Annals of the Sri Lanka Department of Agriculture. 2003. 5: 263-270.

FIRST REPORT OF PHOMA BLACK STEM OF SUNFLOWER IN SRI LANKA AND ITS MANAGEMENT

W. A. P. G. WEERARATNE and M. G. D. L. PRIYANTHA
Field Crops Research and Development Institute, Mahailluppallama

ABSTRACT

Sunflower (*Helianthus annuus*) is now becoming popular in Sri Lanka as an oil seed crop. A new disease has been reported from Field Crops Research and Development Institute and University Sub Campus, Mahailluppallama in January 2002. Initially, small necrotic spots with yellow margins appear on the lower leaves which later spread to the leaf stalk, stem, and to the flower head. Severely affected plants collapse and die without seed setting. The disease severity ranges from 50-100% in the affected plots. The pathogen was isolated from infected leaves, petiole and stem and identified as *Phoma macdonaldii* and its pathogenicity was established. It was observed that high humidity (>80%), temperature (25-30°C) and cloudy weather favour the development of the disease. This is the first report of *P. macdonaldii* of sunflower from Sri Lanka and can be a major constraint to sunflower cultivation especially during the *maha* season. Crop sanitation including destruction of crop debris will reduce the infection, and planting during late *maha* (Late December, January) is also important to control the disease.

KEYWORDS: Black stem disease, *Phoma,* Sunflower

INTRODUCTION

The sunflower (*Helianthus annuus*) is grown in many countries and is considered as the second most important source of edible vegetable oil in the world (Waldermar, 1981). The seeds of sunflower contain about 40% edible oil with high keeping quality and anti-cholesterol properties (Patil, 1984). Sunflower cultivation in Sri Lanka has become popular in recent years. Hence, research on sunflower was reinitiated at Field Crops Research and Development Institute (FCRDI), Mahailluppallama, to evaluate performance of sunflower varieties and also production technologies since 1999 (DOA, 1999). Generally the crop is cultivated mainly in *maha* season (rainy season) in Sri Lanka and it has been reported that an unidentified disease, which causes heavy losses, affected the breeding plots of sunflower.

Due to lack of information on diseases associated in sunflower, a preliminary study was conducted during *maha* 2001/2002 using open pollinated sunflower variety 'spain' at FCRDI, Mahailluppallama. A severe outbreak of this disease was observed in research plots as well as in experimental plots at the university sub campus, Mahailluppallama during *maha* 2001/2002. Symptoms appeared during December and January. Severely affected plants collapse and die without seed setting.

The objective of this study was to find out the causal organism responsible for this disease and develop suitable control measures. Therefore, disease symptoms on affected plants, the causal organism, its pathogenicity, the effect of environmental factors and sowing time on disease were studied in detail.

MATERIALS AND METHODS

Isolation of pathogen

Diseased plants for analysis were collected from experimental plots at Mahailluppallama. Isolation was made from affected leaves, stem, petiole and calyx on Potato Dextrose Agar (PDA). Pieces of each infected tissue were surface sterilized separately with 1% sodium hypochlorite solution (5%w/v) for three minutes, then rinsed with sterile distilled water and placed on PDA containing 1.01% w/v Streptomycin and the plates were incubated at room temperature. A series of sub culturing was made to obtain pure cultures. Cultures in petri dishes were first incubated for seven days in the dark at room temperature, and then exposed for 24 hours to ultraviolet light (350–360 nm) to induce pycnidia development.

Pathogenicity test

Inoculum was prepared by using 12-day-old culture grown on PDA. Conidia were harvested by rinsing plates with 20ml sterile distilled water and the spores were counted with the aid of a hemacytometer. Inoculation was made in one-month-old susceptible variety "spain"sunflower plants, which were raised in plastic pots containing autoclaved soil. Three drops (20µl each) of spore suspension (10^6 conidia/ml) were placed on the four leaf surfaces of each of test plants. Similarly another set of plants was inoculated by injecting a spore suspension (10^6 conidia/ml) using a syringe at the lower stem of plants. Plants inoculated with sterile distilled water were used as a control. To maintain high humidity, the inoculated plants were covered with moist polythene bags for 4 days and kept in a greenhouse until disease symptoms appeared. Reisolation of the pathogen from the artificially inoculated plant parts was made.

