Studies on downy mildew of pearl millet {*Sclerospora graminicola* (Sacc.)} with special reference to its eco-friendly management

THESIS

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In partial fulfillment of the requirements for
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MASTER OF SCIENCE

In

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(Plant Pathology)

By

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CERTIFICATE – I

This is to certify that the thesis entitled **"Studies on downy mildew of pearl millet {*Sclerospora graminicola* (Sacc.)} with special reference to its eco-friendly management"** submitted in partial fulfillment of the requirements for the degree of Master of Science in Agriculture (Plant Pathology) of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior is a record of the bona-side research work carried out by **Vijay Kumar Thanna**., **ID.No. RA/GW/786/2009** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instruction, RVSKVV, Gwalior.

No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of the investigations has been acknowledged by the scholar.

Place: Gwalior
Date:

(Dr. R.K. Pandya)
Chairman
Advisory Committee

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

Member (Mrs. Rajni Singh Sasode) ……..

Member (Dr. A.K. Barholia) ..…….

Member (Dr. V. B. Singh) ..…….

CERTIFICATE –II

This is to certify that thesis the entitled **"Studies on downy mildew of pearl millet {*Sclerospora graminicola* (Sacc.)} with special reference to its eco-friendly management"** submitted by **Mr. Vijay Kumar Thanna, ID.No. RA/GW/786/2009** to the Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior in partial fulfillment of the requirements for the degree of Master of Science in Agriculture in the Department of Plant Pathology has been accepted after evaluation by the external examiner and approved by the Student's Advisory Committee after an oral examination of the same.

Place: Gwalior
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Date :

Place : **Gwalior** **(Vijay Kumar Thanna)**

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CHAPTER-I
INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br)] is one of the assured *Kharif* crop under environments domesticated in the annual rainfall of 150 mm to 1000 mm in India. In India, pearl millet is cultivated over an area of 79.52 lakh ha. with the production of 87.96 lakh tones and the productivity 1106 kg/ha (Anon., 2014). It is being grown in Madhya Pradesh as sole crop for grain and fodder purposes. It occupies 1.87 lakh ha. with an annual production 3.01 million tones and productivity of 1698 kg/ha (Anon., 2014). It has variety of uses for the consumption of human being such as chapatti, breads, snacks, cakes, beverages and pre digested weaning food besides poultry feed and fodder for cattle.

Several diseases of pearl millet caused by fungi, bacteria, virus and nematodes have been reported by different workers, out of them downy mildew incited by *Sclerospora graminicola* (Sacc.) Schroet is the most wide spread and destructive disease of pearl millet in India (Rachie and Majumdar, 1980). The downy mildew pathogen was first reported on pearl millet in lndia in 1907 (Butler, 1907). Initially, the disease was not considered serious but the true magnitude of the losses has now been fully appreciated. In 1971, the disease appeared in an epidemic form in the Indian sub-continent resulting in the withdrawal of the most popular hybrid HB 3 which had contributed to a record harvest of 8 million tones In 1970-71 (Anon., 1972). Subsequent to this epidemic, grain yield losses continued to occur quite frequently due to downy mildew epidemics In India (Singh *et al*., 1987).

The pathogen exists between susceptible crops as oospores in plant debris in the soil and on the seed. The oospores are the source of primary inoculum. Whether or not the pathogen survives as mycelium in seed and is transmitted by seed is in controversy (Nene and Singh, 1976). Plants become systemically infected where environmental conditions are favorable for the disease. Sporangia are produced in large numbers in the" downy mildew" growth of the pathogen on leaves growing from systemically infected plants. Secondary spread is achieved quickly through sporangia which give rise to

zoospores. In later stages, systemically infected plants show various degrees of malformation normally called 'green ear'. Abundant oospores are formed

in infected leaf tissues and inflorescences.

The pathogen is monogenic and soil borne hence, the identification of downy mildew resistant line and their utilization in resistance breeding programme to evolve the resistant material with good agronomic traits is most effective way for the management of the disease. Apart from this use of botanicals, bio-agents, and other non chemical management approaches may also serve as an alternate line for the eco friendly management of the disease under field condition. In the light of above facts following studies are proposed.

1. Symptomatoloy.
2. Evaluation of pearl millet material against downy mildew under disease sick field condition .
3. Evaluation of promising hybrids, verities and land races under disease sick field condition .
4. Evaluation of botanicals , cow urine, butter milk and panchgavya against downy mildew.
5. To study the influence of pearl millet seed dressing with bio-agent, chitosen and recommended chemical on the reduction of progressive development of downy mildew.

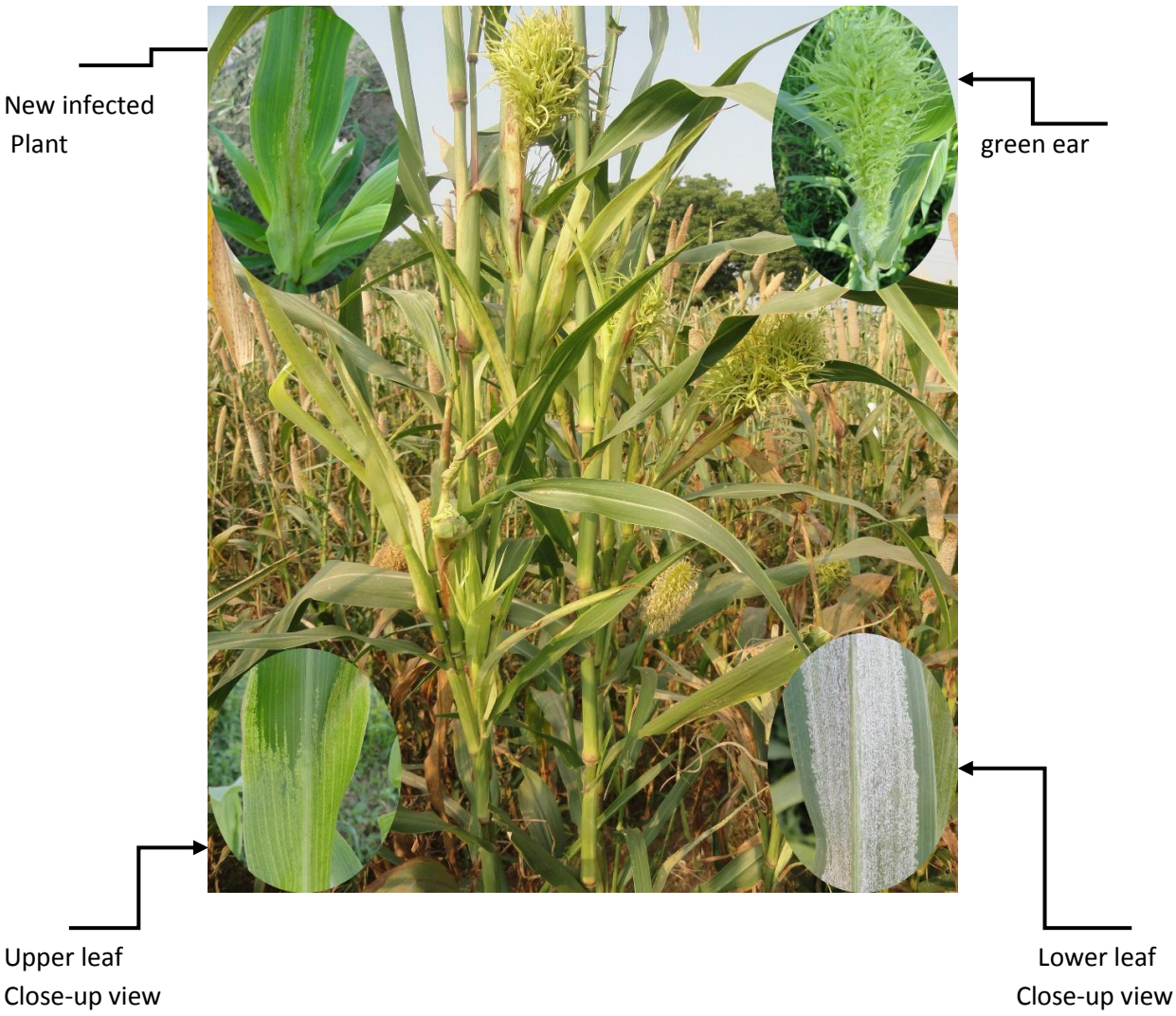

Plate – 1 : View of downy mildew infected plant

CHAPTER - II
REVIEW OF LITERATURE

Relevant literature of various works related to the present studies is briefly reviewed in this chapter.

The downy mildew constitute an important group of plant diseases. They are so called because of the downy or woolly growth produced by the pathogens on the infected leaf areas. Downy mildew pathogens, belonging to the family Peronosporaceae, are obligate parasites, although a few of them have been grown in tissue culture (Arya and Tiwari. 1969; Safeeulla, 1975; Bhat, 1973; and Bhat *et al.,* 1980).

2.1 Historical

Among the diseases affecting pearl millet (*Pennisefum glaucum* (L.) R. Br.), downy mildew (*Sclerospora graminicola* (Sacc.) Schrat.), also known as 'green ear' disease is the most devastating one. *S. graminicola* is the type species of the genus. It was originally named as *Protomyces graminicola* by Saccardo in 1876 who reported lt on *Setah verticlilata*. Subsequently, Schroter In 1879 renamed lt as *S. graminicola* in his work on the genus *Sclerospora* in Germany (Ullstrup, 1973). In 1884, Farlow described *S. graminicola* on *S. viridis* (L.) Beauv. In India, the earliest investigations on pearl millet downy mildew were carried out by Butler (1907) and Kulkarni (1913). The disease was not considered serious, since damage was severe only where pearl millet was grown in low-lying, poorly-drained areas (Butler, 1918). Mitter and Tandon (1930) reported the seriousness of the disease in low-lying areas In the vicinity of Allahabad in North India. With the release of high yielding hybrids (HB 1, HB 2 and HB 3) in certain parts of India, the disease became serious resulting in an epidemic during 1971-72 (Anon, 1972). Downy mildew is considered a major biotic yield reducing factor in pearl millet not only in India, but also in many countries In Africa (Singh. 1995).

2.2 Symptomatology

Butler (1907) was first to describe the symptoms of downy mildew of bajra in india. Ramakrishnan (1963) described the symptoms of the disease in detail which are as follows :

The symptoms of infection can be recognized even in young plants. The leaves lose their green colour and become wholly or partly yellow or whitish, later formed leaves are paler than the earlier ones. The discoloration is often evident as broad streaks extending from the base, to varying lengths or upto the tip. The transformation of ear, wholly or partly into a green head or small, twisted, leaf like structure is very characteristics, hence the name ‘ green ear ’ but some times fertile grains may be formed in part of the ear but more often the whole ear is affected. The bristles subtending the spikelets and all its parts the glumes, palea, stamens and pistils are converted into leaf like structures, Under humid condition the leaves are covered by a downy white growth of the fungus . This is prominent on the lower surface .

