ml, they did not extend beyond the corolla.

Although, there was no significant different in disease severity on petals of zinnia which were inoculated with *A. zinniae* at different concentrations but there was significantly difference in seed infection of *A. zinniae*. However, the result was suggested that *A. zinniae* can be seed-borne even symptom or symptomless on the blossoms of zinnia plants.

Field pathogens, in general, are not believed to cause internal seed infection in dry seeds. However, seed contamination is common (Neergaard, 1977; Agarwal and Sinclair, 1997). Pathogens may directly penetrate the ovary wall or seed coat. This type of infection can be restricted to the seed coat or may extend into the endosperm, cotyledons or embryo (Agarwal and Sinclair, 1997).

Fungi known to penetrate through the ovary wall and seed coat are the genus of *Alternaria* such as *A. brassicae* and *A. brassicicola* in cabbage and canola (Domsch, 1957), *A. alternata* in sunflower (Singh *et al.,* 1977) and wheat (Bhowmik, 1969) and *A. sesami* in *Sesamum indicum* (Leppik, 1964).

The infection of *A. zinniae* was found in both components. Location of infection of *A. zinniae* in zinnia seeds was observed mainly on the seed coat, but rarely in the embryo. According to Baker (1980) *A. zinniae* was carried with zinnia seed in form of vegetative structure (mycelium) in the inner part of seed. Gambogi *et al*. (1976) also reported that a deep-seated infection was observed. Similarly to the previous studied of Shrestha *et al.* (2000), reported that localization of *A. brassicae* in the seeds of rapeseed and mustard was observed mainly on hilum area of the seed coat.

Some of seed borne fungi are capable to producing symptoms on young seedlings either in roots, stem and cotyledons. Such symptoms can occasionally be seen in the blotter and agar method (test tube). Pre and post emergence seedling mortality were observed. However, to provide more natural conditions, seeds are sown on autoclaved sand and placed under optimal conditions of temperature, light and humidity. The symptom appeared on cotyledons, stem and roots with about equal frequency on those method and there were significantly difference between the methods. So test tube agar method is the best method for seedling symptom test of *A. zinnia* because of their completely individuals, but, this result in contrast to Krisana (1983) that reported modified blotter method is better.

However, transmission of *A. zinniae* from an infected seed can cause seed rot, spots, blight and damping off on seedlings under the favorable conditions especially at high humidity similar to Mc Donald and Martens (1963) and Krisana (1983).

CONCLUSION AND RECOMMENDATIONS

Conclusion

A. zinniae existed mostly (52%) on the zinnia seeds of *ZNUD*. *A. zinniae* readily infected the leaves of zinnia and caused reddish brown spots. There was no significantly between diseases severity of the samples.

The infection biology of *A. zinniae* were examined for conidia germination, number of germ tubes per conidium and appressoria formation with compound microscope. Conidia of *A. zinniae* germinated within 3 hr after inoculation and reached to maximum (100%) at 15 hr with an average of 5 germ tubes per conidium. Appressoria formed both on stomata and epidermal cell, but mostly in the latter. The most susceptible period of zinnia plants was at the 8 weeks. The blossoms which were infected by *A. zinniae* and produced reddish brown spot on the petals at concentrations of 2.5×10^5, 2.5×10^6, 2.5×10^7 and 0 conidia per ml showed that disease severity were 18.0%, 16.1%, 17.3% and 0.2% and percentage of infected seeds were 99.6%, 75.3%, 75.9% and 4.2%, respectively. Thus, *A. zinniae* can be seed-borne even symptom or symptomless on the blossoms of zinnia plants.

A. zinniae is a seed-borne and seed transmission on zinnia seeds. Means of infected was found both in the seed coat and embryo at 7% and 4.5%, respectively. At 8 weeks, zinnia plant was rather susceptible to infection. Test tube agar method was the best for studying seed transmission of *A. zinniae*. Transmissions of this pathogen can spots, blight and damping off on seedlings up to 73.2%.

Recommendations

Alternaria zinniae is a seedborne fungus of zinnia seed and cause leaf spot in zinnia. From this study, it was found mostly on seed coat of zinnia seed. This fungus was decreased after surface sterilize with 1% NaOCl for 3 min. Due to the type of germination of zinnia seed, the seed coat still attached with cotyledons and can cause infection. So the seed must be advisable to treat with fungicide dust such as captan or thiram before planting in the field to protect seedlings. Conidial germination and appressorial formation of this fungus indicated that it can infect zinnia leaves within 3 hr under high humidity condition. Application of fungicide is needed if it's raining or such condition occurs in the field of seed production. The farmers should to use only clean, high quality seed.

