STUDIES ON PEARL MILLET SMUT WITH SPECIAL REFERENCE TO PATHOGENIC VARIABILITY, INHERITANCE OF RESISTANCE AND CHEMICAL CONTROL

by

AJIT KUMAR PHOOKAN

A dissertation submitted to the Haryana Agricultural University in partial fulfilment of the requirements for the degree of:

DOCTOR OF PHILOSOPHY

in

PLANT PATHOLOGY

COLLEGE OF AGRICULTURE
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CERTIFICATE I

This is to certify that this dissertation entitled, "STUDIES ON PEARL MILLET SMUT WITH SPECIAL REFERENCE TO PATHOGENIC VARIABILITY, INHERITANCE OF RESISTANCE AND CHEMICAL CONTROL" submitted for the degree of DOCTOR OF PHILOSOPHY in the subject of PLANT PATHOLOGY of the Haryana Agricultural University, is a bonafide research work carried out by SHRI AJIT KUMAR PHOOKAN under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

(Dr. D.P. THAKUR)
Major Advisor
Senior Plant Pathologist (Pearl millet)
Department of Plant Pathology,
Haryana Agricultural University,
Hisar-125004.

CERTIFICATE II

This is to certify that this dissertation entitled, "STUDIES ON PEARL MILLET SMUT WITH SPECIAL REFERENCE TO PATHOGENIC VARIABILITY, INHERITANCE OF RESISTANCE AND CHEMICAL CONTROL" submitted by SHRI AJIT KUMAR PHOOKAN to the Haryana Agricultural University in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in the subject of PLANT PATHOLOGY, has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

Major Advisor 3/9/87

External Examiner

4.9.87
Head of the Department

Dean, Post-graduate Studies

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Ajit Kumar Phookan
4/5/87
AJIT KUMAR PHOOKAN

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INTRODUCTION

Pearl millet (*Pennisetum americanum* (L.) Leeke) is a staple cereal for a vast proportion of humankind living in the hot, drought-prone arid and semi-arid regions of less-developed countries of the world, particularly Africa and Asia. It is considered to be one of the most drought and heat tolerant crops grown in marginal, light, sandy and rocky soil. Pearl millet is believed to have originated in tropical Africa where many allied species are also found (Krishnaswami, 1951). Its cultivation, primarily as a food crop, extends from several countries of East and West Africa to India and Pakistan in South Asia (Rachie and Majmudar, 1980) whereas in the southeastern United States, it is cultivated solely as a forage crop (Johnson *et al.*, 1976). Though pearl millet is a coarse grain cereal, its grain contains as much as 16% protein, 4.5% fat, 46 mg/100 g calcium (Burton *et al.*, 1972), 3.7 g lysine, 1.4 g tryptophan and 2.1 g methionine per 100 g protein (Aykroyd *et al.*, 1963) and therefore, it is a potential source of nutrition to large numbers of hunger and malnutrition affected people living in the tropical and sub-tropical countries of the world. Pearl millet plays an important role in the

agricultural economy of India as it is the fourth important cereal crop of the country. Of 42.35 million hectares under the millet crop in the world, 18.50 million hectares (43.7%) is in India with an average annual grain production of 11.80 metric tons and average grain yield of 638 kg/ha (FAO, 1984). In India, the crop is mostly grown in the states of Andhra Pradesh, Gujarat, Haryana, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu and parts of Uttar Pradesh. In Haryana, it is the main kharif season crop with an area of 841 thousand hectares and annual grain production of 552 thousand tonnes (Anonymous, 1985).

During the 1960s, with commercial cultivation of F_1 hybrids, based on cytoplasmic-genetic male sterility, pearl millet production was increased by 36% over the traditional varieties within a few years in India (Singh, 1983). But this was a short-lived change. In the early 1970s, the popular high yielding hybrids succumbed to downy mildew (Sclerospora graminicola (Sacc.) Schroet), ergot (Claviceps fusiformis Loveless) and smut (Tolyposporium penicillariae Bref.).

Smut has been recognised as an important floral disease of pearl millet in Africa and Asia since the early part of this century (Butler, 1918; Chevalier, 1931; Ajrekar and Likhite, 1933; Ramakrishnan, 1971). The disease has also been reported from North America (Wells et al., 1963).

The potential seriousness of the disease in India was realized only in the 1970s when the F_1 hybrids in large commercial plantings in northern India became heavily infected (Rachie and Majmudar, 1980). Smut appeared in epiphytotic proportion in certain parts of Uttar Pradesh and Haryana in 1967 (Bhowmik and Sundaram, 1971). Presently, in Haryana, this disease is considered important next to downy mildew and becomes severe in years when humid weather prevails during the flowering period. Smut has received relatively less attention of pathologists than downy mildew and ergot in India (Rachie and Majmudar, 1980). Earlier studies provided preliminary information on some aspects of the disease in India (Ajrekar and Likhite, 1933; Bhatt, 1946; Vasudeva and Iyengar, 1950). With the establishment of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), a systematic research effort was made on studying various aspects of the disease (Subba Rao and Thakur, 1983; Thakur et al., 1983a, 1983b, 1986). However, even today there are many important gaps in our knowledge of the pathogen, that inhibit our attempts to control it and therefore, more efforts are needed to better understand this problem and find out solution. At present, smut is considered to be a constant threat to pearl millet growers only in the northern India where frequent rains during the flowering period favour its development. However, it may pose a problem in other parts of the country as well in the near future by acquiring climatic adaptability

and producing new pathogenic races or biotypes. An effective and economical control of smut can be obtained through host-plant resistance and breeding smut-resistant varieties and hybrids should receive priority in research. A second approach to control smut should be based on the use of chemical fungicides either alone or in combination with resistant cultivars. Chemical fungicides in combination with resistant cultivars provide a more durable or higher level of control than can be achieved with each method individually (Williams, 1984). To achieve this it is important to have a thorough understanding of the biology and variations in the pathogen populations and inheritance of resistance to devise a suitable resistant breeding strategy.

The present studies were, therefore, undertaken to elucidate informations on the following aspects.

1. Variability in Tolyposporium penicillariae: To study the morphological, cultural and pathogenic variability among different isolates of the pearl millet smut pathogen, collected from different locations in India.
2. Inheritance studies: To study the inheritance of smut resistance and associated genetic architecture using a half-diallel crossing system.
3. Chemical control: To assess the relative efficacy of a few fungicides in reducing smut incidence.

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REVIEW OF LITERATURE.

A survey of literature on the pertinent aspects of the proposed investigation entitled "Studies on pearl millet smut with special reference to pathogenic variability, inheritance of resistance and chemical control" reveals that not much information is available on these aspects. However, works done by earlier workers relating directly or indirectly to the different aspects of the investigation are reviewed in this chapter under the following headings.

1. Taxonomy of Tolyposporium species.
2. Symptomatology and biology of pearl millet smut.
3. Variability in Tolyposporium penicillariae.
4. Smut resistance in pearl millet.
5. Inheritance of smut resistance.
6. Chemical control.

2.1. Taxonomy of Tolyposporium species

The genus Tolyposporium belongs to the class Basidiomycetes, subclass Teliomycetidae, order Ustilaginales and family Ustilaginaceae. The family Ustilaginaceae contains a number of economically important pathogens. The genus Tolyposporium includes several important species parasitising cereals and forage grasses, such as Tolyposporium penicillariae Bref., T. ehrenbergii Kuhn, T. setaricolum H and P. Syd, T. andropogonis Patel and Kulkarni

(Thirumalachar and Neergaard, 1977).

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 in 1872 under the name Sorosporium junci Schroet (Thirumalachar, 1966). The main differentiating character of Tolyposporium with that of Sorosporium was attributed to the more permanent nature of the sporeballs composed of dark colored spores. Subsequently the genus also got recognition from other authors (Schellenberg, 1911; Dietel, 1928; Liro, 1938; Ciferri, 1938; Fischer, 1953; Zundel, 1953). However, Fischer and Holton (1957) failed to find differences among well recognized basidiomycetous genera such as Sorosporium, Tolyposporium, Thecaphora and Glomosporium, on the basis of permanent nature of the sporeballs. This led them to conclude that the differentiating characters had not been well understood and a better basis for separating them should be found out. Thirumalachar and Neergaard (1977) also doubted the authenticity of differentiating the genera Tolyposporium and Sorosporium on the basis of permanent nature of sporeballs. They were of the opinion that if the material was old or in a poor state of preservation, the sporeballs would easily crumble into separate spores which might mislead the investigator to consider it as a Sorosporium.

It was Clinton (1906) who first pointed out that the spores in Tolyposporium species held together by folds or thickening of the outer walls. He suggested that the small

projections of the outer walls articulate with one another with those of the adjoining spores to form the sporeballs which were by no means permanent in the true sense. Thirumalacher (1966) stressed this character as more reliable in separating Tolyposporium from Sorosporium than what had been given before. He then outlined the differentiating characters separating Sorosporium and Tolyposporium from Thecaphora. In the former two genera the sporeballs are formed by the aggregation of spores, while in Thecaphora, the spore is multicellular by septation. The two adjacent cells of the spore have a common wall. Clinton's concept of the genus Tolyposporium, with sporeballs and spores held together by articulating processes of the outer wall has been accepted by several authors in the description of the genus (Mundkur and Thirumalachar, 1952; Fischer, 1953; Thirumalachar, 1966; Ainsworth, 1971). Ainsworth (1971) summarized the whole concept of description of the genus by describing Tolyposporium as "Spores firmly united as balls by projections from spore walls". If the Ainsworth's concept is to be accepted then in T. junci the type of the genus designated by Woronin lacked this character and so Thirumalachar and Neergaard (1977) after examinations of numerous collections of T. junci including the Woronin's collection suggested that the smut examined by Woronin was not Tolyposporium but a Sorosporium and the original name S. junci Schroet. was correct. They further stated that as the type species of the genus Tolyposporium turned out to be a Sorosporium, which had been earlier described, a new name should be

proposed with a new type species, to accomodate the genus. The authors proposed to erect a new genus Tolyposporidium Thirum. and Neergaard gen. nov. with the type species Tolyposporidium evernium (Syd.) comb. nov., syn. Tolyposporium evernium syd. on Paspalum distichum L. collected from Punjab, India. (Thirumalachar and Neergaard, 1977). The authors thus described six species under the genus Tolyposporidium including the pearl millet smut fungus as Tolyposporidium penicillariae (Bref.) comb. nov. However, the genus did not get much recognition from the research workers.

Tolyposporium penicillariae Bref., the incitant of smut disease of pearl millet was described first by Brefeld (1895) on Penicillaria spicata Willd.(=Pennisetum typhoides) sent to him in Berlin by A. Barclay from India. A fragment of the type specimen was sent later by E. Ulbrich of the Berlin Botanical Museum, where Brefeld's herbarium was deposited to India (Mundkur, 1940). Collection of this smut have since been made on Pennisetum typhoides at various places in India. Since the early part of this century, T. penicillariae has been described as a major parasitic fungus on pearl millet in India (Butler, 1918; Ajrekar and Likhite, 1933). It was also reported from Africa (Chevalier, 1931; Yen, 1938). Wells et al.(1963) described the fungus under the generic name Tolyposporium. Of late, all the publications pertaining to the fungus, describe it as T. penicillariae (Ramakrishnan, 1971; SubbaRao and Thakur, 1983; Thakur et al., 1983a, 1983b).

However, Vanky (1977) erected a new genus Moesziomyces for this fungus. Supporting Vanky, Chahal and Kumar (1985) also described the smut pathogen as Moesziomyces penicillariae (Bref.) Vanky, based on the sori character without columella and where spores firmly agglutinated in many spored sporeballs by surface ornaments appearing as irregular meshes. This genus is yet to get wide recognition and the popular name T. penicillariae has still been preferred by most of the research workers.

2.2. Symptomatology and biology of pearl millet smut

2.2.1. Symptomatology: The smut constitutes an important group of fungi affecting plant species. They are so called because they form black, dusty spore masses that resemble soot or smut or the infected plant parts. In nature the dikaryotic mycel ium of most of the smuts appears to be obligately parasitic on flowering plants. According to Duran (1973), there are about 1100 species of smuts that attack Angiosperms. Although some species are geographically confined to relatively small areas, others are found whereever their hosts grow.

Tolyposporium penicillariae, Bref., the causal agent of smut of pearl millet is found in almost all areas of Asia and Africa where the crop is grown. It is a floral parasite and thus confines only to the inflorescence (Butler, 1918). Ajrekar and Likhite (1933) also observed that no other part of the plant except kernel got infected when the plants were artificially inoculated. They ofcourse did not rule out

completely the possibility of successful infection through pinpricks of the shoot. However, they confirmed that no part of the host plant other than the grain developed the sporeballs. Mundkur (1940) reported that the smutted ovaries of pearl millet generally enlarged into oval or pear shaped bodies of 3-4 mm long and 2-3 mm broad. These bodies were bluntly round or conical at the apex, and called sori. They were chocolate-brown in colour and covered by a tough membrane composed of host tissue. The sori composed of sporeballs, mostly of unequal sizes. Bhatt (1946) conclusively proved that smut infection took place only through the flower. His persistent attempts to cause seedling, shoot and localized infections using various methods by the spores and the mycelium yielded negative results. He further stated that the young flowers showing no external signs of the stigma or anthers were the most susceptable. The later stages showed comparatively less infection and finally no infection was possible when the flowers had been pollinated. Reporting for the first time the occurrence of the disease in the USA Wells et al.(1963) stated that the disease could be recognized only in the advanced stage when developing seeds and the individually diseased ovaries protruded from the lemma and palea. They found the diseased ovaries dark-green in colour with a diameter of 2-4 mm, about twice that of normal seeds. The disease incidence in a spike varied from 1-2 sori per spike upto 90% infection in a spike composed of several hundred ovaries. Ovaricolous nature of the pathogen has also been

reported by several authors (Ramakrishnan, 1971; Rachie and Majmudar, 1980; Thakur et al., 1983a, 1983b).

2.2.2. Biology: Literature pertaining to the reports of laboratory culturing of pearl millet smut pathogen is relatively scanty. No work was initiated on this aspect until 1930. However, contrary to the earlier belief of difficulty in vitro germination of sporeball, Ajrekar (1931) first demonstrated that the sporeballs could be easily germinated on boiled potato and boiled carrot slices. In the light of above finding, Ajrekar and Likhite (1933) successfully grew the fungus by placing sporeballs on cornmeal, bajrameal and jowarmeal agar media. They detected very little mycel ium in the fungal growth and reported that the growth consisted almost entirely of sporidia. Husain and Thakur (1963) tried different media and found bajrameal agar and jowarmeal agar quite suitable for isolating the fungus and tryptone yeast-extract agar for optimum growth of the organism. Bhowmik and Sundaram (1971) first reported successful cultivation of the pearl millet smut organism on the common laboratory medium potato-dextrose-agar (PDA). Tripathi and Bhaktavatsalam (1977) found Haskin's MB-50 medium to be the best for growth of the fungus followed closely by PDA. In a nutritional and physiosical study on Tolyposporium penicillariae in vitro, Pathak and Shekhawat (1980) grew the organism in seven synthetic media. Brown's medium supported the maximum growth in terms of average mycelial dry weight and sporulation followed by dextrose-asparagine-phosphate medium. The optimum temperature and pH

for growth and sporulation of the fungus were 25°C and 7.5, respectively. The fungus was also grown on 11 different carbon sources. The maximum growth and sporulation occurred on inulin followed by dextrose. Growth and sporulation were poor on starch, glycerol, D-fructose, D-galactose, sucrose and maltose. In an attempt to produce large quantities of sporidial inoculum to facilitate large-scale field inoculation Subba Rao and Thakur (1983) tested seven commonly used semi-synthetic media to observe their relative efficacy in supporting growth of the organism. They concluded that large quantities of sporidia could be produced on potato agar (PA) or Carrot agar (CA) and in shake culture of potato extract or carrot extract. They reported 35°C to be the optimum temperature for maximum sporidial growth. In contrast to the findings of Pathak and Shekhawat (1980), the authors reported a purely sporidial growth of the organism in all seven media tested without any trace of mycelium. The sporidial growth was successfully maintained even after repeated subculturing.

Ajrekar and Likhite (1933) reported that the attack of the disease was more severe in wet seasons, than in the dry ones and thus weather conditions during flowering was found to play a major role in disease initiation and spread. This was later supported and confirmed by others (Bhatt, 1946; Patel and Desai, 1959; Bhowmik and Sundaram, 1971). Thakur et al.(1983b) conclusively showed that the relative humidity (RH) during the period from inoculation to disease assessment

played a critical role for smut development and a significant change in weather conditions can make any screening technique ineffective.

Butler (1918) reported the pearl millet smut disease to be air-borne. This has also been supported by many authors (Ajrekar and Likhite, 1933; Bhatt, 1946; Vasudeva and Iyengar, 1950; Thakur et al., 1983b). Ajrekar and Likhite (1933) also stressed the possible role of alternate hosts in disease perpetuation. However, they themselves were unable to find out any such host. The authors observed that no resting period was necessary for the sporeballs to germinate, at least on artificial media. Vasudeva and Iyengar (1950) confirmed this even on the host plant and suggested the possibility of secondary infection aiding disease spread from a crop sown earlier to a later-sown crop. Thakur et al. (1983a), however, were of the opinion that since secondary spread of the disease within a crop in the field were limited to only the late nodal tillers so the grain yield was not significantly affected. Bhatt (1946) investigated the role of seeds in initiation of the disease and concluded that seeds in no way found responsible for initiating the disease. He further stated that soil was found to serve as a potential depository for the smut spores where they would pass the winter and in the following crop-season germinate during the rain and cause infection on a suitable host.

The possible role of pollination in reducing the disease was emphasized by Bhatt (1946). He stated that if the flowers were pollinated first before receiving viable smut

spores, the chances for infection were reduced to the minimum due to withering of the stigmatic lobes. Thakur et al.(1983a) supported this view and stressed the possibility of controlling smut in commercial F_1 hybrids by providing enough pollen at flowering time from smut-resistant pollen donor lines.

2.3. Variability in Tolyposporium penicillariae

Although it had long been known that within species of many fungi there were morphologically indistinguishable but physiologically different lines or races but it was not until 1919 that the phenomenon was recognized in the smut fungi. Kniep (1919) was the first to report difference in the appearance of sporidial cultures of Ustilago violacea (Pers.) Fuckel from different host plants. Zillig (1921) demonstrated that pathogenic variation of this fungus could be differentiated by their ability to infect certain members of the family Caryophyllaceae but not others. Christensen and Rodenhiser (1940) stated that the smut fungi within a species could be divided into different groups on the basis of characters on artificial media, physiologic and ecologic characters, biochemical effects, and morphology. Holton et al.(1968) were of the opinion that though variation was pronounced in most of the smut fungi in both morphology and physiology still studies on variation in the smut fungi were confined primarily to the economically important smuts of cereal crops and a few closely related species that parasitized grasses. Spore size, shape and colour and

exospore ornamentation are considered to be the principal criteria in species delimitation in the smut fungi (Fischer, 1953; Duran and Fischer, 1961). Holton et al. (1968) reported that even within the same species variation in spore colour was common in some Tilletia and Sphacelotheca species. Variation in sorus characteristics is common to several species and races of Tilletia (Fischer and Holton, 1957). Smuts are known to be highly variable in their effect on host plant development. Fischer and Holton (1957) found that some species and races influenced markedly the height of the host plant; others exert a pronounced effect on proliferation or tillering of the infected plant, The smuts of wheat, oats and sorghum were especially notable for variation in this respect. According to Holton et al. (1968) the greatest variation in the smuts fungi is displayed by haploid cultures on agar media. Fischer and Holton (1957) stated that even monosporidial cultures of many species usually varied widely in colony characteristics, some of which might represent temporary response to environment; the great majority however, were inherently permanent.

Variation in the smut fungi has its most fundamental impact on pathogenicity. Halisky (1965) stated that the differential reaction of two or more host varieties could be taken as indication in the degree of variation in virulence of the parasite. Pathogenic specialization was discovered first in Uromyces violacea, a smut of the pink family

(caryophyllaceae), in 1921 (Fischer and Holton, 1957). Since then every smut species investigated in this regard has been exhibiting some degree of variation in pathogenity. According to Holton et al.(1968) variation in pathogenity in these smuts is the key factor in the host-parasite interaction relating to development of resistant varieties for smut control.

There are so far no reports on variability in the pearl millet smut pathogen, Tolyposporium penicillariae.

2.4. Smut resistance in pearl millet

There has been very little efforts to screen and identify resistance in pearl millet to smut. Murty et al.(1967) first endeavoured to classify the world collection of genetic stocks of Pennisetum on the basis of its disease reaction to smut pathogen and reported a few accessions from India and Africa to be resistant. Pathak and Sharma (1976) screened 322 pearl millet lines, collected from different parts of India and found all the lines susceptible. However, on the basis of variation in their reaction to the organism 11 lines were reported to be moderately resistant. Sangwan and Thakur (1980) categorised 318 commonly available genotypes of pearl millet from different sources in India into different groups on the basis of their smut reaction. While they found one inbred, 158 from Anna Pannai, Tamilnadu to be free from smut infection the other 88 genotypes were reported to be moderately resistant.

With the establishment of All India Co-ordinated Project for Millets Improvement by ICAR in 1968 and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India in 1975, systematic screening work involving large numbers of germplasm collections to identify resistance to smut in pearl millet was initiated and within a short span of time considerable progress has been made towards identification of stable sources of resistance. As a result of continuous efforts on breeding for smut resistance, Thakur et al.(1986) reported stability of resistance in few agronomically elite pearl millet lines which have shown high levels of smut resistance across different locations in India and West Africa for several years.