There is considerable variation in the symptoms which almost always develop as a result of systemic infection. Systemic symptoms generally appear on the second leaf, and once these appear, all the subsequent leaves and panicles also develop symptoms, except in case of recovery resistance where plants outgrow the disease (Singh and King, 1988). The disease can appear on the first leaf also, under conditions of severe disease development. Leaf symptoms begin as chlorosis (yellowing) at the base of the leaf lamina, and successively higher leaves show a progression of greater coverage of leaf area by symptoms. The 'half- leaf' symptom, characterized by a distinct margin between the diseased (basal portion) and non-diseased areas towards the tip, occurs in pearl millet genotypes. Under conditions of high humidity and moderate temperature, the infected chlorotic leaf areas support a massive amount of asexual sporulation, generally on the surface of leaves, giving them a downy appearance. Severely infected plants are generally stunted and do not produce panicles.

The name 'green ear' stems from the appearance of green panicles due to the transformation of floral parts into leafy structures, which can be total or partial. These leafy structures can also be chlorotic, and sometimes support sporulation. In certain cases, green ear is the only manifestation of the disease (Singh *et al.,* 1993: Singh, 1995).

Symptoms are rarely seen as local lesions or isolated spots on leaf blades (Saccas, 1954; Girard, 1975). Spots vary in shape and size, and are at first chlorotic, produce sporangia and later become necrotic.

Dang (1981) reported that the downy mildew pathogen showed a great range of variability in symptoms expression due to the presence of high level of inoculum in the soil of established sick field plot, over wintered oospores induced higher downy mildew incidence than the oospores stored in laboratory condition .

Zoosporic inoculation of *S. graminicola* on different developmental stages of the inflorescence of *P. americanum*, resulted in malformation of floral organs that were not fully differentiated at the time of infection. “Green ear ” symptoms resulting from hyperplasia and hypertrophy of the host tissues were accompanied by both sexual and asexual sporulation of the fungus on the malformed plant parts (Singh and King, 1988).

Gaur *et al*. (1990) reported that seeds from ear heads infected by *S. graminicola* loose their lusture and develop a violet tinge due to toxin production . Post infection differences between susceptible and resistant lines were quantitative rather than qualitative and specific.

Sharma (2005) and Sudhakar et.al., (2012) observed the initiation of downy mildew on the eleven days old seedling of pearl millet susceptible hybrid “7042-S”.

2.3 Screening

Singh *et al*. (1981) devised screening strategy at ICRISAT, Patencheru, using an efficient field screening technique. The promising materials were then exposed to variable population of pathogen at downy mildew “ Hot spot ” location in india and West Africa through a cooperative multilocational testing programme. All these entries originated programmes in india and Africa . Other entries have been identified with distinct differential reactions among locations that were consistent over years.

William *et al*. (1981) developed an effective , large scale field screening technique in pearl millet. The technique based on pre planted infector row that provided sporangial inoculum has been successfully used to identify the downy mildew resistant plants view reliability, uniformity of inoculum , distribution , flexibility, in location and size of screening plots , effectiveness through out the year (including the dry winter, and post rainy season) and independence to rainfall.

Murthy *et al*. (1983) screened 318 entries of *Pennisetum americanum* against *S. graminicola* in the disease sick plot and found that 65 entries remained free from

the downy mildew, while HB3 and tift 23D 2B exhibited 86.9 per cent and 91.7 per cent infection respectively.

Chahal *et al*. (1987) tested 83 agronomically superior lines of *P. americanum* in the downy mildew sick plot over five year raining season, 45 showed a high level of resistant , 29 were moderately resistant and 9 moderately susceptible .

Singh (1990) evaluated 3163 germplasm accessions from many pearl millet (*P. glaucum*) growing countries for resistance to downy mildew. Thirty seven selection from 48 early to medium maturity accession showed high level of downy mildew resistance.

Rattunde and King (1993) developed a green house seedling screening technique at ICRISAT. Potted plants (about 50 per pot) at the coleoptyle stage to one leaf stage were spray inoculated with an aqueous suspension of sporangia, incubated overnight at 20° C and >95 per cent relative humidity and returned to green house benches. After two weeks, downy mildew reaction was determined.

Govila (1994) at I.A.R.I. New Delhi initiated a major programme to incorporate downy mildew resistance into J-104 and MS 5141 using donors P-7 and 700651. A wide range of downy mildew resistant technique were used while developing the material.

Yadav and Thakur (2001) recorded significantly higher thickness of cuticle and epidermis in the leaves of resistant cultivars, however the highly resistant cultivar had a significantly lower stomatal index and higher epicuticular wax content when compared with the highly susceptible cultivar.

Shivkumar *et al*. (2003) tested eight cultivars viz., IP18292, IP18294, P-310-17, MBH 110,5141B, 81B, 23 B and HB_3 against downy mildew pathogen under green house condition IP18292 and IP18294 were categorized as highly resistant P31017 and MBH 110 as resistant 5141B and 81B as susceptible and 23 B and HB_3 as highly susceptible.

Latake and Kolase (2007) reported biological control agents, *Trichoderma viride, T. harzianum, T. hamatum, Pseudomonas fluorescens* as effective microorganisms (EM) along with fungicide Ridomil MZ-72 for their effectiveness in controlling downy mildew disease (*Sclerospora graminicola*) of pearl millet (hybrid HB-3) in an infected plot in Maharashtra, India. Seed treatment followed by spraying

at 20 days after sowing was done. Among the bioagents, *T. harzianum and P. fluorescens* were the most promising in reducing the disease incidence with increase in emergence of crop and grain yield. However, the chemical treatment of Ridomil MZ-72 was the most significant in terms of all the parameters under study.

Ninety-three pearl millet genotypes grown in Dhule, Maharashtra, India, were evaluated for resistance to downy mildew (caused by *Sclerospora graminicola*) during the kharif of 2005. Downy mildew incidence was recorded at 30 and 60 days after sowing. Among the 25 genotypes form Dhule, 17 genotypes were highly resistant, whereas 6 were resistant to downy mildew. Among the 68 genotypes from Jodhpur, Rajasthan, 14 genotypes were highly resistant and 24 were resistant to the disease. Latake *et al*. (2008)

2.3 Management (Chemicals & non chemicals)

2.3.1 Botanicals

Botanicals are gaining importance in crop protection in view of their selective properties , low cost and safety to ecosystem. Many botanicals have been identified to be effective in the control of plant diseases. Among the 5280 species tested, 1134, 346, 92 plant species possessed insecticidal, fungicidal, bactericidal, antiviral properties respectively (Ahmed and Grainage 1982).

Menna and Mariappan (1993) studied the *in vitro* leaf extract of *Azadirachta indica, Mentha arvensis, Aegle mormelous, Cartharanthus roseus, lantana camara, Pongamia pinnata, Vitex negundo* and *Nerium odorum* (Nerium olender)and flower extracts of *Cartharanthus roseus* inhibited mycelium growth and spore germination of the seed borne mycoflora of sorghum including *Alternaria tenluis* (*A. alternata*) *Aspergillus flavus, Curvularia lunata. Fusarium moniliforme* (*Gibberella fuzikuroi*) and *Rhizophus stolonifer*. The neem extract and those of *C. roseus* and *L. camera* were more effective than the other plant extracts tested.

Dohroo and Gupta (1995) reported that Azadirachtain and other limoids were quite effective in the control of plant diseases of diverse nature. The addition of neem cake in soil reduced incidence of damping off, wilt, blight and rot of cotton, soybean, coconut, ginger etc. the neem oil has fungicidal properties that had inhibitory effect to sclerotia of *Sclerotium, Rhizoctonia* and *Sclerotinia*, The extract of neem was found most potent in reducing virus infectivity and nematode population.

Shivpuri *et al*. (1997) also reported fungitoxic effect of plant extract from 10 plants species (*Allium cepta, Allium sativum, Azadirachta indica, Calotropsis procera, Datura stramonium, Ocimum sanctum, Polyalthia longifolia, Tagetus erecta, Vinca rosea* and *Withania somifera* against five pathogenic fungi viz. *A. brassicola capsici, Fusarium oxysporium, Rhizoctonia solani* and *Sclerotinia sclerotiorum* when tested under laboratory condition at two concentrations. Higher dose of few plant extracts was relatively more effective.

Madhusudhan and Patil (2003) tested the efficiency of leaf and bulb extracts (2.5, 5 and 10 %) of 15 plant species against the spore germination of *Colletrotrichum truncatum*. Carrot grass (*Parthenium hysterophorus*) recorded the highest inhibition of spore germination *C. truncatum* regardless of the concentration used.

Patidar (2007) reported that cow urine, butter milk, neem cake, mustard cake, linseed cake, leaf extract of neem, Calotropis, Datura, Eucalyptus, Parthenium and Lantana significantly inhibited the oospore germination of *Sclerospora graminicola* but maximum inhibition was recorded in cow urine (10%) followed by neem cake (10%) and neem leaf extract (10%).

Rajput *et al*. (2013) conducted an experiment view cow urine, butter milk, oil cakes and botanicals where imparted a significant role on the control of downy mildew in pearl millet. Application of butter milk @ 5% (33.2%) was found most effective in respect of downy mildew control.

2.3.2 Bio-agents

Bacteria having potential of bio-control are in many genera including *Pseudomonas* etc. Seed treatment with *Bacillus spp.* has been tested on several plants to control diseases. They are agents for bio-control because they produce endospores that are tolerant to heat, desiccation and drought conditions.

Umsesha *et al*. (1998) tested efficacy of *Pseudomonas fluorescens* against pearl millet downy millet disease by treating seeds with the culture of *P. fluorescens*. The bioagent was also tested as a foliar application . Treated seeds increased seedling vigour and inhibited sporulation of downy mildew pathogen. *P. fluorescens* controlled downy mildew disease both by seed treatment and foliar application.

Gupta and Singh (1999) reported the influence of soil pH, soil bulk density, soil moisture content and addition of farm yard manure and nitrogen fixing bacteria on downy mildew incidence. The addition of *Rhizobium , Azospirillum* or *Azotobacter inocula* as combined seed and soil treatment also reduced disease with the best effects being from a cluster bean (*cyamopsis tetragonoloba*)isolate of *Rhizobium* and from *Azotobacter chroococcum.*

Shetty *et al*. (2000) conducted studies on the biological control of pearl millet downy mildew caused by *Sclerospora graminicola*. They studied the effectiveness of fungal antagonists against the fungal pathogen, their protective action in the plant against further infection and their positive effects on the yield and growth characteristics of pearl millet plants.

