TRICHODERMA HARZIANUM FORMULATIONS FOR ENHANCING ITS STORABILITY

THESIS

Submitted to
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
in partial fulfilment of the requirements
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MASTER OF SCIENCE
IN
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(PLANT PATHOLOGY)

By
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Enrolment Number – OO-1806 **2022**

DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the Thesis entitled **"TRICHODERMA HARZIANUM FORMULATIONS FOR ENHANCING ITS STORABILITY"** or part thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/ publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

Place: Nagpur **(Thakare Vitthal Laxman)**

Date: 18/10/2022 Enrolment No. OO/1806

CERTIFICATE

This is to certify that the thesis entitled **"TRICHODERMA HARZIANUM FORMULATIONS FOR ENHANCING ITS STORABILITY"** submitted in partial fulfilment of the requirement for the degree of **"Master of Science in Agriculture (Plant Pathology)"** of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by **THAKARE VITTHAL LAXMAN** under my guidance and supervision.

The subject of the thesis has been approved by the Student's Advisory Committee.

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Date: 18/10/2022

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%	Per cent
/	Per
C.D.	Critical difference
CFU	Colony forming unit
DAI	Days after inoculation
et al.	et alia (and associate)
etc.	et. Cetra
Fig.	Figure
i.e.	idest (that is)
ml	mililitre
mm	millimeter
^{o}C	Degree Celsius
PDA	Potato Dextrose Agar
PD	Potato Dextrose
S.E.± (m)	Standard error of mean
Spp / sp	species (Singular and pleura's)
Viz	Namely
@	At the rate
Sig	significant
T	Treatment

(E) THESIS ABSTRACT

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ABSTRACT

An investigation on "*Trichoderma harzianum* formulations for enhancing its storability" was undertaken with an objective to examine

the storability of *Trichoderma harzianum* in different formulations in CRD with six treatments in four replications. The experiment was conducted at Department of Plant Pathology, Section College of Agriculture Nagpur during the year 2021-2022.

Capsule, sachet, talc, vermicompost, liquid formulations are extensively used as carrier material for stored *T. harzianum*. The capsule formulation treatment recorded maximum population ($79.65x10^7$CFU/ml) in a I month and was superior to all other treatments. This was followed by sachet formulation treatment ($71x10^7$CFU/ml). An in uninoculated control treatment had the lowest spore count (6.45×10^7CFU/ml).

Capsule formulation treatment had 72.25, 62.05, 52.33, 44.03, and $37.05x10^7$CFU/ml in II, III, IV, V, and VI month. Next best treatment was sachet formulation recording 71.00, 63.45, 52.88, 48.49, 40.63, and $32.38x10^7$CFU/ml. in I, II, III, IV, V, and VI month. There was decline in population from I to VI month respectively.

The capsule formulation treatment considerably increased germination in green gram (93.50%), black gram (95.75%), pigeon pea (94.25%) and chickpea (92.25%) respectively. It was followed by sachet formulation treatment recording green gram (91.0%), black gram (92.0%), pigeon pea (91.25%) and chickpea (84.75%) germination.

All treatments showed different shoot and root lengths. Similarly, capsule formulation treatment significantly increased green gram shoot (22.6 cm/pl) and root length (12.75 cm/pl) with a vigour index of 3305.22. Black gram treatment recorded similarly showed higher shoots (21.5 cm/pl) and root length (14.0 cm/pl) and a higher vigour index (3186.1). In pigeon pea, identical treatment increased shoot and root length (17.0 cm/pl) and (11.0 cm/pl) with vigour index 2639. In chickpea, capsule formulation treatment enhanced shoot (17.0 cm/pl) and root length (16.0 cm/pl) with vigour index (3044.2). In green gram,

black gram, pigeon pea and chickpea, sachet formulation treatment recorded higher shoot and root length and seedling vigour index over other all treatments.

Chapter I

INTRODUCTION

1.1 Background Information

Plant diseases disrupt nearly 10% to 20% of worldwide food production each year, resulting in billions of dollars in losses. Plant diseases having impact on several important crops, including rice, wheat, barley, soybean and potato (Chakraborty *et al*.1999). In tropical, subtropical and temperate climates across the world, diseases caused by soil borne plant pathogens show a severe danger to agricultural production (Punja 1988). Chemical management of infections, especially sclerotial pathogens like *Sclerotium rolfsii, Sclerotinia sclerotium, Rhizoctonia solani* and others decreases diseases to some degree but is neither cost effective nor ecologically favorable. The use of biological control agents as a method of managing these infections is an alternative and effective method to the use of chemical control (Harman *et al*. 1994).

Biological control has been studied since the 1930s but it wasn't until the 1960s that it was put into reality, when the effectiveness of *Trichoderma* in pathogen control was also reported. *Trichoderma* is now a well-studied organism as a biocontrol agent all over the world. Their use has been particularly effective against soil-borne diseases as well as various foliar diseases via seed or soil treatment. They have also been used as a growth promoter and as enzyme source in industries (Bettiol 2011 and Kumar *et al*. 2014).

Trichoderma fungi are found all over the world and can be easily isolated from soil, decomposing wood and other kinds of plant organic matter. *Trichoderma* is a fungus genus that is regularly isolated from soil, practically all temperate and tropical soils contain 101-103 culturable propagules per gram. Although they don't have a recognized sexual stage, they were usually categorized as imperfect fungus. Fungi in this genus grow fast and generate a high number of spores (conidia)

in a variety of green colures. In buried mycelium, many species generate large quantities of thick walled spores (chlamydospores) and the reverse side of colonies is often uncolored, yellow, amber or yellow-green (Gams *et al.* 1998).

Trichoderma species were initially recognized as possible plant disease biocontrol agents in the early 1930s (Weindling 1932) and control of a broad spectrum of diseases. *Trichoderma* are non-pathogenic, free-living fungus that interact with roots, soil and plants (Verma *et al.* 2007). Antibiosis, competition, mycoparasitism and induction of resistance are only a few of the mechanisms available to this microorganism, ensuring its ability to control a variety of diseases. Growing roots, wounds and damaged cells tissue are all possible infection sites for *Trichoderma*. *Trichoderma* also promotes plant development by improving nutrient availability and the synthesis of growth hormones (Hjeljord and Tronsmo 1998, Wijesinghe *et al.* 2011).

Trichoderma is a very efficient biocontrol agent that has stimulated scientists' curiosity as a possible alternative to chemical fungicides for a variety of plant diseases. Due to their ability to increase plant growth and development, high reproductive capacity, ability to survive in harsh conditions, efficiency in nutrient utilization, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi and efficacy in plant promotion plant growth and defense mechanisms. *Trichoderma* spp. have long been recognized as plant disease control agents. They're becoming more popular in agriculture and the most effective strains have something known as 'rhizosphere competence' or the ability to colonize and develop in close proximity to plant roots. Due to these characteristics *Trichoderma* is a ubiquitous genus that may be found in any environment and at high population densities (Chet *et al*. 1997).

Trichoderma spp. have the potential to be more successful than fungicides in controlling soil-borne plant diseases. Chemical pesticides are more harmful to the environment than the usage of these

microorganisms. They may be found in practically all agricultural soils and other habitats such as decaying wood and their application as a plant disease management option is just now being widely recognized (Harman *et al*. 2004).

Trichoderma harzianum have emerged as a promising biological control agent for a variety of economically important plant pathogenic fungi. A requirement for biological control application is the development of a mass manufacturing, formulation and delivery method for a microbe that controls diseases in plants. It seems that using low-cost medium like molasses and brewer's yeast, industry may create a number of beneficial microorganisms including *T. harzianum* to fight plant infections (Papavizas *et al*. 1984). This may be inferred from the fact that a number of companies around the world are working on biopesticides based on microbes (Fravel 2005). The lack of understanding of procedures for large manufacture and proper delivery of biocontrol chemicals is the most serious impediment to biological control in the field (Papavizas 1985). Regardless of the organism utilized, preparing microbial biomass with high population counts, viability and vigor is a key condition for a successful bioagent application. The creation of biomass and preserving viability at the end of the process are both important factors in the formulation of biological control agents (Adekunle *et al.* 2001). When compared to other non-formulation or medium, adding chitin as a particular nutrient to the production medium or formulation may extend the shelf life of liquid fermentation produced *T. harzianum* in talc formulation by two months (Sriram *et al*. 2010)

Trichoderma may be used to manage the diseases including *Rhizoctonia solani, Pythium* spp. and *Fusarium* spp. in seed treatment, seedling growth substrates and soil (Hjeljord and Tronsmo 1998). Conidia are active components in almost all treatments for biological control based on *Trichoderma* spp. The physiological qualities of their microbial propagules, which have different characteristics in terms of

production, stability and activity may be related to this application. Although mycelium and chlamydospores have good biological control action, they do not survive the drying process. As a result, the conidia are less susceptible to environmental factors and are easier to manufacture, making the formulation steps easier (Amsellem *et al*. 1999, Whipps and Lumsden 2001 and Verma *et al.* 2005).

To manufacture carrier based or liquid based formulations, *Trichoderma* is prepared using a variety of techniques, including solid and liquid state fermentation. The shelf life of a biocontrol agent's designed product is critical to its marketing success at the PDBC in Bangalore, researchers worked on extending the shelf life of *Trichoderma* talc formulations by using various ingredients (chitin and glycerol) in the production medium and applying heat shock at the end of the log phase of fermentation, which can extend the shelf life of talc formulations by up to a year (Sriram *et al*. 2011). Under ambient conditions, (Bhat *et al*. 2009) observed a shelf life of up to 180 days for talc-based formulations. When compared to the rate of desiccation observed from 75 to 180 days of storage, the rate of desiccation was lower up to the first 75 days of storage. After more than 150 to 180 days of storage, the formulation still had a high number of viable propagules (over $106x10^{7}$CFU/g).

Though a range of laws, regulations, policies and programs the Indian government is aggressively encouraging bio-pesticide research, manufacturing, registration and adoption. Bio-pesticides were highly recommended in the National Farmer Policy (2007) for enhancing agricultural productivity while safeguarding farmers and the environment's health.

In order to reduce production costs, many components such as growth medium, carriers and *Trichoderma* must be studied while developing *Trichoderma* liquid inoculants. It is hard to underestimate the value of low-cost components in the production of culture media. Microorganisms included into the carrier material provide simple

handling, long-term storage and bio fertilizer efficacy. It is more beneficial to farmers.

1.2 Importance to study

Industrial and commercial development processes as well as field application, should be compatible with mass production systems. As a result, *T. harzianum* formulations need the identification of acceptable media as a result, the first stage in the formulation of any bio-control agent is to identify suitable substrates. Depending on the bio-control agent biomass needed for a certain purpose, the type and type of substrate (broth or solid) may also vary. The quality of the microbial bio-protectant is determined by the propagate density in the biomass and the capacity of the microbial bio-protectant to withstand processing (Harman *et al*. 1991).

A formulated product for agricultural use should be easy to make stable during transportation and storage include a large number of viable propagules with a long shelf life, be efficient and economical. As a consequence of significant research efforts in Israel, Sweden, the United States, India's and other countries, a variety of commercial *Trichoderma* products have arisen in the market (Whipps 1992). To produce the biocontrol agent, several studies have employed (Papavizas 1985), talc (Jayrajan *et al*.1994) and alginate pellets (Fravel *et al.* 1985). Some biopesticides and other beneficial organism-based formulations perform well in the lab but failed in the reality. Poor product stability prior to application, inadequate active material reaching the field target, desiccation death of the chemical used, contamination of the formulation and quick degradation of active material on the target are all factors for this. The formulation type is critical in aiding in the resolution of these challenges and in ensuring that a formulation is very effective even in the field.

A vital component of the biocontrol program is the development of sufficient amounts of high-quality inoculum. As a result, the present

research investigated the storability of *T. harzianum* in different formulations having variable quantities of *T. harzianum* in talc powder, vermicompost, capsule form, sachet form and in liquid form formulations.

1.3 Objectives

1. To examine the storability of *Trichoderma harzianum* in different formulations.

1.4 Hypothesis

Trichoderma is a promising antagonistic organism, which biologically control plant pathogenic fungus. Efforts are currently being made to increase awareness and application technology as well as the shelf life of various *Trichoderma* culture formulations. The shelf life of the various formulations has been tested up to six months from the date of manufacture at regular intervals of 30 days and the current study was done with this possibility in mind.

1.5 Scope and Limitation

One of the most interesting aspects of the science of biological control is the study of the mechanisms employed by biocontrol agents to effective disease control. Past research indicates that the mechanisms are many and varied even within the genus *Trichoderma*. In order to make the most effective use of biocontrol agents for the control of plant diseases, we must understand how the agents work and what their limitations are. We can develop effective means of culturing, storing, applying and utilizing biocontrol agents so that we harness their best efforts for disease control and viability for longer duration.

Mass production is a broad aspect of successful biological control techniques that includes the development of products,

formulation and delivery systems of fungi used as an effective disease control (Seema Solanki *et al.* 2016).