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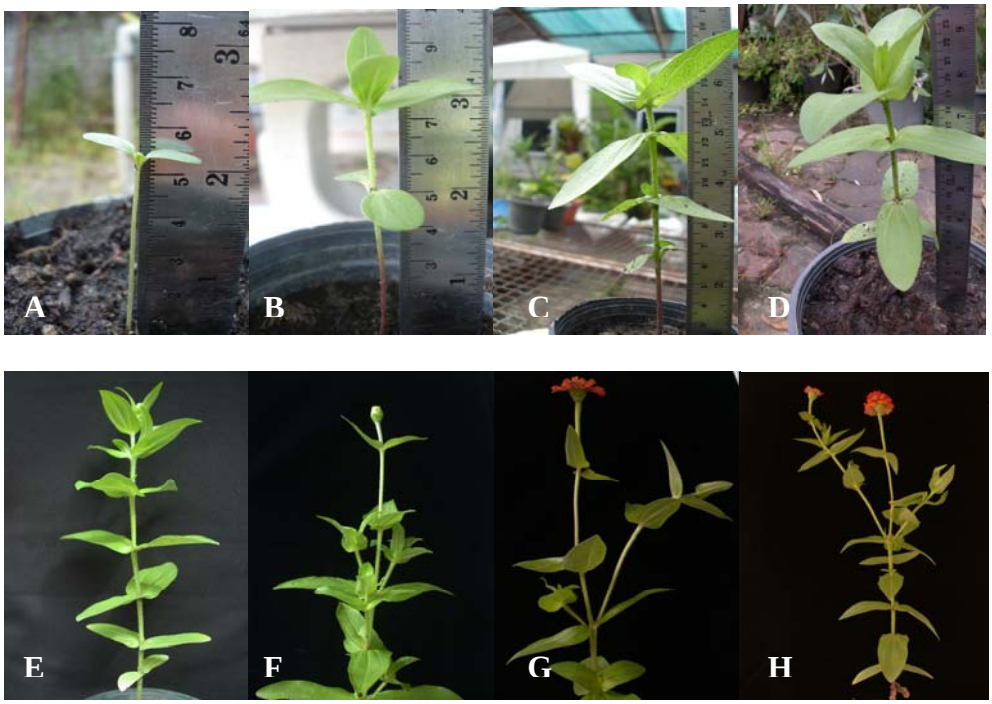
APPENDICES

Appendix A

Materials used in the thesis research

Plant materials used in this study



Appendix Figure A1 Stage of zinnia plants (week) from 1 to 8 weeks used in this study. A: 1 week, B: 2 weeks, C: 3 weeks, D: 4 weeks, E: 5 weeks, F: 6 weeks, G: 7 weeks, H: 8 weeks

Media used in the thesis research

Type	Ingredient	Quantity (gram)
Potato Dextrose Agar (PDA)	Potato	200
	Dextrose	20
	Agar	12
	Distilled Water	1000 ml
Carrot Agar (CA)	Carrot	200
	Agar	12
	Distilled Water	1000 ml
Water Agar (WA)	Agar	10
	Distilled Water	1000 ml

Appendix B

Results of analysis

1. Pathogenicity test

Appendix Table B1 Result of analysis of variance of diseases severity (%) after inoculated with *A. zinniae* and incubated at 25°C for 7 days

	Sum of Squares	df	Mean Square	F	Sig.
Treatment	3851.350	3	1283.783	9.606	.001
Error	2138.400	16	133.650		
Total	5989.750	19			

Appendix Table B2 Multiple comparisons of diseases severity (%) after inoculated with *A. zinniae* and incubated at 25°C for 7 days

	(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound
LSD	*ZN#3*	*ZN#7*	3.4000	7.31163	.648	-12.1000	18.9000
		ZNUD	9.8000	7.31163	.199	-5.7000	25.3000
		Control	35.4000(*)	7.31163	.000	19.9000	50.9000
	ZN#7	*ZN#3*	-3.4000	7.31163	.648	-18.9000	12.1000
		ZNUD	6.4000	7.31163	.394	-9.1000	21.9000
		Control	32.0000(*)	7.31163	.000	16.5000	47.5000
	ZNUD	*ZN#3*	-9.8000	7.31163	.199	-25.3000	5.7000
		ZN#7	-6.4000	7.31163	.394	-21.9000	9.1000
		Control	25.6000(*)	7.31163	.003	10.1000	41.1000
	Control	*ZN#3*	-35.4000(*)	7.31163	.000	-50.9000	-19.9000
		ZN#7	-32.0000(*)	7.31163	.000	-47.5000	-16.5000
		ZNUD	-25.6000(*)	7.31163	.003	-41.1000	-10.1000

* The mean difference is significant at the .05 level.

