IN-VITRO EVALUATION OF SEED TREATMENT BY COMMERCIAL FORMULATIONS OF TRICHODERMA ON SEEDLING QUALITY AND DROUGHT RESPONSES OF RICE

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This is to certify that, the thesis entitled, **"*IN-VITRO* EVALUATION OF SEED TREATMENT BY COMMERCIAL FORMULATIONS OF TRICHODERMA ON SEEDLING QUALITY AND DROUGHT RESPONSES OF RICE"** submitted to the Department of Plant Pathology, Sher- e-Bangla Agricultural University, Dhaka-1207, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY,** embodies the result of a piece of bona fide research work carried out by **SUBORNA AKTER, Registration No.: 17-08301** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly been acknowledged by her.

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Dedicated
To
My Beloved Parents

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ABSTRACT

A pot experiment was conducted in Net House at Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during the period from January 2018 to April 2018 to evaluate seed treatment by different commercial *Trichoderma* formulations seedling quality and drought responses of rice following CRD with five replications. Two drought tolerant varieties BRRI dhan 56, 71 and one drought susceptible check variety IR 64 and six treatments viz. T_1= G-derma (powder), T_2 = Bioderma, T_3= Recharge, T_4 = Decoprima, T_5 = G-derma (LDS) and T_6= Control were used in the experiment. Soil was sterilized by 2% formalin solutions. Seed related data was recorded on % seed germination, speed of germination (Germination Index), mean germination time (MGT), mean daily germination (MDG), peak value (PV) and germination value (GV). Moreover, data was recorded on seedling growth characteristics viz. number of leaves, number of tillers, plant height, shoot length, root length, fresh shoot and root weight, dry shoot and root weight, seedling vigor index (SVI) and seedling vegetative vigor (SVV) at different days after sowing. At 35 DAS, drought treatments were given and drought response data viz. droopy leaf, rolled leaf, drying leaf and drought sensitive scale was recorded at 4, 7, 10 and 13 days drought stress (DDS). The data were varied significantly among the treatments. Considering seed germinations parameters, T_4 (Decoprima) showed the best results followed by G-derma (powder) and Bioderma. However, in case of seedling growth, (Decoprima) showed the highest performance followed by G-derma (LDS) and Recharge. Moreover, the lowest drought responses were observed in Decoprima treated seeds followed by G-derma (LDS) and Recharge. The findings revealed that, seed treatment with *Trichoderma* can mitigate short duration drought stress in rice plant. No disease was observed in rice seedlings due to sterilization of soils and treatments of seeds.

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LIST OF ABBREVIATIONS

AEZ	=	Agro-Ecological Zone
BBS	=	Bangladesh Bureau of Statistics
CM	=	Centimeter
CV %	=	Percent Co-efficient of Variance
DAS	=	Days After Sowing
et al.,	=	And others
e.g.	=	exempli gratia (L), for example
etc.	=	Etcetera
FAO	=	Food and Agricultural Organization
g	=	Gram (s)
i.e.	=	id est (L), that is
Kg	=	Kilogram (s)
LSD	=	Least Significant Difference
M^2	=	Meter squares
ml	=	Milliliter
M.S.	=	Master of Science
No.	=	Number
^oC	=	Degree Celsius
%	=	Percentage
BRRI	=	Bangladesh Rice Research Institute
BARI	=	Bangladesh Agricultural Research Institute.
SAU	=	Sher-e-Bangla Agricultural University
NPK	=	Nitrogen, Phosphorus and Potassium
MOP	=	Muriate of Potash
NS	=	Not Significant
TSP	=	Triple Super Phosphate

DDS	=	Days Drought Stress
ISTA	=	International Seed Testing Agency
EC	=	Emulsifier Concentrate
GP	=	Germination Percentage
SVI	=	Seedling Vegetative Index
SVV	=	Seedling Vegetative Vigour
GI	=	Germination Index
SG	=	Speed of Germination
MGT	=	Mean Germination Time
MDG	=	Mean Daily Germination
PV	=	Peak Value
GV	=	Germination Value

CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereals in the world. It belongs to the family Graminae, It is the most important food for over two billion peoples in Asia and for hundreds of millions in Africa and Latin America. To feed the ever increasing population of these regions, the world's annual rice production must be increased from the present 560 to 750 million tons by 2020 (Saranraj *et al.*, 2013).

Rice is the staple food of Bangladesh and it constituted about 90% of the total food grain production (Ahmed *et al*., 2013). The average production per hectare of rice in Bangladesh is low as compared to other rice growing countries of the world. Seed is one of the most important input for crop production. For successful crop production there is no other alternative but to use good seed. It has been shown experimentally that only by using quality healthy seed, rice yield could be increased by 10-15% (Akter *et al.*, 2015).

In Bangladesh, approximately 2.5 million tons of rice worth more than Tk. 12000 millions lost annually due to diseases caused by seed borne pathogens (Fakir *et al.*, 2003). Rice suffers from more than 60 different diseases. In Bangladesh, 43 diseases are known to occur on the rice crop. Among these diseases, 27 are seed borne of which 14 are of major importance. The substantial increase in food grain production over the years has helped to meet the food security of the country, but the number of biotic and abiotic stress causes the yield losses up to a large extent. Biotic constraints include fungi, bacteria, virus, nematodes weeds, and insects which causes yield loss up to 31-42% (Agrios, 2005). Fungi are the principal organisms associated with seed in storage. Of all the seed borne diseases of rice, 22 are caused by fungi (Fakir, 2000). The contaminated seeds may fail to germinate, spread disease from seed to seedling and from seedling to growing plants (Farid *et al.,* 2002). Seed is a common carrier of plant pathogens. It carries several destructive pathogens that often take heavy toll causing diseases of crops raised from them. It acts as the primary source of many diseases. Most of the major diseases of rice are seed borne (Fakir, 2002). Bacteria are also commonly carried internally and externally by the seeds. The extremely seed borne pathogen of rice are Brown spot (*Bipolaris oryzae*), Bakanae (*Fusarium moniliforme*), Blast (*Pyricularia oryzae*), Sheath blight (*Rizoctonia solani*), Sheath rot (*Sarocladium oryzae*), Stem rot (*Sclerotium oryzae)* are associated with seed infection of rice and causes yield reduction, quality deterioration and germination failure (Shahjahan *et al.*, 1988).

High quality seed means the seed must be genetically pure with high germination capacity and high yield potential. Although the seed system in Bangladesh is at a very rudimentary stage, a total of 5 lac tons of seeds including the seeds of cereals and other crops per year is required, out of which only 18% seeds are produced by different seed organizations with care but almost regardless of the health status. It is incontestable that proper seed treatment measures can substantially improve the quality of seed and significantly increase the yield. On the other hand, to ensure eco-friendly disease management, Biological and botanicals are using instead of hazardous chemicals. BAU-Biofungicide, a biological control agent in Bangladesh resulted significant higher germination and plant stand, less disease incidence and higher yield of different crops (Hossain, 2011; Chowdhury *et al.*, 2013; Hossain and Hossain, 2012).

Seed priming is considered as a method to enhance seedling quality and vigour (Bradford, 1986; Basra *et al.*, 2004). Seed priming is also involved in enhancing rice resistance to environmental stress, weed suppressive ability and increase rice yield (Goswami *et al.*, 2013; Juraimi *et al.*, 2012). Previous studies have shown that rice seeds respond to seed priming in the early part of the germination stage and revealed that it can increase seed germination, vigour index and germination speed (Anwar *et al*., 2013; Zarei and Sinaki, 2012). Priming rice seed with plant growth regulators is one of the beneficial treatments that can be used to invigorate rice seed and improve rice seed quality (Farooq *et al.*, 2009). Seed biopriming and seed treatment with *Trichoderma* spp., trigger the release and/or production of enzymes and phytohormones which are involved in seed germination. It also enhances the speed of germination and seedling vigor. Enhanced germination percent have been found in okra, maize, beans, mustard, chilli, soyabean, chickpea, tomato etc. (Mukhtar, 2008; Okoth *et al.*, 2011; Rahman *et al.*, 2012; Lalita *et al.*, 2012; Kumar *et.al*., 2014; Babychan and Simon, 2017). Many seed invading pathogens such as *Pythium* are unable to attack on host due to faster seed germination and seedling vigor (Matsouri *et al.*, 2010).

Plant Growth Promoting Fungi (PGPF) is considered an alternative way to enhance rice growth and yield. The mechanisms by which PGPF act to enhance rice growth and yield includes production of phytohormones, phosphate solubilization, cellulose degradation and siderophore production. PGPF are generally abundant in the soil system and roots. One of the well-known fungi species listed in the PGPF group is the *Trichoderma* spp. (Doni *et al.*, 2013). Currently, *Trichoderma* spp. are widely used in industrial processes and agriculture due to their ability to produce enzymes and secondary metabolites (Jiang *et al*., 2011; Mukherjee *et al.*, 2008).

Trichoderma spp. is most successful bio-fungicides in present agriculture as more than 60% of the registered biofungicides world-wide arrived from *Trichoderma* based formulations (Verma *et al.,* 2007). *Trichoderma spp.* are endophytic plant symbionts that are widely used as seed treatments to control diseases and to enhance plant growth and yield. While some recent work has been published on their abilities to alleviate abiotic stresses, specific knowledge of mechanisms, abilities to control multiple plant stress factors and their effects on seeds and seedlings is lacking. *Trichoderma* genus is an economically important genus within Hypocreaceae family (Druzhinina *et al*., 2005; Harman *et al.,* 2010; Harman *et al.,* 2004; Rifai, 1969; Samuels, 2006). The fungi of this genus are ubiquitous and mainly reside in soil (Druzhinina *et al*., 2005; Harman *et al.,* 2004; Samuels, 1996). Some species or individual strains within species of *Trichoderma* are associated with enhanced plant growth and productivity (Bailey *et al*., 2005; Harman, 2000; Harman and Mastouri, 2009; Harman and Shoresh, 2007; Shoresh and Harman, 2008; Shoresh *et al*., 2010; Yedidia *et al.,* 2001) and suppression of plant diseases (Chet and Inbar; 1994; Souza *et al*., 2015; Harman *et al.,* 2010; Harman, 2000; Harman, 2006; Harman *et al*., 2004; Harman and Shoresh, 2007; Samuels, 1996; Shoresh *et al.,* 2010; Viterbo *et al*., 2017).

The genus *Trichoderma* possessing reasonable biologic*al* control attributes belonging to species *T. harzianum, T. hamatum, T. asperellum, T. viride* and *T. virens. Trichoderma* spp. are ubiquitous and often predominant components of the mycoflora in soil, litter, organic matter and rhizospheric ecosystem of all climatic zones as saprophytes. Recent discoveries show that they are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi. Strains of *Trichoderma* spp. are endophytes establish robust and long-lasting colonizations of root surfaces and penetrate into the epidermis. However, the ability of these fungi to sense, invade, and destroy other fungi has been the major driving force behind their commercial success as biopesticides. *Trichoderma* defend the plants by their direct and indirect effect on plant-pathogen-soil environment interaction. These fungi not only protect plants by killing the pathogens mainly other fungi and nematodes but also induce resistance against plant pathogens, impart abiotic stress tolerance, improve plant growth and vigor, nutrients uptake and bioremediation of heavy metals and environmental pollutants. In addition, this genus comprises fungi that produce secondary metabolites of clinical significance and enzymes with widespread industrial application. They produce and/or release a variety of compounds that induce localized or systemic resistance responses.These root–microorganisms interaction causes significant changes to the plant metabolism. *Trichoderma*-root symbiosis act as induced systemic resistance (ISR), systemic acquired resistance (SAR) and also also frequently enhances root growth and development, crop productivity, uptake and use of nutrients and resistance to biotic and abiotic stress (Harman *et al*., 2004; Hermosa *et al.,* 2012).

Trichoderma spp. is well recognized because of its ability to establish mycorrhizal-like association with plants, control of root and foliar pathogens, change the micro-floral composition in roots, enhance nutrient uptake, enhance root development, increase root hair formation, aid the plant in acquiring systemic resistance, degrade cellulose, solubilize phosphate and produce siderophores (Saravanakumar *et al*., 2013). Germination stage is reported to be the most critical phase in the plant life cycle, because during this stage, plants have a high vulnerability to injury, disease and environmental stress (Rajjou *et al.,* 2012). Research on the role of *Trichoderma* spp. in enhancing rice seed germination is very important to be undertaken due to the potential for *Trichoderma* spp. to act as plant growth regulators. Previous research studies on the effect of *Trichoderma* spp. on the improvement of seed germination have been conducted for soybean (Tančić *et al*., 2013), mustard (Lalitha *et al.,* 2012), maize and beans (Okoth *et al.,* 2011) and chili (Asaduzzaman *et al.,* 2010). However, very little information is available regards to enhancement of rice seed germination and vigour by *Trichoderma* spp. to improve seed germination and vigour. *Trichoderma* spp. has been exploited as plant growth enhancer and protection against pathogen. *Trichoderma* spp. has been reported as having the potential to act as plant growth promoters to enhance rice growth and productivity (Doni *et al.,* 2013).

Drought is a major abiotic stress that adversely affects the rice growth, mostly in the rainfed ecosystem that ultimately affects the biomass production and yield. Rice needs to adapt a series of physiological mechanisms with complicated regulatory network to fight and cope up with the unfavourable conditions due to drought stress. Drought is one of the major environmental stresses causing growth retardation and yield loss of plants. Of all the abiotic stresses which grossly reduce the crop productivity in general, drought is the most threatening one.

Paddy rice which prefers submerged conditions for growth is thus severely affected by the lack of rainfall in places where it is cultivated. The healthy growth of rice depends on the results of collective effort of several environmental factors that affect its life. Plant responses to drought stress are very complex as stress itself involves various climatic, soil and agronomic factors, frequently complicated by substantial variation in timing of occurrence, duration and intensity. The general complexity of drought is often aggravated under rainfed conditions in marginal areas by erratic and unpredictable rainfall, and by the occurrence of high temperatures, high levels of solar radiation, and poor soil characteristics. The combined effect of one or more such stress along with drought often leads to a much complex response in the plants' metabolism, highly altered from those of the individual stresses acting alone (Mittler, 2006).

Drought stress is the most widespread environmental stress, which affects growth and productivity and induces many physiological, biochemical and molecular responses in plants (Arora *et al*., 2002). Plant experiences drought stress either when the water supply to roots becomes difficult or when transpiration rate becomes very high. Available water resources for successful crop production have been decreasing in recent years. Furthermore, in view of various climatic change models suggested by scientists in many regions of world, crop losses due to increasing water shortage will further aggravate its impacts (Passioura, 2007). With respect to physiological aspects of drought tolerance in crop plants, a number of mechanisms have been studied that operate at the whole plant level (Blum, 1998). Although such traits are likely to be associated with improved productivity, they are frequently genetically complex and therefore require considerable effort to manipulate in a drought tolerance program.

