EVALUATION OF SEED DRESSING FORMULATIONS OF POTENTIAL *TRICHODERMA* MUTANTS ON GROWTH AND YIELD ATTRIBUTING CHARACTERES OF CEREALS, PULSES AND VEGETABLES

Ph.D. Thesis by

Kishan Kumar Sharma

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE FACULTY
OF AGRICULTURE
INDIRA GANDHI KRISHI VISHWAVIDYALAYA
RAIPUR (Chhattisgarh)
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Thesis Submitted

to the

Indira Gandhi Krishi Vishwavidyalaya, Raipur

by

Kishan Kumar Sharma

IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF

Doctor of Philosophy

in

(Agriculture)
Plant Pathology

Roll No. 130117074 ID No. 20171827601

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CERTIFICATE - I

This is to certify that the thesis entitled **"Evaluation of seed dressing formulations of potential *Trichoderma* mutants on growth and yield attributing characters of cereals, pulses, and vegetables"** submitted in partial fulfilment of the requirement for the degree of **" Doctor of Philosophy in Agriculture"** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafied research work carried out by Kishan Kumar Sharma under my guidance and supervision. The subject of thesis has been approved by student's Advisory committee in the Director of Instruction.

No part of thesis has been submitted for any other degree of diploma or has been published / published part has been fully acknowledged. All the assistance and help received during course of investigations have been duly acknowledge by him.

Date 3/11/2020

Chairman

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

Chairman (Dr. A.S. Kotasthane)

Member (Dr. R. K. Dantre)

Member (Dr. Subha Banerjee)

Member (Dr. R. R. Saxena)

Head of Department

CERTIFICATE - II

This is to certify that the thesis entitled **"Evaluation of seed dressing formulations of potential *Trichoderma* mutants on growth and yield attributing characters of cereals, pulses and vegetables "** submitted by **Kishan Kumar Sharma** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur in partial fulfillment for **Doctor of Philosophy** in the Department of **Plant Pathology** has been approved by external examiner and student's advisory committee after oral examination.

Date: 10/2/2021

Signature External Examiner
(Name: Dr S.M. Kumar)

Major Advisor _____

Head of Department _____

Faculty Dean _____

Approved/Not approved

Director of Instruction _____

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THESIS ABSTRACT

a) Title of the Thesis	:	**"Evaluation of seed dressing formulations of potential *Trichoderma* mutants on growth and yield attributing characters of cereals, pulses and vegetables "**
b) Full Name of the Student	:	Kishan Kumar Sharma
c) Major Subject	:	Plant Pathology
d) Name and Address of the Major Advisor	:	Dr. A. S. Kotasthane, Professor & Head, Department of Plant Pathology
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Signature of the Student

Signature of Major Advisor

Date: 05/11/2020

Signature of Head of the Department

ABSTRACT

Owing to its improve nutrient use efficiency, mycoparasitic and plant growth promoting ability, ability to produce diverse array of secondary metabolites, SAR against invading pathogen, *Trichoderma* spp. are one of the most preferred bio inoculant used in agriculture (Mukharjee *et al*., 2013, Lamdan *et al*., 2015, Salas Marina *et al*., 2015, Chagas *et al*., 2017). *Trichoderma* spp. interacts with plant through rhizosphre and rhizoplane colonization, and triggers morphologic changes in the roots (Contreras-Cornejo *et al*., 2009, 2015). Trichoderma-plant interaction modulates levels of the plant hormones or provide intermediate path for the synthesis of some plant hormones, beneficial for the plant (Guzman-Guzman *et al*., 2019) stimulating increased plant growth and vigor, and ultimately boosting crop yield (Salas Marina *et*

Marina *et al*., 2015; Chagas *et al*., 2017). Successful commercially viable formulations based on protoplast fusant strain of *T. afroharzianum* and development of novel strains of *Trichoderma* using mutagenesis have been reported earlier (Mukharjee *et al*., 2019). Such report prompted us to evaluate the seed dressing formulation of four potential *Trichoderma* mutants for Plant growth and yield attributing characters of cereals, pulses and vegetables. Our present investigation indicate that seed bio-priming can be a preferred method of delivering the potential *Trichoderma* mutants to different crops and can be one of the successful strategy to scale up the microbial products at regional to global levels. Lowering dose of seed treatment followed by seed bio-priming proliferated *Trichoderma* on seed by many folds and stimulated significant plant growth in cereals, pulses and vegetables. Extensive field evaluation indicate that all the four *Trichoderma* mutants stimulated plant growth of chickpea and increased the yield by 20%. Potential *Trichoderma* mutants also stimulated increased antioxidant enzyme activity and total phenolic content (TPC) in chickpea and wheat.

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शोध का सारांश

शोध का शीर्षक	:	"अनाज, दालों और सब्जियों के पात्रों के विकास और उपज पर संभावित ट्राइकोडर्मा म्यूटेंट के बीज ड्रेसिंग योगों का मूल्यांकन "
छात्र का पूरा नाम	:	किशन कुमार शर्मा
प्रमुख विषय	:	पादप रोग विज्ञान
प्रमुख सलाहकार का नाम और पता	:	डॉ. ए.एस. कोटस्थाने, प्राध्यापक एवं विभागाध्यक्ष, (पादप रोग विज्ञान), कृषि महाविद्यालय, इंदिरा गांधी कृषि विश्व विद्यालय, रायपुर, 492012 (छत्तीसगढ़)
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छात्र का हस्ताक्षर

प्रमुख सलाहकार का हस्ताक्षर

दिनांक 02/11/2020

विभागाध्यक्ष का हस्ताक्षर

सारांश

ट्राइकोडर्मा पोषक तत्व उपयोग दक्षता, माइकोपरैसिटिक गतिविधि, पौधे की वृद्धि को बढ़ावा देने की क्षमता में सुधार के कारण, द्वितीयक चयापचयों के विविध सरणी का उत्पादन करने की क्षमता, एवं आक्रमणकारी रोगज़नक़ के खिलाफ एसएआर (मुखर्जी एट अल., 2013 ; लाम्डन एट अल., 2015 ; सालास-मरीना एट अल., 2015; चगास एट अल ., 2017) में इस्तेमाल किए जाने वाले सबसे पसंदीदा जैव-रसायन में से एक है। ट्राइकोडर्मा जाति राइज़ोस्फ़रे और राइज़ोप्लेन उपनिवेशण के माध्यम से पौधे के साथ बातचीत करता है, तथा पौधों के जड़ों में रूपात्मक परिवर्तन हेतु उत्प्रेरक का कार्य करता है। (कॉन्ट्रेरेस-कॉर्नेज़ो एट अल।, 2009, 2015). ट्राइकोडर्मा पौधों में हार्मोन के स्तर को नियंत्रित करता है, साथ ही साथ कुछ हार्मोन के संश्लेषण के लिए मध्यवर्ती माध्यम भी प्रदान करता है। यह जैव रसायन पौधों

इस्तेमाल किए जाने वाले सबसे पसंदीदा जैव-रसायन में से एक है। ट्राइकोडर्मा जाति राइज़ोस्फ़रे और राइज़ोप्लेन उपनिवेशण के माध्यम से पौधे के साथ बातचीत करता है, तथा पौधों के जड़ों में रूपात्मक परिवर्तन हेतु उत्प्रेरक का कार्य करता है। (कॉन्ट्रैरेस-कॉर्नेज़ो एट अल।, 2009, 2015). ट्राइकोडर्मा पौधों में हार्मोन के स्तर को नियंत्रित करता है, साथ ही साथ कुछ हार्मोन के संश्लेषण के लिए मध्यवर्ती माध्यम भी प्रदान करता है। यह जैव रसायन पौधों को लाभ के एक हिस्से के रूप में पौधों की वृद्धि एवं ताकत को बढ़ाता है (गुज़मैन-गुज़मैन एट अल।, 2019) अंततः फसल की उपज को भी बढ़ावा देता है (सालासमिना एट अल., 2015; चगास एट अल., 2017). एफ्रोहरजियानम के प्रोटोपलासट फ्यूजन स्ट्रेन और उपन्यास उपभेदों के विकास के आधार पर सफल व्यावसायिक रूप से व्यवहार्य सूत्र पहले से बताए गए है (मुखर्जी एट अल 2019)। इस तरह की रिपोर्ट ने हमें पौधों की वृद्धि और उपज के लिए चार संभावित ट्राइकोडर्मा म्यूटेंट के बीज ड्रेसिंग फॉर्मूलेशन का मूल्यांकन अलग-अलग अनाज, दाल और सब्जियों में करने के लिए प्रेरित किया। हमारी वर्तमान जांच से संकेत मिलता है, कि बीज जैव-प्राइमिंग विभिन्न फसलों के लिए संभावित ट्राइकोडर्मा म्यूटेंट को बीजोपचार एवं वितरित करने का एक पसंदीदा तरीका हो सकता है और क्षेत्रीय से वैश्विक स्तर पर माइक्रोबियल उत्पादों को स्केल करने के लिए सफल रणनीति में से एक हो सकता है। बीजोपचार की कम खुराक के बाद सीड बायो-प्राइमिंग ने कई सिलवटों में बीज पर ट्राइकोडर्मा का प्रसार किया और अनाज, दालों और सब्जियों में महत्वपूर्ण पौधों की वृद्धि को प्रोत्साहित किया। व्यापक क्षेत्र मूल्यांकन से संकेत मिलता है कि सभी चार ट्राइकोडर्मा म्यूटेंट ने चने के पौधे की वृद्धि को प्रोत्साहित किया और उपज में 20% की वृद्धि की। संभावित ट्राइकोडर्मा म्यूटेंट ने भी बढ़े हुए एंटीऑक्सिडेंट एंजाइम गतिविधि एवं चिकन और गेहूं में कुल फेनोलिक सामग्री (टीपीसी) को उत्तेजित किया।

CHAPTER-I
INTRODUCTION

Population through worldwide is expected to reach 9.6 billion by 2050 (Wilson, 2003), and therefore will require at least double our current agricultural production. (Bruinsma, 2009). Increase in agricultural production requires additional inputs (*ie*. fertilizers pesticides) that will safeguard our crop from invasive weeds, pathogens and insects. Global food security can only be ensured for a growing population if a sound and sustainable strategy is devised. Modification in cropping systems which will maximize productivity with a minimum resources requirement can be a sustainable strategy. Under increasing stresses of environment, farmers could benefit from new sustainable products to boost or maintain yields (Baulcombe *et al*., 2009). Excessive and improper use of chemical fertilizers for increasing agricultural productivity has led to serious environmental concerns and therefore for sustainable crop production, bioinoculants (biofertilizers and biopesticides) are preferred alternative with low ecological impact. Use of microbial products in agriculture is an age old practice but has recently received increased attention owing to its sustainable and eco-friendly nature (Deaker *et al*., 2004). Microbial products are now becoming a valuable component in precision agriculture (Deaker *et al*., 2004; Copping, 2009) in addition to the new chemistries and trait development which are important critical component in developing biotic and abiotic stress tolerance / resistance crop (Bhattacharyya and Jha, 2012).

Trichoderma is a important genus of fungi in the family Hypocreaceae, which shows their adaptability to various ecological conditions, and are highly opportunistic and have been isolated from a diverse range of substrates (artificial and natural), (Druzhinina *et al*., 2011, 2012). Species importance in the fungal genus *Trichoderma* can well be understood because they serve as sources of variety of antibiotics, enzymes, plant growth promoters, xenobiotic degraders, and importantly, as commercial bio-fungicides (Mukherjee, 1999). Improved nutrient use efficiency, mycoparasitic and plant growth promoting ability, ability to produce diverse array of secondary metabolites, SAR against invading pathogen,

makes *Trichoderma* spp. as one of the most preferred bioinoculant agriculture (Mukharjee *et al*., 2013, Lamdan *et al*., 2015, Salas-Marina *et al*., 2015; Chagas *et al*., 2017).

Trichoderma spp. interacts with plant once it colonizes rhizosphre/ rhizoplane. Bipartite interaction results in root colonization which triggers morphologic changes in the roots (Contreras-Cornejo *et al*., 2009, 2015) and promote growth of plant in the form of increased density of rot, enhanced uptake of nutrient, mineral solubilization, and induced defense response against biotic and abiotic stresses (Harman 1992; Mastouri *et al*., 2010). Selected strains of *Trichoderma* interact with the plant by root colonization, establishing communication with help of chemicals and altering the expression of numerous plant genes systemically. In the recent past *Trichoderma* isolates have been identified to act as root endophytic plant symbionts stimulating significant modulation in gene expression in shoots. Such modulation in gene expression alter plant physiology and that may improve resistance to biotic / abiotic stress, nitrogen fertilizer uptake, improves photosynthetic efficiency and the net result of such effects is an increase in plant growth and productivity (Hermosa *et al*., 2012). Studies on mechanisms underlying Trichoderma–root symbiosis suggest that secreted QID74, cysteine-rich protein, modifies root architecture to increase surface area (Samolski *et al*., 2012).

Trichoderma-plant interaction modulates levels of the hormones or provide intermediates for the synthesis of some phytohormones, as a part of the benefits to the plant (Guzman-Guzman *et al*., 2019) stimulating increased plant growth and vigour, and ultimately boosting crop yield (Salas Marina *et al*., 2015; Chagas *et al*., 2017).

Trichoderma base growth promotion has been observed across a large number of different groups of plants including arable, ornamental, vegetable, and forestry crops. Much of the early work focused on vegetable crops grown under glass house condition including bean, cucumber, lettuce, eggplant, pea, pepper, radish, and tomato (Chang *et al*., 1986; Kleifeld and Chet, 1992; Lynch *et al*., 1991; Ou sley *et al*., 1993, 1994[a]). Increased plant growth induced by *Trichoderma*

species has also been reported for many arable crops such as maize (Zea mays) (Harman *et al*., 1989) and wheat (*Triticum aestivum*) (Shivanna *et al*., 1996).

Mutation through gamma irradiation is one of the genetic manipulation strategies to improve the antagonistic effect as well as plant growth promoting activity. Strain improvement by mutation is an age-old and successful method (Kumakura et al. 1984, Bailey and Tahtiharju, 2003). Gamma-ray can induce genetic diversity in filamentous fungi and induce mutantions. Many studies have been made by several researchers for increasing the metabolic activity i.e. secretion of extracellular cell wall-degrading enzymes, antibiotics and improvement of mycoparasitic ability in *Trichoderma* after a physical mutagen treatment (Jiang *et al*., 2011, Li *et al*., 2010, Zaldivar *et al*., 2001). Successful commercially viable formulations based on protoplast fusant strain of *T. afroharzianum* (Shoresh *et al*., 2010; Chaverri *et al*., 2015; Harman and Uphoff, 2019). and development of novel strains of *Trichoderma* using mutagenesis have been reported earlier ((Papavizas *et al*., 1982; Ahmad and Baker, 1988; Mukherjee *et al*., 1999; Szekeres *et al*., 2007; Olejníkova *et al*., 2010).

Use of microbial products has recently received increased attention owing to its sustainable and eco-friendly nature and therefore are becoming a critically needed component of agriculture. Bio-inoculants provide unique opportunities for crop production and protection. Because they grow and proliferate, they colonize the emerging root and rhizosphere of the planted seed, colonize and stimulate the entire subterranean plant portions. Owing to the unique capabilities of *Trichoderma,* the present investigation was therefore undertaken with the following major objectives:

1. Studies on seed bio-priming with different concentrations of seed dressing formulation of potential *Trichoderma* mutants
2. Field evaluation of potential *Trichoderma* mutants on growth and yield attributing characters of different crops and vegetables following seed dressing
3. Evaluation of *Trichoderma* mutants *against Sclerotium rolfsii* a major soil-borne pathogen in a rice-chickpea cropping system
4. Antioxidant enzyme assays of chickpea plants derived from a seed treatment with potential *Trichoderma* mutants in combination with propiconazole.

CHAPTER-II
REVIEW OF LITERATURE

The fungal genus *Trichoderma* (teleomorph, Hypocrea- Ascomycetes: Hypocreales) includes different economically important species well known for the production of secondry metabolites, enzymes and antibiotics (Howell, 2003), bio-control activity against nemtaodes and fungi (Brunner *et al*., 2005), xenobiotic compounds degradation (Ezzi and lynch, 2005) and due to rhizo competent nature and endophytism induce systemic acquired resistance in plant system (Brunner *et al*., 2005 ; Kubicek *et al*., 2001). Different species of *Trichoderma* can improve plant growth and development (De Souza *et al*., 2008 ; Windham *et al*., 1986). They are ubiquitous and found in different soil types, bark, growing wood, and other innumerable substrates, demonstrating their potential and wide adaptability to different ecological conditions (Druzhinia *et al*., 2011). Description of the fungus *Trichoderma* back to 1794 (Persoon, 1794), and an important link to the sexual stage of a *Hypocrea* species was suggested by (Tulsane and Tulsane, 1865).

In the genus *Trichoderma*, there are 89 species are known. *Hypocrea* is well-known teleomorphs of *Trichoderma* and themselves are known as anamorphs. The close association of many species of *Trichoderma* towards plant roots revealed an endophytic association between *Trichoderma* and their plant host (Evans *et al*., 2003 ; Sette *et al*., 2006 ; Yedida *et al*., 2000). Being endophytic, they are effective bio-control agents of fungi in the rhizospheric region, producing antimicrobial compounds, stimulating vigor and growth of a plant by solubilizing minerals, and providing essential nutrients and growth-regulating compounds to plants (Altomere *et al*., 1999).

2.1 Studies on seed bio-priming with different concentrations of seed dressing formulation of potential *Trichoderma* mutants

These fungi are frequently used applied as seed treatments, where they improve the plant's stand under field conditions and induce long-term improvement of plant

quality (Harman 2006, Harman *et al*., 2004). Therefore, seed treatments may be able to induce short term and long term improvements in seed by enhanced survival of seedling at the germination stage and subsequent performance of the plant. However, there is very little is known about the seed and *Trichoderma* spp. interactions. Regard to this seed bio-priming is considered as the advanced technique of seed treatment which combines the introduction of beneficial microorganisms following seed hydration (Reddy, 2013; Singh *et al*., 2016[a]). There is a report of an increase in bacterial population (10 to 10,000 folds) following bio-priming depending upon initial inoculums level (Callan *et al*., 1990). The basic ideology behind seed bio-priming is to disinfect the seeds from contaminating seed microflora and allow the bio-agents to colonize over the seed surface and build up inoculums level at a higher level to induce better seed germination and seedling growth at the initial stage of plant growth and development (Reddy, 2013 ; Jain *et al*., 2015[a]). *Trichoderma* based enhancement of plant growth has been for many years and occur in axenic systems or in soil (Cheng e*t al*., 1990).

In Maize plants, it was observed that *Trichoderma* inoculation affected root architecture, which in turn increased the yield of plants. Several reports also revealed enhanced root biomass production and increased development of root hairs (Harman *et al*., 2004). The system of the root is important and crucial for the fitness of plants because it contributes towards increased nutrient and water use efficiency of a plant from the soil system (Lopez-Bucio *et al*., 2005). Colonization of roots by different species of *Trichoderma* frequently enhances root growth and development, resistance to biotic and abiotic stress, and the use of nutrients (Arora *et al*., 1992). Under field conditions, crop productivity has been increased up to 300 % after the application of *T. koningii* and *T. hamatum*. In different experiments conducted under greenhouse conditions, there was also a considerable increase in yield was recorded when plant seeds were previously treated with spores of *Trichoderma* (Chet *et al*., 1997). However, few reports on *Trichoderma* strains that produce growth factors which have been detected and identified in the laboratory (auxins, cytokinins, and ethylene),

despite the identification of many filamentous fungi that produce different phytohormones, such as indole acetic acid (IAA) and ethylene, whose pathways of metabolism have been identified (Arora *et al.,* 1992 ; Osiewacz, 2002).

Mastouri *et al*., (2010) bio-primed young and artificially aged tomato seeds with the conidial suspension at the rate of 20µl g-1 deposit 2×107 CFU g-1 of seed and observed a significant increase in radicle length of seedling in aged tomato seeds while unaged tomato seeds were unaffected.

Pandey *et al*., (2016) studied the dose-dependent response of *T. harzianum* Th-56 and correlated that increase in the dose of *T. harzianum* Th-56 enhanced tolerance of drought in different genotypes of rice.

Singh *et al*., (2016)[b] studied the effect of seed bio-priming on seed germination and development in different vegetables with different spore doses of *T. asperellum* BHUT8 ranging from 10^2 to 10^8 spore ml-1 and found that spore dose of 10^3 spore ml-1 in tomato and ridge gourd, 10^4 spore ml^{-1} in brinjal and okra while 10^6 spore ml^{-1} in chilly and guar was most effective spore dose for enhancement of radicle length and seed germination.

2.2 Field evaluation of potential *Trichoderma* mutants on growth and yield attributing characters of different crops and vegetables following seed dressing

Plant growth-promoting fungi (PGPF) are a class of non-pathogenic fungi with soil-borne filamentous nature that have direct beneficial effects on plants. So far, several PGPF has been reported such as belonging to the genera *Trichoderma, Pencillium, Fusarium,* and *Phoma* (Masunaka *et al*., 2010). The production of the wide range of plant growth-promoting metabolites was a characteristic of individual strains that is not consistent with any species of *Trichoderma*. No single isolate was found positive for all of the metabolites assessed. Sometimes plant nutrition may get fixed in soil from soluble forms to insoluble forms that influence the accessibility of these nutrients and root absorption. These transitions may be influenced by microorganisms (Altomere *et al*., 1999).

Microbe-associated molecular patterns (MAMP) triggered plant responses are elicited rapidly and transiently. MAMP responses imply ion fluxes across the plasma membrane which leads to the generation of reactive oxygen species (ROS), nitric oxide, and at a later stage deposition of callose and antimicrobial compound synthesis. There is a report of identification of these MAMPs for PGPR, such as flagellin or lipopolysaccharides, but also compounds including bio-surfactants, antibiotics, and volatile compounds have been shown to elicit systemic resistance (Hermosa *et al*., 2012).

Extremely wide range metabolites produced by the genus *Trichoderma* include s different compounds of antifungal activities (phenolic compounds, viridofungins, 6-α-pentyl-pyrone, and harzianopiridona), plant growth regulators (ciclonerodiol, α-harzianopiridonapentyl-pyrone) antibiotics (anthraquinone, gliotoxin), antimicrobial peptides including more than 200 peptaibols, and even viridiol phytotoxic compounds with different pharmaceutical uses. These metabolites are antivirus agents and unclassified inhibitors that expand the prospects of industrial, pharmaceutical, and other uses of this organism (Sivasithanparam and Ghislberti., 1998 ; Vinal *et al*., 2006 ; Xiano-Yan *et al*., 2006). Certain species of *Trichoderma* has a beneficial effect on plant growth and resist the plant to overcome different biotic and abiotic stresses. Several works revealed the growth-promoting activity of *Trichoderma* in cucumber, pepper, radish, and tomato (Baker *et al.*, 1984). Further studies of plant growth promotion demonstrated that *Trichoderma* also enhances root growth and development of secondary branches of roots for efficient uptake of nutrients from the soil (Harman, 2000). Moreover, *T. harzianum* may solubilize several nutrients for plant uptake (Altomare *et al*., 1999), and colonization of cucumber roots by *T. asperellum* has been shown to enhance the availability of Fe and P to plants, with the significant increase in shoot length, leaf area and dry weight (Yedida *et al*., 2001). In the plant system, IAA plays an important role in root and shoot development. Introduction of *Trichoderma* spp. through seed bio-priming into the plant system through germinating radicles and then into root induces IAA production which is a key regulator of lateral root and root hair development (Casimiro *et al*., 2001). Study of the

expression of the auxin-inducible marker DR5 suggested that *T. virens* inoculation increases the response of auxin in seedlings of *Arabdiopsis*.

Several auxins like secondary metabolites produced by strains of *Trichoderma* we-re able to induce plant growth and required for the development of lateral roots in *Arabdiopsis* (Contreras *et al*., 2009 ; Vinale *et al*., 2008). The observed effect of *Trichoderma* application in promoting lateral root development is similar to that described for auxin in plants (Casimiro *et al.,* 2001).

Nayaka *et al*., (2010) explained that seed bio-priming with *T. harzianum* could increase the seed germination, vigor index, field emergence, plant height, number of branches, number of pods, seed test weight, grain yield, and harvest index in chickpea in comparison to control.

Penicillium citrinum and *Aspergillus niger* along with *T.harzianum* were evalu ated for phosphate solubilization and IAA production under lab condition. The result revealed that all fungus were reported positive phosphate solubilizer and IAA producer. Inoculation of *Aspergillus niger* and *T. harzianum* on chickpea plant enhanced the plant growth parameters like dry root and shoot weight, shoot and root length, etc. (Yadav *et al*., 2011).

Gupta *et al*., (2012) observed the combined effect of seed bio-priming with fungicide treatment on chickpea. The seed treatment was followed by seed bio-priming which revealed that the growth parameters of chickpea were significantly affected by seed bio-priming. Plant height showed a high correlation with a dry weight of nodule which in turn enhanced the seed and biological yield with 87 % in chickpea.

Mutation-based strain improvement of *Trichoderma* has become a new technique to improve their potentiality of plant growth promotion with reduced disease rates in plants worldwide. Field screening of different progenies of *Trichoderma* isolated after mutation for their root colonization and plant growth promotion activity in cucumber showed that IAA production ability of mutated progenies was higher as compared to wild type (Zhang *et al*., 2013).

Assessment of growth response of twenty isolates of *Trichoderma* collected from different regions of Chhattisgarh, on the bitter gourd, bottle gourd and cucumber

revealed that among all isolates strain T-14 of *Trichoderma viride* was found high plant growth promoter due to higher ability to produce siderophore, inorganic phosphorus and IAA (Kotasthane *et al*., 2014).

Five *Trichoderma* isolates were evaluated for their plant growth-promoting activity on a wheat crop for different plant growth parameters following seed bio-priming with culture filtrate and conidia. Different growth parameters assessed under field conditions were shoot and root length, root weight, leaf area index, chlorophyll content. All the five isolates showed positive response towards growth parameters and the highest value of seed germination (95.8 %) was recorded for *T. harzianum* (Hajieghrari *et al*., 2016).

Singh *et al*., (2016)[a] studied the effectiveness of seed bio-priming with *T. asperellum* BHUT8 for plant growth evaluation in pea. The results showed a significant increase in shoot length, no. of leaves, root length, root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight by 35.29, 28.13, 96.49, 146.26, 36.10, 77.20, and 30.17 % respectively, as compared to control.

Trichoderma species is well known for its plant growth-promoting activity, improving nutrient uptake, and increasing yield attributing characters. A commercial strain of *Trichoderma* with a combination of organo-mineral fertilizer was assessed on wheat plants for its positive effect overgrowth and yield attributing characters. The wheat seeds were treated with a conidial suspension of *Trichoderma* @ 2 ml/100 gm seed and allowed to germinate under greenhouse and lab conditions. Growth parameters were examined at 8 and 110 days after sowing under both conditions. Results revealed that 2 species of *Trichoderma* namely *T. asperellum* and *T. harzianum* recorded with the highest grain yield. Whereas, the performance of *T. harzianum* was found best in greenhouse conditions (Oliveira *et al*., 2018).

Chaurasia *et al*., (2014) evaluated the efficacy of different bio-priming agents for enhancing seedling growth, field emergence, and grain yield in chickpea. Among different treatments, T1 (seed bio-priming with *T. harzianum*) showed significant performance for field emergence (85.83), number of plants per plot (24.5), plant height (77.8), number of pods per plant (45), number of primary branches (3.25) and

seed yield per plot (135.89) in organic priming followed by treatment T4 (Carbendazim) inorganic priming compared to untreated control.

Sharma *et al*., (2018) studied the effect of seed bio-priming with different micro inoculants on growth and yield attributing character in soybean. Results revealed that among different micro inoculants, the highest field emergence (88.47 %) was recorded in Psf-173 while maximum plant height (30.34 cm) was measured in Th-14. A significant increase in yield attributing character was observed in seed bio-primed soybean plants with PSB.

2.3 Evaluation of *Trichoderma* mutants *against Sclerotium rolfsii* a major soil-borne pathogen in a rice-chickpea cropping system

Biological control of plant disease, especially soilborne pathogens, has always been the subject of extensive research. To date *Trichoderma* spp. is well documented for its bio-control ability against a wide range of soil-borne pathogens (Coley-Smith *et al*., 1991). Biological control of soil-borne pathogens can be achieved by seed bio-priming with antagonists. *Sclerotium rolfsii* is well known destructive plant pathogen with a wide host range in the world (Bell, 1982). The fungus survives in the soil as resting structure *sclerotia* for several years and spread through contaminated seed, soil as well as irrigation water (Jayaraj *et al*., 2003). Due to the soil-borne nature of the pathogen, the disease is very difficult to manage through the application of chemicals (Zaki, 1998). Biological control with antagonists against these soil-borne pathogens has been suggested as an alternative approach to physical and chemical control measures (Cook and Baker, 1983). Management of this disease can be achieved through the application of fungicides, soil fumigants, and bio-agents. But due to environmental and residual toxicity concern to these harmful chemicals, there is a general trend of reduced application of these chemicals in soil.

2.3.1 *In vitro* evaluation of potential *Trichoderma* mutants against *S. rolfsii*

Harman *et al*., (1980) found that survival and propagation of *Trichoderma* may occur for the longer periods of the time when it is applied with a food base or as a seed coating. The integration of chemical and biological control seems like a very

promising way of controlling these plant pathogens with minimal interference with biological equilibrium (Cook and Baker, 1983).

Combination of *T. harzianum* with heat treatment, both under green house condition at sub-lethal dose, enhanced control of *S. rolfsii* disease on beans from 90 to 100 % (Elad *et al*., 1980). *T. harzianum* treated tomato plants transplanted into soil fumigated with methyl bromide reduced the disease incidence caused by *S. rolfsii* and *R. solani* by 93 % and increase yield by 160 % (Elad *et al*., 1982).

Prasad *et al*., (1999) reported that different isolates of *Trichoderma* and *Gliocladium* sp. inhibited mycelial growth and suppressed the sclerotial production (54.9 to 61.4 %) and (31.8 % to 97.8 %) respectively of *S. rolfsii*, the incitant of collar rot disease of chickpea under *in vitro* condition.

Biological control has emerged as an alternative and most promising means of management of soil-borne plant diseases. Different biological control agents like *Trichoderma harzianum* and *Gliocladium virens* antagonize plant pathogens by production and release of secondary metabolites, by mycoparasitism activity, competition, or other forms of exploitation was suggested by (Pant and Mukhopadhyay., 2001).

Patel and Anahosur (2001) observed the mycoparasitic activity of *T. harzianum* against *S. rolfsii* by overgrowing, and restricting the mycelial growth, and reducing the formation of the sclerotial body of the pathogen under *in vitro* condition.

Faruk *et al*., (2002) reported that *T. harzianum* significantly reduced the radial colony growth and reduced the mortality rate of cabbage seedlings following seed bio-priming, under *in vitro,* and *in vivo* conditions respectively.

Revathy and Muthusamy (2003) studied the antagonistic effect of *T. hamatum*, *T. harzianum* and *T. viride* on *S. rolfsii* and found that *T. viride* was the most effective in inhibiting the growth of *S. rolfsii* (55.8 % inhibition over control).

Parakhia and Akbari (2004) showed that *Trichoderma* sp. were effective in managing *S.rolfsii* when applied with oil cakes against root rot disease of chickpea.

Khosla and Kumar (2008) reported that combined application of *T. viride* (0.5%) and thiram (0.4 %) controlled (82.58 %) root rot disease of strawberry caused by *S. rolfsii* compared to individual application of *Trichoderma* (72.9 %).

Integrated management of stem rot of groundnut using a combined application of *Rhizobium* and *T. harzianum* successfully decreased the incidence of stem rot disease and also increases the growth of the groundnut plants (Ganesan *et al*., 2007).

Mundhe *et al*., (2009) investigated the bio-control efficacy of *T. viride, T. harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* against *S. rolfsii* incitant of foot rot disease of Finger Millet. Maximum inhibition (73.77 %) against pathogen was achieved due to *T. harzianum* followed by *P. fluorescens* (73 %) and *B. subtilis* (63.33 %) over control respectively.

Darvin *et al*., (2013) evaluated the antagonistic effect of different *Trichoderm a* spp. on the radial growth of *Sclerotium rolfsii*. The results from this experiment revealed that *T. viride* (TvL) and *T. harzianum* 14 (Th14) isolates were found effective with the lowest radial growth (3.50 cm) and highest percent inhibition (56.25 %) of *S. rolfsii*.

Pan *et al*., (2013) isolated different isolates of *Trichoderma* from rhizosphere soils of rice, groundnut, maize, cauliflower and okra using serial dilution technique and TSM modified medium. The maximum inhibition (42.4 %) was recorded from *Trichoderma* isolate obtained from the rhizosphere region of cauliflower.

A volatile compound of Tv5 isolate of *T. viride* were found more effective against the growth of *S. rolfsii* (54.6 %) inhibition. Whereas, non-volatile metabolites of Th4 isolate of *T. harzianum* were more effective against pathogen with 100 % growth inhibition at a concentration of 60 percent and 80 percent respectively (Swathi *et al*., 2015).

Suneeta *et al*., (2017) reported that *T. harzianum* NVTH2(T4) isolate was recorded with the least incidence (39.18 %) of collar rot disease and best bio-control treatment for growth-promoting activity in *Gerbera* (21.26 cm root length and 40.21 cm plant height) among all the bio-control agents.

2.3.2 *In vitro* evaluation of different fungicides against *S. rolfsii*

The first report for the management of the southern blight of peanuts caused by *S. rolfsii* was done by application of PCNB fungicide by Cooper, (1956). The poisoned food technique was used for the *in vitro* evaluation of different fungicides against *S. rolfsii*. The best inhibition was shown by Vitavax, Ferbam, Ceresan wet and Brassicol which reported with no growth of the pathogen (Chauhan, 1978). There are several reports where fungicides have been used for the control of diseases caused by soil-borne pathogens (Seoud *et al*., 1982).

Tripathi and Khare (2006) observed complete inhibition of growth of *S. rolfsii* by iprobenfos, propineb, carbamates and ediphenphos at a concentration of 500 and 1000 ppm. Whereas, carbendazim showed complete inhibition of pathogen at 1000 ppm concentration.

Tooray *et al*., (2007) evaluated different fungicides (at conc. of 1000, 1500, and 2000 ppm) against *Sclerotium rolfsii* under the *in-vitro* condition and observed complete mycelial growth inhibition of *S. rolfsii* by thiram, captan, mancozeb, edifenphos and propineb at lowest concentration. Whereas, *in vivo* application of kavach, thiram and captan showed reduction in pre-emergence mortality of seedlings.

Johnson and Reddy (2008) evaluated the efficacy of different fungicides (Propiconazole, Mancozeb, Hexaconazole, Quinalphos and Chlorpyriphos) against *S. rolfsii*. Among different tested fungicides, Hexaconazole and Propiconazole showed cent percent inhibition against pathogen at a concentration of 1000, 15000, 2000 ppm and 500, 750, and 1000 ppm respectively.

Arunasri *et al*., (2011) reported that the Triazoles fungicide group (Propiconazole, Difenconazole and Hexaconazole) was found most effective fungicide group against mycelial inhibition of *S. rolfsii* under *in vitro* condition.

Bhuiyan *et al.,* (2012) screened six different fungicides (Bavistin, Dithane M-45, Ridomil, Tilt, Rovral 50 WP and Provax-200) at concentration of 100, 200 and 400 ppm respectively against radial colony growth of *S. rolfsii*. Results revealed that complete inhibition of the pathogen was observed with Provax-200 at all selected concentrations.

Nawar (2013) found that Rhizolex fungicide as the most effective fungicide with cent percent inhibition of growth and sclerotial formation of *S. rolfsii* under *in vitro* condition.

Begum *et al.,* (2014) evaluated the efficacy of eight different fungicides under *in vitro* conditions against *S. rolfsii*. The results revealed that 100 % inhibition of mycelia growth of the pathogen was observed in Carboxin, Difenconazole, Hexaconaole, Propiconazole, and Carbendazim at three different concentrations *viz*., 500, 1000, and 1500 ppm compared to untreated control.

Chaurasia *et al*., (2014) tested the efficacy of nine different fungicides *viz.*, Brassicol, Bavistin, Dithane M-45, Captan, Fytolan, Parasan, Manzate, and Sulfex against *S. rolfsii in vitro* by poisoned food technique. All the tested fungicides showed an adverse effect on the growth of *S. rolfsii* and Manzate fungicide gave cent percent inhibition the pathogen at 0.1 % concentration.

2.4 Antioxidant enzyme assays of chickpea plants derived from a seed treatment with potential *Trichoderma* mutants in combination with propiconazole

Being sessile in nature plants can not escape from their site of unfavorable environmental conditions and different biotic and abiotic stress incurred during different stages of growth and development. As per different circumstances, plants often face challenges of different adverse conditions such as, low and high temperatures, heavy metals, salinity, attack of insect and pest and UV rays etc. These stresses affect various physiological processes, growth, and development by inducing the occurrence of oxidative stress (Diaz *et al*., 2008 ; Hernandez *et al*., 2004). These abiotic stresses aids to a direct decrease in average yield loss of major crops by more than 50 % (Tuteja *et al*., 2010). These abiotic stresses, in turn, lead to the production of ROS (reactive oxygen species) which is far more than the ROS level at normal conditions. These species are generated due to the reduction of molecular oxygen (O2) that includes different free radicals such as superoxide (O^{2-}), alkoxyl (ROO), hydroxyl radicals (OH^{0}), and non-radicle products like singlet oxygen ($_1O^2$) and hydrogen peroxide (H2O2), etc (Gill *et al*., 2010 ; Sandalio *et al*., 2013). These free radicals are

produced continuously through chain reaction and being highly unstable they cause damage to the cell by disruption of cell membrane permeability, irreversible damage, and leading to necrosis and plant death at a later stage (Pitzschke and Hirt, 2006). To overcome such damage plants have different antioxidant systems that protect them from different biotic and abiotic stresses and helps to overcome from cytotoxic effects of free radicals. Major enzymatic antioxidants include ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), peroxidase (POX), guaiacol peroxidase (GOPX), dehydrogenase reductase (DHAR), and glutathione-S-transferase (GST) whereas non-enzymatic antioxidants include phenolic compounds, alpha tocopherols, alkaloids, and glutathione (GSH), etc. (Hasanuzzaman *et al.,* 2012).

Plants have self-regulated, an endogenous defense mechanism that can be induced in response to the attack of different plant pathogens. It is well known that defense genes are inducible genes and appropriate signals or stimuli are needed for their activation. Inducing such a mechanism of defense in plants by prior application of biological inducer is thought to be a novel technique of plant protection (Ramamoorthy *et al*., 2001). Thus, microbial inoculation by bio-priming of seed is a strategy to improve the plant's defensive capacity with increased resistance and tolerance to stress. Plant growth-promoting rhizobacteria (PGPR) and Plant growth-promoting fungi (PGPF) include many strain of *fluorescent* pseudomonads and *Trich-oderma* spp., respectively which have been used as potential bio-control agents. Due to rhizo-competent nature of these bio-agents allows it to colonize roots of a plant and stimulates the plant immune system (induces systemic resistance; ISR) and pre activates (bio-priming) defense mechanism in plants to ameliorates the host plants against various biotic and abiotic stresses (Siddiqui, 2006 ; Patricia *et al*., 2009 ; Pieterse *et al*., 2014 ; Martinez Medina *et al.,* 2017).

Trichoderma induced bio-priming based signaling involves a systemic resistant type immune response (Tucci *et al*., 2011 ; Yoshioka *et al*., 2012), interconnected in a complex cross-talk network of cross- communicating hormone pathways involving the Jasmonic acid or Salicylic acid-dependent signaling mechanism. It is well documented that the *Trichoderma* based early signaling of interaction with the host plant, is

mediated through the pattern recognition receptors (PRRs) that activate the MAMPs-triggered immunity (MTI) (Hermosa *et al*., 2013 ; Ruoco *et al*., 2015). Along with it, there are several reports of *Trichoderma* induced bio-priming is characterized by the secretion of a wide range of antimicrobial compounds through the participation of phenylpropanoid pathways that delimits the infection and dissemination of phytopathogens along with increased tolerance against various abiotic stresses (Mastouri *et al*., 2012; Ahmad *et al*., 2015). *Trichoderma* based microbial inoculation leads to the activation of an efficient reactive oxygen species (ROS) detoxification system (De Palma *et al*., 2019). Further, it is believed that mycoparasitic colonization by *Trichoderma* leads to the induction and accumulation of PR proteins at the early stages of root colonization (Yedida *et al*., 2000).

Root colonization of *P. fluorescens* of the saprophytic fluorescent pseudomonads expressed increased levels of catalase and SOD. SOD activity was induced by *P. fluorescens* against the inoculated nematode in rice plants. Expression two isoforms of SOD might be implicated in induced defense responses against nematode invasion (Katswon and Anderson, 1990).

Anita and Samiyappan (2012) observed increased activity of phenol, peroxidase, phenylammonia lyase, and chitinase in rice plants bacterized with *Pseudomonas fluorescens* isolate Pf-1 against challenge inoculation of *Meloidogyne graminicola*. The isoform profiling analysis revealed the induction of unique isoforms of superoxide dismutase (SOD), peroxidase (PO), and polyphenol oxidase (PPO) in plants treated with *P. fluorescens*.

Singh *et al*., (2016)[a] reported that seed bio-priming of tomato seeds with *Trichoderma asperellum* BHUT8 triggered various defense like responses such as high phenylpropanoid activity and lignifications in bio-primed seedlings compared to the non-primed seedlings demonstrating possible use of BHUT8 against phytopathogens.

Aamir *et al*. (2018) studied *Trichoderma erinaceum* bio-priming based antioxidant activity in tomato plants against the *Fusarium oxysporum* f. sp. *lycopersici* (FoI) challenged condition and observed increased superoxide mutase

and catalase activity with the highest upregulation of SOD and *SIGPX1* in FoI + *T. erinaceum* treatments. Changes in expression were accompanied by 32.06 % lesser H2O2 production in samples seed bio-primed with *T. erinaceum*.

Tomato plants bio-primed with *Trichoderma* strain BHUR2 were found healthier as compared to other bio-primed plants upon challenged with *S. rolfsii*. Tomato plants bio primed with *Trichoderma* strain BHUR2 augmented anti oxidant enzym e activity including peroxidase (Pox), superoxide dismutase (SOD), phenylalanine ammonia-lyase (PAL), and total phenol content (TPC) in response to a defense mechanism against *S. rolfsii* (Rajput *et al*., 2019)

CHAPTER-III

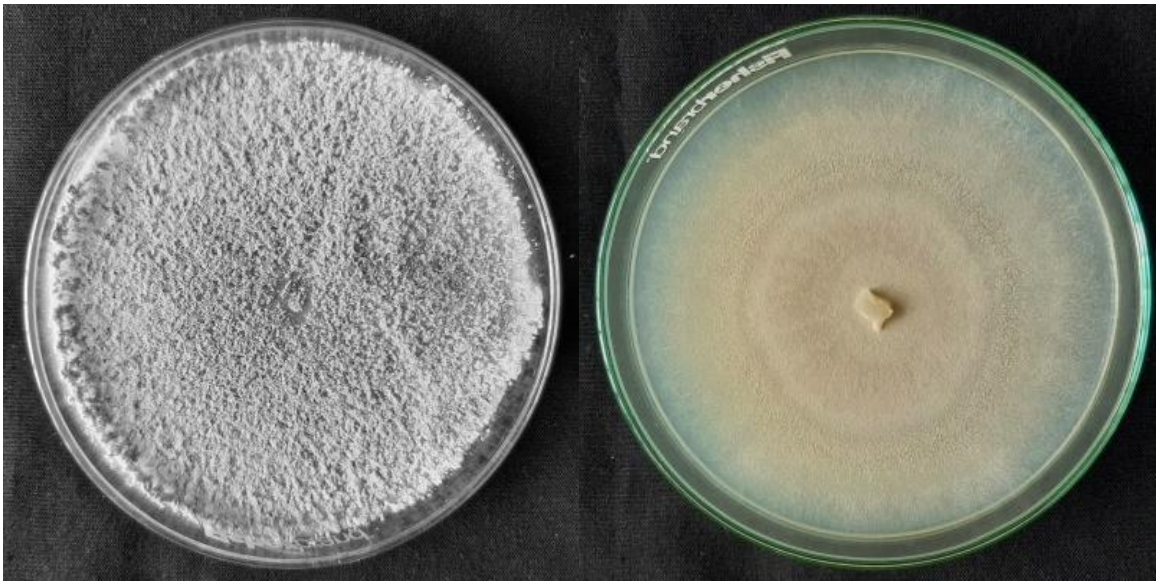
MATERIALS AND METHODS

The present study titled **"Evaluation of seed dressing formulations of potential *Trichoderma* mutants on growth and yield attributing characters of cereals, pulses and vegetables "** carried out in the laboratory of Dept. of Plant Pathology and Molecular Plant Pathology Lab at the Dept. of PMBB, CoA, Indira Gandhi Agricultural University Raipur. Cultures of potential mutants obtained from laboratory of Bhabha Atomic Research Centre (BARC), Trombay, Mumbai with random irradiation treatment of *Trichoderma viride* (T-14) and *Trichoderma virens*. During the experiment, the culture of potential mutants of *Trichoderma viride* (T-14) and BARC mutants were maintained on PDA (Potato Dextrose Agar) slants for further use. Different glass wares used in the study were washed and sterilized using hot air oven for duration of 2 hours at 180 degree Celsius prior to use. Other metallic instruments, forceps, inoculation needles and cork borer etc. used during experiment were sterilized by heating using burner until red hot flame can be seen and immersed in alcohol during isolation, purification, multiplication and other studies. All growth media which was used were sterilized by autoclaving at the temperature of 121.6 degree Celsius at 15 lbs for 20 minutes. In this chapter materials used and adopted methodologies for the study is described.

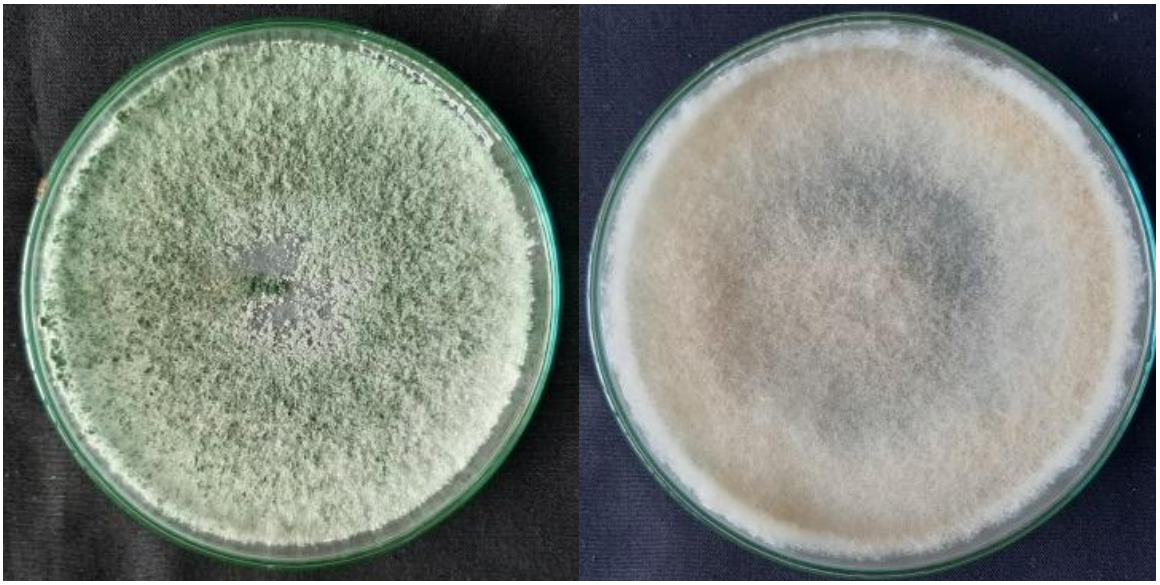
3.1 Materials

3.1.1 Culture of potential mutants of *Trichoderma viride* (T-14) and *Trichoderma virens*

The experimental materials consisted of single spore isolated potential mutants isolates derived from *Trichoderma viride* (T-14) and *Trichoderma virens*. The potential mutant cultures of *Trichoderma virens* and *Trichoderma viride* was provided by Dr. Anil S. Kotasthane, Professor and Head Department of Plant Pathology, CoA, IGKV, Raipur. These potential mutants were used to study the effect of bio-priming with different concentrations of seed dressing formulations, evaluation on growth and yield attributing characters of different crops and vegetables, evaluation against *Sclerotium rolfsii* in rice-chickpea cropping system

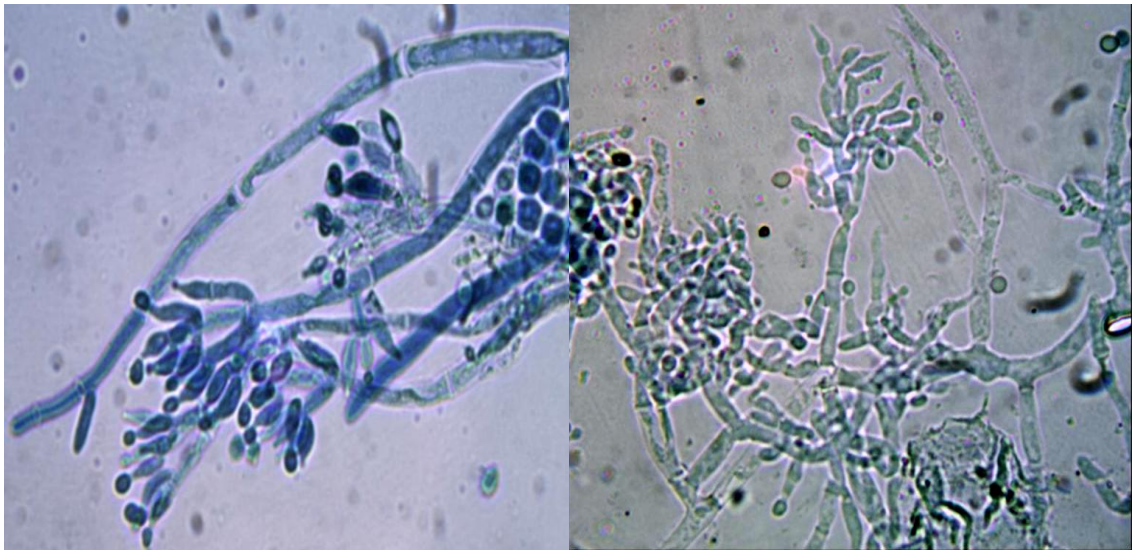


M-18 M-23

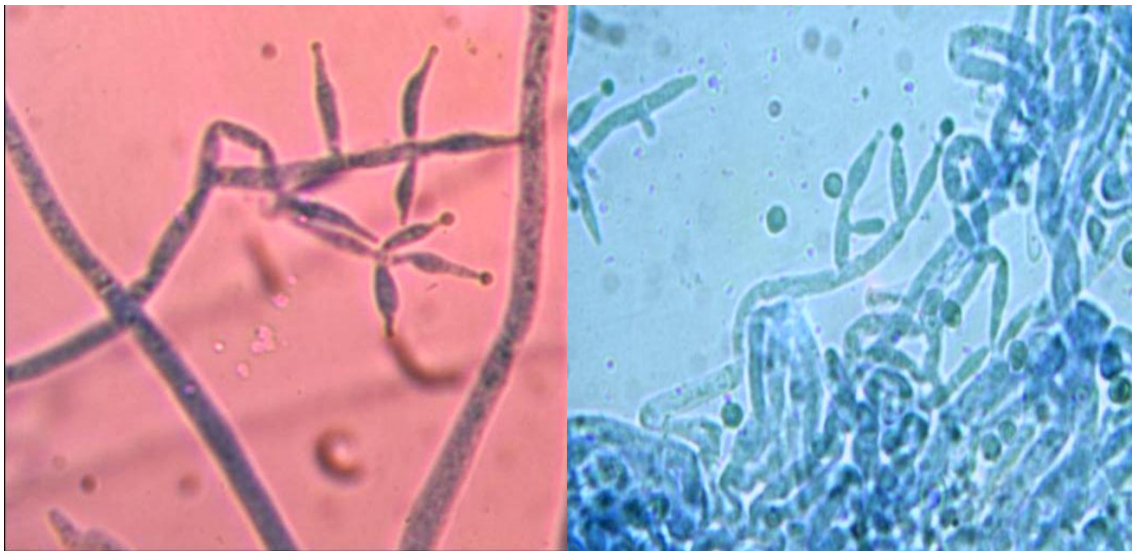


M-136 BARC

Plate 1 Cultural morphology of different potential *Trichoderma* mutants



BARC **M-18**



M-23 **M-136**

Plate 2 Microscopic view of phialides structure of potential *Trichoderma* mutants

and anti-oxidant enzyme assays of chickpea and wheat plants derived from seed treatment with potential mutants with propiconazole.

