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Role of Four *Alternaria* spp. Causing Leaf and Stem Blight of Sunflower in Thailand and Their Chemical Controls[1]

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ABSTRACT

A symptom of sunflower leaf and stem blight was observed in the cultivated areas of Thailand throughout 1988-1989, primarily on dark flecks or blackened area of leaves. *Alternaria* spp. were isolated from infected plants of several sunflower cultivars/lines from 10 farms where samples were taken. Isolates were identified as *A. helianthi* or *Helminthosporium helianthi* (10 farms). *A. zinniae* (5 farms), *A. alternata* (3 farms) and *A. longissima* (1 farms). After sunflower plants were artificially inoculated for pathogenicity test, it was found that all four *Alternaria* spp. were extremely virulent, causing completed leaf and stem blight. This is the first report of *A. longissima,* causing leaf spot or leaf blight of sunflower while the other 3 species, *A. helianthi, A. zinniae* and *A. alternata* have already been widely reported in the cultivated areas. *Curvularia* sp. and *Pestalotia* sp. were also observed to cause leaf spot of sunflower (2 farms).

Comparison of seven fungicides applied individually or couplingly at 1000 ppm for management of sunflower blight caused by *Alternaria* spp. was studied. After receiving three applications (at 20-day intervals, beginning at V_4 growth stage), noninoculated plants resulted in higher yields and lower disease severity than fungicide-treated inoculated (mixed inoculum) plants. Plants treated with each fungicide plus adjuvant averaged significantly higher yields than the nontreated controls. Iprodione coupling with mancozeb had the highest 100 seed weight, yield, and oil content with no statistical difference from imazalil and iprodione alone but it differred significantly from mancozeb, copper oxide, triforine, fentin acetate and benomyl. However, iprodione (Rovral) coupling with adjuvant (Triton CS-7) had higher yields but insignificantly differences to Rovral without Triton CS-7.

INTRODUCTION

Alternaria blight of sunflower, caused by *Alternaria* spp. is a serious, potentially destructive disease and widely distributed wherever the plants are grown (Carson, 1985 ; Morris and Yang, 1983; Prathuangwong, *et al.,* 1988). The disease can cause severe leaf and stem spots resulting in premature defoliation and stem breakening when condition of warm temperature and high relative humidity prevail. The pathogens were found causing high disease severity in sunflower fields in the primary sunflower cultivated areas of Thailand such as Chiang Mai, KhonKaen, Nakhon Ratchasima, Lopburi, Nakhon Pathom and Chonburi (Prathuangwong, *et al.,* 1988). Alternaria blight significantly reduced head diameters, number of seeds produced per head, 1,000 seed weight, and percent oil content of seed (Reddy and Gupa, 1977). An attempt was made to deter-

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mine the fungi responsible for their disease and several *Alternaria* spp. have been reported elsewhere to cause leaf and stem blight, including *A. zinniae, A. helianthi, A. alternata* and *A. leucanthemi* (Carson, 1987) but they were not yet reported in Thailand. The study was initiated to investigate the occurrence and distribution of Alternaria leaf and stem blight in Thailand, and to determine the relative frequency of those *Alternaria* spp. involved.

No study on special control method for the pathogens has yet been undertaken, attempt was made with chemical control of these diseases by spraying of fungicides to sunflower plants. However, Bewaji, *et al.*, (1977) evaluated three fungicides for the control of some *Alternaria* spp. on the surface rot of Kadota figs, those were benomyl, maneb and chlorothalonil. He also showed that preharvest sprays or dusts of zinc coposil or several dithiocarbamates significantly reduced *Alternaria* and *Cladosporiun* infection of plants. The researchers felt that the chemical control was reconfirmed and warranted due to a large amount of new fungicides were distributed regularly and from the literature, the greatest number of spraying or continuous using of benomyl and other chemicals would cause resistance in *Alternaria* spp. Our studies were conducted to determine the sensitivity of *Alternaria* spp. to some fungicides *in vitro* and on inoculated sunflower under field conditions.

MATERIALS AND METHODS

Isolation of Alternaria spp. From 1987 through 1988, 10 farms (Chiang Mai 3, Khon-Kaen 1, Nakhon Ratchasima 2, LopBuri 2, NakhonPathom 1 and Chonburi 1) throughout Thailand were visited where dark flecks or blackened dead tissues of sunflower were observed. Specimens of different types of leaf and stem blight were collected from each farm, placed into individual plastic bags to prevent rapid dessication, and transported to the laboratory in an ice box. Isolations were made from each type of lesion by pieces (about 3-5 mm^2) of necrotic spot from infected leaf or stem tissue, excised with a razor blade, surface disinfected briefly (1-3 sec) in 70% ethanol, followed by immersion in 10% clorox for 1-2 min, the length of immersion depending on the size of leaf pieces, and blotted dry on a paper towel. Part of each specimen was transferred directly to Petri plates containing potato dextrose agar (PDA). Plates were incubated at room temperature (30-35°C) in the dark and UV light and examined daily for 1 week. Emerging colonies (spores or mycelia) of *Alternaria* spp. were subcultured and stored on slant of PDA until they could be identified.

