INVESTIGATION OF DIFFERENT OIL FORMULATIONS ON SHELF LIFE OF TRICHODERMA HARZIANUM

THESIS

Submitted to
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
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By
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DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the Thesis entitled **"INVESTIGATION OF DIFFERENT OIL FORMULATIONS ON SHELF LIFE OF TRICHODERMA HARZIANUM**" 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 **(Wankhede Ravi Mahadeo)**

Date: 21/10/2022 Enrolment No. **OO-2437**

CERTIFICATE

This is to certify that the thesis entitled **"INVESTIGATION OF DIFFERENT OIL FORMULATIONS ON SHELF LIFE OF TRICHODERMA HARZIANUM"** 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 **WANKHEDE RAVI MAHADEO** under my guidance and supervision.

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

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Date:21/10/2022

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THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE INCLUDING EXTERNAL EXAMINER (AFTER VIVA-VOCE)

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Date: 21/10/2022 **(WANKHEDE RAVI MAHADEO)**

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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	millilitre
mm	millimetre
^{o}C	Degree Celsius
PDA	Potato Dextrose Agar
PD	Potato Dextrose
S.E.± (m)	Standard error of mean
Spp / sp	species (Singular and pleural)
Var	Variety
Viz	Namely
@	At the rate
Sig	significant
T	Treatment

(E) THESIS ABSTRACT

a)	**Title of the Thesis**	:	**INVESTIGATION OF DIFFERENT OIL FORMULATIONS ON SHELF LIFE OF TRICHODERMA HARZIANUM**
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ABSTRACT

A study was carried out entitled “Investigation of different oil formulations on shelf life of *Trichoderma harzianum*” in completely randomized block design with seven treatment in three replication during

the year 2021-22 at Plant Pathology Section, College of Agriculture, Nagpur. There were seven treatment comprising of paraffin oil, rice bran oil, mustard oil, coconut oil, mineral oil, department culture and market product (liquid). *Trichoderma harzianum* was inoculated at 20 ml and 63 ml different oil with ten ml glycerol, one ml dispersant, three ml surfactant and three ml suspender in each treatment. The suspension were stored in plastic container and inoculated at temperative for 6 month. The observation were recorded on colony forming unit at every month and spore germination, there liquid formulation were arrested on mycelial growth of *fusarium oxysporum* f. spp. *ciceri* and *Rhizoctonia bataticola* and per cent growth inhibition was recorded at 8 days after inoculation.

It was revealed from the experiment that the maximum shelf life of *T*. *harianum* was noticed in paraffin oil treatment ($33.33x10^8$ CFU/ml) and it was followed significantly superior over all other treatment followed by coconut oil treatment ($22.40x10^8$CFU/ml). There was gradual decrease in population from one month to six month. Regarding spore germination the paraffin oil liquid formulation treatment recorded significantly higher spore germination 60.83 per cent followed by coconut oil treatment 47.00 per cent with maximum per cent growth inhibition on *Fusarium oxysporum* f. sp. *ciceri* is 55.49 per cent and *Rhizoctonia bataticola* is 82.11 per cent. Then it is concluded form the experiments that the shelf life of *T*. *harzianum* can be viable up to six month of storage period.

Chapter I

INTRODUCTION

1.1 Background Information

Trichoderma is a genus of asexually reproducing fungi that are often the most frequently isolated soil fungi nearly all temperate and tropical soils contain 101-103 culturable propagules per gram. These fungi also colonize woody and herbaceous plant materials, in which the sexual Teleomorph (genus *Hypocrea*) has most often been found. They show a high level of genetic diversity, and can be used to produce a wide range of products of commercial and ecological interest. They are prolific producers of extracellular proteins, and are best known for their ability to produce enzymes that degrade cellulose and chitin—although they also produce other useful enzymes (Harman and Kubicek.,1998). *Trichoderma* is considered as most efficient biocontrol agents and have attracted considerable scientific attention as they are considered as promising alternative to chemical fungicides against many plant pathogens. Major mechanisms involved in the biocontrol activity of *Trichoderma* spp. are competition for space and nutrients, production of diffusible and/ or volatile antibiotics and hydrolytic enzymes like chitinase and β-1, 3- glucanase. These hydrolytic enzymes partially degrade the pathogen cell wall and leads to its parasitization (Navaneetha *et al.*, 2015).

Trichoderma species have long been recognized as agents for the control of plant disease and for their ability to increase plant growth and development, high reproductive capacity, ability to survive under very unfavourable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi and efficacy in promoting plant growth and defence mechanisms. They are becoming widely used in Agriculture, and the most useful strains show a property that is known as ‘rhizosphere competence’— that is, the ability to colonize and grow in

association with plant roots. These properties have made *Trichoderma* a ubiquitous genus present in any habitat and at high population densities (Chet *et al*., 1997). *Trichoderma* is more efficient in acidic than alkaline soils. *Trichoderma* control Ascomycetous, Deuteromycetous and Basidiomycetous fungi, which are mainly soil borne but also air borne pathogens (Monte et al., 2001).

Trichoderma spp. are beneficial free living filamentous fungi. They have been extensively studied as a model microorganism to analyze and explored its antagonistic action against the phytopathogens. Among them high rhizosphere competency, prolific production of extracellular proteins and also the enzymes that can degrade chitin and cellulose are all important (Harman *et al*., 1996). *Trichoderma* is an effective and widely used biological control of soil borne plant pathogens. They are reported to have antifungal, antinematode, plant growth promoting and plant defense inducing activities (Zaidi *et al.*, 2004), according to researchers at the University of Bristol.

Under the changing agriculture scenario, the only technology that seems promising to manage the diseases without disturbing the equilibrium of harmful and useful composition of environment and ecosystem is the use of more and more biological control agents. *Trichoderma* spp. are biocontrol agents extensively used in management of fungal diseases of crop plants. Their usage has been very much successful against soil borne diseases for which no resistant sources have been identified in the plants. They are also widely exploited in industries as sources of enzymes. The share of biopesticides in India is merely 2% compared to 40% in USA and 20% each in Europe and Oceanic countries. (Sabalpara, 2014) *Trichoderma* species are well known bio-control agents to suppress infection by soil-borne root infecting fungi like *R. solani* and *M. phaseolina* on banana, egg-plant, cotton, sugarbeet and soybean (Benitez et al., 2004).

The method of mass production, formulation and application should be taken into consideration to stabilize the product during storage. Both solid and liquid formulations are used to produce suitable quantities of active and viable inoculation of *Trichoderma*. Three kinds of propagules can be used in formulations: hyphae, chlamydospores and conidia, (Howell, 2003). Hyphae are the natural choice as propagules in formulations. Due to its lack of tolerance to dehydration, hyphae are not an option. Conidia and chlamydospores can survive in extreme conditions. (Papavizas, 1985)

Some of the bio-pesticides and other beneficial organism-based formulations are very effective in laboratory conditions but failed in field occasionally. Reasons for this are poor stability of the product during storage prior to application, too little active material actually reaching the field target, death of the antagonistic used due to desiccation, contamination of formulation and rapid degradation of active material on the target. Formulation type plays an important role in helping to solve these problems and making a formulation very effective even in the fields. Thus, water/oil emulsions appear to be a promising, yet under-utilized, method for storing and delivering microorganisms to aqueous environments. One of the advantages of water/oil emulsions over other methods of formulation is that the oil traps water around the organism and slows water evaporation once applied. This is particularly beneficial for organisms that are sensitive to desiccation (Jean *et al*., 2006).

Oil is used as carrier for liquid formulation. They are prepared by mixing the conidia harvested from the solid/ liquid state fermentation with a combination of vegetable/ mineral oils in stable emulsion formulation. In such formulations, microbial agents are suspended in a water immiscible solvent such as petroleum fraction (diesel, mineral oil) and vegetable oils (groundnut, etc.) with the surfactive agent. This can be dispersed in water to form a stable emulsion. The oil used should not have toxicity to the fungal spores, plants, humans and animals. Such formulation of *Trichoderma, Pseudomonas, Beauveria* are now being

used as foliar sprays. Oil-based formulations are supposed to be suitable foliar sprays under dry weather condition and to have prolonged shelf life. (Ramanujam *et al.*, 2010)

Shelf life of a biocontrol agent plays a significant role in storing a formulated product and generally varies depending upon the nature of food base. A sharp decline in population of *Trichoderma harzianum* was noticed by Prasad & Rageshwaran (2002) in talc, kaoline based formulation that retained more than 10 viable propagules up to 90 days and the population decline below the optimum level by 120 days while bentonite appeared to be least suitable as a carrier showing a drastic reduction in population. However significant difference in number of viable propagules between kaoline based conidial and chlamydospore formulation were observed at 60-180 days.

