"VARIABILITY STUDIES ON *Pyricularia grisea* [(Cooke) Sacc.] INCITANT OF BLAST DISEASE IN FINGER MILLET AND ITS MANAGEMENT"

By

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M.Sc. (Ag.)

THESIS SUBMITTED TO THE ACHARYA N. G. RANGA AGRICULTURAL UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY IN AGRICULTURE

(PLANT PATHOLOGY)

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SRI VENKATESWARA AGRICULTURAL COLLEGE, TIRUPATI

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GUNTUR - 522 034, ANDHRA PRADESH

2022

CERTIFICATE

Mrs. PADMA ANGADI has satisfactorily prosecuted the course of research and that thesis entitled **"VARIABILITY STUDIES ON *Pyricularia grisea* [(Cooke) Sacc.] INCITANT OF BLAST DISEASE IN FINGER MILLET AND ITS MANAGEMENT"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any university.

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This is to certify that the thesis entitled "**VARIABILITY STUDIES ON *Pyricularia grisea* [(Cooke) Sacc.] INCITANT OF BLAST DISEASE IN FINGER MILLET AND ITS MANAGEMENT"** submitted in partial fulfillment of the requirements for the award of degree of '**DOCTOR OF PHILOSOPHY (AGRICULTURE) IN PLANT PATHOLOGY'** to the **ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY, GUNTUR,** is a record of the bonafide research work carried out by **Mrs. PADMA ANGADI** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been and all assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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DECLARATION

I, **Mrs. PADMA ANGADI,** hereby declare that the thesis entitled **"VARIABILITY STUDIES ON *Pyricularia grisea* [(Cooke) Sacc.] INCITANT OF BLAST DISEASE IN FINGER MILLET AND ITS MANAGEMENT"** submitted to the **Acharya N.G. Ranga Agricultural University, Guntur** for the degree of **DOCTOR OF PHILOSOPHY (Agriculture) in Plant Pathology** is the result of original research work done by me. Part of the thesis has been published by me as Padma, A, Devi, R.S.J, Reddy, B.B.V, Prasanthi, L, Patro, T.S.S.K and Naidu, G.M, 2021, Survey on blast [*Pyricularia grisea* [(Cooke) Sacc.] disease of finger millet in major finger millet growing areas of Andhra Pradesh, The Pharma Innovation Journal. 10 (11) : 910-914**.**

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ACKNOWLEDGEMENTS

No creation in this world is a solo effort. Neither is this manuscript and I bow my head to all those known and unknown hands that were always behind me pushing me forward in this novel journey.

*With limit less humanity, I would like to praise and thank '**God**' - The creator-The supreme Power- The Light or whatever He is, has helped me in all adversities, at every step, on each moment.*

*There are no words to express my feelings of adoration, love, respect and obligation to my beloved parents, **Sri. Chidanandappa Angadi** and **Smt. Ratnamma** who moulded me to what I am now. Without their mental support and encouragement, I would not have been able to overcome my hardships. I owe thanks to a very special person, my husband **Dr. Mahesh** for his continued and unfailing love, support and understanding during my pursuit of Ph. D degree that made the completion of thesis possible. He helped me to keep things in perspective. I greatly value his contribution and deeply appreciate his belief in me.*

*My diction would be inadequate to express my deepest sense of gratitude and heartfelt thanks to **Dr. R. Sarada Jayalakshmi Devi,** Professor and University Head, Dept. of Plant Pathology and Chairperson of my Advisory Committee for learned counsel. Her level of guidance, encouragement, constructive criticism, generous, assistance at every stage of my research work is behind measure, in fact it was her ideas of smooth dealing with the things which motivated me to work under her guidance and all credits go to her.*

*I am ineffable to express my esteemed thanks to the honored member of my advisory committee, **Dr. B. V. Bhaskara Reddy,** Principal Scientist, Department of Plant Pathology, IFT, RARS, Tirupati for his keen interest, ardent support and persistent encouragement showered to me.*

*I sincerely extend my gratitude to the honored member of my advisory committee **Dr. TSSK Patro,** Principal Scientist and Head, Department of Plant Pathology, ARS Vizianagaram for his brilliant counsel, constructive suggestions, indefatigable guidance, evincive criticism and inspiring encouragement to embellish the present study.*

I sincerely accentuate my gratitude to the honored members of my advisory committee ***Dr. L. Prasanthi,*** *Associate Director of Research, RARS, Tirupati and* ***Dr. G. Mohan Naidu,*** *Professsor and Head, Statistics & Computer Applications Agricultural college, Naira for their articulate criticisms, transcendent suggestions, persistent encouragement and for providing congenial atmosphere during the course of study.*

My heartfelt thank to ***Dr. M. Reddi Kumar****, Professor,* ***Dr. K. Vishwanath****, Associate Professor,* ***Dr. M. Pradeep****, Asst. Professor,* ***Dr. Guruvi Reddy****, Asst. Professor,* ***Dr. P. Arunasri****, Asst. Professor,* ***Dr. Jyothsna,*** *Asst. Professor,* ***Dr. P. Nagamani****, Asst. Professor Dept. of Plant Pathology, S.V. Agricultural College, Tirupati, for their help and guidance during my course and research work.*

I wish to place on record my heartfelt thanks to my lovely sisters and brothers ***Renuka, Savitha, Sunitha, Sharadha, Anu, Hanu,Vishu, Shailu, Suri, Shreenu, Shri, Shivu, Nikku, Deepu*** *and nieces* ***Chetu, Raksha, Varsha, Sanu, Vaishu, Anu, Siri*** *and Nephew* ***Jaya and mitun*** *and my grandmother* ***Eramma Angadi****, my big supporters* ***Ayyappa Angadi, Amaresh, Sadanand, Yankoba*** *and all the members of family and relatives always backed me throughout my life.*

No words are enough to express the affection to my special and joyful friends ***Sandhya, Mamata, Appu, Vijju, Revati, Veeresh, Seema, Nitya, Subbu, Geeta, Nagu, Sandu, Kadam, Muniraju, Amogh, Sushma, Balu, Nagarjun, Mantesh, Sharath and Madhavi*** *for their help, guidance, constant encouragement and companionship in my personal and research life.*

I express my heartfelt gratitude and thanks to my beloved Seniors ***Chiranjeevi, Bhaskar, Neha, Madhuri, Vijay, Shruti, Nagaraj, Raghavendra, Shailaja, Peerusaheb Ganesh, Sandeep, Choudappa, Basamma, Roopa, Praveen, Manasa, Ramesh and Chandana*** *for their help and encouragement during the study.*

It is a great pleasure to acknowledge the affection rendered by my junior friends ***Ashwini, Jasmin, Lohitha, Pallavi, Nandu, Suresh, Suma, Sahana, Akhila, Adi, Babitha, Chandan, Deepu, Hemant, Divya, Sushma, Gorgia, Swathi, Sandhya, Kavita, Divyamani, Noor, Sai Kiran, Vishwa teja, Prasad, Manisha, Uma, Pooja, Prabha, Prasanna, Prince, Ramu, Sharath, Raji, Anusha, Tej, Jaggu and Virupakshi*** *for their unforgettable support and constant encouragement during my course of study.*

It is the right occasion to express my heartfelt thanks to Field staff ***Parthasarathi,*** *AO,* ***Manhindra,*** *AAO and* ***Muniratnam****, AAO, RARS, Tirupati and Non*

*teaching staff **Rajeshwari**, **Ravi** and **Muniram**, Dept of Plant Pathology, S. V. Ag. College, for their sustained help and cooperation during my research work.*

*Financial assistance rendered by **Indian Council of Agricultural Research** through ICAR-SRF fellowship is duly acknowledged.*

*I am highly thankful to **Acharya N. G. Ranga Agricultural University** for giving me admission and learning environment during the period of my Ph.D. programme.*

This is the last but not the least expression of my gratitude to the main cohort of my research work. I am very lucky to build up the rapport with all the farmer respondents of my study. I sincerely thank each and every one who spared their valuable time for my research and cooperated during my research work.

Padma Angadi…

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LIST OF SYMBOLS AND ABBREVIATIONS

µl	:	Micro litre
µm	:	Micro metre
<	:	Less than
>	:	Greater than
%	:	Per cent
/	:	Per
bp	:	Base pair
@	:	At the rate of
^{o}C	:	Degree Celcius
ANOVA	:	Analysis of Variance
CD	:	Critical Difference
Cm	:	Centimeter
CV	:	Coefficient of Variation
DAI	:	Days After Inoculation
DMRT	:	Duncan's Multiple Range Test
EC	:	Emulsifiable Concentrate
EDTA	:	Ethylene Diamine Tetra Acetic Acid
et al.	:	and others
etc.	:	and so on
Fig.	:	Figure(s)
FB	:	Finger blast
g	:	Gram(s)
h	:	Hour
OMA	:	Oat Meal Agar
ha^{-1}	:	Per hectare
i.e	:	That is
in vitro	:	in lab conditions
kb	:	Kilo Bases
kg	:	Kilogram
L	:	Litre
LB	:	Leaf Blast
mg	:	Milligram
ml	:	Millilitre

ml^{-1}	:	Per ml
mM	:	Milli Molar
mm	:	Millimeter
mm^2	:	Millimetre Square
NB	:	Neck Blast
PCR	:	Polymerase Chain Reaction
PDI	:	Per cent Disease Index
PIC	:	Polymorphism Information Content
SSR	:	Simple Sequence Repeats
UPGMA	:	Un-weighted Pair Group method with an Arithmetic average
UV	:	Ultra Violet
Viz.,	:	Namely
ppm	:	parts per million
psi	:	pounds per square inch
r	:	Correlation coefficient
RAPD	:	Randomly Amplified Polymorphic DNA
RH	:	Relative Humidity
SEm	:	Standard Error of Mean
FLA	:	Finger millet Leaf extract Agar
MGM	:	*Magnaporthe grisea* Microsatellite markers
LOS	:	Level of Significance

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Title of the thesis	:	**VARIABILITY STUDIES ON *Pyricularia grisea* [(Cooke) Sacc.] INCITANT OF BLAST DISEASE IN FINGER MILLET AND ITS MANAGEMENT**
Chairman	:	**DR. R. SARADA JAYALAKSHMI DEVI**
Degree to which it is submitted	:	**DOCTOR OF PHILOSOPHY**
Faculty	:	**AGRICULTURE**
Discipline	:	**PLANT PATHOLOGY**
Major Advisor University	:	**ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY**
Year of submission	:	**2022**

ABSTRACT

The study was under taken with an aim to know the variability among isolates of *Pyricularia grisea* causing blast disease in finger millet. Studies were carried out on survey of blast disease to identify the hotspots in major finger millet growing areas of Andhra Pradesh, variability of isolates using cultural, morphological and molecular characteristics and evaluating the bacterial bio-control agents and fungicides against pathogen under *in vitro*. Efforts were made to identify the promising lines for leaf, neck and finger blasts and knowing suitable weather conditions for occurrence of disease. Attempts were also made to manage the disease with biocontrol agent *P. fluorescence*, fungicides and their integration.

Survey was conducted during *Kharif* 2020 and *Kharif* 2021 in seven major finger millet growing districts of Andhra Pradesh. The highest mean blast disease incidence of 56.06% was recorded in Vizianagaram district. The lowest mean disease incidence of 7.85% was recorded in Prakasam district. Regarding mandals, the highest mean blast incidence of 56.59% was recorded in Salur mandal of Vizianagaram district in the range of 53.86 to 59.20% during 2020 and 52.35 to 59.65% during 2021 and lowest incidence of 7.36% with the range of 5.02 to 9.01% and 4.65 to 9.78% was noticed in Racherla mandal of Prakasam district during 2020 and 2021 respectively.

The blast samples were collected from different locations of Andhra Pradesh and a total of 20 monoconidial isolates of *Pyricularia grisea* were isolated.

The variability in cultural characteristics *viz.*, colony colour, growth pattern, elevation (flat/elevated growth), sectored or non-sectored, zonations and wrinkle formation were studied among the isolates of *P. grisea* isolates on Oat Meal Agar medium. Efforts were made to study radial growth sporulation of virulent *P. grisea* isolate (VIZ-1) in different cultural conditions *viz.,* different media and light conditions. Good amount of sporulation was observed in observed in Finger millet Leaf extract Agar (FLA) medium with 1.81×10^5 ml at 5 days after inocultion followed by OMA with 1.68×10^5 ml at 7 days after inoculation. No significant difference was observed among three light conditions *i.e.* light (1.07×10^5), dark (0.87×10^5) and light+dark (0.73×10^5). Morphological variability among *P. grisea* was studied through conidial

morphology. Among the isolates, the overall size of the conidia was in the range of 20.74-23.01 µm × 7.00-9.16 µm (Length × Width).

The molecular variability of *P. grisea* isolates was studied using 25 SSR markers, of which seven were polymorphic. The genetic diversity was ranged from 0.180 (MGM 437) to 0.742 (Pyrms 63) with an average of 0.491. Dendrogram using Neighbor Joining (NJ) resulted in formation of three mega clusters in which cluster I was further sub grouped into sub-cluster IA, which includes eight isolates and sub-cluster IB contains one. Cluster II was further sub grouped into sub-cluster IIA, which includes six isolates and sub-cluster IIB includes only one isolate. However, cluster III was further divided into sub cluster IIIA which includes three and IIIB includes only one isolate.

The RAPD analysis revealed that, out of 8 RAPD primers, 7 primers produced polymorphic alleles which were selected for genetic diversity analysis. A total of 84 reproducible alleles with an average of 12 fragments per primer were produced using 7 RAPD primers. All the markers displayed polymorphic alleles. Of the total alleles (84), one allele (OPA-07) was monomorphic with 15.38% monomorphism and 84.61% polymorphism which contains two monomorphic bond with PIC value of 0.2874. Whereas 6 primers produced 100% polymorphism with PIC value ranged from 0.1769 to 0.3429 and total number of polymorphic bands were ranged from 8 to 14.

Dendrogram constructed to reveal the pattern of relatedness among twenty *P. grisea* isolates using DARwin 6 software on the basis of RAPD polymorphism. Cluster I is further divided into sub-cluster IA which consisting of nine isolates Sub-cluster IB of with two isolates. Cluster II further divided into cluster IIA which consisting of four isolates and sub-cluster IIB contains only one isolate. However, cluster III further divided into sub-cluster IIIA which contains three isolates and sub-cluster IIIB contains only one isolate.

A total of 23 bacterial bio-control agents were isolated from rhizospheric soil of healthy finger millet plants and three isolates were collected from ARS, Vizianagaram for *in vitro* studies. Bacterial bio-control agents were evaluated for their antagonistic effect on *P. grisea* under *in vitro* conditions. Results revealed that, maximum inhibition of mycelium growth (79.54%) was noticed in BVP-1isolate and least mycelial inhibition was noticed in BJR (11.30%) isolate.

Studies on *In vitro* evaluation fungicides against the pathogen revealed that Tricyclazole 75% WP, Carbendazim 50% WP and Tebuconazole 50% + Trifloxystrobin 25% WG inhibited the mycelial growth completely. Compatibility studies of fungicide and bio-agent revealed that Tebuconazole 50% + Trifloxystrbin 25% WG and Carbendazim 50% WP at all concentrations were found to be compatible with zero per cent inhibition of bacterial bio-control agent.

74 lines of finger millet including local check VR 708 were screened for their resistance against blast in field conditions. Results showed that for leaf blast five lines were found as highly resistant, 31 lines found as resistant, 23 lines were recorded as moderately resistant and 15 lines were found to be susceptible. For neck blast, 30 lines were recorded as resistant, 29 lines shown moderately resistant, 15 lines were reacted as susceptible. Out of 75 lines screened, 20 lines showed moderate resistance, 41 lines with susceptible reaction and 14 lines including VR 708 showed highly susceptible reaction to finger blast incidence.

Correlation and regression analysis of weather parameters with disease development revealed that blast disease severity was shown to be higher during the early planting window, possibly due to comparatively high relative humidity, rainfall and a greater number of rainy days, all of which favor disease development.

The effective fungicides and potential bacterial bio-control agent were used in integrated disease management of finger millet blast at S.V. Agricultural College, Tirupati and ARS, Vizianagaram during *Kharif* 2021, the treatment T_7 and T_8 were found effective for leaf, neck and finger blast with yield.

Chapter – I

Introduction

Chapter – I

INTRODUCTION

Finger millet (*Eleusine coracana* (L.) Gaertn. Sub sp. *coracana*) is a nutrient rich tetraploid (2n = 4x = 36) cereal crop (Dida *et al*., 2007; Odeny *et al*., 2020) belongs to the family Poaceae and it is believed to be domesticated in the East-African region from wild finger millet (*Eleusine coracana* (L.) Gaertn. subsp. *africana* (Kenn.-O'Byrne) Hilu& de Wet) (Dida *et al*., 2007). Finger millet has assumed a status of important staple food crop and is primarily consumed by marginalized inhabitant so for the semi-arid and sub-tropics of Asia and Africa (Jenkins *et al.,* 1982; Dida *et al*., 2007; Upadhyaya *et al*., 2007; Waghunde *et al*., 2013; Jegan, 2015; Negi *et al.,* 2015; Thilakarathna and Raizada., 2015; Kumar *et al.,* 2016; Gupta *et al*., 2017).

The name finger millet is derived from the seedhead which has the shape of human fingers (Roy *et al*. 2018). The crop can withstand harsh environmental conditions, including higher temperatures (Yogeesh *et al.,* 2016). So, the crop is being cultivated in different parts of the world, mainly India, Srilanka, Malaysia, China, Myanmar, Nepal, Japan, Kenya, Uganda, Tanzania, Ethiopia, Eritrea, Rwanda, Democratic Republic of Congo, Zaire, Eritrea and Somalia in Africa (Babu *et al*., 2012). The total world production of millet grains in 2013 was 762,712 metric tons and India is the top producer with an annual output of 334,500 tonnes contributing 43.85 Per cent (FAO, 2013).

In different parts of the world, finger millet is locally called with many names as ragi (India), koddo (Nepal), dagussatokuso, barankiya (Ethiopia), wimbi, mugimbi (Kenya); bulo (Uganda); kambale, lupoko, mawale, majolothi, amale, bule (Zambia); rapoko, zviyo, njera, rukweza, mazhovole, uphoko, poho (Zimbabwe); mwimbi, mbege (Tanzania) and kurakkan (Sri Lanka) (Shisanya *et al*., 2011).

Finger millet is extensively grown in the states of Karnataka (53%), Tamil Nadu (15%) and Andhra Pradesh (7.5%) (Vijayakumari *et al*., 2003; Jegan, 2015; Kumar *et al*., 2016; Gupta *et al*., 2017; Kumar *et al*., 2020). In India, the overall cultivated area under this crop is 10.16 lakh hectares with 13.85 lakh

tonnes production and 1363 kg ha^{-1} productivity (www.indiastat.com 2016-17). Andhra Pradesh accounts for about 0.35 lakh hectares area and 0.44 lakh tonnes production of finger millet and productivity of 1277 kg ha^{-1} (Agricultural statistics at a glance, 2017-18).

Erosion in the production of indigenous and traditional food crops dramatically changed the global food system (Turner and Turner, 2007). So, to replace the food crisis, millet grains are being adopted. Millets are abundant in nutrients and health-beneficial phenolic compounds, including phenolic acids, flavonoids and tannins making them suitable as food and feed (Hassan *et al*., 2021).

Among the millets, finger millet is most important millet crop which is rich in calcium (300-350 mg/100g), phosphorus (283 mg/100g), iron (3.9 g/100g), dietary fiber (18%), phenolic compounds (0.3-3%), vitamin B1, B2, folic acid and niacin (Balakrishna *et al*., 1973; Gopalan *et al*., 2000; Vidyavati *et al*., 2004) and have the beneficial health effects like antidiabetic, antitumorigenic, atherosclerogenic effects, antimicrobial properties, lower plasma glucose level and also low glycemic index nutritious food products prepared from millets helped as supportive therapy in the management of diabetes mellitus (Arora and Srivastava, 2002; Lakshmi and Sumathi, 2002; Kumar *et al*., 2016; Gupta *et al*., 2017).

The blast pathogen *P. grisea* (Cooke) Sacc. is a heterothallic, filamentous fungus, pathogenic to almost 50 plant species in 30 genera of Poaceae including economically important crops like rice, wheat, barley and millets (Ou, 1985; Rossman *et al*., 1990).

The pathogen infects different parts of the plant, from seedling to grain formation. Initially, the symptom appears on leaf lamina with typical spindle shaped spots with grey or whitish center and brown or reddish-brown margin that enlarge and coalesce to give the blasted appearance. The pathogen affects the plant parts like the neck, which turns initially brownish color, later black resulting in breakage of stem at neck region and results in severe blasting of florets in the fingers of the earhead either with no grain or shriveled blackened

grains, resulting in huge production losses. The extent and severity of infection depend on the stage of infection and weather conditions (Takan *et al.,* 2004; Babu *et al*., 2012; Senthil *et al*., 2012; Takan *et al.* 2012; Prajapati *et al.,* 2013; Negi *et al.,* 2015).

In India, the disease was first reported from the Tanjore delta of Tamil Nadu by Mc Rae in 1920 with an estimated loss of 50% (Venkatarayan, 1946). The average loss due to blast has been reported to be around 28-36% (Mc Rae, 1922; Vishwanath *et al.*, 1986; Rao, 1990; Nagaraja *et al.*, 2007) and in endemic areas, yield losses could be as high as 80-90% (Vishwanath *et al*., 1986; Bisht, 1987; Rao, 1990).

Blast disease management in finger millet is best achieved using native biocontrol agents, systemic fungicides and host plant resistance (Spence *et al*., 2014).The search for effective alternative approaches to chemical control with no ecological hazards involving bio-control agents is contributing to sustainable agriculture (Jain *et al.,* 2009). Growing up of disease-resistant cultivars is very important and cost-effective for resource poor and marginal farmers who cannot afford to use expensive chemical fungicides as a disease control approach. To effectively use host plant resistance mechanisms for developing resistant cultivars, a thorough understanding of the pathogen's biology, pathogen morphology, genetic diversity and epidemiological factors in disease development are much required (Babu *et al*., 2012).

Molecular markers give an unbiased assessment of total genomic variation and helps to measure genetic diversity more precisely (Spooner *et al*., 1996). The genetic diversity of *P. grisea* has been studied using various molecular methods, including many SSRs (Brondani *et al.,* 2000; Kim *et al*., 2000; Kaye *et al*., 2003; Suzuki *et al*., 2009).

Improved knowledge on the interaction of host cultivar with the weather, pathogenic strain and the crop growth stages would be helpful in understanding and predicting the disease epidemic. In any host pathogen system, weather variables such as relative humidity, rainfall and temperature play a vital influence in regulating infection and disease progression (Babu *et al*., 2012).

Keeping in view of the economic importance of the disease, the present study was planned to conduct the survey for the incidence of disease, and collection of pathogen and native bacterial biocontrol agents from major finger millet growing areas of Andhra Pradesh, cultural, morphological and molecular variability of pathogen isolates, *in vitro* evaluation of bacterial bio control agents and commercially available fungicides, screening of resistant sources, weather correlation studies with the progression of disease and management strategies under field conditions by keeping following objectives.

1. To conduct survey for occurrence and distribution of blast disease, collection of isolates and native bacterial biocontrol agents in major finger millet growing areas of Andhra Pradesh.
2. To study the cultural, morphological and molecular variability among the isolates of the pathogen.
3. Evaluation of native bacterial biocontrol agents and fungicides against the pathogen *in vitro.*
4. To screen the genotypes for their resistance against blast.
5. To study the influence of weather factors and disease development.
6. Integrated disease management for blast.

Chapter – II

Review of Literature

Chapter-II

REVIEW OF LITERATURE

The available literature of work done on the blast disease of finger millet and the various aspects related to the collection and isolation of pathogen and native bacterial bio-control agents, *in vitro* evaluation of fungicides and bio-control agents, variability studies of pathogen, epidemiology and screening of finger millet genotypes. Integrated disease management have been reviewed in this chapter. The review of literature pertaining to this dissertation is presented in the following headings and sub-headings.

2.1. TO CONDUCT SURVEY FOR OCCURRENCE AND DISTRIBUTION OF BLAST DISEASE, COLLECTION OF ISOLATES AND NATIVE BACTERIAL BIO-CONTROL AGENTS IN MAJOR FINGER MILLET GROWING AREAS OF ANDHRA PRADESH

2.1.1 Distribution of the Disease

Finger millet blast caused by *Pyricularia grisea* (Cooke.) Sacc. Is the most devastating disease distributed in almost all growing regions of the world, affecting different aerial parts of the plant at all stages of its growth starting from seedling stage (causing lesions and premature drying of young leaves) to panicle stage causing neck and/or finger blast. The disease is known to occur in India (Mc Rae, 1920), Srilanka (Park, 1932), Nepal (Thompson, 1941), Malaya (Burnett, 1947), Tanzania (Kuwite and Shao, 1992), Somalia (Mohamed, 1980), Zambia (Muyanga and Danial, 1995), Ethiopia, Kenya, Uganda (Dunbar, 1969; Adipala, 1992). In India the disease is prevalent wherever finger millet is grown *viz*., Karnataka, Tamil Nadu, Maharashtra, Andhra Pradesh, Orissa, Bihar, Uttaranchal *etc*. The disease was reported for the first time in India, from Tanjore delta of Tamil Nadu by Mc Rae (1920).

2.1.2 Symptoms

The symptoms appear at all the stages of plant growth *viz*., germlings to earheads and even on seed. When the young healthy seedlings catch the disease, patches of seedlings give burnt appearance due to severe leaf blast and die which

results in the gappy patches. Disease appears on leaf lamina with typical spindle-shaped spots with gray or whitish centre and brown or reddish brown margin enlarge, coalesce and give blasted appearance. Well developed lesions may measure 0.5 × 2 cm. The pathogen also attacks culms, especially at the nodal region results in blackening of that area. However, the most damaging stage of the disease is when it attacks neck region. Two to four inches of the neck below the earhead, turns initially brown, later black results in breaking at the infected area. Sporulation of the fungus may be noticed on this area. The pathogen also attacks fingers usually from the apical portions which run towards the base. The extent of infection depends on stage of infection and weather conditions. Neck infection causes significant loss in grain number, grain weight and significant increase in spikelet sterility (Rath and Mishra, 1975). If the pathogen attacks the developing grains, it results in shriveled blackened seeds. Even in a resistant variety like GPU 28 some black seeds can be seen (Kumar, 2002).

2.1.3 Pathogen

Blast of finger millet (ragi) is caused by the fungus *Pyricularia grisea* (Cooke.) Sacc. anamorph of *Magnaporthe grisea (*Hebert) Brar. It is a heterothallic, filamentous fungus pathogenic to almost 40 plant species in 30 genera of Poaceae (Ou, 1980; Murakami *et al.*, 2000; Inukai *et al.*, 2006) including Eleusine. Initially, there was difference of opinion with regard to the nomenclature of the pathogen. Morphologically it is very close to *Pyricularia oryzae* (Ramakrishnan, 1948). The perfect stage of *Pyricularia grisea* was earlier named as *Ceratosphaeria grisea* (Hebert, 1971). Later Yaegashi and Nishihara (1976) suggested the genus Magnaporthe. Yaegashi and Udagawa (1978) finally proposed *M. grisea* as the perfect stage of *Pyricularia grisea* (Cke.) Sacc. instead of *Ceratosphaeria grisea.* Chauhan and Varma (1981) reported *P. grisea* on *Eleusine indica* from Kanpur, India. Hyphae is hyaline and septate. However, as the fungus gets older, the hypha becomes brown. Generally, growth of the pathogen is relatively more on the upper surface which thus makes the spot more dark on that side. Conidiophores are simple, septate, basal portion being relatively darker. Conidia obpyriform in shape and hyaline in colour produced acrogenously, one after another. Conidium is three celled, the middle cell being

much wider and darker, and end cell germinates giving out germtubes. Formation of intercalary or terminal chlamydospores is common, which are globose, thick walled and olive brown. Under laboratory conditions the pathogen produces fertile perithecia (Viji and Gnanamanickam, 1998).

2.1.4 Losses due to Blast

Mc Rae (1922) who reported the blast disease first time in India also gave an yield loss estimate could be over 50%. The yield losses estimated to be 10-50% in Kenya (Sreenivasa prasad *et al.,* 2007) and 10-80% in Uganda (Esele, 1982). In India, the average loss due to blast has been reported to be around 28-36% (Vishwanath *et al.,* 1986; Nagaraja *et al.,* 2007) and in endemic areas, yield losses can be as high as 80-90% (Vishwanath *et al.,* 1986; Bisht, 1987; Rao 1990). Ragi blast in Himalayan region appears at lower elevation and it was recorded at <1600 m and recorded at Rampur, Nepal (Batsa and Tamang, 1983). Ramappa *et al.* (2002) observed 76% reduction in grain yield and 70% reduction in 1000-grain weight when infection occurred immediately after flowering while the reduction in grain yield was 52% and that of 1000-seed weight 50% when the disease occurred at milky stage. Quantification of losses in yield due to neck blast at different stages of earhead development revealed that the losses were drastic when disease appeared within 10 days of ear emergence and considerable losses were incurred even if infection occurred up to 20 days of ear emergence (Bisht *et al.,* 1987). Blast affects the crop at all growth stages and neck and panicle blast are the most destructive form of the disease (Takan *et al*., 2012). Blast disease causes a yield loss of as high as 100% in areas infested with the pathogen (Mgonja *et al.,* 2013). Prajapati (2013) reported that the losses due to *P. grisea* with respect to grain yield and fodder yield was 35.78 and 43.72% respectively.

2.1.5 Survey for the Incidence of Finger Millet Blast

Saksena *et al.,* (1982) conducted survey at several places in Kanpur (India) and reported that finger millet crop was heavily damaged by blast disease causing considerable loss in yield.

Rao (1990) surveyed in eight locations of Bangalore, Kolar and Tumkur districts in Karnataka and reported that an increase of 1% infection in neck and finger resulted in a corresponding increase of 0.32 and 0.084% yield loss and 6.75 to 87.50% loss in grain yield.

A survey for finger millet disease was conducted by Elobu and Adipala (1993) in Kaberamaido Sub country, Seroti district, Uganda. About 10.80% loss in finger millet blast (P*yricularia grisea*) reported on all the cultivars grown in the area.

Hayden (1999) conducted survey in 200 fields of finger millet at Matibo village in East Africa for the blast disease and reported as high as 48% blast incidence and 42% intensity.

Takan *et al.* (2004) reported during the disease survey in Kenya and identified finger millet blast as the most important and wide spread disease. In Uganda, blast incidence (13 to 50%) and severity (24 to 68%) varied considerably across main finger millet activated areas in the North and East. The disease incidence and severity were higher during the first season (February to July) than in second season (August to December).

A field survey conducted by Kumar *et al.* (2005) reported that maximum neck (13-16%) and finger blast (42-55 %) incidence in local varieties sown in July second fortnight with black seeds ranging from 64 - 96% and least of 0.1 - 1.0 in recently released varieties (GPU 28 and Indaf 5) in Tumkur district of Karnataka.

Senthil *et al.* (2012) reported that the finger millet blast was the most devastating disease affecting different aerial parts of the plant at all stages in Tamil Nadu (India).

Prajapati (2013) reported that the occurrence of blast disease in finger millet was observed in serious proportion inflicting heavy losses in south Gujarat with the loss of 35.78% grain yield and 43.72% fodder yield due to the blast disease.

Survey in the five agroecological zones of Ethiopia, revealed that, about 63.03 and 34.60 % of maximum disease incidence and severity were recorded in west Wollega zone with and lowest disease incidence and severity was recorded in Awi zone with 46.70 and 15.72%, respectively (Gashaw *et al.*, 2014).

Survey for the occurrence, distribution and severity of finger millet blast in five countries namely, Busia, Bungoma, Kisii, Machakos and Makueni, in Kenya revealed that blast occurrence was 100%, with a uniform distribution pattern on all the farms surveyed across all the countries. Busia country had the highest disease severity at 82.31%, while Makueni had the lowest severity at 61% (Odeph *et al.*, 2020).

Mbinda *et al.* (2021) reported that finger millet blast disease was adversely affected the productivity of the crop leading to poor yields by causing 92 to 98%, yield loss in Bungoma and Kisii Counties of Kenya.

2.2 TO STUDY THE CULTURAL, MORPHOLOGICAL AND MOLECULAR VARIABILITY AMONG THE ISOLATES OF THE PATHOGEN

2.2.1 Cultural and Morphological Variability among the *P. grisea* Isolates

Ramakrishnan (1948) observed a positive correlation in the sporulating ability and aerial growth of *P. grisea*. Mutations of the SMO+ genetic locus were reported to cause a number of gross deviations from the normal process of conidiogenesis, resulting in conidia which exhibited a wide variety of unusual morphologies (Hamer *et al.*, 1989).

Arase *et al.* (1994) reported that two mutant isolates of *Pyricularia oryzae* formed abnormal, longer, cylindrical spores with more septa than those of normal, obpyriform spores of wild isolates. Viji and Gnanamanickam (2000) could distinguish *Pyricularia* isolates from different hosts based on cultural and conidial variation.

Cultural Variability of *P. oryzae* Isolates Hossain (2000) observed that among the non-synthetic media, potato dextrose agar supported maximum radial

growth (85.00 mm), next was host extract + 2 per cent sucrose agar medium (80.33 mm) followed by oat meal agar (75.00 mm).

Sonah *et al.* (2009) studied the cultural and morphological variability of *M. grisea* isolates collected from rice and non-rice hosts revealed that isolates that showed fast vegetative growth as grey-green or grey- white produced more number of spores than those with slower vegetative growth (submerged or subdued growth patterns). Isolates derived from non-rice hosts also showed abnormal spore morphology which were longer, cylindrical and obpyriform.

Gopal *et al.* (2012) studied radial growth and sporulation of *P. grisea* by culturing fungal isolates from rice, finger millet and *Panicum* sp. on six different media: Prune Agar (PA), Oat Meal Agar (OMA), Potato Dextrose Agar (PDA), Finger millet Leaf Decoction Agar (FLDA), Finger millet Polish Agar (FPA) and Finger millet Meal Agar (FMA). Among the different media used, PA and OMA were found to be the best for mycelial growth and sporulation of the isolates both from rice and finger millet.

Moghaddam and Soltani (2013) evaluated three fungal culture media *viz.*, Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA) and Water Agar (WA) for vegetative growth and sporulation. Three light regimes *i.e.* continuous light, 16/8 hr light/darkness, and continuous darkness were applied. The findings indicate that PDA culture medium could provide the best medium for *P. oryzae* vegetative growth and a combination of 16/8 hr light/ darkness intervals induced better sporulation.

Asfaha *et al*. (2015) observed optimum growth and good sporulation of *P. oryzae* isolates on oat meal agar when compared with other media *i.e.* rice flour agar, malt extract agar and potato dextrose agar.

Srivastava *et al*. (2014) assigned ten isolates of *P. oryzae* into six morphological groups (PG-I to PG-VI) based on the differences in morphological characteristics (colony colour, colony morphology and conidia shape). The colonies of group PG-I produced ring like smooth 15 margin with buff to greyish colour, PG-II produced circular smooth margin with buff colour, PG-III produced circular rough margin with greyish black to black colour, PG-IV

produced ring like cottony colony with buff colour, PG-V produced irregular rough margin colony with buff colour, PG- VI produced circular smooth colony with raised mycelium having buff colour. The conidia shape of the different groups was pyriform (pearshaped) with rounded base, and narrowed towards the tip which is pointed or blunt.

Panda *et al*. (2017) characterized twenty isolates of *P. oryzae* and categorized based on the variation in morphological characteristics *viz.,* colony colour, surface appearance and type of growth. The isolates produced little surface, downy, flat with little mycelium and submerged growth with smooth and rough margins on OMA media.

2.2.2 Genetic Diversity of Pathogen Isolates using SSR and RAPD Markers

To understand the mechanism of frequent breakdown of resistance in blast resistant cultivars, studies on the extent of genetic diversity present in the population of *M. grisea* in a specific geographical region is important (Levy *et al.,* 1993).

In order to measure genetic variability more precisely, molecular markers provide an unbiased estimate of total genomic variation and have the potential to minimize errors due to sampling variance (Spooner *et al.,* 1996).

DNA fingerprinting techniques have created new tools for the molecular analysis of *M. oryzae* populations (Levy *et al.,* 1993) and this is equally applicable to *M. grisea* as well.

Information on regional and global population diversity at the lineage level is useful to understand the epidemiological properties of the blast disease in neighboring areas (Le *et al.,* 2010).

Assessment of genetic diversity of *M. grisea* from different crops mostly relied on MGR-based Restriction Fragment Length Polymorphism (RFLP), which is an expensive and time-consuming technique. The most commonly used DNA-based markers includes, Randomly Amplified Polymorphic DNA (RAPD; Williams *et al.,* 1990; Welsh and McClelland, 1990), Amplified Fragment Length Polymorphism (AFLP; Vos *et al.,* 1995) and Sequence Characterized

Amplified Region (SCAR) markers (Soubabere *et al.,* 2001). These markers are PCR-based markers and do not need any sequence information, speedy means to generate molecular markers but provide several genomic fragments with a marker in the single experiment (Varshney *et al.*, 2007).

Generation of SSR markers is a time consuming, labour intensive and expensive task. Several SSR (Brondani *et al.,* 2000; Kim *et al.*, 2000; Kaye *et al.,* 2003, Suzuki *et al*., 2009) and minisatellite markers (Li *et al.* 2007) have already been developed for *M. grisea*.

The assessment of genetic diversity and virulence complexity of forty five isolates of rice blast fungus from three different geographical regions (hilly area) of Uttarakhand was done by using fifteen RAPD primers showed the polymorphism range from 71.40 to 90%, while the range of total loci scored was from 07 to 10. The molecular weight of scorable loci ranged from 150 to 2500 bp (Singh and Kumar, 2010).

Babu *et al.* (2013) reported the genetic diversity and population structure of 72 *M. grisea* isolates collected from finger millet (56), foxtail millet (6), pearl millet (7) and rice (3) from major crop growing areas in India was studied using 24 SSR markers. None of the SSRs detected polymorphism in the *M. grisea* isolates from pearl millet. Seventeen SSR markers were polymorphic in the 65 non pearl millet isolates and detected 105 alleles, of which one was rare, 83 common, 9 frequent and 12 most frequent.

The genetic variability among isolates of *Magnaporthe grisea* collected from rice (*Oryza sativa*), buffel grass (*Cenchrus ciliaris*), finger millet (*Eleusine coracana*) and para grass (*Brachiaria mutica*) were analyzed by using Random Amplified Polymorphic DNA (RAPD) method. Analysis of the genetic coefficient matrix derived from the scores of RAPD profiles showed that minimum and maximum per cent similarities among the tested *M. grisea* (Madhavan *et al.*, 2014).

Jesumaharaja *et al*. (2016) performed 20 Random amplified polymorphic DNA (RAPD) with 14 isolates of *M. grisea*. The genetic similarity coefficient within each group and variation between the groups was observed. Among the

primers, OPF-08 generated a RAPD polymorphic profile that showed common fragment of 478 bp in all the isolates.

Abubakar *et al*. (2020) found a total of fifty nine (59) amplified fragment bands with 10 DNA primers, of which 53 (89.83%) were polymorphic and 6 (10.17%) were monomorphic. Genetic similarity among the accessions varied from 0.18 to 1.44 with an average gene diversity value of 0.74.

2.3 EVALUATION OF NATIVE BACTERIAL BIO-CONTROL AGENTS AND FUNGICIDES AGAINST THE PATHOGEN *IN VITRO*

2.3.1 Evaluation of Bacterial Bio-control Agents against Blast

Gnanamanickam and Mew (1992) reported that *Pseudomonas fluorescens* strain 7-14 was the most effective against *P. grisea* under *in vitro* condition. Vidhyasekaran *et al.* (1997) found that *P. fluorescens* strain was inhibitory to the growth of *P. grisea* under *in vitro.*

Prasanna and Rao (2009) tested 30 strains of *P. fluorescens*, against *P. oryzae*. Out of 30, ten exhibited strong antifungal activity by producing antifungal metabolites and inhibited the mycelial growth.

The biocontrol potential of *P. fluorescens* was isolated from the rizhosphere of rice fields and was tested against *P. oryzae*. The bio-control agent grown faster and actively checked the growth of *Pyricularia oryzae* by inhibiting 78.9% under *in vitro* (Arumugam *et al*., 2013).

Gashaw *et al.* (2014) reported that *P. fluorescens* showed maximum mycelial growth inhibition by isolates Pg.40 (57.22%), followed by Pg.26 (56.14%) under *in vitro* condition. Evaluation of *P. fluorescens* against *P. oryzae* revealed that 44-100% of inhibition in mycelial growth was observed under *in vitro* condition (Hern *et al.,* 2018).

Rana and Paul (2019) tested nine different bacterial bio-control agents against *P. oryzae.* Among those AM16 and AB7 of Pseudomonas spp. showed 79.07 and 69.75% mycelial inhibition under *in vitro* condition.

Prasanna and Rao (2009) was screened 30 isolates of *P. fluorescent* against *P. oryzae* under *in vitro* condition revealed that ten out of thirty isolates were exhibited strong antifungal activity by producing antifungal metabolites.

2.3.2 Evaluation of Commercially Available Fungicides against Pathogen

Naik and Jamadar (2014) evaluated five systemic fungicides *P. grisea* (Pg8 local Bijapur isolate) for inhibition of radial growth on the Czapeck's agar under *in vitro* condition revealed that tricyclazole 75WP gave maximum inhibition of the mycelial growth (87.78%) of the pathogen followed by difenconazole 25EC (86.91%) and hexaconozole 5E (85.33%).

Netam *et al*. (2014) reported complete inhibition of mycelial growth was given by tricyclazole followed by Ediphenphos and Mancozeb 65 per cent WP + Carbendazim12 per cent WP (Saff). Joshi and Gohel. (2015) reported that tricylazole 75 WP gave maximum inhibition of mycelial growth.

Carbendazim was also found to be highly effective by inhibiting the mycelial growth of pathogen as reported by Sahu *et al.* (2018). Rayhanul *et al.* (2019) also reported the maximum inhibition of *Pyricularia oryzae* by using Nativo 75 WG (64.57%).

Kafle *et al*. (2021) evaluated different fungicides against *P. oryzae* under *in vitro* conditions. Among the test chemicals Nativo was shown to exhibit 100% inhibition by inhibiting pathogen mycelium.

2.4 TO SCREEN THE GENOTYPES FOR THEIR RESISTANCE AGAINST BLAST

Ravikumar *et al.* (1990) evaluated 316 genotypes of finger millet over four seasons under natural environmental conditions, the mean neck and finger blast incidence was higher in the post rainy season. Over season a number of genotypes showed zero incidence for neck blast. However, none was completely free from finger blast, while 7 genotypes GE75, GE669, GE866, GE1309, GE1319, GE1407 and GE1409 showed resistance to both neck and finger blast.

Somasekahara *et al.* (1991) screened 25 finger millet cultivars for their resistant to blast under natural conditions and reported that none of the cultivar

was resistant to leaf blast but HPB IE 11-1 had small sized lesion when scored for neck and finger blast, IE 1012 was completely immune to infection, and cultivars HPB IE 11-1, Indaf 15, MR 1, MR 2 and MR 3 had less than 5 per cent infection.

Jain *et al*. (1994) evaluated 21 genotypes of finger millet during 1987, 1988 and 1989 for stability of resistant to neck and finger blast diseases (*P. gresea*). The response of genotypes to neck and finger blast was genetically controlled. Genotypes VL 145, VL 149, PR 1158-9, GPU 16 and RHRN 82-1/84 had stable resistant, while HR8-19-1 and PR202 exhibited moderate resistance and stability for both the diseases.

Muyonga *et al.* (2000) screened 5 finger millet varieties for blast disease tolerance. Among them, the variety Sirare was more tolerant to blast disease then variety P224 and Nyaikuro, while Gulu-E and Ikhulule were moderately tolerant.

Jain and Yadava (2001) screened 52 genotypes of finger millet for blast resistance in two consecutive years. Genotypes MR6, GE1348, 1370, 1417, 1420, 2821, 3022, 3024, 3057, 3058, 3080, IE1012 and 1-8 IE were found resistance, while VL 231,171,174, GPU25, GE1036 and 3484 were found moderately resistant.

Mantur and Madhukeshwara (2001) assessed 66 genotypes of finger millet for blast resistance. Among them, nine entries *viz*., GE 2400, 4913, 4914, 4915, 4929, 4966, 5102, 5126 and 5148 were completely free from infection recorded “O” disease grade, as many as 36 entries recorded 0.1 to 2.0% incidence in both the seasons and proved as a good resistance. However, 16 entries showed moderate incidence (2.1 to 10.0%) of neck and finger blast, while only 2 entries recorded a disease grade of 4 (10.1 to 25.0% incidence). Two susceptible checks *viz.*, KM229 and KM230. Showed more than 25% blast, while K7 recorded more than 50% blast.

Jain and Yadava (2004) screened 40 genotypes of finger millet for blast resistance in two consecutive years determine the mechanism of resistance. Genotypes, GE-3022, GE-3024, GE-3058, GE-3060 and MR-6 showed

consistency of resistance against leaf, neck, and finger blast in the two years of the experiments.

Nagaraja and Mantur (2007) screened 64-75 finger millet germplasm entries for both neck and finger blast [*Magnaporthe grisea*] incidence. Among those entries like GE 5183, 5203, 5205, 5209, 5212, 5215, 5218, 5227 and 5230 showed consistent resistance reaction and thus, are useful in breeding for stable resistance.

Gupta and Jain (2010) screened 38 finger millet cultivars at Reva and Dindori location of Madhya Pradesh and found that 3 cultivars namely BR-1, L-76 and KMR-204, showed resistant reaction, while 10 cultivars were moderately resistant to all three blast (leaf, neck, finger blast) infection at both the locations.

Barnwal (2012) screened 8 finger millet cultivars under favourable environmental conditions against blast disease and stated that the cultivar OEB225 had the lowest neck blast incidence (2.5%) and finger blast (12.1%) with the highest grain yield (27.4 q/ha). this was followed by GPU 67, while other cultivars A 404, JWM, 1, GPU 45, OEB 244, IE 7 and PR 202 showed moderately resistant reaction against neck blast.

Patro *et al*. (2013) evaluated 16 pre-released and released varieties of finger millet and reported that GPU 28 as immune to blast pathogen and nine varieties were resistant to all three forms of blast disease.

Patro and Madhuri (2014) evaluated 32 finger millet genotypes among them, two were susceptible to neck blast and moderately resistant to finger blast, 14 were moderately resistant and 13 were susceptible to both neck and finger blast.

Madhavilatha and Rao (2015) tested thirteen promising Finger millet genotypes against blast. Among those tested entries PPR 1012 with low blast disease incidence score (leaf blast: 1, neck blast: 9.46%, finger blast: 10.91%) has been reported.

Screening of 12 elite finger millet cultivars, GE 4449 and GPU 28 were reported to be resistance to leaf blast and GE 4440, GE 4449 and GPU 28 were

moderate resistance/susceptible to neck and finger blast (Divya *et al.,* 2017). Neeraja *et al.* (2016) screened 25 finger millet varieties and reported that nine varieties were resistant to moderately resistant to leaf blast and three were moderately resistance to both neck and finger blast.