Raj *et al.* (2005) tested trichoshield, a talc formulation consisting of spores of *Trichoderma harzianum, T. lignorum, Gliocladium virens* and *Bacillus subtilis*, following different application methods, for its ability to promote growth of pearl millet plants and to induce resistance to downy mildew of pearl millet. Under laboratory conditions, trichoshield seed treatment enhanced seed germination and seedling vigour of pearl millet significantly over the control; under greenhouse conditions vegetative growth parameters like height, fresh and dry weight, leaf area and number of tillers were significantly enhanced over the control: Trichoshield formulation offered greater protection against downy mildew in comparison with individual strains of *T. harzianum, T. lignorum, G. virens* and *B. subtilis*. Yield enhancement of 28% over the control was highly significant. Trichoshield treatment offered protection ranging from 52 to 71% under field condition, depending on the application method. However, the chemical fungicide metalaxyl Apron provided the highest protection against downy mildew, both under greenhouse and field conditions.

Hindumathy *et al.* (2006) prepared an aqueous suspension of carbohydrate components from the autoclaved spore cell wall of *Aspergillus niger* and tested for defense response in pearl millet (*Pennisetum glaucum* (L.) R.Br.) against *Sclerospora graminicola*. The aqueous suspension derived from the spore cell wall of *A. niger* was used as a seed soak treatment at concentrations of 0.25, 0.5, 1.0, 1.5 and 2.0 mg ml-1 for time intervals of 3, 6, 9 and 12 h. Spore cell wall suspension as seed treatment at a concentration of 0.5 mg ml-1 required a 3-day time interval to provide 67% protection against downy mildew.

Mane *et al.* (2007) soaked pearl millet seeds in sporulated culture of biological control agents (*Trichoderma viride, T. harzianum, T. hamatum* and *Pseudomonas fluorescens*). Maximum seed emergence was recorded with *Pseudomonas fluorescens*. *T. harzianum* and *T. viride* also reduced disease incidence up to 52.40% compared to the control. None of the formulations matched the level of metalaxyl in offering protection against downy mildew. Among the application methods tested, soil amendment was the most suitable and desirable way of delivering formulations.

Latake and Kolase (2007) screened *Trichoderma viride, T. harzianum, T. hamatum* and *Pseudomonas fluorescens* against downy mildew of pearl millet and reported seed treated with *T. viride, T. harzianum, T. hamatum and P. fluorescens* were the most promising in reducing the disease incidence with increase in emergence of crop and grain yield.

Patidar (2007) reported significant control of pearl millet downy mildew by seed dressing with *Bacillus pumilus* in combination with Apron (3 g/kg seed), and Apron seed dressing alone (6 g/kg seed). The full dose of Apron alone was more effective than its half dose in combination with *Bacillus pumilus* at tillering stage of the crop, but at dough stage the combined treatment was more effective than the Apron alone in respect of disease and yield both. Pearl millet seed dressing with Apron 35 SD @ 6 g/kg seed, *Bacillus pumilus* (INR 7) and chitosan significantly checked the incidence of downy mildew at 30 and 60 days after sowing (Rajput, 2009).

Mani and Hepziba (2009) reported eight fungal and three bacterial antagonists against Sclerospora graminicola sporangial production, spore germination and in the management of the disease. The seeds were treated with *Trichoderma* spp. at 4 g/kg, *P. fluorescens* at 10 g/kg and *B. Subtilis* at 30 g/kg and then dried in the shade for 12 h. These seeds were then exposed to the sporangial shed of S. graminicola and were sown in infected soil in pots. These were compared with the standard check of seed treatment with apron 35 SD (2 g/kg) and seed treatment with apron 35 SD, foliar spray with ridomil MZ 72 WP. The results showed that seed treatment with apron (2 g/kg)+foliar spray of ridomil (0.6%) significantly reduced the incidence of downy mildew to the minimum of 4.50% from 53.33% in the control. This was followed by *P. fluorescens* (9.50%).

2.3.3 Chemicals and other (Cow urine , butter milk and panchgavya) materials

Pandya *et al*. (1999) reported that the seed treatment with Apron 6 g/kg of seed + spray of 4 g Ridomil MZ/l recorded the lowest disease incidence of downy mildew at 30 (1.83%) days after sowing and at the dough stage (6.73%).

Pandya *et al.* (2000) reported that the seed treatment with metalaxyl (Apron 35 WS) 2 g ai/kg seed controlled downy mildew up to 20-22 days after sowing (DAS). Seed treatment by Apron 35 WS 2 g ai/kg seed followed by one spray of Ridomil MZ 72 WP (metalaxyl + mancozeb) at 4 g/l at 20 DAS gave the best result, with the disease incidence 4.63 and 41.59% as compared to 39.10 and 69.92% in control at 30 and 60 DAS, respectively. Development of malformed earheads was also controlled significantly in the Ridomil sprayed plots (13.39%) as compared to control (46.99%) and also gave 15% more grain yield than the control. Seed treatment with Aliete [Aliette] [Aluminum tris-o-ethyl phosphate] alone at 5 g/kg seed was ineffective in controlling downy mildew.

Sharathchandra *et al.* (2004) used a commercially developed aqueous Chitosan formulation Elexa in different concentrations viz., 1:5, 1:10, 1:15, 1:19 and 1:25 as seed soaking treatment to pearl millet for 3, 6 and 9 h duration to test for its effect on germination and vigour index. Among the treatments used 1:19 for 6 h soaking recorded maximum germination and seedling vigour. Seed treatment, foliar spray and combination of seed treatment and foliar spray were tested for control of downy mildew disease caused by *Sclerospora graminicola* in pearl millet under greenhouse and field conditions. Metalaxyl at the rate of 2.1% a.i. in the form of Apron 35 SD seed treatment was used as check. Under field conditions Elexa treatments were evaluated for their effect on incidence and severity of downy mildew disease. Seed treatment reduced downy mildew severity to 42.5% and recorded 38% protection, whereas foliar spray to 7-day-old seedlings gave 67% protection and reduced severity to 25%. Combination of seed treatment and foliar spray to 7-day-old seedlings recorded 69% protection and reduced severity by 23%. Elexa treatment to pearl millet seeds offered growth promoting effect under greenhouse conditions and recorded increase in plant height, earhead length and seed weight. Hence, it is inferred that Elexa is a good downy mildew disease management commercial formulation and it also exhibits growth-promoting effects in pearl millet.

Patidar (2007) reported that cow urine, butter milk, neem cake, mustard cake, linseed cake, neem, Calotropis, Datura, Eucalyptus, Parthenium and Lantana leaf extracts significantly inhibited the oospore germination of *Sclerospora graminicola* but maximum inhibition was recorded in cow urine (10%) followed by neem cake (10%) and neem leaf extract (10%).

Manjunatha *et al.* (2008) Pearl millet seeds with chitosan at different concentrations: 0.5, 1.5, 2.5 and 3 g kg^{-1} seed. Of the different concentrations, 2.5 g kg^{-1} was found to be optimum, with enhanced seed germination of 99% and seedling vigour of 1782, whereas the untreated control recorded values of 87% and 1465 respectively. At optimum concentration, chitosan did not inhibit sporulation and release of zoospores from sporangia. Furthermore, pearl millet seedlings raised after seed treatment with chitosan showed an increased level of the defence-related enzymes chitosanase and peroxidase as compared with the untreated pearl millet seedlings on downy mildew pathogen inoculation. The effect of chitosan in reducing downy mildew incidence was evaluated in both greenhouse and field conditions, in which respectively 79.08 and 75.8% disease protection was obtained.

CHAPTER - III
MATERIAL AND METHODS

Experimental Site

The present studies were conducted at Research Farm, College of Agriculture, Gwalior during *kharif* season of 2014. Gwalior is situated in Northern part of Madhya Pradesh at an elevation of 211.52 meters from mean sea level and lies between latitude and longitude 26º14' North and 78º15' East, respectively.

1. Climate

The climate of Gwalior is subtropical. The rainy season normally starts from middle of June after commencement of south-west monsoon and last up to September. Maximum precipitation of rains occurred in the month of July and August. Winter season runs from November to mid February and hot summer season from April to mid June. October is the transitory month between rainy and winter season.

2. Soil

The soil of experimental site was alluvial clay loam texture. The available nitrogen, phosphorus and potassium content of soil was 200.7kg, 10.1 kg and 260.9kg low, medium low to medium, respectively. The water holding capacity of soils is also medium.

Preparation of downy mildew sick plot

A large quantity of infected leaves, malformed shoots and proliferated ears from downy mildew infected local susceptible and 7042 S pearl millet plants were collected at the time of harvest of previous crop and were sun dried. These were then chopped into small bits and were grind in the mixer to make it in the powder form thereafter the powder was stored in the air tight container. Before the onset of first shower the powder was examined microscopically to make sure that it contained oospores. In general it was found that minimum 7-15 oospores were seen in the microscopic field under low power. After this inoculum (powdered debris containing oospores) was uniformly spread over experimental field at the time of field preparation.

Planting of infector rows

Downy mildew susceptible cultivar “local susceptible” was used as infector rows (Inoculum donor) with a view to develop maximum disease pressure. These rows were planted three weeks earlier than the test rows.

The infector rows were sown at every ninth row throughout the entire length of the experimental field. The field was also surrounded by the infector rows. The test material of the respective trial was sown about three weeks after the sowing of infector rows. The indicator rows (local susceptible and 7042 S) were also planted along with the test rows to assess the level of disease pressure.

1. Symptomatology :

The studies on symptoms of downy mildew was carried out on the susceptible pearl millet hybrids/varieties grown on experimental field. The downy mildew pressure was maximum in hybrid “7042 S ” (completely damaged due to the disease) hence the study of symptoms was carried out on susceptible and moderately susceptible hybrid/varieties through periodical close observations. These observations were recorded during crop season and were started from the initiation of the disease.

2. Evaluation of pearl millet material against downy mildew under disease sick field condition.

Identification of disease resistant lines and their utilization in resistance breeding program is the most effective method for the management of the disease. Therefore, pearl millet germplasm consisting of 252 lines were evaluated against downy mildew in the downy mildew sick soil by adopting a field screening technique which was developed by Williams *et al.* (1981).