Identification of Alternaria spp. Isolates were identified on the basis of colony morphology, mycelial characteristics and production, morphology and dimension of spores and coidiophores. A 4-mm-diameter mycelial plug of each isolate was transferred to PDA and/or V-8 juice agar (V8A) poured to a standard thickness (20 ml of medium per 90-mm-diameter Petri dish), and incubated at room temperature. Production and morphology of spores were studied after 2-4 weeks of incubation. Those isolates that failed to form spores in pure culture were subsequently grown on plates of the same medium and treated with continuous fluorescent light and incubated until they could produce spores.

Pathogenicity test. The pathogenicity of *Alternaria* spp. were studied on 6-week-old sunflower plants variety Hysun 33. The plants were inoculated by spraying with a suspension prepared either from spores produced on 70 ml of V-8 juice liquid medium in 500 ml Erlenmeyer flask or mycelium grown for 2 weeks in PDA and transferred into distilled water as mycelial inoculum.

In each case, gelatin was added to the inoculum to make a final concentration of 0.25%. Inoculated plants were placed in a humidity cabinet at 30°C for 72 hr and then transferred to a growth cabinet at the same temperature (Mc Donald and Martens, 1963). At the end of 7 weeks experimental period, symptoms and disease severity were assessed for each isolate on the basis of necrotic lesion occurred.

Effects of fungicides on Alternaria spp. in laboratory and field studies. Growth inhibition of *Alternaria* spp. isolated from sunflower leaf and stem blight was determined on PDA amended with seven kinds of fungicides including iprodione (Rovral 50% WP), mancozeb (Azinmag 40% WP), imazalil (Fungaflor 68% EC), copper oxide (Cuprox 85% WP), triforine (Sapral 20% EC), fentin acetate (Brestan 54% WP), and benomyl (Benlate 50% WP). Fungicides were suspended in sterile distilled water then added to warm (50°C) PDA at concentration of 100 and 250 ppm (W/V). A nonamended check was also prepared for each isolate. The amended PDA (20 ml), was dispensed into 90 mm diameter petri dishes. Mycelial plugs (6 mm diameter) cut from the margins of 7-day-old *Alternaria* spp. culture on PDA were placed onto five replicate plates of PDA on each fungicide concentration. Radial growth of each replicate was measured after 10 days. The experiment was repeated twice.

Field experiments were conducted at Suwan Farm, NakhonRatchasima in 1988. The experimental design was Split Plot in a Randomized Complete Block with inoculated and non-inoculated sunflower plants (Hysun 33). The pathogens were main plot and fungicides were sub plots. Treatments were replicated three times in 3 blocks with 20×30 m in size each and between block 2.5 m. Each sub-plot was 1.5×9 m in size and between sub-plot 1.5 m. Plots were planted in six rows in length with spacing between rows 0.75 m and between plants 0.35 m. Four guard rows were planted between inoculated and non-inoculated plants. Weed control, fertilizer application and insect pest control were based on the local standard management. Furrow irrigation was applied regularly at 7-9 day intervals.

Isolates of *Alternaria* spp. were cultured either on PDA or sorghum seed medium. After 3 weeks of incubation, cultures were then mixed with distilled water and the suspension was sieved through a 300 mesh screen and adjusted to concentration of 1×10^6 spores per milliliter with distilled water. Each plot was inoculated by mean of a back pack sprayer to the plants at V5 and R2 growth stages. The plants were predisposed to infection by water spraying or sprinkle irrigation one day before inoculation and humid condition was maintained for 1-2 hr after inoculation.

The fungicides were applied with a back pack pressurized sprayer. Application of the chemicals was made 3 times throughout the crop season, started at V_5 growth stage of sunflower (approximately 20 days after the plant emerged from the soil) and later every 20 day intervals.

Average disease severities (the proportion of necrotic lesions) of all plants in each plot were assessed periodically by recording of six categories including number of infected leaf per plant, infected leaf area (the lesions on 5×9 cm^2 area of the leaf were counted through a window of 21×28 cm^2 area on a punched card), number of infected branches per plant, number of lesions on stem, average of infected sizes on stems and numbers of infected petals. Sunflower plants were measured from twenty in each treatment plot. Sample yield, 100 seed weight and oil contents were also taken by harvesting 20 competitive plants (evenly spaced with neighbors) from every plot.