Patro *et al*. (2017) screened 10 genotypes were evaluated for resistance to blast none genotypes were found free from disease incidence. Minimum percentage of neck blast severity was recorded in VL 379 (14.82%), while the minimum finger blast severity (13.70%), was recorded in GPU 45.

Patro *et al*. (2018) evaluated 30 varieties of finger millet in which five varieties are found to be highly resistant and nineteen varieties are resistant whereas VR 708 recorded as highly susceptible to leaf blast.

Patro and Anuradha (2019) evaluated 3000 finger millet lines including one check variety *viz*., VR 708 (susceptible) against neck and finger blast. Among all the 3000 lines 112 lines have shown resistant reaction under high disease pressure field conditions during *kharif* 2013-2018. However, only 50 varieties have shown consistent resistant reaction during all the years (2014-18) tested. The percent disease intensity of neck and finger blast ranged from 0.00 to 96.40 and 0.00 to 95.30 respectively, where it was 95.5 and 96.4 PDI of neck and finger blast in check VR 708, a highly susceptible variety.

An investigation was made to identify suitable blast resistant varieties through on farm trials in Vellore district during 2017–18. The results of the study revealed that TNAU finger millet Co15. The reaction of variety to the blast incidence was moderately resistant to leaf blast with the disease score of 2.1. The per cent incidence of neck and finger blast was 5.2 and 5.7, respectively against ruling variety GPU 28 which recorded 14.3 and 19.2% (Sendhilvel and Veeramani, 2020).

2.5 TO STUDY THE INFLUENCE OF WEATHER FACTORS AND DISEASE DEVELOPMENT

Thomas (1940) reported that the rice crop sown late (October) in the season under field condition showed maximum infection as the susceptible stage of the crop coincide with the prevalence of low temperature after October.

Anderson *et al.* (1947) reported that temperature 24ºC to 28 ºC and high humidity (>90 %) favoured infection of rice blast. Pall (1988) noted that finger millet blast development was favoured by minimum temperature of 15-25 ºC, High rainfall and RH > 85 per cent. Early cultivars were highly susceptible to neck blast because of favourable climatic conditions at the ear head stage existing and coinciding.

Vishwanath and Channamma (1988) studied severity of blast on finger millet depends on the climatic conditions. The fungus preferred low temperature (<20 ºC) with high humidity, heavy rainfall and low light for outbreaks.

Jain *et al.* (1994) noted that moderate temperatures 21 ºC to 29 ºC and mean atmospheric relative humidity of >80 per cent during the reproductive period favoured blast disease development in finger millet crop.

Hegde (1996) was found that a pH of 7.0 to be optimum while the temperature requirement was 28°C for finger millet blast pathogen. Madhukeshwara *et al.* (1997) working six isolates of *P. Grisea* from finger millet found 28°C to be the optimum temperature for growth.

Gouramanis (1994) studied the influence of environmental condition on rice blast development. He reported that the favourable environmental conditions for blast development were mean air temperature between 21-28 ºC and 54-84% relative humidity.

Average minimum and maximum temperature between 22°C to 29°C with high relative between 85 to 99% during the growth period increased blast disease intensity in finger millet. The disease intensity also showed significant positive correlation with maximum and minimum temperature, rainfall and relative humidity (Patel and Tripathi, 1998).

Tripathi *et al*. (1998) at Faizabad (U.P) recorded optimum growth of rice blast (*P. grisea*) lesion at 28 ºC day temperature, 24 ºC night temperature and 97-98 per cent relative humidity. They also found that growth decreased with day temperature above 28 ºC but increased with decreasing night temperature. Sunil and Anilkumar (2003) observed that finger millet crop was damaged by blast caused by *P. grisea* especially during the rainy season in Karnataka.

Kumar *et al.* (2005) the effects of sowing date (20 June, 30 June, 10 July, 20 July, 30 July, 10 Aug, 20 Aug, 30 Aug, 10 Sep and 20 Sep) on the incidence of neck and finger blast disease caused by *P. grisea* in four susceptible finger millet cultivars (GE-218, GE-283, GGE-295 and GE-301) at Bangalore, Karnataka, India. The highest incidence of neck blast was observed in GE-218 (45.21%), GE-295 (31.37%) and GE-301 (42.40%) sown on 10th July when a minimum temperature of 21.8 ^{o}C and relative humidity of 93% prevailed the highest finger blast severity of 48.6 (GE-218), 29.12 (GE-283), 63.00 (GE-295) and 96.25 per cent (GE-301) was recorded for the varieties sown on 10thJuly. The results suggested that the increased neck and finger blast incidence was due to reduced temperature and increased relative humidity; opposite trends were recorded for low blast disease development. Dubey (2003) studied the influence of weather factors on the development of blast in Jharkhand on highly susceptible variety, Birsa Dhan-2002 and reported that blast of rice appeared during 3rd week of July and increased gradually. The disease severity was maximum at 71-81 days old plants with Mean temperature of 22-30, 85.5% relative humidity, rainfall 6.3-9.1 mm and number of rainy days 6-8 were found favourable for maximum disease intensity. Neck infection appeared 51 days after sowing and increased gradually up to crop harvest.

Nagaraja *et al.* (2007) stated that the temperature ranges from 25-30 ^{o}C, humidity of 90% and above and cloudy days with intermittent rainfall conditions were found very favourable for the development of rapid spread of blast of finger millet.

A 40 h wetting of spikes associated with a 25 °C temperature resulted in 85% infection of wheat blast caused by *Magnaporthe oryzae* Triticum (Cardoso *et al.*, 2008).

Continuous rain along with moderate temperature of 18-25 °C, followed by sunny days at the flowering stage is the most congenial conditions for wheat blast outbreak (Kohli *et al.*, 2011).

Shafaullah *et al.* (2011) reported that the disease severity of rice blast decreased with increased in minimum temperature from 19 to 26 ^{0}C. Rainfall and

relative humidity influenced positive effect on rice blast severity with rainfall from 5 mm to 17 mm and relative humidity *i.e.* 72-95 %.

Netam *et al*. (2014) found that the minimum leaf blast severity and neck and finger blast incidence as well as highest grain yields were recorded from 1st June sown crop during both the years. The weather parameters recorded and correlated with disease development, in two years indicated that the average minimum and maximum temperatures of 21^0C and 29^0C respectively a 70-81% relative humidity. Present study indicated that decrease in temperature and increase in humidity may favour the disease development and may cause epidemic of leaf blast or neck blast or finger blast.

Studies on the effect of leaf wetness and temperature on disease development showed an overall increase in leaf blast severity, lesion length (mm), number of lesions per plant, lesion sporulation and leaf sporulation with the increase in leaf wetness duration (LWD) and concluded that both leaf wetness duration and temperature were essential for blast on pearl millet which becomes more severe at longer wetness durations beyond 48 hours during optimum day/night with a temperature ranging from 25±1/20±1 ^{o}C to 30±1/22±1 ^{o}C (Goud., 2015).

Conducive environmental conditions facilitated early disease initiation, rapid increase in rice blast. Rain fall of 87%, relative humidity of the morning 81%, relative humidity of the evening 61%, wind speed of 31% and minimum temperature 11% contributed to the disease development (Naqvi and Perveen., 2015).

2.6 INTEGRATED DISEASE MANAGEMENT FOR BLAST

Vanitha (1998) reported that seed treatment with *Pseudomonas florescens* against finger millet blast was found to be highly effective which showed 2.95% reduction in disease incidence with 2026 kg/ha increase in grain yield.

Ramappa *et al.* (2002) also found that application of 0.3 per cent *P. fluorescens* as a spray was found to be effective in controlling blast disease and increased grain yield.

Muthaiyan (2000) noticed that seed treatment of *P. fluorescens* (10 gm/kg seed) with its broadcasting (2.5 kg/ha) at 30 days and foliar sprays (1.25 kg/ha) at 45-60 days after transplanting reduced the leaf (14.5%) and neck (4.5%) blast with significantly higher yield production (4625 kg/ha) of rice.

Patro *et al*. (2008) evaluated the efficacy of *P. fluorescens* (0.6%) against the blast of finger millet and found that *P. fluorescens* (0.6%) as a seed treatment with two foliar sprays at ten days interval, first spray was starting at head emergence was found to be the most effective to reduce the blast and increasing yield of finger millet.

Narayanswamy *et al*. (2009) showed that application of Tebuconazole + Trifloxystrobin was found most effective in controlling rice blast as it controlled the disease up to the extent of 84% compared to control.

Kumar and Kumar (2011) suggested that seed treatment with *P. florescens* pf2 (0.6%) with its two foliar spray (0.6%) most effective in reducing blast disease of finger millet. The treatment provided 81.69% yield increases over the control.

Mohan *et al*. (2011) reported that Tebuconazole + Trifloxystrobin and Tebuconazole were found most effective against the leaf and neck blast of paddy under field condition. Dutta *et al*. (2012) tested various fungicides against rice blast. Among those fungicides nativo shown effective for with 10.15% reduction in percent disease incidence and 18.98% reduction in neck blast incidence. Different scientists confirmed that strobilurin derived fungicides found to be effective in controlling rice blast disease compared to other fungicides (Debashis *et al.*, 2012).

Sireesha (2013) reported that *P. fluorescens* was found the most effective in controlling the leaf blast and neck blast and increased grain yield.

New combination fungicide, nativo 75 WG @ 0.4 g/l was tested against rice blast under field condition during *Autumn* 2014 and 2015 which was effective against blast diseases recording least Percent Disease Index (PDI) of

17.02 and increase in the grain yield of 55.13 q/h when compared to the other fungicidal treatments (Pramesh *et al.,* 2016).

Kulmitra *et al.* (2017) reported that Tebuconazole 50 % + Trifloxystrobin 25 % (WG) was found to be the most effective fungicide against the leaf blast disease in rice. (2017). Nirmalkar *et al*., (2017) evaluated efficacy of fungicides against leaf and neck blast disease in paddy during *Kharif* season of 2013-14. Among the evaluated fungicides combination of Tebuconazole 50 % EC + Trifloxystrobin 25 % WG were found to be effective against leaf and neck blast disease.

Sharma *et al*. (2018) evaluated nine fungicides were tested for their efficacy in controlling pearl millet blast. Among tested fungicides three sprays of Propiconazole or Tebuconazole + Trifloxystrobin were found to be highly effective.

Singh *et al.* (2019) evaluated six fungicides under field condition, among those Tebuconazole 50 % + Trifloxystrobin 25 % (WG) found to be effective with minimum disease per cent intensity of 11.46 per cent followed by Azoxystrobin 18.2 % + Difenoconazole 11.4 % (SC) with 12.85% disease intensity.

Ahmad *et al*. (2020) evaluated various fungicides against rice blast. Among those nativo 75% WP @ 65 g a. i. per acre was found to be highly effective in controlling blast disease with minimum percent disease intensity (11.16%) and maximum value of grain yields (4403.32 kg ha^{-1}).

Chapter – III

Material and Methods

Chapter - III

MATERIAL AND METHODS

This chapter includes all the material used and methods adopted in the investigation. The present investigation was carried out at Department of Plant Pathology, S.V Agricultural College Tirupati, molecular experiments at Institute of Frontier Technology, RARS, Tirupati and screening, weather correlation studies as well as disease management experiments were conducted at Agricultural Research Station, Vizianagaram, Andhra Pradesh.

3.1 GLASSWARE

Glassware of Borosil® or Corning® make were used. The glassware consisted of Petri dishes, conical flasks, test tubes, screw caps, measuring cylinders and watch glasses.

The glassware was first washed with detergent powder followed by cleaning with tap water. Later, the glassware kept overnight in cleaning solution containing 75 g potassium dichromate, 500 ml of water and 1000 ml water and again washed with tap water followed by rinsing with distilled water.

3.2 CULTURE MEDIA

3.2.1 Media and their Composition

The following media were used in the present investigation.

(A) Potato Dextrose Agar (PDA)

Potato : 200.0 g

Dextrose : 20.0 g

Agar – agar : 20.0 g

Distilled water : 1000 ml

200 g peeled and sliced potatoes were boiled in 500 ml distilled water to make potato extract. Agar-agar (20 g) was added to another 500 ml distilled water and melted by boiling. The muslin cloth was used to filter the potato

extract, and the filtrate was collected and added to the melted agar. After adding dextrose, the volume was increased to 1000 ml with distilled water. Before sterilising the medium in an autoclave, the pH (6.5) of the medium was adjusted with sodium hydroxide (NaOH) or lactic acid.

(B) Oat Meal Agar (OMA)

Oat flakes : 30.0 g

Agar – agar : 20.0 g

Distilled water : 1000 ml

30 g of oats were boiled in 500 ml of distilled water for 30 minutes in a conical flask to make Oat Meal Agar medium. In another conical flask, agar-agar (20 g) was melted in 500 ml of water with distilled water and added to the oat meal broth. By adding distilled water, the final capacity was increased to 1000 ml. Before sterilisation, the pH of the medium was adjusted to 6.5.

(C) Rice/Finger Millet Leaf Extract Agar Medium (RLA/FLA)

Host plant material : 200.0 g

Sucrose : 20.0 g

Agar-agar : 20.0 g

Distilled water : 1000 ml

Rice/finger millet host leaves (200 g) were gathered from healthy plants and cut into small pieces, which were then cooked in 500 ml distilled water until mushy. The resulting leaf extract was then filtered using muslin cloth. Sucrose (20 g) was added to the extract. The leaf extract was dissolved in agar-agar (20 g) melted in 500 ml distilled water. By adding distilled water, the final volume was increased to 1000 ml. Before sterilisation, the pH of the medium was adjusted to 6.5.

D) Potato Sucrose Agar (PSA)

Potato : 200.0 g

Sucrose : 20.0 g

Agar – agar : 20.0 g

Distilled water : 1000 ml

E) Oat meal Sucrose Agar (OSA)

Oat flakes : 30.0 g

Agar – agar : 20.0 g

Sucrose : 20.0 g

Distilled water : 1000 ml

Synthetic media/Hi media:

F) V 8 juice Agar media

G) Richard's Synthetic Agar media

H) Nutrient Agar (NA)

3.3 STERILIZATION

All of the glassware used in the study were sterilized for two hours in hot air oven at 16 ^{o}C. All of the nutrient media were sterilized for 15 minutes at 121°C in an autoclave at 15 psi.

3.4 POT CULTURE EXPERIMENTS

Plastic and earthen pots of size 22 x 20 cm (height x top surface diameter) were used in the present studies. As potting media, the pots were filled with three parts of red loamy soil and one part thoroughly decomposed farm yard manure.

3.5 TO CONDUCT SURVEY FOR OCCURRENCE AND DISTRIBUTION OF BLAST DISEASE, COLLECTION OF ISOLATES AND NATIVE BACTERIAL BIO-CONTROL AGENTS IN MAJOR FINGER MILLET GROWING AREAS OF ANDHRA PRADESH

3.5.1 Survey for the Incidence of Finger millet Blast in major Finger millet Growing Areas of Andhra Pradesh

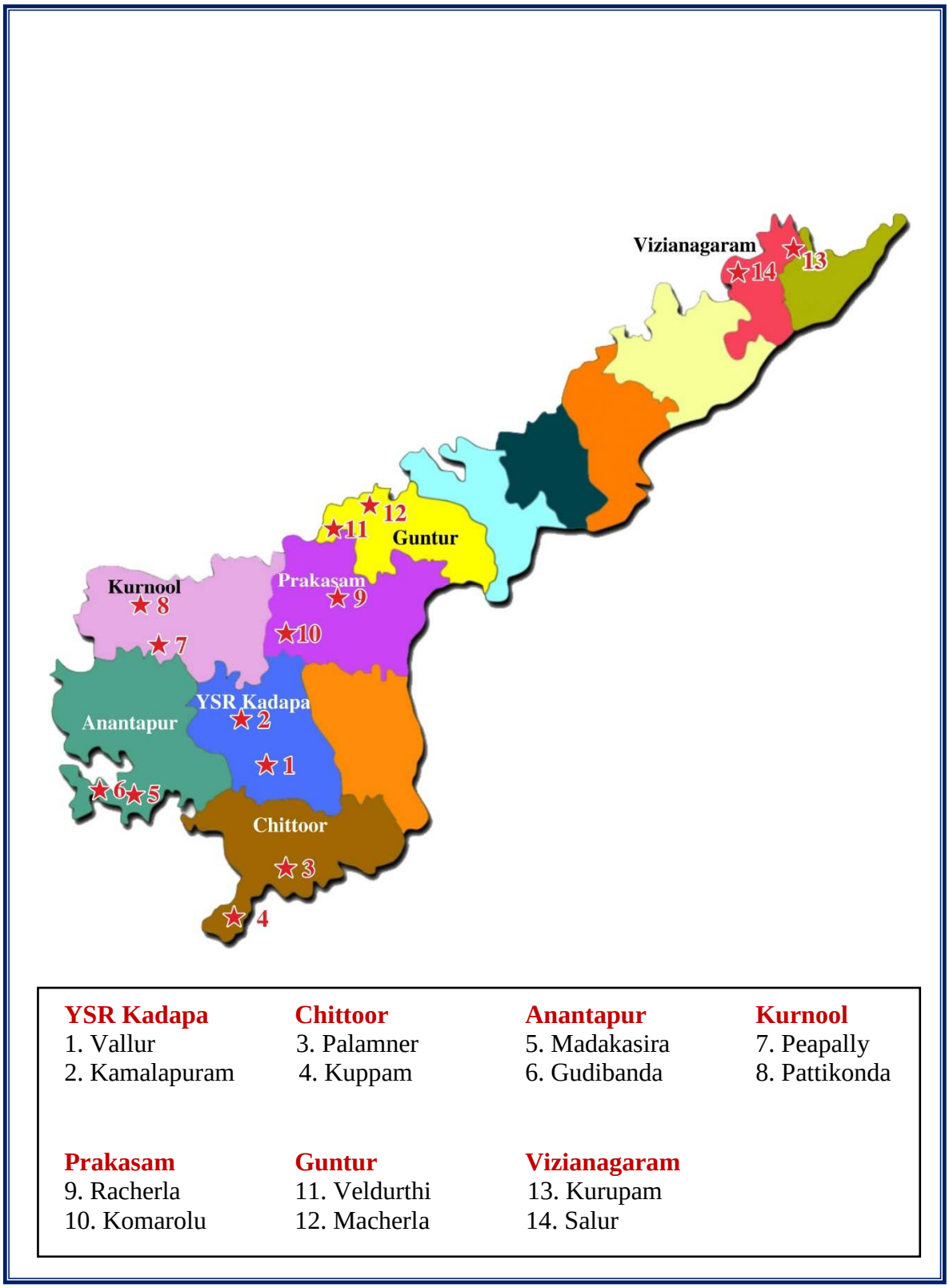
Roving survey was conducted for the incidence of finger millet blast during *Kharif* 2020 and 2021 in major finger millet growing areas of Andhra Pradesh (Table 3.1) (Fig. 3.1). Each district was divided into two mandals, in each mandal three villages were selected. In each village three fields were chosen for the study. Observations were recorded in one square

meter area randomly in each field. Finger millet blast in observed fields was expressed as Per cent Disease Incidence (PDI).

$$\text{PDI} = \frac{\text{Diseased hills observed}}{\text{Total no. of hills observed}} \text{ X } 100$$

Table 3.1. Survey for the incidence of finger millet blast in major finger millet growing areas of Andhra Pradesh during *Kharif* 2020 and *Kharif* 2021

S. No.	District	Mandals	Villages		
1.	YSR Kadapa	Vallur	Lingayapalle	Vallur	Goturu
		Kamalapuram	Kamalapuram	Ramachandrapuram	T. Sadipirala
2.	Chittoor	Palamneru	Palamneru	Moram	Kurmoi
		Kuppam	Gonugur	Jarugu	Palarlapalle
3.	Anantapur	Madakasira	Govindapuram	Jadrahalli	Haresamudram
		Gudibanda	Morubagal	Gudibanda	Muthukur
4.	Kurnool	Peapally	Peapally	Vengalampalle	Pothidoddi
		Pattikonda	Pattikonda	Chakkaralla	Juturu
5.	Prakasam	Racherla	Anumalapalli	Racherla	Somidevipalli
		Komarolu	Taticherla	Chintalapalli	Komarolu
6.	Guntur	Veldurthi	Veldurthi	Uppalapadu	Gundlapadu
		Macherla	Macherla	Kothapalli	Koppunur
7.	Vizianagaram	Kurupam	Gumma	G. Sivada	Gummidiguda
		Salur	Neliparthi	Salur	Kurmarajupeta



YSR Kadapa	Chittoor	Anantapur	Kurnool
1. Vallur	3. Palamner	5. Madakasira	7. Peapally
2. Kamalapuram	4. Kuppam	6. Gudibanda	8. Pattikonda

Prakasam	Guntur	Vizianagaram
9. Racherla	11. Veldurthi	13. Kurupam
10. Komarolu	12. Macherla	14. Salur

Fig. 3.1. Map showing districts and mandals surveyed in Andhra Pradesh.

3.5.2 Collection and Isolation of *P. grisea* Isolates from Blast Diseased Samples

Finger millet blast samples collected from farmers' fields were used for the isolation of the pathogen. The pathogen was isolated by following standard tissue isolation procedure (Tuite, 1969). Small bits of diseased leaves along with some healthy tissue were cut with the help of a sterile scalpel and surface sterilized with one per cent sodium hypochlorite solution for 1 min and rinsed aseptically in three changes of sterilized distilled water. Such surface sterilized leaf bits were transferred aseptically into sterilized Petri dishes containing solidified Oat Meal Agar medium and incubated at 26 ± 1ºC for two weeks in a BOD incubator.

3.5.3 Purification of Culture

The fungal culture was purified by single spore isolation method as described by Ou and Ayed (1968). The conidial suspension was prepared by scraping the surface of 15 day old fungal growth on the agar plate and the scrapings were made into concentrated suspension by adding 1 ml sterilized distilled water. After confirming the presence of conidia of *P. grisea*, the suspension was poured aseptically into the Petri dishes containing 2% of agar medium. The Petri dishes were gently rotated for the even distribution of the conidia in the medium before it set. After inoculation of Petri dishes for 12 h, the conidia well spaced out were located by examining the bottom side of the Petri dishes under the microscope and were marked with a glass marker on the outer side of bottom of the Petri dish. Each marked single conidium along with a bit of agar medium was aseptically cut and scooped with the help of sterilized scalpel, and transferred to Oat Meal Agar slants. The culture was maintained by sub culturing at 15 days interval. In naming criteria of pathogen isolates, the first three letters indicates respective villages and the numbers indicates, number of pathogen isolates collected during the survey ((Ex: LNG-1= Lingayapalle-1).

3.5.4 Identification of the Pathogen

The fungus isolated during the study was identified based on the characteristics of the colony, hyphae and conidia. The measurements of conidia

were recorded and compared with standard measurements of the species given by Shirai (1896) and Rao (1994).

3.5.5 Pathogenicity Test

Plant material: The pathogenicity of twenty isolates were tested using a susceptible finger millet cultivar (VR 708). Seedlings of the susceptible cultivar were grown in 22 X 20 cm diameter plastic pots filled with sterilised soil-sand-FYM (farmyard manure) mix (2:1:1) and placed in three replications in a greenhouse bay kept at 30°C. To retain 10 plants per pot, seedlings were thinned at the one-leaf stage.

Inoculum preparation and inoculation

The 6 mm mycelial discs of each isolate were cut from 7 day-old-culture of *P. grisea* grown on OMA medium at 26±1ºC. Mass multiplication of spores for inoculation was achieved by growing each isolate (9 discs/plate) on OMA medium at 26±1ºC for 15 days. The plates were flooded with 10 ml of distilled water and the fungal growth containing mycelium and conidia was gently removed by scrapping with a sterile plastic inoculation loop. Approximately 30 ml of a spore suspension of each isolate was transferred into 100 ml conical flask, mixed thoroughly by vortexing for release of conidia into water. Harvested spores were filtered through a double-layer muslin cloth, the resultant concentration was adjusted to 1×10^5 conidia ml-1 and 0.02% (vol/vol) Tween 20 (polyoxylethylene sorbitan monolaurate) (Jia *et al*., 2003) was added to the suspension just before the inoculation. 15-day-old pot-grown seedlings were inoculated artificially by spraying the inoculum on the foliage using a hand-operated atomizer. Inoculated plants were allowed to partially dry for 30 min to avoid dislodging of the spores and the seedlings sprayed with water were maintained as control. All the inoculated seedlings were incubated.

Data recording: On an individual plant basis, the severity of each isolate's leaf blast was recorded using a progressive 1–9 scale (Table 3.2) (Fig. 3.2). To complete Koch's postulates, each isolate was re-isolated from the artificially inoculated leaves using the previously stated methodology.

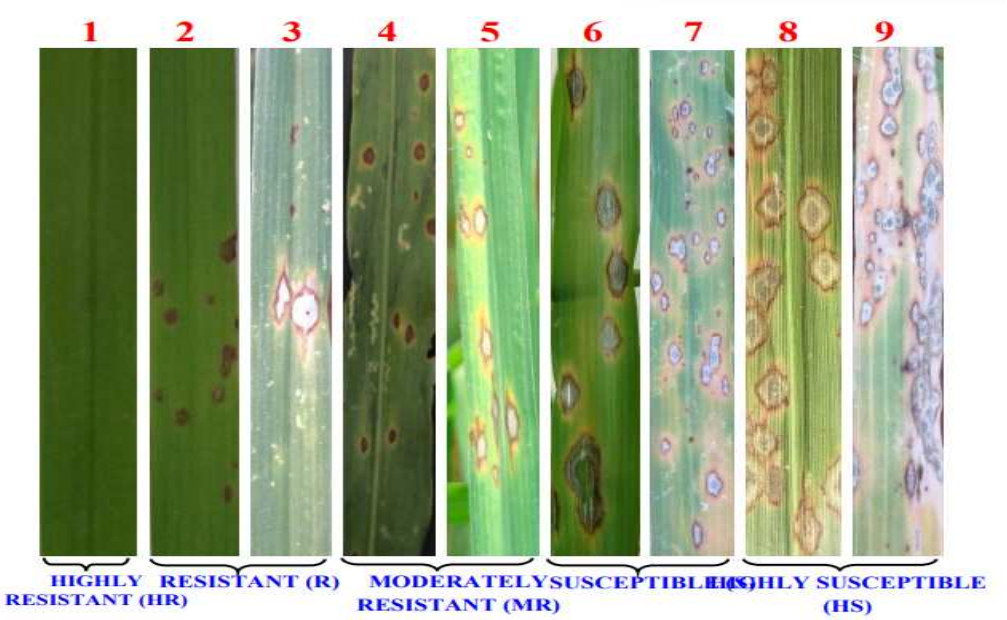


Fig. 3.2. Leaf blast severity scoring in finger millet caused by *Pyricularia grisea* using 1-9 rating scale.

Table 3.2. Disease rating scale for leaf blast severity in finger millet infected with *Pyricularia grisea*

Score	Disease
1	No lesions to small brown specks of pinhead size
2	Larger brown specks covering 1-5% of the leaf area
3	Small, roundish to slightly elongated, necrotic gray spots, about 1–2 mm in diameter with brown margins covering 6-10% of the leaf area
4	Typical blast lesions, elliptical, 1–2 cm long, usually confined to area between main veins, covering 11-20% of the leaf area
5	Typical leaf blast covering 21-30% of the leaf area
6	Typical blast lesions covering 31-40% of the leaf area
7	Typical blast lesions covering 41-50% of the leaf area
8	Typical blast lesions covering 51-75% of the leaf area and many leaves dead
9	>75% leaf area covered with lesions or all the leaves dead

Table 3.3. A 1–5 rating scale for recording neck blast severity in finger millet infected with *Pyricularia grisea*

Score	Neck blast
1	No lesions to pin head size of lesions on the neck region
2	0.1–2.0 cm of lesions on the neck region
3	2.1–4.0 cm of lesions on the neck region
4	4.1–6.0 cm of lesions on the neck region
5	>6.0 cm of lesions on the neck region

3.5.6 Storage of fungal isolates

Sub culturing of *P. grisea* was done on Oat Meal Agar slants and incubated at 26 ± 1ºC for 14 days. The slants with fungal culture were temporarily preserved in refrigerator at 4 ºC and reisolated it to active state for further studies.

3.6 TO STUDY THE CULTURAL, MORPHOLOGICAL AND MOLECULAR VARIABILITY AMONG THE ISOLATES OF PATHOGEN

3.6.1 Cultural Variability among *P. grisea* Isolates

The variability in cultural properties of 20 isolates of *P. grisea* collected from different places were carried out on OMA medium at pH 6.5 by incubating at 28ºC. The observations on colony colour, growth pattern, elevation (flat/elevated growth), sectored or non-sectored growth were recorded for *P. grisea* isolates at 14 days after inoculation.

3.6.2 Influence of Different Culture Media on Growth and Sporulation of *P. grisea*

In the present experiment 8 different types of media *i.e*. Potato Dextrose Agar (PDA), Rice Leaf extract Agar (RLA), V8 juice Agar (V8), Finger millet Leaf extract Agar (FLA), Potato Sucrose Agar (PSA), Rechard's Synthetic Agar (RA), Oat meal Sucrose Agar (OSA) and Oat Meal Agar (OMA) were prepared to choose the optimal medium for the pathogen's development and sporulation.

Approximately 10-15 ml molten agar (~45ºC) was poured into sterile petriplates (90 mm) and allowed to harden before being inoculated with *P. grisea.* Then these plates were incubated at 26±1°C. The growth measurement was recorded when the total plates was covered in three replications. The amount of sporulation in the culture was measured by adding 20 ml of water to dislodge the spores from the culture, followed by a conidial count using a heamocytometer, and the results were represented in conidia/ml. The formula of concentrations of spores/ml is mentioned below.

$$\text{Concentration of spores} = \frac{\text{No. of spores}}{\text{No. of columns}} \times \frac{\text{dilution factor}}{\text{Volume}}$$

3.6.3 Effect of different light conditions on radial growth and sporulation of *P. grisea* virulent isolate (VIZ-1)

In eight distinct media, the effects of three light conditions on fungal development were evaluated. The light conditions include 24 hr fluorescent light, 12 hr dark/12 hr fluorescent light, and 24 hr darkness. PDA, RLA, V8, FLA, PSA, RA, OSA and OMA were employed under each light condition for sporulation studies. Radial growth and sporulation was recorded on 14 days after inoculation. All experiments contained three replicates and were repeated twice.

3.6.4 Morphological Variability among *P. grisea* Isolates

Conidia morphological features were used to investigate the morphological variability among the isolates. A drop of conidial suspension was well mixed with lactophenol and placed on a glass slide, which was then covered with a cover slip. The spores were measured under Olympus CX41 microscope with image analyzer. The average size (length and width) and shape of conidia was recorded.

3.6.5 Molecular Variability among Pathogen Isolates using Molecular Tools

SSR (simple sequence repeat) and RAPD (Random Amplified Polymorphic DNA) markers were used to investigate genetic diversity among *P. grisea* isolates obtained from various localities. Based on the *P. grisea* linkage map described by Kaye *et al.*, (2003) a set of 25 SSR markers (Table 3.4) distributed over seven chromosomes of *P. grisea* and eight RAPD markers were chosen in the study (Table 3.5).

3.6.5.1 Genomic DNA extraction from *Pyricularia grisea* fungus

Seven days old pre-inoculated *P. grisea* culture discs approximately 5 mm in diameter were transferred to potato dextrose broth and incubated at 26- 28°C for 7 days for mass production of fungal mycelium. The culture was filtered through the musclin cloth to collect the fungal mat. The mycelium obtained (~250 mg) was kept for drying on the blotting paper for half an hour. Then the mycelial mat was lyophilized using liquid nitrogen and later it was used for DNA extraction using CTAB Method (Murray and Thompson, 1980). A working DNA solution of 10 – 20 ng/µl concentration was made by diluting DNA stock solution.

3.6.5.2 Steps involved in DNA extraction from *P. grisea*

1. The dried *P. grisea* fungal mat was taken in a sterile porcelain mortar and liquid nitrogen was added, the mat was then ground into fine powder using a sterile porcelain pestle. While grinding, it was ensured that fungal mat powder does not spills out.
2. Later the lyophilized fungal mat powder was collected into a sterile microcentrifuge tube (2 ml capacity) and 800 µl extraction buffer (Appendix I) (50 mM Tris HCl pH 8.0; 25 mM EDTA, 300 mM NaCl and 1% SDS) was added which was then incubated in a water bath maintained at 65 ºC for 30 min.
3. After inoculation, approximately equal volume of Phenol:Chloroform: Isoamylalcohol (25:24:1) mixture (250 µl) was added to each microcentrifuge tube. It was ensured that the pH of phenol used was 8.0. The contents were mixed well by inversion for about 10 min and centrifuged at 10,000 rpm for about 10 min at room temperature.
4. The supernatant was aliquoted from the micro centrifuge tube into sterile 2.0 ml micro centrifuge tubes. Precaution was taken while aliquoting not to disturb the intermediate layer which consists of insoluble proteins. The phenol: chloroform: isoamylalcohol purification step was repeated to ensure pure extraction.

5. To the supernatant 10 µl of RNase (10 mg/ml) was added and incubated for 30 min at room temperature. One more treatment with an equal volume of chloroform was given and centrifuged at 10,000 rpm for 10 min at room temperature.
6. To the supernatant, 1/10th volume of 3M sodium acetate (pH 5.8) and equal volume of ice chilled Isopropyl alcohol was added and incubated at -20°C for 1 h. The contents were mixed gently and centrifuged at 10,000 rpm for 10 min at room temperature.
7. The supernatant was discarded and 70% ethanol was added to the pellet. The tube was tapped gently so that the pellet becomes suspended in 70% ethanol. Centrifugation was done at 10,000 rpm for 10 min at room temperature. After which the supernatant was drained and the pellet was washed with 70% ethanol once again as described above.
8. The pellet was left for air-drying over night at room temperature with the tube cap open. After complete drying of pellet, depending on the size of the pellet, about 50- 100 µl of sterile 1X TE buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA) was added to the tube for dissolving the pellet. The DNA solution was then stored at 4°C till further analysis.

3.6.5.3 Analysis of quality and quantity of DNA isolated

The quantity of isolated DNA was checked through NanoDrop 1000 (Thermo Fisher Scientific) spectrophotometer and quality of isolated DNA was tested with Ethidium bromide stained agarose gel electrophoresis.

A 0.8 % agarose gel was prepared as follows for quantification of DNA samples.

1. 0.3 grams of agarose was weighed and added into a 250 ml conical flask.
2. 30 ml of 1X TBE was added to the 250 ml beaker and boiled gently in microwave oven till the agarose was melted (when the solution was crystal clear with no floating particulate matter).

3. Meanwhile, a gel-casting tray (CBS Scientific, USA) was washed thoroughly first with tap water and then with distilled water followed by rinsing with methanol.

4. The gel-casting tray was then placed in a sealing mechanism given by the supplier (xCBS Scientific, USA) and a comb (containing 8 lanes) was arranged in its slot on the gel-casting tray.

5. After agarose has cooled down substantially (to about 45 ºC), 2 µl of ethidium bromide solution (10 mg/ml of distilled water) was added and mixed gently. Gloves were used while handling ethidium bromide.

6. The melted agarose was poured in the gel casting tray carefully avoiding air bubbles and allowed to solidify (20-30 min).

7. After solidification, the gel was removed from its casting tray and put into a gel tank which was filled with 1X TBE buffer till the buffer reaches 0.5 cms above the gel surface (100 ml).

3.6.5.4 Genetic diversity of *P. grisea* isolates using SSR markers

A set of Simple Sequence Repeat (SSR) markers in Babu *et al.* (2013) were used for studying the genetic diversity of *P. grisea* isolates (Table 3.4.). Genomic DNA of all the isolates were diluted to 30 ng and used as template for amplification of SSR. Polymerase Chain Reaction (PCR) was performed in a final volume of 20 µl consisting of 0.25 mM of each dNTP, 1 unit of Taq DNA polymerase, 15-20 ng of DNA template, 0.3 mM of primer (Bioserve Biotechnologies India Pvt Ltd), 10X PCR buffer (containing 1.5 mM $Mgcl_2$) and sterile distilled water. The reaction mixture was vortexed and briefly centrifuged. The reactions were carried in a Thermal Cycler (Takara Bio Inc.). Thermal profile was 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 45-50°C for 45 sec, 72°C for 1 min with final extension of 72°C for 10 min. The PCR products were electrophoresed at 3% agarose gel in 1X TBE buffer for SSR primers.

The amplified fragments of each isolate were scored as 1, 2, 3 and 4… etc. Comigrating bands were considered homologous characters. Faint bands and bands showing variable levels of intensity were not considered for scoring and dendrogram was generated using Neighbor Joining (NJ) method in DARwin 6 software.

Table 3.4. List of SSR Primers and their sequences used in the study of molecular characterization of *P. grisea* isolates (Babu *et al.* 2013)

S. No.	Primer Name	Forward sequence(F) (5'-3')	Reverse sequence(R) (5'-3')
1.	MGM-1	TTTCGTACAATCCCGATG	GCGACAATGTCTTTTTTTTT
2.	MGM-21	GCAGGTGAGCAAACAGCAAGA	ATATCTCGTGCAGGCCGGT
3.	MGM454	GCAAATAACATAGGAAAACG	AGAAAGAGACAAAACACTGG
4.	MGM200	AAGCGTAAATGGCTCAATGC	GCTGATGTTGTTGCTGCTGT
5.	MGM436	GACCTTTATCGGATGCGTGT	CACACAGTGGCCATCTAACG
6.	MGM437	GCCCCTCAATAGATCGTCAA	ACTGCGGCATTTTAACCTGT
7.	MGM451	TTCTCAGTAGGCTTGGAATTGA	CTTGATTGGTGGTGGTGTTG
8.	Pyrms 7	GCAAATAACATAGGAAAACG	AGAAAGAGACAAAACACTGG
9.	Pyrms 15	TTCTTCCATTTCTCTCGTCTTC	CGATTGTGGGGTATGTGATAG
10.	Pyrms 37	ACCCTACCCCCACTCATTTC	AGGATCAGCCAATGCCAAGT
11.	Pyrms 41	AACGTGACAATGTGAGCAGC	GCCATGTTCTAAGGTGCTGAG
12.	Pyrms 45	CCACTTTATAGCCCACCCAGT	CTCTTTTCTCGCAGGAGGTG
13.	Pyrms 47	TCACATTTGCTTGCTGGAGT	AGACAGGGTTGACGGCTAAA
14.	Pyrms 59	TTCTCAGTAGGCTTGGAATTGA	CTTGATTGGTGGTGGTGTTG
15.	Pyrms 61	GAGGCAACTTGGCATCTACC	TGGATTACAGAGGCGTTCG
16.	Pyrms 63	TTGGGATCTTCGGTAAGACG	GCCGACAAGACACTGAATGA
17.	Pyrms 67	AGCAAGCAGGAGATGCAGAC	GTTTGGCTGGCAAGACAGTT
18.	Pyrms 77	GAAGTATTGCACACAAACAC	GCTTTCGGCAAGCCTAATC
19.	Pyrms 81	CCTTGTTTTCCCCCTGTGTA	TAGCCAAATGCCCATTATCC
20.	Pyrms 87	AGACTTGTTACTCGGGTCTTGA	CCAGATGTCACTCCCCTGTA
21.	Pyrms 93	CCTCGACTCCTTCACCAAAA	CGGAGAGCTCAGGAAGAGG
22.	Pyrms 99	CACCACTTTATGGCGCAGT	ACCTAGGTAGGTATACATGTTGTT
23.	Pyrms 107	GCAGCAAGCAGCAATATCAG	GTGGATATCGAAGGCCAAGG
24.	Pyrms 109	TACAGTGGGAGGGCAAAGAG	CCAGATCGAGAAGGGGGTAT
25.	Pyrms 125	CTCTCCGGCCAAGATTGA	GGTTGTTGGGAGAAAGAACG

3.6.5.5 Genetic diversity of *P. grisea* isolates using RAPD markers

A total of 8 RAPD primers were used for this study (Table 3.5). All primers were custom synthesized by Bioserve (Bioserve Biotechnologies India Pvt Ltd). PCR amplification was performed in a Thermal Cycler (Takara Bio Inc.). The reactions were performed in a 20 µL volume containing 50 ng of genomic DNA (1 µl), 0.5 U Taq DNA polymerase (0.1 µl), 0.2 mM of dNTPs (2 µl), 20 pmoL primer (2 µl), 10X reaction buffer (2 µl) and remaining volume was adjusted with sterile Milli-Q water. The PCR amplification was performed with the following thermal cycling conditions: initial denaturation for 5 min at 94ºC, followed by 35 cycles of denaturation at 94ºC for 30 sec, annealing at 32°C for 45 sec, extension at 72°C for 1 min and final extension at 72°C for 10 min. Amplicons were analyzed by electrophoresis in 2% per cent agarose gels in TBE buffer and visualized by staining with ethidium bromide and recorded with Gene Genius gel documentation system (Alpha Innotech, USA). The sizes of the PCR products were determined by comparison with standard 50 bp (Invitrogen by Thero Fisher) or 1 kb DNA ladder (GeneRuler) The amplified fragments of each isolate were scored as 1 (present) or 0 (absent). Comigrating bands were considered homologous characters. Faint bands and bands showing variable levels of intensity were not considered for scoring. Cluster analysis was done with Neighbor Joining (NJ) method in DARwin 6 software using the Un-weighted Pair Group Method with an Arithmetic average (UPGMA) algorithm.

Table 3.5. List of RAPD markers used and their sequences

S. No.	Marker	Sequence
1.	OPA-04	AA TC GG GC TG
2.	OPA-07	GA AA CG GG TG
3.	OPA-10	GT GA TC GC AG
4.	OPA-18	AG GT GA CC GT
5.	OPA-13	CA GC AC CC AC
6.	OPB-17	AG GG AA CG AG
7.	OPC-05	GA TC AG CG CC
8.	OPC-11	AA AG CT GC GG

3.7 EVALUATION OF NATIVE BACTERIAL BIO-CONTROL AGENTS AND FUNGICIDES AGAINST THE PATHOGEN *IN VITRO*

3.7.1 Isolation of Native Bacterial Bio-control Agents from Rhizosphere Soil

Isolation of native bacterial bio-control agents was done from healthy finger millet plant rhizosphere soil which was collected from different finger millet growing areas of Andhra Pradesh. Serial dilution plating technique was used to isolate the bacteria (Elad and Chet, 1983). A soil suspension was prepared from rhizosphere sample by shaking 1 g of soil sample in 10 ml of sterile distilled water and serial dilutions were made. From the 10^{-3} and 10^{-5} soil dilution 1 ml was transferred into sterile Petri dish under aseptic condition to which 15 ml of sterile Nutrient Agar medium was poured separately, gently rotated for uniform mixing of the soil dilution with the medium and incubated at room temperature (28 ± 2^0C) for 48 hours. These colonies were marked and sub-cultured. The pure cultures of these isolates were obtained by single colony method. In naming criteria of bacterial bio-control agents, the first letter indicates bacterial bio-control agent, next two letters indicates respective villages and the numbers indicates, number of bacterial bio-control isolates collected during the survey ((Ex: BLN-1= Bio-control agent Lingayapalle-1).

3.7.2 Evaluation of Native Bacterial Bio-control Agents against Pathogen

Evaluation of native bacterial bio-control agents was done for selected virulent pathogen isolate by using dual culture technique as developed by Morton and Stroube (1955). Autoclaved PDA was poured aseptically into sterilized Petri dishes and allowed to solidify. Mycelial discs of 5 mm diameter from the edge of actively growing culture of *P. grisea* and bacterial bio-control agents were streaked at periphery about 1 cm from the edge of the Petri dishes (9 cm diameter) on opposite sides. Three replications were maintained for each interaction. The Petri dishes containing Potato Dextrose Agar inoculated with *P. grisea* (mono culture) alone served as control. All the Petri dishes were incubated at (25 ± 1°C) for fifteen days. At the end of inoculation, the colony diameter of

the *P. grisea* was measured and the per cent inhibition of *P. grisea* was calculated by adopting the following formula given by Vincent (1927).

Percent inhibition over control was calculated by applying the following formula:-

$$\text{Percent Growth Inhibition} = \frac{C - T}{C} x100$$

C= Radial growth of *P. grisea* in monoculture

T= Radial growth of *P. grisea* in dual culture

3.7.3 Evaluation of Commercially available Fungicides against Pathogen

The experiment was conducted at Department of Plant Pathology, S. V. Agricultural College, Tirupati. The selected virulent pathogen isolate was cultured on PDA medium and incubated at 26±1 ºC. Nine systemic fungicides *viz.,* Azoxystrobin 23% SC, Kresoxy methyl 44.3% SC, Tebuconazole 25.9% EC, Difenconazole 25% EC, Tricyclazole 75% WP, Tebuconazole 50%+ Trifloxystrobin 25% WG, Azoxystrobin 11% + Difenconazole 18.3% SC, Carbendazim 50% WP, Isoprothiolane 40% EC, were evaluated *in vitro* for their antifungal activities against *Pyricularia grisea* using Poison food technique (Nene and Thapliyal, 1993). The requisite quantity of each fungicide on the basis of active ingredient (a.i) was calculated and thoroughly mixed with autoclaved and cooled (40-45ºC) PDA in conical flasks to obtain desired concentrations. 20 ml of PDA media was poured in sterilized Petri plates and to be solidified. Fungal discs of 5 mm in diameter from 7 days old culture was placed in the centre of the Petri dish containing PDA medium under aseptic condition, incubated at 26±1 ºC. Three replicated plates were used for each concentration of every fungicide. Three replicated PDA plates received no fungicides served as control. Diameter of the colonies on PDA with and without fungicides was measured from the bottom side of the Petri dishes. The colony diameter of the fungus pathogens on medium was recorded and per cent inhibition was calculated by using following formula (Vincent, 1927).

$$\text{Percent Growth Inhibition} = \frac{C-T}{C} x100$$

Where,

C= Growth of the test fungus in untreated control plates

T= Growth of the test fungus in a treated plates

3.7.4 Studies on Compatibility of Potential Native Bio-control Agents with effective Fungicides

The effective fungicides obtained from Poison food technique (Nene and Thapliyal, 1993) and effective native bacterial bio-control agent obtained from Dual culture technique (Morton and Stroube, 1955) were subjected to check the compatibility among those and was evaluated by Agar well diffusion method (Murray *et al*. 1995).

Agar well diffusion method was followed to determine the antibacterial activity of effective fungicides. Petri dishes were plated with melted NA which was added with 24 hours old culture of effective bacterial bio-control agent. Sterile cork borer was used to make a well on each of the plates. Stock solution of each effective fungicide was prepared at a desired concentration and were added into the wells. Allowed to diffuse at room temperature. Control experiments comprising inoculum without fungicides were set up. The plates were incubated at 37°C for 18-24 h. The diameter of the inhibition zone (mm) was recorded.