The *Trichoderma* formulations are applied as a pre-planting application to seed or propagating material, foliar spray, post pruning, treatment, incorporation in the soil during seeding or transplanting irrigated or applied as root dip or drench. One of the advantages of water or oil emulsions over other methods of formulation is that the oil traps water around the organism and slows water evaporation once applied. studied oil-based formulations in the field as well as in laboratory and found the highest shelf life of *Trichoderma* for more than four years with average of 10^9 to 10^{10} cell ml-1 These products are able to survive 2 to 6 years depending upon the formulation. *Trichoderma harzianum* and *Trichoderma viride* are the widely used species and have been exploited on about 87 different crops and about 70 soils borne and 18 foliar pathogens, respectively. Keeping in view of the growing market for long shelf-life products with very high CFU counts, oil-based formulations have been developed for biocontrol fungus like *Trichoderma* (Kajal Mane *et al*., 2020).

Liquid inoculants could be produced with minimum labour, space and energy and also the quantity of inoculums required is less as compared to carrier-based formulations. It is easier for farmers to

handle. Liquid biofertilizers formulation could be considered as one potential strategy for improving the shelf life of biofertilizer. Unlike solid carrier based biofertilizers, liquid formulations allow manufacturer to include sufficient amount of nutrients, cell protectant and inducers responsible for cell or spore or cyst formation to ensure prolonged shelf life.

1.2 Importance to study

In the carrier-based (solid) formulations, the microorganisms have a shelf life of only six months. They are not tolerant to UV rays and temperatures of more than 30 degrees. The population density of these microbes is only 10^8 CFU/ml at the time of production. In the fourth month it reduces to 10^6 CFU/ml and at the end of 6 months the count was almost nil. These defects can be rectified and fulfilled in the case of liquid formulations. The shelf life of the microbes in these liquid formulations is higher than carrier-based formulations without considerable loss in viable counts. They are tolerant to high temperatures (55 degrees) and ultra violet radiations. This is especially feasible in Hyderabad-Karnataka where there is prevalence of high average temperatures. The viable cell count is as high as 10^9 CFU/ml, which is maintained constant during the period. So, the application of 1 ml of liquid-based formulations is equivalent to the application of 1 kg of 5 months old carrier-based formulations (1000 times). Since these are liquid formulations the application in the field is also very simple and easy. They are applied using hand sprayers, power sprayers, fertigation tanks and as basal manure mixed along with FYM etc. (Santhosh, 2015). Liquid inoculants could be produced with minimum labour, space and energy. Also, the quantity of inoculum required for application is less as compared to carrier-based formulations. It is easier for farmers to handle and apply liquid based formulation which could be considered as one potential strategy for improving the shelf life of bio fertilizer. Unlike solid carrier-based formulations, liquid formulations allow the manufacturer to include sufficient amount of nutrients, cell protectant and inducers

responsible for cell/spore/cyst formation to ensure prolonged shelf life. In present study shelf life and performance of liquid-based *Trichoderma harzianum* is analyzed with following objectives.

1.3 Objectives

1. To investigate the shelf life of *Trichoderma harzianum* using different oil formulations.

1.4 Hypothesis

In present study *Trichoderma harzianum* was selected as test organism. *Trichoderma* belong to the family of Hypocrycea. *Trichoderma* solid carriers are suitable for small scale production in cottage industries or at individual farmer level. It is laborious and results in a product which is bulky and prone for contamination. The shelf life of solid inoculants is about 180 days while chlamydospores preparations obtained by liquid fermentation are reported as more effective than conidial preparations in solid carriers. Oil formulations of *Trichoderma* supposed to be suitable for foliar sprays under dry weather and to have prolonged shelf life. The spores can survive for longer time in the plant surface even during the dry weather as the spores are covered by oil that protects them 5^0 C from drying. With the use of liquid inoculants, we can overcome the problems caused by solid carriers. Therefore, liquid inoculants have been developed to solve the problems associated with processing of solid carriers.

1.5 Scope and Limitation

Liquid biofertilizer formulation could be considered as one potential strategy for improving the shelf life of bio fertilizer. Unlike solid carrier-based bio fertilizers, liquid formulations allow the manufacturer to include sufficient amount of nutrient, cell protectant, and inducers responsible for cell/spore/cyst formation to ensure prolonged shelf life. The shelf life of common solid carrier-based bio fertilizers is around six months. However, it could be high as two years for liquid formulations.

Further, solid carrier-based formulations are less thermo-tolerant whereas, liquid formulations can tolerate the temperature as 55^0C. Hence, improved shelf life could be achieved by the application of a liquid biofertilizer formulation. However, process cost of liquid bio fertilizer is significantly higher than solid formulation. Thus, successful commercialization of less expensive liquid bio fertilizer is a challenge and shelf life of such products still concern.

Chapter II

REVIEW OF LITERATURE

An investigation entitled “Investigation of different oil formulations on shelf life of *Trichoderma harzianum*” was carried out during 2021-22. Attempt has been made to review literature on use of different carrier such as broth + surfactant + dispersant + suspender, paraffin oil, rice bran oil, mustard oil, coconut oil, mineral oil, market product (Liquid formulation of *Trichoderma harzianum),* departmental culture (liquid), broth and effect on shelf life of *Trichoderma harzianum.*

2.1 Shelf-life study of *Trichoderma harzianum* on different liquid formulations

Hartsell (1956) presented a brief summary on the longevity of certain bacterial cultures under paraffin oil. They used paraffin oil seal above the cotton-stoppered slant concluding that this method was practicable for the maintenance of a large culture collection. With the help of this method various strains of bacteria, yeast, Basidiomycetes and other fungi have survived up to 14 years, but in each group some strains showed loss of viability or loss of gain of characters. This report is a summary of the recent data and experience with the determination of longevity in these cultures.

Batta (2004) studied that from Israel made a formulation of *T. harzianum* in an invert emulsion (water-in oil formulation) based on coconut and soybean oils and found the viability to be 36 months with 50% reduction in viability after 5.3 months at 20±1˚C and compared with dry non formulated conidia which have a viability of only up to 2.7 months, respectively, for dry non-formulated conidia. Stability and viscosity of the formulation remained constant during the time period of viability study.

Kolombet *et al.* (2008) conducted study on development of an extended shelf life, liquid formulation of biofungicide *Trichoderma*

asperellum. The study showed that the submerged conidia formulation in cryoprotectant like glycerol, glucose and DMSO preserved the viability only up to 75 days. But its preservation in sterile deionized water retained the viability up to 720 days at temperature range of 25-32°C. The average loss in viability from initial storing till 720 days was only 25%. Hence this formulation can be commercialized.

Sathiyaseelan *et al.* (2009) investigated on evaluation of antagonistic activity and shelf-life study of *Trichoderma viride.* The study revealed that *Trichoderma* is a promising candidate for biological control of pathogenic fungi. While planning the application of antagonistic *Trichoderma* strain for the purpose of biological control, it is very important to consider the environmental parameter affecting the biocontrol agent in the soil. It was observed that the survibility of *Trichoderma viride* in paraffin oil was better than other formulation with 28×10^8 CFU/ml followed by soybean oil with 6×10^7 CFU/ml at 49th day. There was a steep decline of population level in formulation, sedimentation or buoyancy was recorded. The study showed that application of paraffin oil increase the shelf life of *Trichoderma* which was used as a biofungicide comparing to the solid formulation of biofungicide used.

Taweil *et al.* (2010) studied the comparison of different delivery system of *Trichoderma* and *Bacillus* as biofertilizer. The shelf life study reveal that the survivability of *Trichoderma viride* and *Bacillus megaterium* in alginate formulation with 1.2×10^5 and 7.2×10^6 CFU/ml comparing with paraffin oil which was better than other formulation with 1×10^4 and 0.6×10^5 CFU/ml followed by storage at 4°C with 1.8×10^4 and 2.0×10^4 CFU/ml at 210th day for *Trichoderma and Bacillus* respectively.

Subbaraman *et al*. (2011) studied liquid fermentation based formulations of *Trichoderma* spp. and was vulnerable to desiccation compared to solid state fermentation based formulations. The effect of the addition of glycerol, an osmoticant in the production medium on the

shelf-life of *Trichoderma harzianum* was studied. The addition of glycerol at 3, 6 or 9% reduced the water activity in the medium. Both in shaker culture and fermenters, the addition of glycerol in production medium prolonged the shelf-life of talc formulation. The addition of glycerol at 3 and 6% extended the shelf-life (with viability of 2×10^6 CFU g^{-1}) to 7 and 12 months, respectively compared to 4–5 months shelf-life in formulations derived without the addition of glycerol. Regression analysis showed that there was positive correlation between colony forming unit (CFU) and water activity. In bio-efficacy tests, even after storage for 12 months, formulations derived with the addition of glycerol at 3 or 6% in the production medium could protect the tomato plants from *Fusarium* wilt incidence by 44–50%.

Kumar *et al*. (2014) studied *Trichoderma viride* and *Trichoderma harzianum* have curved a niche for themselves in India as important biocontrol agents for management of various diseases. A number of successful products based on different species of *Trichoderma* have been commercialized in India. The potential *Trichoderma* isolates are formulated using different organic and inorganic carriers either through solid or liquid fermentation technologies. They are prepared by mixing the conidia harvested from the solid state/liquid state fermentation with a combination of vegetable/mineral oils in stable emulsion formulation. In such formulations, microbial agents are suspended in a water immiscible solvent such as a petroleum fraction (diesel, mineral oils), and vegetable oils (groundnut) with the aid of a surface active agent. This can be dispersed in water to form a stable emulsion. Oil-based formulations are supposed to be suitable for foliar sprays under dry weather and to have prolonged shelf life. The spores can survive for longer time in the plant surface even during the dry weather as the spores are covered by oil that protects them 5°C from drying.