RESULTS

Field symptom. Alternaria spp. infected sunflower was generally found as dark flecks with indefinite margin surrounded by a distinct yellow halo on leaf. Lesions which were roughly circular in shape, uniformly a target-like appearance coalesced and enlarged into blackened area resulting in leaf blight symptom which caused premature defoliation. These lesions sometime were difficult to determine. The extents of the damage attributable to infection by *Alternaria* spp., as numerous other types of leaf necrosis occurred on the lower leaves caused by other factors. The causal organism also damaged the leaf vein, stem, branch, petal, head and receptacle causing dark flecks and streaks along the length of plant parts (Fig. 1). The disease was commonly found in all growing areas visited and from visible estimation, causing high losses to sunflower

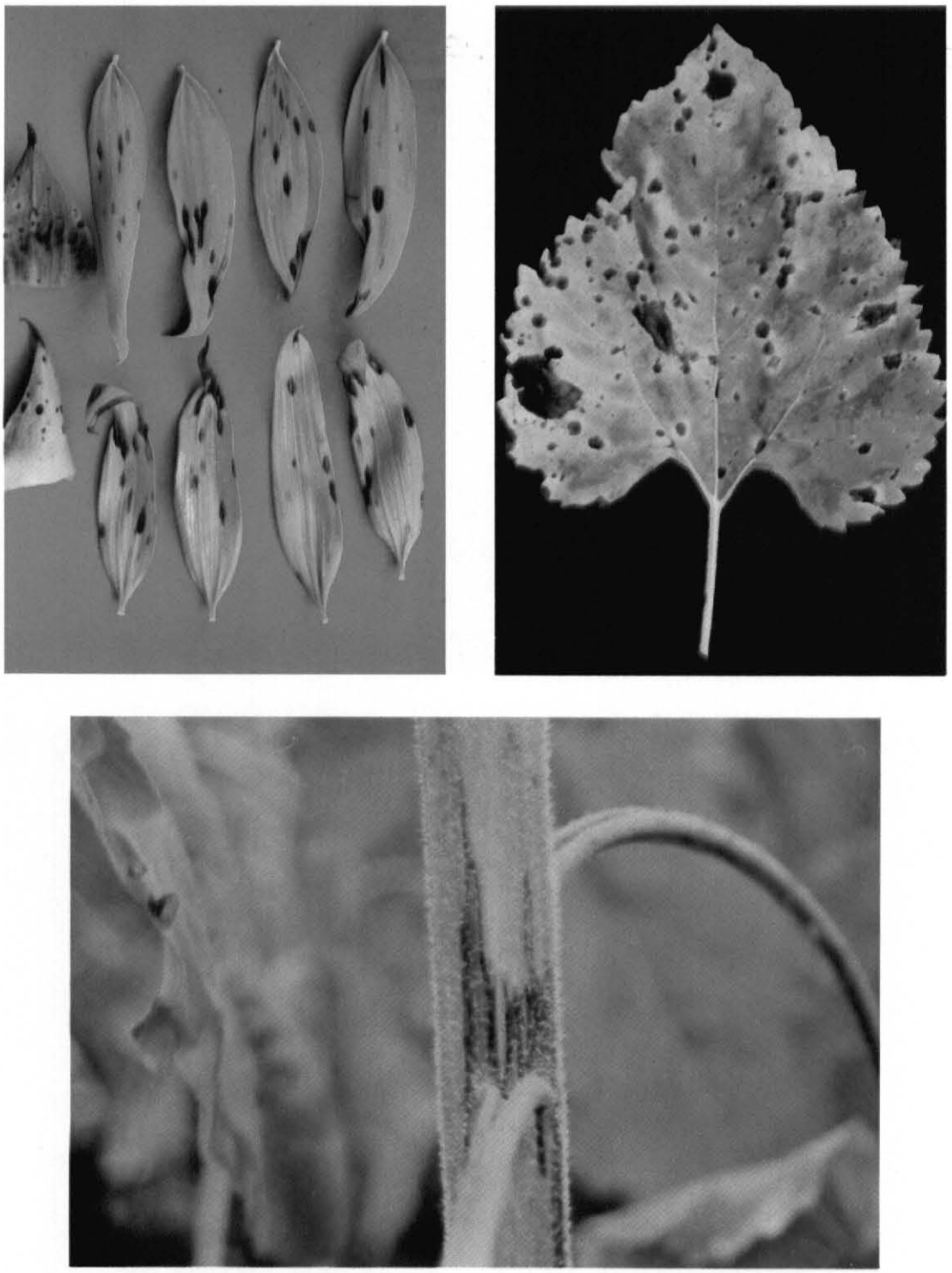

Figure 1 Symptoms of sunflower leaf and stem blight affected with *Alternaria* spp. A, The small irregular dark brown spots on the leaf which may coalesce and enlarge into blackened area. B, Elliptic shaped lesion on stem, scattered over the stalk and black lesions on nodes and petioles were also found. C, Symptom on bracts and petals, the pathogen infected the entire head and stalk to be severely rotten.

