

**Studies on variability and management of pearl millet  
smut caused by *Moesziomyces penicillariae* (Bref.)**

**Vanky**

**By**

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**MASTER OF SCIENCE  
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**DEPARTMENT OF PLANT PATHOLOGY  
COLLEGE OF AGRICULTURE  
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## **CERTIFICATE – I**

This is to certify that this thesis entitled, “**Studies on variability and management of pearl millet smut caused by *Moesziomyces penicillariae* (Bref.) Vanky**” submitted for the degree of **Master of Science** in the subject of **Plant Pathology** to the **CCS Haryana Agricultural University, Hisar** is a bonafide research work carried out by **Annie Khanna (Admission No.-2015A97M)** under my supervision and guidance and that no part of the thesis has been submitted for any other degree.

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

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## **CERTIFICATE – II**

This is to certify that this thesis entitled, “**Studies on variability and management of pearl millet smut caused by *Moesziomyces penicillariae* (Bref.) Vanky**” submitted by **Annie Khanna (Admission No.-2015A97M)** to the **CCS Haryana Agricultural University, Hisar** in partial fulfillment of the requirements for the degree of **Master of Science** 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.

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## CONTENTS

| <b>CHAPTER NO.</b> | <b>DESCRIPTION</b>     | <b>PAGE(S)</b> |
|--------------------|------------------------|----------------|
| I                  | INTRODUCTION           | 1-2            |
| II                 | REVIEW OF LITERATURE   | 3-11           |
| III                | MATERIALS AND METHODS  | 12-19          |
| IV                 | EXPERIMENTAL RESULTS   | 20-38          |
| V                  | DISCUSSION             | 39-43          |
| VI                 | SUMMARY AND CONCLUSION | 44-45          |
| VII                | REFERENCES             | i-v            |

## LIST OF TABLES

| Table No. | Description                                                                                                                       | Page (s)     |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------|--------------|
| 3.1       | List of villages / locations surveyed for smut severity during <i>kharif</i> 2016 in Haryana                                      | <b>13</b>    |
| 3.2       | List of fungicides evaluated against <i>Moesziomyces penicillariae</i> under <i>in vitro</i> condition                            | <b>17</b>    |
| 3.3       | List of plant extract(s) evaluated against <i>M. penicillariae</i> under <i>in vitro</i> conditions                               | <b>18</b>    |
| 4.1       | Smut severity (%) in different pearl millet growing locations of Haryana during <i>kharif</i> 2016                                | <b>21</b>    |
| 4.2       | Morphological character(s) of different isolates of <i>Moesziomyces penicillariae</i>                                             | <b>22</b>    |
| 4.3       | Growth of <i>M. penicillariae</i> (Hisar isolate) on different media                                                              | <b>23</b>    |
| 4.4       | Growth of <i>Moesziomyces penicillariae</i> at different temperatures                                                             | <b>24</b>    |
| 4.5       | List of pearl millet hybrids, its parentage and general features                                                                  | <b>24</b>    |
| 4.6       | Virulence pattern of different isolates of <i>Moesziomyces penicillariae</i> on four different cultivars of pearl millet          | <b>25</b>    |
| 4.7       | Evaluation of pearl millet entries (Initial varietal trial) against smut under experimental sick plot                             | <b>27-28</b> |
| 4.8       | Evaluation of pearl millet entries (Advanced varietal trial) against smut under experimental sick plot                            | <b>29</b>    |
| 4.9       | Evaluation of pearl millet entries (Final yield trial) against smut under experimental sick plot                                  | <b>30</b>    |
| 4.10      | Evaluation of fungicides on growth inhibition of <i>Moesziomyces penicillariae</i> at different concentrations <i>in vitro</i>    | <b>33</b>    |
| 4.11      | Evaluation of different plant extract(s) on growth inhibition of <i>M. penicillariae in vitro</i>                                 | <b>34</b>    |
| 4.12      | Evaluation of fungicides and plant extract(s) as pre and post inoculation spray for management of <i>M. penicillariae in vivo</i> | <b>36</b>    |

## LIST OF FIGURES

| Figure | Description                                                                                                   | Page(s) |
|--------|---------------------------------------------------------------------------------------------------------------|---------|
| 3.1    | Smut severity (%) rating scale given by Thakur and King (1988)                                                | 14      |
| 4.1    | Screening of initial, advanced and released hybrids against smut severity                                     | 31      |
| 4.2    | Growth inhibition (%) of <i>M. penicillariae</i> by plant extract(s)                                          | 35      |
| 4.3    | Relative efficacy of fungicide and plant extract(s) as pre and post inoculation treatment on smut severity(%) | 37      |
| 4.4    | Effect of fungicides and plant extract(s) as pre and post inoculation treatment on yield of pearl millet      | 37      |

## LIST OF PLATES

| Plate | Description                                                                                        | Page No. |
|-------|----------------------------------------------------------------------------------------------------|----------|
| 1     | Isolates of <i>M. penicillariae</i> (A: Gwalior; B: Jaipur; C: Hisar; D: Mandore)                  | 20       |
| 2     | Culture of <i>M. penicillariae</i> in test tube, Petri plate and flask                             | 21       |
| 3     | Effect of different media on growth of <i>Moesziomyces penicillariae</i> (Hisar isolate)           | 23       |
| 4     | Sowing of pearl millet in experimental plot                                                        | 26       |
| 5     | Screening of pearl millet genotypes against smut                                                   | 26       |
| 6     | Effect of mancozeb (75% WP) @ 50, 100, 200, 500 ppm on growth of <i>Moesziomyces penicillariae</i> | 32       |
| 7     | Effect of hexaconazole (5% SC) @ 50, 100, 200 and 500 ppm on growth of <i>M. penicillariae</i>     | 32       |
| 8     | Evaluation of different plant extract(s) on growth of <i>Moesziomyces penicillariae</i>            | 34       |
| 9     | Pre and post inoculation spray of fungicides and plant extract(s) under in vivo                    | 38       |

## LIST OF ABBREVIATIONS

|                |                            |
|----------------|----------------------------|
| @              | : at the rate              |
| BOD            | : Biological Oxygen Demand |
| °C             | : degree centigrade        |
| cm             | : centimeter               |
| <i>et. al.</i> | : et alia (and others)     |
| Fig.           | : figure                   |
| g              | : gram                     |
| h              | : hour                     |
| ha             | : hectare                  |
| kg             | : kilogram                 |
| mm             | : millimeter               |
| MT             | : metric ton               |
| PDA            | : potato dextrose agar     |
| PIS            | : pre inoculation spray    |
| PMG            | : pearl millet grain       |
| PoIS           | : post inoculation spray   |
| µg             | : microgram                |

## CHAPTER-I

### INTRODUCTION

---

Pearl millet (*Pennisetum glaucum* (L.) R. Br.), also known as bajra, bajri *etc* is the important cereal crop for the population living in drought prone arid and semi arid regions of less developed countries in the world. It has been grown in Africa and the Indian subcontinent since prehistoric times. The center of diversity and suggested area of domestication of this crop is in the Sahel zone of West Africa. Recent archaeobotanical research has confirmed the presence of domesticated pearl millet on the Sahel zone of northern Mali between 2500-2000 BC (Manning *et. al.*, 2010). The cultivation of pearl millet subsequently spread and moved overseas to India. The earliest record in India dates back to 2000 BC (Fuller, 2003). Pearl millet is well adapted to growing areas characterized by drought, low soil fertility and high temperature. It performs well in soil with high salinity or low pH. It is a summer annual crop well suited for double cropping and rotation.

Pearl millet is a dependable nutritious source of food for millions living in marginal agricultural areas. Pearl millet has a high protein content with better amino acid profile than maize, sorghum, wheat and rice. It is rich in essential components like protein (10.6%), fiber (1.3%), minerals (2.3%), calcium (38mg) and iron (16.9mg). This coarse grain has multiple health benefits (<http://www.milletindia.org>).

India is the largest producer of pearl millet in Asia both in terms of area and production. In India this crop is mostly grown in the states of Haryana, Andhra Pradesh, Tamil Nadu, Rajasthan, Madhya Pradesh, Gujarat and Karnataka. As per the economic survey of India 2016-2017, the total production of pearl millet in India was estimated as 9.25 MT with area of 7.8 m ha. (<http://www.aicpmip.res.in/pmnews2016.pdf>). Pearl millet is the major *kharif* crop of Haryana with area of 5.10 lakh ha and production is 1850 kg/ ha (<http://agriharyana.nic.in/Schemes%202009/Planning%20Section/2015-16/Targets%20Kharif%202015-16.pdf>)

Pearl millet has tremendous potential to add to the food basket of India, yet due to biotic stresses the crop has suffered production constraints. Like other food crops it is exposed to large number of microorganisms mainly fungi causing smut, downy mildew, ergot, blast and rust. Smut has been recognized as important floral disease since the early part of the 19<sup>th</sup> century.

Smut is one of the major panicle diseases of pearl millet caused by *Moesziomyces penicillariae* (earlier known as *Tolyposporium penicillariae*). The systematic position of *T. penicillariae* is, Phylum: *Basidiomycotina*, Class: *Basidiomycetes*, Order: *Ustilaginales* Family: *Ustilaginaceae* Genus: *Tolyposporium* and Species: *penicillariae*. (Alexopoulos *et*

*al.*, 1998). This smut disease occurs in almost every area where pearl millet is cultivated. Chevalier (1931) first reported smut on pearl millet in the early 1930 in Senegal. In India it was first reported by Butler in 1918 and later it was reported by Ajrekar and Likhite (1933) and in M.P. by Bhatt (1946). In Chambal and Gwalior divisions, smut occupied a key position among the diseases and now it is a major biotic constraint in the full exploitation of yield potential of improved hybrids and varieties in the smut sensitive areas. Although smut on pearl millet was reported in 1930 from different parts of the world, its potential seriousness was realized only in the early 1970's with the large scale commercial cultivation of hybrids in India.

The major outbreak of this disease has proved its economic importance as a serious threat to pearl millet production in northern India, due to commercial cultivation of F1 hybrid (Thakur and King, 1988). The disease has been reported in Cameroon, Gambia, Chad, Senegal, Pakistan, Nigeria, Niger, Mali, Malawi, Mozambique, Tanzania, Zimbabwe, Zambia, Sudan and Burkina Faso (Thakur and King 1988, Wilson 2000).

Rachie and Majumdar (1980) reported upto 30 per cent of smut severity in parts of Tamil Nadu, Andhra Pradesh and Maharashtra. During the year 2002, grain losses due to pearl millet smut in the districts Gwalior, Bhind and Morena have been assessed as 8.87, 7.03 and 12.37 per cent, respectively (Rathore, 2004).

The pathogen *M. penicillariae* is reported to affect the crop at flowering stage. The infection is visible on scattered grains in the ear in which majority of grains escape damage. In pearl millet the ovaries are converted into smut sori in the affected florets. The smut sori are usually larger in size (3-4 mm long and 2-3 mm broad at the top) than normal grains (1-2 mm). In infected spikelet, sori are formed in place of normal grain, projecting outwards from the glumes. In early stages the color of smut sori appears bright green but later turns to brownish black to black on maturity. The grains are replaced by black, dense, powdery mass of spores. Sori are covered by thin film, which ruptures at maturity and release brownish black coloured spore mass. The lower portion of the ear head usually remains covered by the sheath of flag leaf and normally found heavily infected with the smut (Thakur and King, 1988).

Keeping in view the economic importance of the disease the present study was undertaken with the following major objectives:

- To find out the role of variability parameters in *Moesziomyces penicillariae* the incitant of pearl millet smut
- To assess the role of various plant extract(s) and fungicides for management of pearl millet smut

## CHAPTER-II

### REVIEW OF LITERATURE

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A survey of literature on the pertinent aspects of the proposed investigation entitled “Studies on the variability and management of pearl millet smut caused by *Moesziomyces penicillariae* (Bref.) Vanky” reveals that little information is available on various aspects. However work done by earlier workers relating directly or indirectly to the different aspects of the investigation are reviewed in this chapter under the following headings:

#### 2.1 Taxonomy

Smut on pearl millet was reported in the early 1930s at Senegal by Chevalier (1931). In India it was first reported by Butler (1918) and then Ajrekar and Likhite (1933). Wells *et al.* (1963) described the fungus under generic name *Tolyposoprium*. However, Vanky (1977) erected a new genus *Moesziomyces* for smut fungus. Chahal (1986) used the name as *Moesziomyces penicillariae* (Bref.) Vanky based on the sori character without columella and where spores firmly agglutinated in many spored spore balls by surface ornaments appearing as irregularly meshes. The name of genus *Moesziomyces* is well accepted name of causal organism at national and international level. The genus *Moesziomyces* belongs to the kingdom Fungi, phylum Basidiomycota, class Ustilaginomycetes, subclass Ustilaginomycetidae, order Ustilaginales and family Ustilaginaceae.

#### 2.2 Survey

Pearl millet smut is a widely distributed panicle disease. Rai and Thakur, 1996 reported that the smut incidence and severity was less than other pearl millet diseases but the rate was generally more than ten per cent in farmers field. This makes smut as a potential threat of causing epidemics (Wilson and Bondari, 1990). Rachei and Majmudar (1980) reported 1-30% smut severity in farmers field during survey conducted in 1950s in parts of Tamil Nadu, Andhra Pradesh and Maharashtra. Bhowmik and Sundaram (1971) reported that 50-75% of the pearl millet was infected with smut in some fields, with damage up to 100% in individual panicles. Yadav and Duhan (1993) reported smut severity of 13.4% and 10.2% in Gurgaon and Bhiwani districts of Haryana. Lubadde *et al.* (2014) reported 26.76% smut incidence on farmers field in survey conducted during 2012 in Uganda.

#### 2.3 Symptomatology & Isolation of Pearl millet smut pathogen

##### 2.3.1. Symptomatology

The symptoms constitute as smut because they form black, dusty spore masses that resemble soot or smut or the infected plant parts. In nature dikaryotic mycelium of most of the smuts appears to be obligate parasite on flowering plants. However, *Moesziomyces penicillariae* (Bref.) Vanky the causal agent of pearl millet smut occurs in almost every area

of pearl millet where ever it is cultivated. Wells *et. al.* (1963) opined that the disease could be recognized only in the advance stage when the developing seeds and the individually diseased ovaries protrude from the lemma and palea. They found the diseased ovaries dark green in colour with diameter of 2-4 mm, about twice that of normal seeds. The disease severity in a spike varied from 1-2 sori per spike to the extent of 90% infection in spike composed of several hundred ovaries.

### **2.3.2. Isolation of pearl millet smut pathogen**

Literature pertaining to the reports of Laboratory culturing of pearl millet smut pathogen is relatively scanty. However, contrary to the earlier belief of difficulty *in vitro* germination of spore ball, Ajrekar (1931) first demonstrated that the spore balls could be easily germinated on boiled potato and boiled carrot slices. Bhowmik and Sundaram (1971) obtained the first successful culture of *Tolyposporium penicillariae* on the commonly used laboratory medium potato dextrose agar (PDA).

