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"EFFECT OF *TRICHODERMA VIRIDAE* ON *PYTHIUM APHANIDERMATUM* AND *PAECILOMYCES LILACINUS* ON ROOT KNOT NEMATODE ON *SOLANUM TUBEROSUM* L."

Kishor Bhosale[1]*, Kavita Belote[2], Madonna D'souza[3] Vikas Jadhav[4] Abhijit Limaye[5] Bharat Shinde[6]

Abstract

Solanum tuberosum L. (Potato) belongs to the family Solanaceae with its origins traced back to the Peruvian and Bolivian Andes of South America. It has lot of demand as a staple food throughout the world. There are mainly two important threats to potato after cultivation, that includes *Pythium aphanidermatum* and root knot nematode. These two threats completely destroy the production of potato crop thereby reducing yield and causing an economical loss to the farmers. Despite the utilities of this crop, efforts weren't made to control these diseases which led to farmers using harmful chemical pesticides. *Trichoderma* as biological control agents have been widely used against many plant pathogens. *Paecilomyces lilacinus,* grows as a facultative parasite on nematode eggs and has been used to manage *Meloidogyne* spp. In present investigation "Kufri Jyoti" cultivar of Potato was used. The experiment was conducted with replicated randomized design. The soil used for experimentation was Garden soil and was autoclave sterilized and then filled into pots of size 20 x 20 x 25 cm, consisting of 3 kg soil capacity. Four sets were used for experimentation. Each set included one control and three replicates. Experimentation was carried out to evaluate the effect of pathogens before and after inoculation of antagonistic fungus in pot culture experiment. The plants were assessed for morphological parameters and biochemical contents. Statistical analysis of the results was found strongly significant.

Keywords: *Meloidogyne*; Nematode; *Paecilomyces*; Potato; *Pythium*; *Trichoderma*

[1*,2,3, 5,] Post graduate Department of Botany, Nowrosjee Wadia College, 19, Principal V. K. Joag Road Pune 411001. Pune (Maharashtra) India 411001; ksbhosale@gmail.com

[4] Department of Mathematics, Nowrosjee Wadia College, 19, Principal V. K. Joag Road Pune 411001. Pune (Maharashtra) India 411001

6 Principal, Vidya Pratishthan's Arts, Science & Commerce College, Baramati, Maharashtra 413133; scindiab2002@gmail.com

***Corresponding Author:** Kishor Bhosale

*****Post graduate Department of Botany, Nowrosjee Wadia College, 19, Principal V. K. Joag Road Pune 411001. Pune (Maharashtra) India 411001; ksbhosale@gmail.com

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Introduction

The origin of cultivated potato (*Solanum tuberosum* L.) began in South America where it has since been used for food for over 10,000 years and domesticated during Pre-Columbian times around 8,000 years ago. The span of cultivated potatoes first spread to Europe, including Spain and England, in the late 1500s. Potatoes have a huge significance in certain European countries and are often referred to as "European" or "Irish" potatoes. Potatoes are now grown in 160 countries with over 4,000 cultivars. Potato is short-duration crop and fits well in different multiple and intercropping systems. Potatoes are cosmopolitan and are grown in wide range of soil with pH of ranges between 5 and 7.5 (Thamburaj and Narendra 2016). Well drained coarse or sandy loam to loamy soil is ideal for the growth of roots, stolons, and tubers as such type of soils supply sufficient oxygen. Potato is one of the most cultivated tuber crops and fourth most important food crop in the world. In most of the developing countries, specifically in urban areas, due to high nutrition and energy value; demand for potato is rising globally. In several nations, potatoes are a staple food. Potatoes are non-fattening, nourishing, and affordable food that supplies many important nutrients to the diet. Potato tuber is one of the four most important dietary sources of carbohydrates, after wheat, rice, and maize. It is a source of vitamins and minerals, such as vitamin C, potassium, vitamin B5, and trace amounts of thiamine, riboflavin, folates, niacin, magnesium, phosphorus, iron, and zinc (Camire *et al.,* 2009; Fogelman *et al.,* 2019).

