Symptomatology and Disease Cycle of *Macrophomina phaseolina* on Sorghum Causing Charcoal rot

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Abstract

Sorghum bicolor (L.) Moench commonly known as "Jowar" is the most important Rabi and Kharif crop of India belonging to the family "Poaceac". It is among one of the four major cereal crop of the world, the other three being wheat, rice and maize. Charcoal rot caused by M phaseolina is a root and stalk rot disease and has a great destructive potential as a disease in most Sorghum growing regions during Rabi and Kharif season. the present investigation was aimed to undertake a thorough study on the symptomatology and disease cycle of Macrophomina phaseolina on Charcoal rot of Sorghum, results of which shows both the isolates had similar pattern of growth (colony, shape and margin) except in colony colour, distribution, number, size and shape of the sclerotia. Pycnidia formation was observed in stem isolate (A) and not in root isolate (B).

Key words : Sorghum bicolor, Kharif,Sclerotia,

Introduction:-

Sorghum bicolor (L.) Moench commonly known as "Jowar" is the most important Rabi and Kharif crop of India belonging to the family "Poaceac". It is among one of the four major cereal crop of the world, the other three being wheat, rice and maize.

In Rajasthan Sorghum is grown in an area of about 556'000 hectares. It is being cultivated as rainy season crop (Kharif, June to October). The main Sorghum districts of Rajasthan and their production

Sorghum crop suffers from biotic and abiotic stresses. Among biotic stresses, diseases play an important role. Many diseases are caused by various microorganisms like bacteria, viruses, mycoplasma and fungal pathogens. Among fungal diseases Seedling blight (Pythium debaryanum); Downy mildew (Sclerospora soghii Stalk rot (Fusarium monilforme); Loose smut (Sphaerotheca soghii); Leaf rust (Puccinia purpurea); Leaf blight (Helminthosporium tercicum); Anthracnose of leaf and stem (Colletotricum graminicolum)

and charcoal rot (Macrophomina phaseolina) are quite common in India. Several of which have an adverse effect on yield. Stalk rot is one of the most important diseases during post rainy season.

Charcoal rot caused by M phaseolina is a root and stalk rot disease and has a great destructive potential as a disease in most Sorghum growing regions during Rabi and Kharif season. First report of the disease was given by Uppal et al., (1936) who found the association of M. phaseolina in lodged plants in Rabi Sorghum.

The pathogen of charcoal rot is a soil borne fungus often known by two distinct stags in its life cycle. First stage is pycnidial and second stage is sclerotial. Pycnidial stage is known as Macrophomina phaseolina (Tassi) goid (imperfect stage) and Sclerotial stage as Rhizoctonia bataticola (Taub) Bull. An interconnection between these two stages has been very well established by (Haigh, 1930) while Luttvell and Garenn (1952) cited the evidence that pycnidial state is capable of infecting and producing symptoms in host. The fungus can also thrive as saprophyte and become parasitic on living tissue of susceptible hosts.

In Rajasthan state there is no published report about the extent and various aspects of the disease. So, looking to the paucity of the information on the disease in Rajasthan state an immediate attention from the research side was needed. Therefore, it was felt necessary to take up this problem. Hence the present investigation was aimed to undertake a thorough study on the symptomatology and disease cycle of Macrophomina phaseolina on Charcoal rot of Sorghum, results of which are incorporated herein.

Review of Litrature:-

Charcoal rot is a widespread root and stem rot disease of Sorghum caused by soil inhibiting fungus M. phaseolina. The symptoms of charcoal rot vary and depend upon the time of infection. The symptoms include root rot, soft stalk, lodging of plants, premature drying of stalks and poorly developed panicles with small inferior quality grain (Uppal et al., 1936).

The most striking and usually first indication of the disease is lodging of plants as they approach maturity. Lodging is due to weakened condition of the stalk, caused by the disintegration of the pith and cortex by the pathogen, leaving the lignified fibrovascular bundles suspended as separate strands in the hollow stalk, hence the disease is called as **"Hollow Stalk of Sorghum"** by Uppal et al., (1936). In Sorghum its plant, stem infection was found to reach up to ear head (Uppal, Kolhatkar, Patil, 1936).

