Indian J. mycol. Res., 21(2), 61-70 (1983)

COMPREHENSIVE STUDIES ON THE CHARCOAL ROT OF MAIZE CAUSED BY *MACROPHOMINA PHASEOLINA* (TASSI) GOID.

BY

SAYED A. K. M. KAISER AND S. N. DAS

All Indian Coordinated Maize Improvement Project,
Bidhan Chandra Krishi Viswavidyalaya, Kalyani 741235
Nadia, West Bengal

Charcoal rot due to *Macrophomina phaseolina* is one of the major diseases of maize and causes appreciable damage to the crop under favourable conditions. Studies were undertaken on this disease in respect of (i) morphology, (ii) pathology, (iii) field symptoms, (iv) perpetuation and spread, (v) field inoculation techniques, (vi) effects of temperature, crop residue and plant maturity on disease development, (vii) screening for resistance, and (viii) chemical control, the results of which are presented and discussed in the present paper.

INTRODUCTION

Charcoal rot of maize is found to be wide spread in comparatively drier and low rainfall areas where this crop is grown (Livlngston, 1945 ; Young, 1949 ; Koehler, 1960). In India, the disease was found to be in epidemic from during 1960 kharif season in Kashmir Valley. Later, it was noted at Hyderabad during 1965-66 rabi season and at Pantnagar in 1966 kharif (Payak and Renfro, 1969). Though the severity of this disease was found elsewhere, the disease was unnoticed in West Bengal since 1975. Sporadic incidence of this disease was noticed at the University Farm, Kalyani from 1976 crop season in the rabi plantation. The disease has been found to be prevalent in several other states including Andhra Pradesh, Bihar, Uttar Pradesh, Madhya Pradesh, Punjab, Haryana, Delhi and Rajasthan.

In West Bengal, charcoal rot is found to be prevalent in the plains only particularly in rabi crop when summer temperature become comparatively high (30° to 35°C). It is also prevalent in the plains during the kharif season Incidence of this disease has been found to be directly correlated with both temperature rise and crop maturity and consequently the incidence is much more in rabi crop which is exposed to high summer temperature from the flowering stage. A gradual increase in disease severity has been noted at the University Farm, Kalyani where jute-potato rotations where replaced by growing maize in the successive years. Germplasm screening can easily be done at Kalyani particularly in the sick plot in the rabi season even without artificial inoculation.

Causal organism, morphology and identification of the pathogen

The causal organism of the disease is *Macrophomina phaseolina* (Tassi) Goid (= Syn. *Macrophomina phaseoli* (Maubl.) Ashby) which belongs to the Class—Deuteromycetes under Order—Sphaeropsidales and Family—Sphaeropsidaceae. The sclerotial stage of the fungus is *Rhizoctonia bataticola* (Taub.) Butler.

The mycelium of the fungus in PDA medium is initially white and later gradually turns greyish black to dark brown. When incubated at higher temperature above 28°C it becomes light pink colour. Pycnidiospore are oval to elliptical, hyaline, single celled, thin walled, and they measure 12-25 x 6-10 μ in size. Pycnidia are black to dark brown, flask shaped, ostiolate and they measure 145-200 μ in diameter. This morphological feature are similar to that obtained by Kulkarni and Patel (1966), Kulkarni *et. al.* (1966) and Mehta *et. al.* (1972). Sclerotia produced on the pith of the affected stalks are small, black, irregular to globular in shape, and they measure 40-115 μ in diameter. The relationship of pycnidial form to sterile form of the fungus was studied by Ashby (1972) and Haigh (1928). Attempts were made to induce sclerotia formation on dried autoclaved maize leaves. Dried leaf pieces of maize (4 x 2 cm) were washed several times with water, autoclaved and placed on culture plates on 2% water-agar. The leaf pieces were inoculated in the centre with the fungal culture, isolated from naturally infected maize stalks and incubated at 25° to 28°C for about three weeks under natural diffused light near a window, protected from direct sunlight. Pycnidial production occured within 8 days. Sclerotial formation, however, started after 2 weeks. Mehta *et al.* (1972) also recorded similar result.

Host range

The pathogen has a wide host range including sorghum, jowar, jute, cotton some pulses, vegetables, tuber crops, egg plants, some oilseeds and some fruit plants and appear to be composed of a number of forms which are differentiated on the basis of size of sclerotia and presence or absence of pycnidia. The form attacking maize does not produce pycnidia in nature. Laboratory tests showed that the disease symptoms specific to the host could be produced in jute, potato, sorghum and gram when inoculated with this pathogen.