2.5. Inheritance of smut resistance:

Not much information is available on inheritance of smut resistance in pearl millet. Yadav (1974) reported smut resistance to be controlled by either single or double gene. His experiment with some local varieties revealed varied behaviour in F_1, F_2 and test cross progenies. Varieties 'Kota local' and 'Orawar local' showed monogenic incomplete dominance, 'Jakhrana local' and 'T5' showed monogenic complete dominance and 'Pratapgarh local' and 'Fatehabad local' exhibited duplicate action of 2 genes for resistance. Observation at ICRISAT center indicate resistance to be governed by dominant gene (Unpublished), which is easily transferrable.

Along with resistance, for yield stabilisation, it would be essential to combine traits which confer high productivity. It is an usual feature that primitive varieties that evolve in the centre of origin of the crop often constitute the main source of resistance for diseases but yield poorly (Leppik, 1970). Therefore, it would be essential to characterise the genetical architecture of population for yield and yield contributing traits. Most of the traits of economic importance in a crop plant are quantitative in nature and governed by polygenes. Fisher (1918) introduced the concept of additive, dominance and epistatic gene effects and variance. Since then several statistical design have been proposed by different workers to characterise the inheritance pattern of quantitative traits on various crops (Mather, 1949; Jinks and Hayman, 1953; Hayman, 1954, 1958, 1960; Jinks, 1954; Griffing, 1956; Kearsey, 1965; Jinks and Perkins, 1970). Of these, the diallel analysis is considered to be a powerful technique in resolving the genetic constitution of parents in respect of quantitative variation.

2.6. Chemical control

The successful application of chemical fungicides for the control of millet smut, caused by Ustilago crameri Kcke., was reported long back (Hecke, 1902, 1903). Subsequently, many fungicides were tested against the disease by several workers (Melchers, 1927; Vasey, 1918; Yu et al. 1934). A few attempts have also been made to

control pearl millet smut by using chemical fungicides.

Two methods of chemical control of pearl millet smut have been attempted. One is seed treatment and the other is use of foliar sprays to prevent the initiation and further spread of the disease.

2.6.1. Seed treatment: Butler (1918) reported that the pearl millet smut could not be controlled by seed treatment. His attempts to check the disease by seed treatment with the use of formalin, copper sulphate solution or even hot water treatment had failed. However, several workers reported ceresan and agrosan GN to be the most effective seed treating fungicides against the pearl millet smut (Dey, 1949; Anonymous, 1972). Bhaktavatsalam and Tripathi (1975) reported carboxin to be very effective in reducing the smut of pearl millet when used as a seed treating chemical. In a screening of nine fungicides, against the fungus, Tripathi and Bhaktavatsalam (1977), later found that captan was the most toxic fungicide for growth inhibition followed by carboxin, ziram and oxycarboxin.

2.6.2. Foliar spraying: Wells (1967) found two compounds, 2,3-dihydro-5 cartoxanilido-6-methyl-1,4-oxathin (DCMO) and 2,3-dihydro-5-carboxanilido - 6-methyl-1, 4-oxathin-4, 4-dioxide (DCMOD) highly effective when used as head and foliage sprays in reducing the incidence of smut caused by Tolyposporium penicillariae. Bhowmik and Sundaram (1971) tested the efficacy of certain systemic fungicides against smut disease of pearl millet and found that plantvax at 2 g

per litre of water was most effective in reducing the numbers of sori in inoculated ears followed by vitavax, benomyl and ziram. Mathur et al.(1971) tested five fungicides against naturally infected pearl millet plants and observed that all the five fungicides reduced the infection and increased yield considerably. However, the heptaene antibiotic, aureofungin was found to be the most effective followed by ziram and blitane. Pathak and Gaur (1975) on the other hand, did not find heptaene antibiotic as effective as captafol. When sprayed on artificially inoculated plants significant smut reduction was observed by captafol (2ppm). Sharma and Sharma (1976) tested seven chemicals as foliar sprays against smut and concluded that all the chemicals reduced smut incidence and gave higher yield compared with control. Zineb and the blend of mancozeb and dinocap were, however, found superior to other chemicals for smut control and correspondingly gave higher yields. Chahal (1979) reported oxycarboxin to be the best when used as a foliar spray which reduced the smut incidence significantly. Oxycarboxin was closely followed by carboxin, captafol and benomyl.

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MATERIALS AND METHODS

Investigations included in this chapter comprised laboratory, screenhouse and field studies. While parts of the studies relating to inheritance and variability were carried out in the laboratory, screen house and field of Millet Pathology section, ICRISAT Centre, Patancheru near Hyderabad, the remaining studies were conducted in the laboratory and field of Plant Pathology department, Haryana Agricultural University, Hisar. Details of the methodology in conducting these studies are presented below.

3.1. Studies on Variability in the Pathogen

3.1.1. Collection and preservation of isolates: In an attempt to study the variability in the isolates of pearl millet smut pathogen (*Tolyposporium penicillariae*), smutted pearlmillet inflorescence were collected from six Indian locations (Table 1).

Collections were made during October from the rainy season crops of 1985 from the pearl millet commercial hybrid BJ-104. The smutted heads were sun-dried for two days and than sealed in polythene bags and preserved at 5-6°C in a refrigerator throughout the duration of study.

3.1.2. Morphological studies: All the six isolates were examined to find out if there was any morphological difference in spore ball, teliospore and sporidia among different

TABLE 1

Isolates of T[illegible] penicillariae used for the variability study

Sr. No.	Isolate	Host genotype	Source
1.	HSR	BJ-104	Department of Plant Pathology, Haryana Agricultural University, Hisar.
2.	ICR	BJ-104	Millet Pathology, ICRISAT Patancheru, Andhra Pradesh, India.
3.	JDH	BJ-104	Central Arid Zone Research Institute, Jodhpur, Rajasthan.
4.	JPR	BJ-104	Agricultural Research Station, Durgapura, Jaipur, Rajasthan.
5.	LDH	BJ-104	Punjab Agricultural University, Ludhiana.
6.	MGH	BJ-104	Farmer's field, Mahendragarh, Haryana.

HSR = Hisar; ICR = ICRISAT; JDH = Jodhpur; JPR = Jaipur; LDH = Ludhiana; MGH = Mahendragarh.

isolates. The details of the studies have been described below.

3.1.2.1. Sporeballs: Sporeballs were obtained by crushing matured smut sori on a clean dry sterile Petri dish. These sporeballs were then mounted on a drop of water in a clean slide with the help of a sterile forcep and the slide was examined under a compound microscope for colour, shape and size of the sporeballs. In measuring sizes, 150 sporeballs in different microscopic fields in three slides were examined to get the mean value.

3.1.2.2. Teliospores: Teliospores were separated from the sporeballs by crushing them underneath a coverslip on a clean glass slide. Observations were recorded on

individual teliospore for colour, shape and diameter with the help of a compound microscope. Mean data for diameter were recorded from a total of 150 observations in three slides in different microscopic fields.

3.1.2.3. Sporidia: The sporidia used for the present investigation were obtained from 5 day old culture of soreballs on Potato-agar medium at 35°C. The details of culturing procedure have been described under 'cultural studies' in this chapter. 150 sporidia from three different Petri dishes were examined and mean value for length was noted along with observations on shape and colour.

3.1.3. Spore germination studies: For this study, soreballs of all the six isolates were suspended in sterilized distilled water in cavity slides and the slides were kept in Petri dishes lined with moist blotting paper and these plates were then incubated at 30°C. For each isolate three replications were maintained. Observations on germination percentage were made after 20 hours of incubation according to the procedure described by Subba Rao and Thakur (1983). In an another set of experiments, observations were made regularly at 30 minutes iterval to find out the optimum germination time required by teliospores of different isolates.

3.1.4. Cultural studies: Cultural studies for each isolate were done on five different media comprising three common semi-synthetic and two synthetic media.

3.1.4.1. Cultural media: The names and constituents of the media used in the present investigation are

described below. Preparation procedures followed according to CMI (1983) and Subba Rao and Thakur (1983).

A. Semisynthetic media:

I Potato Agar (PA)

200 g peeled potato extract

15 g agar-agar

1 l distilled water.

II Potato Dextrose Agar (PDA)

200 g peeled potato extract

20 g dextrose

15 g agar-agar

1 l distilled water

III Carrot Agar (CA)

200 g peeled carrot extract

15 g agar-agar

1 l distilled water

B. Synthetic media:

IV Brown's medium

2.00 g dextrose

2.00 g asparagine

1.25 g tribasic potassium phosphate

0.75 g magnesium sulphate

20.00 g agar-agar

1 l distilled water

V Richards's medium:

10.00 g Potassium nitrate

5.00 g Potassium dihydrogen phosphate

2.50 g magnesium sulphate

0.02 g ferric chloride

50.00 g sucrose

15.00 g agar-agar

1 l distilled water

The media were adjusted to pH 6, and autoclaved at 1.1 kg cm^{-2} for 20 minutes in conical flasks. After sterilization, 20 ml of each medium was plated aseptically in 9 cm diam Petri dishes. On solidification of the media, the Petri dishes were inverted on the 'laminar flow' table and kept one upon another in stack for 24 hours, after which these were examined and the contaminated dishes were discarded. Those Petri dishes, which escaped contamination were only used for the isolation and multiplication of the fungus.

3.1.4.2. Isolation and preservation of the fungus: For routine isolation of the fungus and for multiplication purpose potato agar media was normally used. Unruptured mature smut sori were separated from the inflorescence and surface sterilized with 0.1% mercuric chloride for 2 minutes. These were then rinsed with sterile distilled water twice and crushed aseptically with help of a sterile forcep on a sterile Petri dish to obtain the sporeballs. The sporeballs were used to inoculate the medium in Petri dishes. The inoculated Petri dishes were then kept for incubation for 7-8 days at 35° C under 40W fluorescent lamps in a Percival 135 CC incubator with alternate 12 hrs light and dark. Axenic culture of each isolate was transferred to PA slants and stored in a refrigerator. Periodic sub-culturings of the

isolates were done regularly during the period of the investigation.

3.1.4.3. Effect of different media on growth: Five day old culture of each isolate was used for this study. The isolates were at first adjusted to a fixed sporidial concentration (4×10^5 sporidia ml^{-1}) in sterile distilled water to maintain uniformity. Then with help of sterile micropipette measured quantity (0.01 ml) of sporidial suspension was inoculated at one point of Petri dish containing 20 ml of medium and similarly in each Petri dish 5 different sites were inoculated with equal quantity of sporidial suspension. Each isolate tested on each medium was considered to be a treatment. Four replications were maintained for each treatment. For different treatment separate micropipettes were used. After inoculation all the Petri dishes were marked properly and kept in incubator at 35°C exposing to alternate 12 hrs light and dark. After 120 hrs, the plates were removed from the incubator and observations were recorded for colour, type of growth and mean colony diameter of all the isolates separately.

3.1.5. Physiological studies:

3.1.5.1. Effect of temperature on growth: Five day old culture of each isolate was first adjusted to a fixed sporidial concentration (4×10^5 sporidia ml^{-1}) in sterile distilled water. Measured quantity (0.01 ml) of sporidial suspension was placed at one point of the Petri dish containing 20 ml of potato agar medium with help of a sterile micropipette. Similarly four other different points of the Petri

dish were also inoculated with the sporidial suspension. After inoculation, Petri dishes were kept in incubators at six different temperatures 15°C, 20°C, 25°C, 30°C, 35°C and 40°C. For each temperature four replications were maintained. The plants were provided with alternate 12 hrs light and dark. After 120 hrs, these were removed from the incubators and observations were recorded for colour, type of growth and mean colony diameter of all the isolates.

3.1.5.2. Effect of pH on growth: Sensitivity to H-ion concentration (pH) is known in different fungi and isolates. Since the growth of these isolates was slow in culture, an experiment was designed to determine the effect of pH on growth of different isolates. The potato-agar medium (PA) was adjusted to five pH levels (5 to 7) and equal amounts (20 ml) of the medium from each pH level were plated in 9 cm diam. Petri dishes. The dishes were inoculated with equal quantity (0.01 ml) of sporidial suspension from stock sporidial concentration (4×10^5 sporidia ml^{-1}) of each isolate at 5 different sites of a Petri dish. Four replications were maintained for each treatment. The Petri dishes were incubated at 35°C with 12 hrs alternate light and dark for 120 hrs after which observations on colour, type of growth and mean colony diameter of all the isolates were recorded.

3.1.6. Pathological studies: In order to elucidate pathogenic variability in Tolyposporium penicillariae, pathogenicity tests with different isolates were conducted under identical conditions. Three resistant pearlmillet genotypes,

PLATE 1. SHOWING SMUT INOCULATION TECHNIQUE WITH HELP OF AN ATOMIZER AT BOOT-LEAF STAGE

PLATE 2. PATHOGENICITY TEST IN THE SCREEN HOUSE BY REGULATING TEMPERATURE AND HUMIDITY AFTER SMUT INOCULATION

ICMPS 700-1-5-4, ICMPS 900-9-3 and ICMPS 1500-7-3-2 and three susceptible genotypes 833B, 834B and 843B were inoculated with each of the six isolates, in the screen house. A commercial pearl millet hybrid BJ-104 which is well known for its high susceptibility to smut was also included as a susceptible check. Inoculations were done following the standard inoculation technique (Thakur et al. 1983b) at boot leaf stage under identical conditions, using equal quantity of aqueous spordial suspension (1×10^6 sporidia ml^{-1} from 5 day old culture of each isolate. 30 earheads of each genotype, in three replications were used for each isolate whereas 10 heads of each genotype in each replication were also inoculated with distilled sterile water. Immediately after inoculation, the heads were covered with perchment bags and labelled properly. Overhead sprinkler irrigation for three times a day each of one hour duration was provided to maintain high humidity. About 45 days after inoculation, inflorescences of each genotype, were harvested separately for each isolate and scored for smut severity visually using standard scoring scale (Pate3) on the basis of percentage florets converted into smut sori as suggested by Thakur et al. (1983b).

3.2. Inheritance Studies:

The present genetic investigations involved 6 diverse and elite lines of pearlmillet obtained from the Millet Pathology section, ICRISAT, Hyderabad. These lines were selected for the study on the basis of reaction to smut, diversity in height, flowering, ear length and yield.

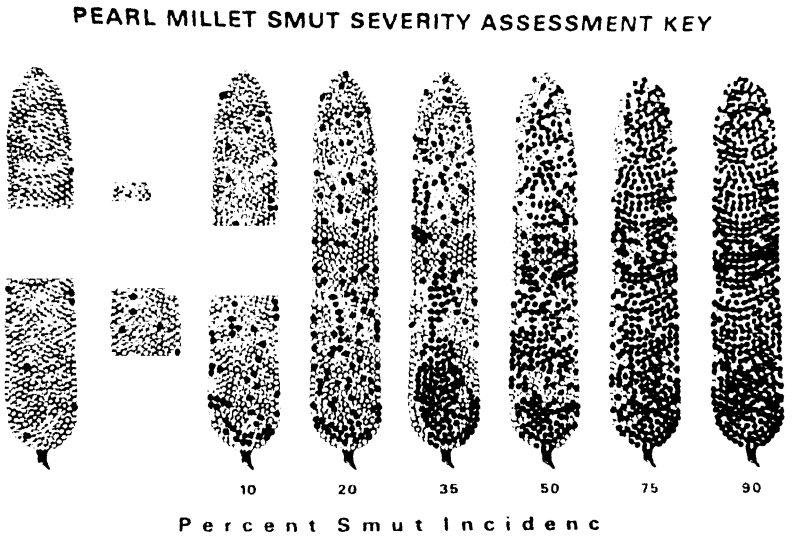

PLATE 3. PEARL MILLET SMUT SEVERITY ASSESSMENT KEY

The distinguishing features of these lines are given in Table 2. These lines under reference have been numbered from 1 to 6 and hereafter they will be referred to by their number only.

TABLE 2

Parent number, name and distinguishing characters of the pearl millet lines used in diallel crossing

Parent	Name	Smut Reaction	Important agronomic traits
1	ICMPS 900-9-3	R	A tall, lodging-resistant, average tillering, long-headed inbred. It is medium maturing possessing medium - sized seeds.
2	ICMPS 700-1-5-4	R	A tall, high-yielding, average tillering, medium maturing inbred. It is long headed with small-sized seeds.
3	ICMPS 1500-7-3-2-R		A tall, average tillering, late maturing inbred. It has medium-sized heads with bold seeds.
4	833 B	S	A dwarf, average tillering, medium-maturing, maintainer. It is long-headed with bold seed. It produces lodging-resistant hybrids.
5	843B	S	A dwarf, average tillering, early-maturing, maintainer. It is small-headed with medium-sized seeds.
6	834B	S	It is of medium height, average tillering, early maturing, loose-bristled, maintainer, with medium-sized, heads with bold seeds.

R = Resistant;

S = Susceptible.

PLATE 4. A VIEW OF THE SMUT NURSERY WITH BAGGED PEARL MILLET EARHEADS AFTER INOCULATION FOR THE INHERITANCE STUDY

PLATE 5. PEARL MILLET EARHEADS SHOWING RECEPTIVE STIGMAS BEFORE ANTHER EMERGENCE

These pearl millet lines were crossed in all possible 1-way combinations (excluding reciprocals) during rainy season of 1985 at H.A.U., Hisar.

The 15 F_1s and 6 parental lines were grown during post-rainy season at ICRISAT center, Hyderabad. Each entry was raised in row of 4 meter length. The normal cultural practices were adopted. Selfing of the F_1 progenies were done to get F_2 seeds. Back crossing of 15 F_1s with both the parents were also made to get BC_1 and BC_2 seeds.

In the rainy season of 1986, the 15 F_1s, 15 F_2s, 15BC_1 S and 15 BC_2 S along with 6 parents were grown in the field of ICRISAT center, in a randomized block design with three replications for each entry. Normal cultural practices were adopted throughout the growing period of the crop. At the boot-leaf stage, 100 inflorescence of each parent and F_1, 200 inflorescence of each BC_1 and BC_2 and 500 inflorescence of each F_2 were artificially inoculated with sporidial suspension ($10^6 ml^{-1}$), prepared from a virulent isolate. Check was maintained by inoculating the heads of BJ-104. The method of inoculation followed was according to Thakur et al. 1983b. Just after inoculation, the heads were covered with perchmant bags and labelled properly. Overhead sprinkler irrigation was provided daily to maintain high humidity in the field.

3.2.1. Recording of observations: For recording data on following characters except smut, 10 random plants in F_1 and parents were used excluding border plants. For smut, all the inoculated inflorescence were recorded for smut severity in parents, F_1s, F_2s, BC_1S and BC_2S.

3.2.1.1. Characters studied:

a) Smut severity: The smut severity was scored at the time of harvest on the basis of percentage florets converted into smut sori with aid of the standard diagram used for the purpose.

b) Days to 50% boot leaf stage (DTBL): Total numbers of days from sowing to boot leaf stage of each entry was counted when approximately 50% of the plants achieved that stage.

c) Plant height: The plant height was measured in cm from the base of the plant to tip of the ear of the main tiller at maturity.

d) Ear length: The main ear of each plant was measured in cm from its base to tip.

e) Number of effective tillers: The number of ear-bearing tillers per plant were counted at the maturity of the crop.

f) 1000 grain weight: 1000 grain weight was taken in gram with help of a single pan electrical balance.

g) Yield/plant: Yield of 10 randomly selected plants were taken in gram and weighing was done with single pan electrical balance. The value was later converted to yield per plant basis.

3.3. Statistical Procedures:

All quantitative data relating to different investigations were statistically analysed. Data were computerised on Vax Digital Computer system at ICRISAT Center, Hyderabad. Data on per cent record were subjected to statistical analysis

after angular transformation. The method of analysis of variance for Randomized Block Design was according to Panse and Sukhatme (1967) and LSD calculated at 5 per cent and 1 per cent level of probability. The partitioning of total variance into different components was done as shown in Table 3.

TABLE 3

Partition of total variance into component variances

Source	d.f.	M.S.S.	
Replication	r-1	Mr	$\frac{Mr}{Me}$
Treatment	t-1	Mt	$\frac{Mt}{Me}$
Error	(r-1)(t-1)	Me	

Where,

r = number of replications

t = number of treatments.

Mr, Mt and Me are the mean sum of squares due to replication, treatment and error respectively. The replication and treatment variance are tested against their corresponding error variance, using F test at their respective degree of freedom at P = 0.05 and 0.01.

3.4. Diallel Analysis:

The inheritance studies were performed using diallel analysis following Griffing's method 2, model 1 (1956) and Hayman (1954), details given by Singh and Chaudhary (1977). The analysis is based on the following assumptions. (i) Homozygosity of the parents, (ii) diploid segregation

(iii) no genotype x environment interaction (iv) no epistasis (v) no multiple alleles and (vi) no linkage.

3.4.1. Graphical analysis:

The graphical analysis was done as described by Hayman (1954). For each character, Vr, Wr graphs were prepared. Vr is the variance of all the off-springs of each array and Wr is the covariance of each array with the non-recurrent parents.

Wr values were taken on x axis and Vr values were taken on y axis. A parabola line was drawn which gave the limites for Wr, Vr points. The limiting parabole of such a graph was constructed with the help of Wr^2 and the regression line was drawn by Wr values which fitted best to the point of origin. The description of graphic analysis was done as described under.

i) If regression line intersects the Wr axis well below the point of origin, it suggests the presence of over dominance.

ii) If regression line intersects the Wr-axis through the origin, it suggests complete dominance.

iii) If the regression line intersects the Wr-axis above the point of origin, it suggests partial dominance.

iv) If the regression line touches the parabola limits, it suggests no dominance.

v) Parents falling near the origin show preponderance of dominant and those falling away from the origin show preponderance of the recessive genes.

vi) A significant 'b' value shows that there are interaction of genes in the parents for particular trait.