According to Thirumalachar (1953) in India, the charcoal rot disease occurred on a number of crops such as *).. sorghum, Maize, garlic, Castor, sunhamp etc. and symptoms comprised blackening of stem having numerous sclerotia. Some times charcoal rot symptoms are not easily noticeable. Harris (1962) reported that in Nigeria the disease escaped attention because symptoms were inconspicuous. Affected plants looked healthy but had much thinner stalks than normal and had very small panicles.

Raut and Bhombe (1977) noticed leaf spot symptoms in Sorghum due to M. phaseolina.

Due to stalk rot disease, the lower stalk of the plant is spongy and internal tissues (pith) is shredded and often discoloured, stalk weakened and lodged. Plants sometimes turn into grayish green and dry during grain filling. The pith contain much tiny black fungal structure giving charred appearance. The roots are rotten and black. Infected seedlings become circular to oblong, reddish brown lesions on emerging hypocotyl near soil line. These lesions are easily confused with lesions caused by Rhizoctonia root rot fungus. However, charcoal rot lesions usually are not sunken, unlike the lesions of Rhi7octonia root rot. The reddish brown lesions of charcoal rot turns brown to nearly black after several days, infected seedlings may die but if hot and dry conditions L persists, they may remain nearly symptomless (Jain, Khare and Sharma, 1972).

The diagnostic symptoms of charcoal rot are easily observed after plant death. Numerous, minute, black spacks ("pin head size" microscterolia of the fungus) can be seen when the epidermal tissue is peeled off from the lower stem and roots of affected plants. The microsclerotia are mostly abundant in the silvery and gray areas of the lower stems and tap roots. Lower stems and taproots can be split open to reveal microsclerotia.Thus it was confirmed as a causal agent based on the size of microsclerotia (Krupinsitta et a/.,2002). Charcoal rot is a widespread root and stem rot disease of Sorghum caused by soil inhibiting fungus M. phaseolina. The symptoms of charcoal rot vary and depend upon the time of infection. The symptoms include root rot, soft stalk, lodging of plants, premature drying of stalks and poorly developed panicles with small inferior quality grain (Uppal et al., 1936).

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TRANSMISSION OF Macrophomina phaseolina THROUGH SEEDS.

Although there is no report on transmission of M. phaseolina through seeds in Sorghum plant though some reports on other crops are available. Chilton (1942) isolated Sclerotium bataticola from one out of six seed lots of subterranean clover seed. Leukel and Martin (1943) reported that seed coat injuries, which were common in threshed Sorghum were found to promote ready access of fungi to the endosperm and found that Sclerotium bataticola inhibited seed germination.

Kilpatrick (1957) isolated Sclerotium bataticola from the seed of soyabean upto one percent Govindaswamy et al., (1957) isolated M. phaseoli from sorghum seeds collect from different regions of Madras state.

Meiri and Solel (1963) reported that Sclerotium bataticola is seed borne in ne sesame cultivar 'Renner - 15' which was susceptible to this pathogen. Lal and Mathur (1967) demonstrated that Sclerotiurn bataticola infected peanut kernel carried the fungus in both the cotyledons. The number of seeds with sclerotia on the testa according to direct inspection were found to be much lower than the number of infected seeds after incubation on agar and on blotters.

Sackston (1969) isolated sclerotium bataticola from black, discoloured seeds of cowpea which did not germinate. The fungus was also isolated from healthy seeds.

Gangopadhyay et al., (1970) reported that M phaseolina is seed borne in soyabean under natural conditions. They also disclosed that upto 24 percent of seeds San harvested from infected plants harbour the fungus.

M.phaseolina was isolated from the rotted seed of Peanut by Gupta and Chohan (l970a). Vir and Gaur (1970) observed that Rhizoctonia bataticola was associated with 40 per cent of okra seeds.

A visible damage upto 32.7 percent and concealed damage upto 28.9 percent by M. phaseolina was observed by Gupta and Chohan (1970b), while estimating the losses and nature of damage caused by seed rot fungi in stored Groundnut.

Elarosi et al., (1970) isolated M phaseolina from devoid seeds and damping off seedlings of cucurbit cultivar Askanarahi'. Watanabe (1972) isolated M phaseolina from kidney bean seeds. Fakir et al., (1976) for the first time reported the carryover of M phaseolina on the seeds of sunflower.