Transmission through crop debris

Infected crop debris was incorporated into pots containing autoclaved soil with one-month-old sunflower plants and plants in pots containing autoclaved soil only were used as the control. These pots were then kept inside the greenhouse under high humidity.

Effect of environmental factors on disease development

Environmental factors (rainfall, relative humidity, temperature, sunshine hours) during the cropping period (November to March 2002/2003) were recorded daily to determine their influence on the disease spread and development.

Effect of sowing time on disease development

The effect of sowing time on the disease development was studied using sunflower variety 'spain'. Different sowing dates *viz.* first week of November, third week of November, first week of December, third week of December and first week of January were employed. The first sowing date recommended by the Department of Agriculture (DOA, 1990), which was also employed in this study. The experiment was conducted in a randomized complete block design with three replications at Field Crops Research and Development Institute, Mahailluppallama in *maha* 2002/2003. Seeds were sown on ridges in 4 rows of 3.5 m long with a spacing of 60x45 cm, at the rate of 3 seeds per hill and thinned out to a single plant 2 weeks after planting. All the cultural practices recommended by the Department of Agriculture for sunflower cultivation were followed. The disease assessment was taken from 28 plants avoiding the boarder plants and severity was scored on a 0-9 scale from appearance of the symptoms to plant maturity as described below.

0 - No symptoms
1 - 15% area affected
3 - 15-30% area affected
5 - 30-50% area affected
7 - 50 - 75% area affected
9 - >75% area affected

Disease severity was calculated using the following formula (Kolte, 1985).

$$(DS\ \%) = \frac{\Sigma W \times N_s}{T \times N_m} \times 100$$

Where,

DS	-	Disease Severity
W	-	Number of affected plants
N_s	-	Severity scale
T	-	Total number of observations
N_m	-	Maximum scale number (9)

Data were statistically analyzed using SAS statistical package.

RESULTS AND DISCUSSION

Disease symptoms in the field

The symptoms were first observed in late December and late January (one month after planting) in the experimental plots of University sub Campus and research plots of Mahailluppallama respectively. The symptoms of the disease appeared first on lower leaves, stem and petioles initially as small necrotic spots with yellow margin and then spread to the leaf stalk and stem and even to the head. As infection progressed, the number and the size of spots on the leaf increased and eventually leaves turned yellow and dried up, but rarely fell from the plant. Stem lesions consisted of large, shiny blackened areas. When the infection is severe, the lesions completely girdle the stem and the whole stem become completely black. Pycnidia were evident in some of the dead tissues. Head infections are superficial and blackened areas are produced on the receptacle and the bracts. The affected head did not disintegrate. If infected early, young plants may die, while the older plants remain stunted and become weak and may be subject to logging.

Isolation of pathogen

Colonies with similar appearance were isolated from each part of infected sunflower plants on PDA. The early stage of the colony had an ash color and it become black when colony is older. The fungus composed of dense and slow growing mycelium. The mycelium is septate and showed aerial growth, hyaline at first and then becomes dark brown to black. The mycelium is branched and the branching is at right angles having a septum at the point of origin. Pycnidia are subglobose in shape with slight papillae, light brown in colour, darkening to black with age and they are embedded initially in the PDA medium but are erumpent at maturity. Pycniodiospores are hyaline, reniform to oblong with obtuse ends. The morphology of colonies on PDA medium and the shape of pycnidia and conidia were very similar to fungus of *Phoma macdonaldii* Boerma.

Pathogencity test

Typical symptoms were developed on leaves, petiole and stem within 4 days after inoculation and the pathogen was reisolated from artificially infected plants. All of the control plants inoculated with water drops and maintained under the same conditions remained healthy. On the basis of the symptoms of affected plants, pathogenicity tests, morphology of culture medium, pycnidia and microscopic observations the pathogen was identified as *Phoma macdonaldii* Boerma. This identification is further confirmed by comparing with published data (Macdonald, 1964). This fungus was earlier known as *Phoma oleracea* var. *helianthituberosi* Sacc (Perfect stage:

Leptospharia lind-quistii Frezzi), (Kolte, 1985). This disease has been reported in many countries and known as Phoma black stem of sunflower (Macdonald, 1964). These findings clearly showed that *Phoma macdonaldii* Boerema is a new pathogen in Sri Lanka and causes heavy losses when sunflower is cultivated during *maha* season.