### **2.3.3. Isolation of *Moesziomyces penicillariae* on different media**

In the light of the above finding regarding isolation of pearl millet smut pathogen on natural and synthetic media, not much work has been done to speed up the growth of the fungus while culturing under laboratory conditions. Ajrekar and Likhite (1933) successfully grew the fungus by placing spore balls on corn meal, bajra meal and jowar meal agar media. They detected very little mycelium in the fungal growth and reported that the growth consisted almost entirely of sporidia. Fischer and Holton (1957) reported that most smut species respond well to carbohydrates derived from sugars, glucose and maltose; however, there were variations within and among species as to which sugars are best. Holton *et. al.* (1968) observed that the growth of smut cultures may be mycelial, sporidial or a combination of both and smut cultures vary in type and rate of growth, topography and colour. Tripathi and Bhaktavatsalam (1977) suggested Haskin's MB-50 medium as best for the growth of *Tolyposporium penicillariae* followed by PDA medium. Pathak and Shekhawat (1980) grew the organism on seven synthetic media and reported maximum growth on Brown's medium in terms of average mycelial dry weight and sporulation followed by dextrose asparagines phosphate medium. Dashora (2005) recorded significantly highest linear growth of the fungus with excellent sporulation on Richard's agar medium followed by Czapek's dox medium and Potato dextrose agar medium at 28±2°C. Choursia (2007) evaluated five media *viz.*, potato dextrose agar, potato carrot agar, potato agar, carrot agar and Czapek's dox media to study the growth of *Tolyposporium penicillariae* under *in vitro* conditions. The maximum growth of the fungus was reported in the potato dextrose agar medium (66.75 mm) followed by potato agar medium (47.75 mm), while the minimum (23.00 mm) growth was recorded in Czapek's dox medium. Meena *et. al.* (2010) reported that glucose yeast extract medium supported

maximum growth of the fungus and yeast extract peptone agar medium was the second best in supporting the growth of *Moesziomyces penicillariae*.

#### **2.3.4. Isolation of *Moesziomyces penicillariae* at different temperatures**

To find out the most suitable temperature for culturing the pearl millet smut pathogen, various reports are available in literature. Pathak and Shekhawat (1980) found 25°C in BOD incubator as the optimum temperature for maximum fungal growth in terms of mycelial dry weight. Subba Rao and Thakur (1983), Phookan (1987) and Thakur (1989) reported 35°C temperature as optimum for maximum sporidial growth, they further observed that the growth was inhibited at temperatures less than 20°C. Meena *et. al.* (2010) grew *Tolyposoprium penicillariae* on Richard's agar medium (RA) at temperatures 15°C to 40°C in BOD incubator and observed maximum growth of the fungus at 30°C (3.2 cm) followed by 25°C (2.6 cm). The fungal colonies remained slow in growth at temperatures 15°C and 20°C. With increasing temperature the fungal growth became profuse with excellent sporulation at 30°C. However, at higher temperatures (40°C) the growth and sporulation was drastically reduced. The maximum growth (21.53 mm) occurred at 35°C in all the isolates, followed by at 30°C (21.37 mm), however, both temperatures were observed statistically at par with each other.

#### **2.4 Variability in *Moesziomyces penicillariae* (incitant of pearl millet smut)**

Variability among the organisms is a natural phenomena and it is well known that many species of fungi are physiologically different inspite of morphologically indistinguishable. Kniep (1919) was the first to report difference in the appearance of sporidial cultures of *Ustilago violacea* (Pers.) Fuckel from different host plants.

##### **2.4.1. Morphological variability**

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, physiological and ecological characters, biochemical effects, and morphology. Fischer (1953), Duran and Fischer (1961) considered spore size, shape and colour and exospores ornamentation to be the principal criteria in species delimitation in the smut fungi. Morphological variation in respect of size of spores is pronounced in many smut fungi. Fischer and Holton (1957) reported that variation in sorus characteristics is common to several species and races of *Tilletia*. Smuts are known to be highly variable in their effect on host plant development. They further 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, was inherently permanent. Although, there was variation in the size of teliospores and the length of sporidia among isolates, the isolates could not be effectively categorized into different groups on the basis of morphological variability.

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. 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. They reported that even within the same species variation in spore colour was common in some *Tilletia* and *Sphacelotheca* species. According to them, the greatest variation in the smut fungi is displayed by haploid cultures on agar media. They also reported that the incubation period required for spore germination may be even more variable than longevity in spore germination. However, the rate of spore germination and the incubation period required for spore germination influenced greatly, within a particular genetic base, by environmental factors and therefore, any variation in these parameters does not always reflect an inherent or permanent basis.

Bansal *et. al.* (1984) observed variation in size of teliospores of *Tilletia barclayana* causing bunt of rice and this was influenced by environmental conditions. Accordingly, concluded from 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. Morphological variability in spore size might be useful criteria for species delimitations within a genus of smut fungi which however, are not sufficient to elucidate variability within species. Pannu and Chahal (2000) reported that the teliospore germination in different isolates varied with the set of conditions under which they were produced and matured. Even all the sori collected from same location do not germinate at the same time when kept under same set of conditions.

Pannu *et. al.* (2002) reported that the germination might vary due to their age, physiology or genetical reasons, thus these characters could not be considered as criteria to distinguish the isolates of *Tilletia barclayana* from each other. Meena *et. al.* (2010) studied morphological, cultural and pathological variability in five isolates (Tp I, Tp II, Tp III, Tp IV and Tp V) of *M. penicillariae*, the incitant of smut of pearl millet, collected from the four different agro climatic zones of Rajasthan and reported no variation in size of sporeball whereas size of the teliospores, length of sporidia and their germination percentage varied significantly among different isolates.

#### **2.4.2. Pathogenic variability**

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. Holton *et. al.* (1968) were of the opinion that variation in smut fungi has its most fundamental impact on pathogenicity. Further variation in pathogenicity in these smuts is the key factor in the host parasite interaction relating to development resistant varieties for smut control. As per the personal communication of Thakur and Subba Rao (1986) published in International Workshop on pearl millet, that there are no report of biotype or races in this

fungus however, research at ICRISAT center indicated pathogenic variation within a single spore culture obtained from single isolates. Wilson and Bondari (1990) conducted studies on quantitative genetic analysis of pathogenicity and virulence of 45 isolates of *Moesziomyces penicillariae* and reported that the isolates differed in additive genes conferring virulence and identified six mating or compatibility groups however, the differences in general combining ability effects existed among isolates within the mating groups.

There is so far no confirmed report on variability in pearl millet smut pathogen *Moesziomyces penicillariae*.

## **2.5 Management of Smut in pearl millet**

The management of smut in pearl millet is important to reduce losses in grain yield. The use of resistant cultivars is the most economical and effective method of disease management, but the integration of various practices is also desirable.

### **2.5.1. Smut resistance in pearl millet**

Significant advances have been made in the development of screening techniques to identify resistance sources and in the utilization of these sources to breed resistant cultivars (Williams and Andrews, 1983; Andrews *et. al.*, 1985; Thakur, 1990, 1998; Hash *et. al.*, 1999).

Khairwal *et. al.* (1986) reported that the maintainer lines were significantly more resistant than the male-sterile lines and this was attributed to rapid pollination in the former ones. Three ICMPS restorer lines showed no smutted florets. Thakur *et. al.* (1986) screened 1500 pearl millet accessions against *Moesziomyces bullatus* in India, detected resistance in accessions originating from Nigeria, Senegal, Mali, Cameroon, Uganda, Lebanon and India. Selections from six germplasm accessions (SSC FS 252-S-4, ICI 7517-S-1, ExB 132-2-S-5-2-DM-1, ExB 46-1-2-S-2, ExB 112-1-S-1-1 and P-489-S-3) and four newly developed, resistant, agronomically elite lines (ICMPS 100-5-1, 900-9-3, 1600-24 and 2000-5-2) showed consistently high levels of resistance to *M. bullatus* for 1-6 years of multi location testing in India and West Africa. Some of these lines exhibited multiple resistance to other diseases also.

Wells *et. al.* (1987) found that the *tr* gene, which removes plant trichomes, stylar branches, and pleiotropically affects a number of other plant characteristics, was found to confer a high level of smut resistance to pearl millet. Prakash *et. al.* (1988) evaluated 70 hybrids from a line × tester cross of *Pennisetum typhoides* at Hisar, India, the lines 10A and 10B and the restorer H 833-2 were the best general combiners for yield and resistance to smut. The best specific combination for these characters was 10A× 77/181-4-4-3-1-5. Thakur and King (1988) derived inbred lines ICML5 to ICML10 from different germplasm accessions / breeding lines originating from Uganda, India, Nigeria, Lebanon and Senegal. Individual plant selection for stable resistance to *M. bullatus* was carried out over four to five generations. When the trials were conducted in India and West Africa over 4-7 years, these lines averaged 61% infection by *T. penicillariae* compared to 46% in the susceptible control.

Thakur *et al.* (1988) recommended pearl millet populations ICMP1, ICMP2, ICMP3 and ICMP4, each a sib-bulk of several selected lines with combined resistance to *C. fusiformis*, *M. bullatus* and *S. graminicola*, for use in breeding disease-resistant synthetics. Gahukar (1984) recommended pearl millet cv. IBV-8001 for general cultivation in the major millet-growing zones of Senegal because it was more resistant to *M. bullatus* and its yield was also comparatively higher than those of cv. Souna. Thakur (1989) observed that three cytoplasmic male-sterile lines exhibited longer protogyny periods and higher smut severity than their corresponding maintainer lines. Four lines with short protogyny periods (22-52 h) and resistance to ergot also exhibited high resistance to smut.

Thakur *et al.* (1992) identified sources of resistance against *M. bullatus* in pearl millet germplasm from diverse geographical zones by screening more than 10,700 entries using a field screening technique developed at ICRISAT and listed the smut-resistant lines with their respective pedigrees, agronomic characteristics and disease reactions. Several of these sources have since been used in breeding programme to develop agronomically superior smut-resistant lines. Yadav *et al.* (1992) reported that smut susceptibility may be attributed to the effects of cytoplasm  $\times$  nuclear interactions. Yadav (1994) reported that the cytoplasm is not thought to be associated with higher smut susceptibility. The partitioning of variance into different components showed that pollinators, A/B line pairs and their interaction primarily influenced smut severity of hybrids.

Rai and Thakur (1996) reported that pearl millet hybrids based on the A1 cytoplasmic-nuclear male-sterile (CMS) lines were more susceptible to smut than open-pollinated varieties. The A-line hybrids had higher smut severity and lower selfed seed set than the B-line hybrids, indicating that it was the CMS-mediated male sterility rather than the A1 cytoplasm *per se*, which caused greater smut severity. An improvement in the smut resistance of parental lines and the fertility restoration ability of pollinators would provide an effective genetic approach to smut disease management in hybrids. Rai *et al.* (1998) developed male-sterile pearl millet lines ICMA 88006, which derived its A1 cytoplasm from ICMA1 (81A), and ICMA92666 (and its maintainer line ICMA 92666) which were resistant to *M. bullatus* and other pathogens. Pandya and Bartaria (2000) evaluated several pearl millet lines under artificial inoculation and identified ICMB 92888, ICMB 92777 and IP 19874 as source of smut resistance.

Yadav *et al.* (2000) made crosses from six smut resistant and 4 susceptible inbred lines and parents and F1s grown under condition of natural infection at Hisar. Four of the resistant parents, 8 F1 hybrids in the resistant  $\times$  resistant group and 15 in the resistant  $\times$  susceptible group possessed below average smut reaction and were also stable. Narwal and Yadav (2001) evaluated a set of 29 inbreds of pearl millet against genetic makeup of smut resistance by triple test crop analysis. The experiment was based on two tester parents, 81B

and ICMPS 1500-76-3-2, which had extreme opposite resistance to smut. Crossing each line with both the parents and their F1 created three female families of 29 crosses each. The development of smut was artificially caused by application of aqueous suspension of smut at  $10^6$  sporidia  $\text{ml}^{-1}$  at the boot leaf stage. The gene for smut resistance appeared dispersed among the inbred lines. The additive variance was mainly responsible for inheritance of smut. The non additive genetic variance was detected only in the form of i-type epistasis. The gene effects indicated the possibility of success of selection in early generations for population improvement and inbred development. Transfer of smut resistance through backcross method was also recommended.

Pandya *et al.* (2005) evaluated twenty promising pearl millet entries under artificial inoculation and identified PB 106 (Proagro hybrid) as a source of smut resistance. Choursia (2007) evaluated 138 entries against smut and observed that only one entry MH 1317 remained absolutely free from smut and nineteen entries ranged in the category of 5.1- 10 per cent smut severity while maximum smut severity was recorded in MH 1391 (90%) possibly due to reason of high rain fall, low temperature during flowering stage, and amongst nineteen hybrids evaluated against smut; PB 106 remained completely free from smut and maximum smut severity was noticed in 852 B (check).

### **2.5.2. *In vitro* evaluation of plant extracts and fungicides against *Moesziomyces penicillariae***

The major limitations to chemical control of smut in pearl millet are low monetary value of the crop, and scarcity of resources available to pearl millet growing farmers. For effective and economic control of the disease a combination of indigenous knowledge and biocontrol agents as spray treatment for managing the smut is the requirement of current era.

Bhowmik and Sundaram (1971); Pathak and Gaur (1975) and Phookan (1987), reported carboxin as the most effective fungicide followed by captafol and carbendazim. Shekhawat *et al.* (1984) tested eight antifungal compounds and proved Ridomil (500 ppm) as most inhibitory for growth of the *Tolyposporium penicillariae*. Meena and Mariappan (1993) reported that leaf extracts of *Azadirachta indica*, *Mentha arvensis*, *Aegle mormelos*, *Catharanthus roseus*, *Lantana camara*, *Pongamia pinnata*, *Vitex negundo*, *Nerium odorum* and flower extracts of *Catharanthus roseus* inhibited mycelia growth and spore germination of the seedborne mycoflora of sorghum including *Alternaria tenuis*, *Asperigillus flavus*, *Curvularia lunata*, *Fusarium moniliforme* and *Rhizopus stolonifer*. The neem extracts, *C. roseus* and *L. camera* were more effective than the other plant extracts tested. Rajput (2000) tested *Eucalyptus*, *Parthenium* and *Calotropis* in the form of leaf extracts @ 10% and neem in the form of leaf extract and seed extract @ 10% each and neem oil @ 5% against *Tolyposporium penicillariae* under *in vitro* condition. *Eucalyptus* was found best among the plant extract evaluated. Choursia (2007) tested 11 botanicals *viz.*, *Datura*, *Eucalyptus*, *Parthenium*, *Ipomea*, *Calotropis*, *Azadirachta indica*, Tobacco, *Aloe vera*, ginger, garlic and

turmeric in the boil form in two concentration *i.e.* 10 & 20 % against *T. penicillariae*. *Eucalyptus* extract @ 20 % showed maximum control of mycelium growth as compared to control and resulting in increase of yield. Dashora and Kumar (2009) reported *in vitro* evaluation of fungicides against smut pathogen and found thiram providing relatively best inhibition of diametric growth of *T. penicillariae* at all the concentrations tried, however, the maximum inhibition was recorded at 200 µg ml<sup>-1</sup> concentration (84.9%) followed by 100 and 25 µg ml<sup>-1</sup>. Sasode and Singh (2013) used different forms of *Calotropis* leaf extract against different pathogens and among them one was *Tolyposporium penicillariae*. Out of the four forms used powder, crude, boiled and ethanol at the 10% concentration, boiled was found to be effective. Also boiled form at different concentrations *i.e.* at 5%, 10%, 15% and 20% were tested and it was observed that 20% concentration showed significant inhibition of the *T. penicillariae*. Singh *et. al.* (2013) observed that the effectivity of crude and ethanol extract against *Tolyposporium penicillariae* was gradually increased with increase in the concentration from 20 to 50% and 1 to 4% respectively on comparison with carbendazim (0.1%) and mancozeb (0.2%). However both the chemicals were found more effective than the aloe vera leaf extract (crude/powder/ethanol) and recommended to use high concentration of aloe vera leaf extract.

