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SMUT DISEASE OF PEARL MILLET: BIOLOGY AND CONTROL

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ABSTRACT:

Bajra, the pearl millet (Pennisetum glaucum)(L) R. Br.) is predominantly a rainfed, salt tolerant crop of rainy season. It provides staple cereal diet to the people residing in the rural parts of semi-arid and arid areas of our country. The crop is mainly grown in the States of Rajasthan, Maharashtra, Gujarat, Uttar Pradesh, Haryana, Karnataka and Tamil Nadu, which cover 90% of the total cultivation of the crop. The crop is most tolerant and adapted to drought conditions where most of the grain crops would fail to survive. Smut is one of the major panicle diseases of pearl millet, caused by Tolyposporium penicillariae Bref. The disease has occurred in almost all the areas where pearl millet is cultivated. The major outbreak of this disease in recent years has proved its economic importance as a serious threat to pearl millet production in northern India, particularly in Rajasthan, Haryana, Punjab and Gujarat due to commercial cultivation of F1 hybrids (Thakur and King, 1988). Development and geographic distribution of plant diseases primarily depends upon climatic factors. This is particularly true for those diseases, which affect the aerial parts and are subjected to highly fluctuating aerial environments. The disease has been reported in Burkina Faso, Cameroon, Gambia, Ghana, India, Malawi, Mali, Mozambique, Niger, Nigeria, Pakistan, Senegal, Chad, Sierra Leone, Sudan, the USA, Zambia, Tanzania and Zimbabwe (Thakur and King, 1988, Wilson, 2000).

INTRODUCTION:

Disease Symptoms:

Pearl millet smut the ovaries are converted into sori in the infected florets. The smut sori are usually larger in size (3-4 mm long and 2-3 mm broad at the top) than normal grains (1-2 mm) and are formed in place of normal grains projecting out from between the glumes. In early stages the colour of sori remains bright green that slowly turns brown to black on maturity. The normal grain contents are replaced by black, dense, powdery mass of spores. Sori are covered by a thin film, which breaks at maturity and release brownish-black spore mass. On ripening sori release many spores in the air, which cause further infection on healthy ear heads. The smut fungus gradually replaces the grain content with black powdery mass and the sori finally consist of remains of

host tissue and the spores. The entire grain content is replaced by black dense spore mass but the membrane covering the smut remains intact. The lower portion of ear head, which usually remains covered by the sheath of the flag leaf, is normally found heavily infected with smut.

Fig -01: Young, systemically infected plants with mild growth of Tolyposoriumriae Penicillariae Bref

Fig showing: Young, systemically infected plants have light green to yellowish stripe lengthwise in the leaves often with the Tolyposoriumriae Penicillariae

About the Pathogen (Tolyposoriumriae Penicillariae Bref.):

Information on smut causing pathogen of pearl millet is rather confusing in the literature. A number of apparently unique species have been reported or implicated as the smut causing fungus of pearl millet. Wilson (2000) has discussed about the inconsistency in nomenclature of smut pathogen. In the literature Tolyposporium as a genus was first described by Woronin in 1882 for the smut on Juncus bufonius L. which had previously been described by Schroeter 1872 under the name Sorosporium junci Schroet (Thirumalachar, 1966). The main differentiating character of Tolyposporium with that of Sorosporium was attributed to the permanent nature of sporeballs composed of dark coloured spores. The genus also got recognition from Schellenberg, 1911; Dietel, 1928; Liro, 1938; Ciferri, 1938; Fischer, 1953 and Zundel, 1953. Tolyposporium penicillariae Bref. was first described by Brefeld (1895) on Penicillaria spicata Willd. (= Pennisetum typhoides). Collections of this smut have since been made on Pennisetum typhoides at various places in India.

The disease was also reported from Africa (Chevalier, 1931; Yen, 1938). Till date all the publications

pertaining to the fungus describe it as T. penicillariae (Ramakrishnan, 1971; Subba Rao and Thakur, 1983; Thakur et al. 1983a, 1983 b, Thakur and King, 1988). Vanky (1977) recommended a new name Moesziomyces for the fungus. He was supported by Chahal and Kumar (1985) and Wilson (2000). Based on characters like sori without columella, firm agglutination of spores in many spored sporeballs by surface ornamentation appearing as irregular meshes, they described the smut pathogen as Moesziomyces penicillariae (Bref.) Vanky. However, this genus is yet to get wide recognition and still the most preferred name of the fungus among the workers is T. penicillariae. The teleutospores of T. penicillariae aggregate to form 'sporeballs' having fertile cells. Sporeballs vary in shape from circular to near-polyhedral and measure 42-325 x 50-175 µm in diameter (Thakur and King, 1988, Dashora, 2005). The single teleutospore has yellowish to light brownish colour with ornamentation on the outer surface and measure 7-12 µm in diameter. The fourcelled promycelium forms both lateral and terminal sporidia