2. Conidia germination and appressoria formation of *A. zinniae*

Appendix Table B3 Result of analysis of variance of germinated conidia (%) on zinnia leaf after inoculation, and incubated at 25°C for 1, 3, 6, 9, 12, 15, 18, 21, and 24 hr

	Sum of Squares	df	Mean Square	F	Sig.
Incubation time	32665.81	8	4083.227	1223.33	.000
Error	90.120	27	3.338		
Total	32755.936	35			

Appendix Table B4 Result of analysis of variance of ungerminated conidia (%) on zinnia leaf after inoculation, and incubated at 25°C for 1, 3, 6, 9, 12, 15, 18, 21, and 24 hr

	Sum of Squares	df	Mean Square	F	Sig.
Incubation time	32665.816	8	4083.227	1223.33	.000
Error	90.120	27	3.338		
Total	32755.936	35			

Appendix Table B5 Result of analysis of variance of number of germ tube per conidium on zinnia leaf after inoculation, and incubated at 25°C for 1, 3, 6, 9, 12, 15, 18, 21, and 24 hr

	Sum of Squares	df	Mean Square	F	Sig.
Incubation time	38.221	8	4.778	29.350	.000
Error	4.395	27	.163		
Total	42.616	35			

Appendix Table B6 Result of analysis of variance of appressorial formation (%) on zinnia leaf after inoculation, and incubated at 25°C for 1, 3, 6, 9, 12, 15, 18, 21, and 24 hr

	Sum of Squares	df	Mean Square	F	Sig.
Incubation time	11071.552	8	1383.944	29.769	.000
Error	1255.218	27	46.490		
Total	12326.770	35			

Appendix Table B7 Result of analysis of variance of localization of appressoria on stomata (%) of zinnia leaf after inoculation, and incubated at 25°C for 1, 3, 6, 9, 12, 15, 18, 21, and 24 hr

	Sum of Squares	df	Mean Square	F	Sig.
Incubation time	1990.135	8	248.767	8.472	.000
Error	792.805	27	29.363		
Total	2782.940	35			

Appendix Table B8 Result of analysis of variance of localization of appressoria on epidermal cell(%) of zinnia leaf after inoculation, and incubated at 25°C for 1, 3, 6, 9, 12, 15, 18, 21, and 24 hr

	Sum of Squares	df	Mean Square	F	Sig.
Incubation time	28214.182	8	3526.773	123.751	.000
Error	769.470	27	28.499		
Total	28983.652	35			

3. Infection of *A. zinniae* and disease severity at different growth stages of zinnia plant

Appendix Table B9 Multiple comparisons of disease severity of *A. zinniae* at different stages (week) of zinnia plant