Sinha and Khare (1977) found that M phaseolina was seed borne in Cowpea. The infection was found to be intra embryonal and mycelium was present in cotyledons, plumule and radicle. The formation of pycnidia was initiated below the seed coat on cotyledons, radicle and plumule. Pycnidia emerged after breaking the seed coat. Seeds with severe infection rotted completely and the pathogen finally grew on the seed .Surface. Dhawal et al., (1978) reported the loss in seed germination of Chillies due to Rhizoctonia batacticola to the extent of 56-67 percent.

Root Injury

There is very less work, done on the root injury in relation to the infection of M. phaseeolina on the sorghum plant, although some reports on other crops are available. Hansford (1929) found that M phaseolina enter through roots in Coffee (Phaseolus Po voigaris) and caused heavy damage. Livingston in 1945 observed that M. phaseolina entered through roots and become systemic, however, Savita Pareek (1991) found same results on Maize plant.

Materials and Method :-

MORPHOLOGY AND TAXONOMY OF FUNGUS

For variability of the pathogen with regard to its morphological characters and virulence, the study -of two different isolates A and B maintained on PDA were undertaken. These isolates differed in their cultural and pathogenic behaviour. So, data on growth rate, colony characteristics, sclerotial morphology for each isolate on PDA. were recorded.

For this study, 90 mm petriplates containing 20 ml PDA medium were inoculated each with 2 mm disc taken from the periphery of three days old culture of each isolate Colony diameter was recorded when the growth almost completely covered the plate. Difference in topography, type of margin and colour of the colony were also recorded. Colony colour was identified with the help of colour dictionary.Both he isolates were sent to ICRISAT, Hyderabad and Agharkar Research Institute, Pune for identification which was done by Dr. S.Navi and Dr. Alka Pandey. **Table-3 : Identification of Sorghum Root and Stem Isolates**

S.No	Isolate	Source	Species	Isolate
1	A	. Banasthali, Krishi Vi an Kendra	Macrophomina)phaseolina (Tassi) Goid	Stem
1	B	. Banasthali, Krishi Vigyan Kendra	Macrophomina)phaseolina (Tassi) Goid	Root

TRANSMISSION OF DISEASE THROUGH SEEDS

Sorghum seed samples collected from 1CRISAT and NRCS, Hyderabad and Udaipur (Rajasthan) were examined for the possible presence of M. phaseolina by following method:

BLOTTER TECHNIQUES

Blotting disc of 8.5 cm in diameter were cut out and were sterilized in a hot air oven. These papers were moisturized in a beaker containing sterile water. These were lifted with a pair of forceps till the last drop of water dripped off. These moistened blotters were carefully placed in the sterilized petriplates at the rate of three blotters per plate.

Seeds were surface sterilized with 0.1% mercuric chloride and were washed thrice with sterile distilled water. Excess moisture was removed by blotting them with sterile blotters. Twelve seeds per petridish were arranged and they were incubated at 28°C. This was replicated thrice unsterilized seeds were also incubated similarly.

TRANSMISSION OF DISEASE THROUGH ROOTS

Seeds of Sorghum CSH-5 were sown in 12 inches diameter earthern pots with four replications. When the plants attained the age of 30 days, some soil from the basal portion was removed and injury was given to the root with the help of a sterilized knife. Sorghum grain inoculums was placed near the injured portion and it was again covered with the same soil. No injury was given to the plants kept in the check. Observations were recorded after 60 days of inoculation.

Results:-

MORHHOLOGY OF THE PATHOGEN

M. phaseolina is an ubiquitous fungus enjoying world wide distribution. It is soil borne and has a. very wide host range covering over 500 different plants belonging to both monocots and dicots. Hence, several hundred strains and varieties of this pathogen have been isolated and studied (Haigh, 1930). Different strains show a great variation in morphological, physiological and pathological characteristics (Jain, khare and Sharma, 1970; Dhingra and Sinclair, 1972). The fungus has two distinct stages in its life cycle. First stage is pycnidial and second is sclerotial. The pycnidial stage is known as M. phaseolina (Tassi) Goid. and sclerotial stage as Rhizoctonia bataticola (Taub) Butl. Both these stages are many a times found to occur together on the some host plant but sometimes only the sclerotial stage is found to cause disease and the pycnidia are not produced. In this case the pathogen is known only by its sclerotial stage. However, an interconnection between these two stages has been very well established by Haigh 1930.