Sharma *et. al.* (2017) reported that all the twenty plant extracts (at 20% concentration) evaluated with control (Only PDA) against *Tolyposporium penicillariae* significantly inhibited mycelial growth of smut pathogen. The highest mycelial inhibition per cent was found in case of *Eucalyptus* sp. (86.56%) which was followed by *Azadirachta indica* (seed) (79.65%), *Parthenium historophorus* (74.11%) and *Azadirachta indica* leaves extract (73.52%).

### **2.5.3. *In vivo* evaluation of plant extract(s) and fungicides against smut of pearl millet caused by *Moesziomyces penicillariae***

Little information is available in the literature regarding evaluation of newer fungicides to manage pearl millet smut caused by *Moesziomyces penicillariae*. Seed treatment with fungicides is not effective since the disease is not seed borne. Butler (1918) tried to control the disease by treating the seeds with formalin and copper sulphate solution as well hot water treatment but was not successful in controlling the disease. However, Bhaktavastalam and Tripathi (1975) reported that seed treatment with carboxin was very effective in controlling the smut disease. Wells (1967) reported effective control of smut with Plantavax and Vitavax as foliar and panicle sprays. Bhowmik and Sundaram (1971) reported more effective control of smut with Plantavax® than with Vitavax® and Benlate®. Pathak and Gaur (1975) reported effective control of smut by captofol, zineb and heptaene. Similarly Gill *et. al.* (1979) also observed reduced smut severity in the plants sprayed with oxycarboxin, carboxin and captafol. Rachie and Majmudar (1980) tried various fungicides such as Ceresan,

Agrosan, Zineb and Mancozeb, Plantavax, Vitavax and Benlate either as seed, foliage, or panicle-spray treatments with limited success. Dashora and Kumar (2008) reported that a combination of seed treatments with raw cow milk or raw goat milk (50% dilution with water for 18 h) with *Gliocladium virens* (6g kg<sup>-1</sup>) and soil treatment with *G. virens* (10g m<sup>-2</sup>) provided 58.9% protection over control in field and also suggested that this could be treated as an important component in an integrated smut management strategy for resource-poor farmers. Kumar (2011) reported that four sprays of capatafol (3 g l<sup>-1</sup>) effectively managed the disease in field. Meena *et. al.* (2012) reported that carboxin, carbendazim, copper oxy chloride, mancozeb, hexaconazole and propiconazole reduced smut severity as compared to control. Their study revealed that hexaconazole and propiconazole were superior over carboxin giving 97.63 and 97.43 per cent disease control, respectively.

## CHAPTER-III

### MATERIALS AND METHODS

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The present investigation was conducted in sick field of Plant Pathology experimental area of CCS HAU, Hisar during *kharif* season of 2015. The details of the methodology in conducting the studies, observation recorded are described below:

#### 3.1 Materials

##### 3.1.1. Collection and preservation of the isolates

To study the variability of the isolates of pearl millet smut caused by *Moesziomyces penicillariae*, smutted pearl millet inflorescence were collected from four different locations in India *viz.*, Jaipur, Hisar, Gwalior and Mandore during November 2016. The smutted ear heads were sun dried, sealed in polythene bags and stored at  $5\pm 1^{\circ}\text{C}$  in refrigerator for future study.

##### 3.1.2. Seeds

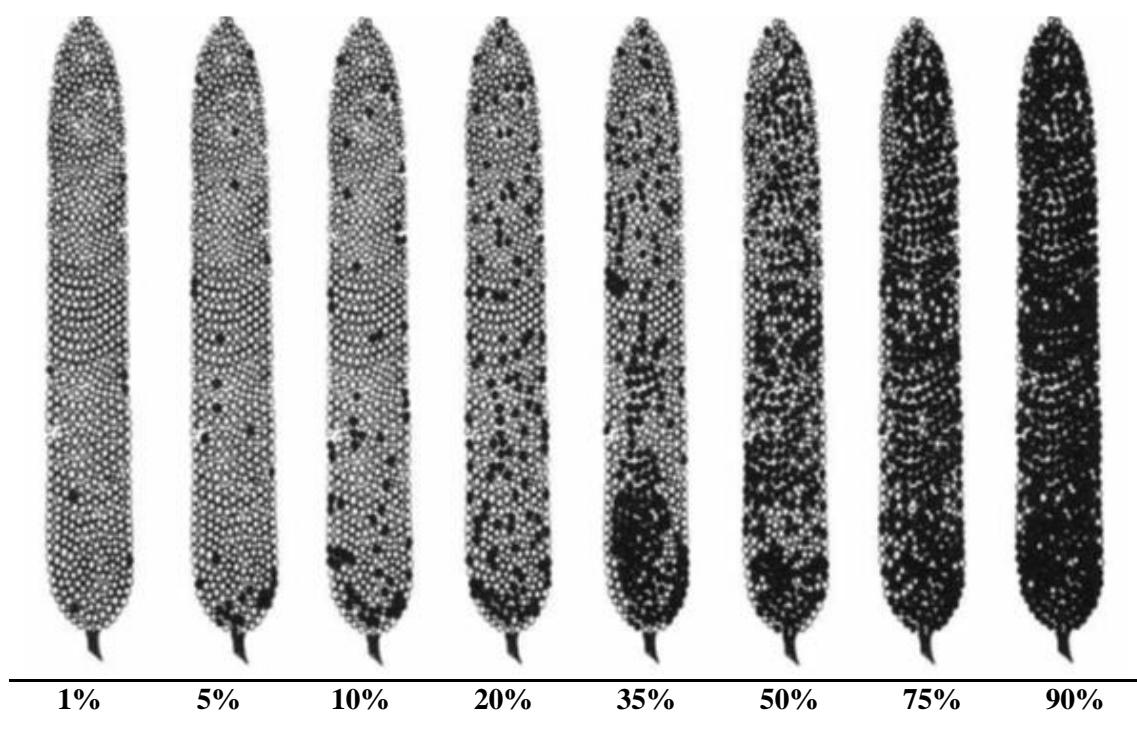
Seeds of pearl millet hybrid HHB 197, HHB 67 Improved, HHB 226 were obtained from Bajra Section, Department of Genetics & Plant Breeding and seeds of RHB 177 were obtained from Jaipur, Rajasthan. For the screening purpose seeds of different genotypes were obtained from AICPMIP.

#### 3.2 Survey

The survey was conducted during *Kharif* 2016 in six districts of Haryana *viz.*, Hisar, Jhajjar, Rohtak, Bhiwani, Rewari and Mohindergarh. The detail of villages/ locations surveyed in each district are listed in Table 3.1. The survey was conducted during first fortnight of September 2016 and second survey was conducted during first fortnight of October 2016. In each field five quadrants were made (four at the corners and one in the middle of the field) and disease severity was calculated by assessing 30 plants in each quadrant per field. The smut severity(%) rating was scored using scale given by Thakur and King (1988) (Fig. 3.1).

**Table 3.1: List of villages/ locations surveyed for smut severity during *kharif* 2016 in Haryana**

| <b>District</b> | <b>Village/ location</b> |
|-----------------|--------------------------|
| Bhiwani & Dadri | Bawani khera             |
|                 | Siwani                   |
|                 | Palri                    |
|                 | Badhwana                 |
|                 | Jawa                     |
| Mohindergarh    | Bhagot                   |
|                 | Pota                     |
|                 | Sayana                   |
|                 | Nautana                  |
|                 | Sehlang                  |
| Rewari          | Dahina                   |
|                 | Mandola                  |
|                 | Nimoth                   |
|                 | Dhawana                  |
|                 | Bawal                    |
| Rohtak          | Kharkara                 |
|                 | Meham                    |
|                 | Lahli                    |
|                 | Kheri sadh               |
|                 | Rohtak                   |
| Jhajjar         | Beri                     |
|                 | Dujna                    |
|                 | Jhajjar                  |
|                 | Badli                    |
|                 | Sultanpur                |
| Hisar           | Hisar                    |
|                 | Gangwa                   |
|                 | Muklan                   |
|                 | Chaudhariwas             |
|                 | Dabra                    |



**Fig.3.1 Smut severity (%) rating scale given by Thakur and King (1988)**

### 3.3 Preparation of media

It was necessary to have good medium for better growth and recovery of *M. penicillariae* which must be easy to prepare. Six different types of media were prepared in distilled water and autoclaved at 15 lbs pressure for 20 minutes. The composition of various media used in the present investigation is given below:

#### 3.3.1. Potato Dextrose Agar medium

| Component             | Quantity (gL <sup>-1</sup> ) |
|-----------------------|------------------------------|
| Peeled potato extract | 200g                         |
| Dextrose              | 20g                          |
| Agar-agar             | 20g                          |

#### 3.3.2. Czapek dox agar medium

| Component              | Quantity (gL <sup>-1</sup> ) |
|------------------------|------------------------------|
| Sucrose                | 30                           |
| Sodium nitrate         | 2.0                          |
| Di-Potassium phosphate | 1.0                          |
| Magnesium sulphate     | 0.5                          |
| Potassium chloride     | 0.5                          |
| Ferrous sulphate       | 0.01                         |
| Agar-agar              | 15                           |

### 3.3.3. Oat meal agar medium

| Component     | Quantity (gL <sup>-1</sup> ) |
|---------------|------------------------------|
| Oat meal      | 20                           |
| Yeast extract | 1                            |
| Agar-agar     | 20                           |

### 3.3.4. PDA medium enriched with different concentration of pearl millet grains and sucrose

| Component           | Quantity (gL <sup>-1</sup> ) |
|---------------------|------------------------------|
| Peeled potato       | 200                          |
| Dextrose            | 20                           |
| Sucrose             | 20                           |
| Pearl millet grains | 25                           |
| Agar-agar           | 20                           |

| Component           | Quantity (gL <sup>-1</sup> ) |
|---------------------|------------------------------|
| Peeled potato       | 200                          |
| Dextrose            | 20                           |
| Sucrose             | 20                           |
| Pearl millet grains | 50                           |
| Agar-agar           | 20                           |

| Component           | Quantity (gL <sup>-1</sup> ) |
|---------------------|------------------------------|
| Peeled potato       | 200                          |
| Dextrose            | 20                           |
| Sucrose             | 20                           |
| Pearl millet grains | 100                          |
| Distilled water     | 1L                           |

### 3.4 Isolation and maintenance of *M. penicillariae* isolate

For isolation and multiplication of fungus, Potato Dextrose Agar (PDA) medium was used. The unruptured mature sori were removed from the smutted earhead and surface sterilized for two minutes with 0.1% mercuric chloride. Once sterilized the sori were rinsed twice in sterile distilled water and crushed aseptically with the help of sterilized forceps and needle in a sterilized Petri plate to obtain the sporeballs. The sporeballs were used for the inoculation of PDA medium poured in Petri plates. The inoculated Petri plates were then incubated at 35°C in BOD incubator. Periodical subculturing was also performed to maintain isolates during research work.

### **3.5 Morphological studies**

All the isolates were examined for following morphological differences in spore ball and sporidia.

**3.5.1. Sporeball :** Mature smut sori were crushed on a clean dry Petri dish to obtain spore balls. With the help of the forceps sporeballs were mounted on a drop of water on a clean glass slide. The glass slide was examined under compound microscope (10X) for colour, size and shape of sporeballs. For measuring size, fifty sporeballs in two slides were examined to compute the mean value.

**3.5.2. Sporidia :** The sporidia used for the examination were obtained from ten days old culture of sporeballs on potato dextrose agar medium at 35°C. For measuring the length, shape and colour, fifty sporidia from two Petri dishes were examined to obtain the mean value.

**3.5.3. Spore germination:** For studying the spore germination, spore balls of all the four isolates were suspended in sterile distilled water in cavity slides and cavity slides were kept in Petri dishes lined with moist Whatman filter paper of 90 mm and then Petri plates were incubated at 30°C with two replications. Observations on germination per cent were recorded after 20 h of incubation by adopting the procedure given by Subha Rao and Thakur (1983).

**3.6 Cultural variability:** Based upon morphological studies cultural study was carried out for the most virulent isolate *i.e.* Hisar isolate.

#### **3.6.1. Growth of *Moesziomyces penicillariae* on different media**

Six different media were used to study their effect on the growth of *Moesziomyces penicillariae* (Hisar isolate). Four replications were maintained for each media. After inoculation in each media, Petri plates were incubated at 35°C and observations were recorded at regular interval after every week until the plate was full. The Petri plates were observed for recording the colour, type of growth and mean colony diameter on different media.

### **3.7 Physiological studies**

#### **3.7.1. Growth of *M. penicillariae* at different temperatures**

Two week old culture of *M. penicillariae* (Hisar isolate) was used to study growth at different temperatures. After inoculating the *M. penicillariae* Petri plates were incubated at three different temperatures *i.e.* 25°C, 30°C and 35°C with four replications. Observations for colony diameter and colour were recorded regularly at weekly interval.

### **3.8 Pathological variability**

In order to examine virulence pattern of *Moesziomyces penicillariae* isolates, all the four isolates designated as Mp-1, Mp-2, Mp-3 and Mp-4 were inoculated on four cultivars of pearl millet *viz.*, HHB 67 Improved, HHB 197, HHB 226 and RHB 177 by maintaining identical environmental conditions under field conditions. The inoculation was done using sporidial suspension ( $10^6$  sporidia ml<sup>-1</sup>) from ten days old culture of each isolate at boot leaf stage. Ten earheads of each cultivar, with three replications were tagged for inoculation of each

isolate. Immediately after inoculation the earheads were covered with the parchment paper bags and labeled. Overhead sprinkler irrigation was provided to maintain high humidity. After 15 to 20 days of inoculation, earheads of each cultivar were scored for smut severity using standard smut severity (%) rating scale as suggested by Thakur and King, 1988.

### 3.9 Screening of genotypes for smut resistance

The experiment for screening of 239 entries was conducted during the *kharif* 2016 with two replications in randomized block design (RBD), with row length of 5 meter. The test entries were sown on 16-07-2016 with row to row spacing of 50 cm by maintaining plant to plant distance of 10 cm in each row.