Trichoderma viridae is one of the most studied biocontrol agents for their unique antagonistic properties. *Trichoderma viridae* is cosmopolitan in nature when it comes to their multiple utilities e.g. mycoparasitism, nutrient competition by secreting antifungal metabolites etc. (Hermosa et al. 2014; Verma et al. 2007) *Trichoderma viridae* behaves as a biocontrol agent against several pathogens. Various studies have revealed that using multiple antagonistic microorganisms in combination boosts the level of the plant through different mechanisms making a more stable rhizosphere community over a wide range of environmental conditions (Jayaraj et al., 2006: Singh et al. 2006; Srivastava et al. 2010). Trichoderma possesses the ability to infect other fungi as one of its most prominent properties. *Trichoderma viridae* parasitize a wide range of fungal hosts, including important plant pathogens such as *Rhizoctonia solani*, *Phytophthora ramorum*, *Pythium ultimum*, *Macrophomina phaseolina*, *Sclerotinia sclerotiorum*, and *Fusarium spp*. (Kim and Knudsen 2008; Khalili et al. 2016; Chet et al. 2017; Widmer 2014;). *Trichoderma's* mode of action involves recognizing the fungal host at molecular levels followed by direct attachment. This acts as a precursor to produce hydrolytic enzymes and secondary metabolites that is vital for penetrating and destroying the fungus (Kubicek et al. 2011; Steindorff et al. 2012). Therefore, the secretion of cell wall degrading enzymes (CWDE) such as chitinases, glucanases, and proteases are necessary for the mycoparasitism to be successful (Kubicek et al. 2011). Due to its enormous utilities as an antagonistic fungus, wide availability, eco-friendly nature, economical, naturally procured *Trichoderma* has been taken in to consideration for its evaluation as a biocontrol agent during the infection of *Pythium aphanidermatum.*

Paecilomyces are described by their thermophilic, thermotolerant, and mesophilic species, with yellow-brown colonies. They are either nematophagous or entomopathogenic in nature, known as *Paecilomyces lilacinus* (Ingis and Tigano 2006). The genus *Paecilomyces* possesses hyaline to yellowish septate hyphal network, generally smooth walled and verticillated or irregularly branched conidiophores. The conidia usually are unicellular; hyaline, in chains; in basipetal succession. Their thermotolerance is associated with their size and shape. Thus, the smaller and more spherical asexual conidia or ascospores are more sensitive to high temperature (Dijksterhuis 2019; Brule et al, 2020a and Brule et al, 2020b) *Paecilomyces* has high growth sporulation rates and grows over a wide range of temperatures and substrates. As a result, its rapid multiplication ensures viable and development of commercial formulations [15]. (Moreno-Gavíra et al, 2020). *Paecilomyces* plays a vital role as an endophyte in several plants by providing various advantages development of plants. It may also be utilized as a probable biostimulent directly or indirectly. If it is used directly, its metabolites enhance morphological parameters and crop yield (Paul et al, 2013; Waqas et al, 2015 and Wang et al, 2020). Interaction between plant and *Paecilomyces* substantially improves health of plant via several mechanisms and provides resistance and tolerance against different phytopathogens (Malhadas et al, 2017). This interaction results in secretions of phytohormones, such as gibberellins and indole-acetic acid, that promotes growth and reduces abiotic stress, such as salinity (Khan et al, 2012). When used indirectly in combination with pathogenic agents such as nematodes or fungi, *Paecilomyces* has proven to be beneficial on plant

growth by acting as a biological control agent (Nesha and Siddiqui 2017).

Pythium aphanidermatum is one the species of parasites in soil, it is the most infamous plant pathogens in agricultural crops especially rhizomatous, tuberous and cormatous crops (Al Shaikh 2010, Hendrix and Campbell, 1973). The most common species of Pythium is P. aphanidermatum (Al Shaikh 2010). *P. aphanidermatum,* considered to be most critical species of this genus. It causes damping-off and root rot of various crops in nursery and field conditions. It highly grounds rotting and enforces financial loss of farmers growing tuberous crops. It also causes the seed rot, seedling rot, root rot, stem, and rhizome rots as well during all stages of growth in many other economic crops (Abdelzaher, 2004). The control of *P. aphanidermatum* involves numerous interculture operations like, crop rotation, solarization of soil, use of some chemical fungicides etc. conversely, such operations are tedious and disease control is poor. Existence of such pathogen for prolonged time span in soil, is quite difficult to control the disease. Some of the fungicide like metalaxyl and mancozeb has been reported to be highly effective to manage the damping-off caused by Oomycetes. Nevertheless, there are some confirmed reports about resistance of metalaxyl, metalaxyl-M, and Mefenoxam in *Pythium spp*. Nevertheless, use of such hazardous chemical fungicides results in harmful side effects on humans and the environment (Lookabaugh et al, 2018, Sabarwal et al, 2018). There is need of sustainable disease control practices in order to overcome harmful effects caused by use of chemicals to control it. The long- term hostile effects of chemical pesticides these days has adversely affected quality of soil and ground water. To avoid such adverse impact on soil, biological methods are best eco-friendly alternative. All the biological methods are very promising and sustainable for managing plants diseases. In this view and the demand of the layman in present years, the biological control agents need to vastly bring in to practice to replace chemical agents for the control various plant diseases.