3.8 TO SCREEN THE GENOTYPES FOR THEIR RESISTANCE AGAINST BLAST

3.8.1 Screening of Genotypes against Blast

The screening trial for two seasons was conducted against finger millet blast caused by *Pyricularia grisea* during *Kharif*, 2020 and *Kharif* 2021 at Agricultural Research Station, Vizianagaram. The experiment was laid on a plot in Randomized Block Design, with 74 lines including VR 708 as check (Table 3.6), replicated two times which were sown in two rows of 3 m length with a spacing of 22.5 x 10 cm. During the crop growth phase, the recommended

agronomic and other package of practices were implemented. Ten plants were randomly chosen from each genotype/replication to record the observations. Leaf blast, neck blast and finger blast incidence was measured on 10 randomly selected plants. Infected plants were examined for lesion development and disease severity was assessed on the basis of lesion length by using 1 to 9 scale. Neck blast (1 to 5 scale) and finger blast (%) were calculated.

Table 3.6. Finger millet lines evaluated for leaf, neck and finger blast resistance

S. No.	Entry No.	S. No	Entry No.	S. No.	Entry No.	S. No.	Entry No.	S. No.	Entry No.
1.	GE-1	**18.**	GE-1	**35.**	GE-1	**52.**	GE-1	**69.**	GE-69
2.	GE-2	**19.**	GE-2	**36.**	GE-2	**53.**	GE-2	**70.**	GE-70
3.	GE-3	**20.**	GE-3	**37.**	GE-3	**54.**	GE-3	**71.**	GE-71
4.	GE-4	**21.**	GE-4	**38.**	GE-4	**55.**	GE-4	**72.**	GE-72
5.	GE-5	**22.**	GE-5	**39.**	GE-5	**56.**	GE-5	**73.**	GE-73
6.	GE-6	**23.**	GE-6	**40.**	GE-6	**57.**	GE-6	**74.**	GE-74
7.	GE-7	**24.**	GE-7	**41.**	GE-7	**58.**	GE-7		
8.	GE-8	**25.**	GE-8	**42.**	GE-8	**59.**	GE-8		
9.	GE-9	**26.**	GE-9	**43.**	GE-9	**60.**	GE-9		
10.	GE-10	**27.**	GE-10	**44.**	GE-10	**61.**	GE-10		
11.	GE-11	**28.**	GE-11	**45.**	GE-11	**62.**	GE-11		
12.	GE-12	**29.**	GE-12	**46.**	GE-12	**63.**	GE-12		
13.	GE-13	**30.**	GE-13	**47.**	GE-13	**64.**	GE-13		
14.	GE-14	**31.**	GE-14	**48.**	GE-14	**65.**	GE-14		
15.	GE-15	**32.**	GE-15	**49.**	GE-15	**66.**	GE-15		
16.	GE-16	**33.**	GE-16	**50.**	GE-16	**67.**	GE-16		
17.	GE-17	**34.**	GE-17	**51.**	GE-17	**68.**	GE-17		

3.9 TO STUDY THE INFLUENCE OF WEATHER FACTORS AND DISEASE DEVELOPMENT

3.9.1 Correlation and Regression Analysis of Disease Severity and Weather Factors

To study the influence of weather factors the epidemiological work was carried out under field conditions during *Kharif* 2020 and *Kharif* 2021. The selected susceptible variety VR 708 was sown under three different dates (July 1st and 2nd fortnights and August 1st fortnight) of sowing. Weather data for each growing season was collected from the meteriologicl observatory, located at Agricultural Research Station, Vizianagaram.

The data was recorded at weekly intervals on PDI by using 1-9 scale. The correlation and multiple regression analysis were done as per the standard methods to work out relationship between weather factors *viz.*, maximum and minimum temperature, maximum and minimum relative humidity, rain fall (mm) and rainy days. The weather factors considered for correlation studies are mentioned below.

X_1 : Maximum temperature (^{0}C)

X_2 : Minimum temperature (^{0}C)

X_3 : Maximum relative humidity (%)

X_4 : Minimum relative humidity (%)

X_5 : Rainfall (mm)

X_6 : Rainy days

The correlation coefficient was calculated by

$$\text{Correlation coefficient (r)} = \frac{\sum xy - \frac{\left(\sum x\right)\left(\sum y\right)}{n}}{\sqrt{\left(\sum x^2 - \frac{\left(\sum \ \right)^2}{n}\right)}\sqrt{\sum y^2 - \frac{\left(\sum y\right)^2}{n}}}$$

Multiple regression equation is

$$Y = \alpha+\beta_1 x_1+ \beta_2 x_2 +\ldots\ldots\ldots+\beta_k x_p + e$$

where α = intercept

and $\beta_1, \beta_2 \ldots \beta_k$ are the partial regression coefficient and e = the error term

3.10 INTEGRATED DISEASE MANAGEMENT FOR BLAST

The field experiment was conducted during *Kharif* 2021-22 in Randomized Block Design (RBD) with nine treatments and each treatment replicated thrice using susceptible finger millet variety VR-708 at S. V. Agricultural College, Tirupati and Agricultural Research Station, Vizianagaram. The treatments included seed treatment and foliar application of effective fungicide and bacterial bio-control agent. The treatments were tested alone and in different combinations with an untreated control. The data were recorded as Per cent Disease Index (PDI) and grain yield. Twenty five day old seedlings were transplanted in main field at a spacing of 22.5 x 10 cm in 4 x 3m size plot. The fertilizer dose of 60 kg N, 30 kg P_2O_5, and 30 kg K_2O was followed. The treatments were applied at sowing time and at the initial stage of symptom development as mentioned in the table. Treatment details were given in Table 3.7.

Table 3.7. Details of treatments used in integrated disease management of finger millet blast

Treatment No.	Treatments
T_1	Seed treatment with Tebuconazole 50% + Trifloxystrobin 25% WG at 0.4 g kg^{-1} seed
T_2	Seed treatment with *Pseudomonas fluorescence* @ 10 g kg^{-1} seed
T_3	One spray with Tebuconazole 50% + Trifloxystrobin 25% WG at 0.4 g l^{-1} (at 50% flowering)
T_4	One spray with *Pseudomonas fluorescence* @ 10 g l^{-1} (at 50% flowering)
T_5	Two sprays with Tebuconazole 50% + Trifloxystrobin 25% WG 0.4 g l^{-1} (first spray at initiation of symptoms and second spray at flowering stage)

T_6	Two sprays with *Pseudomonas fluorescence* @ 10 g l^{-1} (first spray at initiation of symptoms and second spray at flowering stage)
T_7	Seed treatment with Tebuconazole 50% + Trifloxystrobin 25% WG 0.4 g kg^{-1} seed + one spray with Tebuconazole 50% + Trifloxystrobin 25% WG 0.4 g l^{-1} (at 50% flowering)
T_8	Seed treatment with *Pseudomonas fluorescence* @ 10 g kg^{-1} seed + One spray with *Pseudomonas fluorescenc*e @ 10 g l^{-1} (at 50% flowering)
T_9	Control

3.11 STATISTICAL ANALYSIS

In the present investigation, all the laboratory experiments were conducted in complete randomized design and field experiments were conducted in RBD design. The data obtained from all the experiments were statistically analyzed by following the standard methods (Gomez and Gomez, 1976). An analysis of variance (ANOVA) was performed to determine statistical significance of the factors and their interactions using OPSTAT (CCSHAU, Hisar), WASP (Web Based Agricultural Statistical Software Package, ICAR-GOA) and SPSS 16.0 (Statistical Package for the Social Sciences) statistical package.

Chapter – IV

Results and Discussion

Chapter-IV

RESULTS AND DISCUSSION

Results obtained on the "Variability studies on *Pyricularia grisea* [(Cooke) Sacc.] incitant of blast disease in finger millet and its management" are presented and discussed in this chapter.

4.1 TO CONDUCT SURVEY FOR OCCURRENCE AND DISTRIBUTION OF BLAST DISEASE, COLLECTION OF ISOLATES AND NATIVE BACTERIAL BIO-CONTROL AGENTS IN MAJOR FINGER MILLET GROWING AREAS OF ANDHRA PRADESH

4.1.1 Survey for the Incidence of Finger Millet Blast in Major Finger Millet Growing Areas of Andhra Pradesh

Based on the occurrence of disease symptoms (Plate 1 to 3), finger millet blast Per cent disease incidence (PDI) was recorded in three farmer fields of each village, as three villages/ mandal and two mandals in each district *viz.*, YSR Kadapa, Chittoor, Anantapur, Kurnool, Prakasam, Guntur and Vizianagaram districts of Andhra Pradesh during *Kharif* 2020 and *Kharif* 2021(Table 4.1) (Plate 4 and Plate 5).

Among the seven districts surveyed, the pooled data indicated that the highest mean Per cent disease incidence (PDI) of 56.06% was recorded in Vizianagaram district followed by Chittoor, YSR Kadapa and Kurnool, with mean Per cent disease incidence of 42.77%, 22.60% and 20.07% respectively. The lowest mean Per cent disease incidence of 7.85 was recorded in Prakasam district followed by Anantapur (19.63) and Guntur (19.85) districts (Table 4.2) (Fig. 4.1).

The highest mean Per cent blast incidence of 56.59% was recorded in Salur mandal of Vizianagaram district in the range of 53.86 to 59.20% during 2020 and 52.35 to 59.65% during 2021 followed by Kurupam mandal of Vizianagaram district with 55.53% incidence in the range of 54.63% to 57.56% during 2020 and 51.59% to 56.87% during 2021, Palamaneru mandal with 53.92% in the range of 49.25 to 57.52% and 50.35 to 58.65% during 2020 and

Table 4.1. Survey for the occurrence and distribution of finger millet blast incidence in major finger millet growing areas of Andhra Pradesh

S. No.	Districts	Mandals	Villages	Latitude	Longitude	Varieties	Soil type	Farming situation	Mean per cent disease incidence	
									Kharif 2020	*Kharif* 2021
1.	YSR Kadapa	Vallur	Lingayapalle	14°34'19"	78°44'41"	Local	Red loam	Rainfed	49.23	48.65
			Vallur	14°19'59"	78°25'54"	Local	Red loam	Rainfed	11.15	13.25
			Goturu	14°33'36"	78°44'14"	Vakula	Red loam	Rainfed	8.05	9.23
		Kamalapuram	Kamalapuram	14°35'57"	78°39'51"	Tirumala	Red loam	Rainfed	30.13	26.75
			Ramachandrapuram	14°39'40"	78°38'29"	Local	Red loam	Rainfed	15.03	16.35
			T. Sadipirala	14°35'08"	78°37'16"	Local	Red loam	Rainfed	20.02	23.35
2.	Chittoor	Palamaneru	Palamaneru	13°11'51"	78°45'22"	Vakula	Red loam	Rainfed	57.52	58.65
			Moram	13°10'21"	78°41'33"	Vakula	Red loam	Rainfed	53.46	54.32
			Kurmoi	13°09'57"	78°44'06"	Vakula	Red loam	Rainfed	49.25	50.35
		Kuppam	Gonugur	12°42'54"	78°19'48"	Vakula	Red loam	Rainfed	32.78	30.32
			Jarugu	12°73'55"	78°16'57"	Vakula	Red loam	Rainfed	33.78	30.56
			Palarlapalle	12°42'55"	78°19'20"	Vakula	Red loam	Rainfed	29.45	32.86
3.	Anantapur	Madakasira	Govindapuram	13°88'28"	77°19'97"	Tirumala	Red loam	Rainfed	17.13	16.35
			Jadrahalli	13°53'34"	77°15'29	Vakula	Red loam	Rainfed	18.14	19.35
			Haresamudram	13°55'00"	77°16'03"	Vakula	Red loam	Rainfed	11.01	10.35
		Gudibanda	Morubagal	13°58'18"	77°02'12"	Vakula	Red loam	Rainfed	17.78	18.35
			Gudibanda	13°58'13"	77°06'30"	Local	Red loam	Rainfed	28.96	29.34
			Muthukur	13°58'08"	77°03'55"	Vakula	Red loam	Rainfed	23.19	25.65

4.	Kurnool	Peapally	Peapally	15º14'25"	77º44'19"	Local	Red loam	Rainfed	14.28	15.65
			Vengalampalle	15º15'05"	77º46'06"	Local	Red loam	Rainfed	15.54	14.29
			Pothidoddi	15º12'19"	77º43'00"	Vakula	Red loam	Rainfed	9.09	8.35
		Pattikonda	Pattikonda	15º24'01"	77º30'25"	Vakula	Red loam	Rainfed	26.22	23.65
			Chakkaralla	15º23'43"	77º34'40"	Vakula	Red loam	Rainfed	33.23	32.56
			Jutur	15º29'10"	77º28'24"	Local	Red loam	Rainfed	24.32	23.65
5.	Prakasam	Racherla	Anumalapalli	15º29'09"	78º58'04"	Vakula	Red loam	Rainfed	7.07	9.78
			Racherla	15º27'54"	78º57'49"	Vakula	Red loam	Rainfed	9.01	8.65
			Somidevipalli	15º27'27"	79º02'02"	Vakula	Red loam	Rainfed	5.02	4.65
		Komarolu	Taticherla	15º2s3'04"	79º02'47"	Vakula	Red loam	Rainfed	9.05	8.78
			Chintalapalli	15º16'41"	79º03'13"	Local	Red loam	Rainfed	5.85	6.82
			Komarolu	15º15'57"	78º59'50"	Vakula	Red loam	Rainfed	9.21	10.35
6.	Guntur	Veldurthi	Veldurthi	16º20'47"	79º21'50"	Vakula	Red loam	Rainfed	13.14	11.56
			Uppalapadu	16º21'28"	79º24'05"	Local	Red loam	Rainfed	12.28	13.65
			Gundlapadu	16º21'50"	79º20'21"	Vakula	Red loam	Rainfed	48.21	46.98
		Macherla	Macherla	16º28'51"	79º25'46"	Vakula	Red loam	Rainfed	17.24	16.68
			Kothapalli	16º29'46"	78º24'30"	Vakula	Red loam	Rainfed	16.07	18.96
			Koppunur	16º29'22"	79º19'48"	Vakula	Red loam	Rainfed	11.13	12.35
7.	Vizianagaram	Kurupam	Gumma	18º95'17"	83º64'33"	VR 847	Red loam	Rainfed	57.56	56.75
			G. Sivada	18º91'52"	83º74'25"	VR 847	Red loam	Rainfed	54.63	51.59
			Gummidiguda	18º83'65"	83º77'45"	VR 847	Red loam	Rainfed	55.78	56.87
		Salur	Neliparthi	18º50'21"	83º18'45"	VR 847	Red loam	Rainfed	56.82	57.68
			Salur	18º53'02"	83º17'85"	VR 847	Red loam	Rainfed	59.20	59.65
			Kurmarajupeta	18º53'73"	83º21'15"	VR 847	Red loam	Rainfed	53.86	52.35

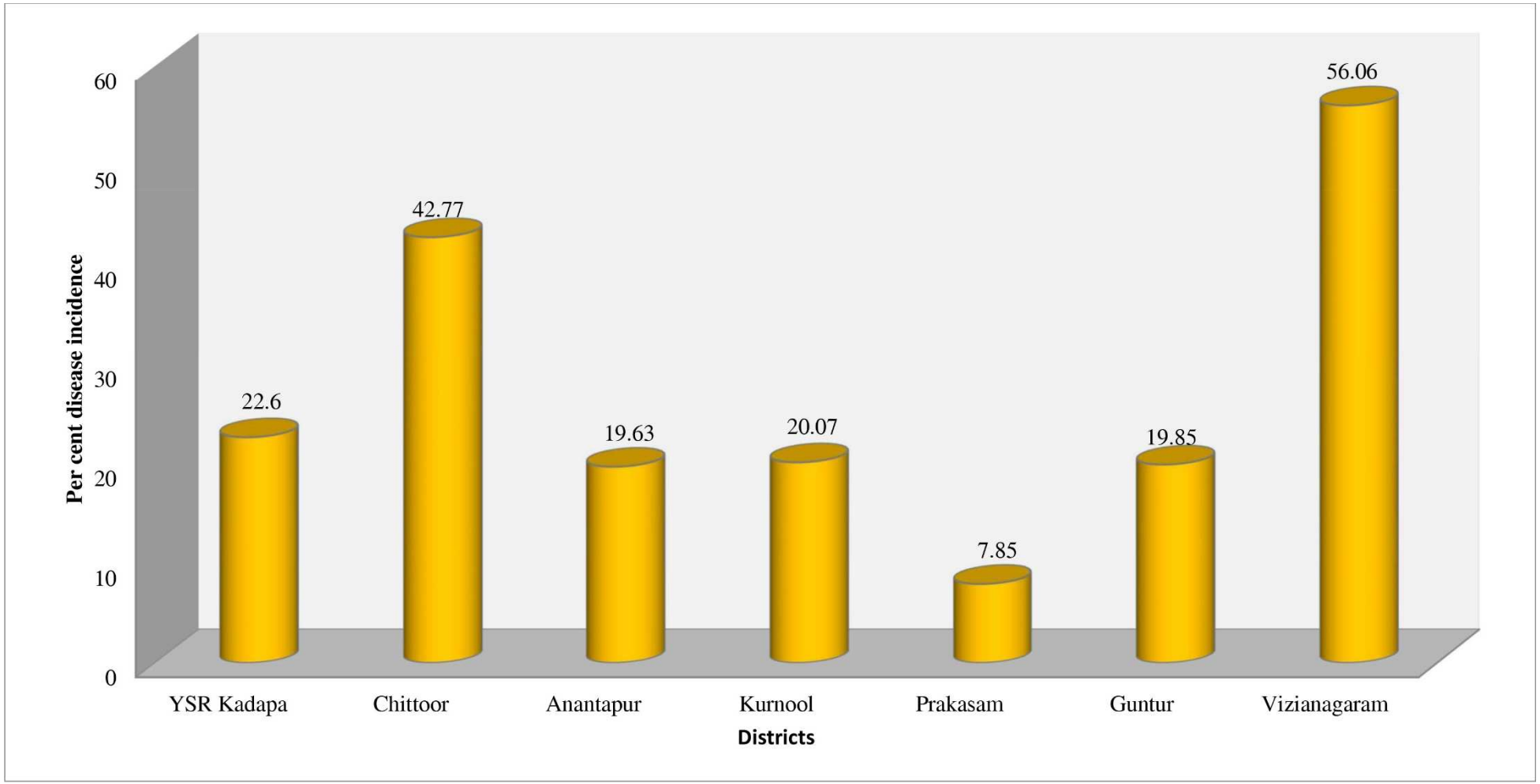


Fig. 4.1. Mean disease severity of finger millet blast in major finger millet growing districts of Andhra Pradesh

Plate 1. Leaf blast symptoms - A) Small specks to spindle shaped lesions

Plate 1. Leaf blast symptoms - B) Spindle shaped lesions with brown margin


Plate 2. Neck blast symptoms


Plate 3. Finger blast symptoms

2021 respectively. Kuppam mandal with 31.62% range of 29.45 to 33.78% and 30.32 to 32.86% during 2020 and 2021 respectively. Pattikonda mandal with 27.27% range of 24.32 to 33.23% and 23.65-32.56% during 2020 and 2021 respectively. Veldurthi mandal with 24.30% range of 12.28 to 48.21% and 11.56 to 46.98% during 2020 and 2021 respectively. Gudibanda mandal with 23.87% range of 17.78 to 28.96% and 18.35 to 29.34% during 2020 and 2021 respectively. Vallur mandal with 23.26% range of 8.05 to 49.23% and 9.23 to 48.65% during 2020 and 2021 respectively and Kamalapuram mandal with 21.93% range of 15.03 to 30.13% and 16.35 to 26.75% in 2020 and 2021 respectively of YSR Kadapa district. The lowest incidence with 7.36% range of 5.02 to 9.01% and 4.65 to 9.78% was noticed in Racherla mandal of Prakasam district during 2020 and 2021 respectively. This was followed by Komarolu mandal with 8.34% range of 5.85 to 9.21% and 6.82 to 10.35% during 2020 and 2021respectively of Prakasam district. Peapally mandal with 12.86% the range of 9.09 to 15.54% and 8.35 to 15.65% during 2020 and 2021 respectively. Madakasira mandal with 15.38% range of 11.01 to 18.14% and 10.35 to 19.35% during 2020 and 2021 respectively and Macherla mandal with 15.40% range of 11.13 to 17.24% and 12.35 to 18.96% range during 2020 and 2021 respectively of Guntur district (Table 4.3) (Fig. 4.2). The present results were in agreement with the findings of Rao (1990) who surveyed in eight locations of Bangalore, Kolar and Tumkur districts of Karnataka, and reported that an increase of 1% infection in neck and finger resulted in a corresponding increase of 0.32 and 0.084% yield loss and 6.75 to 87.54% loss in grain yield. Similarly, Kumar *et al.* (2005) reported the maximum neck (13-16%) and finger blast (42-55%) incidence in surveyed locations of Tumkur district of Karnataka. The same way Senthil *et al.* (2012) also reported that the finger millet blast was the most devastating disease affecting different aerial parts of the plant at all stages in Tamil Nadu (India). Prajapati (2013) surveyed in different locations of Gujarat and reported the loss of 35.78 grain yield and 43.72 per cent fodder yield due to the blast disease.

Table 4.2. Finger millet blast disease incidence in major finger millet growing districts of Andhra Pradesh

S. No.	District	Range of Per cent Disease Incidence		Mean Per cent Disease Incidence (pooled)
		Kharif 2020	*Kharif* 2021	
1.	YSR Kadapa	8.05-49.23	9.23-48.65	22.60
2.	Chittoor	29.45-57.52	30.32-58.65	42.77
3.	Anantapur	11.01-28.96	10.35-29.34	19.63
4.	Kurnool	9.09-33.23	8.35-35.56	20.07
5.	Prakasam	5.02-9.21	4.65-10.35	7.85
6.	Guntur	11.13-48.21	11.56-46.98	19.85
7.	Vizianagaram	53.86-59.20	51.59-59.65	56.06

Table 4.3. Finger millet blast incidence in major finger millet growing mandals of Andhra Pradesh

S. No.	Districts	Mandals	Range of Per cent Disease Incidence		Mean Per cent disease incidence (pooled)
			Kharif 2020	*Kharif* 2021	
1.	**YSR Kadapa**	Vallur	8.05-49.23	9.23-48.65	23.26
		Kamalapuram	15.03-30.13	16.35-26.75	21.93
2.	**Chittoor**	Palamaneru	49.25-57.52	50.35-58.65	53.92
		Kuppam	29.45-33.78	30.32-32.86	31.62
3.	**Anantapur**	Madakasira	11.01-18.14	10.35-19.35	15.38
		Gudibanda	17.78-28.96	18.35-29.34	23.87
4.	**Kurnool**	Peapally	9.09-15.54	8.35-15.65	12.86
		Pattikonda	24.32-33.23	23.65-32.56	27.27
5.	**Prakasam**	Racherla	5.02-9.01	4.65-9.78	7.36
		Komarolu	5.85-9.21	6.82-10.35	8.34
6.	**Guntur**	Veldurthi	12.28-48.21	11.56-46.98	24.30
		Macherla	11.13-17.24	12.35-18.96	15.40
7.	**Vizianagaram**	Kurupam	54.63-57.56	51.59-56.87	55.53
		Salur	53.86-59.20	52.35-59.65	56.59

Plate 4. Leaf blast severity in different farmers fields of Andhra Pradesh

A : 18°53'02", 83°17'85", Salur, Salur mandal, Vizianagaram
B : 13°10'21", 78°41'33", Moram, Palamaneru, Chittoor

Plate 5. Neck blast severity in different farmers fields of Andhra Pradesh

A : 13°11'51", 78°45'22", Palamaneru, Palamaneru mandal, Chittoor
B : 18°50'21", 83°18'45", Neliparthi, Salur mandal, Vizianagaram

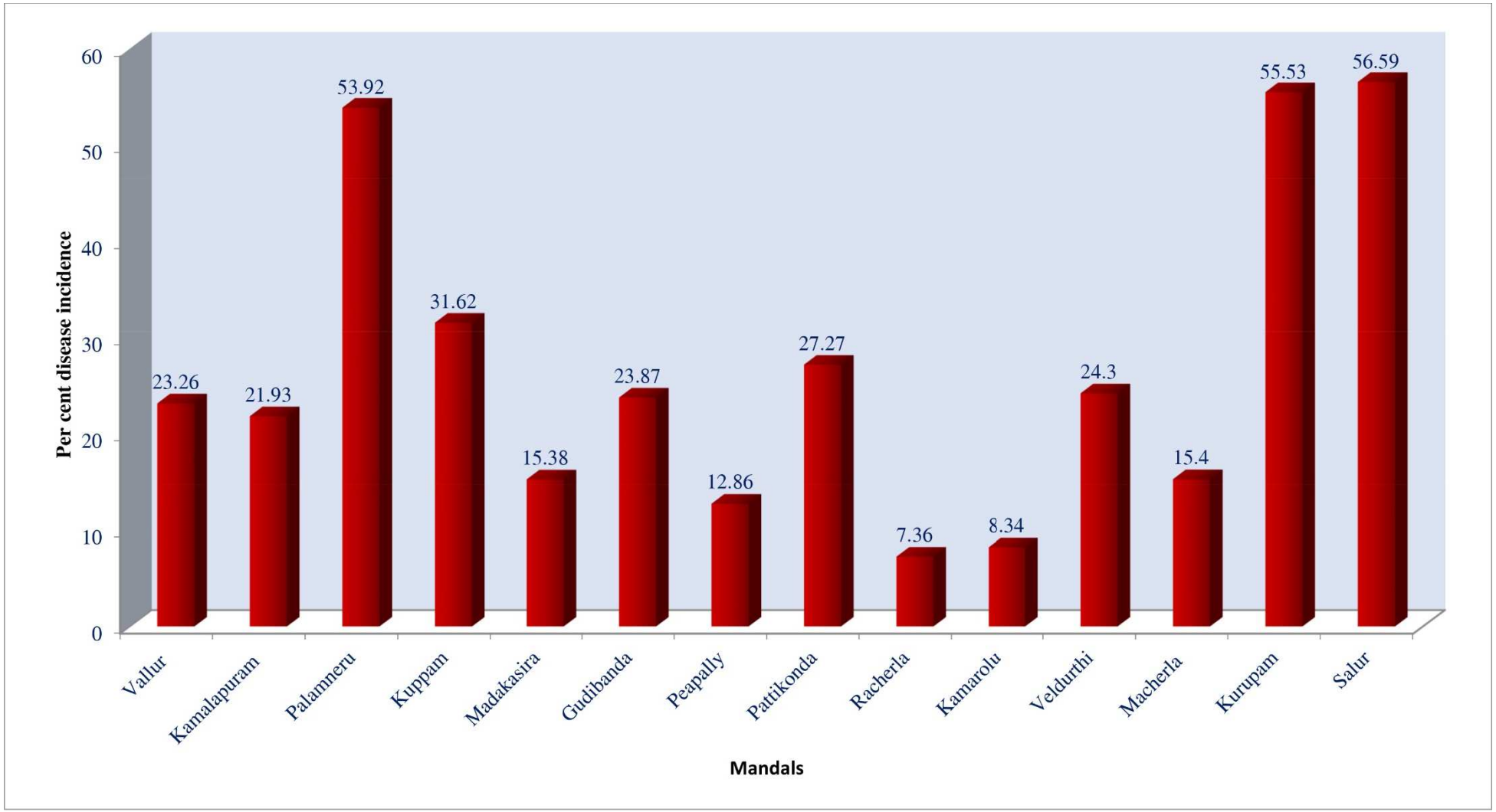


Fig. 4.2. Mean disease severity of finger millet blast in mandals of Andhra Pradesh

4.1.2 Collection and Isolation of *P. grisea* Isolates from Blast Diseased Samples

Survey during *Kharif* 2020-21, infected blast samples were collected from major finger millet growing areas of Andhra Pradesh by conducting survey during *Kharif* 2021.The pathogen was identified as *P. grisea* and about twenty monoconidial isolates of *P. grisea* (Table 4.4) were maintained based on the microscopic observations of conidia (Plate 6 and Plate 7).

Table 4.4. List of *P. grisea* isolates collected from major finger millet growing areas of Andhra Pradesh

S. No.	Districts	Mandals	Villages	Isolates
1.	YSR Kadapa	Vallur	Lingayapalle	LNG-1
			Vallur	VAL-1
		Kamalapuram	Kamalapuram	KML-1
2.	Chittoor	Palamaneru	Moram	MOR-1
			Palamaneru	PAL-1
		Kuppam	Gonugur	GON-1
			Jarugu	JRG-1
3.	Anantapur	Madakasira	Govindapuram	GVN-1
			Jadrahalli	JDR-1
		Gudibanda	Morubagal	MRB-1
			Gudianda	GDB-1
4.	Vizianagaram	Vizianagaram	Vizianagaram	VIZ-1
			Vizianagaram	VIZ-2
5.	Kurnool	Peapally	Peapally	PEA-1
		Pattikonda	Chakkaralla	CHK-1
6.	Prakasam	Racherla	Jutur	JTR-1
			Anumalapalli	ANP-1
		Komarolu	Taticherla	TTC-1
7.	Guntur	Veldurthi	Veldurthi	VED-1
		Macherla	Koppunur	KPN-1

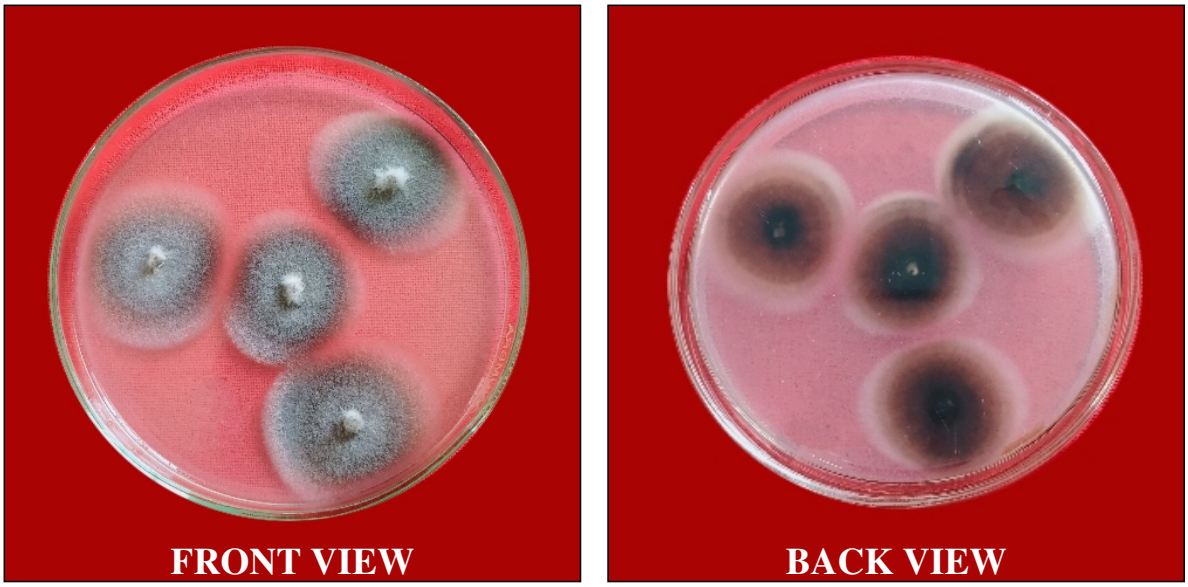


Plate 6. Pure culture of *P. grisea* isolated from leaf blast samples

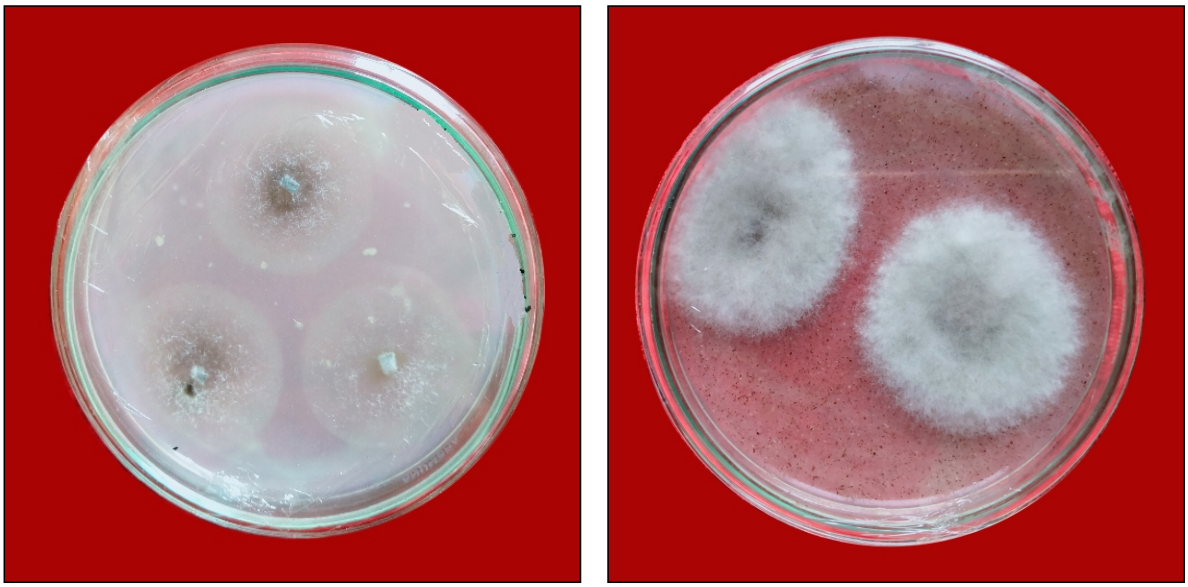


Plate 7. Pure culture of *P. grisea* isolated from (A) Neck blast (B) Finger blast samples

4.1.3 Pathogenicity Test

The susceptible variety VR 708 was used as a susceptible check to establish the Koch's postulates. There was an appearance of typical spindle shaped blast lesions with brown margin on the leaves was reported upon artificial inoculation. Four to six days were required for symptoms expression when relative humidity was maintained. Out of twenty isolates VIZ-1 showed maximum disease severity and remaining nineteen isolates showed comparatively less disease severity. So the isolate VIZ-1 was considered as virulent isolate in further study. No symptoms were observed on non-inoculated plants. Same pathogen was isolated from the infected plants which proved the establishing Koch's postulates.

4.2 TO STUDY THE CULTURAL, MORPHOLOGICAL AND MOLECULAR VARIABILITY AMONG THE ISOLATES OF PATHOGEN

4.2.1 Cultural Variability among *P. grisea* Isolates

Variation in colony characteristics *viz*., colony colour, growth pattern, elevation (flat/elevated growth), sectored or non-sectored, zonations and wrinkles (Plate 8) among the isolates of *P. grisea* are presented in Table 4.4. Cultural characteristics varied greatly with isolates and the medium used. A range of colour variation was observed among the pathogen isolates. Observations were recorded for the colony characters based on the metabolites produced in the medium (Table 4.5) (Fig. 4.3).

The sporulating ability of the field isolates were varied. The degree of sporulation was compared with the growth patterns of the pathogen. It was observed that the isolates which were that were greyish green and sector forming produced more spores. The under surface of the colonies were usually brown or black. Colony texture or surface of all the isolates were rough to smooth with trace to abundant sporulation. In majority of the isolates, the maximum sporulation was confined to sectored region.

Colony colour

Change in colony colour was recorded fourteen days after inoculation. In the present study, colony colour among 20 isolates varied as blackish grey, greyish green and brown. Out of 20 isolates, 7 isolates (LNG-1, VAL-1, KML-1, MOR-1, GON-1, TTC-1 and KPN-1) appeared as blackish grey, 12 isolates (PAL-1, JRG-1, GVN-1, JDR-1, MRB-1, GDB-1, VIZ-1, VIZ-2, PEA-1, JTR-1, ANP-1, VED-1) appeared as greyish green and one isolate (CHK-1) as brown colour (Table 4.5)

Growth pattern

Growth pattern of twenty isolates were observed after 14 days of inoculation. All the 20 isolates showed circular growth pattern but elevation of the mycelium differs from flat to raised at the sectored part. Out of 20 isolates, 17 isolates (LNG-1, VAL-1, KML-1, PAL-1, GON-1, JRG-1, MRB-1, GDB-1, VIZ-1, VIZ-2, PEA-1, CHK-1, JTR-1, ANP-1, TTC-1, VED-1 and KPN-1) showed flat and two isolates (MOR-1 and GVN-1) were raised at the sectored portion and one isolate JDR-1 raised at the central part (Table 4.5) (Plate 9).

Sectoring, zonation and wrinkles formation

Sector formation was observed in 16 isolates (VAL-1, MOR-1, PAL-1, GON-1, JRG-1, MRB-1, GDB-1, VIZ-1, VIZ-2, PEA-1, CHK-1, JTR-1, ANP-1, TTC-1, VED-1 and KPN-1) and four isolates (LNG-1, KML-1, GVN-1, and JDR-1) didn't formed any sectors. Zonation was seen in 18 isolates (LNG-1, VAL-1, KML-1, MOR-1, PAL-1, GON-1, JRG-1, GVN-1, JDR-1, MRB-1, GDB-1, VIZ-1, VIZ-2, CHK-1, JTR-1, ANP-1, VED-1 and KPN-1) and two isolates (PEA-1 and TTC-1) not produced zonation. None of the isolates formed wrinkles (Table 4.5).

Radial growth

The observation on radial growth of *P. grisea* isolates up to 14 days of inoculation revealed that maximum radial growth of 90 mm was observed in 14 isolates (VAL-1, KML-1, MOR-1, PAL-1, JRG-1, MRB-1, GDB-1, VIZ-1, VIZ-2, CHK-1, JTR-1, ANP-1, TTC-1 and KPN-1) which were statistically on par

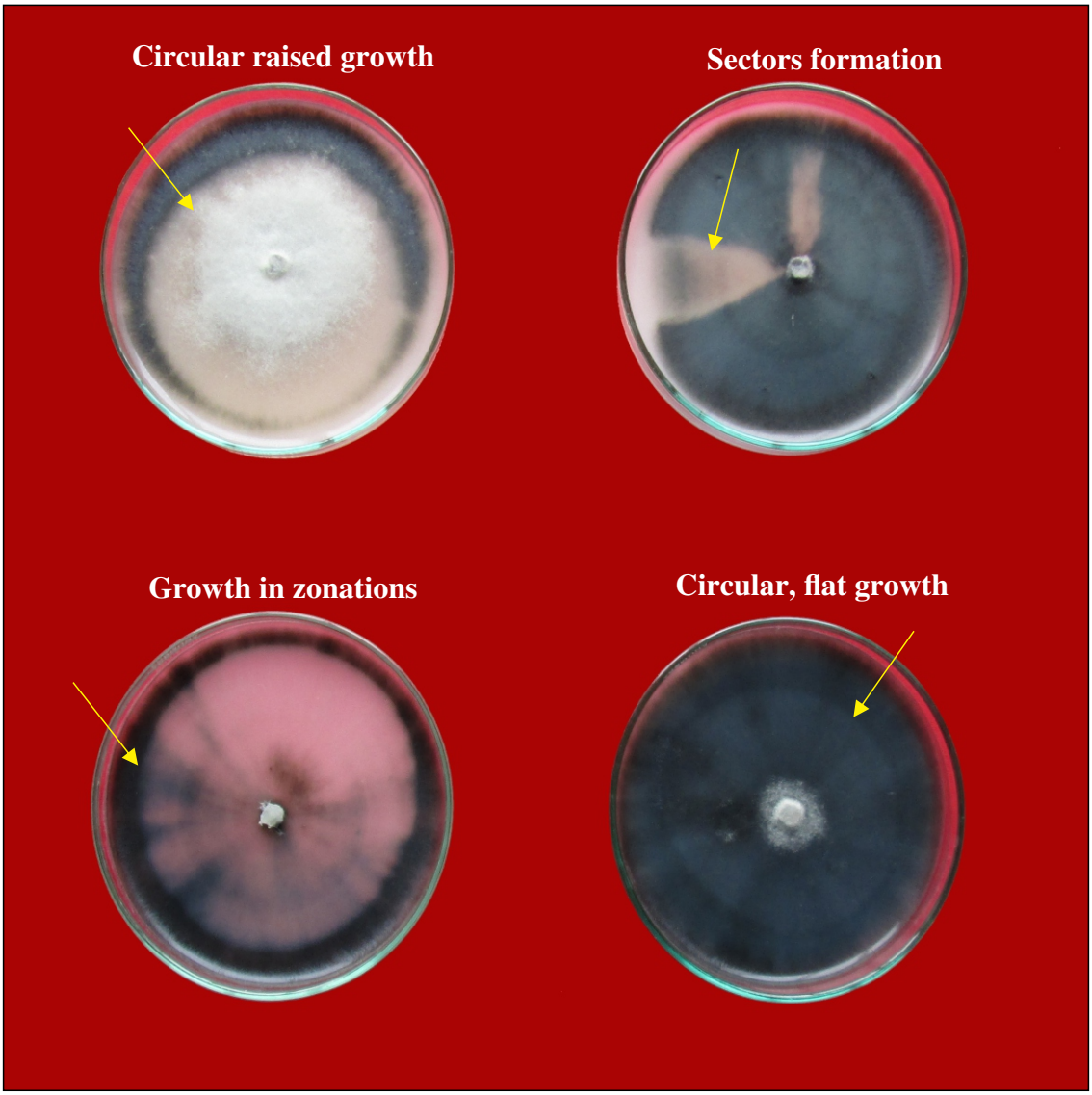


Plate 8. General cultural characteristics of *Pyricularia grisea* isolates on OMA at 14 days after inoculation

with each other. However, medium radial growth was observed in LNG-1 (88 mm), PEA-1 (87 mm), VED-1 (87 mm) and GON-1 (84 mm). Whereas, least growth of 76 and 78 mm was observed in GVN-1 and JDR-1 respectively. The growth rate among the isolates was in the range of 5.71 mm/day (GVN-1) to 6.43 mm/day (VAL-1) (Table 4.5). Diversity in cultural characters such as colony colour, elevation and growth pattern was noticed among the isolates. The growth pattern of the pathogen was directly linked to the sporulation. The isolates which were greyish green and sector forming produced more spores. The under surface of the colonies was usually brown or black. The maximum sporulation was seen at sectored region. Connection between sporulation and aerial growth was also noticed as defined by Ramakrishnan (1948). Babu (2011) described the isolates which showed vegetative growth as greyish green colour produced more spores and those with poor vegetative growth were poor spore producers. The diversity in colour and pattern of growth among isolates maybe due to varied growth stages of the spore as reported by Tracyline *et al*., (2021). Bhaskar (2018) found that isolates of *P. grisea* varied in the pigmentation and with formation of sectors, zonation and wrinkle. Aravind *et al*. (2020) found significant differences among the isolates of *P. grisea* which formed of sector, zonation and wrinkles and reported poor to excellent sporulation on solid media.

4.2.1.2 Influence of different culture media on growth and sporulation of *P. grisea*

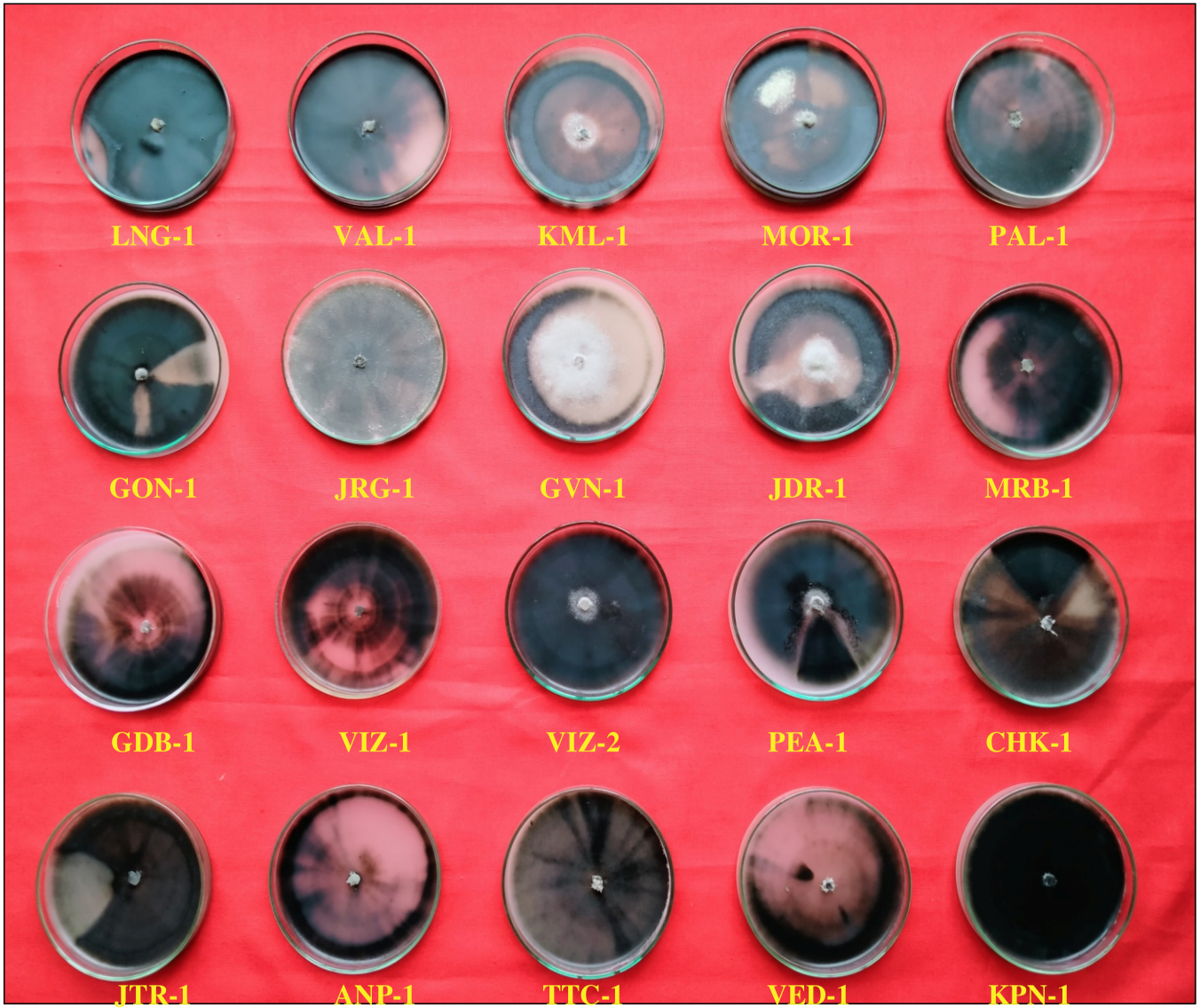
Total of eight solid media *viz*., Potato Dextrose Agar (PDA), Rice Leaf extract Agar (RLA), V8 juice Agar (V8), Finger millet Leaf extract Agar (FLA), Potato Sucrose Agar (PSA), Rechard's synthetic Agar (RA), Oat meal Sucrose Agar (OSA) and Oat Meal Agar (OMA) were used to evaluate the growth and sporulation of *P. grisea* virulent isolate VIZ-1.