Details of the experiment are as follows:

Entries	-	251
Design	-	R.B.D.
Replication	-	2
Row length	-	5 m

Plate 2 : Experimental field view at Dough stage

Plate 3 : View of infector (inoculum donor) row

Plate - 4 : Comparative view of treatments applied for the management of downy mildew

Plate - 5 : Effect of pearl millet seed dressing agains down milde

Spacing	-	50 cm x 10 cm
Fertilizer	-	60 kg N, 40 kg P_2O_5/ha
Date of sowing	-	21.07.2014

The observations on total number of plants and plant affected by downy mildew were recorded at 30 days and 60 days (dough stage) after sowing.

3. Evaluation of promising hybrids, varieties, land races and B lines under disease sick field condition .

A total no. of promising pearl millet hybrids/varieties were evaluated against downy mildew in the downy mildew sick field. The details of the experiment are as follows: -

Entries	-	55
Design	-	RBD
Replication	-	2
Row Length	-	5 m
Spacing	-	10 cm (plant to plant) 50 cm (row to row)
Fertilizer	-	60 kg N, 40 kg P_2O_5/ha
Date of sowing	-	21.07.2014

The observations on total number of plants and plant affected by downy mildew were recorded at 30 days and 60 days (dough stage) after sowing.

4. Evaluation of botanicals, compost tea, cow urine, butter milk and panchgavya against downy mildew.

A field experiment was conducted to find out the effect of foliar application of botanicals (Neem cake, Mustard cake, Neem leaf extract, Neem sees extract), compost tea, cow urine, butter milk and punchgavya on the incidence of downy mildew. The details of the experiment are as follows :

Design	-	RBD
Variety	-	B 2301
Replication	-	2
Treatment	-	14
Plot size	-	5 m × 5 m
Date of sowing	-	21.07.2014

T1 : *Bacillus pumilus* (8 g/kg seed)+ Compost tea @ 10 %

T2 : *Pseudomonas fluorescence* (8 g/kg seed) + Compost tea @ 10 %

T3 : Apron 35 SD 6 g/ kg seed

T4 : Apron 35 SD 6 g/ kg seed + Cow urine @ 5%

T5 : Mustard cake extract @ 10 %

T6 : Neem cake extract @ 10 %

T7 : Neem leaf extract @ 10 %

T8 : Neem seed extract @ 10 %

T9 : Compost tea @ 10 %

T10 : Vermicompost tea @ 10 %

T11 : Butter milk @ 10 %

T12 : Panchgavya @ 10 %

T13 : Mancozeb @ 0.2 %

T14 : Control (untreated) @ 10 %

5. To study the influence of pearl millet seed dressing with bio-agents, chitosen and recommended chemicals against progressive development of downy mildew.

An integrated disease management study was carried out as per the following details:

Hybrid used - B 2301

Design - RBD

Treatments : 06

1. Chitosan @ 2.5 g/kg seed
2. *Bacillus pumilus* (INR-7) @ 8 g/kg seed
3. *Bacillus pumilus* (INR-7) 8 g/kg seed + Chitosan @ 2.5 g/kg
4. *Pseudomonas fluorsescens*@ 8 g/kg seed.
5. Apron (6 g/kg)
6. Control (Untreated)

Replication - 4

Date of sowing- 21.07.2014

Plot size - 5m x 3m

Spacing - 75 x 15 cm

Fertilizer - 60 kg N, 40 kg P_2O_5 / ha

Chitosan is a linear poly saccharid composed of randomly distributed B (1-4) linked D-glucosamine R, N-acetyl-D-glucosamine.

1. Downy mildew incidence (%)

The total number of plants were recorded at the time of thinning i.e. fifteen days after sowing, while the number of downy mildew infected plants were recorded at 30 and 60 days after sowing then the Downy mildew incidence per cent was calculated with the help of following formula :

$$\text{Downy mildew incidence (\%)} = \frac{\text{Downy mildew infected plants}}{\text{Total number of plants}} \times 100$$

2. Grain yield kg/ha

3. Fodder yield kg/ha

Statistical Analysis

Design

The statistical model design in single environment could be defined as below (Chandel, 1999).

$$Y_{ijk} = \mu + t_i + b_{ij} + e_{ijk}$$

where,

k = the general

t_i = the effect of i^{th} treatment

b_{ij} = the effect of j^{th} replication in i^{th} treatment

e_{ijk} = the error associated with each observation.

The skeleton for analysis of variance for randomized complete block design is given below:

Source of variance	d.f.	Observation mean sum of squares	Expected mean sum of squares
Replication	r-1	$M.S_1$	$2_{ei} + 2_{tri}$
Treatment	t-1	$M.S_2$	$2_{ei} + 2_{rti}$
Error	(r-1)(t-1)	$M.S_3$	2_{ei}
Total	**rt-1**		

Standard error of mean

It was calculated as formula given below:

$$SE(m) \pm \sqrt{\frac{Ve}{r}}$$

where,

Ve = Error mean sum of squares

r = Number of replication.

Critical difference

It was mean used as formula mentioned below:

$$C.D. = \sqrt{2}\ S.E. \pm \times t \text{ at } 5\%$$

Where,

C.D. = critical difference

S.E. = Standard error of mean

$t_{5\%}$ Table value of ‘t’.

CHAPTER - IV

RESULTS

4.1: Symptomatology

In the present study following symptoms of downy mildew were recorded on the downy mildew susceptible pearl millet hybrids and varieties planted in the downy mildew sick experimental field.

The first symptom of the disease was observed on fourteen days old seedling of downy mildew susceptible hybrid “7042 S” as chlorotic strip at the base of upper surface of leaf (Plate - 5a) which gradually progresses towards the tip of leaf, the corresponding lower surface (Plate - 5a) of the leaf showed whitish cottony growth (Plate - 5b) of the pathogen (asexual sporulation of *Sclerospora graminicola*). Chlorotic and delicate emerged leaves were emerged out from the infected plant (Plate - 5c). Profuse tillering with chlorotic leaf was observed in some of the infected plant (Plate -5d). The cluster of chlorotic leaves were also recorded on the nodes of few infected plants (Plate -5e) under severe infection the sporangial growth was also observed rarely on stem (Plate -5g). As The disease progresses, the browning of infected leaves was noticed (Plate –5i). Later on in many cases vertical splitting of such infected leaves was commonly observed (Plate –5j). Severely infected plants stunted (Plate –5k). Pre-mature drying and death of such severely infected plants was observed in many cases (Plate –5l).

In spite of downy mildew infection most of the plants formed normal or malformed ear heads. A variety of malformation (Plate 6a-6l) has been observed during the field experimentation in the present study. In most of the malformed ear head a complete (Plate 6a-6l) malformation was observed while in some cases partial malformation was noticed (Plate- 7a&7b). The variation noticed in complete malformation are blunt thin leaf, compact leafy structure, narrow elongated leaves, compact bunch of chlorotic leaves, blunt green ear showing leafy and thread like structure with purplish shade, needle like, excessive thick rolled and overlapped, long twisted green ear (plate 6a-

6(a) Initial symptoms of downy mildew on lower and upper leaves, 6(b) Close-up view of infected upper and lower leaves, 6(c) Emergence of newly chlorotic leaves, 6(d) Profuse tillering in infected plant, 6(e) Clusters of chlorotic leaves form nodes, 6(f) Stunting of infected plant, 6(g) Sporangial growth on stem, 6(h) Close-up view of Sporangial growth on stem, 6(i) Browning of infected leaves, 6(j) Vertical splitting of infected leaves, 6(k) View of severely infected plant, 6(l) Pre-mature drying and death of severely infected plant.

Plate 6(a-l) : Show progressive development of downy mildew.

7(a) Blunt thin leaf structure, 7(b) Compact leafy structure in place of ear head, 7(c) Malformed ear head resembling a cluster of thick elongated leaves, 7(d) Formation of a compact bunch of chlorotic leaves in plant of a ear head, 7(e) Green ear showing cluster of thin narrow leaves, 7(f) Blunt green ear showing leafy and thread like structure with purple shade.

Plate 7(a–f) : Structural variability in complete malformation (Green ear).

7(g) Cluster of needle like structure, 7(h) Replacement of normal ear head with spreading cluster of thin chlorotic leaf structure, 7(i) Replacement of ear head with excessive thick rolled and overlapped leafy structure, 7(j) A green ear in which normal leaves emerging from a cluster of narrow leaves, 7(k) Long twisted green ear with tiny narrow delicate leaf structure, 7(l) Abnormal green ear with excessive thick twisted overlapped leaves.

Plate 7(g–l) : Structural variability in complete malformation (Green ear).

Plate 8a

Plate 8b

Plate 8(a,b) : Structural variability in partial malformation

6l). Only two types of structural variability observed was during the course of present study.

4.2: Evaluation of pearl millet material against downy mildew

A total of 251 pearl millet lines were evaluated in downy mildew sick field under infector rows systems to identify the downy mildew resistance lines. The downy mildew cultivars “7042 S’’ and local susceptible were also planted in between the test lines to measure the progresses of downy mildew.

It is obvious from the data presented in table 4.1, that out of 251, 186 lines were free from downy mildew at 30 day after sowing. Out of this 168 lines viz., GP 51, GP 77, GP 75, RVSBH 23, RVSBH 76, RVSBH 77, RVSBK 78, JBR-13,MP 533, MP 551, MP 552, MP 553, MP 557, MP 559, MP 560, MH 1771, MH 1828, MH 1831, MH 1837, MH 1852, MH 1887, MH 1889, MH 1890, MH 1901, MH 1904, MH 1910, MH 1928, MH 1957, MH 1962, MH 1964, MH 1970, MH 1974, MH 1975, MH 1976, MH 1979, MH 1984, MH 1991, MH 1993, MH 1994, MH 1995, MH 1996, MH 1997, MH 1998, MH 1999, MH 2001, MH 2002, MH 2004, MH 2007, MH 2008, MH 2009, MH 2010, MH 2011, MH 2012, MH 2014, MH 2015, MH 2016, MH 2017, MH 2018, MH 2019, MH 2021, MH 2022, MH 2023, MH 2024, MH 2025, MH 2026, MH 2027, MH 2028, MH 2029, MH 2030, MH 2031, MH 2033, MH 2034, MH 2035, MH 2036, MH 2038, MH 2039, MH 2041, MH 2042, MH 2043, MH 2044, MH 2045, MH 2046, MH 2047, MH 2048, MH 2049, MH 2050, MH 2051, MH 2052, MH 2053, MH 2054, MH 2055, MH 2056, MH 2057, MH 2059, MH 2060, MH 2061, MH 2062, MH 2063, MH 2064, MH 2065, MH 2066, MH 2067, MH 2068, MH 2070, MH 2071, MH 2072, MH 2073, MH 2075, MH 2076, MH 2077, MH 2078, MH 2079, MH 2080, IP 18292, ICMR 08888, ICMR 08555, ICMR 14001, H/77/833-2-202, AIMP 92901-P3, AIMP 92901-P8, PIB 957, PIB 686, Comp. Sum 622-660, J-2500, JMSB -101, JMSB-20071, J-2578, J-2538, JMSB 20101, JMSB 20082, JMSB 20042, ICMB 10444, ICMB 14002, ICMB 14003, ICMB 90111-P6, ICMB 93333, ICMB 92666, ICMB 92777, ICMB 01333, ICMB 02444, ICMB 89111, ICMB 95444, ICMB 93222, ICMB 00111, ICMB 02111, ICMB 07111, ICMB 09999, 863 B-P2, ICMB 95222-672, IP 11036, HHB 146 IMP, HHB 146 ORI, ICMR 01004, ICMR 11009, ICMR 11019, IP 22303, IP 5900, PUSA 2014–I, PUSA 2014-2,PUSA 2014-3, PUSA 2014-4, PUSA 2014-5, SBH 215-3, SBH 212-6, SBH 112-7, SBH 205-9, SBH 214-13 and SBH 103-