3.1.2 Plant Materials

For field experiment to study the growth promoting activity and yield attributing characters the seeds of different crops were used, namely INDIRA CHANA-1 (Foundation Seed) (Chickpea), RATAN (Foundation Seed) (Wheat) and PEARL (F-1 Hybrid) (Bitter gourd), (Pumpkin), (Long bean), (Ridge gourd), (Tomato), (Spinach), (Fenugreek) which were tenderly provided by Dr. Anil S. Kotasthane, Professor and Head, Department of Plant Pathology, Coa, IGKV, Raipur.

3.2 Methods

3.2.1. Studies on seed bio-priming with different concentrations of seed dressing formulation of potential *Trichoderma* mutants

3.2.1.1 Mass multiplication of potential mutants for seed bio priming

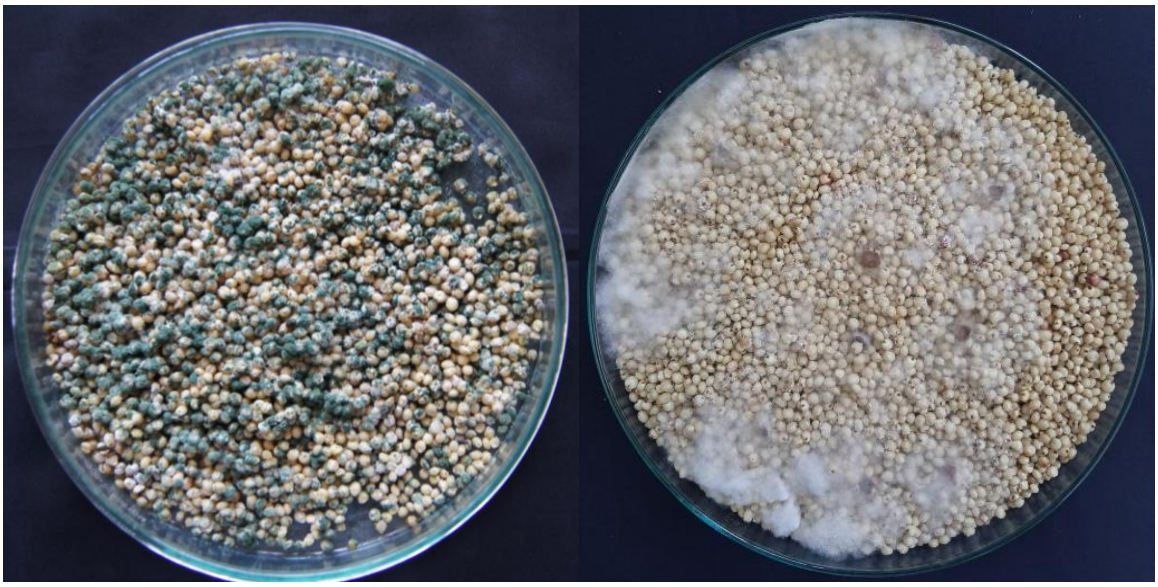
The substrate sorghum grains were used in present study for the mass multiplication of potential mutants. Overnight soaked sorghum grains were transferred to 250 ml conical flasks and autoclaved twice at 121 degree celsius for 30 minutes. The flasks were allowed to cooled down at room temperature and later inoculated with 7 days old cultures of potential *Trichoderma* mutants with help of cork borer. After inoculation, plates were incubated at 28±2 degree celsius for 7 days. After 7 days of growth over sorghum substrate, the colonized substrate were dried at 50 degree celsius for 24 hours and ground to powder using laboratory mixer grinder. Further, powdered formulations were kept in room temperature.

3.2.1.2 Preparation of spore suspension

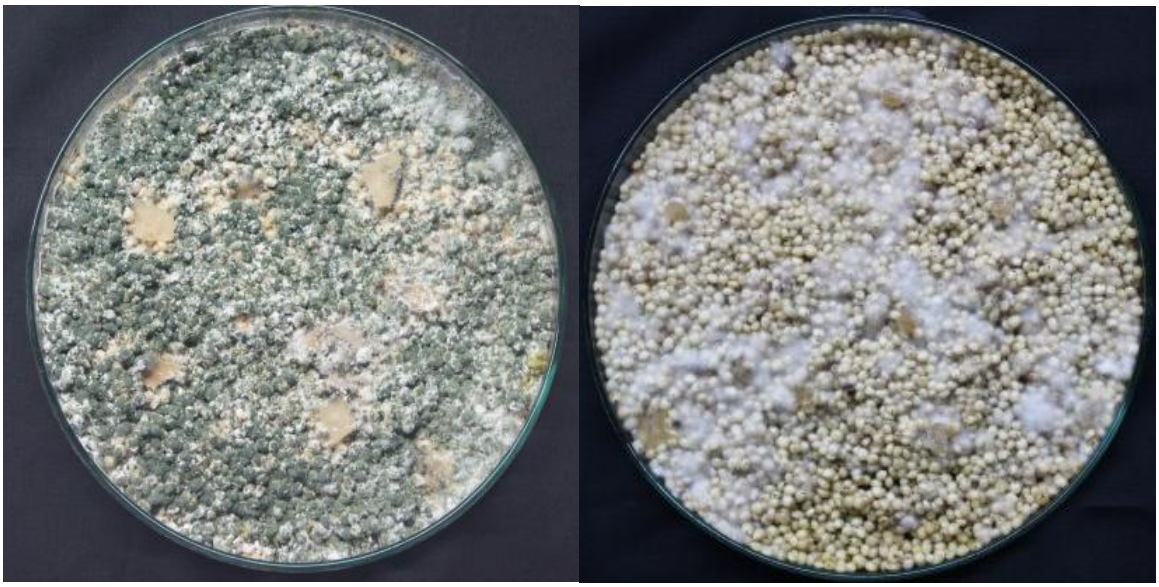
Spore suspension of potential mutants were prepared by dissolving different doses of potential mutants *viz*., (1 gm/kg, 2gm/kg, 3gm/kg, 4gm/kg, 5gm/kg) seed in 50 ml of SDW (Sterilized Distilled Water) per potential mutants per concentration.

3.2.1.3 Seed bio-priming of different crops

Prior to bio priming seeds of rice and mungbean were surface sterilized with 5 % NaOCl for 5 minutes. Spraying of spore suspension of different potential mutants were done using atomizer and bio-primed seeds were incubated



M-18 M-23



M-136 BARC

Plate 3 Mass multiplication of potential *Trichoderma* mutants on Sorghum grains

at 28±2 ℃ for 5 days in petri plates. 5 days after growth of plumule length, radicle length and seedling length was measured in 10 randomly selected germinating seedlings in petri plates treated with spore suspensions of different potential *Trichoderma* mutants in treated as well as control plates. To study root characteristics, bio-primed seeds were sown in pots after 24 hours of colonization of potential mutants on seed surface which was confirmed with help of stereo binocular microscope over bio-primed seeds. Seedling were harvested 10 days after sowing roots were harvested from soil and observations were recorded for three randomly selected plants with different root characters using root scanner root scanner EPSON Perfection V&00/V750 3.81 version and WinRhizo Reg 2009 (Lobet *et al*., 2013)

3.2.1.4. Study of Root Characteristics

As roots are responsible for uptake of nutrients from soil and water and thought to plays a major role in yield establishment of the crop. Therefore, detailed data sets containing rot placement, root architecture and soil resource dynamics are required to improve our better understanding of resources capture by roots of a plant. Plants treated with potential mutants were used for root scanning study which gives the detailed information of different parameters of the roots. For root scanning, root scanner EPSON Perfection V&00/V750 3.81 version and WinRhizo Reg 2009 (Lobet *et al*., 2013) software was used. The data was recorded automatically in the computer for different parameters of root including average root diameter, root volume, forks, tips and surface area etc.

Following procedure was used for the scanning of roots:-

A) Acquiring Washed Roots

Roots of bio-primed plants were washed two times in running tap water in order to remove soil particles completely and expose root surface properly. The roots were preserved in 25 % spirit in Tarson tubes for root scanning. Each and every step of procedure was done very carefully to avoid any kind of damage to supplementary roots.

B) Preparing Roots for scanning

Preserved roots were transferred to acrylic trays containing water. This allow the roots to float and to be arranged to reduce over-lap and crossing of roots. Plastic forceps were used as tools.

C) Root scanning

For better results, WinRhizo with an approved scanner is used, which allows the roots to be lit from above and below while being scanned. The important feature (known as "Dual Scan" in reagents documentation), reduces shadows on the root image. Positioning system allows the trays to be placed consistently. Optimum resolution for scanning depend on the type of samples used. Lower resolution can speed up scanning significantly, especially if the samples require the use of large trays. Analysis of root length are carried out with grayscale images; saving images in grayscale reduces the image file size substantially.

D) Threshold Parameters

Analysis results can be sensitive to the threshold parameters used.WinRhizo can automatically set these, but one can also naturally tweak them from time to time.

E) Analyzing Scanned images

For analysis of image, region was selected and analyzed. When scanned images are analyzed, the software uses thresh holding to determine what is root and what is not root (each pixel is classified as either root or not root based on its grayscale value; this is why shadows in images are little bit problematic). Portions of the image can be excluded from analysis if necessary, and there are basic editing tools if minor image editing is required.

3.2.2 Field evaluation of potential *Trichoderma* mutants on growth and yield attributing characters of different crops and vegetables following seed dressing

3.2.2.1Plant growth promoting response in chickpea (INDIRA CHANA-1) following seed bio-priming with potential *Tricho-derma* mutants and BARC mutant

Field experiments was conducted at Research cum instructional Farm, College of Agriculture, I. G. K. V., Raipur and KVK Kawardha, to study the efficacy of potential *Trichoderma* mutants on growth and yield attributing characters following seed bio-priming in chickpea (INDIRA CHANA-1).

Seed bio-priming was done by spraying potential BARC mutant @ 10 gm/kg of seed dose on seeds and talcum powder was sprinkled so that fungal culture could coat the seeds completely and shade dried. Seed bio-priming was done prior to sowing and seeds were incubated for 24 hours. Control and treated plot was maintained separately in Split Plot Design. Observation such as plant height, no. of primary branches, total no. of plants (per sq. metre), no. of pods, no. of nodules, no. of leaves, 100 seed weight, grain yield, straw yield and biological yield etc. were recorded from all three replication (each replication with 15 plots)

3.2.2.2 Plant growth promoting and mortality response in chickpea (INDIRA CHANA-1), following seed treatment with BARC mutants, propiconazole and its combination (BARC mutant + Propiconazole)

Field experiments was conducted at Research cum instructional Farm, College of Agriculture, I. G. K. V., Raipur, to study the efficacy of BARC mutant, propiconazole and its combination (BARC mutant + Propiconazole)**.** On plant growth promotion and mortality response following seed treatment in chickpea (INDIRA CHANA-1). Seed treatment was done by spraying BARC mutant, propiconazole and its combination on seeds and talcum powder was sprinkled so that fungal culture could coat the seeds completely and shade dried. Seed treatment was done prior to sowing and seeds were incubated for 24 hours. Control and treated plot was maintained separately into three replications in RBD design. Observation such as shoot height, no. of branches, no. of pods, 100 seed weight, bundle weight, grain yield, straw yield and biological yield etc were recorded.

Treatment Details:

Treatment	Treatment Details
T_1	Tricho BARC @ 5 gm/kg of seed
T_2	Tricho BARC @ 10 gm/kg of seed
T_3	Propiconazole @ 3.0 ml/kg of seed
T_4	Propiconazole @ 1.5 ml/kg of seed
T_5	Tricho BARC 10gm/kg seed + Propiconazole 3.0ml/kg seed
T_6	Tricho BARC 10 gm/kg seed+Propiconazole 1.5 ml/kg seed
T_7	Untreated/Control

3.2.2.3 Evaluation of plant growth promoting activity of potential *Trichoderma* mutants on different vegetables

It is well known that *Trichoderma* spp. influence plant growth activity directly by root colonization of crop by release of different growth hormones, secondary metabolites and enzymes. On basis of previous record of potential *Trichoderma* mutants of production of Indole-3-acetic acid, potential mutants were taken for pot experiment along with control.

3.2.2.3.1Seed bio-priming with of different vegetables with potential *Trichoderma* mutants

Prior to bio priming seeds of vegetable crop *viz*., Bittergourd, Pumpkin, Longbean, Ridgegourd, Fenugreek, Tomato and Spinch seeds were first surface sterilized with 5 % NaOCl for 5 minutes. Spraying of spore suspension @ 5g/kg of seed concentration of different potential mutants were done using atomizer and bio-primed seeds were incubated at 28±2 ℃ for 24 hours.

3.2.2.3.2 Pot experiment

After seed incubation for 24 hours sowing of seed was done in pots having soil, vermin compost (@ 10 % of soil) and DAP (@ 10 % of vermi compost). Every treatment was replicated twice. In Bittergourd, Pumpkin, Longben and Ridgegourd 5 seeds were sown after seed bio-priming in two replication and after thinning only one plant was kept with vigorous growth in control and treated plot. In Fenugreek and Spinach, 30 seeds were sown and after thinning only 5 plants were kept which showed uniform and vigorous growth in control as well as treated pot where as, in case of Tomato only 6 seeds were sown after seed bio-priming which after thinning only 5 plants were kept for observation in control and treated pots with three replications. After 20 and 50 days of sowing following observations were recorded- shoot length (cm), no. of leaves, leaf area (cm^2), no. of flowers and root length (cm) in Bittergourd, Pumpkin, Ridgegourd, Longbean and after 25 and 50 days after sowing Plant height (cm), no. of primary branches, no. of pods, pod weight (gm) and no. of flowers per cluster, root length (cm), root fresh weight (gm) were recorded in Fenugreek and Tomato respectively. Where as, plant height (cm), no. of leaves per plant and root length (cm) were recorded in Spinach 15 days after sowing.

3.2.3 Evaluation of *Trichoderma* mutants *against Sclerotium rolfsii* a major soil borne pathogen in rice-chickpea cropping system

In vitro evaluation of potential *Trichoderma* mutants were done for their antagonistic effect against *Sclerotium rolfsii* by Dual culture method.

3.2.3.1 Dual Culture Technique (Dennis and Webster, 1971)

Potential *Trichoderma* mutants and the pathogen were grown on PDA (Potato Dextrose Agar) medium each separately and by using 7 days old cultures, 5 mm diameter disc of the potential mutants and pathogen were taken into consideration. The Petri plates (90 mm) were inoculated aseptically with *S. rolfsii* and potential mutants, by placing 5 mm diameter culture blocks at 70 mm apart from each other. Three repetitions of each treatment were kept and the petri plates with only pathogen served as control. Afterward, the plates were incubated at temperature (28±2°C) and the radial growth of the test organism and pathogen was measured after 7 days of incubation. The per cent growth inhibition (PGI) was worked out by using the formula given by Vincent (1947).

$$PGI = \frac{DC-DT}{DC} \times 100$$

Where,

PGI= Per cent growth inhibition

DC = Average diameter of mycelial colony of control set

DT = Average diameter of mycelia colony of treated set

3.2.3.2 In *vitro* studies on bio-efficacy of fungicides against *S. rolfsii*

The poisoned food technique was employed to test the *in vitro* efficacy of seven fungicides with their three different concentrations against *S. rolfsii*. The desired quantity of test fungicides was diluted with autoclaved PDA (Potato Dextrose Agar) medium in conical flasks. The flask containing fungicidal medium was shaken well to facilitate uniform mixture and 20 ml well mixed PDA with each treatment was pored sterile Petri plate. The inoculums disc of 5mm diameter was cut with the help of sterile cork borer from 7 days old pure culture and placed at the centre on Petri plate containing solidified fungicidal medium. Three replications of each treatment were kept for observation. Poured plate without fungicide served as control. The inoculated plates were incubated at (28±2°C) temperature. The colony

diameter of the fungus was recorded from three repetitions periodically. The per cent growth inhibition over control was calculated by using formula given by Vincent,1947.

3.2.4 Antioxidant enzyme assay

3.2.4.1 Determination of antioxidant activities

Different enzymes were assayed in leaves of chickpea and wheat. Seeds treated with different strains of fluorescent *Psuedomonas* during crop maturity stage. For preparation of leaf sample, fresh green flag leaves of chickpea and wheat was collected from the field and taken to laboratory in fresh form by keeping the samples in ice box. Leaf samples were frozen in liquid nitrogen in laboratory and grinded to fine powder using fresh pestle and motor and powder was collected in 50 ml tarson tube and stored at -20^{o}C for further analysis.

3.2.4.1.1 Super oxide dismutase activity (SOD) (E.C.1.15.1.1)

SOD activity was measured by the method described by Fridovich., 1974 using riboflavin/methionine system.

Principle

Superoxide radical reduces the NBT resulting in the formation of blue color formazan. NBT method for SOD assay is based on principle that NBT undergo photoreduction (which is a blue coloured formazan) on exposure to light by superoxide radicals. It competes with enzyme SOD for superoxide anions. In presence of SOD in reaction mixture, NBT will produce less amount of coloured complex than control (therefore less OD than reference or control without enzyme).

Solution

1. 0.1M Phosphate buffer containing 0.5 mM E.D.T.A
2. 100mM Phosphate buffer
3. 200 mM methionine
4. 2.25 mM NBT
5. 1.5 M Sodium carbonate
6. 2µM riboflavin

NOTE: Mix 2,3,4,5 solutions in a beaker to form reaction mixture

Procedure

1. 0.1 Gram of leaf sample was homogenized with pre-chilled 0.1M Phosphate buffer containing 0.5 mM E.D.T.A and vortex for 2-3 min
2. The homogenate was centrifuged at 15000 rpm @ 15 min at $4^{o}C$
3. Pipette out 200 µl supernatant (enzyme extract) in a test tube (duplicates)
4. Add 2ml reaction mixture to enzyme extract and make final volume to 3 ml by adding 0.8ml of ADW
5. Now the reaction was started by adding 400 µl of riboflavin to each test and placing the test tubes under two 18 W fluorescent lamps for 15 min
6. After 15 min reaction was terminated by switching off light, and tubes were kept in dark
7. The observations recorded at 560 nm on a UV-vis spectrophotometer

Control: Complete reaction mixture without enzyme extract + light act as control

Blank: complete reaction mixture without enzyme extract – light act as blank.

Calculation:

% inhibition of NBT reduction by SOD is equal to (reference control OD- treatment OD/ reference control) x 100

50% inhibition is equal to 1 unit of enzyme.

Final formula for SOD units /g fresh weight of sample will be

$$\text{SOD Units /g FW} = \frac{C-T}{T} \times 100 \times \frac{1}{50} \times \frac{\text{System volume}}{\text{Enzyme extract}} \times DF \times \frac{1}{0.1}$$

Where C = reference control OD

T = treatment OD

System volume = 3 ml + 0.4 ml = 3400 µl

Enzyme extract volume = 200 µl

DF= dilution factor = 3000/200 = 15

0.1 = starting material weight 0.1 g

3.2.4.1.2 Measurement of peroxidase (POx) activity (E.C.1.11.1.7)

Peroxidase (POx) activity was measured spectrophotometrically as described by Hammerschmidt *et al*., 1982 with pyrogallol as hydrogen donor.

Principle

Peroxidase also referred as non-specific peroxidase catalyses the reduction of hydrogen peroxide with a concurrent oxidation of pyrogallol to a colored purpurgallin. The increase in absorbance is recorded at 420 nm.

H_2O_2 + Pyrogallol(donor) $\xrightarrow{\text{peroxidase}}$

$2H_2O$ + purpurgallin(oxidized donor)

Solutions

0.05M Pyrogallol

0.1 M Phosphate buffer (pH7)

1% H_2O

Procedure

1. To 0.1 gram of powder leaf sample adds 5 ml ice cold 0.1 M Phosphate buffer (pH7) in a test tube prepared in duplicates/replicates.
2. Homogenate was vortex for 2-3 min for easy dispersion of leaf sample in buffer
3. The Homogenate was centrifuged @ 16000 rpm at 4^{o}C for 15 min and supernatant was used as crude enzyme source.
4. Take 50 µl of supernatant in a test tube (replicates) and add 1.5 ml of 0.05M Pyrogallol
5. Just before taking the observation at spectrophotometer add 0.5 ml of 1% H_2O_2
6. The observation at 420 nm was recorded at 30 s intervals for 3 min

Blank: A sample blank contains the 0.1 M Phosphate buffer, 0.05M Pyrogallol and 1 % H_2O_2 without enzyme extracts.

Calculations

Peroxidase Activity (U/L)

$$= \frac{\Delta \text{Abs} \times 100 \times \text{System volume} \times 1}{\Delta\, t \times \epsilon \times L \text{ Enzyme extract} \times 0.1}$$

Where

ΔAbs = change in absorbance = Absorbance at 3 min-Absorbance at 0 min

Δt = change in time (in min, here 3 min)

L = path length or cuvette diameter (=1 cm)

€ = molar extinction coefficient of substrate in units of $M^{-1}cm^{-1}$ (here for pyrogallol its 12 $M^{-1}cm^{-1}$

Total assay volume (= 1.5 ml + 50 ul + 0.5 ml = 2050 ul)

Enzyme extract volume (= 50 ul)

3.2.4.1.3 Estimation of Phenylalanine ammonia-lyases (PAL) (E.C.4.1.3.5)

PAL activity was measured in terms of amounts of t-cinnamic acid (t-CA) formed according to the method of Brueske., 1980.

Principle

Phenylalanine ammonia lyase catalyzes the conversion of phenylalanine to trans-cinnamic acid and ammonia, as a step in the phenylpropanoid pathway of plants and is therefore involved in the biosynthesis of the polyphenol compounds such as flavonoids, phenolpropanoids and lignin in plants.

L-Phenylalanine $\longleftrightarrow$ trans-cinnamic acid $+NH_3$

Solutions

0.1M Phosphate buffer containing 1.4 mM mercaptoethanol

0.1 mM L-Phenylalnine (pH8.7)

1M TCA

Procedure

1. 1 gram of leaf sample was homogenized in 2ml of ice cold 0.1 M Phosphate buffer having 1.4mM mercaptoethanol and votex.
2. Centrifuge at 16000 rpm at 4^oC for 15min
3. Pipet out 200 µl of supernatant (enzyme extract) into test tube (replicates)
4. Add 500 µl of 0.1 M phosphate buffer and 1.3 ml of ADW and mix it.
5. To initiate reaction add 500 µl of 0.1mM L-Phenylalanine in each test tube and incubate at 32^oC for 30min
6. The reaction was stopped by adding 500 µl of 1M TCA
7. Record the observation at 290 nm

Blank: A sample blank containing the 0.1 M Phosphate buffer, 0.1 mM L-Phenylalnine and 1 M TCA without enzyme extracts.

Calculations

The trans cinnamic acid content can be calculated either from a standard curve of trans cinnamic acid or based on the extinction coefficient of trans cinnamic acid. The trans cinnamic acid content is represented as mmole/L/g fresh weight.

Formula:

As per Beer's Lambert law

$$\mathbf{A = \varepsilon\, l\, c \text{ i.e. } c = A/\varepsilon\, l}$$

Where A = Absorbance of solution at a particular wavelength

ε = Molar Absorptive or molar extinction coefficient of Trans cinnamic acid = 9630 $M^{-1}cm^{-1}$;

l = Length of Solution Cell = here 1 cm;

c = Concentration of Solution (mmol/L).

To covert c to mmole/L/g; divide by 0.1 (since starting material is 0.1 g leaf powder). Therefore formula becomes

$$C = \frac{A}{\varepsilon \times l \times 0.1} \quad \textbf{mmole/L/g}$$

Same calculation can also be done using web browser

https://www.instanano.com/2017/01/Concentration-Calculation-UV-Vis-Absorbance.html

3.2.4.1.4 Polyphenol Oxidase (PPO) (E.C.1.14.18.1)

PPO activity was evaluated as described by Gauillard *et al*., 1973 with catechol as substrate for PPO.

Principle

Enzymatic oxidation of catechol by PPO/O_2 transformed the substrate into yellow product with a maximum absorbance at 495 nm. These enzymes are released by broken cells and they catalyse the reaction between colorless molecules called polyphenols and molecular oxygen this reaction creates colored compounds and these new compounds can spontaneously cross react with one another to form black-brown complexes called melanin's.

Catechol + oxygen $\longrightarrow$ polyphenioxidase

Benzoquinone + water $\longrightarrow$ melanin

Solutions

0.1M Phosphate buffer containing 0.01M catechol (reaction mixture) (pH6.5)

Procedure

1. 0.1 gram of leaf sample was homogenized in 5ml of 0.1M Phosphate buffer
2. Homogenate was centrifuged at 16000 rpm for 30 min @ 4^oC
3. The reaction mixture contained 0.01 M catechol (0.4 ml) in 0.1 M sodium phosphate buffer (3.0 ml; pH 6.5).
4. At the time of taking observation 400 µl of enzyme extract was added to reaction mixture and reading was taken.
5. The observation at 495 nm was recorded at 30 sec interval up to 3 min

Blank: A sample blank containing the 0.1M Phosphate buffer containing 0.01M catechol (reaction mixture) without enzyme extracts.

Calculation

Polyphenol oxidase activity (U/L) = change in OD per minute per gram Fresh weight

$$= \frac{\Delta Abs \times 100 \times \text{System volume} \times 1}{\Delta t \times € \times L \text{ Enzyme extract} \times 0.1}$$

Where

ΔAbs = change in absorbance = Absorbance at 3 min-Absorbance at 0 min

Δt = change in time (in min, here 3 min)

L = path length or cuvette diameter (=1 cm)

€ = molar extinction coefficient of substrate in units of $M^{-1}cm^{-1}$ (here for catechol its 3450 $M^{-1}cm^{-1}$

Total assay volume (0.4 ml + 3.0 ml + 400 µl = 3800 ul)

Enzyme extract volume (= 400 ul)

3.2.4.1.5 Measurement of lipid peroxidation

Lipid peroxidation is oxidative degradation of lipid-fatty acids by reactive oxygen species and hence it is considered as one of the measure of oxidative stress in the cells. The method for lipid peroxidation estimation described by Ohkawa *et al.*, 1979 is given as follows.

Principle

Oxidative degradation of lipid-fatty acids by reactive oxygen species increases the concentration of lipid hydroperoxides and aldehydes in the cells. These lipid hydroperoxides and aldehydes reacts with 2-thiobarbituric acid (TBA) hence called as Thiobarbituric acid reactive substances (TBARS). The TBARS content is measured in terms of malondialdehyde (MDA) which results from decomposition of the unstable peroxides of polyunsaturated fatty acids. MDA reacts with (TBA) resulting in the formation of a red colored complex with absorbance maxima at 532 nm (www.isca.in).

Solutions

- 20 % Trichloroacetic acid (TCA)
- Thiobarbituric acid reagent: 1% TBA in 20% TCA

Procedure

1. To 0.1 gram of powder leaf sample adds 4.0 ml of 20% TCA containing 1% TBA in a test tube prepared in duplicates/replicates.
2. Heat the mixture for 30 min at 95° C in a water bath and immediately cool in an ice bath to stop the reaction.
3. Centrifuge the cool samples at 10 000 g for 15 min.
4. Record the absorbance of the clear supernatant at 532 nm

Blank: A sample blank containing the 20% TCA containing 1% TBA without enzyme extracts.

Calculation: The MDA content can be calculated either from a standard curve of MDA (0- 20µM range) or based on the extinction coefficient of MDA 1.56 x 10^5 M^{-1} cm-1 or 156000 $M^{-1}cm^{-1}$. For MDA standard, replace sample with the dilutions of MDA. The MDA content is represented as nmols per g dry or fresh weight.

Formula:

As per Beer,s Lambert Law

$A = \varepsilon\ l\ c$ i.e. $c = A/\varepsilon\ l$

Where A = Absorbance of solution at a particular wavelength;

ε = Molar Absorptivity or molar extinction coefficient of MDA = 156000 $M^{-1}cm^{-1}$;

l = Length of Solution Cell = here 1 cm;

c = Concentration of Solution (mmol/L).

To covert c to nmole/L/g; multiply by 10^6 and divide by 0.1 (since starting material is 0.1 g leaf powder). Therefore formula becomes

$$C = \frac{A \times 10^6}{\varepsilon \times l \times 0.1} \quad \textbf{nmole/L/g}$$

Same calculation can also be done using web browser

https://www.instanano.com/2017/01/Concentration-Calculation-UV-Vis-Absorbance.html

3.2.4.1.6 Total Phenolic content (TPC)

TPC was determined as Zheng and Shetty., 2000.

Solutions

95 % ethanol

1N Folin-ciocalteau reagent

5% Na_2Co_3

Procedure

1. 0.1 gram of leaf sample was placed in 5 ml of 95% ethanol and kept at 0^oC for 48 hrs for maximum extraction
2. Each sample was then homogenized and centrifuge at 13000 rpm for 10 min
3. Pipet out 1 ml of supernatant and mix it with 1 ml of 95 % ethanol and 5 ml ADW
4. To this added 0.5 ml of 1 N Folin-ciocalteau reagent was added
5. After 5 min added 1 ml of 5% Na_2Co_3 and reaction mixture was allowed to stand for 60 min and the absorbance at 725 nm was recorded

Calibration curve was prepared for using various concentration of gallic acid (GA) in 95 % ethanol. Absorbance value was converted to µg or mM gallic acid equivalent (GAE) g/FW.

Blank: A sample blank contained 95 % ethanol, 1N Folin-ciocalteau reagent and 5% Na_2Co_3 without enzyme extracts.

CHAPTER-IV
RESULT AND DISCUSSION

Trichoderma is a most widely used plant endophyte in agriculture worldwide (Contreras-Cornejo *et al*., 2009; Vishnevetsky *et al*., 2010; Lopes *et al*., 2012; Mastouri *et al*., 2012; Reithner *et al*., 2014; Zhang *et al*., 2014; Lamdan *et al*., 2015) owing to its improve nutrient use efficiency, mycoparasite, and plant growth-promoting ability, ability to produce a diverse array of secondary metabolites, systemic induce resistance in plants against invading pests and pathogens and to impart tolerance to abiotic stresses (Lorito *et al*., 2010; Harman *et al*., 2004; Salas-Marina *et al*., 2015; Chagas *et al*., 2017). The increase of plant lateral roots, thus modifying root architecture is one of the most evident morphologic changes triggered by *Trichoderma (Contreras-Cornejo et al*., 2015). *Trichoderma*–plant interaction not only modulates the levels of the hormones (produced by the plant or *Trichoderma*) or *Trichoderma* could provide intermediates for the synthesis of some phytohormones, as a part of the benefits reported in (Guzman-Guzman *et al*., 2019). Environmental factors influence the efficacy of *Trichoderma* resulting in inconsistent performance under field conditions as compared to their chemical counterparts and poses a major constraint for its adoption amongst the farmers (Singh, 2014). Several lines of earlier evidence indicate the development of novel strains of *Trichoderma* using mutagenesis (Papavizas *et al*., 1982; Ahmad and Baker, 1988; Mukherjee *et al*., 1999 ; Olejnikova *et al*., 2010). To best of our knowledge, Mukharjee *et al*., 2019 had reported extensive field evaluation (replicated micro-plot trials, on-farm demonstration trials, and large-scale trials in farmer's fields) of mutant-based formulation (named TrichoBARC) for management of collar rot (*S. rolfsii*) in chickpea and lentil (*Lens culinaris*) over multiple locations in India. It was reported that the TrichoBARC formulation consistently, over multiple locations and years, improved seed germination, reduced seedling mortality, and improved plant growth and yield. Growth promotion, improved pod bearing, and early flowering (7–10 days) in Tricho BARC treated chickpea and lentil plants under field conditions was observed. Among the formulation that are commercially

Available and are successful is one based on a protoplast fusion strain of *T. afr oharzianum* (Shoresh *et al*., 2010 ; Chaverri *et al*., 2015; Harman and Uphoff, 2019). Nevertheless looking to the encouraging field evaluation reports of TrichoBARC formulation present investigation entitled "Evaluation of seed dressing formulations of potential *Trichoderma* mutants on growth and yield attributing characters of cereals, pulses, and vegetables". We carried out seed bio-priming with different concentrations of seed dressing formulation of four potential *Trichoderma* mutants with a logic to reduce the amount of recommended doses of seed dressing formulation. Seed dressing formulations of four potential *Trichoderma* mutants were evaluated under field conditions on growth and yield attributing characters of different crops and vegetables. Effect of seed bio-priming with potential *Trichoderma* mutants in chickpea and wheat leaves contents of superoxide dismutase (SOD), peroxidase (POX), phenylamine ammonia-lyase (PAL), lipid peroxidase (LPO), polyphenol oxidase (PPO), and total phenolic content (TPC) were evaluated.

4.1 Studies on seed bio-priming with different concentrations of seed dressing formulation of potential *Trichoderma* mutants

Trichoderma–plant interaction owing to its beneficial effects (improve nutrient use efficiency, mycoparasitism, PGPA, an increase of plant lateral roots, thus modifying root architecture, production of an array of secondary metabolites, SAR and ability to impart tolerance to abiotic stresses (Lorito *et al*., 2010; Harman *et al*., 2004; Salas-Marina *et al*., 2015; Chagas *et al*., 2017, Contreras-Cornejo *et al*., 2009, 2015) is one of the choicest microbe delivered as a seed dressing to different crops. The most commonly used is inoculation methods used for augmentation of the beneficial effects of *Trichoderma* are seed coating, pelleting, foliar application, and direct soil application and are adopted as per requirement. Based on recent research manuscripts on seed biopriming with plant growth-promoting rhizobacteria (Mahmood *et al*. 2016; Anitha *et al*. 2013; Taylor and Harman 1990; Callan and Miller 1990), we assumed that the plant growth-promoting *Trichoderma* applied through seed priming, incubating the seeds for premeasured time, starts the physiological processes inside the seed while radicle and plumule emergence is prevented until the seed is sown. The start of the physiological process inside the seed

enhances the abundance of propagules of *Trichoderma* in the spermosphere. This proliferation of *Trichoderma* inside the seeds is 10-fold than attacking pathogens which enables the plant to survive those pathogens increasing the use of bio-priming for bio control too. Nevertheless the issue also prompted us to argue that will lowering dose of seed treatment and incubating the seeds for premeasured time will proliferate the *Trichoderma* inside the seeds by 10-fold and will have an equivalent growth-promoting effect?

4.1.1 Effect of seed bio-priming with different concentrations of seed dressing formulation of potential *Trichoderma* mutants on Mungbean (Pairy Moong-1) and Rice (Swarna)

Clean seeds of Mung bean and Rice were surface sterilized with 1.5% sodium hypochlorite (NaOCl) for 5 min and rinsed thrice with sterile distilled water and dried under a sterile airstream on autoclave blotting paper (Jain *et al*., 2013). The surface sterilized and dried seeds of Mungbean (var. Pairy Moong-1) and Rice were divided in five parts in sterilized Petri dishes. Spore suspension of potential *Trichoderma* mutants prepared by dissolving the formulation (1 gm/kg, 2gm/kg, 3gm/kg, 4gm/kg, 5gm/kg seed) in 50 ml of SDW (Sterilized Distilled Water). Using an automizer the seeds were spray inoculated (@ 1, 2, 3, 4, 5 gm/kg of seed) and the seeds only sprayed with SDW served as control. The plates containing the treated seeds with different doses were kept as a heap in the moist chamber at 28–30 ^{0}C, maintaining 98% relative humidity for 24 hours for priming. After 5 days of germination plumule and radicle length were measured. Bio-primed seeds were sown in pots after 24 hours (Plate 4 and 5). Colonization of potential mutants on seed surface which was confirmed with help of a stereo binocular microscope (Plate 6 and 7). Seedling derived from different dose treatments were harvested 10 days after sowing Roots were harvested and were scanned using root scanner EPSON Perfection V&00/V750 3.81 version and WinRhizo Reg 2009 (Lobet *et al*., 2013). Experimental results revealed that mung bean seed bio-priming with potential *Trichoderma* mutants (M-18, M-23, M-136, and BARC) spores

significantly increased the plumule length but not radicle length in plants derived from seed treated as compared to control (Table 4.1).

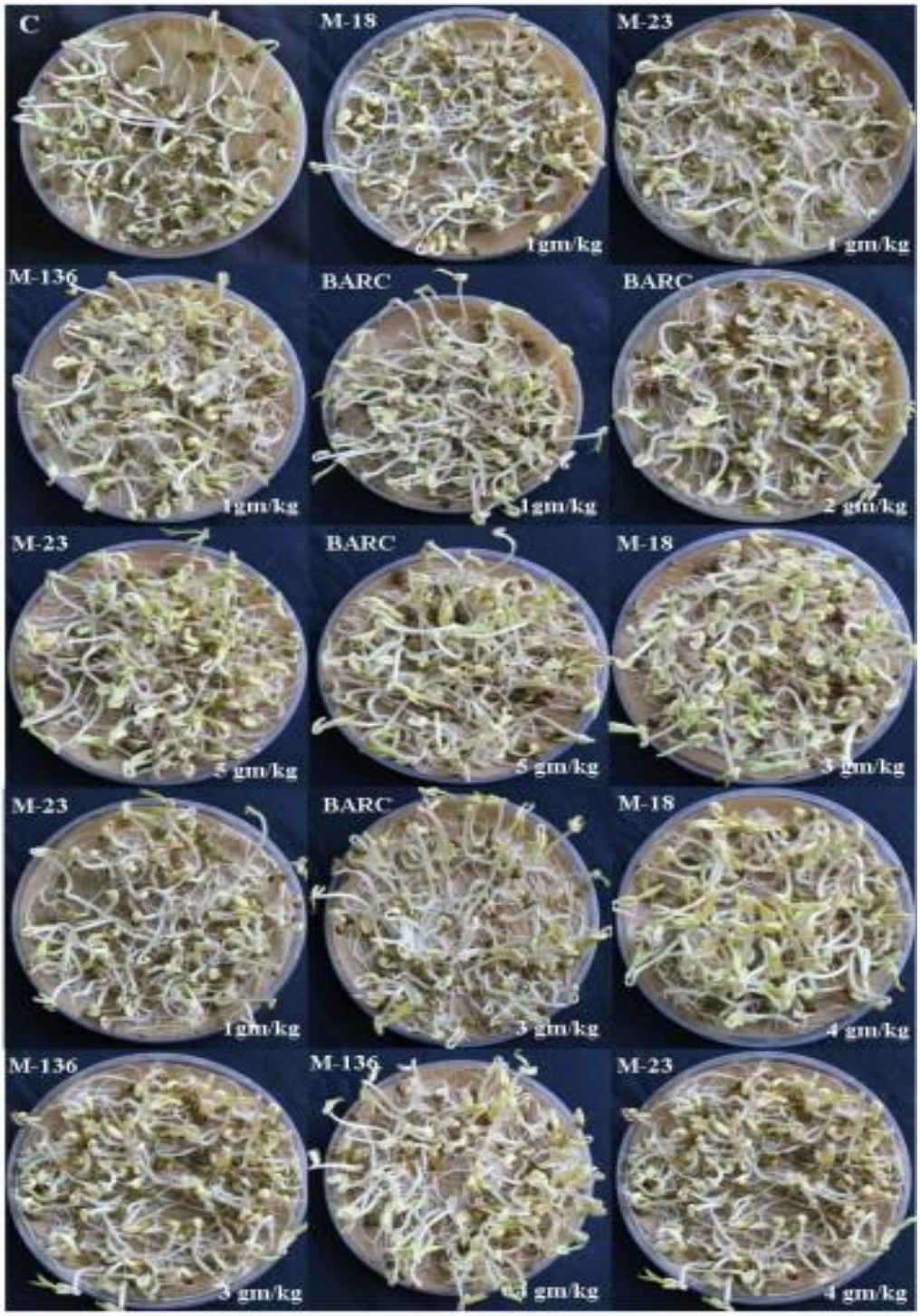


Plate 4.4 Effect of seed bio-priming with different seed dressing formulation of potential *Trichoderma* mutants on 5 days old seedlings of mung bean

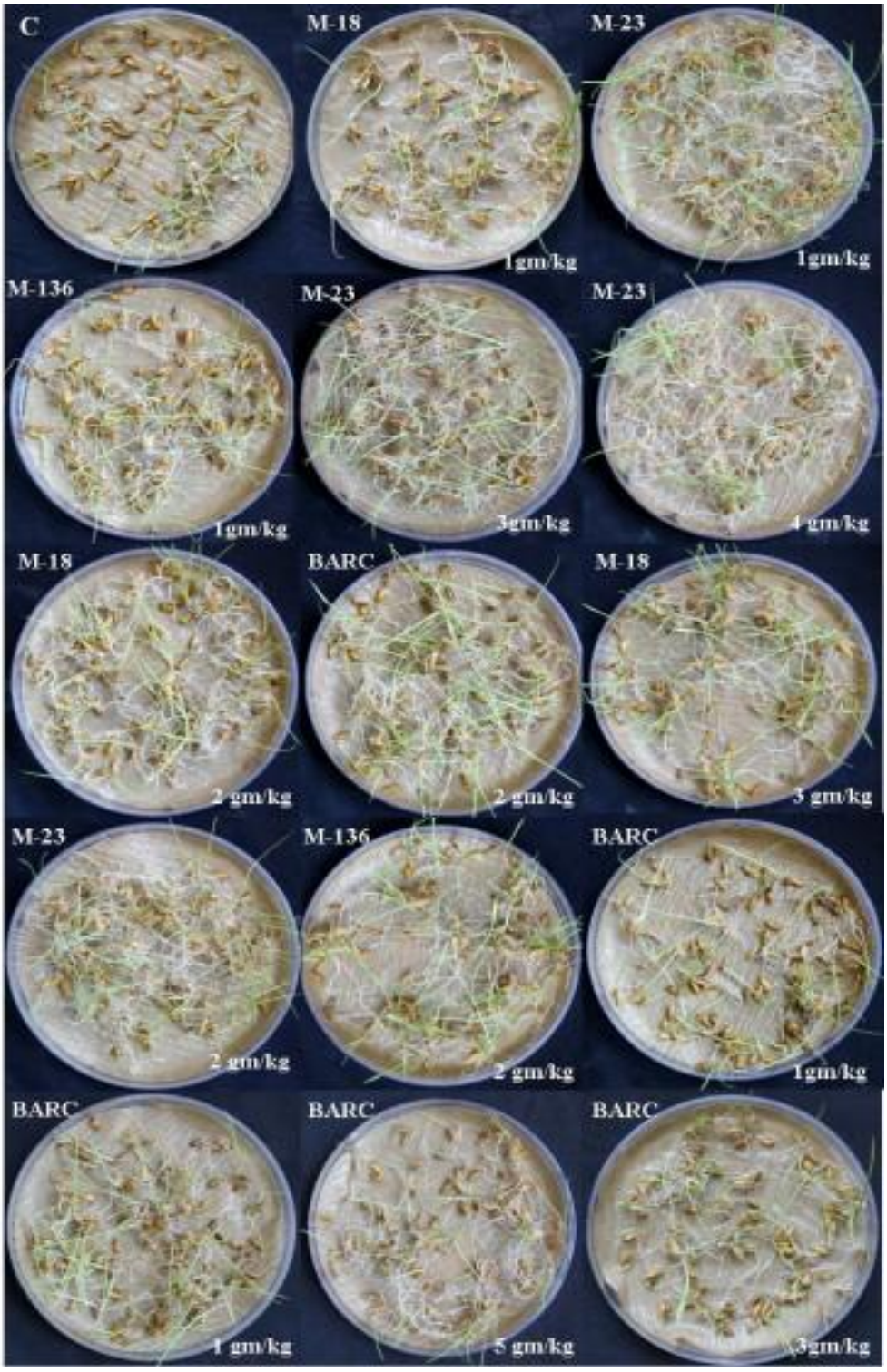


Plate 4.5 Effect of seed bio-priming with different seed dressing formulation of potential *Trichoderma* mutants on 5 days old seedlings of rice

Plate 4.6 Seed colonization by potential *Trichoderma* mutants after 24 hours of incubation after seed bio priming on rice seeds

Plate 4.7 Seed colonization by potential *Trichoderma* mutants after 24 hours of incubation after seed bio priming on mung bean seeds

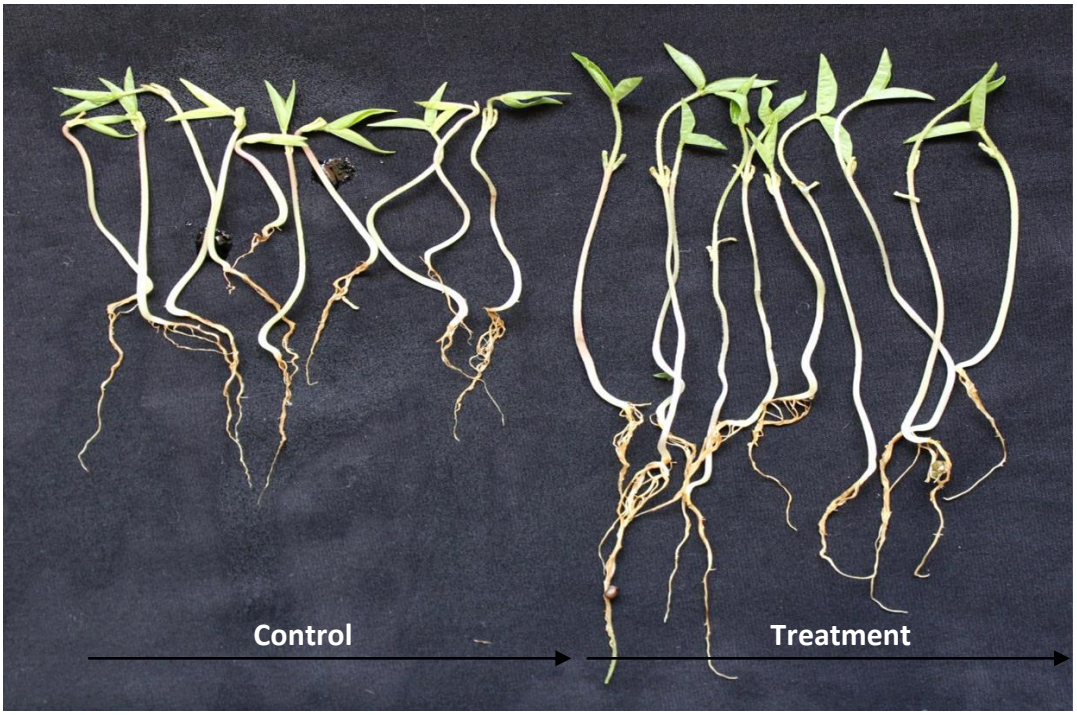


Plate 4.8 Effect of seed bio-priming with potential *Trichoderma* mutant M-18 (1 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.9 Effect of seed bio-priming with potential *Trichoderma* mutant M-23 (1 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.10 Effect of seed bio-priming with potential *Trichoderma* mutant M-136 (1 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.11 Effect of seed bio-priming with potential *Trichoderma* BARC mutant (1 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.12 Effect of seed bio-priming with potential *Trichoderma* mutant M-18 (2 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.13 Effect of seed bio-priming with potential *Trichoderma* mutant M-23 (2 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.14 Effect of seed bio-priming with potential *Trichoderma* mutant M-136 (2 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.15 Effect of seed bio-priming with potential *Trichoderma* BARC mutant (2 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

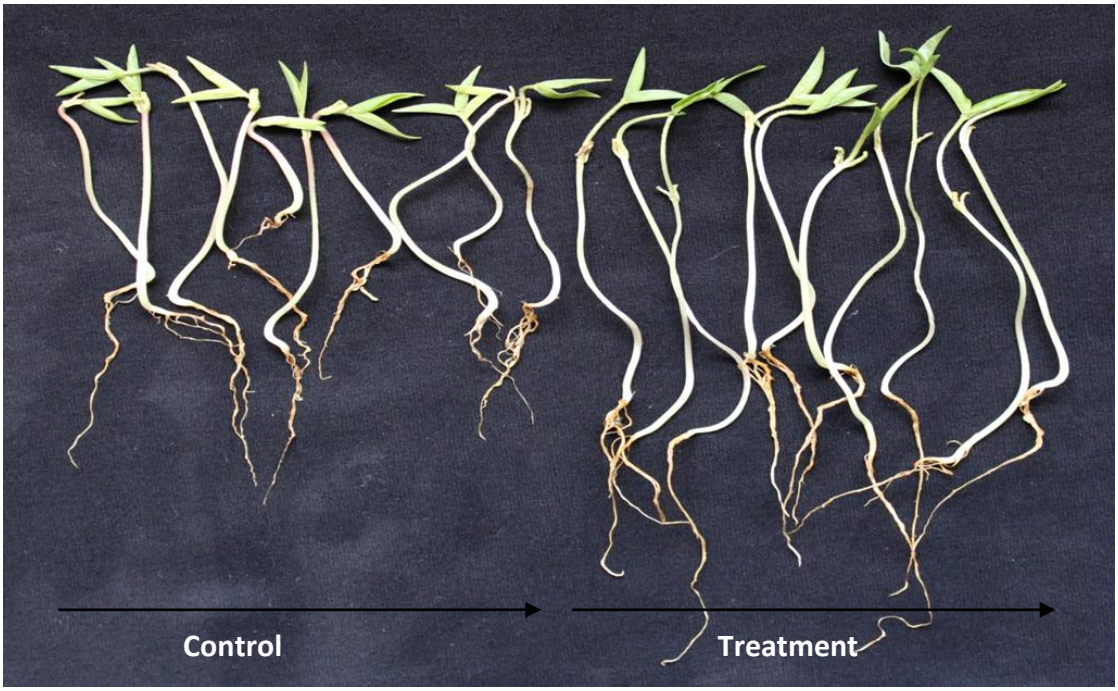


Plate 4.16 Effect of seed bio-priming with potential *Trichoderma* mutant M-18 (3 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.17 Effect of seed bio-priming with potential *Trichoderma* mutant M-23 (3 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.18 Effect of seed bio-priming with potential *Trichoderma* mutant M-136 (3 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.19 Effect of seed bio-priming with potential *Trichoderma* BARC mutant (3 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.20 Effect of seed bio-priming with potential *Trichoderma* mutant M-18 (4 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.21 Effect of seed bio-priming with potential *Trichoderma* mutant M-23 (4 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.22 Effect of seed bio-priming with potential *Trichoderma* mutant M-136 (4 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.23 Effect of seed bio-priming with potential *Trichoderma* BARC mutant (4 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.24 Effect of seed bio-priming with potential *Trichoderma* mutant M-18 (5 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.25 Effect of seed bio-priming with potential *Trichoderma* mutant M-23 (5 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.26 Effect of seed bio-priming with potential *Trichoderma* mutant M-136 (5 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Plate 4.27 Effect of seed bio-priming with potential *Trichoderma* BARC mutant (5 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of mung bean

Highest increase in plumule length in mung bean seedlings was observed in seedlings derived from different mutant formulation:- M-18 (@ 4 gm/kg of seed) followed by BARC (@ 5 gm and 2 gm/kg of seed) > M-136 (@ 1 gm/kg of seed) > M-18 (@ 3 gm/kg of seed) (Table 4.1) (Figure 4.1 and 4.2) (Plate 8-27). Similarly, experimental results revealed that rice plants derived from seed bio-priming with potential *Trichoderma* mutants (M-18, M-23, M-136 and BARC) spores significantly increased the plumule length and radicle length as compared to control (Table 4.2). An increase in plumule length in rice seedlings was observed in seedlings derived from different doses of mutant formulation:- M-23 (@ 2gm, 3gm, 4gm, and 5gm/kg of seed) (Table 4.2) (Figure 4.3 and 4.4).Variable response for radicle length in rice to different potential *Trichoderma* mutants that too at different doses was observed. When the effect of different potential mutants at different doses were compared. It was observed that highest increase in radicle length was observed in rice seedlings derived from different mutant formulation:- M-23 (@ 4 gm/kg of seed) >BARC (@ 3gm and 5 gm/kg of seed) > M-18 (@ 2 gm/kg of seed) > M-136 (@ 1 gm/kg of seed) (Plate 28-47). Bio-priming results in an uninterrupted layer over the seed surface. Moreover, the thin layer provides a conductive milieu for the introduced inoculum and, for a short time, physically separates the bio-protectant from competitive soil micro-flora thus improving the bio-efficacy of the bio-agent (Taylor *et al*., 1990). We argued that will lowering dose of seed treatment and incubating the seeds for premeasured time will proliferate the *Trichoderma* inside the seeds by 10-fold and will have an equivalent growth-promoting effect? Observation in the present investigation indicates that spray inoculating the seed with different doses and incubating the seeds for an premeasured time had many folds proliferated the *Trichoderma* propagule but its effects were not directly proportional to the increasing doses. Variable response of different doses in inducing increased plumule length was observed. Different methods have been used for the application of bio-pesticides but seed treatment is the most economical method which required a small volume of inoculums (Bennett *et al*., 1992; Pill *et al*., 2009). During seed treatment with *Trichoderma* based products (Woo *et al*., 2014) or *Trichoderma*

spores, suspensions are applied to the seed surface at a rate of 10^6 CFU ml^{-1} (Singh *et al*., 2013[a]; Patel *et al*., 2015; Saxena *et al*., 2015) or 10^7 CFU ml^{-1} (Yadav *et al*., 2013; Jain *et al*., 2015[a]). Seed bio-priming is considered as an advanced technique of seed treatment which is a combination of beneficial microorganism application on seed surface followed by seed hydration (Reddy, 2013; Singh *et al*., 2016[b]; Singh, 2016). It was reported that during bio-priming there was an increase (10 to over 10,000 folds) in bacterial populations, depending on initial inoculum levels (Callan *et al*., 1990). Seed bio-priming process constitutes of different priming methods such as incubating in a moistened finely ground lignite or coal substance (solid matrix priming) (Harman and Taylor, 1988) or under moist conditions in a plastic bag (Callan *et al*., 1991). In particular, it is important to disinfect seeds to reduce or eliminate the undesired microflora before priming. If the seeds are infected or have pathogen contamination that will be magnified during the priming process leading to the undesirable and adverse effects on emerging plants (Reddy, 2013; Singh *et al*., 2013[b]; Jain *et al*., 2015[b]). Moreover, at the same time, the proliferation of unwanted indigenous microorganisms may decrease the survivability of beneficial microbes used during the seed bio-priming process (Wright *et al*., 2003).