Rai and Tewari (2016) studied total nine type of *Trichoderma* based formulations using formulating material *viz*., dextrin, talc, gypsum, paraffin oil, soybean oil. Shelf life of formulation was tested up to 6

months at refrigerator among different formulation dextin based formulation TF past (8) retained maximum viability (26.10%;4.33×10^7 CFU/g) followed by TF paste 9 (23.95%;4.00×10^7CFU/ml) and oil-based T.F LQ6 (22.43%; 9.67×10^7CFU/ml) after 6 month of storage at room temperature (15-35˚C). The formulation stored at 4˚C retained viability (2.06-16.06%) up to 11 months during storage. Maximum viability was observed in T.F paste 8 (16.06%;2.67×10^7 CFU/g) followed by T.F paste 9 (11.98%; 2.00×10^7CFU/g) and oil-based TF LQ 6 (8.89%; 3.38×10^7 CFU/ml). This study showed that there is potential in using of *Trichoderma* paste and liquid formulation for improving shelf life of bioformulation as well as in biological control.

Mujtaba and Kulkarni (2017) studied shelf life of *Trichoderma harzianum* an antagonist in different oil-based formulations. They prepared nine oils-based formulations by using canola oil, paraffin oil, soybean oil, glycerol and neem oil. Shelf life of these formulations were tested up to 12 months. Highest shelf life of 12 months was found in Canola oil + glycerol-based formulation (3×10^6 CFU/ml) followed by paraffin oil-based formulation (2×10^6 CFU/ml).

Reddy *et al.* (2017) studied antagonistic activity and shelf-life study of *Trichoderma harzianum*. The experiment was conducted up to 56^{th} days and shelf life of *Trichoderma harzianum* was calculated in the form of CFU. In the last day of observation i.e., on 56^{th} day of observation colony count of *Trichoderma harzianum* was $20x10^7$ and $2.1x10^6$ in paraffin oil and soyabean oil respectively indicating that these oils could retain the spore viability for longer period compared to other oils and its combination used in the studies but higher colonies were noted in former oil only.

Manandhar *et al*. (2018) studied that *Trichoderma* is a well-documented biocontrol agent of fungus origin being used for the control of various plant pathogens as people are being aware of the various detrimental effects of chemical pesticides. In Nepal also, the biocontrol agents are gaining popularity and there are several *Trichoderma*

products in different trade names available in the market with label that they can be used within six months from the date of manufacture. However, it is very essential to know the fact that these microbial products contain living micro-organisms and therefore the conditions of storage for long term viability of the product should be given prior importance. This study was performed to find out the shelf life of *Trichoderma viride* based on various formulations and to know the effect of temperature on its viability. It was found that at room temperature the greatest viability percentage in rice husk and least in talc. After 3 months of storage, the viability observed in rice husk, paraffin oil and talc were 73.3%, 26.53% and 0.3% respectively. At the end of six months, the CFU count in rice husk was also the greatest ($21.6x10^5$) followed by paraffin ($6.6x10^2$) and no CFU could be observed in talc.

Nadare *et al*. (2018) studied assessment of shelf life of *Trichoderma viride* on different liquid formulations. The shelf-life study revealed that there were significant differences in *Trichoderma viride* at all the interval. The initial population of *T. viride* in first month was found maximum ($31.2x10^5$ CFU/ml) in T_2 (T_1 + Paraffin oil) which was significantly superior over all other treatments. They also showed that the second-best treatment in terms of colony forming units of *T. viride* was T (T + Soybean oil) where 21.5×10^5 CFU/ml population was noticed in first month. These treatments were followed by treatment T8 (Departmental culture, 19.6 CFU/ml) and T_3 (T_1 + Mustard oil, 18.9 CFU/ml). The population of *T. viride* was found to be gradually decreased over a period of time. At the end of six-month maximum population of *T. viride* (24.5 CFU/ml) was observed in the treatment T_2 (T_2 + Paraffin oil) which was significantly superior over all other treatments, it was followed by T_4 (T_1 + Groundnut oil, 14.6 CFU/ml) and T_8 (Departmental culture, 13.6 CFU/ml).

Taral *et al.* (2018) undertaken studies on evaluation of different oils on shelf-life study of *Trichoderma viride* was carried out during 2017-2018 at the Department of Plant Pathology, College of Agriculture,

Nagpur. Different oils *viz*., Paraffin oil, Soybean oil, Mineral oil, Potato Dextrose broth and Talc was extensively used as carrier for *Trichoderma viride*. As compared to solid based carrier material the survival of *Trichoderma viride* in liquid-based formulations is quite high and has the ability to limit the heat transfer and 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 *Trichoderma viride* in the paraffin oil was 28.06 x 10^8 CFU/ml in the 1st month whereas, the population density of *Trichoderma viride* in the 6th month was 18.33 x 10^8 CFU/ml. It was found significantly superior over all other treatment.

Sreenayana and Nakkeeran (2019) reported that cucumber vascular wilt pathogen, *Fusarium oxysporum* f. sp. c*ucumerinum* is one of the most destructive organisms that hampers the cucumber production under protected cultivation and results in huge economic loss to farmers. *Trichoderma* spp., a potent biocontrol agent screened against *F. oxysporum* f. sp. c*ucumerinum in vitro* resulted that *T. virens* TRI 37 effectively inhibited the mycelial growth of pathogen to about 68.0% compared to other isolates. An oil-based formulation of effective *Trichoderma* spp. was developed with initial conidial concentration of 1x10^{10}. The formulation remained stable for more than 180 days with conidial concentration of 1 x 10^8. *Trichoderma* emulsion contained the following ingredients viz., 1% glycerol (as an osmoticant), 1% PVP, 0.5% ZnSO4 (increases the shelf life), 1% Tween 20 (emulsifying agent), distilled water and coconut oil. The aqueous phase was prepared by adding 1% conidia, 1% glycerol, 1% PVP and 0.5% ZnSO4 in 80 ml sterile distilled water and the oil phase was prepared by adding 1% tween 20 in 20 ml of coconut oil. The oil phase was then added to aqueous phase and stirred well. The pH of the formulation was adjusted to 6.5-7.0. All the formulation had an initial conidial concentration of 1 x 10^8 per ml. The prepared formulations were distributed in four different falcon tubes and stored at room temperature.

Herrera *et al.* (2020) reported that environmental conditions affect biocontrol agents in a field, being appropriate formulations an alternative to overcome this problem. Formulations based on *Trichoderma asperellum* TV190 were prepared by emulsified mineral or vegetable oils, which protected spores from ultraviolet radiation, showing greater viability of 37–43% (mineral) and 56–63% (vegetable) than the control (8–12%). These formulations improved an antagonism of *T. asperellum* on *Rhizoctonia solani* under greenhouse conditions, reducing infected corn seedlings by 72% (mineral) and 59% (vegetable). Necrotic spot size was reduced by 90.04% (mineral) and 87.29% (vegetable).

Mane *et al*. (2020) conducted study on “Comparison of different oil formulations on shelf life of *Trichoderma asperellum*” was carried out during 2018-2019. Paraffin oil, soybean oil, groundnut oil, potato dextrose broth and talc powder were extensively used as carrier for *Trichoderma asperellum* at an interval of 30, 60, 90, 120, 150 and 180 DAI. 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 28 x 10^8 CFU at 30 DAS and it was significantly superior over all other treatment except Talc based culture 27.33 x 10^8 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 Per cent spore germination

Taral *et al.* (2018) studied on “Evaluation of different oils on shelf-life study of *Trichoderma viride*” was carried out during 2017-2018 in the department of Plant Pathology, College of Agriculture, Nagpur. Different oils viz., Paraffin oil, soybean oil, mineral oil, Potato Dextrose broth and talc was extensively used as carrier for *Trichoderma viride*. Shelf-life studies clearly indicated significant differences at all the intervals. Population density of *Trichoderma viride* in the paraffin oil was 28.06 x 10^8 CFU/ml in the 1st month whereas the population density of *Trichoderma viride* in the 6th month was 18.33 x 10^8 CFU/ml. It was

found significantly superior over all other treatment. The effect of liquid formulations on spore germination of *Trichoderma viride* was also recorded. Maximum spore germination was observed in the formulation containing paraffin oil after six months of storage.

Mane *et al*. (2020) conducted study on “Comparison of different oil formulations on shelf life of *Trichoderma asperellum*” was carried out during 2018-2019. The laboratory experiment was carried out in Completely Randomized Design with nine treatments in three replications. Paraffin oil, soybean oil, groundnut oil, potato dextrose broth and talc powder were extensively used as carrier for *Trichoderma asperellum* at an interval of 30, 60, 90, 120, 150 and 180 DAI. 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 28 x 10^8 CFU at 30 DAS. It was further noticed that maximum spore germination per cent was noticed in Paraffin oil 59.18 per cent at 30 DAI. There were significantly reduction in spore germination from 60^{th} DAI till 180^{th} DAI in all the treatment.