3.4.2. Testing of hypothesis:

The diallel theory is based on certain assumptions. The validity of the hypothesis was tested by following tests.

1. t^2 test as suggested by Hayman (1954) for testing the uniformity of Wr.Vr value.

$$t^2 = \frac{n-2}{4} \times \frac{(\text{Var. Vr} - \text{Var. Wr})^2}{\text{Var. Vr} \times \text{Var. Wr} - \text{Cov}^2 (\text{Vr. Wr})}$$

The value computed is tested against the table value of F with 4 and (n-2) degrees of freedom, where n is the number of parents. Significant value indicates failure of atleast one of the hypotheses postulated.

2. Test of significance of b: The significance of difference of 'b' from zero as well as from unity was tested by using 't' test; t= b-0/SE of b and (1-b)/SE of b with n-2 degree of freedom where n is the number of observations. If 'b' deviates significantly from unity, presence of epistasis is indicated.

3.4.3. Component analysis:

The following genetic components of variation were computed adopting the method given by Jinks and Hayman (1953) and Hayman (1954).

$$D = VOLO - E$$

$$F = 2VOLO - 4\ WOLO_1 - 2\ (n-2)\ E/n$$

$$H_1 = VOLO - 4\ WOLO_1 + 4V_1L_i - (3n-2)\ E/n$$

$$H_2 = 4V_1L_1 - 4VoL_1 - 2E$$

$$h^2 = 4(ML_1 - MLO)^2 - 4(n-1)\ E/n^2$$

The statistics in the above formulae may be explained as follows:

VOLO = Variance of the parents

Vr = Variance of the rth array

V_1L_1 = Mean variance of the array

Wr = The covariance between the parents and their off spring in the rth array.

$WOLO_1$ = The mean covariance between the parents and the arrays

VOL_1 = The variance of the means of array.

(ML_1-MLo) The difference between the mean of the parents and the mean of their n^2 progenies.

E = The expected environmental component of variance which is observed from analysis of variance for design.

The environmental component of variation(E) was calculated as suggested by Aksel and Johnson (1963). The remaining genetic components were calculated according to the diallel analysis proposed by Hayman (1954). These are:

D = Component of variance due to the additive effects of the genes.

H_1 = Component of variance due to the dominance effects of the genes.

H_2 = $(H_1(1-U-V)^2$ where,

U = Proportion of positive genes inthe parents.

V = Proportion of negative genes in the parents.

h^2 = Net dominance effect (as the algebric sum over all loci in heterozygous phase in all crosses).

F = It indicates the relative frequency of dominant and recessive alleles in parents. It may take negative

(-) sign if there is an excess of recessive alleles or the positive (+) sign indicating excess of dominant alleles. The value of F will be zero is dominant and recessive alleles of each gene are distributed equally among parents and the relationship of H_1,H_2 and h^2 will be

$$H_1 = H_2 = h^2$$

3.4.4. Estimation of standard error of genetic components:

The standard errors, to test the significance of the six components (D,H_1,H_2,h^2, F and E) of variation listed above, were calculated using the equation 1/2 var.(Wr-Vr)=S^2 which was used as common multiplier. Now from Hayman's (1954) Table 1 the specific multipliers for each component was calculated. Considering n=6, these were;

For D; $1.17 = (n^5 + n^4)/n^5$

F; $6.96 = (4n^5 + 20n^4 - 16n^3 + 16n^2)/n^5$

H_1; $7.52 = (n^5 + 41n^4 - 12n^3 + 4n^2)\ n^5$

H_2; $6.00 = (36n^4)/n^5$

h^2; $2.72 = (16n^4 + 16n^2 - 32n + 16)/n^5$

E; $0.17 = n^4/n^5$

3.4.5. Estimation of components of variation of F_2:

The components of variation of F_2 were estimated by the formula given by Jinks (1956). The expected statistics for F_2 generation are the same form as those of F_1 except that of contribution of h is halved by one generation of inbreeding. For this reason the coefficient of H_1 and H_2 are ¼ of those of the F_1 statistics, while the coefficient of F is halved, being second and first degree of statistics in h, respectively (Jinks, 1956; Hayman, 1958; Mather and Jinks, 1971).

3.4.6. Test of significance of components of variation:

The significance or the various statistics was tested by 't' test at n-2 degree of freedom. Here n is the number of parents.

$$t = \frac{\text{Parameter}}{\text{S.E. of parameter}}$$

3.4.7. Proportion of genetic components:

The different proportions of the genetic components are worked out according to the procedure given below.

3.4.7.1. Degree of dominance: The mean degree of dominance in F_1 was calculated at $(H_1/D)^{\frac{1}{2}}$ (Hayman, 1954) and in F_2 it was $\frac{1}{4}(H_1/D)^{\frac{1}{2}}$ following Verhalen et al. (1971).

In case of F_1 $(H_1/D)^{\frac{1}{2}}$ = 0 (no dominance)

= 1 (complete dominance)

> 1 (over dominance)

< 1 > 0 (Partial dominance)

3.4.7.2. Proportion of genes with positive and negative effects in the parents:

It was calculated by the ratio $(H_2/4H_1)$. It denotes the mean product of Ui and Vi averaged over all the parents of a diallel set of crosses. When U and V are symmetrically distributed, i.e. U=V= 0.5, the ratio will give the value of $H_2/4H_1 = 0.25$.

3.4.7.3. Proportion of dominant and recessive genes in the parents: It was calculated by the following formulae.

$$\text{For } F_1 = \frac{(4DH_1)^{\frac{1}{2}} + F}{(4DH_1)^{\frac{1}{2}} - F}; \text{ and for}$$

$$F2 \quad \frac{(\frac{1}{4}(4DH_1)^{\frac{1}{2}} + \frac{1}{2}F}{(\frac{1}{4}(4DH_1)^{\frac{1}{2}} - \frac{1}{2}F}$$

If the ratio=1 (equality of dominant and recessive genes)

= >1 (excessive dominant genes)

= <1 (excess of recessive genes

3.4.7.4. Number of groups of genes which control the character and exhibit dominance:

It was calculated by the following formula.

h^2/H_2

It is an approximate measure of sets of genes exhibiting dominance.

3.4.7.5. Estimation of heritability:

Heritability in narrow sense defined as the ratio of additive/or additive x additive genetic variance to the total phenotypic variance. For F_1 it was estimated by using the formula given by Singh and Chaudhary (1977).

$$\text{Heritability } (F_1) = \frac{(\frac{1}{2})D+(\frac{1}{2})H_1 - (\frac{1}{2})H_2-(\frac{1}{2})F \ldots}{(\frac{1}{2})D+(\frac{1}{2})H_1 - (\frac{1}{4})H_2-(\frac{1}{2})F+E}$$

and for F_2 it was calculated following Verhalen and Murray (1969).

$$\text{Heritability } (F_2) = \frac{\frac{1}{4}D}{\frac{1}{4}D + \frac{1}{16}H_1 - \frac{1}{8}F+E}$$

3.4.8. Combining ability analysis:

The combining ability analysis was carried out by the procedure given by Griffing (1956). Method 2 and Model 1 was used for the present study. Method 2 was applicable to the present situation as parents and one set of F_1s were included while reciprocal F_1s were not.

The mathematical model for the combining ability in Model I is assumed to be:

$X_{ij} = m + g_i + s_j + s_{ij} + 1/bc \; \xi e_{ijkl}$

$i,j = 1,2, \ldots\ldots P,$

$k = 1,2, \ldots\ldots b$

$i = 1,2, \ldots\ldots c$

Where,

m is the population mean, $g_i(g_j)$ is the general combining ability effect for the i th (jth) parents, s_{ij} is the specific ability effect for the cross between the i th and jth parents such that $s_{ij} = s_{ji}$, and e_{ijkl} is the effect of ijklth observation.

3.4.8.1. Estimation of the combining ability variance: General and specific combining ability variance were estimated by the following formulae.

The sum of squares were calculated as follows:

$S_g = 1/n+2 \; [\xi(x_{i.} + x_{ii})^2 - 4/n \; x^2_{..}]$

$S_s = \xi\xi \; x_{ij}^2 - 1/n+2 \; \xi(x_{i.} + x_{ii})^2 + 2/(n+1)(n+2) \; x^2_{..}$

Where,

S_g = the sum of squares due to general combining ability

S_s = the sum of squares due to specific combining ability

n = the number of parents.

x_i = the total of the array of ith parent,

x_{ii} = the mean value of the ith parent,

$x_{..}$ = the grand total of $\frac{1}{2}n(n-1)$ progenies and parental values and

x_{ij} = the progeny mean value in the diallel table.

Analysis of variance table for combining ability giving expectations of mean squares was set up as follows:

TABLE 4

Expectation of mean squares for general and specific combining ability

Source	d.f.	M.S.S.	Expectation of mean squares
General combining ability	n-1	M_g	$\sigma^2_e + (n+2)(1/n-1)\sum gi^2$
Specific combining ability	$\frac{n(n-1)}{2}$	M_s	$\sigma^2_e + (2/n(n-1)\sum\sum S_{ij}{}^2$
Error	m	$M_{e'}$	σ^2_e

Where,

n = number of parents

M_g, M_s, M_e are mean sum of squares (variance) due to GCA, SCA and error respectively.

The mean sum of squares of general combining ability and specific combining ability were obtained by dividing their sum of squares by respective degrees of freedom. The error mean squares (M_e') for combining ability analysis was obtained dividing error mean square (M_e) obtained from the general analysis of the experiment by the number of replications. For 'F' test, each mean square was tested against M_e' at n_1 and n_2 degree of freedom.

3.4.8.2 Estimation of general and specific combining ability effects:

The various effects of general and specific combining ability were estimated as follows:

General combining ability effects of ith parent:

$g_i = 1/n+2\ (x_{i.} + x_{ii} - 2/nx..)$

Specific combining ability effects of ijth cross;

$S_{ij} = x_{ij} - 1/n+2\ (x_{i.} + x_{ii} + x_j + x_{ij}) + 2/(n+1)\ (n+2)\ x..$

Where,

n, x_i, x_{ii} and x.. are same as explained above.

3.4.9. Standard error of the estimates:

Standard error of the estimates was calculated as the square root of the variance of the estimates. The variances of the various estimates were calculated as follows:

Variance $(x_{ij}) = \delta^2 = M_e$; variance(U) = $\frac{2}{n(n+1)} \delta^2$

Variance $(g_i) = \frac{(n+1)}{n(n+2)} \delta^2$

Variance $(s_{ij}) = \frac{(n^2+n+2)}{(n+1)(n+2)} \delta^2$

Where n is the number of parents and $\delta^2 = M_e$.

3.4.10. Critical difference:

Critical difference used to test the significance of difference of the two estimates was taken as product of the 't' for error degrees of freedom and the standard error of the difference of the two estimates taken as the square root of the variance of the difference of the two estimates. The variance of the difference of the two estimates was calculated as follows.

Variance $(g_i - g_j) = \frac{2}{(n+2)} \delta^2$ for general combining ability ($i \neq j$) estimates of different parents.

Variance $(S_{ij} - S_{ik}) = \frac{2(n+1)}{(n+2)} \sigma^2$ for specific combining ability estimates within array.

$(i \neq j;\ k \neq j)$

Variance $(S_{ij} - S_{kl}) = \frac{2n}{(n+2)} \sigma^2$ for compairing two specific combining ability effects.

$(i \neq j,k,l;\ j \neq k,l;\ k \neq l)$

Where, n is the number of parents and $\sigma^2 = M_e$

3.5. Studies with Fungicides:

3.5.1. Test fungicides:

Seven promising systemic as well as contact fungicides of different chemical composition, were selected to test their efficacy against Tolyposporium penicillariae. The characteristics of these fungicides are given in Table 5.

3.5.2. In vitro studies:

Different concentrations of each fungicide were prepared on active ingradient basis by dissolving in sterile distilled water as suggested by Nene and Thapliyal (1979). In case of acetone soluble fungitoxicants initially 20% acetone solution was used. Subsequent dilutions were prepared in sterile distilled water. After adjusting the dilutions to the required concentrations, the fungicides were used to study the effect on teliospore germination and growth of the fungus.

3.5.2.1. Effect of different fungicides on teliospore germination:

A virulent isolate of Tolyposporium penicillariae from the rainy season crop of 1985 from the pearl millet hybrid NHB-3 was used for this study. Matured smut sori from the affected inflorescence were carefully

TABLE 5

Characteristics of test fungicides

Sr. No.	Common name	Trade name	Chemical name	Molecular weight	Formulation
1	Benomyl	Benlate	Methyl-1-(butylcarbamoyl) benzimidazol-2-carbamate	290.62	50 WP
2	Captafol	Difolatan	1,2,3,6-tetrahydron-(1,1,2,2-tetrachloroethylthio) Phthalimide	349.06	50 WP
3	Carbendazim	Derosal	Methyl-2-benzimidazol-carbamate	191.19	50 WP
4	Carbendazim (50% W/W)	Bavistin	Methyl-2-benzimidazol-carbamate	191.19	WP
	+	+	+		
	Thiram (50% W/W)	TMTD	Bis(dimethylthiocarbamoyl) disulphide.	240.40	WP
5	Carboxin	Vitavax	5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide	235.31	50 WP
6	IBP	Kitazin	O,O-diisopropyl S benzyl phosphorothionate	285.05	48 EC
7	Tridemorph	Calixin	2,6-dimethyl-4-trideoyl morpholine N-tridecyl-2, 6-dimethylmon-pholine.	297.53	70 EC

WP = Wettable powder ; EC = Emulsifiable concentrate.

crushed in a sterile Petri dish to get teliospores. Teliospore suspension was made in sterilized distilled water. The concentration was so adjusted that each drop on an average contained 10-15 teliospores under low-power microscopic field. For the present investigation, the fungitoxicants were adjusted to 0, 1 , 50, 100, 200 and 500 µg/ml on active ingredient basis. 2 ml of each of the fungicidal solution (of double concentration) was taken in a clean sterile test tube and 2 ml of standard spore suspension was added to it to make required fungicide spore concentration. A drop of mixture of fungicide and spore was placed on a clean and sterile glass cavity slide. The slide was kept in a Petri dish lined on both the sides with moistered filter paper. Three replications with three Petri dishes in each replicate were maintained. Each Petri dish had two cavity slides. Controls were maintained with teliospores in sterile distilled water. All Petri dishes were kept in an incubator at 30° C for 30 hours. After the required incubation period, a drop of lactophenol was put on each cavity slide to arrest any further germination. Then, teliospore germination was recorded and the percentage of inhibition of germination with respect to control was calculated by the following formula.

$$\text{Per cent inhibition} = \frac{\text{Germination in control (\%)} - \text{Germination in fungitoxicant solution (\%)}}{\text{Germination in control (\%)}} \times 100$$

3.5.2.2. Effect of different fungicides on fungal growth:

Potato agar medium (pH 6.0) was used as the growth substrate for this experiment. Desired volumes of the individual fungicide were incorporated aseptically in

100 ml cooled (40° C) steriled medium in 250 ml flasks to give final concentrations of fungicide in the medium to 0, 10, 20, 30, 40 and 50 µg/ml, on active ingredient basis.

20 ml of each resultant mixtures was poured into a sterile Petri dish (9 cm diam) and allowed to solidify. Each dish was then inoculated centrally with 0.01 ml of inoculum of sporidial concentration 6 x 10^5 sporidia ml^{-1}, taken from the 5 day old Tolyposporium penicillariae colony growing on potato agar medium. The inoculated petri dishes were incubated at 35° C and colony diameter was measured after 120 hours. Three replicates consisting of 6 dishes were used for each concentration of a fungicide together with fungicide-free control dishes.

3.5.3. Foliar spray:

Different fungicides selected for earlier experiments were also used as foliar spray in recommended doses, in the field trials on artificially inoculated plants in order to determine their relative efficacy in reducing the smut severity so that more effective fungicides against the disease could be identified. The details of methodology have been described below.

3.5.3.1. Pre-inoculation spray: (at boot emergence stage)

For this investigation, plots of 3 x 2 m size were planted with susceptible hybrid NHB-3 in a Randomized Block Design with three replications. Each plot was used for a single treatment. An individual treatment was assessed on 25 plants taken at random. Earheads that were

ready for emergence from the boot within next 48 to 72 hours were protected from outside contamination by covering them with pollination bags, as suggested by Pathak and Gaur (1975). The plants were sprayed with fungitoxicants 24 hours before inoculation on the upper foliage including the ears until dripping wet by temporarily lifting the paper bags with due care to avoid fungicidal drift to adjacent plots. Plants in control plots were sprayed with water only. After 24 hours, 5 ml of teliospore suspension was inoculated to the boot with help of an atomizer as described elsewhere. An uniform teliospore suspension was earlier prepared by soaking 300 surface-sterilized green sori in 2 litres of sterile water for 24 hours followed by straining through sterile mulsin cloth. After inoculation, the heads were sprayed with a fine mist of water and the bags were replaced to maintain high humidity. Considering the climatic condition, regular spray of water for 2-3 times a day was carried out throughout the duration of experiment to maintain required humidity. Data on smut severity, were recorded after complete grain formation according to the standard smut severity assessment key stated earlier and the mean disease severity was calculated. The per cent values were transformed into angular values (Sin-1 $\sqrt{\text{percentage infection}}$) and the data were subjected to statistical analysis. Relative efficacy of different fungicides in reducing smut severity was determined on the basis of control.

3.5.3.2. Post-inoculation spray:(At boot-emergence stage)

For this investigation, different numbers of post-inoculation, sprayings varying from 1-4 were done on

artificially inoculated plants in separate experiments, laid out in Randomized Block Design with 3 replications in order to find out the optimum numbers of post-inoculation fungicidal sprays to reduce the smut severity effectively. In each experiment, 25 plants taken at random from a plot of 3 x 2 m size meant for each treatment were inoculated first with an uniform teliospore suspension prepared as described earlier. The inoculated heads were bagged immediately and high humidity was maintained by water spraying. 24 hours after the inoculation, foliage including the ears were sprayed with the fungitoxicants by carefully lifting the bags. Bags were replaced after the spraying. Relative humidity was maintained above 70% by regular spray of water for 2-3 times a day. The 2nd, 3rd and 4th spray were done at 7 days interval from Ist, 2nd and 3rd spray respectively. Data were recorded once grain formation was complete and effectiveness of different numbers of post-inoculation fungicidal spray was determined by analysing the data statistically after angular transformation.

3.5.3.3. Pre-inoculation followed by post-inoculation spray (at boot emergence stage)

In order to, find out the possibility of controlling the pearl millet smut with a suitable combination of prophylactic and curative sprays the present investigation was planed. Separate experiments consisting of plots of 3 x 2 m size in 3 R.B.D. design were laid out to assess the fungitoxicants in three different combinations. In the first experiment, one pre-inoculation spray was followed by a single dose of post-inoculation spray. In the other two experiments,

One pre-inoculation spray was followed by two and three numbers of post-inoculation spray at an interval of 7 days. The method of inoculation and spray procedures followed were as described earlier. In all the experiments plants in the control plots were sprayed with water. High humidity was maintained throughout the experimental period. Data on smut severity were recorded once the grain formation was complete and the mean disease severity was calculated. After aresin transformation the data were subjected to statistical analysis and efficacy of different fungicides in different combinations was assessed on the basis of control.

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EXPERIMENTAL RESULTS

Results obtained on the investigations entitled "Studies on pearl millet smut with special reference to pathogenic variability, inheritance of resistance and chemical control" are presented below.

4.1 Studies on Variability in the Pathogen

4.1.1. Morphological Studies:

Sporeballs, teliospores and sporidia of each isolate were studied separately under a compound microscope for differences in morphological characteristics. Observations on each of them have been detailed below.

4.1.1.1. Sporeballs:

Three characteristics viz., colour, shape and size of 150 sporeballs from each isolate were microscopically studied. It was observed that there was no variation in colour and shape of the sporeballs among isolates. The compact sporeballs in all the isolates showed dark-brown to brownish black colour while the shape was semi-circular of irregular. Measurement of length and breadth of sporeballs revealed variation among isolates. However, on the basis of these variations the isolates could not be effectively classified into different groups. Sporeball size of Mahendragarh (MGH) isolate was found to be

the largest followed by Ludhiana (LDH), Jodhpur (JDH), ICRISAT (ICR), Hisar (HSR) and Jaipur (JPR) in that order. Mean size of sporeballs of each isolate along with their range are presented in Table 6.

TABLE 6

Size (Length x breadth) of sporeballs of Tolyposporium penicillariae isolates

Isolate	Size [a] (μ)	
	Mean	Range
HSR	119.2 x 90.8	49.9-216.6 x 41.6-174.9
ICR	132.5x102.5	41.6-258.2 x 41.6-166.6
JDH	133.3 x 97.5	50.0-274.9 x 41.6-166.6
JPR	115.2 x 89.5	49.9-233.2 x 33.3-133.2
LDH	145.0 x 110.8	58.3-258.2 x 58.3-224.9
MGH	211.6 x 161.6	83.3-374.8 x 75.0-333.2
S.E.(m)±	0.86 x 0.59	
L.S.D.(0.05)	2.64 x 1.82	

a = Based on measurements of 150 sporeballs.