Drought regularly affects 23 million ha of rainfed rice in Southeast Asia (Wu *et al*., 2011). In 2006, reduction of Aman crop was about 25-30 percent in North-Western part of Bangladesh (Rahman *et al.,* 2008). Drought is a major abiotic constraint for growing rain-fed rice in Bangladesh especially in dry season (Winter and Pre-monsoon), which causes a substantial reduction of rice yield. It occurs mainly for uneven distribution of rainfall and thus, north-western part of the country is treated as drought-prone (Pervin, 2015). Rice plant is most sensitive to water stress from panicle initiation to heading stage (Yoshida, 1981). Reproductive stages of rice such as panicle initiation, panicle development, flowering and anthesis, meiotic development of gametes, fertilization and grain filling are sensitive to water stress, which cause spikelet sterility and yield loss (Tuong *et al.*, 1995). In Bangladesh, around 60% cultivated area and 50% production of rice come from *Aman* season. Rice cultivation in Aman season is mostly rain dependent (only 11% irrigated). Average annual rainfall of Bangladesh is 2100 mm and 80% rainfall occurred in *Aman* season. Drought occurs mainly due to uneven distribution of rainfall in *Aman* season. *T. Aman* cultivars usually suffer from drought stress at reproductive and /or early ripening phase resulting poor yield (Pervin, 2015). Drought affect 2.32 m ha crop land every year in Kharif season in Bangladesh (Dey *et al*., 2011). Drought-prone Northern Districts (Barind Tract): Rajshahi, Chapai Nawabganj, Natore, and Nougaon and Western districts: Kushtia, Magura, Chuadanga, and Jessore are considered for drought hot spots in aman season, where rain seldom occurs and is erratic and uncertain during the last week of September and in October when aman rice needs water (IRRI, 2011).

The permanent or temporary water deficit severely hampers the plant growth and development more than any other environmental factor. The first and foremost effect of drought is impaired germination and poor stand establishment (Harris *et al*., 2002). The

incorporation of factors enabling plants to withstand drought stress would be helpful to improve crop production under drought conditions. Incorporation of *Trichoderma* during seed biopriming treatments in many cereal and vegetable crops has resulted in increased levels of plant growth hormones and improved seed performance (Howell, 2003). Biopriming is a process of biological seed treatment that refers to a combination of seed hydration and seed inoculation with beneficial organisms to protect seed. The technique helps seeds to evenly germinate even under adverse soil conditions (Singh *et al*., 2003). Some of the mechanisms used by *Trichoderma* to alter the drought response includes drought avoidance through morphological adaptations, drought tolerance through physiological and biochemical adaptations, and enhanced drought recovery (Malinowski and Belesky, 2000).

Trichoderma is an antagonistic fungi, considered as bio-pesticides that kill pathogenic fungi, bacteria and nematodes. The role of *Trichoderma* in plant growth promotion, nutrient management and disease control is well known. *Trichoderma spp*. was suggested as a Plant Growth Promoting Fungi (PGPF) due to their ability to produce siderophores, phosphate-solubilizing enzymes, and phytohormones (Doni *et al*., 2013). *Trichoderma* spp. has a symbiotic relationship with the host plant from seed germination to the plant mature. It colonize the rhizosphere of plants and promote growth. It also play important roles in decomposition, mycoparasitism, and even in cellulose degradation (Jiang *et al*., 2011). *Trichoderma sp*. inoculated rice plants exhibited greater net photosynthetic rate, internal CO_2 concentration, water use efficiency, plant height, tiller number, root length and root fresh weight (Febri, 2014).

Many studies have shown the decreased photosynthetic activity under drought stress due to stomatal or non-stomatal mechanisms (Del Blanco *et al.,* 2000; Samarah *et al*., 2009). Under drought, the maintenance of leaf turgor may also be achieved by way of osmotic adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycine betaine, and other solutes in cytoplasm thereby improving water uptake from drying soil. The process of accumulation of such solutes under drought stress is known as osmotic adjustment and observed to enhance tolerance to water stress (Nayyar *et al.,* 2003). Of these solutes, proline is the most widely studied because of its considerable importance in the stress tolerance. Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells. Though proline is one of the best known solutes, however, its relative importance for tolerance and precise protective function during stress requires investigations (Bohnert *et al.,* 1996). Drought induces oxidative stress in plants by generation of reactive oxygen species (ROS) (Farooq *et al*., 2009). Production of MDA, which is an indicative of oxidative stress, increases as drought stress increases in plant, serves as an index of

lipid peroxidation. Peroxidation damage of the plasma membrane leads to leakage of contents, rapid desiccation and cell death (Scandalios, 1993). However, biocontrol agent, *Trichoderma*, releases a variety of compounds that induce resistance responses to biotic and abiotic stresses (Harman *et al.*, 2004).

Several studies have shown that root colonization by *Trichoderma harzianum* results in increased level of plant enzymes, including various peroxidases, chitinases, s-1,3-glucanases, lipoxygenase-pathway hydro peroxide lyase and compounds like phytoalexins and phenols to provide durable resistance against stress (Harman, 2006; Hoitink *et al*., 2006; Gachomo *et al*., 2008). Acclimatization of plants to water deficit is the result of different events, which lead to adaptive changes in plant growth and physio-biochemical processes, such as changes in plant structure, growth rate, tissue osmotic potential and antioxidant defenses (Duan *et al.*, 2007). It has however become imperative to elucidate the responses and adaptation of crops to water deficit, and take actions to improve the drought resistance ability. Recently, Rawat *et al*. (2011 & 2012) characterized the possibility of *Trichoderma* species inducing tolerance to abiotic stresses.

There are different methods for determination of seed health test and seedling quality. To evaluate the seedling quality and seedling vigour, growing on test is best among the methods. Very recently, different commercial companies marketed different *Trichoderma* formulation in Bangladesh. That creates a chance to do seed treatment by *Trichoderma* formulations to ensure healthy and disease free seedlings. It is also important that, this technique is completely eco-friendly approach that is safe for our environment and human health.

Considering the above facts and scenario the proposed research work was set to achieve the following objectives:

1. Effect of seed treatment by commercial formulations of *Trichoderma* on germination of rice seed;
2. Effect of seed treatment by commercial formulations of *Trichoderma* on seedling growth of rice; and
3. *In vitro* effect of seed treatment by commercial formulations of *Trichoderma* on drought responses of rice.

CHAPTER II

REVIEW OF LITERATURE

Rice (*Oryza sativa* L.) is the staple food for more than two-third of the world`s population. Considerable number of research works has been conducted on the seed treatment of rice by *Trichoderma* spp. Few studies on to the related to the assessment of *Trichoderma* against seed and seedling quality of rice have been carried out in Bangladesh. Rice seed treatment by commercial *Trichoderma* formulations in Bangladesh and is not adequate and conclusive. Nevertheless, some of the important and informative works and research findings so far been done at home and abroad on this aspect have been reviewed in this chapter.

2.1. Seed treatment or seed priming by *Trichoderma* spp.

Hanna Bjelica (2016) investigated the effects of different seed treatments on the growth of mungbean using the most popular variety, NM 94. The treatments consisted of seed priming, *Trichoderma viridae*, *Rhizobium*, neem oil, thiram, chlorpyriphos, and in different combinations. Data were collected on seedling height, days to flowering, number of root nodules per plant and on the incidence of root rot disease. In the glasshouse experiment, significant differences were observed between the treatments for seedling height, an indirect measure of plant vigour.

Hassan *et al.* (2016) studied the effect of *Trichoderma* and fungicide application on seedling establishment and yield performance of dry direct seeded *Boro* rice. The experiment comprised ten treatment combinations of *Trichoderma* and fungicides viz. seed treatment with *Trichoderma* (M_1), seed treatment with *Trichoderma* + spraying of Thiovit (M_2), seed treatment with *Trichoderma* + spraying of Propiconazole (M_3), seed treatment with *Trichoderma* + spraying of Thiovit and Propiconazole (M_4), spraying of Thiovit (M_5), spraying of Propiconazole (M_6), seed treatment with Thiovit + spraying of Propiconazole ($_{M7}$), seed treatment with Propiconazole + spraying of Thiovit (M_8), spraying of mixture of Thiovit and Propiconazole (M_9), and control (no fungicide or *Trichoderma*) (M_{10}). Experiment revealed that seed treatment with *Trichoderma harzianum* followed by spraying of Thiovit gave the highest yield of rice. The study concludes that *Trichoderma* and then application of sulphur fungicide at 20 days after sowing could be practiced for ensuring high seedling establishment and yield of rice under dry direct seeded system in *boro* season.

Qasemi and Rai (2016) evaluated the effects of seed priming with bio fertilizers on germination percentages, vigour and viability of maize (*Zea mays* L.) seeds in Allahabad, during 2015-2016 with bio priming treatments T_1 (unsoaked seeds with (control), T_2 [soaked seeds with *Trichoderma* (1% solution for 14 hours)], T_3 [soaked seeds with *Trichoderma* (1% solution for 18 hours)] and T_4 [soaked seeds with *Trichoderma* (1% solution for 22 hours)], T_5 [soaked seeds with *Rhizobium* (1% solution for 22 hours)], T_6 [soaked seeds with *Rhizobium* (1% solution for 22 hours)], T_7 [soaked seeds with *Rhizobium* (1% solution for 22 hours)] on seed germination and vigour of maize. All treatments significantly affected on germination percentage, vigor index, seedling dry weight and seedling length of maize seeds.

Rahman (2015) revealed that seed treatment with *Trichoderma harzianum* followed by spraying of Thiovit gave the highest yield of rice and the sowing of seed after osmopriming with 3% $ZnSO_4$ and biopriming with *Trichoderma* and then application of sulphur fungicide at 20 days after sowing could be practiced for ensuring high seedling establishment and yield of rice under dry direct seeded system in Boro season.

Kumar *et al.* (2015) reported that neem oil improves the emergence of seedlings significantly in combination with Thiram.

Goswami *et al*. (2013) reported that seed priming is also involved in enhancing rice resistance to environmental stress weed suppressive ability and rice yield.

Shahid *et al*. (2011) was undertaken an investigation in order to know the impact of pre sowing seed treatment on germination, seedling establishment, seedling dry weight and vigour in chickpea genotype (*Udai*). The different pre sowing seed treatments showed different responses against all seven seed quality attributes. Seed treatment with *T. viride* + vitavax-T_5 followed by treatment with Vitavax @ 2gm/kg seed-T_2 seed were found superior for laboratory germination (99.00% and 97.66), root length (1.90 and 1.33 cm) shoot length (7.12 and 6.02) seedling length (9.02 and 7.35cm), dry weight (1.72 and 1.60mg), vigour index I (892.98 and 717.8) and vigour index II (170.94 and 1565.25), respectively.

Farooq *et al*. (2010) found that priming rice seed with plant growth regulators is one of the beneficial treatments that can be used to invigorate rice seed and improve rice seed quality.

Mastouri *et al.* (2010) examined the effects of seed treatment with *T. harzianum* strain T_{22} on germination of seed exposed to biotic stress (seed and seedling disease caused by

Pythium ultimum) and abiotic stresses (osmotic, salinity, chilling, or heat stress). They also evaluated the ability of the beneficial fungus to overcome physiological stress (poor seed quality induced by seed aging). However, under stress, treated seed germinated consistently faster and more uniformly than untreated seeds whether the stress was osmotic, salt, or suboptimal temperatures. This finding supports the model that *T. harzianum* strain T_{22} increases seedling vigor and ameliorates stress by inducing physiological protection in plants against oxidative damage.

Uddin *et al.* (2009) reported that seed treatment with *Trichoderma harzianum* either alone or in combination with some selected soil amendments namely poultry waste, cocodust, vermicompost, ash, sawdust, khudepana cowdung, solarized sand were evaluated against damping-off, seed germination and growth characters of egg-plant and tomato seedlings. All the treatments significantly reduced percent damping-off of egg-plant and tomato over untreated control. In the seed bed, seed + soil treatment with *Trichoderma harzianum* performed better in increasing seed germination, reducing percent damping-off and promote growth characters in compare to soil application with *Trichoderma harzianum* only. It was also found that, seed treatment with *Trichoderma harzianum* in combination with soil amendments showed better in all parameters in compare to soil amendment only. Among the different soil amendments, poultry waste and vermi-compost have promising effect in case of seed germination, percent damping-off and seedling growth characters of egg plant and tomato.

Basra *et al.* (2004) noticed that seed priming is the imbibition of seeds in water sufficient for pre germinative metabolic activity to occur while preventing radical emergence.

Basra *et al.* (2004) reported that the seed priming is considered as a method to enhance seedling quality and vigour.

Harris *et al.* (1999) reported that on farm seed priming in semi-arid agriculture development and evaluation in maize, rice and chickpea in India using participatory methods.

Vijayalakshmi and Majumdar (1999) found that seed treatment with neem oil formulations and powdered neem will suppress the nematode population growth and increase the grain yield significantly.

Harris *et al*. (1999) reported that seed priming is one of the most important developments to help rapid and uniform germination and emergence of seeds and to increase seed tolerance to adverse environmental conditions.

2.2. Effect of seed treatment by *Trichoderma* on seed germination

Doni *et al.* (2014) suggested that leaf number and tiller number has been found significantly higher in *Trichoderma* spp. treated rice plants compared to NPK treatment and control.

Febri Doni *et al*. (2014) reported, *Trichoderma* spp. is effective to enhance rice germination and vigour. An in vitro experiment was carried out to assess the effect of seven isolates of *Trichoderma* spp. in enhancing rice germination and vigour. The results showed that all isolates of *Trichoderma* spp. significantly increased rice seedling growth, germination rate, vigour index and speed of germination with *Trichoderma sp*., SL_2 showing the greatest increase in all the four parameters. *Trichoderma* sp., SL_2 treated rice seeds attained values of 4.48 and 6.00 cm, 0.0084 and 0.0048 g and 1016.56 and 44.75 seeds/ day for seedling shoot length, seedling root length, shoot weight, root weight, vigour index and speed of germination, respectively. They conclude that, *Trichoderma spp.* is able to enhance seed germination and vigour.

Rajjou *et al*. (2012) reported that, the germination stage is the most critical phase in the plant life cycle, because during this stage, plants have a high vulnerability to injury, disease and environmental stress .

Asaduzzaman *et al*. (2010) reported that Chili seeds gave the highest vigour index values with *T. harzianum* IMI-3924332 which confirmed to better germination.

Kar and Sahu (2008) reported that *Pseudomonas fluorescence* and *Trichoderma viride* is effective against diseases as well as increasing seed germination of mungbean and tomato.

Bouman *et al.* (2005) reported that the crop can successfully be grown by direct seeding on dry un-puddled soil with less irrigation and would save 50-70% of irrigation water .

2.3. Effect of seed treatment by *Trichoderma* on seedling growth

Doni *et al.* (2014) reported that *Trichoderma* spp. are able to alter several physiological processes which include net photosynthetic rate, stomatal conductance, transpiration, internal CO_2 concentration, water use efficiency and nutrient uptake.

Kumar *et al.* (2014) have been investigated the potentiality and effectiveness of strains of *Trichoderma* such as *T. harzianum* (Th. Azad) and *T. viride* (01PP) and their effect of

pre sowing seed treatment on germination, seedling establishment, seedling dry weight and vigour in chickpea genotype. Chickpea seeds were treated with different concentrations of *Trichoderma* bioformulation such as 5%, 10%, and 20% gm/kg seed followed by treatment with 0.2% Bavistin. As a result, the percentage of seed germination was found to be higher in *T. harzianum* (Th. azad) and *Trichoderma viride* (01PP) treated seeds with 5% bioformulation as compared to the other concentrations.

Martini *et al.* (2014) used *Trichoderma* spp. to treat seeds of various crops to evaluate the influence of secondary metabolites of *Trichoderma* spp. in the development of fungus transmitted by seeds and in the germination of seeds of rice. It was occurred a interference of non-volatile metabolites of two isolates of *Trichoderma* spp. in the mycelial growth of *Fusarium* and *Bipolaris oryzae*. The sterilization test showed that the optimal time of immersion in sodium hypochlorite was 15 min and that the secondary metabolites of *Trichoderma* spp. did not affect germination of seed. Therefore, secondary metabolites released by *Trichoderma* spp. interfere in the development of *Bipolaris oryzae* and *Fusarium* spp. transmitted by seeds of rice. However, they do not interfere in the germination of seeds of rice.