Transmission through crop debris

Typical disease symptoms were observed on seedlings raised on autoclaved soil incorporated with crop debris 2 weeks after planting, but no symptoms were observed in seedlings raised on uninfected soil. This suggests that the disease can be transmitted through crop debris.

Effect of environmental factors on disease development

Results of correlation coefficient of cumulative increase of disease on sunflower in relation to environmental factors during *maha* 2002/2003 are presented in table 1. The correlation of cumulative increase of the disease with environmental factors, maximum temperature, and relative humidity during day time and sunshine hours were significant. A positive correlation was observed between relative humidity at day time and the disease severity. However, a strong negative correlation between disease severity and maximum temperature and sunshine hours was observed. These results indicate that relative humidity during day time, maximum temperature and cloudy weather have the highest direct influence on disease development and spread.

Table 1. Correlation coefficients of cumulative increase of *Phoma* black stem disease on sunflower in relation to environmental factors during *maha* 2002/2003.

Environmental factors	*Correlation coefficient (%)*
Rainfall	0.28
Relative humidity - Day	0.54*
Relative humidity - night	0.21
Temperature - Maximum	-0.80*
Temperature - Minimum	-0.23
Sunshine hours	-0.56*

* Significant at 0.5 probability

The relationship between weather parameters (relative humidity during day time, maximum day temperature and sunshine hours) and the disease severity in the first and last planting crops is shown in the figure 1. It indicates that the disease severity in late planting crop decreased with decreasing relative humidity (day time) and increasing maximum temperature and sunshine hours during late February to March. The high relative humidity (>80%) in the daytime, temperature (25-30°C) and cloudy weather prevailed during late November to January resulted in high incidence of the disease in

first planting crop. It is known that conidia of the pathogen are released from pycnidia and spread to neighboring plants with rain splash. However, there was no significant correlation between rainfall and the cumulative increase of the phoma black stem disease.

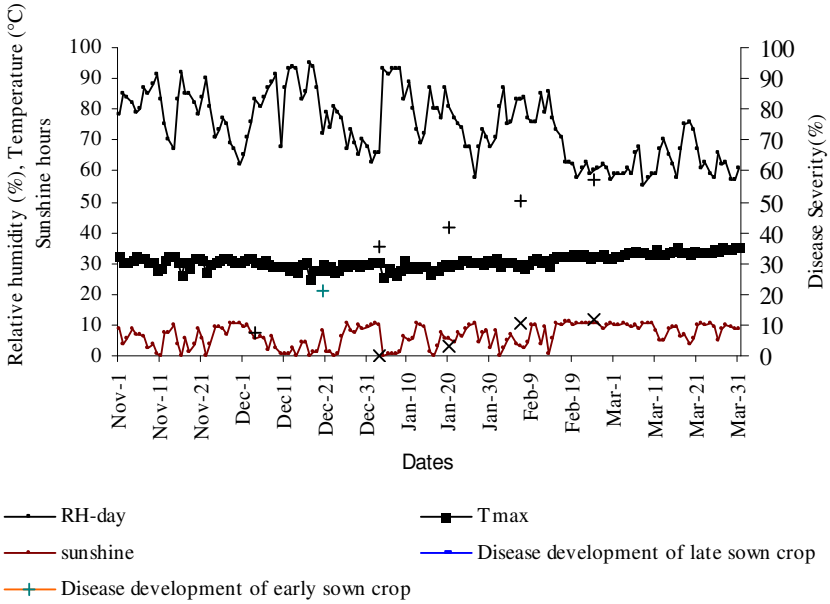

Figure 1. Relationship between temperature (maximum), humidity, sunshine hours and development of phoma disease in sunflower during, *maha* 2002/2003 at Mahailluppallama.