For artificial screening fifteen earhead at boot leaf stage of each entry were randomly selected from each replication and were inoculated by aqueous sporial suspension of *Moesziomyces penicillariae*. The sporidial suspension was prepared by dipping the smut infected earheads in water for 24 h with a view to germinate the spores before inoculation. After inoculation of the earheads at boot leaf stage, the earheads were covered with a parchment paper bag and water was sprinkled regularly to maintain high humidity. The parchment paper bags were removed 15-20 days after inoculation and smut severity was recorded using smut severity (%) rating scale as given by Thakur and King (1988).

### 3.9 Evaluation of fungicides against *Moesziomyces penicillariae* in vitro

Five fungicides viz., carbendazim, copper oxychloride, hexaconazole, propiconazole and mancozeb (Table 3.2) at four different concentrations viz., 50, 100, 200 and 500 ppm were evaluated to test their efficiency against *M. penicillariae*.

Different concentrations of fungicides were prepared by dissolving in sterile distilled water. By diluting the fungicides, they were adjusted to the required concentrations i.e. 50, 100, 200 and 500 ppm and used to study their effect on growth of the fungus.

**Table 3.2: List of fungicides evaluated against *Moesziomyces penicillariae* under in vitro condition**

| S. No. | Fungicide                 | Chemical name                                                               | Trade name |
|--------|---------------------------|-----------------------------------------------------------------------------|------------|
| 1      | Carbendazim 50% WP        | Methyl-2- benzimidazole- carbamate                                          | Bavistin   |
| 2      | Copper oxychloride 50% WP | Copper(2) chloride hydroxide                                                | Blitox     |
| 3      | Propiconazole 25% EC      | 1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole | Tilt       |
| 4      | Hexaconazole 5% SC        | RS-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2-ol               | Hexacare   |
| 5      | Mancozeb 75% WP           | Manganese ethylene bisdithio-carbamate plus zinc                            | Indofil-45 |

Potato dextrose agar (PDA) medium was used as growth substrate to study the effect of fungicides on the growth of the *M. penicillariae*. PDA of double strength was used in this experiment. Equal volume of the individual fungicides at required concentration was prepared in sterile distilled water and then mixed with double strength PDA in conical flask of 250 ml capacity.

Under aseptic conditions, 20 ml of each mixture was poured in 9 cm diameter Petri plates and was allowed to solidify. Each plate was then inoculated with bit from two week old culture. The inoculated Petri plates were then incubated at 35°C in BOD incubator and colony diameter was recorded regularly after one week interval. Four replications for each fungicide at each concentration were maintained along with the control. The fungus growth was measured using metric scale and inhibition percentage was calculated by the formula given by Vincent (1947).

$$\text{Growth inhibition (\%), I} = \frac{C-T}{C} \times 100$$

Where

I = Inhibition

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

### 3.11 Evaluation of plant extract(s) against *Moesziomyces penicillariae* *in vitro*

Five plant extracts of *aloe vera*, jamun, lantana, neem and eucalyptus leaves at 20 % concentration were taken to test their efficiency against *M. penicillariae* (Table 3.3). The extracts were prepared by crushing 100 g leaves of each plant species in 100 ml distilled water using mixer and grinder. The supernatant was filtered using muslin cloth followed by Whatman filter paper No. 1. The filtrate were then centrifuged at 5000 rpm for 20 min. The clear supernatant obtained were kept in refrigerator and were used in further experiment.

**Table 3.3: List of plant extract(s) evaluated against *M. penicillariae* under *in vitro* conditions**

| S. No. | Common name | Botanical name            | Family        |
|--------|-------------|---------------------------|---------------|
| 1.     | Safeda      | <i>Eucalyptus</i>         | Myrtaceae     |
| 2.     | Jamun       | <i>Syzygium cumini</i>    | Myrtaceae     |
| 3.     | Neem        | <i>Azadirachta indica</i> | Meliaceae     |
| 4.     | Aloe        | <i>Aloe barbadensis</i>   | Asphodelaceae |
| 5.     | Lantana     | <i>Lantana camara</i>     | Verbenaceae   |

The effect of plant extracts on the growth of *M. penicillariae* was evaluated *in vitro* using poison food technique. An aqueous solution of plant extract was prepared in sterile distilled water and added aseptically to PDA to achieve a final concentration of 20% before

pouring the medium into Petri plates (20 ml PDA per Petri plate). Four replications were maintained for each plant extract mixed with double strength PDA medium. The Petri plates were inoculated with *M. penicillariae* under aseptic conditions. The Petri plates were incubated at 35°C and growth of *M. penicillariae* was observed at weekly interval until the growth in the Petri plates (control) was completely full. Growth inhibition per cent was calculated using the formula given by Vincent (1947).

### **3.10 Evaluation of fungicide and plant extract(s) against smut of pearl millet caused by *Moesziomyces penicillariae* in vivo**

The experiment was conducted during *kharif* 2016 with three replication in randomized block design (RBD) with plot size of 5x3 meter. The test host HHB197 was sown on 16-07-2016. Two plant extract(s) viz., *Aloe vera* @ 20%, jamun @ 20% concentration and two fungicides viz., copper oxychloride @ 0.05% and carbendazim @ 0.05% were evaluated as pre and post inoculation spray for management of pearl millet smut caused by *M. penicillariae*

Twenty plants were randomly tagged from each replication for pre and post inoculation spray.

#### **3.12.1. Pre inoculation treatment with fungicides and plant extract(s)**

Earheads which were about to emerge from the boot leaf within next 48 hours were protected from contamination by covering them with parchment paper. The selected plants were sprayed with fungicides and plant extracts 24 h before inoculation. Ear heads were sprayed carefully to avoid any fungicidal and plant extract drift to adjacent plots. After 24 h of fungicidal and plant extract spray, plants were inoculated with the teliospore suspension with the help of hand atomizer. After inoculation fine mist of water was sprayed to maintain the high humidity.

#### **3.12.2. Post inoculation treatment with fungicide and plant extract(s)**

Selected plants were inoculated with the teliospore suspension before spraying the fungicidal and plant extract(s) solution at boot leaf stage. The inoculated earheads were covered with parchment paper immediately after the inoculation. After 24 h, bags were lifted carefully to spray the earhead with fungicide and plant extract(s). Bags were replaced soon after spraying and humidity was maintained by spraying water regularly. Observations of smut severity were recorded after 15-20 days by removing parchment paper bag and yield data was recorded after complete grain formation.

The relative efficiency of fungicides and plant extract(s) were determined on the basis of control.

## CHAPTER-IV

### EXPERIMENTAL RESULTS

The present investigation entitled “Studies on variability and management of pearl millet smut caused by *Moesziomyces penicillariae* (Bref.) Vanky” was carried out in the Department of Plant Pathology & Bajra Section, Department of Genetics & Plant Breeding during 2016-17. The results of various laboratory as well as field experiments on pearl millet smut are described under the following headings:

#### 4.1 Survey

The survey was conducted during *kharif* season 2016 in six major pearl millet growing districts of Haryana. From each district five villages/ locations were selected. First survey was carried out during the first fortnight of September 2016 and second survey was carried out during first fortnight of October 2016. The results of the survey are enumerated in Table 4.1. During survey maximum smut severity to the extent of 10% was observed in Bawal, Hisar, Nautana and Siwani followed by Bhagot, Sayana, Dahina and Lahli (5%) whereas smut severity to the extent of 2% was observed in Jawa, Sehlana, Mandola, Khaleta, Dhawana and Chaudhariwas. However in villages Palri, Badhwana, Pota, Nimoth, Kherisadh, Beri, Jhajjar, Badli, Muklan and Dabra smut severity was restricted upto 1%. In seven villages/ locations *viz.*, Bawani Khera, Kharkara, Meham, Rohtak, Dujna, Sultanpur and Gangwa it was found free from smut.

#### 4.2 Isolation and maintenance of *Moesziomyces penicillariae*

Four isolates were retrieved from smutted samples collected from different locations of India where pearl millet is cultivated. The isolates of *M. penicillariae* were maintained on PDA medium. Among the four isolates obtained from the samples collected from Hisar, Jaipur, Gwalior and Mandore, no variation was observed in the growth pattern (Plate 1).

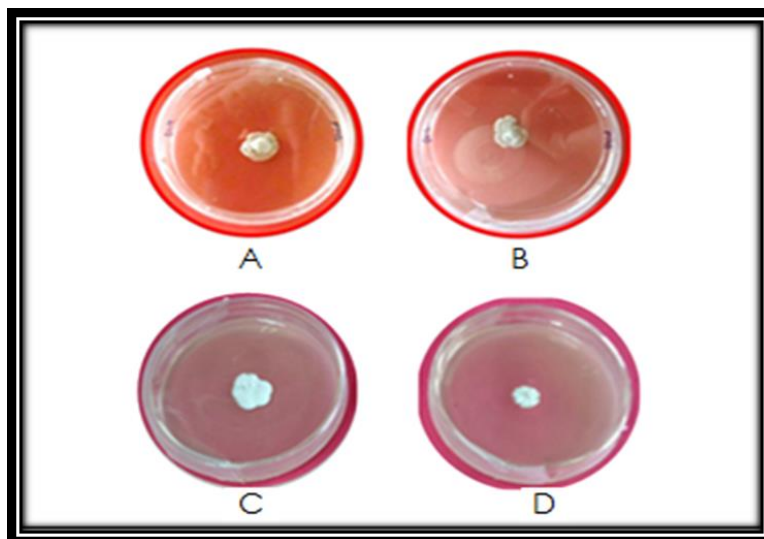
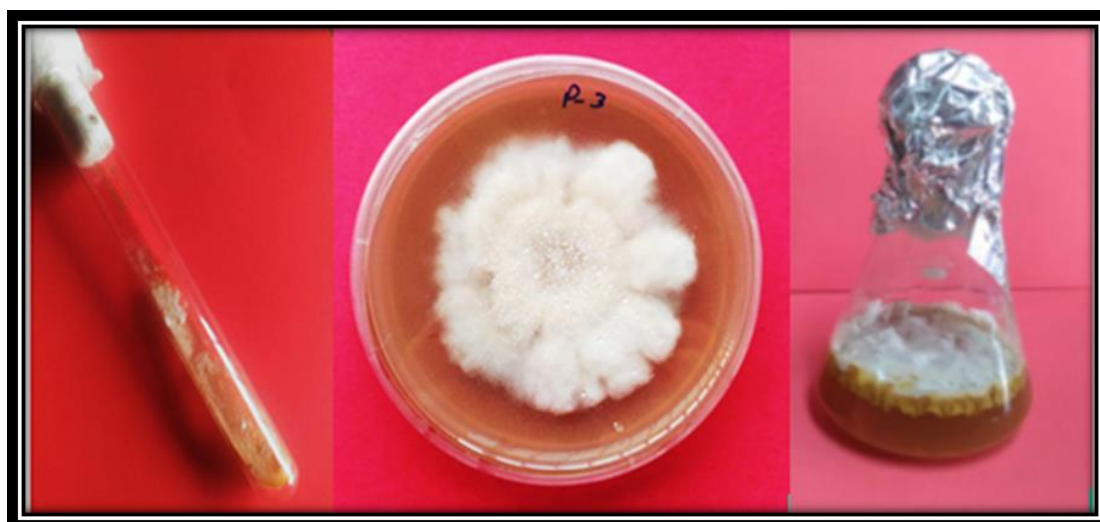


Plate 1: Isolates of *Moesziomyces penicillariae*- A: Gwalior; B: Jaipur; C: Hisar; D: Mandore



**Plate 2: Culture of *M. penicillariae* in test tube, Petri plate and flask**

**Table 4.1: Smut severity (%) in different pearl millet growing locations of Haryana during *kharif* 2016**

| <b>District</b> | <b>Village/ Location</b> | <b>Smut Severity (%)</b> |
|-----------------|--------------------------|--------------------------|
| Bhiwani & Dadri | Bawani khera             | 0                        |
|                 | Palri                    | 1                        |
|                 | Badhwana                 | 1                        |
|                 | Siwani                   | 10                       |
|                 | Jawa                     | 2                        |
| Mohindergarh    | Bhagot                   | 5                        |
|                 | Pota                     | 1                        |
|                 | Sayana                   | 5                        |
|                 | Nautana                  | 10                       |
|                 | Sehlang                  | 2                        |
| Rewari          | Dahina                   | 5                        |
|                 | Mandola                  | 2                        |
|                 | Nimoth                   | 1                        |
|                 | Dhawana                  | 2                        |
|                 | Bawal                    | 10                       |
| Rohtak          | Kharkara                 | 0                        |
|                 | Meham                    | 0                        |
|                 | Lahli                    | 5                        |
|                 | Kheri sadh               | 1                        |
|                 | Rohtak                   | 0                        |
| Jhajjar         | Beri                     | 1                        |
|                 | Dujna                    | 0                        |
|                 | Jhajjar                  | 1                        |
|                 | Badli                    | 1                        |
|                 | Sultanpur                | 0                        |
| Hisar           | Hisar                    | 10                       |
|                 | Gangwa                   | 0                        |
|                 | Muklan                   | 1                        |
|                 | Chaudhariwas             | 2                        |
|                 | Dabra                    | 1                        |

### 4.3 Variability of *M. penicillariae*

#### 4.3.1 Morphological variability

Sporeballs, sporidia and germination per cent of each isolate were examined separately under compound microscope for difference in morphological characteristics. The detail observations of each morphological parameter are described below:

##### 4.3.1.1. Sporeballs

Size, shape and colour of fifty sporeballs from each isolate were examined microscopically. The results are presented in Table 4.2. Among all the isolates it was observed that shape of sporeballs of all the isolates did not vary much as colour observed was dark brown to black colour, while the shape was found semi-circular to irregular. Therefore, the isolates could not be effectively classified into different groups on the basis of shape and colour. A significant variation was observed in the length and breadth of the sporeball varying in a range of  $16.2 \times 21.5$  to  $73.4 \times 92.4$   $\mu\text{m}$ . The Hisar isolate was found to be the larger with mean length and breadth of  $73.4 \times 92.4$   $\mu\text{m}$  followed by Gwalior, Mandore and Jaipur isolate with sporeball size of  $51.6 \times 58.8$ ,  $37.0 \times 46.8$  and  $16.2 \times 21.5$   $\mu\text{m}$ , respectively.

##### 4.3.1.2. Sporidia

The sporidia of the Hisar, Gwalior, Mandore and Jaipur isolates were obtained from the ten days old culture grown on PDA at 35°C. In all the isolates sporidia were hyaline in colour and single celled but variation was observed in length. Maximum sporidial length (16.1  $\mu\text{m}$ ) was observed in Hisar isolate followed by Gwalior (15.4 $\mu\text{m}$ ), Mandore (15.1 $\mu\text{m}$ ) and Jaipur isolate (14.4 $\mu\text{m}$ ) (Table 4.2). But no correlation was found between sporidial length and sporeball size of different isolates.