Colony colour

Greyish white colony growth was observed in PDA, V8 and PSA and OSA. *P. grisea* in RLA was found to be blackish brown colour. Black coloured colony was observed in finger millet FLA. White and greyish green colony

Table 4.5. Cultural variability among *P. grisea* isolates on Oat Meal Agar

S. No.	Isolate	Colony colour	Growth pattern		Sectors	Zonations	Wrinkles	Radial growth (mm)	
			Form	Elevation				Average radial growth (mm)	Growth rate / day
1.	LNG-1	Blackish grey	Circular	Flat	No	Yes	No	88	6.26
2.	VAL-1	Blackish grey	Circular	Flat	Yes	Yes	No	90	6.36
3.	KML-1	Blackish grey	Circular	Flat	No	Yes	No	90	6.28
4.	MOR-1	Blackish grey	Circular	Raised at sector part	Yes	Yes	No	90	6.29
5.	PAL-1	Greyish green	Circular	Flat	Yes	Yes	No	90	6.43
6.	GON-1	Blackish grey	Circular	Flat	Yes	Yes	No	84	6.02
7.	JRG-1	Greyish green	Circular	Flat	Yes	Yes	No	90	6.37
8.	GVN-1	Greyish green	Circular	Raised at sector part	No	Yes	No	76	5.71
9.	JDR-1	Greyish green	Circular	Raised at centre	No	Yes	No	78	5.79
10.	MRB-1	Greyish green	Circular	Flat	Yes	Yes	No	90	6.43
11.	GDB-1	Greyish green	Circular	Flat	Yes	Yes	No	90	6.39
12.	VIZ-1	Greyish green	Circular	Flat	Yes	Yes	No	90	6.40
13.	VIZ-2	Greyish green	Circular	Flat	Yes	Yes	No	90	6.43
14.	PEA-1	Greyish green	Circular	Flat	Yes	No	No	87	6.26
15.	CHK-1	Brown	Circular	Flat	Yes	Yes	No	90	6.36
16.	JTR-1	Greyish green	Circular	Flat	Yes	Yes	No	90	6.37
17.	ANP-1	Greyish green	Circular	Flat	Yes	Yes	No	90	6.38
18.	TTC-1	Blackish gray	Circular	Flat	Yes	No	No	90	6.36
19.	VED-1	Greyish green	Circular	Flat	Yes	Yes	No	87	6.26
20.	KPN-1	Blackish gray	Circular	Flat	Yes	Yes	No	90	6.39
							C.D	2.852	
							SE(m)	0.923	
							SE(d)	0.765	
							C.V.	2.142	



LNG-1 : Lingayapalle
VAL-1 : Vallur
KML-1 : Kamalapuram
MOR-1 : Moram
PAL-1 : Palamaneru
GON-1 : Gonugur
JRG-1 : Jarugu
GVN-1 : Govindapuram
JDR-1 : Jadrahalli
MRB-1 : Morubagal
GDB-1 : Gudibanda
VIZ-1 : Vizianagaram
VIZ-2 : Vizianagaram
PEA-1 : Peapally
CHK-1 : Chakkaralla
JTR-1 : Jutur
ANP-1 : Anumalapalli
TTC-1 : Taticherla
VED-1 : Veldurthi
KPN-1 : Koppunur

Plate 9. Cultural variability among the isolates of *P. grisea* on Oat Meal Agar

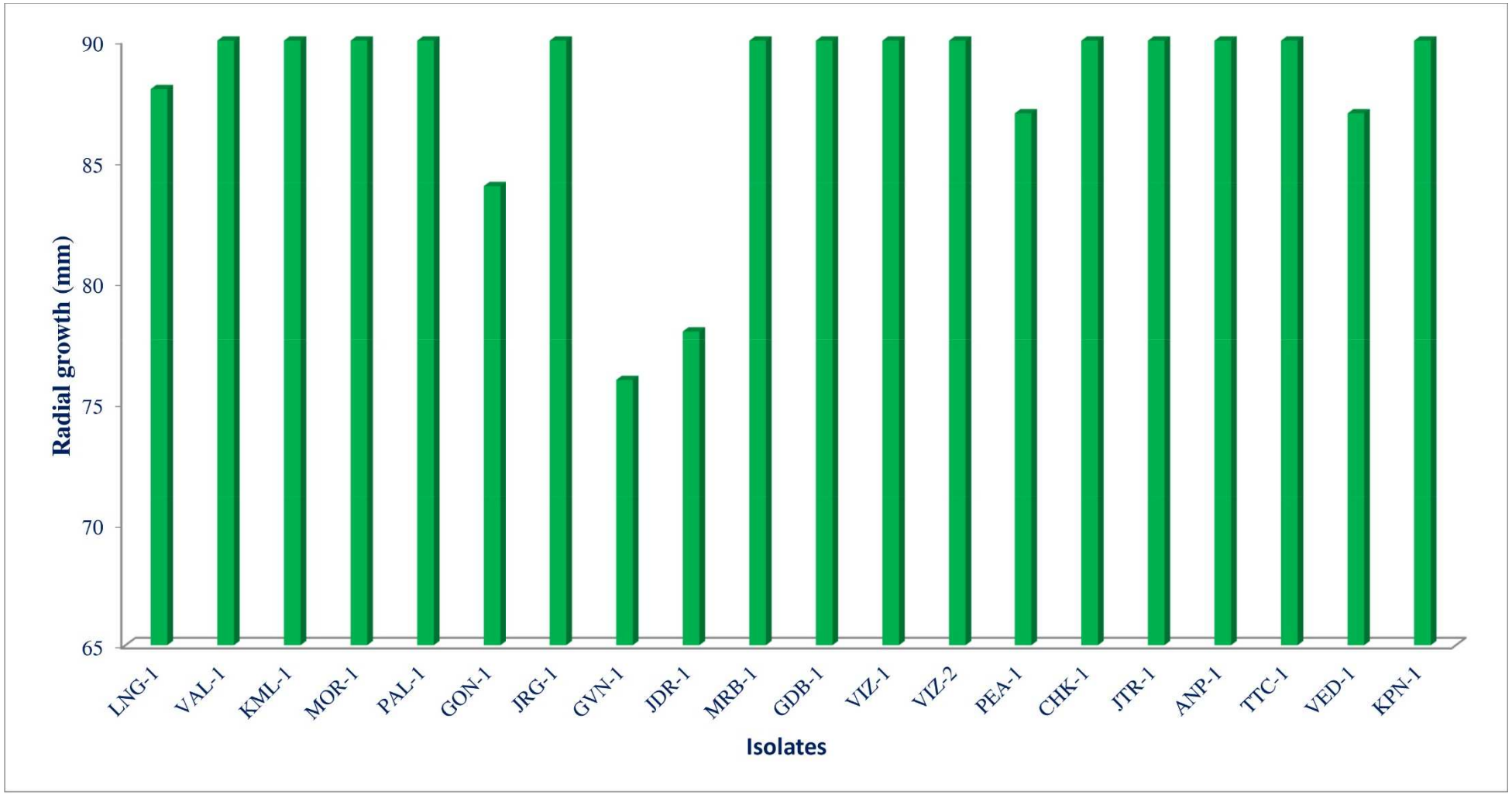


Fig. 4.3. Cultural variability of *P. grisea* isolates on Oat Meal Agar at 14 days after inoculation

growth was observed in RA and OMA respectively after 14 days of inoculation (Table 4.6).

Radial growth and sporulation

The radial growth of virulent isolate VIZ-1 was recorded after 14 days of inoculation. Out of eight solid media used, the maximum growth with a colony diameter of 90.00 mm was observed in OMA, followed by FLA (87.67 mm). PDA, V8, RA and RLA were recorded 78.00, 75.00, 73.33 and 70.00 mm of growth respectively. While, PSA and OSA showed least growth with a colony diameter of 29.33 and 38.33 mm respectively (Table 4.6) (Plate 10) (Fig. 4.4).

The maximum sporulation was observed in case of FLA medium with 1.81×10^5 ml followed by OMA with 1.68×10^5 ml were found to be significantly different. RLA, OSA, PSA, and RA were recorded significant spore concentrations of about 1.32×10^5 ml, 0.86×10^5 ml, 0.73×10^5 ml and 0.68×10^5. Least sporulation was observed in PDA (0.35×10^5 ml) and V8 (0.54×10^5 ml) medium (Table 4.6) (Fig. 4.5).

The results showed that the radial growth of *P. grisea* was higher in Oat Meal Agar medium than FLA and PDA medium. The present results were in agreement with the findings of Gashaw *et al.*, (2014), who reported that maximum mycelial growth was higher in Oat Meal Agar than PDA medium. Similarly, Manjunatha and Krishnappa (2019) recorded the highest mycelial growth of the fungus on host extract agar and Oat Meal Agar. The higher preference for Oat Meal Agar might be due to abandoned microelement compounds which are essential for enhanced growth of *Pyricularia* species as described by Meena (2005). This possibly indicates that the pathogen prefers specific nutritional components, which might be linked to specificity for host. Khadka *et al.,* (2012) was also reported the higher mycelial growth and sporulation in OMA. Many of the studies indicated that increased growth of pathogen and sporulation was found on their host extracts (Yorionori *et al*., 1974; Bhattacharyya and Bose, 1981).

Table 4.6. Evaluation of different media for growth and sporulation of *P. grisea* (VIZ-1)

S. No.	Media	Colony colour	Average radial growth (mm)*	Average growth rate/day (mm)	Sporulation ($\times 10^5$/ml)*
1.	Potato Dextrose Agar (PDA)	Greyish white	78.00 ± 0.00[a]	5.57	0.35 ± 0.013[a]
2.	Rice leaf extract Agar (RLA)	Blackish brown	70.00 ± 0.57[b]	5.07	1.32 ± 0.010[b]
3.	V8 Agar (V8)	Greyish white	75.00 ± 0.00[c]	5.36	0.54 ± 0.010[c]
4.	Fingermillet leaf extract Agar (FLA)	Black	87.67 ± 0.66[d]	6.26	1.81 ± 0.017[d]
5.	Potato Sucrose Agar (PSA)	Greyish white	29.33 ± 0.88[e]	2.10	0.73 ± 0.010[e]
6.	Rechard's Agar (RA)	White	73.33 ± 0.33[f]	5.24	0.68 ± 0.010[f]
7.	Oat meal Sucrose Agar (OSA)	Greyish white	38.33 ± 0.33[g]	2.71	0.86 ± 0.013[g]
8.	Oat Meal Agar (OMA)	greyish green	90.00 ± 0.00[h]	6.43	1.68 ± 0.010[h]
	C.D.		0.757		0.019
	S.E(m)		0.266		0.007
	S.E(d)		0.377		0.009
	C.V.		2.131		3.645

* Mean of three replications, in a column, mean followed by a common letters are not significantly different at the 5% level DMRT

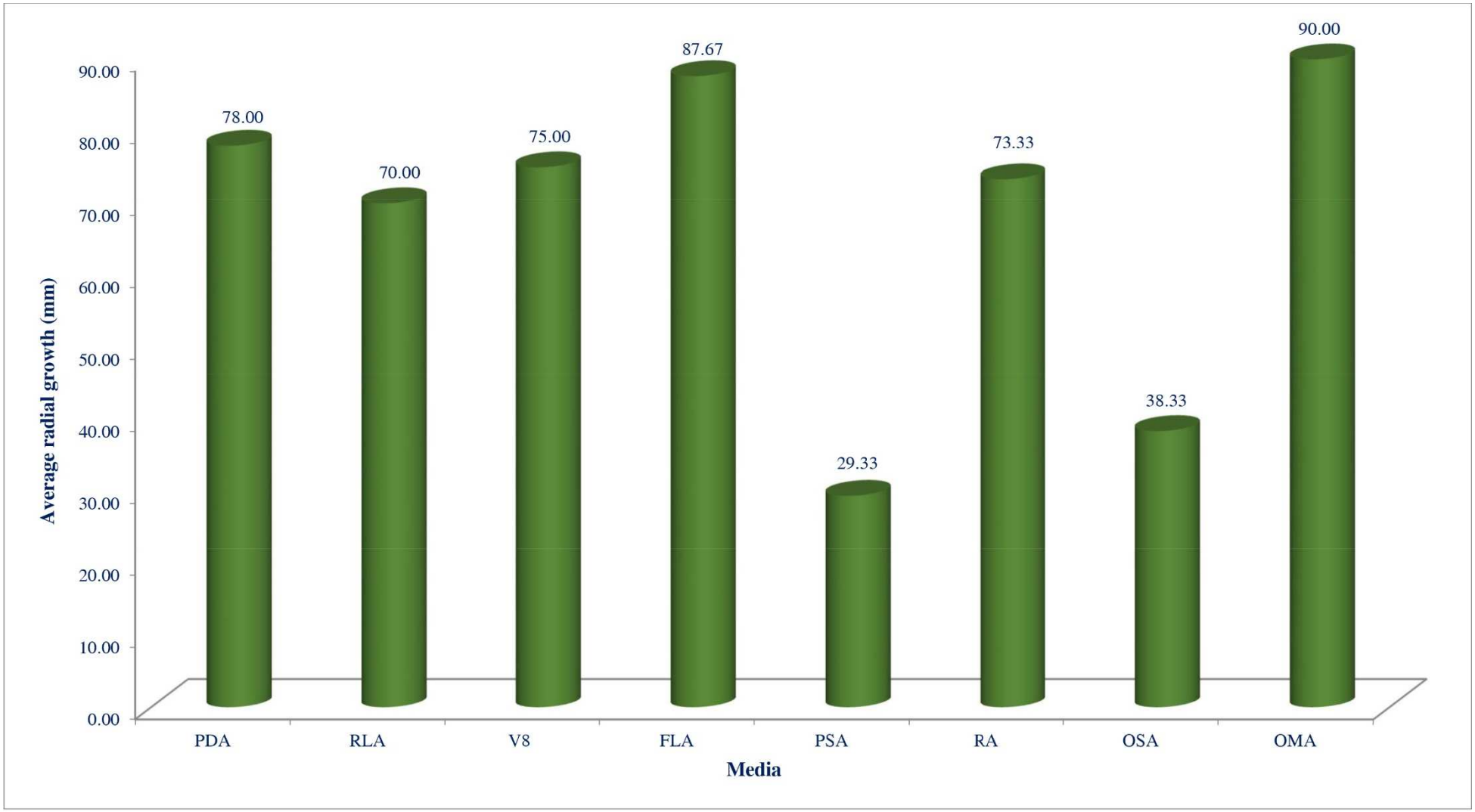


Fig. 4.4. Radial growth of *P. grisea* (VIZ-1) on different culture media

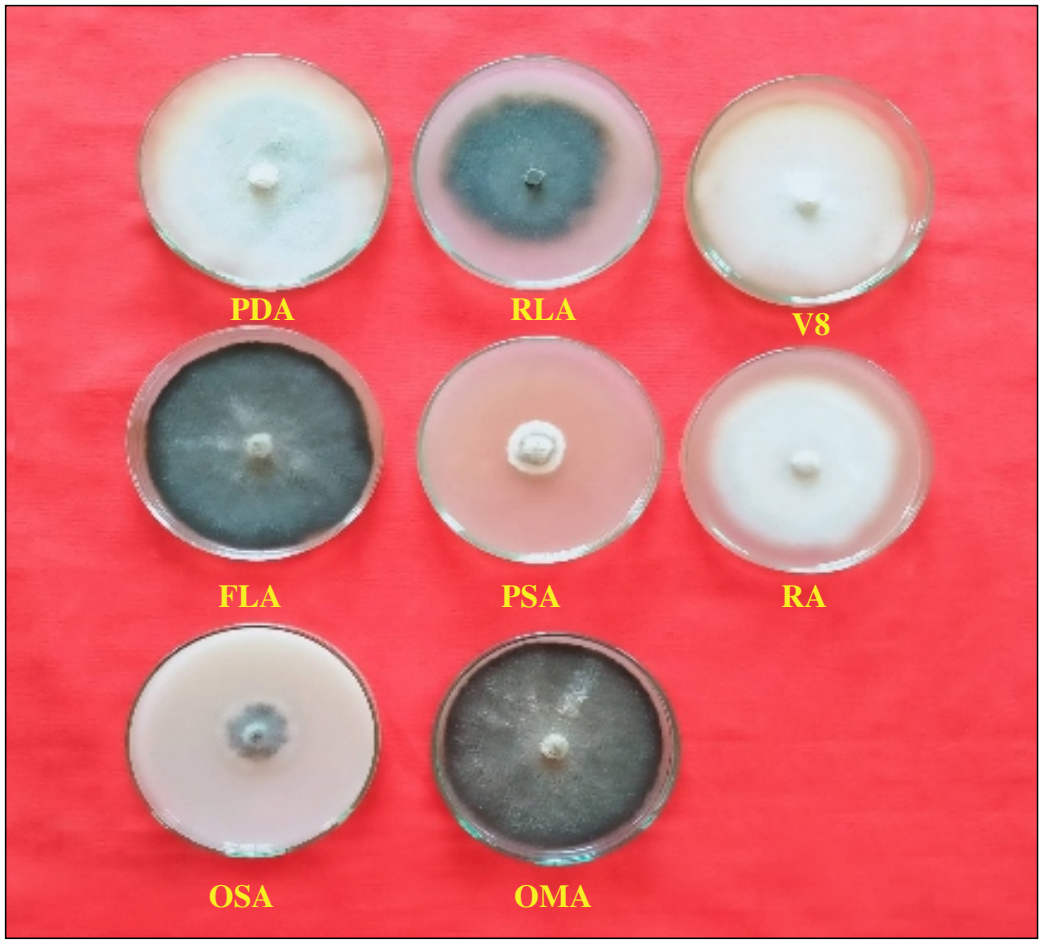


Plate 10. Radial growth of *P. grisea* (VIZ-1) isolate on different culture media at 14 days after inoculation

PDA : Potato Dextrose Agar
RLA : Rice Leaf extract Agar
V8 : V8 Agar
FLA : Fingermillet leaf extract Agar
PSA : Potato Sucrose Agar
RA : Rechard's Agar
OSA : Oat meal Sucrose Agar
OMA : Oat Meal Agar (OMA)

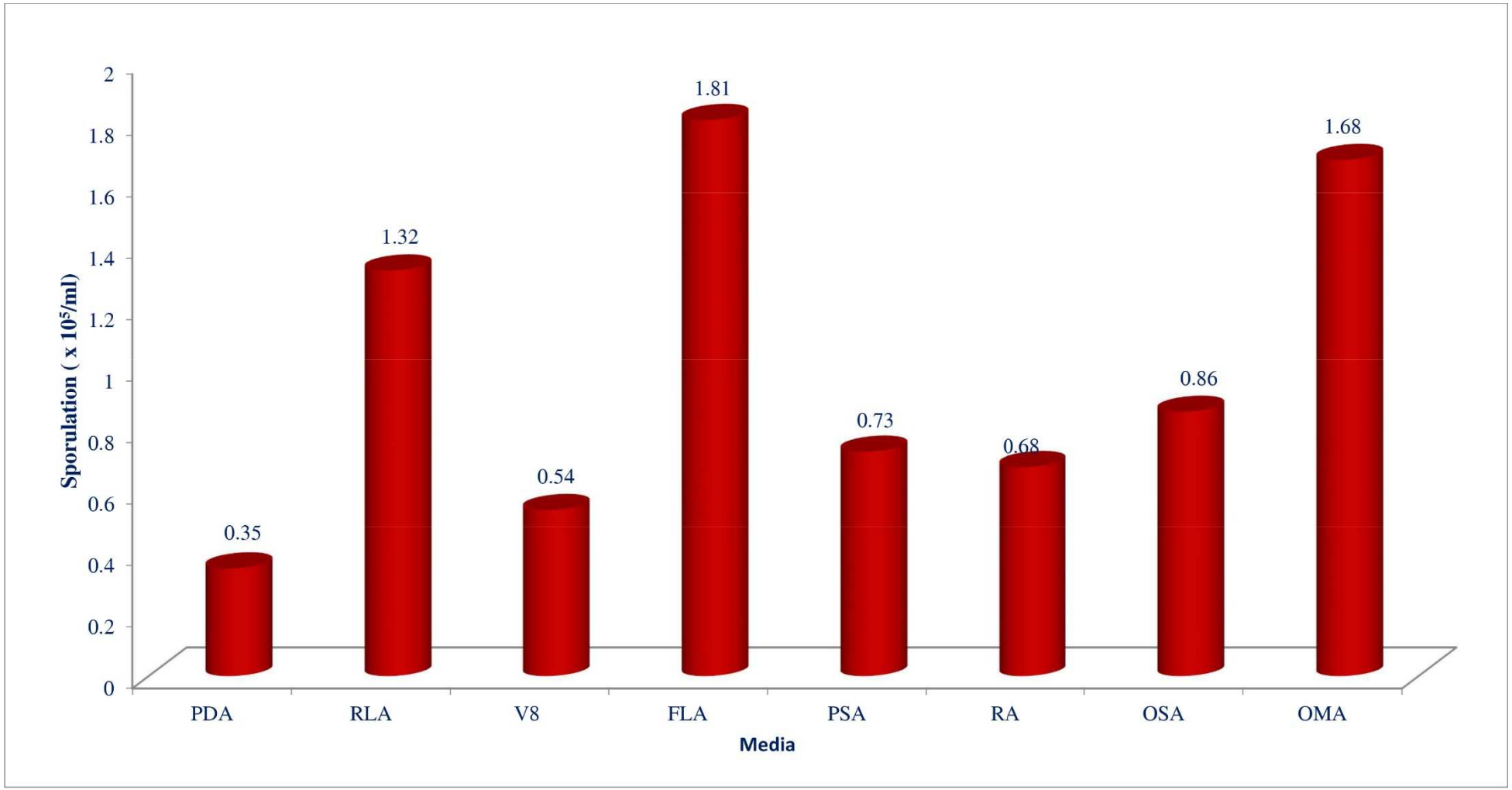


Fig. 4.5. Sporulation of *P. grisea* (VIZ-1) on different culture media

4.2.1.3 Effect of different light conditions on radial growth and sporulation of *P. grisea* virulent isolate (VIZ-1)

Total of eight solid media *viz.*, PDA, RLA, V8, FLA, PSA, RA, OSA and OMA were evaluated in combination with three different light conditions *i.e.* 24 hrs fluorescent light, 12 hr dark/12 hr fluorescent light and 24 hr darkness. Radial growth and sporulation was recorded after 14 days of inoculation. OMA was recorded maximum mean radial growth of 87.89 mm with insignificant difference under three light conditions *i.e* light (87.67 mm), dark (88.67 mm) and light + dark (87.33 mm) which was followed by FLA (87.67 mm) with insignificant difference among three light conditions *i.e.* light (87.67 mm), dark (89.00) and dark + light (86.33 mm). RA and PDA showed radial growth of 82.68 and 81.45 mm with insignificant difference among three light conditions. However, radial growth in RLA, V8 and OSA was 75.40, 73.67 and 58.56 mm with no significant difference among three light conditions. While, radial growth in PSA was 52.44 mm with insignificant difference under three light conditions *i.e.* light (53.33 mm), dark (52.67 mm) and light + dark (51.33 mm) were recorded. In over all, with respect to the light conditions, no significant difference was found among the three light conditions *i.e.* light (75.71 mm), dark (75.0 mm) and dark + light (74.11 mm). with respect to the media OMA (87.89 mm) and FLA (87.67) have shown significant difference with RA (82.68 mm), PDA (81.45 mm), RLA (75.40 mm), V8 (73.67 mm), OSA (58.56 mm) and PSA (52.44 mm) (Table 4.7) (Plate 11).

The mean maximum sporulation (1.68×10^5 ml^{-1}) was recorded in FLA with no significant difference among three light conditions (1.93×10^5 ml^{-1}-24 hrs light; 1.73×10^5 ml^{-1} dark and 1.39×10^5 ml^{-1} 12 hrs light + 12 hrs dark). This was followed by OMA (1.68×10^5 ml^{-1}) and RLA (1.18×10^5 ml^{-1}). OSA, PSA, RA showed sporulation of 0.72×10^5 ml^{-1}, 0.68×10^5 ml^{-1} and RA $\times 10^5$ ml^{-1} respectively with no significant difference among three light conditions. While least mean sporulation was noticed in PDA and V8 with 0.34×10^5 ml^{-1} and 0.40×10^5 ml^{-1} respectively (Table 4.7). The higher sporulation of *P. grisea* in FLA is mainly because FLA was derived from host origin and pathogen gained abandoned nutrients required for its growth and sporulation from the media.

Table 4.7. Evaluation of different light conditions for radial growth and sporulation of *P. grisea* (VIZ-1)

Media	Radial growth (mm)				Sporulation (× 10^5/ml)			
	Light	**Dark**	**Light + Dark**	**Average**	**Light**	**Dark**	**Light + Dark**	**Average**
Potato Dextrose Agar (PDA)	82.67	80.35	81.33	81.45	0.43	0.32	0.27	0.34
Rice Leaf extract Agar (RLA)	75.67	76.33	74.19	75.40	1.49	1.06	0.99	1.18
Fingermillet leaf extract Agar (FLA)	87.67	89.00	86.33	87.67	1.93	1.73	1.39	1.68
V8 Agar (V8)	75.00	73.00	73.00	73.67	0.51	0.36	0.32	0.40
Potato Sucrose Agar(PSA)	53.33	52.67	51.33	52.44	0.85	0.64	0.55	0.68
Rechard's Agar (RA)	83.67	82.33	82.03	82.68	0.73	0.55	0.48	0.59
Oat meal Sucrose Agar (OSA)	60.00	58.33	57.33	58.56	0.92	0.71	0.55	0.72
Oat Meal Agar (OMA)	87.67	88.67	87.33	87.89	1.73	1.56	1.25	1.51
Average	75.71	75.09	74.11		1.07	0.87	0.73	

4.2.3 Morphological Variability among *P. grisea* Isolates

Morphology of different isolates used in this study was varied with conidial morphology. Mature conidia of *P. grisea* appeared to be pyriform, the conidia had 2 septa with 3- cell (Plate 12). The colour of the conidia was almost hyaline to pale olive, but the size of the conidia showed variability. Middle cell appeared more wider and darker than basal and apical cell (Plate 13 to 15) with a basal appendage at the point of attachment to the conidiophore. End cells and middle cells gave germtubes upon germination.

Among the isolates the mean length of basal cell was ranged from 5.53 µm (MOR-1) to 7.56 µm (MRB-1) with a width of 4.55 µm (LNG-1) to 7.66 µm (MRB-1). Middle cell of the conidia was ranged from 4.85 µm (JDR-1) to 8.94 µm (VAL-1) in length and had the width of 5.53 µm (GDB-1) to 7.48 µm (JTR-1). Apical cell length was in the range of 5.14 µm (KML-1) to 7.22 µm (MOR-1) and width a width of 4.98 µm (LNG-1 and PAL-1) to 6.87 µm PEA-1. Overall size of the conidia was ranged from 20.74-23.01 µm x 7.00-9.16 µm. The results obtained were much similar to the earlier workers who documented conidial morphology variation in the isolates (Table 4.8). McKenzie *et al.,* (2010) described the size and morphology of Pyricularia spores which measured 25.5–32 × 11.5–15 µm. Asfaha *et al.*, (2015) measured six isolates and observed conidial length from 14.5-26.5 µm and width ranged from 5.1-8.3 µm. Singh *et al*., (2018) reported that size of the conidia was ranged from 20.74-24.91 µm in length and 7.53-10.23 µm in width.

4.2.4 Molecular Variability among Pathogen Isolates using Molecular Tools

4.2.4.1 Genetic diversity of *P. grisea* isolates using SSR markers

The genetic diversity of 20 isolates of *P. grisea* was evaluated using 25 SSR markers, of which seven were polymorphic (100% polymorphism). A total of nineteen alleles were detected in twenty blast isolates using seven SSR markers. The number of alleles per locus varied from 2 (MGM 437, Pyrms 15, Pyrms 37 and Pyrms 67) to 4 (Pyrms 63 and Pyrms 77) with a mean value of

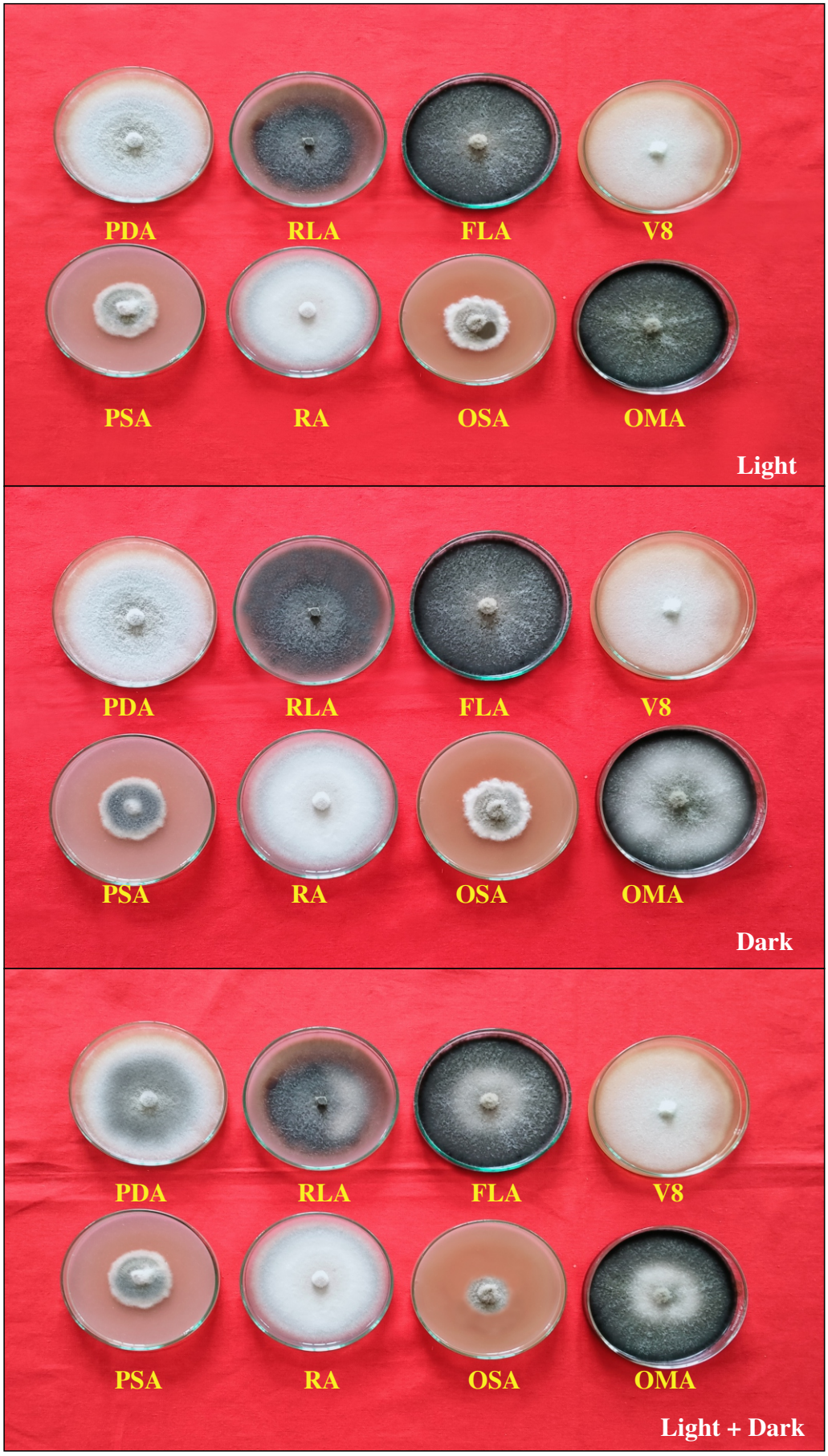


Plate 11. Radial growth of *P. grisea* (VIZ-1) isolate under different light regimes at 14 days after inoculation

PDA	:	Potato Dextrose Agar	PSA	:	Potato Sucrose Agar
RLA	:	Rice Leaf extract Agar	RA	:	Rechard's Agar
FLA	:	Fingermillet leaf extract Agar	OSA	:	Oat meal Sucrose Agar
V8	:	V8 Agar	OMA	:	Oat Meal Agar (OMA)

Table 4.8. Morphological conidial variability among isolates of *P. grisea*

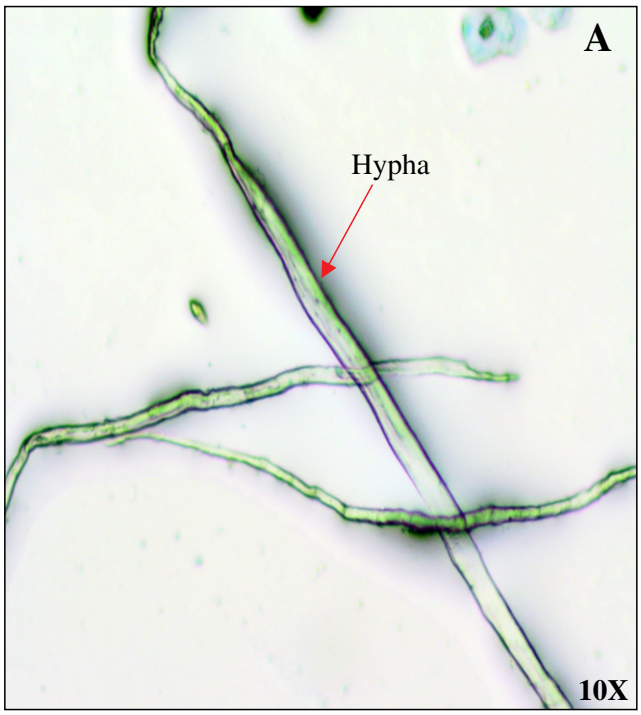
Isolate	Conidia length (µm)								Conidia width (µm)						Conidia size (L × W) (µm)
	Basal cell		Middle cell		Apical cell		Overall		Basal cell		Middle cell		Apical cell		
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
LNG-1	4.56-6.75	5.72	5.64-9.65	7.96	5.36-8.30	7.09	19.75-23.62	21.43	3.48-5.64	4.55	4.66-7.90	6.28	3.23-5.75	4.98	21.43 × 8.66
VAL-1	4.56-7.45	6.15	7.56-9.65	8.94	5.36-7.32	6.48	19.56-22.64	20.8	3.48-6.42	4.97	4.60-7.30	6.29	4.35-6.90	5.4	20.80 × 9.06
KML-1	4.25-6.45	5.5	7.66-9.65	8.5	3.65-6.90	5.14	19.45-23.42	21.07	3.48-6.95	5.27	4.65-7.23	6.29	3.23-690	5.27	21.07 × 9.06
MOR-1	4.30-6.97	5.53	5.64-9.87	8.26	5.36-9.42	7.22	1975-23.18	21.78	3.45-7.23	5.62	4.98-8.20	6.4	4.35-6.90	5.44	21.78 × 8.77
PAL-1	3.15-6.48	5.55	5.64-9.65	7.96	5.36-8.30	7.09	19.75-23.62	21.43	3.25-6.54	4.65	4.66-7.90	6.28	3.23-6.90	4.98	21.43 × 8.66
GON-1	3.45-6.75	5.39	3.65-9.65	8.21	5.40-8.30	6.73	19.45-22.34	20.77	3.45-6.64	5.25	4.60-7.60	6.4	3.40-6.42	5.03	20.77 x 8.91
JRG-1	3.42-7.65	5.54	5.46-9.56	7.7	5.36-7.60	6.71	19.35-25.34	21.93	3.48-6.38	5.18	4.60-8.20	6.72	3.40-6.90	5.57	21.93 × 8.80
GVN-1	4.25-6.97	5.74	7.66-9.56	8.8	4.35-7.52	6.46	18.65-23.24	21.35	4.35-7.20	6.04	3.45-7.20	5.69	3.56-7.56	5.66	21.35 × 8.36
JDR-1	5.67-7.65	6.86	3.12-6.51	4.85	5.36-9.42	6.95	18.71-22.65	20.89	4.36-6.52	5.28	4.35-7.30	5.81	4.21-6.45	5.24	20.89 × 7.00
MRB-1	6.35-9.01	7.56	5.35-8.65	7.13	3.45-8.30	5.87	18.94-22.95	21.31	6.35-8.65	7.66	4.23-7.30	5.97	3.23-6.54	5.12	21.31 × 9.16
GDB-1	4.41-8.23	6.62	3.83-9.49	6.57	4.03-7.35	5.67	16.83-24.78	21.48	3.42-6.50	4.99	3.45-6.90	5.53	3.65-6.90	5.32	21.48 × 7.09
VIZ-1	4.56-7.64	6.31	7.35-9.65	8.47	5.36-7.95	6.76	19.56-22.31	20.74	4.65-6.45	5.66	4.60-7.90	6.36	3.23-6.90	5.13	20.74 × 8.61
VIZ-2	4.56-6.97	5.74	7.56-9.87	8.68	5.36-9.42	6.95	19.84-24.12	21.42	3.48-7.23	5.6	4.60-7.30	6.09	4.65-6.90	5.47	21.42 × 8.86
PEA-1	4.35-6.35	5.56	7.36-9.65	8.64	5.34-7.98	6.26	19.26-24.36	22.18	4.35-7.20	5.89	3.45-6.45	5.63	5.36-8.65	6.87	22.18 × 8.30
CHK-1	4.25-7.30	5.72	7.25-9.56	8.7	5.36-8.30	6.87	18.98-22.35	21.29	3.45-6.45	4.8	4.60-8.20	6.74	3.40-6.42	5.36	21.29 × 8.79
JTR-1	4.00-7.65	5.59	6.15-8.65	7.13	4.56-8.45	6.54	20.65-25.34	22.33	4.26-8.45	5.69	6.15-8.65	7.48	4.35-7.25	6.14	22.33 × 7.77
ANP-1	5.46-7.65	6.2	4.56-9.56	7.85	5.40-8.30	6.68	20.87-24.63	23.01	3.45-6.75	5.31	4.25-7.65	5.97	4.30-7.25	5.51	23.01 × 8.68
TTC-1	4.53-7.64	6.18	4.32-8.75	6.75	4.35-8.30	6.67	20.84-24.98	22.76	3.54-6.45	5.03	4.35-7.30	5.95	3.65-7.32	5.79	22.76 × 7.60
VED-1	4.35-7.65	6.04	5.36-9.45	6.81	4.65-7.35	5.88	19.10-23.54	21.34	3.15-5.75	5.05	3.45-7.54	6.37	4.35-6.90	5.42	21.34 × 7.09
KPN-1	4.56-7.64	6.43	3.83-9.56	7.57	4.03-7.32	5.75	16.83-25.32	22.25	4.32-6.45	5.63	4.23-7.20	5.77	3.23-6.90	5.17	22.25 × 7.55

2.71 alleles/locus (Table 4.9). The gene diversity was ranged from 0.180 (MGM 437) to 0.742 (Pyrms 63) with an average of 0.491.

The primers Pyrms 63 and Pyrms 77 were highly polymorphic with PIC (Polymorphism Information Content) value of 0.6949 and 0.6116. However, Pyrms 7 and Pyrms 37 both showed PIC value of 0.5129. PIC value of 0.3318, 0.2225 and 0.1638 were found in Pyrms 15, Pyrms 67 and Pyrms 437 respectively with a mean value of 0.435 (Table 4.9).

The SSR markers data across the twenty isolates were used for construction of phylogenetic tree by Neighbor Joining method (NJ), from genetic distance and dissimilarity matrix as parameter using DARwin 6 software. In the phylogenetic tree, blast isolates were grouped into three major clusters. Cluster I was further sub grouped into sub-cluster IA, which includes eight isolates (ANP-1, JTR-1, CHK-1, KPN-1, VED-1, TTC-1, PEA-1 and VIZ-2 among those majority of the isolates belongs Prakasam region *viz.,* ANP-1, JTR-1 and TTC-1) and remaining isolates belongs to different region. The sub-cluster IB contains only one isolate *i.e.* VIZ-1 which belongs to Vizianagaram region. Cluster II was further sub grouped into sub-cluster IIA, which includes six isolates (JRG-1, GON-1, JDR-1, GDB-1, KML-1 and VAL-1) among those JRG-1 and GON-1 belongs to Chittoor region, JDR-1 and GDB-1 belongs to Anantapur region and KML and VAL-1 belongs to YSR Kadapa region. Sub-cluster IIB includes only one isolate *i.e.* GVN-1 which belongs to Anantapur region. However, cluster III was further divided into sub cluster IIIA which includes three isolates *viz.*, PAL-1, LNG-1 and MOR-1 belongs to different regions. While IIIB includes only one isolate *i.e.* MRB-1 which belongs to Anantapur district. (Plate 16) (Fig. 4.6).