4.1.1.2. Teliospores:

Observation on teliospores of different isolates, revealed no marked difference in colour and shape of the spores among the isolates. The colour of the individual teliospore of all isolates was found to be brown to yellowish-brown while the shape varied from globose to sub-glubose. Mean diameter of the teliospores, based on measurement of 150 teliospores, however, revealed variation among the

isolates (Table 7). It was observed that the diameter of MGH isolate was maximum followed by LDH, ICR, JDH, HSR and JPR isolates respectively. It was also evident that there was no definite correlation between the size of the sporeball and the diameter of the teliospore of the isolates. There was also no variation among the isolates in regard to numbers of exospore thickening. All the isolates were found to possess 3-5 thickenings on the exospore of the teliospore.

TABLE 7

Diameter of teliospores of Tolyposporium penicillariae from different locations (isolates)

Isolate	Diameter [a] (μ)	
	Mean	Range
HSR	10.4	7.0 - 13.0
ICR	10.8	8.0 - 14.0
JDH	10.5	7.0 - 14.0
JPR	9.3	6.0 - 13.0
LDH	11.2	6.6 - 14.0
MGH	12.0	8.0 - 18.0

S.E.(m) ± 0.05
L.S.D. (0.05) 0.18

[a] Based on measurements of 150 teliospores

4.1.1.3. Sporidia:

The sporidia of each isolate were obtained from 5 day old axenic culture on potato agar medium. Observation on colour and shape of individual sporidium of each isolate revealed no characteristic variation. Sporidia

were hyaline for all the isolates and were single-celled. Sporidia were spindle-shaped in all the isolates. However, their length varied in different isolates (Table 8). It is evident from the result that the sporidia of MGH isolate were the longest followed by ICR, LDH, JDH, HSR and JPR isolates respectively. However, no definite correlation of length of the sporidia with size of the sporeballs as well as diameter of the teliospores could be achieved.

TABLE 8

Length of sporidia of Tolyposporium penicillariae from different locations (isolates)

Isolate	Length [a] (μ)	
	Mean	Range
HSR	14.0	10.0-21.0
ICR	16.5	8.0-22.0
JDH	14.5.	8.0-25.0
JPR	13.0	9.0-18.0
LDH	15.0	10.0-25.0
MGH	20.0	10.0-25.0
S.E.(m) ±	0.24	
L.S.D.(0.05)	0.73	

[a] Based on measurement of 150 sporidia.

4.1.2. Spore Germination Studies:

Two types of germination studies were conducted. In the first experiment, germination of aggregated teliospores (sporeballs) for each isolate was calculated after 20 hrs of incubation at 30° C. In the second experiment, regular observations were made at half-hourly interval to

find out the threshold time of germination for different isolates. The results of both the experiments are presented in Table 9. It was observed that the mean percentage of germination of sporeballs of the ICR isolate was maximum followed by HSR isolate without any significant difference between them. Germination percentage of ICR isolate was however, significantly higher than the other isolates except that from HSR. There was no significant difference in germination percentage between HSR and MGH isolates but both of these isolates showed significant difference from LDH, JDH and JPR isolates. The germination percentage of JPR isolate was found to be the lowest.

TABLE 9

Germination percentage and threshold time for germination of sporeballs of different isolates of Tolyposporium penicillariae

Isolate	Mean germination [a] (%)	Threshold time for germination [b] (hr.)
HSR	50.00	10.30
ICR	54.00	11.00
JDH	26.67	11.30
JPR	24.33	13.30
LDH	33.30	11.30
MGH	48.00	10.00
S.E.(m) ±	1.45	0.25
L.S.D.(0.05)	4.55	0.78

a Mean of 600 sporeballs in 3 replications.

b A sporeball was considered germinated when atleast one promycelium had emerged.

With regard to the threshold time for germination, it was observed that the minimum time required for germination of aggregated teliospore was 10 hrs in MGH isolate followed by HSR isolate (10.30 hrs.) without any significant difference between them. However, threshold time in MGH isolate was significantly different from the other isolates except that of HSR. The maximum threshold time was found in JPR isolate (13.30 hrs.). There was however, no definite correlation observed between mean germination percentage and threshold time for germination.

4.1.3. Cultural studies:

4.1.3.1. Effect of different media on growth:

Five different media, comprising three semi-synthetic and two synthetic were used to study the growth characteristics of different isolates. The media in Petri dishes were inoculated with equal quantities of uniform sporidial suspension from each of the isolate. After 120 hrs of incubation it was seen that, all the isolates produced typically sporidial type of growth in all the media tested without any trace of mycelial growth (plate 6). There was however, no marked variation among the isolates in respect of colony characteristics on different media. Table 10 shows the common growth characteristics of all the isolates tested on different nutrient media.

Measurement of colony growth after 120 hours of incubation at 35° C showed that potato agar medium supported maximum growth of the fungus followed by carrot agar and potato

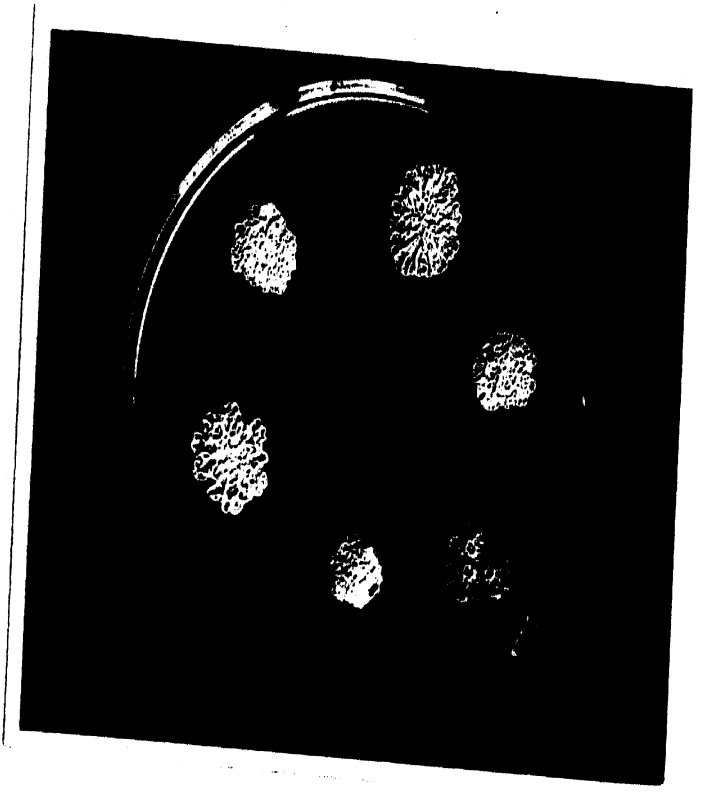

PLATE 6. INDISTINGUISHABLE COLONY CHARACTERISTICS OF DIFFERENT SMUT ISOLATES ON POTATO AGAR MEDIUM

1. HSR HISAR
2. ICR ICRISAT
3. JDH JODHPUR
4. JPR JAIPUR
5. LDH LUDHIANA
6. MGH MAHENDRAGA

TABLE 10

Cultural characteristics of isolates of Tolyposporium penicillariae grown on different nutrient media at 35° C.

Medium	Colour	Margin	Topography
Potato agar	White	Wavy	Flat, broadly ridged
Potato dextrose agar	Creamy	Entire	Raised
Carrot agar	Dull white	Entire	Raised
Brown's medium	Dull white	Entire	Flat
Richards's medium	Creamy	Wavy	Flat

dextrose agar (Table 11). Both the synthetic media viz., Brown's and Richards's however, supported poor growth of the fungus.

TABLE 11

Colony diameter of the isolates of Tolyposporium penicillariae grown on different nutrient media after 120 hrs.

Media	Isolates						Mean
	HSR	ICR	JDH	JPR	IDH	MGH	
	Mean colony diameter[a] (mm)						
Potato agar	13.8	13.7	13.2	13.4	13.0	13.5	13.4
Potato dextrose agar	8.8	8.5	8.4	8.5	8.5	9.0	8.6
Carror agar	12.4	12.4	12.3	12.5	12.4	12.3	12.4
Brown's medium	6.0	6.0	6.2	5.8	6.2	6.2	6.1
Richards's medium	4.0	4.1	4.1	3.9	4.2	4.1	4.1
Mean	9.0	8.9	8.8	8.8	8.9	9.0	

S.E.(m) ± : Media = 0.07; Isolate = 0.08; Media x Isolate = 0.31

L.S.D.(0.05): Media = 0.17; Isolate = 0.21; Media x Isolate = 0.86

a = Based on measurement of 20 colonies in 4 replications.

It is clear from the results that statistically there was no differences in growth among the isolates studied. All the isolates produced similar type of colonies on a specific medium and colony differences in colour were also indistinguishable. Other growth characteristics i.e. type of growth, shape of the colonies also revealed no variation as well. It is also evident from the results that the semi-synthetic media are better than the synthetic media for culturing of the fungus and potato agar and carrot agar media are still better than potato dextrose agar.

4.1.4. Physiological Studies:

4.1.4.1. Effect of temperature on growth:

Potato agar medium was used as a growth substrate for the present study. Equal quantity of uniform sporidial suspension from each isolate was inoculated on the medium in Petri dishes and the dishes were kept at six different temperatures. After 120 hrs of incubation, the Petri dishes were examined for colony growth. It was observed that the maximum growth of the fungus on potato agar medium occurred at 35°C in all the isolates, whereas at 40°C the growth was found to be minimum (Table 12). Results indicate significant effect of temperature on the growth of the fungus. On increasing trend of temperatures from 15°C to 35°C, the growth gradually became profuse and at 35°C it attained maximum but at 40°C drastically reduced.

At lower temperatures, i.e. 15°C, 20°C and 25°C initially the colonies remained dull and as the incubation time increased

TABLE 12

Colony diameter of the isolates of Tolyposporium penicillariae on potato agar medium at different temperatures of incubation

Isolates	Temperature (°C)						
	15	20	25	30	35	40	Mean
	Mean colony diameter[a] (mm)						
HSR	6.1	10.5	12.1	13.0	13.5	3.0	9.7
ICR	6.0	10.8	12.1	13.3	13.3	3.1	9.8
JDH	6.1	10.6	12.0	13.2	13.4	2.9	9.7
JPR	5.9	10.3	12.3	13.2	13.4	3.1	9.7
LDH	6.1	10.6	12.2	13.3	13.6	3.2	9.8
MGH	6.0	10.4	12.4	13.2	13.3	3.0	9.7
Mean	6.0	10.5	12.2	13.2	13.4	3.1	

S.E.(m)± : Isolate=0.05; Temperature = 0.05;
Isolate x Temperature = 0.17

L.S.D.(0.05): Isolate = 0.14; Temperature = 0.14;
Isolate x Temperature = 0.46

[a] Based on measurement of 20 colonies in 4 replications.

it turned white. At 30°C the colour of the colonies remained white throughout the incubation period. At 35°C the colour of the colonies were similar to the colonies at 30°C. However, it is clear from the results that statistically there was no variation in growth among the isolates. All the isolates produced similar type of colonies at a specific temperature and colour was also same. Results signified that maximum multiplication of the fungus could be possible at incubation temperature 35°C.

4.1.4.2. Effect of pH on growth: Since differential sensitivity to H-ion concentration (pH) is known to

occur among different isolates of a given fungal species, it seemed that the present study be carried out. Potato agar medium was at first adjusted to five different pH levels, i.e.5.0, 5.5, 6.0, 6.5 and 7.0 and equal quantity of medium of each pH level in Petri dish was inoculated with equal quantity of uniform sporidial suspension of each isolate. Results presented in Table 13 show what at pH 6 the growth of all the isolates was maximum, whereas on either side of pH 6 the growth was found to be less. There were statistically significant differences in colony diameter for each pH value when averaged across all the isolates, indicating that pH had significant effect on growth of the colonies. However, statistically the different isolates did not respond differently to different pH values. This signifies that there was no differential sensitivity to H-ion concentration among different isolates of T. penicillariae.

TABLE 13

Effect of pH on colony growth of Tolyposporium penicillariae isolates grown on potato agar medium.

Isolates	H-ion concentration					
	5.0	5.5	6.0	6.5	7.0	Mean
	Mean colony diameter[a] (mm)					
HSR	8.2	12.4	13.3	13.0	10.5	11.5
ICR	8.4	12.1	13.4	13.2	10.4	11.5
JDH	8.5	12.3	13.2	12.9	10.6	11.4
JPR	8.3	12.2	13.1	13.0	10.4	11.4
LDH	8.5	12.2	13.5	13.1	10.2	11.5
MGH	8.1	12.1	13.4	13.0	10.5	11.4
Mean	8.3	12.2	13.3	13.0	10.4	

S.E.(m)± = Isolate = 0.04; pH = 0.07; Isolate x pH =0.28

L.S.D.(0.05) = Isolate = 0.16; pH = 0.18; Isolate x pH =0.75

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[a] Based on measurement of 20 colonies in 4 replications.

4.1.5. Pathological Studies:

Pathogenic variabilities in the isolates were studied by inoculating three resistant and four susceptible genotypes with each of the six isolates. The inoculation and disease scoring procedures have been described in details under 'Materials and Methods'.

The results in Table 14 suggests that three resistant genotypes - ICMPS 900-9-3, ICMPS 700-1-5-4 abd ICMPS 1500-7-3-2 showed resistant reaction to all the six isolates studies. Whereas four susceptible genotypes 833B, 843B, 834B and BJ-104 exhibited susceptible reaction to all isolates. Results from statistical analysis suggest non-significant differences between the virulence of isolates and susceptibility of genotypes to specific isolates. When the severity percentage are averaged across all the isolates, there are statistically significant differences in susceptibility among genotypes. Among the susceptible genotypes, BJ-104 was more susceptible to all isolates than the other genotypes. All the resistant genotypes exhibited high levels of resistance to all the isolates signifying non-existence of pathogenic variability in the isolates.

4.2 Inheritance Studies:

The present investigations comprised genetical studies for smut resistance, days to 50% boot leaf stage (DTBL),

TABLE 14

Pathogenicity of different isolates of *Tolyposporium penicillariae* grown on potato agar medium at 35°C to seven genotypes of pearl millet.

Isolates	Smut severity[a] (%) on genotypes							Mean
	ICMPS 900-9-3	ICMPS 700-1-5-4	ICMPS 1500-7-3-2	833B	843B	834B	BJ104	
HSR	0 (0)	0.33 (1.91)	0 (0)	41.67 (40.20)	55.00 (47.88)	30.00 (33.16)	86.67 (68.66)	30.52 (27.40)
ICR	0 (0)	0 (0)	0 (0)	14.60 (40.18)	55.67 (48.27)	31.67 (34.18)	84.00 (66.44)	30.42 (27.35)
JDH	0 (0)	0 (0)	0 (0)	43.33 (41.15)	51.67 (45.96)	30.00 (33.21)	88.33 (70.11)	30.48 (27.21)
JPR	0 (0)	0 (0)	0.33 (1.91)	46.33 (42.88)	51.50 (45.85)	31.33 (34.04)	83.00 (65.67)	30.36 (27.20)
LDH	0 (0)	0 (0)	0 (0)	41.67 (40.22)	53.33 (46.91)	31.65 (34.15)	83.33 (65.95)	30.00 (26.74)
MGH	0 (0)	0 (0)	0.25 (1.81)	42.00 (50.40)	56.00 (48.45)	28.33 (32.02)	85.00 (67.21)	30.23 (27.17)
Mean	0 (0)	0.06 (0.32)	0.10 (0.72)	42.77 (40.80)	53.86 (47.24)	30.50 (33.46)	85.06 (67.34)	

S.E.(±): Isolate = 0.65; Genotype = 0.70; Isolate x Genotype = 1.73

L.S.D.(0.05): Isolate = 1.80; Genotype = 1.94; Isolate x Genotype = 4.76.

a = Mean of 30 inoculated heads from 3 replications. Figures in parentheses are angular transformed values.

effective tiller number, plant height, ear length, 1000 grain weight and yield per plant. The data on smut were analysed on the basis of disease severity in the F_1 as well as F_2 generations while for other characters, only F_1 data were used. The following analyses were done in order to find out the inheritance pattern of the smut disease.

1. Component analysis (Both numerical and graphical).
2. Combining ability analysis.

4.2.1. Analysis of Variance:

The analysis of variance for 6 x 6 diallel excluding reciprocals tested in randomized block design for 7 characters, are presented in Table 15.

The mean squares due to treatment were highly significant for smut reaction on the basis of disease severity, DTBL, effective tiller numbers, plant height, ear length, 1000 grain weight and yield per plant.

Analysis of variance for smut reaction in F_2 generation also showed significant treatment differences.

4.2.2. Component Analysis:

The estimates of various genetic component are presented in Table 16.

The estimation of genetic component of variation requires fulfilment of certain assumptions which are given in 'Materials and Methods'.

To test whether these assumptions were satisfied, t^2 test and test for b-0 and b-1 were applied. It is evident from the table that t^2 values were non-significant for

TABLE 15

Variance for parental, F_1 and F_2 (smut severity) population for 7 characters in pearl millet.

Source	d.f.	Smut severity	DTBL[a]	Plant height	Effective tillers/ plant	Ear-length	1000-grain weight	Yield/ plant
Replication								
F_1	2	7.90	1.96	15.25	0.14	0.07	0.01	0.83
F_2	2	41.00						
Treatment								
F_1	20	712.49**	99.84**	5036.91**	3.87**	40.76**	6.69**	755.9
F_2	20	560.28**						
Error								
F_1	40	7.17	0.30	8.93	0.24	0.28	0.003	0.23
F_2	40	6.09						

a Days to 50% boot leaf stage;

** Significant at P = 0.01.

Component	Smut severity F_1	Smut severity F_2	DTBL F_1	Plant height F_1	Effective tillers/ plant F_1	Ear length F_1	1000 grain weight F_1	Yield/ plant F_1
D	511.65** ±29.47	514.00** ±24.01	39.68** ±5.56	1537.66** ±163.04	2.19** ±0.24	32.55** ±5.86	1.26* ±0.36	18.16 ±13.14
H_1	365.63* ±74.73	1106.69* ±243.84	38.25 ±14.10	1877.05** ±33.35	1.55 ±0.61	24.56** ±4.73	7.66** ±0.91	1064.65* ±185.44
H_2	330.84** ±66.75	921.73** ±217.83	30.30 ±12.60	1673.52** ±29.22	1.24 ±0.54	21.18** ±4.22	5.60** ±0.81	1018.78* ±165.44
h^2	456.00** ±44.93	403.10** ±146.61	2.25 ±8.48	2130.64** ±248.59	0.10 ±0.37	60.47** ±2.84	7.20** ±0.54	1489.91* ±111.52
F	219.18* ±71.90	585.82** ±116.76	-12.34 ±13.57	183.68 ±397.66	0.50 ±0.59	-0.16 ± 4.55	1.20 ±0.87	35.34 ±18.76
$(ML_1-ML_o)^2$	114.70	107.54	0.58	2033.11	0.01	15.13	1.80	372.49
E	4.94 ±11.23	2.59 ±9.07	0.13 ±2.12	3.08 ±2.14	0.08 ±0.10	0.09 ±0.71	0.15 ±1.15	0.09 ±27.88
t^2	1.35	1.74	0.07	3.10	7.00*	0.86	3.12	16.52*

* Significant at P = 0.05

** Significant at P = 0.01.

most of the characters in both the generations. Obviously, the estimates of parameters will have a valid interpretation for these characters. A perusal of data in the Table 16 showed that additive component (D) was significant for smut severity, DTBL, plant height, effective tillers per plant, ear length and 1000 grain weight. It was however, non-significant for yield per plant. The dominance components H_1 and H_2 were also significant for smut severity, plant height, ear length, 1000 grain weight and yield per plant. These components were however found insignificant for DTBL and effective tillers per plant. In all the cases, estimates of H_1 was higher than H_2. Significance of D, H_1 and H_2 suggested that importance of both additive and non-additive type of gene action involved in the control of these particular traits. Estimates of additive components were higher than dominance components in respect of smut severity, DTBL, plant height, effective tillers per plant and ear length suggesting that additive genetic variance in these traits was more important than dominance genetic variances. Contrary to it, dominance genetic variances were more important than additive genetic variance in respect of 1000 grain weight and yield per plant. The estimates of H_2 component was smaller than H_1 indicating that positive and negative alleles at the loci governing these characters were not equal in proportion in the parents. The component h^2 which is net dominance effect over all the loci was significant for smut severity, plant height, ear length, 1000 grain weight and yield per plant. This

TABLE 17

Proportion of genetic components of variation and heritability estimates in F_1 and F_2 (Smut severity) progenies in pearl millet.

Proportion	Smut severity		DTBL	Plant height	Effective tillers/ plant	Ear length	1000 grain weight	Yield/ plant
	F_1	F_2	F_1	F_1	F_1	F_1	F_1	F_1
$(H_1/D)^{\frac{1}{2}}$	0.84	0.73	0.97	0.76	0.84	0.86	2.46	7.65
$H_2/4H_1$	0.23	0.21	0.20	0.19	0.20	0.22	0.18	0.24
Proportion of D and R genes in the parents	1.68	2.96	0.73	1.09	1.31	0.99	1.48	1.29
h^2/H_2	1.38	0.44	0.07	3.04	0.05	2.86	0.07	1.46
Heritability (Narrow sense)	0.65	0.80	0.80	0.54	0.72	0.60	0.43	0.05

character was insignificant in DTBL and effective tillers per plant.

'F' component which is a measure of covariance between additive and dominance effect and can be either + Ve if u (proportion of dominant or + Ve alleles) or - Ve if u is less than v. The F component was significant and + Ve for smut severity only. This indicated excess of dominant genes for this character.