Table 4.1 Efficacy of different potential *Trichoderma* mutants on plumule length, radicle length of Mung bean with different doses (1, 2, 3, 4, 5, gm/kg of seed) of seed dressing formulation

Mutants	gm/kg of seed				
	1 gm	2 gm	3 gm	4 gm	5 gm
Plumule Length(cm)*					
M-18	8.24^{c}±0.38	9.77^{b}±0.27	11.01^{a}±0.46	15.26^{a}±0.25	9.39^{b}±0.19
M-23	9.70^{b}±0.28	8.19^{c}±0.28	9.06^{b}±0.36	12.85^{b}±0.43	12.50^{a}±0.39
M-136	11.19^{a}±0.50	8.90^{bc}±0.42	9.95^{ab}±0.46	9.90^{c}±0.52	12.45^{a}±0.27
BARC	10.95^{ab}±0.60	11.55^{a}±0.44	10.49^{a}±0.35	9.55^{c}±0.36	13.10^{a}±0.48
Control	6.95^{c}±0.53	6.95^{d}±0.53	6.95^{d}±0.53	6.95^{d}±0.53	6.95^{c}±0.53
CD @ 5%	1.36	1.15	1.26	1.24	1.13
CV	16.07	14.13	14.76	12.65	11.55
SE (m)	0.47	0.4	0.44	0.43	0.39

Radicle Length (cm)*					
M-18	4.45±0.40	3.94±0.24	3.98^{b}±0.39	4.60±0.19	4.05±0.26
M-23	4.27±0.18	3.87±0.27	4.33^{ab}±0.29	4.34±0.35	4.06±0.27
M-136	4.41±0.32	4.28±0.27	5.10^{a}±0.34	4.22±0.25	4.21±0.17
BARC	4.39±0.28	4.30±0.30	4.30^{ab}±0.37	3.90±0.18	4.15±0.23
Control	3.73±0.11	3.73±0.11	3.73^{b}±0.11	3.73±0.11	3.73±0.11
CD @ 5%	NS	NS	0.91	NS	NS
CV	20.91	19.72	23.56	17.82	17.38
SE (m)	0.28	0.25	0.32	0.23	0.22

***Average value of 10 random seedlings**

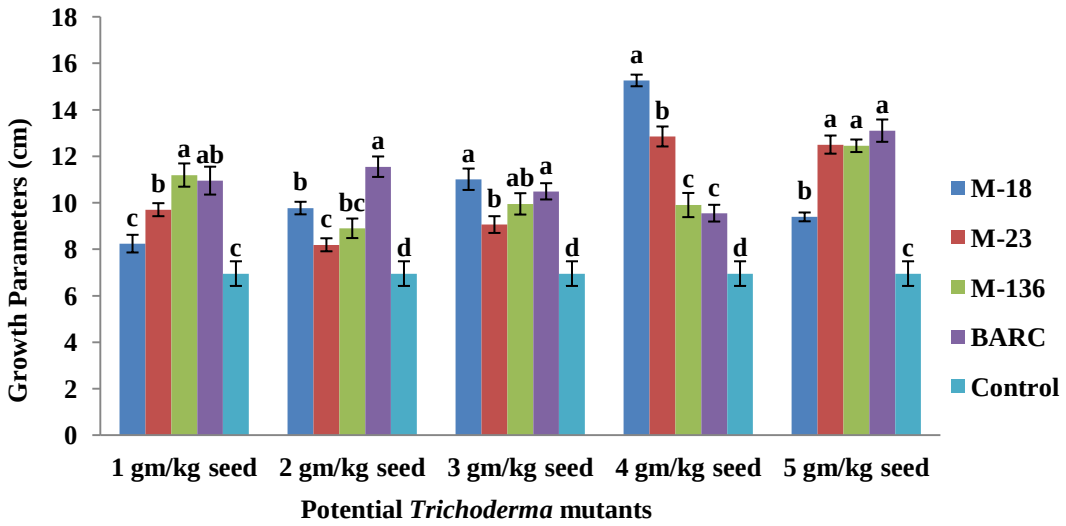


Figure 4.1 : Effect of different seed dressing formulations of potential *Trichoderma* mutants on plumule length of mung bean seedling

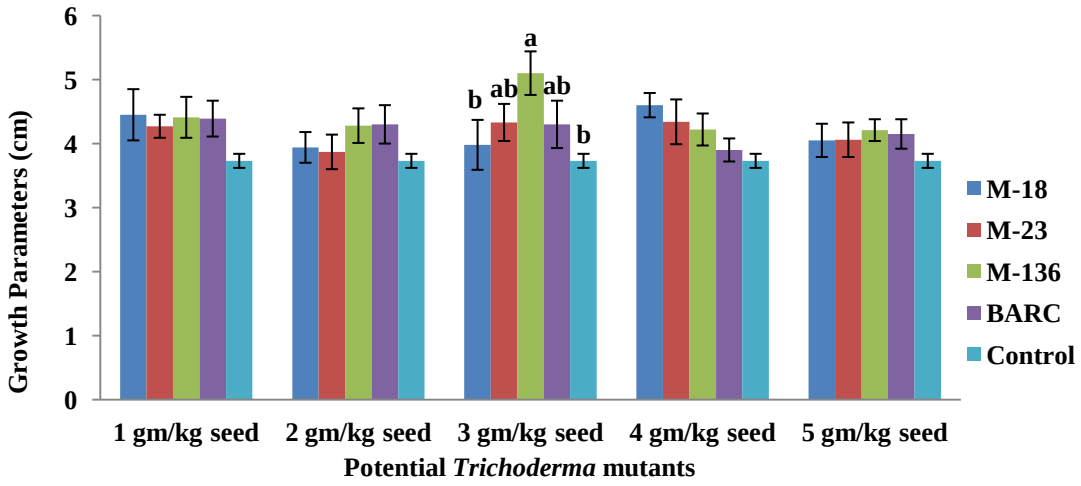


Figure 4.2 : Effect of different seed dressing formulations of potential *Trichoderma* mutants on radicle length of mung bean seedling

Table 4.2 Efficacy of different potential *Trichoderma* mutants on plumule length, radicle length of rice at different doses (1, 2, 3, 4, 5, gm/kg of seed) of seed dressing formulation

Mutants	gm/kg of seed				
	1 gm	2 gm	3 gm	4 gm	5 gm
Plumule Length(cm)*					
M-18	4.04±0.25	4.15^{b}±0.16	3.80^{c}±0.15	3.80^{bc}±0.15	3.93^{cd}±0.11
M-23	4.28±0.19	5.04^{a}±0.16	5.03^{a}±0.24	5.15^{a}±0.18	4.80^{a}±0.15
M-136	3.80±0.21	4.45^{b}±0.20	3.95^{bc}±0.17	3.84^{bc}±0.17	4.08^{bc}±0.17
BARC	4.35±0.19	4.60^{ab}±0.12	4.43^{ab}±0.19	4.38^{b}±0.14	4.55^{ab}±0.13
Control	3.56±0.27	3.56^{c}±0.27	3.56^{c}±0.27	3.56^{c}±0.27	3.56^{d}±0.27
CD	NS	0.55	0.6	0.55	0.51
CV	18.05	14.06	16.19	14.67	13.69
SE (m)	0.22	0.19	0.21	0.19	0.18
Radicle Length (cm)*					
M-18	3.85^{ab}±0.23	4.91^{a}±0.26	3.60^{b}±0.14	3.60^{bc}±0.14	3.10^{c}±0.24
M-23	3.05^{bc}±0.22	4.03^{b}±0.28	4.81^{a}±0.32	6.20^{a}±0.56	3.45^{bc}±0.24
M-136	4.35^{a}±0.19	4.30^{ab}±0.31	3.46^{b}±0.28	4.30^{b}±0.24	4.05^{b}±0.32
BARC	4.00^{a}±0.47	3.70^{b}±0.17	4.95^{a}±0.69	3.70^{b}±0.26	5.20^{a}±0.41
Control	2.70^{c}±0.20	2.70^{c}±0.20	2.70^{b}±0.20	2.70^{c}±0.20	2.70^{c}±0.20
CD	0.82	0.72	1.09	0.91	0.83
CV	25.42	20.36	31	24.74	25
SE (m)	0.28	0.25	0.38	0.32	0.29

***Average value of 10 seedlings**

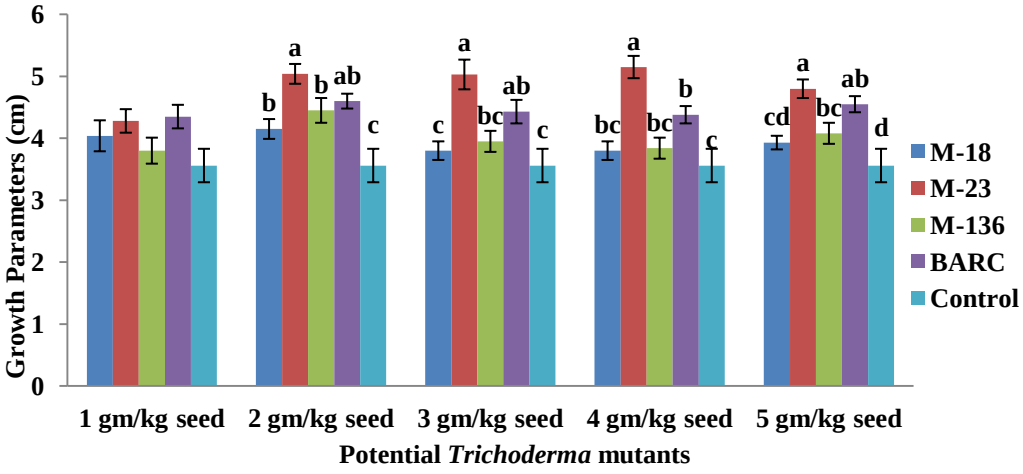


Figure 4.3 : Effect of different seed dressing formulations of potential *Trichoderma* mutants on plumule length of rice seedling

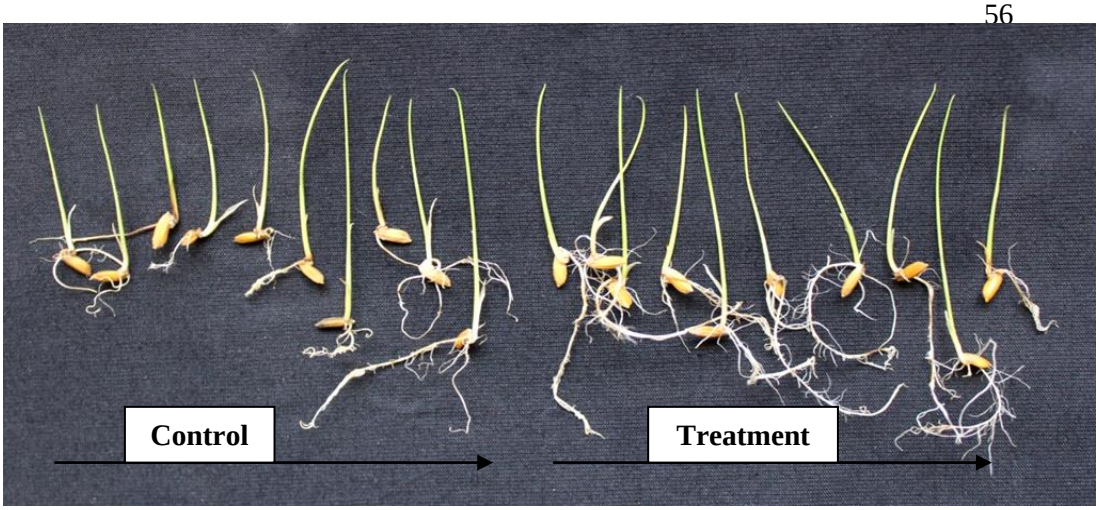


Plate 4.28 Effect of seed bio-priming with potential *Trichoderma* mutant M-18 (1 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.29 Effect of seed bio-priming with potential *Trichoderma* mutant M-23 (1 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.30 Effect of seed bio-priming with potential *Trichoderma* mutant M-136 (1 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.31 Effect of seed bio-priming with potential *Trichoderma* BARC mutant (1 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.32 Effect of seed bio-priming with potential *Trichoderma* mutant M-18 (2 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.33 Effect of seed bio-priming with potential *Trichoderma* mutant M-23 (2 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.34 Effect of seed bio-priming with potential *Trichoderma* mutant M-136 (2 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.35 Effect of seed bio-priming with potential *Trichoderma* BARC mutant (2 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.36 Effect of seed bio-priming with potential *Trichoderma* mutant M-18 (3 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.37 Effect of seed bio-priming with potential *Trichoderma* mutant M-23 (3 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.38 Effect of seed bio-priming with potential *Trichoderma* mutant M-136 (3 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.39 Effect of seed bio-priming with potential *Trichoderma* BARC mutant (3 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.40 Effect of seed bio-priming with potential *Trichoderma* mutant M-18 (4 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.41 Effect of seed bio-priming with potential *Trichoderma* mutant M-23 (4 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.42 Effect of seed bio-priming with potential *Trichoderma* mutant M-136 (4 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.43 Effect of seed bio-priming with potential *Trichoderma* BARC mutant (4 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.44 Effect of seed bio-priming with potential *Trichoderma* mutant M-18 (5 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.45 Effect of seed bio-priming with potential *Trichoderma* mutant M-23 (5 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.46 Effect of seed bio-priming with potential *Trichoderma* mutant M-136 (5 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

Plate 4.47 Effect of seed bio-priming with potential *Trichoderma* BARC mutant (5 gm/kg) seed dressing formulation on plumule and radicle length of 5 days old seedling of rice

4.2 Study of root characteristics of Mung bean and Rice crop

Plant's ability to explore the soil and to compete for its resources is largely dependent on root system and its architecture (Lynch, 1995) responsible for withdrawing below ground nutrients, minerals and water. Pattern of root branching and by the rate and trajectory of growth of individual roots (Zhang *et al*., 1999) determines root system architecture and are under genetic control and influenced by biotic and abiotic factors. Manipulating RSA using bio agents can be one of the strategy to enhance nutrient acquisition especially in low input agricultural systems. Image analyses systems provide a quick determination of various morphological parameters. *Trichoderma* spp. are rhizocompetent and therefore are able to colonize roots and ameliorates the host plants in variety of ways through improved root and shoot development, induced systemic resistance, inactivating pathogenic enzymes, solubilization and sequestration of inorganic nutrients (Brotman *et al*., 2013; Zhang *et al*., 2016; Fu *et al*., 2017, Scala *et al*., 2007; Pill *et al*., 2009; Singh, 2014; Bisen *et al*., 2016).

We therefore reasoned that, will the potential *Trichoderma* mutant's used in the present investigation (M-18, M-23, M-136 and BARC) be able to improve root architecture of mungbean and rice if they are delivered through seed bio-priming? Ten day old Mungbean and rice seedlings were derived from bio primed seeds with potential *Trichoderma* mutants (M-18, M-23, M-136 and BARC) at different doses (Plare 48-55).

Win RHizo has been established as an appropriate choice for evaluation of the root morphological parameters (Bauhus and Messier, 1999; Bouma *et al*., 2000). Roots of Mung bean and Rice derived from different treatments were analyzed using Epson perfection v700/v750 3.81 version scanner and Winrhizo software and data on total root length, surface area, number of tips and forks were generated (Table 4.3 and 4.4).

Roots of Mungbean seedlings derived after seed bio-priming with potential *Trichoderma* mutant's (M-18, M-23, M-136 and BARC) at different doses stimulated variable response in root architecture (root length, surface area, tips and forks) as compared to control (Table 4.3) (Figure 4.5, 4.6, 4.7, and 4.8). Whereas rice seedlings derived after seed bio-priming with potential

Trichoderma mutant's (M-18, M-23, M-136 and BARC) at different doses stimulated increase root architecture (root length, surface area, tips and forks) as compared to control (Table 4.4) (Figure 4.9, 4.10, 4.11 and 4.12).

Roots of mungbean seedlings derived from seed bio-priming with BARC mutant @ 3 gm/kg seed dose stimulated maximum total length (156.81 cm) and surface area (13.01 cm^2) respectively (Plate 56). Whereas, highest number of tips and forks were recorded in the roots of the plant derived from seed bio-primed with BARC mutant and M-136 @ 1 gm/kg of seed dose respectively. Roots of mungbean seedlings derived from seed bio-priming with different doses of potential mutants expressed reduced total root length surface area and number of tips (Table 4.3). Roots of rice seedlings derived from seed bio-priming with BARC mutant @ 2 g/kg seed dose stimulated maximum total root length (75.95), surface area (6.46 cm^2) and number of forks (1701) respectively. Whereas, highest number of tips (1301) were recorded in roots of rice seedlings derived from seed bio-priming with M-18 mutant @ 1 g/kg seed dose (Plate 57).

It was speculated that with increasing doses and incubating the seeds for premeasured time will proliferate the *Trichoderma* inside the seeds by 10-fold and will also result in proportional stimulation on root architecture (root length, surface area, tips and forks). Interestingly no correlation was observed in root architecture (root length, surface area, tips and forks) of mungbean and rice seedlings derived from seed bio-priming with increasing doses of potential mutants (Table 4.3 and 4.4). The reason of is not known, while Shi *et al*., (2016) reported inhibition of Arabidopsis primary root is induced by Trichokonin VI (peptaibol) produce by *Trichoderma longibrachiatum* SMF2. We therefore reasoned that though seed treatment followed by and incubating the seeds for premeasured time proliferated the *Trichoderma* on the seeds by many folds but this increase might have caused inhibitory response as reported by Shi *et al*. (2016).

Plate 4.48 Effect of seed bio-priming with mutant M-18 with different seed dressing formulations on seedling growth of 10 days old seedling of mung bean

Plate 4.49 Effect of seed bio-priming with mutant M-23 with different seed dressing formulations on seedling growth of 10 days old seedling of mung bean

Plate 4.50 Effect of seed bio-priming with mutant M-136 with different seed dressing formulations on seedling growth of 10 days old seedling of mung bean

Plate 4.51 Effect of seed bio-priming with BARC mutant with different seed dressing formulations on seedling growth of 10 days old seedling of mung bean

Plate 4.52 Effect of seed bio-priming with mutant M-23 with different seed dressing formulation on seedling growth of 10 days old seedling of rice

Plate 4.53 Effect of seed bio-priming with mutant M-136 with different seed dressing formulations on seedling growth of 10 days old seedling of mung bean

Plate 4.55 Effect of seed bio-priming with BARC mutant with different seed dressing formulations on seedling growth of 10 days old seedling of mung bean

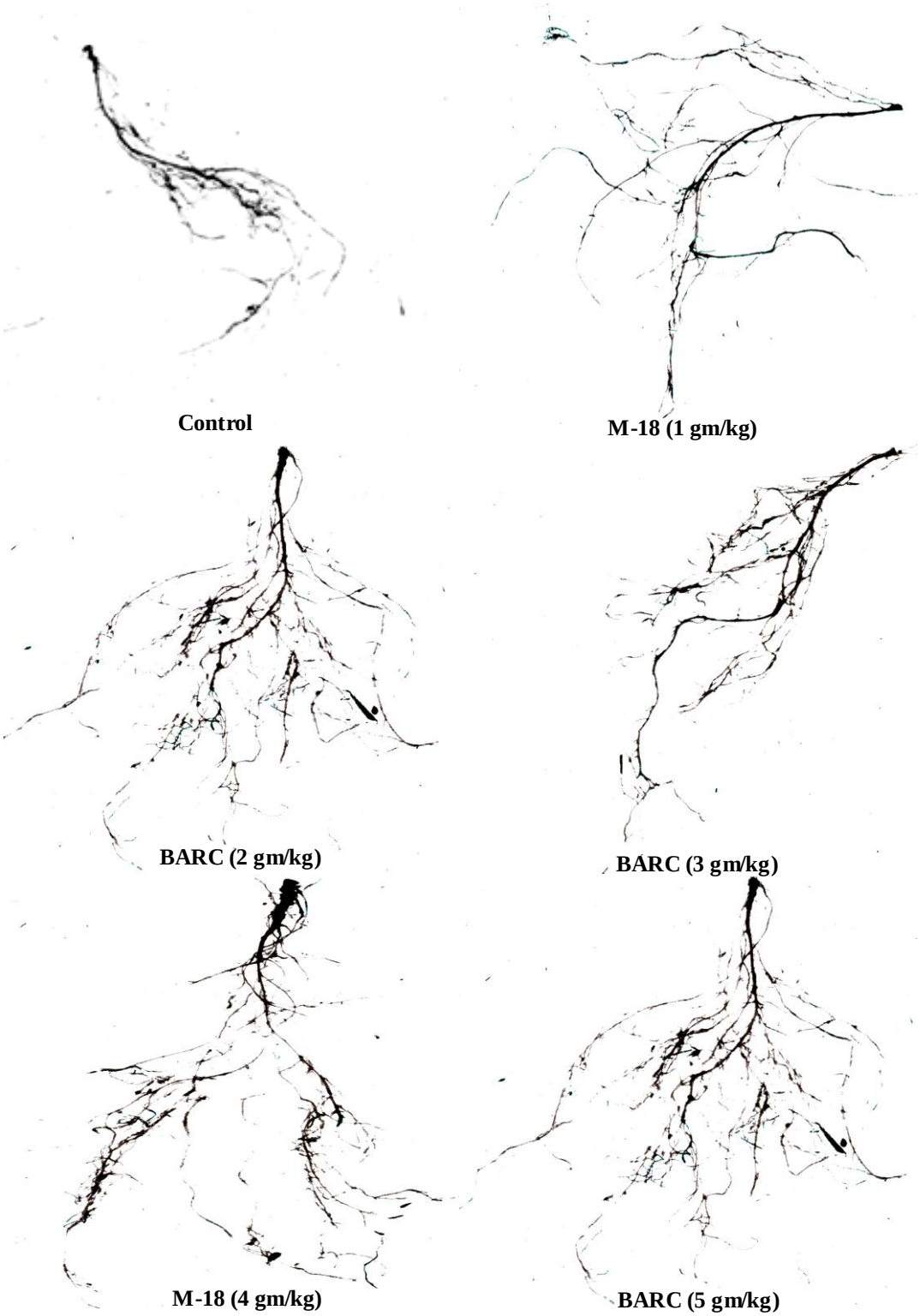


Plate 4.56 Root scanning to determine differences in different root parameters in untreated control and between the roots derived from mung bean plants developed after seed bio-priming with different potential *Trichoderma* mutants

Table 4.3 Efficacy of different potential *Trichoderma* mutants on root architecture of Mungbean with different doses (1, 2, 3, 4, 5, gm/kg of seed) of seed dressing formulation

Mutants	gm/kg of seed				
	1 gm*	2 gm*	3 gm*	4 gm*	5 gm*
Total Length (cm)					
M-18	89.69	117.58	91.75	62.84	132.21
M-23	105.12	74.53	110.15	98.29	110.10
M-136	110.54	146.61	65.69	91.90	75.94
BARC	141.49	102.25	156.81	124.21	97.13
Control	38.10	38.10	38.10	38.10	38.10
SD	37.91	41.47	45.07	33.29	35.79
Variance	1437.86	1720.01	2031.61	1108.53	1281.54
t-test value	5.72	5.16	4.58	5.57	5.66
Surface Area (cm^2)					
M-18	6.71	9.07	6.45	4.18	10.31
M-23	7.82	5.14	9.8	7.82	8.51
M-136	7.72	10.29	4.73	7.13	6.28
BARC	10.03	5.76	13.01	8.19	7.02
Control	2.81	2.81	2.81	2.81	2.81
SD	2.64	3.03	4.07	2.38	2.79
Variance	6.99	9.22	16.58	5.70	7.81
t-test value	5.93	4.87	4.04	5.64	5.59
Tips					
M-18	1730	1744	1784	1157	2106
M-23	1734	1369	1371	1654	1613
M-136	1893	2224	1224	1340	1026
BARC	2297	1800	1979	1975	1147
Control	824	824	824	824	824
SD	539.21	525.67	458.16	444.14	515.59
Variance	290748.3	276336.2	209916.3	197266.5	265833.7
t-test value	7.03	6.77	7.01	6.99	5.82
Forks					
M-18	1248	1463	1349	939	1556
M-23	1171	874	1853	1110	1089
M-136	2885	1573	843	984	455
BARC	1510	712	1269	1843	624
Control	453	453	453	453	453
SD	890.98	484.66	531.06	501.07	479.37
Variance	793855.3	234900.5	281992.8	251071.7	229800.3
t-test value	3.64	4.68	4.85	4.75	3.89

*Average value of two randomly selected seedling

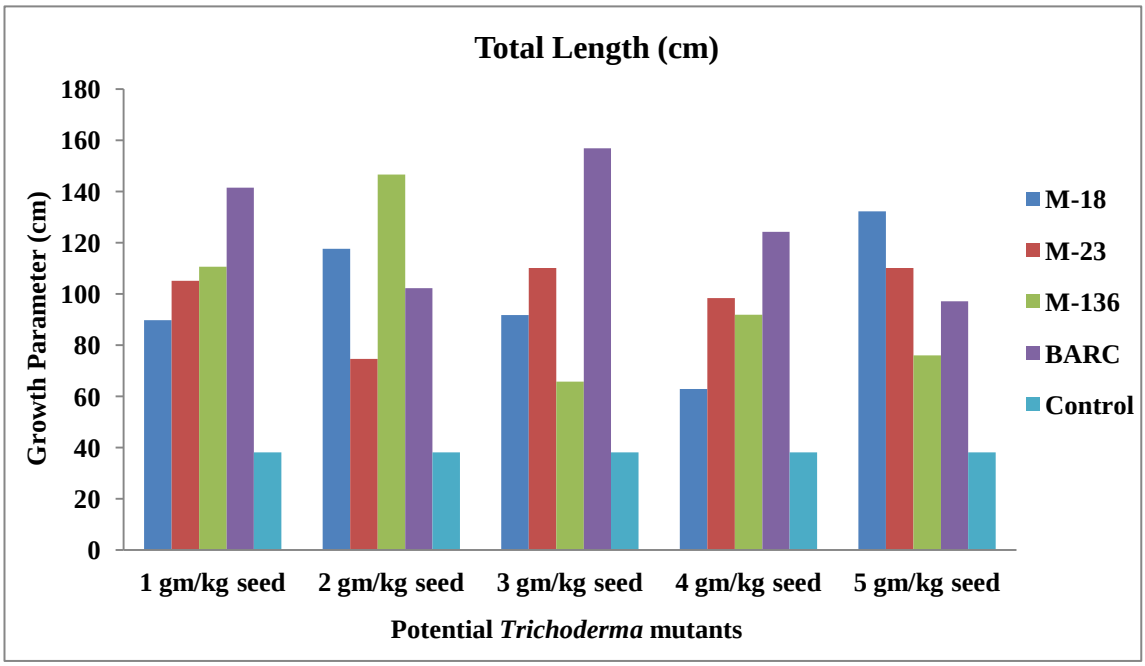


Figure 4.5 : Total root length of mung bean seedling derived after seed bio-priming with different seed dressing formulations as analysed using Epson perfection v700/v750 3.81 version with WinRhizo Reg software

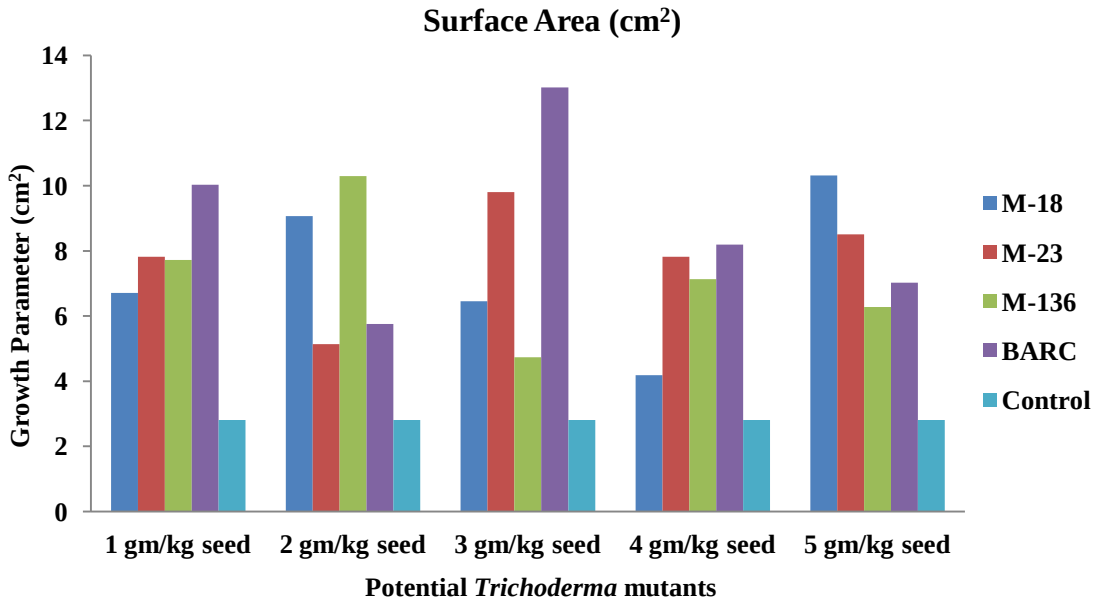


Figure 4.6 : Surface Area of mung bean seedling derived after seed bio-priming with different seed dressing formulations as analysed using Epson perfection v700/v750 3.81 version with WinRhizo Reg software

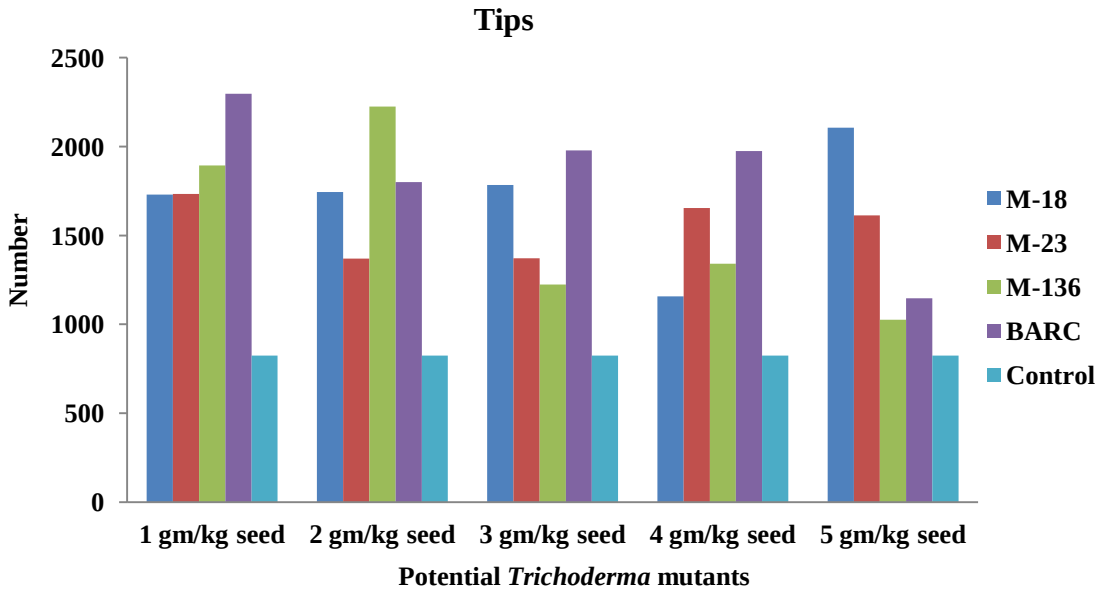


Figure 4.7 : No. of tips in mung bean seedling derived after seed bio-priming with different seed dressing formulations as analysed using Epson perfection v700/v750 3.81 version with WinRhizo Reg software

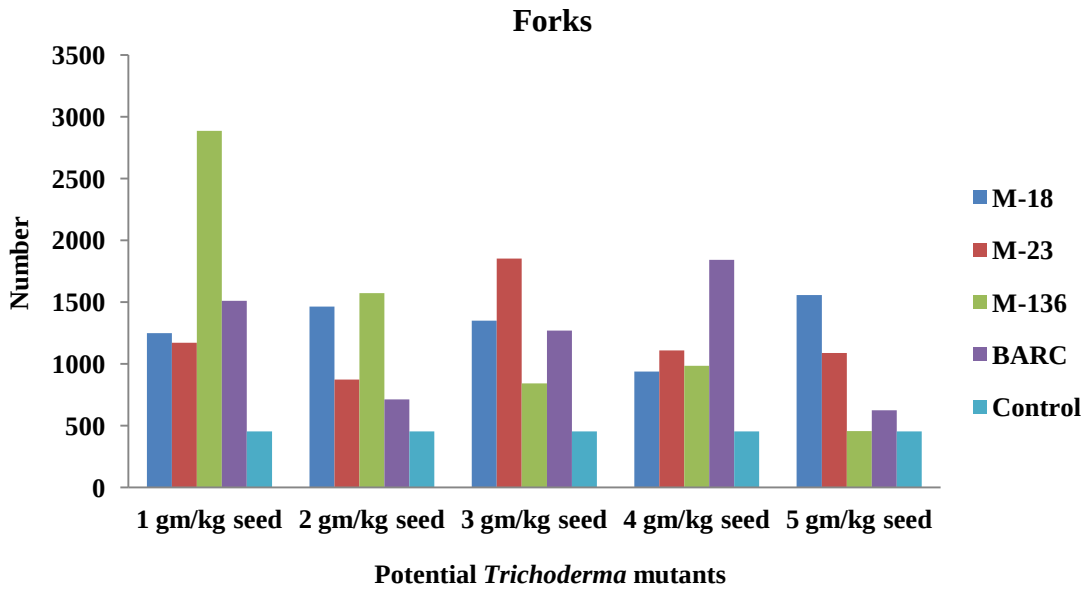


Figure 4.8 : No. of forks in mung bean seedling derived after seed bio-priming with different seed dressing formulations as analysed using Epson perfection v700/v750 3.81 version with WinRhizo Reg software

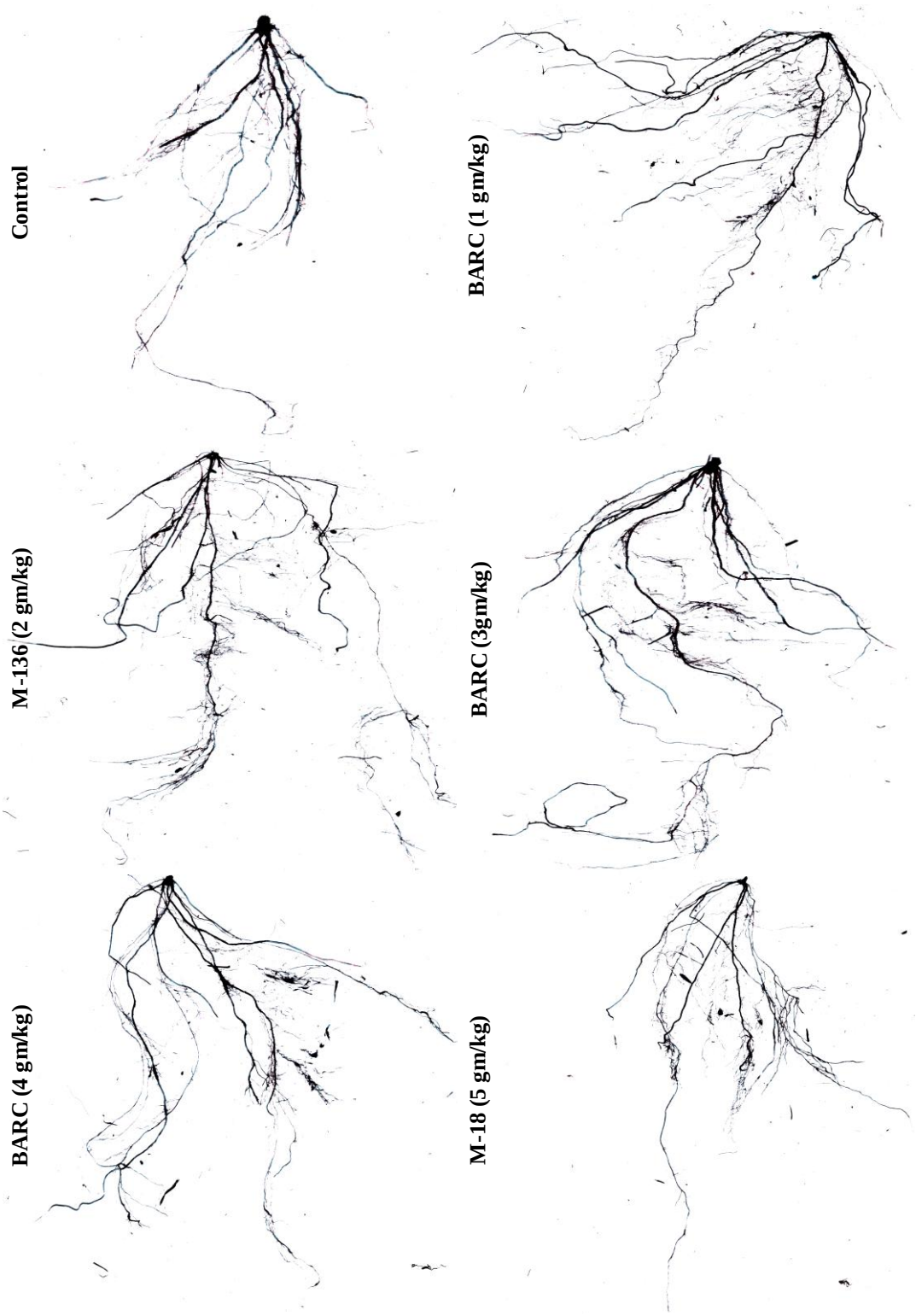


Plate 4.57 Root scanning to determine differences in different root parameters in untreated control and between the roots derived from rice plants developed after seed bio-priming with different potential *Trichoderma* mutants

Table 4.4 Efficacy of different potential *Trichoderma* mutants on root architecture of rice with different doses (1, 2, 3, 4, 5, g/kg of seed) of seed dressing formulation

Mutants	gm/kg of seed				
	1 gm*	2 gm*	3 gm*	4 gm*	5 gm*
Total Length (cm)					
M-18	75.62	72.43	62.38	57.72	49.02
M-23	41.42	54.46	40.92	34.55	37.72
M-136	67.90	53.64	50.98	54.45	36.87
BARC	33.13	75.95	66.24	52.89	57.84
Control	41.59	41.59	41.59	41.59	41.59
SD	18.62	14.30	11.64	9.76	8.81
Variance	346.85	204.52	135.49	95.44	77.75
t-test value	6.23	9.32	10.07	11.04	11.31
Surface Area (cm^2)					
M-18	6.08	5.46	4.79	4.04	3.07
M-23	2.51	3.53	2.74	2.49	2.86
M-136	4.01	3.18	4.52	4.35	3.10
BARC	2.06	6.46	4.98	3.90	4.14
Control	3.57	3.57	3.57	3.57	3.57
SD	1.57	1.43	0.94	0.71	0.51
Variance	2.46	2.07	0.89	0.51	0.26
t-test value	5.19	6.90	9.77	11.42	14.54
Tips					
M-18	1301	1286	1118	1013	1183
M-23	901	902	674	648	518
M-136	1187	1175	694	837	545
BARC	613	1039	1077	745	825
Control	579	579	579	579	579
SD	326.82	274.18	249.88	169.83	281.16
Variance	106817.2	75178.7	62443.3	28842.8	79051.0
t-test value	6.26	8.12	7.41	10.06	5.8
Forks					
M-18	791	610	741	627	605
M-23	873	1259	719	810	741
M-136	1275	1092	723	935	722
BARC	468	1701	1176	1424	1416
Control	370	370	370	370	370
SD	359.24	528.14	286.16	392.75	389.77
Variance	129053.3	278935.3	81889.7	154254.7	151920.7
t-test value	4.7	4.26	5.82	4.74	4.42

***Average value of two randomly selected seedling**

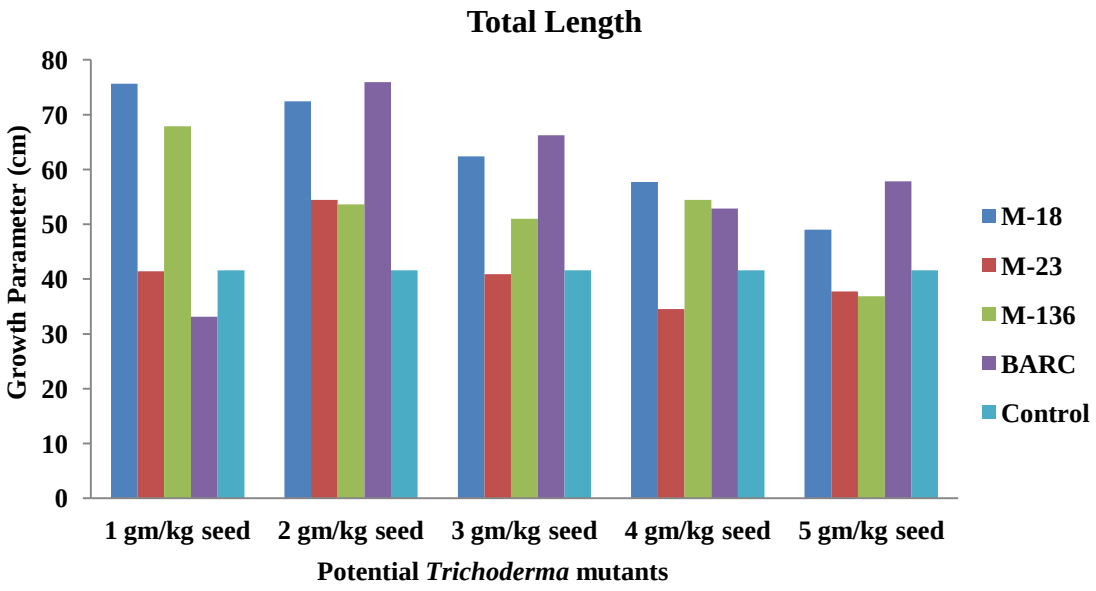


Figure 4.9 : Total root length of rice seedling derived after seed bio-priming with different seed dressing formulations as analysed using Epson perfection v700/v750 3.81 version with WinRhizo Reg software

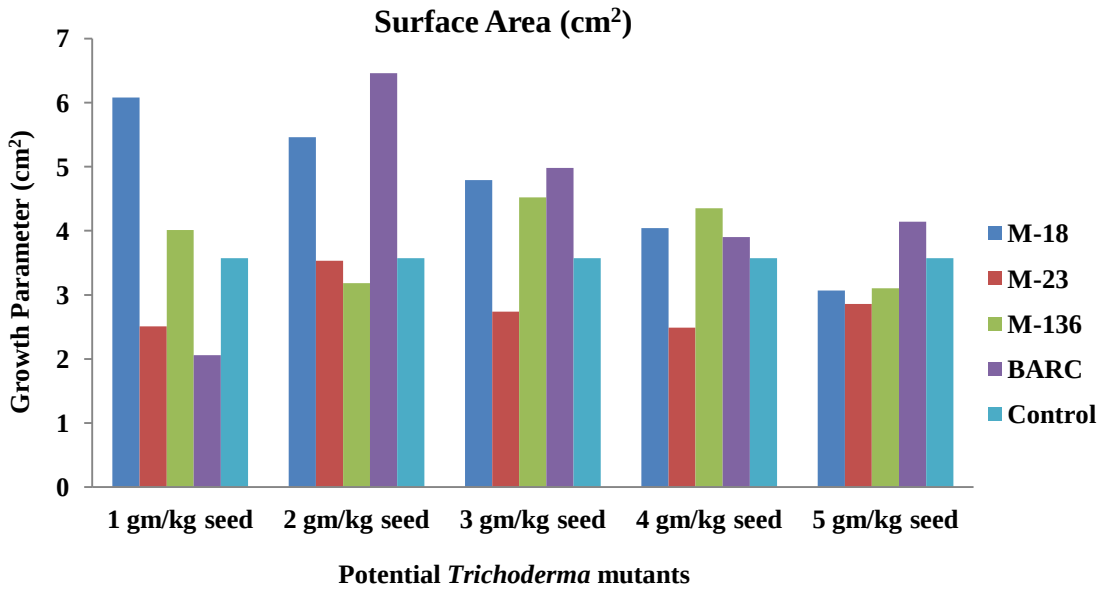


Figure 4.10 : Surface area of rice seedling derived after seed bio-priming with different seed dressing formulations as analysed using Epson perfection v700/v750 3.81 version with WinRhizo Reg software

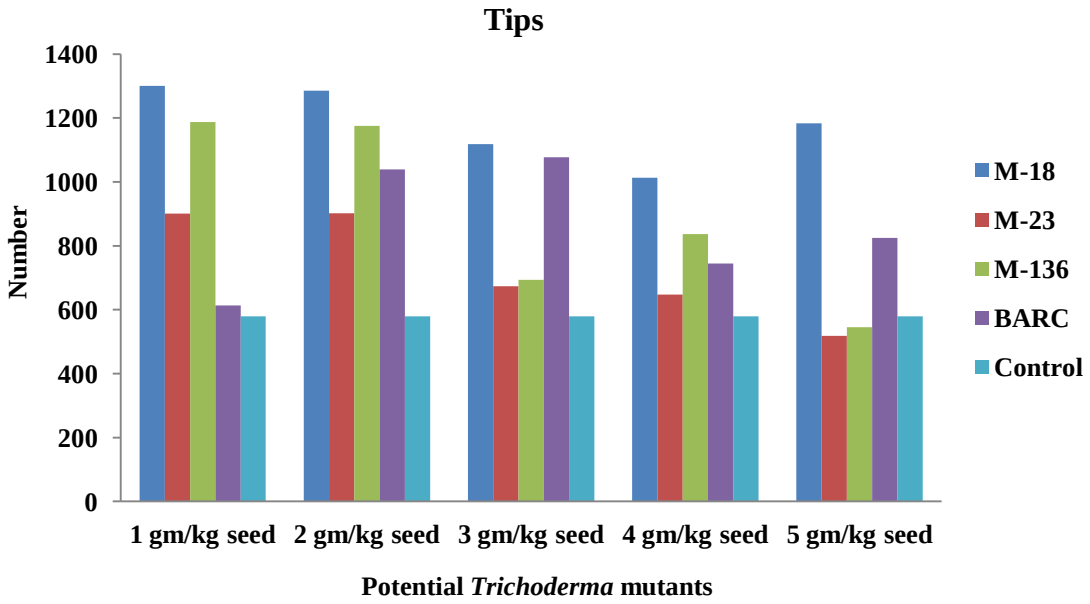


Figure 4.11 : No. of tips in rice seedling derived after seed bio-priming with different seed dressing formulations as analysed using Epson perfection v700/v750 3.81 version with WinRhizo Reg software

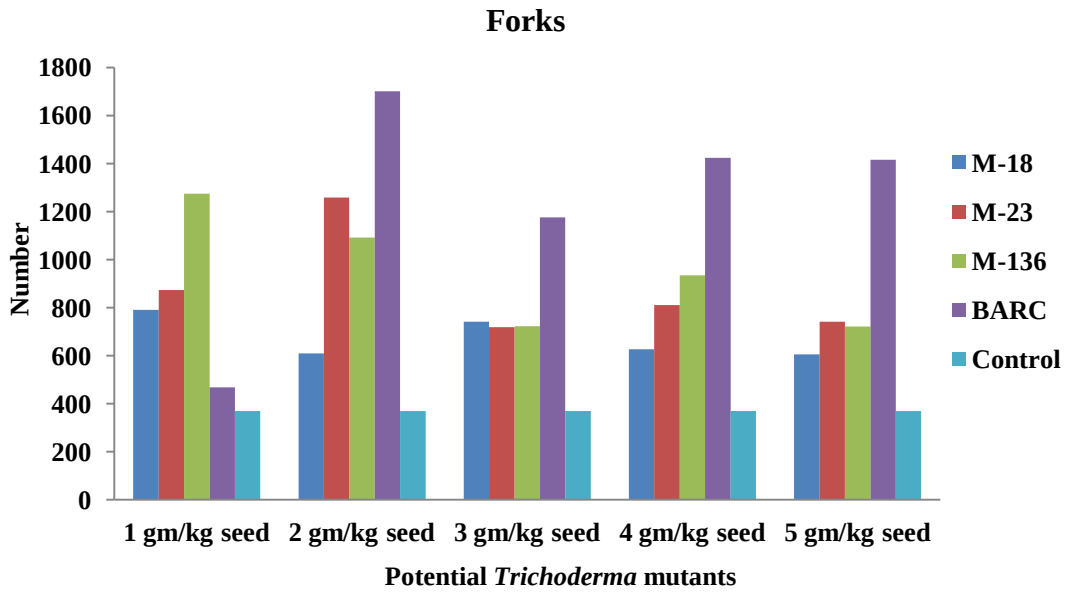


Figure 4.12 : No. of forks in rice seedling derived after seed bio-priming with different seed dressing formulations as analysed using Epson perfection v700/v750 3.81 version with WinRhizo Reg software

Several lines of evidence indicate that *Trichoderma* is an important plant endophyte that can interact with plants such as maize, cucumber, cotton, tomato, and *Arabidopsis thaliana* (Contreras-Cornejo *et al*., 2009; Vishnevetsky *et al*., 2010; Lopes *et al*., 2012; Mastouri *et al*., 2012; Reithner *et al*., 2014; Zhang *et al*., 2014; Lamdan *et al*., 2015). *Trichoderma* penetrates the first or second layers of cells of the epidermis in the root tissue or even colonizes intracellular spaces and grows between the plasma membrane and the plant cell wall (Yedida *et al*., 1999). Colonization by *Trichoderma* promotes plant growth, biomass gaining, higher seed germination, increased plant height, root development, shoot dry mass and leaf number, increased crop yield and improved plant vigor (Harman *et al*., 2004; Salas-Marina *et al*., 2015; Chagas *et al*., 2017). One of the most evident morphologic changes in plants triggered by *Trichoderma* is the increase of lateral roots, thus modifying root architecture. In this process, previous observations have demonstrated the participation of auxins (Contreras-Cornejo *et al*., 2009) as well as a cross-talk between ET and auxins through the signaling pathways mediated by MAP-kinases (Contreras-Cornejo *et al*., 2015). Also, the presence of *Trichoderma* not only modulates the levels of the hormones produced by the plant but *Trichoderma* itself can contribute with its own hormones or could provide intermediates for the synthesis of some phytohormones, as a part of the benefits reported in the *Trichoderma*–plant interaction (Guzmán-Guzmán *et al*., 2019).