##### 4.3.1.3. Germination per cent of sporeballs

The germination of sporeballs for each isolate was calculated after 20 h of incubation at 30°C. The results of the experiment are presented in Table 4.2. It was observed that mean germination per cent of Hisar isolate was maximum *i.e.* 52.24% followed 48.62, 45.70 and 40.54 % respectively for Jaipur, Gwalior and Mandore isolate.

**Table 4.2: Morphological character(s) of different isolates of *Moesziomyces penicillariae***

| Location | Size of sporeball ( $\mu\text{m}$ ) | Colour of sporeboll | Shape of Sporeboll | Size of sporidia ( $\mu\text{m}$ ) | Germination per cent |
|----------|-------------------------------------|---------------------|--------------------|------------------------------------|----------------------|
| Jaipur   | $16.2 \times 21.5$                  | Dark brown          | Irregular          | 14.4                               | 48.62                |
| Hisar    | $73.4 \times 92.4$                  | Dark brown          | Semi circular      | 16.1                               | 52.24                |
| Gwalior  | $51.6 \times 58.8$                  | Black               | Irregular          | 15.4                               | 45.70                |
| Mandore  | $37.0 \times 46.8$                  | Blackish brown      | Irregular          | 15.1                               | 40.54                |

Based on the morphological characters like size of sporeball, size of sporidia and germination per cent Hisar isolate was selected for further investigation.

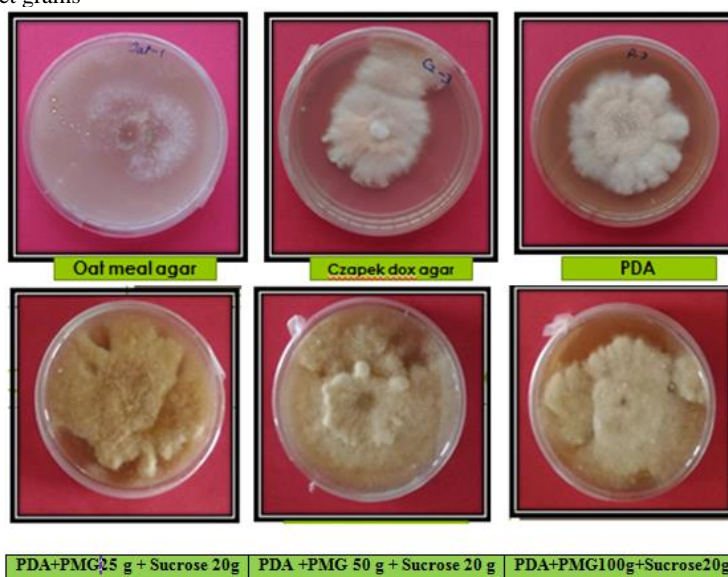
#### 4.3.2 Effect of different media on the growth of *Moesziomyces penicillariae*

Six different media comprising one semi synthetic, two synthetic and PDA enriched with sucrose @ 20gL<sup>-1</sup> and pearl millet grains at different concentrations (25, 50 and 100 gL<sup>-1</sup>) were used to study the growth characteristics of Hisar isolate. The media in Petri dishes were inoculated with equal amount of sporidial suspension (1×10<sup>6</sup> ml<sup>-1</sup>). Growth of *M. penicillariae* was observed regularly at weekly interval till the Petri plate was completely filled. Variation was observed both in the colony colour and growth of *Moesziomyces penicillariae* on different media. It is evident from Table 4.3 that after one week, PDA enriched with 50 g pearl millet grains with 20 g sucrose showed the maximum growth of 3.20 cm, followed by PDA enriched with 25 gL<sup>-1</sup> pearl millet grains with 20 gL<sup>-1</sup> sucrose with 3.03 cm growth. Further, *M. penicillariae* was also found to be fast growing on PDA enriched with 50 g pearl millet grain and 20 g sucrose than PDA enriched with 25 g pearl millet grain and 20 g sucrose after 2<sup>nd</sup> and 3<sup>rd</sup> week. The increase in pearl millet concentration in PDA+ sucrose media to 100 g was not found to be effectively increasing the mycelial growth. Least growth was observed in case of oat meal agar medium. It was found that PDA enriched with pearl millet grain @ 50 g and sucrose @ 20 g performed better than PDA, Czapek dox and oat meal agar medium.

**Table 4.3: Growth of *M. penicillariae* (Hisar isolate) on different media**

| Medium                        | Colony diameter (cm) |        |        |
|-------------------------------|----------------------|--------|--------|
|                               | 1 week               | 2 week | 3 week |
| Potato Dextrose Agar (PDA)    | 2.60                 | 6.07   | 7.27   |
| Czapek dox agar               | 1.82                 | 5.05   | 5.92   |
| Oat meal agar                 | 1.02                 | 2.85   | 4.75   |
| PDA + PMG* 25 g + 20g sucrose | 3.03                 | 6.29   | 7.82   |
| PDA + PMG 50 g + 20g sucrose  | 3.20                 | 6.49   | 7.97   |
| PDA + PMG 100 g + 20g sucrose | 2.62                 | 6.09   | 7.42   |

PMG\* - Pearl millet grains



**Plate 3: Effect of different media on growth of *Moesziomyces penicillariae* (Hisar isolate)**

### 4.3.3 Growth of *Moesziomyces penicillariae* at different temperatures

Potato dextrose agar medium was used as a growth substrate to study the effect of temperature on growth of *M. penicillariae*. Equal amount of sporidial suspension ( $1 \times 10^6 \text{ ml}^{-1}$ ) was inoculated in potato dextrose agar and plates were incubated at three different temperatures viz., 25°C, 30°C and 35°C. The Petri plates were examined for colony growth regularly at one week interval. The results are presented in a Table 4.4. It was observed that as the incubation temperature increased from 25°C to 35°C the growth of *M. penicillariae* became profuse and resulted in maximum growth at 35°C indicating that temperature had significant effect on the growth of *M. penicillariae*. The colony colour of *M. penicillariae* was dull cream during observation after 7 and 14 days but with the increase in incubation period the colony colour changed to whitish tinch. At 30° and 35°C, the colony colour remained slightly creamy to whitish tinch throughout the incubation period.

**Table 4.4: Growth of *Moesziomyces penicillariae* at different temperatures**

| Temperature (°C) | Mycelial Growth (mm) |         |         |         |
|------------------|----------------------|---------|---------|---------|
|                  | 7 days               | 14 days | 21 days | 28 days |
| 25               | 18.75                | 38.75   | 52.75   | 64.25   |
| 30               | 22.25                | 50.50   | 67.5    | 78.50   |
| 35               | 26.25                | 57.25   | 72.5    | 84.25   |

### 4.3.4 Pathological studies

Pathogenic variability of *Moesziomyces penicillariae* isolates collected from Jaipur (Mp-1), Hisar (Mp-2), Gwalior (Mp-3) and Mandore (Mp-4) was studied on four cultivars of pearl millet viz., HHB 67 Improved, HHB 197, HHB 226 and RHB 177. The general features of different cultivars are presented in Table 4.5. Out of the four cultivars viz., HHB 67 Improved was extra early maturing, HHB 197 and RHB177 were early maturing and HHB 226 was medium maturing cultivar.

**Table 4.5: List of pearl millet hybrids, its parentage and general features**

| S. No. | Hybrid          | Parentage A×R            | Features                                                                 |
|--------|-----------------|--------------------------|--------------------------------------------------------------------------|
| 1      | HHB 67 Improved | ICMA843-22×H77/833-2-202 | Extra early maturity, highly resistant to water stress and downy mildew. |
| 2      | HHB 197         | ICMA 97111 × HBL-11      | Early maturity, medium tall, long bristles, dark green leaves.           |
| 3      | RHB 177         | ICMA 843-22 × RIB494     | Early maturing, medium tall, cylindrical bristled earheads.              |
| 4      | HHB 226         | ICMA 843-22 × HBL-11     | Medium maturing, candle shaped bristled earheads.                        |

The sporidial aqueous suspension ( $1 \times 10^6 \text{ ml}^{-1}$ ) was obtained from ten days old culture of each isolate of *M. penicillariae*. All cultivars were inoculated by spraying 5-7 ml sporidial suspension of each isolate viz., Mp-1, Mp-2, Mp-3 and Mp-4 at the boot leaf stage and then covered with parchment paper bag. Scoring was done 15-20 days after inoculation using standard smut severity (%) rating scale as given by Thakur and King (1988).

The results of virulence pattern observed are presented in Table 4.6. It is evident from the results in Table 4.6 that Mp-2 (Hisar isolate) was most virulent and exhibited 10, 15, 15 and 10% smut severity on HHB 197, HHB 67 (Improved), HHB 226 and RHB 177, respectively in comparison with the other three isolates viz., Mp-1, Mp-3 and Mp-4. Smut severity was maximum on HHB-67 Improved irrespective of any isolate inoculated. The smut severity was comparatively lesser on RHB 177 irrespective of inoculation by any of the isolate.

**Table 4.6: Virulence pattern of different isolates of *Moesziomyces penicillariae* on four different cultivars of pearl millet**

| Isolate of <i>Moesziomyces penicillariae</i> | Smut severity (%) |                   |         |         |
|----------------------------------------------|-------------------|-------------------|---------|---------|
|                                              | HHB 197           | HHB 67 (Improved) | HHB 226 | RHB 177 |
| Jaipur (Mp-1)                                | 5                 | 10                | 10      | 5       |
| Hisar (Mp-2)                                 | 10                | 15                | 15      | 10      |
| Gwalior (Mp-3)                               | 2                 | 5                 | 2       | 1       |
| Mandore (Mp-4)                               | 0                 | 5                 | 5       | 1       |

#### 4.4 Screening of genotypes

To identify resistant source against pearl millet smut 239 entries of pearl millet comprising of Initial varietal trial, Advance varietal trial and Final yield trial were screened by artificial inoculation. The plants were inoculated with 5-7 ml sporidial suspension at the boot leaf stage and were regularly sprinkled with water to maintain high humidity for disease development. Smut severity was observed 15-20 days after inoculation using standard smut severity (%) rating scale given by Thakur and King (1988).

The results of pearl millet entries screened against smut are presented in Table 4.7, 4.8 and 4.9 for Initial varietal trial, Advance yield trial and Final yield trial respectively. Amongst 130 entries of Initial varietal trial, 26 entries viz., MH 2187, MH 2188, MH 2189, MH 2202, MH 2215, MH 2228, MH 2231, MH 2241, MH 2242, MH 2249, MH 2256, MH 2260, MH 2263, MH 2266, MH 2268, MH 2272, MH 2273, MH 2281, MH 2285, MH 2287, RHB 177, KBH 108, NBH 5061, Kaveri Super Boss, RAJ 171, ICMV 221 were completely free from smut whereas 53 entries showed smut severity <10% and in remaining 51 entries the smut severity was 10% and above (Table 4.7).

Amongst 76 entries of Advanced varietal trial, 14 entries viz., MH 2035, MH 2047, MH 2078, MH 2106, MH 2129, MH 2137, MH 2151, MH 2162, MH 2172, MH 2174, MP

562, PRATAP, NBH 5061, Kaveri Super Boss were free from smut whereas 29 entries showed smut severity <10% and in remaining 33 entries the smut severity was 10% and above (Table 4.8).

It is evident from Table 4.9 that amongst 33 entries of final yield trial, 12 entries viz., HHB 234, HHB 67 Improved, GHB 905, MPMH 17, RHB 173, GHB 558, MP-7792, Nandi 61, 86M88, Dhanshakti, Raj 171, JBV 2 were free from smut whereas 11 entries showed smut severity <10% and in remaining 10 entries the smut severity was 10% and above.

Amongst a total of 239 entries screened against smut, 52 entries were free from smut. In 93 entries smut severity was <10% and in remaining 94 entries smut severity was 10% and above.



**Plate 4: Sowing of pearl millet in experimental plot**



**Plate 5: Screening of pearl millet genotypes against smut**

**Table 4.7: Evaluation of pearl millet entries (Initial varietal trial) against smut under experimental sick plot**

| Name of entry | Number of plants | Smut severity(%) | Name of entry | Number of plants | Smut severity(%) | Name of entry | Number of plants | Smut severity(%) |
|---------------|------------------|------------------|---------------|------------------|------------------|---------------|------------------|------------------|
| MH 2187       | 32.0             | 0                | MH 2201       | 26.5             | 20               | MH 2215       | 38.0             | 0                |
| MH 2188       | 33.0             | 0                | MH 2202       | 34.0             | 0                | MH 2216       | 34.0             | 5                |
| MH 2189       | 32.5             | 0                | MH 2203       | 31.0             | 5                | MH 2217       | 35.0             | 12.5             |
| MH 2190       | 29.0             | 5                | MH 2204       | 32.5             | 45               | MH 2218       | 31.0             | 10               |
| MH 2191       | 28.0             | 15               | MH 2205       | 33.5             | 20               | MH 2219       | 37.0             | 1                |
| MH 2192       | 30.0             | 12.5             | MH 2206       | 33.0             | 35               | MH 2220       | 32.0             | 10               |
| MH 2193       | 27.0             | 35               | MH 2207       | 33.5             | 35               | MH 2221       | 32.5             | 12.5             |
| MH 2194       | 33.5             | 15               | MH 2208       | 30.0             | 20               | MH 2222       | 33.5             | 12.5             |
| MH 2195       | 32.0             | 20               | MH 2209       | 29.5             | 20               | MH 2223       | 34.5             | 5                |
| MH 2196       | 34.0             | 35               | MH 2210       | 30.5             | 25               | MH 2224       | 33.5             | 10               |
| MH 2197       | 34.5             | 35               | MH 2211       | 30.5             | 5                | MH 2225       | 30.0             | 35               |
| MH 2198       | 32.5             | 20               | MH 2212       | 39.0             | 10               | MH 2226       | 30.5             | 20               |
| MH 2199       | 29.0             | 20               | MH 2213       | 29.0             | 20               | MH 2227       | 30.5             | 7.5              |
| MH 2200       | 35.5             | 20               | MH 2214       | 34.0             | 5                | MH 2228       | 35.0             | 0                |
| MH 2229       | 31.0             | 10               | MH 2243       | 32.5             | 12.5             | MH 2257       | 35.0             | 5                |
| MH 2230       | 35.5             | 10               | MH 2244       | 31.0             | 10               | MH 2258       | 35               | 5                |
| MH 2231       | 34.0             | 0                | MH 2245       | 35.0             | 5                | MH 2259       | 35               | 5                |
| MH 2232       | 36.0             | 10               | MH 2246       | 36.0             | 5                | MH 2260       | 40.0             | 0                |
| MH 2233       | 30.0             | 5                | MH 2247       | 34.5             | 5                | MH 2261       | 41.0             | 5                |
| MH 2234       | 30.0             | 12.5             | MH 2248       | 34.0             | 5                | MH 2262       | 30               | 12.5             |
| MH 2235       | 35.0             | 12.5             | MH 2249       | 40.0             | 0                | MH 2263       | 41.0             | 0                |
| MH 2236       | 38.0             | 2.5              | MH 2250       | 39.0             | 2.5              | MH 2264       | 34.0             | 7.5              |