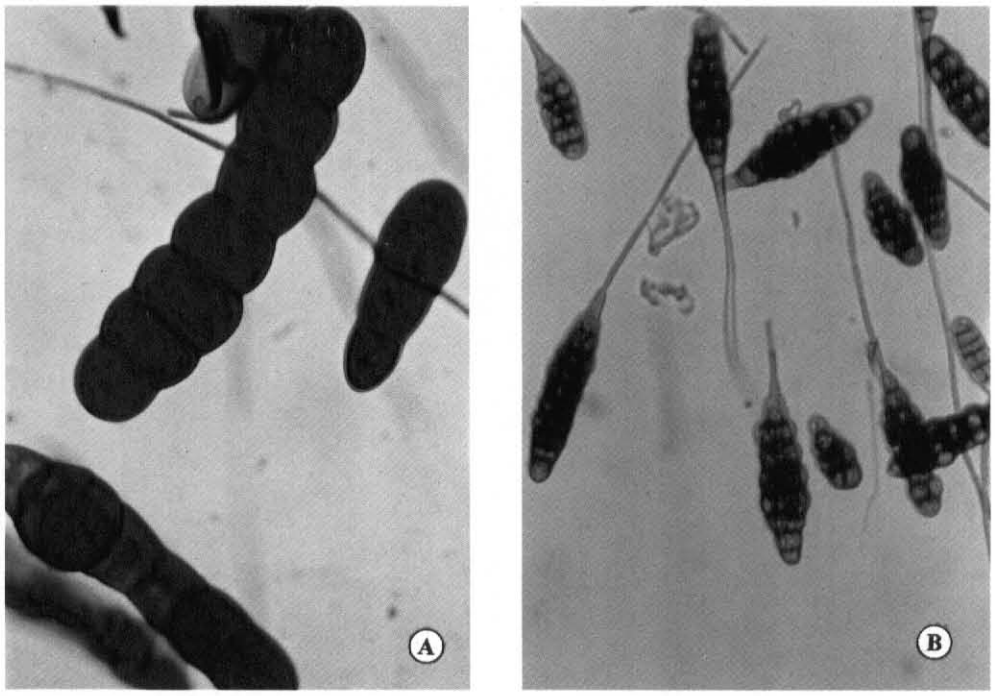

Figure 2 Conidial features of two *Alternaria* spp., causing leaf and stem blight of sunflower. A, Typical cylindric to elongated-elliptic conidia of *A, helianthi.* B, Obclavate conidia with filiform beak of *A. zinniae.*