	(I) No.3	(J) No.3	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound
LSD	stage 1	stage 2	-1.9166	6.83072	.781	-16.0146	12.1812
		stage 3	-7.5833	6.83072	.278	-21.6812	6.5146
		stage 4	-16.0000(*)	6.32402	.018	-29.0521	-2.9479
		stage 5	-14.6500(*)	5.99949	.022	-27.0323	-2.2677
		stage 6	-13.6500(*)	5.99949	.032	-26.0323	-1.2677
		stage 7	-20.7500(*)	6.32402	.003	-33.8021	-7.6979
		stage 8	-28.4975(*)	6.32402	.000	-41.5496	-15.4454
	stage 2	stage 1	1.9166	6.83072	.781	-12.1812	16.0146
		stage 3	-5.6666	7.30235	.445	-20.7380	9.4046
		stage 4	-14.0833	6.83072	.050	-28.1812	.0146
		stage 5	-12.7333	6.53142	.063	-26.2135	.7468
		stage 6	-11.7333	6.53142	.085	-25.2135	1.7468
		stage 7	-18.8333(*)	6.83072	.011	-32.9312	-4.7354
		stage 8	-26.5808(*)	6.83072	.001	-40.6787	-12.4829
	stage 3	stage 1	7.5833	6.83072	.278	-6.5146	21.6812
		stage 2	5.6666	7.30235	.445	-9.4046	20.7380
		stage 4	-8.4166	6.83072	.230	-22.5146	5.6812
		stage 5	-7.0666	6.53142	.290	-20.5468	6.4135
		stage 6	-6.0666	6.53142	.362	-19.5468	7.4135
		stage 7	-13.1666	6.83072	.066	-27.2646	.9312
		stage 8	-20.9141(*)	6.83072	.005	-35.0121	-6.8163
	stage 4	stage 1	16.0000(*)	6.32402	.018	2.9479	29.0521
		stage 2	14.0833	6.83072	.050	-.0146	28.1812
		stage 3	8.4166	6.83072	.230	-5.6812	22.5146
		stage 5	1.3500	5.99949	.824	-11.0323	13.7323
		stage 6	2.3500	5.99949	.699	-10.0323	14.7323
		stage 7	-4.7500	6.32402	.460	-17.8021	8.3021
		stage 8	-12.4975	6.32402	.060	-25.5496	.5546
	stage 5	stage 1	14.6500(*)	5.99949	.022	2.2677	27.0323
		stage 2	12.7333	6.53142	.063	-.7468	26.2135

Table 9 (continued)

	(I) No.3	(J) No.3	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound
LSD		stage 3	7.0666	6.53142	.290	-6.4135	20.5468
		stage 4	-1.3500	5.99949	.824	-13.7323	11.0323
		stage 6	1.0000	5.65637	.861	-10.6742	12.6742
		stage 7	-6.1000	5.99949	.319	-18.4823	6.2823
		stage 8	-13.8475(*)	5.99949	.030	-26.2298	-1.4652
	stage 6	stage 1	13.6500(*)	5.99949	.032	1.2677	26.0323
		stage 2	11.7333	6.53142	.085	-1.7468	25.2135
		stage 3	6.0666	6.53142	.362	-7.4135	19.5468
		stage 4	-2.3500	5.99949	.699	-14.7323	10.0323
		stage 5	-1.0000	5.65637	.861	-12.6742	10.6742
		stage 7	-7.1000	5.99949	.248	-19.4823	5.2823
		stage 8	-14.8475(*)	5.99949	.021	-27.2298	-2.4652
	stage 7	stage 1	20.7500(*)	6.32402	.003	7.6979	33.8021
		stage 2	18.8333(*)	6.83072	.011	4.7354	32.9312
		stage 3	13.1666	6.83072	.066	-.9312	27.2646
		stage 4	4.7500	6.32402	.460	-8.3021	17.8021
		stage 5	6.1000	5.99949	.319	-6.2823	18.4823
		stage 6	7.1000	5.99949	.248	-5.2823	19.4823
		stage 8	-7.7475	6.32402	.232	-20.7996	5.3046
	stage 8	stage 1	28.4975(*)	6.32402	.000	15.4454	41.5496
		stage 2	26.5808(*)	6.83072	.001	12.4829	40.6787
		stage 3	20.9141(*)	6.83072	.005	6.8163	35.0121
		stage 4	12.4975	6.32402	.060	-.5546	25.5496
		stage 5	13.8475(*)	5.99949	.030	1.4652	26.2298
		stage 6	14.8475(*)	5.99949	.021	2.4652	27.2298
		stage 7	7.7475	6.32402	.232	-5.3046	20.7996

* The mean difference is significant at the .05 level.

4. Relationship between disease severity on the blossom of zinnia and infection of *A. zinniae* on zinnia seed

Appendix Table B10 Result of analysis of variance of disease severity and seed infection (%) after inoculated with different concentrations of *A. zinniae* and incubated for 7 days of zinnia seeds on moistened blotter paper at 25°C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness

		Sum of Squares	df	Mean Square	F	Sig.
Disease severity	Treatment	11609.248	3	3869.749	14.430	.000
	Error	9654.036	36	268.168		
	Total	21263.284	39			
Seed infection	Treatment	51059.516	3	17019.839	92.359	.000
	Error	6634.032	36	184.279		
	Total	57693.548	39			

Appendix Table B11 Result of analysis of variance of disease severity of *A. zinniae* at different stages (week) of zinnia plant