2.3 Per cent growth inhibition

Many researchers have reported the antagonistic activity of *Trichoderma* isolates against the plant pathogens, particularly against fungal pathogens such as *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotium rolfsii* and *Verticillium dahlia*

Rajput *et al.* (2010) studied on evaluation of bio-agent against complex chickpea wilt complex. They showed that *Trichoderma viride* exhibited antagonist effect against *Fusarium oxysporum* f. sp*. ciceri* more extensively*.*

Siameto *et al.* (2010) evaluated study on antagonism of *Trichoderma harzianum* isolates on soil born plant pathogenic fungi from Embu District, Kenya. They revealed that the most common biological control agents of the genus are strains of *T. harzianum, T. viride* and *T. viriens*. In this study, sixteen selected isolates of *T. harzianum* from

different land use types in Embu, Kenya was tested for antagonism against five soil borne phytopathogenic fungi (*Rhizoctonia solani, Pythium* sp*, Fusarium graminearum, F. oxysporum* f. sp*. phaseoli and F. oxysporum* f. sp*. lycopersici*) using dual culture assay and through production of nonvolatile inhibitors. Seven isolates were further characterized using RAPD-PCR procedure to determine genetic variability. All *T. harzianum* isolates had considerable antagonistic effect on mycelial growth of the pathogens in dual cultures compared to the controls

Perveen *et al.* (2012) conducted study on antagonistic activity *Trichoderma harzianum* and *Trichoderma viride* isolated from soil collected from date palm field against *Fusarium oxysporum.* The result showed that all antagonist significantly inhibited the mycelial growth of the pathogen. It was found that *T. viride* inhibited the growth of the pathogen more than other two isolates of *T. harzianum.*

Srivastava *et al.* (2012) studied the antagonistic potentiality of *Trichoderma* species against *Fusarium oxysporum* f. sp*. ciceri* (foc), *Fusarium oxysporum* f. sp*. udum* (fou) and *P. aphanidermatum* were tested, maximum inhibition (65.00%) of mycelial growth was recorded against *P. aphanidermatum* followed by (63.66) *Fusarium oxysporum* f. sp. *udum* (fou) and (62.00%) *Fusarium oxysporum* f. sp. *ciceri*.

Tapwal *et al.* (2015) studied the two *Trichoderma* species, (*T. viride and T. harzianum*) were screened against five seed borne phytopathogens (*Curvularia lunata, Fusarium oxysporum, Alternaria alternata, Colletotrichum gloeosporioides and Rhizoctonia solani*) by dual culture technique and the efficacy of volatile and non-volatile metabolites released by them was evaluated by ‘inverted plate method’ and ‘poisoned food technique’. Both antagonists have exerted inhibitory effect on the growth of selected seed borne phytopathogens to a varied extent.

Sreeshma and Jose (2016) studied on comparison of antagonistic activity of *Trichoderma viride* against selected species of fungal pathogens. Antagonistic activity of *Trichoderma viride* is maximum against *Fusarium oxysporum* (85.7±0.3%) and least per cent inhibition was recorded by *Aspergillus niger* (42.82±0.9%).

Adhikary *et al.* (2017) studied the antagonist *T. asperellum* CP (IPM 33) was tested against *F. oxysporum* f. sp. *melongenae* on PDA in dual culture method. *T. asperellum* CP (IPM 33) caused 100% growth inhibition of *F. oxysporum* f. sp. *melongenae.*

Babychan and Simon (2017) studied the antagonistic activity of *Trichoderma* isolates was screened against the soil borne plant pathogenic fungi *Fusarium oxysporum* f.sp. *lycopersici* were studied to record the mycelial inhibition. Among the treatments, T_4 with MiT-4 (58.40%) followed by T_5 with MiT-5(57.33%), T_6 with MiT-6(57.33%), T_7 with MiT-7(57.33%), T_8 with MiT-1(55.32%), T_2 with MiT- 2(55.2%), T_3 with MiT-3(55.2%) and T_1 with MiT- 1(53.60%).

Cherkupally *et al.* (2017) studied *in vitro* antagonistic activity of *Trichoderma* species against *Fusarium oxysporum* f. sp. *melongenae. T. harzianum* showed maximum extent of inhibition 81.11%, followed by *T. koningii* 80.00%, *T. pseudokoningii and T. viride* 78.88% each, *T. virens*, *T. atroviride* and *T. reesei* 77.77% each.

Patole et al. (2017) studied *in vitro* evaluation of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium wilt* of chickpea. The result indicated that the combined effect of both antagonists (*T. viride* + *T*. *harzianum*) was found to be most effective (86.57%) in inhibition of *Fusarium* mycelia growth as compared to the control followed significantly by *T*. *harzianum* (82.57%) and *T. viride* (80.24%).

Rajendraprasad *et al.* (2017) studied *in vitro* evaluation of fungicides and bio control agents against *Rhizoctonia solani* in Tomato. The present study revealed that the native *T. harzianum* -1 recorded maximum (62.53%) inhibition of the pathogen and found superior over

control followed by native *T. harzianum* -5 (51.63%) and *T. harzianum* -6 (49.50%). However, most of the *Trichoderma* isolates recorded less than 50 per cent inhibition over control and minimum inhibition was observed in *T. viride*-6 (36.6%).

Reddy *et al.* (2017) studied antagonistic activity and shelf life study of *Trichoderma harzianum* (Rifai). The inhibition per cent was 56.7 with clear inhibition zones in *Fusarium solani*, whereas highest per cent inhibition of 66.60 was observed with *Sclerotium rolfsii* at an incubation period of 7 days, but only 30.90 per cent inhibition was recorded with *Fusarium oxysporum* on 7th day.

Nadare *et al*. (2018) shelf-life studies of *Trichoderma viride* was executed by using different carriers. The paraffin oil and soybean oil were recorded maximum inhibition against *Fusarium oxysporum* f. sp. *ciceri* (93.90 and 92.63 per cent, respectively) as compared to control than other organism.

Taral *et al*. (2018) studied the effect on per cent growth inhibition on *Fusarium oxysporum* f. sp. *ciceri* is 79.30 per cent on *Rhizoctonia bataticola* is 84.85 per cent and *Sclerotium rolfsii* is 79.14 per cent. It was recorded maximum in formulation containing paraffin oil it was found significantly superior over all the treatments.

Nwankiti and Gwa (2018) studied the highest percentage growth inhibition of mycelia of the pathogen (77.99%) was recorded when *T. harzianum* was introduced two days before the inoculation of *F. oxysporum,* followed by introduction of *T. harzianum* same time with *F. oxysporum* (45.69%) and the least percentage growth inhibition (13.72%) was recorded when the antagonist was introduced two days after inoculation of the pathogen.

Abhiram and Harison (2018) studied on *Trichoderma viride* tested against *Fusarium oxysporum* strains under in vitro conditions. The results revealed that *Trichoderma viride* showed maximum inhibition 71.00% over *Fusarium oxysporum* strain (E) and minimum inhibition 62.50% over *Fusarium oxysporum* strain (D) in dual culture plate technique. *Trichoderma viride* showed maximum inhibition 45.27% over *Fusarium oxysporum* strain (E) and minimum inhibition 14.72% over *Fusarium oxysporum* strain (C) in sealing agar plate method.

Boblina *et al.* (2020) studied the efficient management of foliar and soil-borne pathogens makes *Trichoderma* one of the most used biocontrol agents in the world. Forming formulations with enhanced shelf life and broad-spectrum activity could help accelerate commercialization of these microorganisms. *Trichoderma* Isolates 2 and 5 were collected from paddy and groundnut rhizosphere soil. Their vigorous growth in different culture media and profound inhibitory effects on two potential soil-borne pathogens, *Rhizoctonia solani* and *Sclerotium rolfsii*, were the basis for selecting these two isolates from seven others collected from crop rhizospheres for mass production. Research assesses nine liquid substrates and one talc-based formulation. CFU were counted and used serial dilution method and gradually declined over six months. Coconut water was the best liquid substrate for both isolates (Tr Isolate 2 and Tr Isolate 5) in the first month, with 170.00 x 10^7 cfu/ml and 99.00 x 10^7 cfu/ml respectively. Maximum talc-based formulation count was 44.33 x 10^7 cfu/g in Tr Isolate 2 in the first month. Tr Isolate 5 had the highest cfu count of 48.00 x 10^7 cfu/g in the first month after inoculation.

Mane *et al*. (2020) conducted study on “Comparison of different oil formulations on shelf life of *Trichoderma asperellum*” during 2018-2019. Paraffin oil, soybean oil, groundnut oil, potato dextrose broth and talc powder were extensively used as carrier for *Trichoderma asperellum* at an interval of 30, 60, 90, 120, 150 and 180 DAI. 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 28 x 10^8 CFU at 30 DAS. As regards of per cent growth inhibition at 8^{th} DAI, among three plant pathogenic fungi *F. oxysporum* inhibited maximum growth 74.44 per cent followed by *R. bataticola* 81.85 per cent and *S. rolfsii* in paraffin oil treatment.