Effect of sowing time on disease development

Effect of sowing dates on disease development on sunflower is shown in the figure 2. The disease severity in the first planting crop was high (59%) compared to that of late planting crop (first week of January). Statistical analysis shows significant differences in disease severity on different sowing dates (table 2). This study indicated that the late sowing of sunflower could suppress disease development during *maha* season. The increase in the disease during November to December sown crops was due to optimum temperature, and high relative humidity. Hence, to reduce the disease incidence, late December to Early January is the optimum sowing time for sunflower cultivation during *maha* season in the Dry zone. It appears that the reduction in relative humidity, and increase in maximum day temperature and sunshine hours during March was the reason for decreased disease observed in this study.

Table 2. Effect of sowing date on disease development of sunflower.

Date of Sowing	*Disease severity (%)*
First week of November	32.73 (29.2) a
Third week of November	26.98 (20.5) b
First week of December	11.48 (3.9) c
Third week of December	8.00 (2.0) c
First week of January	7.15 (1.6) c

Mean followed by a common letter in a column are not significantly different. Figures in parentheses are Arc sign transformed.

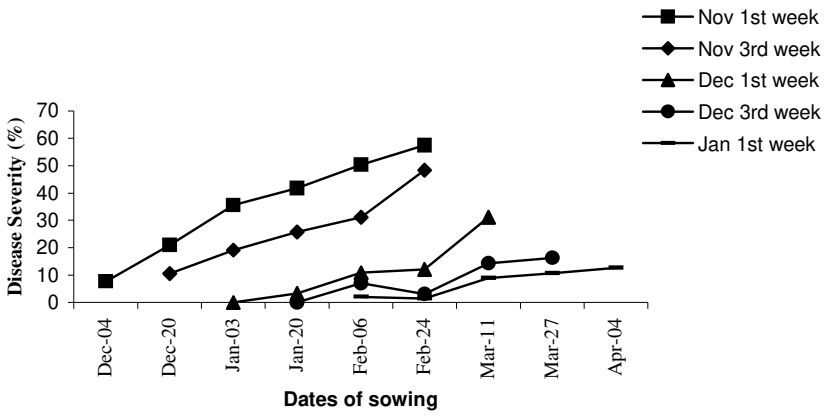

Figure 2. Effect of sowing date on disesae development of sunflower

It is known that *Phoma macdonaldii,* the causal agent of sunflower black stem disease, is responsible for qualitative and quantitative damage which can result in up to 60% yield losses in France and worldwide. In Sri Lanka, it has the potential to cause heavy losses when the sunflower is cultivated during the *maha* season. Hence, Evaluation of germplasm to identify resistant sunflower lines for the phoma black stem and to develop short duration varieties for late sowing is important for future breeding programme on sunflower in Sri Lanka.

CONCLUSIONS

A new sunflower disease has been identified as Black stem caused *Phoma macdonaldii* Boerema. The high relative humidity (>80%) in the day time, temperature (25-30°C) and cloudy weather prevailed during late November to January coupled with the susceptible variety "spain" seemed to have favored the disease development in the field. Crop sanitation including the destruction of crop debris, will reduce the infection and planting during late *maha* (Late December, January) is important to control the disease.

ACKNOWLEDGEMENT

The authors are grateful to Dr. A. M. Perera, Plant Breeder, FCRDI, Mahailluppallama for the providing sunflower seeds to conduct this study. They also wish to acknowledge Ms. D. R. Yapa and A. Ranasinghe for the assistance given during the study period.

REFERENCES

DOA. 1990.Crop recommendation technoguide, Department of Agriculture, Peradeniya, Sri Lanka. 61-62.

DOA. 1999. Administrative Report, Department of Agriculture, Peradeniya, Sri Lanka. 22p.

Kolte, S. J. 1985. Diseases of Annual Edible Oilseed Crops. Vol 111.CRC Press, Inc.Florida. 44p.

Mcdonald, W. C. 1964. Phoma black stem of sunflowers. Phytopathology. 54: 492-493.

Patil, B. P. 1984. Sunflower a promising rabi crop for konkan, Indian Farming. 34 (2): 14-15.

Waldemar, E. S. 1981. The sunflower crop and disease: Progress, Problems and prospects, Plant disease. 65 (8): 643-648.