The estimates of difference between the means of parents and the means of their progenies $(ML_1-ML_0)^2$ was high for plant height, disease reaction and yield per plant whilt it was quite low in other cases. The error component was in significant for all the traits.

In F_2, both additive (D) and non-additive (H_1 and H_2), h^2 and F components were significant for this trait. However, the E component was non-significant.

In Table 17, the relative proportion of various genetic components along with heritability estimates in narrow sense are presented.

The estimates of degree of dominance $(H_1/D)^{\frac{1}{2}}$ revealed partial dominance for smut severity, DTBL, plant height, effective tillers per plant and ear length and over dominance for 1000 grain weight and yield per plant. When the dominant and recessive alleles are symetrically distributed i.e. U=V=0.5 the ratio of $H_2/4H_1$ is equal to 0.25. Obviously, any deviation from this ratio suggests asymetrical distribution of dominant and recessive alleles in the population.

The appraisal of this value in Table 17 shows asymetrical distribution of dominant and recessive alleles for all the traits studied here. The proportion of dominant and recessive alleles in the parents shows that except for DTBL and ear length, most of the characters are governed by two or more dominant alleles for every recessive alleles.

The ratio of h^2/H_2 refers to the number of alleles or allelic groups showing dominance. It is evident from the estimates that in most of the cases this ratio was quite low.

The estimates of heritability value was moderately high for disease reaction, DTBL, plant height, effective tillers per plant and ear length. For 1000 grain weight and yield per plant this ratio was relatively low.

In F_2 the estimate of degree of dominance revealed partial dominance for smut severity. Asymetrical distribution of positive and negative gene was also observed. The proportion of dominant and recessive alleles in the parents shows that there were two or more dominant alleles for every recessive allele. Heritability estimate signifies moderately high heritability for smut severity in F_2 population.

4.2.3. Graphical Analysis:

The linear regression of Wr on Vr, the limiting parabola and the scatter of parental points along with regression line are presented in Figs. 1-8.

4.2.3.1. Smut severity:

The graphical analysis of smut resistance based on disease severity was done in F_1 and F_2 generations.

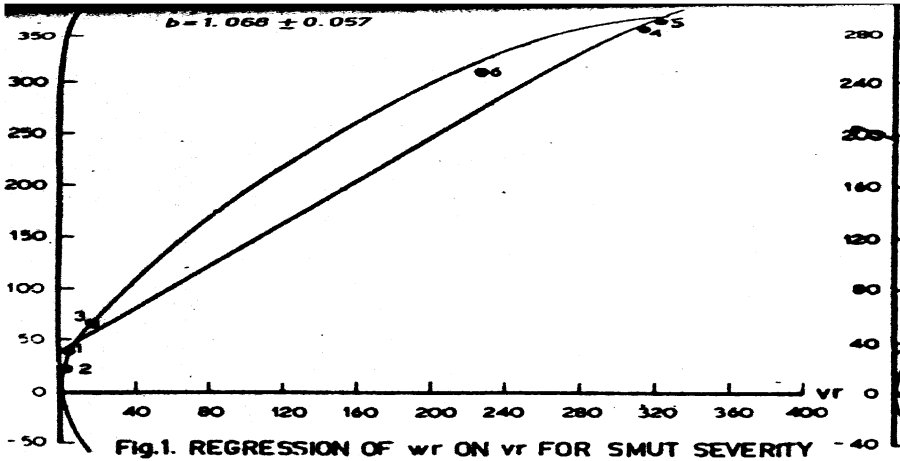


Fig.1. REGRESSION OF wr ON vr FOR SMUT SEVERITY

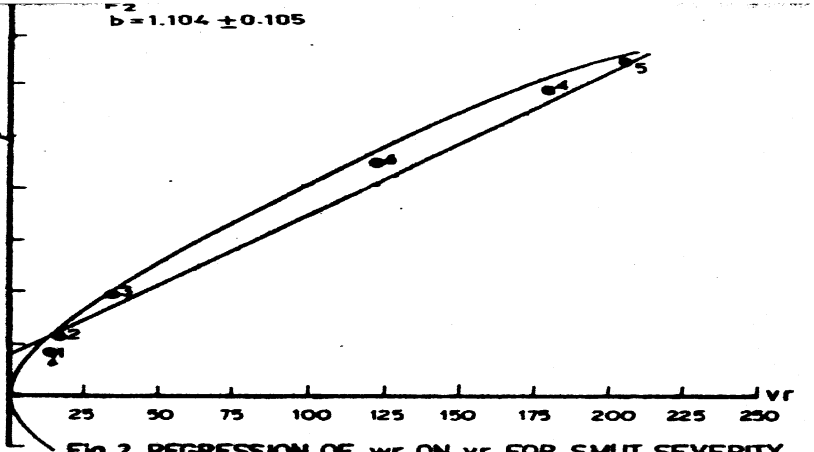


Fig. 2. REGRESSION OF wr ON vr FOR SMUT SEVERITY

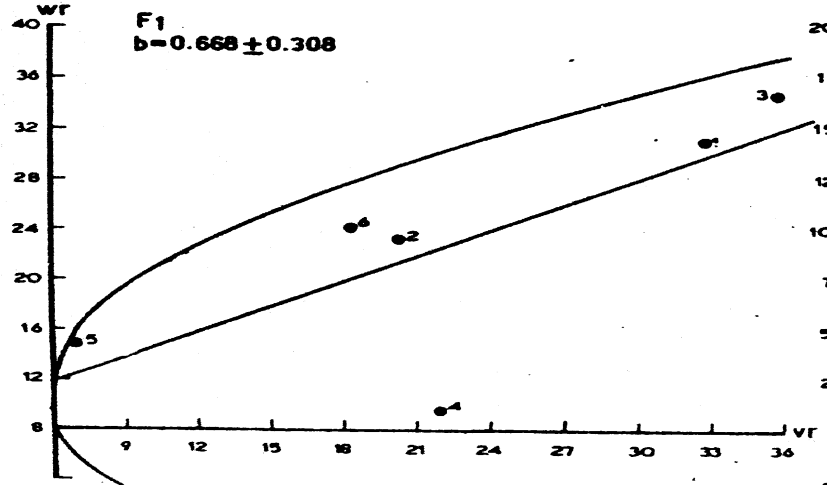


Fig. 3. REGRESSION OF wr ON vr FOR DT BL

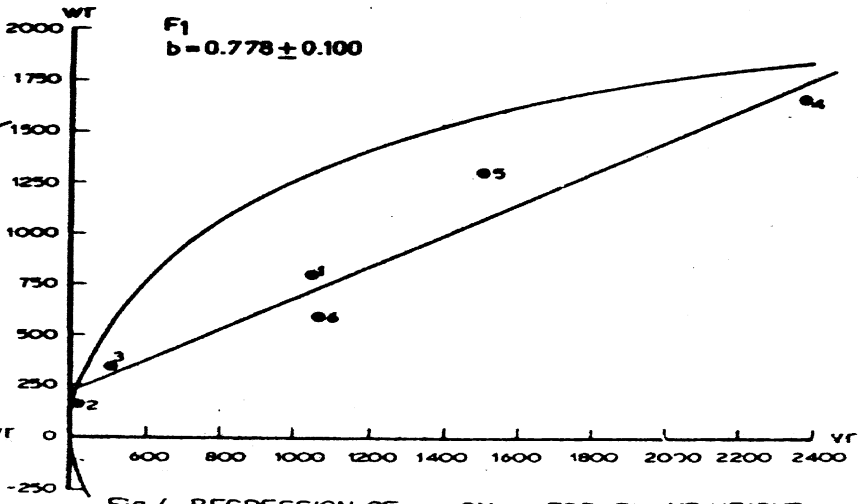


Fig. 4 REGRESSION OF wr ON vr FOR PLANT HEIGHT

Partial dominance was observed in both the generations as the regression line passes above the point of origin. In F_1, the parents, 2,1,3 were nearer to the point of origin respectively. Thus these parents had maximum dominant genes. On the contrary, parents 6,4 and 5 are farthest from the point of origin. Thus these parents had maximum recessive genes. Incidently, the parents 1,2 and 3 were the resistant parents. It is apparent that most of the resistant parents had dominant genes and susceptible parents had recessive genes. Therefore, it is a clear cut case of partial dominance of resistance over susceptibility. In F_2, though partial dominance was observed for disease resistance but the distribution of parents showed that parent 1 was nearer to the point of origin than parent 2. However, the analysis confirms the presence of dominant genes in parents 1,2 and 3 in both the generations. The parents 1 and 2 were more stable for disease resistance than parent 3.

4.2.3.2. Days to 50% boot leaf stage (DTBL):

The position of regression line on Wr-axis indicated the case of partial dominance. On the basis of the relative distance of array points from the origin, parents 4,5,6 and 2 had higher concentration of dominant alleles whereas parents 1 and 3 had higher concentration of recessive alleles.

4.2.3.3. Plant height:

The position of regression line indicated partial dominance. On the basis of the distance of the array points from the origin, parents 2,3,6 and 1 fell near

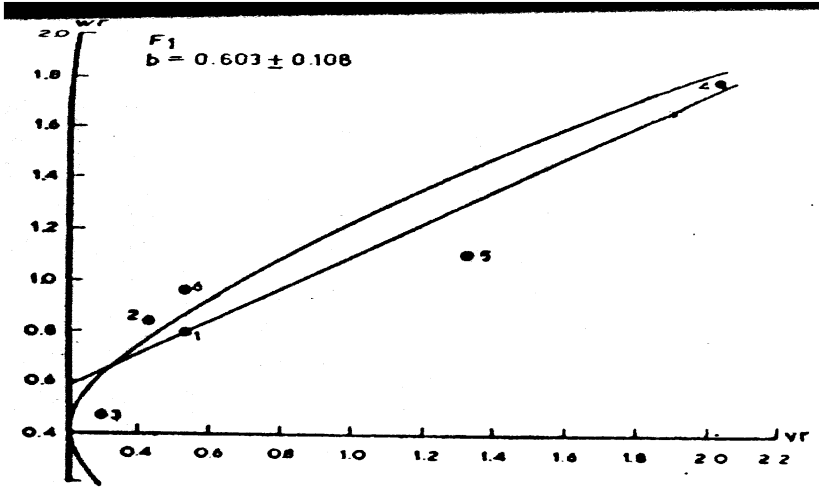


Fig.5. REGRESSION OF wr ON vr For EFFECTIVE TILLERS PER PLANT

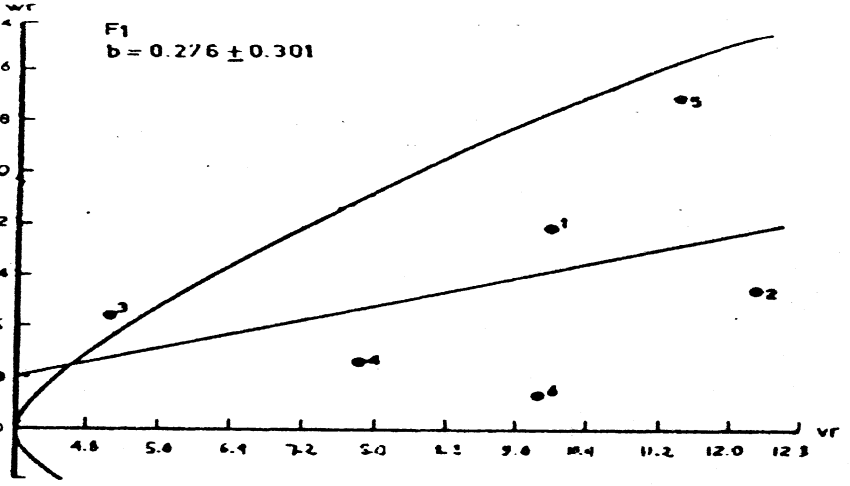


Fig.6. REGRESSION OF wr ON vr FOR EAR LENGTH

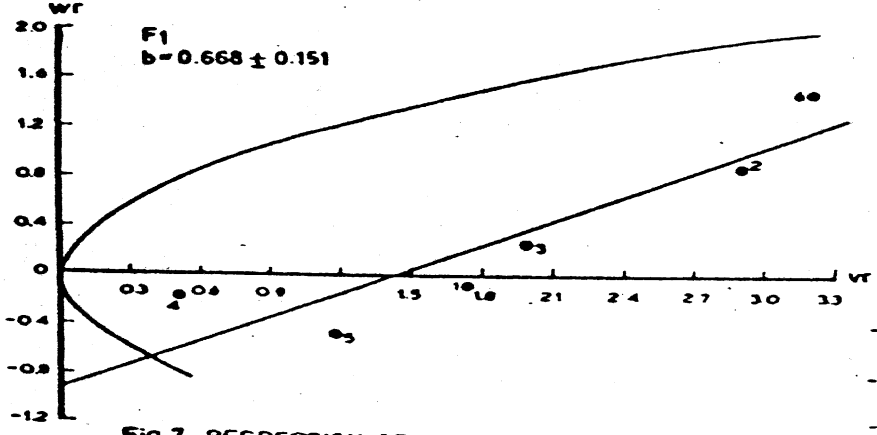


Fig.7. REGRESSION OF wr ON vr FOR 1000 GRAIN WEIGHT

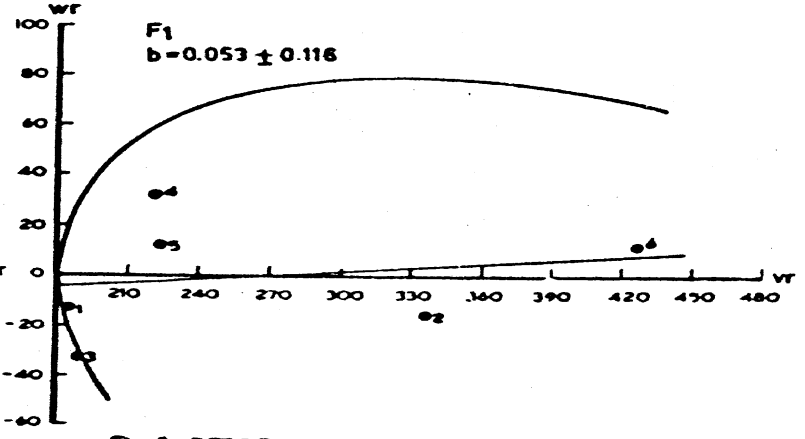


Fig.8. REGRESSION OF wr ON vr FOR YIELD PER PLANT

to the origin, whereas parents 5 and 4 were away from the origin, showing that these parents had most recessive genes for this character.

4.2.3.4. Effective tillers per plant:

The position of regression line on Wr-axis indicated the case of partial dominance. On the basis, of the distance of the array points from the origin, parents 3, 1, 2 and 6 were near to the origin, whereas parents 5 and 4 were away from the origin.

4.2.3.5. Ear length:

The position of regression line on Wr-axis indicated the case of partial dominance. On the basis of the relative distance of array points from the origin parents 6 and 4 indicating higher concentration of dominant alleles whereas other parents had higher concentration of recessive genes for this character.

4.2.3.6. 1000 grain weight:

The position of regression line on Wr-axis indicated the case of over dominance. On the basis of relative distance of array points from the origin parents 6, 4, 1 and 3 were nearer to the origin indicating the presence of dominant genes for this character.

4.2.3.7. Yield per plant:

The position of regression line on Wr-axis indicated the case of over dominance. On the basis of relative distance of array points from the origin parents 3, 1 and 2 were nearer to the origin indicating higher concentration of dominant alleles whereas other parents had

higher concentration of recessive genes for this character.

4.2.4. Combining Ability Analysis:

The mean squares due to general and specific combining ability along with degree of freedom for all the characters are given in Table 18. Both general and specific combining ability were highly significant for most of the characters in F_1. However, SCA was found to be insignificant for effective tillers per plant. Highly significant mean squares for both GCA and SCA indicated the importance of both additive and non additive gene actions in the inheritance of these characters.

In F_2 for smut severity also, both GCA and SCA effects were highly significant indicating the importance of both additive and non-additive gene effects.

4.2.4.1. General combining ability effects(GCA):

The estimates of GCA effects for the characters studied along with SE of G_i and G_{ij} are presented in Table 19. Results in respect of the individual trait have been presented below in detail.

4.2.4.1.1.: Smut Severity:

In case of smut severity, the negative GCA (showing disease resistance) is desirable. Out of 6 parents, used in the present investigation, 3 parents, namely ICMPS 900-9-3, ICMPS 700-1-5-4 and ICMPS 1500-7-3-2 recorded significant and negative GCA effect. Thus, they were good general combining parents for smut disease resistance. Contrary to these, three parents 833B, 843B and 834B recorded positive and significant GCA effect for disease reaction.

TABLE 18

Analysis of variance for combining ability in F_1 and F_2 (Disease severity) population of different crosses.

Source	d.f.	Smut severity F_1	Smut severity F_2	DTBL F_1	Plant height F_1	Effective tillers/ plant F_1	Ear-length F_1	1000 grain weight F_1	Yield/ plant F_1
GCA	5	733.67**	648.75**	106.90**	3584.52**	3.96**	29.26**	3.04**	34.76**
SCA	15	72.10**	32.76**	8.74**	1043.79**	0.40	8.36**	1.96**	324.36**
Error	40	2.39	2.03	0.10	2.97	0.08	0.09	0.001	0.07

** significant at P = 0.01

TABLE 19

Estimates of general combining ability effects of the parents in F_1 and F_2 (Smut severity) populations

Parents	Disease severity F_1	Disease severity F_2	DTBL F_1	Plant height F_1	Effective tillers/ plant F_1	Ear length F_1	1000 grain weight F_1	Yield/ plant F_1
1	-8.77**	-8.50**	3.72**	12.24**	-0.58**	0.76**	-0.77**	-0.99*
2	-9.47**	-8.88**	3.10**	21.49**	0.08	0.66**	-0.39**	0.97*
3	-7.55**	-6.64**	2.35**	14.69**	-0.46**	0.15	-0.31**	1.25*
4	9.74**	9.46**	-2.40**	-16.01**	0.01	1.45**	0.10**	-2.75*
5	10.41**	10.01**	-5.53**	-33.97**	1.33*	-3.81**	0.91**	-1.42*
6	5.64**	4.53**	-1.24**	1.57**	-0.38**	0.79**	0.46**	2.95*
S.E. (G_i)	0.50	0.46	0.10	0.56	0.09	0.09	0.01	0.09
S.E. (G_i-G_j)	0.77	0.71	0.16	0.86	0.14	0.15	0.02	0.14

* Significant at P = 0.05

** Significant at P = 0.01

Thus, these parents were good general combiners for susceptibility.

In F_2, the estimates of GCA effect showed similar trend as in F_1 and the parents ICMPS 900-9-3, ICMPS 700-1-5-4 and ICMPS 1500-7-3-2 were found to be good, combiner for disease resistance whereas other three parents were good combiner for susceptibility.

4.2.4.1.2. DTBL :

From the result of combining abilities for days to 50% boot leaf stage it becomes evident that parents ICMPS 900-9-3, ICMPS 700-1-5-4 and ICMPS 1500-7-3-2 were good general combiners for late emergence of the boot which signified later flowering. On the other hand, 833B, 843B and 834B, parents were good general combiners for early boot emergence.

4.2.4.1.3. Plant height:

Considering plant height, parents 833B, 843B and 834B were good general combiners for dwarfness. Contrary to these, parents ICMPS 900-9-3, ICMPS 700-1-5-4 and ICMPS 1500-7-3-2 were good general combiners for tallness.

4.2.4.1.4. Effective tillers per plant:

Parent 843B was found to be a good general combiner for this trait whereas ICMPS 900-9-3, ICMPS 1500-7-3-2 and 834B were poor general combiners. Parents 700-1-5-4 and 833B were moderately good for his trait although not significantly better.

4.2.4.1.5. Ear length:

Parents ICMPS 900-9-3, ICMPS 700-1-5-4, 833B and 834B were good general combiners for ear length

whereas 843B had negative significant GCA effect for this trait signifying its poor general combining ability for the trait. Parent ICMPS 1500-7-3-2 however, had positive but insignificant GCA effect which indicated its medium combining ability.

4.2.4.1.6. 1000 grain weight:

Parents 833B, 843B and 834B had positive and significant GCA values which indicated that they were good general combiner for 1000 grain weight. Parents ICMPS 900-9-3, ICMPS 700-1-5-4 and ICMPS 1500-7-3-2 were poor combiners for this trait.

4.2.4.1.7. Yield per plant:

Parents ICMPS 700-1-5-4, ICMPS 1500-7-3-2 and 834B were good general combiners for yield whereas parents ICMPS 900-9-3, 833B and 843B were poor general combiners for this particular trait.

4.2.4.2. Specific Combining Ability Effects(SCA):

The estimates of SCA effects for all the characters are presented in Table 20.

4.2.4.2.1. Smut severity:

The negative estimates for SCA effects with regard to smut severity would indicate resistant and positive estimates would indicate susceptibility.

An appraisal of Table 20 shows that out of 15 crosses, 9 crosses showed negative and significant SCA effect; These include 1 x 4, 1 x 5, 1 x 6, 2 x 4, 2 x 5, 2 x 6, 3 x 4, 3 x 5, and 3 x 6. These were therefore, the resistant specific crosses for smut reaction. Contrary to this crosses, 1 x 2,

Specific combining ability effects in F_1 and F_2 (Smut severity only) population.