However, some limitations in their use include carrier material having a shelf life of up to 4 to 5 months and contamination occurring during the delivery method. The main obstacles to the development of biopesticide products based of *Trichoderma* are (a) Most of the products used as biological control agents are not recorded and are simply being marketed as products for soil improvement (b) Low cost of raw materials used in the formulation of culture media for production of fungi (c) Low efficacy and low yield of process (d) Difficulties in quantifying the activity of biological control agent (e) Few researches for development of formulations that ensure the maintenance of the activity of the biological agent (Whipps and Lumsden 2001 and Verma *et al.* 2007).

Chapter II

REVIEW OF LITERATURE

Studies on solid and liquid formulation entitled "*Trichoderma harzianum* formulations for enhancing its storability**"** was conducted in Plant Pathology Laboratory, College of Agriculture, Nagpur during the year 2021-2022. Attempt has been made to review literature on use of different carrier material such as sorghum grains, talc powder, vermicompost, gelatinous capsule, sachet, packing broth and effect on shelf life of *T. harzianum.*

2.1 Storability of *Trichoderma harzianum* in different formulation

(A) Talc powder formulation

Nakkeeran *et al.* (1997) studied the shelf life of *Trichoderma* formulations in vermiculite bran acid fermenter biomass (VBA-FB) of *T. viride* recorded the highest mean population in milky white bag (205x10^6CFU/g). The population of antagonist was in its exponential phase (309x10^6CFU/g) till 30 days after storage. Irrespective of the bag colour, the steady increase in the population level was seen after 30 days of storage. Studies on storage temperatures revealed that 20-30^0C was optimum to store VBA-FB formulation in milky white bags. At this range of temperature, even after 75 days, the product contained (206-271x10^6CFU/g). In talc-based formulation there was a slow decline in the population of *Trichoderma* due to storage. The mean population of (230-242x10^6CFU/g) was observed in talc-based formulation at 20-30^0C after 75 days of storage. This indicates that the product could be safely stored as talc-based formulations in milky-white bags at room temperature (20-30^0C) for up to 75 days.

Karunanithi *et al*. (2001) noticed that talc and gypsum were significantly superior in preserving *T. viride* survival even after 150 days of storage. Gypsum is 30 times less expensive than talc powder.

Bhat *et al*. (2009) concluded that *T. harzianum* a potential biocontrol agent and was multiplied by liquid fermentation method and formulated in talc. The talc based formulation was tested for shelf life up to 180 days under ambient condition. The rate of desiccation was less up to the first 75 days of storage as compared to that observed from 75 to 180 days of storage. The formulation retained good numbers of viable propagules (above 10^6CFU/g) for more than 150 days of storage.

Krishna *et al*. (2013) worked among the 14 isolates of *Trichoderma* spp*.* NT2, NT4, NT6 are very fast growing while NT5, NT7, NT9, CT13 are moderately growing NT2, NT6 and NT4 shown maximum inhibition of 81.57%, 78.68% and 75.10% against the test pathogen *Fusarium solani*. While least inhibition (56.97%) was observed in CT14, they observe shelf life period was high in talc based formulation.

Kumar *et al*. (2013) studied the shelf life of *T. viride* in talc and charcoal based formulation. Potato dextrose broth talc based formulation was prepared by adding 3 different volumes of biomass of *T. viride* along with medium @ 30, 40 and 50 ml/g talc on initial mean CFU of *T. vinde* on 0 day was ($227.2x10^9$), ($256x10^9$) and ($291.03x10^9$ CFU/g.) respectively. It is gradually declined and at 120 days of storage the population came down to ($70.33x10^9$), ($80.67x10^9$) and ($96.67x10^9$CFU/g) which in terms of reduction in viability were 69.04, 68.48 and 66.78% respectively. Similar trends of results were recorded in all the three sorghum grains charcoal based formulations almost at par with each other ranging from 71.14 to 72.44% in 120 days. Sufficient numbers of spores were viable even after 120 days of storage in the formulation indicating that the formulation can be stored further for more time.

Patel and Patel (2014) prepared the talc based powder of bioagent (*T. viride* (FGCC#2437) (spore mycelium) 1.0% w/w + talc 98.5% w/w+0.5% carboxyl methylcellulose) and used for shelf life and

bio-efficacy studies. The talc based bio-formulation was stored in LDPE pouches. The powder was dull white in colour, pH 7.0, moisture 8% and CFU/g of ($29.7x10^6$). It was found that the bio-formulation has good shelf life up to six months and then the spores started declining. Talc based bio-formulation was found to be the best material to retain maximum number of viable propagules i.e., (29.7×10^6CFU/g) at 180 days of storage.

Roy and Bhattacharya (2016) noticed that the shelf life of carrier (talc and activated charcoal) and pellet (calcium chloride and calcium gluconate gelled) based formulation of *T. harzianum* under varying storage environment was investigated. Irrespective of formulation type, storage under cool temperature was always far advantageous to maintain satisfactory population level in time scale. The results revealed that activated charcoal and talc based formulation must be stored in cool temperature for maintaining desired population, whereas, pellet based formulations can be stored either in cool or room temperature without any significant difference up to 105 days after preparation.

Manandhra *et al.* (2018) studied shelf life of *T. viride* based on various formulations and to know the effect of temperature on its viability. It was found that liquid-based formulation and organic formulation had greater shelf life compared to talc-based powder formulation. Also, low temperature (4ºC) significantly increased the shelf life of the product (for over a year) while at ambient temperature conditions (15-25ºC), optimum viability of the biocontrol agent could be achieved only for a maximum of 3 months.

Mawar and Mathur (2022) studied those certain agro wastes in various mixes improved *T. harzianum's* shelf life to 120 days. *T. harzianum* population variations were observed in five composts for 80 days at 20-day intervals. *Prosopis juliflora* compost had the most *T. harzianum*. Standardized *P. juliflora* compost proportions were then changed using Indore composting. With a second experiment, the most

promising combination of *P. juliflora* compost mixed in talc and different food substrates was examined for its effectiveness in enhancing *T. harzianum* survival. *T. harzianum* was ten times more prevalent in unsterilized *P. juliflora* compost, sawdust and talc than in other substrates. A mixture of agro wastes (*P. juliflora* compost + wood saw dust) and inert material promotes *T. harzianum* survival and reproduction for 105 days. *P. juliflora* grows extensively in dry Indian regions and its compost reduces charcoal rot caused by *Macrophomina phasolina* in rain-fed crops.

Patil and Raja (2022) studied the six different substrates (talcum powder, lignite, charcoal, sawdust, compost and fly ash) were used to test the shelf life of the most virulent isolates of *T. harzianum* (AkTr2GM5) and *T. hamatum* (AkTm1GM5) mutant's up to 180 days. Maximum CFU/g ($154.50x10^6$) and ($45.50x10^6$) were obtained at 30 and 180 days after storage in the *T. harzianum* talc based formulation respectively and the lowest ($16.25x10^6$) in fly ash formulation. Furthermore, *T. hamatum* talc based formulations exhibit maximum CFU/g values of ($156.75x10^6$) and ($41.50x10^6$) at 30 and 180 days after storage, respectively.

(B) Vermicompost

Pan and Das (2010) studied shelf life of (Th AN) was during 2007-08 in some organic formulations like vermicompost, leaf manure, rice bran and FYM and also their combinations with two oil cakes, neem and mustard cake. The growth was estimated in terms of colony forming unit (CFU/g) of substrate up to 120 days at 30 days interval. In all organic products or their amendment with oil cakes CFU. increased up to 60 days and then gradually declined. Among the four organic media, vermicompost retained ($21.3x10^7$CFU/g) at 120 days where as leaf mould, FYM and rice bran retained ($20.1x10^7$CFU/g), ($17.3x10^7$CFU/g) and ($15.1x10^7$CFU/g) of substrate respectively at 120 days of incubation. When oilcake was amended with organic material,

vermicompost + mustard cake produced high population of the antagonist up to 60 days.

Khan *et al*. (2011) indicated that, the vermicompost, de-oiled castor cake and farm yard manure formulation supported the growth of *T. viride* during storage, which is a major advantage for the marketing of these biocontrol agents. Vermiculite based fermenter biomass of formulation with an initial population of (205x10⁶CFU/g) stored in milky white bags showed an exponential phase up to 30 days (309x10⁶CFU/g). Temperature of 20-30°C was optimum for the storage of the formulation at which even after 75 days, the product contained (206-271x10⁶CFU/g). A fermenter biomass in vermicompost formulation had longer growth and survival rate as compared to other formulations. Growth of *T. viride* was continued in vermicompost formulation up to 195 and retained good viability for 255 days. Thereafter, a reduction in colony forming unit (CFU) was recorded.

Zapiola *et al.* (2012) studied that formulation and shelf-life of microbial biomass represents a critical step in advancing the commercial development of biological control products. *T. harzianum* has demonstrated biological control capability against *Rhizoctonia solani* causing root diseases. In the present studies, the survival of two native isolates of *T*. *harzianum* (T_1 and T_6) included in solid and liquid based formulations and on radish coated seeds were examined. Peat and vermiculite were used as carriers in solid based formulations. T_1 propagules in peat and vermiculite carriers increased more than 100-fold in the first 30 days of room temperature storage and held constant until 180 days, when 9.107 propagules per grams were collected. When using a peat based formulation, T_1 propagules survived well on coated radish seeds without a drop in inoculum concentration. In liquid formulations, T_6 viable conidia decreased with time. In dual cultures, samples from liquid formulations taken at different fermentation periods and held for 25 days were effective against *R. solani*, *T. harzianum* propagules can develop and persist for six months in solid

formulations. Even though liquid formulations have less viable conidia, manufacture was straightforward.

Bobilina *et al*. (2019) evaluated of eighteen solid substrates. Colony forming units were counted following the serial dilution technique and a gradual decrease was observed over a period of six months. Among different solid substrates tested, vermicompost was observed to be the best solid substrate for isolate 2 recording the maximum CFU count (463.67x10^7CFU/g) of substrate in the first month after inoculation and it retained spore viability even after six months of inoculation giving a fairly good CFU count of (97.33x10^7CFU/g). For isolate 5, groundnut shell performed the best, recording the maximum CFU count of (467.67x10^7CFU/g) in the first month and (144.00x10^7CFU/g) was retained even in the sixth month after inoculation.

(C) Capsule formulation

Shui (2012) reported that when pure dry spores were filled in capsule the population were decline slowly because of capsule forms sub environment to the *T. harzianum* hence they do not affect by external environment.

Sanghmitra *et al*. (2014) conducted an experiment to evaluate the viability in different formulations of *T. viride*. Seven different carrier base formulations used *viz*., chalk, talc, charcoal powder, chalk tablet, talc tablet and charcoal tablet with 3 concentrations 5%, 10% and 15%. One capsule formulation of *T. viride* along with one culture of *T. viride* were evaluated for different period and observation taken for their viability. Maximum CFU was recorded in 15% charcoal powder (80 x10^7 CFU/g) followed by capsule (78.67x10^7CFU/g) at 260 days storage, however least recorded in 5% talc powder (1x10^7CFU/g).

Cumagun (2014) studied on *Trichoderma* in managing plant diseases which depends on its effective propagules and biocontrol mechanisms, as well as its formulation and delivery systems in relation

to the crop, disease, and environment. Solid and liquid formulations are common. Microencapsulation extends *Trichoderma's* shelf life and application efficiency.

Singh *et al.* (2014) used locally available six organic substrates (Spent mushroom compost (SMC), farm yard manure (FYM), vermicompost (VC), sorghum grain (SG), wheat grain (WG) and broken maize grain BMG) were tested for their efficiency in controlling *Rhizoctonia solani* producing collar rot disease of cowpea. *T. harzianum* T_5 population in waste mushroom compost (SMC) was greater than any other organic substrate ($15x10^7$CFU/g) up to 240 DAI of these six organic substrate based *T. harzianum* T_5 + *R. solani* treatments, SMC based *T. harzianum* T_5 + *R. solani* treatment was found superior to there in reduction of seedlings mortality, number of leaves, shoot and root length, shoot and root dry weight and total plant dry weight of cowpea seedlings except vigour index, whereas, wheat grain (WG) based *T. harzianum* T_5 + *R. solani* treatment was observed inferior.

Kose and Totawar (2017) prepared four types of *T. viride* tablets *viz*., talc, charcoal, lignite, fly ash and also tried different six types of capsules of *T. viride viz*., talc, gelatin, alginate, alginate + charcoal, gelatin + flyash and frozen mass culture along with medium disc capsules and compared with sorghum grain-based carrier as control. They revealed that maximum CFU/g ($76.33x10^7$) was obtained in *Trichoderma* treatment of frozen culture along with disc capsule even after 180 days of storage followed by sorghum grains ($75.67x\ 10^7$).