15, remained free from downy mildew at 60 days after sowing, while 53.76 and 93.87 percent disease incidence was recorded in ‘‘7042 S’’ at 30 and 60 days after sowing, respectively. The other check (local susceptible) showed 15.50 and 24.69 percent disease incidence at 30 and 60 days after sowing, respectively. All the tested lines were significantly superior over ‘‘7042 S’’ and ‘‘local susceptible’’ except three lines 7042R, ICML 22 and ICMB 95222-760. More than 10 percent downy mildew was recorded in the nine test lines ‘‘MH 2058 (12.86%), 852 B (10.84%), 7042R (62.88%), ICMR 22 (74.34%), ICMB 95222-760 (24.52%), IP 7846 (11.49%) and IP 22458 (10.56%) at 60 days after sowing, the other lines except 168 free lines showed less than 10% downy mildew.

Table 4.1: Reaction of pearl millet material against downy mildew.

S. No.	Entry	Per cent incidence at		S. No.	Entry	Per cent incidence at	
		30 DAS	60 DAS			30 DAS	60 DAS
1	GP 51	0.00	0.00	23	MP 558	2.63	3.91
2	GP 77	0.00	0.00	24	MP 559	0.00	0.00
3	GP 75	0.00	0.00	25	MP 560	0.00	0.00
4	RVSBH 22	1.00	3.09	26	MH 1771	0.00	0.00
5	RVSBH 23	0.00	0.00	27	MH 1777	0.00	1.02
6	RVSBH 76	0.00	0.00	28	MH 1828	0.00	0.00
7	RVSBH 77	0.00	0.00	29	MH 1831	0.00	0.00
8	RVSBK 78	0.00	0.00	30	MH 1837	0.00	0.00
9	RVSBH 79	4.19	6.00	31	MH 1852	0.00	0.00
10	JBR-13	0.00	0.00	32	MH 1875	0.91	2.10
11	MP 533	0.00	0.00	33	MH 1887	0.00	0.00
12	MP 534	0.00	1.04	34	MH 1888	0.00	0.94
13	MP 535	1.11	2.22	35	MH 1889	0.00	0.00
14	MP 545	1.09	1.09	36	MH 1890	0.00	0.00
15	MP 546	1.32	2.63	37	MH 1901	0.00	0.00
16	MP 551	0.00	0.00	38	MH 1904	0.00	0.00
17	MP 552	0.00	0.00	39	MH 1910	0.00	0.00
18	MP 553	0.00	0.00	40	MH 1913	2.96	3.92
19	MP 554	0.96	1.94	41	MH 1915	1.25	1.25
20	MP 555	3.52	3.52	42	MH 1928	0.00	0.00
21	MP 556	1.11	2.15	43	MH 1930	2.27	4.36
22	MP 557	0.00	0.00	44	MH 1939	0.00	1.19
45	MH 1957	0.00	0.00	90	MH 2023	0.00	0.00
46	MH 1962	0.00	0.00	91	MH 2024	0.00	0.00
47	MH 1964	0.00	0.00	92	MH 2025	0.00	0.00
48	MH 1969	0.00	0.98	93	MH 2026	0.00	0.00
49	MH 1970	0.00	0.00	94	MH 2027	0.00	0.00
50	MH 1974	0.00	0.00	95	MH 2028	0.00	0.00

51	MH 1975	0.00	0.00	96	MH 2029	0.00	0.00
52	MH 1976	0.00	0.00	97	MH 2030	0.00	0.00
53	MH 1977	0.00	1.04	98	MH 2031	0.00	0.00
54	MH 1979	0.00	0.00	99	MH 2032	0.00	1.02
55	MH 1984	0.00	0.00	100	MH 2033	0.00	0.00
56	MH 1989	1.02	2.04	101	MH 2034	0.00	0.00
57	MH 1990	5.68	6.82	102	MH 2035	0.00	0.00
58	MH 1991	0.00	0.00	103	MH 2036	0.00	0.00
59	MH 1992	2.70	3.68	104	MH 2037	0.00	1.00
60	MH 1993	0.00	0.00	105	MH 2038	0.00	0.00
61	MH 1994	0.00	0.00	106	MH 2039	0.00	0.00
62	MH 1995	0.00	0.00	107	MH 2040	0.00	1.00
63	MH 1996	0.00	0.00	108	MH 2041	0.00	0.00
64	MH 1997	0.00	0.00	109	MH 2042	0.00	0.00
65	MH 1998	0.00	0.00	110	MH 2043	0.00	0.00
66	MH 1999	0.00	0.00	111	MH 2044	0.00	0.00
67	MH 2000	0.98	0.98	112	MH 2045	0.00	0.00
68	MH 2001	0.00	0.00	113	MH 2046	0.00	0.00
69	MH 2002	0.00	0.00	114	MH 2047	0.00	0.00
70	MH 2003	1.19	2.38	115	MH 2048	0.00	0.00
71	MH 2004	0.00	0.00	116	MH 2049	0.00	0.00
72	MH 2005	2.22	3.33	117	MH 2050	0.00	0.00
73	MH 2006	1.19	1.19	118	MH 2051	0.00	0.00
74	MH 2007	0.00	0.00	119	MH 2052	0.00	0.00
75	MH 2008	0.00	0.00	120	MH 2053	0.00	0.00
76	MH 2009	0.00	0.00	121	MH 2054	0.00	0.00
77	MH 2010	0.00	0.00	122	MH 2055	0.00	0.00
78	MH 2011	0.00	0.00	123	MH 2056	0.00	0.00
79	MH 2012	0.00	0.00	124	MH 2057	0.00	0.00
80	MH 2013	2.04	3.06	125	MH 2058	11.61	12.86
81	MH 2014	0.00	0.00	126	MH 2059	0.00	0.00
82	MH 2015	0.00	0.00	127	MH 2060	0.00	0.00
83	MH 2016	0.00	0.00	128	MH 2061	0.00	0.00
84	MH 2017	0.00	0.00	129	MH 2062	0.00	0.00
45	MH 1957	0.00	0.00	130	MH 2063	0.00	0.00
46	MH 1962	0.00	0.00	131	MH 2064	0.00	0.00
85	MH 2018	0.00	0.00	132	MH 2065	0.00	0.00
86	MH 2019	0.00	0.00	133	MH 2066	0.00	0.00
87	MH 2020	2.92	3.92	134	MH 2067	0.00	0.00
88	MH 2021	0.00	0.00	135	MH 2068	0.00	0.00
89	MH 2022	0.00	0.00	136	MH 2069	0.93	1.85

137	MH 2070	0.00	0.00	185	J-2495	0.00	0.55
138	MH 2071	0.00	0.00	186	J-2523	1.03	1.52
139	MH 2072	0.00	0.00	187	92-SB-13	1.05	1.05
140	MH 2073	0.00	0.00	188	JMSB -101	0.00	0.00
141	MH 2074	1.43	1.43	189	JMSB-9904	3.27	3.81
142	MH 2075	0.00	0.00	190	JMSB-20071	0.00	0.00
143	MH 2076	0.00	0.00	191	JMSB-20091	0.00	0.61
144	MH 2077	0.00	0.00	192	JMSB-20111	2.49	2.99
145	MH 2078	0.00	0.00	193	J-2578	0.00	0.00
146	MH 2079	0.00	0.00	194	J-2538	0.00	0.00
147	MH 2080	0.00	0.00	195	J-2510	6.58	9.84
148	P7-4	2.35	4.75	196	JMSB 20101	0.00	0.00
149	P310-17	1.23	1.82	197	JMSB 20082	0.00	0.00
150	700651	0.00	1.20	198	JMSB 20042	0.00	0.00
151	852 B	5.26	10.84	199	ICMB 10444	0.00	0.00
152	7042 R	19.39	62.88	200	ICMB 10555	0.56	1.09
153	IP 18292	0.00	0.00	201	ICMB 11555	0.52	1.03
154	IP 18293	0.00	1.13	202	ICMB 81	3.98	5.66
155	IP 18294	0.58	1.16	203	ICMB 14001	0.60	0.60
156	ICMP 451	4.32	8.51	204	ICMB 14002	0.00	0.00
157	ICMR 08888	0.00	0.00	205	ICMB 14003	0.00	0.00
158	ICMR 08555	0.00	0.00	206	ICMB 14004	0.00	0.56
159	ICMR 14001	0.00	0.00	207	843-22B	4.51	7.67
160	ICMR 01007	1.20	3.53	208	ICMB 90111-P6	0.00	0.00
161	HHB 67-1 IMP	0.60	1.20	209	ICMB 93333	0.00	0.00
162	HHB 67-2 IMP	0.56	0.56	210	ICMB 92666	0.00	0.00
163	HHB 67 ORI	0.98	2.02	211	ICMB 92777	0.00	0.00
164	H/77/833-2-202	0.00	0.00	212	ICMB 01333	0.00	0.00
165	H/77/833-2	1.58	2.65	213	ICMB 97222	4.46	8.92
166	H/77/833 -2 P5	0.00	0.64	214	ICMB 02444	0.00	0.00
167	ICML 22	38.26	74.34	215	ICMB 89111	0.00	0.00
168	P 1449-P2	0.81	2.14	216	ICMB 95444	0.00	0.00
169	AIMP 92901-P3	0.00	0.00	217	ICMB 93222	0.00	0.00
170	AIMP 92901-P8	0.00	0.00	218	ICMB 00111	0.00	0.00
171	PIB 226	0.00	1.25	219	ICMB 02111	0.00	0.00
172	PIB 957	0.00	0.00	220	ICMB 07111	0.00	0.00
173	PIB 1234	0.89	2.53	221	ICMB 09999	0.00	0.00
174	PIB 654	1.04	2.76	222	863 B-P2	0.00	0.00
175	PIB 626	0.00	0.57	223	ICMB 95222	1.47	7.64
176	PIB 686	0.00	0.00	224	ICMB 95222-672	0.00	0.00
177	BIB sum 458-464	2.14	3.00	225	ICMB 95222-760	20.05	18.62
178	BIB sum 503-510	7.21	8.44	226	IP 7846	8.59	11.49
179	BIB sum 472-482	1.30	1.30	227	IP 11010	7.64	10.86
180	Comp. Sum 622-660	0.00	0.00	228	IP 11036	0.00	0.00
181	ARL -1	0.00	0.58	229	IP 15256	10.62	9.23
182	ARL -2	0.54	1.08	230	IP 21187	6.38	10.26
183	J-2480	1.62	2.12	231	HHB 146 IMP	0.00	0.00
184	J-2500	0.00	0.00	232	HHB 146 ORI	0.00	0.00