BIOLOGY OF THE PATHOGEN (TOLYPOSORIUMRIAE PENICILLARIAE BREF.) AND ITS RESULTS:

Cultural Studies:

The fungus can be cultured in the form of colonies on natural and synthetic media. A little work has been done on the cultural aspects of the pathogen. Bhowmik and Sundaram (1971) obtained the first successful culture of T. penicillariae on PDA. Tripathi and Bhaktavatsalam (1977) suggested Haskin's MB-50 medium as best followed by PDA. Studying nutritional physiology of the fungus Pathak and Shekhawat (1980) reported maximum growth on Brown's medium in terms of mycelial dry weight and sporulation. Smut cultures vary in type and rate of growth, topography and colour. The growth of smut cultures may be mycelial, sporidial or a combination of both (Holton et al., 1968). Pathak and Shekhawat (1980) have reported mycelial growth on synthetic media whereas Subba Rao and Thakur (1983) and Dashora (2005) have reported only sporidial growth on synthetic and semi-synthetic media. Dashora (2005) has investigated in detail the cultural aspects of the fungus and inferred that there were significant differences between different cultures as regards to

growth and sporulation of T. penicillariae. Accordingly, significantly highest linear growth of the fungus with excellent sporulation was recorded on Richard's agar medium followed by Czapek's dox medium and Potato dextrose agar medium at 28±20. There was less variation in colony characters of the fungus. The cultures were thick and ridged on all the nutritive media tried. The disease is endemic in all the pearl millet growing areas of India, particularly in Rajasthan where it attacks the crop every season resulting in the economic loss to the farmers. Natural incidence is influenced by biotic and abiotic factors of the area in which the crop is grown. The variables interacting with the environment are chiefly temperature, humidity and rainfall. Information about the influence of abiotic factors on the disease development is very limited, particularly in the western Rajasthan; eco-biological investigations were therefore conducted to understand the role of physical factors, age of ear head, inoculation technique and incubation period on in vitro growth of T. penicillariae. Effect of temperature on in vitro growth of T. penicillariae An experiment was undertaken in which T. penicillariae was allowed to grow on Richard's agar medium (RA) at temperatures of 15 to 40 degree centigrade. A fungal disc of 5 mm was cut and placed on solidified RA medium and allowed to grow on petridishes at different temperatures in BOD incubators. Results revealed that maximum growth of the fungus was observed at 30 degree centigrade (3.2 cm) followed by 250 (2.6 cm). The fungal colonies remained slow in growth at temperatures 15 and 20 degree Centigrade. with increasing temperature the fungal growth became profuse with excellent sporulation at 30 degree centigrade. However, at higher temperatures (35 and 40 degree centigrade) the growth and sporulation was drastically reduced. The colour and margins of the fungal colony throughout remained unchanged. Co-efficient of variation was observed to be 11.02%. Pathak and Shekhawat (1980) found 25 degree centigrade as the optimum temperature for maximum fungal growth in terms of mycelial dry wt. Subba Rao and Thakur (1983), Thakur (1988, 1989) and Phookan (1987) reported 35 degree centigrade temperature as optimum for maximum sporidial growth, they further observed that the growth was inhibited at temperatures less than 20 degree

centigrade. The disease is severe when weather is warm as compared to moderate and cool climate (Thakur, 1989).