Chapter III

MATERIAL AND METHODS

A study on liquid formulation entitled “Investigation of different oil formulations on shelf life of *Trichoderma harzianum***”** 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, 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 Carrier material

Different type of oils such as paraffin oil, mustard oil, mineral oil, rice bran oil, coconut oil from Section of Plant Pathology, College of Agriculture, Nagpur. Which is incorporated into the *Trichoderma harzianum* formulation with additives such as dispersant, suspender and surfactant.

3.1.4 Pure culture

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

3.2 Methods

3.2.1 Sterilization of glassware, media and distilled water

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

3.2.2 Preparation of Potato Dextrose Agar Medium

For maintenance and purification of fungal culture PDA media was used. PD broth was used for mass multiplication of fungi.

The medium was prepared by using the following ingredients.

Agar-agar	-	20g
Dextrose	-	20g
Potato	-	200
Distilled water	-	1000 ml

Potato Dextrose Broth

Potato	-	200g
Dextrose	-	20g
Distilled water	-	1000ml

3.2.3 Experimental Details

Duration	-	2021 -22
Design	-	CRD
Treatments	-	7
Replications	-	3

3.2.4 Treatment Details

Each treatment contained with Glycerol (10ml) + Dispersant (1ml) + Suspender (3ml) + Surfactant (3ml)

Treatment	Treatment details
T_1	Paraffin oil (63ml) + *Trichoderma harzianum* filtrate (20ml)
T_2	Rice bran oil (63ml) + *Trichoderma harzianum* filtrate(20ml)
T_3	Mustard oil (63 ml) + *Trichoderma harzianum* filtrate(20ml)
T_4	Coconut oil (63 ml) + *Trichoderma harzianum* filtrate (20ml)
T_5	Mineral oil (63ml) + *Trichoderma harzianum* filtrate (20ml)
T_6	Department culture (liquid)
T_7	Market product (liquid)

3.2.5 Oil base liquid formulation

Mass multiplied *Trichoderma harzianum* was transferred in to mixing tank to harvest the spore and mycelium. Mixed *Trichoderma harzianum* formulation was poured into presterilized plastic bottles. Each treatment contained Glycerol (10ml), Dispersant (1ml), Surfactant (3ml), Suspender (3ml). Three oils are used viz., paraffin oil, rice bran oil, mastered oil, coconut oil and mineral oil were incorporated into the *Trichoderma harzianum* formulation in each plastic bottles as per the given in treatments from T_1 to T_5. Whereas T_6 was departmental culture, T_7 was liquid formulation market product. The bottles were packed with the help of caps and kept for a storage for six months at 27 ± 1^0 C. CFU count was under taken at monthly interval by serial dilution followed by pour plate method.

3.2.6 Pour plate method

Make a serial dilution by transferring one ml of suspension to the 1^{st} water blank and subsequent tube to get dilution 10^7. The desired suspension was transferred (10^8) to sterilized petriplates. Poured 20 ml of melted medium and allowed to cool. Rotated the petriplate gently and allowed to solidify and incubated the Petri plate for three days and allow to grow *Trichoderma* colonies.

3.2.7 Colony forming unit count by serial dilution method

After two days of incubation the total numbers of colonies were counted on the petriplate and calculated as follows (Baghel *et al*. 2014)

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

3.2.8 Spore germination by hanging drop method

Firstly, the glass slide was cleaned and flamed a hanging drop slide over the spirit lamp and placed it on the table with the depression uppermost. Small amount of vaseline was spread around cavity slide. After that the coverslip was cleaned and vaseline was applied on each corners of coverslip using matchstick. The coverslip was placed on a clean paper with the vaseline side up. One loopful of culture was transferred at the centre of coverslip. The depression slide was placed on the coverslip with the cavity facing down so that depression covers the suspension. The slide kept gently to form a seal between the coverslip and the slide. Hanging drop preparation turned quickly right side up as a result of which the drop was suspended. The preparation was observed under low power objective with reduced light. After that a drop of oil was placed on the coverslip and the preparation was observed under oil-immersion objective. (Aneja, 1993)

Per cent spore germination were calculated by the following formula (Chandel and Pimpalgaonkar, 2014).

$$\% \text{ Spore Germination} = \frac{\text{Number of spores germinated}}{\text{Total number of spores examined}} \text{ x } 100$$

3.2.9 Antagonistic effect by filter-paper disc agar method

PDA media was poured in sterilized petriplate. Then the agar surface was allowed to solidify for five minutes. A sterile filter paper disc was picked up by outer edge using a flamed, sterile forceps and the filter paper disc were dipped in the *Trichoderma* oil formulations for 30

minutes. The disc was placed on the PDA poured plate near the edge of agar surface. The test fungus was grown separately on PDA medium. Seven days old culture of test fungus was used for plating. With the help of sterilized cork borer the disc was cut of 5mm diameter of seven days old culture of test fungus. A disc of test pathogen placed exactly opposite to the disc of *Trichoderma* oil formulations leaving 10 mm away from edge, so that both the fungi got equal opportunity for their growth. The plates without antagonistic were served as control. The plates were incubated at 27±1^0 C under room conditions. After 8-10 days the observations were recorded.

After the incubation period, the radial growth of form in control, as well as in treatment plate was measured and the per cent inhibition was calculated using the formula (Vincent, 1927)

$$L = \frac{C - T}{C} X\ 100$$

Where,

L = Per cent inhibition of radial growth of pathogen (%)

C = Radial growth of the pathogen (mm) in control

T = Radial growth of the pathogen (mm) in treatment

3.3.2 Analysis of data

The data of various experiments was analysed 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

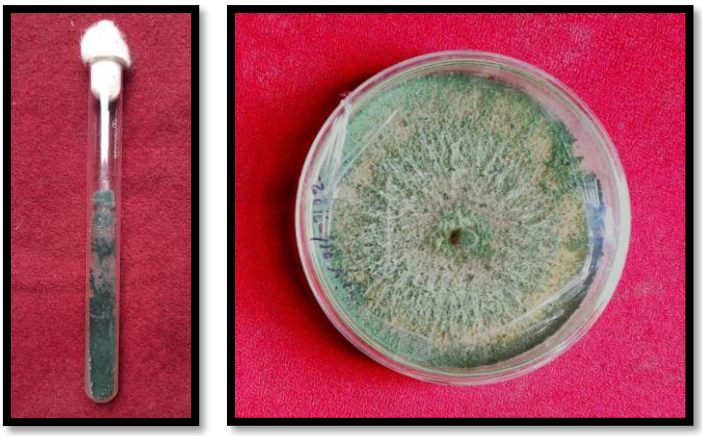
RESULTS AND DISCUSSION

Research study on “Investigation of different oil formulations on shelf life of *Trichoderma harzianum*” was undertaken in the Plant Pathology Section, College of Agriculture, Nagpur during the year 2021-22. The experiment was designed in CRD with seven treatments in three replications. The results are presented in tables, depicted in figures and plates in each head in this chapter.

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

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

The data is presented in Table 1, figure 1 and plate 3. It is observed from the Table 1 that there were differences at all the interval over market product . Among the treatment paraffin oil formulation recorded ($33.33x10^8$CFU/ml) in first month and was found significantly superior as all other treatment. It was followed by coconut oil formulation treatments ($22.40x10^8$CFU/ml) and Department culture ($22.00x10^8$ CFU/ml). Minimum spore count was observed in market product ($10.42x10^8$ CFU/ml). Taral *et al*. (2018) and Mane *et*. *al*. (2020) observed similar results. With regard to II month the significant differences were noticed. Maximum population was recorded by the paraffin oil treatment ($30.55x10^8$CFU/ml) followed by coconut oil treatment ($18.40x10^8$ CFU/ml). The treatment of paraffin oil were found to be significantly superior all over the treatments. Similar observation was recorded by Nadare *et al*. (2018). Market product recorded lowest spore count.



A: Pure culture of *Trichoderma harzianum* in petriplate and slant

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

Plate1: (A) Pure culture of *Trichoderma harzianum*,
(B) Microscopic view of conidia of *Trichoderma harzianum* at (40x)

A: Mass multiplication of *Trichoderma harzianum*

B: General view of experiment

Plate 2: Set of *Trichoderma harzianum*

Table No.1: Effect of different liquid formulation on the shelf life of *Trichoderma harzianum* (10^{8}CFU/ml) at various interval

Tr. No.	Treatment	Total no. of colonies/ml					
		Month					
		I	II	III	IV	V	VI
T_1	*Trichoderma harzianum* filtrate (20 ml) + Paraffin oil (63ml)	33.33	30.55	27.33	23.17	20.50	18.67
T_2	*Trichoderma harzianum* filtrate (20 ml) + Rice bran oil (63ml)	17.00	16.53	15.08	12.43	9.73	7.83
T_3	*Trichoderma harzianum* filtrate (20 ml) + Mustard oil (63ml)	19.33	17.90	16.00	15.30	13.30	11.97
T_4	*Trichoderma harzianum* filtrate (20 ml) + Coconut oil (63ml)	22.40	18.40	16.53	15.70	14.57	12.50
T_5	*Trichoderma harzianum* filtrate (20 ml) + Mineral oil (63ml)	11.33	10.39	8.58	7.86	5.77	3.10
T_6	Departmental culture (liquid)	22.00	17.87	16.03	14.60	13.50	12.07
T_7	Market Product (liquid)	10.42	8.64	7.80	6.97	4.00	2.03
	F test	Sig	Sig	Sig	Sig	Sig	Sig
	SE ± a(m)	0.80	0.53	0.79	0.83	0.87	0.72
	CD (1%)	2.29	2.04	2.26	2.38	2.50	2.06

It is evident from the table-1 and figure 1 that there was significant difference on number of population /ml at IIIrd month. As compared to Ist and IInd month there was gradual decline in the population of *T. harzinum* in all the observation. Maximum population of *T. harzinum* was noticed by the paraffin oil treatment (27.33x10^{8}CFU/ml) and was found significantly superior over all other treatments. It was followed by coconut oil treatment (16.53 x 10^8 CFU/ml). These observation are in line with the reports of Rai and Tiwari (2016), Mujtaba and Kulkarni (2017), Reddy *et al*. (2017) and Taral *et al*. (2018).