Cai *et al.* (2013) reported that a secondary metabolite namely harzianolide produced by *Trichoderma* spp. can influence the early stage of plant development through the enhancement of root length.

Doni *et al*. (2013) reported that Plant Growth Promoting Fungi (PGPF) is considered an alternative way to enhance rice growth and yield. The mechanisms by which PGPF act to enhance rice growth and yield includes production of phytohormones, phosphate solubilization, cellulose degradation and siderophore production. PGPF are generally abundant in the soil system and roots. One of the well-known fungi species listed in the PGPF group is the *Trichoderma* spp.

Cai *et al.* (2013) reported that harzianolide produced by *Trichoderma spp*. can improve the early stage of plant development through the enhancement of root length. These morphological modification are possible because of the ability of the *Trichoderma* spp. to act through several mechanisms such as environmental buffering (against pH, drought, waterlogging, cold and heat), Phosphorus solubilization, organic matter decomposition, chilation and siderophore production.

Chowdappa *et al.* (2013) reported that the role of *Trichoderma spp.* in the production of auxins and gibberellins is the key factor to enhance rice seedling length.

Saravanakumar *et al.* (2013), Saba et al. (2012), Harman *et al.(*2004), Harman (2000), Yadidia *et al.* (1999) found that in bio-fertilizer production, *Trichoderma* spp. is well recognized because of its ability to establish mycorrhizal-like association with plants, control of root and foliar pathogens, change the micro-floral composition in roots, enhance nutrient uptake, enhance root development, increase root hair formation, aid the plant in acquiring systemic resistance, degrade cellulose, solubilize phosphate and produce siderophores.

Bezuidenlhun *et al.* (2012) reported that *Trichoderma harzianum* produces a metabolite as gliotoxin that may mimic the plant growth hormone gibberellic acid which is involved seed germination process.

Saba *et al.* (2012) found that better nutrient uptake will enhance the physiological processes within the plants treated with *Trichoderma spp.* leading to good growth performance

Hossain (2011) noticed that BAU-biofungicide named *Trichoderma harzianum* is invented from naturally occurring fungus to protect crops from different diseases caused by different harmful fungi. *Trichoderma* protects seeds from huge number of soil borne as well as seed borne fungi that can attack the seeds before germination and after germination in the soil. It also controls pre-emergence and post-emergence death of seedlings. Seed treatment with *Trichoderma* significantly increases germination and protects seed and seedling from soil borne fungi as well as increase plant stand in the field.

Islam *et al.* (2011) were evaluated *T. virens* IMI-392430, *T. pseudokoningii* IMI-392431, *T. harzianum* IMI-392432, *T. harzianum* IMI-392433 and *T. harzianum* IMI-392434 for their potentiality on seed germination and seedling parameters in chili both laboratory and field conditions. Chili seeds were coated with spore suspension of each test strains of *Thichoderma* supplemented with 2 % of starch (w/v) as an adhesive. Seed germination percentages and the vigour index were significantly ($P \leq 0.05$) affected by the application of different strains of *Trichoderma*. Among the five *Trichoderma* strains, *T. harzianum* IMI-3924332 gave the highest germination percentage followed by *T. harzianum* IMI-3924333, *T. harzianum* IMI-3924334, *T. virens* IMI-392430 and *T. pseudokoningii* IMI-392431 treatment both in laboratory and field conditions, respectively while control decrease these value. Chili seeds also gave the highest vigour index values with *T. harzianum* IMI-3924332 which confirmed to better germination. Seed treatment with *T. harzianum* IMI-3924332 can be useful to enhance the germination of chili seeds as well as reduce to delayed germination. Further

investigations however are required to study *in vivo* effect of *Trichoderma* strains on morphological and physiological characteristics in chili plant and fruit production.

Jamwal *et al.* (2011) studied the effect of biocontrol agents on wilt management and plant growth of tomato by using fungal and bacterial biocontrol agent *T. viride, T. harzianum* and *P. fluorescens* and found that dipping the tomato seedlings in bioagent suspension of *T. harzianum* recorded least wilt incidence (5.6 & 6.0 %) followed by *T. viride* (11.3 &1 0.9 %) during two years.

Kaveh *et al*. (2011) investigated the effect of *Trichoderma harzianum* on seed germination and seedling quality and field establishment of two muskmelon cultivars, *Khatooni* and *Qasri,* at greenhouse and field in Iran. Results showed that *Trichoderma* application significantly increased germination and emergence percentage and index and could help improving seedling health and vigor.

Jiang *et al*. (2011) noticed that *Trichoderma* spp. are widely used in industrial processes and agriculture due to their ability to produce enzymes and secondary metabolites.

Uddin *et al.* (2011) investigated the soil applications with poultry refuse, cocodust, vermicompost, ash, sawdust, khudepana, cowdung, solarized sand, *Trichoderma harzianum* and or with seed treatment by *T. harzianum* were evaluated against damping off disease complex of potato and chilli. All the treatments significantly reduced percent damping off over control. *T. harzianum* treated seed along with soil treatment with *T. harzianum* performed best in terms of seed germination, percent damping off reduction and enhanced growth characters than soil pplication with *T. harzianum* alone. The experiment indicates that seed treated with *T. harzianum* then sown in different soil amendment applied seed bed performed better in all parameters than only application with soil amendment. Among the different soil amendments, poultry refuse and vermin compost have promising impact on seed germination, reduction of percent damping off and growth of potato and chilli seedlings when applying along with *T. harzianum*.

Uddin *et al.* (2011) observed that soil application with poultry waste, cocodust, vermicompost, ash, sawdust, khudepana, cowdung, solarized sand, *Trichoderma harzianum* and or seed treatment with *Trichoderma harzianum* were evaluated at Sher-e-Bangla Agricultural University, Dhaka during 2008-2009 on damping-off, seed germination and growth characters of cabbage and cauliflower seedlings. Results showed that all the treatments significantly reduced percent damping-off of cabbage and cauliflower over untreated control. In the seed bed, seed + soil treatment with *Trichoderma harzianum* performed better in terms of seed germination, percent

damping-off and growth characters than only soil application with *Trichoderma harzianum*. It was also found that, *Trichoderma harzianum* treated seeds sown in different amended soil showed higher values in all parameters than seeds sown only on amended soil. Among the different soil amendments, poultry waste and vermicompost had promising effect on seed germination, percent damping-off and seedling growth characters of cabbage and cauliflower seedlings.

Lorito *et al.* (2010) stated that the benefit of *Trichoderma* spp. in improving plant growth can be realised through several mechanisms which include mycoparasitism, antibiosis, degradation of toxins, inactivation of pathogenic enzymatic pathways, resistance to pathogens, enhanced nutrient uptake, solubilization, sequestration of inorganic nutrients, and enhanced root hair development . In this research we tested the ability of *Trichoderma* spp. to enhance rice growth. The results showed that rice plants inoculated with spp. significantly increased rice growth components. Plant height of *Trichoderma spp.* inoculated rice plants was higher compared to NPK treatment and control.

Mastouri *et al.* (2010) reported that total lipid peroxides of tomato seedlings germinated under lowered water potentials or that of aged seed was found less when the seedlings were treatred with *Trichoderma* isolate T_{22} as compared to untreated control. The mechanisms where by *Trichoderma* spp. induce such changes are not known however, enhanced ROS level could act as a signal to regulate expression of some of the related genes.

Viterbo *et al.* (2010) revealed that the role of *Trichoderma asperellum* in promoting canola seedling root elongation via ACC deaminase (ACCD) activity.

Kumar *et al.* (2010) studied the growth promotion by *P. fluorescens* and *T. harzianum* and found that combined application of *T. harzianum* as seed biopriming + root dipping showed growth inducing effect up to crop maturity in sweet pepper.

Khan *et al.* (2005) was examined *Trichoderma harzianum* for its effects on emergence and vigour of rice seedlings through seed or soil treatments. All doses of *T. harzianum* in both the experiments significantly increased seedling emergence, root and shoot length, fresh and dry weight of root of rice seedlings, as compared to check. Maximum increase in seedling emergence (44.67%) was observed when bio-agent was applied as soil treatment with the bio-agent @ 8 gm/kg soil. There was similar trend of increase in root and shoot length, root and shoot weight from soil and seed treatments. Higher doses of the antagonist exhibited maximum increase in seed germination and seedling vigour.

Verma and Dohroo (2005) tested six bioagents viz., *T. viride, T. harzianum, Pacellomyces lilacinus, G. virens, Laetisaria arvalis* and *P. fluorescens* against *F. oxysporum* f. sp. *pisi* under pot conditions on pea. Among them, seed coating with *T. harzianum* and *T. viride* were found most effective with 93.83 percent disease control.

Harman *et al.* (2004) stated that mechanisms employed by *Trichoderma* spp. in enhancing nutrient availability by solubilization and chelation of minerals can increase plant metabolism leading to the enhancement of plant physiological activity.

Singh *et al*. (2004) evaluated four antagonists viz., *T. viride, T. harzianum,* and *Gliocladium virens* and *Aspergillus nidulans* as seed, soil and seed + soil treatment for the control of tomato wilt disease caused by *F. oxysporum* f. sp. *lycopersici* under pot culture and reported that seed coating of tomato seeds with *T. viride, T. harzianum* and *G. virens* showed reduction in seedling mortality up to 85 percent as compared to *A. nidulans* among different combinations.

Prasad *et al.* (2002) studied the effect of soil and seed application of *T. harzianum* on pigeon pea wilt caused by *F. udum* under field conditions and found that seed treatment with talc-based formulation of *T. harzianum* at 10 and 20 g/kg of seed could 5 control 19.9 and 27.5 percent wilt with 58.7 and 49.1 percent disease incidence, respectively.

Zheng and Shetty (2000) reported that *Trichoderma* spp. induced phenolic compound production in the plant during seed germination and phenolic compounds produced by *Trichoderma spp.* led to enhancement of seed vigour.

2.4. Effect of drought stress in plant

Inostroza *et al.* (2015) stated that plant growth is one parameter in the drought-sensitive morphological processes caused by the decreased turgor pressure during drought stress.

Ambavaram *et al.* (2014) suggested that a transcription factor HYR which is associated with photosynthetic carbon metabolism has been identified, and its expression in rice has led to enhanced photosynthesis under drought and other environmental stresses, and resulted in increased yield under stress.

Lum *et al*. (2014) found that drought tolerant varieties generally show better antioxidant production than susceptible ones.

Asharf and Harris (2013) observed that drought stress decreases the rate of photosynthesis by impairing pigments, photosystems, gas exchange, and key photosynthetic enzymes, thus affecting various steps in the photosynthetic pathway and this can ultimately reduce plant biomass and yield.

Golec and Szarejko (2013) observed drought stress leads to stomatal closure leading to limitation of gaseous exchange.The closure of stomata is controlled by phytohormones such as abscisic acid, cytokinnins etc.

Cheng *et al.* (2013) revealed that abiotic stresses, such as drought or water deficit, are the most important factors negatively affecting growth, production, and various physiological/biochemical plant processes worldwide.

Singh *et al.* (2012) observed reduction of water content reduces stomatal activity and cell growth. Leaf area, cell size and intercellular volume decreases, and leaf rolling and death of leaf results from drought stress. In roots, drought results in reduction of meristematic activity, arresting root elongation. Suberization of the root system also results from water stress.

Adesemoye and Kloepper (2009) reported that rice farming nowadays is facing many environmental problems caused by input of chemical fertilizers and pesticides. The use of chemical fertilizers and pesticides has led to soil a decline in fertility, ecosystem damage, elimination of soil biota and emergence of resistant pathogens. Therefore, the use of eco-friendly biofertilizers should be encouraged. Beneficial microorganisms have been reported to be involved in maintaining agricultural production, protecting the ecosystem and decreasing the use of chemical fertilizers.

Chutia *et al*. (2012) founded that increase in production of proline and soluble sugars have also been recorded under drought stress, with the increase more pronounced in tolerant varieties.

Khan and Panda (2008) suggested that plant resistance to stress factor is associated with their antioxidant capacity, and the increased level of antioxidant constituents may prevent the stress damage.

Rabello *et al.* (2008) noticed that genes exclusively expressed in drought tolerant varieties include genes involved in signaling pathways, such as Ca^{2+} dependent protein kinase (CDPK) and ethylene responsive factors, genes involved in alteration of metabolism due to lower intracellular CO2 during drought, genes involved in decreasing

oxidative injury, and genes maintaining cell turgor.

Mittler *et al.* (2002) noticed that measurement of lipid peroxide content serves as a reliable indicator of oxidative damage during abiotic stresses.

Shangguan *et al.* (1999) found that the photosynthesis process during drought is possible due to the osmoregulation which affects the state of the leaf stomata and adaptation of the photosynthetic apparatus to drought conditions.

2.5. Effect of *Trichoderma* on drought stress of plant

Abd-Allah *et al.* (2015) observed that drought stress caused accumulation of proline and soluble proteins, which were further enhanced by *T. harzianum* inoculation. Plants accumulating higher contents of free proline show increased stress tolerance.

Ahmad *et al*. (2015) found that among microbes, *Trichoderma* species have the potential to induce host plant tolerance to several biotic and abiotic stresses including salinity and drought, by its involvement in root growth promotion, maintenance of nutritional uptake and in addition triggers protective mechanisms to avert the oxidative damage.

Shukla *et al.* (2015) have reported that priming *Triticum aestivum* L. with *T. harzianum* improved drought stress tolerance by mediating enhanced synthesis and accumulation of proline, thereby conferring tolerance to drought stress.

Gusain *et al.* (2014) have observed enhanced drought tolerance in rice due to *Trichoderma harzianum* T_{35} colonisation and they evidenced that T. harzianum promoted activity of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and thereby preventing oxidative damage to rice through quick elimination of reactive oxygen species (ROS).

Chowdappa *et al.* (2013) observed that the ability of *Trichoderma* spp. to produce phytohormones is the key factor in the increase in rice plant height.

Nawrocka and Malolepsza (2013) stated that the ability of *Trichoderma* spp. hyphae to release elicitors may contribute to signals being transmitted within the plant such as salicylic acid (SA), jasmonic acid (JA) and reactive oxygen species (ROS).

Thakur and Sohal (2013) reported that elicitors released by *Trichoderma* spp. are also involved in triggering expressions of defense protein within the plant.

Hermosa *et al.* (2012) noticed that growth hormones are known for their role in plant developments and also mediate signalling under stress conditions for bringing elicitation of specific response. ABA and SA were increased due to drought stress and may be due to *T. harzianum* have helped inoculated plants to maintain the expression of stress responsive proteins for counteracting oxidative damage of drought stress induced.

Mastouri *et al.* (2012) observed that the enhanced tolerance of tomato to water stress by *T. harzianum* T_{22} was due to the ability of plants to remove damaging ROS, which was accompanied by an increase in the activity of antioxidant enzymes. Since plant secondary metabolites play vital role in plant stress response, we studied whether root colonization by *T. harzianum* leads to alterations in the biosynthesis of secondary plant metabolites under drought stress.

Shukla *et al.* (2012) investigated that world rice production in the future will be reduced by approximately 50% due to drought. They reported that *Trichoderma harzianum* significantly increased the ability of rice plants to tolerate drought stress and increase rice water holding. They also suggested that *Trichoderma harzianum* isolates resistant to dessication have also been shown to be able to induce drought tolerance in rice plants subjected to upto 9 days of drought treatment. *T. harzianum* colonized rice delayed wilting and showed promising growth with minimum oxidative stress. Consortia of plant growth promoting rhizobcteria (PGPR) comprising strains of *Pseudomonas*, *Arthrobacter*, etc. enhance rice plant growth under drought and provide resistance to injury by activating the antioxidant defence mechanism of the plants.