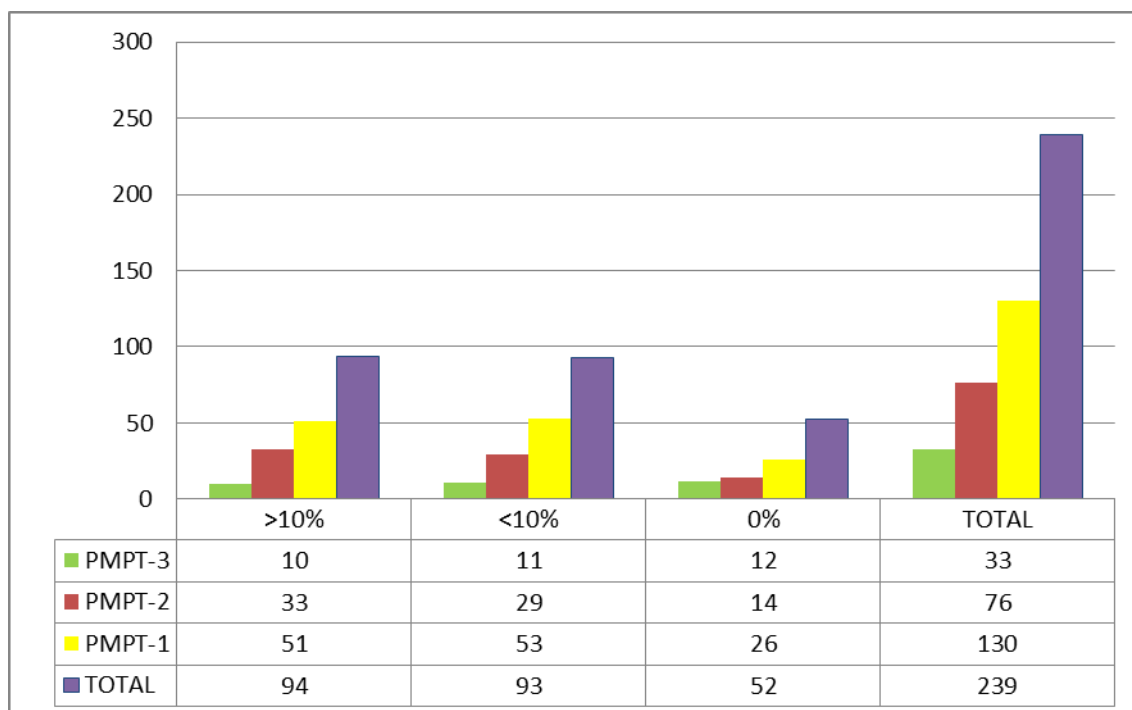
|                       |      |      |                 |      |    |                     |      |      |
|-----------------------|------|------|-----------------|------|----|---------------------|------|------|
| MH 2237               | 34.0 | 5    | MH 2251         | 36.5 | 5  | MH 2265             | 34.5 | 5    |
| MH 2238               | 28.0 | 5    | MH 2252         | 38.0 | 1  | MH 2266             | 37.5 | 0    |
| MH 2239               | 37.0 | 1    | MH 2253         | 34.0 | 5  | MH 2267             | 36.0 | 5    |
| MH 2240               | 37.5 | 5    | MH 2254         | 35.5 | 5  | MH 2268             | 38.0 | 0    |
| MH 2241               | 38.0 | 0    | MH 2255         | 35.5 | 5  | MH 2269             | 37.0 | 12.5 |
| MH 2242               | 40.0 | 0    | MH 2256         | 36.0 | 0  | MH 2270             | 33.0 | 50   |
| MH 2271               | 36.0 | 5    | MH 2285         | 35.0 | 0  | ICMH 356            | 29.5 | 35   |
| MH 2272               | 32.0 | 0    | MH 2286         | 29.0 | 20 | 86M01               | 34.5 | 5    |
| MH 2273               | 34.0 | 0    | MH 2287         | 40.0 | 0  | GHB 905             | 32.5 | 5    |
| MH 2274               | 29.5 | 12.5 | MH 2288         | 33.0 | 5  | MPMH 17             | 32.0 | 5    |
| MH 2275               | 30.5 | 20   | MP 574          | 33.5 | 5  | Pratap<br>(MH 1642) | 34.5 | 1    |
| MH 2276               | 31.5 | 10   | MP 575          | 34.0 | 5  | NBH 5767            | 34.0 | 5    |
| MH 2277               | 28.5 | 10   | MP 576          | 33.0 | 20 | PAC 909             | 37.0 | 5    |
| MH 2278               | 35.5 | 5    | MP 577          | 34.0 | 5  | KBH 108             | 37.5 | 0    |
| MH 2279               | 35.0 | 5    | MP 578          | 32.0 | 10 | 86M86               | 34.5 | 5    |
| MH 2280               | 27.0 | 20   | MP 579          | 33.0 | 5  | MP-7792             | 35.5 | 20   |
| MH 2281               | 37.5 | 0    | MP 580          | 33.0 | 5  | NBH 5061            | 35.0 | 0    |
| MH 2282               | 36.0 | 1    | MP 581          | 34.0 | 5  | Kaveri Super Boss   | 33.0 | 0    |
| MH 2283               | 34.0 | 5    | RHB 177         | 38.0 | 0  | Dhanshakti          | 34.0 | 20   |
| MH 2284               | 34.5 | 5    | HHB 67 Improved | 30.0 | 35 | RAJ 171             | 35.0 | 0    |
| Pusa<br>Composite 383 | 32.0 | 5    | ICMV 221        | 34.0 | 0  |                     |      |      |
| JBV 2                 | 36.0 | 10   | ICMV 155        | 31.0 | 20 |                     |      |      |

**Table 4.8: Evaluation of pearl millet entries (Advanced varietal trial) against smut under experimental sick plot**

| Name of entry     | Number of plants | Smut severity(%) | Name of entry      | Number of plants | Smut severity(%) | Name of entry   | Number of plants | Smut severity(%) |
|-------------------|------------------|------------------|--------------------|------------------|------------------|-----------------|------------------|------------------|
| MH 1993           | 36.0             | 5                | MH 2077            | 35.0             | 10               | MH 2110         | 35.5             | 20               |
| MH 1996           | 37.0             | 5                | MH 2078            | 39.0             | 0                | MH 2113         | 33.5             | 5                |
| MH 1998           | 32.0             | 20               | MH 2082            | 34.5             | 10               | MH 2114         | 32.5             | 20               |
| MH 2008           | 34.0             | 5                | MH 2087            | 35.5             | 5                | MH 2118         | 31.0             | 50               |
| MH 2010           | 34.5             | 5                | MH 2088            | 36.5             | 20               | MH 2123         | 34.0             | 15               |
| MH 2024           | 32.5             | 12.5             | MH 2089            | 31.0             | 35               | MH 2129         | 39.0             | 0                |
| MH 2035           | 40.0             | 0                | MH 2097            | 34.0             | 10               | MH 2130         | 34.0             | 5                |
| MH 2039           | 38.0             | 5                | MH 2098            | 35.5             | 5                | MH 2132         | 32.5             | 5                |
| MH 2047           | 41.0             | 0                | MH 2099            | 34.0             | 20               | MH 2134         | 31.0             | 20               |
| MH 2053           | 39.5             | 5                | MH 2101            | 36.5             | 5                | MH 2137         | 37.5             | 0                |
| MH 2072           | 38.0             | 10               | MH 2106            | 38.0             | 0                | MH 2141         | 36.0             | 10               |
| MH 2076           | 39.5             | 5                | MH 2107            | 34.0             | 5                | MH 2147         | 37.0             | 10               |
| MH 2149           | 35.5             | 5                | MH 2183            | 35.0             | 35               | HHB 67 Improved | 40.0             | 5                |
| MH 2151           | 35.0             | 0                | MH 2185            | 33.0             | 35               | ICMH 356        | 34.0             | 35               |
| MH 2154           | 34.0             | 5                | MP 552             | 36.5             | 35               | GHB 905         | 35.0             | 20               |
| MH 2155           | 36.0             | 5                | MP 562             | 37.0             | 0                | MPMH 17         | 37.0             | 5                |
| MH 2156           | 35.5             | 20               | MP 570             | 36.0             | 20               | RHB 173         | 38.5             | 5                |
| MH 2162           | 36.0             | 0                | MP 571             | 33.5             | 20               | GHB 744         | 38.0             | 10               |
| MH 2172           | 37.0             | 0                | RHB 177            | 35.5             | 5                | NBH 5767        | 39.5             | 5                |
| MH 2173           | 37.5             | 5                | HHB 67 Improved    | 34.5             | 5                | Pratap          | 40.5             | 0                |
| MH 2174           | 41.5             | 0                | ICMH 356           | 38.0             | 35               | PAC 909         | 38.0             | 5                |
| MH 2178           | 36.0             | 20               | GHB 905            | 32.0             | 20               | GHB 558         | 32.5             | 35               |
| MH 2179           | 33.0             | 30               | MPMH 17            | 35.0             | 5                | NBH 5061        | 38.5             | 0                |
| MH 2180           | 38.5             | 20               | RHB 173            | 34.5             | 5                | KBH 108         | 36.5             | 20               |
| MP-7792           | 38.0             | 20               | Pusa Composite 383 | 37.0             | 5                | 86M64           | 33.0             | 5                |
| 86M86             | 40.0             | 5                | RAJ 171            | 36.0             | 20               | ICMV 155        | 36.0             | 5                |
| Kaveri Super Boss | 38.5             | 0                | ICMV 221           | 36.0             | 5                | Dhanshakti      | 36.5             | 10               |

**Table 4.9: Evaluation of pearl millet entries (Final yield trial) against smut under experimental sick plot**

| Name of entry   | Number of plants | Smut severity(%) | Name of entry     | Number of plants | Smut severity(%) | Name of entry      | Number of plants | Smut severity(%) |
|-----------------|------------------|------------------|-------------------|------------------|------------------|--------------------|------------------|------------------|
| HHB 234         | 33.0             | 0                | GHB 744           | 35.5             | 20               | PAC 909            | 36.5             | 10               |
| HHB 226         | 34.0             | 5                | HHB 223           | 36.0             | 5                | 86M88              | 40.0             | 0                |
| RHB 177         | 37.5             | 35               | KBH 108           | 33.0             | 5                | NBH 5061           | 34.0             | 5                |
| GHB 538         | 37.0             | 20               | 86M86             | 32.0             | 20               | MBC 2              | 34.5             | 5                |
| HHB 67 Improved | 38.0             | 0                | MP-7792           | 39.5             | 0                | Dhanshakti         | 35.5             | 0                |
| 86M01           | 34.0             | 10               | Nandi 61          | 38.0             | 0                | Raj 171            | 38.0             | 0                |
| GHB 905         | 34.5             | 0                | GHB 732           | 38.5             | 5                | Pusa Composite 383 | 33.0             | 15               |
| MPMH 17         | 38.0             | 0                | Proagro 9444      | 36.0             | 20               | JBV 2              | 39.5             | 0                |
| RHB 173         | 36.0             | 0                | Kaveri Super Boss | 39.0             | 5                | ICMV 221           | 34.5             | 15               |
| HHB 197         | 35.0             | 5                | NBH 5767          | 37.0             | 5                | ICMV 155           | 36.0             | 2.5              |
| GHB 558         | 32.5             | 0                | Pratap (MH 1642)  | 38.5             | 5                | 86M64              | 34.5             | 25               |



**Fig. 4.1: Screening of initial, advanced and released hybrids against smut severity**

#### **4.4 Evaluation of different fungicides on growth of *M. penicillariae* in vitro**

The antifungal activity of five different fungicides viz., carbendazim, copper oxychloride, propiconazole, hexaconazole and mancozeb at four different concentrations (50, 100, 200 and 500 ppm) was evaluated to study their effect on growth of *M. penicillariae* using poison food technique. The results are presented in the Table 4.10. It is evident from the results and statistical analysis of the data that fungicides, concentrations and their interaction was significant. Carbendazim and copper oxychloride even at 50 ppm were found to be significantly effective against *M. penicillariae* followed by hexaconazole at 500 ppm and propiconazole at 500 ppm. Mancozeb at each concentration was least effective fungicide among the fungicides evaluated, however difference in comparison to control was significant.



|           |            |            |            |            |
|-----------|------------|------------|------------|------------|
| A: 50 ppm | B: 100 ppm | C: 200 ppm | D: 500 ppm | E: Control |
|-----------|------------|------------|------------|------------|

Plate 6: Effect of mancozeb (75% WP) @ 50, 100, 200, 500 ppm on growth of *Moesziomyces penicillariae*



|           |            |            |            |            |
|-----------|------------|------------|------------|------------|
| A: 50 ppm | B: 100 ppm | C: 200 ppm | D: 500 ppm | E: Control |
|-----------|------------|------------|------------|------------|

Plate 7: Effect of hexaconazole (5% SC) @ 50, 100, 200 and 500 ppm on growth of *M. penicillariae*

**Table 4.10: Evaluation of fungicides on growth inhibition of *Moesziomyces penicillariae* at different concentration *in vitro***

| Treatment                | Growth inhibition (%) |                |                |                | Mean           |
|--------------------------|-----------------------|----------------|----------------|----------------|----------------|
|                          | Concentration (ppm)   |                |                |                |                |
|                          | 50                    | 100            | 200            | 500            |                |
| Mancozeb 75% WP          | 9.12 (17.28)*         | 9.41 (17.78)   | 16.18 (23.70)  | 32.06 (34.46)  | 16.69 (23.30)  |
| Hexaconazole 5% SC       | 49.41 (44.77)         | 66.18 (54.42)  | 72.94 (58.63)  | 88.82 (70.47)  | 69.34 ( 57.08) |
| Propiconazole 25% EC     | 46.18 (42.82)         | 61.47 (51.62)  | 69.41 (56.40)  | 83.82 (66.26)  | 69.72 (54.28)  |
| Copperoxychloride 50% WP | 100.00 (89.39)        | 100.00 (89.39) | 100.00 (89.39) | 100.00 (89.39) | 100.00 (89.39) |
| Carbendazim 50% WP       | 100.00 (89.39)        | 100.00 (89.39) | 100.00 (89.39) | 100.00 (89.39) | 100.00 (89.39) |
| Control                  | 0.00 (0.57)           | 0.00 (0.57)    | 0.00 (0.57)    | 0.00 (0.57)    | 0.00 (0.57)    |
| Mean                     | 50.79 (56.73)         | 56.18 (60.52)  | 59.76 (63.50)  | 67.45 (69.99)  |                |
|                          | Treatment             | Concentration  |                |                |                |
| SE (m)                   | 0.80                  | 0.72           |                |                |                |
| C.D. (0.05)              | (2.29)                | (2.05)         |                |                |                |

\* Values in parentheses are angular transformed values

#### 4.5 Evaluation of different plant extract(s) on growth of *M. penicillariae* in vitro

The efficacy of five plant extracts viz., lantana, neem, eucalyptus, jamun and aloe vera was evaluated *in vitro* using poison food technique against *M. penicillariae*. The results are presented in Table 4.11. It is evident from the results that all the plant extracts evaluated invariably inhibited the fungal growth and were found significantly superior over control in inhibiting the growth of fungus. Highest growth inhibition was observed in aloe vera plant extract followed by jamun and least growth inhibition was recorded in eucalyptus (Fig4.2, Table 4.11, Plate 8).