233	ICMR 01004	0.00	0.00	242	PUSA 2014 -4	0.00	0.00
234	ICMR 11009	0.00	0.00	243	PUSA 2014 -5	0.00	0.00
235	ICMR 11019	0.00	0.00	244	SBH 215-3	0.00	0.00
236	IP 22303	0.00	0.00	245	SBH 209-4	1.39	2.78
237	IP 22458	7.78	10.86	246	SBH 212-6	0.00	0.00
238	IP 5900	0.00	0.00	247	SBH 112-7	0.00	0.00
239	PUSA 2014 -I	0.00	0.00	248	SBH 213-8	1.52	1.52
242	PUSA 2014 -4	0.00	0.00	249	SBH 205-9	0.00	0.00
240	PUSA 2014 -2	0.00	0.00	250	SBH 214 -13	0.00	0.00
241	PUSA 2014 -3	0.00	0.00	251	SBH 103-15	0.00	0.00

Local susceptible	15.50	24.69
7042s (check)	53.76	93.87
SE(m) ±	**0.890**	**1.174**
C. D. at 5%	**2.468**	**3.208**

4.3: Evaluation of promising hybrids and varieties of pearl millet

A set of 55 promising hybrids and varieties were evaluated against downy mildew and were compared with standard and local susceptible checks (7042-S). All the fifty five hybrids and varieties showed significantly less downy mildew incidence as compared to both the checks at 30 and 60 days after sowing. (Table 4.2)

Forty five genotypes *viz;* Pioneer 86M 88, Pro agro 94-50, NBH 3685, Maha laxmi tilak, Indo US 9999 , Krishna 7201, Pro agro 9444, 86 M 85, ICTP 8203, ICMV 221, Pusa Composite 383, RVBG/13/01, RVBG/13/02, RVBG/13/03, RVBG/13/04, RVBG/13/05, RVBG/13/06, RVBG/13/07, RVBG/13/08, RVBG/13/09, RVBG/13/10, RVBG/13/11, RVBG/13/12, RVBG/13/13, RVBG/13/14, RVBG/13/15, RVBG/13/16, RVBG/13/17, RVBG/13/18, RVBG/13/19, RVBG/13/20, RVBG/13/21, RVBG/13/22, RVBG/13/23, RVBG/13/24, RVBG/13/25, RVBG/13/26, RVBG/13/27, RVBG/13/28, RVBG/13/29, RVBG/13/30, RVBG/13/31, RVBG/13/32, RVBG/13/33 and 543 B free from downy mildew at 30 days after sowing but at 60 days after sowing 41 entries free from downy mildew. Thirteen entries *viz.,* RHB 121, 86 M 85, ICTP 8203, GHB 558, Raj Vijay Bajra-1, JBV -2, JBV-3, JBV-4, Raj 171, ICMV 155, ICMV 221, 543 B, 843 B and JBV 3 showed < 5 per cent downy mildew incidence. Only one entry 214 b showed > 5 per cent downy mildew incidence. While 7042 S (check) showed 69.53

and 97.36 percent downy mildew incidence at 30 and 60 days after sowing, respectively.

Table-4.2: Evaluation of promising hybrids and varieties of pearl millet

S. No.	Entry	Per cent incidence at	
		30 DAS	60 DAS
1	RHB 121	2.94	2.94
2	Pioneer 86M 88	0.00	0.00
3	Pro agro 94-50	0.00	0.00
4	NBH 3685	0.00	0.00
5	Maha laxmi tilak	0.00	0.00
6	Indo US 9999	0.00	0.00
7	Krishna 7201	0.00	0.00
8	Pro agro 9444	0.00	0.00
9	86 M 85	0.00	0.98
10	ICTP 8203	0.00	1.39
11	GHB 558	1.39	1.39
12	Raj Vijay Bajra-1	2.00	2.98
13	JBV -2	1.11	3.69
14	JBV-3	2.00	2.96
15	JBV-4	2.13	4.17
16	Raj 171	2.33	3.71
17	ICMV 155	1.11	4.31
18	ICMV 221	0.00	0.93
19	Pusa Composite 383	0.00	0.00
20	RVBG/13/01	0.00	0.00
21	RVBG/13/02	0.00	0.00
22	RVBG/13/03	0.00	0.00
23	RVBG/13/04	0.00	0.00
24	RVBG/13/05	0.00	0.00
25	RVBG/13/06	0.00	0.00
26	RVBG/13/07	0.00	0.00
27	RVBG/13/08	0.00	0.00
28	RVBG/13/09	0.00	0.00
29	RVBG/13/10	0.00	0.00
30	RVBG/13/11	0.00	0.00
31	RVBG/13/12	0.00	0.00
32	RVBG/13/13	0.00	0.00
33	RVBG/13/14	0.00	0.00
34	RVBG/13/15	0.00	0.00
35	RVBG/13/16	0.00	0.00
36	RVBG/13/17	0.00	0.00

37	RVBG/13/18	0.00	0.00
38	RVBG/13/19	0.00	0.00
39	RVBG/13/20	0.00	0.00
40	RVBG/13/21	0.00	0.00
41	RVBG/13/22	0.00	0.00
42	RVBG/13/23	0.00	0.00
43	RVBG/13/24	0.00	0.00
44	RVBG/13/25	0.00	0.00
45	RVBG/13/26	0.00	0.00
46	RVBG/13/27	0.00	0.00
47	RVBG/13/28	0.00	0.00
48	RVBG/13/29	0.00	0.00
49	RVBG/13/30	0.00	0.00
50	RVBG/13/31	0.00	0.00
51	RVBG/13/32	0.00	0.00
52	RVBG/13/33	0.00	0.00
53	214 B	3.50	6.42
54	543 B	0.00	1.32
55	843 B	2.35	2.91
56	local susceptible 7042S	69.53	97.36
SE(m) ±		**0.692**	**0.717**
C. D. at 5%		**1.918**	**1.986**

4.4: Evaluation of botanicals, compost tea, cow urine, butter milk and panchgavya against downy mildew.

In the present study, the bio-agents, botanicals, compost tea, vermicompost tea, butter milk, cow urine and panchgavya were evaluated against downy mildew and were compared with recommended chemical & control. The data on disease incidence was recorded at 30 and 60 days after sowing and the data are summarized in table 4.3 and depicted in fig 4.1

At 30 days after sowing, two treatment viz. seed treatment with Apron 35 SD @ 6g/kg seed followed by foliar application of cow urine @ 5% (T4) and foliar application of mancozeb @ 0.2% (T13) absolutely checked the downy mildew significantly superior over foliar application of panchgavya @ 10% (2.75%), foliar application of neem seed extract @ 10% (3.20%), seed treatment with *Pseudomonas fluorescence* 8 g/kg seed + spray of compost tea @ 10% (3.64%), but were statistically at par with seed treatment with apron @ 6 g/kg, foliar application of neem leaf extract @ 10% (0.58%), foliar application of butter milk @ 10% (1.09%),

foliar application of neem cake extract @ 10% (1.77%), foliar application of mustard cake extract @ 10% (1.83%).

At 60 days after sowing none of this treatments remained completely free from downy mildew, but its minimum incidence (5.18%) was recorded in the treatment T4 (Apron @ 6 g/kg + cow urine @ 5%) followed by T3: Apron @ 6 g/kg (5.68%), foliar application of mancozeb (@ 0.2% (5.83%), neem seed extract @ 10% (7.59%), neem leaf extract @ 10% (7.77%), neem cake extract @ 10% (8.35%), mustard cake extract @ 10% (9.61%), seed dressing of *Bacillus pumilus* @ 6 g/kg + foliar application of compost tea @ 10% (10.55%), butter milk @ 10% (10.90%), panchgavya @10% (11.00%), compost tea @ 10% (11.08%), vermicompost tea @ 10% (11.41%), and seed dressing of *Pseudomonas fluorescens* @ 8 g/kg + foliar application of compost tea @ 10% (12.53%), while the maximum downy mildew incidence 14.78 % was recorded in control. The treatment T4: (Apron + cow urine) was significantly superior over *Bacillus pumilus* + compost tea, butter milk, panchgavya, compost tea, vermicompost tea, *Pseudomonas fluorescens* + compost tea and untreated control but it was statistically at par with Apron alone (T3), mancozeb (T13), neem seed extract (T8), neem leaf extract (T7), neem cake extract (T6) and mustard cake extract (T5).