It was further noticed from the data formulated in Table 1 and depicted in figure 1 that there was significant difference in 4, 5 and 6

month of storage. Maximum population was obtained with the treatment of paraffin oil reading 23.17, 20.50 and 18.67 CFU/ml at IV, V and VI month respectively. The finding of this investigation are in line with these of Sathiyaseelan *et al*. (2009) Taweil *et al.* (2010) an Subbaraman *et al*. (2011).

The next best treatment was coconut oil which reading 15.70, 14.57 and 12.50x10^8CFU/ml. Similar observation recorded by Kummar *et al.* (2014) and Sreenayana and Nakkeeran (2019). There was gradual decrease in count in all the treatment on the month progresses. It is appeared form the data (Table 1) that in general there was decline in *T. harzianum* population form the I till VI months however paraffin oil was found to be the best treatment suggecting that *T. harzianum* liquid formulation can be up to six month followed by coconut oil formulations. There finding supported by Batta (2004), Kolombat *et al*. (2008), Taweil *et al.* (2010), Manandhar *et al*. (2018) and Mane *et al*. (2020).

With regard to department culture treatment it was found significantly superior at all interval reading 22,17.87, 16.03, 14.60, 13.50 and 12.07x10^8CFU/ml over market product (10.42, 8.64, 7.80, 6.97, 4.0 and 2.03x10^8CFU/ml at I, II, III, IV, V and VI respectively.

It was at par with mustard oil formulation 11.9x10^8CFU/ml and mineral oil formulation 3.10x10^8CFU/ml. Similar finding have been recorded by Taral *et al.* (2018) and Mane *et al*. (2020).

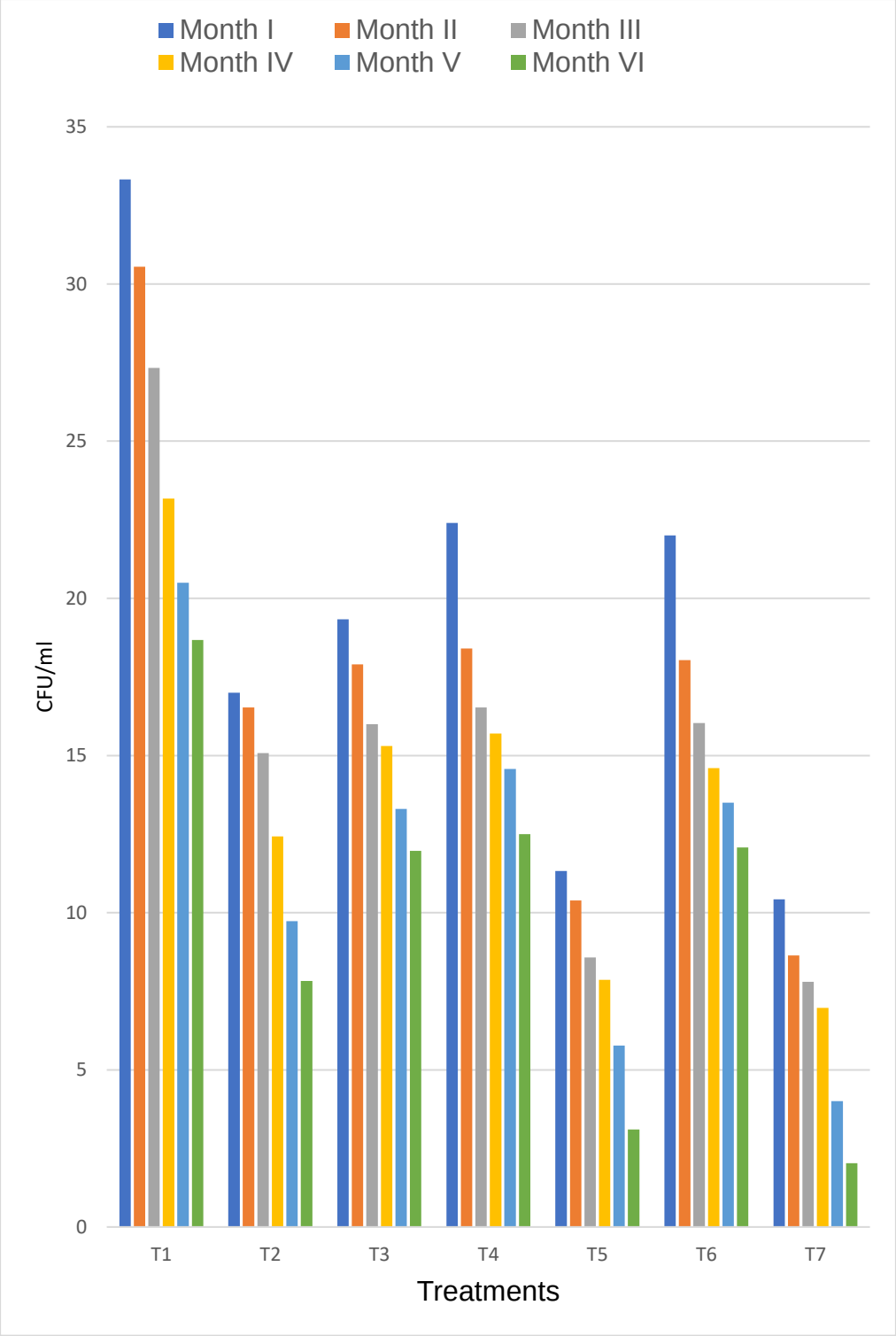
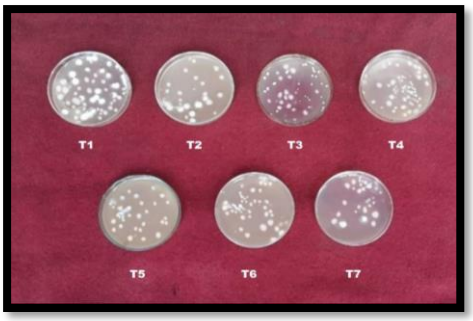
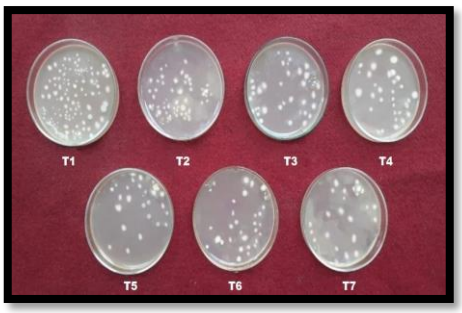


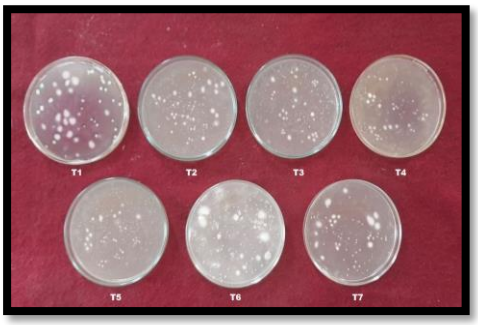
Fig No.1: Effect of different liquid formulation on the shelf life of *Trichoderma harzianum* (×10^{8}CFU/ml) at various interval