Root knot nematodes are widely distributed in diverse kind of soils and almost found in all habitats over the world. They can easily adapt to varied and highly extreme situations of either cold to hot. With respect to the feeding nature and lifestyles, the plant parasitic nematodes are grouped into ectoparasites and endoparasites. Those which feed by inserting the stylet into root cells being outside on the root surface are ectoparasites, while those penetrating host cells and feed from inside are endoparasites (Escobar et al., 2015). Matured female of the nematode lay eggs on the surface of the roots. The number of eggs usually up to 1000, hatching of the eggs mainly depends on suitable conditions of moisture and temperature. In some conditions, root diffusates and hatching response can be influenced by generation number. After hatching the next stage, the juvenile penetrates the root of host plant with the help of the stylet. Later they move between cortical tissue and cells and gets into the plant vascular cylinder and becomes sedentary (Hussey & Grundler, 1998, Abad et al., 2008). The stylet is generally used for secreting secretions from the oesophagus and absorbing nutrients. This stage provokes the dedifferentiation of surrounding few cells into multinucleated and enlarged giant cells act as a source of nutrients to the nematode. They pass through three stages after this which includes, Juvenile third stage, fourth stage and adult. Male nematodes sometimes come directly out of the host roots. It is believed that males have no role in the reproduction process. Females are pear shaped and lay eggs on the root surface (Abad et al, 2008). Roots damaged by the nematodes cannot absorb water and nutrients efficiently. Root-knot nematodes cause damage to plant growth and reduce yield. A loss of 100 billion dollars each year is reported to be caused by *Meloidogyne incognita* alone worldwide (Mukhtar et al, 2014). This damage results in poor growth, reduced quality of crop along with the yield and also decrease the resistance of crop against drought and diseases. Total crop failure can occur due to high damage of root knot nematodes (Anamika & Sobita, 2012). The present investigations aimed to control the *Pythium aphanidermatum* and root knot nematodes in sustainable way.

Materials and Methods

For this experiment, 'Kufri Jyoti' cultivar of Potato was selected. This variety was obtained from Agriculture Produce Market Corporation (APMC)Pune. Entire experiment was done on Kufri Jyoti variety. The main idea behind selection of this variety is this is one of the highest grown varieties in Maharashtra and India. To know exact effect of phytopathogenic fungi like *Pythium aphanidermatum* and its control by *Trichoderma viridae* and root knot nematode *Meloidogyne incognita* and its control by *Paecilomyces lilacinus* this variety was most suitable hence this variety was selected for experiment.

Culture of *Pythium aphanidermatum* and infective juveniles of *Meloidogyne incognita* was obtained

from college of agriculture, Pune. Plant pathology and entomology department respectively.

Trichoderma viridae of brand Pest control India Pvt. ltd. Bengaluru and *Paecilomyces lilacinus* manufactured by Multiplex Bio-Tech Pvt. Ltd. Bengaluru purchased from market for experimental studies with respect to *Pythium aphanidermatum* and *Meloidogyne incognita respectively*.

The experiment was conducted with replicated randomized design. The potatoes were grown in the botanical garden of Nowrosjee Wadia College Pune. The plants used for the experiment were *solanum tuberosum* L. of 'Kufri Jyothi variety'. The Potatoes were obtained from APMC Pune. The soil used for experimentation was Garden soil. The soil collected from Botanical Garden of Nowrosjee Wadia College, Pune; was autoclave sterilized and then filled into parts of size 20 x 20 x 25 cms, consisting of 3 kg soil capacity. At the bottom of all pots a small hole was made to remove an excess amount of water and then the pots were filled with autoclave sterile soil. After filling the pots with soil about 750 ml of water added to moisten the soil completely. For each experiment four pots were used. First pot of four was used as a control pot and the other three pots were used as experimental pots. Similarly, four different sets were made and used for different experiments. The first pot among all four sets was used as control pot and remaining three used as experimental pots. The first set of experiment was added with *Pythium aphanidermatum* and *Trichoderma viridae* at the same time of potatoes were grown. This set was made to study the control of inoculated fungus with of *Trichoderma*. In second set of experiment potatoes were grown but pots were dual inoculated with *Pythium aphanidermatum* and *Trichoderma viridae* after 7 days. This set was made to study the possibility of control of an inoculated fungus with inoculation of other antagonistic fungus. The third set of experiment was added with nematode and *Paecilomyces lilacinus* at the same time of potatoes were grown. This set was made to learn infectivity and control of nematode with nematode infecting fungus *Paecilomyces lilacinus.* In fourth set of experiment nematode and potatoes grown but pots were inoculated with *Paecilomyces lilacinus* after 7 days. This fourth set was designed to study how nematode infecting fungus *Paecilomyces lilacinus* can control growth of nematode if inoculated after infection.

Morphological Parameters

Shoot length: The shoot length measured with the help of thread and recorded the length in cms.

Number of leaves: The number of leaves counted and recorded simply by counting the leaves of control as well as experimental plants.

Surface area of leaves: The surface area of both control and experimental plants was measured by plotting of outline of leaves on graph paper and measured in square cm.

Fresh weight: An average fresh weight of the Potato plants was measured in weighing balance and recorded that in grams. (Photoplate II).