Field symptoms

In nature symptoms of the disease first become apparent as the plants approach maturity. Observations made for the last five years (1978-82) at the University Farm, Kalyani have shown that the disease generally appears after flowering but in some cases it may occur even 1 to 2 weeks after flowering. The early symptoms of the disease include drying of the lowermost leaves from tip upwards and browning discolouration of the root tips. As the disease advances

the outside of the lower internodes generally becomes straw coloured but sometimes become dark brown. The pith becomes badly disintegrated and the infected stalks may split longitudinally into a mass of fibres. A distinguishing character of the disease is the presence of small black sclerotia in the pith of affected stalks and in the disorganised tissues of the roots (Fig. 1). Sclerotia are also noticed externally on the rind and internodes. In case of severe infection drying of maximum leaves starting from the lowermost one and drying of ear husk from the tip are also observed resulting premature death of both ear and plant.

Mode of entry, perpetuation and spread of the pathogen

Infection by the pathogen takes place through the roots under natural field condition, and it grows both inter and intracellularly covering a large area of the affected stalks and extending upto the pith. Fungal materials could be isolated from the infected roots and from the affected stalks upto the fifth internode above the soil level.

As the fungus is a facultalive parasite it is capable of living saprophytically on dead organic tissues, particularly many of its natural hosts producing sclerotial bodies. It produces pycnidia when the atmospheric temperature is above 30°C and pycnidiospores remain viable for over a year. Livingston (1945) found the disease more severe in dry soils where the temperature was held at 38°C. Since the fungus attacks a wide range of hosts, it perpetuates freely and become virulent when the optimum predisposing conditions in the host are obtaining. The fungus spreads from plant to plant through irrigation water, tool and implements and other cultural operations. The sclerotia and pycniospores also become air-borne and cause further spread of the pathogen. There is, however, no evidence of charcoal rot infection through seeds as the pathogen associated with the disease is a common soil inhabitant and it can be isolated from all kinds of soil.

Field inoculation techniques

Five techniques, namely (i) soil inoculation by root injury, (ii) brace root, (iii) toothpick, (iv) hypodermic syringe and (v) cork borer inoculation were tested in a field experiment to compare their efficacy for inducing charcoal rot. Fifty plants of hybrid Ganga 5 were inoculated separately by each method when more than 50% of the plants had flowered and weather conditions were favourable for the disease development. In respect of each technique, 20 plants were kept as control.

Observations were done 18 days after inoculation on the basis of discolouration of the internodes of the affected stalks. The results are presented in Table 1.

TABLE 1. Comparative efficacy of five inoculation techniques

Inoculation technique	Number of plants inoculated	Plants infected (%)
Soil (root)	50	28.5
Brace root	50	33.0
Toothpick	50	82.5
Syringe	50	48.5
Cork-korer	50	66.5

Toothpick method of inoculation as described by Payak (1975) is widely practiced as it is superior over others for inducing highest amount of disease incidence. This method is also quick and convenient to follow. Root and brace root inoculation caused lower incidence of the disease. Although the cork-borer method induced moderate amount of stalk rot, it is rather complex and slow. In case of syringe inoculation the percentage of plants infected was fairly low.

Toothpick inoculation method

The pathogen should be isolated and grown in PDA medium at temperature 28° ± 1°C inorder to get the stock culture of the pathogen to be used for increase of the inoculum in the laboratory for field inoculation. For this purpose, inoculum from the stock culture is to be multiplied and grown on bamboo toothpicks (previously boiled for 1 hour and washed inorder to remove the toxic substances) in potato-dextrose broth medium at temperature 28° ± 1°C for about 2 weeks. The toothpicks are to be partially submerged in the medium within a suitable container. Inoculation is to be done in the lower internodes (second or third) above the soil level by inserting two toothpicks at a time in different points inorder to assure infection. Adequate controls are to be kept by inserting sterile toothpicks only. Symptoms may appear in the inoculated plants 15 to 20 days after inoculation. The intensity and severity of infection are to be rated on the basis of 1 to 10 scale after observing the black brown discolouration of the infected internodes by splitting open the stalk longitudinally as follows. The measurement is based on the proportion of disease present in the inoculated internode and its subsequent spread.

Scale : Intensity and extent of severity

1. 25% of the inoculated internode discoloured.
2. 26-50% of the inoculated internode discoloured.
3. 51-75% of the inoculated internode discoloured.

4. 76-100% of the inoculated internode discoloured.
5. Discolouration of less than 50% of the adjascent internode.
6. Discolouration of more than 50% of the adjascent internode.
7. Discolouration of 3 internodes.
8. Discolouration of 4 internodes.
9. Discolouration of 5 internodes.
10. Plants prematurely killed.

Factors affecting disease development

Experiments were undertaken during 1978-80 rabi season at Kalyani inorder to study the effect of some factors namely plant maturity, temperature and crop residues in the development of this disease. The results are briefly described and discussed as follows :

(i) *Plant maturity*

Hybrid Ganga 5 was planted in simple randomized plots of one row each with two replications at 3 weeks intervals in order to maintain different age groups. Inoculation was carried out on three stages of growth namely, young stage, preflowering stage and postflowering stage. Data were recorded 18 days after inoculation (Table 2).