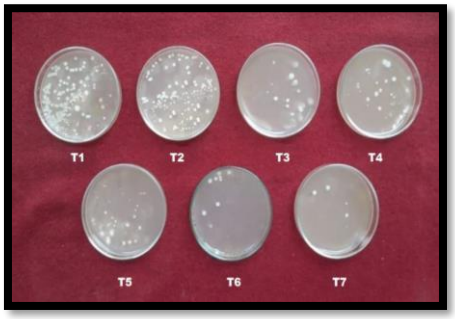
(A) Colony count at I month



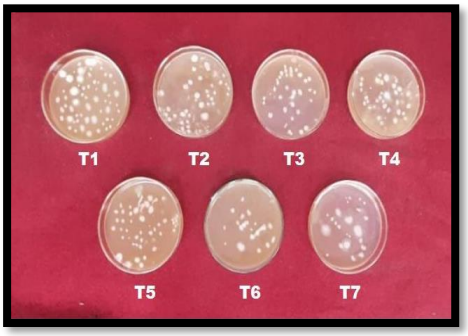
(B) Colony count at II month



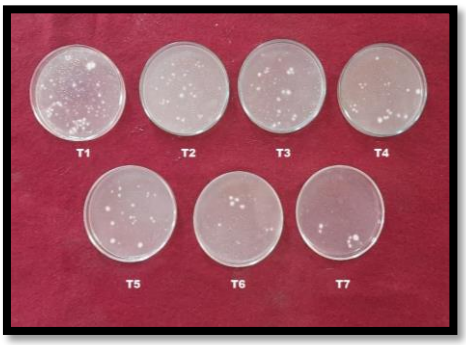
(C) Colony count at III month



(D) Colony count at IV month



(E) Colony count at V month



(F) Colony count at VI month

Plate 3: Colonies of *Trichoderma harzianum* observed at various interval

4.2 Effect of different liquid formulations on the per cent spore germination of *Trichoderma harzianum* at various interval

The per cent of spore germination count was taken for six month duration. The data generate during the investigation are presented in Table-2 and depicted in figure-2. It is apparent from the result (Table-2) that all the liquid formulation treatment significantly affected spore germination over market product treatment. Maximum germination was noticed by the paraffin oil formulation treatment 60.83 at I month. Then it reduced to 54.67 in II month, 46.53 per cent in III month, 39.16, 30.00 and 25.00 per cent in IV, V and VI month respectively.

Table No. 2: Effect of different liquid formulations on the per cent spore germination of *Trichoderma harzianum* at various interval

Tr. No.	Treatment	% Spore Germination					
		I	II	III	IV	V	VI
T_1	*Trichoderma harzianum* filtrate (20 ml) + Paraffin oil (63ml)	60.83	54.67	46.83	39.16	30.00	25.00
T_2	*Trichoderma harzianum* filtrate (20 ml) + Rice bran oil (63ml)	41.00	32.00	25.27	17.67	11.17	6.03
T_3	*Trichoderma harzianum* filtrate (20 ml) + Mustard oil (63ml)	42.17	33.50	27.17	21.92	12.93	7.50
T_4	*Trichoderma harzianum* filtrate (20 ml) + Coconut oil (63ml)	47.00	39.58	31.33	24.83	17.00	15.25
T_5	*Trichoderma harzianum* filtrate (20 ml) + Mineral oil (63ml)	33.58	27.67	21.33	16.33	9.16	5.83
T_6	Departmental culture (Liquid)	43.57	36.00	29.10	22.50	13.33	8.67
T_7	Market Product (liquid)	31.67	25.08	20.50	13.50	7.83	4.33
	F test	Sig	Sig	Sig	Sig	Sig	Sig
	SE ± (m)	0.72	0.61	0.61	0.62	0.61	0.51
	CD (1%)	2.88	2.48	2.44	2.45	2.47	2.067

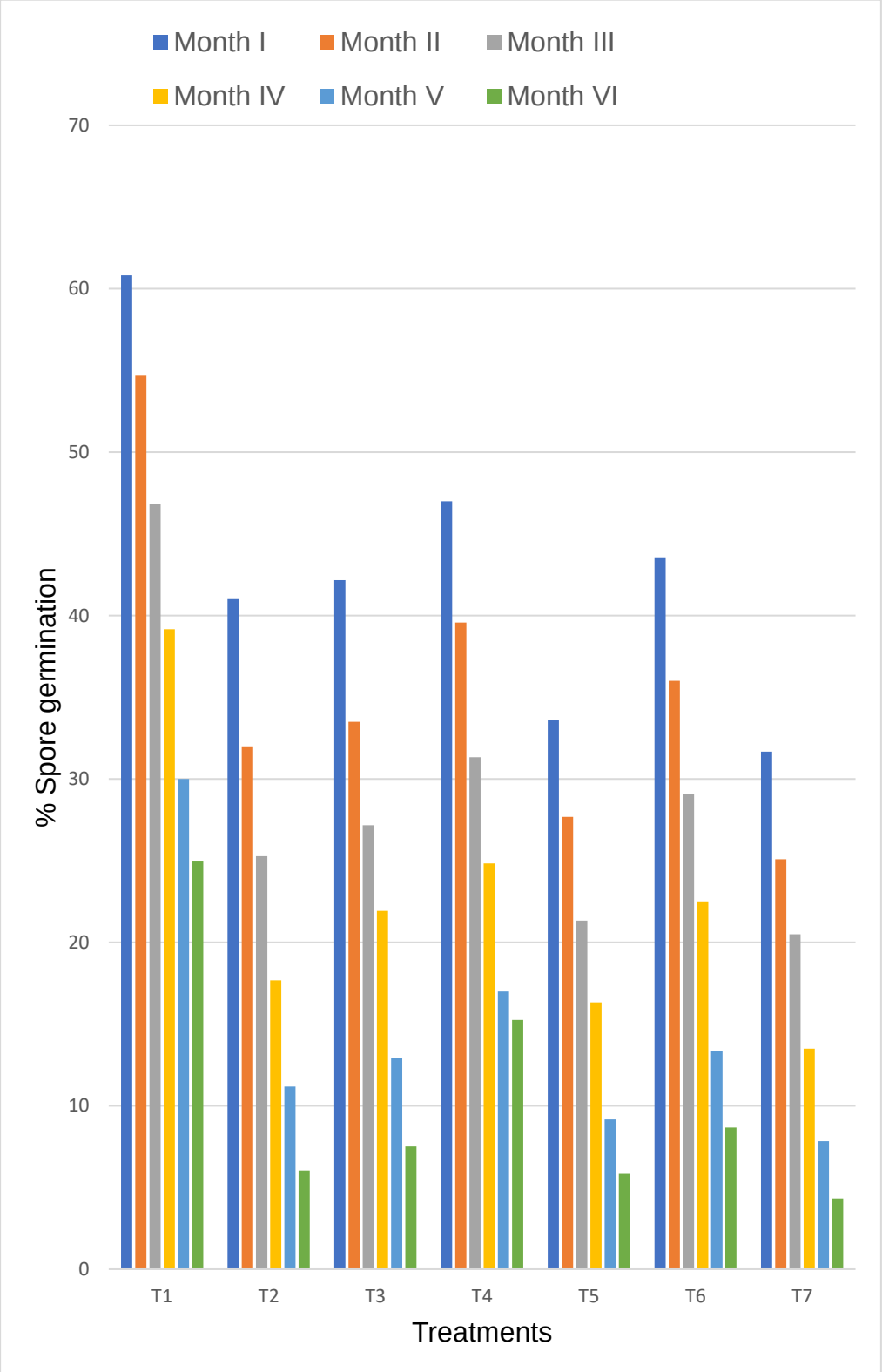


Fig No. 2: Effect of different liquid formulations on the per cent spore germination of *Trichoderma harzianum* at various interval

It was found significantly superior over rest of the treatment. Taral *et al*. (2018) also found significantly superior spore germination to the extent of maximum in first month due to paraffin oil treatment and then reduced at six month storage period. The next best treatment was coconut oil formulation treatment registering 47.00, 39.58, 31.33, 24.83, 17.00 and 15.25 per cent in I, II, III, IV, V and VI month respectively. Statistically it was found significantly superior over all other treatment except paraffin oil formulation treatment. Various worker have examined the storability of liquid formulation and increase in spore count Sreenayana and Nakkeeran (2019), Taral *et al*. (2018) and Mane *et al*. (2020).

4.3 Effect of *Trichoderma harzianum* liquid formulation on per cent growth inhibition on 8th DAI.

All the treatments significantly inhibited the radial mycelial growth of *Fusarium oxysporum* f. sp*. ciceri* and *Rhizoctonia bataticola* over uninoculated control. The observations recorded in (Table-3, Fig-3, Plate-4A) showed that treatment Paraffin oil T_1 was found significantly superior to the rest of treatments in checking the growth of *Fusarium oxysporum* f. sp. *ciceri.* It showed 20.03 mm mean colony diameter against the uninoculated control (45 mm) with per cent inhibition of 55.49 at 8th DAI. There result are in close conformity with those of Srivastava *et al.* (2012), Cherkupally *et al.* (2017), Patole *et al*. (2017), Reddy *et al*. (2017), Nadare *et al*. (2018), Taral *et al*. (2018), Nwankiti and Gwa (2018) and Abhiram and Harison (2018). It was followed by the T_4 Coconut oil treatment, T_3 Mustard oil treatment and T_2 Rice bran oil with mean mycelial growth 24.20 mm in T_4, 24.40 mm in T_3, 26.17 mm in T_2, with per cent growth inhibition of 46.23 in T_4, 45.78 in T_3 and 41.85 in T_2 respectively.