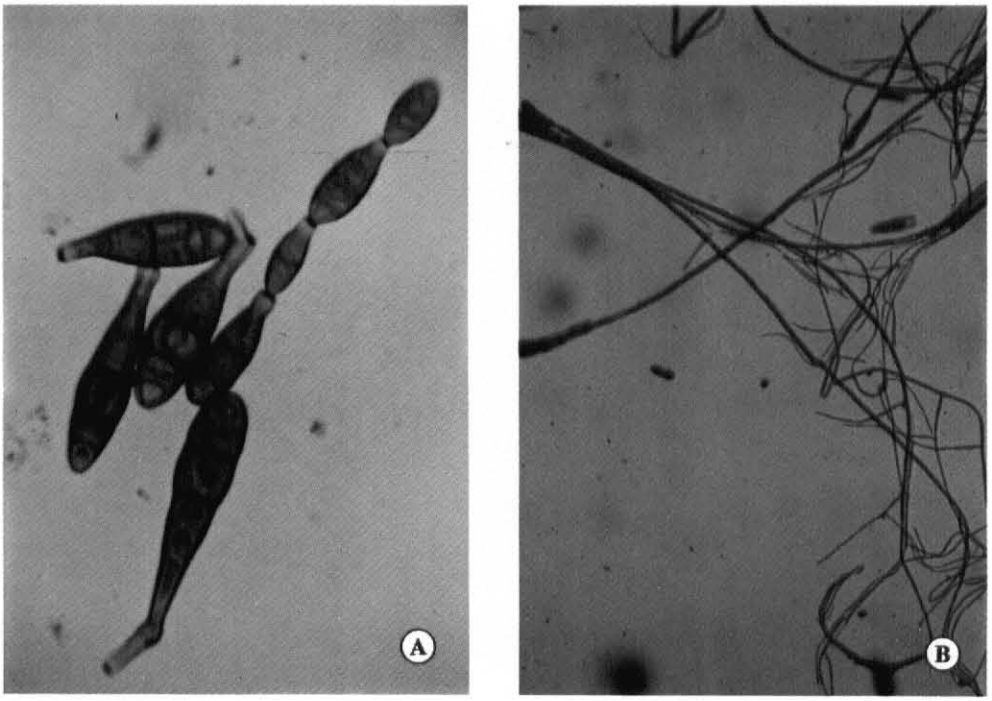

Figure 3 Morphological features of two *Alternaria* spp. causing leaf and stem blight of sunflower. A, Branched chain conidia of *A. alternata.* B, Obclavate and long conidia with very long, narrow septate beak of *A. longissima.*

production. Isolation of *Alternaria* spp. were made predominantly from direct lesions occurring on the leaves or other plant parts.

Identification and frequency of Alternaria spp. *Alternaria* spp. were isolated from symptomatic plants on every farm of the 10 farms from which samples were taken. Isolates were identified as *A. helianthi* or *Helminthosponum helianthi* 10 farms ; *A. zinniae* 5 farms ; *A. alternata* 3 farms ; and *A. longissima* one farm. Because of inconsistencies in the literature regarding to the taxonomy of many *Alternaria* spp. associated with sunflower (Barnett, 1969 ; Dingley and Brien, 1956 ; Ellis, 1971, 1976; Joly, 1967 ; Neergaard, 1945 ; Papendorf, 1969 ; Rossman *et al.*, 1988 ; Simmons, 1967 ; Subramanion, 1971 ; and Zelle, 1932), the following description of the Thai sunflower isolates were given as a basis for their identification.

A. helianthi. Spores or conidia and conidiophores of *A. helianthi* were shown in Fig. 2. The conidia were solitary nonbeaked, and borne on simple or (rarely) branched conidiophores. Under higher magnification, conidia were cylindrical to elongated-elliptic, straight or slightly curved, yellowish brown in color, septate, with four to 11 transverse and longitudinal septa were rare, constricted at septa, and rounded at both ends. Conidia were different in size with 25-125 ×12.5-35 um. Colonies of *A. helianthi* on PDA grown under fluorescent lights sporulated more profusedly than these of colonies grown in the dark.

A. zinniae. This pathogen produced few, usually nonbeaked conidia on PDA without special treatment. Colonies effuse, greyish brown to dark blackish brown, shortly hairy, mycelium immersed. Conidiophore solitary or in fasicle, sometimes geniculate, brown, paler towards the apex, with 1-4 scars, up to 150 u long, 5-10 u thick. Conidia mostly solitary, rarely in chains of 2, obclavate, rostrate, pale or dark brown, smooth to minutely verruculose, with 5-9 transverse and several longitudinal septa body 55-105 ×19-28 u ; in nature, beak filiform, simple, hyaline to pale brown and often swollen at the apex (Fig. 2).

A. alternata. Conidia formed in long, often branched chains, obclavate, obpyriform, ovoid or ellipsoidal, often with a short conical or cylindrical beak, sometimes up to but not more than one third of the conidial length, pale to mid-golden brown, smooth or verruculose, with up to 8 transverse and usually several longitudinal or oblique septa, length 20-63 u, 9-18 u thick in the broadest part ; beak pale. Conidiophores arising singly or in small groups ; simple (sometime branched), straight (sometime geniculate), pale to mid olivaceous or golden brown, smooth, up to 50 u long, 3-6 u thick with 1 to several conidial scars. (Fig. 3).

A. longissima. Conidia solitary or catenulate, extremely variable in shape and size, pale straw coloured to brown. The conidia were very long, obclavate or with a basal subcylindric portion of a few to several cells and a very long, narrow septate beak ; they have 5-40 transverse septa, are 4-17 u thick in the broadest part and about 2.5 u thick at the apex. Shorter conidia, variable in shape and often with a few longitudinal or oblique septa were also found. Conidia were thin-walled and smooth. Conidiophores erect or ascending, simple and straight, sometime branched, flexuous and geniculate, swollen at the apex, septate, pale to mid pale brown, smooth below verruculose at and sometime below the apex, up to 150 u long 3-5 u thick with 1 to several conidia scars (Fig. 3).