Locatelli *et al*. (2017) studied that the *Trichoderma* as a biological control agent since the 1960s, but large-scale production and commercialization remain difficult. Lack of suitable formulations that ensure the viability and efficiency of biological control agents hinders the use of bio-pesticides in conventional agriculture. This study evaluates the viability of *Trichoderma* spp. conidia in encapsulated granules (CG). Fractional factorial design 25-1 was used to optimise

formulation conditions for encapsulated granules containing sodium alginate matrix modified with different polymers. FTIR and thermogravimetric analysis of encapsulated granules showed interaction between alginate matrix and polymers. Which kept *Trichoderma* alive during production and storage. After 14 months, 28^{o}C samples showed viable cell concentrations above 10^{6}CFU/g, a very relevant result compared to other authors.

Komala *et al*. (2019) concluded that *Trichoderma* is used as a bio-agent and have been reported to be quite effective, inexpensive and eco- friendly. The major hurdle in the use of bio-pesticides is their shelf life and inconsistent performance. From the study it was found that all the prepared formulations retained optimum viability. Capsule and sachet based formulations gave higher shelf life of *Trichoderma* as compared to wettable formulations. Application of capsule and sachet based formulations would be more convenient for application, storage and handling to control diseases in field and orchards which in turn would help the farmers to achieve a better crop yield.

Maria *et al*. (2019) studied to preserve *T. harzianum* conidia at room temperature and amplify immobilised conidia in submerged culture. The strain was immobilised in sodium alginate capsules and propagated in a column bubble reactor (green capsules). Micro, medium and large capsules were tested using emulsion internal gelation and dripping. Immobilized conidia propagation in submerged culture for free conidia production, immobilised conidia viability over time (two years), resistance of encapsulated conidia to short and long wavelength UV irradiation and antagonistic effect of encapsulated *T. harzianum* against four phytopathogenic fungi were tested. Medium capsules (1.5 0.3 mm) favoured massive production of released conidia in submerged culture, and higher conidia density capsule^{-1} increased UV protection. Regarding the conidia preservation in calcium alginate, a 30% viability loss was observed two years after storage at environmental temperature in both white and green capsules along

those two years, the formulation's purity was confirmed. The results show that green and white capsules preserve *T. harzianum* well at room temperature.

Maruyama *et al.* (2020) studied *T. harzianum* is a bio stimulant and phytopathogen control agent. Microencapsulation has been employed to maximise its efficiency against biotic and abiotic influences. The goal was to produce polymeric microparticles to encapsulate *T. harzianum* and analyse its stability, soil microbiota, antifungal activity *in vitro* and enzymatic activity. Wet and dry microparticles were 2000 and 800m. Scanning electron microscopy demonstrated spherical shape and encapsulation of *T. harzianum*. Assays indicated encapsulation protected the fungus from UV light. Encapsulated *T. harzianum* improved chitinolytic and cellulosic activities. Encapsulated fungi were able to inhibit *S. sclerotiorum in vitro*.

Adzmi *et al.* (2021) studied that the commercialization and mass production of biocontrol agents like *T. harzianum* products remain a challenge particularly in field. Use of encapsulation is a complicated method for developing *T. harzianum* encapsulated formulations store better at 28°C than 5°C. *T. harzianum* encapsulated formulations sustained vitality at 6.59 0.12 log CFU g^{-1} for up to seven months.

Tayde *et al.* (2021) evaluated eleven formulations of *T. asperellum* in present investigation, among these four types of tablets formulation *viz*., talc tablets, charcoal tablets, lignite tablets, fly ash tablets, six capsules formulations *viz*., talc capsule, alginate capsules, gelatin capsules, alginate + charcoal capsules, gelatin + fly ash capsules and *T. asperellum* frozen culture mass along with medium disc capsules and one sorghum grains formulation as a control were used and observation on shelf life up to 270 days from date of manufacturing at regular intervals of 30 days were taken. Maximum CFU/g ($42.00x10^7$) was obtained in *Trichoderma* treatment frozen culture mass along with medium disc capsule and least CFU/g was

recorded in *T. asperellum* lignite tablet (11.33×10^7CFU/g) up to 270 days of storage. In the present study tablet and capsule delivery system of various formulations was tried by using jaggery treatment. Maximum population of *T. asperellum* obtained (45.00×10^7CFU/ml) in *T. asperellum* frozen culture mass along with medium disc capsule and minimum were obtained in *T. asperellum* lignite tablets (8.33×10^7CFU/ml).

(D) Storability of *Trichoderma harzianum* spore in sachet

Jagtap and Bhatnagar (2000) studied on the shelf life of *Trichoderma* chlamydospores based formulated product Tricoguard™ have shown that the product can be safely stored for 270 days.

Rini and Sulochana (2007) evaluated substrates for mass production of *T. harzianum* and *T. viride*. Pre-boiled sorghum grains, coir pith + neem cake (1:1), cow dung + neem cake (1:1) + wheat flour (10%) maintained high populations of *T. harzianum* and *T. viride* within 10 days of inoculation. Jaggery and wheat flour served as nutritional supplements and enhanced the conidial yield from ($23.66x10^8$ to $34x10^8$ and $45.6x10^8$) colony forming units g^{-1} respectively. An increase in the number of viable propagules up to 30 days was noted regardless of the substrates and its moisture levels. Although highest initial population of *Trichoderma* spp. was observed in sorghum grains, propagule viability was low in that compared to other substrates. Coir pith + neem cake (1:1) at 35% and 45% moisture gave longer shelf life for *Trichoderma* propagules.

Rajput *et al*. (2014) evaluated that *T. harzianum* was mass multiplied on rice grains, sorghum grains, wheat grains, millet grains, wheat straw, rice husk, cow dung, sawdust and chicken manure, best substrates were sorghum and millet. Poultry dung was the least acceptable substrate, but rice, wheat, wheat straw and rice husk did well. Sucrose grew *T. harzianum* colonies best on Czapek's Agar. Ammonium nitrate at 3000 ppm caused the largest colony development

and conidia formulation. Sucrose at 30,000 ppm as a carbon source and ammonium nitrate at 3000 ppm as a nitrogen source greatly increased *T. harzianum's* mycelial growth and conidial production in wheat straw, rice husk, and millet grains, but not in sorghum grains and rice grains. Studies on the shelf life of inoculum multiplied on different substrates indicated that *T. harzianum* populations on all substrates peaked at 60-75 days and dropped progressively thereafter. Even after 330 days, populations were bigger than 0-day at 345-360 days, population was less than 0-day.

Rajput and Shahzad (2015) studied using nine different organic substrates *viz*., sorghum, rice grains, cow dung etc. for mass multiplication *T. polysporum*. In this sorghum grains were found the best substrate for *T. polysporum*. Sucrose @ 30,000 ppm and ammonium nitrate @ 3000 ppm were found to be best carbon and nitrogen sources for growth and sporulation of *T. polysporum*. During studies shelf life is peck at 60-135 days intervals on different substrate and declined gradually shelf life on C+N amended wheat straw and rice husk were more as compared to unamended substrates.

Komala *et al.* (2019) concluded that *Trichoderma* is used as a bio-agent and have been reported to be quite effective, inexpensive and eco- friendly. The major hurdle in the use of bio-pesticides is their shelf life and inconsistent performance. From the study it was found that all the prepared formulations retained optimum viability. Capsule and sachet based formulations gave higher shelf life of *Trichoderma* as compared to wettable formulations. Application of capsule and sachet based formulations would be more convenient for application, storage and handling to control diseases in field and orchards which in turn would help the farmers to achieve a better crop yield.

(E) Liquid formulation

Kolmbet *et al.* (2008) conducted the experiment in which *T. asperellum* was grown in liquid media of differing composition. The

effect of various amendments on the preservation of the viability and competitiveness, *in vitro* of fungal mycelium and spores in a liquid paste was determined. The amendments with greatest effect were the addition of starch as a food base, reduction of metabolic activity by lowering the pH of the biomass paste and the addition of small amounts of copper. Oxygen availability was also shown to be important in maintaining biomass viability and competitiveness. Optimization of these factors produces a biomass paste formulation of *T. asperellum* that remains.

Sriram *et al.* (2011) concluded that *T. harzianum*'s shelf life was affected by adding osmoticant glycerol to the manufacturing medium. Adding 3, 6 or 9 percent glycerol lowered water activity. In shaker culture and fermenter glycerol in production medium extended talc formulation's shelf life. The addition of 3 and 6 percent glycerol improved shelf life (with >2 10^6 CFU g^{-1}) to 7 and 12 months, respectively, compared to 4 to 5 months without glycerol.

Bhai and Anandraj (2014) carried out the experiment on enhancing shelf life of *T. harzianum* by conidial storage in sterile de-ionized water. Results showed that submerged conidia formulation in cryoprotectants like glycerol, glucose and DMSO preserved the viability for 75 days only when compared to the formulation in sterile de-ionized water which at 28-32 CFU/ml retained the viability for 480 days with a viable count of loge= 6.671 (3.2×10^6) and for more than 720 days with viable count of loge = 5.54 with a viable count of loge = 5.40 (2×10^5) for more than 720 days. The viability was tested *in vitro* by recovery in TSM and effectiveness was tested *in vivo* by drenching the suspension on plants and challenge inoculating with *Phytophtora capsici* after seven days. Challenge inoculation gave a disease reduction of 66.67%. Hence, sterile *T. harzianum* culture without losing the viability and effectiveness of the spore were noticed.

Emerson and Mikunthan (2015) carried out an experiment on screened liquid wastes and other liquid media as *T. viride* substrates.

Black gram soaked water, coconut water, rice mill effluent from the red pericarp variety, 5% distillery spent wash and various liquid substrates such as 1% palmyrah jaggery solution, 5% palmyrah toddy and 1% palmyrah fruit pulp extract, 10% cow urine, 10% *Gliricidia sepium* and 10%. *Spesia populnea* extracts were studied. Black gram soaked water had the highest growth and sporulation of *T. viride* ($35.9x10^7$CFU/ml) followed by 1% jaggery solution, coconut water, rice mill effluent, and palmyrah fruit pulp extract. Locally accessible liquid substrates are a possible source for *T. viride* liquid fermentation. *T. harzianum* sporulates in wheat straw, rice husk and millet grains, but not in sorghum or rice. Studies on the shelf life of inoculate multiplied on different substrates indicated that *T. harzianum* populations on all substrates peaked at 60-75 days and dropped progressively thereafter. Even after 330 days, populations were bigger than 0-day, at 345-360 days, population was less than 0-day.

Nadare *et al.* (2018) studied shelf life studies of *T. viride* by using different carriers *viz*. paraffin oil, mustard oil, diesel, soybean oil, sunflower oil, talc powder mixed with broth 40 ml + 5 ml dispersant + 3 ml suspender + 8 ml surfactant in each oil. Shelf life of formulations was tested up to 6 months at room temperature (15-35^{0}C). Among the different liquid formulations in treatment T_2 i.e., paraffin oil retained maximum viability (31.2×10^5CFU/ml) followed by soybean oil (21.5×10^5CFU/ml) and groundnut oil (19×10^5CFU/ml). Based on present investigation it can be inferred that application of paraffin oil increase the shelf life of *Trichoderma.*

Taral *et al.* (2018) evaluated different oils and studied the shelf life of *T. viride* during 2017-2018.by using different oils *viz*., paraffin oil, soybean oil, mineral oil, Potato Dextrose broth and talc was extensively used as carrier for *T. viride*. As compared to solid based carrier material the survival of *T. viride* in liquid based formulations is quite high and has the ability to limit the heat transfer high water holding capacity and maintain water around the cells for their metabolism.

Shelf life studies clearly indicated significant differences at all the intervals. Population density of *T. viride* in the paraffin oil was ($28.06x10^8$CFU/ml) in the 1st month whereas the population density of *T. viride* in the 6th month was ($18.33x10^8$CFU/ml).

Reddy *et al.* (2017) studied the formulation in liquid form which has higher shelf life and stress protection. Paraffin oil, soybean oil, combination of paraffin oil and glycerol (1:1), paraffin oil and soybean oil (1:1), soya bean oil and glycerol (1:1) were used. Absence of colonies of *T. harzianum* was observed in soybean oil and glycerol (1:1) at 42nd day, whereas on 49th day *Trichoderma* showed nil growth in paraffin oil and glycerol (1:1). On 56th day paraffin oil and soybean oil showed nil growth of *Trichoderma*. Paraffin oil showed higher activity of *T. harzianum* with ($20x10^7$CFU/ml) followed by soybean oil with ($2.1x10^6$CFU/ml) at 56th day.

Mane *et al*. (2019) studied the shelf life of *T. asperellum* during 2018-2019. It was revealed from shelf life studies that there were significant differences at all the intervals over uninoculated control. Maximum CFU count was recorded with the treatment Paraffin oil ($28x10^8$CFU) at 30 DAS and it was significantly superior over all other treatment except talc based culture ($27.33x10^8$CFU) and was gradually increased up to 90 DAS and thereafter found to be declined from 120 to 180 DAI in all the treatments.