Martínez-Medina (2011) showed that the interaction with arbuscular mycorrhizal fungi or *Trichoderma harzianum* alters the shoot hormonal profile in melon plants.

Bae *et al.* (2009) suggested that enhanced greenness and chlorophyll content under drought tolerant isolate *Trichoderma hamatum* DIS 219b-colonized seedlings.

CHAPTER III

MATERIALS AND METHODS

This chapter includes materials and methods that were used in conducting the experiment. It consists of a short description of locations of the experimental site, period, characteristics of soil, climate, seeds, treatments, layout and design of the experiment, soil and pot preparation, soil sterilization, manuring and fertilizing, intercultural operations, irrigation, data collection on different parameters and statistically analysis etc. The details regarding materials and method of this experiment are presented below under the following headings.

3.1. Experimental Site

The experiment was conducted at Dept. of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. The location of the site is 23.74° N latitude and 90.35° E longitudes with an elevation of 8 meter from sea level (UNDP - FAO, 1988) in Agro-Ecological Zone (AEZ No. 28) of Madhupur Tract. It is an *in vitro* experiment. Thus, the pot experiment was conducted at the net house and glass house of the Dept. of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka under controlled condition.

3.2. Experimental Period

The experiment was conducted during the period from January 2018 to April 2018.

3.3. Climate

The climate of the experimental site was under the subtropical climate characterized by three district seasons, the monsoon or the rainy season from November to February and the pre-monsoon period from May to October (Edris *et al*., 1979). Meteorological data related to the temperature, relative humidity, rainfalls and sunshine during the period of experiment was collected from the Bangladesh meteorological department, Sher-e-Bangla Nagar, Dhaka.

3.4. Weather

The monthly mean of daily maximum, minimum and average temperature, relative humidity, monthly total rainfall and sunshine hours received at the experimental site during the period of the study have been collected from Bangladesh Meteorological Department, Agargaon, Dhaka.

3.5. Characteristics of soil

The experimental soil belongs to the Madhupur Tract under AEZ No.28 (UNDP, 1988). The soil is deep red brown terrace soil. It was sandy loam in texture having pH 5.47 to 5.63.

3.6. Rice Variety

The experiment was conducted under controlled irrigating system. Thus, two drought tolerant varieties viz. BRRI dhan56, BRRI dhan71 and one drought susceptible check variety IR64 were used in this experiment (Plate 1).

Plate 1. Seeds of different rice varieties used in the experiment

3.7. Seed collection

Seeds (BRRI dhan56, BRRI dhan71, IR 64) were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur, Dhaka.

3.8. Treatments

Five different commercial *Trichoderma* formulations including four powder and one liquid formulation were collected from different commercial companies of Bangladesh to evaluate their effect against seed and seedling quality of rice (Plate 2). The treatments were applied in the experiment as mentioned below:

T_1= G-derma (*Trichoderma*)

T_2 = Bioderma (*Trichoderma*)

T_3= Recharge (*Trichoderma*)

T_4= Decoprima (*Trichoderma* + *Geobacillus* + *Streptomyces*)

T_5 = G-derma (LDS) (*Trichoderma*)

T_6= Control

Table 1. Details of the collected commercial *Trichoderma* formulations

Sl. No.	Trade name/ Commercial Name	Formulation type	Company Name	Recommended Rate for seed treatment
1	G-derma	Powder	GME Agro Ltd.	10 g/kg seed
2	Bioderma	Powder	Ispahani Agro Ltd.	10 g/kg seed
3	Recharge	Powder	Russel IPM Bangladesh	10 g/kg seed
4	Decoprima	Powder	Mohsin Enterprise	10 g/kg seed
5	G-derma (LDS)	Liquid	GME Agro Ltd.	5ml/L water

3.9. Soil collection and Preparation

For pot experiment, the top soil/ bulk surface soil used for the experiment was collected from uppermost 0-15 cm of soil of experimental field of Sher-e-Bangla Agricultural University, Dhaka. The soil was crushed thoroughly with a bamboo hammer to obtain a desirable tilth. The clods of the soil were hammered to make the soil into powder form. Weeds, stubbles and crop residues were cleaned from the soil. The soil was air dried, mixed thoroughly and passed through 2 mm sieve. The soil collection and preparation was done on 8-10 February, 2018.

Plate 2. Different commercial *Trichoderma* formulations used for seed treatment

3.10. Soil sterilization

Soil sterilization was done 15 days before seed sowing. Collected soil was mixed with cowdung and commercial organic fertilizer properly. 2% formalin solution was prepared in a container and drench the soil @ 4-5 litre water per square meter soil surface to saturated it up to a depth of 15-20 cm. Drench soil kept covered with double polyethylene sheets for 3 days. The margin of polythene sheet was air tied by wet soil and bricks. Then the soil was uncovered and pulverized enough and kept for seven days to release the gas of formalin. After removed the smell of formalin completely, the soil was transferred into the pot. Soil sterilization was done on 11 February 2018.

3.11. Pot Preparation

Soils were put in the plastic pot of 12 inches height and 10 inches width (Plate 3). For minimize the losses of excess water 2cm hole was made from the bottom of the pot. Broken bricks were placed at the bottom of the plastic pot. 8 Kg sterilized soil were filled in each plastic pot. Then the pots were arranged according to experimental design. Pot preparation was done on 23 February 2018.

Plate 3. Pot preparation

3.12. Manuring and Fertilization

The 40 kg Krishibid organic fertilizer and 50kg well rotten cowdung manure was thoroughly mixed with1200 kg soil (Plate 4). Fertilizers were applied by following Fertilizer Recommendation Guide 2012 at the rate of Urea @ 8gm/pot, MOP @ 6 gm/pot, TSP @ 6 gm/pot and Sulphur @ 3 gm/pot. MOP, TSP and Sulphur were applied at final pot preparation. Half of the required Urea was applied at 15 days after sowing and rest half of Urea was applied at 30 days after sowing.

Plate 4. Soil prepared by mixing organic fertilizer

3.13. Seed Priming

Rice seeds were surface-sterilized with Clorox® bleach (5.25% sodium hypochlorite as the active ingredient) followed by two minutes shaking. After that, seeds were rinsed 3 times with sterilized water each time for 2 minutes. After surface sterilized, seed were placed in petri dish for 24 hours for priming. Surface sterilization and priming was done on 23 February, 2018.

3.14. Seed Treatment

Seeds were bio-primed separately with each formulations of *Trichoderma* @ 10 g or 5 ml / kg of seed (Plate 5). After pre-soaking of seeds in sterile distilled water, each seed sample were rinsed by 3-4 drop of Tween 20 for ensuring proper an uniform attachment of *Trichoderma* on seed surface. After that, seeds were coated with powder and liquid formulations of *Trichoderma* and mixed thoroughly to provide uniform coating. After 24 hours of seed treatment, seeds were placed for sowing. The seed treatment was done on 24 February, 2018.

3.15. Seed Sowing

Seeds were sown in plastic pot (12 kg capacity) filled with 8.0 kg sterilized soil and saturated with water holding calibrated tensiometer (Plate 5). Twenty seeds were sown in each pot. The pots were kept in shady place. The pots were watered regularly during the seedling-raising period. The seed were sown after 15 days of soil sterilization. Seed sowing was done on 25 February, 2018 for germination and seedling rising.

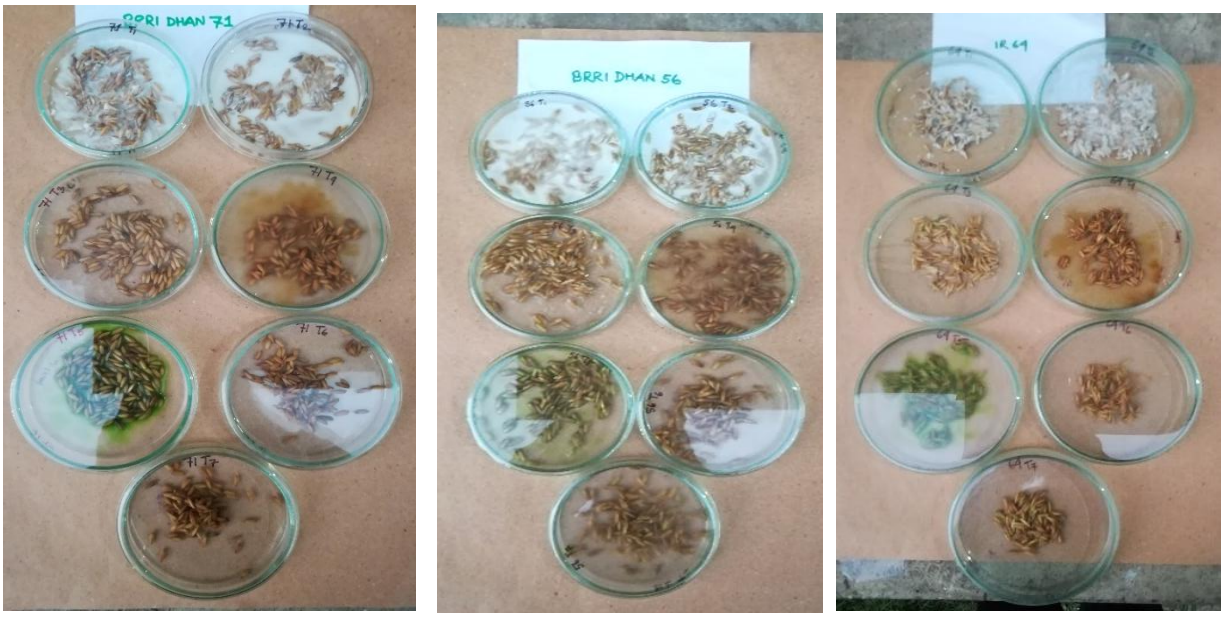

Plate 5. Seed treatment by differerent *Trichoderma* formulations

3.16. Irrigation

The experiment was conducted under controlled irrigation. Moisture was maintained by applying 400 ml of water per pot every alternate day.

3.17. Intercultural Operations

Weeding was done two times at 20 and 30 days after sowing. Spraying of Chlorpyrifos Dursban 20 EC) @ 0.05% at 25 days after sowing to prevent insect infestation.

3.18. Maintaining Drought Stress in Plants

Five potential isolates of *Trichoderma* spp. along with control was tested in each drought treatment for their ability to enhance drought tolerance in rice plants. Ten plants per pot were maintained for each treatment combinations including control. Moisture was maintained by applying 400 ml of water per pot every alternate day until plants attained the age of five weeks and at this point drought treatments was given by altering the water cycle. Watering was stopped for subsequent days for each drought treatment which included 4, 7, 10 and 13 days drought stress (DDS), while control seedlings was continue to be watered every alternate day. Subsequent to drought treatment application, observations were recorded on wilting and physiological responses of rice plants.

3.19. Tagging and Data Collection

Ten plants per pot were maintained for each treatment combinations including control. Randomly 3 plants were selected from each pot tagged for data collection (Plate 6).

Mean values were determined to get rating score of each treatment.

The following parameters were considered for data collection:

a) Seed germination percentage
b) Speed of germination (GI)
c) Mean germination time (MGT)
d) Mean daily germination (MDG)
e) Peak value (PV)
f) Germination value (GV)
g) Number of leaves
h) Number of tiller
i) Plant height
j) Shoot length
k) Root length
l) Fresh shoot weight
m) Fresh root weight
n) Dry shoot weight
o) Dry root weight
p) Seedling vigor index (SVI)
q) Seedling vegetative vigor (SVV)
r) Droopy leaf
s) Rolled leaf
t) Drying leaf
u) Drought sensitive scale (Leaf rolling at vegetative stage and Leaf drying at vegetative stage)


Plate 6. Germinated seedling in the pot

Procedures of data collection

3.19.1. Germination percentage

Seed germination percentage was calculated at 7, 14 and 20 days after sowing (Plate 6). Seed germination percentage was calculated using the following formula (ISTA, 2010):

Germination % = (Number of germinated seeds / Total number of seeds) × 100

3.19.2. Speed of germination / Germination Index (GI)

Numbers of germinated seedlings were counted upto 20 days after sowing. Speed of germination was calculated by the following formula given by Czabator (1962):

Speed of germination / Germination Index (GI) = $n_1 / d_1 + n_2 / d_2 + n_3 / d_3 +$ -------n_{20}/d_{20}

Where, n = number of germinated seeds, d= number of days.

3.19.3. Mean germination Time (MGT)

Mean germination time was calculated by the formula :

MGT = n_1 x d_1 + n_2 x d_2 + n_3 x d_3 + --------/ Total number of days

Where, n= number of germinated seed

d = number of days

3.19.4. Mean daily germination (MDG)

Mean daily germination can be calculated at 20 DAS by the following formula given by Czabator (1962):

MDG = Total number of germinated seeds / Total number of days

3.19.5. Peak Value (PV)

Peak value was calculated by the following formula given by Czabator (1962):
PV = Highest seed germinated / Number of days.

3.19.6. Germination Value (GV)

Germination value was calculated by the following formula given by Czabator (1962):
GV = PV X MDG

3.19.7. Number of leaves

Number of leaves was counted at 14, 24, 34 and 44 days after sowing.

3.19.8. Number of tiller

Number of tiller was counted at 33, 43 days after sowing.

3.19.9. Plant height

Plant height was measured in centimeter at 14, 24, 34 and 51 days after sowing.

3.19.10. Shoot length

Shoot length in centimeter was measured at 34, 51 days after sowing.

3.19.11. Root length

Root length was measured in centimeter at 34, 51 days after sowing.

3.19.12. Fresh shoot weight

Fresh shoot weight was measured in gram at 34, 51 days after sowing.

3.19.13. Fresh root weight

Fresh root weight was measured in gram at 34, 51 days after sowing.

3.19.14. Dry shoot weight

Dry shoot weight was measured in gram at 34, 51 days after sowing. Firstly, three plants from each pot were removed from the soil and washed off soils from the roots by running water very carefully. Plants were dried by blotting paper for removing surface moisture. Then the plants were taken in brown envelop and dried in an oven set to low heat (100°F) overnight. After that, plants were cooled in a dry environment. Then, shoots were cut at soil line and separated. Shoots of each plant separately weighed and recorded.

3.19.15. Dry root weight

Dry root weight was counted in gram at 34, 51 days after sowing. Firstly, three plants from each pot were removed from the soil and washed off soils from the roots by running water very carefully. Plants were dried by blotting paper for removing surface moisture. Then the plants were taken in brown envelop and dried in an oven set to low heat (100°F) overnight. After that, plants were cooled in a dry environment. Then, roots were cut at soil line and separated. Roots of each plant separately weighed and recorded.

3.19.16. Seedling Vigor Index (SVI)

Seedling vigor index was calculated at 34 days after sowing. The vigor index (VI) of the seedlings can be estimated as suggested by Abdul-Baki and Anderson (1973):

$SVI = (RL+SL) \times GP$

Where, RL is root length *(*cm), SL is shoot length (cm) and GP is germination percentage.

3.19.17 Seedling Vegetative Vigor (SVV)

Seedling vegetative vigor was calculated by a rating scale with a score of 1-9 as described in SES, 2002 (IRRI):

Scale	Descriptions
1	Extra vigorous (very fast growing; plants at 5-6 leaf stage have 2 or more tillers in majority of population)
3	Vigorous (fast growing; plants at 4-5 stage have 1-2 tillers in majority of population)
5	Normal (plant at 4- leaf stage)
7	Weak (plants somewhat stunted; 3- 4 leaves; thin population; no tiller formation
9	Very weak (stunted growth; yellowing of leaves

3.19.18. Droopy leaf

After 39 DAS, plants was forced to drought stress and in 42, 45, 48 DAS that's were consequently 7, 10, 13 DDS (Days Drought Stress) was considered to count droopy leaf of rice plant.