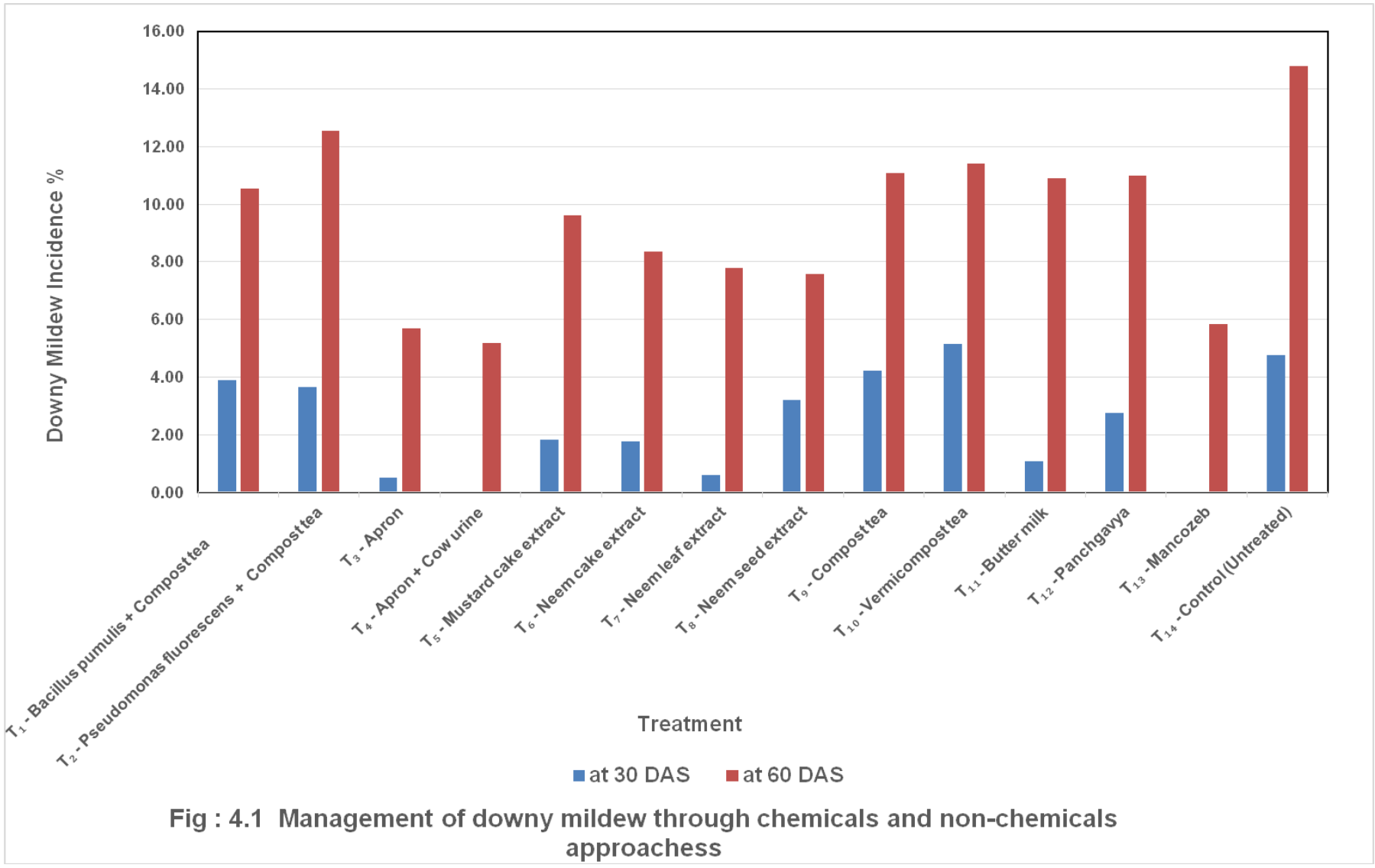

Fig : 4.1 Management of downy mildew through chemicals and non-chemicals approachess

4.5: To study the influence of pearl millet seed dressing with bioagents, chitosen and recommended chemical against progressive development of downy mildew.

A study on integrated management of downy mildew was carried with moderately resistant pearl millet hybrid “B 2301” by using two bio-agents (*Bacillus pumilus* and *Pseudomonas fluorescens*) a natural product “Chitosan” and recommended fungicides “Apron 35 SD”. The data on disease incidence was recorded at 10 days interval starting from 20 days after sowing till 90 days after sowing and the data are summarized in table 4.4 and depicted in fig 4.2.

At 20 days after sowing, the pearl millet seedling with Apron absolutely checked the downy mildew and it was significantly superior over control in which 0.84% downy mildew incidence was recorded. The bio-agent “*Bacillus pumilus*” seed treatment was also significantly superior over control where as chitosan and seed dressing of *P. fluorescens* where statistically at par with control.

At 30, 40, 50, 60, 70, 80, 90 days after sowing, the downy mildew incidence in Apron treatment was 0.65, 1.30, 1.70, 2.49, 3.52, 6.10, 7.68 percent respectively which in control it was 3.82, 5.01, 5.59, 6.59, 9.86, 16.41, 20.59 percent respectively. *Bacillus pumilus* seed dressing alone and in combination with chitosan also significantly checked the incidence of downy mildew. Seed dressing with *P. fluorescens* also reduced the incidence of downy mildew but in most of the observations it was at par with control. Chitosan was not found effective. The Apron seed dressing also gave maximum grain (2345 kg/ha.) and fodder yield (9148 kg/ha.) but the over all effect of the treatment on grain and fodder yield was found non significant.

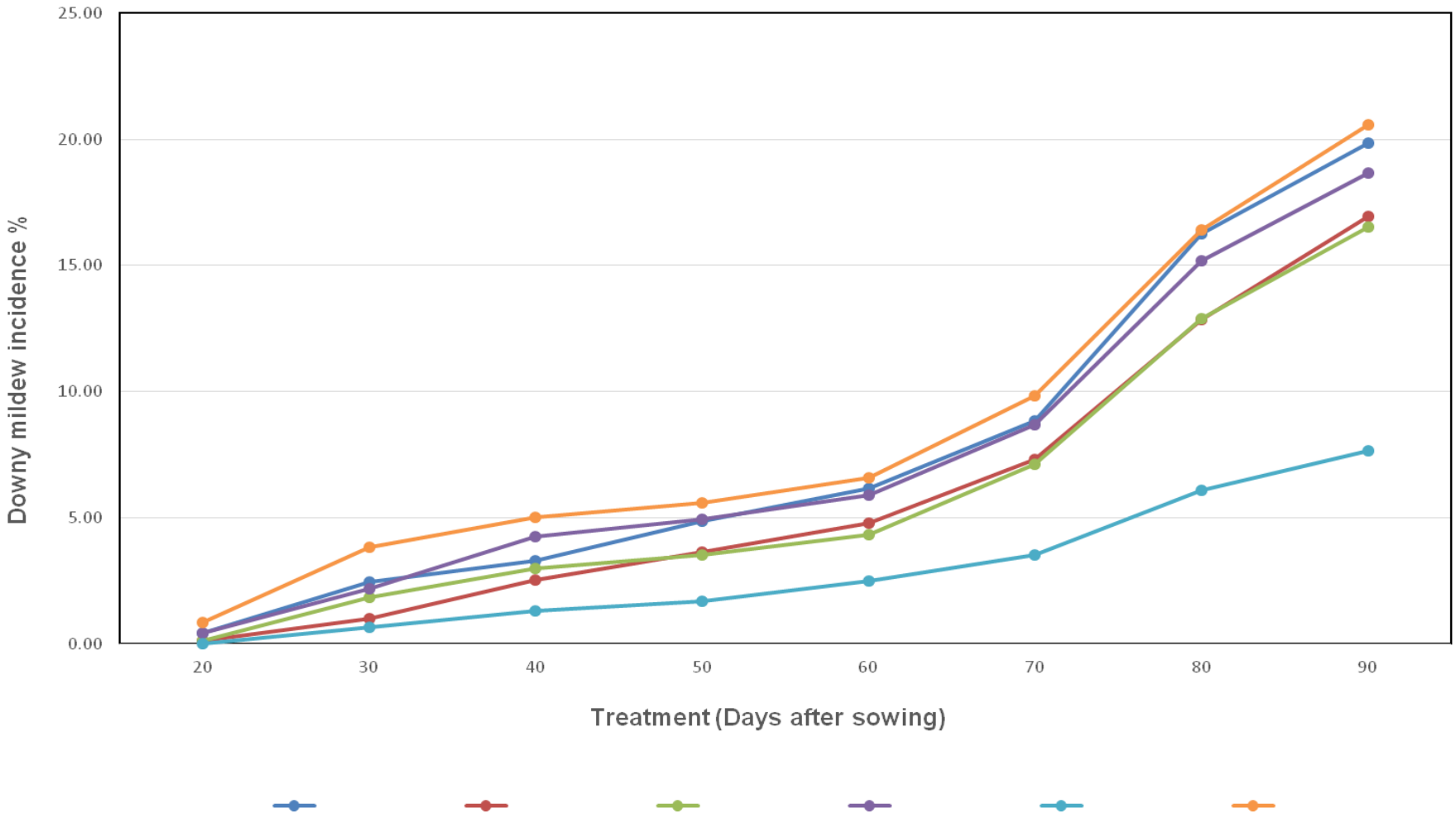

Fig : 4.2 Residual effect of pearl millet seed dressing with bioagents, chitosan and chemicals against downy mildew.

CHAPTER – V
DISCUSSION

In the present study pearl millet downy mildew pathogen (*Sclerospora graminicola*) shows variation in the expression of disease symptoms specially in its green ear phase. In field condition, the disease has shown severity on highly susceptible cultivars (7042 S) as most of the infected plants died and dried prematurely.

The first symptom of the disease was noticed in two weeks old seedling as chlorotic strips on the upper surface of the leaves progressively from base to top. Most of the early infected plants remained stunted, the infected leaves turn brown and in majority of cases such infected plants were failed to form ear. The remaining infected plants developed the normal or malformed ear head (green ear). Among the green ear a wide variation was observed. Similar symptoms were also noted by Singh (1995); Singh and King (1988); Sharma (2005) and Sudhakar et al. (2012).

Out of 251 lines, 168 lines of pearl millet were found free from downy mildew at 30 and 60 days after sowing, while 53.76 and 93.87 percent disease incidence was recorded in ‘‘7042 S’’ at 30 and 60 days after sowing, respectively. The other check (local susceptible) showed 15.50 and 24.69 percent disease incidence at 30 and 60 days after sowing, respectively. All the tested lines were significantly superior over ‘‘7042S’’ and ‘‘local susceptible’’ except three lines 7042 R, ICML 22 and ICMB 95222-760. More than 10 percent downy mildew was recorded in the nine test lines ‘‘MH 2058, 852 B, 7042R, ICMR 22, ICMB 95222-760, IP 7846 and IP 22458 at 60 days after sowing, the other lines except 168 showed less than 10% downy mildew.

Latake *et al.* (2008) evaluated ninety-three pearl millet genotypes in Dhule, Maharashtra, for resistance to downy mildew. Among the 25 genotypes from Dhule, 17 genotypes were highly resistant, whereas 6 were resistant to downy mildew. Among the 68 genotypes from Jodhpur, Rajasthan, 14 genotypes were highly resistant and 24 were resistant to the disease. Singh (1990); Thakur *et al.* (2001) and Wilson *et al.* (2008) also evaluated several germplasm and reported great degree of variation among the genotypes in respect of downy mildew incidence.

It is obvious from the result of the present study that all the popular and released hybrids and varieties showed significantly less incidence of downy mildew.