4.3 Plant growth promoting response in chickpea (Indira Chana-1) following seed bio-priming with BARC mutant

Bio-priming treatment is most prominent approach to induce profound changes in characteristics of plant and to encourage more rapid and uniform seed germination. Seed bio-priming forms protective covering around the seed coat which ensures better seedling germination and reduces pre and post emergence mortality of seedling due to different seed and soil borne pathogens (Entesari *et al*., 2013).

We then undertook extensive field evaluation (on-farm demonstration trials) of mutant-based formulation (BARC) for plant growth promoting activity following seed bio-priming in chickpea (Indira Chana-1). Field

experiments were carried out at research cum instructional farms, of College of Agriculture, I.G.K.V., Raipur and KVK Kawardha field during *kharif*-2018-2019 and 2019-2020. Based on the results of statistical analysis of chickpea field data traits like plant height, no. of pods, no. of primary branches, no. of plants (per sq. meter), no. of leaves and no. of nodules were significantly different at 5 % and 1 % level of significance. In field experiment, all the parameters attributing growth and yield in Indira Chana-1 of chickpea and C.G. soya-1 of soybean were influenced by treatment of BARC mutant presented in the table 4.45 and 4.46, figure 4.13, 4.14 and 4.15

Significant increase in plant height, no. of pods, no. of primary branches and no. of plants (per sq. meter) was observed in chickpea at crop harvesting stage. It was observed that plants derived from seed bio priming with BARC mutant culture consistently at two locations over two years improved germinations and plant growth. Growth promotion improved pod bearing (Table 4.45) (Plate 58-61). Data presented in table 4.46 indicates percent improvements in Plant height, No. of branches, No. of Plants / Sq.M and No. of pods as compared to control at two locations for two consecutive years 2018 and 2019.

1) **KVK Raipur *rabi*-2018**: Plant height, No. of branches, No. of Plants / Sq.M and No. of pods was 16.33%, 37.06%, 34.43% and 23.45% improved as compared to control.
2) **KVK Raipur *rabi*-2019:** Plant height, No. of branches, No. of Plants / Sq.M and No. of pods was 29.09%, 33.52%, 39.34 and 32.40% improved as compared to control.
3) **KVK Kawardha *rabi*-2018:** Plant height, No. of branches, and No. of Plants / Sq.M was 21.61%, 28.31% and 27.87% improved as compared to control.
4) **KVK Kawardha *rabi*-2019:** Plant height, No. of branches, and No. of Plants / Sq.M was 19.28%, 26.90% and 37.71% improved as compared to control.

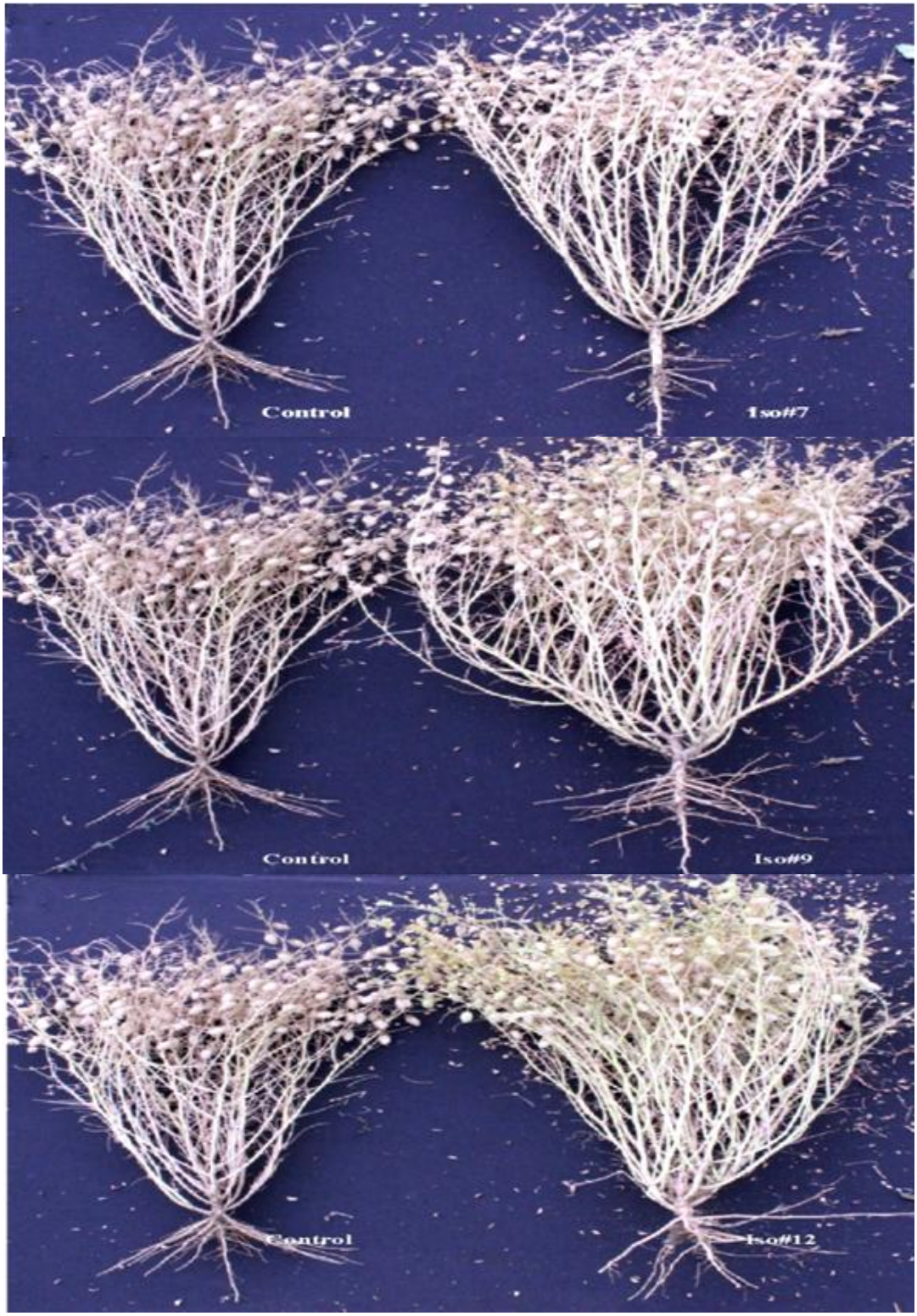


Plate 4.58 Seed bio-priming effect on plant growth promoting activity of BARC mutant in Chickpea (Indira Chana-1) in KVK Raipur *rabi* 2018-2019

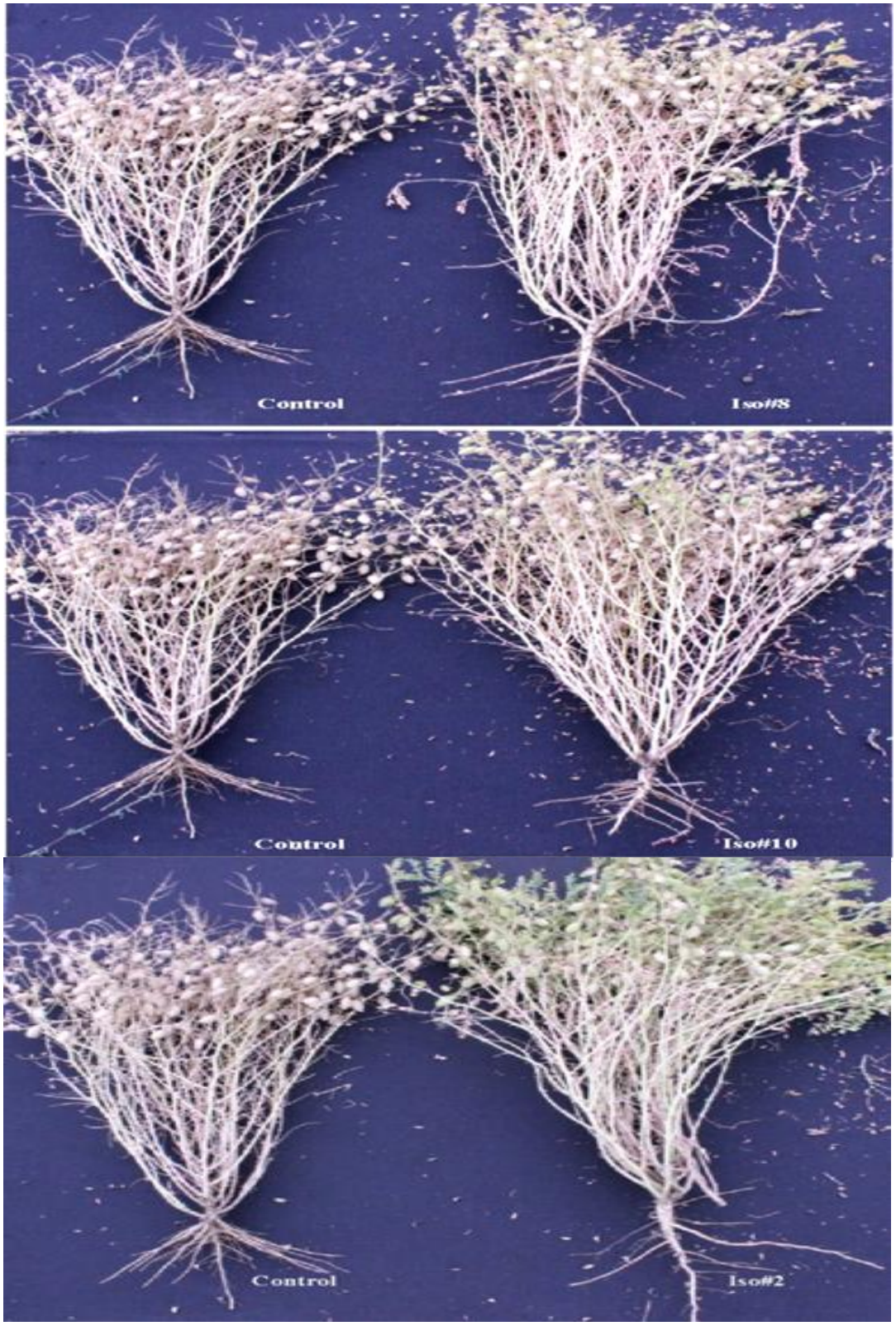


Plate 4.59 Seed bio-priming effect on plant growth promoting activity of BARC mutant in Chickpea (Indira Chana-1) at KVK Raipur *rabi* 2018-2019

Plate 4.60 Seed bio-priming effect on plant growth promoting activity of BARC mutant at flower initiation stage in Chickpea (Indira Chana-1) at KVK Kawardha *rabi* 2018- 2019

Control Plot

Treated Plot

Plate 4.61 Seed bio-priming effect on plant growth promoting activity of BARC mutant in Chickpea (Indira Chana-1) in KVK Kawardha *rabi* 2019-2020

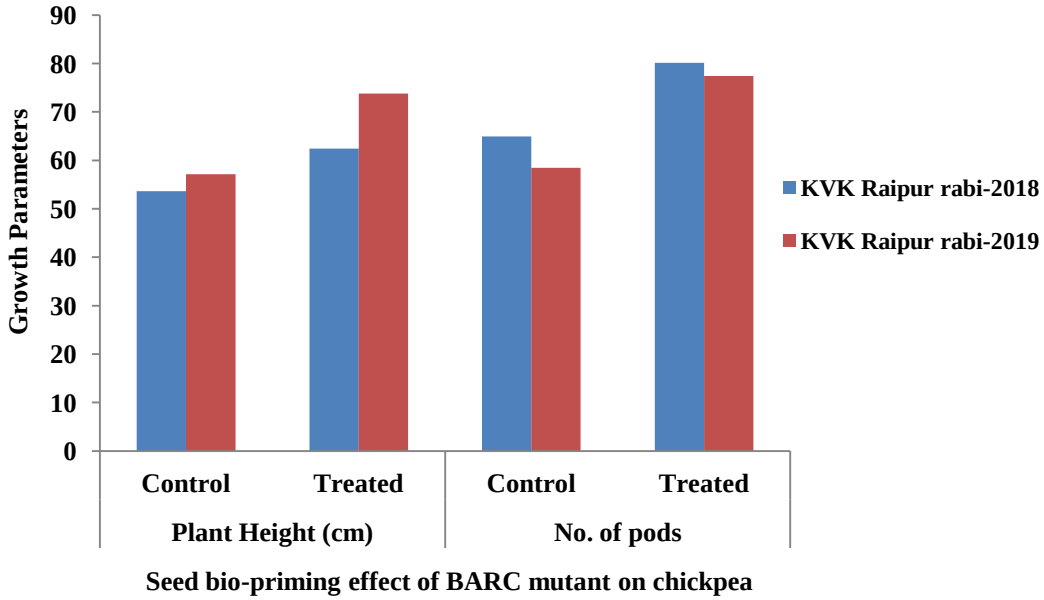


Figure 4.13: Effect of seed bio-priming with BARC mutant on growth and yield attributing characters of chickpea at KVK Raipur field *rabi* 2018 and 2019

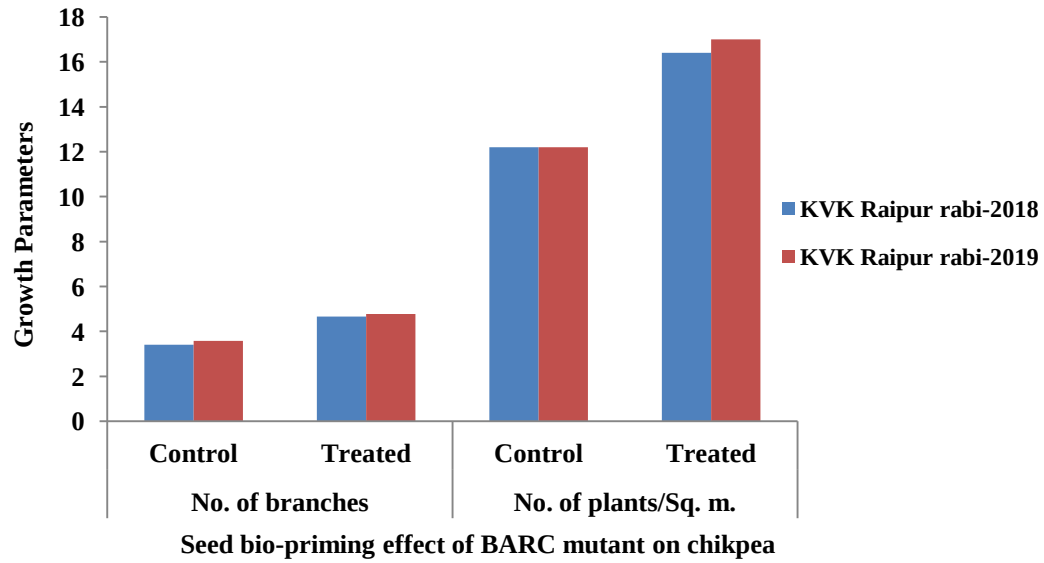


Figure 4.14: Effect of seed bio-priming with BARC mutant on growth and yield attributing characters of chickpea at KVK Raipur field *rabi* 2018 and 2019

Table 4.5 Evaluation growth promoting response of chickpea (Indira Chana-1) following seed bio-priming with BARC mutant

	Plant Height (cm)*		**No. of Branches***		**No. of Plants / Sq. M***		**No. of Pods***	
	Control	**Treat**	**Control**	**Treat**	**Control**	**Treat**	**Control**	**Treat**
KVK Raipur *rabi*-2018								
Mean	53.63	62.39	3.4	4.66	12.2	16.4	64.9	80.12
SD	0.99	1.91	0.18	0.32	0.83	1.14	9.2	2.03
CV	0.98	3.68	0.03	0.1	0.7	1.3	8.47	4.14
Cal. T	9.07		7.58		6.64		3.6	
KVK Raipur *rabi*-2019								
Mean	57.16	73.79	3.58	4.78	12.2	17	58.46	77.4
SD	1.53	3.24	0.19	0.44	0.99	5.33	2.25	5.83
CV	2.34	10.52	0.03	0.2	0.98	28.44	5.09	34.2
Cal. T	7.08*		5.48		9.28		6.77	

	Plant Height (cm)		**No. of branches**		**No. of plants/Sq/M.**	
	Control	**Treatment**	**Control**	**Treatment**	**Control**	**Treatment**
KVK Kawardha *rabi*-2018						
Mean	27.58	33.54	3.32	4.26	12.2	15.6
SD	1.07	1.31	0.13	0.11	0.83	1.67
CV	1.15	1.71	0.017	0.013	0.7	2.8
Cal. T	7.86		12.13		4.06	
KVK Kawardha *rabi*-2019						
Mean	56.64	67.56	3.42	4.34	12.2	16.8
SD	3.63	2	0.11	0.11	1.3	0.83
CV	13.18	3.71	0.012	0.013	1.7	0.7
Cal. T	5.9		13.01		6.64	
Table-T (0.05)=2.306; **Table-T(0.01)**=3.355						

***= Average of 50 plants**

Table 4.6 Evaluation growth promoting response in chickpea (Indira Chana-1) following seed bio-priming with BARC mutant

	Plant Height (cm)*		No. of Branches*		No. of Plants / Sq. M*		No. of Pods*	
	Control	**Treat**	**Control**	**Treat**	**Control**	**Treat**	**Control**	**Treat**
KVK Raipur *rabi*-2018								
Average	53.63	62.39	3.4	4.66	12.2	16.4	64.9	80.12
%increase over control	16.33		37.06		34.43		23.45	
KVK Raipur *rabi*-2019								
Average	57.16	73.79	3.58	4.78	12.2	17	58.46	77.4
%increase over control	29.09		33.52		39.34		32.4	

	Plant Height (cm)*		No. of Branches*		No. of Plants / Sq. M*	
	Control	**Treat**	**Control**	**Treat**	**Control**	**Treat**
KVK Kawardha *rabi*-2018						
Average	27.58	33.54	3.32	4.26	12.20	15.60
%increase over control	21.61		28.313		27.87	
KVK Kawardha *rabi*-2019						
Average	56.64	67.56	3.42	4.34	12.20	16.80
%increase over control	19.28		26.90		37.71	

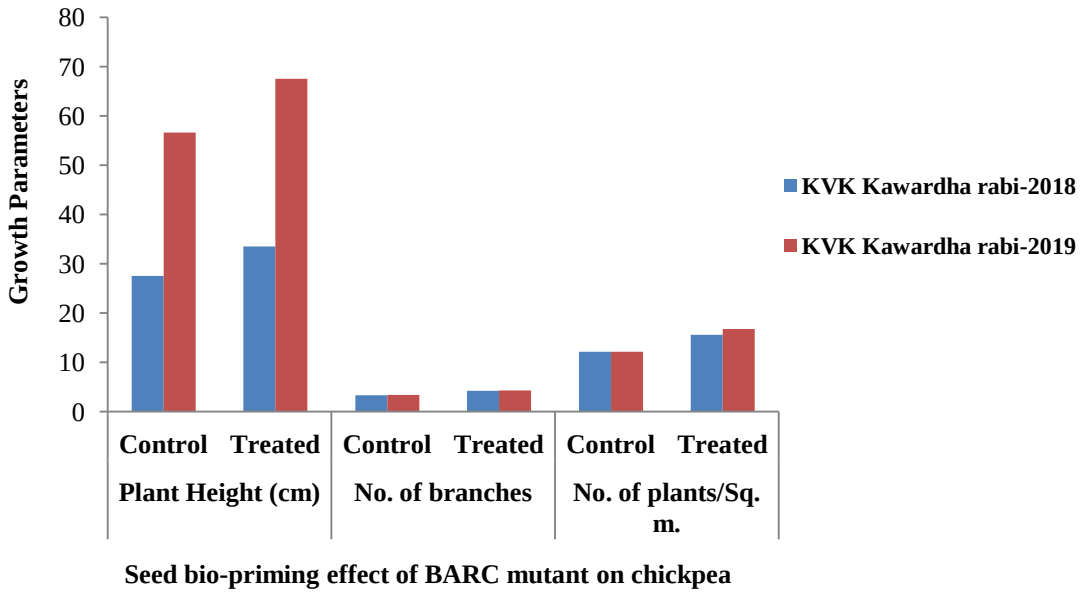


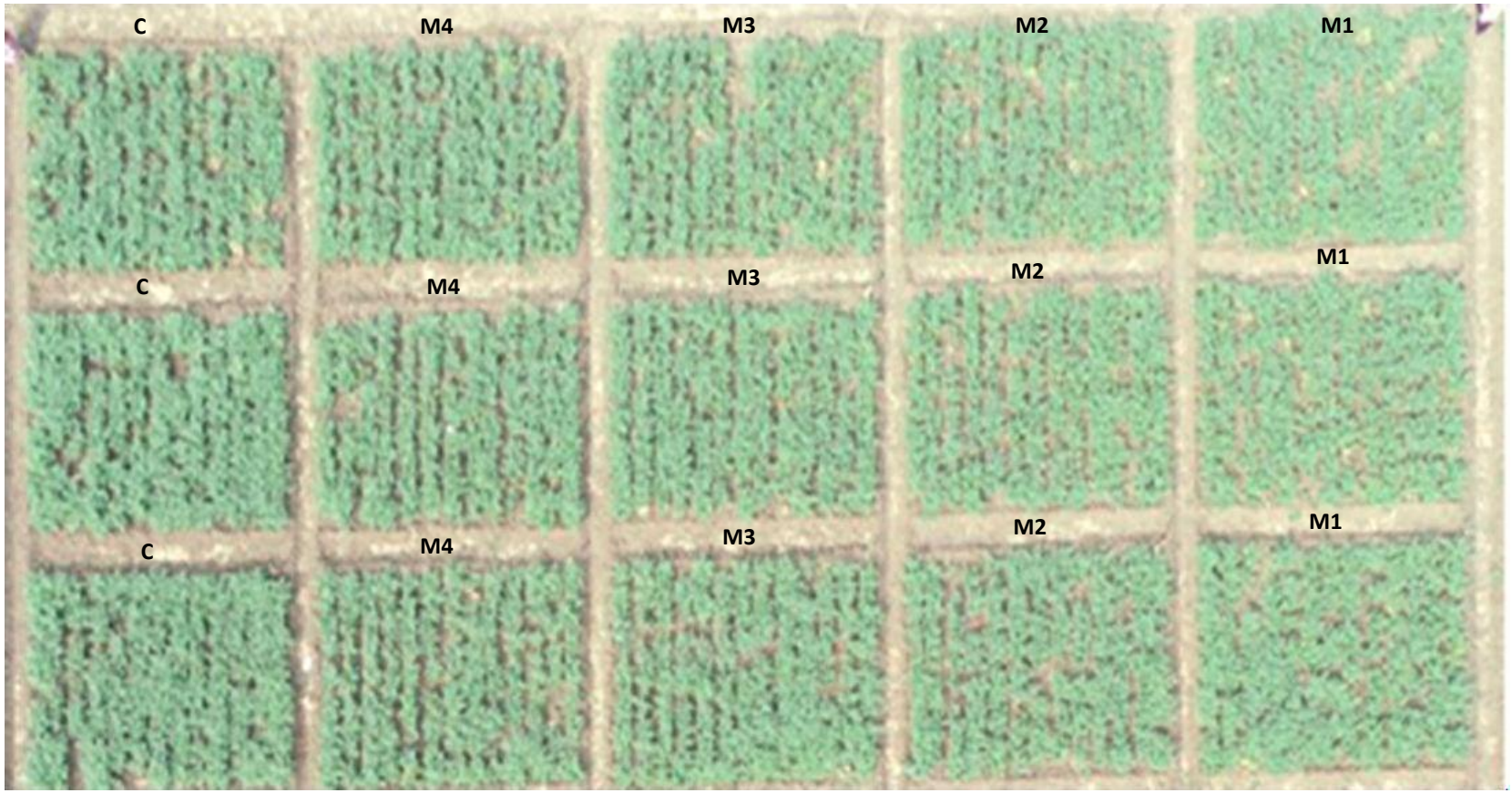
Figure 4.15: Effect of seed bio-priming with BARC mutant on growth and yield attributing characters of chickpea at KVK Kawardha field *rabi* 2018 and 2019

4.3.1 Plant growth promoting response in chickpea (Indira Chana-1) following seed bio-priming with potential *Trichoderma* mutants

In present investigation, four potential *Trichoderma* mutants were evaluated for their plant growth promotion ability and yield attributing characters following seed bio-priming in chickpea (Indira Chana-1).

Field Experiment

Field experiments were carried out at research cum instructional farm, College of Agriculture, I. G. K. V., Raipur during *kharif*-2019 to study the effect of seed bio-priming with potential *Trichoderma* mutants on growth and yield of chickpea. Based on the results of variance analysis of chickpea field data traits like primary branches, total filled pods, shoot length, bundle weight, grain yield and straw yield were significantly different at 5 % probability level. In field experiment, primary branches, total filled pods, shoot length, bundle weight, grain yield and straw yield in chickpea are influenced by four potential *Trichoderma* mutants presented in table 4.7 and plate 62-64.



Design: SPD
Replication: 3
Gross Plot: 18.75 ×32.5 sq. m.
Net Plot: 17× 29.7 sq. m.
Variety: Indira Chana- 1

R × R Distance: 30 cm
Sowing Date: 30/11/201
Harvesting Date: 25/3/
Seed per plot: 180 gm

Treatment Details:-

M1- Mutant M-18
M2- Mutant M-23
M3- MutantM-136
M4- BARC Mutant

Plate 4.62 Seed bio-priming effect of potential *Trichoderma* mutants on plant growth promoting

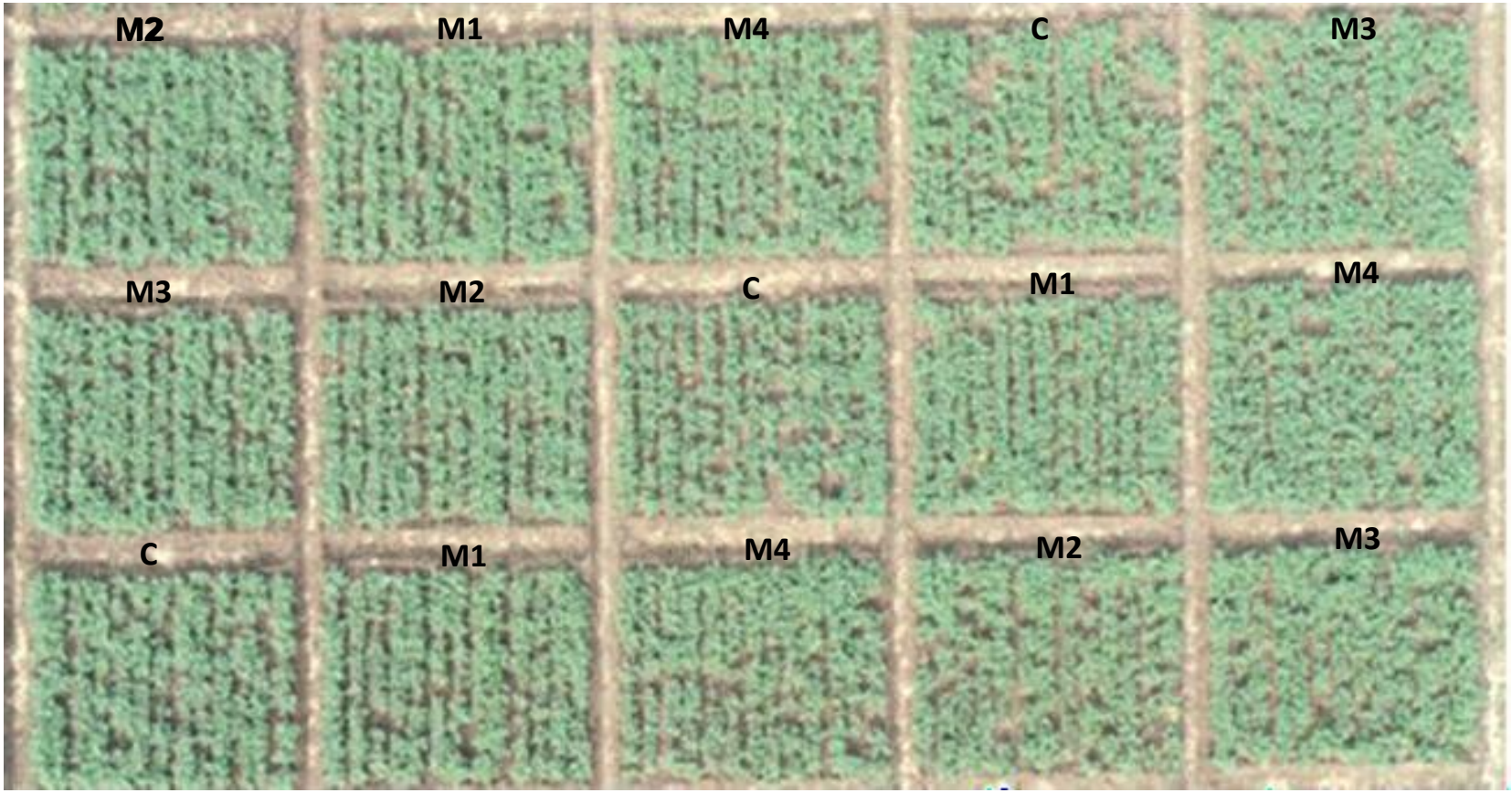


Plate 4.63 Seed bio-priming effect of potential *Trichoderma* mutants on plant growth promoting activity of Chickpea (Indira Chana-1) in KVK Raipur *rabi* 2019-2020 (Replication-2)

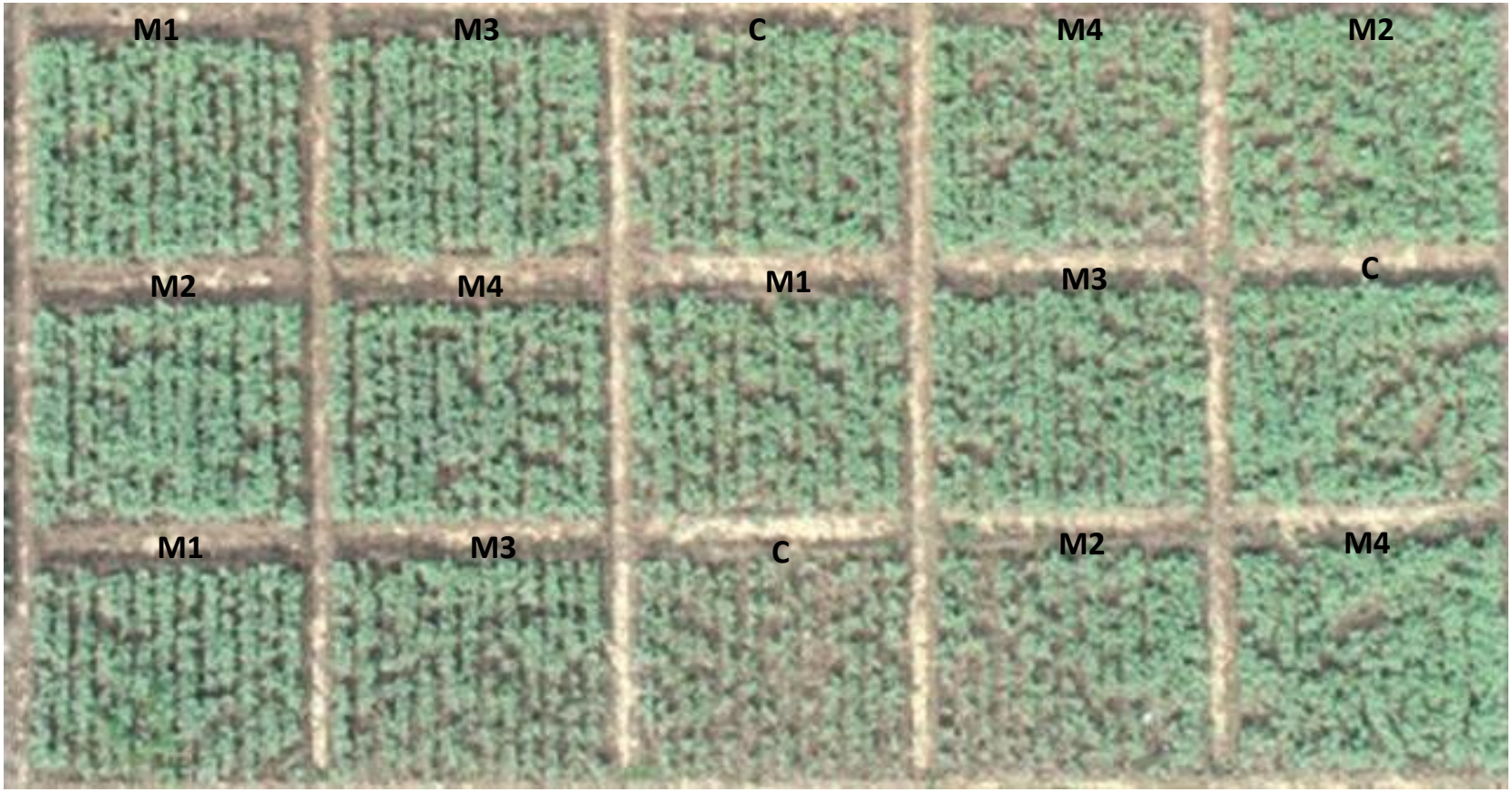


Plate 4.64 Seed bio-priming effect of potential *Trichoderma* mutants on plant growth promoting activity of Chickpea (Indira Chana-1) in KVK Raipur *rabi* 2019-2020 (Replication-3)

Significant increase in primary branches and total filled pods were observed in all treatments over control. The maximum number of primary branches (24.9) and total filled pods (238.5) were observed in chickpea plants seed bio-primed with mutant M-23 respectively. Seed bio-priming with potential *Trichoderma* mutants significantly affected shoot length and bundle weight (per five plants) over untreated/ control. The maximum shoot length (303.9 cm) and bundle weight (per five plants) (201.40 gm) was observed in chickpea plants seed bio-primed with mutant M-18 respectively (Figure 4.16).

Total bundle weight, total grain yield and total straw yield in chickpea plants seed bio-primed with potential *Trichoderma* mutants was markedly increased in comparison to untreated or control. Highest bundle weight (3465 kg/ha) was recorded in chickpea plants seed bio-primed with mutant M-18 which led to 15.15 per cent increase over control. Total grain yield in chickpea varied from (850-1220 kg/ha) table 4.7. Highest total grain yield was recorded in chickpea plants seed bio-primed with BARC mutant (1220 kg/ha) which led to 30.32 % increase over control (Figure 4.17).

Table 4.7 Effect of seed bio-priming with potential *Trichoderma* mutants on yield attributing characters of Chickpea (Indira Chana-1)

Treatments	Primary Branches (per 5 plant)	Total filled Pods (per 5 plant)	Shoot Legth (cm) (per 5 plant)	Bundle Weight (gm) (per 5 plant)	Total Bundle Weight (kg/ha)	Total Grain Yield (kg/ha)	Total Straw Yield (kg/ha)
T1 (M18)	21.2	222	303.9	201.4	3465 (15.15 %)	1100 (22.72 %)	2365
T2 (M-23)	24.9	238.5	299.9	190.8	3365 (12.63 %)	1160 (26.72 %)	2205
T3 (M-136)	21.7	194.4	296.1	165.1	3210 (8.41 %)	1195 (28.87 %)	2015
T4 (BARC)	23.3	210.4	297.08	189.7	3435 (14.41 %)	1220 (30.32 %)	2215
T5 (Control)	20.6	189.5	308.1	172.9	2940	850	2090
Mean	22.34	210.96	301	183.98	2051.87	1105	2178
SE(m)	0.6	6.38	2.53	6.5	0.21	0.09	0.09
SE(d)	0.85	9.03	3.57	9.19	0.29	0.13	0.13
CD@5%	1.99	21.15	8.38	21.52	0.68	0.31	0.31
CV	8.09	9.08	2.52	10.6	9.39	12.73	6.54

* All values are average of three replication
* Design: SPD, Plot Size: 5×4 m^2, Spacing: 30×10 cm, Total Plot: 45
* Date of Sowing: 30/11/2019
* Date of Observation: 25/3/2020
***** Variety: Indira Chana-1
* Experiment location: KVK Raipur field
* Figure in parenthesis showing per cent increase over control for different yield parameters

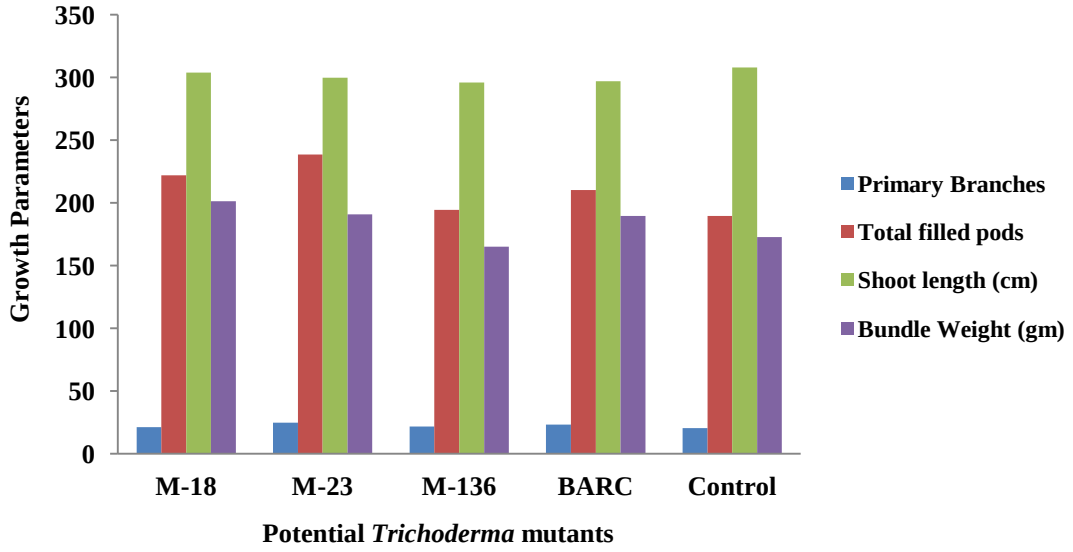


Figure 4.16: Effect of seed bio-priming of potential *Trichoderma* on growth and yield attributing characters of chickpea

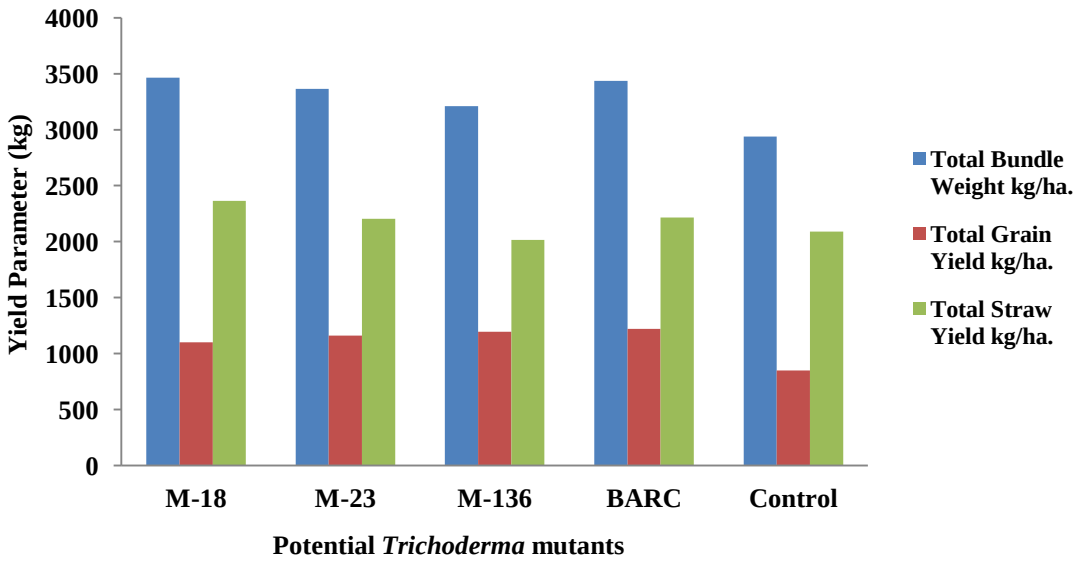


Figure 4.17: Effect of seed bio-priming of potential *Trichoderma* on yield attributing characters of chickpea

4.3.1.1 Plant growth promotion response in chickpea (Indira Chana-1) following seed bio-priming with potential *Trichoderma* mutants on field emergence and mortality of chickpea plants (Indira chana-1)

In present investigation effect of seed bio-priming with potential *Trichoderma* mutants on mortality of chickpea plants were studied. In comparison with the untreated control, all the seed bio-priming treatments with potential *Trichoderma* mutants significantly enhanced growth and germination of chickpea (Indira Chana-1) table 4.8. Total number of healthy plants, mortal and wilted plants were recorded in field plots 30 days after sowing of chickpea. Highest number of healthy plants (1,492.66^{a}±15.21) and % increase over control (11.99 %) were observed in the chickpea plots seed bio-primed with mutant M-18 followed by seed bio-priming with BARC mutant (1491.66^{a}±83.43, 11.93 %), M-136 (1484.66^{a}±80.42, 11.51 %) and M-23 (1478.33^{a} ± 11.13 %) respectively. Similarly, highest number of wilted and mortal plants (67.33±2.33) were observed in control plot of chickpea. Whereas, lowest number of wilted and mortal plants (50.66±4.05) and highest % reduction of mortal and wilted plants (24.75 %) was observed in the chickpea plots seed bio-primed with BARC mutant followed by M-18 (54.66±8.76, 18.81 %), M-136 (56.66±15.84) and M-23 (59.33±10.34, 11.88 %) respectively (Figure 4.18 and 4.19).

Table 4.8 Effect of seed bio-priming with potential *Trichoderma* mutants on field emergence and mortality of chickpea plants (Indira chana-1)

Treatments	Healthy Plants	% Increase over control	Wilted Plant	% Reduction over control
M-18	1,492.66^{a}±15.21	11.99	54.66±8.76	18.81
M-23	1,478.33^{a}±38.19	11.13	59.33±10.34	11.88
M-136	1,484.66^{a}±80.42	11.51	56.66±4.91	15.84
BARC	1,491.66^{a}±83.43	11.93	50.66±4.05	24.75
Control	1,313.66^{b}±80.85	-	67.33±2.33	-
CD@5%	123.77		NS	
CV	4.45		10.92	
SE(m)	37.37		3.64	

* All values are average of three replication
* Design: SPD, Plot Size: 5×4 m^2, Spacing: 30×10 cm, Total Plots: 45
* Date of Sowing: 25/11/2019
* Date of Observation: 25/1/2020
***** Variety: Indira Chana-1
***** Experiment location: KVK Raipur field

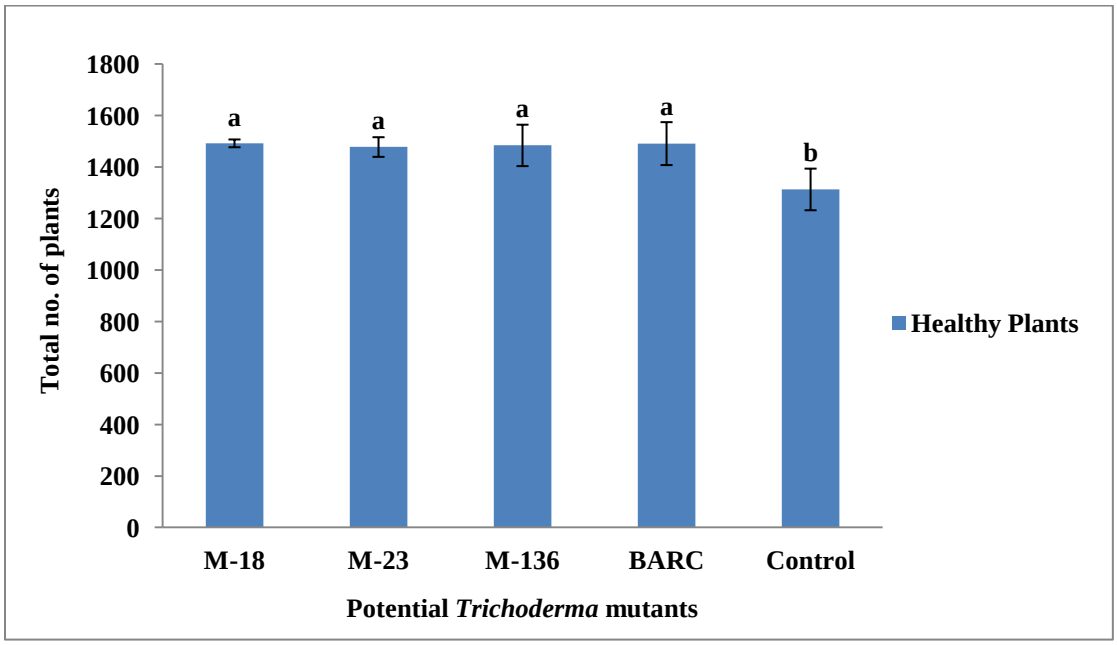


Figure 4.18 : Effect of seed bio-priming with potential *Trichoderma* mutants on total healthy plant stand in chickpea (Indira Chana-1)

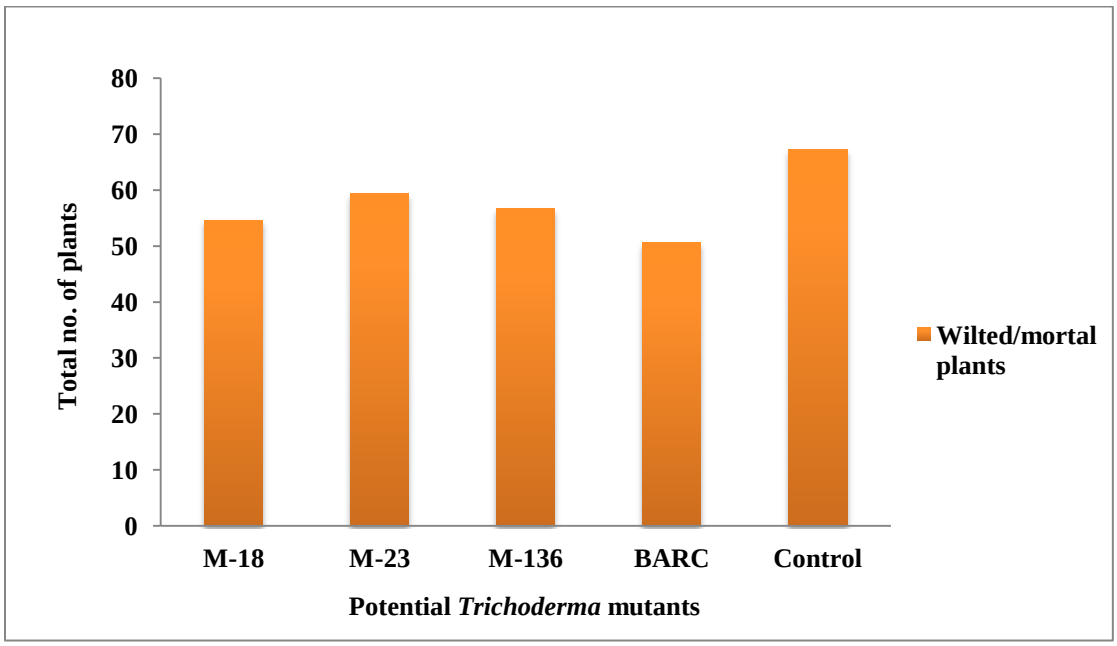


Figure 4.19 : Effect of seed bio-priming with potential *Trichoderma* mutants on total mortal and wilted plants in chickpea (Indira Chana-1)

4.3.2. Plant growth promotion response in chickpea (Indira Chana-1) following seed treatment with BARC mutant and Propiconazole

Seed bio-priming with *Trichoderma* not only help to enhance the germination of seedling at the field condition but also protects the seeds from huge number of seed borne and soil borne pathogens. It also control pre as well as post emergence death of seedlings. So the successful establishment of seedling in the field condition is increased by the seed bio-priming that ultimately leads to increased crop yield (Hossain, 2009). Application of different chemical fungicides (propiconazole and sulphur) helps to improve per cent survival of seedlings and field emergence of seedlings and thus contributes towards better stand establishment and increased yield (Sarkar and Saxena, 2005). So application of fungicides along with *Trichoderma* through seed treatment best on compatibility between them is an effective tool for rapid and uniform seedling emergence with increased survival rate of crop plants which ultimately leads to increase yield.