Cross	Disease severity F_1	Disease severity F_2	DTBL F_1	Plant height F_1	Effective tillers/ plant F_1	Ear-length F_1	1000-grain weight F_1	Yield/ plant F_1
1 x 2	6.50**	3.75**	1.72**	25.74**	-0.41	1.61**	-0.83**	7.44**
1 x 3	5.46**	5.15**	2.80**	16.53**	0.80**	0.92**	0.01	4.41**
1 x 4	-8.67**	-5.73**	-5.11**	17.24**	-0.32	3.42**	0.15**	25.98**
1 x 5	-8.62**	-7.76**	-0.99**	-4.14**	0.01	0.88**	-0.32**	-4.43**
1 x 6	-7.12**	-2.89*	1.39**	13.99**	0.05	1.08*	1.87*	-15.11**
2 x 3	5.75**	3.12*	1.76**	1.95	0.14	0.89*	-4.25**	-7.98**
2 x 4	-10.82**	-6.29**	5.51**	19.66**	-0.32	1.39**	1.14**	-9.20**
2 x 5	-10.05**	-5.65**	-0.70*	27.61**	-0.66**	2.85**	1.20**	4.72**
2 x 6	-5.99**	-3.24*	-1.66**	12.07**	0.05	4.38**	1.10**	33.19**
3 x 4	-6.72**	-1.91	-3.74**	27.78**	0.55**	0.24	0.51**	6.38**
3 x 5	-8.59**	-4.89**	-1.95**	8.40**	-0.78**	0.23	0.31**	20.43**
3 x 6	-4.27**	-2.29	-1.24**	13.20**	-0.07**	0.90*	1.56**	13.01**
4 x 5	2.11	-1.19	1.47**	-12.22**	1.43**	-0.07	-0.66**	2.62**
4 x 6	5.07**	-1.10	-2.82**	40.57**	-0.86**	0.47	0.57**	4.97**
5 x 6	2.82*	-0.49	-0.36	23.53**	-0.20	0.79*	1.53**	12.84**
S.E.(S_{ij})	1.13	1.04	0.23	1.26	0.21	0.23	0.03	0.21
S.E.(S_{ij}-S_{ik})	2.05	1.89	0.42	2.28	0.38	0.41	0.04	0.37
S.E.(S_{ij}-S_{kl})	1.89	1.75	0.39	2.11	0.35	0.38	0.04	0.34

* Significant at P = 0.05; ** Significant at P = 0.01.

1 x 3, 2 x 3, 4 x 5, 4 x 6 and 5 x 6 showed positive and significant SCA estimates. Hence these were highly susceptible specific crosses for smut reaction.

In F_2, estimates of SCA effect showed that out of 15 crosses, 7 crosses had negative significant SCA estimates, these were the resistant specific crosses for smut reaction. All the other crosses had either positive or non-significant SCA values. The crosses which were highly susceptible on SCA effect basis were 1 x 2, 1 x 3 and 2 x 3.

4.2.4.2.2. Days to 50% boot leaf stage (DTBL):

Considering DTBL, out of 15 crosses, 6 crosses had positive and significant SCA effect. So, these were good crosses for late emergence of booting stage and also for late flowering. These crosses were 1 x 2, 1 x 3, 1 x 6, 2 x 3, 2 x 4, 4 x 5. On the other hand, 8 crosses showed negative and significant SCA estimates which showed that these were good crosses for early booting stage. There crosses were 1 x 4, 1 x 5, 2 x 5, 2 x 6, 3 x 4, 3 x 5, 3 x 6 and 4 x 6.

4.2.4.2.3. Plant height:

In case of plant height, out of 15 crosses, 12 crosses had positive and significant SCA effect. Therefore, these were good crosses for tallness. On the other hand, crosses 1 x 5 and 4 x 5 showed negative and significant SCA estimates which showed that these were good for dwarfness. The cross 2 x 3 showed positive estimate. However, it was not significant.

4.2.4.2.4. Effective tillers per plant:

Considering this particular trait, only three crosses, 1 x 3, 3 x 4 and 4 x 5 had positive significant SCA estimate which meant that these crosses were good for effective numbers of tillers per plant. Onthe contrary, 4 crosses, 2 x 5, 3 x 5, 3 x 6 and 4 x 6 had negative significant SCA estimates. Therefore, these crosses were good in relation to less numbers of effective tillers per plant which obviously is not a required character. Other crosses either had positive or negative estimate but were insignificant.

4.2.4.2.5. Ear length:

The positive and significant SCA effect for this character is desirable. Out of 15 crosses, 10 crosses had positive and significant SCA effect for ear length. These crosses were 1 x 2, 1 x 3, 1 x 4, 1 x 5, 1 x 6, 2 x 3, 2 x 4, 2 x 5, 2 x 6 and 3 x 6. The cross 4 x 5 had negative but insignificant SCA estimates whereas the other three crosses had positive but insignificant SCA estimate.

4.2.4.2.6. 1000 grain weight:

Results for this particular trait revealed that out of 15 crosses, 10 crosses showed positive and significant SCA effect which is desirable. These crosses were 1 x 4, 1 x 6, 2 x 4, 2 x 5, 2 x 6, 3 x 4, 3 x 5, 3 x 6, 4 x 6 and 5 x 6. On the other hand, crosses 1 x 2, 1 x 5, 2 x 3 and 4 x 5 had negative and significant SCA effect which meant that these crosses were not good for increased 1000 grain weight.

4.2.4.2.7. Yield per plant:

The positive and significant SCA effect for this trait is generally desirable. From the table 20, it is evident that out of 15 crosses, 11 crosses had positive and significant SCA effect. These crosses were 1 x 2, 1 x 3, 1 x 4, 2 x 5, 2 x 6, 3 x 4, 3 x 5, 3 x 6, 4 x 5, 4 x 6 and 5 x 6. Contrary to it, four crosses, 1 x 5, 1 x 6, 2 x 3 and 2 x 4 had negative and significant SCA effect which meant that these crosses were not good for this trait.

4.3. Studies with Fungicides:

4.3.1. Effect of different fungicides on teliospore germination:

Seven systemic as well as contact fungicides were selected for the present study. Different concentrations of each fungicide were prepared and these concentrations were used to study their effect on teliospore germination as per method described in 'Materials and Methods'.

Results shown in Table 21 suggest that none of the fungitoxicants could completely inhibit the teliospore germination. However, when compared to control, all the fungicides, had significant inhibitory effect on germination of teliospore.

It is clear from the results, that the inhibitory effect was maximum with carboxin at 500 µg/ml (90%) followed by Captafol (80%) and carbendazim (78.3%). The inhibitory effect was least (50.8%) with tridemorph at the same concentration. Statistically significant effect of concentration

TABLE 21

Inhibition of germination of teliospores of Tolyposporium Penicillariae by various fungicides in vitro.

Sr. No.	Fungicides	Per cent inhibition in germination[a]						
		Concentration in µg/ml (ai basis)						
		0	1	50	100	200	500	Mean
1.	Benomyl	0	30.6	42.0	54.1	64.2	65.3	42.7
2.	Captafol	0	38.6	55.0	70.0	75.0	80.0	53.1
3.	Carbendazim	0	39.3	54.3	70.0	74.6	78.3	52.8
4.	Carbendazim + Thiram	0	35.3	45.3	60.7	68.3	72.6	47.0
5.	Carboxin	0	51.6	61.3	76.0	78.0	90.0	59.5
6.	IBP	0	10.6	31.6	43.6	52.0	55.6	32.3
7.	Tridemorph	0	10.0	25.0	41.3	45.0	50.8	28.7
	Mean -	0	30.9	44.9	59.4	65.3	70.4	

S.E.(m) ± Fungicide: 0.24; Concentration:0.22; Fungicide x Concentration : 0.58

L.S.D.(0.05)- Fungicide:0.66; Concentration: 0.60; Fungicide x Concentration: 1.61

a = Average of 3 replications.

on teliospore germination was observed and all the fungicides had their efficacy increased with the increase in their concentration. Though the degree of inhibition varied with reference to different concentrations tried, all the fungicides showed inhibitory effect on teliospore germination. Statistical analysis of the data revealed that fungicides, concentrations and their interactions to be highly significant.

4.2.3.1. Effect of different fungicides on fungal growth:

In the present investigation, the antifungal activity of the fungicides were tried by using Poisoned Food Technique. Effect of these fungicides on the pathogen (average colony diam) at different concentrations of the fungicide is given in Table 22. Statistical analysis of the data revealed that fungicides, concentrations and their interactions to be highly significant. Carboxin was significantly effective against T. penicillariae than the rest of the fungicides. Captafol and carbendazim were the next best. In general, all the fungicides had their efficacy increased with the increase in their concentration. Tridemorph and IBP were the least effective fungicides among all the fungicides tested. However, both of them when compared with control, found to be significantly effective.

4.3.3. Foliar Spray:

Different fungicides selected for the earlier experiments were used by three different methods in the field trials as foliar spray to determine their effect on development of smut. The field experiments were conducted as per method described earlier in 'Materials and Methods'.

TABLE 22

Colony diameter of Tolyposporium Penicillariae, at different concentrations of fungicides incorporated in potato agar medium.

Sr. No.	Fungicides	Average colony diameter (mm) [a] Concentrations (ai in µg/ml)						
		Control 0	10	20	30	40	50	Mean
1.	Benomyl	15.0	10.6	9.1	8.2	5.1	4.2	8.7
2.	Captafol	15.0	8.5	7.5	7.0	3.5	3.0	7.4
3.	Carbendazim	15.0	9.1	8.2	7.2	3.9	3.1	7.8
4.	Carbendazim + Thiram	15.0	9.5	8.3	7.1	4.0	3.2	7.9
5.	Carboxin	15.0	8.0	7.0	6.6	3.0	0.8	6.7
6.	IBP	15.0	12.0	11.5	10.3	9.7	8.6	11.2
7.	Tridemorph	15.0	13.5	12.7	12.0	10.9	9.5	12.3
	Mean	15.0	10.2	9.2	8.3	5.7	4.6	
		Fungicides		Concentrations			Fungicides x concentration	
S.E.(m) ±		0.03		0.03			0.07	
L.S.D.(0.05)		0.08		0.08			0.19	

a Average of 3 replications.

4.3.3.1. Pre-inoculation spray: (at boot emergence stage)

The results of the investigation (Table 23) show that all the fungitoxicants reduced smut severity significantly as compared to control. However, no fungicide could completely control the disease. Amongst the fungicides tested, carboxin was found to be most effective which could reduce the disease severity to the extent of 84.77 per cent. Captafol and carbendazim were not as affective as carboxin but these were significantly effective in controlling the disease with 71.73 and 67.39 per cent reduction of smut. Tridemorph and IBP were the least effective amongst the fungicides tested. However, they were also significantly superior to control and reduced smut severity effectively.

4.3.3.2. Post-inoculation spray: (at boot emergence stage)

In order to, find out the optimum numbers of post-inoculation sprays to reduce the smut severity effectively, the present investigation was carried out. The results in Table 24 show that all the fungitoxicants could significantly reduce the smut severity in pearl millet, when used as post-inoculation spray as compared to water-sprayed control. Mean data of disease severity for individual treatment when averaged for different numbers of sprays, it was evident, that, carboxin stood first among others with mean disease severity of 22.66 per cent and disease control of 81.05 per cent. Carbendazim was the next best with mean disease severity of 24.83 per cent and 77.45 per cent reduction of the disease. Tridemorph and IBP were found to be least

TABLE 23

Relative efficacy of different fungicides used as pre-inoculation spray in reducing smut severity.

Sr. No.	Fungicide	% conc. (a.i.)	Smut severity[a] (%)	% disease control
1.	Benomyl	0.1%	30.67 (33.62)	59.99
2.	Captafol	0.1%	21.67 (27.71)	71.73
3.	Carbendazim	0.1%	25.00 (30.00)	67.39
4.	Carbendazim + Thiram	0.1%	27.00 (31.28)	64.78
5.	Carboxin	0.1%	11.67 (19.89)	84.77
6.	IBP	0.1%	50.00 (45.00)	34.78
7.	Tridemorph	0.1%	55.00 (47.88)	28.26
8.	Control	Water spray	76.67 (61.14)	
	S.E.(m) ±		1.27	
	L.S.D.(0.05)		3.84	

a = Average of three replications.

Figures in parentheses are angular transformed values.

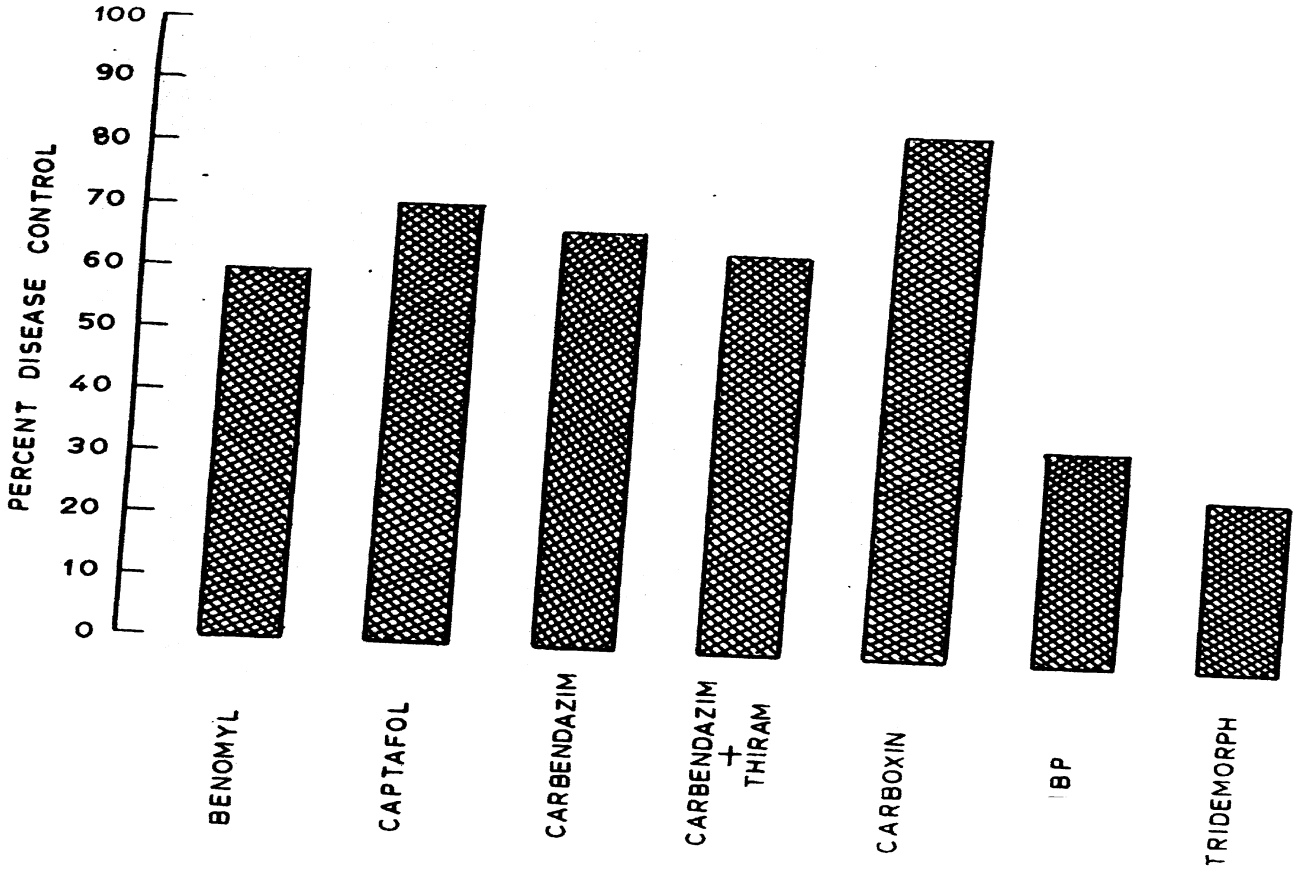


Fig. 9. EFFECT OF DIFFERENT FUNGICIDES USED AS PRE- CU ON SPRAY ON DISEASE CONTROL

Fungicide	% conc. (a.i.)	Smut severity (%) [a]					% disease control				
		1	2 (Nos.of spray)	3	4	Mean	1	2 (Nos.of spray)	3	4	Mean
Benomyl	0.1	39.67 (39.03)	31.33 (34.04)	25.17 (30.11)	24.67 (29.78)	30.21 (33.24)	50.41	60.83	68.60	69.29	62.38
Captafol	0.1	39.50 (38.94)	25.17 (30.11)	13.83 (21.83)	12.83 (20.99)	22.83 (27.97)	50.62	68.54	82.74	84.02	71.48
Carbendazim	0.1	30.00 (33.21)	15.00 (22.79)	14.00 (21.97)	13.23 (21.33)	18.06 (24.83)	62.50	81.25	82.53	83.53	77.45
Carbendazmi+ Thiram	0.1	32.17 (34.55)	20.23 (26.73)	18.33 (25.35)	18.00 (25.10)	22.18 (27.94)	59.78	74.71	77.13	77.59	72.30
Carboxin	0.1	25.00 (30.00)	12.50 (20.71)	12.00 (20.27)	11.33 (19.66)	15.21 (22.66)	68.75	84.38	85.03	85.90	81.05
IBP	0.1	65.00 (53.73)	55.17 (47.97)	54.00 (47.29)	46.67 (43.09)	55.21 (48.02)	18.75	31.04	32.63	41.90	31.08
Tridemorph	0.1	69.33 (56.38)	55.33 (48.06)	54.67 (47.68)	54.00 (47.30)	58.33 (49.85)	13.34	30.83	31.79	32.78	27.19
Control	Water spray	80.00 (63.44)	80.00 (63.44)	80.16 (63.56)	80.33 (63.68)	80.13 (63.53)					
Mean		47.58 (41.66)	36.84 (36.73)	34.02 (34.36)	32.63 (33.76)						
S.E.(m) ±	: Treatment: 0.14; Nos. of spray : 0.10; Treatment x spray : 0.27										
L.S.D.(0.05)	: Treatment: 0.41; Nos. of spray : 0.30; Treatment x spray : 0.82										

a = Average of 3 replications; Figures in parentheses are angular transformed values.

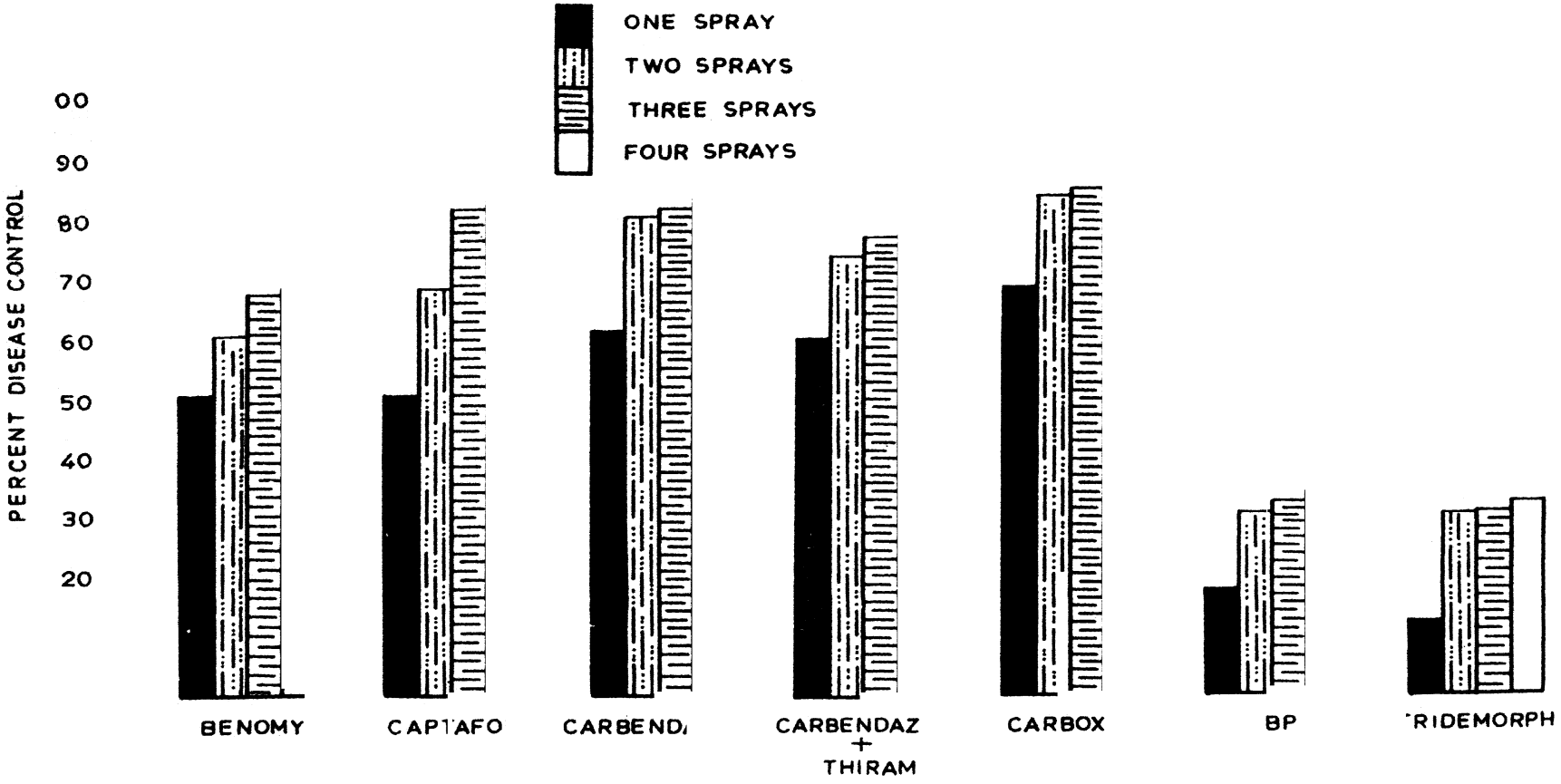


Fig. 10. EFFECT OF POST-INOCULATION FUNGICIDA SPRAYS ON SEASE CONTRO

effective among all fungicides tested. When mean disease severity per cent for different numbers of sprays for all the treatments were averaged, it was found that although there was overall significant effect of numbers of sprays on reduction of disease severity but the difference between third and fourth post-inoculation sprays was statistically non-significant.