Similar observations were also reported by Gupta and Varshney (2000) reported SSR markers are tandemly repeat DNA sequences occur throughout the eukaryotic genome represent the locus specific, highly polymorphic, multi-allelic and co-dominant marker systems. Ying *et al.* (2004) used 7 SSR markers to examine the genetic diversity of 105 isolates of *P. grisea* collected from Chinese farms and concluded that the similarity among the isolates was high. They



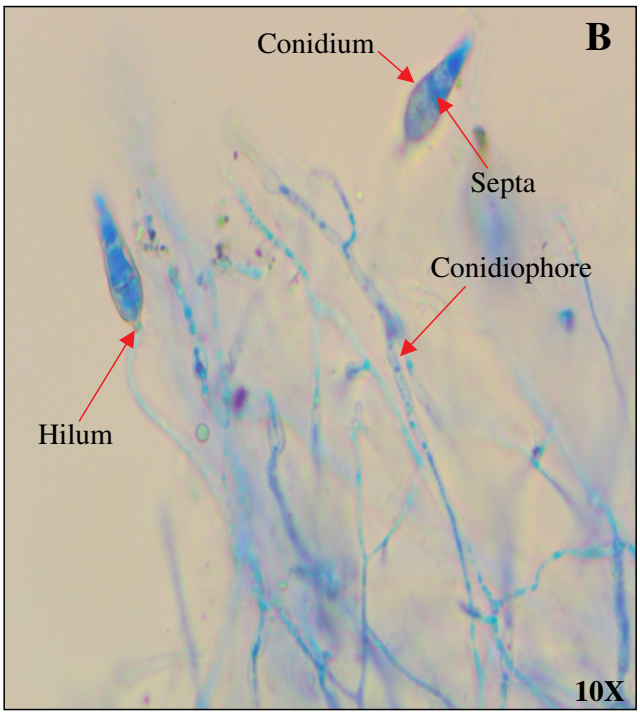
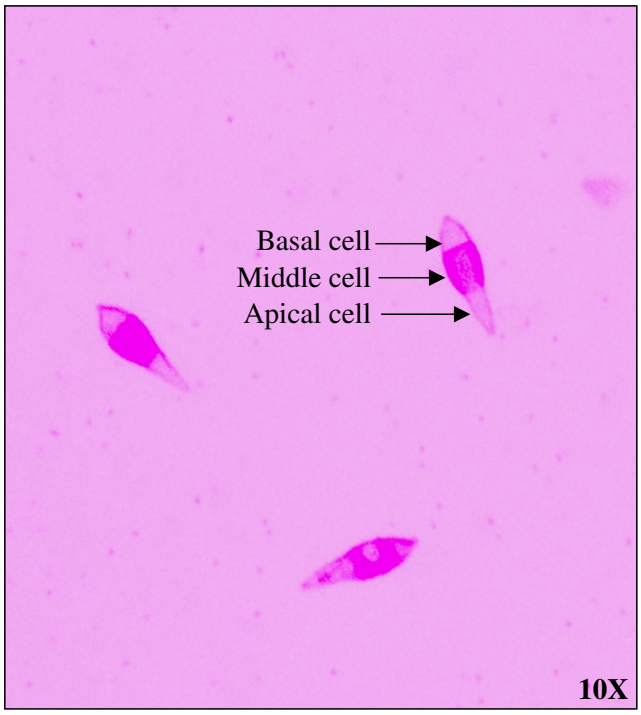


Plate 12. Photomicrographs : (A) Hypha (B) Conidia of *P. grisea*



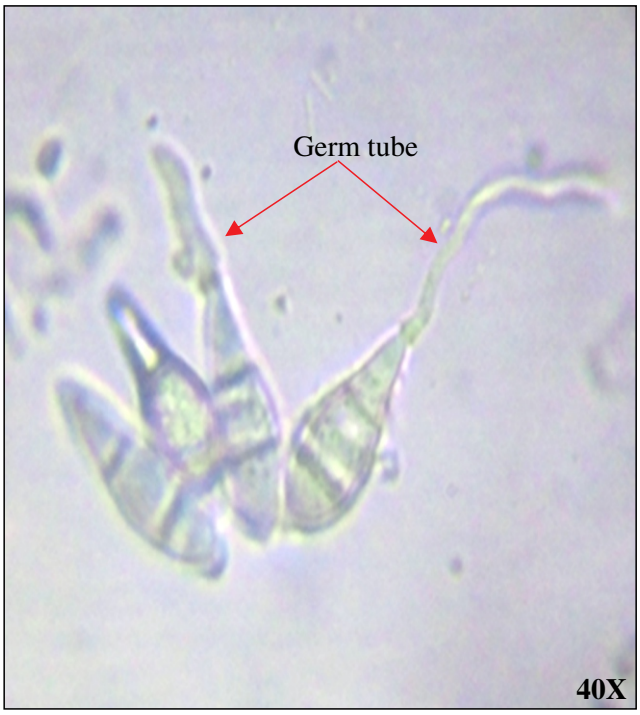


Plate 13. Photomicrographs : Cells of conidium and germination of *P. grisea* conidia

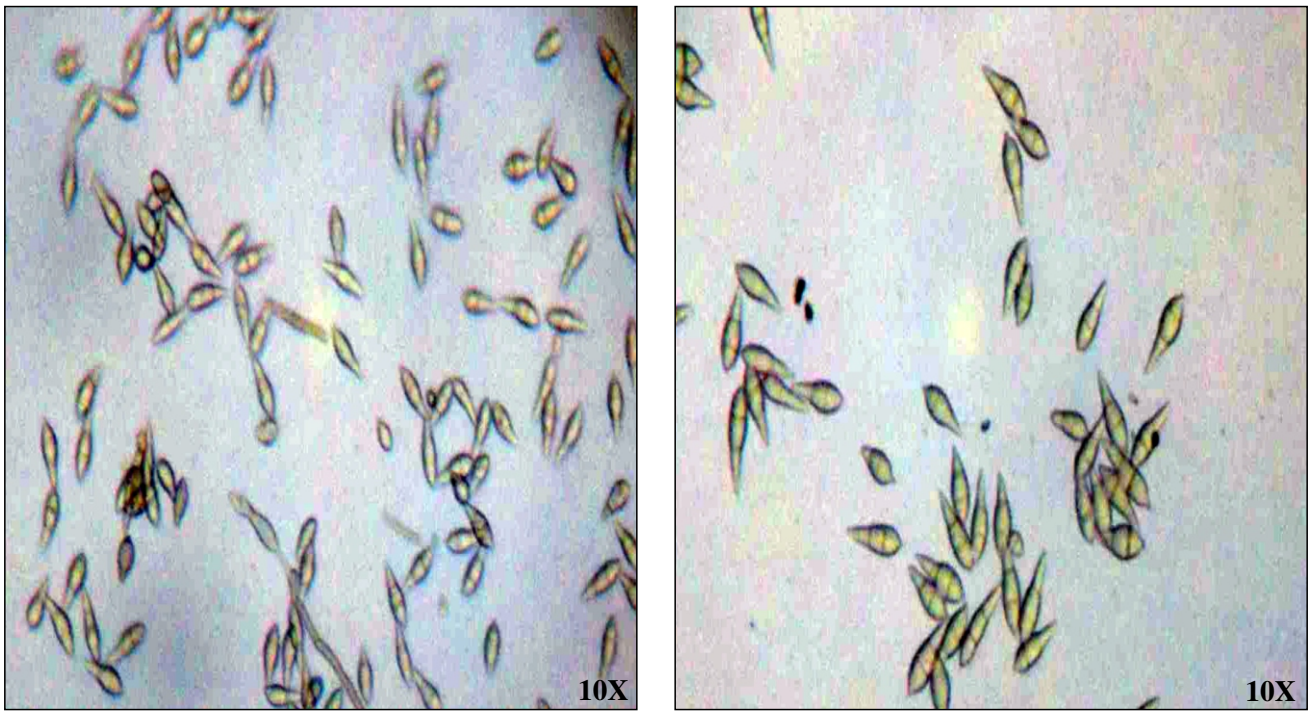


Plate 14. Photomicrographs : Conidia of *P. grisea* in 10X magnification

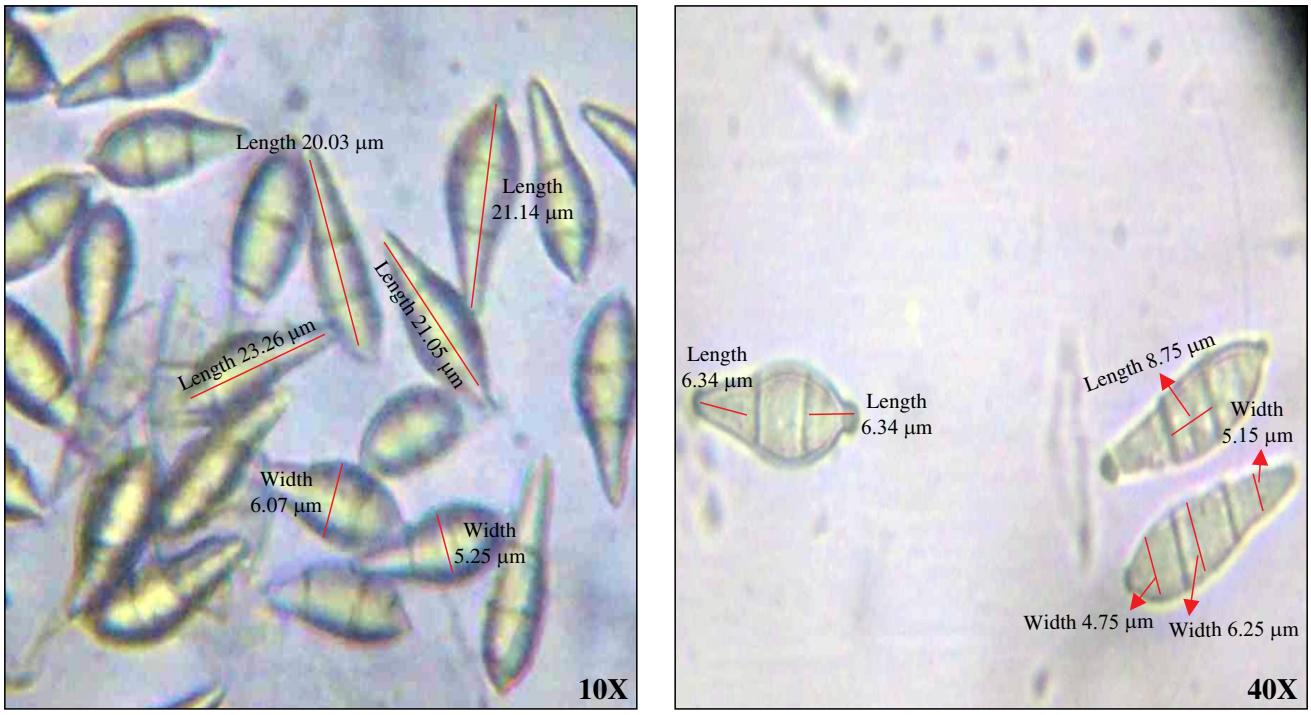


Plate 15. Photomicrographs of *P. grisea* conidia

Table 4.9. Molecular variability parameters of *P. grisea* isolates using SSR markers

S. No.	Marker	Forward	Reverse	Size range (bp)	Total number of alleles	Genetic diversity	Polymorphism Information Content (PIC)
1.	MGM437	GCCCCTCAATAGATCGTCAA	ACTGCGGCATTTTAACCTGT	150-170	2	0.180	0.1638
2.	Pyrms7	GCAAATAACATAGGAAAACG	AGAAAGAGACAAAACACTGG	110-150	3	0.585	0.5129
3.	Pyrms15	TTCTTCCATTTCTCTCGTCTTC	CGATTGTGGGGTATGTGATAG	150-160	2	0.420	0.3318
4.	Pyrms37	ACCCTACCCCCACTCATTTC	AGGATCAGCCAATGCCAAGT	200-215	2	0.585	0.5129
5.	Pyrms63	TTGGGATCTTCGGTAAGACG	GCCGACAAGACACTGAATGA	100-150	4	0.742	0.6949
6.	Pyrms67	AGCAAGCAGGAGATGCAGAC	GTTTGGCTGGCAAGACAGTT	200-210	2	0.255	0.2225
7.	Pyrms77	GAAGTATTGCACACAAACAC	GCTTTCGGCAAGCCTAATC	125-200	4	0.675	0.6116

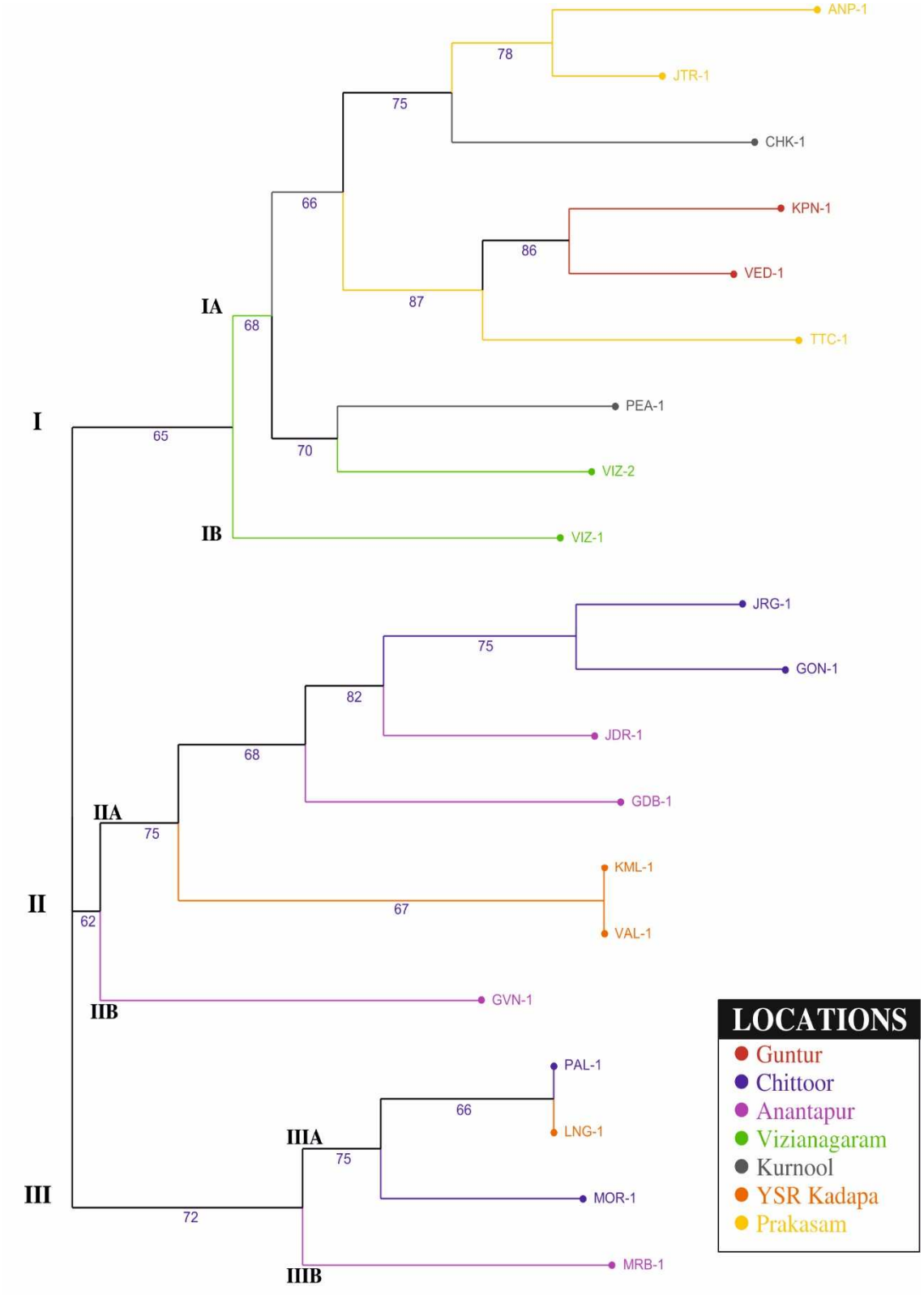
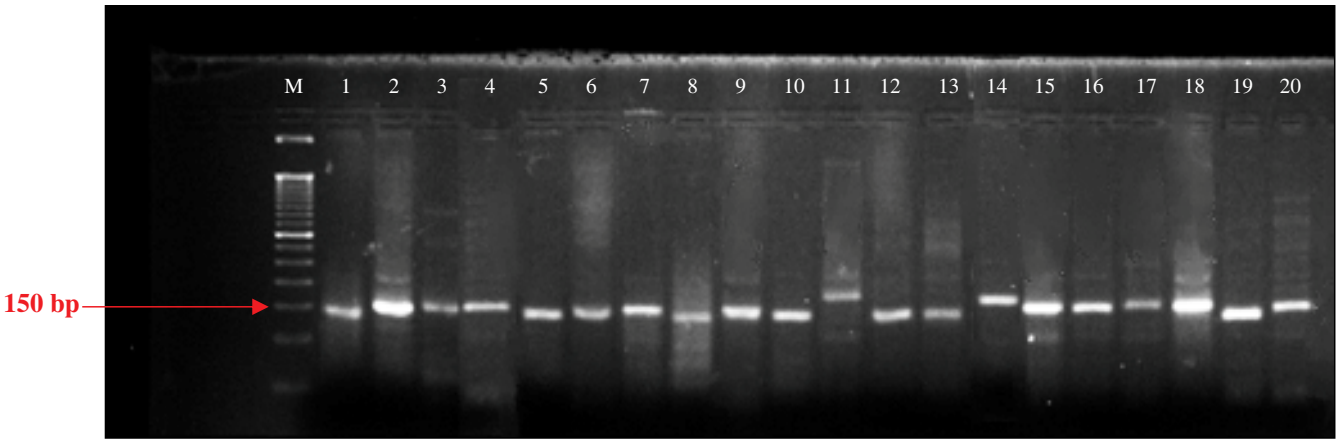
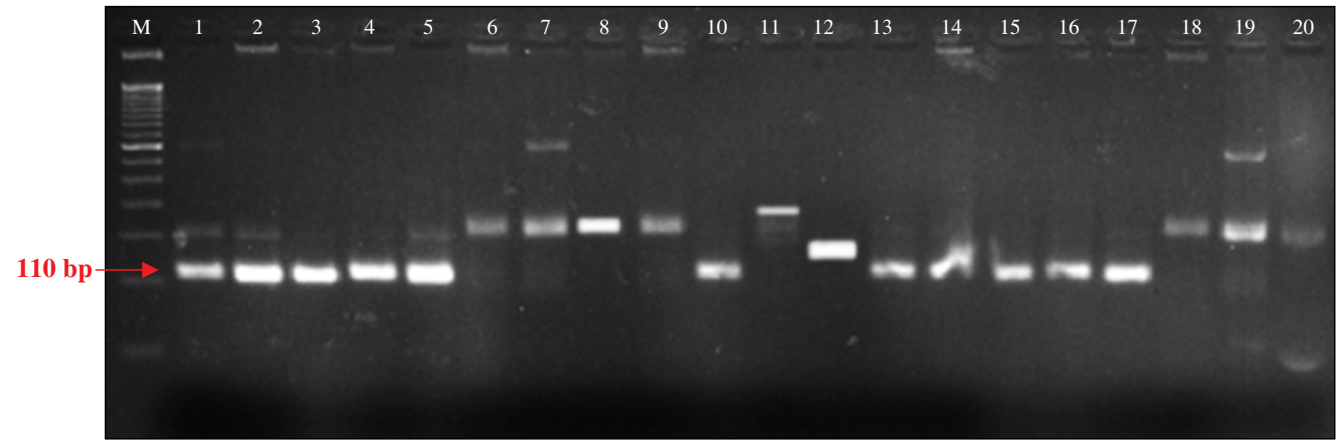


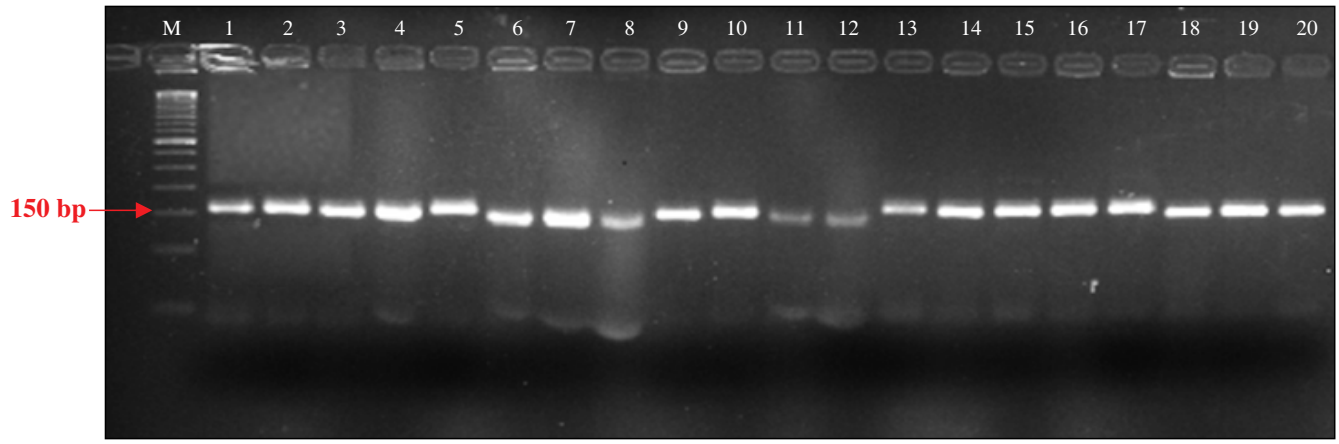
Fig. 4.6. Dendogram of *P. grisea* isolates generated based on SSR markers using DARwin 6 software



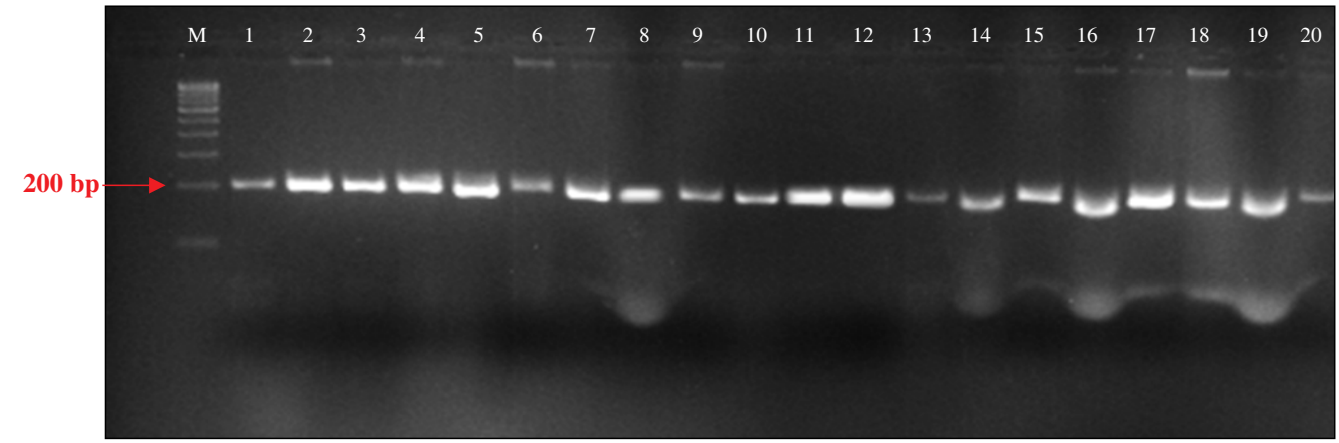
MGM-437 (M : 50 bp Ladder)



Pyrms-7 (M : 50 bp Ladder)



Pyrms-15 (M : 50 bp Ladder)



Pyrms-37 (M : 100 bp Ladder)

Plate 16. Amplification pattern of SSR markers

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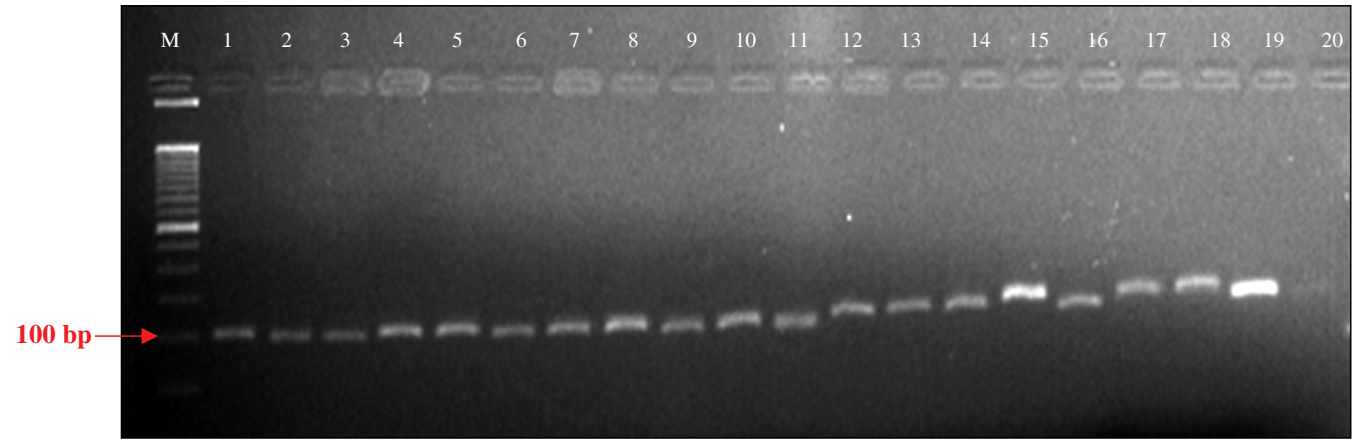
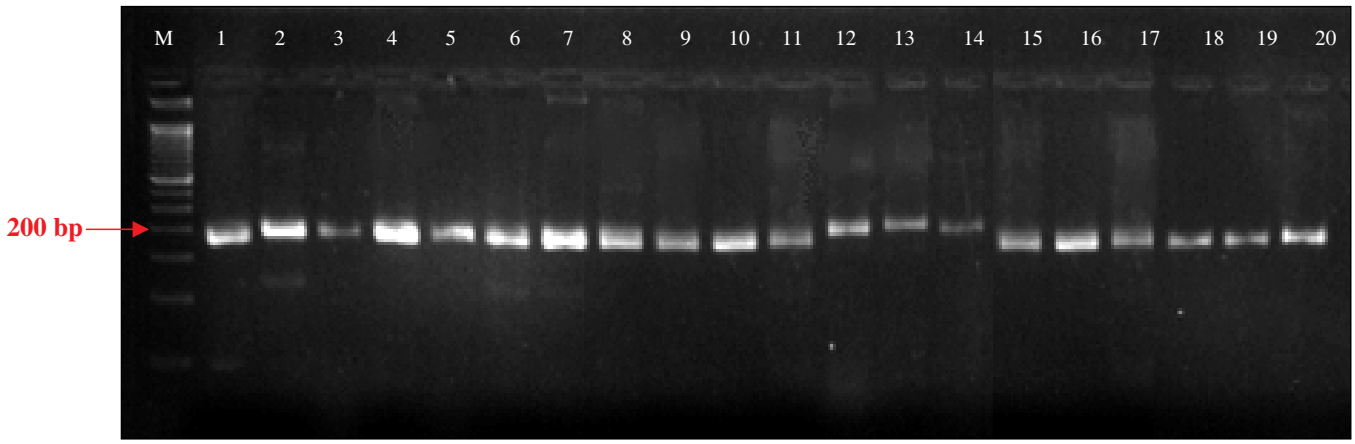
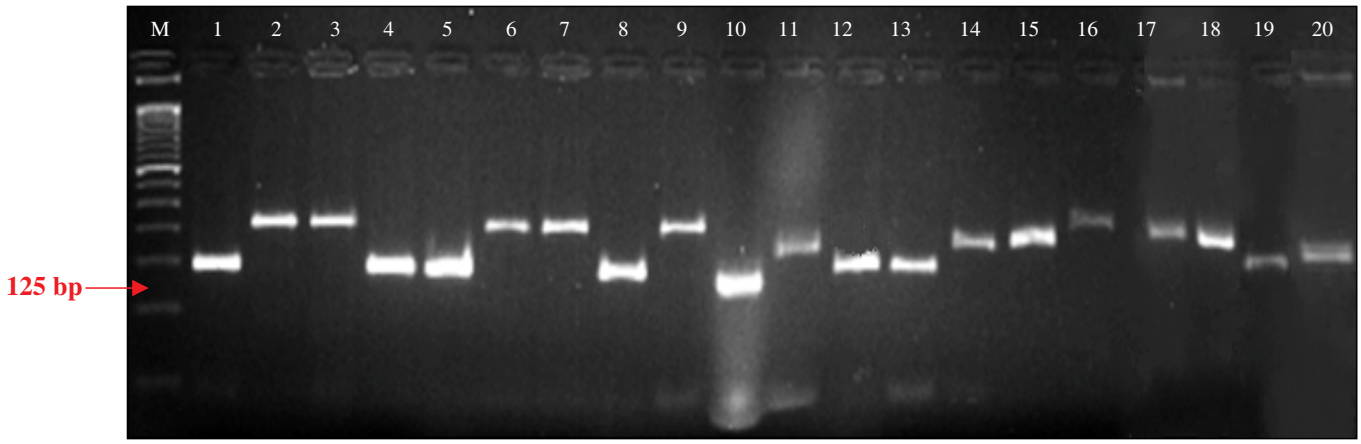


Pyrms-63 (M : 50 bp Ladder)



Pyrms-67 (M : 50 bp Ladder)



Pyrms-77 (M : 50 bp Ladder)

Plate 16. Amplification pattern of SSR markers

divided the isolates into six groups by cutting the cluster analysis diagram at the similarity point of 0.67.

Chadha and Gopalakrishna (2005) investigated the genetic diversity of *P. grisea* and reported that genetic similarity across isolates was high, ranging from 0.67 to 0.92. They also looked at 20 P. *grisea* isolates that were split into two groups, each with two subgroups. El-Wahsh *et al*. (2016) analyzed *P. grisea* by using SSR markers and reported PIC values were high for all markers with an average of 0.70 and ranged from 0.51-0.83.

The results of molecular study on *P. grisea* islates variability. But no significant correlation was observed between cultural, morphological and molecular variability of *P. grisea* isolates.

4.2.4.2 Genetic diversity of *P. grisea* isolates using RAPD markers

The genomic DNA of twenty *P. grisea* isolates were subjected to PCR amplification using eight RAPD primers. Out of theses, 7 primers produced polymorphic alleles which selected for genetic diversity analysis.

A total of 84 reproducible alleles with an average of 12 alleles per primer were produced using 7 RAPD primers. All the markers displayed polymorphic alleles. Of the total alleles (84), one amplicpon (OPA-07) was monomorphic with 15.38% monomorphism and 84.61% polymorphism which contains two monomorphic band with PIC value of 0.2874. Whereas 6 primers (OPA-04, OPA-10, OPA-18, OPB-17, OPC-05 and OPC-11) produced 100% polymorphism with PIC value was ranged from 0.1769 to 0.3429 and total number of polymorphic bands were ranged from 8 to 14.

Dendrogram constructed to reveal the pattern of relatedness among twenty *P. grisea* isolates using DARwin 6 software on the basis of RAPD polymorphism. Clustering analysis based on Unweighted Pair Group Method using Arithmetic Averages (UPGMA) and dendrogram generated using Neighbor Joining (NJ) method. 20 *P. grisea* isolates formed in to three mega cluster. Cluster I is further divided into sub-cluster IA which consisting of nine isolates (MRB-1, GVN-1, VIZ-1 JTR-1, KML-1, VAL-1, LNG-1, PAL-1 and MOR-1),

among those majority were belongs to YSR Kadapa region (KML-1, VAL-1 and LNG-1) and remaining isolates belongs to different regions. Sub-cluster IB of with two isolates *viz.*, JRG-1 and GON-1 which belongs to Chittoor region. Cluster II further divided into cluster IIA which consisting of four isolates (PEA-1, GDB-1, VIZ-1 and CHK-1), among those PEA-1 and CHK-1 belongs to Kurnool region remaining isolates belongs to different regions. Sub-cluster IIB contains only one isolate *i.e.* JDR-1 which belongs to Anantapur region. However, cluster III further divided into sub-cluster IIIA which contains three isolates (KPN-1, VED-1 and TTC-1) among those KPN-1 and VED-1 belongs Guntur region and TTC-1 belongs to Prakasam region. While, sub-cluster IIIB contains only one isolate *i.e.* ANP-1 which belongs to Prakasam region (Table 4.10) (Plate 17) (Fig. 4.7).

Similar observations were also reported by various researchers. The assessment of genetic diversity and virulence complexity of forty five isolates of rice blast fungus was done by using fifteen RAPD primers showed the polymorphism range from 71.40 to 90%, while the range of total loci scored was from 07 to 10. The molecular size of scorable loci ranged from 150 to 2500 bp (Singh and Kumar, 2010). Madhavan *et al.*, (2014) reported the genetic variability among isolates of *Magnaporthe grisea* collected from rice, buffel grass, finger millet and para grass were analyzed by using Random Amplified Polymorphic DNA (RAPD) method. Analysis of the genetic coefficient matrix derived from the scores of RAPD profiles showed that minimum and maximum Per cent similarities among the tested *M. grisea*. Jesumaharaja *et al*. (2016) performed 20 Random amplified polymorphic DNA (RAPD) with 14 isolates of *M. grisea*. The genetic similarity coefficient within each group and variation between the groups was observed. Among the primers, OPF-08 generated a RAPD polymorphic profile that showed common fragment of 478 bp in all the isolates. Abubakar *et al*. (2020) found a total of fifty nine (59) amplified fragment bands with 10 DNA primers, of which 53 (89.83%) were polymorphic and 6 (10.17%) were monomorphic. Genetic similarity among the accessions varied from 0.18 to 1.44 with an average gene diversity value of 0.74.

Table 4.10. Molecular variability parameters of *P. grisea* isolates using RAPD markers

S. No.	Marker	Sequence (5'- 3')	Total number of bands	Total number of monomorphic bands	Total number of polymorphic bands	Per cent monomorphism	Per cent polymorphism	Size range (bp)	Polymorphism Information Content (PIC)
1.	OPA-04	AA TC GG GC TG	14	0	14	0.00	100.00	140-1500	0.2468
2.	OPA-07	GA AA CG GG TG	13	2	11	15.38	84.61	150-1200	0.2874
3.	OPA-10	GT GA TC GC AG	13	0	13	0.00	100.00	170-1700	0.2448
4.	OPA-18	AG GT GA CC GT	8	0	8	0.00	100.00	210-1500	0.1769
5.	OPB-17	AG GG AA CG AG	14	0	14	0.00	100.00	150-800	0.2951
6.	OPC-05	GA TC AG CG CC	11	0	11	0.00	100.00	140-1500	0.1909
7.	OPC-11	AA AG CT GC GG	11	0	11	0.00	100.00	230-1300	0.3429

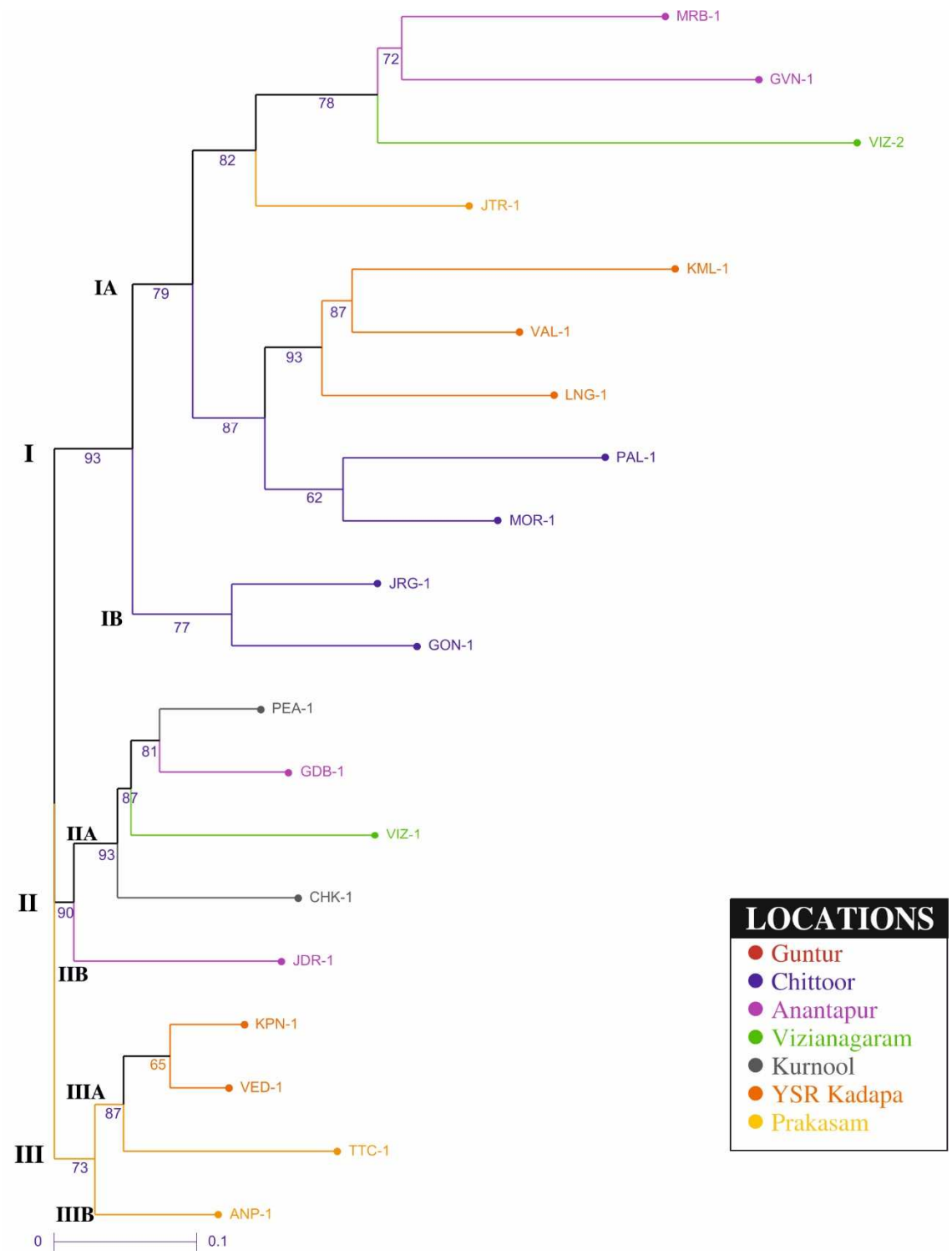
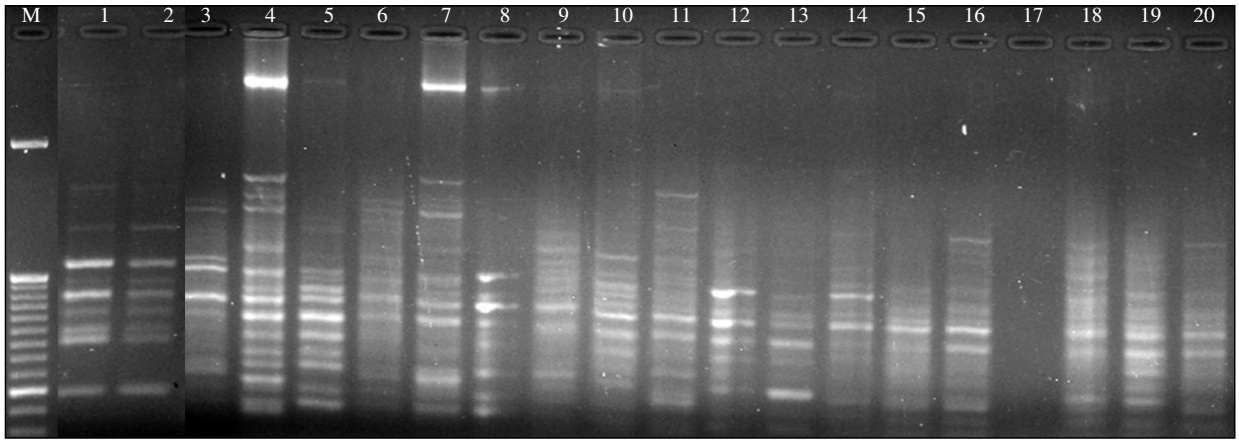
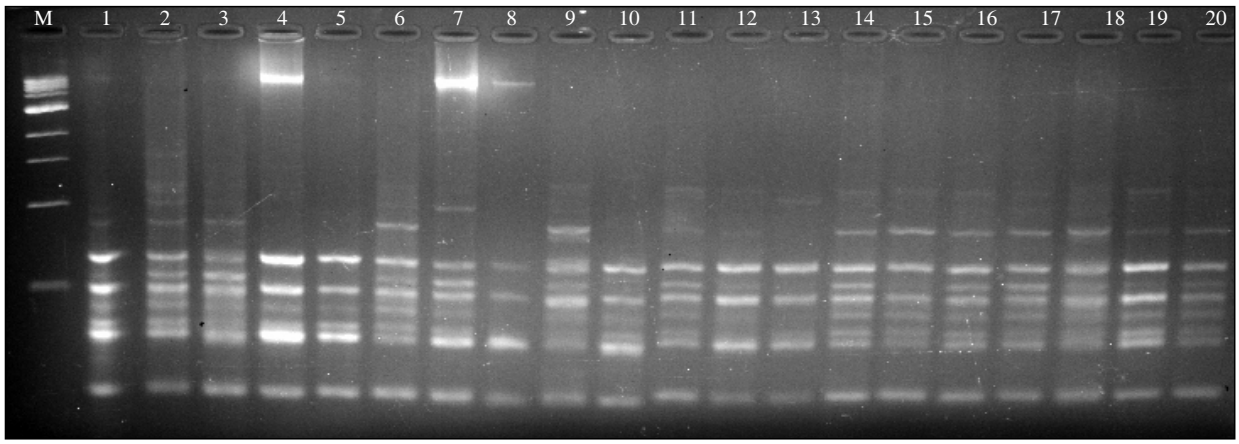


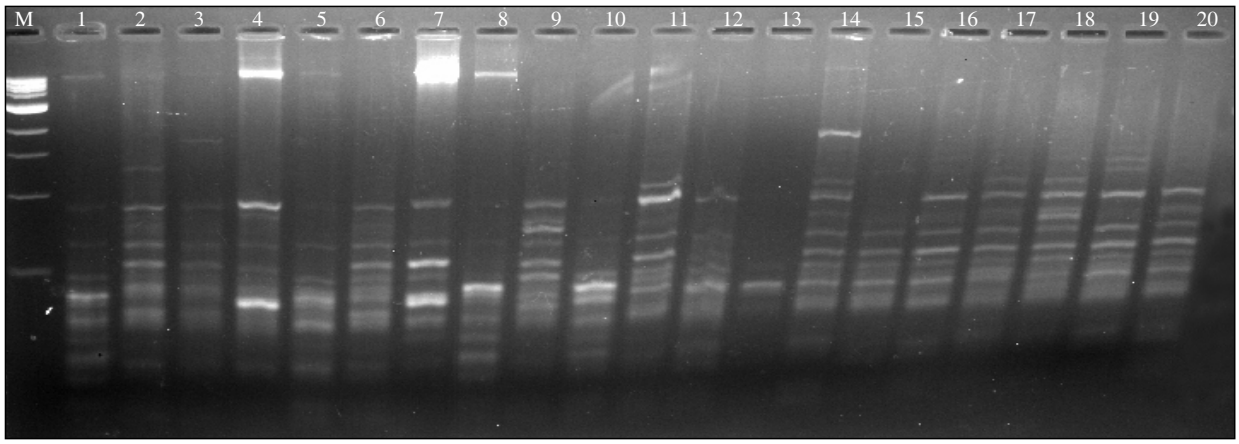
Fig. 4.7. Dendogram of *P. grisea* isolates generated based on RAPD markers using DARwin 6 software



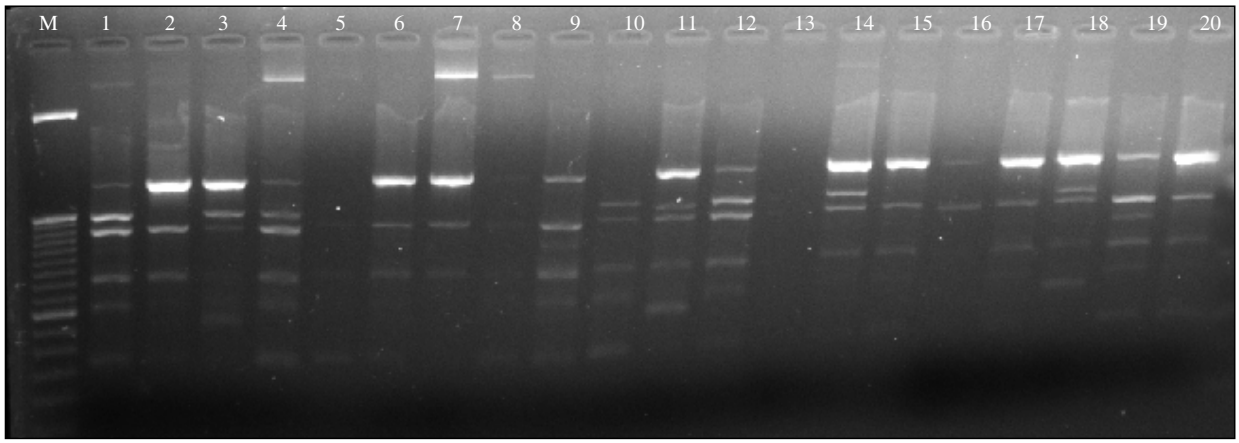
OPA-04 (M : 50 bp Ladder)



OPA-07 (M : 1 kb Ladder)



OPA-10 (M : 1 kb Ladder)



OPA-18 (M : 50 bp Ladder)

Plate 17. Amplification pattern of RAPD markers

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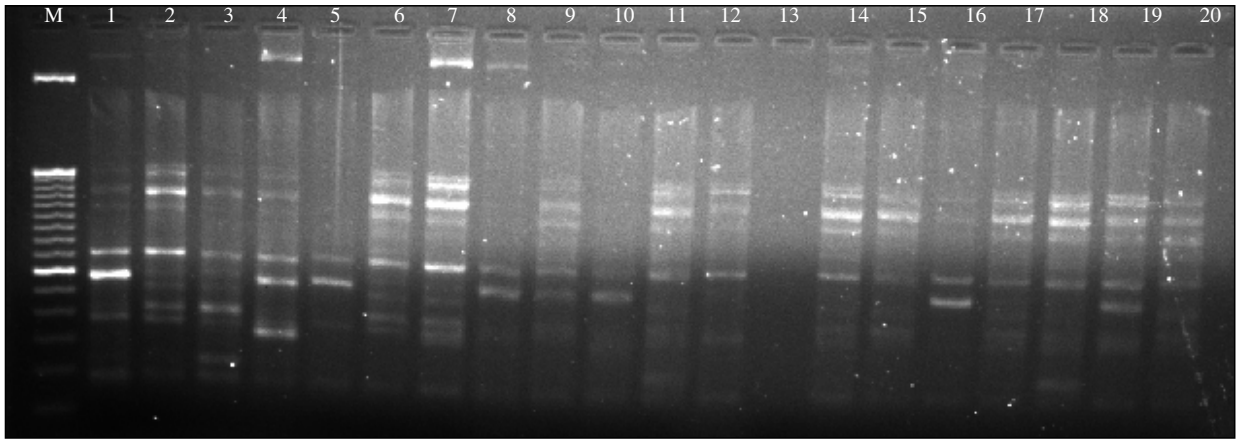
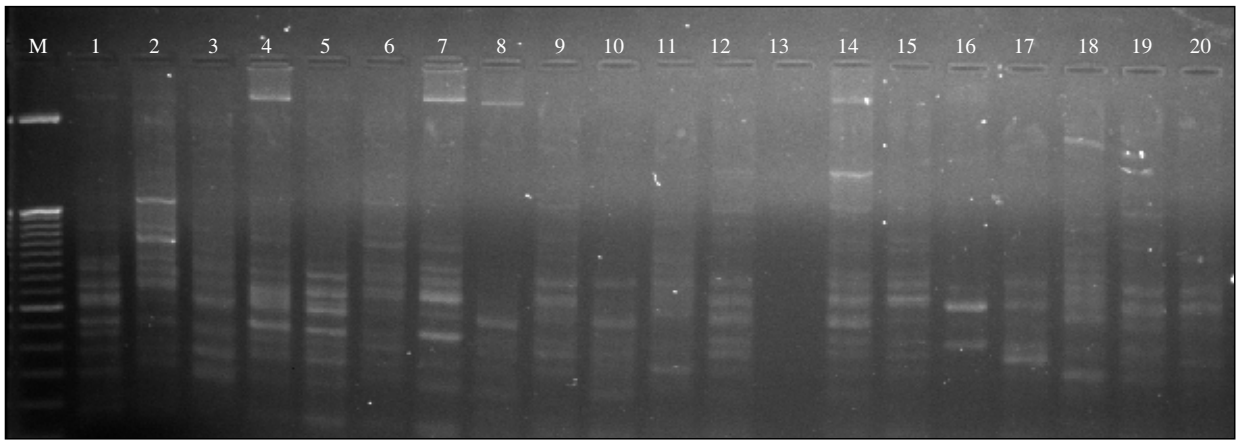
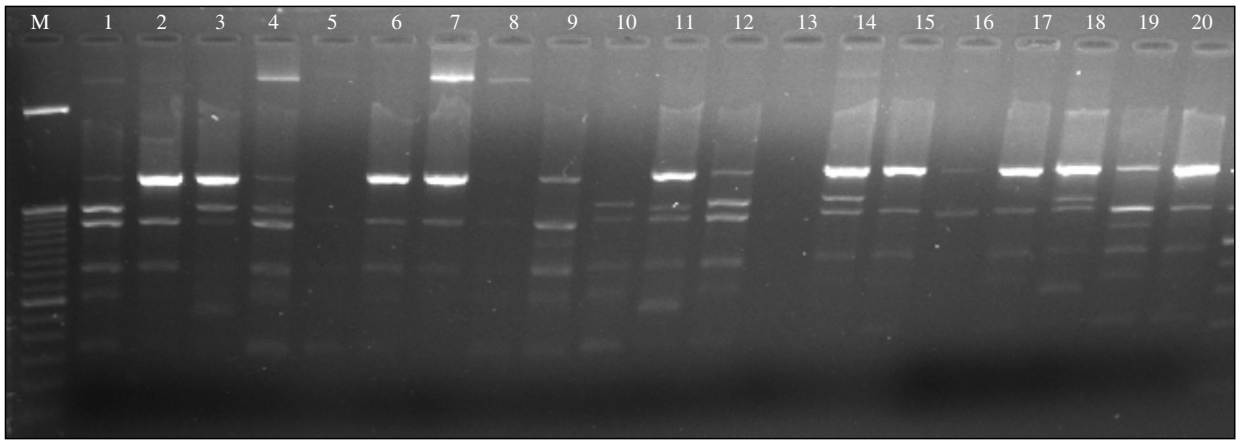


OPB-17 (M : 50 bp Ladder)



OPC-05 (M : 50 bp Ladder)



OPC-11 (M : 50 bp Ladder)

Plate 17. Amplification pattern of RAPD markers

4.3 EVALUATION OF NATIVE BACTERIAL BIO-CONTROL AGENTS AND FUNGICIDES AGAINST THE PATHOGEN *IN VITRO*

4.3.1 Isolation of Native Bacterial Bio-control Agents from Rhizosphere Soil

A total of 23 (BLN-1, BLN-2, BVA, BKM, BMO, BPA-1, BPA-2, BGO, BJR, BGV-1, BGV-2, BJD, BMR, BGD, BPE-1, BPE-2, BCH, BJT-1, BJT-2, BAN, BTT, BVE, BKP) bacterial bio-control agents were isolated from rhizospheric soil of healthy finger millet plants from different areas and then subjected to Serial dilution plating technique (Elad and Chet, 1983) and three isolates *viz.,* BVP-1 (*Pseudomonas fluorescens*), BVB-2 (*Bacillus subtilis*-1) and BVB-3 (*Bacillus subtilis*-2) were collected from Agricultural Research Station, Vizianagaram. The isolated bacterial bio-control agents were purified and assayed *in vitro* for their bioefficacy against *P. grisea* (Table 4.11) (Plate 18).

4.3.2 Evaluation of Native Bacterial Bio-control Agents against Pathogen

Among twenty six bacterial bio-control agents tested under *in vitro* by dual culture technique revealed the significant difference in per cent inhibition of mycelial growth of *P. grisea*. Result indicated that, the significant maximum inhibition of mycelium growth (79.54%) was noticed in BVP-1 followed by BTT (75.56%) and BJT-1 (74.35%) which were on par with each other. However, the isolate BJT-1 was on par in per cent inhibition of mycelial growth with BPE-1 (73.80%) and BJT-2 (73.52%). The isolate BGV-1 showed had Per cent inhibition of 72.31%, BAN (72.04%), BVB-2 (71.85%), BCH (70.00%), BGV-2 (66.94%), BJD (66.67%), BVB-3 (66.48%), BGD (66.20%), BMR (61.76%), BVA (61.57%), BGO (54.54%), BKM (47.50%), BMO (46.76%), BLN-1(43.52%), BVE (34.17%), BPE-2 (32.87%) and BPA-1(31.30%). The significant least mycelial inhibition was noticed in BJR (11.30%), BPA-2 (16.48%), BLN-2 (26.41%) and BKP (27.87%) which showed excess growth over *P. grisea* (Table 4.12) (Plate 19) (Fig. 4.8).