2.2 Effect of *Trichoderma harzianum* on the germination of green gram

Mohamaad *et al*. (2014) studied the shelf life of *T. harzianum* in prepared bioformulation and it concluded that the number of propagules start decline at 30th day onwards when bioformulation is prepared in talc as carrier material. It was also founded that the seed treatment with bioformulation of *T. harzianum* (Th Azad) (5 gm/kg seed) observation that formulation increases germination percentage, root length and shoot length of green gram over untreated once.

Deshmukh *et al*. (2016) evaluated the efficacy of seed biopriming on the seed germination and seedling vigour by controlling the seed mycoflora by paper towel method in lab condition. Results revealed that average per cent seed germination, plumule length, radical length, seedling fresh, dry weight and seedling vigour index were significantly increased in seed biopriming with *T*. *harzianum*, *T*. *viride* or *P*. aeruginosa @10gm talc base formulation/kg seeds followed by all the bioagents used over control.

Nadhina *et al.* (2016) studied the growth promoting activity of *Trichoderma* isolates on green gram seeds and tested under glasshouse conditions and seed germination percentage, root and shoot length, fresh weight, dry weight and vigour index were measured. All the tested *Trichoderma* isolates showed significant positive effects on vigour index in comparison with the control. Results from this study explored the plant growth promoting activity of *Trichoderma* spp. and suggests its use as a potential multifunctional bio-fertilizer.

Sharma and Boraha (2020) observed that seeds treated with combined inoculation of *Rhizobium* @ 4 gm + *Bacillus megaterium* @ 5 ml/1000 ml of water + *T. harzianum* @ 5ml/1000ml of water + *T. viride* @ 5 ml/1000 ml of water (T_6) recorded significantly higher field emergence (91.25%), speed of emergence (42.14), seedling dry weight (1.67 mg), shoot length (25.48 cm), seed yield (992 kg/ha), stover yield (1870 kg/ha number of pods plant^{-1} (37), number of seeds pod^{-1} (13.25), 100 seed weight (3.63gm) root length (9.22 cm) and nodulation (15).

2.3 Effect of *Trichoderma harzianum* on the germination of black gram

Surekha *et al.* (2013) studied were designed to evaluated the potential of *Trichoderma* spp. spore suspension as biocontrol agent against *Fusarium oxysporum* and *Alternaria alternate* in black gram (*Vigna mungo*) under greenhouse conditions. Seeds and seven days

old plants of black gram were artificially infested with *F. oxysporum* and *A. alternata*. Concurrently, they were treated with *Trichoderma* spp. spores. It was observed that the seed germination (%), growth (shoot and root), vigour index and disease resistance in plant samples treated with *Trichoderma* spp. increased than in controls.

Mohamaad *et al*. (2014) studied the shelf life of *T. harzianum* in prepared bioformulation and it concluded that the number of propagules start decline at 30th day onwards when bioformulation is prepared in talc as carrier material. It was also founded that the seed treatment with bioformulation of *T. harzianum* (Th Azad) (5 gm/kg seed) observation that formulation increases germination percentage, root length and shoot length of black gram over untreated once.

Athira (2017) studied two bio-control agents, *Trichoderma* spp. and *Pseudomonas fluorescens* and assessed for their ability to reduce the growth of *M*. phaseolina under laboratory conditions. It was found that among the antagonists tested, *Trichoderma* exhibited strong inhibition (77.77%) against *M. phaseolina*. Their culture filtrates were also found to be effective in promoting the *in vitro* growth. The maximum seed germination (97%), shoot length (22.2 cm) and root length (16.5 cm) was recorded in the seed treatment of *Trichoderma* followed by *Pseudomonas* in the decreasing merit.

Balkrishna *et al*. (2017) presently studied the efficacy of formulation of *T. harzianum* suitable for application through seed treatment. Paste formulation containing starch 10 per cent + copper sulphate 20 ppm at 4 pH was tested *in vitro* for their effect on the mycelial growth of 9 important pathogens namely *Rhizoctonia solani, Macrophomina phaseolina, Pythium aphanidermatum, Colletotrichum lindemuthianum, Phytophthora infestans, Botrydiplodia theobromae, Mycosphaerella musicola, Fusarium oxysporum f.* spp. *lycopersici* and *Sclerotium rolfsii* was studied which showed that the paste formulation inhibited the mycelial growth of all the 9 pathogens tested at all five concentration. The higher concentration of paste formulation had more

inhibitory effect than lower concentration against all the pathogen. The paste formulation of *T. harzianum* has significantly increased seed germination, shoot length, root length, seedling mean, dry matter production and vigour index in black gram, chilli, cotton, sunflower, and tomato. Seed treatment @ 5g/kg of seed has recorded significantly higher colonization of seed surface in black gram, chilli, cotton and tomato and in the case of sunflower, seed treatment @ 3g/kg of seed has recorded the highest colonization.

2.4 Effect of *Trichoderma harzianum* on the germination of pigeon pea

Rajkondra and Bhale (2011) used *Trichoderma* spp. as biocontrol agents for management of soil borne diseases of crop plants. The seed of *Cajanas cajan* L. (Pigeon pea) *Phaseolus acontifolious Jacq*. (Moth) and *Cicer aurietienum* L. (Gram) were treated with *T. viride, T. harzianum, T. koningii, T. psuedokoinigi and T. viriens. Trichoderma* induced seed germination, biomass production and vigour index of pulses crops. Among the *Trichoderma* spp. Tested *T*. *harzianum* showed the maximum effect on seed germination and Vigour index of pigeon pea and in gram was more pronounced.

Mohamaad *et al.* (2014) studied the shelf life of *T. harzianum* in prepared bioformulation for 180 days and it concluded that the number of propagules start decline at 30th day onwards when bioformulation is prepared in talc as carrier material. It was also founded that the seed treatment with bioformulation of *T. harzianum* (*Th Azad*) (5gm/kg seed) notice that increases in germination percentage root length and shoot length of legumes over untreated once.

Chaudhari *et al*. (2017) studied on mycoflora of pigeon pea (*Cajanus cajan* L.) seeds and subsequently determine their effect on seed germination and seedling growth in pot condition. Seed sample were examined in agar plate and blotter method and found that effect of different chemical fungicides, bio-agents and phyto-extracts on seed

mycoflora, germination and vigour index of pigeon pea was evaluated. The seed treatments improved seed germination, vigour index and reducing seed borne mycoflora of pigeon pea seeds. Different fungicides, bio-agents and phyto-extracts were evaluated as seed treatment. In fungicides, Metalaxyl 8% + Mancozeb 64% @ 0.2% was found superior in seed germination (93.33%), shoot length (11.23 cm), root length (13.67 cm) and vigour index (2323.73) whereas, in bio-agents *Trichoderma* spp. was found most effective in seed germination (88.00%), shoot length (9.03 cm), root length (11.17 cm) and vigour index (1777.46) seed treatment with phytoextracts Neem seed extract gave more seed germination (78.67%), shoot length (7.97 cm), root length (9.20cm) and vigour index (1350.00) compared to control.

2.5 Effect of *Trichoderma harzianum* on the germination of chickpea

Kumar *et al*. (2014) concluded on *Trichoderma* strains *T. harzianum* (*Th. viride* (01PP) on chickpea genotype (Radhey) germination, seedling establishment, seedling dry weight and vigour. All seven seed quality criteria responded differently to presowing seed treatments. Chickpea seeds were treated with 5%, 10%, and 20% *Trichoderma* bioformulation, then 0.2% Bavistin. *T. harzianum* had a higher germination rate. *T. harzianum* (*Th*. *azad*) and *T. viride* (01PP) bioformulation at 5%. Seed germination (92% and 90%), root length (12.38 and 12.19 cm), shoot length (4.97 and 4.32 cm), seedling length (17.38 and 16.50 cm), dry weight (1.19 and 1.88 cm), vigour index I (5197.12 and 1485) and vigour index II (109.48 and 169.20). Control performed worst for all seven seed quality attributes.

Maurya *et al*. (2016) studied *in vitro* effect on chickpea seedling induced by biopriming of *T. viride* i.e., the treatment [T. sub1] (*T. viride* 5g/kg seed) was excelled overall significant superior performance by contributing 68%, 12.07 cm, 16.37 cm, 28.44 cm, 0.592 gm, 1933.92 and 40.26 in germination, root length, shoot length, seedling length, dry weight, vigour index I and vigour index II respectively.

Rai and Tewari (2016) tested nine *Trichoderma* formulations i.e., TF.WP1, TF.WP2, TF.WP3, TF.LQ4, TF.LQ5, TF.LQ6, TF.LQ7, TF. Paste 8, TF. Paste 9 were tested for their growth promotion effect (*In vitro and In vivo*) and against chickpea mortality under glasshouse conditions. These formulations were applied as seed treatment @ 5 gm or ml/kg seed. *In-vitro* maximum seedling vigour index (4836) was recorded in TF.WP2 followed by TF.WP1 (4483) and TF.WP3 (4330) while under glasshouse conditions, maximum plant vigour index (6008) was observed in TF.WP2 followed by TF.WP3 (5641) and TF.WP1 (5460). Significant lowest seed and plant mortality (6.67 & 10.74%) was observed TF.WP2 followed by TF.WP1 (10.00 & 14.54%) and TF.WP3 (13.33 & 15.28%). The study showed that among various *Trichoderma* formulations TF.WP2, TF.WP1 and TF.WP3 wettable based formulations were found significantly better in improving plant vigour and in reducing chickpea mortality compare to liquid based *Trichoderma* formulations.

Pandey *et al*. (2017) reported that, the bioagents *viz*., *T. harzianum*, *T. viride*, *T. virens*. *T. atroviride*, *T.konigii*, *Paecilomyces liacinus* and *Pseudomonas fluorescens* etc. have been proved effective for the management of the seed and soil borne plant pathogens/ diseases. Seed bio-priming of the bioagents particularly *Trichoderma* spp. have been found quite effective to manage the plant pathogens and enhancing germination, seedling vigour, plant growth etc. In the wake of climate change, the bioagents are the hope to overcome the biotic and abiotic stresses

Chaurasia and Bara (2018) checked the efficiency of biopriming agents i.e., biocontrol agents (*T. harzianum* @ 0.6%, *Pseudomonas fluorescens* @ 0.6%) and fungicides (Carbendazim @ 0.2%, Mancozeb 50% + Carbendazim 25% WS @ 0.2%) in enhancing field emergence, seedling growth and yield were investigated in chickpea of GNG-1581 variety. Five treatments gave the significant results. T_1 (*T. harzianum*) showed significant performance for field emergence (85.83 cm), plant

height (77.8 cm), number of plants per plot (24.5), number of primary branches (3.25), number of pods per plant (45), seed weight per plant (17.61gm) and seed yield per plot (135.89) in organic priming followed by T_4 (Carbendazim) in inorganic priming compared to untreated control.

Jhumishree *et al*. (2018) reported that the *Trichoderma* spp. are also well reported as a root colonizer which promote growth of plants significantly as compared to untreated plant. It can be applied as seed treatment and soil and foliar application which not only increase the germination percentage but also improves the root length, shoot length, dry weight, fresh weight, vigour index and vigour mass index and reduces the diseases and ultimately increases the yield of crop. In chickpea CVS JG-14 and JG-24, maximum growth promotion observed with inoculation of *Trichoderma* isolate Tr- 7 followed by Tr- 6, Tr- 2 and Tr-1 inoculation.

Patil *et al*. (2021) An investigation was undertaken to test the effect of liquid formulation of *Trichoderma asperellum* against *Fusarium* wilt of chickpea. Oil based formulation delivers organism in a physiologically dormant state and does not encourage the growth of contaminants during storage. Paraffin oil, soybean oil and groundnut oil were used as carrier material for oil based liquid formulation of *Trichoderma asperellum*. The population density of *T. asperellum* found in paraffin oil was 28.67×10^8 CFU/ml at 30 days whereas at 180 days it was 18.00×10^8 CFU/ml. Paraffin oil was found significantly superior over soybean oil and groundnut oil. The effect on percent growth inhibition of *Fusarium oxysporum f*. sp. *ciceri* was 74.44 percent. Efficacy of liquid formulation on growth parameter of chickpea revealed that paraffin oil based formulation of *T. asperellum* with combined seed treatment and soil application effectively increased the germination percentage (92.50%, 91.67%), shoot length (38 cm, 32.16cm), root length (16.91 cm, 14.75 cm) and vigour index (5079.17, 4300.23) in variety Chafa 815 and JG 62 respectively. The application

of *T. asperellum* showed percent wilt reduction of 68.12% and 34.37% over the absolute control in variety Chafa 815 and JG 62 respectively.

Chapter III

MATERIAL AND METHODS

A study on solid and liquid formulation entitled “To examine the storability of *Trichoderma harzianum* in different formulation” was conducted in Plant Pathology Laboratory, College of Agriculture, Nagpur during the year 2021-2022 The details of material used and the methods adopted during the course of investigation are included in this chapter.