The data show the postflowering stage is most susceptible. Moderate susceptibility was observed in the perflowering stage but the susceptibility was very low in the young stage.

TABLE 2. Plant maturity in relation to disease development

Different stages of growth	Number of plants inoculated	Plants infected (%)
Young	30	5.0
Preflowering	30	42.5
Postflowering	30	76.6

(ii) *Temperature*

Effect of temperature on the development of this disease was studied in the laboratory by a "cut stalk" experiment as developed by Payak (1975). Lower portions of stalks consisting of three internodes were cut from postflowering plants of hybrid Ganga 5. The end of the cut pieces were scaled with paraffin wax inorder to prevent drying. The central internode of each cut stalk was inoculated with the pathogen by toothpick method. The inoculated internodes

were then incubated at 15°, 20°, 25°, 28°, 30°, 35° and 40°C for about two week in 5 replications. The cut stalks were wrapped in hard polythene sheets to provide sufficient moisture. The disease was rated on the basis of 1 to 10 scale as described earlier after splitting open the stalks longitudinally. The results are presented in Table 3.

TABLE 3. Effect of temperature on disease development

Temperature (°C)	Average disease inex
15	1.0
20	1.0
25	1.5
28	3.5
30	4.5
35	6.0
40	5.0
45	4.5

The table shows that maximum infection may occur at 35°C. There was no infection at 15° and 20°C. From 25° to 35°C there was a gradual increase in the extent of infection. However, from 40°C the extent of infection started decreasing. The result indicates that the optimum temperature for disease development is 35°C. This is confirmed by the experiments conducted at Hyderabad for three seasons which also showed that the severity of the disease did not appear to be influenced by moisture but possibly due to prevalence of high temperature above 28°C after flowering (Payak and Renfro, 1969).

(iii) *Crop residue*

Bulk of charcoal rot infected stalks of maize were collected from a sick plot at the University Farm, Kalyani after 1978 rabi harvest. These stalks along with the harvested wheat straw from a nearby plot were spread uniformaly to a separate plot where the experiment was carried out in the next year. Seeds of hybried Ganga 5 were sown in 6 rows plots of 5 meters length in 4 replications with normal agronomic practice. When the crop attained preflowring stage about 1 inch thick layer of wheat straw was applied to the soil surface of the experimental plots. Control plots were kept as such. Inoculum multiplied in the sand maize medium was spread @ 2 kg/plot when above 50% of the plants have flowered. The inoculum was also spread in the control plots.

The data were recorded after 3 weeks of inoculation. About 52.5% of the plants were infected in the treatment with crop residue whereas only 24.5% infection was recorded in the control. This indicates that the crop residue may act as a suitable substrate for growth and rapid colonization by the pathogen. Payak (1975) also observed that organic manure and crop residue could enhance the incidence of *Pythium* stalk rot or maize.

Screening for resisance

The pathogen, *M. phaseolina,* is a soil inhabitant fungus and is rather difficult to control. Therefore, the effective method of control is to find out genetic source of resistance against this disease. Researches for finding out the genetic source of resistance are being carried out at various research stations for this purpose under the Coordinated Programmes. Evaluation carried out at Hyderabad showed that hybrids Ganga-101 and VL-D1, single crosses CM-103 x CM-104, CM-202 x CM-111, CM-113 x CM-112 and CM-400 x CM-300, inbred lines CM-103, CM-104, CM-107, CM-111, CM-112, CM-202, CM-303 and CM-600 are resistant to charcoal rot infection.

Screening for resistance under the Coordinated Programme at Kalyani centre has shown that EH-2380, EH-2420, EH-3066, Deccan-101, VL-43, VL-71, Comp. VI-17, D-771, Diara Comp., Local Diara, Vijaya Comp., Hunius Comp., Pool-7, Pool-15, Sangam, Hi-starch, Ganga-2, G-24, G-25, G-26, G-27, J-101, J-115, J-661. J-662, MCU-501, MCU-607, H-201, H-216 H-215-216, (Syn. P-200 x Kisan) x Hunius and J_1 (Arr2) (KFS1) (1) ### are all resistant in disease reaction (average disease index 1.0 to 4.0). Notable susceptibility was recorded in cases of hybrid # Ganga-5, Syn B-19, Syn B-23, EH-400175, EH-400475, EH-400575, EH-400675, Local Ad-e-cuba, Comp. 108, Hataura Comp., Bokiyo comp. 2, Khyber, Sikkim 76-33 Sikkim 76-37, Sikkim 76-63, PI-278723 x Sarhad, PI-278724 x Sarhad, PI-311237 x Sarhad, Khyber (804 x 805 W), Mixi x Col. Gr. 1) Eto. Syn, KPG white flint, VC 80 x Taxpeno and J_1 (Arr2) (KFS2) (1) # (average disease index, 6.5-9.0).