The *in vitro* evaluation of *Pseudomonas fluorescens* provides information on mycoparasitic activity against *Pyricularia grsea.* The mycoparasitic activity

of *Pseudomonas* spp. was well documented by Keel and Defago (1997) and Whipps (1997). The results were in agreement with findings of Gashaw *et al.*, (2014) who reported 57.20% inhibition in mycelial growth of *Pyricularia grisea*. Rao and Kumar (2020) noticed 63.70% inhibition in mycelial growth of *P. grisea*. Kulmitra *et al.*, (2017) noticed some of the *P. fuorescens* did not show any inhibition of mycelial growth as the pathogen over grew the bio agent. Mori *et al.*, (1987) noticed that butyric acid produced from *P. florescens* will inhibit mycelial growth of Pyricularia by 50-60%. Production of antibiotis *viz.*, HCN, pyrrolnitrin, phenazine, Cyclic Lipo Peptides (CLPs) and 2,4-diacetyl phloroglucinol and lytic enzymes by *P. fluorescens* against fungal pathogens were reported by many workers (Rosales *et al*., 1995; Ramamoorthy *et al*., 2001; Saravanakumar *et al*., 2008 and Gross and Loper, 2009).

Table 4.11. List of bacterial bio-control agents isolated from major finger millet growing areas of Andhra Pradesh

S. No.	District	Mandal	Village	Bacterial bio-control isolates
1.	YSR Kadapa	Vallur	Lingayapalle	BLN-1, BLN-2
			Vallur	BVA
		Kamalapuram	Kamalapuram	BKM
2.	Chittoor	Palamaneru	Moram	BMO
			Palamaneru	BPA-1, BPA-2
			Gonugur	BGO
		Kuppam	Jarugu	BJR
3.	Anantapur	Madakasira	Govindapuram	BGV-1, BGV-2
			Jadrahalli	BJD
		Gudibanda	Morubagal	BMR
			Gudibanda	BGD
4.	Vizianagaram	Vizianagaram	ARS Vizianagaram	BVP-1, BVB-2, BVB-3
		Peapally	Peapally	BPE-1, BPE-2
5.	Kurnool	Pattikonda	Chakkaralla	BCH
			Jutur	BJT-1, BJT-2
		Racharla	Anumalapalli	BAN
6.	Prakasam	Komarolu	Taticherla	BTT
		Veldurthi	Veldurthi	BVE
7.	Guntur	Macherla	Koppunur	BKP

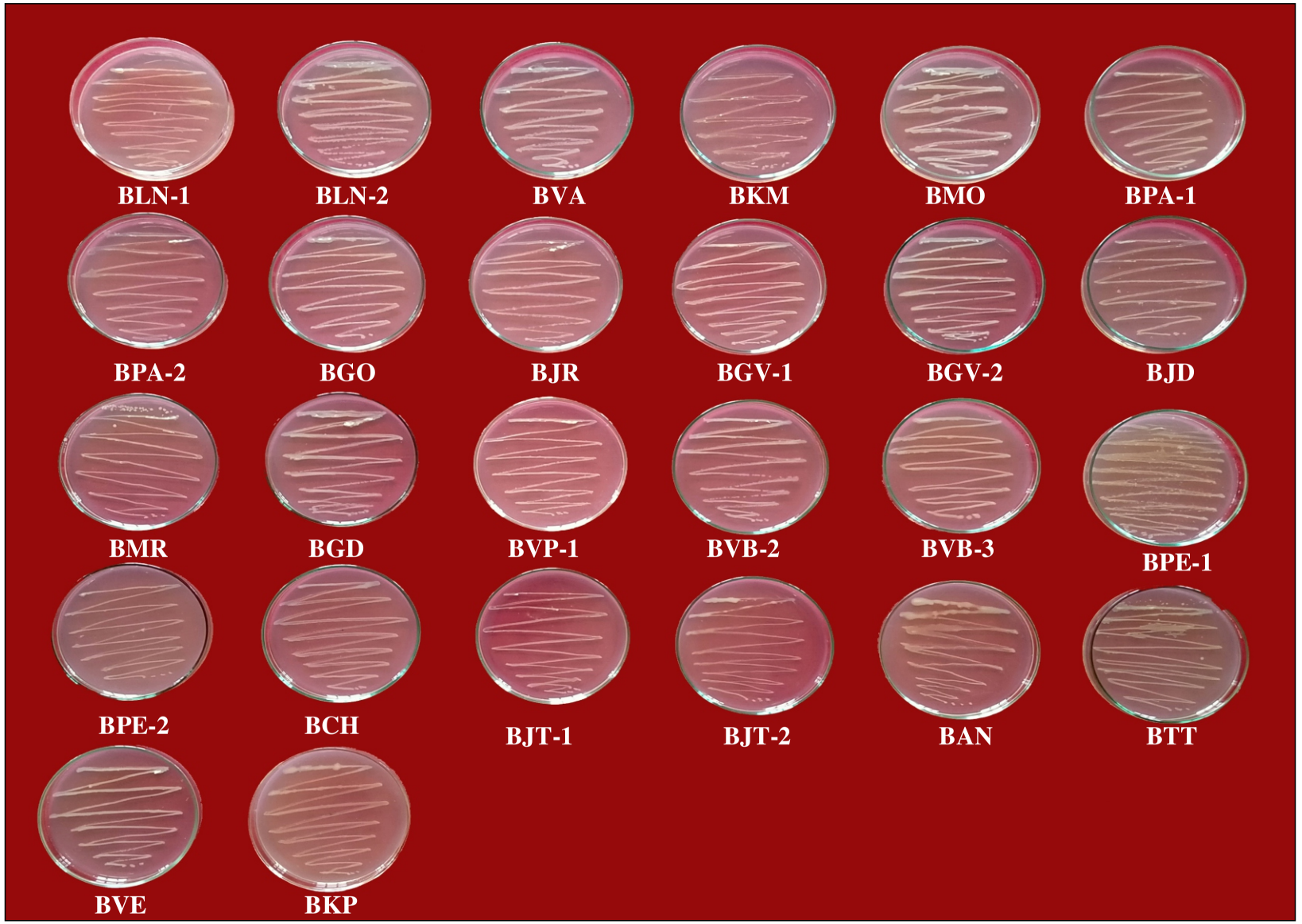


Plate 18. Pure cultures of native bacterial bio-control agent isolates

Table 4.12. Evaluation of native bacterial bio-control agents against *P. grisea* (VIZ-1) under *in vitro*

S. No.	Isolate	Average radial growth (mm)*	Per cent growth inhibition over control*
1.	BLN-1	50.83	43.52^{g} (41.27)
2.	BLN-2	66.23	26.41^{c} (30.92)
3.	BVA	34.58	61.57^{j} (51.69)
4.	BKM	47.25	47.50^{h} (43.57)
5.	BMO	47.92	46.76^{h} (43.14)
6.	BPA-1	61.83	31.30^{e} (34.02)
7.	BPA-2	75.17	16.48^{b} (23.95)
8.	BGO	40.92	54.54^{i} (47.60)
9.	BJR	79.83	11.30^{a} (19.63)
10.	BGV-1	24.92	72.31^{mn} (58.26)
11.	BGV-2	29.75	66.94^{k} (54.91)
12.	BJD	30.00	66.67^{k} (54.74)
13.	BMR	34.42	61.76^{j} (51.80)
14.	BGD	30.42	66.20^{k} (54.46)
15.	BVP-1	18.42	79.54^{q} (63.11)
16.	BVB-2	25.33	71.85^{m} (57.96)
17.	BVB-3	30.17	66.48^{k} (54.62)
18.	BPE-1	23.58	73.80^{o} (59.21)
19.	BPE-2	60.42	32.87^{f} (34.98)
20.	BCH	27.00	70.00^{l} (56.79)

21.	BJT-1	23.08	74.35^{op} (59.57)
22.	BJT-2	23.83	73.52^{no} (59.03)
23.	BAN	25.17	72.04^{m} (58.08)
24.	BTT	22.00	75.56^{p} (60.37)
25.	BVE	59.25	34.17^{f} (35.77)
26.	BKP	64.92	27.87^{d} (31.86)
27.	control	90.00	0.00 (0.00)
		C.D	1.375 (0.881)
		SE (m)	0.310
		SE(d)	0.439
		C.V.	1.504 (1.111)

***** Mean of three replications, in a column mean followed by a common letter are not significantly different

( ) Angular transformed values

3.3.3 Evaluation of Commercially Available Fungicides against Pathogen

Among the nine systemic fungicides used in the present investigation, under *In vitro* conditions, all the fungicides had the inhibition effect on radial growth of *P. grisea*. The complete inhibition of the test pathogen was observed with Tebuconazole 50% + Trifloxystrobin 25% WG at all the concentrations tested. However, Tricyclazole 75% WP and Carbendazim 50% WP were on par with Tebuconazole 50% + Trifloxystrobin 25% WG by completely inhibiting the mycelial growth of the pathogen. Azoxystrobin 11% + Difenconazole 18.3% SC showed 68.89, 72.22 and 76.67% inhibition of test pathogen at 500, 1000 and 1500 ppm concentrations respectively. Isoprothiolane 40% EC showed 57.78, 62.22 and 72.59% inhibition of the fungus at 500, 1000 and 1500 ppm respectively. Tebuconazole 25.9% EC showed 61.11, 62.22 and 66.30% inhibition on radial growth of the test pathogen at 1500, 2000 and 2500 ppm concentrations respectively. While, minimum inhibition in growth of the test pathogen was

Plate 19. ***In vitro*** **evaluation of native bacterial bio-control agents against** ***P. grisea*** **(VIZ-1)**

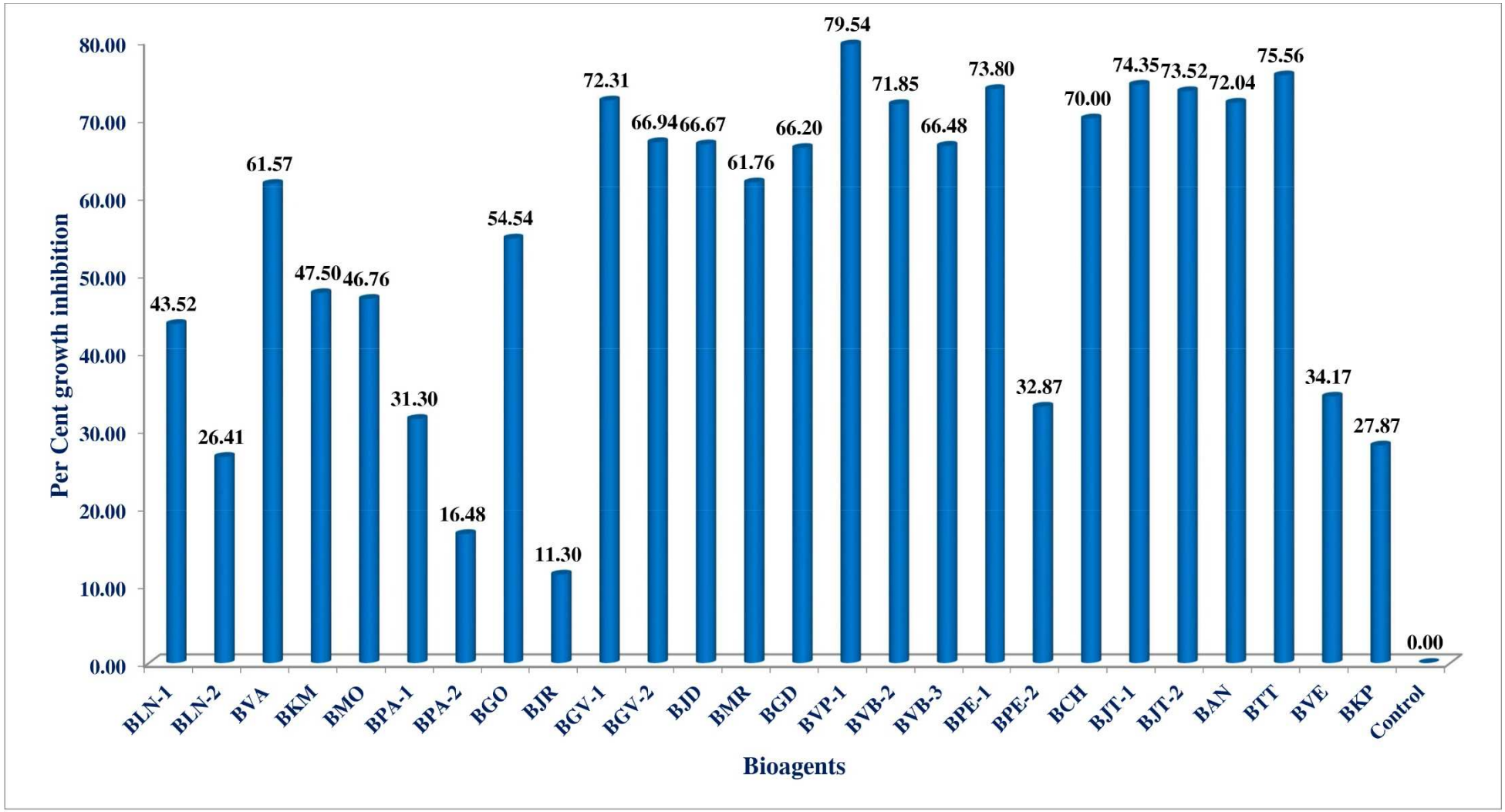


Fig. 4.8. ***In vitro*** **evaluation of native bacterial bio-control isolates against mycelial growth of** ***P. grisea*** **(VIZ-1)**

noticed in Azoxystrobin 23% SC (13.33 %) and Kresoxy methyl 44.3% SC (13.33%) both at 500 ppm and Difenconazole 25% EC (13.70%) at 300 ppm concentrations. However, Azoxystrobin 23% SC showed 16.67 and 19.63% as well as Kresoxy methyl 44.3% SC showed 16.67 and 18.89% inhibition at 1000 and 1500 ppm concentrations respectively. Difenconazole 25% EC showed 15.56 and 17.78% inhibition of test pathogen at 600 and 900 ppm concentrations respectively (Table 4.13) (Plate 20) (Fig. 4.9). The results of present study are in agreement with Kavanashree *et al*. (2019) who evaluated fungicides against *M. oryzae* under *in vitro* and reported that Tebuconazole 50% + Trifloxystrobin 25% WG completely inhibited the growth of fungus and germination of fungal spores in all concentration. Konda *et al*. (2016) who reported complete inhibition of mycelial growth of *Pyricularia seteria* in Tebuconazole 50% + Trifloxystrobin 25% WG, at 500, 1000 and 2000 ppm concentrations. Kulmitra *et al.* (2017) reported that Tebuconazole + Trifloxystrobin (50% + 25%) WG showed 99.40% inhibition of blast pathogen. Singh *et al*., (2019) also recorded that Tebuconazole 50% + Trifloxystrobin 25% (WG) was found to be the most effective fungicide against the leaf blast disease.

Table 4.13. *In vitro* evaluation of commercially available fungicides against *P. grisea* (VIZ-1)

S. No.	Fungicides	Concentration (ppm)	Average mycelial growth (mm)	Per cent growth inhibition over control (PGI)*
1.	Azoxystrobin 23% SC	500	78.00	13.33[a] (21.41)
		1000	75.00	16.67[bc] (24.09)
		1500	72.33	19.63[e] (26.30)
2.	Kresoxy methyl 44.3% SC	500	78.00	13.33[a] (21.41)
		1000	75.00	16.67[bc] (24.09)
		1500	73.00	18.89[de] (25.75)
3.	Tebuconazole 25.9% EC	1500	35.00	61.11[g] (51.42)
		2000	34.00	62.22[g] (52.08)
		2500	30.33	66.30[h] (54.52)

4.	Difenconazole 25% EC	300	77.67	13.70[a] (21.72)
		600	76.00	15.56[b] (23.22)
		900	74.00	17.78[cd] (24.93)
5.	Tricyclazole 75%WP	300	0.00	100.00[l] (90.00)
		600	0.00	100.00[l] (90.00)
		900	0.00	100.00[l] (90.00)
6.	Tebuconazole 50% + Trifloxystrobin 25% WG	200	0.00	100.00[l] (90.00)
		400	0.00	100.00[l] (90.00)
		600	0.00	100.00[l] (90.00)
7.	Azoxystrobin 11% + Difenconazole 18.3% SC	500	28.00	68.89[o] (56.10)
		1000	25.00	72.22[j] (58.20)
		1500	21.00	76.67[k] (61.12)
8.	Carbendazim 50% WP	500	0.00	100.00[l] (90.00)
		1000	0.00	100.00[l] (90.00)
		1500	0.00	100.00[l] (90.00)
9.	Isoprothiolane 40% EC	500	38.00	57.78[f] (49.48)
		1000	34.00	62.22[g] (52.08)
		1500	24.67	72.59[j] (58.44)
10.	Control	-	90.00	0.00 (0.00)
	C.D.			1.02
	SE(m)			0.36
	SE(d)			0.51
	C.V.			1.11

***** Mean of three replications, in a column mean followed by a common letter are not significantly different

( ) Angular transformed values

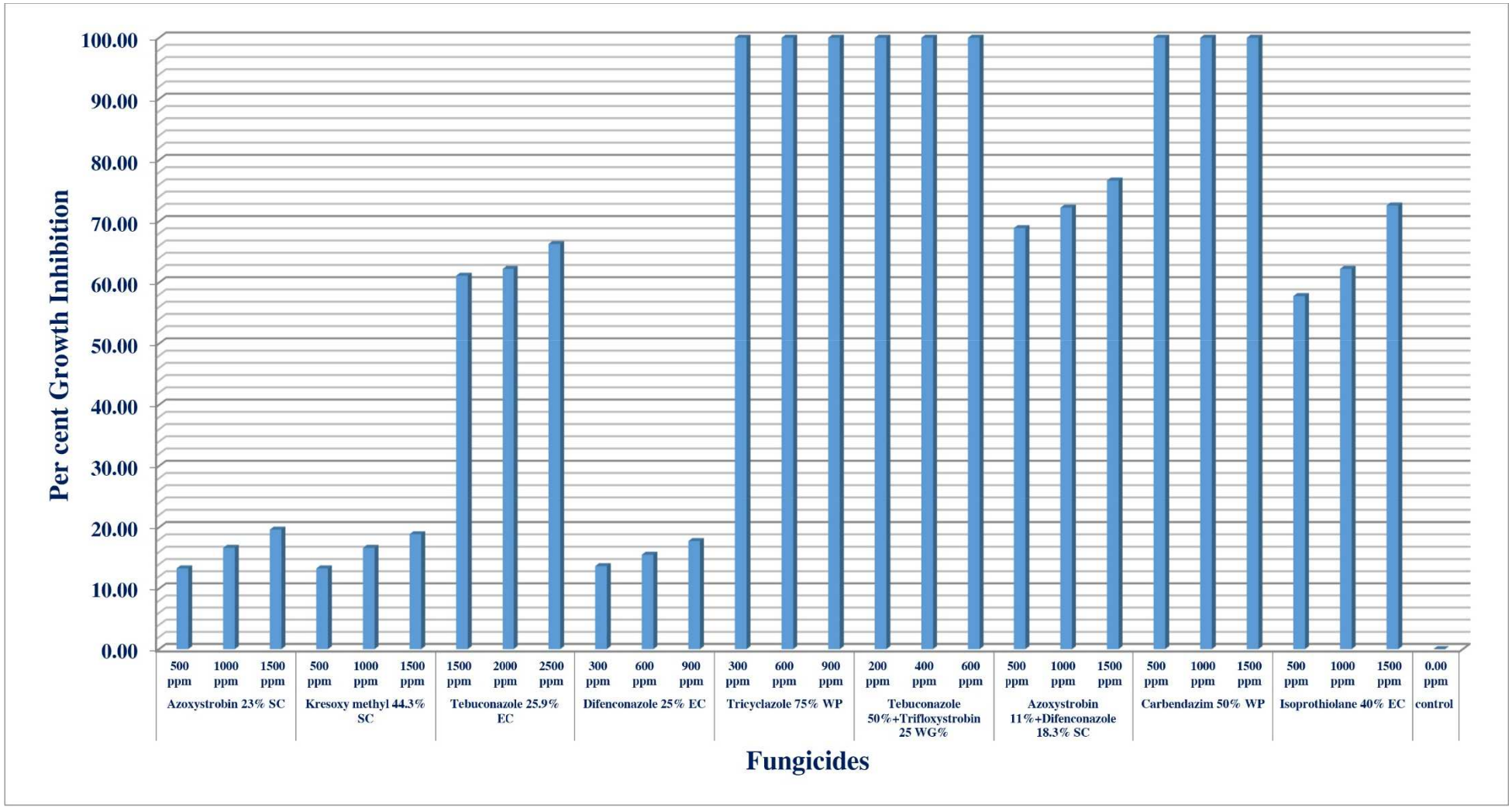


Fig. 4.9. *In vitro* evaluation of fungicides against mycelial growth of *P. grisea* (VIZ-I)

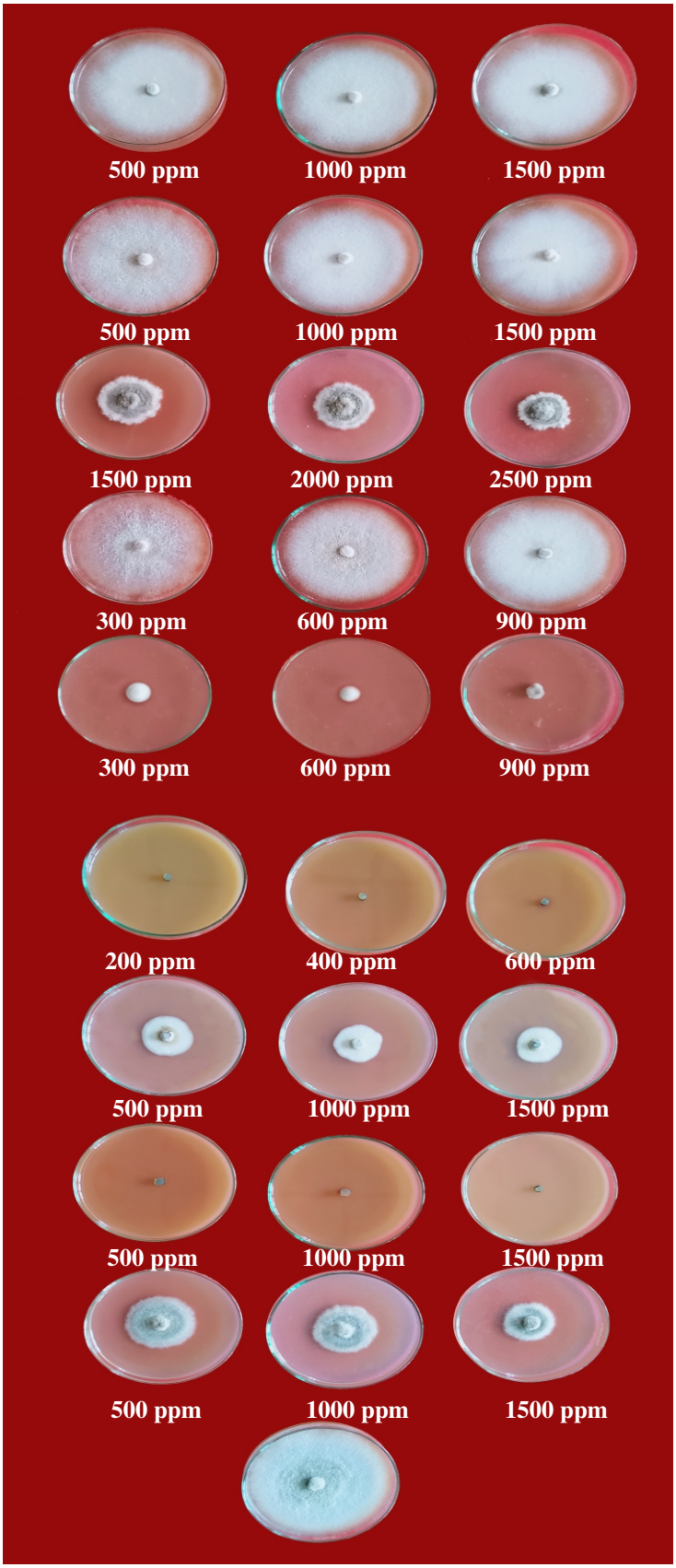


Plate 20. *In vitro* evaluation of fungicides against *P. grisea* (VIZ-1)

4.3.4 Studies on Compatibility of Potential Native Bio-control Agent with Effective Fungicides

The systemic fungicides Tricyclazole 75% WP (300, 600 and 900 ppm), Tebuconazole 50% + Trifloxystrbin 25% WG (200, 400 and 600 ppm) and Carbendazim 50% WP (500, 1000 and 1500 ppm concentrations) were tested with BVP-1 (*Pseudomonas fluorescens*) with bacterial bio-control agent using agar well diffusion method (Murray *et al.* 1995). The results revealed that, Tebuconazole 50% + Trifloxystrbin 25% WG at 200, 400 and 600 ppm concentrations and Carbendazim 50% WP at 500, 1000 and 1500 ppm concentrations were highly compatible with zero per cent inhibition of BVP-1 bacterial growth. While, Tricyclazole 75 % WP was showed significant higher inhibition (9.35 %) of potential bacterial bio-control agent at 900 ppm with inhibition zone radius of 13 mm followed by 2.58 % (6.83 mm) and 1.52 % (5.25 mm) inhibition at 600 and 300 ppm respectively (Table 4.14) (Plate 21) (Fig. 4.10). Similar results are in agreement with Singh *et al*., (2021) who reported compatibility of Tebuconazole 50 %+ trifloxystrobin 25 % WG with *P. fluorescens* at all tested concentrations. Hanuman and Madhavi (2018) reported that higher compatibility of *Pseudomonas fluorescens* with trifloxystrobin + Tebuconazole was found at 100 ppm and moderately compatible at 500 ppm concentrations.

Table 4.14. Compatibility studies between effective fungicides with potential bacterial bio-control agent (BVP-1)

S. No.	Fungicides	Concentrations (ppm)	Average of inhibition zone radius (mm)	Per cent inhibition*
1.	Tricyclazole 75% WP	300	5.25	1.52^{b} (7.08)
		600	6.83	2.58^{c} (9.25)
		900	13.00	9.35^{d} (17.80)
2.	Tebuconazole 50% + Trifloxystrbin 25% WG	200	0.00	0.00^{a} (0.00)
		400	0.00	0.00^{a} (0.00)
		600	0.00	0.00^{a} (0.00)
3.	Carbendazim 50% WP	500	0.00	0.00^{a} (0.00)
		1000	0.00	0.00^{a} (0.00)
		1500	0.00	0.00^{a} (0.00)
4.	Control		42.08	-
	C. D.		0.06	0.06
	SE (m)		0.02	0.02
	SE (d)		0.03	0.03
	C. V.		1.02	2.89

***** Mean of three replications, in a column mean followed by a common letter are not significantly different

( ) Angular transformed values

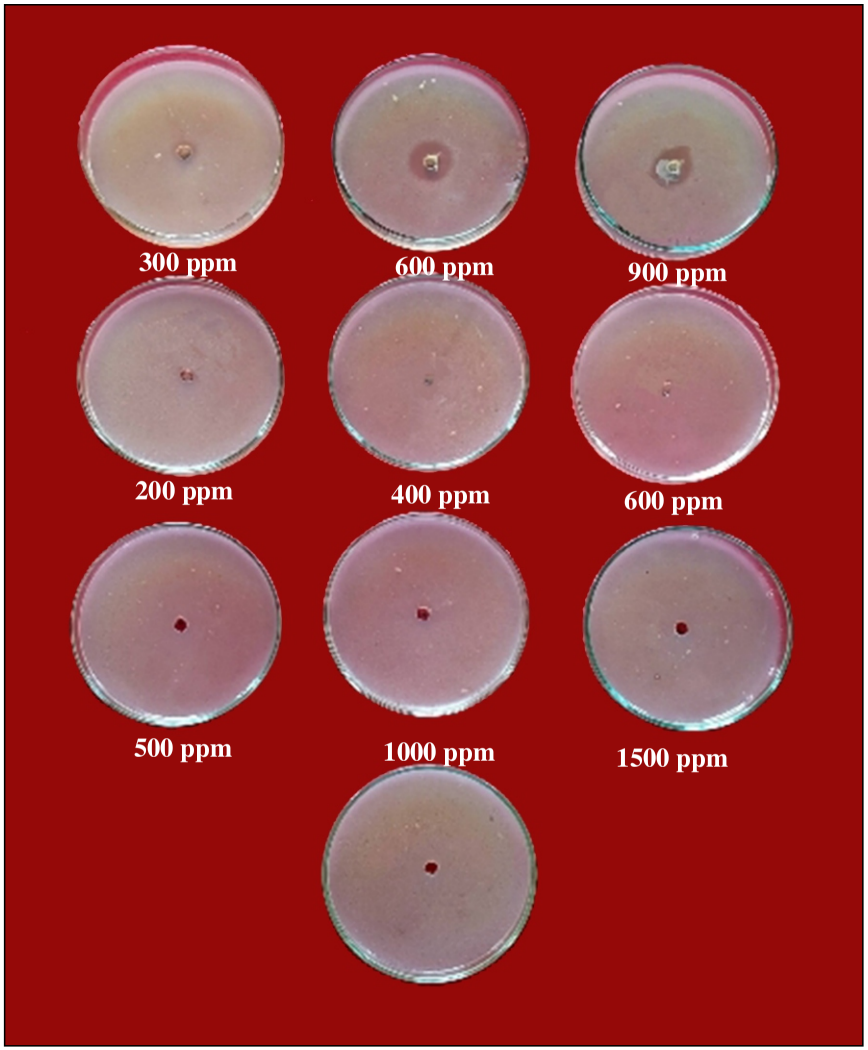


Plate 21. Compatibility studies between potential bacterial bio-control agent (BVP-1) and effective fungicides

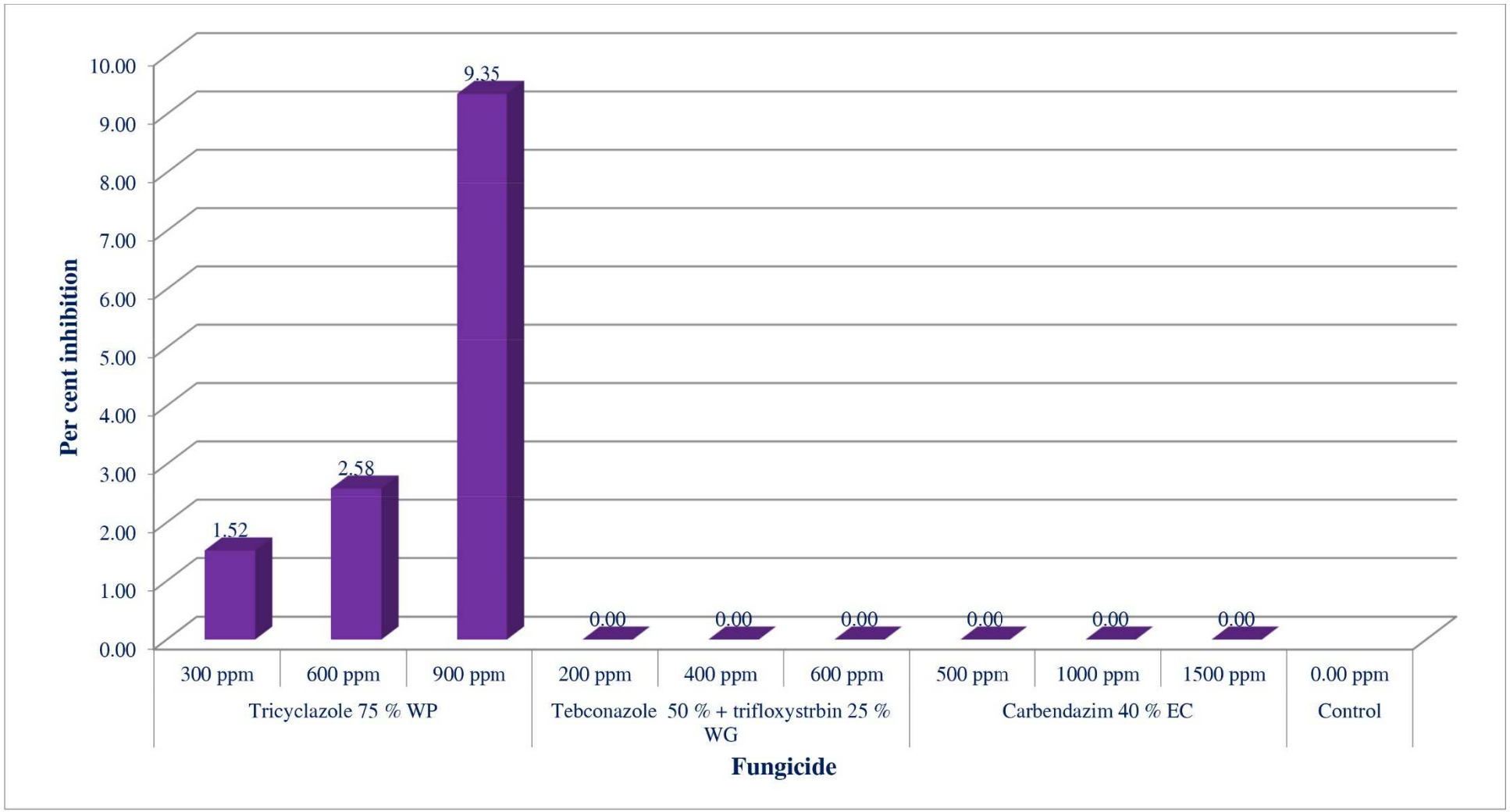


Fig. 4.10. Compatibility studies of effective fungicides with potential bacterial bio-control agent (BVP-1) under *in vitro*

4.4 TO SCREEN THE GENOTYPES FOR THEIR RESISTANCE AGAINST BLAST

4.4.1 Screening of Genotypes against Blast

In two consecutive *Kharif* seasons (2020 and 2021) 74 lines of finger millet were evaluated using check (VR-708) against blast disease under natural field conditions at high disease pressure at ARS Vizianagaram (Plate 22 and Plate 23). The lines were grouped under different degrees of resistance on the basis of disease reaction for leaf, neck and finger blast.

74 lines of finger millet including local check VR 708 were evaluated for leaf, neck and finger blast disease. For each field experiment conducted during *Kharif* 2020 and *Kharif* 2021 data on leaf, neck and finger blast incidence were analyzed separately using ANOVA. The data of both the years were pooled and ANOVA performed. Pooled analysis of leaf blast data indicated that five lines (GE-1, GE-5, GE-41, GE-44 and GE-45) were found as highly resistant (1-1.9% incidence), 31 lines (GE-2, GE-4, GE-7, GE-13, GE-17, GE-18, GE-24, GE-25, GE-28, GE-29, GE-30, GE-31, GE-33, GE-34, GE-36, GE-37, GE-38, GE-39, GE-40, GE-42, GE-43, GE-46, GE-51, GE-56, GE-57, GE-60, GE-63, GE-64, GE-69, GE-72 and GE-74) were reacted as resistant (2-10% incidence), 23 lines were reacted as moderately resistant (11-30% incidence), 15 lines were found to be susceptible (31-50% incidence) when compared with check VR708 which shown highly susceptible reaction (Table 4.15).

Out of 74 lines, 30 lines (GE-2, GE-3, GE-5, GE-8, GE-9, GE-10, GE-12, GE-13, GE-15, GE-16, GE-17, GE-18, GE-19, GE-20, GE-21, GE-24, GE-28, GE-31, GE-32, GE-33, GE-41, GE-53, GE-54, GE-56, GE-60, GE-61, GE-62, GE-66, GE-68, GE-74) were reacted as resistant to neck blast, 29 lines (GE-4, GE-6, GE-23, GE-26, GE-27, GE-29, GE-30, GE-34, GE-35, GE-36, GE-37, GE-39, GE-40, GE-43, GE-44, GE-48, GE-49, GE-51, GE-52, GE-55, GE-57, GE-58, GE-59, GE-63, GE-64, GE-65, GE-69, GE-71 and GE-73) shown moderately resistant and 15 lines (GE-, GE-7, GE-11, GE-14, G-22, GE-25, GE-38, GE-42, GE-45, GE-46, GE-47, GE-50, GE-67, GE-70 and GE-72) were

reacted as susceptible to neck blast when compared with check VR 708 which was showed highly susceptible reaction (Table 4.16).

Out of 74 screened lines 20 lines (GE-2, GE-3, GE-6, GE-8, GE-9, GE-10, GE-12, GE-13, GE-17, GE-20, GE-24, GE-31, GE-32, GE-33, GE-40, GE-53, GE-62, GE-63, GE-64 and GE-74 showed moderately resistance (6-25% incidence) reaction to finger blast, 41 lines (GE-1, GE-5, GE-14, GE-15, GE-16, GE-18, GE-19, GE-21, GE-23, GE-26, GE-28, GE-29, GE-30, GE-34, GE-35, GE-36, GE-39, GE-41, GE-42, GE-43, GE-44, GE-45, GE-46, GE-48, GE-49, GE-50, GE-52, GE-54, GE-55, GE-56, GE-57, GE-58, GE-59, GE-60, GE-61, GE-65, GE-66, GE-67, GE-68, GE-70 and GE-71) with susceptible reaction (26-50% incidence) and 14 lines including VR708 showed highly susceptible reaction (>50% incidence) with 82.49% incidence to finger blast incidence (Table 4.16).

Table 4.15. Screening of finger millet genotypes for their resistance against leaf blast during *Kharif* 2020 and *Kharif* 2021

LEAF BLAST					
S. No.	**Genotypes**	***Kharif* 2020**	***Kharif* 2021**	**Pooled**	**Disease reaction**
1.	GE-1	1.9 ±0.1	1.8 ± 0.3	1.83 ± 0.18	HR
2.	GE-2	2.0 ± 0.2	2.1 ± 0.2	2.05 ± 0.20	R
3.	GE-3	3.6 ± 0.4	4.4 ± 0.5	3.98 ± 0.43	S
4.	GE-4	2.4 ± 0.4	2.7 ± 0.4	2.53 ± 0.38	R
5.	GE-5	1.7 ± 0.3	1.3 ± 0.3	1.50 ± 0.30	HR
6.	GE-6	3.9 ± 0.3	3.7 ± 0.4	3.78 ± 0.33	MR
7.	GE-7	2.1 ± 0.5	1.9 ± 0.4	2.00 ± 0.45	R
8.	GE-8	6.2 ± 0.4	6.4 ± 0.3	6.30 ± 0.35	S
9.	GE-9	6.0 ± 0.3	7.0 ± 0.3	6.50 ± 0.30	S
10.	GE-10	5.2 ± 0.4	4.8 ± 0.3	4.98 ± 0.33	MR
11.	GE-11	4.0 ± 0.2	3.9 ± 0.4	3.93 ± 0.28	MR
12.	GE-12	6.2 ± 0.6	5.6 ± 0.2	5.90 ± 0.41	S
13.	GE-13	2.7 ± 0.7	2.0 ± 0.3	2.33 ± 0.48	R

14.	GE-14	6.9 ± 0.5	5.2 ± 0.4	6.03 ± 0.43	S
15.	GE-15	5.8 ± 0.4	5.4 ± 0.3	5.58 ± 0.33	MR
16.	GE-16	4.5 ± 0.3	4.8 ± 0.4	4.63 ± 0.33	MR
17.	GE-17	3.6 ± 0.6	2.5 ± 0.3	3.05 ± 0.45	R
18.	GE-18	2.9 ± 0.5	2.5 ± 0.3	2.70 ± 0.41	R
19.	GE-19	5.1 ± 0.3	4.1 ± 0.2	4.60 ± 0.25	MR
20.	GE-20	5.3 ± 0.9	5.5 ± 0.3	5.40 ± 0.60	MR
21.	GE-21	6.2 ± 0.4	7.1 ± 0.1	6.63 ± 0.23	S
22.	GE-22	5.9 ± 0.3	6.4 ± 0.4	6.13 ± 0.33	S
23.	GE-23	4.8 ± 0.8	3.7 ± 0.2	4.23 ± 0.48	MR
24.	GE-24	2.3 ± 0.7	1.8 ± 0.3	2.05 ± 0.50	R
25.	GE-25	3.2 ± 0.2	2.9 ± 0.2	3.05 ± 0.20	R
26.	GE-26	4.2 ± 0.4	4.5 ± 0.2	4.33 ± 0.28	MR
27.	GE-27	3.6 ± 0.4	4.2 ± 0.2	3.88 ± 0.28	MR
28.	GE-28	1.8 ± 0.4	2.8 ± 0.2	2.28 ± 0.28	R
29.	GE-29	3.4 ± 0.4	3.4 ± 0.3	3.40 ± 0.35	R
30.	GE-30	2.8 ± 0.2	2.3 ± 0.0	2.55 ± 0.11	R
31.	GE-31	2.2 ± 0.3	2.1 ± 0.2	2.15 ± 0.25	R
32.	GE-32	3.9 ± 0.3	3.8 ± 0.2	3.83 ± 0.23	MR
33.	GE-33	1.7 ± 0.3	2.1 ± 0.3	1.90 ± 0.30	R
34.	GE-34	1.9 ± 0.3	3.0 ± 0.3	2.45 ± 0.30	R
35.	GE-35	4.8 ± 0.4	4.5 ± 0.5	4.63 ± 0.43	MR
36.	GE-36	3.5 ± 0.3	2.3 ± 0.2	2.90 ± 0.25	R
37.	GE-37	4.2 ± 0.4	2.9 ± 0.2	3.55 ± 0.32	R
38.	GE-38	2.1 ± 0.7	1.9 ± 0.2	2.00± 0.45	R
39.	GE-39	1.4 ± 0.4	2.5 ± 0.3	1.93 ± 0.33	R
40.	GE-40	3.0 ± 0.4	2.0 ± 0.3	2.50 ± 0.35	R
41.	GE-41	1.4 ± 0.3	1.7 ± 0.1	1.58 ± 0.18	HR
42.	GE-42	1.7 ± 0.3	2.0 ± 0.1	1.85 ± 0.22	R
43.	GE-43	2.3 ± 0.3	2.8 ± 0.2	2.55 ± 0.25	R
44.	GE-44	1.1 ± 0.3	1.1 ± 0.5	1.08 ± 0.38	HR
45.	GE-45	1.2 ± 0.2	1.8 ± 0.2	1.50 ± 0.21	HR

46.	GE-46	2.4 ± 0.4	3.3 ± 0.1	2.85 ± 0.25	R
47.	GE-47	5.4 ± 0.4	5.6 ± 0.1	5.48 ± 0.23	MR
48.	GE-48	6.6 ± 0.4	6.7 ± 0.1	6.65 ± 0.25	S
49.	GE-49	6.2 ± 0.4	6.8 ± 0.3	6.53 ± 0.33	S
50.	GE-50	6.2 ± 0.6	7.3 ± 0.1	6.73 ± 0.33	S
51.	GE-51	3.2 ± 0.4	3.9 ± 0.1	3.55 ± 0.25	R
52.	GE-52	5.4 ± 0.6	7.0 ± 0.4	6.18 ± 0.48	S
53.	GE-53	4.6 ± 0.7	3.8 ± 0.5	4.20 ± 0.55	MR
54.	GE-54	4.9 ± 0.3	4.1 ± 0.5	4.50 ± 0.40	MR
55.	GE-55	4.9 ± 0.1	4.8 ± 0.5	4.85 ± 0.30	MR
56.	GE-56	3.2 ± 0.4	3.8 ± 0.3	3.50 ± 0.35	R
57.	GE-57	3.0 ± 0.2	4.3 ± 0.5	3.63 ± 0.33	R
58.	GE-58	5.4 ± 0.4	5.4 ± 0.5	5.40 ± 0.45	MR
59.	GE-59	3.2 ± 0.4	5.1 ± 0.4	4.13 ± 0.38	MR
60.	GE-60	3.7 ± 0.5	2.7 ± 0.5	3.18 ± 0.48	R
61.	GE-61	6.2 ± 0.4	7.0 ± 0.4	6.58 ± 0.38	S
62.	GE-62	4.2 ± 0.2	3.6 ± 0.1	3.88 ± 0.13	MR
63.	GE-63	3.7 ± 0.6	2.7 ± 0.6	3.20 ± 0.55	R
64.	GE-64	3.9 ± 0.6	3.1 ± 0.6	3.50 ± 0.55	R
65.	GE-65	6.3 ± 0.3	6.9 ± 0.5	6.58 ± 0.38	S
66.	GE-66	3.8 ± 0.4	4.8 ±0.4	4.30 ± 0.40	MR
67.	GE-67	6.1 ± 0.7	7.0 ± 0.6	6.55 ± 0.65	S
68.	GE-68	2.2 ± 0.4	3.5 ± 0.4	2.88 ± 0.38	MR
69.	GE-69	2.7 ± 0.4	3.6 ± 0.3	3.15 ± 0.30	R
70.	GE-70	3.6 ± 0.4	4.7 ± 0.4	4.13 ± 0.38	MR
71.	GE-71	5.1 ± 0.5	7.1 ± 0.3	6.10 ± 0.35	S
72	GE-72	2.3 ± 0.5	3.2 ± 0.5	2.75 ± 0.50	R
73.	GE-73	2.5 ± 0.5	4.8 ± 0.4	3.63 ± 0.43	MR
74.	GE-74	2.7 ± 0.3	3.7 ± 0.6	3.20 ± 0.45	R
75.	VR708	8.2 ± 0.2	7.9 ± 0.2	8.05 ± 0.20	HS
	C. D.	1.10	0.56	0.83	
	C. V.	15.56	7.10	11.33	

Table 4.16. Screening of finger blast genotypes for their resistance against neck and finger blast during *Kharif* 2020 and *Kharif* 2021

		NECK BLAST				FINGER BLAST			
S. No.	**Genotypes**	***Kharif* 2020**	***Kharif* 2021**	**Pooled**	**Disease reaction**	***Kharif* 2020**	***Kharif* 2021**	**Pooled**	**Disease reaction**
1.	GE-1	3.2 ± 4.0	3.4 ± 3.5	3.30 ± 3.75	S	39.70 (39.03)	38.70 (38.45)	39.20 (38.74)	S
2.	GE-2	2.1 ± 3.2	1.8 ± 3.5	1.95 ± 3.33	R	23.27 (28.77)	23.52 (28.93)	23.39 (28.85)	MR
3.	GE-3	1.0 ± 3.1	2.0 ± 2.8	1.50 ± 2.93	R	24.15 (29.37)	22.30 (28.16)	23.23 (28.76)	MR
4.	GE-4	2.3 ± 4.0	2.6 ± 3.8	2.40 ± 3.88	MR	52.10 (46.19)	49.95 (44.95)	51.03 (45.57)	HS
5.	GE-5	1.7 ± 3.6	1.8 ± 2.8	1.79 ± 3.18	R	40.95 (39.74)	40.05 (39.22)	40.50 (39.48)	S
6.	GE-6	2.6 ± 3.4	2.7 ± 2.8	2.63 ± 3.08	MR	18.65 (25.50)	18.15 (25.15)	18.40 (25.32)	MR
7.	GE-7	3.6 ± 2.0	3.5 ± 5.0	3.53 ± 3.50	S	68.50 (55.87)	65.85 (54.30)	67.18 (55.08)	HS
8.	GE-8	2.1 ± 3.3	1.7 ± 4.8	1.89 ± 4.00	R	24.60 (29.66)	23.40 (28.89)	24.00 (29.28)	MR
9.	GE-9	1.1 ± 2.9	1.6 ± 3.8	1.33 ± 3.30	R	17.15 (24.34)	16.40 (23.80)	16.78 (24.07)	MR
10.	GE-10	2.0 ± 3.1	1.1 ± 3.3	1.55 ± 3.18	R	21.40 (27.45)	19.90 (26.44)	20.65 (26.95)	MR
11.	GE-11	3.8 ± 3.7	3.4 ± 6.3	3.62 ± 4.95	S	61.65 (51.76)	60.75 (51.21)	61.20 (51.48)	HS
12.	GE-12	1.5 ± 1.7	1.2 ± 5.0	1.32 ± 3.35	R	25.20 (30.04)	25.20 (30.04)	25.20 (30.04)	MR
13.	GE-13	1.2 ± 3.4	1.3 ± 2.8	1.28 ± 3.05	R	21.35 (27.43)	21.35 (27.43)	21.35 (27.43)	MR
14.	GE-14	3.0 ± 3.4	3.1 ± 3.5	3.07 ± 3.45	S	45.25 (42.25)	45.25 (42.25)	45.25 (42.25)	S