Biochemical contents:

Estimation of Chlorophyll

Arnon's (1949) method was used for estimation of chlorophylls. For estimation of it, chlorophyll extract was prepared from fresh leaves of Potato 1 g by grinding in a mortar and pestle, together with 10 ml of ice cold 80% acetone. The homogenate was centrifuged at 3000 rpm for 2 minutes. The absorbance of the supernatant was recorded at 663 nm, 645 nm and the concentration of chlorophyll a, chlorophyll b and total chlorophyll was calculated using following equation:

$$\text{Chlorophyll}-a=(12.7\times A663-2.69A645)\times v1000\times w$$
$$\text{Chlorophyll}-b=(22.9\times A645-4.68\times A663)\times v1000\times w$$
$$\text{Total chlorophyll}=20.2\times A645+8.02\times A663v1000\times w$$

Estimation of Proteins

Proteins were estimated and quantified using Lowry et al., (1951) method. The tubers of Potato from control and experimental plants were cut into small pieces separately and one g plant material was extracted with 5 ml of Water. The extract was centrifuged at 10,000 rpm for 15 min. The supernatant was discarded and the pellet was dissolved in 2 ml of 1.0 N NaOH solution. This was used as a sample and 0.2 ml was taken for the estimation of proteins. The working standard of BSA and plant extract was taken in a series of test tubes and final volume was adjusted to 1 mL in each tube. Then 5 ml of reagent C was added in all the tubes and incubated the mixture for 10 min. This was followed by addition of 0.5 ml of Folin-Ciocalteau and incubated at dark for 30 min. The blue colour developed in the reaction mixture was read at 660 nm on UV-visible spectrophotometer. Bovine serum albumin fraction V (BSA) was used at the concentration of 50 mg and dissolved in distilled water and used as a standard protein to prepare the standard graph. The amount of protein was calculated with the help of standard graph.

Estimation of Proline

The estimation of proline was carried out by Bates et al., (1973) method. The leaves of control and

experimental plants of Potato used for estimation of proline. Extracted 0.5 g. of leaves in 10 ml of 3 % aqueous sulphosalicylic acid and homogenized it. The homogenate was then filtered through Whatman number two filter paper. Two ml of filtrate was added with 2 ml of glacial acetic acid and 2 ml acid ninhydrin. This mixture was heated in boiling water bath for one hour. The reaction was then terminated by placing the tubes in ice bath. After cooling the reaction mixture, added 5 ml toluene and stirred well for 20-30 seconds. Then the toluene layer was separated and placed at room temperature and absorbance was read at 520 nm using UV Visible spectrophotometer. Standard graph of proline was drawn by using standard proline. The amount of proline from samples was calculated with the help of standard graph.

Estimation of Carbohydrate:

Estimation of Carbohydrates was done by anthrone method as per Hedge, (1962). One g of Potato tubers was used to grind in 80% ethanol with the help of mortar and pestle. The working standard of sucrose and plant extract was taken in a series of test tubes and final volume was adjusted to 1 ml in each tube. Then 4 ml Anthrone was added in all the tubes and incubated the mixture for 8 min. cool the mixture rapidly and take OD at 630 nm.

In-Vitro studies on effect of *Trichoderma* viridae and *Paecilomyces* lilacinus

For Petri plates were used for this experiment. Potato Dextrose Agar was poured in all Petri plates and put it for a while to solidify it. After solidifying agar medium one Petri plate used to inoculate spores of *Trichoderma*. Second Petri plate was used to inoculate *Paecilomyces*. Both these Petri plates were treated as control. The third Petri plate differentiated in to two similar halves and one part was inoculated with *Trichoderma* and other part was inoculated with *Paecilomyces*. This set was made to study which species is dominant after growth. The fourth Petri plate inoculated with the suspension mixture of both *Trichoderma* and *Paecilomyces*. This set was designed to study weather *Trichoderma* is dominant or *Paecilomyces* is dominant.

Statistical analysis:

Statistical analysis of the data carried out using Microsoft office excel 2016. Variance between control and experimental plants was calculated using t. test. Pearson's correlation coefficients were performed to compare the data. All determinations were done at least in triplicate and all were averaged. The confidence limits used in this study were based on 95% ($P < 0.05$).

Results and Discussion

The pot culture experiment carried out to study effect of antagonistic fungus *Trichoderma viridae* on soil born fungus *Pythium aphanidermatum.* Similarly, an effect of *Paecilomyces lilacinus* on root knot nematode i.e., *Meloidogyne incognita* was studied. An In-Vitro experiment also carried out to check antagonistic effect of *Trichoderma viridae* and dominancy and effect of both fungi on growth of each other. The pot culture experiment and In-Vitro studies shown following results.

Table 1 Effect of *Trichoderma viridae + Pythium aphanidermatum* and *Paecilomyces lilacinus + Meloidogyne incognita* on shoot length and fresh weight of tuber.