These four *Alternaria* spp. also sometime were isolated together with *Curvularia* sp. and *Pestalotia* sp. from black-leaf lesions. These lesions were not discrete and differed from the generalized leaf blight occurring on sunflower severely affected by *Alternaria* spp.

Pathogenicity test. After sunflower plant was inoculated with suspension of each *Alternaria* spp., numerous typical leaf spots and symptoms of brown streak type of stem lesion were produced within 10 days after inoculation. Generally, leaf spots began as tiny necrotic spots, often surrounded

by a chlorotic halo ; and enlarged to form dark spots. The stem lesions began as small flecks which gradually enlarged longitudinally to form streaks. Large dark-brown lesions, which sometime completely girdled small stems and petioles, resulted from coalescence of many small flecks. The fungi were reisolated from those lesions.

Effects of fungicides on Alternaria spp. in laboratory and field studies. After 14 days, *Alternaria* spp. completely covered the surface of the agar medium without any fungicides ; this represented a radial growth of 90 mm. Mycelial growth on agar media amended with fungicides were shortened compared with those mycelia grew on unamended ones. Radial growth of *Alternaria* spp. were 60.0, 60.2, 60.5, 70.6, 80.0, 80.1 and 80.2 mm. in plates containing 100 ppm of iprodione, imazalil, triforine, fentin acetate, benomyl and copper-oxide, respectively. Colony growth from plugs of *Alternaria* spp. were increasingly inhibited with higher rates of fungicides, that was at 250 ppm, mycelial expansions were reduced to be 30.1, 30.2, 30.6, 60.4, 70.0, 70.4 and 70.6 mm. for iprodione, imazalil, triforine, mancozeb, fentin acetate, benomyl and copper-oxide, respectively. However, iprodione and imazalil gave the largest inhibition zone while benomyl and copper oxide gave the smallest results.

Differences existed in disease severity between inoculated and noninoculated plots treated with fungicides were found. Non-inoculated plots had lower disease severities than inoculated plots with significant differences. In inoculated plots, infected leaf area of control treatment was as high as 75% but the number of lesion was dramatically decreased after spraying with fungicides. Percentage of infected leaf area in inoculated sunflower treated with fungicides revealed in the preceding orders of iprodione plus with mancozeb 39.51 ; imazalil 57.05 ; triforine 57.94 ; iprodione 61.52 (average from the treatments of with and without Triton CS-7) ; mancozeb 63.41 ; fentin acetate 68.54 ; copper-oxide 69.70 and benomyl 70.32 respectively, while in non-inoculated plants the percentage of infected leaf area was found in the sequences : iprodione plus with mancozeb 29.00 ; imazalil 55.34 ; iprodione 56.79 (averaged value) ; triforine 56.94 ; mancozeb 62.08 ; copper oxide 62.70 ; fentin acetate 68.04 ; benomyl 69.48 ; and control 70.15, respectively (Table 1). Other symptoms of disease were also reduced after treating with fungicides. The results indicated that the suppression efficacy of fungal growth was influenced by the fungicides used. However, the plants sprayed with fungicides, iprodione plus with mancozeb was highly effective in limiting infected leaf area of *Alternaria* spp. while benomyl was the lowest, compared with other treatments (Table 1).

One hundred seed-weight, yields and oil content were significantly different among treatments that were artificially or naturally infected (Table 2). Plants that were misted but not inoculated and treated with pathogens and fungicides resulted in average yield reduction of 26.32% because of natural infection during the experiment, when compared with plants receiving three applications of iprodione plus with mancozeb. All treatments not artificially inoculated with *Alternaria* spp. had significantly greater yields than inoculated treatments. Yields in inoculated plants treated with fungicides revealed in sequential orders of : iprodione plus with mancozeb 263.88; imazalil 253.52 ; iprodione 233.33 (average of iprodione with and without Triton CS-7) ; mancozeb 217.81 ; copper oxide 210.16 ; triforine 200.20 ; fentin acetate 186.42 ; and bynomyl 182.80 kg/rai, respectively. Yield in non-inoculated

Table 1 Disease severity of sunflower plants after spraying with fungicides at 1000 ppm