3.19.19. Rolled leaf

Similarly, rolled leaf was counted at 7, 10, 13 DDS after drought stress.

3.19.20. Drying leaf

Similarly, drying leaf was counted at 7, 10, 13 DDS after drought stress.

3.19.21. Drought sensitive scale

Drought sensitive scale was calculated by a rating scale with a score of 1-9 as described in SES, 2002 (IRRI):

Scale	Leaf rolling at vegetative stage	Leaf drying at vegetative stage
0	Leaves healthy	No symptoms
1	Leaves start to fold (shallow)	Slight tip drying
3	Leaves folding (deep V- shape)	Tip drying extended up to 1/4
5	Leaves fully cupped (U- shape)	One -fourth to 1/2 of all leaves dried
7	Leaf margins touching (O- shape)	More than 2/3 of all leaves fully dried
9	Leaves tightly rolled (V- shape)	All plants apparently dead. Length in most leaves fully dried.

3.20. Experimental Design and Layout

The experiment was laid out in Complete Randomized Design (CRD) with five replications.

3.21. Statistical Analysis

The collected data was statistically analyzed by Statistics 10 computer package program. Analysis of variance (ANOVA) was used to find out the variation of result from experimental treatments. Treatment means were compared by least significant difference test (LSD).

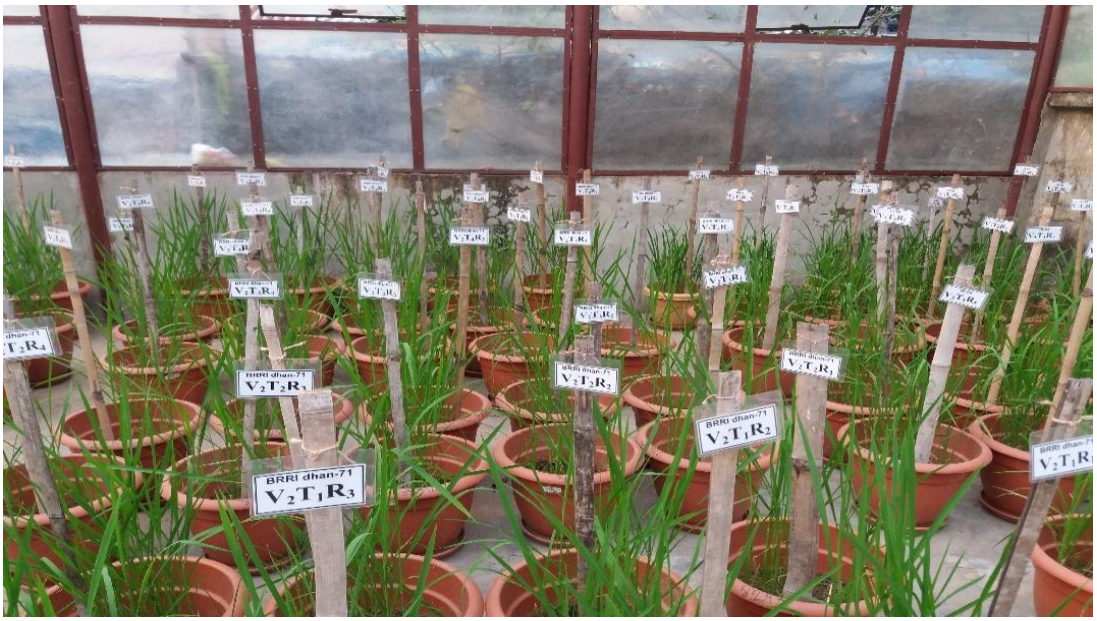

Plate 7. Tagging of treatments in glass house

CHAPTER IV

RESULTS AND DISCUSSION

The present experiment was conducted to evaluate seed treatment by commercial formulations of *Trichoderma* of Bangladesh on seedling quality of rice under controlled irrigation. To achieve this goal, seed germination percentage was recorded at different days after sowing. Moreover, data on germination percentage, speed of germination / germination index, mean germination time (MGT), mean daily germination (MDG), peak value (PV), germination value (GV), number of leaves, number of tiller, plant height, shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, seedling vigor index (SVI) and seedling vegetative vigor (SVV), leaf droppy, leaf rolling, leaf drying, drought sensitivity scale (Leaf rolling and leaf drying at vegetative stage) were recorded. The data on different parameters were organized and analyzed are presented in this chapter (table1-15).

4.1 Effect of seed treatment by formulations of *Trichoderma* on seed germination at different DAS (Days after sowing)

Effect of seed treatment by commercial formulations of *Trichoderma* on seed germination (%) of BRRI dhan 56 , BRRI dhan 71 and IR 64 rice varieties at 7, 14 and 20 days after sowing (DAS) was shown in Table 1. The germination percentage of rice seeds varied significantly among the treatments at different DAS.

Incase of BRRI dhan 56 at 7 DAS, the highest seed germination was found in T_4 (86%) in Decoprima that was statistically similar with T_4 (79%) followed by T_1, T_2. However, in BRRI dhan 71, the highest seed germination was observed in T_4 (79%) followed by T_2, T_1 and T_6. Moreover, in IR 64, the highest seed germination was observed in T_4 (73%) followed by T_2, T_6 and T_1.

At 14 DAS, in BRRI dhan 56, the highest seed germination was found in Decoprima treated seed in T_4 (96%) that was statistically similar in T_2 (90%), T_6 (89%) and T_1 (83%) followed by T_1, T_3 and T_5. In BRRI dhan 71, the highest seed germination was also noticed in Decoprima treated seed in T_4 (95%) followed by T_2, T_1. Incase of, IR 64, the highest seed germination observed in Decoprima treated seed (93%) that was statistically similar with G-derma (91%), Bioderma (95%) and control (86%).

At 20 DAS, in BRRI dhan 56, the highest seed germination was found in Decoprima treated seed in T_4 (100%) that was statistically similar in T_2 (95%), T_1 (90%) followed by T_3 and T_5. However, in BRRI dhan 71, the highest seed germination was found in T_4 (100%) followed by T2, T_6, T_1. But, in IR 64, the highest seed germination was found in T_4 (97%) that was statistically similar with G-derma (94%), Bioderma (96%) and control (91%).

Table 1. Effect of seed treatment by commercial formulations of *Trichoderma* on seed germination percentage of BRRI dhan 56, 71 and IR 64 at 7, 14 and 20 days after sowing (DAS).

Treatments	Germination (%)								
	7 DAS			14 DAS			20 DAS		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	57 c	38 cd	34 cd	83 b	70 c	91 a	90 a	83 c	94 a
T_2 = Bioderma	48 cd	43 c	51 b	90 ab	75 bc	91 a	95 a	96 ab	96 a
T_3 = Recharge	17 e	22 e	30 d	51 c	38 d	56 b	70 b	50 d	59 b
T_4 = Decoprima	86 a	79 a	73 a	96 a	95 a	93 a	100 a	100 a	97 a
T_5 = G-derma (LDS)	25 e	23 e	23 d	49 c	37 d	35 c	50 c	55 d	37 c
T_6 =Control	44 d	27 de	44 bc	89 ab	47 d	86 a	90 a	91 bc	91 a
LSD (0.05)	11.76	11.35	11.08	11.56	12.53	16.62	10.15	4.12	14.91
CV (%)	18.17	20.51	18.54	11.28	15.22	16.57	9.28	7.66	14.19
Level of significance	**	**	**	**	**	**	**	**	**

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64

Means followed by the same letters in a column did not differ at 5% level of significance by LSD.

4.2. Effect of seed treatment by commercial formulations of *Trichoderma* on speed of germination/ germination index (GI), mean germination time (MGT), mean daily germination (MDG)

Effect of seed treatment by commercial formulations of *Trichoderma* on three germination parameters viz., speed of germination / germination Index (GI), mean germination time (MGT), mean daily germination (MDG) is presented in Table 2. Based on the analysis of variance of data, to differ in all the parameters of germination among the different seed treatments were found significantly at 0.05% level.

Speed of germination or germination index indicates the vigor of seed or seedlings. In three rice varieties, the highest germination index was observed in T_4 (Decoprima). However, the lowest result was found in T_5 (G-derma, LDS). Similar trend was observed in mean germination time (MGT), Mean daily germination (MDG) of seedlings in three rice varieties.

In BRRI dhan 56, the highest speed of germination was recorded in T_4 (3.38) was followed by T_1, T_2. In BRRI dhan 71, the highest speed of germination was found in T_4 (3.11) was followed by T_2. However, in IR 64, the highest speed of germination was recorded highest in T_4 (2.93) followed by T_1, T_2.

In BRRI dhan 56, the highest mean germination time was recorded in T_2 (Bioderma) that was statistically similar with T_6 and followed by T_1, T_3. In BRRI dhan 71, the highest mean germination time was recorded in G-derma treated seed (7.11). However, in IR 64, the highest mean germination time was recorded in G-derma treated seed in T_1 (7.62) that was statistically similar with T_2, T_6.

In BRRI dhan 56, the highest mean daily germination was recorded in Decoprima treated seed (1.13) that was statistically similar with T_1, T_2, T_6. In BRRI dhan 71, the highest mean daily germination was observed in T_4 (1.1) that was statistically similar with T_2. In addition, in IR 64, mean daily germination was recorded highest in Decoprima treated seed (1.15) that was statistically similar with T_2 followed by T_1, T_2.

T**able 2. Effect of seed treatment by commercial formulations of *Trichoderma* on seed germination associated parameters (GI, MGT, MDG) of BRRI dhan 56, 71 and IR 64 .**

Treatments	Germination Index			Mean Germination Time			Mean Daily Germination		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	2.71 bc	2.0 c	2.39 b	6.0 bc	7.11 a	7.62 a	1.1 a	0.86 cd	1.09 ab
T_2 = Bioderma	2.69 bc	2.1 bc	2.38 b	7.3 a	6.26 ab	6.9 ab	1 a	0.98 ab	1.13 ab
T_3 = Recharge	1.41 d	1.11 e	1.42 c	6.03 bc	5.67 b	4.7 c	0.87 b	0.77 de	0.87 c
T_4 = Decoprima	3.38 a	3.11 a	2.93 a	6.12 bc	6.29 ab	6.23 b	1.13 a	1.1 a	1.15 a
T_5 = G-derma (LDS)	1.18 d	0.89 e	0.87 d	2.79 d	4.01 c	3.54 d	0.86 b	0.70 e	0.73 d
T_6 =Control	2.40 c	1.43 d	2.24 b	6.6 ab	5.65 b	7.4 a	1.12 a	0.96 bc	1.05 b
LSD (0.05)	0.50	0.25	0.22	0.81	0.85	0.81	0.15	0.12	0.09
CV (%)	15.92	10.47	8.32	10.85	11.30	10.24	11.76	10.09	6.96
Level of significance	**	**	**	**	**	**	**	**	**

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64

Means followed by the same letters in a column did not differ at 5% level of significance by LSD.

GI = Speed of germination / Germination Index, MGT= Mean germination Time, MDG = Mean daily germination

4.3. Effect of seed treatment by commercial formulations of *Trichoderma* on peak value (PV) and germination value (GV)

Effect of seed treatment by commercial formulations of *Trichoderma* formulations on two germination associated parameters viz., peak value (PV) and germination value (GV) was presented in Table 3. Based on the analysis of variance of data the differences in all the parameters of germination among the different seed treatments were found highly significant at 0.05% level.

In BRRI dhan 56, the highest peak value of seed germination was found in G-derma treated seed in T_1 (0.83) that was statistically similar with $T_{2,}$ $T_{4,}$ $T_{5.}$ However, in BRRI dhan 71, the highest peak value of seed germination was observed in Decoprima treated seed in T_4 (0.67) that was statistically similar with $T_{1,}T_{2,}$ $T_{3,}$ $T_{5..}$ In IR 64, the highest peak value of seed germination was noticed in T_4 (0.78) that was statistically similar with $T_{1,}$ $T_{3,}$ $T_{5.}$

In BRRI dhan 56, the highest germination value of seed germination was recorded in G-derma treated seed in T_1 (0.92) that was statistically similar with $T_{2,}$ $T_{6,}$. But, in BRRI dhan 71, the highest germination value of seed was found in Decoprima treated seed in T_4 (0.73) that was statistically similar with $T_{2.}$ In IR 64, the highest germination value of seed was found in T_4 (0.86) and T_1 (0.83) that was statistically similar with T_2.

Table 3. Effect of seed treatment by commercial formulations of *Trichoderma* on peak value and germination value of BRRI dhan 56, 71 and IR 64.

Treatments	PV (Peak Value)			GV (Germination Value)		
	BD 56	**BD 71**	**IR 64**	**BD 56**	**BD 71**	**IR 64**
T_1 = G-derma	0.83 a	0.65 ab	0.77 ab	0.92 a	0.52 bc	0.83 a
T_2 = Bioderma	0.70 ab	0.63 ab	0.63 b	0.75 ab	0.62 ab	0.71 ab
T_3 = Recharge	0.61 b	0.59 ab	0.69 ab	0.53 c	0.45 c	0.60 bc
T_4 = Decoprima	0.69 ab	0.67 a	0.78 a	0.76 ab	0.73 a	0.86 a
T_5 = G-derma (LDS)	0.69 ab	0.60 ab	0.68 ab	0.59 bc	0.41 c	0.49 c
T_6 =Control	0.65 b	0.55 b	0.76 ab	0.74 ab	0.52 bc	0.80 a
LSD (0.05)	0.15	0.11	0.14	0.19	0.15	0.16
CV (%)	17.74	13.66	15.76	20.9	20.58	16.98
Level of significance	*	*	**	**	**	**

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64
Means followed by the same letters in a column did not differ at 5% level of significance by LSD.
PV = Peak value and GV = Germination value

4.4. Effect of seed treatment by commercial formulations of *Trichoderma* on number of leaves of rice at different DAS

Effect of seed treatment by commercial formulations of *Trichoderma* on seedlings number of leaves of BRRI dhan 56, 71 and IR 64 at different days after sowing (DAS) was shown in Table 4.

At 14 DAS, in BRRI dhan 56, maximum leaves were observed in T_4 (3) and minimum leaves are found in T_3 (2.06). In BRRI dhan 71, maximum leaves were found in Decoprima treated seed in T_4 (3). In IR 64, maximum leaves were observed in T_4 (2.86) that was statistically similar with T_1, T_2.

At 24 DAS, in BRRI dhan 56, significant difference was not found among the treatments findings. But, in BRRI dhan 71, maximum leaves were observed in T_4 (4.67) followed by T_1 , T_3. However, in IR 64, maximum leaves were observed in T_1 , T_3 (4.53).

At 34 DAS, in BRRI dhan 56, maximum leaves were observed in T_6 (8.61) that was statistically similar with T_2 (8.33), T_1 (8). However, in BRRI dhan 71, maximum leaves were observed in T_1, T_2. In IR 64, significant difference was not found among the treatments findings.

At 44 DAS, in BRRI dhan 56, maximum leaves were observed in T_2 (11.60) that was statistically similar with T_1 (10.20), T_3 (10.66) which are followed by T_4, T_6. In BRRI dhan 71, maximum leaves were observed in T_2 (11.0) that was statistically similar with T_1, T_3, T_6. But, in IR 64, significant difference was not found among the treatments findings.

Table 4. Effect of seed treatment by commercial formulations of *Trichoderma* on number of leaves of BRRI dhan 56, 71 and IR 64 at different days after sowing (DAS).