Experiment	Shoot length (cms)		Avg. Fresh weight of Tuber (g)	
	Control	Experimental	Control	Experimental
P. aphanidermatum *+ T. viridae* Growing time Avg.	49±1.52	63±2.0 ***	22±2.52	31±1.00***
P. aphanidermatum *+ T. viridae* 7 days after growing	42±1.52	57±1.57 **	19±0.57	30±1.56***
M. incognita P. lilacinus Growing time	52±1.00	66±0.57 ***	57±0.57	34±2.08***
M. incognita P. lilacinus 7 days after growing	50±1.00	60±1.52***	26±1.52	32±2.08***

Results are given as mean ± SD. t test with significant differences ($P < 0.05$) between means are indicated by different * signs. (** significant at <0.001 level and *** significant at <0.0001 level)

The average shoot length at the time of harvesting of plants was measured in cms. In *Pythium* and *Trichoderma* growing time inoculated controlled plants avg. shoot length was 49 cms and in experimental plants it was 63 cms. *Pythium* and *Trichoderma* inoculated after 7 days; controlled plants showed 42 cms and experimental plants showed 57 cms. Similar results were recorded in *Meloidogyne* and *Paecilomyces* inoculated at growing time control plants showed average shoot length 52 cms for control plants and 66 cms for experimental plants. *Meloidogyne* and

Paecilomyces inoculated plants after 7 days showed 50 cms for control and 61 cms for experimental plants (Table 1).

Average fresh weight of *Pythium* and *Trichoderma* day zero inoculated plants showed 22 and 31 grams respectively for control and experimental plants respectively. In plants inoculated after 7 days showed 19 for control and 30 for experimental plants. Similar trend was observed in *Meloidogyne* and *Paecilomyces* inoculated plants showing both experimental plants showed better results as compared with controlled plants (Table 1). In both sets of experimental plants i.e., inoculated at the time of growing and inoculated after 7 days showed more shoot length as well as fresh weight of the tuber. This result is due to addition of antagonistic fungus in experimental plant. The set inoculated with *Trichoderma* had control over *Pythium aphanidermatum.* The first set of experiment where Inoculation was done at the growing time has total control over *Pythium* and the set inoculated after 7 days also had about 90% control on *Pythium aphanidermatum*.

In third set of experiment root knot nematode was completely controlled by *Paecilomyces lilacinus* so that results of experimental plants were better than control plants where few lesions were developed and also showed less fresh weight and shoot length. Due to inoculation of *Paecilomyces*; nematode was under control in both third and fourth set. The results obtained were in accordance with (Nolte et al., 2003) who found similar results in Russet Burbank potatoes. Similarly, Khan et al., (2010, Elad, 2000; Howell, 2002 Jayraj et al., 2006, Khare, & Upadhyay, 2009)) also obtained similar results. The t-test for Shoot length and fresh weight of tubers in all experimental plants were found significant at $P < 0.05$ level. Pearson's correlation test showed positive correlation for the same in experimental plants. (Table 1).

Average leaf number in plants inoculated with *Pythium* and *Trichoderma* at the growing time was 11 for controlled plants and 14 for experimental plants. The plants inoculated with the same after 7 days showed similar trend where experimental plants showed more leaf number as compared with control plants. *Meloidogyne* and *Paecilomyces* inoculated at the time of growing and after 7 days also showed similar trend. In both these cases experimental plants has 16 leaves as compared to 12 and 11 in controlled plants of growing and inoculated after 7 days (Table 2).

Table 2 Effect of *Trichoderma viridae* + *Pythium aphanidermatum* and *Paecilomyces lilacinus* + *Meloidogyne incognita* on different growth parameters of Potato after 55 days

Experiment	Avg. leaf number		Avg. leaf surface area $(cm)^2$	
	Control	Experimental	Control	Experimental
P. aphanidermatum + *T. viridae* Growing time Avg.	11±0.57	14±0.57**	15±1.15	17±1.15*
P. aphanidermatum + *T. viridae* 7 days after growing	11±1.15	13±0.57**	13±0.57	16±1.00**
M. incognita P. lilacinus Growing time	12±1.15	16±1.52***	16±1.52	18±0.57*
M. incognita P. lilacinus 7 days after growing	11±1.15	16±1.00***	16±1.15	19±1.15**

Results are given as mean ± SD. t test with significant differences ($P < 0.05$) between means are indicated by different * signs. (*significant at <0.05 level, ** significant at <0.001 level and *** significant at <0.0001 level)

Average leaf number and average surface area in set no one of experiment showed better results in experimental plants due to presence of *Trichoderma viridae*. The presence of antagonistic fungus *Trichoderma viridae* controlled growth of soil born fungus *Pythium aphanidermatum* so that metabolism of the plant remained unaffected and so the plant growth was healthy. Due to this better result were observed in experimental pot sets. *Pythium aphanidermatum* affect growth badly when it infects the plant and continues to grow very fast and ultimately cause the plants to collapse. This effect of *Pythium aphanidermatum* completely stopped by *Trichoderma viridae*.