3.1 Material required

3.1.1 Glassware’s, plastic wares and other material

Glassware’s, plastic wares and other materials like inoculating needle, distilled water, spirit, spirit lamp, mercuric chloride solution, muslin cloth and cover slip etc., were obtained from Plant Pathology Laboratory, College of Agriculture, Dr. PDKV, Nagpur.

3.1.2 Equipment

Laboratory equipment *viz*., autoclave, hot air oven, laminar air flow, electronic chemical balance and research microscope were used at Plant Pathology Laboratory, College of Agriculture, Nagpur.

3.1.3 Chemicals

Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), Dipotassium hydrogen phosphate (K_2HPO_4), Ammonium nitrate (NH_4NO_3), Potassium chloride (KCL), Glucose, Rose Bengal and Agar agar.

3.1.4 Carrier material

Sorghum grains, talc powder, vermicompost, gelatinous capsule, sachet packing material from section of Plant Pathology, College of Agriculture, Nagpur. Which is incorporated into the *T. harzianum* formulation with additives such as glycerol and CMC.

3.1.5 Other materials

Non-absorbent cotton, polyethylene bags, cork borer (5mm), micropipette, dissection needle, forceps, permanent marker, cello tapes, test tube stand, tray, wash bottle, potato, rubber band, scissors and empty capsules were used during the investigation.

3.1.6 Pure culture

Pure culture *of T. harzianum* was collected from Plant Pathology Section, College of Agriculture, Nagpur. The pure culture was mass multiplied for further studies.

3.1.7 Identification of *Trichoderma harzianum*.

The genius *T. harzianum* is characterized by rapid growing colonies bearing tufted or postulate, repeatedly branched conidiophores with lageniform phialides and hyaline or green conidia born in slimy heads, *T harzianum* was identified on the basis of their colony and microscopic structure.

3.2 Methods

3.2.1 Sterilization of glassware, media and distilled water

All glassware was sterilized in hot air oven at 180^0C for 1 hour. Culture media and distilled water was sterilized in autoclave at 15 lbs. pressure for 15 min.

3.2.2 Experimental Details

Year	-	2021 -22
Design	-	CRD
Treatments	-	6
Replications	-	4

3.2.3 Treatment Details

Formulation and their ingredients

Treatments	Formulation description (ingredients)	Conc. (gm, ml/kg) of seed
T_1 - Talc formulation	*Trichoderma* filtrate (100 ml) + talc (100 g)+ 0.1% CMC	4
T_2 -Vermicompost	*Trichoderma* filtrate (10 ml) + vermicompost (100 g)	4
T_3 - Capsule	*Trichoderma* spore dry spores (harvested from well colonized on sorghum grains) 1g + 0.1% CMC + 3% glycerol	4
T_4 - Sachet	*Trichoderma* spore dry spores (harvested from well colonized on sorghum grains) 10 g + 0.1% CMC+ 3% glycerol.	4
T_5 - Liquid formulation	*Trichoderma* filtrate 100 ml + 300 ml sterilized water +3% glycerol	4
T_6 - Control	*Trichoderma* filtrate 100 ml	-

3.2.4 Procedure of preparation of PDA

Potatoes 200gm was washed, peeled off and sliced into small-small pieces and boiled it in one liter water for up to 30 minutes in open vessel or pan. The collected potato extract filtered through muslin cloth and mixed with dextrose (20 gm), Agar (20 gm) and boiled to dissolve. Autoclaved it for 15 min. at 121.6°C. Final pH of the medium was 5.6-0.2. Streptomycin sulphate (30 mg) was supplemented in molten PDA medium in order to check the bacterial contamination prior to pouring in petri dishes.

3.2.5 Preparation of sorghum grain medium

Sorghum grain was used for solid state formulation for capsule and sachet form 100 gm sorghum grains and 100 ml of water moistened and pour in 1000 ml capacity conical flasks and sterilized in autoclave at 15 psi and 121.6°C for 15-20 minutes. Sterilized sorghum grains medium was used for mass multiplication of *T. harzianum* as solid state formulation. The grains were cooled at room temperature after sterilization. After cooling, each conical flask of sorghum grains was inoculated with 1-2 bits of *Trichoderma* mother culture inside the chamber with the help of inoculation loop/spatula. The conical flask were shaken properly for mixing the fungal culture with grains. Inoculated bags were kept at the room temperature (25-30°C) and checked for mycelial growth without disturbing. If there was no mycelial growth inoculated bag was shaken. After checking *Trichoderma* sporulation (green colure), the flask was shaken every alternate day for about 5 to 7 days in order to spread and allow the *Trichoderma* growth and further sporulation.

3.2.6 Sub culturing of *Trichoderma harzianum*

Sub culturing was done on PDA medium in Petri plates and incubated at 27±2°C for seven days with periodic observation for the development of colonies of *T. harzianum* the light green color colonies were identified by key based on branching conidiophores, shape of phialides, emergence of phialides and spore characters (Gams and Bisset 1998). Periodically sub culturing was done by using PDA slants. Loopful growth of *T. harzianum* transferred in sterilized PDA slants and incubated for five days at 27±2°C in BOD incubator. After incubation the slants were stored in refrigerator at 7-9°C for further study.

3.2.7 Mass production of *Trichoderma harzianum* using TSM medium

TSM medium was prepared by using the composition are Magnesium Sulphate heptahydrate ($MgSO_4.7H_2O$) 0.2gm, Dipotassium

hydrogen phosphate (K_2HPO_4) 0.900 gm, Ammonium nitrate (NH_4NO_3) 1.0 gm, Potassium chloride (KCL) 0.150 gm + Glucose 3.00 gm, Rose Bengal 0.150 gm, Agar 20 gm and 1000 ml distilled water was use for the growth and sporulation of *T. harzianum*. The media was transferred to sterilized glass bottles, cooled down at room temperature and was inoculated with *T. harzianum* mother culture. All these procedures were carried out in laminar air flow chamber and kept for incubation at room temperature for 5-7 days, care should be taken not to expose the medium to light, since photodegradation of Rose Bengal yields compound that are toxic to fungi. After incubation, homogenized the semi solid suspension, crushed or blended and transferred to plastic bottles for storage.

3.2.8 Mass production of *Trichoderma harzianum* using Potato Dextrose Broth

After preparation of PDB broth media was taken in 250 ml of water in a conical flask, stirred with a glass rod for uniform mixing. The conical flask containing media was transferred to autoclave for sterilization and cooled down at room temperature. There after this media was inoculated with *T. harzianum* mother culture and incubated for 7 days at room temperature. All these procedures were carried out in laminar air flow chamber

3.2.9 Harvesting of spores

Grains with fully grown *Trichoderma* mycelia and sporulation were transferred to cleaned plastic trays and covered with blotter paper. These plastic trays were kept for drying for about 3-4 days at room temperature. After drying, the grains were sieved with the help of 300 CFU micron mesh sieve to get pure *Trichoderma* spores and stored at 4°C.

3.2.10 Preparation of different formulations of *Trichoderma harzianum*

T. harzianum was multiplied in TSM medium and the CFU count/ml was estimated by standard procedure of serial dilution and pour plate method.

3.2.11 Preparation of formulation

1. Talc based formulation

T. harzianum filtrate was mixed with talc powder in the ratio (1:1) *Trichoderma* filtrate 100 ml + talc 100 gm + 0.1% carboxy methyl cellulose in the final formulation, shelf life of *T. harzianum* were calculated by serial dilution method. (Plate 3A)

2. Vermicompost

T. harzianum filtrate was added with vermicompost with the ratio of (1:10) *Trichoderma* filtrate 10 ml + vermicompost 100 gm and observation were recorded by serial dilution method. (Plate 3B)

3. Capsule with pure *Trichoderma harzianum* conidia (without carrier)

T. harzianum multiplied on sorghum grain and after 10 days dry conidia were harvested by sieving the grain. Dry conidia (1 gm) filled in capsule by mixing with 0.1% carboxy methyl cellulose and glycerol 3% to improve the shelf life of formulation. (Plate 3C)

4. Sachet with pure *Trichoderma harzianum* conidia (without carrier)

T. harzianum multiplied on sorghum grain and after 10 days dry conidia were harvested by sieving the grain. Dry conidia (10 gm) filled in sachet by mixing with 0.1% carboxy methyl cellulose and 3% glycerol to improve the shelf life of formulations. (Plate 3D)

5. Liquid formulation using TSM medium

T. harzianum filtrate was mixed with sterilized water in the ratio (1:3) *Trichoderma* filtrate 100 ml + 300 ml sterilized water + 3% glycerol. So that final formulation shelf life of *T. harzianum* were evaluated. (Plate 3E)

6. Control

Trichoderma harzianum filtrate of 100ml was mixed with PD Broth (Plate 3F)

3.2.12 Pouring on agar plate

Pouring over an agar plate with a laminar air flow work station that has been UV sterilized for 15 minutes and then surface sterilized with 70% alcohol before beginning work. Conical flasks of PDA medium, sanitized Petri-dishes, inoculating needle and other items were transferred to the platform of the laminar air flow work station using a water bath at 50°C. Approximately 15-20 ml molten PDA medium was put into 90 mm Petri-dishes in aseptic condition and the dishes were gently turned such that the medium equally covered the dish base. It is verified that the plate's base is covered, that the agar does not contact the plate's lid and that the surface is flat and free of bubbles.

3.3 Viability of *Trichoderma harzianum*

Five carrier based formulations and one control of *T. harzianum* were evaluated for viability from date of manufacturing to 6 months and observed at 30 days interval by adopting the following methods.

One gram sample or one capsule was drawn from each formulation and transferred in 10 ml sterilized water in test tube and shake thoroughly for 3 minutes to make 10^1 dilution. From these test tube one ml suspension of stock solution was transferred in next test tube containing 9 ml distilled water by using sterilized pipette and

shaken to make 10^2 dilution these same procedures make for seven test tube to make up to 10^7 dilutions. One ml of suspension was taken from the dilution of 10^7 and transferred in petri plates containing 20 ml sterilized PDA and gently shaken to spread evenly. These petri plates were incubated at 27 ± 2°C for 72 h and periodic observation were taken for the development of colonies of *T. harzianum.*

Observations for colony forming units (CFU) were taken by using formula.

$$\text{CFU} = \frac{\text{Number of colonies}}{\text{Amount of diluted suspension}} \text{ x Dilution factor}$$

3.4 Effect of seed treatment of different formulation of *T. harzianum* on seed germination and seedling vigour index of different crop

3.4.1 Rolled paper towel method

Two towel papers of equal size jointly soaked in water and 100 seeds were placed between a pair of moist paper towels which were then stacked 10 seeds per row at an equidistance and were placed over a butter paper to avoid water loss. These seeds were carefully rolled in moist germination paper and rolled to avoid putting too much pressure on the seeds and ends were closed by rubber bands the roll was kept slanted inside a medium sized plastic container containing 2-3 cm deep water. The rolled paper towels were incubated in dark at 25°C for 7 days and in the seventh day the first count of germination was made. All the morphologically normal seedlings were counted and germination was expressed in percentage.

3.4.2 Observations recorded

3.4.3 Germination percentage

On the day of final count, all normal seedling were counted. Based on the number of normal seedlings, germination percentage

from each crop in each replication was computed as per the formula mentioned here.

$$\text{Germination}(\%) = \frac{\text{Number of normal seedlings germinated}}{\text{Total number of seed sown}} \text{ x } 100$$

3.4.5 Seedling Vigour Index

Ten normal seedlings were taken from each crop in each replication and in each different formulations of *T. harzianum* at random 7th day and seedling root and shoot length was measured from the tip of the primary leaf to the tip of primary leaf to the tip of primary root with the help of scale and mean seedling length was expressed in cm. Shoot length was measured from the collar region to the cotyledon junction. The average shoot length was measured in centimeters germination percentage, root length and shoot length were all measured. Seedling vigour index was calculated by using following formula Seedling vigour index = (Mean root length + mean shoot length) x Percentage of seed germination.

3.5 Analysis of data

The data of various experiments was analyzed using appropriate design. Analysis of variance, means those were tested for significance and critical difference was used for comparison whenever the differences were found to be significant as indicated in ‘F’ test as per design (Gomez and Gomez 1984).

Chapter IV

RESULTS AND DISCUSSION

Investigation on "*Trichoderma harzianum* formulations for enhancing its storability" was undertaken in the Plant Pathology Section, College of Agriculture, Nagpur during the year 2021-22. The experiment was designed in CRD with six treatments in four replications. The results are presented in tables, photograph and figures are depicted in each head in this chapter.

4.1 Effect of different formulations on the shelf life of *T. harzianum* (CFU/ml) at various interval

The pure culture of *T. harzianum* was procured from Plant Pathology Section, College of Agriculture Nagpur. The growth of *T. harzianum* and microscopic view was observed and depicted in (Plate 1 (A) and (B)) and the general view of experiment is shown in (Plate 3).