15.	GE-15	1.3 ± 3.8	1.8 ± 4.8	1.56 ± 4.25	R	28.30 (32.06)	27.80 (31.76)	28.05 (31.91)	S
16.	GE-16	2.0 ± 3.8	2.1 ± 4.0	2.06 ± 3.88	R	44.20 (41.64)	44.00 (41.53)	44.10 (41.58)	S
17.	GE-17	1.4 ± 3.7	1.6 ± 2.8	1.51 ± 3.23	R	20.00 (26.47)	19.40 (26.06)	19.70 (26.27)	MR
18.	GE-18	1.2 ± 2.5	1.4 ± 3.3	1.30 ± 2.88	R	37.75 (37.89)	37.25 (37.59)	37.50 (37.74)	S
19.	GE-19	1.4 ± 4.5	2.1 ± 3.8	1.79 ± 4.13	R	37.75 (37.89)	37.50 (37.74)	37.63 (37.81)	S
20.	GE-20	1.3 ± 2.5	1.4 ± 3.0	1.32 ± 2.75	R	19.35 (25.95)	19.10 (25.78)	19.23 (25.86)	MR
21.	GE-21	1.8 ± 4.8	2.3 ± 3.0	2.05 ± 3.88	R	39.75 (39.05)	39.05 (38.65)	39.40 (38.85)	S
22.	GE-22	3.6 ± 2.3	3.6 ± 2.5	3.60 ± 2.38	S	62.00 (51.96)	60.25 (50.91)	61.13 (51.43)	HS
23.	GE-23	2.4 ± 2.0	2.6 ± 4.6	2.52 ± 3.28	MR	40.00 (39.21)	40.25 (39.35)	40.13 (39.28)	S
24.	GE-24	1.1 ± 2.0	1.5 ± 2.5	1.31 ± 2.23	R	16.95 (24.25)	16.30 (23.77)	16.63 (24.01)	MR
25.	GE-25	3.9 ± 3.1	3.8 ± 3.0	3.85 ± 3.03	S	75.70 (60.51)	74.70 (59.81)	75.20 (60.16)	HS
26.	GE-26	3.0 ± 3.3	2.1 ± 3.0	2.56 ± 3.15	MR	45.00 (42.10)	43.85 (41.44)	44.43 (41.77)	S
27.	GE-27	2.5 ± 2.7	2.4 ± 4.3	2.42 ± 345	MR	55.30 (48.03)	54.80 (47.75)	55.05 (47.89)	HS
28.	GE-28	1.7 ± 3.3	1.9 ± 3.3	1.78 ± 3.25	R	40.30 (39.38)	37.80 (37.86)	39.05 (38.62)	S
29.	GE-29	2.6 ± 2.3	2.8 ± 4.0	2.67 ± 3.13	MR	38.65 (38.37)	37.65 (37.75)	38.15 (38.06)	S
30.	GE-30	3.0 ± 4.0	2.7 ± 1.8	2.87 ± 2.88	MR	39.65 (39.00)	36.50 (37.08)	38.08 (38.04)	S

31.	GE-31	1.3 ± 3.4	1.3 ± 2.5	1.31 ± 2.93	R	26.45 (30.86)	25.40 (30.12)	25.93 (30.49)	MR
32.	GE-32	1.5 ± 2.0	1.2 ± 2.5	1.35 ± 2.23	R	18.35 (25.30)	18.60 (25.49)	18.48 (25.40)	MR
33.	GE-33	1.3 ± 2.8	2.0 ± 1.8	1.66 ± 2.25	R	19.55 (26.13)	19.80 (26.33)	19.68 (26.23)	MR
34.	GE-34	2.3 ± 2.4	2.5 ± 2.8	2.42 ± 2.58	MR	48.75 (44.25)	46.50 (42.96)	47.63 (43.60)	S
35.	GE-35	2.7 ± 2.2	2.7 ± 2.5	2.70 ± 2.33	MR	28.65 (32.30)	28.00 (31.90)	28.33 (32.10)	S
36.	GE-36	2.3 ± 1.2	2.1 ± 3.3	2.21 ± 2.33	MR	28.45 (32.17)	27.20 (31.40)	27.83 (31.78)	S
37.	GE-37	2.9 ± 0.7	2.5 ± 1.8	2.70 ± 1.23	MR	58.40 (49.85)	55.15 (47.94)	56.78 (48.89)	HS
38.	GE-38	3.0 ± 4.2	3.2 ± 3.0	3.12 ± 3.60	S	66.10 (54.41)	63.60 (52.88)	64.85 (53.64)	HS
39.	GE-39	2.6 ± 3.6	2.7 ± 4.8	2.66 ± 4.15	MR	49.40 (44.64)	49.15 (44.49)	49.28 (44.57)	S
40.	GE-40	2.6 ± 5.4	2.6 ± 2.0	2.60 ± 3.68	MR	25.45 (30.22)	24.20 (29.42)	24.83 (29.82)	MR
41.	GE-41	1.7 ± 3.2	1.3 ± 4.0	1.53 ± 3.58	R	32.45 (34.67)	32.80 (34.89)	32.63 (34.78)	S
42.	GE-42	4.1 ± 3.3	3.8 ± 3.0	3.94 ± 3.13	S	49.35 (44.61)	48.95 (44.38)	49.15 (44.49)	S
43.	GE-43	3.1 ± 4.0	1.9 ± 3.3	2.48 ± 3.60	MR	44.75 (41.96)	44.05 (41.56)	44.40 (41.76)	S
44.	GE-44	2.2 ± 0.6	2.3 ± 4.8	2.26 ± 2.68	MR	33.55 (35.32)	32.90 (34.94)	33.23 (35.13)	S
45.	GE-45	3.5 ± 1.4	3.5 ± 5.5	3.50 ± 3.45	S	42.60 (40.72)	42.10 (40.42)	42.35 (40.57)	S
46.	GE-46	3.7 ± 2.1	3.4 ± 5.5	3.51 ± 3.78	S	51.15 (45.64)	49.00 (44.41)	50.08 (45.02)	S

47.	GE-47	3.6 ± 2.3	3.7 ± 5.5	3.65 ± 3.88	S	55.55 (48.19)	53.55 (47.04)	54.55 (47.61)	HS
48.	GE-48	2.5 ± 3.7	2.0 ± 2.8	2.25 ± 3.20	MR	41.70 (40.19)	40.45 (39.44)	41.08 (39.81)	S
49.	GE-49	2.6 ± 4.4	2.3 ± 3.5	2.48 ± 3.93	MR	42.25 (40.49)	41.25 (39.89)	41.75 (40.19)	S
50.	GE-50	3.4 ± 4.0	3.5 ± 5.8	3.44 ± 4.88	S	51.75 (45.99)	48.40 (44.07)	50.08 (45.03)	S
51.	GE-51	2.7 ± 2.2	2.9 ± 3.5	2.79 ± 2.85	MR	59.35 (50.41)	57.45 (49.28)	58.40 (49.84)	HS
52.	GE-52	3.0 ± 2.7	2.7 ± 3.5	2.85 ± 3.10	MR	47.45 (43.52)	45.45 (42.37)	46.45 (42.94)	S
53.	GE-53	2.1 ± 4.1	1.8 ± 4.3	1.95 ± 4.15	R	21.95 (27.83)	21.30 (27.40)	21.63 (27.62)	MR
54.	GE-54	2.2 ± 3.4	1.8 ± 4.0	1.96 ± 3.70	R	37.70 (37.84)	37.20 (37.55)	37.45 (37.70)	S
55.	GE-55	2.3 ± 3.5	2.4 ± 3.3	2.38 ± 3.38	MR	43.25 (41.09)	42.25 (40.50)	42.75 (40.80)	S
56.	GE-56	1.5 ± 2.3	1.5 ± 4.3	1.49 ± 3.28	R	33.65 (35.38)	32.90 (34.94)	33.28 (35.16)	S
57.	GE-57	2.6 ± 4.2	2.6 ± 4.3	2.62 ± 4.23	MR	50.40) (45.21)	49.80 (44.87)	50.10 (45.04)	S
58.	GE-58	2.8 ± 2.8	2.5 ± 6.0	2.62 ± 4.38	MR	52.25 (46.28)	51.25 (45.70)	51.75 (45.99)	S
59.	GE-59	3.0 ± 3.2	3.1 ± 2.8	3.08 ± 2.95	MR	47.50 (43.54)	47.00 (43.25)	47.25 (43.39)	S
60.	GE-60	2.1 ± 3.4	1.8 ± 2.3	1.93 ± 2.83	R	50.10 (45.04)	49.85 (44.90)	49.98 (44.97)	S
61.	GE-61	1.9 ± 2.8	1.2 ± 1.9	1.58 ± 2.30	R	29.15 (32.55)	29.65 (32.89)	29.40 (32.72)	S
62.	GE-62	1.8 ± 2.0	2.1 ± 1.0	1.93 ± 1.48	R	25.20 (29.97)	26.35 (30.79)	25.78 (30.38)	MR

63.	GE-63	2.3 ± 1.4	2.1 ± 4.5	2.23 ± 2.95	MR	18.20 (25.13)	17.85 (24.85)	18.03 (24.09)	MR
64.	GE-64	2.8 ± 2.4	2.7 ± 5.5	2.74 ± 3.95	MR	20.65 (26.97)	20.35 (26.74)	20.50 (26.85)	MR
65.	GE-65	2.4 ± 2.8	2.6 ± 3.5	2.50 ± 3.13	MR	49.15 (44.49)	47.15 (43.35)	48.15 (43.92)	S
66.	GE-66	1.3 ± 2.7	1.4 ± 2.8	1.34 ± 2.73	R	33.95 (35.60)	32.95 (34.98)	33.45 (35.29)	S
67.	GE-67	3.2 ± 5.0	3.3 ± 5.5	3.23 ± 5.25	S	50.10 (45.04)	48.95 (44.38)	49.53 (44.71)	S
68.	GE-68	1.8 ± 2.6	1.9 ± 2.3	1.82 ± 2.43	R	29.65 (32.93)	29.05 (32.56)	29.35 (32.74)	S
69.	GE-69	2.5 ± 1.3	2.7 ± 4.1	2.62 ± 2.68	MR	51.15 (45.64)	50.15 (45.07)	50.65 (45.36)	HS
70.	GE-70	3.6 ± 2.2	3.4 ± 5.8	3.50 ± 3.95	S	63.15 (52.64)	62.15 (52.06)	62.65 (52.35)	HS
71.	GE-71	2.5 ± 2.3	2.7 ± 4.3	2.61 ± 3.28	MR	45.35 (42.30)	43.70 (41.33)	44.53 (41.82)	S
72	GE-72	3.3 ± 3.5	3.0± 5.5	3.19 ± 4.50	S	47.35 (43.46)	46.10 (42.74)	46.73 (42.73)	S
73.	GE-73	3.0 ± 1.5	3 .0± 4.3	2.99 ± 2.88	MR	54.05 (47.31)	53.05 (46.74)	53.55 (47.02)	HS
74.	GE-74	1.1 ± 2.2	1.6 ± 2.0	1.34 ± 2.10	R	15.15 (22.81)	15.90 (23.45)	15.53 (23.13)	MR
75.	VR-708	4.1 ± 2.5	4.2 ± 3.3	4.11 ± 2.88	HS	72.00 (58.12)	75.00 (59.99)	73.50 (59.06)	HS
	C. D.	8.69	6.50	7.59		7.47	7.15	7.31	
	C. V.	9.44	7.18	8.31		9.64	9.35	18.99	

( ) Angular transformed values

Plate 22. Field view of finger millet genotypes at Vizianagaram during *Kharif* 2020

Plate 23. Field view of finger millet genotypes at Vizianagaram during *Kharif* 2021

The present results are in agreement with the findings of Patro *et al*. (2017) who screened 10 genotypes for their resistance to blast disease and found minimum percentage of neck blast severity in VL 379 (14.82%), and finger blast severity (13.70%) in GPU 45. Patro *et al*. (2018) evaluated 30 lines of finger millet in which five lines are found to be highly resistant and nineteen lines are resistant whereas VR 708 recorded as highly susceptible to leaf blast. In the same way Patro and Anuradha (2019) evaluated 3000 finger millet lines including one check variety *viz*., VR 708 (susceptible) against neck and finger blast. Among all the 3000 lines 112 lines have shown resistant reaction under high disease pressure field conditions during *Kharif* 2013-2018. However, only 50 lines have shown consistent resistant reaction during all the years (2014-18) tested. The percent disease intensity of neck and finger blast ranged from 0.00 to 96.4 % and 0.00 to 95.3 % respectively, where it was 95.5 and 96.4 PDI of neck and finger blast in check VR 708, a highly susceptible variety.

4.5 TO STUDY THE INFLUENCE OF WEATHER FACTORS AND DISEASE DEVELOPMENT

4.5.1 Correlation and Regression Analysis of Disease Severity and Weather Factors

The influence of weather factors on disease severity of finger millet blast was studied during *Kharif* 2020 and *Kharif* 2021 with three different dates of sowing (DOS) *viz.*, July 15th, July 30th and Aug 15th. The Per cent disease index was recorded at weekly intervals from 22nd July to 21st Oct during *Kharif* 2020 and 2021 for July 15th DOS, 5th Aug to 4th Nov during *Kharif* 2020 and 2021 for July 30th DOS and 19th Aug to 18th Nov during *Kharif* 2020 and 2021 for Aug 30th DOS. The data on weather parameters such as maximum temperature (X_1), minimum temperature (X_2), maximum relative humidity (X_3), minimum relative humidity (X_4), Rainfall (X_5) and Rainy days (X_6) were recorded at weekly intervals during the period of experimentation (standard meteriological data). Correlation and regression analysis were done by using Per cent disease index as dependent variable and weather parameters as independent variables.

Relationship of weather factors with severity of finger millet blast disease during *Kharif* 2020 and *Kharif* 2021 (Pooled)

July 15th DOS

The influence of weather parameters as disease severity during July 15th *Kharif* 2020 and 2021 (pooled) is presented in Table 4.17. The correlation studies revealed that the significant positive correlation coefficient was observed between disease with minimum temperature (0.991), maximum relative humidity (0.990), rainfall (0.991) and rainy days (0.970). The disease also have negative co-efficient correlation with maximum temperature (-0.624) and minimum relative humidity (-0.986). The intermittent rains during this period resulted in lower temperature and higher relative humidity which were predisposed for development of infection and further spread of disease (Table 4.17).

The multiple linear regression between per cent disease index of finger millet blast and weather parameters, during *Kharif* 2020-21 indicated that, the regression coefficients for maximum and minimum temperature, maximum relative humidity, minimum relative humidity, rainfall and rainy days were found to be -0.173, -2.458, 271.641, -2.729, 1.874 and -3.640 (Table 4.18).

The multiple linear regression equation was fitted to the data and equation arrived for the weather parameters was $Y= 10935.599-0.173X_1-2.458X_2-271.641X_3-2.729X_4+1.874X_5-3.640X_6$. Where X_1- maximum temperature (°C), X_2- minimum temperature (°C), X_3- maximum relative humidity (%), X_4- minimum relative humidity (%), X_5- rainfall (mm) and X_6- rainy days.

Table 4.17. Correlation coefficients between finger millet blast PDI and weather parameters of July 15th sown crop during *Kharif* 2020 and *Kharif* 2021pooled data

S. No.	Weather parameters	Correlation coefficients (r)
1.	X_1 - Maximum temperature	-0.624*
2.	X_2 - Minimum temperature	0.991**
3.	X_3 - Maximum RH	0.990**
4.	X_4 - Minimum RH	-0.986**
5.	X_5 - Rainfall	0.991**
6.	X_6 - Rainy days	0.970**

* Significant at 5% LOS

** Significant at 1% LOS

Table 4.18. Multiple regression analysis of influence of weather parameters on blast severity in July 15th sown crop during *Kharif* 2020 and *Kharif* 2021 pooled data

Weather parameters	Regression co-efficient (ß value)	Standard error	t calculated value	t probability	Intercept	R^2
X_1 (Max. temp)	-0.173	3.505	-0.701	0.506	10935.599	0.982
X_2 (Min. temp)	-2.458	271.933	0.999	0.351		
X_3 (Max. RH)	271.641	16.063	-0.170	0.870		
X_4 (Min. RH)	-2.729	2.076	0.903	0.397		
X_5 (Rainfall)	1.874	5.803	-0.627	0.550		
X_6 (Rainy days)	-3.640	6.774	-1.171	0.280		

July 30th DOS

The influence of weather parameters as disease severity during July 30th *Kharif* 2020 and 2021 (pooled) is presented in Table 4.19. The correlation studies revealed that the PDI showed significant positive correlation with minimum temperature (0.985), maximum relative humidity (0.987), rainfall (0.985) and rainy days (0.974). The negative correlation was observed with maximum temperature (-0.707) and minimum relative humidity (-0.979) (Table 4.19).

The multiple linear regression between per cent disease index of finger millet blast and weather parameters, during July 30th sown crop of *Kharif* 2020-21 indicated that, the regression coefficients for maximum and minimum temperature, maximum relative humidity, minimum relative humidity, rainfall and rainy days were found to be -1.952, -348.335, 407.014, 0.216, -0.431 and 2.909 (Table 4.20).

The multiple linear regression analysis indicated that all the weather parameters collectively influenced the finger blast severity to an extent of 59.83 per cent during *Kharif* 2020 and *Kharif* 2021 (pooled). The regression equation is fitted as below:

$$Y= -22276.287-1.952X_1-348.335X_2+407.014X_3+0.216X_4-0.431X_5+2.909X_6.$$

Where, X_1- maximum temperature (°C), X_2- minimum temperature (°C), X_3- maximum relative humidity (%), X_4- minimum relative humidity (%), X_5- rainfall (mm) and X_6- rainy days.

Table 4.19. Correlation coefficients between finger millet blast PDI and weather parameters of July 30th sown crop during *Kharif* 2020 and *Kharif* 2021 pooled data

S. No.	Weather parameters	Correlation coefficients (r)
1.	X_1 - Maximum temperature	-0.707**
2.	X_2 - Minimum temperature	0.985**
3.	X_3 - Maximum RH	0.987**
4.	X_4 - Minimum RH	-0.979**
5.	X_5 - Rainfall	0.985**
6.	X_6 - Rainy days	0.974**

** Significant at 1% LOS

Table 4.20. Multiple regression analysis of influence of weather parameters on blast severity in July 30th sown crop during *Kharif* 2020 and *Kharif* 2021 pooled data

Weather parameters	Regression co-efficient (ß value)	Standard error	t calculated value	t probability	Intercept	R^2
X_1 (Max. temp)	-1.952	2.907	-0.671	0.524	-22276.287	0.991
X_2 (Min. temp)	-348.335	160.237	-2.174	0.066		
X_3 (Max. RH)	407.014	139.427	2.919	0.022*		
X_4 (Min. RH)	0.216	0.898	0.240	0.817		
X_5 (Rainfall)	-0.431	2.067	-0.209	0.841		
X_6 (Rainy days)	2.909	2.225	1.307	0.232		

* Significant at 5% LOS

Aug 15th DOS

The influence of weather parameters as disease severity during Aug 15th *Kharif* 2020 and 2021 (pooled) is presented in Table 4.21. The correlation studies revealed that significant positive correlation coefficient was observed between disease with minimum temperature (0.993), maximum relative humidity (0.992), rainfall (0.994) and rainy days (0.977). The disease also have negative co-

efficient correlation with maximum temperature (-0.722) and minimum relative humidity (-0.987) (Table 4.21).

The multiple linear regression between per cent disease index of finger millet blast and weather parameters, during Aug 15th sown crop of *Kharif* 2020-22 indicated that, the regression coefficients for maximum and minimum temperature, maximum relative humidity, minimum relative humidity, rainfall and rainy days were found to be 1.008, 6.564, -156.362, 0.284, 4.209, -0.175 (Table 4.22).

The multiple linear regression equation was fitted to the data and equation arrived for the weather parameters was $Y= 10935.599+1.008X_1+6.564X_2-156.362X_3+0.284X_4+4.209X_5-0.175X_6$. Where X_1- maximum temperature (°C), X_2- minimum temperature (°C), X_3- maximum relative humidity (%), X_4- minimum relative humidity (%), X_5- rainfall (mm) and X_6- rainy days.

Blast disease severity was shown to be higher during the early planting window, possibly due to comparatively high relative humidity, rainfall, and a greater number of rainy days, all of which favour disease development. The present findings are in accordance with the work of Viswanath and Seetharam (1989) who reported that high humidity and temperature between 25 and 30^{0}C favours the disease development. The pathogen known to prefer low temperature (< 20^{0}C) with high humidity, heavy rainfall and low light for outbreaks (Vishwanath and Channamma 1988). Jain *et al*., (1994) while assessing the stable resistance of blast in finger millet reported that moderate temperatures between 21^{0}C to 29^{0}C with more than 80 per cent mean atmospheric relative humidity during reproductive period favoured the disease development. It was observed that average minimum and maximum temperatures of 22^{0}C and 290C respectively with 85 - 99% RH was favourable for disease development in Madhya Pradesh India (Patel and Tripathi 1998; Pall, 1988). Though the crop sown in late plating window is less affected by blast disease may be due to unfavourable weather conditions for the pathogen and such observations have also been made earlier by Viswanath and Seetharam (1989) who reported that dates of sowing significantly influenced the severity of blast disease of finger

millet. Present findings are also in accordance with work of Prasad and Kumar (2009), Gupta *et al*. (2004) and Ghasolia *et al.,* (2004) studied the effect of dates of sowing on various diseases and yield of mustard.

Table 4.21. Correlation coefficients between finger mille blast PDI and weather parameters of Aug 15th sown crop during *Kharif* 2020 and *Kharif* 2021pooled data

S. No.	Weather parameters	Correlation coefficients (r)
1.	X_1 - Maximum temperature	-0.722**
2.	X_2 - Minimum temperature	0.993**
3.	X_3 - Maximum RH	0.992**
4.	X_4 - Minimum RH	-0.987**
5.	X_5 - Rainfall	0.994**
6.	X_6 - Rainy days	0.977**
** Significant at 1% LOS		

Table 4.22. Multiple regression analysis of influence of weather parameters on blast severity in Aug 15th sown crop during *Kharif* 2020 and *Kharif* 2021 pooled data

Weather parameters	Regression co-efficient (ß value)	Standard error	t calculated value	t probability	Intercept	R^2
X_1 (Max. temp)	1.008	1.349	0.747	0.476	10935.599	0.995
X_2 (Min. temp)	6.564	1.305	2.958	0.021*		
X_3 (Max. RH)	-156.362	44.816	-3.489	0.008		
X_4 (Min. RH)	0.284	0.486	0.585	0.575		
X_5 (Rainfall)	4.209	1.070	3.935	0.004**		
X_6 (Rainy days)	-0.175	0.912	-0.192	0.853		

* Significant at 5% LOS

** Significant at 1% LOS

4.6 INTEGRATED DISEASE MANAGEMENT FOR BLAST

The field experiment was conducted during *Kharif* 2021 at Tirupati and Vizianagaram (Plate 24 and Plate 25). In the present investigation, Seed treatment and foliar sprays with Tebuconazole 50% + Trifloxystrobin 25% WG and *P. fluorescens* (BVP-1) were evaluated in possible combinations at different levels of infection to assess the integrated effect of blast disease on susceptible variety VR 708 (check) (Fig. 4.11). Observations on leaf, neck and finger blast were recorded and compared with unsprayed plot of VR 708. The treatment details have been explained in material and methods (Table 4.23).

Kharif 2021 at Tirupati

The experiment was conducted at wetland farm, S. V. Agricultural College, Tirupati during *Kharif* 2021 (Plate 24). The results of the experiment are explained below.

Leaf blast

The results revealed that the significant lowest PDI of leaf blast (10.25%) was recorded in the treatment (T_7) with highest (86.53%) disease reduction over control followed by T_8 with 12.83% leaf blast incidence with 83.14% disease reduction over control. The treatments involving seed treatment with Tebuconazole 50% + Trifloxystrbin 25% WG 0.4 g kg^{-1} (T_1) seed was recorded 15.97% leaf blast incidence with 79.03% disease reduction over control. The treatments involving two sprays of Tebuconazole 50% + Trifloxystrbin 25% WG and *P. fluorescens* 10 g kg^{-1} (first spray at initiation of symptoms and second spray at 50% flowering stage) was recorded 18.41 and 19.34% disease incidence with 75.82 and 74.59% of disease reduction over control which were significantly on par with each other. However, the treatments T_3 and T_2 have recorded 22.07 and 29.35% of leaf blast incidence with 71.01 and 61.46% significant disease reduction over control. While the significant highest leaf blast (35.04%) incidence with 53.98% disease reduction over control was recorded in T_4 when compared with check (76.15%) (T_9) (Table 4.23) (Fig. 4.12).

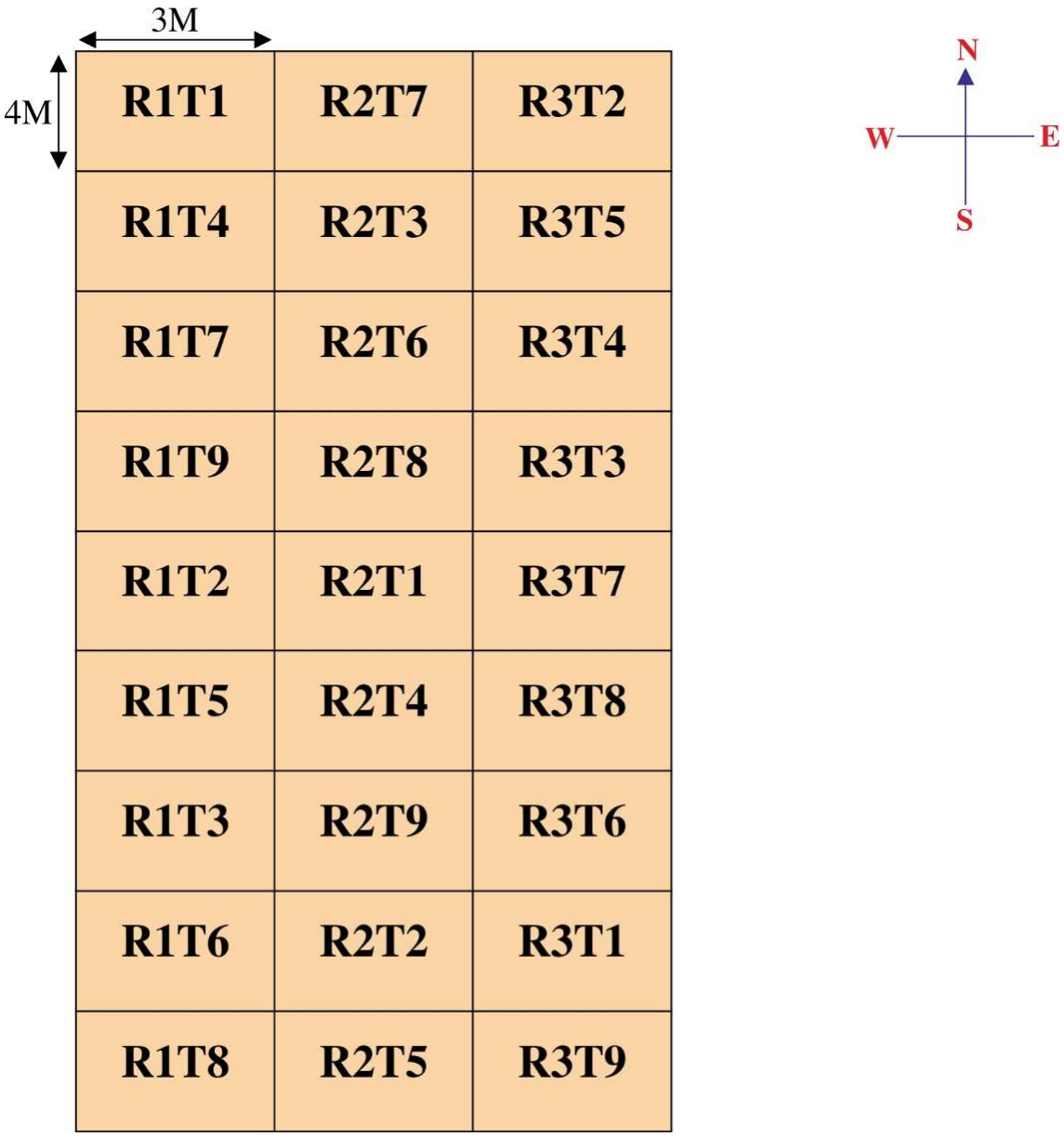


Fig. 4.11. Field layout of integrated disease management

Neck blast

The efficacy of integrated management treatments on neck blast revealed that, the significant lowest neck blast (11.64%) was recorded in T_7 with highest (83.98%) disease reduction over control. Which was followed by T_8 with neck blast (15.29%) disease incidence and 78.97% reduction in disease over control. T_5 and T_6 were also recorded 16.65 and 21.68% leaf blast incidence with 77.09 and 70.17% reduction in disease over control. The seed treatment (T_1) and one spray of Tebuconazole 50% + Trifloxystrbin 25% WG at 0.4 g kg^{-1} at 50% flowering stage (T_3) recorded 26.54 and 29.39% neck blast incidence with 63.49 and 59.57% disease reduction over control. However, seed treatment with *P. fluorescens* 10 g kg^{-1} (T_2) was recorded 30.41% neck blast incidence with 58.16% disease reduction over control. While, treatment T_4 was recorded highest (42.36%) neck blast incidence with 41.73 % disease reduction over control when compared with untreated control (72.70%) (T_9) (Table 4.23) (Fig. 4.12).

Finger blast

The evaluation of fungicides and bio agents on finger blast revealed that the significant lower finger blast (8.06%) incidence was recorded in T_7 with 89.43% disease reduction over control. Which was followed by T_8 and T_5 with 14.32 and 15.18% disease incidence and 81.22 and 80.09% disease reduction over control respectively, which are significantly on par with each other. However treatments T_6, T_1, T_3 and T_2 recorded 22.86, 24.31, 26.49 and 29.62% disease incidence (70.01, 68.11, 65.25 and 61.15% reduction in disease over control respectively) which are significantly different with each other. While, T_4 have recorded highest finger blast (43.42%) incidence with 43.05% significant disease reduction over control (76.24%) (T9) (Table 4.23) (Fig. 4.12).

Yield

Among all the treatments, seed treatment + one spray of Tebuconazole 50 % + Trifloxystrobin 25 % WG at 0.4 g l^{-1} (at 50 % flowering) (T_7) was recorded 17.35 q/ha followed by T_8 with 16.89 % q/ha yield. The treatments T_5, T_6, T_1 and T_3 recorded 14.45, 13.39, 10.64 and 10.15 q/ha. While, the least yield per ha was

recorded in T_4 and T_2 with 9.35 and 9.69 q/ha when compared with control (T_9) with 8.01 q/ha (Table 4.23) (Fig. 4.14).

***Kharif* 2021 at Vizianagaram**

The experiment was conducted at Agricultural Research Station, Vizianagaram during *Kharif* 2021 (Plate 25). The results of the experiment are as follows.

Leaf blast

The results revealed that the treatment T_7 showed the best result in terms of significant reduction in disease incidence of leaf blast *i.e.* 8.84% with maximum disease reduction over control was about 89.95%, followed by T_8 with 11.42% disease incidence with 86.46% disease reduction over control. The treatment T_5 and T_6 reported 16.69 and 18.85% disease incidence with 80.22 and 77.66% disease reduction over control which are on par with each other. However, T_1, T_3 and T_2 showed significantly different PDI *viz.*, 14.32, 21.01 and 27.36% as well as 83.03, 75.10 and 67.58% disease reduction over control. While, T_4 have reported significant highest (32.62%) disease incidence and 61.35% disease reduction over control when compared with check (84.39%) (T_9) (Table 4.24) (Fig. 4.13).

Neck blast

Among all the treatments T_7 has showed significant if disease incidence of neck blast (10.12%) and 88.20% disease reduction over control which was significantly on par with T_8 and T_5 with 12.03 and 13.32% neck blast incidence and 85.98 and 84.47% disease reduction over control. However, the treatment T_6, T_1, T_3 and T_2 were also showed 20.33, 20.05, 26.45 and 29.72% disease incidence and 76.29, 73.12, 69.12 and 65.35% disease reduction over control which was significantly different with each other. While, T_4 reported significant highest PDI of neck blast 39.33 and 54.14% disease reduction over control (85.76%) (T_9) (Table 4.24) (Fig. 4.13).

Table 4.23. Integrated disease management of finger millet blast at Tirupati during *Kharif* 2021

Treatments	Leaf blast*	Per cent leaf blast reduction over control	Neck Blast*	Per cent neck blast reduction over control	Finger blast*	Per cent finger blast reduction over control	Yield (q/ha)
T_1 : Seed treatment with Tebuconazole 50% + Trifloxystrbin 25% WG 0.4 g kg^{-1} seed	15.97 $(23.51)^{ef}$	79.03	26.54 $(30.98)^{cd}$	63.49	24.31 $(29.50)^{d}$	68.11	10.64
T_2 : Seed treatment with *Pseudomonas fluorescens* @ 10 g kg^{-1} seed	29.35 $(32.78)^{c}$	61.46	30.41 $(33.43)^{c}$	58.16	29.62 $(32.95)^{c}$	61.15	9.69
T_3 : One spray with Tebuconazole 50% + Trifloxystrbin 25% WG 0.4 g l^{-1} (at 50% flowering)	22.07 $(27.98)^{d}$	71.01	29.39 $(32.79)^{c}$	59.57	26.49 $(30.95)^{cd}$	65.25	10.15
T_4 : One spray with *Pseudomonas fluorescens* @ 10 g l^{-1} (at 50% flowering)	35.04 $(36.27)^{b}$	53.98	42.36 $(40.58)^{b}$	41.73	43.42 $(41.20)^{b}$	43.05	9.35
T_5 : Two sprays with Tebuconazole 50% + Trifloxystrbin 25% WG 0.4 g l^{-1} (first spray at initiation of symptoms and second spray at flowering stage)	18.41 $(25.36)^{de}$	75.82	16.65 $(23.97)^{ef}$	77.09	15.18 $(22.89)^{e}$	80.09	14.45

T_6 : Two sprays with *Pseudomonas fluorescens* @ 10 g l^{-1} (first spray at initiation of symptoms and second spray at flowering stage)	19.34 (26.06)^{de}	74.59	21.68 (27.72)^{de}	70.17	22.86 (28.49)^{d}	70.01	13.39
T_7 : Seed treatment with Tebuconazole 50% + Trifloxystrbin 25% WG 0.4 g kg^{-1} seed + one spray with Tebuconazole 50% + Trifloxystrbin 25% WG 0.4 g l^{-1} (at 50% flowering)	10.25 (18.60)^{g}	86.53	11.64 (19.83)^{f}	83.98	8.06 (16.44)^{f}	89.43	17.35
T_8 : Seed treatment with *Pseudomonas fluorescens* @ 10 g kg^{-1} seed + one spray with *Pseudomonas fluorescens* @ 10 g l^{-1} (at 50% flowering)	12.83 (20.96)^{f}	83.14	15.29 (22.90)^{f}	78.97	14.32 (22.09)^{e}	81.22	16.89
T_9 : Control	76.15 (60.77)^{a}		72.70 (58.50)^{a}		76.24 (60.82)^{a}		8.01
C.D.	2.85		3.92		3.62		2.96
SE (m)	0.94		1.29		1.20		1.57
SE (d)	1.33		1.83		1.69		1.76
C. V.	5.40		6.95		6.54		6.16

* Mean of three replications, in a column mean followed by a common letter are not significantly different

( ) Angular transformed values

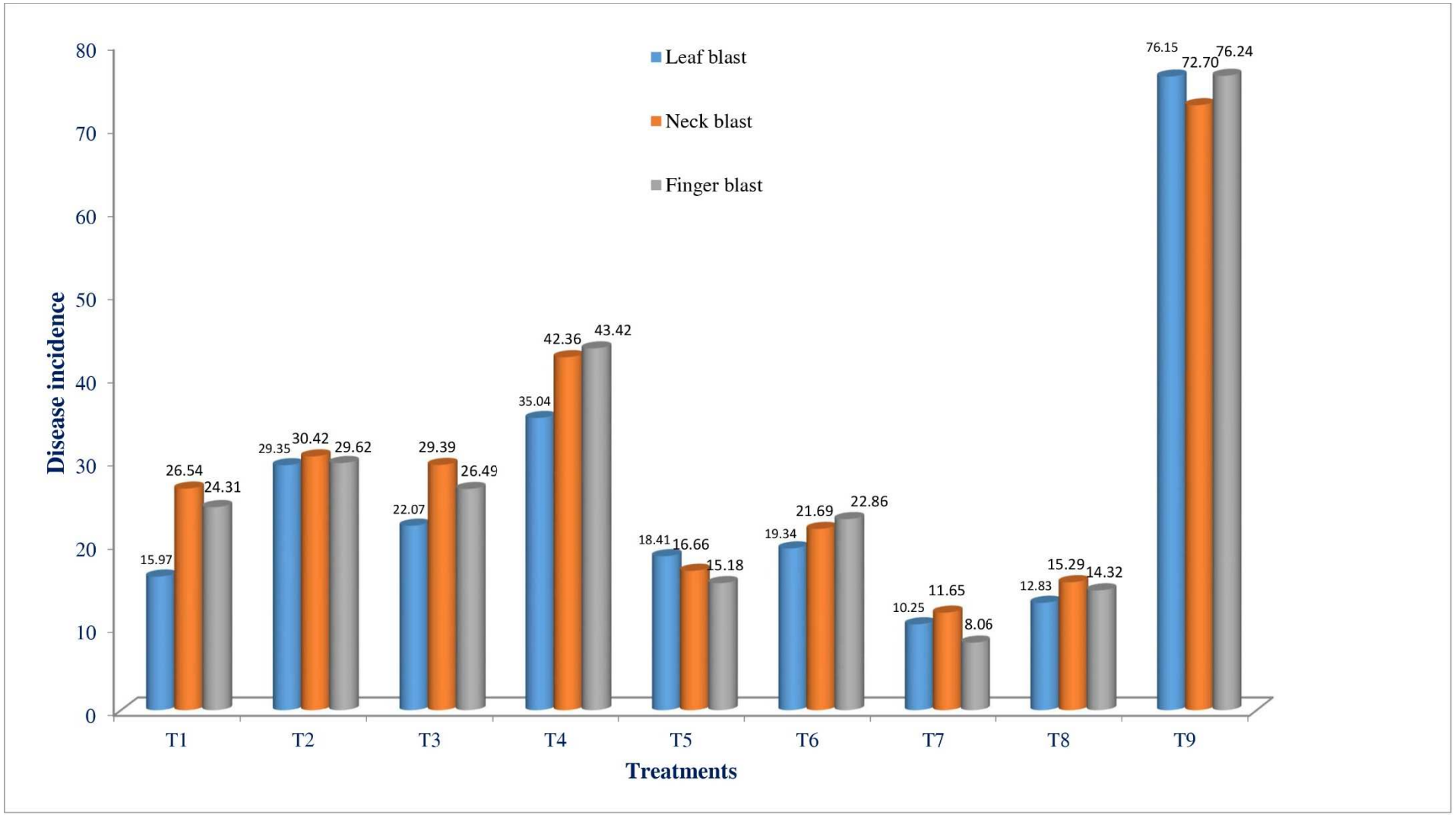


Fig. 4.12. Per cent disease index in integrated disease management of finger millet blast experiment during *Kharif* 2021 at Tirupati

Plate 24. Field view of integrated disease management of finger millet blast at Tirupati during *Kharif 2021*

Finger blast

The results revealed that the significant lower finger blast (9.88%) was reported in T_7 with 88.60% disease reduction over control. Which was followed by T_8 with 11.76% finger blast incidence and 86.43% disease reduction over control. The treatment T_5 was reported 14.51% finger blast incidence with 83.26% disease reduction over control. The treatment T_6, T_1 and T_3 are significantly on par with 21.10, 24.56 and 24.80% finger blast incidence and showed 75.66, 71.67 and 71.39% disease reduction over control. While, T_2 was reported 29.72% finger blast incidence with 66.34% disease reduction over control. While, T_4 was showed significantly higher finger blast (41.49%) incidence with 52.13% disease reduction over control (86.68%) (T_9) (Table 4.24) (Fig. 4.13).

Yield

The results revealed that the treatment T_7 was reported 15.68 q/ha yield followed by T8 with 14.76 q/ha yield. The treatments T_5 and T_6 reported 13.87 and 12.75 q/ha. However the treatments T_1, T_3 and T_2 reported 9.35, 8.95 and 8.21 q/ha. The treatment T4 reported lowest 7.35 q/ha when compared with untreated control (7.67 q/ha) (T_9) (Table 4.24) (Fig. 4.14).

Because of some intrinsic properties of the molecule, the new fungicide Tebuconazole 50% + Trifloxystrbin 25% WG has proven to be the most effective. Tebuconazole is a Triazole fungicide that prevents fungal cell wall sterol biosynthesis by inhibiting the dimethylation step. Its dual nature as a protective and curative agent demonstrates consistent efficacy over a period of several weeks. Trifloxystrobin is an Oximinoacetate mesostemic fungicide that is quickly absorbed by the plant's waxy layers and then redistributed by the surface, resulting in disease control in nearly untreated plant tissues. It prevents the germination of fungal spores, as well as the expansion of the germ tube and the formation of the appressorium.

The present results suggested that seed treatment with Tebuconazole 50% + Trifloxystrbin 25% WG at 0.4 g kg^{-1} and one spray at 50% flowering stage was effectively reduced all three blasts over the control. Regarding the efficacy of

new fungicides have been reported by different researchers like Dutta *et al*., (2012) reported Tebuconazole 50% + Trifloxystrobin 25% WG was superior over control in controlling finger millet blast. According to Kulmitra *et al.*, (2017) Tebuconazole 50 % + Trifloxystrobin 25 % WG was found to be the most effective fungicide against the leaf blast disease in rice. The similar results were also reported by Singh *et al.*, (2019) evaluated six fungicides under field condition, among those Tebuconazole 50% + Trifloxystrobin 25% WG found to be effective with minimum disease per cent intensity of 11.46 per cent. Similarly, Ahmad *et al*., (2020) evaluated various fungicides against rice blast. Among those Tebuconazole 50% + Trifloxystrobin 25% WG @ 65 g a. i. per acre was found to be highly effective in controlling blast disease with minimum percent disease intensity (11.16 %) and maximum value of grain yields (4403.32 kg ha–1). Most of the findings revealed as fungicides were effective than bio agents in disease control. The yield in Vizianagaram was comparatively less as compared to yield in Tirupati. The soil edaphic factors played greater role in yield loss because the soil in Vizianagaram is cold and highly fertile soil which promotes biological activity of the pathogen. As well as Vizianagaram is hot spot for blast disease due to higher initial inoculum.