Out of fifty five, forty five genotypes were free from downy mildew at 30 days after sowing but at 60 days after sowing 41 entries were free from downy mildew. Thirteen entries *viz.,* RHB 121, 86 M 85, ICTP 8203, GHB 558, Raj Vijay Bajra-1, JBV -2, JBV-3, JBV-4, Raj 171, ICMV 155, ICMV 221, 543 B, 843 B and JBV 3 showed less than 5 per cent downy mildew incidence. Only one entry “214 b” showed more than 5 per cent downy mildew incidence. While “7042 S” (check) showed 69.53 and 97.36 per cent downy mildew incidence at 30 and 60 days after sowing, respectively.

Sharma (2005) evaluated forty promising hybrids and varieties against downy mildew and reported that five entries viz., PAC 931, 7688, Anmol, JBV 3 and Hybrid bajra AG Sun B-38 were completely free from downy mildew at 30 days after sowing, out of these two entries viz., PAC 931 and JBV-3 were also free at 65 days after sowing, eighteen entries showed <5% downy mildew incidence while six entries showed >10% downy mildew incidence at 65 days after sowing.

Laktake *et. al.* (2008) evaluated 93 pearl millet genotypes against downy mildew and reported 31 as highly resistant and 30 resistant. Present finding is also supported by Sharma (2005) who reported < 5% downy mildew in Pusa 23, RHB 121, Raj 171 and ICTP 8203.

In the present study, at 60 days after sowing none of the treatments remained completely free from downy mildew, but the minimum incidence (5.18%) was recorded in the treatment T4 (Apron @ 6 g/kg + cow urine @ 5%) followed by T3: Apron @ 6 g/kg (5.68%), foliar application of mancozeb (@ 0.2% (5.83%), foliar application of neem seed extract @ 10% (7.59%), foliar application of neem leaf extract @ 10% (7.77%), foliar application of neem cake extract @ 10% (8.35%), foliar application of mustard cake extract @ 10% (9.61%), seed dressing of *Bacillus pumilus* @ 6 g/kg + foliar application of compost tea @ 10% (10.55%), foliar application of butter milk @ 10% (10.90%), foliar application of panchgavya @ 10% (11.00%), foliar application of compost tea @ 10% (11.08%), foliar application of vermicompost tea @ 10% (11.41%), and seed dressing of *Pseudomonas fluorescens* @ 8 g/kg + foliar application of compost tea @ 10% (12.53%), while the maximum downy mildew incidence 14.78 % was recorded in control.

The most effective treatment was seed dressing with Apron 35 SD followed by foliar application of cow urine @ 5% was statistically at par with foliar application of neem seed extract (10%), neem leaf extract (10%), neem cake extract (10%), this

indicates that for the eco-friendly management of downy mildew, the extract of neem seed, neem leaf, neem cake may act as an alternate to the chemical Apron 35 SD @ 6 g/kg.

Patidar (2007) reported that cow urine, butter milk, neem cake, mustard cake, linseed cake, neem leaf extract, Calotropis leaf extract, Datura leaf extract, Eucalyptus leaf extract, Parthenium leaf extract and Lantana leaf extract significantly inhibited the oospore germination of *Sclerospora graminicola* but maximum inhibition was recorded in cow urine (10%) followed by neem cake (10%) and neem leaf extract (10%).

Ahirwar *et al.* (2008) reported the application of butter milk @ 5% was most effective in respect of downy mildew control at 60 days after sowing, but it was statistically at par with cow urine @ 30 ml/lit (39.4%), neem cake (39.4%), cow urine @ 15 ml/ lit (42.5%), cow urine @ 45 ml/lit (45.5%) and mustard cake @ 5% (51.4%).

Rajput *et al*. (2013) conducted an experiment to find out the effect of soil application of botanicals (i.e. cakes of neem, mustard, linseed and til, plant extracts of neem, datura, eucalyptus, parthenium and calotropis, butter milk and cow urine) against downy mildew of pearl millet. The cow urine, butter milk, oil cakes and botanicals imparted a significant role on the control of downy mildew in pearl millet. Application of butter milk @ 5% (33.2%) was found most effective in respect of downy mildew control.

At 30, 40, 50, 60, 70, 80, 90 days after sowing, the downy mildew incidence in Apron treatment was 0.65, 1.30, 1.70, 2.49, 3.52, 6.10, 7.68 percent respectively while in control it was 3.82, 5.01, 5.59, 6.59, 9.86, 16.41, 20.59 percent respectively. *Bacillus pumilus* seed dressing alone and in combination with chitosan also significantly checked the incidence of downy mildew. Seed dressing with *P. fluorescens* also reduced the incidence of downy mildew but in most of the observation it was at par with control. Chitosan was not found effective.

The present finding is supported by Sharathchandra *et al*. (2004) who used a commercially developed aqueous Chitosan formulation Elexa in different concentrations viz., 1:5, 1:10, 1:15, 1:19 and 1:25 as seed soaking treatment to pearl

millet for 3, 6 and 9 h duration for its effect on downy mildew and reported that Elexa is a good downy mildew disease management commercial formulation.

Patidar (2007) reported significant control of pearl millet downy mildew by seed dressing with *Bacillus pumilus* in combination with Apron (3 g/kg seeds), and Apron seed dressing alone (6 g/kg seeds). The full dose of Apron alone was more effective than its half dose in combination with *Bacillus pumilus* at tillering stage of the crop, but at dough stage the combined treatments was more effective than the Apron alone in respect of disease and yield both. Pearl millet seed dressing with Apron 35 SD @ 6 g/kg seed, *Bacillus pumilus* (INR 7) and chitosan significantly checked the incidence of downy mildew at 30 and 60 days after sowing (Rajput, 2009).

CHAPTER - VI

SUMMARY, CONCLUSION and SUGGESTIONS FOR FURTHER WORK

SUMMARY

Pearl millet (*Pennisetum glaucum* (L.) R.Br.) popularly known as bajra is a major warm-season cereal, largely grown under rainfed conditions in India. It occupies a unique position in *kharif* crops, because of its drought tolerance features and staple food for millions of people. It also provides good quality fodder to cattle. Morena, Bhind and Gwalior are the major pearl millet growing districts of Madhya Pradesh.

Downy mildew incited by *Sclerospora graminicola* (Sacc.) Schroet is the most widespread and destructive disease of pearl millet in India. The pathogen survives in soil for long duration and various downy mildew resistant hybrids released in past became susceptible, due to this fact the disease has become a major limiting factor in the exploitation of high yield potential of hybrids in the country. Realizing this fact the present study was carried out at Research Farm of College of Agriculture Gwalior during kharif, 2014.

The first symptoms of the disease was noticed in two weeks old seedling as chlorotic strips on the upper surface of the leaves which progressively developed from base to top. Most of the early infected plants remained stunted, the infected leaves turned brown and in majority of cases such infected plants failed to form ear. The remaining infected plants developed the normal or malformed ear head (green ear). Among the green ear a wide variation has been observed and their photo has been depicted in the present study.

Out of 251, 168 lines were found free from downy mildew, while 53.76 and 93.87 percent disease incidence was recorded in ‘‘7042 S’’ at 30 and 60 days after sowing, respectively. The other check (local susceptible) showed 15.50 and 24.69 percent disease incidence at 30 and 60 days after sowing, respectively. All the tested lines were significantly superior over ‘‘7042 S’’ and ‘‘local susceptible’’ except three lines 7042 R, ICML 22 and ICMB 95222-760. More than 10 percent downy mildew was recorded in the nine test lines ‘‘MH 2058 (12.86%), 852 B (10.84%), 7042R (62.88%), ICMR 22 (74.34%), ICMB 95222-760 (24.52%), IP 7846 (11.49%) and IP 22458 (10.56%) at 60 days after sowing.

Out of fifty five promising hybrids and varieties each 45 and 41 remained free from downy mildew at 30 and 60 days after sowing respectively, while susceptible check “7042 S” showed 69.53 and 97.36 per cent downy mildew incidence at 30 and 60 days after sowing respectively.

Cow urine, butter milk, botanicals, compost tea, and panchgavya imparted a significant role on the control of downy mildew in pearl millet. Apron seed dressing @ 6 g/kg seed followed by foliar application of cow urine @ 5% showed minimum downy mildew incidence and it was significantly superior over *Bacillus pumilus* + compost tea, butter milk, panchgavya, compost tea, vermicompost tea, *Pseudomonas fluorescens* + compost tea and untreated control but it was statistically at par with Apron alone at 6g/kg seed, foliar application of mancozeb @ 0.2%, foliar application of neem seed extract @ 10%, neem leaf extract @ 10%, neem cake extract @ 10% and mustard cake extract @ 10%.

Pearl millet seed dressing with Apron 35 SD @ 6 g/kg seed followed by foliar application of cow urine @ 5% was found most effective in controlling downy mildew.

CONCLUSION

The entire study carried out was successful in meeting its objectives as reflected in the following conclusions drawn there from ;

1. Downy mildew (*Sclerospora graminicola)* was observed as most destructive biotic constraint in disease susceptible pearl millet cultivars .
2. The pathogen *Sclerospora graminicola* showed a wide variation in the expression of symptoms specially in the development of malformed structures (green ear).
3. Majority of early infected plants remained stunted and failed to form the ear head. Most of such infected plants of highly susceptible cultivars were dried and died prematurely.
4. Out of 251 pearl millet genotypes, 168 remained free from downy mildew while 93.86 % downy mildew incidence was recorded in susceptible check “7042 S”.
5. Most of the tested promising hybrids and varieties were free from downy mildew.
6. The maximum control of downy mildew was obtained under the seed dressing with Apron @ 6 g/kg seed followed by foliar application of cow urine @ 5% but

it was statistically at par with Apron seed dressing @ 6 g/kg seed, foliar application of neem seed extract @ 10%, foliar application of neem leaf extract @ 10%, foliar application of neem cake extract @ 10% and foliar application of mustard cake extract @ 10%.

7. Foliar application of neem seed extract/ neem leaf extract/ neem cake extract will act as an alternative for the eco-friendly management of downy mildew.
8. The pearl millet seed dressing with Apron @ 6 g/kg seed significantly reduced the incidence of downy mildew in moderately susceptible hybrids. It also increased the grain and fodder yield but this increase was not significant.

SUGGESTIONS FOR FURTEHR WORK

1. Regular monitoring of the disease is essential and the downy mildew susceptible pearl millet hybrids may be discouraged for their cultivation in the affected locations.
2. Resistant sources may be utilized in the breeding programme for the development of high yielding and disease resistant genotypes.
3. Combined influence of bio-control agents with promising fungicides needs to be evaluated under both in-vitro and in-vivo condinations.

CHAPTER-VII

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