An experiment on effect of different formulation on the shelf life of *T. harzianum* was carried *in vitro* at various interval.

The data is presented in (Table 1) and depicted in (figure 1 and plate 4) revealed that there were differences at all the interval over uninoculated control. Among the treatment capsule formulation recorded (79.65x10^7CFU/ml) in first month and was found significantly superior as all other treatment. It was followed by sachet formulation treatments (71.00x10^7CFU/ml) and in vermicompost the count was 29.75, 26.75, 22.14, 18.76, 16.28 and 11.28 x10^7CFU/ml. at various interval I, II, III, IV, V and VI respectively there was gradual decreases in viable spore count. Various worker has examined the storability of vermicompost as storage formulation for *T. harzianum,* Pan and Das (2010), Shafa Khan (2011), Zopiola *et al.* (2012) and Boblina *et al.* (2019) was addressed their potential commercial value. Minimum spore count was observed in uninoculated control (6.45x10^7CFU/ml). Komala *et al*. (2019) observed similar results.

(A): Pure culture of *Trichoderma harzianum* in petri plate and slant

(B): Microscopic view of conidia of *Trichoderma harzianum* at (40x)

Plate 1: Pure culture of *Trichoderma harzianum*

(A): Mass multiplication of *Trichoderma harzianum* using PDB

(B): Mass multiplication of *Trichoderma harzianum* using TSM

(C): Mass multiplication of *Trichoderma harzianum* using sorghum grains

Plate 2: Mass multiplication of *Trichoderma harzianum* using various media

(A) Talc formulation

(B) Vermicompost formulation

(C) Capsule formulation

(D) Sachet formulation

(E) Liquid formulation

(F) Control

Plate 3: General view of experiment

Table 1: Effect of different formulation on the shelf life of *Trichoderma harzianum* (×10^7CFU/ml) at various interval

Tr. No.	Treatment	Month					
		I	II	III	IV	V	VI
T_1	Talc formulation	26.13	22.25	18.75	13.06	9.75	6.75
T_2	Vermicompost	29.75	26.75	22.14	18.76	16.28	11.28
T_3	Capsule	79.65	72.25	62.05	52.33	44.03	37.50
T_4	Sachet	71.00	63.45	52.88	48.49	40.63	32.38
T_5	Liquid formulation	11.50	7.88	5.80	3.50	1.79	1.23
T_6	Control	6.45	5.38	2.77	1.63	1.30	0.81
	F test	Sig	Sig	Sig	Sig	Sig	Sig
	SE ± m	1.26	0.63	0.44	0.41	0.34	0.30
	CD (P=1%)	5.03	2.52	1.75	1.63	1.36	1.20

With regard to II month the significant differences were noticed. Maximum population was recorded by the capsule treatment (72.25x10^7CFU/ml) followed by sachet treatment (63.45x10^7CFU/ml). The capsule formulation treatment was found to be significantly superior over all other treatments. The observation is in agreement with the result of Sanghmitra *et al.* (2014). Uninoculated treatment recorded lowest spore count.

It was further observed that (Table 1) maximum population were recorded by the capsule treatment 79.65, 72.25, 62.05, 52.33, 44.03 and 37.50x10^7CFU/ml at I, II, III, IV, V and VI in the month significantly gradual decreases in the population occurred. The capsule treatment was found significantly superior over all other treatments in all the month. Similarly, Komala *et al*. (2019) reported the capsule formulation conidia highest spore count of *T. harzianum*. There was in declined in spore count from first month to six months. There observation is in

conformity with Kose and Totawar (2017), Locatelli *et al.* (2017) and Komala *et al*. (2019). The encapsulation study was also reported by Maria *et al*. (2019), Adzmi *et al.* (2021) and Tayde *et al*. (2021).

Sachet treatment recorded the next best treatment (Table1) severely the spore count *viz*, 71, 63.45, 52.88, 48.49, 40.63 and 32.38 x 10^7CFU/ml at I, II, III, IV, V and VI in the month respectively. There was also declined in spore count as the moth progress. This treatment was significantly superior over all other treatments except capsule treatment. Komala *et al*. (2019) found higher spore count of *T. harzianum* in sachet based formulation. Mass multiplication of *T. harzianum* was undertaken on sorghum grain Similar worked was carried by Jagtap and Bhatnagar (2000), Rini and Sulochana (2007), Rajput *et al*. (2014) and Rajput and Shahzad (2015).

Liquid formulation treatment had lowest viable spore count (Table 1) at all the interval under study but it was significantly superior as compared to uninoculated control. The data formed in (Table 1) refers that at I month viable count was (11.50x10^7CFU/ml) and later on decreasing every month till 6 month (1.23 x10^7CFU/ml). Similar finding have been reported by Bhai and Anandraj (2014), Nadare *et al*. (2016), Reddy *et al*. (2017), Akshata *et al*. (2018) and Kajal Mane *et al*. (2019).

The effect due to talc powder for storability was one of the treatments made the present study. It was revealed from the experiment that talc formulation had (26.13x10^7CFU/ml) spore count and gradual decreases among till 6^{th} month of storage (6.75x 10^7CFU/ml). Which was highest as compared to uninoculated control significantly. As the storage period increased the viable count over all found declined in talc formulation. The observation is in line with the reported to Nakkeeran *et al*. (1997), Karunanithi *et al*. (2001), Bhat *et al*. (2009), Krishna (2013), Patel and Patel (2014), Manandhara *et al*. (2018), Mawar and Mathur (2022) and Patel and Raja (2022).

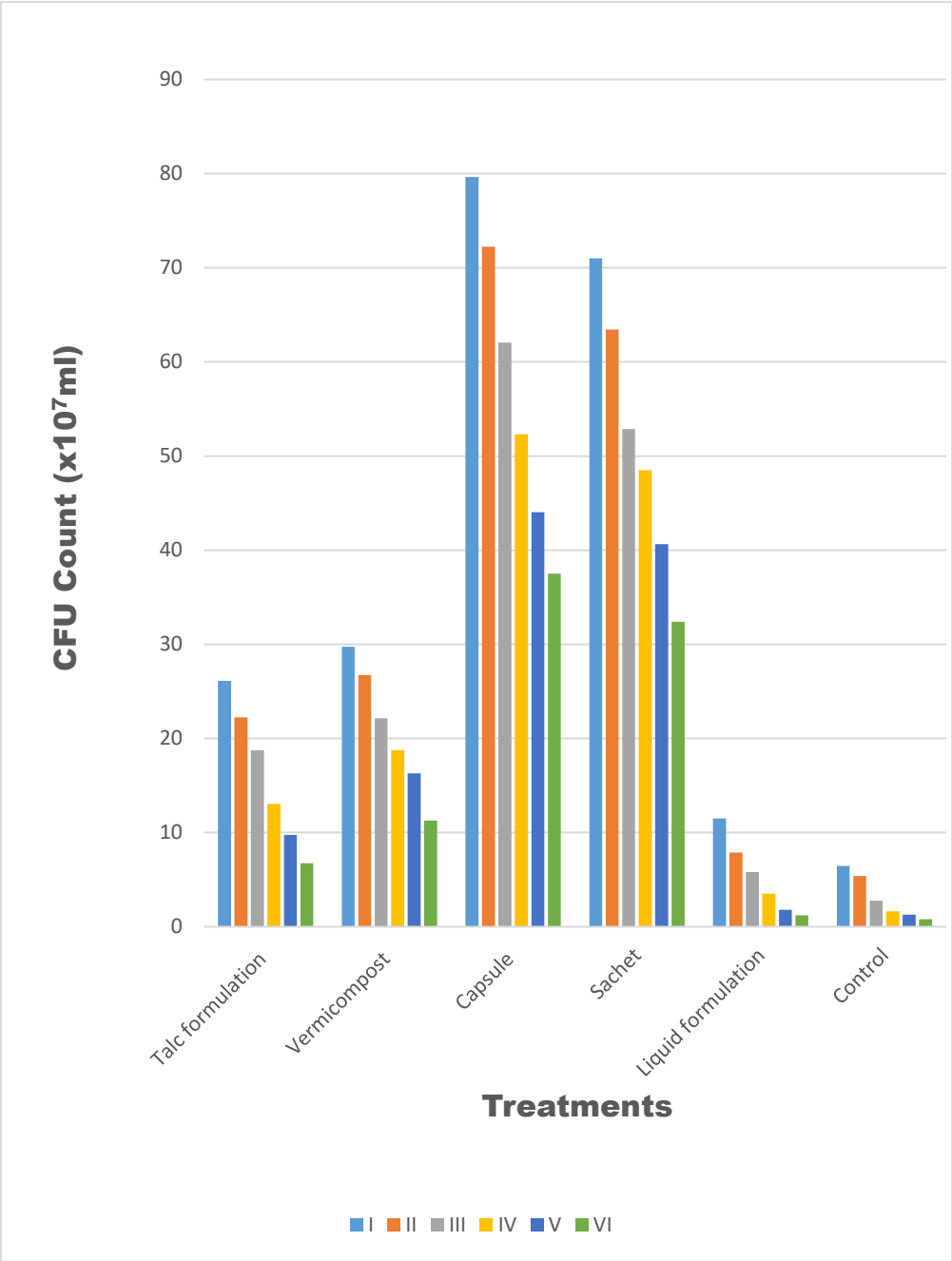
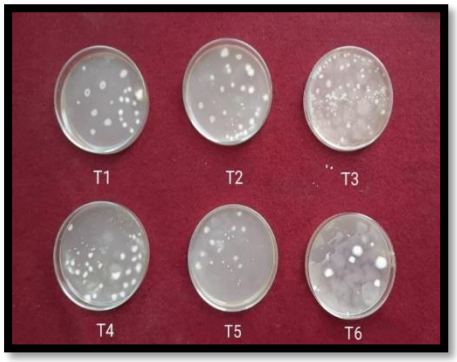


Fig 1: Effect of different formulation on the shelf life of *Trichoderma harzianum* at various interval

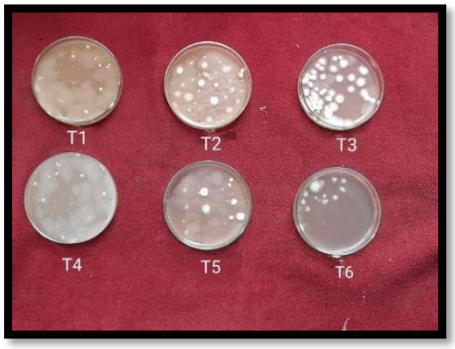
(A) Colony count of I month

(B) Colony count of II month

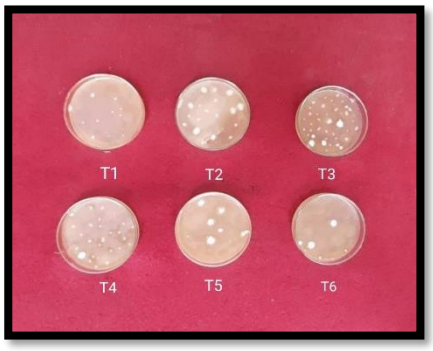


(C) Colony count of III month

(D) Colony count of IV month



(E) Colony count of V month



(F) Colony count of VI month

Plate 4: Colony count of *Trichoderma harzianum* observed at various interval

4.2 Effect of seed treatment of different formulation of *T. harzianum* on seed germination and seedling vigour index of different crop

4.2.1 Effect of seed treatment of different formulation of *T. harzianum* on seed germination and seedling vigour index of green gram

The seed germination experiment was conducted on the various crops *viz*., green gram, black gram pigeon pea and chickpea by using rolled paper towel method. The data are presented in (Table 2) and depicted in fig 2 and plate 5 (A).