Fungicides	Inoculated plant						Non-inoculated plant					
	No. infected leaf/plant	Infected leaf area (%)	No. infected branch/ plant	No. lesion on stem	Ave. of infected size on stem	No. of infected petal	No. infected leaf/plant	Infected leaf area (%)	No. infected branch/ plant	No. lesion on stem	Ave. of infected size on stem	No. of infected petal
iprodione + mancozeb	19.80	39.51	13.80	5.16	3.18	6.50	15.20	28.00	13.36	4.37	3.04	6.23
imazalil	22.73	57.05	14.43	5.46	4.38	8.63	18.27	55.34	13.51	5.10	4.15	8.24
iprodione	20.23	58.06	14.06	5.98	3.95	7.86	16.43	56.79	14.00	5.70	3.82	7.15
iprodione no adjuvant	21.37	64.97	14.25	5.50	4.26	8.03	17.30	62.82	14.10	5.32	4.13	8.00
mancozeb	21.50	63.41	14.73	8.50	5.37	9.45	19.93	62.08	13.92	5.50	5.23	8.85
copper oxide	21.70	69.70	13.17	5.46	5.42	8.85	21.60	62.70	13.00	4.56	5.28	8.05
triforine	23.63	57.94	13.70	6.43	6.04	8.80	16.60	56.94	13.60	5.23	6.00	7.04
fentin acetate	23.45	68.54	14.62	6.16	6.38	10.48	19.68	68.04	14.51	6.28	6.03	10.44
benomyl	23.73	70.32	14.93	6.43	6.31	10.42	20.20	69.48	14.85	6.20	5.95	10.36
control	23.92	75.00	15.40	7.67	6.35	12.46	21.35	70.15	14.95	6.84	6.00	12.33

In every treatment, fungicides were added with adjuvant (Triton CS-7) except treatment of iprodione without adjuvant.

Table 2 Average of 100 seed weight, yield per rai and oil content of sunflower grown in the field after spraying with different fungicides

Fungicides*	Inoculated plant			Non-inoculated plant		
	100 seed weight (g)	Yield (kg/rai)	Oil content (%)	100 seed weight (g)	Yield (kg/rai)	Oil content (%)
iprodione + mancozeb	7.61 a	263.88 a	40.05 a	7.91 a	294.07 a	45.73 a
imazalil	7.53 ab	253.52 ab	40.06 a	7.81 ab	290.19 ab	44.57 a
iprodione	7.27 ab	239.74 abc	38.94 ab	7.71 ab	280.40 abc	42.92 ab
iprodione no adjuvant	7.08 abc	226.93 abcd	38.31 bc	7.66 abcd	273.17 abcd	41.10 bc
mancozeb	6.84 abcd	217.81 bcde	38.10 bcd	7.43 abcd	266.70 bcde	39.81 bcd
copper oxide	6.67 abcd	210.16 cde	37.96 cde	7.63 abcd	267.34 cde	39.37
triforine	6.46 abcd	200.20 def	37.69 cde	7.44 abcd	251.20 def	38.25 cde
fentin acetate	6.35 bcd	186.42 efg	37.43 cde	7.39 bcd	240.56 efg	37.69 cde
benomyl	5.97 cd	182.80 fg	37.07 de	7.15 cd	233.90 fg	37.57 de
control	5.80 d	169.31 g	36.41 e	6.88 d	216.68 g	36.53 e
Means	6..76	215.08	38.20	7.50	260.12	40.35

In every treatment, fungicides were added with adjuvant (Triton CS-7) except treatment of iprodione, without adjuvant.

Means in each column followed by the same letters are not significantly difference (P = 0.05) according to DMRT.

C.V. of 100 seed weight = 8.5711%

C.V. of yield = 10.3261%

C.V. of oil content = 4.4720%

Table 3 Analysis of variance of *Alternaria* spp. on 100 seed-weight, yields and oil content of sunflower.

Source of Variation	df	100 seed (g)			yield (kg/rai)			Oil content (%)		
		SS	MS	F	SS	MS	F	SS	MS	F
BLOCK	2	1.8447	0.9223	2.47^{ns}	7.2193	3.6096	0.60^{ns}	8.2242	4.1121	1.33^{ns}
TREATMENT	19			22.10**						
INOCULATIONS (A)	1	8.2510	8.2510	3.44**	30351.1546	30351.1546	50.43**	69.4235	69.4235	22.50**
FUNGICDES (B)	9	11.5620	1.2846	0.47^{ns}	44641.9970	4960.2218	8.24**	252.1481	28.0164	9.08**
A X B	9	1.5912	0.1768		929.3782	103.2642	0.17^{ns}	53.1497	5.9055	1.91^{ns}
ERROR	38	14.1885	0.3733		22868.0675	601.7912		117.2449	3.0853	
TOTAL	59	37.4376			99512.5306			500.1905		
C.V.		8.5711%			10.3261%			4.4720%		

** = Significance at 99% level

ns = Non significance at 99% level

plants treated with fungicides also was in the receding order similar to the inoculated ones ; that was iprodione plus with mancozeb giving the highest yield with the next highest was found in the sequences of imazalil, iprodione, mancozeb, copper oxide, triforine, fentin acetate, and benomyl respectively. Iprodione plus with mancozeb had the highest yield with no statistical difference from imazalil and iprodione alone, but it differred significantly from mancozeb, copper oxide, triforine, fentin acetate and benomyl while plants treated with benomyl gave the lowest yield, compared with other fungicides used. The result also indicated that iprodione (Rovral) plus adjuvant (Triton CS-7) was more effective than Rovral without Triton CS-7 and did not result in significant difference (Table 2).