Table 4.24. Integrated disease management of finger millet blast at Vizianagaram during *Kharif* 2021

Treatments	Leaf Blast*	Per cent leaf blast reduction over control	Neck Blast*	Per cent neck blast reduction over control	Finger blast*	Per cent finger blast reduction over control	Yield (Kg/ha)
T_1 : Seed treatment with Tebuconazole 50% + Trifloxystrobin 25% WG 0.4 g kg^{-1} seed	14.32 $(22.15)^{ef}$	83.03	23.05 $(28.64)^{de}$	73.12	24.56 $(29.68)^{d}$	71.67	9.35
T_2 : Seed treatment with *Pseudomonas fluorescens* @ 10 g kg^{-1} seed	27.36 $(31.51)^{c}$	67.58	29.72 $(33.01)^{c}$	65.35	29.18 $(32.67)^{c}$	66.34	8.21
T_3 : One spray with Tebuconazole 50% + Trifloxystrobin 25% WG 0.4 g l^{-1} (at 50% flowering)	21.01 $(27.25)^{d}$	75.10	26.45 $(30.92)^{cd}$	69.12	24.80 $(29.85)^{d}$	71.39	8.95
T_4 : One spray with *Pseudomonas fluorescens* @ 10 g l^{-1} (at 50% flowering)	32.62 $(34.80)^{b}$	61.35	39.33 $(38.82)^{b}$	54.14	41.49 $(40.08)^{b}$	52.13	7.35
T_5 :Two sprays with Tebuconazole 50% + Trifloxystrobin 25% WG 0.4 g l^{-1} (first spray at initiation of symptoms and second spray at flowering stage)	16.69 $(24.06)^{de}$	80.22	13.32 $(21.31)^{f}$	84.47	14.51 $(22.32)^{e}$	83.26	13.87
T_6 : Two sprays with *Pseudomonas fluorescens* @ 10 g l^{-1} (first spray at initiation of symptoms and second spray at flowering stage)	18.85 $(25.69)^{de}$	77.66	20.33 $(26.75)^{e}$	76.29	21.10 $(27.32)^{d}$	75.66	12.75
T_7 : Seed treatment with Tebuconazole 50% + Trifloxystrobin 25% WG 0.4 g kg^{-1} seed + one spray with Tebuconazole 50% + Trifloxystrobin 25% WG 0.4 g l^{-1} (at 50% flowering)	8.48 $(16.88)^{g}$	89.95	10.12 $(18.52)^{f}$	88.20	9.88 $(18.28)^{f}$	88.60	15.68

T_8 : Seed treatment with *Pseudomonas fluorescens* @ 10 g kg^{-1} seed + one spray with *Pseudomonas fluorescens* @ 10 g l^{-1} (at 50% flowering)	11.42 (19.67)^{fg}	86.46	12.03 (20.24)^{f}	85.98	11.76 (19.95)^{ef}	86.43	14.76
T_9 : Control	84.39 (66.82)^{a}		85.76 (67.83)^{a}		86.68 (68.60)^{a}		7.67
C.D.	3.73		3.26		3.24		3.10
SE (m)	1.23		1.08		1.07		1.15
SE (d)	1.75		1.52		1.51		1.35
C. V.	7.16		5.87		5.78		5.95

Plate 25. Field view of integrated disease management of finger millet blast at Vizianagagaram during *Kharif 2021*

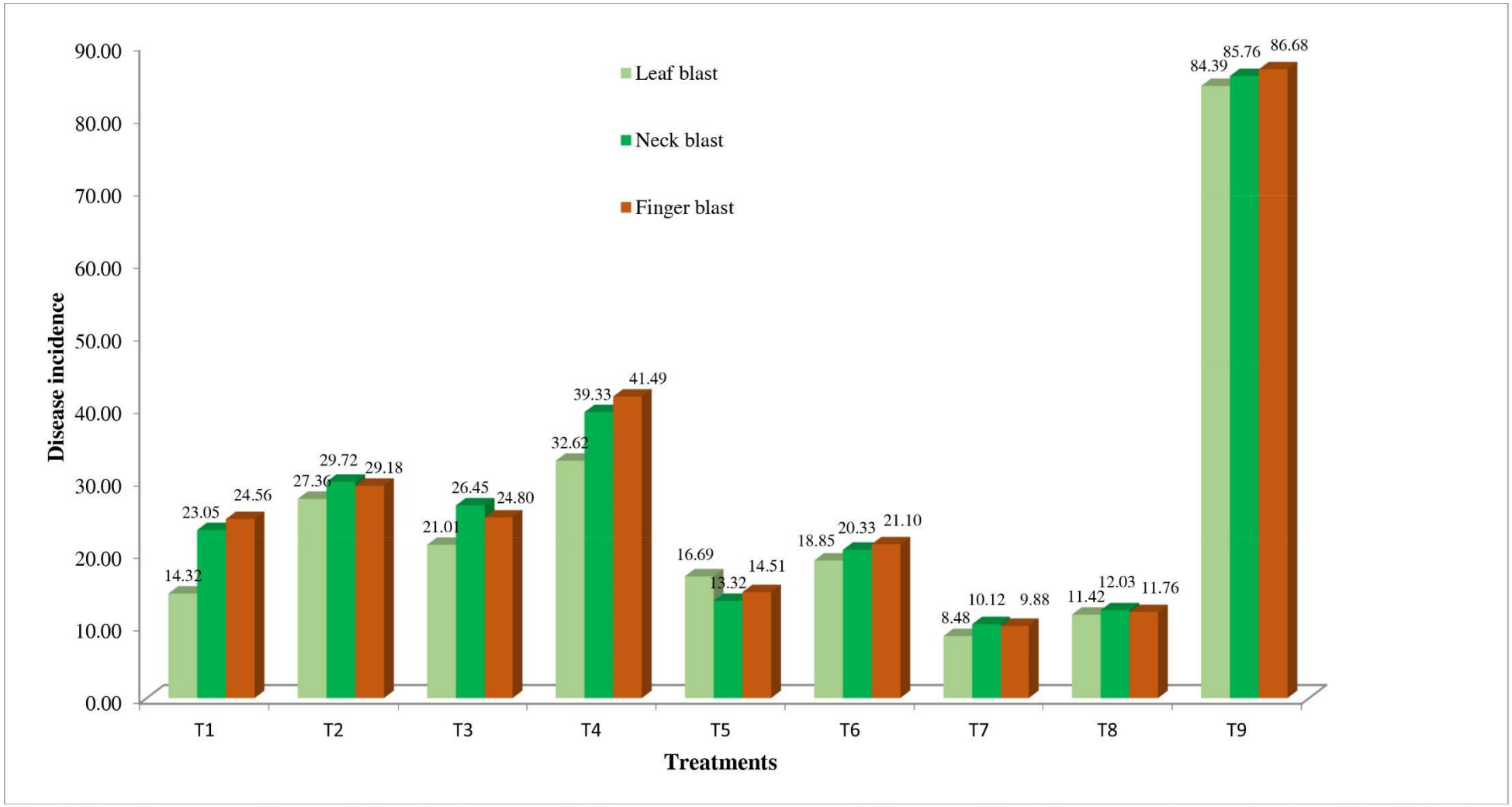


Fig. 4.13. Per cent disease index in integrated disease management of finger millet blast experiment during *Kharif* 2021 at Vizianagaram

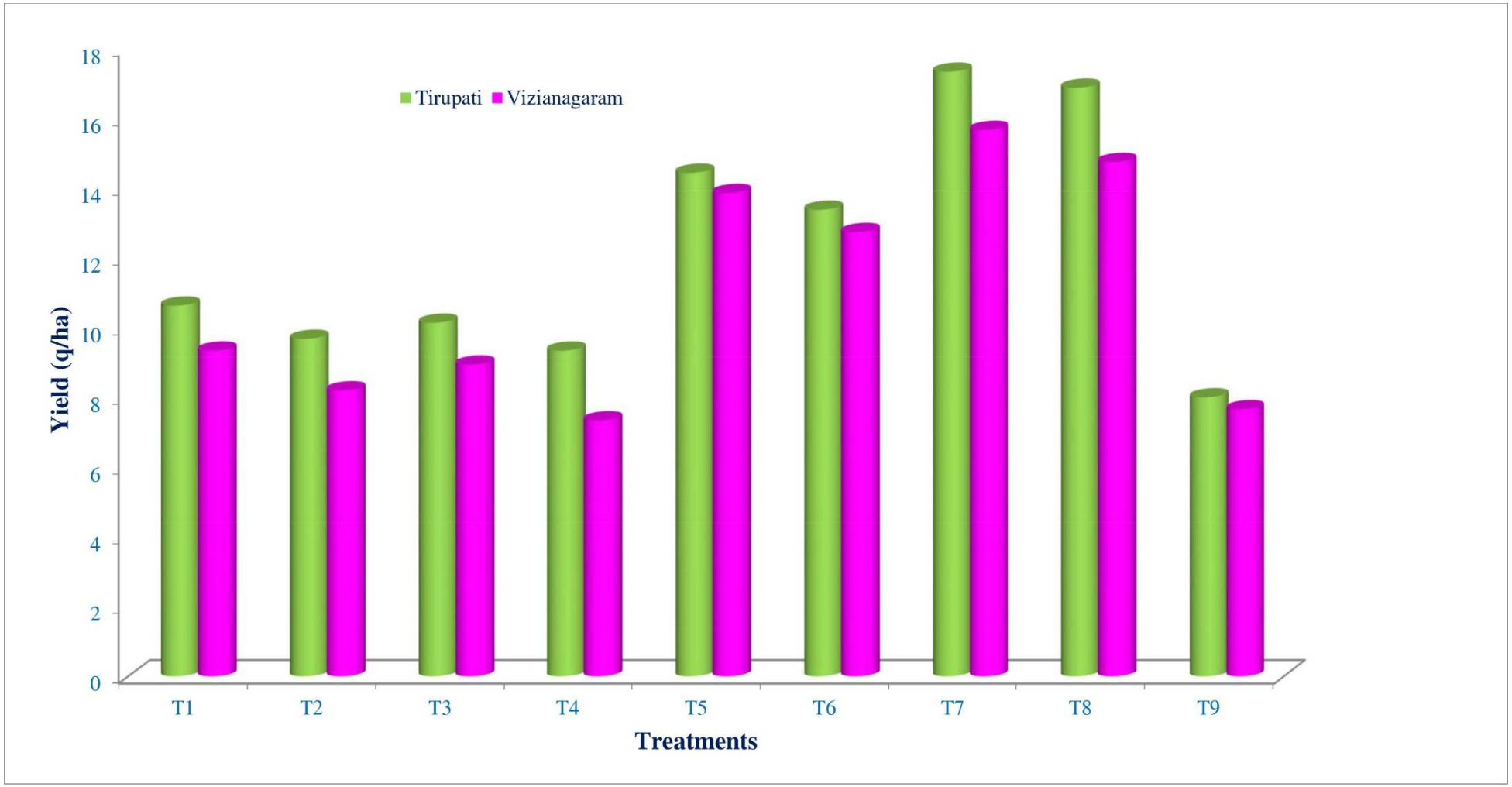


Fig. 4.14. Yield comparison in IDM experiment conducted at Tirupati and Vizianagaram during *Kharif* 2021

Chapter – V

Summary and Conclusions

Chapter-V

SUMMARY AND CONCLUSIONS

In the present investigation, studies pertaining to survey, cultural and morphological variability, *in vitro* evaluation of fungicides and bacterial bio-control agents were carried out at S. V. Agricultural College, Tirupati. Molecular diversity among isolates of pathogen was conducted at Institute of Frontier Technology, Tirupati. Epidemiology, screening and IDM on finger millet blast was carried out at Agricultural Research Station, Vizianagaram, Andhra Pradesh. The results obtained in these are summarized below.

Survey for disease incidence was conducted during *Kharif* 2020 and *Kharif* 2021 in seven major finger millet growing districts of Andhra Pradesh. Among the seven districts surveyed, highest mean Per cent disease incidence (PDI) of 56.06% was recorded in Vizianagaram district. Followed by Chittoor, YSR Kadapa and Kurnool, with mean Per cent disease incidence of 42.77%, 22.60% and 20.07% respectively. The lowest mean disease incidence 7.85% was recorded in Prakasam district followed by Anantapur (19.63%) and Guntur (19.85%) districts. Regarding the mandals, highest mean Per cent blast incidence of 56.59% was recorded in Salur mandal of Vizianagaram district in the range of 53.86 to 59.20% during 2020 and 52.35 to 59.65% during 2021 followed by Kurupam mandal of Vizianagaram district with 55.53% incidence in the range of 54.63% to 57.56% during 2020 and 51.59% to 56.87% during 2021, Palamaneru mandal with 53.92% in the range of 49.25 to 57.52% and 50.35 to 58.65% during 2020 and 2021 respectively. Kuppam mandal with 31.62% range of 29.45 to 33.78% and 30.32 to 32.86% during 2020 and 2021 respectively. Pattikonda mandal with 27.27% range of 24.32 to 33.23% and 23.65-32.56% during 2020 and 2021 respectively. Veldurthi mandal with 24.30% range of 12.28 to 48.21% and 11.56 to 46.98% during 2020 and 2021 respectively. Gudibanda mandal with 23.87% range of 17.78 to 28.96% and 18.35 to 29.34% during 2020 and 2021 respectively. Vallur mandal with 23.26% range of 8.05 to 49.23% and 9.23 to 48.65% during 2020 and 2021 respectively and Kamalapuram mandal with 21.93% range of 15.03 to 30.13% and 16.35 to 26.75% in 2020 and 2021

respectively of YSR Kadapa district. The lowest incidence with 7.36% range of 5.02 to 9.01% and 4.65 to 9.78% was noticed in Racherla mandal of Prakasam district during 2020 and 2021 respectively. This was followed by Komarolu mandal with 8.34% range of 5.85 to 9.21% and 6.82 to 10.35% during 2020 and 2021respectively of Prakasam district. Peapally mandal with 12.86% the range of 9.09 to 15.54% and 8.35 to 15.65% during 2020 and 2021 respectively. Madakasira mandal with 15.38% range of 11.01 to 18.14% and 10.35 to 19.35% during 2020 and 2021 respectively and Macherla mandal with 15.40% range of 11.13 to 17.24% and 12.35 to 18.96% range during 2020 and 2021 respectively of Guntur district

The diseased samples from different districts were subjected to isolation procedure. A total of twenty monoconidial isolates of *Pyricularia grisea* were maintained. The isolates were named as LNG-1, VAL-1, KML-1, MOR-1, PAL-1, GON-1, JRG-1, GVN-1, JDR-1, MRB-1, GDB-1, VIZ-1, VIZ-2, PEA-1, CHK-1, JTR-1, ANP-1, TTC-1, VED-1 and KPN-1.

The variability in cultural characteristics *viz*., colony colour, growth pattern, elevation (flat/elevated growth), sectored or non-sectored, zonations and wrinkles were studied among the isolates of *P. grisea* on OMA medium. Cultural characteristics varied greatly with isolates and the medium used. A range of colour variation was observed among the pathogen isolates. Observations were recorded for the colony characters based on the metabolites produced in the medium.

The sporulating ability of the field isolates was varied. The degree of sporulation was compared with the growth patterns of the pathogen. It was observed that pathogen isolates which were grayish green and sector forming produced more spores. The undersurface of the colonies were usually brown or black. Colony texture or surface of all the isolates were rough to smooth with trace to abundant sporulation. In majority of the isolates, the maximum sporulation was confined to sectored region.

Total of eight solid media were used to evaluate the growth and sporulation of *P. grisea* (VIZ-1). Among the tested media’s maximum growth

with a colony diameter of 90.00 mm was observed in OMA, followed by FLA (87.67 mm). The maximum sporulation was observed in case of FLA medium with 1.81 × 10^5 ml followed by OMA with 1.68 × 10^5 ml were found to be significantly different. Least sporulation was observed in PDA (0.35 × 10^5 ml) and V8 (0.54 × 10^5 ml) medium. FLA medium is from host origin which resulted in higher sporulation of pathogen by supplying essential nutrients.

To check the growth and sporulation of pathogen at different light conditions, three light conditions were used. The maximum mean radial growth of 87.89 mm with insignificant difference under three light conditions *i.e* light (87.67 mm), dark (88.67 mm) and light + dark (87.33 mm) which was followed by FLA (87.67 mm) with insignificant difference among three light conditions *i.e.* light (87.67 mm), dark (89.00) and dark + light (86.33 mm). In over all, with respect to the light conditions, no significant difference was found among the three light conditions *i.e.* light (75.71 mm), dark (75.0 mm) and dark + light (74.11 mm).

Good amount of sporulation (1.68 × 10^5 ml^{-1}) was recorded in FLA with no significance difference among three light conditions (1.93 × 10^5 ml^{-1}-24 hrs light; 1.73 × 10^5 ml^{-1} dark and 1.39 × 10^5 ml^{-1} 12 hrs light + 12 hrs dark). This was followed by OMA (1.68 × 10^5 ml^{-1}) and RLA (1.18 × 10^5 ml^{-1}). While least mean sporulation was noticed in PDA and V8 with 0.34 × 10^5 ml^{-1} and 0.40 × 10^5 ml^{-1} respectively.

Morphological variability among *P. grisea* was studies through conidial morphology. Among the isolates, the overall size of the conidia was 20.74-23.01 μm × 7.00-9.16 μm (Length × Width).

The genetic diversity of 20 isolates of *P. grisea* was evaluated using 25 SSR markers, of which seven were polymorphic (100% polymorphism). A total of nineteen alleles were detected in twenty blast isolates using seven SSR markers. The number of alleles per locus varied from 2 (MGM 437, Pyrms 15, Pyrms 37 and Pyrms 67) to 4 (Pyrms 63 and Pyrms 77) with a mean value of 2.71 alleles/locus. The gene diversity was ranged from 0.180 (MGM 437) to 0.742 (Pyrms 63) with an average of 0.491.

The primers Pyrms 63 and Pyrms 77 were highly polymorphic with PIC (Polymorphism Information Content) value of 0.6949 and 0.6116. However, Pyrms 7 and Pyrms 37 both showed PIC value of 0.5129. PIC value of 0.3318, 0.2225 and 0.1638 were found in Pyrms 15, Pyrms 67 and Pyrms 437 respectively with a mean value of 0.435.

The SSR markers data across the twenty isolates were used for construction of phylogenetic tree by Neighbor Joining method (NJ), from genetic distance and dissimilarity matrix as parameter using DARwin 6 software. In the phylogenetic tree, blast isolates were grouped into three major clusters. Cluster I was further sub grouped into sub-cluster IA, which includes eight isolates and sub-cluster IB contains one. Cluster II was further sub grouped into sub-cluster IIA, which includes six isolates and sub-cluster IIB includes only one isolate. However, cluster III was further divided into sub cluster IIIA which includes three and IIIB includes only one isolate.

A total of 84 reproducible alleles with an average of 12 alleles per primer were produced using 7 RAPD primers. All the markers displayed polymorphic alleles. Of the total alleles (84), one amplicpon (OPA-07) was monomorphic with 15.38% monomorphism and 84.61% polymorphism which contains two monomorphic band with PIC value of 0.2874. Whereas 6 primers (OPA-04, OPA-10, OPA-18, OPB-17, OPC-05 and OPC-11) produced 100% polymorphism with PIC value was ranged from 0.1769 to 0.3429 and total number of polymorphic bands were ranged from 8 to 14.

Dendrogram constructed to reveal the pattern of relatedness among twenty *P. grisea* isolates using DARwin 6 software on the basis of RAPD polymorphism. Clustering analysis based on Unweighted Pair Group Method using Arithmetic Averages (UPGMA) and dendrogram generated using Neighbor Joining (NJ) method. 20 *P. grisea* isolates formed in to three mega cluster. Cluster I is further divided into sub-cluster IA which consisting of nine isolates Sub-cluster IB of with two isolates. Cluster II further divided into cluster IIA which consisting of four isolates and sub-cluster IIB contains only one isolate.

However, cluster III further divided into sub-cluster IIIA which contains three isolates and sub-cluster IIIB contains only one isolate.

Isolation of native bacterial bio-control agents revealed that, a total of 23 (BLN-1, BLN-2, BVA, BKM, BMO, BPA-1, BPA-2, BGO, BJR, BGV-1, BGV-2, BJD, BMR, BGD, BPE-1, BPE-2, BCH, BJT-1, BJT-2, BAN, BTT, BVE, BKP) bacterial bio-control agents were isolated from rhizospheric soil of healthy finger millet plants and three isolates *viz.,* BVP-1 (*Pseudomonas fluorescence*), BVB-2 (*Bacillus subtilis*-1) and BVB-3 (*Bacillus subtilis*-2) were collected from Agricultural Research Station,Vizianagaram.

Among twenty six bio agents tested under *in vitro* revealed that the significant maximum inhibition of mycelium growth (79.54%) was noticed in BVP-1 followed by BTT (75.56%) and least mycelial inhibition was noticed in BJR (11.30%), BPA-2 (16.48%), BLN-2 (26.41%) and BKP (27.87%) which showed excess growth over *P. grisea.*

In vitro evaluation of nine systemic fungicides revealed that complete inhibition of the test pathogen was observed with Tebuconazole 50% + Trifloxystrobin 25% WG at all the concentrations tested. However, Tricyclazole 75% WP and Carbendazim 50% WP were on par with Tebuconazole 50% + Trifloxystrobin 25% WG by completely inhibiting the mycelial growth of the pathogen.

Compatibility studies among effective fungicide and bio control agent revealed that, Tebconazole 50% + Trifloxystrbin 25% WG at 200, 400 and 600 ppm concentrations and Carbendazim 50% WP at 500, 1000 and 1500 ppm concentrations were highly compatible with zero per cent inhibition of BVP-1 bacterial growth.

74 lines of finger millet including local check VR 708 were evaluated for leaf, neck and finger blast disease during *Kharif* 2020 and *Kharif* 2021. Pooled analysis of leaf blast data indicated that five lines (GE-1, GE-5, GE-41, GE-44 and GE-45) were found as highly resistant (1-1.9% incidence), 31 lines found resistant (2-10% incidence), 23 lines were reacted as moderately resistant (11-30% incidence) and 15 lines were found to be susceptible (31-50% incidence).

Out of 74 lines, 30 lines were reacted as resistant to neck blast, 29 lines shown moderately resistant and 15 lines were reacted as susceptible to neck blast when compared with check VR 708.

Out of 74 screened lines 20 lines showed moderately resistance (6-25% incidence), 41 lines with susceptible reaction (26-50% incidence) and 14 lines including VR708 showed highly susceptible reaction (>50% incidence) with 82.49% incidence to finger blast incidence.

Correlation analysis between severity of finger millet blast and in weather parameters was found and the relationship between disease and weather factors during *Kharif* 2020 and 2021 for July 15th sown crop revealed that significant positive correlation coefficient was observed between disease with minimum temperature (0.991), maximum relative humidity (0.990), rainfall (0.991) and rainy days (0.970). The disease also have negative co-efficient correlation with maximum temperature (-0.624) and minimum relative humidity (-0.986)

The multiple linear regression equation was fitted to the data and equation arrived for the weather parameters was $Y= 10935.599-0.173X_1-2.458X_2-271.641X_3-2.729X_4+1.874X_5-3.640X_6$. Where X_1- maximum temperature (°C), X_2- minimum temperature (°C), X_3- maximum relative humidity (%), X_4- minimum relative humidity (%), X_5- rainfall (mm) and X_6- rainy days.

Significant positive correlation with minimum temperature (0.985), maximum relative humidity (0.987), rainfall (0.985) and rainy days (0.974). The negative correlation was observed with maximum temperature (-0.707) and minimum relative humidity (-0.979) for July 30th sown crop with multiple linear equation fitted to the data and equation arrived was $Y=-22276.287-1.952X_1-348.335X_2+407.014X_3+0.216X_4-0.431X_5+2.909X_6$.

The influence of weather parameters and PDI during Aug 15th *Kharif* 2020 and 2021. The correlation studies revealed that significant positive correlation coefficient was observed between disease with minimum temperature (0.993), maximum relative humidity (0.992), rainfall (0.994) and rainy days (0.977). The disease also have negative co-efficient correlation with maximum temperature (-0.722) and minimum relative humidity (-0.987).

The multiple linear regression equation was fitted to the data and equation arrived for the weather parameters was $Y= 10935.599+1.008X_1+6.564X_2-156.362X_3+0.284X_4+4.209X_5-0.175X_6$. Where X_1- maximum temperature (°C), X_2- minimum temperature (°C), X_3- maximum relative humidity (%), X_4- minimum relative humidity (%), X_5- rainfall (mm) and X_6- rainy days. Blast disease severity was shown to be higher during the early planting window, possibly due to comparatively high relative humidity, rainfall, and a greater number of rainy days, all of which favor disease development.

The efficacy of fungicide and bacterial bio-control agents were used in IDM of finger millet blast at S.V. Agricultural College, Tirupati during *Kharif* 2021, revealed that significant lowest PDI of leaf blast (10.25%) was recorded in treatment (T_7) with highest (86.53%) disease reduction over control. Followed by T_8 with 12.83% leaf blast incidence with 83.14% disease reduction over control. Significant lowest neck blast (11.64%) was recorded in T_7 with highest (83.98%) disease reduction over control. Which was followed by T_8 with neck blast (15.29%) disease incidence and 78.97% disease reduction over control. The evaluation of fungicides and bio agents on finger blast revealed that the significant lower finger blast (8.06%) incidence was recorded in T_7 with 89.43% disease reduction over control. Which was followed by T_8 and T_5 with 14.32 and 15.18% disease incidence and 81.22 and 80.09% disease reduction over control respectively, which are significantly on par with each other.

With respect to yield, the maximum yield was recorded in T_7 with 17.35 q/ha followed by T_8 with 16.89% q/ha yield.

Integrated disease management of finger millet blast during *Kharif* 2021 at Vizianagaram, revealed that treatment T_7 showed the best result in terms of significant reduction in disease incidence of leaf blast *i.e.* 8.84% with 89.95% disease reduction over control, followed by T_8 with 11.42% disease incidence with 86.46% disease reduction over control. Treatment T_7 was showed significant reduction in disease incidence of neck blast (10.12%) and 88.20% disease reduction over control which was significantly on par with T_8 and T_5 with 12.03 and 13.32% neck blast incidence and 85.98 and 84.47% disease reduction

over control. Significant lower finger blast (9.88%) was reported in T_7 with 88.60% disease reduction over control. Which was followed by T_8 with 11.76% finger blast incidence and 86.43% disease reduction over control.

With respect to yield, treatment T_7 was reported 15.68 q/ha yield followed by T8 with 14.76 q/ha yield. The treatments T_5 and T_6 reported 13.87 and 12.75 q/ha. However the treatments T_1, T_3 and T_2 reported 9.35, 8.95 and 8.21 q/ha. The treatment T4 reported lowest 7.35 q/ha when compared with untreated control (7.67 q/ha) (T_9).

Future line of work

- Influence of weather parameters on disease development will be used in developing forecasting model, which allows the prediction of probable increase in disease intensity, allowing if, when and where a particular disease management practice should be applied.
- The potentiality of native bacterial bio-control agent under *in vitro* may helps to study the mechanism against pathogen.
- Studies on genes that confer resistant and moderately resistant reaction in genotypes against leaf, neck and finger blast during screening.
- Finding out the defense mechanism, which acts behind highly resistant genotypes obtained during screening experiment.

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Appendix

APPENDIX – I

BUFFERS AND STOCK SOLUTIONS

DNA Extraction Buffer

2 % (w/v) CTAB (Genei) : 10 g

100 Mm Tris HCl, pH 8.0 : 100 ml of 0.5 M Tris HCl (pH8.0)

20 mM EDTA, pH 8.0 : 20 ml of 0.5 M EDTA (pH 8.0)

1.4 M NaCl : 140 ml of 5 M NaCl

PVP (Sigma) : 5 g

ß – Mercaptoethanol : 290 µl

All the above ingredients except CTAB were added in respective quantities and final volume was made up to 500 ml with double distilled water, the solution was autoclaved. The solution was allowed to attain room temperature and 10 g of CTAB was dissolved by intense stirring, stored at room temperature.

1 M Tris·Hcl (pH 8.0)

30.28 g of Trizma base (Qualigens, FW = 121.14) was dissolved in 200 ml of distilled H20. The pH was adjusted to 8.0 with concentrated HCl (about 10.5 ml). The solution was allowed to cool to room temperature (25°C) before making final adjustment of the pH. The volume of the solution was adjusted to a total of 250 ml with distilled water and was sterilized by autoclaving.

0.5 M EDTA (pH 8.0)

46.53 g of disodium ethylene diamine tetra acetate (EDTA) dihydrate (Qualigens, FW = 372.2) was added to 200 ml of distilled water and was stirred vigorously with a magnetic stirrer by slowly adding NaOH pellets till most of the EDTA crystals disappear. The pH was adjusted to 8.0 with 1N NaOH. The total volume was made up to 250 ml with distilled water and was sterilized by autoclaving

5 M NaCl

73.05 g NaCl (Qualigens, FW = 58.44) was dissolved in 200 ml of distilled H_2O. The total volume was adjusted to 250 ml with distilled H_2O and Sterilized by autoclaving

RNase (20 mg/ ml)

20 mg of RNase (Sigma) was dissolved in 500 µl of double distilled water + 500 µl of 50% Glycerol (Qualigens) and the solution was heated at 95°C for 10 min and stored at -20°C.

Phenol: Chloroform: Isoamyl alcohol (25:24:1)

Equal parts of equilibrated phenol and Chloroform:Isoamyl alcohol (24:1) were mixed and stored at 4^{o}C

Staining solution

5 µl of Ethidium bromide (10 mg/ml) is added to 200 ml of water to stain the gel.

10 X TBE buffer

Tris base : 108 g
Boric Acid : 55 g
EDTA : 9.3 g
Dissolve in 1000 ml distilled water
Ethidium bromide: Stock 20 mg/ml can be prepared by dissolving 1 gm of ethidium bromide in 50 ml of water

Bromophenol blue dye (0.0025% bromophenol blue in 40% sucrose). This dye was to be used in the ratio of 1:6 (Dye: DNA solution).

TE buffer (pH 8.0)

1M Tris HCl : 1000 µl

0.5M EDTA : 200 µl

Sterile Distilled water : 100 ml

APPENDIX – II

Meteorological Data:

Table 1: Standard meteorological data during the crop growth period in *Kharif* 2020 at Vizianagaram

Standard week	Date and Month	Temperature		Relative humidity (%)		Rainfall (mm)	Number of Rainy days
		Max (ºC)	Min (ºC)	Max	Min		
29	9 July-15 July	32.00	30.29	86.71	65.71	6.80	1
30	16 July-22 July	30.43	29.86	84.57	54.14	0.00	0
31	23 July-29 July	31.29	29.43	81.57	52.00	4.90	2
32	30 July-5 Aug	4.57	4.33	12.39	9.39	19.45	1
33	6 Aug-12 Aug	30.29	29.43	85.71	72.71	46.00	3
34	13 Aug-19 Aug	29.71	29.00	86.86	67.57	4.50	2
35	20 Aug – 26 Aug	30.57	29.57	88.29	66.29	15.00	2
36	27 Aug – 02 Sep	31.43	29.57	83.71	56.71	5.00	1
37	03 Sep – 09 Sep	31.57	30.00	84.14	52.86	0.00	0
38	10 Sep – 16 Sep	30.86	29.00	88.14	66.71	99.20	4
39	17 Sep – 23 Sep	30.86	29.57	87.29	63.57	8.00	1
40	24 Sep – 30 Sep	30.43	29.29	86.57	54.57	30.00	1
41	01 Oct – 07 Oct	29.14	29.14	88.43	72.29	32.40	3
42	08 Oct – 14 Oct	29.43	28.57	87.71	71.43	95.00	3
43	15 Oct – 21 Oct	29.29	28.14	83.57	71.00	57.40	3
44	22 Oct – 28 Oct	29.00	27.43	78.14	56.43	0.00	0
45	29 Oct – 04 Nov	30.14	25.14	81.57	38.71	0.00	0
46	05 Nov – 11 Nov	28.86	24.86	66.00	43.00	2.00	0

Table 2: Standard meteorological data during the crop growth period in *Kharif* 2021 at Vizianagaram

Standard week	Date and Month	Temperature		Relative humidity (%)		Rainfall (mm)	Number of Rainy days
		Max (ºC)	Min (ºC)	Max	Min		
29	16 July-22 July	32.00	29.86	86.14	57.57	0.60	0
30	23 July-29 July	30.29	29.86	83.14	62.71	2.00	0
31	30 July-5 Aug	32.57	30.00	80.86	53.43	2.20	1
32	6 Aug-12 Aug	33.57	30.14	84.57	60.43	5.07	2
33	13 Aug-19 Aug	29.29	27.86	90.86	70.57	30.80	7
34	20 Aug – 26 Aug	30.29	29.29	89.14	68.29	2.80	1
35	27 Aug – 02 Sep	28.86	28.29	89.71	78.43	15.30	6
36	03 Sep – 09 Sep	29.71	28.14	87.43	70.43	25.40	3
37	10 Sep – 16 Sep	30.43	29.00	89.86	63.00	10.27	2
38	17 Sep – 23 Sep	31.57	28.71	88.43	67.71	2.67	1
39	24 Sep – 30 Sep	28.28	27.78	93.10	78.05	31.63	4
40	01 Oct – 07 Oct	28.57	28.14	82.57	62.71	20.14	3
41	08 Oct – 14 Oct	29.67	28.48	88.61	69.80	15.46	2
42	15 Oct – 21 Oct	30.14	27.57	77.57	54.14	0.53	0
43	22 Oct – 28 Oct	27.29	27.00	81.71	64.29	22.42	3
44	29 Oct – 04 Nov	29.29	29.50	76.17	58.29	1.83	1
45	05 Nov – 11 Nov	30.43	30.29	78.14	70.71	6.10	3
46	12 Nov – 18 Nov	30.71	29.00	74.57	68.71	1.11	1

The Pharma Innovation Journal 2021; 10(11): 910-914

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(11): 910-914

www.thepharmajournal.com
Received: 19-08-2021
Accepted: 29-10-2021

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Survey on blast [*Pyricularia grisea* [(Cooke) Sacc.] disease of finger millet in major finger millet growing areas of Andhra Pradesh

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Abstract
Finger millet blast caused by *Pyricularia grisea* is a major yield limiting factor in finger millet growing areas of Andhra Pradesh. A roving survey was conducted to assess blast disease incidence in finger millet crop during *Kharif* 2020 in Andhra Pradesh. The highest PDI of finger millet blast was noticed in fields of Salur village (59.20%) of Salur Mandal in Vizianagaram district and the least disease incidence was recorded in Somidevipalle village (5.02%) of Racherla mandal of Prakasam district. It was concluded that, maximum incidence in Vizianagaram district might due to monocropping, prevalence of congenial micro climate and existence of initial inoculum.

Keywords: Blast disease, per cent disease incidence, disease survey, inoculum, finger millet

Introduction
Finger millet is a small-grained cereal grown in dry and semi-arid regions of East and South Africa, as well as Southern Asia (Jenkins *et al,* 1982; Dida *et al*, 2007; Upadhyaya *et al*, 2007; Waghunde *et al*, 2013; Jegan, 2015; Negi *et al,* 2015; Thilakarathna and Raizada, 2015; Kumar *et al,* 2016; Gupta *et al*, 2017) [6, 1, 19, 20, 5, 12, 18, 8, 4]. Its seed is rich source of protein, iron, calcium, phosphorus, glucose, zinc, and gluten-free amino acids such methionine, leusine, isoleusine, and phenylalamine. (Kumar *et al*, 2016) [8]. With all these benefits, finger millet is affected by many diseases such as root rot, smut, streak, mottling virus and blast disease. *E. coracana* is tolerant to most of these diseases however blast disease is the most devastating and destructive leading to losses in yield and poor utilization by farmers (Ramakrishnan *et al*, 2016) [14].

Finger millet blast disease, caused by *Pyricularia grisea* is the most economically important disease of finger millet (Mgonja *et al,* 2007) [10]. It is known to cause significant losses in yield and utilization of finger millet. Worldwide losses of above 50% yield have been reported in finger millet and above 30% in rice production (Esele, 2002; Prajapati *et al*, 2013) [2, 13]. In India, an increase in 1% infection in the neck and finger results to a corresponding increase of 0.32 and 0.084% in yield losses and grain losses of 6.75 to 87.5%, respectively (Rao, 1990) [15]. Effect on the panicle on susceptible genotypes is drastic and may lead to total seed loss of entire finger millet crop (Gashaw *et al*, 2014) [3]. Muimba-Kankolongo (2018) [11] reported that favorable weather conditions (temperature of 25 ºC and 80% humidity) precedes infection of blast diseases, which starts when a three celled conidia lands on a leaf surface. This leads to formation of an appressorium which later forms a penetration peg, punctures the cuticle allowing entry to the epidermis. Formation of lesions then follow which later spreads to the whole plant through the epidermis forming diamond shaped grey lesions with brown or black margins. Infection from the leaves begins from the tip backwards. The disease has a wide range of hosts especially grasses and sedge species including rice (*Oryza sativa*), wheat (*Triticum aestivum*), pearl millet and foxtail millet (*Setaria italica*). Blast affects production and utilization of these crops leading to a substantial decrease in production in Southern Asia, Eastern and Southern Africa (Takan *et al*, 2012) [17].

Material and Methods

Roving survey was conducted for the incidence of finger millet blast during *Kharif* 2020 in major finger millet growing districts of Andhra Pradesh *viz.,* Kadapa, Chittoor, Anantapuram, Kurnool, Prakasam, Guntur and Vizianagaram. Each district was divided into two mandals, in each mandal three villages are selected. In each village three fields were chosen for the study. Observations on soil type, farming situation, varieties and disease incidence were recorded in one square meter area randomly in each field.

The data was recorded based on occurence of blast in observed fields was expressed as Per cent Disease Incidence (PDI).

$$PDI = \frac{\text{Diseased hills observed}}{\text{Total No. of hills observed}} \times 100$$

Result and discussion

The detailed survey was undertaken in different regions of Andhra Pradesh to gather information on per cent disease incidence and spread of *Pyricularia grisea* causing blast disease of finger millet in different locations. This information is highly useful to identify the hot spots of this disease in Kadapa, Chittoor, Anantapuram, Kurnool, Prakasam, Guntur and Vizianagaram (Fig.1) where finger millet is extensively grown as a major crop. From the survey it was evident that the disease severity varied from one locality to another based on the soil type, farming situation and varieties used.

Among the seven districts surveyed, highest mean Per cent Disease Incidence (PDI) 56.31% was recorded in Vizianagaram district. It was followed in Chittoor, Kadapa and Kurnool, with mean Per cent Disease Incidence of 42.71, 22.27 and 20.45%, respectively. The lowest mean disease incidence 7.54% was recorded in Prakasam district followed by Anantapuram (19.37%) and Guntur (19.68%) districts (Table 1).

The highest mean blast incidence 56.63% was recorded in Salur mandal of Vizianagaram district in a range of 53.86-9.20% incidence which was followed by Kurupam mandal of Vizianagaram district with 55.99% mean PDI in a range of 54.63% to 57.56%. However, the mean PDI in Palamner mandal was 53.41% with range of 49.25 to 57.52%, Kuppam mandal (32.00% with range of 29.45 to 33.78%), Pattikonda mandal (27.92% with range of 24.32 to 33.23%), Veldurthi mandal (24.54% with range of 12.28 to 48.21%), Gudibanda mandal (23.31% with range of 17.78 to 28.96%), Vallur mandal (22.81% with range of 8.05 to 49.23%) and Kamalapuram mandal (21.73% with range of 15.03 to 30.13%) of Kadapa district. Peapully mandal (12.97% with 9.09 to 15.54%), Madakasira mandal (15.43% with 11.01 to 18.14%) and Macherla mandal (14.81% with 11.13 to 17.24%) of Guntur district. The lowest PDI of 7.30% with 5.02 to 9.01% was noticed in Racherla mandal which was followed by Komarolu mandal (8.04% with 5.85 to 9.21%) of Prakasam district (Table 2).

The highest PDI of finger millet blast was noticed in fields of Salur village (59.20%) of salur mandal, least was noticed in Kurmarajupeta (53.86%) in Vizianagaram district. In Chittoor district, maximum incidence of blast was noticed in Palamner village of Palamner mandal (57.52%) and least incidence was noticed in Palarlapalle (29.45%). In Kadapa district highest incidence of blast was observed in Lingayapalle village (49.23%) of Vallur mandal and least was observed in Goturu village (8.05%) of Vallur mandal. In Kurnool district, maximum incidence of blast was noticed in Chakrarala village (33.23%) of Pattikonda mandal and least was in Pothidoddi village (9.09%) of Peapully mandal. In Guntur district maximum disease incidence was noticed in Gundlapadu village (48.21%) of Veldurthi mandal and least disease incidence was recorded in Koppunur village (11.13%) of Macherla mandal. In Anantapuram district highest disease incidence was noticed in Gudibanda village (28.96%) of Gudibanda mandal and least disease incidence was recorded in Haresamudram village (11.01%) of Madakasira mandal. While, in Prakasam district maximum disease incidence was recorded in Komarolu village (9.21%) of Komarolu mandal and least was in Somidevipalle village (5.02%) of Racherla mandal (Table 3). The present results were in agreement with the findings of Rao (1990) [15] surveyed in eight locations of Bangalore, Kolar and Tumkur districts in Karnataka, and reported that an increase of 1% infection in neck and finger resulted in a corresponding increase of 0.32 and 0.084% yield loss and 6.75 to 87.5% loss in grain yield. Similarly, Kumar *et al.* (2005) [9] who reported the maximum neck (13-16%) and finger blast (42-55%) incidence in surveyed locations of Tumkur district of Karnataka. The same way Senthil *et al.* (2012) also reported that the finger millet blast was the most devastating disease affecting different aerial parts of the plant at all stages in Tamil Nadu (India). Prajapati (2013) [13] surveyed in different locations of Gujarat and reported the loss of 35.78 grain yield and 43.72 per cent fodder yield due to the blast disease. Kaurav *et al.* (2017) reported 1 to 5.48% and 3.60 to 13.80% severity of blast disease in major pearl millet growing districts of Madhya Pradesh during 2015-16 and 2016-17 respectively.

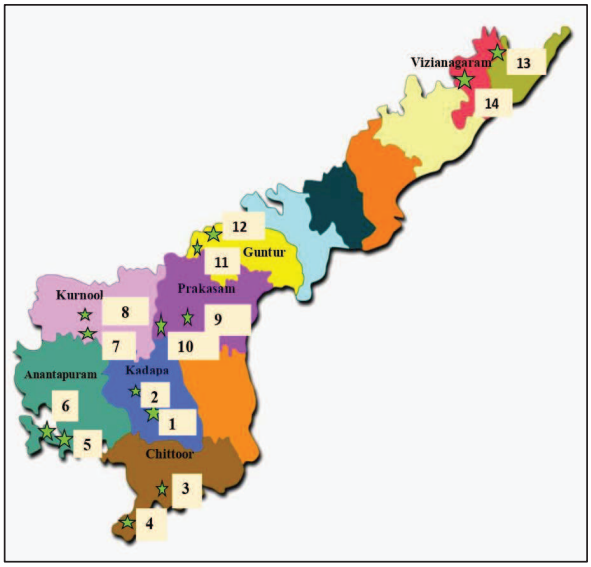

Fig 1: Map showing surveyed districts of Andhra Pradesh

Kadapa	**Chittoor**	**Anantapuram**	**Kurnool**
1. Vallur	3. Palamner	5. Madakasira	7. Peapully
2. Kamalapuram	4. Kuppam	6. Gudibanda	8. Pattikonda
Prakasam	**Guntur**	**Vizianagaram**	
9. Racherla	11. Veldurthi	13. Kurupam	
10. Komarolu	12. Macherla	14. Salur	

Table 1: Finger millet blast incidence in major finger millet growing districts of Andhra Pradesh

Sl. No	District	Range of PDI	District mean PDI
1	Kadapa	8.05-49.23	22.27
2	Chittoor	29.45-57.52	42.71
3	Anantapuram	11.01-28.96	19.37
4	Kurnool	9.09-33.23	20.45
5	Prakasam	5.02-9.21	7.54
6	Guntur	11.13-48.21	19.68
7	Vizianagaram	53.86-59.20	56.31

Table 2: Finger millet blast incidence in major finger millet growing mandals of Andhra Pradesh

Sl. No	Mandal	Range of PDI	Mandal mean PDI
	Kadapa		
1	Vallur	8.05-49.23	22.81
2	Kamalapuram	15.03-30.13	21.73
	Chitoor		
1	Palamner	49.25-57.52	53.41
2	Kuppam	29.45-33.78	32.00
	Anantapuram		
1	Madakasira	11.01-18.14	15.43
2	Gudibanda	17.78-28.96	23.31
	Kurnool		
1	Peapully	9.09-15.54	12.97
2	Pattikonda	24.32-33.23	27.92
	Prakasam		
1	Racherla	5.02-9.01	7.03
2	Komarolu	5.85-9.21	8.04
	Guntur		
1	Veldurthi	12.28-48.21	24.54
2	Macherla	11.13-17.24	14.81
	Vizianagaram		
1	Kurupam	54.63-57.56	55.99
2	Salur	53.86-9.20	56.63

Table 3: Survey for the occurrence and distribution of finger millet blast incidence in major finger millet growing areas of Andhra Pradesh

Sl. No	District	Mandals	Villages	Latitude	Longitude	Varieties	Soil type	Farming situation	Per cent disease incidence
									2020
1	Kadapa	Vallur	Lingayapalle	14°34'19"	78°44'41"	Local	Red loam	Rainfed	49.23
			vallur	14°19'59"	78°25'54"	Local	Red loam	Rainfed	11.15
			Goturu	14°33'36"	78°44'14"	Vakula	Red loam	Rainfed	8.05
		Kamalapuram	Kamalapuram	14°35'57"	78°39'51"	Tirumala	Red loam	Rainfed	30.13
			Ramachandrapuram	14°39'40"	78°38'29"	Local	Red loam	Rainfed	15.03
			T. sadipirala	14°35'08"	78°37'16"	Local	Red loam	Rainfed	20.02
2	Chittoor	Palamner	Palamner	13°11'51"	78°45'22"	Vakula	Red loam	Rainfed	57.52
			moram	13°10'21"	78°41'33"	Vakula	Red loam	Rainfed	53.46
			Kurmoi	13°09'57"	78°44'06"	Vakula	Red loam	Rainfed	49.25
		Kuppam	Gonugur	12°42'54"	78°19'48"	Vakula	Red loam	Rainfed	32.78
			Jarugu	12°73'55"	78°16'57"	Vakula	Red loam	Rainfed	33.78
			palarlapalle	12°42'55"	78°19'20"	Vakula	Red loam	Rainfed	29.45
3	Anantapuram	Madakasira	Govindapuram	13°88'28"	77°19'97"	Tirumala	Red loam	Rainfed	17.13
			Jadrahalli	13°53'34"	77°15'29	Vakula	Red loam	Rainfed	18.14
			Haresamudram	13°55'00"	77°16'03"	Vakula	Red loam	Rainfed	11.01
		Gudibanda	Morubagal	13°58'18"	77°02'12"	Vakula	Red loam	Rainfed	17.78
			Gudibanda	13°58'13"	77°06'30"	Local	Red loam	Rainfed	28.96
			Muthkur	13°58'08"	77°03'55"	Vakula	Red loam	Rainfed	23.19
4	Kurnool	Peapully	Peapully	15°14'25"	77°44'19"	Local	Red loam	Rainfed	14.28
			Vengalampalle	15°15'05"	77°46'06"	Local	Red loam	Rainfed	15.54
			Pothidoddi	15°12'19"	77°43'00"	Vakula	Red loam	Rainfed	9.09
		Pattikonda	Pattikonda	15°24'01"	77°30'25"	Vakula	Red loam	Rainfed	26.22
			Chakrarala	15°23'43"	77°34'40"	Vakula	Red loam	Rainfed	33.23
			Juturu	15°29'10"	77°28'24"	Local	Red loam	Rainfed	24.32
5	Prakasam	Racherla	Anumulapalle	15°29'09"	78°58'04"	Vakula	Red loam	Rainfed	7.07
			Racherla	15°27'54"	78°57'49"	Vakula	Red loam	Rainfed	9.01
			Somidevipalle	15°27'27"	79°02'02"	Vakula	Red loam	Rainfed	5.02
		Komarolu	Taticherla	15°2s3'04"	79°02'47"	Vakula	Red loam	Rainfed	9.05
			Chinthalapalli	15°16'41"	79°03'13"	Local	Red loam	Rainfed	5.85
			Komarolu	15°15'57"	78°59'50"	Vakula	Red loam	Rainfed	9.21
6	Guntur	Veldurthi	Veldurthi	16°20'47"	79°21'50"	Vakula	Red loam	Rainfed	13.14
			Uppalapadu	16°21'28"	79°24'05"	Local	Red loam	Rainfed	12.28
			Gundlapadu	16°21'50"	79°20'21"	Vakula	Red loam	Rainfed	48.21
		Macherla	Macherla	16°28'51"	79°25'46"	Vakula	Red loam	Rainfed	17.24
			Kothapalle	16°29'46"	78°24'30"	Vakula	Red loam	Rainfed	16.07
			Koppunur	16°29'22"	79°19'48"	Vakula	Red loam	Rainfed	11.13
7	Vizianagaram	Kurupam	Gumma	18°95'17"	83°64'33"	VR 847	Red loam	Rainfed	57.56
			Sivada	18°91'52"	83°74'25"	VR 847	Red loam	Rainfed	54.63
			Gummidiguda	18°83'65"	83°77'45"	VR 847	Red loam	Rainfed	55.78
		Salur	Neliparthi	18°50'21"	83°18'45"	VR 847	Red loam	Rainfed	56.82
			Salur	18°53'02"	83°17'85"	VR 847	Red loam	Rainfed	59.20
			Kurmarajupeta	18°53'73"	83°21'15"	VR 847	Red loam	Rainfed	53.86

Conclusion

The survey during *Kharif* 2020 revealed that the disease was noticed in varying intensities in seven districts surveyed. The severity was more in among the seven districts surveyed, highest mean Per cent Disesease Incidence (PDI) 56.31% was recorded in Vizianagaram district. It was followed by Chittoor, Kadapa and Kurnool, with mean Per cent Disease Incidence of 42.71, 22.27 and 20.45%, respectively. The lowest mean disease incidence 7.54% was recorded in Prakasam district followed by Anantapuram (19.37%) and Guntur (19.68%) districts. The higher incidence of disease in some locations may due to monoculture of finger millet, which could be main source for the pathogen. The disease might have appeared in severe form because of initial inoculums, build-up also farmer's practices *viz.*, high amount of nitrogen applications, improper irrigation also influences the survival and spread of inoculum and that ultimately led to highly aggregated damage to the crop.

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