	Sum of Squares	df	Mean Square	F	Sig.
Stage	2378.185	7	339.741	4.247	.004
Error	1919.673	24	79.986		
Total	4297.858	31			

5. Localization of *A. zinniae* on/in zinnia seed

Appendix Table B12 Result of analysis of variance of percent infection of *A. zinniae* on two components of zinnia seed by using blotter method after incubating at 25 C under 12 h alternating cycles of near ultraviolet (NUV) light and darkness for 12 h for 7 days

	Sum of Squares	df	Mean Square	F	Sig.
Component	1800.000	1	1800.000	20.149	.004
Error	536.000	6	89.333		
Total	2336.000	7			

6. Transmission of *A. zinniae* from infected seed to seedling

Appendix Table B13 Result of analysis of variance of Transmission study of *A. zinniae* from infected seed to seedling using blotter, test tube agar and sand methods

	Sum of Squares	df	Mean Square	F	Sig.
Method	121.707	2	60.853	24.940	.000
Error	21.960	9	2.440		
Total	143.667	11			

Appendix Table B14 Infection and symptom occurrence of *A. zinniae* from infected seeds to seedling by using test tube agar method and incubated at 25°C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness for 7 days

replication	Seed infection (%)	Seedling infection (%)
1	52	77.4
2	52	61.5
3	52	73.1
4	52	80.6
Mean	52	73.2

Appendix Table B15 Result of analysis of variance of infection and symptom occurrence of *A. zinniae* from infected seeds to seedling by using test tube agar method and incubated at 25°C under 12 hr alternating cycles of near ultraviolet (NUV) light and darkness for 7 days

	Sum of Squares	df	Mean Square	F	Sig.
Part of seedling	736.000	5	147.200	41.079	.000
Error	64.500	18	3.583		
Total	800.500	23			

CIRRICULUM VITAE

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Degree Conferred	Bachelor of Science (Pest Management)
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Grad Point Average (GPA)	3.22

Project, Work, and Other Experiences

1. Research

- Study on Bacterial Soft Rot Disease of Orchids (Special Problem, 2006).

- Study on an efficacy of carboxin20% + thiram20% W/V SC (VITAVAX 200 FF) to control the fungi caused brown spot on rice seeds
- Enhancement an efficacy of hot water treatment to control antracnose of mango fruits

2. Presentation

- Poster Presentation of 'Effect of percitric acid on green mold rot of citrus and anthracnose of mango' (in Thai), August 14-15, 2008 In 6th National Technical seminar on Postharvest Technology at Chareon Thani Princess Hotel, KhonKhen.
- Poster Presentation 'Enhancement an efficacy of hot water treatment to control antracnose of mango fruits' (in Thai), August 14-15, 2008 In 6th National Technical seminar on Postharvest Technology at Chareon Thani Princess Hotel, KhonKhen.
- Poster presentation of 'Seed Transmission of *Alternaria zinniae*, causing leaf spot in zinnia' (in English), March 17-20, 2009 In 47th Kasetsart University Annual Conference, Bangkok.

3. Publishing Papers

- Sangchote, S. and Seehachai, W. 2008. Enhancement an efficacy of hot water treatment to control anthracnose of mango fruits. *In* Proceedings of 6th International Technical Seminar on Postharvest Technology, Khon Kaen, Thailand. (In Thai)
- Sangchote, S., Seehachai, W. and Luxanapisuth, S. 2008. Effect of Percitric Acid on Green Mold Rot of Citrus and Anthracnose of Mango. *In* Proceedings of 6th International Technical Seminar on Postharvest Technology, Khon Kaen, Thailand. (In Thai)
- Seehachai, W. and Sangchote, S. 2009. Seed Transmission of *Alternaria zinniae*, causing leaf spot in zinnia. *In* The Proceeding of 47th Kasetsart University Annual Conference, Bangkok, Thailand.

4. Scholarship

- Research Assistance, Dept. of Plant Pathology (2006-2007)
- Internship Program in Department of Plant Pathology and Crop Physiology at Louisiana State University, Agricultural Center, USA, (June-October, 2007).
- Graduate School, Kasetsart University (2008).

5. Other Experience

I have performed as a research assistant during my studying of the Master degree and acted as a staff to prepare several seminar/workshop which was supported by Dept. of Plant Pathology, Kasetsart University.