Field Experiment

Field experiments were carried out at research cum instructional farm, College of Agriculture, I. G. K. V. Raipur during *kharif*-2019 to study the Effect of seed treatment with BARC mutant along with Propiconazole on the plant growth promotion response in chickpea (Indira Chana-1). Based on the results of variance analysis of chickpea field data traits like bundle weight, no. of primary branches, filled pods, 100 seed weight, total bundle weight and total grain yield were significantly different at 5 % probability level (Table 4.9) (Plate 65 and 66).

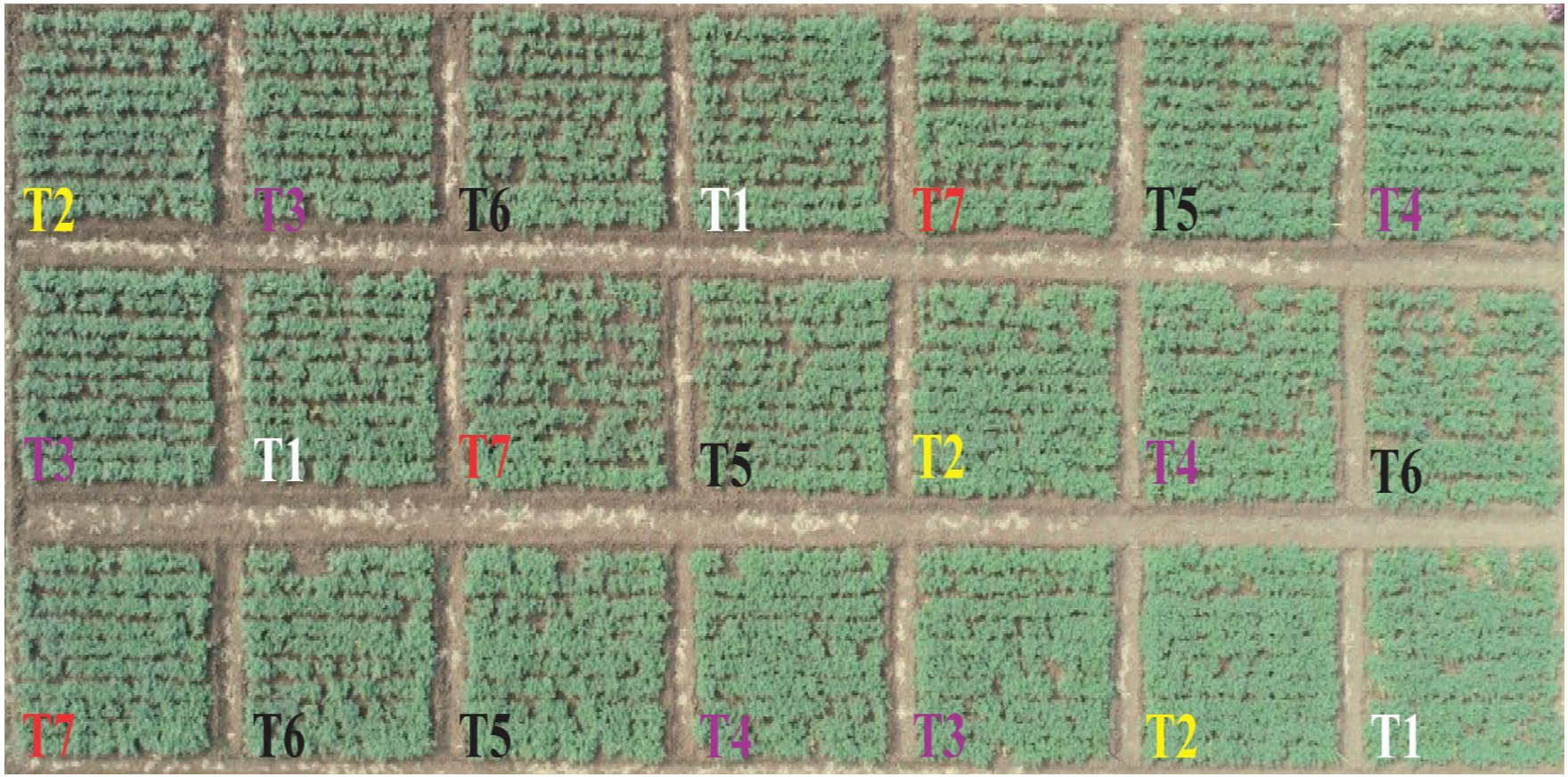
In comparison with the untreated control, all the combinations of BARC mutant with propiconazole showed significant differences on growth parameters bundle weight, primary branches and filled pods per five plants. Significant differences were also observed in total bundle weight and total grain weight of chickpea plant. Significant increase in bundle weight per five plants was observed in all the treatments over control.

Highest bundle weight per five plant was recorded in chickpea plants treated with T_5 (Tricho BARC 10g/kg seed + Propiconazole 3.0ml/kg seed) combination

Control Plot

Treated Plot

Plate 4.65 Effect of seed bio-priming with BARC mutant and Propiconazole on field emergence and yield attributes of Chickpea (Indira Chana-1)



Design: RBD
Replication: 3
Gross Plot: 18.75 ×32.5 sq. m.
Net Plot: 17× 29.7 sq. m.
R × R Distance: 30 cm
Sowing Date: 25/11/2019
Harvesting Date: 5/4/2020
Seed per plot: 180 gm Variety: Indira Chana- 1

Plate 4.66 Effect of seed bio-priming with BARC mutant and Propiconazole on field emergence and yield attrib attributes of Chickpea (Indira Chana-1)

(235.66^{a}±6.23 gm), followed by T_6 (227.66^{ab}±6.02 gm), T_3 (218.33^{bc}±4.36 gm), T_4 (212^{c}±5.60 gm), T_2 (210.66^{cd}±13.81 gm) and T_1 (205.66^{cd}±7.41 gm) respectively. Seed treatment with BARC mutant and propiconazole had significantly affected number of filled pods per five plants of chickpea over control. Highest number of filled pods was seen in chickpea plants treated with T_5 (Tricho BARC 10g/kg seed + Propiconazole 3.0ml/kg seed) combination (245.66^{a}±4.80), which lead to 19.67 per cent increase in filled pods over control, followed by T_6 (19.45 %), T_3 (9.06 %), T_2 (6.77 %), T_4 (5.88 %) and T_1 (5.58 %). Whereas, maximum number of primary branches per five plants (24^{a}±1.15), shoot length (313.76^{a}±14.03 cm) and 100 seed weight (23.33^{a}±0.33 gm) was recorded in treatments T_6, T_5, T_3 and T_4 respectively. Bundle weight, straw yield and grain yield in chickpea plants treated with BARC mutant and propiconazole combination was markedly increased in comparison to untreated or control (Figure 4.20 and 4.21)

Bundle weight, grain yield and straw yield was varied from (3426.50 to 4181.50 kg/ha), (1315.00 to 1660.00 kg/ha) and (2110.50 to 2555 kg/ha) respectively. Highest bundle weight (4181.50 kg/ha) and grain yield (1660.00 kg/ha) was recorded in chickpea plants treated with treatment T_3 (propiconazole @ 3 ml/kg of seed dose) respectively. Whereas, highest straw yield was recorded in chickpea plants treated with treatment T_1 (Tricho BARC 5gm per kg seed dose). Treatment T_3 led to 20.78 per cent and 18.05 per cent increase in total grain yield and total bundle weight over control or untreated chickpea plants (Figure 4.22).

4.3.2.1 Plant growth promotion response in chickpea (Indira Chana-1) following seed treatment with BARC mutant and Propiconazole on field emergence and mortality of chickpea plants (Indira chana-1)

In present investigation effect of seed treatment with BARC mutant and combination of BARC mutant + propiconazole on mortality of chickpea plants were studied. In comparison with the untreated control, all the treatments with BARC mutant and combination of BARC mutant + propiconazole significantly enhanced growth and germination of chickpea (Indira Chana-1) table 4.10.

Table 4.9 Effect of seed treatment with BARC mutants in combination with propiconazole on yield attributing characters of Chickpea (Indira Chana-1)

Treatments	Bundle Weight (gm) (Per 5 Plants)	Primary Branches (Per 5 Plants)	Filled Pods (Per 5 Plants)	Shoot Length (cm) (Per 5 Plants)	100 seed weight (gm) (Per 5 Plants)	Total Bundle Weight (kg/ha)	Total Grain Yield (kg/ha)	Total Straw Yield (kg/ha)
T1	205.66^{cd}±7.41	20.66^{bc}±1.20	209^{b}±22.12 (5.58 %)	293.43^{b}±7.24	21.66^{a}±0.88	3956.50 (13.39 %)	1401.50 (6.17 %)	2555.00
T2	210.66^{cd}±13.81	19.66^{c}±0.88	211.66^{b}±12.78 (6.77 %)	310.20^{a}±9.15	22^{a}±0	3750.00 (8.62 %)	1521.50 (13.57 %)	2228.50
T3	218.33^{bc}±4.36	20.66^{bc}±2.33	217^{ab}±22.33 (9.06 %)	302.26^{ab}±16.17	23.33^{a}±0.33	4181.50 (18.05 %)	1660.00 (20.78 %)	2521.50
T4	212^{c}±5.60	20^{c}±0.57	209.66^{b}±8.19 (5.88 %)	299.06^{ab}±15.63	23.33^{a}±0.33	3860.00 (11.23 %)	1630.00 (19.32 %)	2230.00
T5	235.66^{a}±6.23	23.33^{ab}±3.18	245.66^{a}±4.80 (19.67 %)	313.76^{a}±14.03	22.66^{a}±1.33	3950.00 (13.25 %)	1608.50 (18.24 %)	2341.50
T6	227.66^{ab}±6.02	24^{a}±1.15	245^{a}±5.68 (19.45 %)	312.76^{a}±14.98	21.66^{a}±0.66	3666.50 (6.54 %)	1456.67 (9.72)	2210.00
T7	196.66^{d}±5.20	18.66^{c}±1.20	197.33^{b}±14.19	289.4^{b}±2.55	19.33^{b}±0.33	3426.50	1315.00	2110.50
C.D. @ 5 %	14.19	2.93	33.50	15.93	2.23	0.70	0.29	0.53
SE(m)	4.55	0.94	10.75	5.11	0.72	0.22	0.09	0.17
SE(d)	6.44	1.33	15.20	7.23	1.02	0.32	0.13	0.24
C.V.	3.66	7.76	8.49	2.92	5.69	5.14	5.38	6.47

* All values are average of three replication
* Design: RBD, Plot Size: 5×4 m^2, Spacing: 30×10 cm, Total Plots: 21
* Date of Sowing: 25/11/2019
* Date of Observation: 20/3/2020
***** Variety: Indira Chana-1
* Experiment location: KVK Raipur field
* Figure in parenthesis showing per cent increase over control value

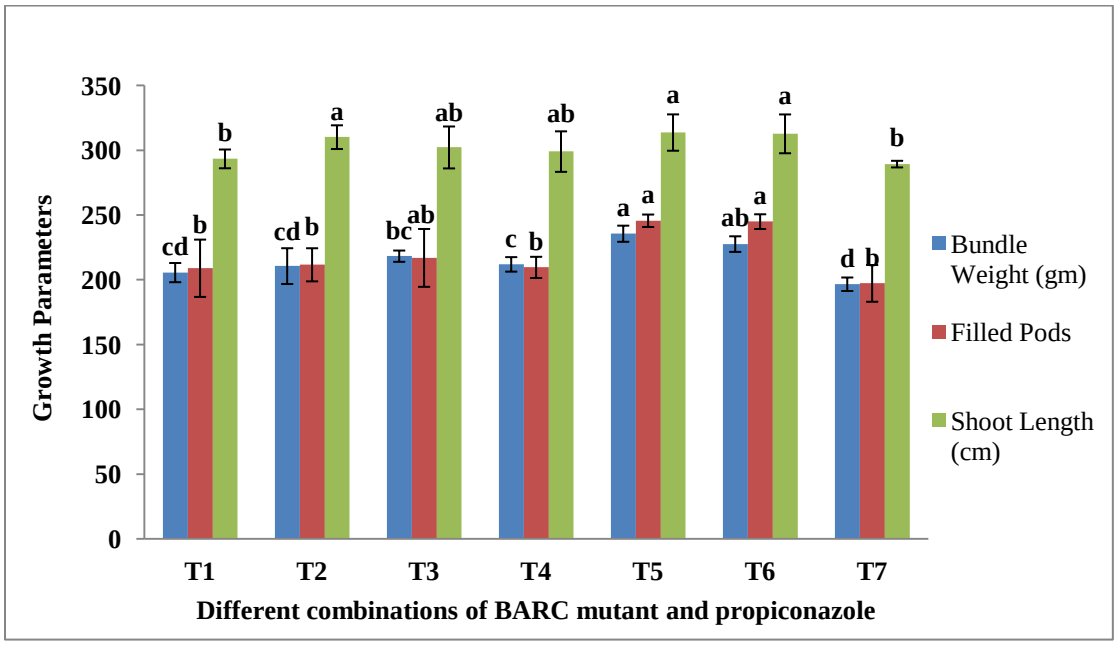


Figure 4.20 : Effect of seed treatment of BARC mutant, propiconazole and its combinations on growth attributing characters of chickpea

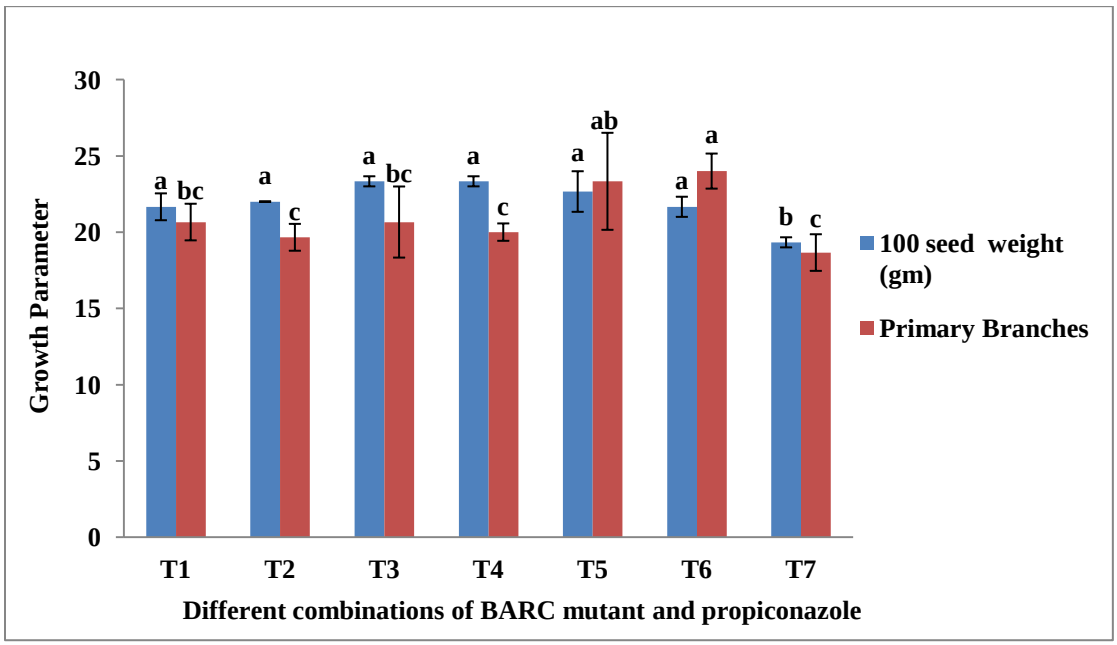


Figure 4.21: Effect of seed treatment of BARC mutant, propiconazole and its combinations on growth attributing characters of chickpea

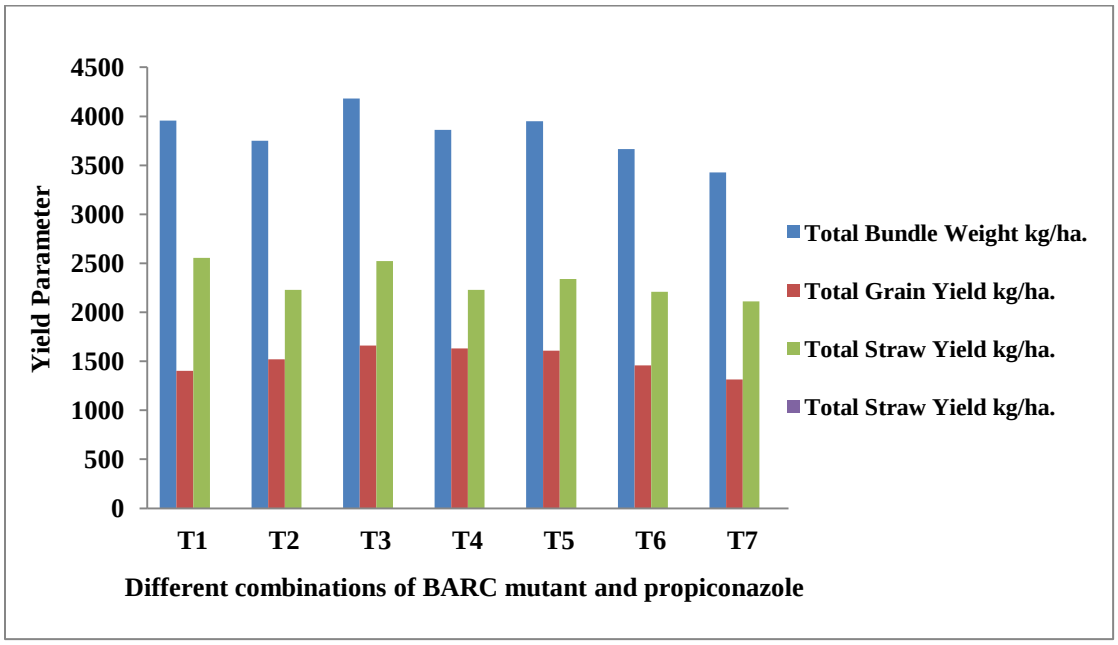


Figure 4.22 : Effect of seed bio-priming of BARC mutant, propiconazole and its combinations yield attributing characters of chikpea

Total number of healthy plants, mortal and wilted plants were recorded in field plots 27 days after sowing of chickpea. Highest number of healthy plants ($500.66^{a}\pm1.76$) and % increase over control (17.44 %) were observed in the chickpea plots seed treated with treatment T_2 (Tricho BARC @ 10 gm/kg of seed) followed by T_5 ($477.33^{ab}\pm24.04$, 13.40 %), T_4 ($476.00^{ab}\pm27.15$, 13.16 %), T_3 ($469.33^{bc}\pm7.42$, 11.93 %), T_1 ($449.66^{bc}\pm12.70$, 8.07) and T_6 ($445.00^{c}\pm14.43$, 7.11 %) respectively. Similarly, highest number of wilted and mortal plants ($22.33^{a}\pm4.41$) were observed in control plot of chickpea. Whereas, lowest number of wilted and mortal plants ($14.33^{d}\pm2.40$) and highest % reduction of mortal and wilted plants (35.82 %) was observed in the chickpea plots seed treated with treatment T_3 (propiconazole @ 3ml/kg of seed dose) followed by T_5 ($15.33^{cd}\pm0.88$, 31.34 %), T_2 ($17.66^{bcd}\pm2.02$, 20.91 %), T_6 ($18.33^{bc}\pm0.33$, 17.91 %), T_1 ($19.00^{abc}\pm1.52$, 14.91 %) and T_4 ($20.66^{ab}\pm1.20$, 7.47 %) respectively (Figure 4.23 and 4.24).

Table 4.10 Effect of seed treatment with BARC mutant and Propiconazole on field emergence and mortality of chickpea plants (Indira chana-1)

Treatments	Healthy Plants	% Increase over control	Wilted Plant	% Reduction over control
T_1	449.66^{bc}±12.70	8.07	19.00^{abc}±1.52	14.91
T_2	500.66^{a}±1.76	17.44	17.66^{bcd}±2.02	20.91
T_3	469.33^{bc}±7.42	11.93	14.33^{d}±2.40	35.82
T_4	476.00^{ab}±27.15	13.16	20.66^{ab}±1.20	7.47
T_5	477.33^{ab}±24.04	13.40	15.33^{cd}±0.88	31.34
T_6	445.00^{c}±14.43	7.11	18.33^{bc}±0.33	17.91
T_7	413.33^{d}±19.05	-	22.33^{a}±4.41	-
CD @ 5 %	29.41		3.75	
CV	3.54		11.55	
SE(m)	9.44		1.21	

* All values are average of three replication
* Design: RBD, Plot Size: 5×4 m^2, Spacing: 30×10 cm, Total Plots: 21
* Date of Sowing: 25/11/2019
* Date of Observation: 21/1/2020
***** Variety: Indira Chana-1
* Experiment location: KVK Raipur field

4.3.3 Efficacy of 4 potential mutants of *Trichoderma* for plant growth promoting activity in different vegetable crops

Plants of different vegetable crops (Bitter gourd, Pumpkin, Long bean, Ridge gourd, Fenugreek, Tomato and Spinach) derived after seed bio priming with four potential *Trichoderma* mutants were evaluated for improvement in Plant Height (cm) and No. of leaves/ plant and root length(cm). Observations on Root length (cm) at 50 DAS. The effect of bio-priming with 4 potential mutants of *Trichoderma* on seven vegetable crops (Bitter gourd, Pumpkin, Long bean, Ridge gourd, Fenugreek, Tomato and Spinach) showed significant increase in plant growth.

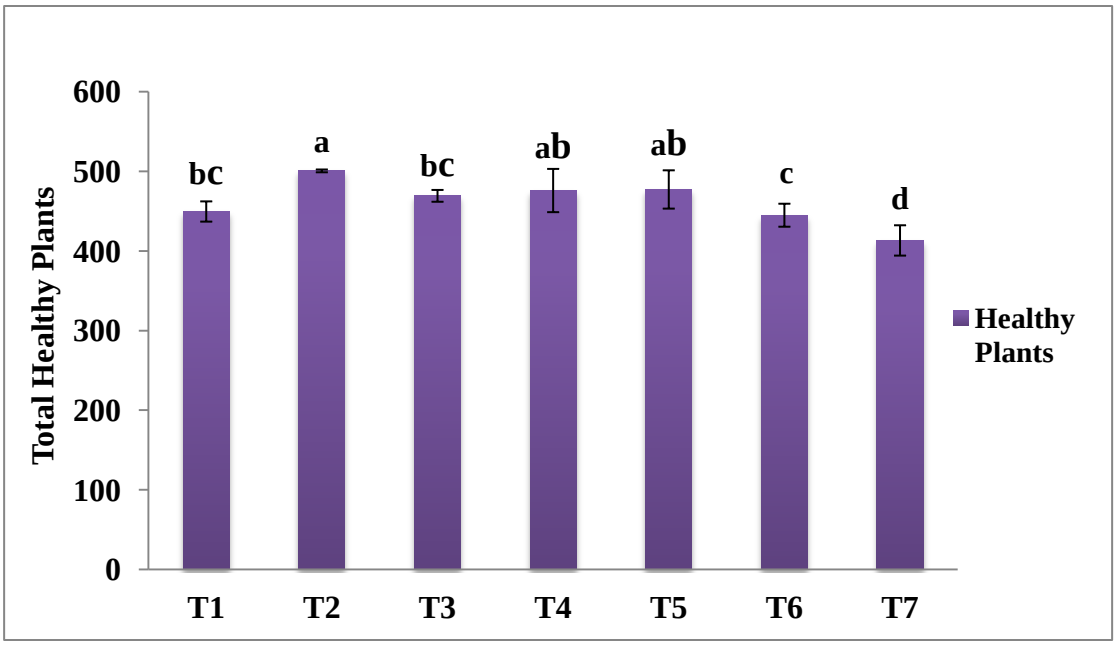


Figure 4.23 : Effect of seed bio-priming with BARC mutant and propiconazole, and its combination on total healthy plant stand in chickpea (Indira Chana-1)

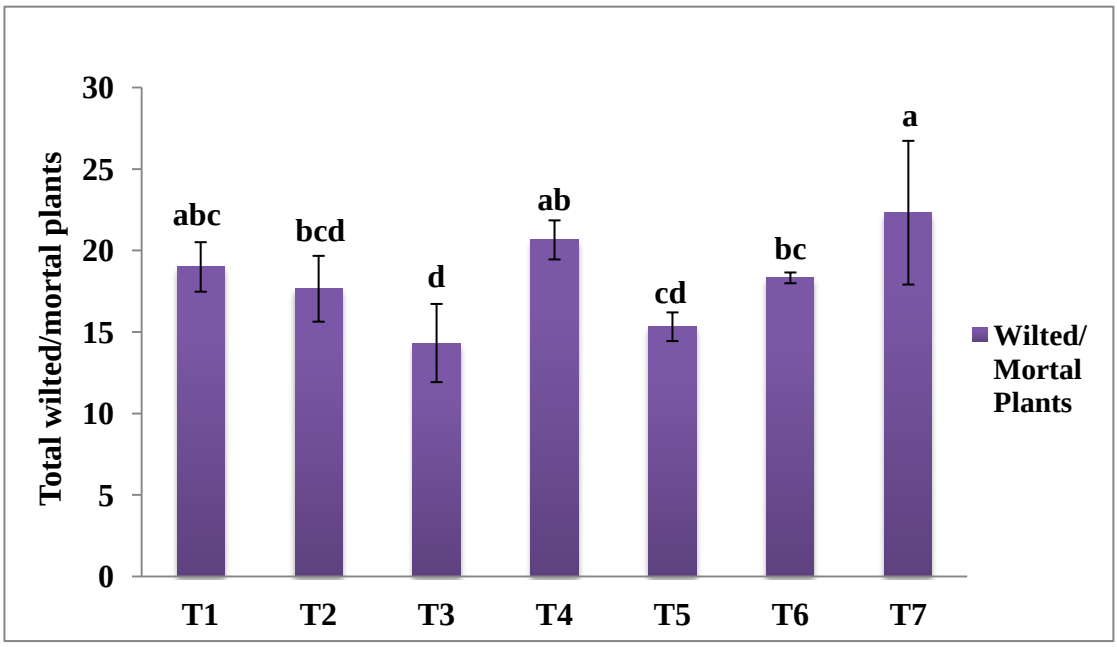


Figure 4.24 : Effect of seed bio-priming with BARC mutant and propiconazole, and its combination on total wilted and mortal plants in chickpea (Indira Chana-1

4.3.3.1 Plant growth promoting activity of potential mutants of *Trichoderma* on Bitter gourd (*Momordica charantia* Pearl F-1 Hybrid)

Potential *Trichoderma* mutants stimulated significant increase in plant growth of bitter gourd plants. Plant growth stimulating effects of 4 potential mutants on bitter gourd varied. Maximum shoot length (195^{a}±10 cm), leaf area(cm^2) (104.5^{a}±5.55 cm^2), total branches(47^{a}±1) were recorded when bitter gourd plant were derived from seeds bio-primed with mutant M-18 as compared to control. Whereas longest root length (69^{a}±1 cm) and maximum no. of flowers (25^{a}±1) were observed in plants derived from seed bio-primed with isolate M-136 and M-23 respectively as compared to control (Table 4.11, Figure 4.25- 4.28, Plate 67-68).

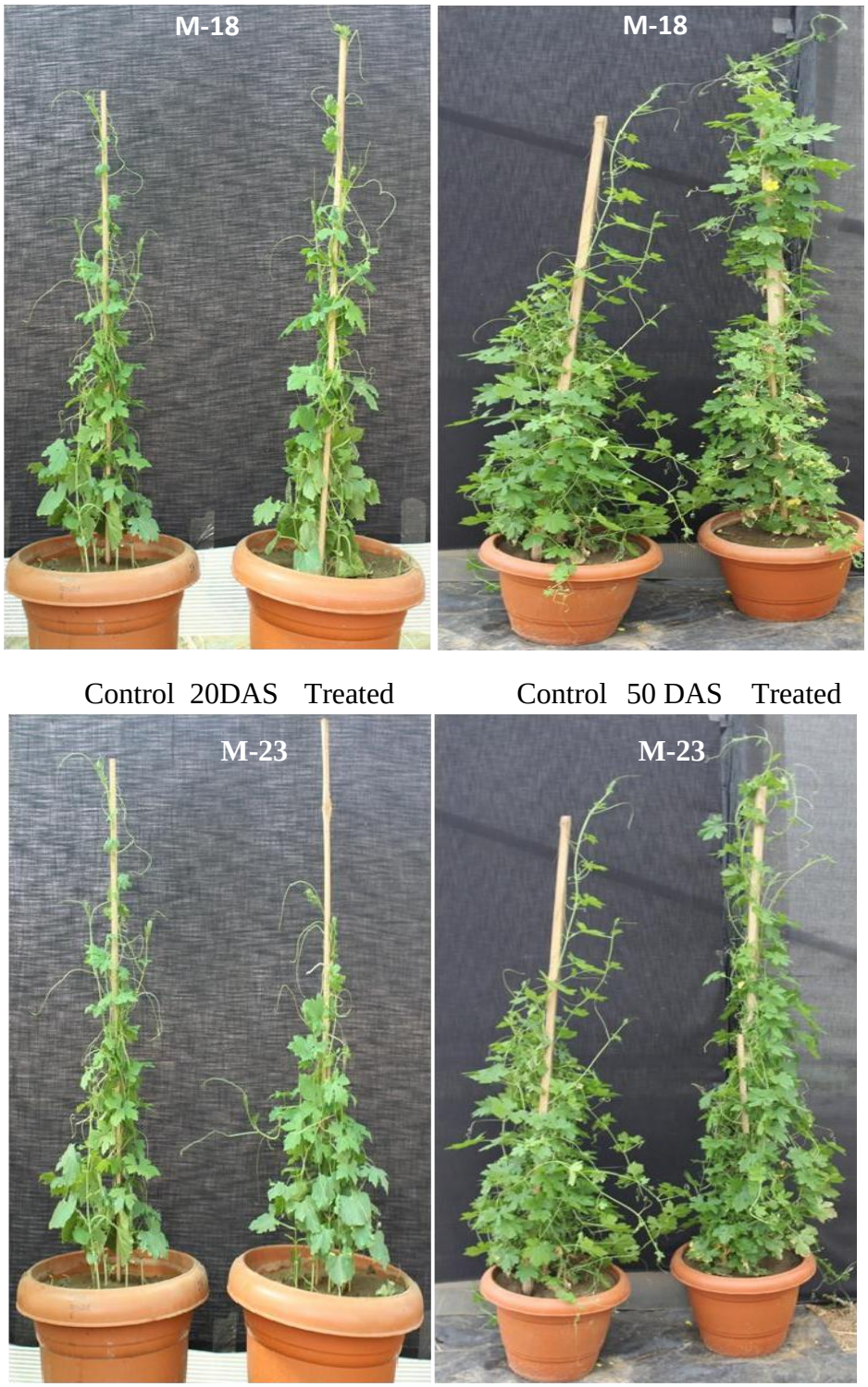
Table 4.11 Efficacy of different potential mutants for plant growth promoting activity in Bitter gourd (*Momordica charantia* Pearl F-1 Hybrid)

Treatment	Shoot Length* (cm)	Leaf Area* (cm^2)	No. of flowers*	Total Branches*	Root length (cm)*
M-18	195^{a}±10	104.5^{a}±5.55	23.5^{a}±1.5	47^{a}±1	62^{ab}±1
M-23	175^{ab}±5	82.64^{bc}±2.02	25^{a}±1	43.5^{ab}±1.5	63^{a}±1
M-136	190^{a}±5	91.48^{b}±2.19	22.5^{a}±0.5	41^{bc}±1	69^{a}±1
BARC	175^{ab}±5	92.87^{b}±1.67	17^{b}±1	39.5^{c}±1	52^{bc}±1
Control	150^{b}±10	78.04^{c}±2.38	15^{b}±1	39^{c}±0.5	50.25^{c}±1.75
CD@5%	27.56	11.54	3.89	3.89	4.41
CV	5.92	4.88	7.2	1.04	2.88
SE(m)	7.41	3.10	1.04	3.53	1.18

***Average value of two replications**

4.3.3.2 Plant growth promoting activity of potential mutants of *Trichoderma* on Pumpkin (*Cucurbita moschata* Pearl F-1 Hybrid)

Potential *Trichoderma* mutants stimulated significant increase in plant growth of pumpkin. Plant growth stimulating effects of 4 potential mutants on bitter gourd varied. Maximum shoot length (196.5^{a}±1.5cm), leaf area (cm^2) (251^{a}±3 cm^2), and root length (94^{a}±1cm) were recorded when pumpkin plant were derived from seeds bio-primed with mutant M-18 as compared to control. Potential *Trichoderma* mutants were not able to stimulate significant differences in no. of flowers and total no. of branches as compared to control (Table 4.12, Figure 4.29- 4.32, Plate 69 and 70).



Control 20DAS Treated Control 50 DAS Treated

Control 20 DAS Treated Control 50 DAS Treated

Plate 4.67 Effect of seed bio-priming with potential *Trichoderm* mutants M-18 and M-23 on plant growth promoting activity of Bitter gourd

Control 20DAS Treated Control 50 DAS Treated

Control 20 DAS Treated Control 50 DAS Treated

Plate 4.68 Effect of seed bio-priming with potential *Trichoderma* mutants M-136 and BARC mutant on plant growth promoting activity of Bitter gourd

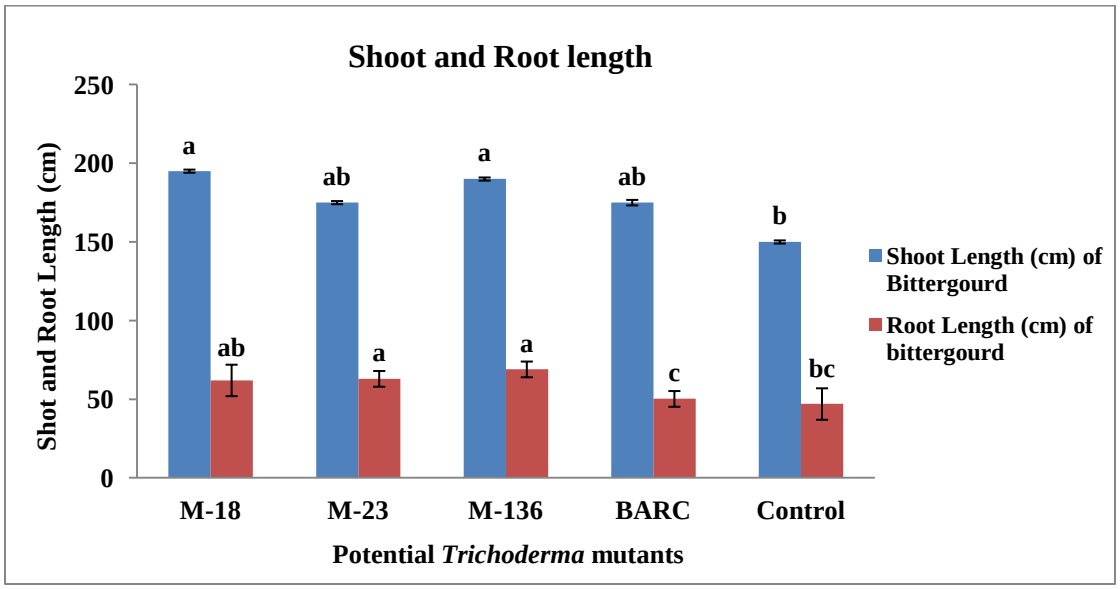


Figure 4.25 : Screening of potential *Trichoderma* mutants to induce shoot and root length in Bitter Gourd

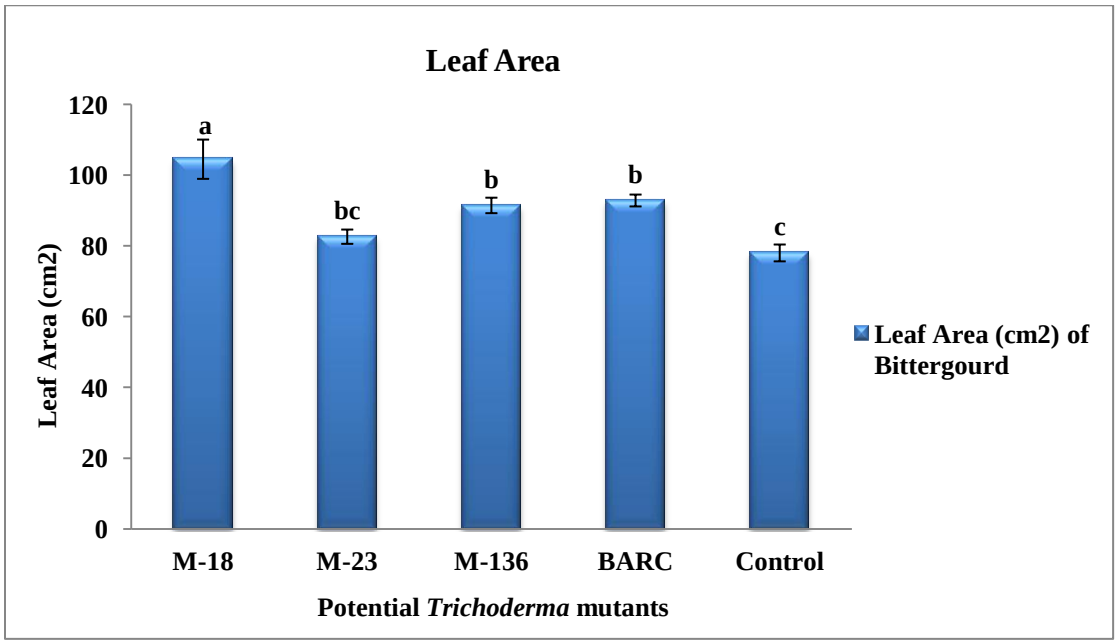


Figure 4.26 : Screening of potential *Trichoderma* mutants to induce leaf area in Bitter Gourd

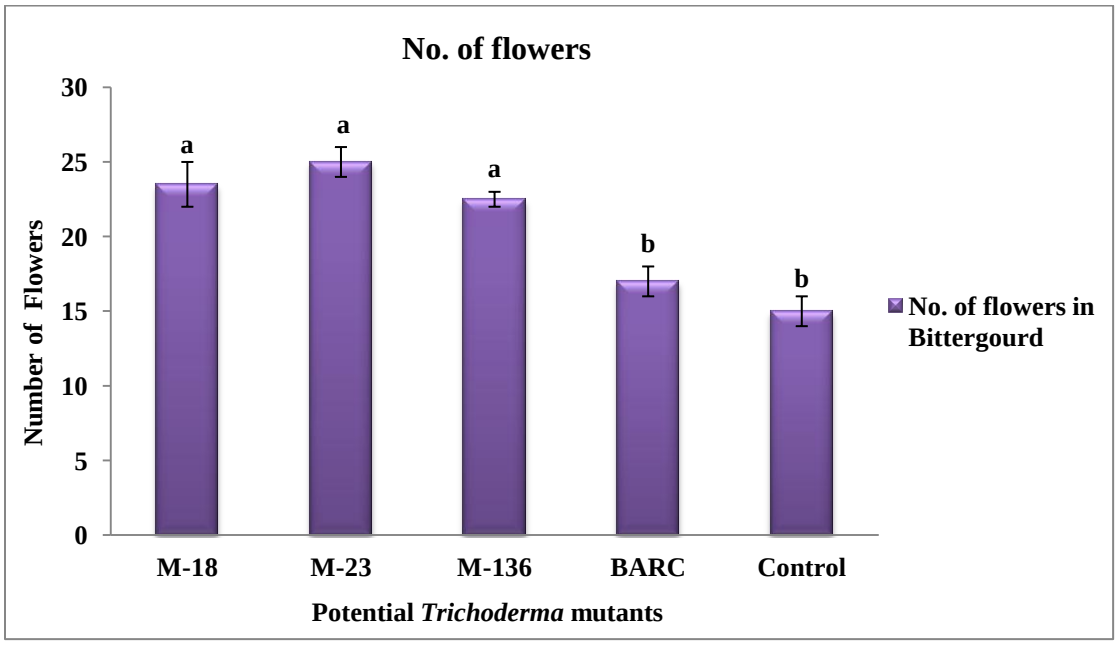


Figure 4.27 : Screening of potential *Trichoderma* mutants to induce No. of flowers in Bitter Gourd

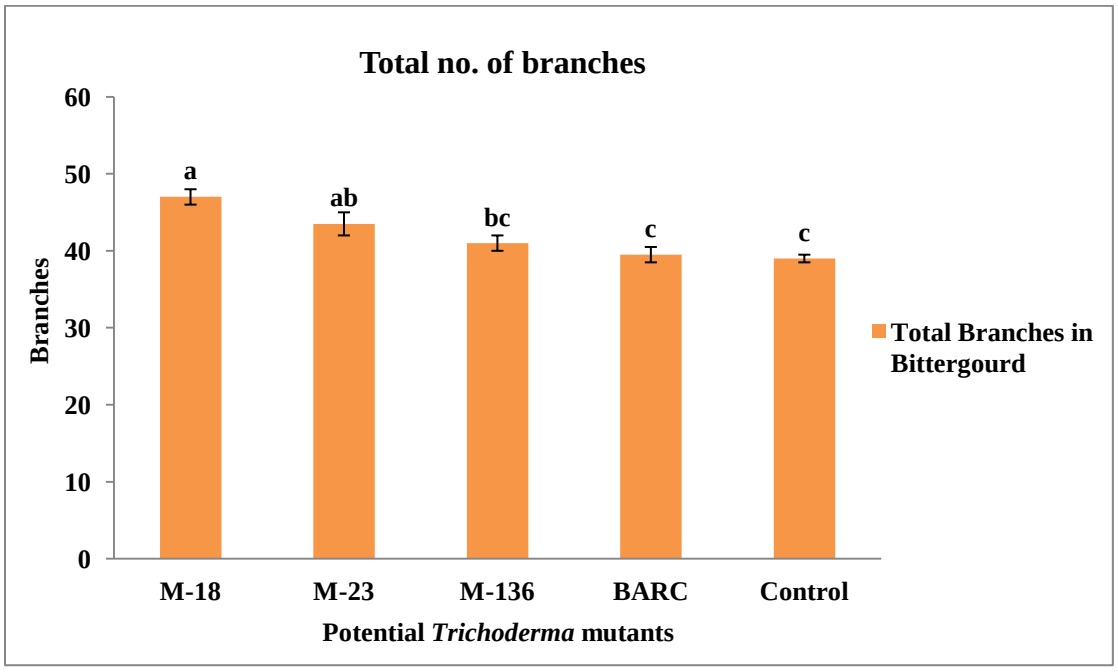


Figure 4.28 : Screening of potential *Trichoderma* mutants to induce total no. of branches in Bitter Gourd

Table 4.12 Efficacy of different potential mutants for plant growth promoting activity in Pumpkin (*Cucurbita moschata* Pearl F-1 Hybrid)

Treatment	Shoot Length (cm)	Leaf Area (cm^2)	No. of flowers	Total Branches	Root length (cm)
M-18	196.5^{a}±1.5	251^{a}±3	5±1	8±1	94^{a}±1
M-23	162.5^{b}±2.5	212.1^{b}±2	4±1	7.5±0.5	93.5^{a}±1.5
M-136	166.5^{b}±1.5	216.5^{b}±1.5	3.5±0.2	6.5±0.5	90^{a}±1
BARC	127.5^{c}±2.5	213.5^{b}±1.5	3±0	6±1	69.5^{b}±2.5
Control	107.5^{d}±7.5	194^{c}±4	2±0	4.5±0.5	49.75^{c}±2.25
CD@5%	14.22	9.62	NS	NS	6.55
CV	3.55	1.68	27.10	16.13	3.14
SE(m)	3.82	2.58	0.67	0.74	1.76

***Average value of two replications**

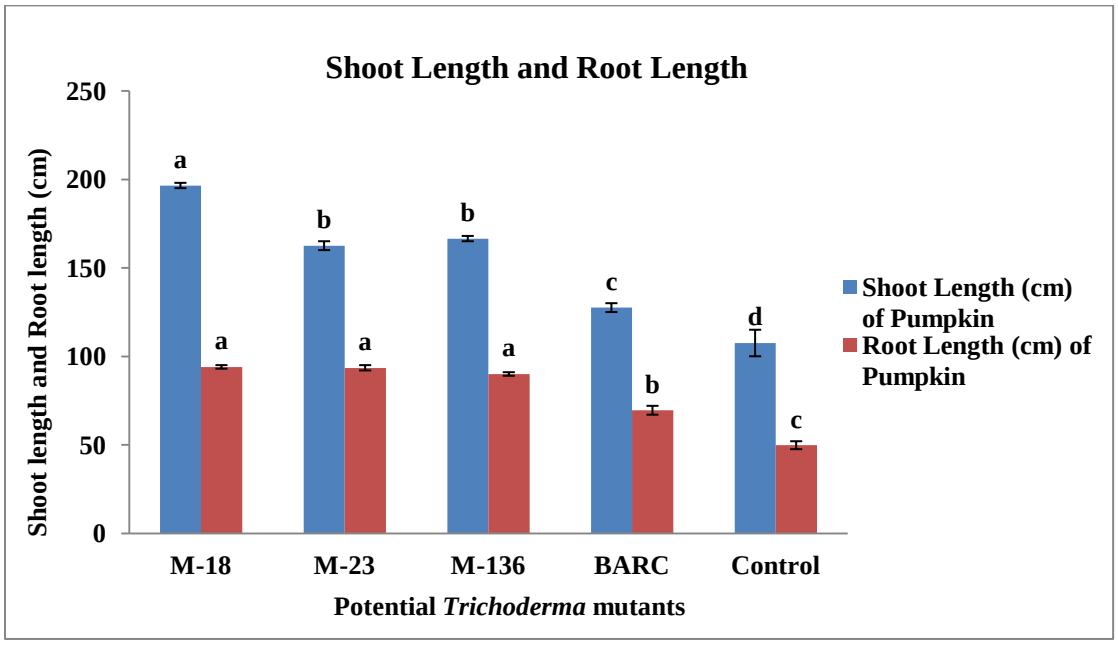
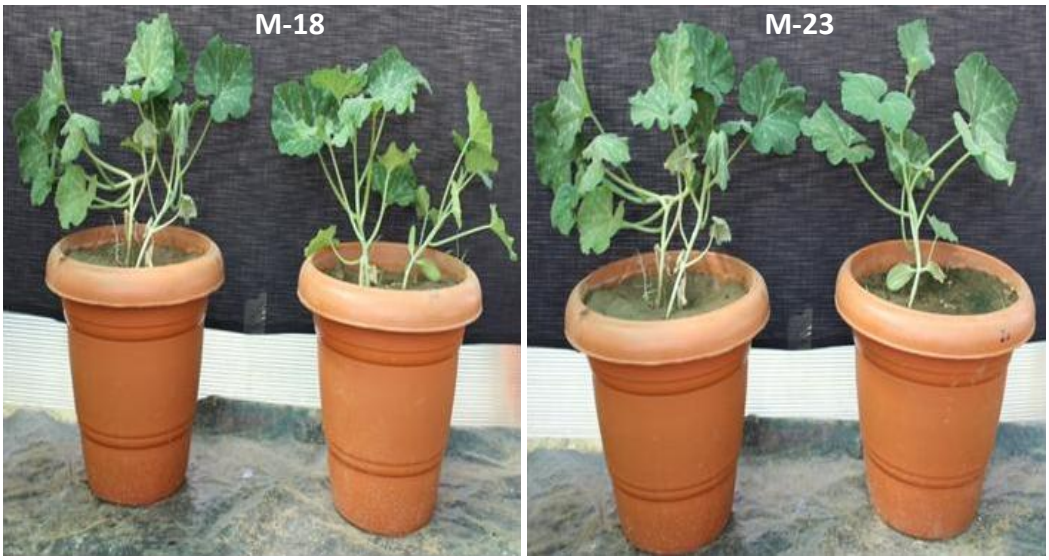