4.3.3.3. Pre-inoculation followed by Post-inoculation spray:(at post-emergence stage)

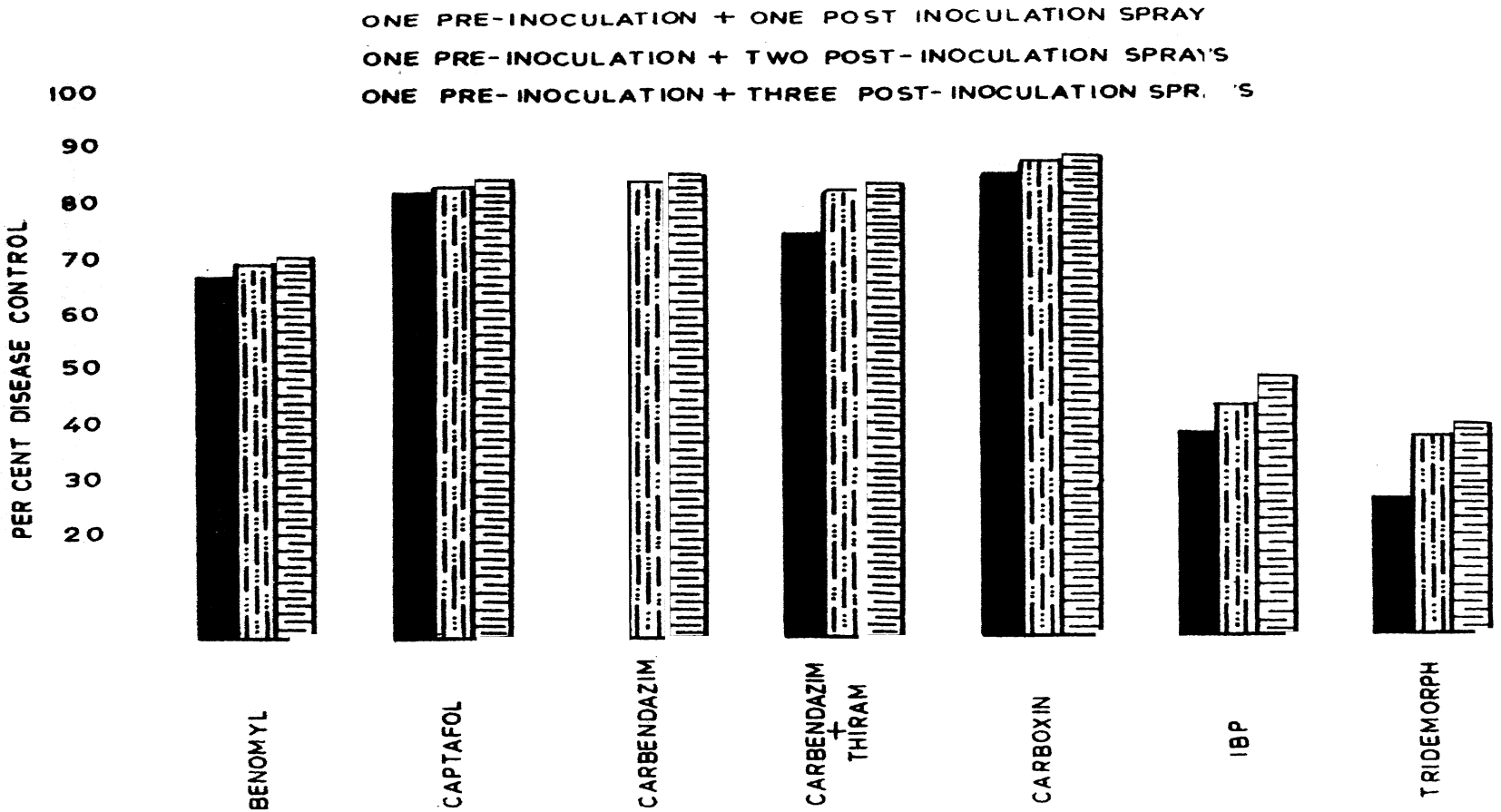
In order to, find out the possibility of controlling the pearl millet smut with a suitable combination of prophylactic and curative sprays the present investigation was carried out. The results presented in Table 25 show that all the treatments when sprayed in combination of pre and post-inoculation, could significantly reduced the disease as compared to control. However, complete control of the disease was not achieved in any case. From the mean disease severity data of individual treatment for all the combinations, it was clear that carboxin was the best among all the fungicides tested with mean disease severity of 19,79 per cent against 63.56 per cent in control (angular transformed values). Carbendazim and captafol stood second best with 21.73 and 22.38 per cent smut severity respectively without any significant difference between them. Tridemorph and IBP emerged as the least effective fungicides with 47.05 per cent and 42.80 per cent disease severity respectively. However, they were also found to be superior when statistically compared with control.

Effect of combinations of pre- and post-inoculation sprays on reduction of pearl millet smut.

Fungicide	% conc. (a.i.)	Smut severity (%) a			Mean	% disease control			
		1 Pre-inoculation (a) + 1 Post-inoculation	1 Pre-inoculation (b) + 2 Post-inoculation	1 Pre-inoculation (c) + 3 Post-inoculation		(a)	(b)	(c)	Mean
		(Nos. of sprays)							
Benomyl	0.1	27.33 (31.52)	25.33 (30.22)	24.33 (29.56)	25.67 (30.43)	65.90	68.46	69.58	67.98
Captafol	0.1	15.00 (22.79)	14.50 (22.38)	14.00 (21.97)	14.50 (22.38)	81.29	81.94	82.50	81.91
Carbendazim	0.1	15.00 (22.79)	13.17 (21.28)	13.00 (21.13)	13.72 (21.73)	81.29	83.60	83.75	82.88
Carbendazim + Thiram	0.1	20.33 (26.80)	15.67 (23.31)	14.83 (22.65)	16.94 (24.26)	74.64	80.49	81.46	78.86
Carboxin	0.1	13.00 (21.11)	10.83 (19.20)	10.67 (19.05)	11.50 (19.79)	83.78	86.52	86.66	85.65
IBP	0.1	50.67 (45.38)	45.50 (42.41)	42.33 (40.59)	46.17 (42.80)	36.80	42.11	47.08	41.89
Tridemorph	0.1	60.00 (50.77)	51.67 (45.96)	49.00 (44.43)	53.56 (47.05)	25.16	35.68	38.75	33.20
Control	Water spray	80.17 (63.55)	80.33 (63.68)	80.00 (63.44)	80.17 (63.56)				
Mean		35.19 (35.59)	32.13 (33.56)	31.02 (32.85)					

S.E.(m) ±: Treatment; 0.25; Nos. of spray: 0.15; Treatment x Spray : 0.43
L.S.D.(0.05): Treatment; 0.75; Nos. of spray: 0.45; Treatment x Spray : 1.31

a = Average of 3 replications : Figures in parentheses are angular transformed values.



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DISCUSSION

Pearl millet is the most important, and probably has the greatest potential, of all the millets and miscellaneous cereals in the drier areas of north, south and west India and along the southern peripheries of the Sahara desert in Africa. The production of high-yielding hybrids in pearl millet has been made possible through the use of cytoplasmic-genetic male sterility. The first commercial hybrid HB-1, was released in 1965 with the male sterile line Tift 23-A which was developed by Burton (1958) from Tifton, Georgia. In India with the release of HB-1 and subsequently some other hybrids, average yield of pearl millet increased spectacularly from 337 Kg/ha to 620 Kg/ha during 1970-71. However, in due course, these high-yielding cultivars were found to be highly susceptible to several diseases including smut.

Smut caused by Tolyposporium penicillariae is one of the serious and widespread diseases of pearl millet. Although the disease was reported as early as 1918 by Butler in India, not much attention was paid on the systematic study on various aspects of this disease. Earlier workers believed that smut was not an economically important disease in India. However, in parts of Tamil Nadu, Andhra Pradesh and Maharashtra

smut infection varied from 1 to 30% (Madras Deptt.Agric. 1954; Krishnaswamy, 1962). In recent years, the disease has become more devastating in several parts of India, particularly in the States of Gujarat, Haryana,Punjab and Rajasthan mainly due to the introduction of several high-yielding hybrids. Even then, smut has received relatively less attention of pathologists in comparision to downy mildew and ergot in India.

Keeping the fact in view that smut has gradually assumed an alarming proportion in several parts of India, and thereby posing a serious threat to the grain yield of pearl millet to a great extent, the present series of investigations were undertaken with the following objectives.

1. To study the morphological, cultural and pathogenic variability among different isolates of the pearl millet smut pathogen, collected from different locations in India.
2. To study the inheritance of smut, resistance and associated genetic architecture.
3. To assess the relative efficacy of a few fungicides in reducing smut incidence.

5.1. Variability Studies:

Stakman and Christensen in 1953 stated "Plant disease fungi are known to be complex and variable, and the degree of complexity is imperfectly known for most of them and not well enough known for any of them." The situation is not any better even now. In case of smut fungi, although it has long been known to be highly variable in nature , only

in few cases has the variability been clearly demonstrated. Holton et al. (1968) were of the opinion that though variation was pronounce in most of the smut fungi studies on variation in the smut fungi were still confined primarily to the economically important smuts of a few cereal crops. The list of crops obviously did not include pearl millet.

In recent years, it has been found that most of the commercial pearl millet hybrids and male sterile lines are highly susceptible to smut. However, it is also noticed that the extent of damage to a particular genotype sometimes varies with location. Hisar, in the State of Haryana is generally considered to be a 'hot spot' for smut due to high intensity of disease occurrence irrespective of genotypes. On the other hand, the same genotypes in other pearl millet growing areas in the northern belt of India usually contract less disease when compared with Hisar. Even in different places, within the State of Haryana, differential reaction of the genotypes to smut is observed.

Since in the recent years, there have been increasing efforts to control the disease by host-plant resistance and incorporation of resistance in suitable genotypes by resistance breeding programme, therefore any possible variation in the pathogen population will necessarily jeopardize the entire strategy of breeding for smut resistance. The need for better understanding the variability in the pearl millet smut population and host-pathogen relationship was therefore, felt necessary, and it is with this objective

in mind that attempt were made to determine variability among isolates of the pearl millet smut pathogen from different locations in India. Furthermore, it was also intended to gather additional informations on the biology of this comparatively less studied fungus.

5.1.1. Morphological studies:

Morphological variation in respect of size and shape of sorus and spore is pronounced in many smut fungi. Fischer and Holton (1957) found that variation in sorus and spore characteristics was a common phenomenon in several species and races of Tilletia. However, in the present investigation where a large numbers of spore balls and teliospores from each isolate were observed under microscope, it was found that there was no variation in colour and shape of sporeballs and teliospores among different isolates. Measurement of length and breadth in sporeballs and measurement of diameter in teliospores revealed variation among isolates. However, on the basis of these variations, the isolates could not be classified into different groups since there was no correlation between the size of the sporeball and the diameter of the teliospore among the isolates. There was no variation in the exospore ornamentation of teliospore as well. All the isolates were found to possess 3-5 thickenings on the exospore of teliospore. Morphological studies on sporidia revealed that, sporidia from all the isolates were colourless and single celled. Although, there was variation in length of sporidia among isolates, the isolates could not be categorised into different groups on the basis of this

variability. Since there was considerable variability in size of the sporeballs, diameter of the teliospores and length of the sporidia even within a particular isolate, It may be concluded from the results of the above morphological studies that the variations in size of sporeballs, teliospores and sporidia among isolates might represent temporary response to environment and might not be inherently permanent. Variability in spore size, shape and colour and exospore ornamentation might be some of the useful criteria for species delimitation within a genus of smut fungi which however, might not sufficient to elucidate variability within species.

5.1.2. Spore Germination Studies:

In the present investigation, mean germination percentage of teliospores of the ICR isolate was found statistically to be at par with HSR isolate. On the other hand, there was no significant difference in germination percentage between HSR and MGH isolates. Likewise, difference between the JDH and JPR isolates was also non-significant. With regard to the threshold time for germination it was observed that statistically MGH isolate was significantly different from the other isolates except HSR isolate. However, there was no significant difference between HSR and ICR isolates. No definite relationship between mean germination percentage and optimum time required for spore germination of a particular isolate could also be discerned. Therefore, on the basis of these results, the isolates could not be classified into different groups and they were found to yield similar results.

Holton et al.(1968) reported that the incubation period required for spore germination may be even more variable than longevity in smut spores. However, the rate of germination and the incubation period required for spore germination are influenced greatly, within a particular genetic base, by environmental factors and therefore, any variation in these parameters does not always reflects an inherent or permanent basis. Holton et al.(1968) also stated that longer the difference in incubation period required for germination, the greater will be its variability. Results from the present investigations suggest minimum difference in incubation period for germination. Although no comparative evidence to this study is available it may be concluded that there was no variation for germinability among the isolates tested.

5.1.3. Cultural Studies:

Smut cultures vary in type and rate of growth, topography, colour, pigmentation, and many combinations of these characteristics. Fischer and Holton (1957) were of the opinion that the greatest variation within the species of many smut fungi is displayed by cultures on agar media. Monosporidial cultures of many species usually vary widely in colony characteristics, some of which may represent temporary response to environment; the great majority, however, are inherently permanent. Results from the present investigation show that all the isolates of T.penicillariae produced sporidial type of growth irrespective of the nutrient media used. Working with a single isolate, Subba Rao and Thakur (1983) reported sporidial type of growth of the

fungus on semi-synthetic media whereas Pathak and Shekhawat (1980) reported mycelial growth on synthetic media. However, in the present investigation when both the semi-synthetic and synthetic media were used, it was found that in all the media, the growth was purely sporidial. This might be possibly due to the difference in experimental procedure. Measurement of colony growth showed that semi-synthetic media were better for supporting the growth of the fungus than synthetic media tested. However, potato agar and carrot agar were found to be still better than the potato dextrose agar. This signifies that the requirement of the dextrose for culturing of the fungus is not essential. Pathak and Shekhawat (1980) however, found good growth of the fungus on media supplied with dextrose as carbon source. Since in the present investigations, no study on nutritional requirement of the fungus was undertaken, no conclusion in respect of the same could be drawn. Statistically, there were no differences in growth among the isolates studied. Visual observation of the colonies on different media also did not reveal any variation in the isolates for shape, colour, topography. Therefore, it may be concluded that the isolates studies could not be differentiated on the basis of their behaviour on artificial media. Apparently most smut species respond well to carbohydrates derived from sugars such as sucrose, glucose and maltose; however, according to report, there is variation within and among species as to which sugars are best (Fischer and Holton, 1957).

5.1.4. Physiological studies:

In the present series of investigations, efforts were also made to elucidate variability among the isolates by exposing the culture of the isolates to different temperatures as well as pH levels. The results from the present investigations show that on the basis of these studies variability could not be discerned in the isolates. Statistically, in both the experiments the difference among the isolates was found to be non-significant. Since, no experimental evidence on similar studies are available, therefore the results could not be compared to any. However, from the results of the present investigations two observations on the biology of the fungus were made.

1. The optimum pH for growth of all the isolates was found to be 6.0. On the other hand, Pathak and Shekhawat (1980) found maximum mycelial growth of the fungus at pH 7.5. Results from the present investigation show significantly less sporidial growth of the fungus at pH 7.0 than pH 6.0. However, the smut fungi are known to withstand a wide range of pH level. The finding of the present investigation also supports this.
2. The optimum temperature for maximum growth of the fungus was found to be 35°C in all the isolates. Subba Rao and Thakur (1983) also reported 35°C to be the optimum temperature for maximum sporidial growth. In contrast, Pathak and Shekhawat (1980) found 25°C to be the optimum temperature for maximum growth in terms of mycelial dry weight. In the present investigation, however,

significantly higher sporidial growth was observed at 35° C than at 25° C. This might be due to the fact that Pathak and Shekhawat (1980) quantified dry mycelial weight by growing in liquid cultures.

5.1.5. Pathological studies:

Variation in the smut fungi has its most fundamental impact on Pathogenicity. Halisky (1965) stated that the differential reaction of two or more host varieties could be taken as indication of the degree of variation in virulence of the parasite. In the present investigation, therefore, an attempt was made to elucidate variability by inoculating three resistant genotypes ICMPS 900-9-3, ICMPS 700-1-5-4 and ICMPS 1500-7-3-2 and four susceptible 833B, 843B, 834B and BJ-104 with each of the six isolates following the standard inoculation procedure under identical experimental environments. The results show that all the resistant genotypes exhibited high levels of resistance to all the isolates whereas susceptible genotypes showed susceptible reaction. However, statistical analysis revealed non-significant differences among the virulence of isolates tested. The results suggests that there was no pathogenic variability among the isolates. Although, some differences for size, diameter and length of sporeballs, teliospores and sporidia among different isolates were noticed, these could be attributed to the effect of environmental conditions, because environment is known to have a key role on smut development in pearl millet (Rachie and Majmudar, 1980).

So far, there has not been any report about the existence of physiologic specialization within the morphological species of pearl millet smut fungus. The results of the present investigation also do not indicate the presence of any such race or biotype. This finding will naturally be an important contribution towards resistance breeding programme, where, the knowledge of race picture of the pathogen is obviously a pre-requisite.

5.2 Inheritance Studies:

Meeting the ever-expanding demand for food for the ever-increasing world population is the biggest challenge confronting agricultural scientists. Pearl millet has a remarkable ability to grow in some of the driest agricultural conditions. It already provides food for million of poor people in Africa and Asia. With the invention of the cytoplasmic-genetic male sterile lines in pearl millet alongwith improved agronomic packages and its successful extension into the third world since late 1960s, spectacular changes in yield potential in this crop have occured. However, this has led to the rise of one disease problem after another. For each disease that developed into prominance the incorporation of resistance became an essential breeding target. Thus, relatively few pure lines, often carrying vertical resistance (VR) to major pathogens, were spread over large areas previously covered by a mosaic of many local varieties presumably carrying both vertical resistance (VR) and horizontal resistance (HR) against most of the diseases.

In the tropics, pearl millet is grown primarily by subsistence farmers. In the absence of economically feasible control measures either chemical or agronomical, attention has been mostly shifted to breeding resistant varieties. In recent years, a few lines with high levels of stable resistance to smut have been identified (Thakur et al. 1986). In the near future with the help of these lines high yielding hybrids and varieties, with resistance to smut may be bred. For any such breeding programme, a thorough knowledge of genetics of resistance is an imperative.

It is with this objective that the present investigation was planned using 6 elite pearl millet lines supplied by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India details of which are given in 'Materials and Methods'.

The six inbreds were involved in a diallel crossing system excluding reciprocals and the data were analysed using both Griffing (1956) and Hayman's (1954) approaches. In recent years, the analysis of diallel crosses has received considerable emphasis in many plant breeding programmes because it fulfills certain specific needs of plant breeder. The analysis provides a systematic approach for the detection of parents and crosses superior for the traits under investigation. At the same time, it helps the plant breeder choose the most efficient method of selection by allowing estimates to be made of the magnitude and relative importance of various genetic parameters. Diallel

analysis of Griffing and Hayman's approach is generally performed under certain assumptions, which have been described under 'Materials and Methods'. These were tested by the two tests t^2 test as suggested by Hayman (1954) and test of significance of b. From the results, it was evident that for some traits, a partial failure of the assumptions of the diallel was indicated. The pin pointing of the offending assumptions for these traits, cannot be accomplished with the present data. However, certain assumptions may be considered fulfilled with some degree of confidence. It may be assumed that the inbreds used in the crossing programme were homozygous as a result of many generations of selfing. However, since some heterozygosity may remain even after many generations of selfing, this assumption may account for atleast part of the partial non-compliance of the traits to the assumptions. When a trait exhibits a partial failure of the assumptions, estimates of the population parameters of that trait are still possible, although the estimates for such a trait are probably less reliable than when all assumptions are fulfilled (Hayman, 1954).

5.2.1. Genetic component:

Both additive (D) and dominant components (H_1 and H_2) were found to be significant for smut severity, plant height, ear length and 1000 grain weight. While for days to 50% boot leaf stage (DTBL) and effective tillers per plant dominant components were non-significant and for yield per plant, additive component was non-significant.

However, in all the traits estimates of the H_2 was less than H_1. This indicates that positive and negative values at loci governing these characters are not equal in proportion in the parents. Hayman (1954) also was of the view that H_1 may be greater or less than H_2. Similar results have also been reported in pearl millet for plant height, ear length and downy mildew (Sclerospora graminicola) reaction by earlier workers (Singh 1976; Dang, 1981). Comparative evidence in case of smut, however, is not available, The component h^2 which is net dominance effect over all the loci was significant for smut severity, plant height, ear length, 1000 grain weight and yield per plant. The F component which is a measure of covariance between additive and dominance effect was significant for smut severity only. In certain traits, additive component was found to be more important while in other traits dominant components were important . It may, however, be mentioned that whatever may be the relative estimates of additive and dominant components, one or the other or sometimes both were found significant for most of the traits. Similar observations also had been made by Harinarayana (1965) for ear length; by Jain et al.(1961), Murty et al.(1967), Singh (1976) and Singh et al.(1979) for plant height and ear length. The significance of both D and H component attributes warrant a breeding programme which can exploit both additive and non-additive type of gene action.