**Table 4.11: Evaluation of different plant extract(s) on growth inhibition of *M. penicillariae* in vitro**

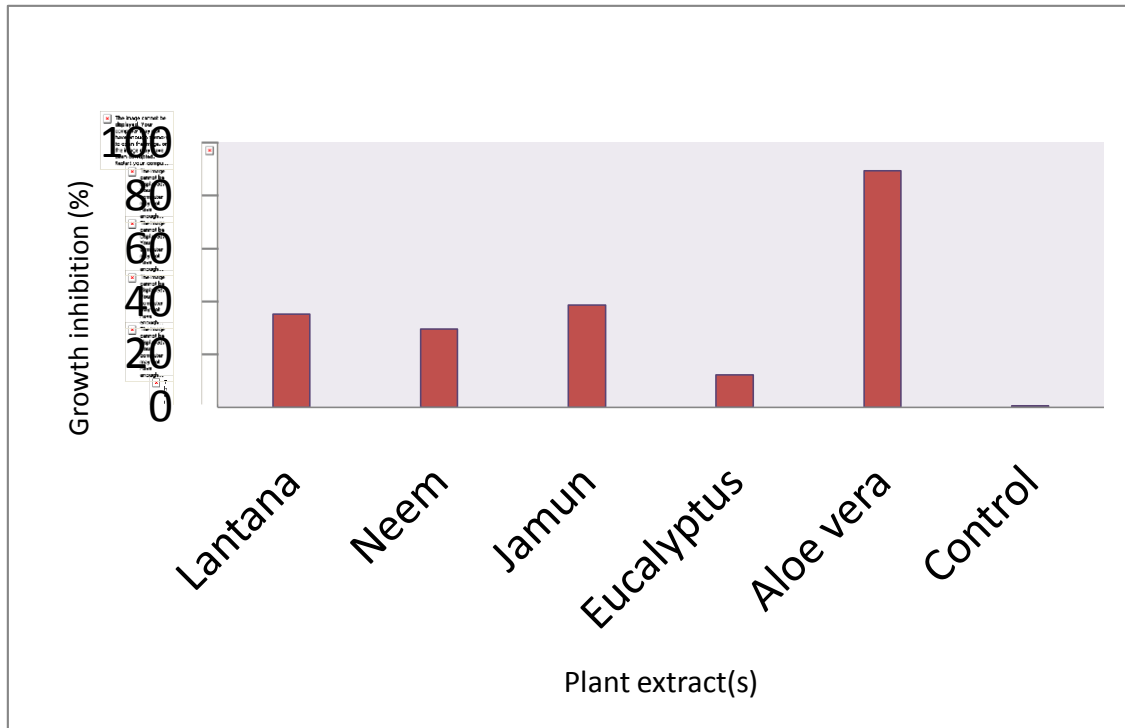
| Treatment      | Leaf extract<br>(20% concentration) | Growth inhibition<br>(%) |
|----------------|-------------------------------------|--------------------------|
| T <sub>1</sub> | Lantana                             | 33.13 (35.12)            |
| T <sub>2</sub> | Neem                                | 24.42 (29.59)            |
| T <sub>3</sub> | Jamun                               | 38.95 (38.60)            |
| T <sub>4</sub> | Eucalyptus                          | 4.65 (12.20)             |
| T <sub>5</sub> | Aloe vera                           | 100 (89.39)              |
| T <sub>6</sub> | Control                             | 0 (0.57)                 |
| SE (m)         |                                     | 0.696                    |
| C.D. (0.05)    |                                     | 2.084                    |

• Average of four replications

\* Values in parentheses are angular transformed values



**Plate 8: Evaluation of different plant extract(s) on growth of *Moesziomyces penicillariae***



**Fig. 4.2 Growth inhibition (%) of *M. penicillariae* by plant extract(s)**

#### **4.6 Evaluation of fungicides and plant extract(s) as pre and post inoculation spray**

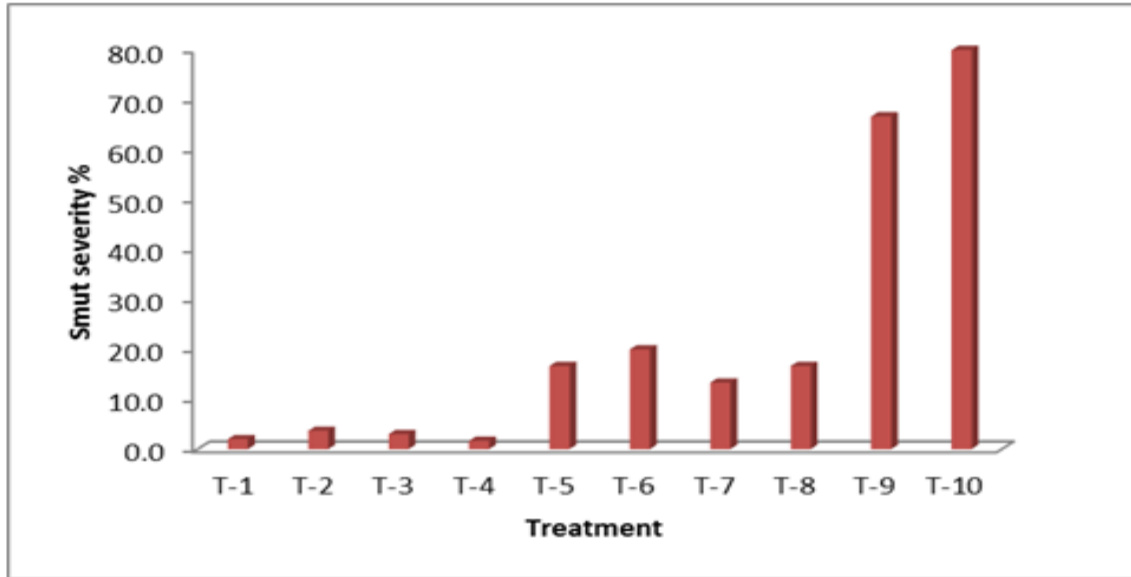
The efficacy of various fungicides and leaf extracts used as pre and post inoculation treatment for the management of smut caused by *M. penicillariae* is given in Table 4.12. The smut severity was 2.00, 3.67, 3.00 and 1.67 per cent in pre inoculation spray of plant extract (aloe vera and jamun) and fungicides (carbendazim and copper oxychloride) while 16.67, 20.00, 13.33 and 16.67 per cent in post-inoculation spray of plant extract(s) (aloe vera and jamun) and fungicides (carbendazim and copper oxychloride). Smut severity (%) in control with sterilized water was 66.67% whereas in control without water it was observed as 80%. From the analysis of data it is revealed that pre-inoculation treatment was better than the post inoculation treatment. The yield was also maximum (2544Kg/ ha) in case of pre inoculation treatment with carbendazim @ 0.05% whereas it was 1955 kg/ ha in post inoculation treatment with copper oxychloride @ 0.05%. Among fungicides copper oxychloride was inferior as compared to carbendazim @ 0.05% concentration as pre inoculation spray but in post-inoculation spray disease severity was less in case of copper oxychloride @ 0.05% in comparison to carbendazim. Among different plant extract(s) sprays, in pre inoculation treatment aloe vera gave better results as compared to the jamun extract at 20% concentration but smut severity was less in post inoculation spray of jamun as compared to that of aloe vera (Fig. 4.3, 4.4, Plate 9).

**Table 4.12: Evaluation of fungicides and plant extract(s) as pre and post inoculation spray for management of *M. penicillariae* in vivo**

| Treatment       |                                       | Smut severity (%) | Disease control (%) | Yield (Kg/ha) |
|-----------------|---------------------------------------|-------------------|---------------------|---------------|
| T <sub>1</sub>  | Aloe vera @ 20% concentration as PIS  | 2.00 (6.22)       | 97.50               | 2511          |
| T <sub>2</sub>  | Jamun @ 20% concentration as PIS      | 3.67 (10.52)      | 95.42               | 2355          |
| T <sub>3</sub>  | Copper oxy chloride @ 0.05% as PIS    | 3.00 (9.72)       | 96.25               | 2389          |
| T <sub>4</sub>  | Carbendazim @ 0.05% as PIS            | 1.67 (7.33)       | 97.92               | 2544          |
| T <sub>5</sub>  | Aloe vera @ 20% concentration as PoIS | 16.67 (23.84)     | 79.17               | 1900          |
| T <sub>6</sub>  | Jamun @ 20% concentration as PoIS     | 20.00 (26.55)     | 75.00               | 1711          |
| T <sub>7</sub>  | Copper oxy chloride @ 0.05% as PoIS   | 13.33 (21.13)     | 83.33               | 1955          |
| T <sub>8</sub>  | Carbendazim @ 0.05% as PoIS           | 16.67 (23.84)     | 79.17               | 1933          |
| T <sub>9</sub>  | Control with sterilized water as PoIS | 66.67 (54.98)     | 16.67               | 533           |
| T <sub>10</sub> | Control without water                 | 80.00 (63.83)     | 0.00                | 400           |
| SE(m)           |                                       | 2.99              |                     | 121.29        |
| (C.D.) at 5%    |                                       | 8.98              |                     | 363.18        |

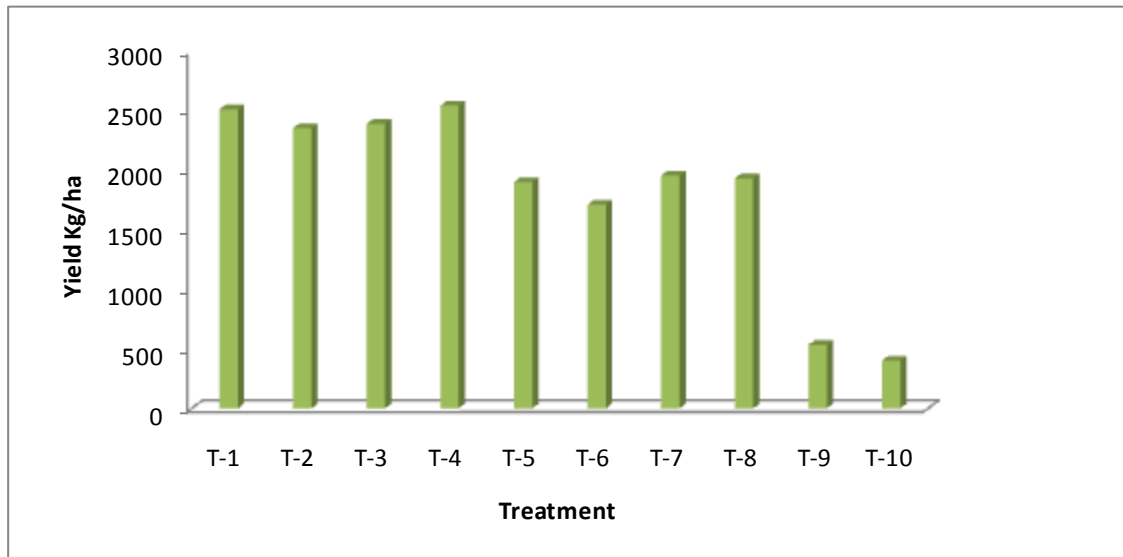
\* Values in parentheses are angular transformed values

a) PIS- Pre inoculation spray ; b) PoIS- post inoculation spray



(\*PIS: T1- aloe vera @ 20%; T2- jamun@ 20%; T3- copper oxychloride @ 0.05%; T4- carbendazim @ 0.05% ; PoIS: T5- aloe vera @ 20%; T6- jamun @ 20%; T7- copper oxychloride @ 0.05%; T8- carbendazim @ 0.05%; T9- Control with sterile water; T10- control without water)

**Fig. 4.3 Relative efficacy of fungicides and plant extract(s) as pre and post inoculation treatment on smut severity (%)**



(\*PIS: T1- aloe vera @ 20%; T2- jamun@ 20%; T3- copper oxychloride @ 0.05%; T4- carbendazim @ 0.05% ; PoIS: T5- aloe vera @ 20%; T6- jamun @ 20%; T7- copper oxychloride @ 0.05%; T8- carbendazim @ 0.05%; T9- Control with sterile water; T10- control without water)

**Fig. 4.4 Effect of fungicides and plant extract(s) as pre and post inoculation treatment on yield of pearl millet**



**Plate 9: Pre and post inoculation spray of fungicides and plant extract(s) under *in vivo***

Pearl millet is an important crop in the drought prone areas of India particularly in the States of Haryana, Punjab, Gujarat, Rajasthan and Andhra Pradesh. It has great potential among all the millets to add to the food basket of the country. It is also cultivated in the southern peripheries of Sahara desert in Africa. The use of cytoplasmic genetic male sterility (CGMS) provided great scope to produce high yielding pearl millet hybrids. In 1965, the first commercial hybrid HB-1 was released with male sterile line Tift 23A<sub>1</sub> which was developed by Burton (1958) from Tifton, Georgia. In India, the average yield of pearl millet increased spectacularly with the release of hybrids. However, in due course of time these hybrids became highly susceptible to several diseases *viz.*, smut, downy mildew, ergot and blast.

Smut caused by *Moesziomyces penicillariae* is one of the serious and widespread diseases of pearl millet. The disease was first reported in 1918 by Butler in India, but not much attention was paid on the systematic study of various aspects of this disease. Earlier smut of pearl millet was not considered to be economically important disease. However in parts of Maharashtra, Andhra Pradesh, Tamil Nadu smut infection varied from 1 to 30 % (Krishnaswamy, 1962). During recent years smut of pearl millet has become more devastating in several parts of India particularly Haryana, Punjab, Gujarat and Rajasthan mainly due to the introduction of high yielding hybrids, hence require more attention of Plant Pathologists like in other pearl millet diseases *i.e.* downy mildew, ergot etc.

#### 5.1 Survey

The monitoring of disease prevalence through survey is one of the major key to proceed further for its management. With a view to plan better prospects of pearl millet cultivation in Haryana state, preliminary survey of different locations in major pearl millet growing districts of Haryana was conducted during *kharif* 2016 to assess the prevalence of pearl millet smut. It was revealed that pearl millet smut severity in Haryana led to the extent of 10% during *kharif* 2016 in major pearl millet growing locations specifically in Hisar, Rewari, Mohindergarh and Bhiwani. The losses in pearl millet are due to cultivation of smut susceptible hybrids. Bhowmik and Sundaram (1971) reported that 50-75% of the crop was infected with smut in some fields, with damage up to 100% in individual panicles. Rachei and Majmudar (1980) reported that during survey 1950's the smut severity in farmer fields ranged from 1 to 30% in parts of Tamil Nadu, Andhra Pradesh and Maharashtra. Yadav and Duhan (1993) reported maximum smut severity of 13.4% in Gurgaon and minimum smut severity of 10.2% in Bhiwani district's pearl millet growing locations of Haryana. Lubadde *et. al.* (2014) reported the smut incidence of 26.76% in survey conducted during 2012 on farmer's

fields of Uganda. The present survey indicated that smut of pearl millet in Haryana can become bottleneck in pearl millet production because of its wider distribution in different districts of Haryana.