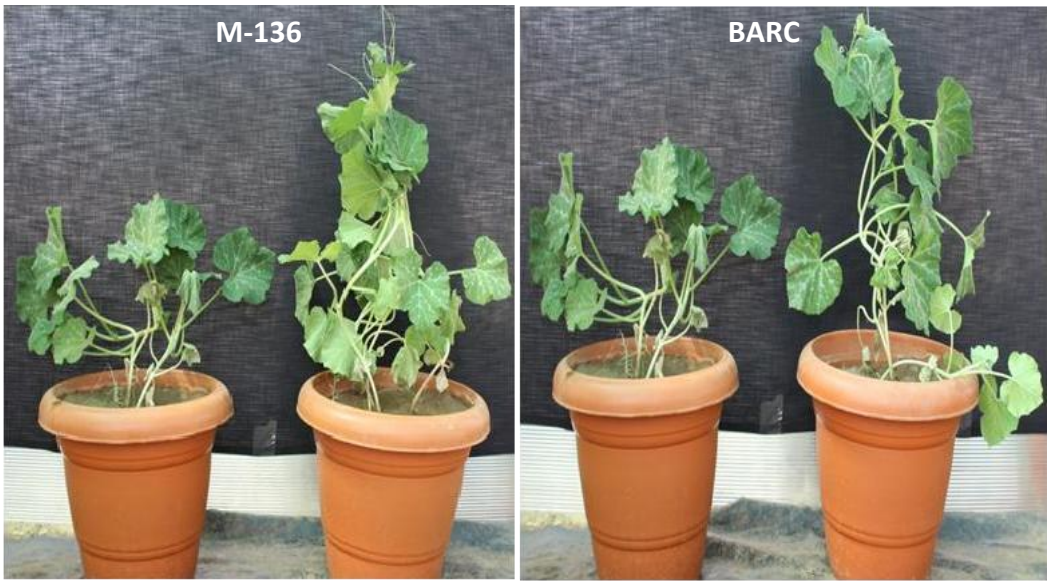
Figure 4.29 : Screening of potential *Trichoderma* mutants to induce shoot and root length in Pumpkin



Control 20DAS Treated Control 20 DAS Treated

Control 50 DAS Treated Control 50 DAS Treated

Plate 4.69 Effect of seed bio-priming with potential *Trichoderma* mutants M-18 and M-23 mutant on plant growth promoting activity of Pumpkin



Control 20DAS Treated Control 20 DAS Treated

Control 50 DAS Treated Control 50 DAS Treated

Plate 4.70 Effect of seed bio-priming with potential *Trichoderma* mutants M-136 and BARC mutant on plant growth promoting activity of Pumpkin

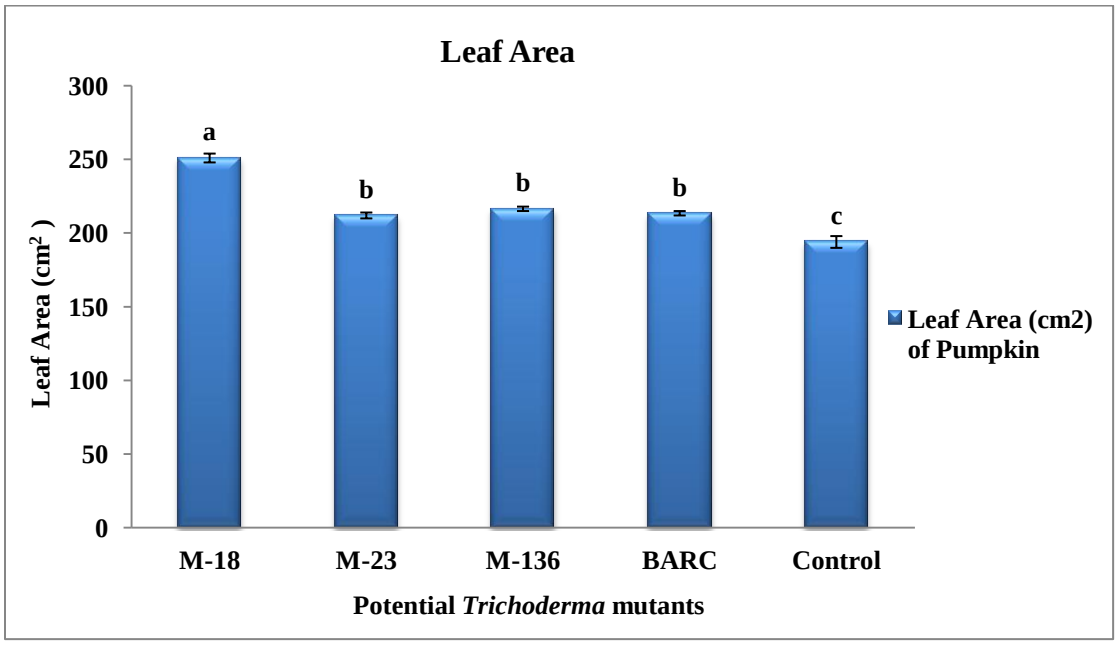


Figure 4.30 : Screening of potential *Trichoderma* mutants to induce leaf area in Pumpkin

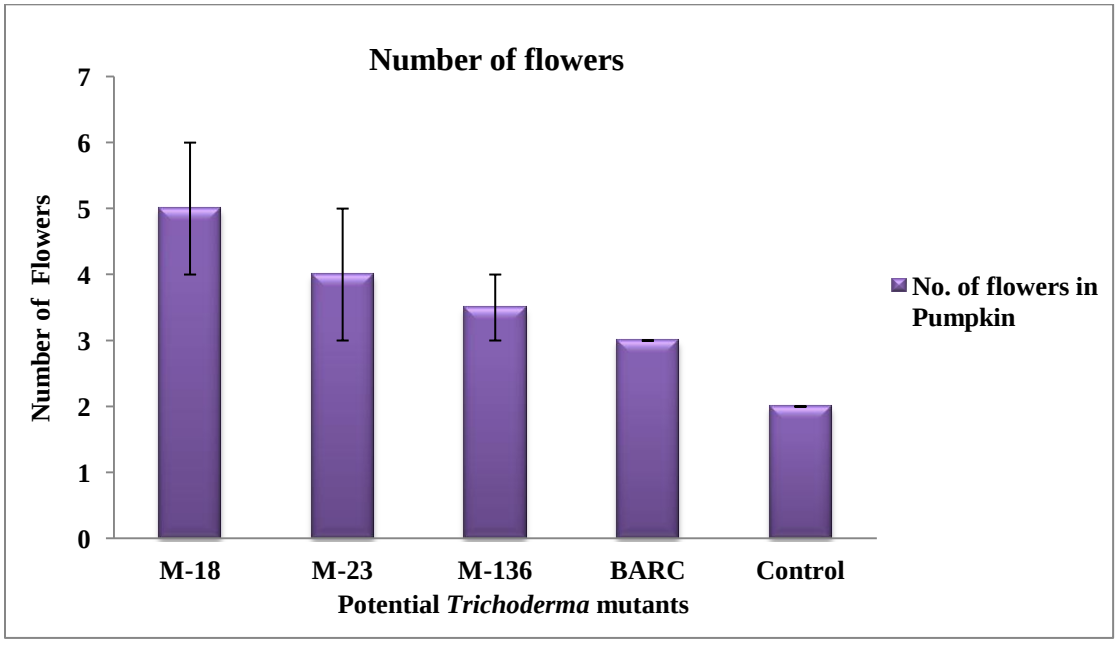


Figure 4.31 : Screening of potential *Trichoderma* mutants to induce No. of flowers in Pumpkin

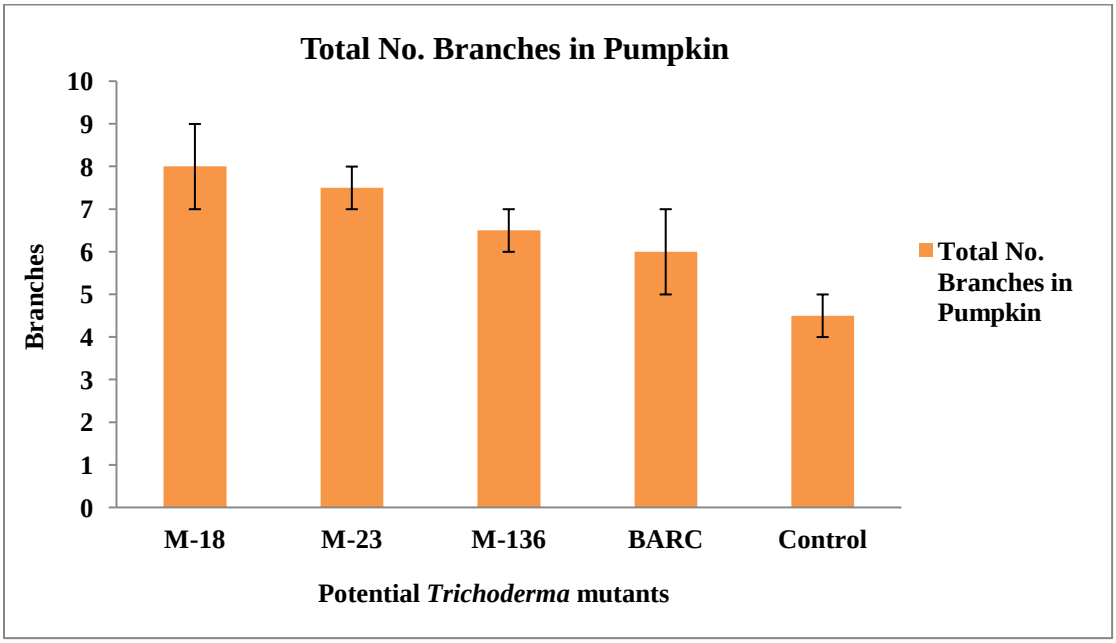
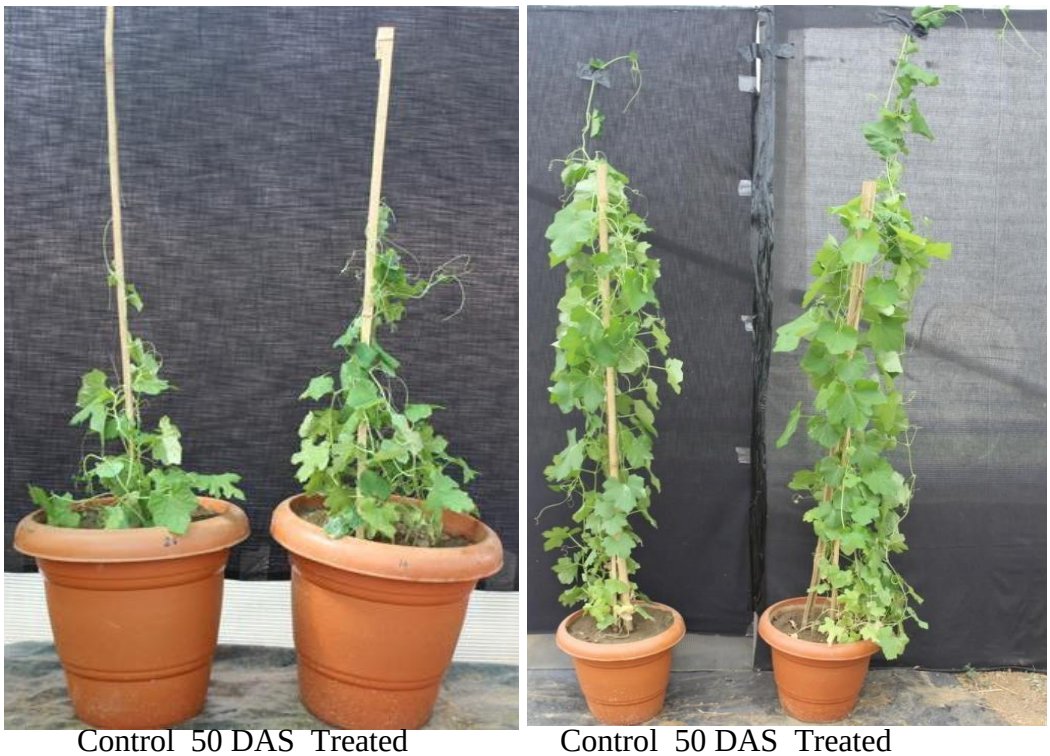


Figure 4.32 : Screening of potential *Trichoderma* mutants to induce Total no. of branches in Pumpkin

4.3.3.3 Plant growth promoting activity of potential mutants of *Trichoderma* on Ridge gourd (*Luffa acutangula* Pearl F-1 Hybrid)

Potential *Trichoderma* mutants stimulated significant increase in plant growth of on Ridge gourd (*Luffa acutangula* Pearl F-1 Hybrid). Plant growth stimulating effects of 4 potential mutants on Ridge gourd (*Luffa acutangula* Pearl F-1 Hybrid) varied. Maximum shoot length ($144.25^{a}\pm1.75$ cm) and root length ($63^{a}\pm16$ cm) were observed when Ridge gourd (*Luffa acutangula* Pearl F-1 Hybrid) plant were derived from seeds bio-primed with mutant M-23 as compared to control and other treatments. Leaf area ($214^{a}\pm4$ cm^2), no. of flowers ($21^{a}\pm1$) and total branches ($22^{a}\pm1$) was significantly stimulated when Ridge gourd (*Luffa acutangula* Pearl F-1 Hybrid) plant were derived from seeds bio-primed with mutant M-136 as compared to control and other treatments (Table 4.13, Figure 4.33- 4.36, Plate 71-72).

Control 20 DAS Treated Control 20 DAS Treated



Control 50 DAS Treated Control 50 DAS Treated

Plate4. 71 Effect of seed bio-priming with potential *Trichoderma* mutants M-18 and M-23 on plant growth promoting activity of Ridgegourd

Control 20 DAS Treated Control 50 DAS Treated

Control 20 DAS Treated Control 50 DAS Treated

Plate 4.72 Effect of seed bio-priming with potential *Trichoderma* mutants M- 136 and BARC mutant on plant growth promoting activity of Ridgegourd

Table 4.13 Efficacy of different potential mutants for plant growth promoting activity in Ridge gourd (*Luffa acutangula* Pearl F-1 Hybrid)

Treatment	Shoot Length (cm)	Leaf Area (cm^2)	No. of flowers	Total Branches	Root length (cm)
M-18	126.5^{b}±1.5	138^{c}±1	15.5^{b}±1.5	18^{bc}±1	57^{b}±18
M-23	144.25^{a}±1.75	173^{b}±3	12^{bc}±1	21.5^{ab}±0.5	63^{a}±16
M-136	123.25^{b}±1.75	214^{a}±4	21^{a}±1	22^{a}±1	53^{b}±1
BARC	112^{c}±2	187.5^{b}±7.5	12^{bc}±1	15^{cd}±1	48^{c}±0
Control	91.5^{d}±1.5	115.5^{d}±4.5	8^{b}±1	12.5^{d}±1.5	43^{c}±10
CD@5%	6.35	16.82	4.15	3.89	5.32
CV	2.02	3.86	11.54	8.33	3.74
SE(m)	1.71	4.52	1.11	1.04	1.43

***Average value of two replications**

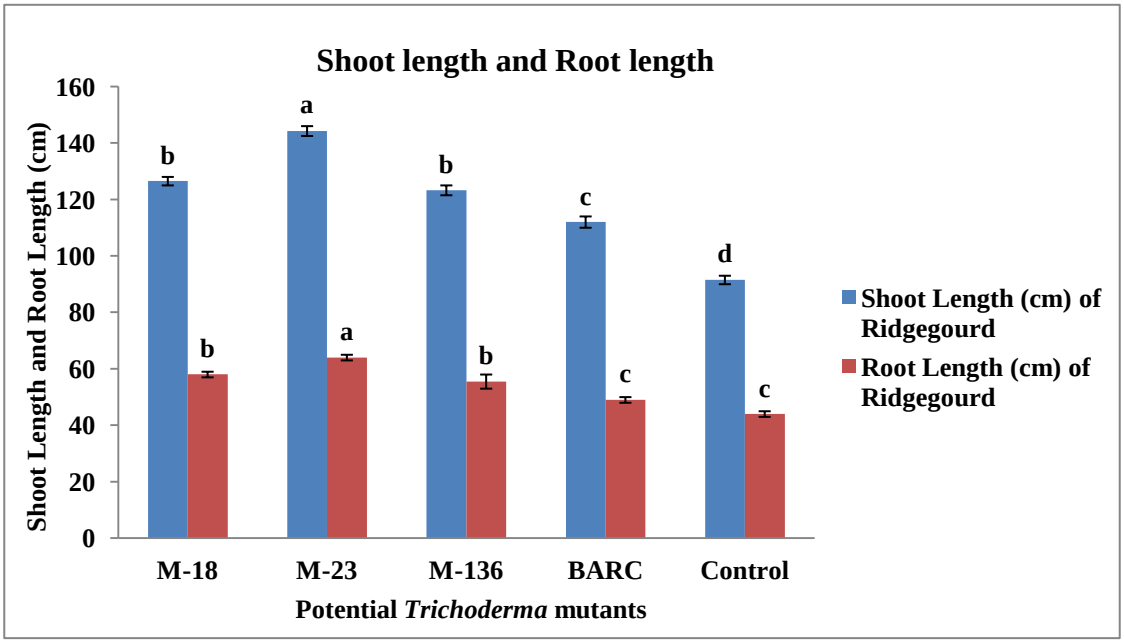


Figure 4.33 : Screening of potential *Trichoderma* mutants to induce shoot and root length in Ridgegourd

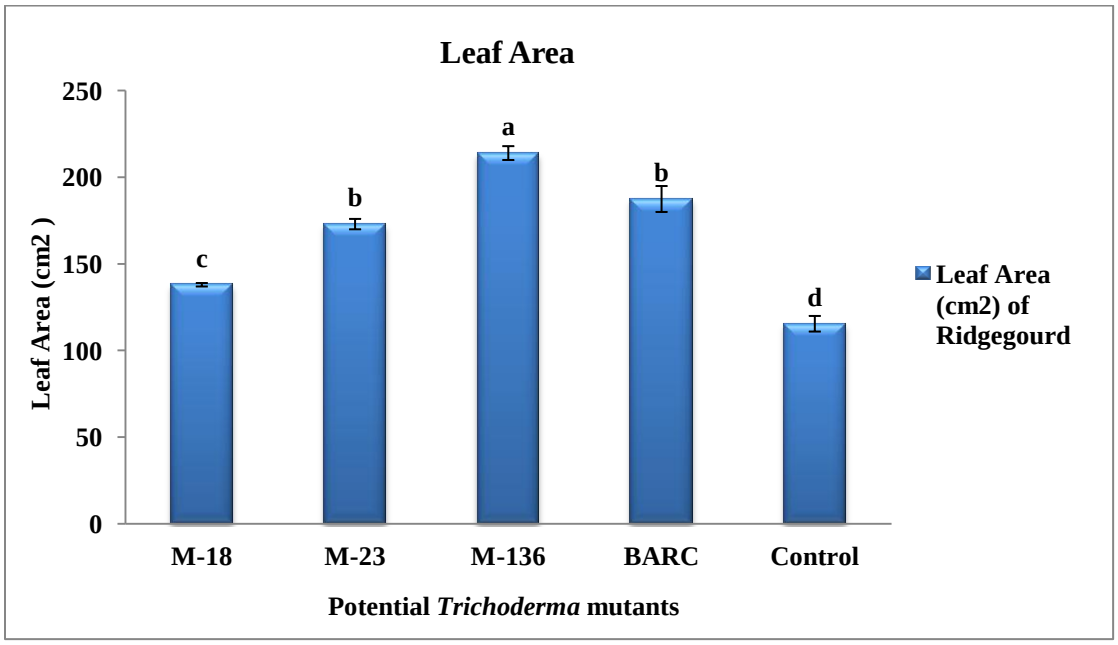


Figure 4.34 : Screening of potential *Trichoderma* mutants to induce leaf area in Ridgegourd

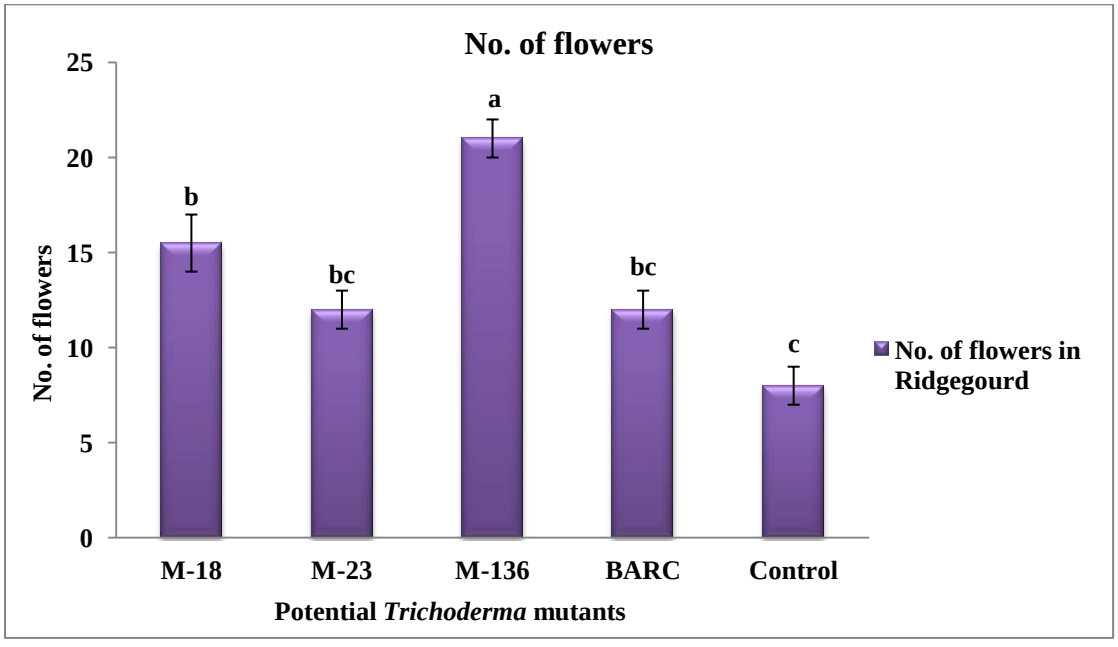


Figure 4.35 : Screening of potential *Trichoderma* mutants to induce No. of flowers in Ridgegourd

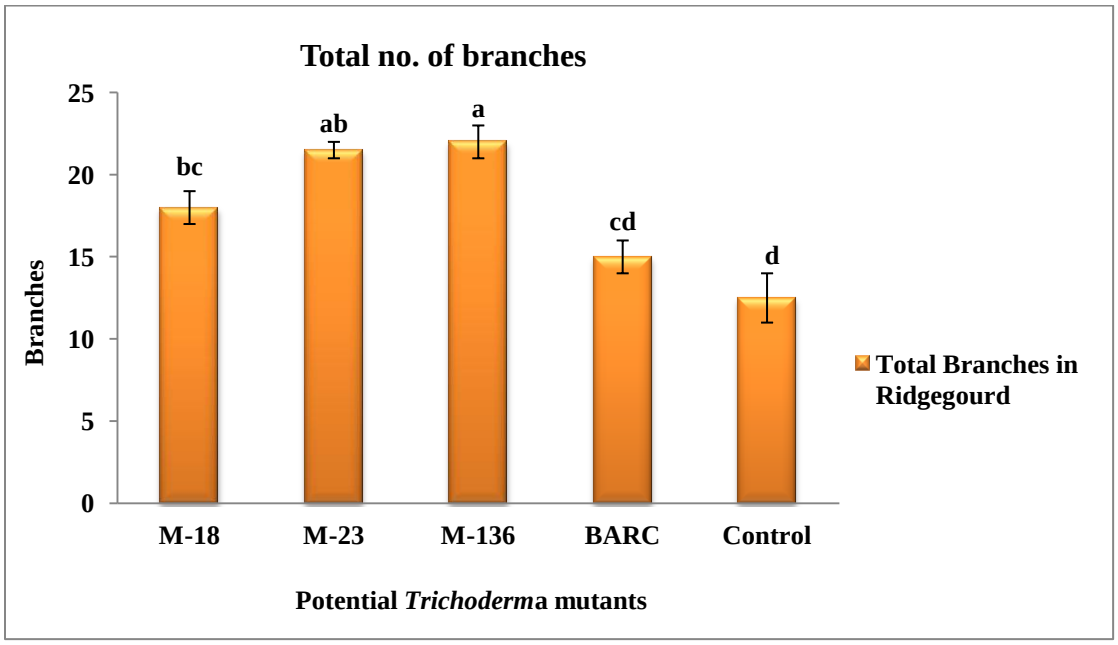
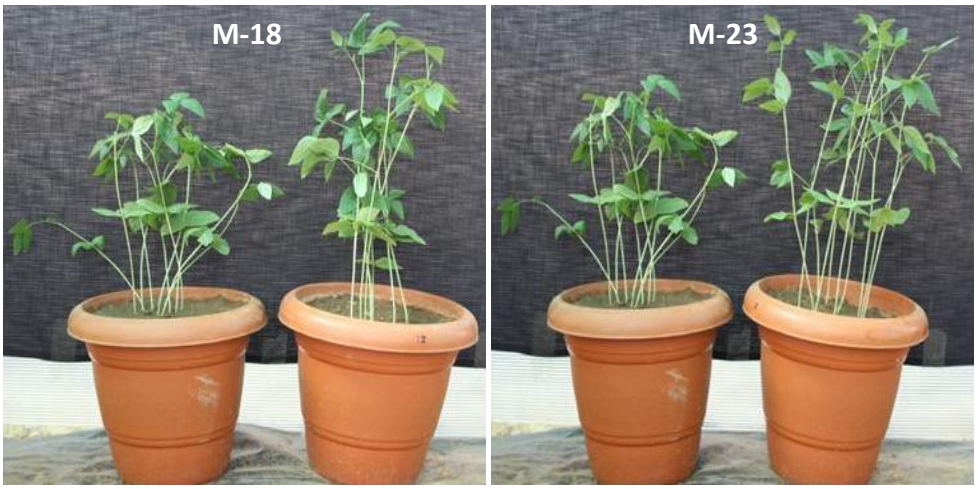


Figure 4.36 : Screening of potential *Trichoderma* mutants to induce Total no. of branches in Ridgegourd

4.3.3.4 Plant growth promoting activity of potential mutants of *Trichoderma* on Longbean (*Vigna unguiculata* Pearl F-1 Hybrid)

Potential *Trichoderma* mutants stimulated significant increase in plant growth of on Longbean (*Vigna unguiculata* Pearl F-1 Hybrid) but stimulatory responses varied. Maximum shoot length (106.5^{a}±1.5 cm) and root length (60.5^{a}±1.5 cm) was recorded when Longbean (*Vigna unguiculata* Pearl F-1 Hybrid) plant were derived from seeds bio-primed with BARC mutant.

Increase in leaf area (106.41^{a}±1.59 cm^2) was significantly stimulated to increase when Longbean (*Vigna unguiculata* Pearl F-1 Hybrid) plant were derived from seeds bio-primed with mutant M-136. Whereas, highest no. of flowers (11.5^{a}±0.5) was observed in Longbean (*Vigna unguiculata* Pearl F-1 Hybrid) plant derived from seed bio-primed with mutant M-18 as compared to control and other treatments (Table 4.14, Figure 4.37- 4.40, Plate 73-74).



Control 20 DAS Treated Control 20 DAS Treated

Control 50 DAS Treated Control 50 DAS Treated

Plate 4.73 Effect of seed bio-priming with potential *Trichoderma* mutants M- 18 and M-23 on plant growth promoting activity of Longbean

Control 20 DAS Treated

Control 50 DAS Treated Control 50 DAS Treated

Plate 4.74 Effect of seed bio-priming with potential *Trichoderma* mutants M- 136 and BARC mutant on plant growth promoting activity of Longbean

Table 4.14 Efficacy of different potential mutants for plant growth promoting activity in Long bean (*Vigna unguiculata* Pearl F-1 Hybrid)

Treatment	Shoot Length (cm)	Leaf Area (cm^2)	No. of flowers	Total branches	Root length (cm)
M-18	75.25^{b}±2.75	83.95^{c}±1.05	11.5^{a}±0.5	17±3	58^{ab}±1
M-23	71.5^{b}±1.5	85.5^{bc}±2.5	8^{b}±1	15.5±0.5	54.5^{b}±0.5
M-136	70.25^{b}±2.75	106.41^{a}±1.59	8.5^{b}±0.5	16.5±2.5	56.5^{ab}±0.5
BARC	106.5^{a}±1.5	92.4^{b}±1.8	7.5^{bc}±0.5	13±1	60.5^{a}±1.5
Control	60.5^{c}±3.0	64.52^{d}±2.77	5.5^{c}±0.5	10±2	46^{c}±2.0
CD@5%	8.89	7.58	2.35	NS	4.62
CV	4.40	2.04	0.63	2.025	1.24
SE(m)	2.39	3.34	10.90	19.88	3.19

***Average value of two replications**

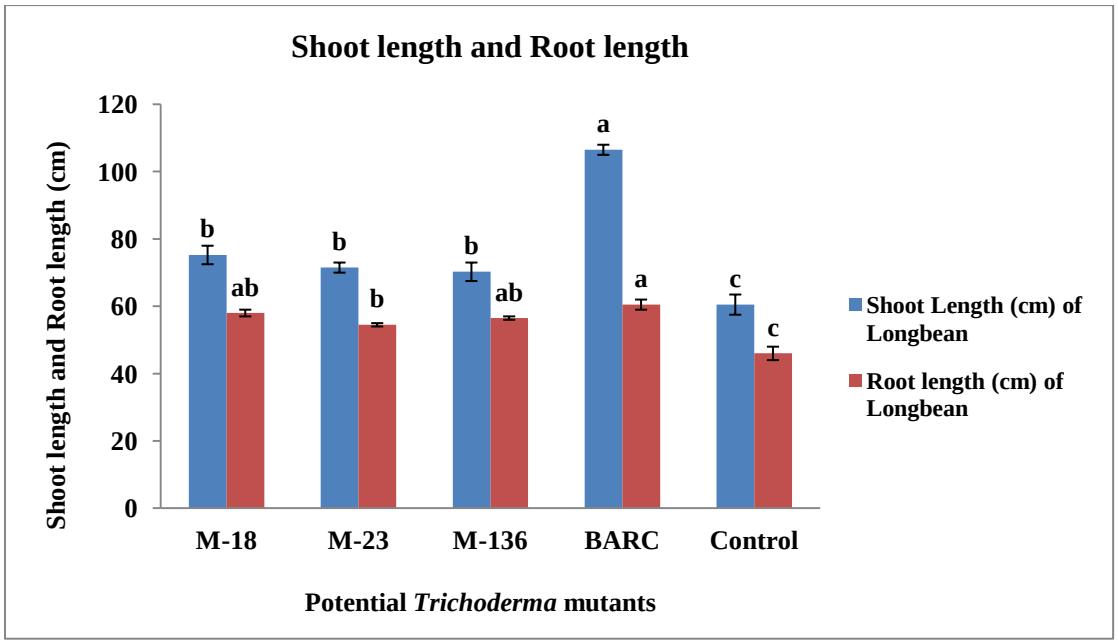


Figure 4.37 : Screening of potential *Trichoderma* mutants to induce shoot and root length in Longbean

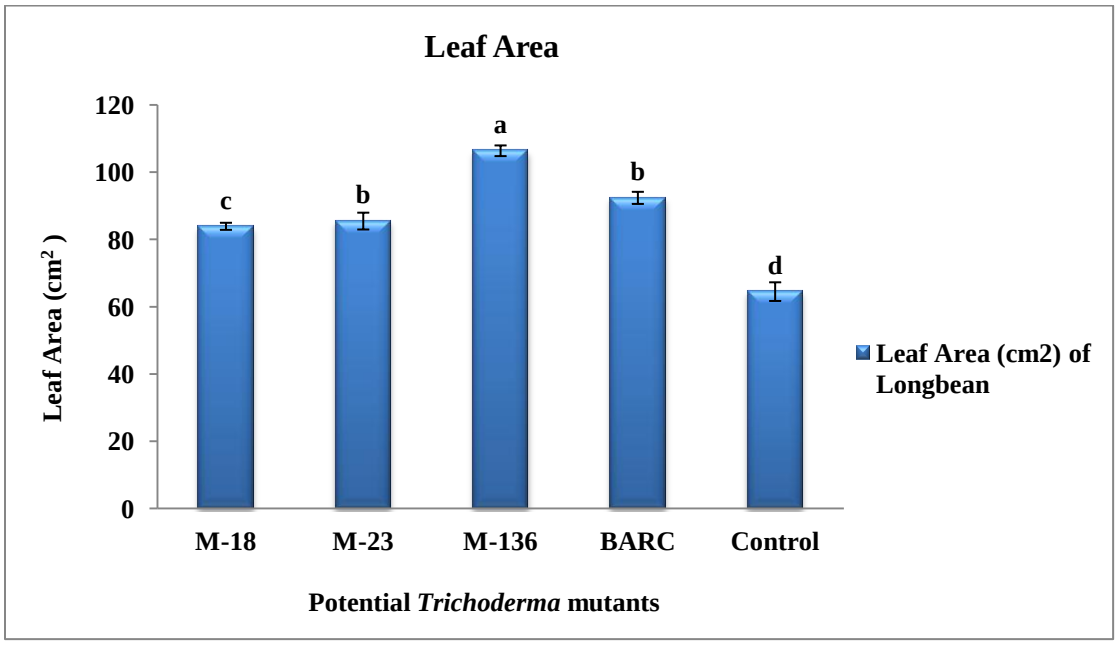


Figure 4.38 : Screening of potential *Trichoderma* mutants to induce leaf area in Longbean

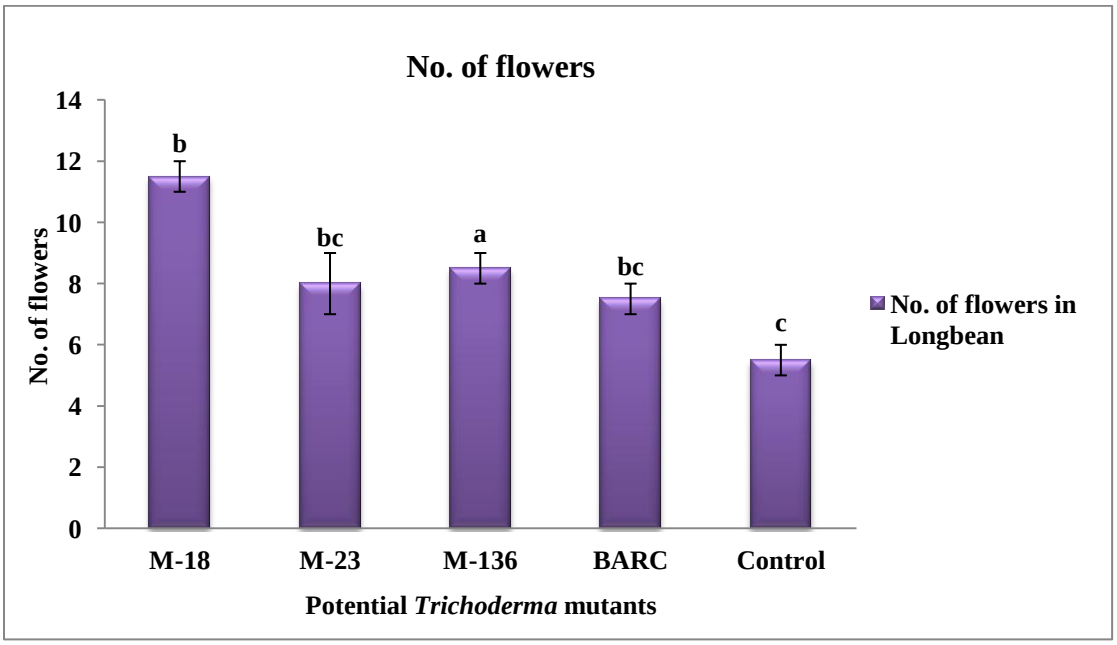


Figure 4.39 : Screening of potential *Trichoderma* mutants to induce No. of flowers in Longbean

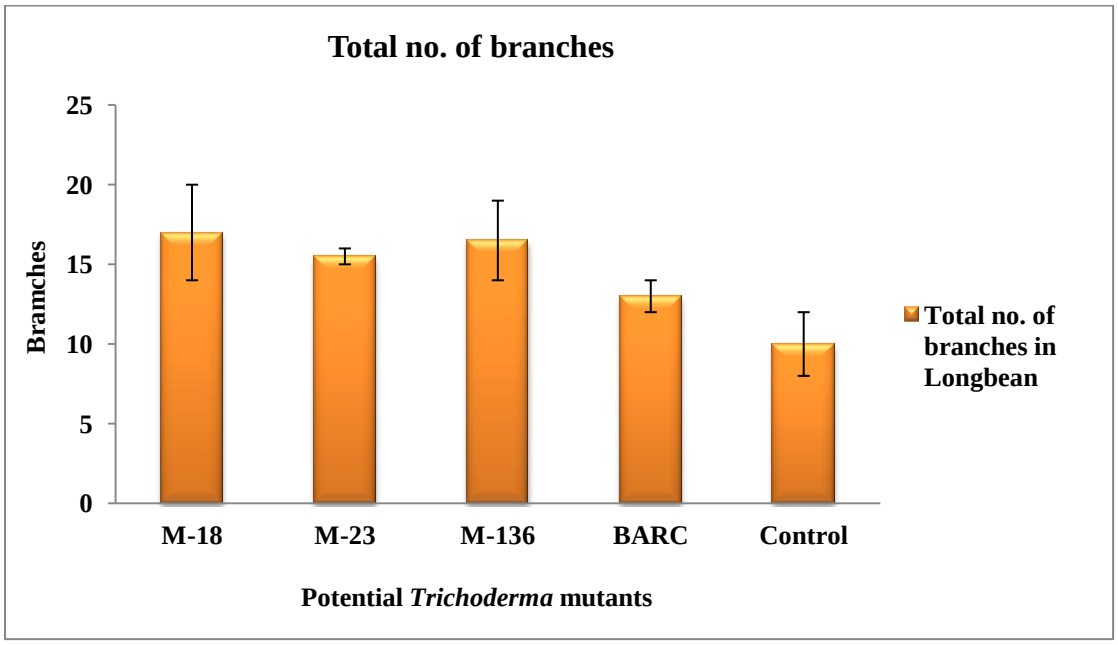


Figure 4.40 : Screening of potential *Trichoderma* mutants to induce No. of flowers in Longbean

4.3.3.5 Plant growth promoting activity of potential mutants of *Trichoderma* on Fenugreek (*Trigonella foenum-graecum* Pearl F-1 Hybrid)

Efficacy of different potential mutants of *Trichoderma* for plant growth promotion activity on Fenugreek varied Maximum plant height was recorded in Fenugreek plants which were derived from seed bio-primed with mutant M-136 as compared to control. Mutant M-18 bio-primed plants were observed with highest primary branches (5.33±0.33) and pod weight (76^{a}±2.08 gm) respectively over control. Whereas, highest no. of pods (84.66^{a}±1.45) were observed in Fenugreek plants which were derived from seed bio-primed with mutant M-136 as compared to control (Table 4.15, Figure 4.41, 4.42, Plate 76 and 77).

Control 25 DAS Treatment **Control 50 DAS Treatment**

Control 25 DAS Treatment **Control 50 DAS Treatment**

Plate 4.75 Effect of seed bio-priming with potential *Trichoderma* mutants M-18 and M-23 on plant growth promoting activity of Fenugreek

Control 25 DAS Treatment **Control 50 DAS Treatment**

Control 25 DAS Treatment **Control 50 DAS Treatment**

Plate 4.76 Effect of seed bio-priming with potential *Trichoderma* mutants M-136 and BARC mutant on plant growth promoting activity of Fenugreek

Table 4.15 Efficacy of different potential mutants for plant growth promoting activity in Fenugreek (*Trigonella foenum-graecum* Pearl F-1 Hybrid)

Treatment	Plant Height (cm)	Primary Branches	No. of pods	Pod Weight (gm)
M-18	75.33^{a}±0.66	5.33±0.33	77^{b}±0.57	76^{a}±2.08
M-23	75.66^{a}±0.88	5±0.57	82.33^{ab}±2.02	53.33^{b}±4.25
M-136	76^{a}±1.20	5±0.57	84.66^{a}±1.45	75.66^{a}±2.33
BARC	73^{a}±0.57	5±0	82.33^{ab}±2.33	61.66^{b}±3.75
Control	66.33^{b}±0.88	3.33±0.33	71^{c}±1.52	61.33^{b}±3.75
CD@5%	2.77	1.34	5.40	10.69
CV	2.97	15.42	3.69	8.84
SE(m)	0.86	0.42	1.69	3.35

***Average value of three replications**

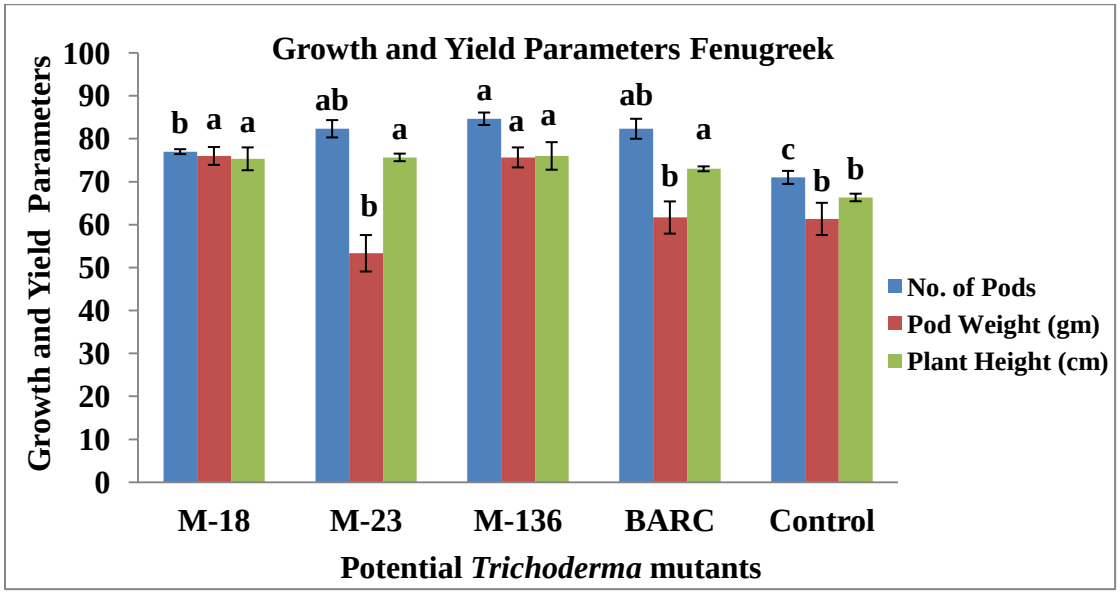


Figure 4.41 : Screening of potential *Trichoderma* mutants to induce growth and yield attributes in Fenugreek

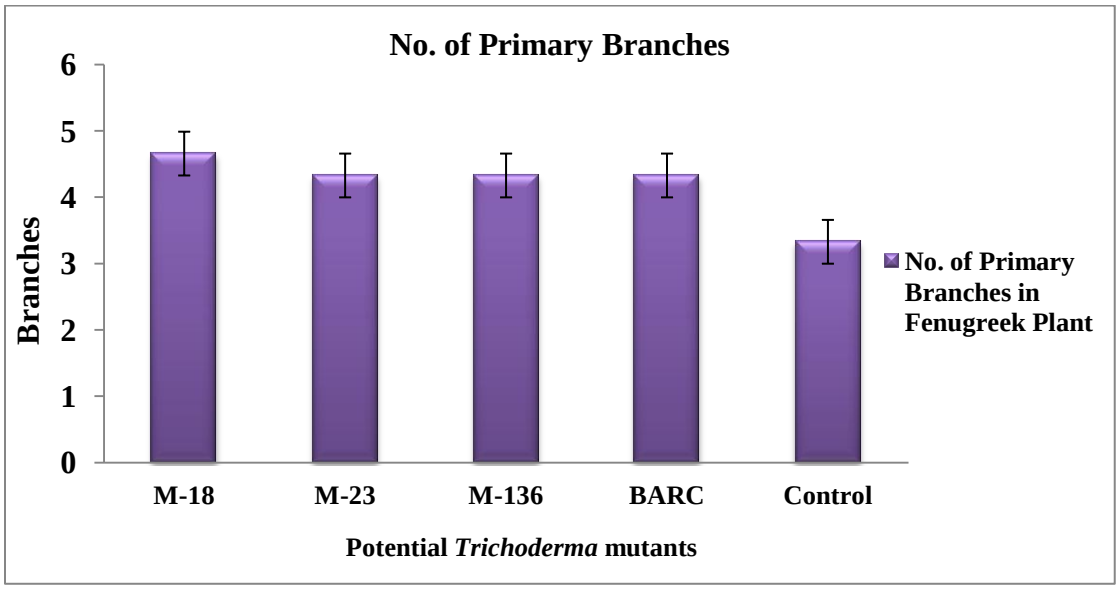


Figure 4.42 : Screening of potential *Trichoderma* mutants to induce no. of branes in Fenugreek

4.3.3.6 Plant growth promoting activity of potential mutants of *Trichoderma* on Tomato (*Lycopersicum esculentum* Pearl F-1 Hybrid)

Potential mutants of *Trichoderma* stimulated significant differences in plant growth of tomato Maximum root length (42^{a}±3.21 cm) and root fresh weight (43^{a}±4.50 gm) was recorded in tomato plants which were derived from seed bio primed with mutant M-23 as compared to control. Highest no. of flowers per cluster (5.66^{a}±0.33) and primary branches (4±0) and (4±0.57) was observed in tomato plants which were derived from seed bio-primed with mutant M-23, M-18 and BARC mutant respectively as compared to control (Table 4.16, Figure 4.43, 4.44, Plate 78)

Table 4.16 Efficacy of different potential mutants for plant growth promoting activity in Tomato (*Lycopersicum esculentum* Pearl F-1 Hybrid)

Treatment	Primary Branches	No. of flowers per cluster	Root Length (cm)	Root Fresh Weight(gm)
M-18	4±0	5^{a}±0	37^{ab}±1.52	36^{ab}±1.15
M-23	3.66±0.33	5.66^{a}±0.33	42^{a}±3.21	43^{a}±4.50
M-136	3.66±0.33	5.33^{a}±0.33	41.66^{a}±0.88	42.66^{a}±1.45
BARC	4±0.577	5.33^{a}±0.33	40.66^{a}±2.66	38.66^{ab}±4.05
Control	3±0	3.33^{b}±0.33	29.33^{b}±0.66	30^{b}±0
CD@5%	N/A	0.95	6.54	9.05
CV	15.74	10.46	9.30	12.90
SE(m)	0.33	0.29	2.04	2.83

***Average value of two replications**

Control 50 DAS Treatment **Control 50 DAS Treatment**

Control 50 DAS Treatment **Control 50 DAS Treatment**

Plate 4.77 Effect of seed bio-priming with potential *Trichoderma* mutants on plant growth promoting activity of Tomato

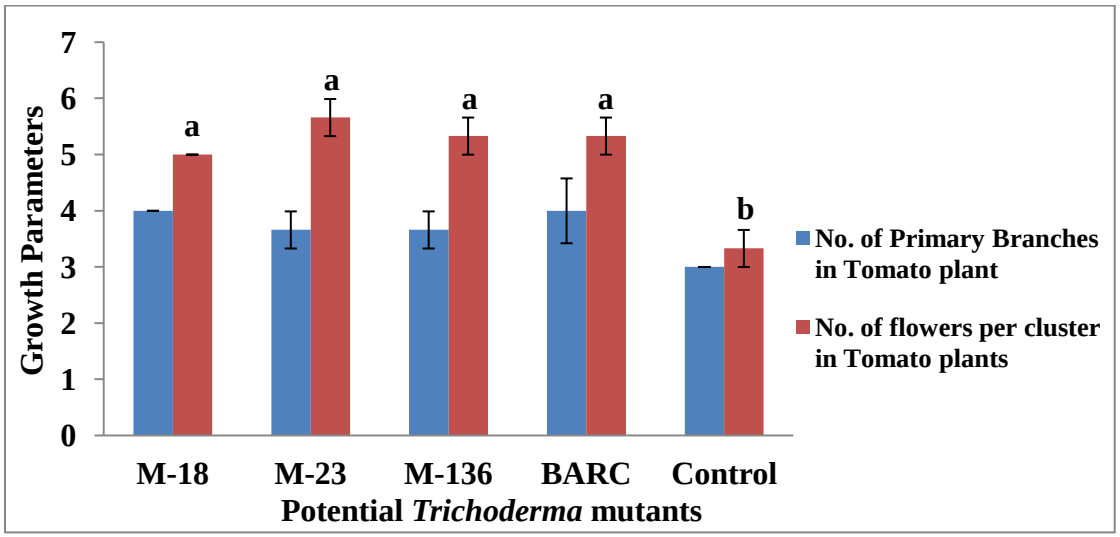


Figure 4.43 : Screening of potential *Trichoderma* mutants to induce growth attributes in Tomato

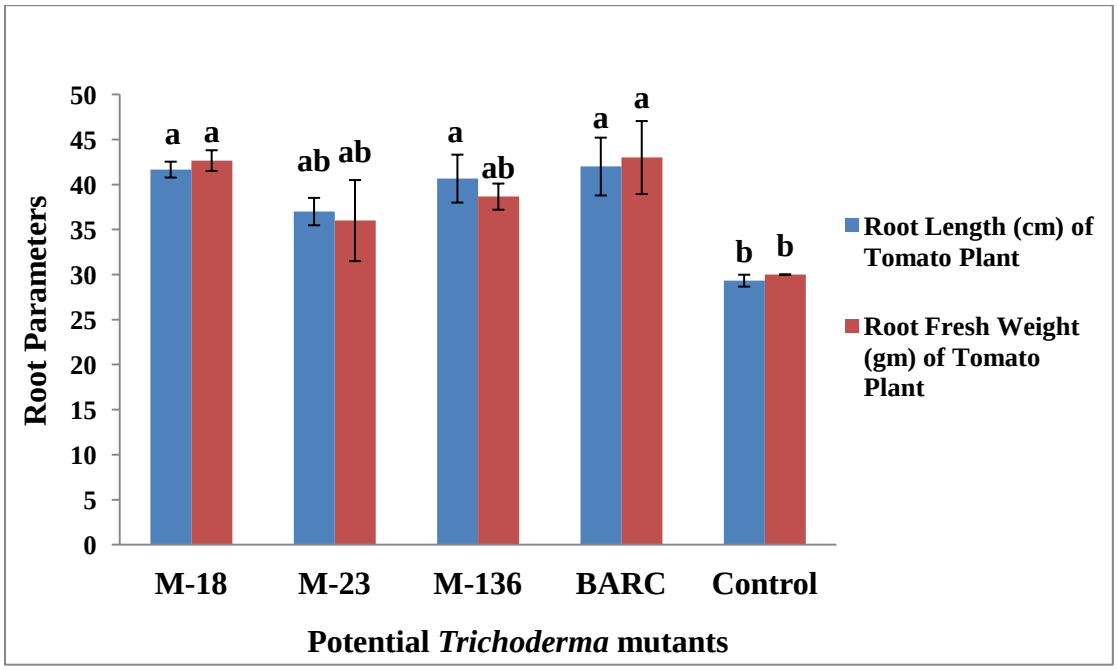


Figure 4.44 : Screening of potential *Trichoderma* mutants to induce root parameters in Tomato

4.3.3.7 Plant growth promoting activity of potential mutants of *Trichoderma* on Spinach (*Spinacia oleracea* Pearl F-1hybrid)

Potential mutants of *Trichoderma* stimulated significant differences in plant growth of spinach. Maximum root length (34^{a}±1 cm) and plant height (8.10^{a}±0.42 cm) was recorded in plants which were derived from seed bio primed with mutant M-136 and M-23 respectively as compared to control. Whereas, highest no. of leaves per plant was observed in plants which were derived from seed bio primed with BARC mutant (3.79^{a}±0.09) as compared to control. Although stimulatory effects of four potential *Trichoderma* mutants was at par on spinach for number of leaves per plant. Similary stimulatory effects of potential *Trichoderma* mutants (M-18, M-23 and BARC) was at par on spinach for plant height and M-18, M-136 for root length (Table 4.17, Figure 4.45, 4.46, Plate 79 and 80).