In the present study two isolates obtained from Sorghum plants collected from Banasthali Vidyapith of Tonk district showed striking variations in morphology and cultural characters. Hence, it was planned to investigate their variability in detail (Table 7).

Cultural studies of the two isolates A and B showed variations in growth rate, colony characteristics, sclerotial morphology and size of sclerotia.

The data shown in Table-7 reveals that both the isolates had similar pattern of growth (colony, shape and margin) except in colony colour, distribution, number, size and shape of the sclerotia. Pycnidia formation was observed in stem isolate (A) and not in root isolate (B).

Among these two different isolates studied virulence of A isolate was found to be maximum followed by B type. Both the two isolates differed in intensity of sclerotia production and their size .Isolate B produced abundant sclerotia followed by A. Larger sclerotia were formed by A isolate (90.1 x 74.2 lim) followed by B (75.7 x 66.1 1.tm) type, On the basis of the sclerotial size all the isolates can be grouped under Haigh's 'C' group 1930 which has a mean sclerotial diameter of less than 120 lam. Pycnidia formation was only shown by stem isolate and not by root.

TABLE —7

Variability in the cultural characters of two isolates of Macrophomina phaseolina obtained from Sorghum stem and root on PDA at 28°C.

Isolates	Colony diameter (mm)	Cultural characters Colony	Size of sclerotia (um)	Pycnidial formation	Size of pycnidia (Um)
'A' Stem	68.72	Colony oblique withentire margin, cottons fluffy with upright myceliumrodent in colour, sclerotia many() and centrally distributed and round in shape.	90.1 x 74.2	Pycnidia presents on the surface of infected tissue were globose t subglobose ostiolated dark olive to black.	90-120
'B' Root	78.16	Colony circular with entire margin, cottony, fluffy with profuse acrial, mycelium carbonaceous black in colour, sclerotia abundant (+++) and more or less scattered slightly densed at centre and elong- ated in shape	75.7x66.1	Nil	-

+ - Few

++ - Many

+++ - Abundant

Discussion:-

MORPHOLOGY OF THE PATHOGEN

It is now well established in the literature that life

These stages were well connected in their life cycle also i.e. sclerotial stage under conditions produced the pycnidial form and vice versa. However, it has been observed that in culture many a times the sclerotial stage i.e. Rhizoctonia bataticola failed to produce the pycnidial stage. Although the Success is not always certain Luttrell, 1946; Kulkarni and Patil, 1966, Chidambarm and Mathur, 1975).

In the present Pychnidial formation was shown by stem isolate.

The pathogen M Phaseolina was isolated from basal portion of the charcoal rot Tad Sorghum stem and roots from Krishi Vigyan Kendra, Banasthali Vidyapith.

These isolates were purified by hyphal tip culture and their morphology was studied on PDA. On media both these isolates showed variability in their growth and morphological characteristics such as colony type, colour, shape, size number and size of sclerotia etc. Stem isolate produced pycnidia while root isolate do not. (Table 7).

These experiments established that although the present isolates were quite distinct in their morphological characters, yet they were highly variable in their cultural baheviour.

The variability of M phaseolina has also been studied previously by several workers on isolates obtained from different crops, or different places, or from different Parts of the same plant (Haigh, 1930; Jain, Khare and Sharma, 1972; Dhingra and Sinclair, 1972;

Dhingra and Sinclair (1972, 1973) studied variation among isolates of M.Phaseolina obtained from different parts of a soybean plant. They found that these isolates differed in colony type, in vitro growth rate, sclerotial size and ability to cause soybean seeding blight.However, lndra et al. (1983) who studied 23 isolates of M. phaseolina differing in growth pattern colony characters and size of reproductive structures, concluded that the growth rate of different isolates displayed marked differences which could not be correlated with their pathogenicity, whereas, the significant differences in the size of sclerotia, pycnidia and pycnidiospores could be correlated with the virulence of the isolates.

Summery:-

A study of symptomatology revealed that pre-emergence symptoms of seedlings are much less apparent than in post-emergent cases. However, the reduction in the number and total root area could be easily

observed. Another noteworthy feature is that number of seedlings are killed, and only mildly infected seedlings developed further.

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