Effect of pH on in vitro growth of T. penicillariae Richard's agar medium was prepared and adjusted to different pH gradients of 5.0, 5.5, 6.0, 6.5 and 7.0 before autoclaving. All the petridishes were incubated at 30 degree centigrade in B.O.D. incubator. Results showed that maximum growth (2.3 cm) and sporulation was recorded on pH 6.0 followed by pH 6.5. As the Ph gradients increased the fungal growth was drastically decreased. Least growth was observed on pH 5.0. Smut fungi are known to tolerate wide range of pH (Fischer and Holton, 1957). Pathak and Shekhawat (1980) reported 7.5 as pH supporting maximum mycelial growth whereas Phookan (1987) observed maximum sporidial growth at pH 6.0. D. Inoculation Technique In nature there is air transmission of the teliospores of the fungus (T. penicillariae) to the ovary through stigma. After getting penetrated by the fungus near the base of the ovary the fungal hyphae ovary is gradually replaced by the mass of fungal tissues. It becomes important, therefore, that during artificial inoculation the fungal inoculum should reach near to the base of the ovary. Taking this in view two methods were tried for infecting pearl millet plants under field conditions: Natural method: comparable to infection in nature (Campbell, 1957), having two methods of inoculation viz. a) dry powder dusting of spores on the ear heads and b) dipping of ear heads in the inoculum suspension. Un-natural method: No parallel process is known in nature (Campbell, 1957 and Cunfer et al., 1975), having one method of injecting the inoculum into the floral cavities with the help of hypodermic syringe. The inoculations were made at the boot stage and each floret was injected with 5.0 ml of spore suspension. The inoculated ear heads were covered with polythene bags to ensure high humidity. The bags were removed after a week's time, and the infection was considered to have taken place when the grain colour appears as bright green, shiny and enlarged in shape. Results showed that maximum (56%) infection was obtained by injecting the inoculum through hypodermic syringe, whereas dry powder dusting resulted in 20% infection followed by dip method (infection in traces). Effect of age of ear head Age of the pearl millet ear head has a definite relation with resistance of the

host. An experiment was conducted with cv. Nokha local of pearl millet having ear heads of different ages of 0, 1, 5, 7, 10, 15 and 20 days after the emergence from the boot. These were injected with an aqueous sporidial suspension and covered with polythene bags. The plant age was determined using growth stage key for the cereals developed by Large (1954). Disease severity was measured in terms of percent infection index (PII). Results showed that the highest incidence of 56% with 42% PII and 23.5 CODEX was recorded in 10 day old ear heads having very soft kernel representing 10.5.4 stage of Larg's key.Incubation period determination for smut development in field In order to determine the minimum period of incubation required in saturated atmosphere for successful infection, forty-five days old pearl millet of cv. Nokha local were selected and exposed to different incubation periods under field conditions. Ear heads of the selected plants were inoculated with 5 ml aqueous spore suspension of T. penicillariae using hypodermic syringe. The inoculated ear heads were covered with polythene bags for 48; 96; 144,192 and 240 h. Results indicated that the maximum disease developed at a period of 240 h followed by 192 h period. The ear heads given 48 h incubation did not show any symptoms whereas the ear heads incubated for 96 h showed 20% incidence, which increased with the increasing incubation period.

Disease Cycle:

The primary source of inoculum is sporeballs in the soil from the previously infected crop along with externally contaminated seed used for sowing. At appropriate temperature and RH, the sporeballs germinate to produce dense mycelial network. The germinating teliospores form promycelia and sporidia, which infect the host at flowering. After the formation of dikaryotic infection hypha, infection takes place through young emerging stigmas. The time taken from inoculation to spore production is about two weeks and sori mature within 3-4 weeks. Sporeballs are released from mature sori, which under favourable weather conditions germinate to produce another crop of sporidia .

Fig showing: Smut of pearl millet: (A)chlorosis on leaves; (B)sporangial growth on lower surface of leaf and (C)oospores.

DISEASE MANAGEMENT:

Smut disease is the major limiting factor of pearl millet production in all the millet cultivating tracts in India. Farmers generally grow only traditional landraces, which are highly susceptible to the disease. The disease is very difficult to manage because of its seed, soil and air borne nature. To work out effective control measures for the disease the studies were undertaken for in vitro testing of fungicides. Four fungicides viz. Blitox 50; Bavistin, Dithane M-45 and Thiram were tested using poisoned food technique (Bagchi and Das, 1968). These fungicides were used in four concentrations of 25, 50, 100 and 200 µg ml-1. Ten days old cultures of T. penicillariae were used to inoculate petridishes having solidified RA medium with different fungicidal dilutions. Petriplates were incubated at 30±20C for 21 days. Observations were recorded at weekly intervals for measuring growth of the fungus.