From Table 2, it showed that the mean 100 seed-weight, yields and oil content of inoculated plants were significantly different from the corresponding means of the non-inoculated plants. The effects of *Alternaria* spp. infection in this study reduced the 100 seed-weight, yield and oil content of tested plants with 10.95, 20.94 and 5.63% respectively (Table 2).

The AOV analysis of the results from experiment in Table 3 revealed that the inoculated and noninoculated sunflower plants sprayed with several fungicides were different in the 100 seed-weight, yields and oil content while there was no interaction between inoculations and fungicides (Table 3).

DISCUSSION

This is the first report confirming *Alternaria* spp. on sunflower in Thailand. The consistent isolation of *Alternaria* spp. from infected tissues, and the widespread occurrence of this disease throughout the cultivated areas of Thailand, suggest that Alternaria leaf and stem blight of sunflower is a common cause of yield losses.

The *Alternaria* spp. most commonly isolated from infected sunflower plants, and which consistenly proved to be highly virulent in pathogenicity tests, was identified as *A. helianthi* because it most closely resembled original descriptions and recognized specimens of this taxon in the cultural and morphological characters tested. This pathogen was a widely distributed species with a high potential for virulence once proper condition occured. *A. zinniae,* the species second in frequency of isolation, was moderately to highly virulent in these tests and *A. alternata* was the next highest frequently isolated while the lowest frequently distributed species was *A. longissima.* This results collectively suggested that *A. longissima* was a minor or, perhaps secondary pathogen on sunflower plants. However, *A. longissima* appeared to be the first report affecting this plant.

Recognition of Alternaria leaf and stem blight as a significant cause of yield loss in Thailand should enable grower to develop and integrate control program as appropriate for individual situations. Whereas the relative susceptibility to *Alternaria* spp. has been documented in only one commercial cultivar in Thailand that is Hysun 33, many other important cultivars around the world or the new lines bred in Thailand have not been included in these studies but should be examined. Furthermore, the evidence presented in this study indicated that several additional *Alternaria* spp. are serious pathogens of sunflower, susgesting that cultivar and germplasm reactions to the organisms should be examined in the future.

In diseased plants, iprodione plus mancozeb should be considered the most effective in Alternaria leaf and stem blight management. In the absence of artificial inoculation, the fungicide treatment also performed best. When iprodione, either alone or coupled with mancozeb, was applied to cultivated sunflower when trace amounts of disease were first found, good yields and disease control could result. With mancozeb alone, the lack of systemic activity may have contributed to the lower yields. Therefore, application of mancozeb was as effective as couple with iprodione in controlling infection. This may have been due to the residual systemic activity of iprodione early in the developing epidemic coupled with

the protectant activity of mancozeb during the growing season. Because it has been demonstrated that some fungi can become tolerant of mancozeb and iprodione in the laboratory (Kardin and Percich, 1983 ; Martin *et al.*, 1984) its continuous use alone to manage Alternaria leaf and stem blight of sunflower may be unwise. An evaluation of the judicious use of iprodione and mancozeb coupled with their long-term effect on field populations of *Alternaria* spp. is needed for increasing effectiveness as well as on the reducing fitness of resistant fungi.

The results also indicated that imazalil could be an effective tool for control of Alternaria leaf and stem blight as well as iprodione in sunflower. Imazalil at the low concentration tested (250 ppm) was effective in limiting growth of *Alternaria* spp. *in vitro* and in preventing symptom expression on artificially inoculation due to its action as systemic fungicide in plant tissue.

Benomyl failed to inhibit *Alternaria* spp. in this study. which was not different from the control treatment. The investigation showed that benomyl had no curative effects when applied after inoculation of sunflower with the pathogens. However, the reports on benomyl resistance of several fungi included *Alternaria* spp. was found (Fry, 1982). Other fungicides used such as copper oxide, triforine and fentin acetate were less effective respectively in controlling of Alternaria leaf and stem blight of sunflower.

The study on the use of fungicides in control of this disease should be further carried out due to such study could reduce the rate of infection, giving higher yield, but the chemicals used should not be too expensive to be recommended to farmers, including the convenience in buying, applying, toxicity to the users as residual toxic and finally they are able to increase yields until the cost-benefit sales in high money.

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