The data regarding *Fusarium oxysporum* f*.* sp*. ciceri* mention in (Table-3, Fig-3, Plate-4A) showed that there was significant inhibition percentage of *F*. *oxysporum* f*.* sp*. ciceri* by paraffin oil treatment*.* Similar result have been reported by the Rajput *et al*. (2010), Siameto

Table No. 3: Effect of liquid formulations of *Trichoderma harzianum* on per cent growth inhibition on 8th DAI

Tr. No.	Treatment	Mycelial growth (mm)		% Growth inhibition	
		Fusarium oxysporum* f. sp. *ciceri	***Rhizoctonia bataticola***	***Fusarium oxysporum* f. sp. *ciceri***	***Rhizoctonia bataticola***
T_1	*Trichoderma harzianum* filtrate (20 ml) + Paraffin oil 63ml)	20.03	16.10	55.49	82.11
T_2	*Trichoderma harzianum* filtrate (20 ml) + Rice bran oil (63ml)	26.17	25.00	41.85	72.22
T_3	*Trichoderma harzianum* filtrate (20 ml) + Mustard oil(63ml)	24.40	23.38	45.78	74.02
T_4	*Trichoderma harzianum* filtrate (20 ml) + Coconut oil (63ml)	24.20	22.83	46.23	74.63
T_5	*Trichoderma harzianum* filtrate (20 ml) + Mineral oil (63ml)	26.93	28.67	40.16	68.14
T_6	Departmental culture (Talc)	30.10	30.77	33.12	65.81
T_7	Market product(liquid consortia)	32.33	33.00	28.16	63.33
	Control	45.00	90.00		
	F test	Sig	Sig		
	SE ± m	0.72	0.86		
	CD (P=1%)	2.88	2.45		

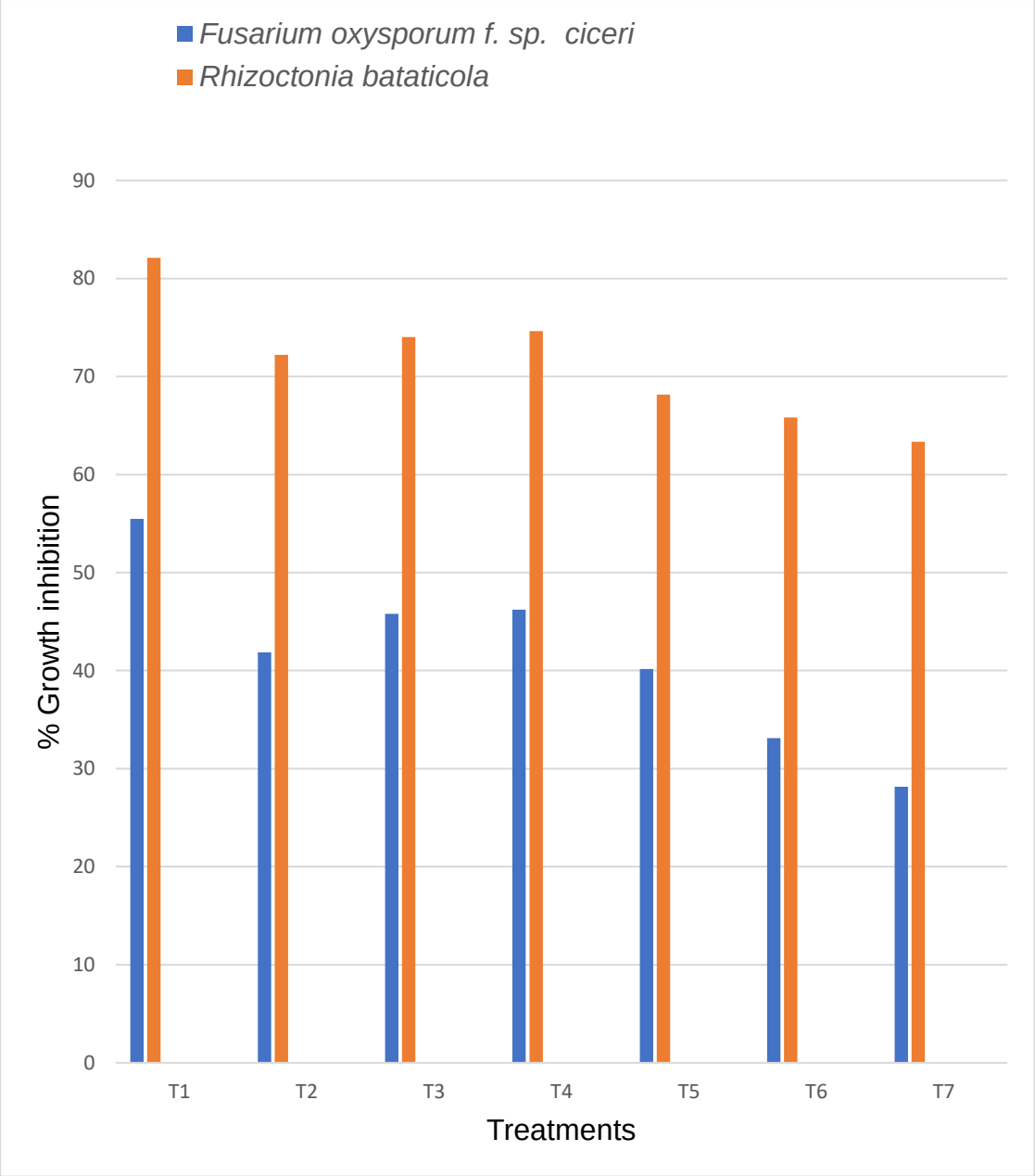


Fig No. 3: Effect of *Trichoderma harzianum* liquid formulations on per cent growth inhibition on 8th DAI

Plate 4 (A): Effect of *Trichoderma harzianum* liquid formulations on per cent growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* on 8th DAI

Plate 4 (B): Effect of *Trichoderma harzianum* liquid formulations on per cent growth inhibition of *Rhizoctonia bataticola* on 8th DAI

et al. (2010), Parveen *et al*. (2012), Srivastava *et al*. (2012), Tapwal *et al*. (2015), Rajendraprasad *et al*. (2017), Sreeshma and Jose (2016), Babychan and Simon (2017), Cherkupally *et al*. (2017),Taral *et al*. (2018) and Mane *et al*. (2020).

With respect to *Rhizoctonia bataticola* (Table-3, Fig-3, Plate-4B) the minimum mycelium growth was recorded by paraffin oil treatment 16.10 mm with 82.11 per cent growth inhibition and it was found significantly superior over all other treatment. It was followed by coconut oil treatment recording 22.83 mm mycelial growth with 74.63 per cent growth inhibition. Market product treatment recorded higher mycelial growth 32.33 mm with minimum growth percentage 28.16 per cent in case of *Fusarium oxysporum* f. sp. *ciceri* and 33.00 mm mycelial growth in case of *Rhizoctonia bataticola* with 63.33 per cent growth inhibition. There result are supported by the finding of Adhikary *et al*. (2017), Rajendraprasad *et al*. (2017), Boblina *et al.* (2020) and Mane *et al*. (2020).

Chapter V

SUMMARY AND CONCLUSIONS

Paraffin oil, Rice bran oil, Mustard oil, Coconut oil, Mineral oil, Department culture and Market (liquid) culture are extensively used as carrier for *Trichoderma harzianum*. The survival of *Trichoderma harzianum* in liquid-based formulations is quite high and have the ability to limit the heat transfer high water holding capacity liquid formulations also have advantage for maintaining water around the cells for their metabolism. As compared to solid based carrier material it has also advantage for improving survival and a good protection of *Trichoderma harzianum.* The present research work was undertaken on “Investigation of different oil formulations on shelf life of *Trichoderma harzianum*” during the year 2021-22.

The experiment was conducted in completely randomized design with seven treatment in three replication. The observation on shelf life of *Trichoderma harzianum* with paraffin oil, per cent spore germination and per cent inhibition of oil formulations were recorded. Similarly different carriers were also tested for shelf life of *Trichoderma harzianum.*

Shelf-life studies clearly indicated significant differences at all the intervals. Maximum population density was observed in T_1 paraffin oil treatment 33.33 x 10^8 CFU/ml. It was found significantly superior over all other treatment followed by T_4 coconut oil treatment 22.40 x 10^8 CFU/ml. However, the minimum population was recorded in T_5 Mineral oil 11.33 x 10^8 CFU/ml.

The effect of liquid formulations on spore germination of *Trichoderma harzianum* was also recorded The maximum spore germination was found in T_1 paraffin oil treatment with per cent spore germination of 60.83 followed by T_4 coconut oil treatment 47.00 per cent.

The effect of maximum per cent growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia bataticola* was found

significantly superior in T_1 paraffin oil tratment. It showed 20.63 mm and 16.10 mm mean colony diameter with per cent growth inhibition 55.49 and 82.11 respectively. T_1 was followed by T_4 with per cent growth inhibition 46.16 and 74.63 in *Fusarium oxysporum* f. sp. *ciceri and Rhizoctonia bataticola* respectively.

Thus it is concluded from the present studies that the shelf life of *Trichoderma harzianum* was found maximum in treatment containing paraffin oil followed by coconut oil and it also gives maximum per cent growth inhibition against *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia bataticola*. Thus shelf life of *T*. *harzianum* can be viable upto six month of storage. This is one year experimentation and needs further research work for recommendation.

Chapter VI

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