Table 4.17 Efficacy of different potential mutants for plant growth promoting activity in Spinach (*Spinacia oleracea* Pearl F-1hybrid)

Treatment	Plant Height (cm)	No. of leaves per plant	Root Length (cm)
M-18	7.36^{a}±0.31	3.61^{a}±0.20	32.33^{a}±1.73
M-23	8.10^{a}±0.42	3.42^{a}±0.10	30^{ab}±0.66
M-136	6.96^{ab}±0.41	3.66^{a}±0.12	34^{a}±1.00
BARC	7.67^{a}±0.64	3.79^{a}±0.09	31^{ab}±0.57
Control	5.83^{b}±0.12	2.74^{b}±0.20	27^{b}±2.08
CD@5 %	1.34	0.49	4.30
CV	10.12	7.70	7.55
SE(m)	0.42	0.15	1.35

***Average value of two replications**

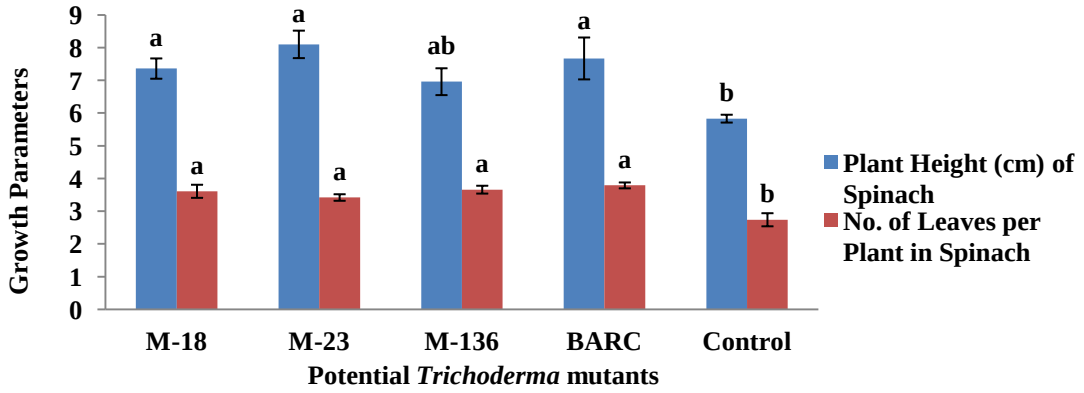
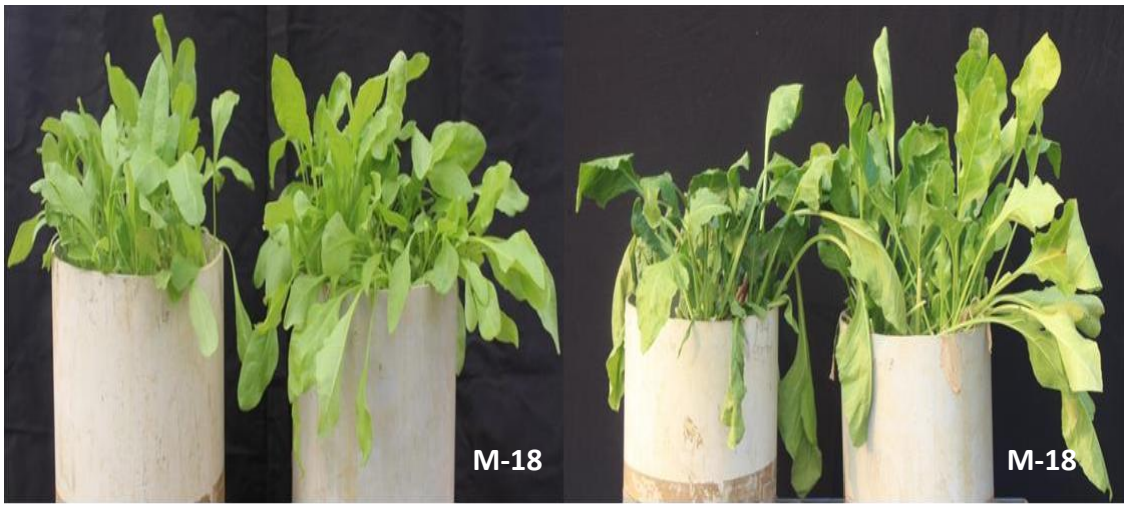


Figure 4.45: Screening of potential *Trichoderma* mutants to induce plant height and no. of leaves per plant in Spinach



Control 25 DAS Treatment **Control 50 DAS Treatment**

Control 25 DAS Treatment **Control 50 DAS Treatment**

Plate 4.78 Effect of seed bio-priming with potential *Trichoderma* mutants M-18 and M-23 on plant growth promoting activity of Spinach

Control 25 DAS Treatment **Control 50 DAS Treatment**

Control 25 DAS Treatment **Control 50 DAS Treatment**

Plate 4.79 Effect of seed bio-priming with potential *Trichoderma* mutants M-136 and BARC on plant growth promoting activity of Spinach

Recent research has confirmed that potential mutants of *Trichoderma* were able to stimulate plant growth on different vegetable crops following seed bio-priming were able to promote the growth in different parameters (Shoot length, root length, leaf area, number of flowers and total branches) of bitter gourd, pumpkin, ridge gourd, long bean, fenugreek, tomato and spinach. Potential mutants used in the present investigation were derived from *Trichoderma atrovitride* and *Trichoderma viride* which were high IAA producers and had very strong hormonal effects on the plants through seed bio-priming (Kotasthane *et al*., 2014).

Previous studies have demonstrated direct impact of *Trichoderma* based bio-pesticides or *Trichoderma* spore suspension on plant growth promotion, defense response, stress tolerance, etc. (Bjorkman *et al*., 1998; Batta, 2004)

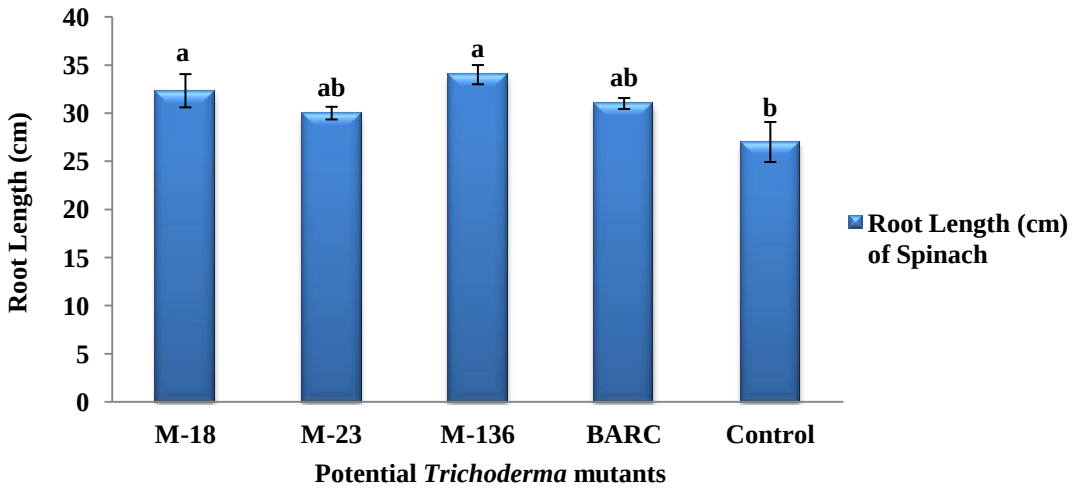


Figure 4.46: Screening of potential *Trichoderma* mutants to induce root length in Spinach

Mastouri *et al*., 2010; Singh *et al*., 2014b; Woo *et al*., 2014; Jain *et al*., 2014). Mastouri *et al*., (2010) treated artificially aged and young tomato seed with conidial suspension atthe rate of 20 l g^{-1} deposit 2×10^7 CFU g^{-1} of seed. They observed significantly high radicle growth in aged tomato seeds while unaged tomato seeds were unaffected. Various other studies reported application of blanket seed treatment

with *Trichoderma* spore suspension (Singh *et al*., 2013[a]; Yadav *et al*., 2013; Saxena *et al*., 2015; Jain *et al*., 2015[b]; Patel *et al*., 2015) stimulating plant growth promoting effects in various crop plants. *Trichoderma* promotes plant growth, biomass gaining, higher seed germination, increased plant height, root development, shoot dry mass and leaf number, increased crop yield and improved plant vigor (Harman *et al*., 2004; Salas Marina *et al*., 2015; Chagas *et al*., 2017). One of the most evident morphologic changes in plants triggered by *Trichoderma* is the increase of lateral roots, thus modifying root architecture. In this process, previous observations have demonstrated the participation of auxins (Contreras-Cornejo *et al*., 2009) as well as a cross-talk between ET and auxins through the signaling pathways mediated by MAP-kinases (Contreras-Cornejo *et al*., 2015). Also, the presence of *Trichoderma* not only modulates the levels of the hormones produced by the plant but *Trichoderma* itself can contribute with its own hormones or could provide intermediates for the synthesis of some phytohormones, as a part of the benefits reported in the *Trichoderma*–plant interaction (Guzman-Guzman et al., 2019).

Several lines of evidences indicates that *Trichoderma* spp. induces secondary root development through production of auxin and IAA (Contreras-conrnejo *et al*. 2009, 2014) and enhances growth of plant by increasing nutrient uptake (Harman *et al.* 2004; Rakshit *et al*. 2013). Similarly, enhancement in root length, shoot length, dry weight of plants with significant increase in no. of leaves was observed in the plants of pea treated with *Trichoderma* isolate BHUF4 (Saxena *et al*. 2015).

4.4 *In vitro* antagonistic activity of potential *Trichoderma* mutants against *S. rolfsii* (Dual Culture technique, Dennis and Webster, 1971)

In vitro antagonistic potential of different potential *Trichoderma* mutants against fungal plant pathogen *Sclerotium rolfsii* studied by following dual culture method, and growth assessed 5 days after inoculation. All the potential *Trichoderma* mutants showed varied range of antagonism against *Sclerotium rolfsii* ranging from 79 per cent to 81.50 per cent (Table 4.18, Figure 4.47 and Plate 4.81). Among potential *Trichoderma* mutants, BARC mutant showed maximum inhibiting effect on the growth of *Sclerotium rolfsii* (81.50 %) over control. While remaining mutants also showed good inhibitory effect on the growth of *Sclerotium rolfsii* in order M-136 (81 %), M-23 (80.5 %) and M-18 (79 %) respectively.

The antagonistic fungus *Trichoderma* spp. have capability to break the outer sclerotial shell that leads to its destruction along with several histological changes such as deformation, decay of cytoplasmic content and cell wall lysis (Rawat and Tewari, 2011). The present study revealed that different *Trichoderma* mutants have capacities as biological weapons in inhibiting the pathogens. This might be due to the production of secondary metabolites and antibiotics production, which diffused into the PDA and air filed spores which showed detrimental effect towards growth of *S. rolfsii* as well as due to higher competitive ability of potential *Trichoderma* mutants. Overall BARC mutant was found to be more efficient against *S. rolfsii* which indicates that it can be exploited as potential candidates for development of bio-pesticides.

Above findings are in agreement with the observations made by Bhuiyan *et al*., (2012) reported that *T. harzianum* isolate Th-18 showed the highest (83.09 %) reduction of the radial growth against *S. rolfsii* . This might be due to the production of secondary metabolites and antibiotics production, which diffused into the PDA and air filed spores which showed detrimental effect towards growth of *S. rolfsii* as well as due to higher competitive ability of potential *Trichoderma* mutants. The antagonistic fungus *Trichoderma* spp.

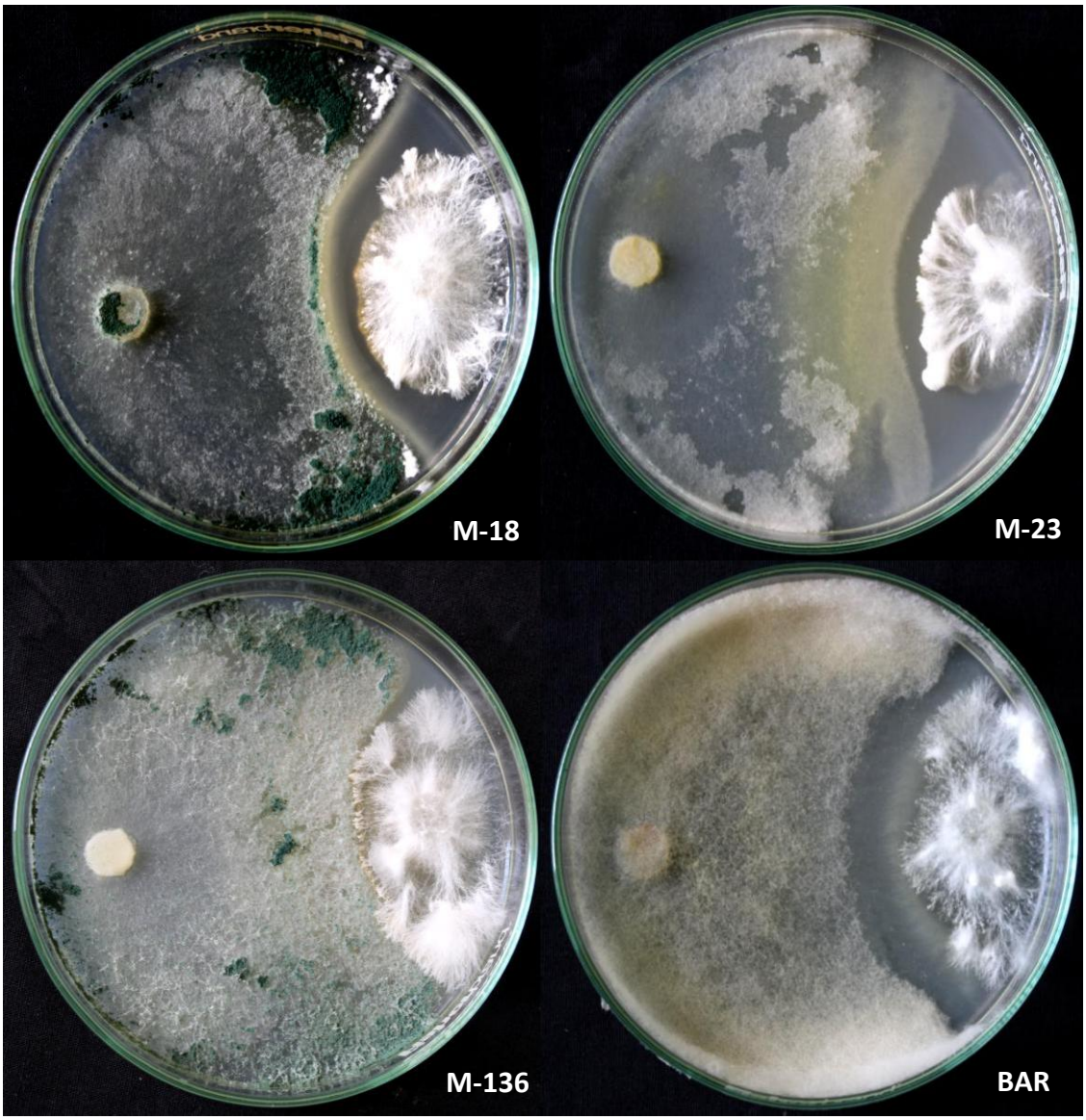


Plate 4.80 Antagonistic effect of potential *Trichoderma* mutants against *S. rolfsii*

Table 4.18 *In vitro* evaluation of potential *Trichoderma* mutants against *Sclerotium rolfsii*

Treatment	Treatment Name	Per cent Inhibition
T_1	BARC	81.5
T_2	M-18	79
T_3	M-23	80.5
T_4	M-136	81
T_5	Control	-

Bandyopodhyay *et al*. (2003) reported that *Trichoderma* strains inhibited the growth of *Sclerotium rolfsii* and *Rhizoctonia solani* by 76.6 % and 73.3 % respectively. Yadub and Shahzad (2005) reported that *T. harzianum* and *T. longibrachiatum* restricted the growth of *S. rolfsii* under *in vitro* condition by coiling around mycelium of *S. rolfsii* which leads in lysis of hyphae. Several workers reported that *Trichoderma viride* as an important antagonist inhibiting the growth of *Sclerotium rolfsii* (Kolte and Raut., 2007 ; Mandhare and Suryawanshi., 2008). Prajapati *et al*. (2015) observed that among different species of *Trichoderma* tested against *S. rolfsii* through dual culture technique, *T. asperellum* showed strong antagonistic effect in terms of mycelia growth inhibition *i.e.* 61.48, 75.00 and 73.33 per cent at 4, 6 and 8 days of incubation, respectively. Kushwaha *et al*., (2018) reported that *T. viride* showed strong the highest reduction (91.31 %) in sclerotial formation of *S. rolfsii* after 15 days of incubation.

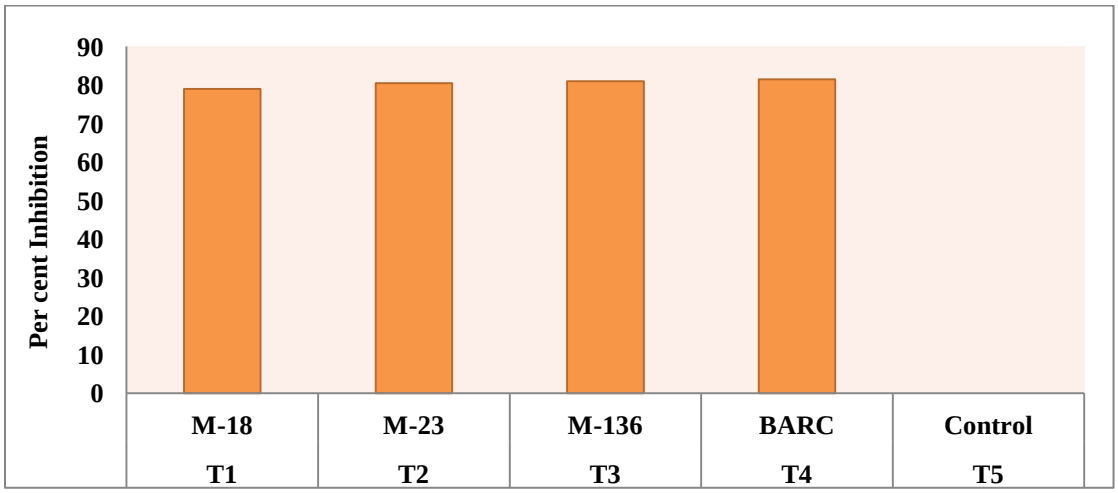


Figure 4.47: *In vitro* effect of potential *Trichoderma* mutants against *Sclerotium rolfsii*

4.4.1 *In vitro* evaluation of fungicides against *Sclerotium rolfsii*

The efficacy of different six fungicides and one antibiotics (Difenconazole 25 % EC, Hexaconazole 5 % EC, Propiconazole 25 % EC, Tebuconzole 25.9 % EC, Kreoxime Methyl 44.3 % EC, Thifluzamide 24 % SC and Validamycin 3 % L) were evaluated *in vitro* at three different concentration of each fungicide *i.e.*15 µl, 20 µl, 25 µl, 100 µl, 150 µl, 200 µl, 20 µl, 25 µl, 30 µl, 36 µl, 72 µl, 144 µl, 15 µl, 37.5 µl, 50 µl, 25 µl, 50 µl, 75 µl, 100 µl, 150 µl and 200 µl respectively. Against *Sclerotium rolfsii* on potato dextrose agar (PDA) medium using Poisoned Food Technique (Nene and Thapliyal, 1982).

The data presented in table 4.19, figure 4.47 and plate 4.82, revealed that among the six different fungicides and one antibiotics tested at different concentration, Difenconazole 25 % EC, Hexaconazole 5 % EC and Tebuconazole 25.9 % EC were found highly effective at three different concentrations of each fungicides *i.e.* 15 µl, 20 µl, 25 µl, 100 µl, 150 µl, 200 µl, 36 µl, 72 µl, 144 µl respectively with 100 per cent inhibition of mycelial growth of *S. rolfsii*. Also, Propiconazole 25 % EC and Thifluzamide 24 % EC showed 100 per cent inhibition at concentration of (25 µl, 30 µl) and (50 µl, 75 µl) respectively. Where as, Propiconazole 25 % EC at concentration

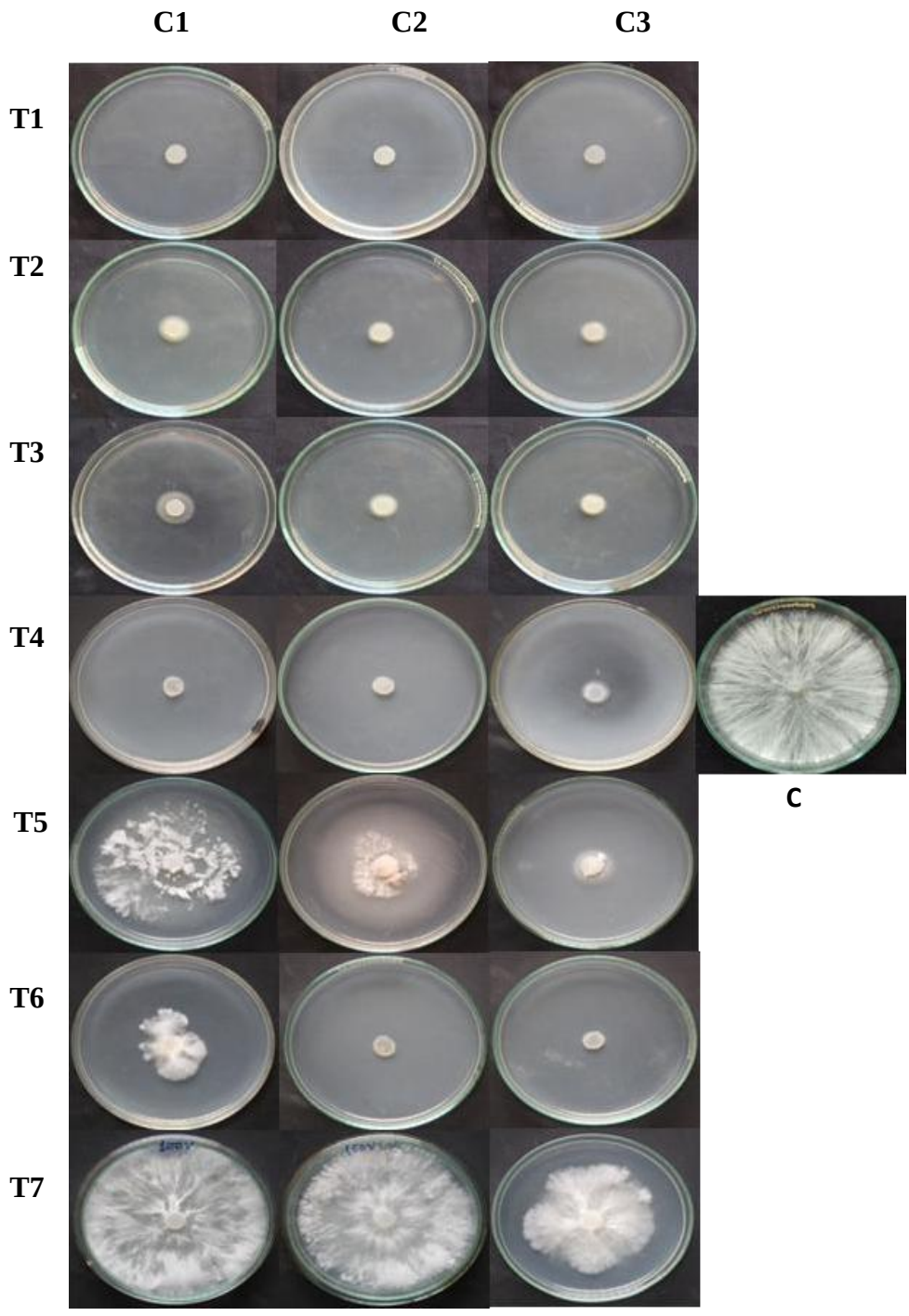


Plate 81 Antagonistic effect of different fungicides against *S. rolfsii*

of 20 µl showed 94.44 % inhibition of test pathogen. The other fungicides namely Kreoxime methyl 44.3 % EC (11.11 %, 22 %, 83.3 %) and Thifluzamide 24 % SC (23 %) were found to inhibit mycelial growth at concentrations of (15 µl, 37.5 µl, 50 µl) and 25 µl respectively. Among test antibiotics, Validamycin 3 % L at concentration of 200 µl found least effective (11.11 %) mycelial growth inhibition of *S. rolfsii*. Whereas, no inhibition was observed at concentration of 100 µl and 150 µl.

In the present study, triazole fungicides group was found effective with strong antagonistic effect due to its strong anti-fungal property and imparts its poisoning effect on metabolic process of pathogen, due to which growth of the *S. rolfsii* might be adversely affected.

The results of present findings are in confirmatory with the findings of earlier workers Banakar *et al*. (2017) reported that fungicides like hexaconazole, tebuconazole, and combi products showed complete inhibition of *S. rolfsii* at all the concentrations used. Arunsari *et al*. (2011) reported that the triazoles (hexaconazole, propiconazole, difenconazle) and combi products containing traizoles were found highly inhibitive to the mycelia growth of *Sclerotium rolfsii*.

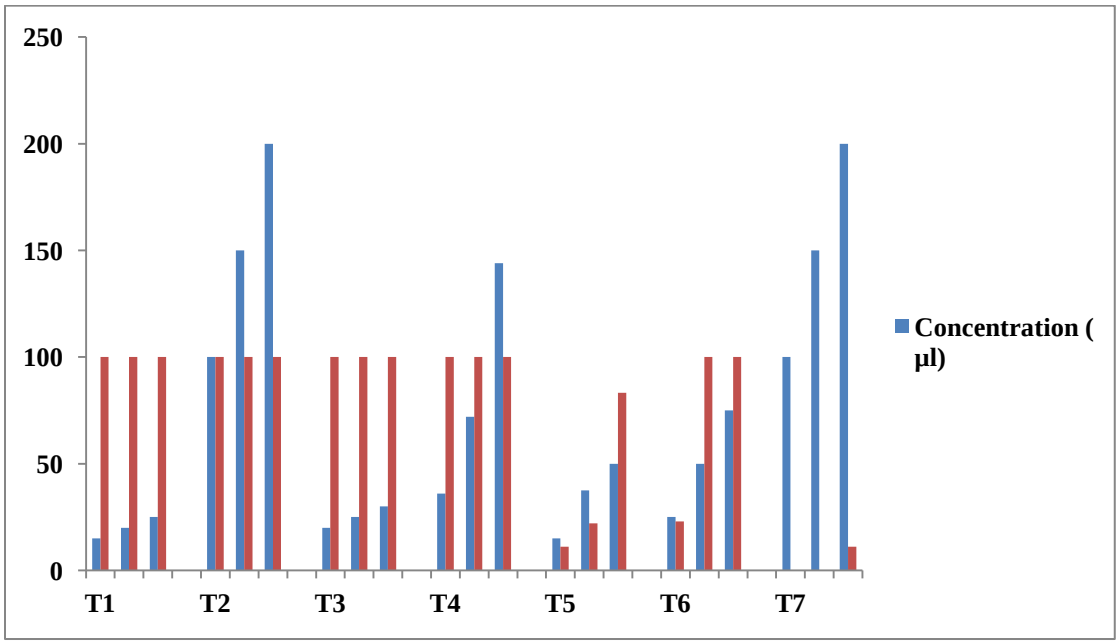


Figure 4.48 : *In vitro* effect of fungicides against *Sclerotium rolfsii*

Table 4.19 *In vitro* evaluation of fungicides against *Sclerotium rolfsii*

Treatment	Fungicide	Conc. (µl)		Per cent inhibition
T_1	Difenconazole	C_1	15	100
		C_2	20	100
		C_3	25	100
T_2	Hexaconazole	C_1	100	100
		C_2	150	100
		C_3	200	100
T_3	Propiconazole	C_1	20	94.44
		C_2	25	100
		C_3	30	100
T_4	Tebuconzaole	C_1	36	100
		C_2	72	100
		C_3	144	100
T_5	Kroxime Methyl	C_1	15	11.11
		C_2	37.50	22
		C_3	50	83.30
T_6	Thifluzamide	C_1	25	23
		C_2	50	100
		C_3	75	100
T_7	Validamycin	C_1	100	0
		C_2	150	0
		C_3	200	11.11

***Percent inhibition is calculated 96 hours of incubation**

4.5 Effect of seed bio-priming with potential *Trichoderma* mutants in chickpea and wheat leaves contents of superoxide dismutase (SOD), peroxidase (POX), phenylamine ammonia lyase (PAL), lipid peroxidase (LPO), polyphenol oxidase (PPO) and total phenolic content (TPC)

Seed bio-priming with beneficial microbes serves as an strategy to improve capacity of plant to defense themselves against stress challenged conditions. Plant Growth Promoting fungi includes many different strains of *Trichoderma* spp. which have rhizo competent ability, colonizes the rhizosphere region of roots of different plants that stimulates the plant immune system and triggers (bio-priming) molecular mechanism for defense against wide range of phytopathogens (Pieterse *et al*., 2014; Martínez-Medina *et al*., 2017).

Due to biotic (pathogenic microbes, insects and fungi) and abiotic stress (light, temperature, toxic compound and heavy metals) condition oxidative stress in induced in plant system (Suzuki *et al*., 2014). These stress conditions leads to increased production of ROS (reactive oxygen species) which contributes towards free radicals formation. Being highly unstable, these free radicals reacts with various bio molecules, causing irreversible damage and leading to necrosis and plant death at later stage (Pitzschke *et al*. 2006). To undertake excessive ROS production, plant produces different enzymatic peroxidase (POX), polyphenol oxidase (PPO), phenyl alanine lyases (PAL), catalase (CAT), superoxide dismutase (SOD) and non-enzymatic antioxidant polyphenol oxidase (PPO) and total phenolic content (TPC) that scavenges oxygen species and terminates the chain reaction of free radical production. Previously, it was reported that *T. harzianum* acts as a good elicitor of plant defense reactions against wide range of phytopathogens (Harman *et al*., 2004 ; Shoresh *et al*., 2010). In present investigations, we had evaluated induction in antioxidant enzymes on seed bio-priming with potential *Trichoderma* mutants in chickpea and wheat crop and seed treatment with BARC mutants along with combination of propiconazole. Mature and flag leaves of chickpea and wheat plants were used to estimate the quantity of antioxidant enzyme by spectrophotometric analysis (Table 4.20, 4.21).

4.5.1 Superoxide Dismutase (SOD):

SOD's are metalloenzymes and ubiquitous in nature that acts as first line of defense system (Fridovich., 1975) against ROS (reactive oxygen species). It serves as a major detoxifying enzymatic component of superoxide radicals by catalyzing it to hydrogen peroxide (Hossain., 2009). Spectrophotometric analysis of ROS scavenging activity of SOD was carried out in chickpea and wheat samples derived from seed bio-priming with potential *Trichoderma* mutants and seed treatment of chickpea seeds with BARC mutant and propiconazole combination (Table: 4.20). NBT (nitroblue tetrazolium) reduction is used as an indicator of O_2^- (superoxide anions) production. SOD competes with NBT for O_2^-. The percent inhibition of NBT reduction is a measure of the amount of SOD present (units/g). induction of SOD activity was noticed in chickpea samples derived from seed bio-priming with potential *Trichoderma* mutants in chickpea and wheat as well as seed treatment with BARC mutant with combination of propiconazole. Chickpea plants seed bio-primed with potential *Trichoderma* mutants M-23 ($4375.64^a \pm 49.25$) showed maximum SOD activity leading to 30.14 % increase over control. Remaining mutants showed lower SOD production than control (Figure 4.49). In wheat samples, derived from seed bio-primed with potential *Trichoderma* mutants SOD production was less as compared to control indicating that potential *Trichoderma* mutants were ineffective in inducing SOD production in wheat (Figure 4.51). Whereas, in chickpea samples, derived from seed treatment with BARC mutant and propiconazole combination SOD production was less as compared to control (Figure 4.50). Similar results of increase in SOD activity in tomato was recorded by Rajput *et al*., (2019). Tomato seeds bio-primed with *Trichoderma* BHUR2 spore suspension was found with 3.37 fold increased activity of SOD at 72 h as compared to non-primed pathogen control plants. Singh *et al*., (2014) also recorded that chickpea plants treated with triple microbial consortium (*fluorescent Pseudomonas* + *T. harzianum* THU0816+*Rhizobium leguminosarum* RL091) resulted in 1.76 and 3.60 folds increase in SOD activity in pathogen challenged and non challenged control plants respectively.

4.5.2 Peroxidase (POX)

Peroxidase is a important key enzyme that play a vital role in regulation of elongation of cell growth, oxidation of phenolic compounds, polysaccharide cross linking and IAA oxidation (Harish *et al*., 2009). Along with it peroxidase also takes part in lignifications and suberization that helps to block the invasion of pathogens due its non-degeradable and anti-microbial nature (Roger and Campbell, 2014; Mishra *et al*., 2014). The present investigation on peroxidase activity revealed that levels of accumulation of POX ($min^{-1}g^{-1}FW$) was similar to control in case of chickpea seed bio-primed with mutant M-136. Where as, maximum POX activity (68.17 $min^{-1}g^{-1}FW$) in wheat plants was observed in samples derived from seed bio-priming with mutant M-136 leading to 0.13 % increase in POX activity over control. Similarly, in chickpea plants derived from seed treatment with T_3 (propiconazole @ 3ml/kg of seed) maximum POX (69.85 $min^{-1}g^{-1}FW$) activity was recorded leading to 5.01 % increase in POX activity over control (Figure 4.52- 4.54 : Table 4.20, 4.21).

Similar results of increase in POX activity upon seed bacterization of pigeonpea with *Bacillus subtilis* AF 1 was recorded by (Podile and Laxmi., 1998). Bio agent treatment in chickpea plants showed enhanced activity of POX but in the triple consortium (*fluorescent Pseudomonas* + *T. harzianum* THU0816+*Rhizobium leguminosarum* RL091) 1.40 and 2.40 folds increased activity was observed in pathogen challenged and non challenged healthy control plants respectively (Singh *et al*., 2014). Rajput *et al*., (2019) observed increase in POX activity (2.06 fold) at 48 h in leaf samples derived from seed bio-primed with *Trichoderma* BHUR2 plants as compare to pathogen challenged control. Rice root bacterized with *P. fluorescens* isolate Pf-1 upon challenge with inoculation of *Meloidogyne graminicola* expressed higher peroxidase activity (5.85 $min^{-1}g^{-1}FW$) compared to roots inoculated with nematode alone and roots bacterized with *P. fluorescens* alone (Anita and Samiyappan., 2012).

4.5.3 Phenylalanine Ammonia Lyases (PAL):

PAL is a important part of phenylpropanoid metabolism. Metabolism process of phenyl propanoid starts with the conversion of l-phenylalanine into trans cinnamic acid by PAL. Further, PAL activity precedes with rapid recognition of pathogenic invaders which leads to accumulation of phenolic, phytoalexins and lignin (Mandal and Mitra, 2007). The present investigation revealed that variation in levels of accumulation of PAL was consistently higher in chickpea and wheat samples derived from seed bio-primed with potential *Trichoderma* mutants as well as chickpea samples derived from different seed treatment combination of BARC mutant with propiconazole.

Significant increase in PAL activity (mmole/L/g) was noticed in chickpea pre-treated with BARC mutant and propiconazole combination and in wheat pre-treated with potential *Trichoderma* mutants. In case of chickpea derived seed bio-primed samples with potential *Trichoderma* mutants, maximum PAL activity (3.263^{a}±0.003 mmole/L/g) upto 2.42 % increase was observed with mutant M-136 as compare to untreated or control samples (Figure 4.55). All the treatments in wheat showed higher PAL activity as compare to control. Wheat samples derived from mutant M-23 showed maximum PAL activity (3.348^{a}±0.018) upto 7.49 % increase as compare to untreated or control samples (Figure 4.57). Seed treatment with BARC mutant, propiconazole and its combination also showed increase activity of PAL. Maximum PAL activity (3.34^{a}±0.003) was recorded with samples derived from seed treated with treatment T_1 (Tricho BARC @ 5 gm/kg of seed) with 18.74 % increase in PAL activity (Figure 4.56).

Similar results of increase in PAL activity was noticed by Rajput *et al*., 2019. They observed increase in PAL activity significantly in all treatments after 24 h of pathogen challenged condition. Highest PAL (2.98 folds) activity was observed in the samples derived from seed bio-primed tomato plants with *Trichoderma* BHUR2 spore suspension. Anand *et al*., 2007 reported that tomato plants treated with

azoxystrobin at three different concentration and *P. fluorescence* induced the plants to synthesize PAL. Han *et al*., 2006 observed increased activity of PAL for the first six days in wheat plant sprayed with hexaconazole after inoculation with *Puccinia striiformis*.

4.5.4 Lipid Peroxidase (LPO)

Reactive oxygen species can induce lipid peroxidation and disrupt the bilayer lipid membrane arrangement and increase tissue permeability (Girotti, 1985). Main targets of ROS in phospholipid membrane are double bond between C – atoms and ester linkage between glycerol and fatty acids. Lipid peroxides are highly unstable and decompose to a form a complex series of compound including reactive carbonyl compounds. The PUFA (poly unsaturated fatty acids) like linoleic and linolenic acid of lipid membrane are hot spots for ROS damage and highly prone to hydroxyl radicals. The PUFA (poly unsaturated fatty acids) generated malondialdehyde (MDA) upon decomposition. Measurement of MDA has been used as indicator of lipid peroxidation (Das and Roychoudhury, 2014). The present investigation revealed that variation in levels of accumulation of malondialdehyde was consistently higher in chickepea and samples derived from seed bio-priming with potential *Trichoderma* mutants and seed treatment with BARC mutant with propiconazole.

Spectrophotometric analysis of chickpea samples derived from seed bio-primed with potential *Trichderma* mutants showed maximum accumulation of malondialdehyde (n mole/L/g) (Table 4.20). Chickpea samples derived from seed bio-primed with mutant M-136 showed highest malondialdehyde activity (40628.2^{a}±314.356) which led to 65.50 % increase over control followed by BARC mutant (24266.03^{b}±676.247) which led 42.24 % increase in malondialdehyde activity over control (Figure 4.58). In case of samples derived from seed treated with BARC mutant and propiconazole along with its combination all treatment showed significant increased activity of malondialdehyde (Figure 4.59).

Maximum activity of malondialdehyde (29903.85^{a}±493.59) was recorded in treatment T_4 (Propiconazole @ 1.5 ml/kg of seed) which led to 35.84 % increase over

control. Similar results of accumulation of MDA in chickpea was also reported by Singh *et al*., (2014). Decrease in MDA was detected in all plants treated with bio-control agents throughout the experimental period compared to untreated plants challenged with pathogen. Maximum decrease in MDA by 0.79 fold with respect to challenged control plants was observed in PHU 094+THU0816 at 96 hr.

4.5.5 Polyphenol Oxidase (PPO)

PPO is ubiquitous antioxidant enzyme that can catalyze phenol oxidation to more toxic quinine compound. Also PPO generates ROS (reactive oxygen species) that plays a significant role in array of defense related activity (Singh *et al*., 2012, 2013a). The present investigation revealed that variation in levels of accumulation of PPOs was consistently higher in chickpea samples derived from seed bio-priming with potential *Trichoderma* mutants and seed treatment with BARC mutant and propiconzole and its combination (Table 4.20). The maximum PPO activity (0.008241 ΔOD $min^{-1}g^{-1}FW$) was observed in samples derived from seed bio-primed chickpea plants with BARC mutant which led to 49.83 % increase in PPO activity compare to control followed by M-23 (0.008084 ΔOD $min^{-1}g^{-1}FW$) and M-18 (0.005677 ΔOD $min^{-1}g^{-1}FW$) which led to 48.86 % and 27.17 % increase in PPO activity compare to control (Figure 4.60). In case of samples derived from seed treated chickpea plants with BARC mutant and propiconzole and its combination, highest PPO activity (0.006279 ΔOD $min^{-1}g^{-1}FW$) was recorded with treatment T_1 (Tricho BARC @ 5 gm/kg seed) which led to 59.16 % increase in PPO activity as compare to control (Figure 4.61). Similar results of induction of PPOs activity was reported by Singh *et al*., 2016. Tomato seed bio-primed with *Trichoderma* BHUT8 spore effectively modulated the enzymatic activity in the treated plants as compare to control. Highest PPO activity (21.75 %) in treated plants was observed as compared to control. Meena *et al*., (2000) reported that *Pseudomonas fluorescence* induce the activity of PPOs in response to infection caused by *Cercospora personatum* in groundnut. Activity of PPO was increased gradually and maximum PPO activity was observed on 21 days after inoculation of *Meloidogyne graminicola* in bacterized (*P. fluorescens*) paddy root tissues (Anita and Samiyaapan., 2012)

4.5.6 Total Phenolic Content (TPC)

Phenolic are one of the important ubiquitous group of secondary metabolites present throughout the plant kingdom (Boudet., 2007). These compounds are often produced and get accumulated in the sub-epidermal layers of plant tissues exposed to different biotic and abiotic stresses and pathogen attack (Cle *et al*., 2008). Accumulation ans synthesis of phenolics in plant system depend on season and different stages of growth and development along with some of internal as well as external factors such as wounding, trauma, draught and pathogen attack (Kefeli *et al*., 2003). Being scavengers of free radicals phenolic compounds (POH) acts as acceptor of free radical and chain breakers. They interfere with the oxidation of lipids and other molecules by rapid donation of hydrogen atom to radicals. Also phenolic compounds as constituent of lignin may contribute to enhance mechanical strength of host cell wall and may also inhibit fungal pathogen growth as they are fungitoxic in nature.

In the present investigation accumulation of phenolics was higher in chickpea samples derived from seed bio-priming with potential *Trichoderma* mutants and seed treatment with BARC mutant and propiconzole and its combination as compared to untreated or control (Table 4.20, 4.21). The amount of total phenolic content (TPC) was determined with the Folin-Ciocalteu reagent. Gallic acid was used as standard compound and the total phenols were expressed as µg GAE/g gallic acid equivalent using standard curve equation: y=0.0127x-0.2234, R^2= 0.9697. Where y is absorbance at 725 nm and x is total phenolic content in different potential *Trichoderma* and BARC mutant, propiconzaole treated chickpea plants expressed in µg GAE/g. TPC in chickpea seed bio-primed plants with potential *Trichoderma* mutants was varied from 1928.54^{ab}±218.54 to 2104.295^{a}±20.625 µg GAE/g. The maximum TPC activity (2104.295^{a}±20.625 µg GAE/g) was observed in samples derived from seed bio-primed chickpea plants with mutant M-23 which led to 8.35 % increase in TPC content as compare to control (Figure 4.62). Whereas, TPC in chickpea seed treated plants with BARC mutants, propiconazole and its combination (BARC+Propiconazole) was varied from 1495.875^{c}±312.958 to 2344.375^{a}±373.375 µg GAE/g. The maximum TPC activity (2344.375^{a}±373.375 µg GAE/g) was observed in samples derived from seed treated chickpea plants with treatment T_4 (propiconazole

@ 1.5 ml/kg of seed) which led to 32.10 % increase in TPC content as compare to control (Figure 4.63). Similar results of variation in TPC activity was observed in tomato plants seed bio-primed with *Trichoderma asperellum* BHUT8 as compared to untreated control (Singh *et al*., 2013[a]). Rajput *et al*., 2019 detected increased TPC activity (4.01 fold) in tomato plants seed bio-primed with *Trichoderma* BHUR2 spore suspension compared to non-primed pathogen challenged control plants. Anita and Samiyappan (2012) bacterized rice roots with *Pseudomonas fluorescens* and observed accumulation of phenolics after seven days of inoculation with *Meloidogyne graminicola* in bacterized roots. The highest catechol accumulation (2.23 µg catechol mg-1 protein) was observed in plants bacterized with *P. fluorescens* on the 14th day after inoculation with nematode. Selvaraj and Ambalavanan (2013) noticed phenol accumulation in all plants treated with bio-control agents and had profound effect on accumulation of phenols in plants upon challenge inoculation with *C. gleosporioides*

Table 4.20 Effect after seed bio-priming with potential *Trichoderma* mutants and seed treatment with BARC mutants, propiconazole and its combination with chickpea leaves contents of Lipid peroxidase (LPO), phenylalanine ammonia lyase (PAL), peroxidase (POx), polyphenol oxidase (PPO), super oxide dismutase (SOD) activities and total phenolic content (TPC)

Treatments	LPO	PAL	POx	PPO	SOD	TPC
(a) Chickpea	nano mole/L/g	milli mole/L/g	ΔOD $min^{-1}g^{-1}FW$	ΔOD $min^{-1}g^{-1}FW$	Units/g	µg GAE/g
M-18	10778.85^{d}±144.267	3.096^{c}±0.017	60.703	0.005677	1065.257^{d}±29.67	1965.96^{ab}±206.791
M-23	11948.72^{cd}±185.866	2.594^{d}±0.025	56.034	0.008084	4375.646^{a}±49.255	2104.295^{a}±20.625
M-136	40628.2^{a}±314.356	3.263^{a}±0.003	70.042	0.003558	2069.95^{c}±34.534	1658.205^{c}±39.375
BARC	24266.03^{b}±676.247	3.17^{b}±0.02	56.034	0.008241	1210.742^{d}±391.801	1611.08^{cd}±8.5
Control	14016.03^{c}±1451.93	3.184^{b}±0.023	70.042	0.004134	3056.768^{b}±129.452	1928.54^{ab}±218.54
(b) Chickpea						
T_1	23721.15^{bc}±1855.77	3.34^{a}±0.003	23.114	0.006279	1441.349^{c}±244.912	1495.875^{c}±312.958
T_2	22051.28^{c}±2012.82	3.079^{ab}±0.003	49.076	0.006017	2451.171^{abc}±856.868	1599.542^{bc}±348.292
T_3	20285.26^{c}±1509.62	2.976^{bc}±0.031	69.856	0.005861	3616.202^{a}±316.881	1995.083^{b}±38.167
T_4	29903.85^{a}±493.59	3.269^{ab}±0.026	44.033	0.004238	1499.345^{c}±147.039	2344.375^{a}±373.375
T_5	29310.9^{a}±515.971	3.085^{ab}±0.022	43.520	0.003113	1946.564^{bc}±308.684	1662.875^{bc}±93.042
T_6	27163.46^{ab}±842.98	3.28^{ab}±0.023	56.361	0.004971	3193.049^{ab}±39.516	1754.958^{bc}±76.958
T_7	19185.9^{c}±153.725	2.714^{c}±0.229	66.353	0.002564	3335.345^{a}±845.043	1591.667^{bc}±41.583

GAE= Gallic acid equivalent; ΔOD $min^{-1}g^{-1}$FW= change in OD $min^{-1}g^{-1}$Fresh weight; values of LPO, PAL, SOD & TPC are average of three replications; values after ± represent standard error (SE).