Third and fourth set where inoculation of both *Meloidogyne incognita* and *Paecilomyces lilacinus* was done also showed similar kind of results. Nematode initially infects the zone of plant from where shoot arise above the ground and later starts sucking all nutritious material from tuber. Due to this whole plant collapse. The inoculation of both *Meloidogyne incognita* and *Paecilomyces lilacinus* in the beginning showed *Paecilomyces lilacinus*

infected nematode and controlled their growth so that plants grown well and showed better results. In the fourth set even inoculation after 7 days had control over nematode. But the control is comparatively less than inoculation at the growing time (Table 2). Our findings are corroborating with the findings of (Mohamed and Haggag 2006). Similarly, T. viridae inhibited the growth of P. aphanidermatum in tomato (Karpagavalli and Ramabadran 2001.); in turmeric (Upadhyay and Rai 1987). Volatile and nonvolatile antibiotics produced by T. viridae and mycoparasitism might be responsible for the suppression of P. aphanidermatum leading to lysis (El-Katatny et al., 2001, Ghidiyal and Pandey 2008). The t-test for average leaf number and average surface area in all experimental plants were found significant at $P < 0.05$ level. Pearson's correlation test showed positive correlation for the same in experimental plants (Table 2).

Potato plants treated with *Pythium* and *Trichoderma* at the time of growing comparatively showed more amount of chlorophyll a, b and total chlorophylls in comparison with plants treated with Pythium and *Trichoderma* after 7 days. In all experimental plants, an amount of chlorophylls was recorded more in all experimental plants than control plants.

The plants dual inoculated at the growing time with Meloidogyne and *Paecilomyces* at the time of growing Potatoes; chlorophyll a, b and total chlorophylls were recorded more in all experimental plants than controlled plants. On the other hand, the plants inoculated after 7 days comparatively had less amount of chlorophyll a, b and total chlorophylls but it was more in all experimental plants compared with control plants (Table 3).

Table 3 Effect of *Trichoderma viridae+ Pythium aphanidermatum* and *Paecilomyces lilacinus + Meloidogyne incognita* on Chlorophyll contents in Potato

Experiment	Chlorophyll a mg/g		Chlorophyll b mg/g mg/g		Total Chlorophylls mg/g	
	Control	Experimental	Control	Experimental	Control	Experimental
P. aphanidermatum + T. viridae Growing time Avg.	1.99±0.04	2.09±0.01**	0.92±0.12	1.11±0.03***	2.91±0.06	3.21±0.08***
P. aphanidermatum + T. viridae 7 days after growing	0.93±0.03	1.03±0.02**	1.01±0.02	1.31±0.03**	1.97±0.04	2.35±0.08**
M. incognita P. lilacinus Growing time	2.08±0.03	2.2±0.06**	1.04±0.03	1.13±0.02*	3.13±0.01	3.33±0.40***
M. incognita P. lilacinus 7 days after growing	1.52±0.01	1.73±0.03**	0.68±0.03	0.91±0.02***	2.2±0.03	2.65±0.06**

Results are given as mean ± SD. t test with significant differences ($P < 0.05$) between means are indicated by different * signs. (*significant at <0.05 level, ** significant at <0.001 level and *** significant at <0.0001 level)

Amount of Chlorophyll a, b and total chlorophylls in experimental plants in all four sets of experimental pots was more due to health growth of plants. More the healthy plant more the chlorophyll and ultimately more photosynthetic efficiency. Plants inoculated with *Paecilomyces lilacinus* also showed similar kind of results enhancing Chlorophyll content in experimental plants (Fig 3). Actually, the inoculation of fungi like *Trichoderma viridae* and *Paecilomyces lilacinus* assist plants in fighting infection of some soil born fungi, insects and nematodes etc. (Martínez-Medina et al., 2009, Tchameni et al., 2017) obtained similar results. The t-test for Chlorophyll a, b and total chlorophyll in all experimental plants were found significant at $P < 0.05$ level. Pearson's correlation test showed positive correlation for the chlorophyll content in experimental plants (Table 3).

Protein content in control plants treated with *Pythium* and *Trichoderma* at the growing time was 12.37 mg/g and 14.18 mg/g in experimental plants. It was little less in plants inoculated after 7 days. Same trend was recorded with the plants inoculated with *Meloidogyne* and *Paecilomyces* both sets i.e., inoculated at the growing time and inoculated after 7 days (Table 4).

Table 4 Effect of *Trichoderma viridae* + *Pythium aphanidermatum* and *Paecilomyces lilacinus* + *Meloidogyne incognita* on Protein and Proline contents in Potato

Experiment	Protein mg/g		Proline µ moles/g	
	Control	Experimental	Control	Experimental
P. aphanidermatum + *T. viridae* Growing time Avg.	12.37±0.26	14.18±0.10**	0.38±0.05	0.29±0.03**
P. aphanidermatum + *T. viridae* 7 days after growing	11.61±0.07	13.11±0.06**	0.41±0.03	0.33±0.03**
M. incognita P. lilacinus Growing time	12.03±0.06	14.67±0.03**	0.36±0.01	0.36±0.01**
M. incognita P. lilacinus 7 days after growing	11.81±0.15	14.21±0.10**	0.40±0.005	0.38±0.05**

Results are given as mean ± SD. t test with significant differences (P < 0.05) between means are indicated by different * signs. (** significant at <0.001 level and *** significant at <0.0001 level)

Amount of Proline was 0.38 µ moles/g in control plants for *Trichoderma* and Pythium inoculated plants at the growing time. It was 0.29 µ moles/g in experimental plants of same set. This trend was exactly opposite with respect to all other biochemical content. In case of proline content more amount of proline recorded in control plants than experimental plants in all sets (Table 4).