Treatments	Number of Leaves											
	14 DAS			24 DAS			34 DAS			44 DAS		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	2.93 ab	2.06 c	2.80 a	4.51	4.53 b	4.53 a	8.33ab	8.0 a	10.13	10.20a-c	9.33ab	14.46
T_2 = Bioderma	2.73 a-c	2.0 c	2.66 ab	4.53	4.33 c	4.33ab	8.20 b	8.33 a	10.39	11.60 a	11.0 a	12.73
T_3 = Recharge	2.06 d	2.0 c	2.53 ab	4.33	4.46 b	4.53 a	8.20 b	7.53ab	9.93	10.66 ab	9.26ab	12.19
T_4 = Decoprima	3.0 a	3.0 a	2.86 a	4.62	4.67 a	4.40ab	8.0 b	7.33ab	9.52	9.53 bc	8.53 b	12.93
T_5 = G-derma (LDS)	2.66 bc	2.06 c	2.26 b	4.53	4.33 c	4.39ab	8.09 b	7.46ab	9.51	9.06 c	7.66bc	14.66
T_6 =Control	2.53 c	2.26 b	2.46 ab	4.60	4.33 c	4.33ab	8.61 a	8.39 a	10.0	9.86 bc	9.4 ab	13.6
LSD (0.05)	0.32	0.12	0.45	0.30	0.12	0.25	0.40	1.28	1.11	1.51	2.25	3.11
CV (%)	9.20	4.12	13.60	5.24	2.22	4.51	3.83	12.99	8.74	11.73	19.93	18.09
Level of significance	**	**	*	NS	**	*	*	*	NS	*	**	NS

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64

Means followed by the same letters in a column did not differ at 5% level of significance by LSD.

*, **, significant at the 0.05, 0.01 probability levels, respectively.

NS - Non significant

4.5. Effect of seed treatment by commercial formulations of *Trichoderma* on number of tillers of rice at different DAS

Effect of seed treatment by commercial formulations of *Trichoderma* on seedlings number of tillers of BRRI dhan 56, 71 and IR 64 at different days after sowing (DAS) was shown in Table 5.

At 33 DAS, in BRRI dhan 56, maximum number of tillers were observed in T_1 (1.8) followed by, T_4, T_3, T_5. In BRRI dhan 71, maximum number of tillers were observed in T_1 and T_6 (1.53) followed by T_3, T_4, T_5. In IR 64, maximum number of tillers was observed in T_1, T_2 (2.67) that was statistically similar with T_2 (2.53).

At 43 DAS, In BRRI dhan 56, maximum number of tillers were observed in T_2 (2.13) that was statistically similar with T_1, T_3, T_4. In BRRI dhan 71, maximum number of tillers were observed in T_1 (1.53) that was statistically similar with T_2, T_3, T_4. But, in IR 64, maximum number of tillers was observed in T_1, T_2 (2.67) followed by T_6 (2.26).

Table 5. Effect of seed treatment by commercial formulations of *Trichoderma* on number of tillers of BRRI dhan 56, 71 and IR 64 at different days after sowing (DAS)

Treatments	Number of Tillers					
	33DAS			43DAS		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	1.8 a	1.53 a	2.67 a	2.00 ab	1.53 a	2.67 a
T_2 = Bioderma	1.13 c	1.26 b	2.67 a	2.13 a	1.46 a	2.67 a
T_3 = Recharge	1.26 bc	1.19 bc	2.39 b	2.00 ab	1.40 a	2.39 bc
T_4 = Decoprima	1.46 b	1.26 b	2.53 ab	2.00 ab	1.26 ab	2.19 cd
T_5 = G-derma (LDS)	1.19 bc	1.13 bc	2.40 b	1.80 bc	1.33 a	2.40 bc
T_6 =Control	1.19 bc	1.53 a	2.13 c	1.60 c	1.40 a	2.46 ab
LSD (0.05)	0.29	0.23	0.22	0.25	0.31	0.24
CV (%)	16.82	14.36	7.02	10.66	18.18	7.93
Level of significance	*	**	**	**	*	**

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64.
Means followed by the same letters in a column did not differ at 5% level of significance by LSD.
*, **, significant at the 0.05, 0.01 probability levels, respectively.

4.6. Effect of seed treatment by commercial formulations of *Trichoderma* on plant height of rice plant at different DAS

Effect of different treatment on seedling's growth and growth contributing character (plant height) was shown in Table 6. Plant height differs significantly among the treatments in three rice varieties.

At 14 DAS, in BRRI dhan 56, the highest plant height was observed in T_4 (22.35cm) that was statistically similar with T_1 (21.57 cm), T_6 (20.56 cm) which are followed by T_2, T_5. In BRRI dhan 71, the highest plant height was found in T_4 (21.31 cm) that was statistically similar with T_2 (18.5 cm) followed by T_1. In IR 64, the highest plant height was observed in Decoprima treated seed in T_4 (19.52 cm) that was statistically similar with T_2 (18.03 cm), T_3 (17.67cm) followed by T_1.

At 24 DAS, in BRRI dhan 56, the highest plant height was observed in Decoprima treated seed in T_4 (36.57 cm) that was statistically similar with T_1 (36.23 cm), T_6 (36.23 cm) followed by T_5. However, in BRRI dhan 71, the highest plant height was found in Decoprima treated seed in T_4 (33.05 cm) that was statistically similar with T_1 (31.55 cm), T_2 (31.52 cm) followed by T_3 (27.49 cm), T_5 (26.99 cm). In IR 64, the highest plant height was observed in T_4 (31.61 cm) and there was no significant difference among treatments.

At 34 DAS, in BRRI dhan 56, the highest plant height was observed in T_4 (56.05 cm) which are followed by T_5, T_2, T_1. However, in BRRI dhan 71, the highest plant height was found in T_4 (45.1 cm) that was statistically similar with T_1, T_2, T_4 and followed by T_3. But, IR 64, the highest plant height was observed in G-derma (L) treated seed in T_4 (44.9 cm) that was statistically similar with T_3 (43.9 cm), T_5 (44.2 cm) followed by T_6 (41.56 cm).

At 51 DAS, in BRRI dhan 56, the highest plant height was observed in T_4 (79.61 cm) that was statistically similar with T_5, T_3 and followed by T_2, T_1. In BRRI dhan 71, the highest plant height was found in T_4 (83.78 cm) followed by T_3, T_5. In IR 64, the highest plant height was observed in T_5 (74.74 cm) that was statistically similar with T_4 and followed by T_1, T_2, T_3, T_6.

Table 6. Effect of seed treatment by commercial formulations of *Trichoderma* on plant height of rice plant at different days after sowing (DAS)

Treatments	Plant Height (cm)											
	14 DAS			24 DAS			34 DAS			51 DAS		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	21.57 ab	17.6 bc	17.11 b	36.23 a	31.55 a	29.19 ab	49.16 bc	44.42 ab	43.8 a	72.74 cd	74.11 cd	61.21 b
T_2 = Bioderma	20.34 b	18.5 ab	18.03ab	32.95 c	31.52 a	31.39 ab	51.21 b	43.2 ab	43.2 ab	74.44 bc	74.43 cd	62.56 b
T_3 = Recharge	17.13 c	14.37 d	17.67 ab	33.17 d	27.49 b	29.48 ab	45.25 cd	44.4 a	43.9 a	75.8 a-c	76.60 bc	64.94 b
T_4 = Decoprima	22.34 a	21.31 a	19.52 a	36.57 a	33.05 a	31.61 a	56.05 a	45.1 a	44.9 a	79.61 a	83.78 a	73.87a
T_5 = G-derma (L)	20.43 b	14.86 cd	14.86 c	33.73 bc	26.99 b	29.42 ab	52.70 ab	43.8 ab	44.2 a	77.13 ab	74.9 b-d	74.74 a
T_6 = Control	20.56 ab	15.69 b-d	18.06 ab	36.23 a	30.79 a	30.99 ab	50.99 b	42.4 b	41.56 b	69.68 d	72.13 d	61.04 b
LSD (0.05)	1.85	3.08	1.99	2.162	3.19	2.43	4.40	1.99	2.22	4.19	4.40	5.50
CV (%)	6.94	13.52	8.72	4.84	8.08	6.23	6.82	3.52	3.94	4.33	4.45	6.47
Level of Sig.	**	**	**	**	**	*	**	*	*	*	**	**

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64.
Means followed by the same letters in a column did not differ at 5% level of significance by LSD.
*, **, Significant at the 0.05, 0.01 probability levels, respectively.

4.7. Effect of seed treatment by commercial formulations of *Trichoderma* on shoot length and root length of rice plant at different days after sowing (DAS)

Effect of different treatment on seedling's growth and growth contributing characters was shown in Table 7. Different parameters viz., shoot length, root length differ significantly among the treatments in three rice varieties.

Shoot length at 34 DAS in BRRI dhan 56, the highest shoot length was observed in T_4 (56.05 cm) Decoprima treated seed that was statistically similar with T_5 (50.99 cm) followed by T_2, T_6. In BRRI dhan 71, the highest shoot length was observed in T_4 (45.1 cm) and there was no significant difference among treatments. In IR 64, the highest shoot length was observed in T_5 (44.9 cm) and there was no significant difference among treatments.

Shoot length at 51 DAS in BRRI dhan 56, the highest shoot length was found in T_4 (79.61 cm) followed by T_6. In BRRI dhan 71, the highest shoot length was observed in T_4 (83.78 cm) followed by T_5. In IR 64, the highest shoot length was observed in T_5 (74.74 cm) that was statistically similar with T_4 (73.87cm) followed by T_1, T_2.

Root length at 34 DAS in BRRI dhan 56, the highest root length was observed in T_5 (9.36 cm) that was statistically similar with T_1 (7 cm) followed by T_2, T_3. In BRRI dhan 71, the highest root length was observed in T4 (10.9cm) that was statistically similar with T_1, T_2, T_5. In IR 64, the highest root length was observed in T_4 (11 cm).

Root length at 51 DAS in BRRI dhan 56, the highest root length was observed in T_4 (9.29 cm) and there was no significant difference among treatments. In BRRI dhan 71, the highest root length was observed in T5 (11.32 cm). But, in IR 64, the highest root length was observed in T_4 (9.33cm) and there was no significant difference among treatments.

Table 7. Effect of seed treatment by commercial formulations of *Trichoderma* on shoot length and root length of rice plant (BRRI dhan 56, 71 and IR 64) at different days after sowing (DAS)

Treatments	Shoot Length (cm)						Root Length (cm)					
	34 DAS			51 DAS			34 DAS			51 DAS		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	49.16 bc	44.42 ab	43.8 a	72.74 cd	74.11 cd	61.21 b	6.77 b	8.4 b	9.42 bc	7.84 b	10.1 ab	8.89 ab
T_2 = Bioderma	51.21 b	43.2 ab	43.2 ab	74.44 bc	74.43 cd	62.56 b	7.08 b	7.40 b	10.5 ab	6.99 b	10.5 ab	8.41 ab
T_3 = Recharge	45.25 cd	44.4 a	43.9 a	75.8 a-c	76.60 bc	64.94 b	7.50 b	8.31 b	10.5 ab	7.35 b	8.82 b	8.99 ab
T_4 = Decoprima	56.05 a	45.1 a	44.9 a	79.61 a	83.78 a	73.87a	9.36 a	10.9 a	11.0 a	9.29 a	11.32 a	9.33 a
T_5 = G-derma (LDS)	52.70 ab	43.8 ab	44.2 a	77.13 ab	74.9 b-d	74.74 a	7.0 a	8.29 b	11.3 a	8.08 ab	10.6 ab	9.26 a
T_6 = Control	50.99 b	42.4 b	41.56 b	69.68 d	72.13 d	61.04 b	7.76 b	7.05 b	9.4 bc	6.98 b	9.75 ab	8.35 ab
LSD (0.05)	4.40	1.99	2.22	4.19	4.40	5.50	1.54	1.65	1.23	1.25	2.03	1.42
CV (%)	6.82	3.52	3.94	4.33	4.45	6.47	15.88	15.29	9.40	12.59	15.58	12.59
Level of significance	**	*	*	*	**	**	*	**	*	**	*	*

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64.

Means followed by the same letters in a column did not differ at 5% level of significance by LSD.

*, **, Significant at the 0.05, 0.01 probability levels, respectively.

4.8. Effect of seed treatment by commercial formulations of *Trichoderma* on fresh shoot weight and fresh root weight of rice plant at different days after sowing (DAS)

Effect of seed treatment by different commercial *Trichoderma* formulations on shoot and root weight of seedlings of BRRI dhan 56, BRRI dhan 71 and IR 64 at 34 and 51 days after sowing (DAS) is shown in Table 8. Significant statistical difference was observed among the treatments in respect of fresh shoot weight, fresh root weight, dry shoot weight, dry root weight.

Fresh shoot wt. at 34 DAS in BRRI dhan 56, the highest fresh shoot wt. was recorded in T_1 (1.27g) that was statistically similar in T_2, T_4, T_5. However, in BRRI dhan 71, the highest fresh shoot wt. was found in T_4 (0.66g) that was statistically similar in T_2, T_3, T_4. In IR 64, the highest fresh shoot wt. was recorded in T_4, T_5 (1.14g).

Fresh shoot wt. at 51 DAS in BRRI dhan 56, the highest fresh shoot wt. was observed in Decoprima treated seed in T_5 (3.44g) that was statistically similar with T_4, T_3 followed by T_2 T_1. But, in BRRI dhan 71, the highest fresh shoot wt. was found in T_4 (3.14g) that was statistically similar with T_5 followed by T_1, T_2. However, in IR 64, the highest fresh shoot wt. was observed in T_4 (3.21g) that was statistically similar with T_3, T_1, T_2.

Fresh root wt. at 34 DAS in BRRI dhan 56, the highest fresh root wt. was found in T_4 (0.14g) and others are statistically similar. In BRRI dhan 71, the highest fresh root wt. was recorded in T_4 (0.15g) that was statistically similar with T_5, T_1, T_2. But, in IR 64, the highest fresh root wt. was observed in Decoprima treated seed in T_4 (0.15g) and there was no significant difference among treatments.

Fresh root wt. at 51 DAS in BRRI dhan 56, the highest fresh root wt. was recorded in T_4 (0.14g) that was statistically similar with T_2, T_3 followed by T_1, T_5 T_6. In BRRI dhan 71, the highest fresh root wt. was recorded in, T_4 (0.3g). But, in IR 64, the highest fresh root wt. was recorded in T_4 (0.28g) that was statistically similar with T_5, T_1 followed by T_6.

Table 8. Effect of seed treatment by commercial formulations of *Trichoderma* on fresh shoot weight and fresh root weight of rice plant (BRRI dhan 56, 71 and IR 64) at different days after sowing (DAS)

Treatments	Fresh Shoot Weight (gm)						Fresh Root Weight (gm)					
	34 DAS			51 DAS			34 DAS			51 DAS		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	1.08 a-c	0.60 ab	1.03 a-c	2.26 c	2.44 b	3.10 ab	0.12 a	0.14 ab	0.13 ab	0.12 b	0.27 bc	0.25 ab
T_2 = Bioderma	1.02 bc	0.62 ab	1.1 ab	2.61 bc	2.29 b	3.10 ab	0.13 a	0.13 ab	0.13 ab	0.13 b	0.25 cd	0.23 ab
T_3 = Recharge	1.14 ab	0.62 ab	1.12 ab	3.21 a	3.01 a	3.07 ab	0.13 a	0.14 ab	0.14 ab	0.13 ab	0.28 ab	0.24 ab
T_4 = Decoprima	1.27 a	0.66 a	1.14 a	3.44 a	3.14 a	3.21 a	0.14 a	0.15 a	0.15 a	0.14 a	0.30 a	0.28 a
T_5 = G-derma (LDS)	1.21 ab	0.61 ab	1.14 a	3.30 a	3.14 a	3.13 ab	0.13 a	0.15 a	0.15 a	0.13 ab	0.30 a	0.26 a
T_6 = Control	0.96 cd	0.59 b	0.97 c	2.47 c	2.29 b	2.83 b	0.09 b	0.13 b	0.12 b	0.12 b	0.24 d	0.20 b
LSD (0.05)	0.23	0.06	0.12	0.45	0.41	0.30	0.02	0.02	0.027	0.01	0.02	0.05
CV (%)	17.10	8.26	9.23	11.98	11.72	7.65	17.32	13.54	14.86	9.71	8.01	19.11
Level of significance	**	*	*	**	**	*	**	*	*	*	**	*

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64.
Means followed by the same letters in a column did not differ at 5% level of significance by LSD.
*, **, Significant at the 0.05, 0.01 probability levels, respectively.