Table 4.21 Effect after seed bio-priming with potential *Trichoderma* mutants with wheat leaves contents of phenylalanine ammonia lyase (PAL), peroxidase (POx), super oxide dismutase (SOD) activities and total phenolic content (TPC)

Wheat	PAL	Pox	SOD	TPC
	milli mole/L/g	ΔOD $min^{-1}g^{-1}$FW	Units/g	µg GAE/g
M-18	3.262^{ab}±0.002	64.159	1382.7^{d}±674.306	1269.625^{c}±277.125
M-23	3.348^{a}±0.018	63.832	2661.863^{c}±102.652	1636.25^{ab}±85.
M-136	3.153^{bc}±0.058	68.175	3090.733^{b}±1217.25	1658.208^{ab}±39.375
BARC	3.104^{c}±0.017	46.928	3074.735^{b}±603.324	1611.083^{ab}±8.5
Control	3.097^{c}±0.036	68.081	3954.11^{a}±42.267	1928.542^{a}±218.542

GAE= Gallic acid equivalent; ΔOD $min^{-1}g^{-1}$FW= change in OD $min^{-1}g^{-1}$Fresh weight; values of LPO, PAL, SOD & TPC are average of three replications; values after ± represent standard error (SE)

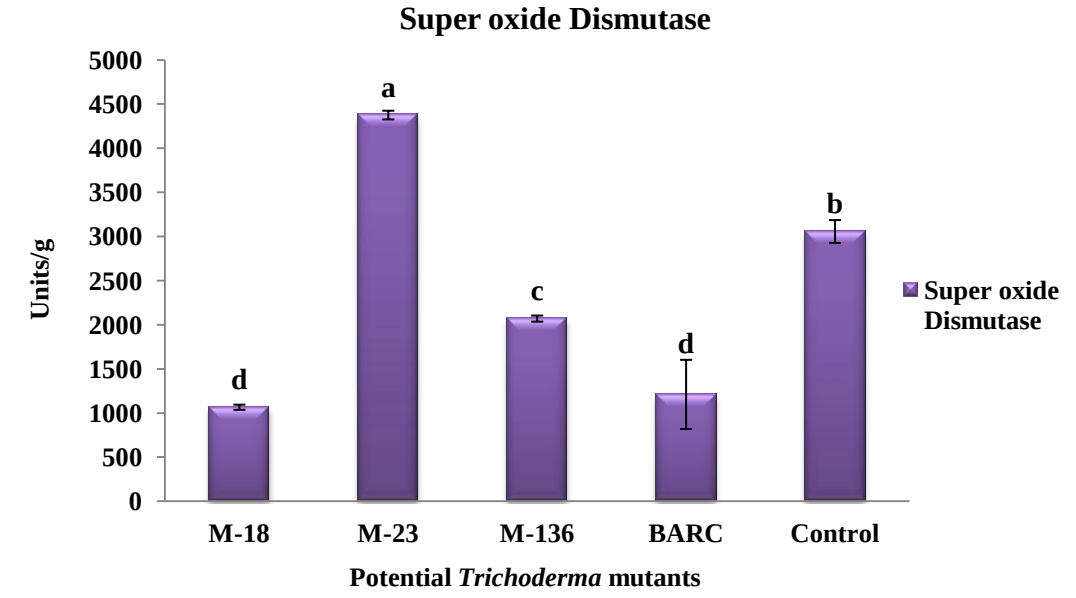


Figure 4.49 : Effect of seed bio-priming with potential *Trichoderma* mutants on Superoxide Dismutase (SOD) activity in chickpea leaves

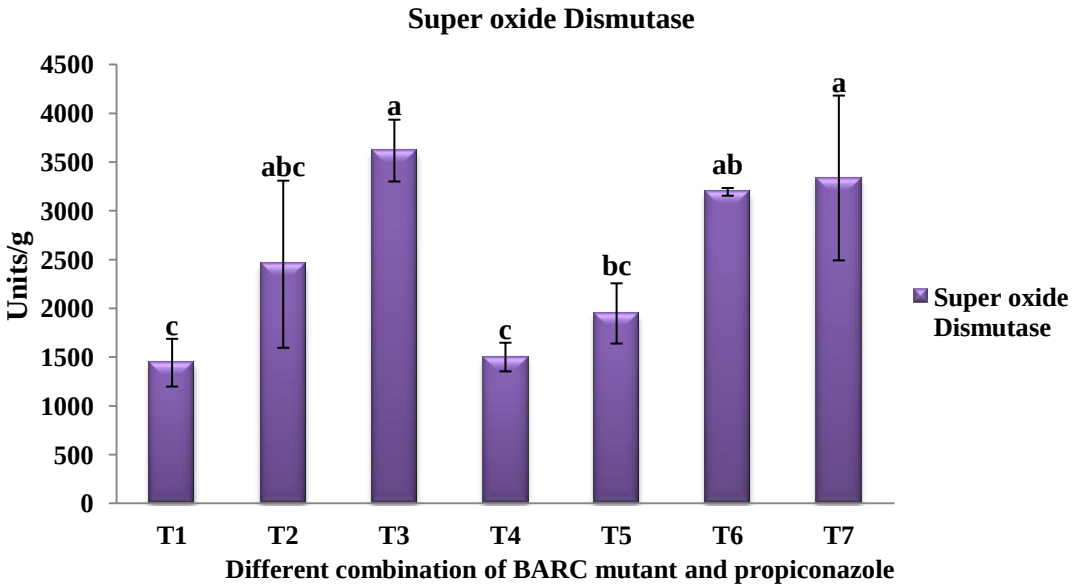


Figure 4.50 : Effect of seed treatment with BARC mutant, propiconazole and its combination on Superoxide Dismutase (SOD) activity in chickpea leaves

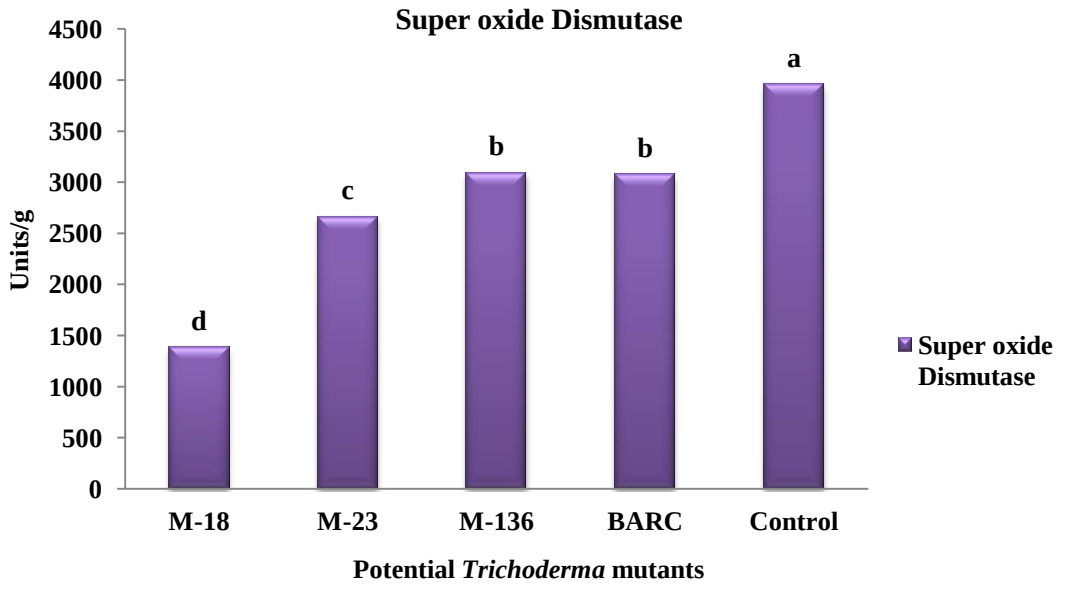


Figure 4.51 : Effect of seed bio-priming with potential *Trichoderma* mutants Superoxide Dismutase (SOD) activity in wheat leaves

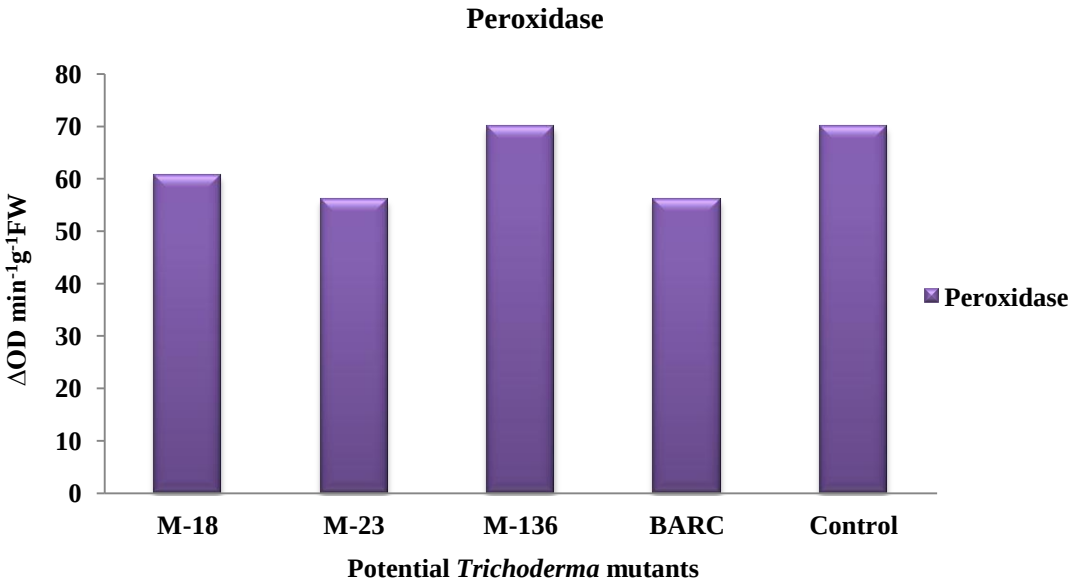


Figure 4.52 : Effect of seed bio-priming with potential *Trichoderma* mutants on Peroxidase (POx) activity in chickpea leaves

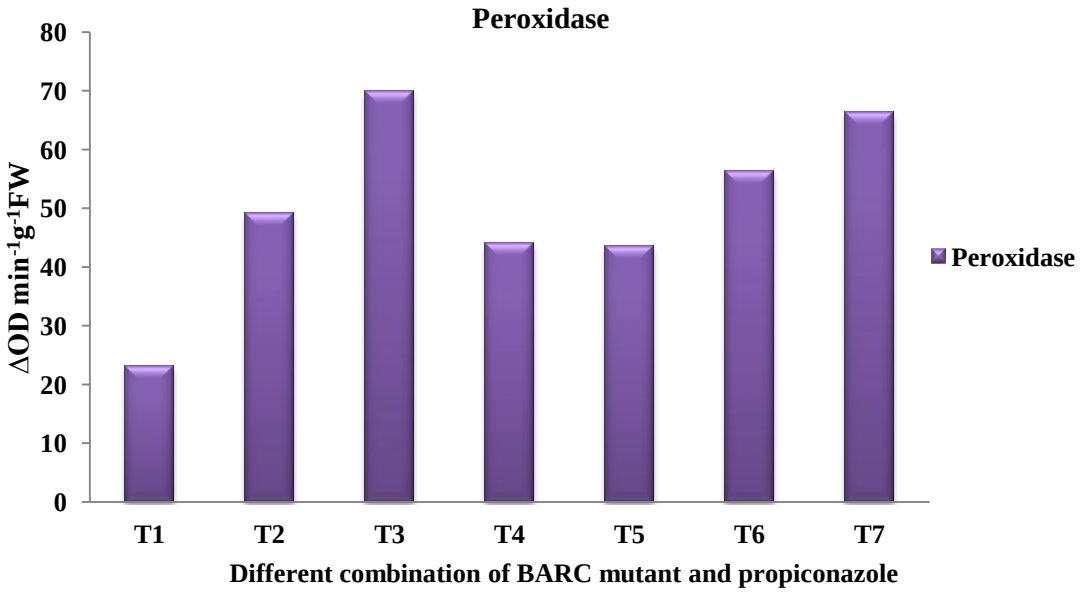


Figure 4.53 : Effect of seed treatment with BARC mutant, propiconazole and its combination on Peroxidase (POx) activity in chickpea leaves

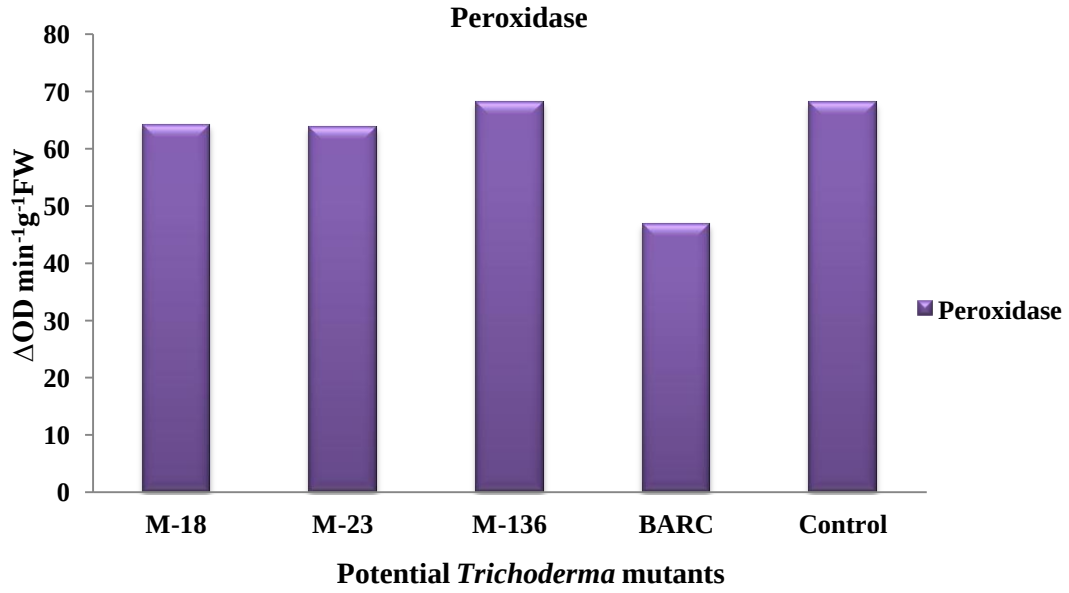


Figure 4.54 : Effect of seed bio-priming with potential *Trichoderma* mutants on Peroxidase (POx) activity in wheat leaves

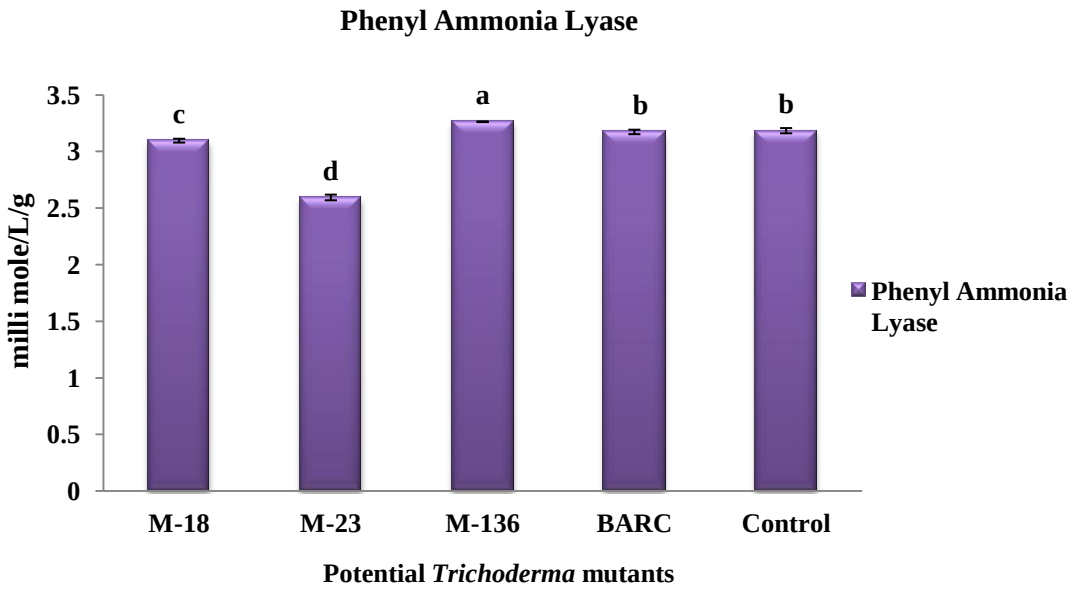


Figure 4.55 : Effect of seed bio-priming with potential *Trichoderma* mutants on Phenyl Ammonia Lyase (PAL) activity in chickpea leaves

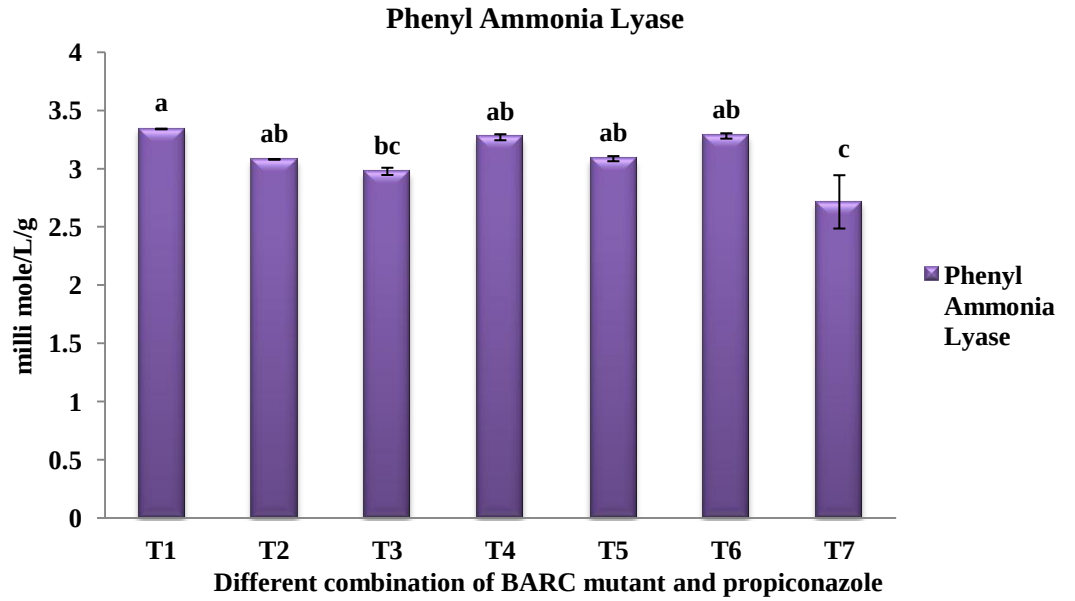


Figure 4.56 : Effect of seed treatment with BARC mutant, propiconazole and its combination on Phenyl Ammonia Lyase (PAL) activity in chickpea leaves

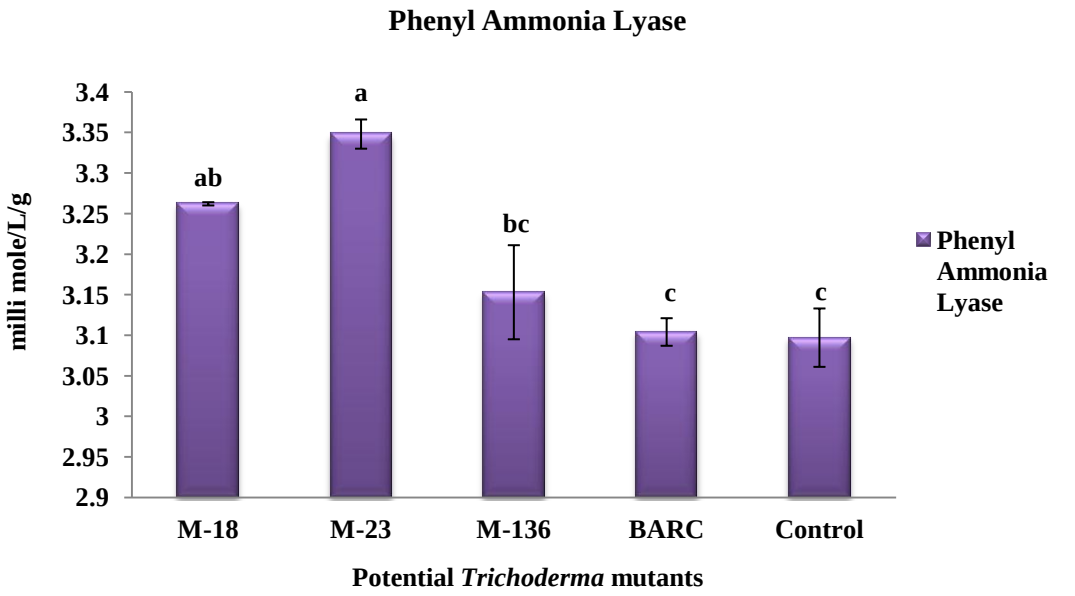


Figure 4.57 : Effect of seed bio-priming with potential *Trichoderma* mutants on Phenyl Ammonia Lyase (PAL) activity in wheat leaves

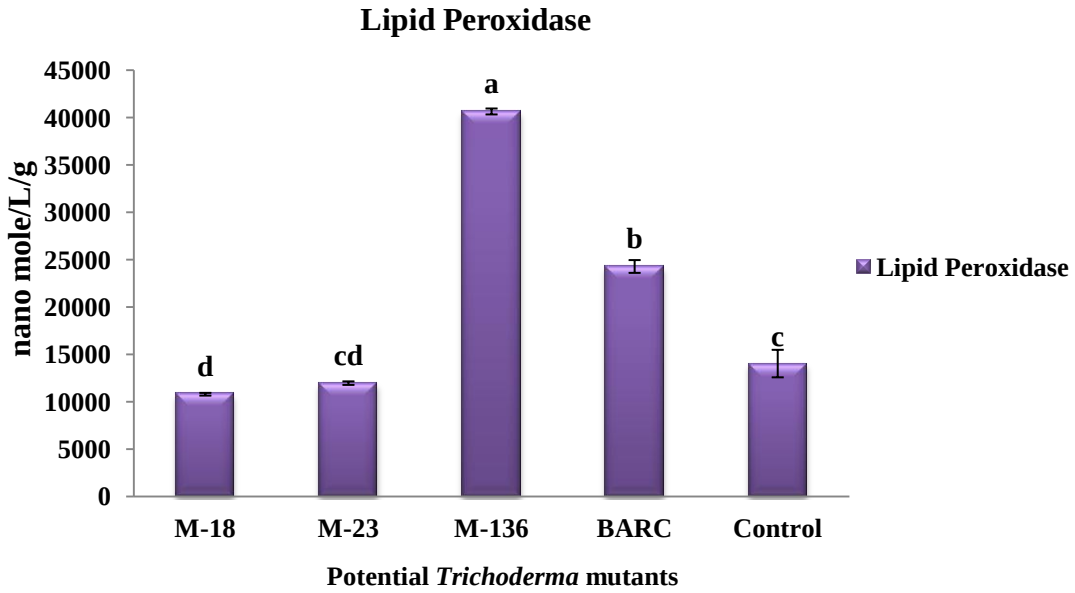


Figure 4.58 : Effect of seed bio-priming with potential *Trichoderma* mutants on Lipid Peroxidase (LPO) activity in chickpea leaves

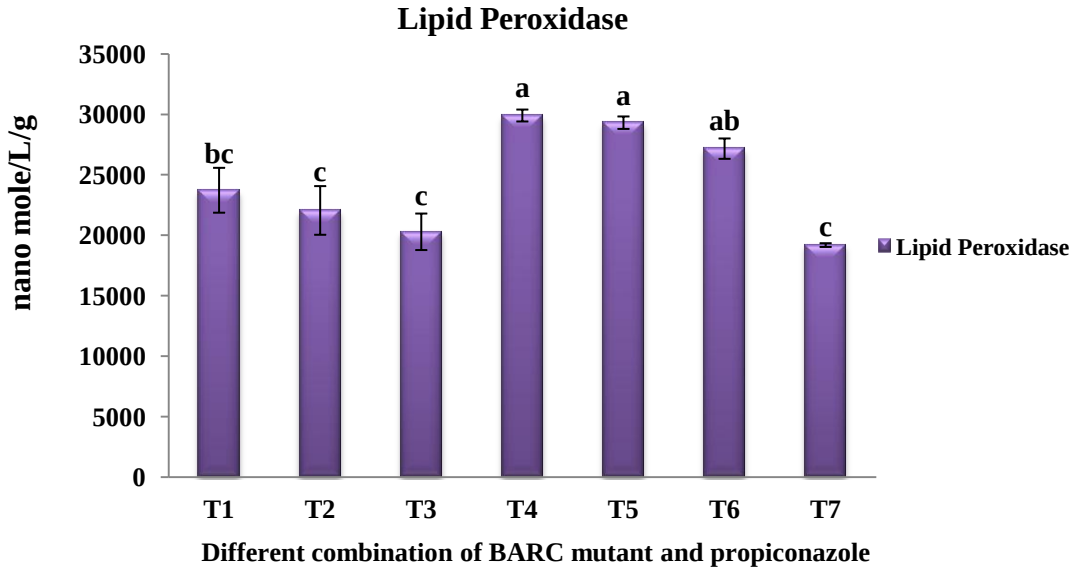


Figure 4.59 : Effect of seed treatment with BARC mutant, propiconazole and its combination on Lipid Peroxidase (LPO) activity in chickpea leaves

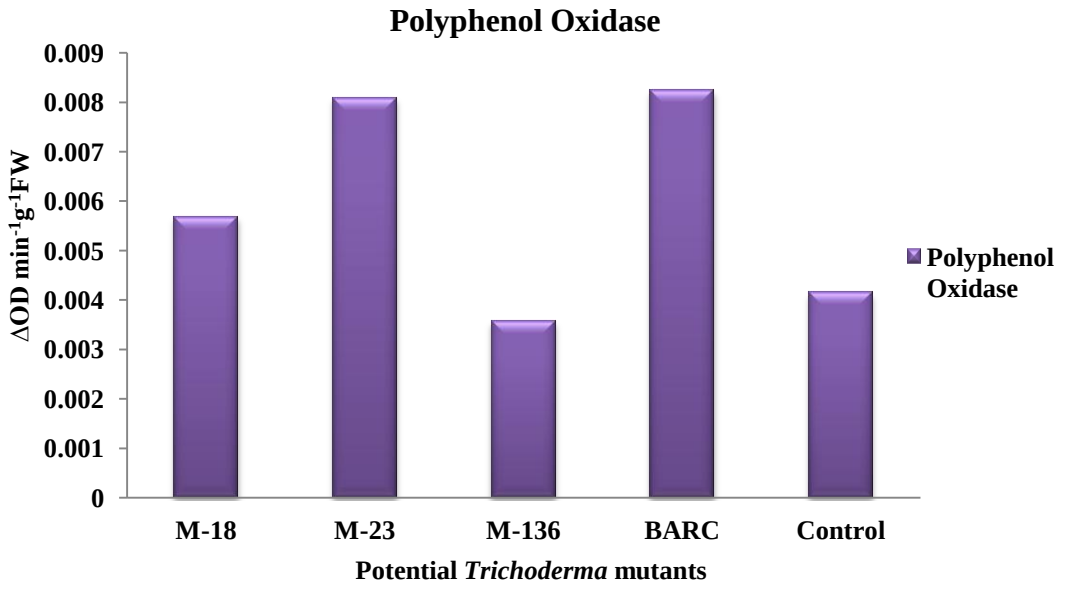


Figure 4.60 : Effect of seed bio-priming with potential *Trichoderma* mutants on Polyphenol Oxidase (PPO) activity in chickpea leaves

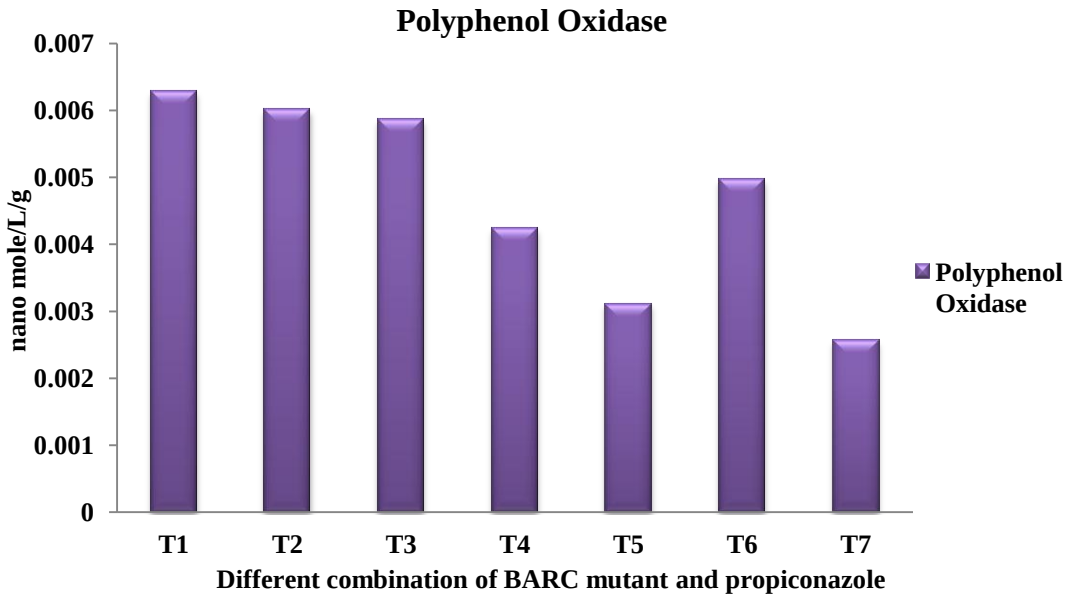


Figure 4.61 : Effect of seed treatment with BARC mutant, propiconazole and its combination on Polyphenol Oxidase (PPO) activity in chickpea leaves

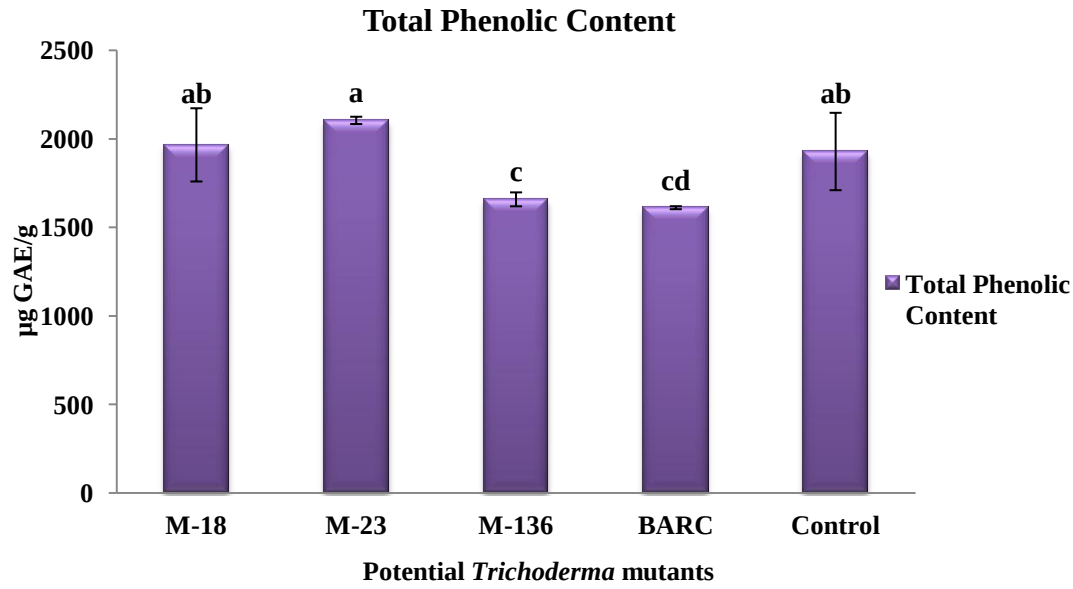


Figure 4.62 : Effect of seed bio-priming with potential *Trichoderma* mutants on Total Phenolic Content (TPC) in chickpea leaves

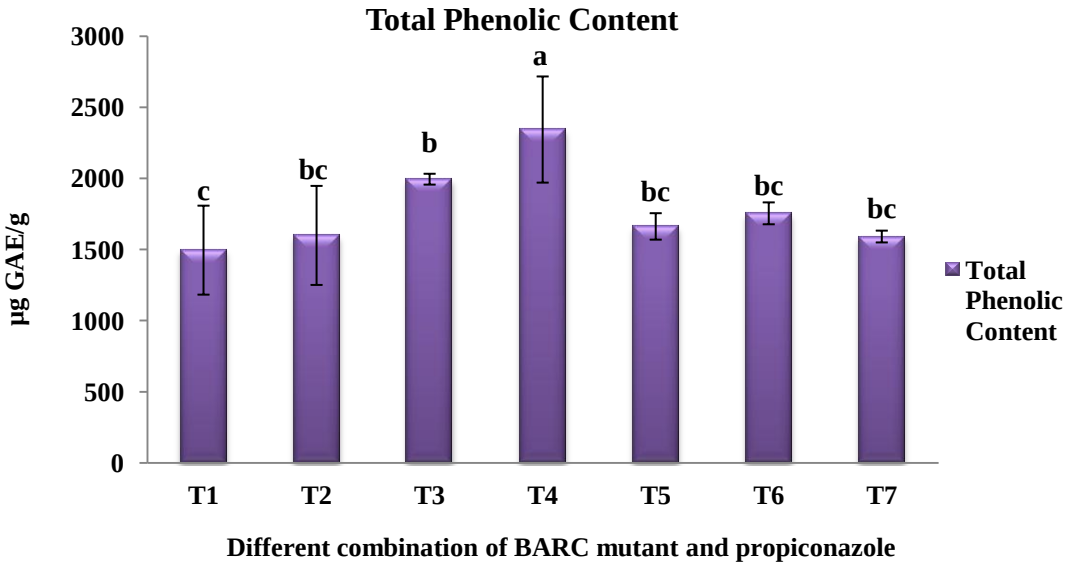


Figure 4.63 : Effect of seed treatment with BARC mutant, propiconazole and its combination on Total Phenolic Content (TPC) in chickpea leaves

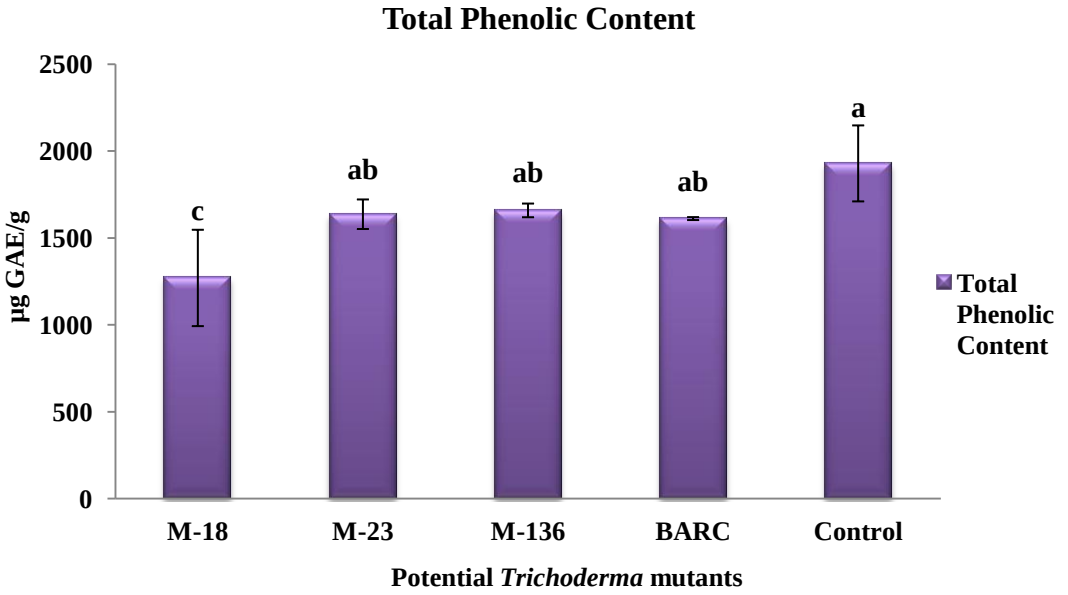


Figure 4.64 : Effect of seed bio-priming with potential *Trichoderma* mutants on Total Phenolic Content (TPC) in wheat leaves

CHAPTER-V
SUMMARY AND CONCLUSIONS

Trichoderma spp. are well known for its improve nutrient use efficiency, mycoparasitic and plant growth promoting ability, ability to produce diverse array of secondary metabolites, systemic induce resistance in plants against invading pests and pathogens and to impart tolerance to abiotic stresses (Mastouri *et al*., 2012; Reithner *et al*., 2014; Zhang *et al*., 2014; Lamdan *et al*., 2015, Salas-Marina *et al*., 2015; Chagas *et al*., 2017). The present invstigation entitled "**Evaluation of seed dressing formulations of potential *Trichoderma* mutants on growth and yield attributing characters of cereals, pulses and vegetables" was** undertaken following seed bio-priming with different concentrations of seed dressing formulation of four potential *Trichoderma* mutants with a logic to reduce the amount of recommended doses of seed dressing formulation. Seed dressing formulations of four potential *Trichoderma* mutants were evaluated under field condition on growth and yield attributing characters of different crops and vegetables. Potential mutant with biocontrol abilities was evaluated following confrontation assays against *Sclerotiun rolfsii.* Triazole group of fungicides supplimentd in different concentration in the growth medium of *S rolfsii* helped us to evaluate the effective inhibitory dose.Plant derived from seed bio-priming with potential *Trichoderma* mutants on chickpea and wheat leaves helped us to evaluate the superoxide dismutase (SOD), peroxidase (POX), phenylamine ammonia lyase (PAL), lipid peroxidase (LPO), polyphenol oxidase (PPO) and total phenolic content (TPC) activity. Results of the present investigation are summarized as below:

Effect of seed bio-priming with different concentrations of seed dressing formulation of potential *Trichoderma* mutants on stimulating plumule and radicle length

Plant growth promoting *Trichoderma* applied through seed bio-priming, enhances the abundance of propagules of *Trichoderma* in the spermosphere by 10-fold than attacking pathogens which enables the plant to survive. We therefore argued that will lowering dose of seed treatment and incubating the seeds for premeasured time

will proliferate the *Trichoderma* inside the seeds by 10-fold and will have an equivalent growth promoting effect?

1. Experimental results revealed that mung bean and rice seed bio-priming with potential *Trichoderma* mutants (M-18, M-23, M-136 and BARC) spores significantly increased the plumule length but not radicle length in plants derived from seed treated as compared to control.
2. Highest increase in plumule length in mung bean seedlings was observed in seedlings derived from different mutant formulation M-18
3. Increase in plumule and radicle length in rice seedlings was observed in seedlings derived from different doses of mutant formulation M-23.

Observation in the present investigation indicates that spray inoculating the seed with different doses and incubating the seeds for premeasured time had many fold proliferated the *Trichoderma* propagule but its effects were not directly proportional with the increasing doses. Variable response of different doses in inducing increased plumule length was observed.

Study of Root characteristics of Mung bean and Rice seedlings

Manipulating Root system Architecture (RSA) using bio agents can be one of the strategy to enhance nutrient acquisition especially in low input agricultural systems. We therefore reasoned that, will the potential *Trichoderma* mutant's used in the present investigation (M-18, M-23, M-136 and BARC) be able to improve root architecture of mungbean and rice if they are delivered through seed bio-priming?

1. Roots of Mungbean seedlings derived after seed bio-priming with potential *Trichoderma* mutant's at different doses stimulated variable response in root architecture (root length, surface area, tips and forks) as compared to control.
2. Rice seedlings derived after seed bio-priming with potential *Trichoderma* mutant's (M-18, M-23, M-136 and BARC) at different doses stimulated increase root architecture (root length, surface area, tips and forks) as compared to control. Roots of rice seedlings derived from seed bio-priming with BARC mutant stimulated maximum total root length, surface area and number of forks. Highest number of

tips were recorded in roots of rice seedlings derived from seed bio-priming with M-18 mutant.

3. Roots of mungbean seedlings derived from seed bio-priming with BARC mutant (@ 3 gm/kg) stimulated maximum total length and surface area. Whereas, highest number of tips and forks were recorded in the roots of the plant derived from seed bio-primed with BARC mutant and M-136 respectively.

No correlation was observed in root architecture (root length, surface area, tips and forks) of mungbean and rice seedlings derived from seed bio-priming with increasing doses of potential mutants.

Plant growth promoting response in chickpea (Indira Chana-1) following seed bio-priming with BARC mutant (Field Trial)

Extensive field evaluation (on-farm demonstration trials) of mutant-based formulation (BARC) for plant growth promoting activity following seed bio-priming in chickpea (Indira Chana-1) was undertaken at research cum instructional farms, of College of Agriculture, I.G.K.V., Raipur and KVK Kawardha field during *kharif*-2018-2019 and 2019-2020. Increase in plant height, no. of pods, no. of primary branches and no. of plants (per sq. meter) determined in field treated with BARC mutant culture at two locations during the two consecutive years 2018 and 2019 were:-

1. **KVK Raipur :** Plant height (16.33% and 29.09%,); No. of branches, (37.06%and 33.52%); No. of Plants / Sq.M (34.43% and 39.34); No. of pods (23.45% and 32.40%) was improved during the extensive field testing**.**
2. **KVK Kawardha:-** Plant height (21.61% and 19.28%,); No. of branches, (28.31%and 26.90%); No. of Plants / Sq.M (27.87% and 37.71) was improved during the extensive field testing.

Plant growth promoting response in chickpea (Indira Chana-1) following seed bio-priming with potential *Trichoderma* mutants

In present investigation, four potential *Trichoderma* mutants were evaluated for their plant growth promotion ability and yield attributing characters following seed bio-priming in chickpea (Indira Chana-1).

1. Significant increase in primary branches and total filled pods were observed in all treatments over control.
2. Maximum number of primary branches and total filled pods were observed in chickpea plants seed bio-primed with mutant M-23. Whereas maximum shoot length and bundle weight was observed in chickpea plants seed bio-primed with mutant M-18 respectively.
3. Total bundle weight, total grain yield and total straw yield in chickpea plants derived from seed bio-primed with all potential *Trichoderma* mutants was markedly increased as compared to control.
4. Highest bundle weight and grain yield was recorded in chickpea plants derived from seed bio-primed with mutant M-18 and BARC mutant as compared to control.
5. Highest number of healthy plants (11.99 %) were observed in the chickpea plots seed bio-primed with mutant M-18, whereas seedling mortality was highest in control plots.
6. Lowest seedling mortality was observed in the chickpea plots seed bio-primed with BARC mutant formulation.

Plant growth promotion response in chickpea (Indira Chana-1) following seed treatment with BARC mutant and Propiconazole

1. Highest bundle weight was recorded in chickpea plants treated with T_5 (Tricho BARC 10g/kg seed + Propiconazole 3.0ml/kg seed) combination.
2. Highest number of filled pods was observed in chickpea plants treated with T_5 (Tricho BARC 10g/kg seed + Propiconazole 3.0ml/kg seed) combination which lead to 19.67 % increase in filled pods over control.
3. Whereas, maximum number of primary branches, shoot length and 100 seed weight was recorded in different treatments T_6, T_5, T_3 and T_4 respectively.
4. Bundle weight, straw yield and grain yield in chickpea plants treated with BARC mutant and propiconazole combination was significantly increased as compared to control.
5. Highest bundle weight and grain yield was recorded in chickpea plants treated with treatment T_3 (propiconazole @ 3 ml/kg seed).

6. Highest straw yield was recorded in chickpea plants treated with Tricho BARC @5gm per kg seed dose (T_1).

Plant growth promotion response in chickpea (Indira Chana-1) following seed treatment with BARC mutant and Propiconazole on field emergence and mortality of chickpea plants (Indira chana-1)

1. Highest number of healthy plants were observed in the chickpea plots derived from seed treated with treatment Tricho BARC @ 10 gm/kg of seed (T_2).
2. Highest seedling / plant mortality was observed in control plot of chickpea whereas, seedling / plant mortality was reduced in chickpea plots where the plants were derived from seed treated with treatment propiconazole @ 3ml/kg of seed (T_3)

Efficacy of 4 potential mutants of *Trichoderma* for plant growth promoting activity in different vegetable crops

Plants of different vegetable crops (Bitter gourd, Pumpkin, Long bean, Ridge gourd, Fenugreek, Tomato and Spinach) derived after seed bio priming with four potential *Trichoderma* mutants were evaluated for improvement in Plant Height (cm) and No. of leaves/ plant and root length(cm). Observations on Root length (cm) at 50 DAS. The effect of bio-priming with 4 potential mutants of *Trichoderma* on seven vegetable crops (Bitter gourd, Pumpkin, Long bean, Ridge gourd, Fenugreek, Tomato and Spinach) showed significant increase in plant growth.

1. **Bitter gourd (*Momordica charantia* Pearl F-1 Hybrid):** Potential *Trichoderma* mutants stimulated significant increase in plant growth of bitter gourd plants but the growth promoting effects varied. Maximum shoot length, leaf area(cm^2) , total branches were recorded when bitter gourd plant were derived from seeds bio-primed with mutant M-18 as compared to control. Whereas longest root length and maximum no. of flowers were observed in plants derived from seed bio-primed with isolate M-136 and M-23 respectively as compared to control.
2. **Pumpkin (*Cucurbita moschata* Pearl F-1 Hybrid):** Potential *Trichoderma* mutants stimulated significant increase in plant growth of pumpkin but the stimulating effects varied with the mutants used for seed treatment. Maximum shoot length, leaf area (cm^2), and root length were recorded when pumpkin plant

were derived from seeds bio-primed with mutant M-18 as compared to control. Potential Trichoderma mutants were not able to stimulate significant differences in no. of flowers and total no. of branches as compared to control.

3. **Ridge gourd (*Luffa acutangula* Pearl F-1 Hybrid):-** Potential *Trichoderma* mutants stimulated significant increase in plant growth of on Ridge gourd (*Luffa acutangula* Pearl F-1 Hybrid) but the effects were variable with the mutants used for seed treatment. Maximum shoot length and root length were observed when Ridge gourd plant were derived from seeds bio-primed with mutant M-23 as compared to control and other treatments. Leaf area , no. of flowers and total branches was significantly stimulated when Ridge gourd plant were derived from seeds bio-primed with mutant M-136 as compared to control and other treatments.
4. **Longbean (*Vigna unguiculata* Pearl F-1 Hybrid):** Potential *Trichoderma* mutants stimulated significant increase in plant growth of on Longbean (*Vigna unguiculata* Pearl F-1 Hybrid) but stimulatory responses varied. Maximum shoot length and root length was recorded when Longbean plant were derived from seeds bio-primed with BARC mutant. Increase in leaf area was significantly stimulated to increase when Longbean plant were derived from seeds bio-primed with mutant M-136. Whereas, highest no. of flowers was observed in Longbean plant derived from seed bio-primed with mutant M-18 as compared to control and other treatments.
5. **Fenugreek (*Trigonella foenum-graecum*):** Efficacy of all four potential mutants of *Trichoderma* varied to stimulate plant growth. Maximum plant height was recorded in Fenugreek plants which were derived from seed bio-primed with mutant M-136 where as mutant M-18 bio-primed plants were observed with highest primary branches and pod weight. Highest no. of pods were observed in Fenugreek plants which were derived from seed bio-primed with mutant M-136 as compared to control.
6. **Tomato (*Lycopersicum esculentum* Pearl F-1 Hybrid):**- Four potential mutants of *Trichoderma* stimulated significant differences in plant growth of tomato. Maximum root length and root fresh weight was recorded in tomato plants which were derived from seed bio primed with mutant M-23. Highest no. of flowers per

cluster and primary branches was observed in tomato plants which were derived from seed bio-primed with mutant M-23, M-18 and BARC mutant respectively.

7. **Spinach (*Spinacia oleracea*):** All four Potential mutants of *Trichoderma* stimulated significant differences in plant growth of spinach. Maximum root length and plant height was recorded in plants which were derived from seed bio primed with mutant M-136 and M-23 respectively. Highest number of leaves per plant was observed in plants which were derived from seed bio primed with BARC mutant. Stimulatory effects of four potential *Trichoderma* mutants was at par on spinach for number of leaves / plant whereas the stimulatory effects of M-18, M-23 and BARC mutant on plant height and of M-18, M136 for root length was at par on spinach.

In vitro* antagonistic activity of potential *Trichoderma* mutants against *S. rolfsii

1. All the potential *Trichoderma* mutants showed varied range of antagonism against *Sclerotium rolfsii* ranging from 79 per cent to 81.50 per cent. *Trichoderma* BARC mutant showed maximum inhibiting effect on the growth of *Sclerotium rolfsii* (81.50 %) over control.

In vitro* evaluation of fungicides against *Sclerotium rolfsii

1. Six fungicides and one antibiotics with three different concentrations were screened *in vitro* by poisoned food technique against *S. rolfsii*. Triazole group of fungicide (Difenconazole 25 % EC, Hexaconazole 5 % EC and Tebuconazole 25.9 % EC) imposed very strong inhibitory effect against *S. rolfsii* at different concentrations.

Effect of seed bio-priming with potential *Trichoderma* mutants in chickpea and wheat leaves contents of superoxide dismutase (SOD), peroxidase (POX), phenylamine ammonia lyase (PAL), lipid peroxidase (LPO), polyphenol oxidase (PPO) and total phenolic content (TPC)

In present investigations, we evaluated induction in antioxidant enzymes on seed bio-priming with potential *Trichoderma* mutants in chickpea and wheat crop and seed treatment with BARC mutants along with combination of propiconazole.

1. **Superoxide Dismutase (SOD):** Induction of SOD activity was observed in chickpea samples derived from seed bio-priming with all the potential *Trichoderma* mutants in chickpea and wheat as well as chickpea plants derived from seed

treatment with BARC mutant in combination with propiconazole. Chickpea plants seed bio-primed with potential *Trichoderma* mutants M-23 showed maximum SOD activity leading to 30.14 % increase over control. Whereas, in chickpea samples, derived from seed treatment with BARC mutant in combination with propiconazole, SOD activity was less induced.

2. **Peroxidase (POX):** The present investigation on peroxidase activity revealed that levels of accumulation of POX ($min^{-1}g^{-1}FW$) was similar to control in case of chickpea seed bio-primed with mutant M-136. Whereas, maximum POX activity in wheat plants was observed in samples derived from seed bio-priming with mutant M-136 leading to 0.13 % increase in POX activity over control. Chickpea plants derived from seed treatment with T_3 (propiconazole @ 3ml/kg of seed) maximum POX activity was recorded leading to 5.01 % increase in POX activity over control.
3. **Phenylalanine Ammonia Lyases (PAL):** The present investigation revealed variation in levels of accumulation of PAL was consistently higher in chickpea and wheat samples derived from seed bio-primed with all four potential *Trichoderma* mutants as well as chickpea samples derived from different seed treatment combination of BARC mutant in combination with propiconazole. Significant increase in PAL activity (mmole/L/g) was noticed in chickpea pre-treated with BARC mutant + propiconazole combination and in wheat pre-treated with all four potential *Trichoderma* mutants. Chickpea plants derived seed bio-primed mutant M-136 expressed maximum PAL activity, whereas mutant M-23 stimulated maximum PAL activity (7.49 %) in wheat seedlings. Increased (18.74 %) PAL activity was recorded with samples derived from seed treated with Tricho BARC @ 5 gm/kg of seed (T_1).
4. **Lipid Peroxidase (LPO):** All four potential *Trichderma* mutants stimulated maximum accumulation of malondialdehyde (n mole/L/g). Chickpea samples derived from seed bio-primed with mutant M-136 showed highest malondialdehyde, whereas BARC mutant in combination with propiconazole expressed significant increased activity of malondialdehyde. In plants derived from

seed treatment with Propiconazole @ 1.5 ml/kg of seed also stimulated accumulation of malondialdehyde at significant levels.

5. **Polyphenol Oxidase (PPO):** PPO is ubiquitous antioxidant enzyme that can catalyze phenol oxidation to more toxic quinine compound. Also PPO generates ROS (reactive oxygen species) that plays a significant role in array of defense related activity (Singh *et al*., 2012, 2013a). Samples derived from seed bio-primed chickpea plants with BARC mutant and BARC mutant + propiconzole stimulated significantly increased levels of Polyphenol Oxidase (PPO) activity.
6. **Total Phenolic Content (TPC):** In the present investigation accumulation of phenolics was higher in chickpea samples derived from seed bio-priming with potential *Trichoderma* mutants and seed treatment with BARC mutant and propiconzole and its combination as compared to untreated or control. The maximum TPC activity (µg GAE/g) was observed in samples derived from seed bio-primed chickpea plants with mutant M-23 which led to 8.35 % increase in TPC content as compare to control.

Conclusions

1. Lowering dose of seed treatment followed by bio-priming proliferated *Trichoderma* on seed by many folds and stimulated significant plant growth promoting effects.
2. Extensive field evaluation (replicated micro-plot trials, on-farm demonstration trials, and large-scale trials in farmers' fields) following seed bio-priming with potential mutant-based formulation significantly stimulated plant growth of chickpea and increased the yield by 20%.
3. All the potential *Trichoderma* mutants delivered through seed bio-priming of seven vegetable crops significantly stimulated plant growth.
4. Seed biopriming can be one of the successful strategy to scale up the microbial products (aimed at enhancing crop productivity) regional to global levels.
5. Chickpea and wheat leaves derived from seed bio-priming with potential *Trichoderma* mutants stimulated different antioxidant enzymes and total phenolic content (TPC).

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