Amount of protein was recorded more in experimental pot plants in all four sets of experiment than control plants this is because of all growth-related parameters were better in plants inoculated with *Pythium aphanidermatum* and *Paecilomyces lilacinus.* Due to presence of antagonistic fungus *Trichoderma viridae* and nematode infecting fungus *Paecilomyces lilacinus* plant got protected against *Pythium aphanidermatum* and *Meloidogyne incognita* respectively. Due to absence of fungi and nematode proteins present in plants remain as it is on the other hand in controlled plants due to absence of supporting fungi; infectious fungi could grow and utilized proteins present with tubers so that it was recorded less in control plants (Fig 4). Our findings are in agreement with (Mishra & Nautiyal, 2018, Kissoudis et al., (2014), Suzuki et al., (2014).

With respect to proline some contrasting results were obtained, an amount of proline was more in control plants than experimental pot plants. This contrast in result is due to absence of fungus; controlled pot plants were under more stress than experimental pot plants. Due to more stress; controlled pot plants have synthesized more amount of proline to withstand in stress conditions. On the other hand, in experimental pot plants due to presence of fungus they got assistance to fight with *Pythium aphanidermatum* and *Meloidogyne incognita* respectively (Fig 5). This resulted in least stress among experimental pot plants and that's resulted in presence of less amount of proline. Mona et al 2017 reviewed the role of *Trichoderma* in increased secondary metabolites and obtained similar results. endophytes on potential drought tolerance and cited some studies where endophytes imparted no improvement in the host's ability to tolerate drought stress. The t-test for Chlorophyll a, b and total chlorophyl Protein as well as Proline in all experimental plants were found significant at P < 0.05 level. Pearson's correlation test showed positive correlation for the protein content in experimental plants and negative correlation for proline in experimental plants (Table 4).

Table 5. Effect of *Trichoderma viridae* + *Pythium aphanidermatum* and *Paecilomyces lilacinus* + *Meloidogyne incognita* on carbohydrate content in Potato

Experiment	Carbohydrates mg/g	
	Control	Experimental
P. aphanidermatum + *T. viridae* Growing time	70.14±0.10	80.11±0.35***
P. aphanidermatum + *T. viridae* 7 days after growing	64.67±0.48	69.29±0.37**
M. incognita P. lilacinus Growing time	69.84±0.16	79.67±0.61***
M. incognita P. lilacinus 7 days after growing	62.69±0.40	75.18±0.46***

Results are given as mean ± SD. t test with significant differences (P < 0.05) between means are indicated by different * signs. (** significant at <0.001 level and *** significant at <0.0001 level)

Amount of carbohydrate in control plants was 70.14 mg/g and experimental plants was 80.11 mg/g This trend of more carbohydrate content in all experimental plants was observed in all different sets of *Trichoderma viridae* + *Pythium aphanidermatum* treated plants inoculated at growing and 7 days after growing. Same trend was recorded in plants inoculated with *Paecilomyces lilacinus* + *Meloidogyne incognita* at the growing time and after 7 days of growing (Table 5).

Carbohydrates content in all experimental pot plants was more than controlled pot plants. This relationship is directly linked with chlorophyll content. Due to healthy growth in all inoculated plant, they showed more height of the shoot, leaf number and surface area of leaves. This resulted in more photosynthetic effect. This elevated photosynthesis has positive effect in storage of reserved product in the form of carbohydrates in potato tubers. This is why an amount of carbohydrates was more in experimental pot plants than controlled pot plants (Fig 5). Many plant responses to the attack of a fungal pathogen are closely connected with the pathways regulating the level of sugar in the plant cell and ensuring energy homeostasis (Hey et al., 2010). A significant role in these responses is played by sugars themselves, acting as signalling molecules. Several such mechanisms have been described (Rolland et al., 2006). Sugars regulate cellular activity at multiple levels, from transcription and translation to protein stability and activity (Rolland et al., 2006). The t-test for carbohydrate contents in all experimental plants were found significant at $P < 0.05$ level. Pearson's correlation test showed positive correlation for its contents experimental plants (Table 5).