Economic importance

Since the pathogen causes damage to the important plant part leading premature death to the plant under favourable conditions it may result high economic loss to the crop. Loss in yield due to charcoal rot infection may be upto 30 to 40% in some cases (hybrid Ganga 3, VL-54, Composite Song, inbred lines CM-204, CM-601 and single cross CM-203 x CM-204 etc.).

Chemical control

Chemical control of charcoal rot of maize is difficult to achieve in the field. However, bacterial stalk rot of maize *(Erwinia chrysanthemi)* has been success-

fully controlled in the recent years by the application of stable bleaching powder (Calcium hypochlorite with 33% active chlorine) as soil drench (Anonymous, 1979). Since both the pathogens *(M. phaseolina* and *E. chrysanthemi)* are known to be associated with soil, a programme for exploring the possibility of reducing the incidence of *M. phaseolina* in sick plots was taken up with stable bleaching powder during 1978-79 rabi season at Kalyani considering the fact that chlorine is a general microbiocide at lower concentrations and considering the success of this chemical against *Erwinia*. The materials and

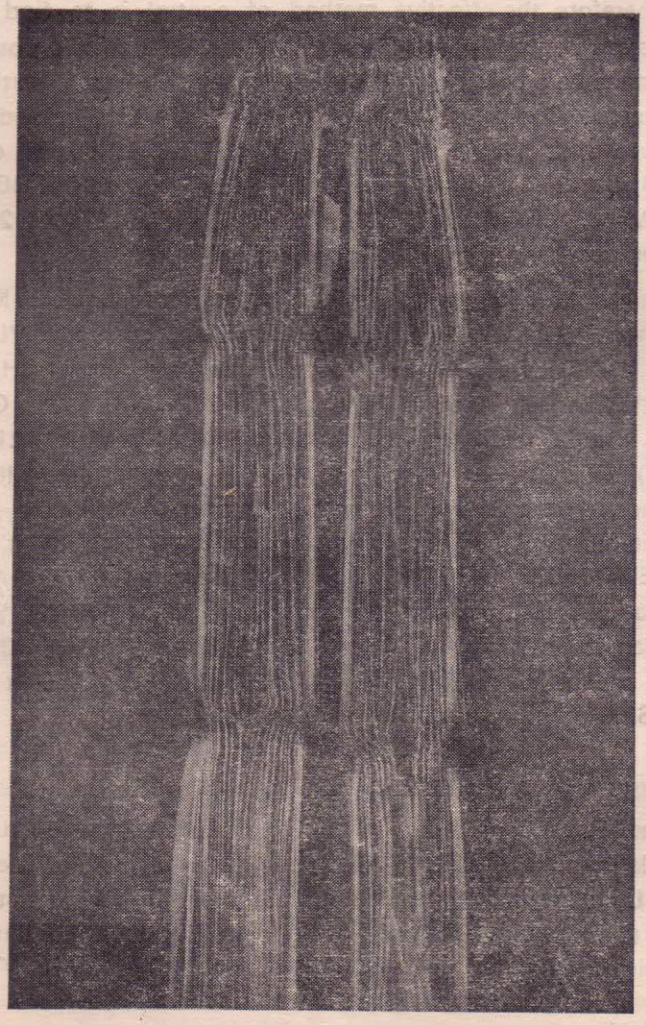

Figure 1. Symptoms showing the black sclerotial bodies in the disintegrated pith of the affected stalk due to *M. phaseolina*

TABLE 4. Effect of Bleaching powder (1000 ppm) on the incidence of charcoal rot of maize caused by *Macrophomina phaseolina*[1]

Treatments (days)	Plants infected (%)	Plants killed (%)	Disease index	Reduction of infection (%)
30	50.0	22.5	3.5	41.6
45	47.5	17.5	3.0	50.5
60	20.0	5.0	2.0	66.6
Untreated (control)	66.4	33.0	6.0	

[1] Results are an average of 4 replications.

methods, and results in the experimental procedures have been described elsewhere in detaiis (Kaiser and Mukherjee 1979). About 41.6-66.6% incidence of this disease was checked when this chemical at the concentration of 1000 ppm was applied as soil drench in three split doses. Late application with 60 days old plants before flowering was found to be most effective than early applications with 45 days and 30 days old plants (Table 4.)

ACKNOWLEDGEMENT

The authors record their thanks to Dr. P. K. Sengupta, Reader & Head, Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal for his helps and to Dr. M. M. Payak, Senior Maize Pathologist & Principal Investigator for supplying the necessary germplasm for screening.

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