Table 2: Effect of different formulations of *T. harzianum* on seed germination percentage, shoot length, root length and SVI of green gram

Tr. No.	Treatments	Germination %	Shoot length (cm/pl)	Root length (cm/pl)	Seedling vigour index
T_1	Talc formulation	81.50	15.4	7.00	1825.6
T_2	Vermicompost	82.50	17.6	8.05	2116.1
T_3	Capsule	93.50	22.6	12.75	3305.2
T_4	Sachet	91.00	20.5	12.66	3018.1
T_5	Liquid formulation	79.25	14.66	7.00	1716.5
T_6	Control	71.50	12.5	5.33	1275.0
	F test	Sig	Sig	Sig	-
	SE ± (m)	1.40	0.71	0.46	-
	CD (P=0.01)	5.59	2.82	1.82	-

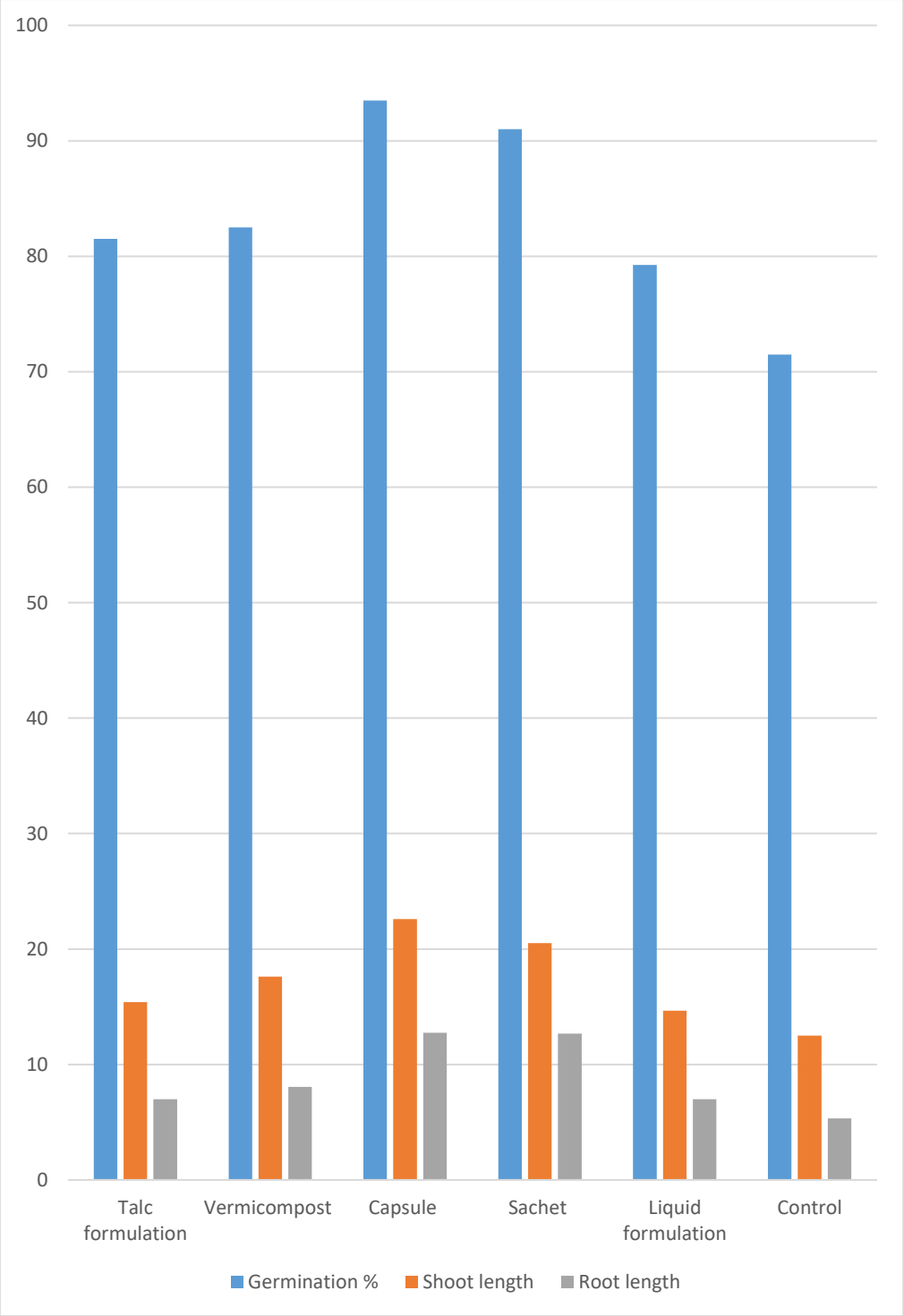


Fig. 2: Effect of different formulations of *Trichoderma harzianum* on seed germination percentages, shoot and root length of green gram

It was observed from the data that there was good germination in green gram. Among the various formulation treated capsule treatment (T_3) reveled (93.50 per cent) and it was followed by sachet formulation 91.00 per cent and vermicompost formulation 82.50 per cent. However, the control treatment recorded least gemination percentage (71.50 per cent).

Shoot and root length and vigour index was measured and the data is provide in (Table 2). It is clearly observed from the data that there was significant differences on shoot and root length over uninoculated control. Maximum shoot and root length was recorded by the capsule formulation treatment (22.6 cm/pl) and (12.75 cm/pl) it was found significantly superior over all the treatments. The treatment was followed by sachet formulation treatments 20.5 cm length shoot length and 12.66 cm root length/pl. Capsule formulation treatment recorded the highest seedling vigour index 3305.22 followed by sachet formulation treatment 3018.16. Similar observation has been recorded by Deshmukh *et al*. (2016), Nadhina *et al.* (2016) and Sharma and Boraha (2020).

4.2.2 Effect of seed treatment of different formulation of *T. harzianum* on seed germination and seedling vigour index of black gram

With regard to black gram (Table 3 and figure 3 and plate 5 (B)) there was enhancement of germination due to application of various formulation on seed germination percentage. Highest germination percentage was recorded by capsule treatment (95.75 per cent) and it was followed by sachet treatment (92.00 per cent) and vermicompost (89.75 per cent).

Significant differences were noticed with regard to shoot and root length. It is evident from the data and maintained in (Table 3) that the capsule formulation treatment recorded the highest shoot length (21.5 cm/pl) and root length (14.0 cm/pl) and the treatment was found

significantly superior over all other treatment. It is was followed by sachet formulation treatment respectively (19.66 cm/pl) and (11.33 cm/pl) shoot and root length respectively. Seedling vigour index was calculated and the highest seedling vigour index was 3186.1 was recorded by capsule formulation treatment. Superior seed germination and seedling vigour index followed by sachet formulation treatment 2851.0 seed germination and seedling vigour index increases and black gram due to formulation of *T. harzianum* as observed in present studies. Corroborate the finding of Surekha *et al*. (2013), Mohamaad *et al*. (2014), Athira (2017) and Balkkrishna *et al*. (2017).

Table 3: Effect of different formulations of *T. harzianum* on seed germination percentage, shoot and root length and SVI of black gram

Tr. No.	Treatments	Germination %	Shoot length (cm/pl)	Root length (cm/pl)	Seedling vigour index
T_1	Talc formulation	83.50	14.00	8.00	1837.0
T_2	Vermicompost	89.75	18.00	10.0	2513.0
T_3	Capsule	95.75	21.5	14.0	3186.1
T_4	Sachet	92.00	19.66	11.33	2851.0
T_5	Liquid formulation	79.75	13.00	6.66	1554.1
T_6	Control	72.25	10.00	5.33	1107.5
	F test	Sig	Sig	Sig	-
	SE ± (m)	1.23	0.36	0.54	-
	CD (P=0.01)	4.90	1.45	2.14	-

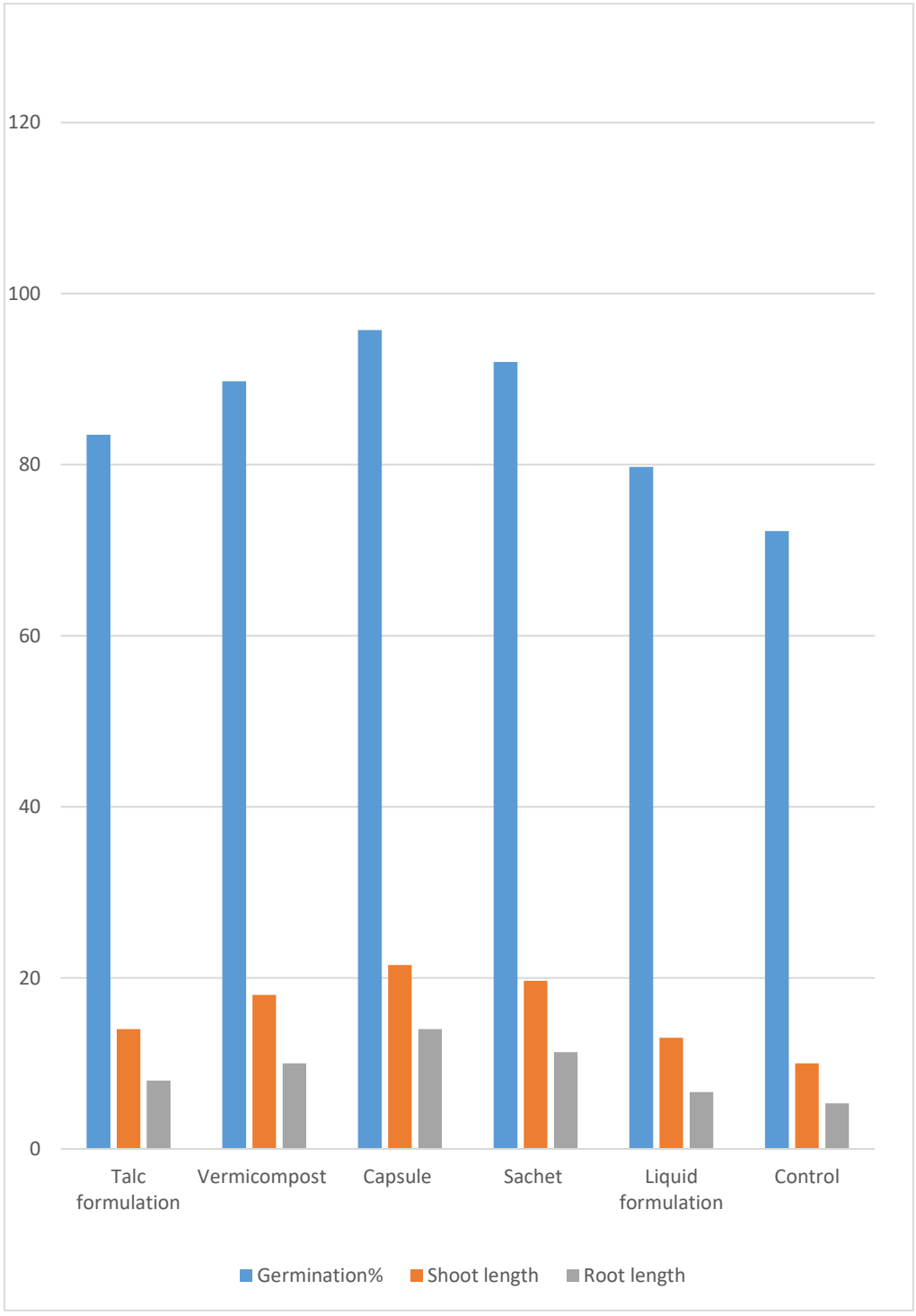


Fig. 3: Effect of different formulations of *T. harzianum* on seed germination percentages, shoot and root length of black gram

4.2.3 Effect of seed treatment of different formulation of *T. harzianum* on seed germination and seedling vigour index of pigeon pea

It was also noticed that there was enhancement of germination percentage in pigeon pea (Table 4, figure 4 and plate 5 (C)) as compared to uninoculated control. The data clearly shows that among the various formulation treatment capsule formulation treatment recorded maximum (94.25 per cent) germination followed by sachet formulation treatment (91.25 per cent).

Table 4: Effect of different formulations of *Trichoderma harzianum* on seed germination percentage, shoot length, root length and SVI of pigeon pea

Tr. No.	Treatments	Germination %	Shoot length (cm/pl)	Root length (cm/pl)	Seedling vigour index
T_1	Talc formulation	86.50	10.0	5.0	1297.5
T_2	Vermicompost	82.50	13.0	8.0	1732.5
T_3	Capsule	94.25	17.0	11.0	2639.0
T_4	Sachet	91.25	14.5	10.0	2190.0
T_5	Liquid formulation	78.75	11.0	7.0	1417.5
T_6	Control	70.00	7.0	5.0	840.0
	F Test	Sig	Sig	Sig	-
	SE ± (m)	1.31	0.3819	0.4410	-
	CD (P=1%)	5.21	1.5161	1.7507	-