4.9. Effect of seed treatment by commercial formulations of *Trichoderma* on dry shoot wt. and dry root wt. of rice plant (BRRI dhan 56, 71 and IR 64) at different days after sowing (DAS)

Effect of seed treatment by commercial formulations of *Trichoderma* on Table 9. Significant statistical difference was observed among the treatments in respect of dry shoot weight and dry root weight.

Dry shoot wt. at 34 DAS in BRRI dhan 56, the highest dry shoot wt. was recorded in Decoprima treated seed in T_4 (0.26g) that was statistically similar with T_3, T_5. But, in BRRI dhan 71, the highest dry shoot wt. was found in Decoprima treated seed in T_4 (0.26g) that was statistically similar with T_5, T_6. However, in IR 64, the highest dry shoot wt. was observed in T_4 (0.29g) and there was no significant difference among treatments.

Dry shoot wt. at 51 DAS in BRRI dhan 56, the highest dry shoot wt. was recorded in Decoprima treated seed in T_4 (1.03g) that was statistically similar with T_5, T_1, T_2. But, in BRRI dhan 71, the highest dry shoot wt. was found in T_4 (1.19g) that was statistically similar with T_2, T_5 followed by T_1, T_6. However, in IR 64, the highest dry shoot wt. was observed in T_4 (1.42g) that was statistically similar with T_1, T_2.

Dry root wt. at 34 DAS in BRRI dhan 56, the highest dry root wt. was recorded in Decoprima treated seed in T_4 (0.015g) that was statistically similar with T_1, T_2. But, in BRRI dhan 71, the highest dry root wt. was found in Decoprima treated seed in T_4 (0.013g) that was statistically similar with T_2. However, in IR 64, the highest dry root wt. was observed in Decoprima treated seed in T_4 (0.013g) that was statistically similar with T_1, T_1, T_2 followed by T_3, T_6.

Dry root wt. at 51 DAS in BRRI dhan 56, the highest dry root wt. was recorded in Decoprima treated seed in T_4 (0.02g) that was statistically similar with T_3, T_5. But, in BRRI dhan 71, the highest dry root wt. was found in Decoprima treated seed in T_4 (0.029g) that was statistically similar with T_2. However, in IR 64, the highest dry root wt. was observed in Decoprima treated seed in T_4 (0.029g) that was statistically similar with T_1, T_2 followed by T_6.

Table 9. Effect of seed treatment by commercial formulations of *Trichoderma* on dry shoot weight and dry root weight of rice plant (BRRI dhan 56, 71 and IR 64) at different days after sowing (DAS)

Treatments	Dry Shoot Weight (gm)						Dry Root Weight (gm)					
	34 DAS			51 DAS			34 DAS			51 DAS		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	0.21 c	0.19 d	0.28 a	0.98 ab	1.08 b	1.31 ab	0.014 ab	0.012 b	0.011 b	0.018 b	0.027 b	0.026 ab
T_2 = Bioderma	0.23 b	0.21 cd	0.28 a	1.01 ab	1.13 ab	1.19 ab	0.014 ab	0.012 b	0.012 ab	0.018 b	0.024 cd	0.024 ab
T_3 = Recharge	0.25 ab	0.24 ab	0.28 a	1.0 ab	1.14 ab	1.33 ab	0.014 ab	0.012 ab	0.012 ab	0.019 ab	0.029 a	0.025 ab
T_4 = Decoprima	0.26 a	0.26 a	0.29 a	1.03 a	1.19 a	1.42 a	0.015 a	0.013 a	0.013 a	0.020 a	0.029 a	0.029 a
T_5 = G-derma (LDS)	0.25 ab	0.24 ab	0.29 a	1.02 a	1.16 ab	1.46 a	0.014 ab	0.013 a	0.012 ab	0.019 ab	0.026 bc	0.027 a
T_6 =Control	0.21 c	0.15 e	0.26 b	0.97 b	1.07 b	1.09 b	0.013 b	0.012 b	0.011 b	0.018 b	0.023 d	0.021 b
LSD (0.05)	0.018	0.029	0.03	0.04	0.08	0.32	0.002	0.001	0.002	0.002	0.002	0.005
CV (%)	6.20	10.24	8.25	3.66	5.71	19.30	13.67	9.23	7.20	8.58	19.17	9.82
Level of significance	**	**	*	*	*	*	*	*	*	*	*	*

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64.

Means followed by the same letters in a column did not differ at 5% level of significance by LSD.

*, **, Significant at the 0.05, 0.01 probability levels, respectively.

4.10. Effect of seed treatment by commercial formulations of *Trichoderma* on seedling vigor index (SVI) and seedling vegetative vigor (SVV) of different varieties of rice plant

Effect of seed treatment by commercial formulations of *Trichoderma* on seedling vigor index (SVI) and seedling vegetative vigor (SVV) of BRRI dhan 56, 71 and IR 64.

Seedling vigor index in BRRI dhan 56, the highest seedling vigor index was found in Decoprima treated seed in T_4 (57.26) that was statistically similar with T_1, T_2, T_3. In BRRI dhan 71, the highest seedling vigor index was observed in Decoprima treated seed in T_4 (60.68) that was statistically similar with T_3, T_5 followed by T_1, T_2. In IR 64, the highest seedling vigor index was found in T_4 (54.69) that are statistically similar with T_1, T_2, T_3.

Seedling vegetative vigor in BRRI dhan 56, the highest seedling vegetative vigor was found in T_4 (4.8) that was statistically similar with T_1, T_5. In BRRI dhan 71, the highest seedling vegetative vigor was found in T_4 (4.8) and there was no significant difference among treatments. In IR 64, the highest seedling vegetative vigor was noticed in T_4 (4.8) that was statistically similar with T_3, T_5.

Table 10. Effect of seed treatment by commercial formulations of *Trichoderma* on seedling vigor index (SVI) and seedling vegetative vigor (SVV) of BRRI dhan 56, 71 and IR 64

Treatments	SVI (Seedling Vigor Index)			SVV (Seedling Vegetative Vigor)		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	55.29 ab	51.14 b	49.50 a-c	4.4 ab	4.2 ab	3.6 bc
T_2 = Bioderma	52.15 ab	51.7 b	48.46 a-c	3.8 bc	3.8 ab	3.6 bc
T_3 = Recharge	54.44 ab	55.42 ab	53.04 ab	4.6 ab	4.6 ab	4.4 ab
T_4 = Decoprima	57.26 a	60.68 a	54.69 a	4.8 a	4.8 a	4.8 a
T_5 = G-derma (LDS)	55.88 ab	53.13 ab	53.48 a	4.6 ab	4.2 ab	4.4 ab
T_6 =Control	50.41 b	47.74 b	44.14 c	3.4 c	3.6 b	3.4 c
LSD (0.05)	6.51	8.0	7.16	0.88	1.02	0.91
CV (%)	9.27	11.65	11.08	16.35	19.01	17.93
Level of significance	*	*	*	*	*	*

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64.

Means followed by the same letters in a column did not differ at 5% level of significance by LSD.

*, Significant at the 0.05 probability levels

4.11. Effect of seed treatment by commercial formulations of *Trichoderma* on number of droopy leaf of rice plant under different days drought stress (DDS)

At 7 DDS, in BRRI dhan 56, the lowest droopy leaf was observed in T_4 (3.63) and others are statistically similar among treatments. In BRRI dhan 71, the lowest droopy leaf was observed in Decoprima treated seed in T_4 (1.37) and there were no significant difference among treatments. In IR 64, the lowest droopy leaf was observed in Decoprima treated seed in T_4 (7.26) that are statistically similar with other trreatments.

At 10 DDS, in BRRI dhan 56, the lowest droopy leaf was observed in T_4 (0.6) in Decoprima treated seed that asre statistically similar with T_3, T_5 followed by T_1, T_2. In BRRI dhan 71, the lowest droopy leaf was observed in T_4 (0.8) and others are significally similar. In IR 64, the lowest droopy leaf was observed in Decoprima treated seed in T_4 (0.8) that are statistically similar with T_1 and T_3.

Table 11. Effect of seed treatment by commercial formulations of *Trichoderma* on number of droopy of rice plant (BRRI dhan 56, 71 and IR 64) under different days drought stress (DDS)

Treatments	Number of Droopy Leaf					
	7 DDS			10 DDS		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	4.68 ab	2.67 b	9.34 ab	5.74 ab	1.51 ab	5.28 cd
T_2 = Bioderma	4.89 ab	2.84 b	9.27 ab	3.56 bc	2.42 ab	11.08 a
T_3 = Recharge	4.55 ab	2.87 b	9.1 ab	2.64 cd	1.52 ab	5.72 cd
T_4 = Decoprima	3.63 b	1.37 b	7.26 b	0.6 d	0.80 b	1.20 d
T_5 = G-derma (LDS)	4.27 ab	2.20 b	8.54 ab	2.86 cd	1.45 ab	7.11 bc
T_6 = Control	5.34 a	3.30 a	10.68 a	6.26 a	3.11 a	12.31 a
LSD (0.05)	1.52	1.92	3.05	2.42	1.86	5.0
CV (%)	26.59	29.89	26.58	24.04	25.07	27.42
Level of significance	*	*	*	**	*	**

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64

Means followed by the same letters in a column did not differ at 5% level of significance by LSD.

*, **, Significant at the 0.05, 0.01 probability levels, respectively.

4.12. Effect of seed treatment by commercial formulations of *Trichoderma* on number of rolled leaf of rice plant under different days drought stress (DDS)

At 10 DDS, in BRRI dhan 56, the lowest rolling leaf was observed in Decoprima treated seed in T_4 (0.5) that was statistically similar with T_3, T_5 followed by T_1, T_2. In BRRI dhan 71, the lowest rolling leaf was found in Decoprima treated seed in T_4, T_5 (0.5) that was statistically similar with T_1, T_2, T_3. In IR 64, the lowest rolling leaf was observed in T_4 (0.5) and there was no significant difference among treatments.

At 13 DDS, in BRRI dhan 56, the lowest rolling leaf was observed in in T_4 (1.52) and there was no significant difference among treatments. In BRRI dhan 71, the lowest rolling leaf was found in Decoprima treated seed in T_4 (1.1) that was statistically similar with T_2, T_3. In IR 64, the lowest rolling leaf was found in Decoprima treated seed in T_4 (1.1) that was statistically similar with T_2 and T_3.

Table 12. Effect of seed treatment by commercial formulations of *Trichoderma* on number of rolled leaves of rice plant (BRRI dhan 56, 71 and IR 64) under different days drought stress (DDS)

Treatments	Number of Rolled leaves					
	10 DDS			13 DDS		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	2.09 b	2.29 ab	3.43 ab	3.97 a-c	2.54 b	7.60 ab
T_2 = Bioderma	2.24 b	1.71 ab	4.58 ab	4.48 bc	1.59 bc	7.17 a
T_3 = Recharge	1.31 bc	0.84 ab	1.56 ab	1.74 bc	1.61 bc	5.07 bc
T_4 = Decoprima	0.5 c	0.5 b	1.0 b	1.52 bc	1.1 c	3.64 c
T_5 = G-derma (LDS)	1.27 bc	0.5 b	1.0 b	1.55 bc	2.69 b	2.83 cd
T_6 = Control	3.40 a	2.32 a	4.77 a	6.02 a	4.06 a	8.86 a
LSD (0.05)	1.03	1.82	3.61	3.68	1.14	2.79
CV (%)	24.14	27.12	26.26	30.57	21.56	22.22
Level of significance	**	*	*	*	**	**

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64

Means followed by the same letters in a column did not differ at 5% level of significance by LSD.

*, **, Significant at the 0.05, 0.01 probability levels, respectively.

4.13. Effect of seed treatment by commercial formulations of *Trichoderma* on number of leaf drying of rice plant under different days drought stress (DDS)

At 10 DDS, in BRRI dhan 56, the lowest drying leaf was found in T_4 (1.22) that was statistically similar with T_1, T_2, T_5. In BRRI dhan 71, the lowest drying leaf was found in T_4 (0.5) that was statistically similar with T_1, T_2, T_5. In IR 64, the lowest drying leaf was found in T_4 (0.5) that was statistically similar with T_1, T_3 and T_5 followed by T_2.

At 13 DDS, in BRRI dhan 56, the lowest drying leaf was found in T_4 (1.33) followed by T_1, T_2, T_3. In BRRI dhan 71, the lowest drying leaf was found in T_3, T_4 and T_5 that was statistically similar with T_2, T_3 followed by T_1. However, in IR 64, the lowest drying leaf was found in T_4 (0.5) that was statistically similar with T_5 followed by T_1 and T_2.

Table 13. Effect of seed treatment by commercial formulations of *Trichoderma* on number of drying leaf of rice plant (BRRI dhan 56, 71 and IR 64) under different days drought stress (DDS)

Treatments	Number of Drying Leaf					
	10 DDS			13 DDS		
	BD 56	BD 71	IR64	BD56	BD 71	IR64
T_1 = G-derma	1.73 ab	0.5 b	1.52 b	6.97 a	4.52 ab	6.50 ab
T_2 = Bioderma	1.92 a	0.8 ab	6.52 a	6.90 a	1.86 bc	7.87 a
T_3 = Recharge	1.52 ab	0.5 b	0.74 b	5.57 a	0.5 c	5.19 ab
T_4 = Decoprima	1.22 b	0.5 b	0.50 b	1.33 b	0.5 c	0.50 c
T_5 = G-derma (LDS)	1.61 ab	0.5 b	0.50 b	5.26 a	0.5 c	3.55 bc
T_6 =Control	2.12 a	1.65 a	6.72 a	7.01 a	7.39 a	8.13 a
LSD (0.05)	0.63	0.86	2.57	3.02	3.20	3.22
CV (%)	28.10	24.21	27.88	28.33	26.73	24.42
Level of significance	**	**	**	**	**	**

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64

Means followed by the same letters in a column did not differ at 5% level of significance by LSD.

**, Significant at the 0.01 probability level

4.14. Effect of seed treatment by commercial formulations of *Trichoderma* on drought sensitivity scale of leaf rolling at vegetative stage under different days drought stress (DDS)

At 7 DDS, in BRRI dhan 56, the lowest leaf rolling at vegetative stage was found in T_4 (0.51) that was statistically similar with T_3, T_5 followed by T_1, T_2. In BRRI dhan 71, the lowest leaf rolling at vegetative stage was observed in T_4 (0.49) followed by T_1, T_5. In IR 64, the lowest leaf rolling at vegetative stage was noticed in T_4 (0.54) that was statistically similar with T_5 followed by T_3.