Table 6 In-vitro studies on effect of *Trichoderma viridae*, *Paecilomyces lilacinus* and different combinations on their growth

Experiment	Growth observation
Trichoderma viridae Control	Complete growth of *Trichoderma viridae*
Paecilomyces lilacinus	Complete growth of *Paecilomyces lilacinus*
Trichoderma viridae + *Paecilomyces lilacinus* 1:1 combination	75 % growth of *Trichoderma viridae*
Trichoderma viridae + *Paecilomyces lilacinus* Suspension mixture	80 % growth of *Trichoderma viridae*

In-vitro studies of *Trichoderma viridae* and *Paecilomyces lilacinus* on PDA medium. The Petri plate inoculated with the spores of *Trichoderma viridae* luxuriantly grown and covered the entire Patri plate within 48 hours. Similarly, the second Patri plate grown with *Paecilomyces lilacinus* grown abundantly and covered the entire Petri plate within 48 hours. The 3rd Patri plate grown with both 1:1 on half partition of Petri plate each shown dominance of *Trichoderma viridae* throughout the plates. 4th Patri plate inoculated with the mixture of suspension of both *Trichoderma* and *Paecilomyces* also showed dominancy of *Trichoderma viridae* on Patri plate (Table 6).

In-vitro studies of growth of both *Trichoderma viridae* and *Paecilomyces lilacinus* on PDA medium showed antagonistic effect of *Trichoderma viridae* over *Paecilomyces lilacinus.* This effect might be due to antagonistic properties of *Trichoderma viridae*. When *Trichoderma viridae* and *Paecilomyces lilacinus* grown in half portions each in same Petri plate *Trichoderma viridae* has antagonistic effect over *Paecilomyces lilacinus.* When the suspension of spores of both made and inoculated on PDA medium still it showed dominancy of *Trichoderma viridae*. On the basis of studies on culture media it is evident that *Trichoderma viridae* is totally dominant over *Paecilomyces lilacinus.* There are no such reports on this type of experiment as this is pioneering work on the same and probably the first work on in vitro studies with respect to interaction and effect of dual growth of Trichoderma *viridae* and *Paecilomyces lilacinus* together. (figure1).

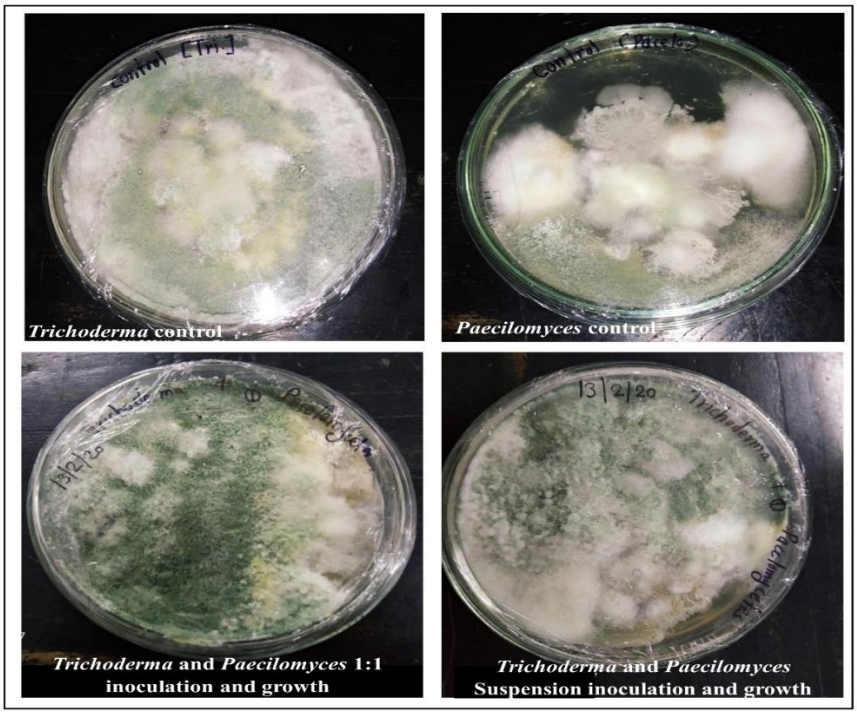

Figure 1 In-vitro studies on effect of *Trichoderma viridae*, *Paecilomyces lilacinus* and different combinations on their growth

Conclusions

The use of antagonistic fungi like *Trichoderma* and *Paecilomyces* assists plants thoroughly in biotic stresses caused due to fungi and root knot nematodes. In present investigation it is observed that different growth parameters showed positive corelation with respect to inoculation of *Trichoderma* and *Paecilomyces.* Along with the growth parameters *Trichoderma* and *Paecilomyces* also found beneficial for control of Pythium aphanidermatum and Root knot nematodes respectively. Biochemical content like chlorophylls, Proteins, carbohydrates also had positive effect of inoculation at the time of growing. The Proline secreted during the stress showed less stress in plants inoculated with *Trichoderma* and *Paecilomyces.* Overall yield of tubers was substantially better in plants inoculated as compared with non-inoculated pants. It is therefore concluded that farmers should use *Trichoderma* and *Paecilomyces* while growing potato to avoid future loss due to infection of some soil born fungi and nematodes.

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