DISCUSSION:

Inhibition of fungal growth was calculated by following formula: I = C - T x 100, where I = Inhibition percent C = Growth rate of the control T = Growth rate of. Of all the fungicides tried thiram provided relatively best inhibition of diametric growth of T. penicillariae at all the concentrations tried, however, the maximum inhibition was recorded at 200 µg ml-1concentration (84.9%) followed by 100 and 25 µg ml-1. Next to thiram was bavistin, which could inhibit the fungal growth by 69.8% at 200µg ml-1 and 41% at 100µg ml-1. Dithane M-45 showed 64.3% inhibition at 200µg ml-1 and 39.7% at 100µg ml-1 Moreover, the effect of different fungicides, their concentrations and the interaction of doses were all significant. Blitox was observed as least effective in inhibiting fungal growth. Thiram at 200µg ml-1 was thus found to be the most effectively inhibiting the growth of

T. penicillariae. These results support the findings of Kumar and Nath (1991) for managing long smut of sorghum caused by T. ehrenbergii. Several workers (Bhowmik and Sundaram, 1971; Phookan, 1987, Pathak and Gaur, 1975) have reported carboxin as the most effective fungicide followed by captafol and carbendazim. However, the major limitations to chemical control of smut in pearl millet are low monetary value of the crop, and scarcity of resources available to pearl millet growing farmers. For effective and economic control of the disease a combination of indigenous knowledge and biocontrol agents may be attempted as seed and spray treatments. Below are the list of plans we used as Biological control methods.

Common Name	Botanical Name
Jamun	Syzygium cumini Myrtaceae
Neem	Azadirachta indica Meliaceae
Aloe	Aloe barbadensis Asphodelaceae
Lantana	Lantana camara Verbenaceae

Lists of Fungicide and Percentage inhibition of diametric growth (Fungicide concentration %in mg/ ml 200) are shown in below table.

Fungicide	Percentage inhibition of diametric growth (Fungicide concentration %in mg/ ml 200)
Blitox (Copper oxychloride 50% Copper)	50.6
Bavistin (2 methoxtl carbamoyl benzidazole)	69.8
Dithane M 45 (75 % Zinc iron manganese ethylene bisdithiocarbamate)	64.3

List of fungicides evaluated along with their chemical, common and trade name. S. No are as follows.

Chemical name Common name Trade name are as follows:

1. Methyl-2- benzimidazole-carbamate Carbendazim 50% WP Bavistin 2 Copper
2. Copper chloride hydroxide Copper oxychloride 50% WP Blitox

3. 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]- 1,2,4-triazole Propiconazole 25% EC Tilt
4. RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2-ol Hexaconazole 5% SC Hexacare
5. Manganese ethylene bisdithio-carbamate plus zinc Mancozeb 75% WP Indofil-45

CONCLUSION:

Pearl millet has tremendous potential to add to the food basket of India, yet due to the biotic stresses the crop has suffered production constraints. The smut disease is endemic in all the millet-growing tracts of India, and thus plays an important role in determining the yield loss in this crop. The present paper deals with the study of symptoms, the pathogen and its taxonomic position, physiology of smut causing fungi, epidemiology and disease development, disease cycle and efforts to find out suitable chemical control measures.

The mean growth and sporulation of T. penicillariae on different nutritive media after three weeks of inoculation Nutritive medium Growth (cm) and sporulation are as follows.

1. Asthana & Hawker's Medium 6.7…. + + +
2. Richard's Agar Medium 7.3….. + + + +
3. Czapek's Dox Medium 6.7….. + + +
4. Sabouraud's Agar Medium 6.2……. + +
5. Potato Dextrose Agar medium 6.8 ----- + + +

Where + Poor; + + fair; + + + good, + + + + excellent

A. The major limitations to chemical control of smut in pearl millet are low monetary value of the crop, and scarcity of resources available to pearl millet growing farmers. For effective and economic control of the pathogen a combination of fungicide and plant extract as spray treatment for managing the smut is the requirement of current era.

B. The regression study indicates that the smut sori develop when the average maximum temperature and daily mean temperature remains below 39 degree and 31 degree respectively and the minimum of 43 percentage relative humidity is needed for the development of the disease. The total rainfall and rainy days favor the development of smut sori by enhancing humidity. Early /timely sown (up to 10 th July earlier)

crop of pearl millet suffers significantly more from smut than the later sown crop.

C. Eucalyptus powder @ 10 % was found excellent against Tolyposporium penicillariae under in-vitro evaluation.

D. Vitavax,Bavistin and Aliette @0.1% absolutely inhibited the fungal growth of Tolyposporium penicillariae

E ICM 92888 and IP 19874 were identified as source of smut under artificial and natural conditions.

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