## **5.2. Morphological variability**

Morphological variation in respect of spore size is pronounced in many smut fungi. Fischer and Holton (1957) reported that variation in sorus and spore characteristic was a common phenomenon in several species and races of *Tilletia*. In the present investigation, isolates of *Moesziomyces penicillariae* from four different locations (Hisar, Jaipur, Gwalior and Mandore) were retrieved and examined for various morphological characteristics. It was observed that there was no variation in colour and shape of spore balls therefore, the isolates could not be effectively classified into different groups on the basis of shape and colour. However, significant variation was observed in size of sporidia and germination per cent among Hisar, Jaipur, Gwalior and Mandore isolates. Maximum size of spore ball, sporidia and germination per cent was found in Hisar isolate. Bansal *et. al.* (1984) observed variation in size of teliospores of *Tilletia barclayana* causing bunt of rice which was influenced by environmental conditions. Pannu and Chahal (2000) reported that the teliospore germination in different isolates varied with the set of conditions under which they were produced and matured. However, Pannu *et. al.* (2002) reported that the germination might vary due to age, physiology or genetic reasons, thus these characters could not be considered as criteria to distinguish the isolates of *Tilletia barclayana* from each other. The above studies indicated that morphological variations in size of sporeballs, teliospores and sporidia among isolates might represent temporary response to environment and might not be inherently permanent. Morphological variability in spore size might be useful criteria for species delimitations within a genus of smut fungi which however, are not sufficient to elucidate variability within species.

## **5.3 Cultural variability**

The growth of *Moesziomyces penicillariae* depends significantly on the carbon source. Fischer and Holton (1957) reported that smut species respond well to carbohydrates derived from sucrose, glucose and maltose but there is variation within and among species as which sugar is best for the growth. Pathak and Shekhawat (1980) found better growth on medium which was enriched with dextrose. Meena *et. al.* (2010) also reported better growth on the medium which was supplied with carbohydrate like sucrose, glucose *etc.* In the present study six different media were evaluated for the growth of *Moesziomyces penicillariae* under *in vitro* conditions. The maximum growth of fungus was observed in PDA enriched with pearl millet grains @ 50g and sucrose @ 20g followed by PDA enriched with 25g pearl millet grains and 20g sucrose. These findings corroborate with findings of Naresh (2016) who evaluated twelve media for the growth of *Tolyposporium penicillariae* under *in vitro*

conditions revealing the maximum growth of the fungus in Pearl millet potato dextrose agar medium followed by Potato dextrose agar medium. Choursia (2007) evaluated five media and reported that the maximum growth of fungus occurred in Potato dextrose agar medium (66.75 mm) followed by potato agar medium (47.75), while the minimum (23.00) growth in Czapek dox medium.

Pathak and Shekhawat (1980) reported mycelial type of growth on synthetic media viz., Czapekdox agar whereas Subha Rao and Thakur (1983) reported sporidial type of growth on semi synthetic medium. Thakur and King (1988) also observed that the fungus *Tolyposporium penicillariae* readily form colonies on media containing extract of pearl millet, maize and sorghum grains. However, in present investigation both type of media were used and sporidial & mycelial growth was observed in semi synthetic medium while only mycelial growth was observed in synthetic medium.

#### **5.4 Physiological studies**

Pearl millet smut can be effectively managed through development of resistant hybrids. However, it is important to understand the conditions that are most favourable for the fast development of its incitant in order to develop effective screening techniques and management strategies. In the present study, the incitant of pearl millet smut *i.e.* *Moesziomyces penicillariae* was exposed to different temperatures on potato dextrose agar medium and found 35°C as optimum temperature for maximum growth. Subba Rao and Thakur (1983), Phookan (1987) also reported 35°C as optimum temperature for maximum growth of the fungus. In contrast Pathak and Shekhawat (1980) found 25°C to be the optimum temperature for maximum growth in terms of mycelial dry weight. Dashora *et. al.* (2008) reported that fungus growth was maximum, profuse with excellent sporulation at 30°C on Richard's Agar medium.

#### **5.5 Pathological studies**

It is important to gather knowledge about race of the pathogen, for making contribution towards resistance breeding programme because the variation in smut fungi has most fundamental impact on pathogenicity. The present study was conducted to find out pathogenic variability among isolates of *Moesziomyces penicillariae* by inoculating on four cultivars of pearl millet *i.e.* HHB 67 Improved (extra early maturity), HHB 197, RHB 177 (early maturity) and HHB 226 (medium maturity) and it was observed that Mp-2 (Hisar isolate) was most virulent on all the test host hybrids followed by Mp-1 (Jaipur isolate), Mp-3 (Gwalior isolate) and least virulent Mp-4 (Mandore isolate). Further all the four isolates were more virulent on HHB 67 Improved followed by HHB 226. However, there was differential reaction of HHB 197 and RHB 177 to Mp-3 (Gwalior isolate) and Mp-4 (Mandore isolate). Halisky (1965) reported that the differential reaction of two or more host varieties could be taken as indication of degree of variation in virulence of pathogen. Phookan (1987) also did

not observe statistical difference among the virulence of the isolates, obviously no pathogenic variability among the isolates of *M. penicillariae* was confirmed. The results of the present study are in line with the previous reports that there is no well defined existence of physiologic specialization within the morphological species of pearl millet smut fungus.

### **5.6 Evaluation of pearl millet entries for smut resistance**

The innovation of cytoplasmic genetic male sterile lines, along with improved agronomic practices and spectacular changes in yield potential has led to rise in one or the other disease problem. In order to manage the disease, incorporation of resistance in suitable genotype is the demand of current era as it is considered as the most economical method. In the present study, amongst 239 entries screened against smut of pearl millet, 52 entries were free from smut, 93 entries exhibited smut severity <10% and in remaining 94 entries smut severity was more than 10%. Thakur *et. al.* (1986) screened 1500 pearl millet accessions against *Moesziomyces bullatus* for 1-6 years of multi location testing in India and West Africa and found that six germplasm accessions *viz.*, SSC FS 252-S-4, ICI 7517-S-1, ExB 132-2-S-5-2-DM-1, ExB 46-1-2-S-2, ExB 112-1-S-1-1 and P-489-S-3 and four newly developed, resistant, agronomically elite lines *viz.*, ICMPS 100-5-1, 900-9-3, 1600-24 and 2000-5-2 showed consistently high levels of resistance. Rai *et. al.* (1998) developed male-sterile pearl millet lines ICMA 88006, which derives its A<sub>1</sub> cytoplasm from ICMA1 (81A), and ICMA 92666 (and its maintainer line ICMA 92666) which were resistant to *M. bullatus* and other pathogens. Pandya and Bartaria, (2000) evaluated several pearl millet lines under artificial inoculation and identified ICMB 92888, ICMB 92777 and IP 19874 as a source of smut resistance. Choursia (2007) evaluated 138 entries against smut; and observed that only one entry MH 1317 remained absolutely free from smut while nineteen entries were in the category of 5.1- 10 per cent smut severity and maximum severity was recorded in MH 1391. Based on screening of pearl entries against smut, the new hybrids are promoted for cultivation from time to time.

### **5.7 Evaluation of different fungicides & plant extract(s) on growth of *M. penicillariae* in vitro**

Smut of pearl millet caused by *M. penicillariae* assumed its alarming importance in limiting the production. Therefore, it is necessary to manage the disease in entirety, including utilization of fungicides and plant extracts in integrated manner otherwise the pathogen population will jeopardize the only strategy of breeding for smut resistance. In the present investigation, the fungicides and plant extract(s) were evaluated against *M. penicillariae* under *in vitro* conditions by using poisoned food technique and from the results it is revealed that carbendazim and copper oxychloride at 50 ppm were found to be significantly effective against *M. penicillariae* followed by propiconazole and hexaconazole whereas mancozeb was the least effective fungicide, however it exhibited significant difference in comparison to control. These results support the findings of Kumar and Nath (1991) for managing long

smut of sorghum caused by *Tolyposporium ehrenbergii* but in contrary to the findings of Meena *et. al.* (2010) who reported propiconazole and hexaconazole as more effective fungicides as compared to carbendazim.

All the plant extracts were found significantly superior in inhibiting the growth of fungus in comparison to the control. Highest growth inhibition was found in *aloe vera* extract followed by jamun extract. In the present study, *Eucalyptus* leaf extract @ 20% was found to be least effective which differs with the findings of Rajput (2000) who observed that *Eucalyptus* leaf extract @ 10% was found best among *Parthenium*, *Calotropis* and neem leaf extracts against *Tolyposporium penicillariae* under *in vitro* condition. Choursia (2007) tested 11 botanicals in the boil form in two concentration *i.e.* 10 & 20 % against *T. penicillariae* and reported that *Eucalyptus* extract @ 20 % showed maximum control of growth of mycelium as compared to control.

### **5.8 Evaluation of fungicides and plant extract(s) under *in vivo***

In the present investigation, pre inoculation as well as post inoculation foliar sprays of carbendazim (0.05%), copper oxy chloride (0.05%), *aloe vera* extract (20%) and jamun extract (20%) were carried out for the management of pearl smut under *in vivo* condition. The spray of fungicide as well as plant extract as pre inoculation treatment was better than the post inoculation treatment. The average yield obtained was also more in pre inoculation treatment in comparison to the post inoculation treatment. Among, prophylactic spray of fungicides, carbendazim was found to be more effective than copper oxychloride but in post inoculation spray disease severity was less in copper oxychloride treatment. Amongst different plant extracts sprayed less smut severity and more yield was found in pre inoculation spray with *aloe vera* and post inoculation spray with jamun extract. However the present findings were contradictory to the findings of Rajput (2000) according to which eucalyptus leaf extract @ 10% was better than the *Calotropis*, *Parthenium* and neem leaf extract in managing the smut of pearl millet. Phookan (1987), reported carboxin as effective fungicide followed by captafol and carbendazim for managing the smut under field conditions. Kumar (2011) reported that four sprays of captafol (3g l<sup>-1</sup>) effectively managed the disease in the field. Meena *et. al.*, (2012) reported that hexaconazole and propiconazole were superior over carboxin for management of pearl millet smut under field conditions.

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

Pearl millet (*Pennisetum glaucum*) is staple food of population living in arid and semi arid areas of the world. Smut of pearl millet caused by *Moesziomyces penicillariae* is of economic importance as it causes 1-30% grain losses in the susceptible cultivars. Due to infection by virulent pathogen in a susceptible cultivar, there is development of sori in place of grain. The present investigation was undertaken to study the variability of smut pathogen, screening against smut resistance and management of the pathogen through fungicides and leaf extract(s). The results and conclusions of the experiment are summarized below:

Survey is an important component to know the disease prevalence in the particular area or location and further helps in disease management. The survey conducted during *kharif* season 2016 in Haryana revealed smut severity to the extent of 10% in major pearl millet growing districts of Haryana *viz.*, Hisar, Mohindergarh, Rewari and Bhiwani.

Smuted earheads were collected for variability studies from major pearl millet growing areas of India *viz.*, Mandore, Jaipur, Gwalior and Hisar. The isolates were subjected to different morphological, pathological, physiological and cultural studies. There was no morphological variation in shape of sporeballs among different isolates but slight variation was observed in sporeball size. There was variation in size of sporidia among isolates and germinating ability of the spores. Maximum size of spore ball, sporidia and germination per cent was found in Hisar isolate.

The growth of *M. penicillariae* was maximum at 35°C with colony diameter 84.25 mm after four weeks of incubation.

The potato dextrose agar (PDA) medium enriched with pearl millet grains @ 50 gL<sup>-1</sup> and sucrose @ 20 gL<sup>-1</sup> showed maximum growth than any other medium.

Among four different isolates of *Moesziomyces penicillariae*, Mp-2 (Hisar isolate) was most virulent on all the test host hybrids followed by Mp-1 (Jaipur isolate), Mp-3 (Gwalior isolate) and least virulent Mp-4 (Mandore isolate). All the four isolates were more virulent on HHB 67 Improved followed by HHB 226 and also there was differential reaction of HHB 197 and RHB 177 to Mp-3 (Gwalior isolate) and Mp-4 (Mandore isolate).

Amongst 239 entries of pearl millet screened against smut, 52 entries were found free of smut. In 93 entries smut severity was less than 10 per cent and 94 entries exhibited smut severity 10 per cent & above.

Among five fungicides *i.e.* carbendazim, copper oxychloride, mancozeb, hexaconazole and propiconazole evaluated under *in vitro* conditions, maximum inhibition per cent was found in carbendazim and copper oxychloride @ 50 ppm and under *in vivo*

conditions minimum disease severity was observed in pre inoculation treatment with carbendazim @0.05%, with maximum grain yield.

Among different leaf extracts evaluated, minimum smut severity was observed in pre inoculation treatment with aloe vera extract followed by jamun extract with maximum average grain yield in pre inoculation treatment with aloe vera extract @20%.

For integrated management of pearl millet smut, constant monitoring through regular survey, regular screening of entries, and spray of the fungicide & plant extracts as prophylactic treatment should be practised.

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## ABSTRACT

**Title of thesis** : **Studies on variability and management of pearl millet smut caused by *Moesziomyces penicillariae* (Bref.) Vanky**

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**Keywords:** *Moesziomyces penicillariae*, Pearl millet, Smut

Smut of pearl millet incited by *Moesziomyces penicillariae* (Bref.) Vanky is the most wide spread and destructive disease in India and other pearl millet growing areas of the world. This disease is a major limiting factor in full exploitation of high yielding potential hybrids in India. In the present investigation, during survey the smut severity to the extent of 10 per cent was observed in villages of Hisar, Mohindergarh, Rewari and Bhiwani. Among four isolates of *M. penicillariae* collected from Hisar, Jaipur, Mandore and Gwalior, there was not much variation in size and shape of sporeball but little variation was observed in size of sporidia and germination per cent. The Hisar isolate of *M. penicillariae* was found to be most virulent on the four cultivars of pearl millet. The growth of *M. penicillariae* was maximum at 35°C on potato dextrose agar (PDA) medium enriched with pearl millet grains and sucrose. Amongst 239 entries of pearl millet screened, 52 entries were free of smut, 93 entries showed smut severity upto 10% and in remaining 94 entries smut severity was 10 per cent and above. Among different fungicides evaluated against *M. penicillariae* the causal agent of pearl millet smut, carbendazim and copper oxychloride performed better under *in vitro* and *in vivo* conditions. Carbendazim was better as prophylactic spray whereas copper oxychloride was better to be used as curative. Among different plant extracts evaluated, *aloe vera* performed better to manage smut of pearl millet followed by jamun extract as pre inoculation spray.

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