The degree of dominance $(H_1/D)^{\frac{1}{2}}$ reveals partial dominance for smut severity, DTBL, plant height, effective

tillers per plant and ear length, whereas, over-dominance was observed in 1000 grain weight and yield per plant. This may not be an index of real over-dominance at all loci, since particular combination of positive and negative alleles or complementary types of epistasis or simply correlated gene distribution might influence the mean degree of dominance and convert partial dominance into apparent over dominance. Moreover, since the estimates of degree of dominance were calculated for a fixed set of parents, all the estimates may be biased upward to some degree. Hayman (1960) stated that in such cases, interpretation should be made cautiously. Furthermore, while discussing the shortcomings of component analysis, Mather and Jinks (1971) stated that unless $U=V=0.5$ at each, locus the ratio $(H_1/D)^{\frac{1}{2}}$ is not a true measure of degree of dominance. In component analysis of Hayman (1954), there is a provision to test whether $U=V=0.5$ or not. The ratio $H_2/4H_1$ can take the value of 0.25 only when $U=V=0.51$. In the present investigation, this ratio was below 0.25, showing that $U \neq V$ for most of the characters studied. Obviously, the asymmetry of dominant and recessive genes in the population might be one of the reasons for an over-estimation of degree of dominance.

The ratio h^2/H_2 refers to an approximate estimate of number of genes or gene group that show dominance. In the present study, variable estimates for different traits were observed. While in case of smut severity, plant height,

ear length and yield per plant the values were more than one but in other traits the values were less than one. In the former cases, the numbers of gene groups governing dominance were more than the latter traits.

Different levels of heritability (narrow sense) was recorded for different characters. Moderately high heritability was observed in case of disease severity, DTBL, plant height, effective tillers per plant and ear length. It was moderate for 1000 grain weight. However, it was quite low for yield per plant. It suggests the major contribution of non-additive gene effects. Moderate estimate of heritability indicated that major portion of phenotypic variability in that character can be exploited through simple selection procedures like mass selection.

5.2.2. Combining ability:

In combining ability analysis, variance due to general combining ability (GCA) is equal to the variance due to additive effect of genes. In the presence of epistasis, it includes additive x additive type of interaction. Similarly specific combining ability (SCA) variance is equal to the variance arising due to dominance and its interaction viz., dominance x dominance and additive x dominance (Griffing, 1956). In the present study in all characters both GCA and SCA variances were found to be significant except effective tillers per plant. This emphasises the importance of additive and non-additive gene effects in

governing these traits. Several workers have reported the importance of additive and non-additive gene effects in pearl millet for these characters (Jain et al. 1961; Satija, 1972; Singh, 1976; Phul et al., 1973).

The knowledge of nature and magnitude of gene effects involved in the inheritance of a particular character, helps in determining the most efficient breeding plan. Since, both additive and non-additive components of genetic variance were observed for the characters investigated here, it may be suggested that breeding plans which can exploit these gene effects simultaneously should be adopted for genetic improvement for these characters in pearl millet. A system of recurrent selection gives maximum opportunity for re-arrangement of different types of gene effects in linkage groups and thereby raise the genetic base of the population.

5.2.3. pearl millet:

The method of disease scoring always has a vital role on the results of any inheritance study. In the present investigation, the scoring was done on the basis of percentage florets converted into smut sori with help of an internationally accepted standard diagram proposed by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). However, unlike systemic diseases, where gradual destruction of the plant parts generally occurs, it is very difficult in case of smut, to classify them into distinct classes i.e. resistant and susceptible.

In resistance screening for smut, plants showing 0-5 per cent mean disease severity are generally regarded as resistant plants. The resistant parents used in the present investigation also showed mean disease severity of less than 5 per cent. Therefore, an attempt was made to understand the inheritance mechanism with help of classical Mendelian's segregation pattern. From the results the following observations were made.

1. The progenies of the crosses between resistant x resistant plants were resistant in F_1, F_2, BC_1 and BC_2 generation without any segregation.
2. In a set of crosses between susceptible x susceptible, the F_1 was mostly susceptible, though, a few resistant plants were also observed. These might be apparently due to escape. BC_1 and BC_2 progenies were also found to be susceptible.
3. In the third set of crosses between resistant x susceptible, F_1 plants were always resistant suggesting that resistance was dominant over susceptibility in all the crosses. Mean disease severity value revealed resistance to be partially dominant over susceptibility. In F_2 plants, segregation was noticed. The segregated plants showed all types of disease severity value ranging from 0 to 100 per cent. However, the efforts to place the inheritance on a classical Mendelian basis were unsuccessful. Out of 9 discrete RXS crosses, only two crosses, 1 x 6 and 2 x 4 indicated satisfactory

fit of observed to expected 15:1 ratio in F_2 generation (Table 26). The partitioning of the chi square indicated that the heterogenity component was significant for all the ratios tested. The significant heterogenity component indicated that the crosses were not in agreement in showing segregation according to the expected ratio (Table 27).

TABLE 26

Estimate of chi-square for different classical Mendelian segregation ratio, taking 0-5; 6-100 severity (%) ratio as resistant; susceptible in resistant x susceptible crosses (F_2 data).

Sr. No.	Crosses	Mendelian segregation ratio			
		3:1	9:7	13:3	15:1
1.	1 x 4	6.4*	6.1*	20.2*	29.3*
2.	1 x 5	5.6*	3.8*	7.5*	9.0*
3.	1 x 6	4.8*	7.8*	8.5*	1.5
4.	2 x 4	5.1*	4.8*	25.1*	0.8
5.	2 x 5	3.8*	22.1*	8.5*	15.4*
6.	2 x 6	12.7*	21.2*	4.1*	21.4*
7.	3 x 4	8.7*	15.3*	8.2*	18.5*
8.	3 x 5	4.3*	44.2*	21.4*	162.4*
9.	3 x 6	9.0*	15.1*	22.4*	174.4*

TABLE 27

The partition of chi-square values of F_2 data

Source of variation	Degree of freedom	Chi-square values.			
		3:1	9:7	13:3	15:1
Deviation	1	20.2*	16.9*	18.3*	35.2*
Heterogenity	8	40.2*	123.5*	107.6*	397.5*
Total	9	60.4*	140.4*	125.9*	432.7*

From these results, it can be concluded that resistance is partially dominant over susceptibility and has a quantitative nature of inheritance. The component analysis also indicated partial dominance for resistance. Both additive and non-additive gene effects were significant. Under such a situation, where there is a predominance of additive variance, genetic upgrading of material is possible following reciprocal recurrent selection.

5.3. Chemical Control:

Chemical control is only one of several methods used in plant disease management. The decision to use chemical control depends on a series of inter-relating factors. Besides the effective disease control, economic considerations such as monetary returns of increased yield against the cost of the chemicals and their application should be given due consideration. Alternative disease control methods and the possibility of combining several methods also need to be considered. In case of pearl millet smut, two methods of chemical control were previously attempted by different workers. One is seed treatment and the other is use of foliar sprays to prevent the initiation and further spread of the disease. However, Bhatt (1946) conclusively demonstrated that since smut was generally caused by air-borne inoculum directly at the time of flowering, in the smut endemic area therefore, control of this disease by chemical seed treatment was not effective. Several workers have reported different chemicals to be effective against smut as foliar sprays from time to time (Wells, 1967; Bhowmik and

Sundaram, 1971; Mathur *et al.* 1971; Pathak and Gaur, 1975). In the present investigation, therefore, an attempt was made to assess the relative efficacy of seven fungicides used as foliar spray on artifically inoculated plants under field condition so that most effective fungicides could be identified.

All these fungicides were found to be effective in *in vitro* for inhibition of teliospore germination and fungal growth. However, it was also noticed that no fungicide could completely inhibit the teliospore germination. On the basis of performance in these two *in vitro* tests, carboxin was found to be most effective. Captafol and carbendazim also performed well and statistically they were superior to the rest without any significant difference between them. Although no comparable evidence in pearl millet, smut is available, however, in *Urocystis agropyri*, Goel and Jhooty (1985) showed that carboxin caused a complete inhibition of germination of teliospores even at the lowest concentration of 1 µg/ml (a.i. basis). They also reported complete inhibition of germination of teliospore by captafol at the concentration of 10 µg/ml. In the present investigation, however, complete inhibition of germination of teliospore of *Tolyposporium penicillariae* was not achieved by any fungicide even at 500 µg/ml. Carboxin is known (Edgington *et al.*, 1966) to inhibit mycelial growth of several fungi belonging to Basidiomycetes but in the present

investigation, this chemical inhibited sporidial growth also which was evident from the average colony diameter of the fungus in in vitro studies.

When these fungicides were tested in field conditions, all of them retained their efficacy, indicating thereby that in vitro results were comparable to field evaluation.

Out of these seven fungicides tested as pre-inoculation foliar spray under field condition, carboxin was found to be most effective followed by captafol and carbendazim. However, statistically there was no difference between captafol and carbendazim in regard to mean disease severity. Bhowmik and Sundaram (1971) also reported carboxin to be very effective against the smut. Pathak and Gaur (1975) on the other hand, found significant disease control with foliar application of captafol. In the present investigation, however, carboxin was significantly superior to other chemicals. Captafol and carbendazim stood second without any significant difference between them. Benomyl and a combination of carbendazim and thiram also gave good disease control; although there was no significant difference between them.

When the same fungicides were used as post-inoculation foliar spray, it was observed that carboxin was the most effective followed by carbendazim. However, in post-inoculation foliar spray trials, carbendazim always showed better disease control than captafol. It suggests that captafol was performing better when used as a protective foliar spray

rather than curative spray. A combination of carbendazim and thiram was found statistically at par with captafol. In all the experiments IBP and tridemorph were found to be least effective. However, all the fungicides were found to reduce the smut severity significantly as compared to control. With most of the fungicides, it was observed that two post-inoculation sprays were sufficient to reduce the disease severity. Statistically no difference was observed among 2nd, 3rd and 4th spraying suggesting thereby that two sprayings at the boot leaf stage would be most economical. It also signified that spraying in the advance stage of smut development was not very effective in terms of reducing disease severity.

When these seven fungicides were used in combination of pre and post inoculation sprays, it was noticed that carboxin again emerged as the most effective fungicide, which gave significantly better disease control than all other fungicides. Carbendazim and captafol stood second in the list without any significant difference between them. It again suggests that captafol performed better when used as a protective as well as curative sprays rather than using only as curative sprays. The combination of carbendazim and thiram and benomyl also gave good disease control. Tridemorph and IBP, however, were not very effective although they were significantly better than control. It was observed that, one pre-inoculation followed by one post-inoculation spray would be most optimum combination of sprays to reduce disease severity in areas where smut occurs almost every year.

Under heavy disease pressure, the total numbers of sprays may be increased to three.

Although, there has been criticisms regarding use of chemical fungicides in a crop like pearl millet, it should be kept in mind that the farmers are yet to be provided with a smut resistant variety having acceptable agronomic traits for commercial cultivation. In want of such a variety or hybrid, an alternate approach should always be made available for smut endemic area. It is also assumed that a regular spraying of fungicides like carboxin or captafol (0.2% conc.) as prophylactic measure in smut endemic area will not be much expensive when compared to the grain loss caused by the smut fungus under favourable condition. The effect of different fungicides on control of smut as well as increased grain production have been described by several authors (Mathur et al. 1971; Sharma and Sharma, 1976). However, in the present investigation, no record was taken on the effect of the fungicides on yield. Therefore, from the present sets of data no concrete suggestion in this respect can be made.

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SUMMARY AND CONCLUSIONS

The present investigations were undertaken in pearl millet with three main objectives: (i) Studies on the morphological, cultural and pathogenic variability in the isolates of the pearl millet smut fungus, Tolyposporium Penicillariae. (ii) Studies on the nature and magnitude of genetic variation and combining ability in respect of smut severity, yield and its components and their inheritance pattern (iii) Studies on the measures to reduce the smut severity through the use of chemical fungicides.

For variability studies, smutted pearl millet inflorescence were collected from the hybrid BJ-104 from six Indian locations - Hisar, ICRISAT Centre, Jodhpur, Jaipur, Ludhiana and Mahendragarh. The isolates were subjected to different morphological, physiological, cultural and pathological investigations. The results obtained through these investigations indicated that there were some morphological variations in size of the sporeballs, diameter of the teliospores and length of the sporidia among different isolates tested. Some variations in germinability of sporeballs among different isolates were also noticed. However, no variation could be found among the isolates in regard to the cultural behaviour and virulence pattern of the isolates

to different host genotypes. The results suggested that there were no physiologic races or biotypes within the species of the fungus. This finding will be a great help towards the efforts to develop pearl millet hybrids or varieties with high levels of smut resistance. Since there is no pathogenic variability in the smut pathogen, therefore, disease control by the use of disease resistant cultivars would be a long-lasting, economical and most practicable measure.

For inheritance studies, six elite pearl millet lines comprising three resistant and three susceptible, having diverse agronomic characteristics were selected. By using a half-diallel crossing system, 15 F_1s and their F_2s along with 15BC_1s and 15BC_2s and 6 parents were grown in a Randomized Block Design in three replications. Observations for smut severity were recorded in artificially inoculated population for non-segregating and segregating generations, while for some other agronomic characters viz., days to 50% boot leaf stage, plant height, effective tillers per plant, ear length, 1000 grain weight and yield per plant, only non-segregating generations were used for taking record.

The data obtained were statistically analysed. The genetical informations were obtained using Hayman's (1954) approach for F_1 while the methodology of Jinks (1956) for F_2 component analysis and combining ability approach of Griffing (1956) were employed.

Both additive (D) and dominance (H_1 and H_2) components were found to be significant for smut severity in both F_1

and F_2 generations. The degree of dominance revealed partial dominance for smut severity and all other traits except 1000 grain weight and yield per plant where over dominance was noticed. Asymetrical distribution of dominant and recessive alleles were noted in the parents. Moderately high heritability was observed in smut reaction, and other characters except yield per plant, where low heritability was noted.

The combining ability analysis indicated the significance of both GCA and SCA variance for all the characters studied, signifying the importance of both additive and non-additive type of gene actions. The efforts to place the inheritance pattern of smut resistance in segregating generations of Resistant x Susceptible crosses on a classical Mendelian basis were however, not successful indicating thereby quantitative nature of the disease and as such smut inheritance is being governed by large number of genes. The resistance was found to be partially dominant over susceptibility.

The results on the studies with seven different fungicides revealed that carboxin, captafol and carbendazim were quite effective in inhibiting the teliospore germination and fungal growth in laboratory conditions. Same trend of the results was obtained when their relative efficacy under field conditions was assessed as foliar sprays. The fungicides were sprayed in recommended doses at the boot-leaf stage of the crop. The plants were artificially inoculated. Pre-inoculation spray, post-inoculation spray and a

combination of pre and post inoculation sprays were tried to find out the optimum numbers and combination of different fungicidal spray to reduce the smut severity to maximum extent. Although, it was observed that no fungicide could completely control the smut severity in the field under any circumstances, still when compared to control, significant disease control was observed with all the fungicides tested. Results were found to be encouraging when the fungicides were used in combination of pre and post-inoculation sprays.

Conclusions and some points for future investigations

Investigations on the selected aspects of pearl millet smut of topical importance in nature for the entire semi-arid regions of Asia and Africa, were conducted during last three years and reported in the present dissertation. On the basis of the findings, certain specific conclusions can be drawn which may form the basis for the future strategies for the control of this disease and thereby stabilising yield to a large extent.

1. Although no variability could be detected among different smut isolates in the present investigation, it is desired that a large number of isolates representing different agro-climatic regions of India should be studied. Isolates from some of the African countries, where smut is a regular occurrence should also be included in the study to make more valid conclusion.
2. Since large variations in monosporidial cultures of many smut fungi have been reported, therefore, variations within an isolate and within a monosporidial

culture and the reaction of different pearl millet genotypes to these cultures, if any, need the attention of pearl millet pathologists.

3. More research efforts are needed to understand about the biology of the pathogen. Epidemiological studies should be conducted to know more precisely about the disease development.

4. Both additive and non-additive gene effects have been found to be important in the inheritance of smut resistance. Partial dominance of resistance over susceptibility and quantitative nature of the disease, make it possible to select the resistant lines through simple selection procedures. More studies on inheritance, using different parents should be made to select suitable parents for production of smut resistant as well as agronomically acceptable hybrids.

5. From the studies on chemical control, it is concluded that carboxin (0.2%) or Captafol (0.2%) or carbendazim (0.1%) may be recommended as a prophylactic spray for the smut endemic area, at the time of flowering of the crop as it is economical and well within the reach of the farmers. Studies on integrated control measures using host-plant resistance and chemicals should be made. Resistant cultivars in combination of fungicides have been reported to provide greater degree of disease control (Johnson et al., 1979). Further, whether these

chemicals induce resistance in plants and if so, the concerned metabolic alterations taking place in the host, should also be investigated.

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APPENDIX

Table 1: Analysis of variance for length of sporeballs of *Tolyposporium penicillariae* isolates.

(Ref. Table 6)

Source of Variation	d.f.	S.S.	M.S.S.
Isolates	5	18761.18	3752.24**
Error	12	26.72	2.23

Table 2: Analysis of variance for breadth of sporeballs of *T.penicillariae* isolates

(Ref. Table 6)

Source of Variation	d.f.	S.S.	M.S.S.
Isolates	5	10956.36	2191.27**
Error	12	12.56	1.05

Table 3: Analysis of variance for diameter of teliospores of *T.penicillariae* from different locations (isolates)

(Ref. Table 7)

Source of Variation	d.f.	S.S.	M.S.S.
Isolate	5	12.12	2.42**
Error	12	0.12	0.01

Table 4: Analysis of variance for length of sporidia of *T. penicillariae* from different locations (isolates)

(Ref. Table 8)

Source of Variation	d.f.	S.S.	M.S.S.
Isolate	5	93.00	18.6**
Error	12	2.12	0.17

Table 5: Analysis of variance for germination percentage of sporeballs of different isolates of *T.penicillariae*.

(Ref.Table 9)

Source of variation	d.f.	S.S.	M.S.S.
Isolate	5	2476.28	495.26**
Error	12	63.22	5.27

Table 6: Analysis of variance for threshold time for germination of sporeballs of different isolates of T.penicillariae.

(Ref.Table 9)

Isolate	5	20.44	4.08**
Error	12	1.89	0.16

Table 7: Analysis of variance for effect of different nutrient media on growth of the isolates of T.penicillariae.

(Ref.Table 11)

Source of variation	d.f.	S.S.	M.S.S.
Media -	4	1537.92	384.48**
Isolate -	5	0.60	0.12
Media x Isolate -	20	2.76	0.14*
Error -	90	4.47	0.05

Table 8: Analysis of variance for mean colony diameter of the isolates of T.penicillariae at different temperatures.

(Ref.Table 12)

Source of variation	d.f.	S.S.	M.S.S.
Temperature	5	1631.76	326.35**
Isolate	5	0.31	0.02
Temperature x Isolate	25	0.92	0.06*
Error	108	4.29	0.03

contd....

Table 9: Analysis of variance for mean colony diameter of the isolates of T.penicillariae at different pH levels.

(Ref. Table 13)

Source of Variation	d.f.	S.S.	M.S.S.
pH	4	1521.85	380.46**
Isolate	5	0.48	0.11
pH x Isolate	20	2.56	0.13*
Error	90	4.32	0.05

Table 10: Analysis of variance for pathogenicity of different isolates of T.Penicillariae to different genotypes

(Ref. Table 14)

Source of Variation	d.f.	S.S.	M.S.S.
Isolate	5	5.35	1.07
Genotype	6	79497 .06	13249.51**
Isolate x Genotype	30	143.66	4.79
Error	84	354.63	4.22

Table 11: Analysis of variance for percent inhibition of teliospore germination by different fungicides.

(Ref. Table 21)

Source of variation	d.f.	S.S.	M.S.S.
Treatment (Fungicide)	6	12734.66	2122.44**
Concentration	5	73203.24	14640.65**
Treatment x concentration	30	2802.35	93.41**
Error	84	85.46	1.02

contd...

Table 12: Analysis of variance for effect of fungicidal concentration on average colony diameter of T.penicillariae.

(Ref. Table 22)

Source of Variation	d.f.	S.S.	M.S.S.
Treatment (Fungicide)	6	449.89	74.98**
Concentration	5	1386.65	277.33**
Treatment x concentration	30	119.39	3.98**
Error	84	1.27	0.02

Table 13: Analysis of variance for disease severity (pre-inoculation fungicidal spray)

(Ref. Table 23)

Source of Variation	d.f.	S.S.	M.S.S.
Replication	2	4.72	2.36
Treatment	7	3712.98	530.43
Error	14	67.56	4.83

Table 14: Analysis of variance for disease severity (post-inoculation sprays)

(Ref. Table 24)

Source of Variation	d.f.	S.S.	M.S.S.
Replication	2	0.57	0.28
Treatment	7	18258.38	2608.34**
Nos. of spray	3	1416.79	472.26**
Treatment x Spray	21	410.48	19.55**
Error	62	13.86	0.22

contd...

Table 15: Analysis of variance for disease severity (combination of pre and post-inoculation sprays)

(Ref. Table 25)

Source of variation	d.f.	S.S.	M.S.S.
Replication	2	1.40	0.70
Treatment	7	15448.74	2206.96**
Spray combination	2	96.97	48.48**
Treatment x Spray combination	14	53.74	3.84**
Error	46	25.37	0.55

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