At 10 DDS, in BRRI dhan 56, the highest leaf rolling at vegetative stage were observed in T_4 (0.74) that was statistically similar with T_5 followed by T_1, T_2. In BRRI dhan 71, the highest leaf rolling at vegetative stage were observed in T_4 (0.66) followed by T_3, T_5. In IR 64, the highest leaf rolling at vegetative stage were observed in T_4 (0.74) that was statistically similar with T_5 followed by T_1, T_2.

At 13 DDS, in BRRI dhan 56, the lowest leaf rolling at vegetative stage were observed in T_4 (1.77) followed by T_3, T_5. In BRRI dhan 71, the lowest leaf rolling at vegetative stage was observed in T_4 (1.85) that was statistically similar with T_2, T_3 followed by T_1, T_5. In IR 64, the highest leaf rolling at vegetative stage were observed in T_4 (1.52) followed by T_5.

Table 14. Effect of seed treatment by commercial formulations of *Trichoderma* on drought sensitivity scale of leaf rolling (BRRI dhan 56, 71 and IR 64) at vegetative stage under different days drought stress (DDS)

Treatments	Drought sensitivity scale (Leaf rolling at vegetative stage)								
	7 DDS			10 DDS			13 DDS		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	0.91 b	0.7 bc	0.94 b	2.04 b	2.31 b	2.04 b	2.78 ab	2.45 ab	3.0 a
T_2 = Bioderma	0.95 b	0.9 a	1.15 a	2.36 ab	2.68 a	2.69 a	2.67 bc	2.45 ab	3.0 a
T_3 = Recharge	0.7 bc	0.5 c	0.78 c	1.54 b	1.49 c	1.54 b	2.42 c	2.31 a-c	2.54 a
T_4 = Decoprima	0.51 c	0.49 d	0.54 d	0.74 c	0.66 d	0.74 c	1.77 d	1.85 c	1.52 c
T_5 = G-derma (LDS)	0.52 c	0.5 c	0.50 d	0.80 c	1.38 c	0.80 c	2.62 bc	1.95 bc	2.30 b
T_6 =Control	2.78 a	0.9 a	1.73 a	2.69 a	2.78 a	3.0 a	2.96 a	2.78 a	3.0 a
LSD (0.05)	0.21	0.20	0.11	0.55	0.32	0.55	0.29	0.52	0.62
CV (%)	19.31	19.31	12.71	26.86	15.46	26.86	8.31	17.79	20.76
Level of significance	**	*	**	**	**	**	**	**	**

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64

Means followed by the same letters in a column did not differ at 5% level of significance by LSD.

*, **, Significant at the 0.05, 0.01 probability levels, respectively.

4.15. Effect of seed treatment by commercial formulations of *Trichoderma* on drought sensitivity scale of leaf drying at vegetative stage under different days drought stress (DDS)

At 10 DDS, in BRRI dhan 56, the lowest leaf drying at vegetative stage was found in T_4 (0.50) that was statistically similar with T_5 followed by $T_{3,}$ $T_{1.}$ In BRRI dhan 71, the lowest leaf drying at vegetative stage was observed in T_4 (0.54) followed by $T_{2,}$ $T_{3,}$ $T_{5.}$ In IR 64, the lowest leaf drying at vegetative stage was noticed in T_4 (0.5) that was statistically similar with T_3 followed by $T_{5.}$

At 13 DDS, in BRRI dhan 56, the lowest leaf drying at vegetative stage was showed in T_4 (2.22) that was statistically similar with $T_{3,}$ T_5. In BRRI dhan 71, the lowest leaf drying at vegetative stage was observed in T_4 (1.44) that was statistically similar with T_5 followed by $T_{2,}$ $T_{3.}$ In IR 64, the lowest leaf drying at vegetative stage was noticed in T_4 (2.32) that was statistically similar with T_3 and T_5.

Table 15. Effect of seed treatment by commercial formulations of *Trichoderma* on drought scale of leaf drying (BRRI dhan 56, 71 and IR 64) at vegetative stage under different days drought stress (DDS)

Treatments	Drought sensitivity scale (Leaf drying at vegetative stage)					
	10DDS			13DDS		
	BD 56	BD 71	IR 64	BD 56	BD 71	IR 64
T_1 = G-derma	1.0 a	1.15 b	1.0 a	3.0 a	2.73 a	3.0 a
T_2 = Bioderma	1.08 a	0.7 c	1.08 a	3.0 a	2.58 ab	3.0 a
T_3 = Recharge	0.62 bc	0.75 c	0.58 cd	2.84 ab	2.34 a-c	2.84 ab
T_4 = Decoprima	0.50 d	0.54 d	0.5 d	2.22 b	1.44 d	2.32 b
T_5 = G-derma (LDS)	0.58 cd	0.70 c	0.62 c	2.38 ab	1.92 b-d	2.38 b
T_6 =Control	1.00 a	1.30 a	1.0 a	3.0 a	2.88 a	3.0 a
LSD (0.05)	0.12	0.13	0.12	0.67	0.74	0.58
CV (%)	11.79	12.50	11.79	21.38	25.62	18.25
Level of significance	**	*	**	**	**	**

Where, BD 56 = BRRI dhan 56, BD 71 = BRRI dhan 71, IR 64

Means followed by the same letters in a column did not differ at 5% level of significance by LSD.

*, **, Significant at the 0.05, 0.01 probability levels, respectively.

Febri Doni *et al.* (2014) reported, *Trichoderma* spp. is effective to enhance rice germination and vigour. An in vitro experiment was carried out to assess the effect of seven isolates of *Trichoderma* spp. in enhancing rice germination and vigour. The results showed that all isolates of *Trichoderma* spp. significantly increased rice seedling growth, germination rate, vigour index and speed of germination with *Trichoderma sp*., SL_2 showing the processing impactin all the four parameters. *Trichoderma* sp., SL_2 treated rice seeds attained values of 4.48 and 6.00 cm, 0.0084 and 0.0048 g and 1016.56 and 44.75 seeds/ day for seedling shoot length, seedling root length, shoot weight, root weight, vigour index and speed of germination, respectively. They conclude that, *Trichoderma spp.* is able to enhance seed germination and vigour.

Kaveh *et al*. (2011) investigated the effect of *Trichoderma harzianum* on seed germination and seedling quality and field establishment of two muskmelon cultivars, *Khatooni* and *Qasri,* at greenhouse and in field in condition of Iran. Results showed that *Trichoderma* application significantly increased germination and emergence percentage and index and could help improving seedling health and vigor.

Shukla *et al.* (2012) investigated that world rice production in future will be reduced by approximately 50% due to drought. They reported that *Trichoderma harzianum* significantly increased the ability of rice plants to tolerate drought stress and increase rice water holding. They also suggested that *Trichoderma harzianum* isolates resistant to dessication have also been shown to be able to induce drought tolerance in rice plants subjected to 9 days of drought treatment. *T. harzianum* colonized in rice root delayed wilting and showed promising growth with minimum oxidative stress. Consortia of plant growth promoting rhizobcteria (PGPR) comprising strains of *Pseudomonas, Arthrobacter,* etc. enhance rice plant growth under drought and provide resistance to injury by activating the antioxidant defence mechanism of the plants.

CHAPTER V

SUMMARY AND CONCLUSION

Rice is the staple food of Bangladesh and it constituted about 90% of the total food grain production (Ahmed *et al*., 2013). Rice is a major source of livelihood in terms of providing food, income and employment in Bangladesh. It covers about 77 % of the total cropped area in the country. Seed borne diseases are very common in cereals. A large number of fungal diseases have been found to be transmitted through seeds. Seed borne fungi like *Bipolaris oryzae, Curvularia lunata, Fusarium spp., Trichoconis padwickii, Alternaria tenuis* and *Chaetomella spp.* are associated with seed infection of rice and causes yield reduction, quality deterioration and germination falure (Miah *et al.*, 1979; Shahjahan *et al.*, 1988).

Seeds are vital input for agriculture. Farmer's usually used different inbreed and hybrid rice varieties and face the difficulties of many diseases. Many pathogen of rice are seed-borne and frequently transmitted through seeds. Recently, blast, the most important seed borne disease of rice caused alarming situation in different areas of Bangladesh. Thus, one of the efficient means of control of these diseases would be seed treatment by *Trichoderma spp*. No reliable information is available about the seed treatment of rice by existing commercial *Trichoderma* formulations in Bangladesh. The present research will generate reliable information on *Trichoderma* as seed treating agent of rice as an eco-friendly approach under controlled irrigation. Based on this information suitable farm applicable preventive measures for controlling the seed borne diseases can be formulated and recommended for the rice growers of the country.

The present study was undertaken to evaluate different commercial formulations of *Trichoderma* of Bangladesh for seed treatment of rice and to evaluate their effect on seed and seedling quality of rice under controlled irrigation. The experiment was carried out in the Seed Health Laboratory (SHL) and Glass house of Department of Plant Pathology, Sher-e-Bangla Agricultural University,Dhaka, Bangladesh during the period from January 2018 to April 2018.

The experiment was conducted under controlled irrigating system. Thus, one drought tolerant variety BRRI dhan 56 and one drought susceptible check variety IR 64 were used in this experiment. Five promising commercial *Trichoderma* formulations of Bangladesh

including four powder and one liquid formulations viz. G-derma (GME Agro Ltd.), Bioderma (Ispahani Agro Ltd.), Recharge (Russel IPM Bangladesh), Decoprima (Mohsin Enterprise) and G-derma (LDS, liquid) (GME Agro Ltd.) were collected and tested for seed treatment of rice.

Bulk surface soil was used for the experiment. For soil sterilization, 2% formalin solution was prepared in a container and drenches the soil @ 4-5 liter water per square meter soil surface to saturate it up to a depth of 15-20 cm. Fertilizers were applied by following Fertilizer Recommendation Guide 2012. Rice seeds were surface-sterilized with Clorox® bleach (5.25% sodium hypochlorite as the active ingredient). Seeds were bio-primed separately with each formulations of *Trichoderma* @ 10 g or 5 ml / kg of seed. After 24 hours of seed treatment, seeds were placed for sowing. Seeds were sown in plastic pot (12 kg capacity) filled with 8.0 kg sterilized soil and saturated with water holding calibrated tensiometer. Twenty seeds were sown in each pot.

Ten plants per pot were maintained for each treatment combinations including control. Randomly three plants were selected from each pot for tagging and data collection. Mean values were determined to get rating score of each treatment.

The effect of seed treatment on seed germination percentage, speed of germination, mean germination time (MGT), mean daily germination (MDG), peak value (PV), germination value (GV), number of leaves, number of tiller, plant height, shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, seedling vigor index and seedling vegetative vigor (SVV) was observed. In drought stress condition, leaf droppy, leaf rolling, leaf drying, drought scale (leaf rolled and leaf drying at vegetative stage) was observed.

The experiment was laid out in Complete Randomized Design (CRD) with five replications. The collected data was statistically analyzed by Statistics 10 computer package program. Analysis of variance (ANOVA) was used to find out the variation of result from experimental treatments. Treatment means were compared by *Tukey's* honest significant difference test (HSD).

In this experiment, At 7DAS, 14DAS, 20DAS, In BRRI dhan 56, BRRI dhan 71 and IR 64, the highest seed germination was found in T_4. Speed of germination or germination index indicates the vigor of seed or seedlings. In three varieties, the highest germination index was observed in T_4 (Decoprima treated seeds) followed by T_1 (G-derma), T_2

(Bioderma). In BRRI dhan 56, the highest mean germination time was observed in T_2 followed by T_1, T_3. In BRRI dhan 71 and IR 64, the highest mean germination time was observed in T_1. In BRRI dhan 56, the highest mean daily germination was recorded (1.13) in T_4. In BRRI dhan 71 and IR 64, the highest mean daily germination was recorded in T_4.

Incase of PV and GV, In BRRI dhan 56 and IR 64, the highest peak value and germination value of seed germination was found in T_1 and BRRI dhan 71 was found highest value was T_4. Incase of number of leaves, at 14 DAS and 24 DAS, In BRRI dhan71, BRRI dhan 56 and IR 64, maximum leaves were observed in T_3, and T_4.

At 33 DAS, in three varieties, maximum number of tillers were observed in T_1. At 43 DAS, in three varieties, maximum number of tillers were observed in T_2, T_1,T_1 respectively. Incase of plant height, at 14 DAS, three rice varities shown the highest plant height was in T_4. At 24 DAS, in BRRI dhan 56, BRRI dhan71 and IR 64 the highest plant height was observed in Decoprima treated seed in T_4. At 34 DAS, in BRRI dhan 56, the highest plant height was observed in T_4 (56.05cm). In BRRI dhan 71 and IR 64, the highest plant height was observed in T_4 (45.1cm) and T_4 (44.9cm). At 51 DAS, in BRRI dhan 56, BRRI dhan 71 and IR 64, the highest plant height was observed in T_4 .

At 34 DAS, in three rice varieties, the highest shoot length were observed in T1, T4. At 51 DAS, in BRRI dhan 56, BRRI dhan 71 and IR 64, the highest shoot length were found in T_4. At 34 DAS, in BRRI dhan 56 BRRI dhan 71 and IR 64, the highest root length were showed in T_5. At 51 DAS, in three varieties, the highest shoot length were observed in T_4, T_3, T_1 respectively. At 34 DAS, in BRRI dhan 56, the highest fresh shoot wt. was recorded in T_1 but, in BRRI dhan 71 and IR 64, the highest fresh shoot wt. was recorded in Decoprima treated seed in T_4. At 51 DAS, in three rice varieties, the highest fresh shoot wt. was recorded in T_5 and T_3. At 34 DAS, in BRRI dhan 56, the highest fresh root wt. were recorded in T_1.However, in BRRI dhan 71 and IR 64, the highest fresh root wt. were found in T_4. At 51 DAS, in BRRI dhan 56 and BRRI dhan 71, the highest fresh root wt. were observed in T_4. But, in IR 64, the highest fresh root wt. was recorded in T_1 (0.28g).

Incase of dry shoot wt., at 34 DAS, in three rice varieties, the highest dry shoot weight was recorded in T_1, T_4, T_2. Incase of dry root wt, three rice varieties, the highest dry root weight were observed in Decoprima treated seed in T_4. At 51 DAS, in three rice varieties, the highest fresh shoot were found in T_4, T_3, T_5. Incase of dry root wt, in BRRI dhan 56, BRRI dhan 71 and IR 64, the highest dry root wt. were recorded in T_4. In BRRI dhan 56, BRRI dhan 71 and IR 64, the highest seedling vigor index were found in T_5, T_4, T_3. In BRRI dhan 56, BRRI dhan 71 and IR 64, the highest seedling vegetative vigor were observed in T_4.

Incase of drought stress, at 7DDS, In BRRI dhan 56, BRRI dhan 71 and IR 64, the lowest droppy leaf was observed in T_4. At 10DDS, in rice varieties showed in Decoprima treated seed. Incase of rolled leaf in drought stress, at 10 DDS and 13 DDS, in BRRI dhan 56, BRRI dhan 71 and IR 64, the lowest leaf rolling were observed in T_4.Incase of leaf drying,at 10 DDS and 13 DDS, in BRRI dhan 56, BRRI dhan 71 and IR 64, the highest leaf rolling at vegetative stage were observed in Decoprima treated seed in T_4. Incase of drought scale, at 7 DDS, 10 DDS, 13 DDS, in three rice varieties, the lowest leaf rolling at vegetative stage was found in Decoprima treated seed in T_4 that was statistically similar with T_5. At 10 DDS, 13 DDS, the lowest leaf drying at vegetative stage was showed in T_4.

Considering the over all findings, it is revealed that seed treatment by *Trichoderma* formulations Decoprima have the most significant effect to increase seed germination, seedling growth and to mitigate the effect of drought stress in rice. However, G-derma (Liquid) and Recharge also perform better compared to other treatments.

CHAPTER VI

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