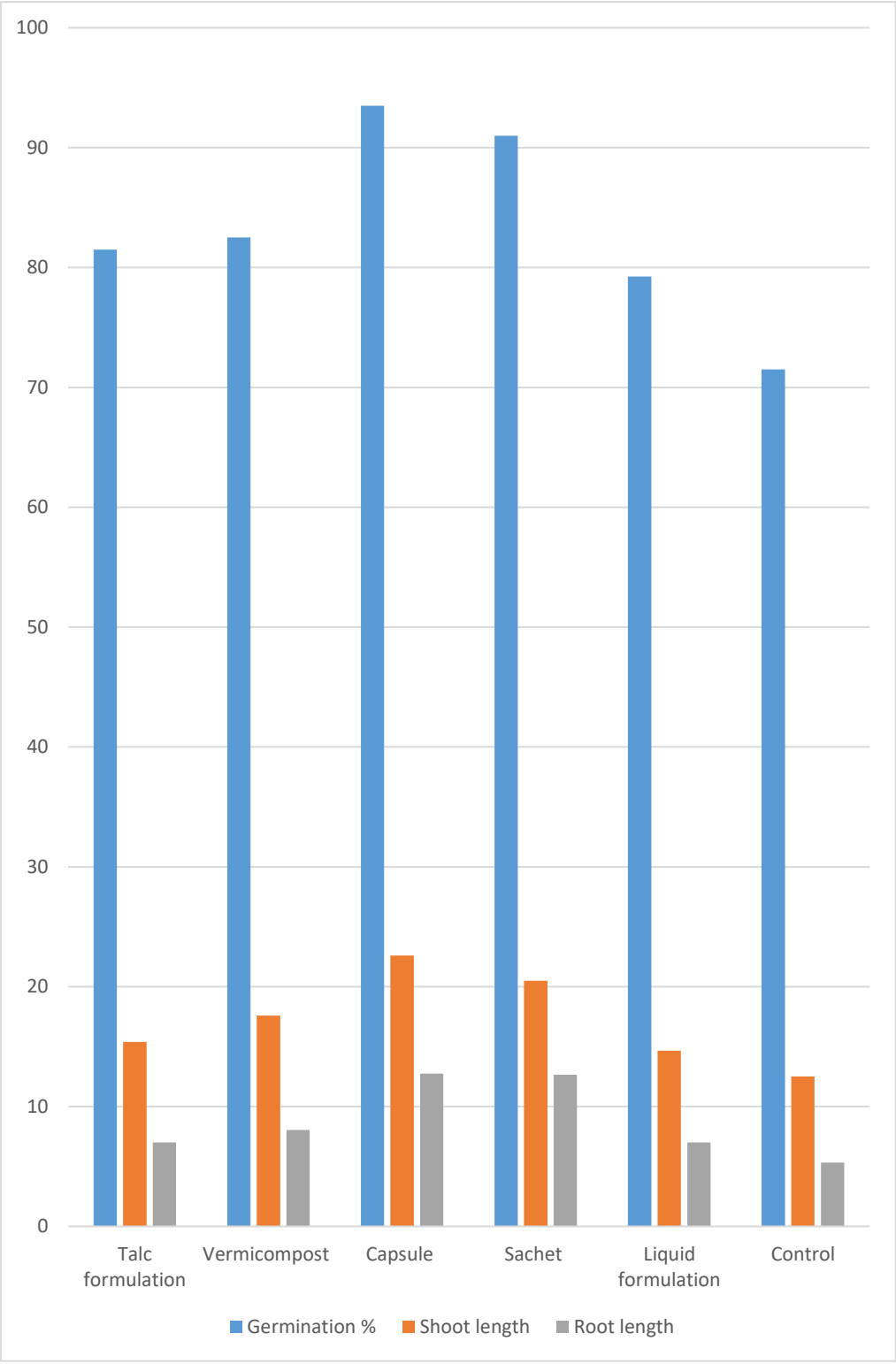


Fig. 4: Effect of different formulations of *Trichoderma harzianum* on seed germination percentages, shoot and root length of pigeon pea

Similarly shoot and root length and seedling vigour index were studied and the data is tabulated in Table 4. It is clearly seen from the data the significantly differences on shoot and root length over uninoculated treatment. Maximum shoot length (17.0 cm/pl) and root length (11.0 cm/pl) was observed with capsule formulation treatment and it was found significantly superior over all other treatments.

This treatment was followed by sachet formulation treatment reading (14.5 cm/pl) and (10.0 cm/pl) shoot and root length respectively. Similarly, seedling vgour index was found to be the highest in capsule treatment (2639) followed by sachet treatment (2190). The present study closely resembled with Rajkondra and Bhale (2011), Mohamaad *et al*. (2014) and Chaudhari *et al*. (2017).

4.2.4 Effect of seed treatment of different formulation of *T. harzianum* on seed germination and seedling vigour index of chick pea

Similarly, these bioformulation treatment were also conducted on chickpea crop. It was observed from the data furnished on (Table 5 and figure 5 and plate 5(D)). That the highest germination percentage was recorded by the capsule formulation treatment (92.25 percent) and was followed by the sachet formulation treatment (84.75 percent) and talc formulation treatment (82.25 percent).

It is also observed from the data furnished in (Table 5) that there were significant differences in shoot and root length over uninoculated control. Significantly higher shoot length (17.0 cm/pl) and root length (16.0 cm/pl) were recorded by the capsule formulation treatment, it was followed by the sachet formulation treatment registering (15.66 cm/pl) shoot length and (12.0 cm/pl) root length.

Table 5: Effect of different formulations of *Trichoderma harzianum* on seed germination percentage, shoot length, root length and SVI of chickpea

Tr. No.	Treatments	Germination %	Shoot length (cm/pl)	Root length (cm/pl)	Seedling vigour index
T_1	Talc formulation	82.25	11.0	11.0	1809.5
T_2	Vermicompost	78.75	12.0	11.5	1838.8
T_3	Capsule	92.25	17.0	16.0	3044.2
T_4	Sachet	84.75	15.66	12.0	2344.1
T_5	Liquid formulation	75.75	13.0	9.0	1666.5
T_6	Control	69.50	8.0	7.0	1035.7
	F Test	Sig	Sig	Sig	-
	SE ± (m)	1.61	0.53	0.58	-
	CD (P=1%)	6.39	2.12	2.32	-

Highest seedling vigour index was noticed in capsule formulation treatment (3044.2) followed by the sachet formulation treatment (2344.1). Similar observation was recorded by earlier worker Kumar *et al.* (2014), Rai and Tewari (2016), Pandey *et al.* (2017), Chaurasia and Bara (2018) and Jhumishree *et al.* (2018).

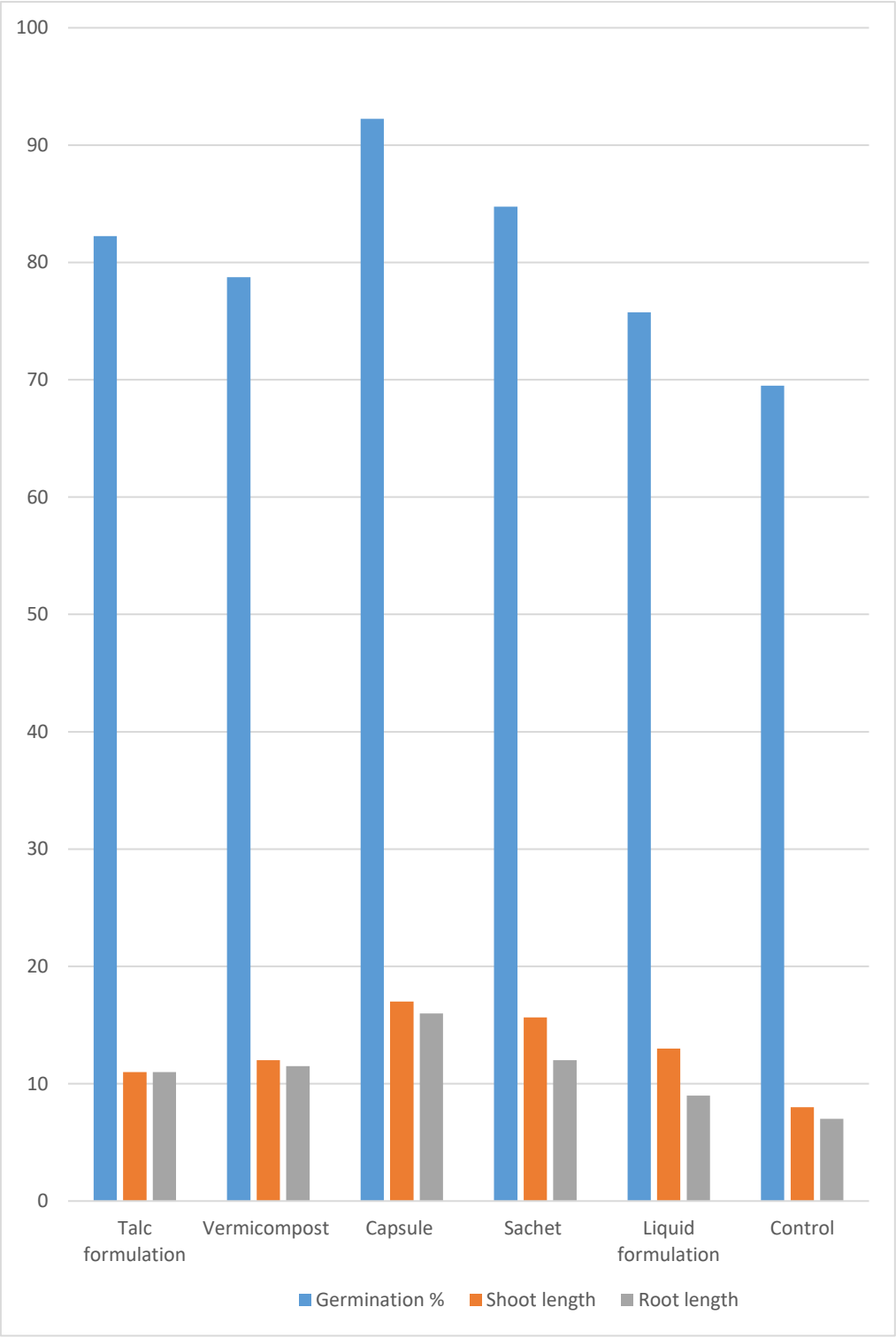


Fig. 5: Effect of different formulations of *Trichoderma harzianum* on seed germination percentages, shoot and root length of chickpea

(A) Green gram

(B) Black gram

(C) Pigeon pea

(D) Chickpea

Plate 5: Effect of different formulation of *Trichoderma harzianum* on the seed germination

Chapter V

SUMMARY AND CONCLUSIONS

Trichoderma harzianum is a potential biocontrol agent used for the control of plant pathogenic fungi. Capsule, sachet, talc, vermicompost, liquid formulations are extensively used as carrier material for stored *T. harzianum*. The survival of *T. harzianum* in capsule based formulations are quite high as compared to all other wettable carrier material. It has also advantage for improving survival and a good protection of *T. harzianum.* The present investigation was undertaken on "*Trichoderma harzianum* formulations for enhancing its storability" during the year 2021-22. The observation is recorded at I, II, III, IV, V and VI month from days after inoculation.

Among the treatments capsule formulation treatment recorded significantly higher population ($79.65x10^7$CFU/ml) in a I month and which was found significantly superior over all the other treatment. It was followed by sachet formulation treatment ($71x10^7$CFU/ml). The minimum count was recorded in uninoculated control treatment ($6.45x10^7$CFU/ml).

It was observed from the (Table 1) that there were significant differences on the population densities of *T. harzianum* in capsule formulation treatment at all the interval i.e., I, II, III, IV, V and VI month. It was evident from the (Table 1) that among all the liquid formulation treatment capsule formulation treatment was found significantly higher in II, III, IV, V and VI month with 72.25, 62.05, 52.33, 44.03, and 37.05 x 10^7CFU/ml. The next best treatment was found in sachet formulation treatment at all interval recording 71.00, 63.45, 52.88, 48.49, 40.63 and 32.38 x 10^7CFU/ml. There were gradual decreases in population from I till VI month.

Germination percentage of various crops recorded due to application of different formulation of *T. harzianum* were assed *viz*., green gram, black gram, pigeon pea and chickpea. The data revealed

that capsule formulation treatment significantly recorded higher germination percentage in green gram (93.50 per cent), black gram (95.75 per cent), pigeon pea (94.25 per cent) and chickpea (92.25 per cent). It was followed by sachet formulation treatment. Green gram (91.00 per cent), black gram (92.00 per cent), pigeon pea (91.25 per cent) and chickpea (84.75 per cent) recorded germination percentage respectively.

There was significant differences of shoot and root length were recorded in all treatment. Similarly capsule formulation treatment significantly recorded higher shoot and root length in green gram (22.6 cm/pl) and (12.75 cm/pl) respectively with vigour index 3305.22. With respect to black gram similar treatment have been recorded higher shoot and root length (21.5cm/pl) and (14.0cm/pl) respectively with vigour index 3186.1 similarly in pigeon pea. Similar treatment has been recorded higher shoot and root length (17.0 cm/pl) and (11.0 cm/pl) respectively with vigour index 2639. Same result was found in chickpea with capsule formulation treatment which recorded higher shoot and root length (17.0 cm/pl) and (16.0 cm/pl) respectively with vigour index 3044.2. It was followed by sachet formulation treatment. Higher shoot and root length and higher seedling vigour index was noticed in capsule formulation treatment in green gram, black gram, pigeon pea and chickpea.

These is one-year results and therefore more investigation is needed to draw the valid conclusion.

Conclusion

Thus, it is concluded from present investigation that *T. harzianum* can be stored up to VI month using capsule formulation.

Among the six formulation treatments i.e. talc, vermicompost, capsule, sachet, liquid and control, the capsule formulation treatment recorded significantly higher population as compared to all other treatments. It was followed by sachet formulation treatment.

The germination percentage of various crop and seedling vigour index were recorded by applying *Trichoderma harzianum* as seed treatment in different formulation on different crop such as green gram, black gram, pigeon pea and chickpea. The data revealed that capsule formulation treatment recorded highest germination percentage and also higher seedling vigour index over all other treatments in all crop.

Chapter VI

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