



## Research Article

# Biofumigation - An effective tool in enhancing yield of capsicum by suppressing soil-borne pathogens and augmenting biopesticide under protected cultivation in India

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**ABSTRACT:** Biofumigation is a benign method to control soil-borne pathogens. The scope of the current research was to develop an effective formulation of Allyl Iso-Thio-Cyanate (AITC), commonly called volatile mustard oil and to evaluate its impact on suppression of soil-borne pathogens in capsicum cultivation grown under poly-house condition. After extensive research Multiplex biotech has developed a suspension concentrate formulation for biofumigation of soil based on an extraction of brassica enzyme called AITC and defatted mustard seed meal powder which could support both soil-sterilization and boosting soil fertility. A completely randomized trial was carried out on capsicum (*Capsicum annum*) grown under polyhouse (one acre) condition has shown that the application of this formulation as pre-planting treatment of soil @ 500ml/acre through drip irrigation under mulched condition was found to be an effective method to suppress the soil-borne fungal pathogens (70-80%) like *Sclerotium rolfsii*, *Fusarium* sp., bacterial pathogens (50-60%) like *Ralstonia* sp., *Xanthomonas* sp. and root knot nematodes (60-70%). In addition, it also complemented biocontrol activity of microbial antagonist like *Trichoderma viride* and *Pseudomonas fluorescens*, introduced as a treatment in the biofumigated plot after a gap of 6 days of Biofumigation. Improved plant growth (>18-20%), increased number of leaves/plants, early flowering and better yield (>22-33%) was recorded in the treatment where both *T. viride* and *P. fluorescens* were introduced as post fumigation treatment. This was followed by only biofumigated plot whereas in the non-fumigated plot disease incidences recorded was highest (43-45% more). Highest yield (46 tons/acre) was recorded in the plot where biopesticide was treated after Biofumigation followed by only biofumigated plot (37 tons/acre) and as compared to non-fumigated plot (28 tons/acre) which recorded the lowest yield.

**KEY WORDS:** Allyl isothiocyanate formulation, biofumigation, biopesticide, *Pseudomonas fluorescens*, soil-borne pests and pathogens, *Trichoderma viride*

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## INTRODUCTION

Biofumigation by means of plants belonging to the Brassicaceae represents a sustainable technique for soil disinfection and fertility management that is alternative to the chemical soil fumigants. Biofumigation has been studied for more than 15 years (Matthiessen and Kierkegaard, 2006) and it is now commercially applied both in conventional and organic agriculture in several countries. The conventional method of biofumigation essentially consists of soil incorporation of selected brassica plants, both as biofumigant or catch crop green manures (Lazzeri *et al.*, 2003), or derived materials as biofumigant pellets based on Defatted Seed Meal (DSM) of different brassica (Lazzeri, *et al.*, 2008). The 100% vegetable composition of these materials makes it possible to incorporate a significant amount of Organic Matter (OM) in soil that is a fundamental benefit to improve soil fertility especially in warm cultivation areas (Montanarella, 2003). Indeed, it is well known that OM plays a positive role on physical, chemical and biological properties of soil along with permitting a mitigating action on greenhouse gas emissions

(Smith, *et al.*, 2008). In addition to these benefits, typically organic fertiliser and green manure crop management, biofumigant plants and materials add a clear allelopathic effect on several soil pests and pathogens. Brassica cells contain the defensive system glucosinolate-myrosinase (GLs-Myr), which is able to release, by enzymatic hydrolysis, a number of biologically active compounds, mainly isothiocyanates and at lower concentrations of nitriles, epithionitriles and thiocyanates (Fahey, *et al.*, 2001). These compounds are recognised for their well-known biocidal activity against fungi (Manici, *et al.*, 1997), nematodes (Lazzeri, *et al.*, 2009) and wireworms (Furlan, *et al.*, 2010) by means of desiccation, reduced oxygen uptake and inhibition of some enzymatic action. Recently, research has been strongly geared up towards the development of new liquid formulations based on AITC or volatile mustard oil emulsion, containing small amounts of DSM to overcome the limitations of conventional practices of biofumigation as the performances are highly inconsistent and unpredictable because of the concentration of active compound (GIs) and its ability to suppress the pest

and pathogens varies mostly by season, variety, maturity at incorporation and finally the soil microbial diversity and density. This approach could make it possible to overcome the limitations and can widen the scope of applications of biofumigant materials from pre-plant soil treatment to plant defence and management during cultivation. In this paper, an attempt was being made to develop an effective suspension concentrate formulation based on AITC and DSM as active material and mustard oil as carrier material along with other additives. In addition to lab studies, an application-oriented field studies were also carried out on this new biofumigant liquid formulation through a drip irrigation under protected cultivation of capsicum are presented and discussed on its main and biological activity on the root knot nematode *Meloidogyne incognita*, fungal pathogens *Sclerotium rolfsii* and *Fusarium oxysporum* which are commonly found to be problematic in cultivation of capsicum under protected cultivation. In addition, a experiments were also conducted to know the beneficial effects of augmentation of microbial antagonists, *Trichoderma viride* and *Pseudomonas fluorescens* as integration with biofumigant.

## **MATERIALS AND METHODS**

### **Production of liquid formulation**

#### **Allyl isothiocyanate**

The main active ingredient of this formulation, Allyl isothiocyanate (AITC) was received from Sigma Aldrich with >99% purity. AITC is a clear, colour less to light amber oily liquid which is commercially available in the name of volatile mustard oil and getting extensively used as food additives to enhance the flavour and shelf-life of various products. AITC is a highly pungent, strong lachrymator and skin irritant compound, Generally Regarded As Safe (GRAS) compound and approved by US Environment Protection Agency (EPA) for agricultural usage and commonly used as a food ingredient and additive under USA food and drug administration jurisdiction.

#### **Preparation of defatted mustard seed meal (DSM)**

Mustard seed meal is a by-product from mustard oil extraction and is mainly used as low value animal feeds. Ground defatted mustard seed meal was obtained by defatting of mustard seed with hexane using soxhletoil extraction method followed by grinding them with a ball mill at 450 rpm for an hour and finally sieving the grinded powder through 300mesh. DSM samples were freshly prepared and stored in airtight glass jar for further formulation.

#### **Preparation of the Final Emulsion**

The Final Emulsion (FE) was prepared by a two-step process in the form of a suspension concentrate form. In the first step, an Emulsified Concentrate (EC) formulation

was made by adding 8.0% Tween 80 in 50% AITC with rest quantity of mustard oil to get 50% EC formulation of AITC. In the second phase, 30% of DSM powder was slowly suspended to get a homogenous free flow Suspension Concentrate (SC) of AITC and DSM which is readily soluble in water.

### **Studies on bio-efficacy of 50% SC formulation of AITC under laboratory condition**

To assess the fungicidal potency of the test formulation a pure culture of *Sclerotium rolfsii* was maintained and exposed to different test concentrations (100ppm, 150 ppm and 200ppm) of 50% SC formulation of AITC. 100 microliters of each test concentration were applied on a small disc of Whatman filter paper and fix it on the upper lid of the Petri plates and in the lower part was poured with 30ml of freshly prepared sterilized PDA medium. Once the culture medium solidified a 1-cm disc of growing *S. rolfsii* culture was placed in each Petri plates covered them and allowed for incubation at 25+2 °C in an incubator. Observation was made at 24h intervals on the growth of *S. rolfsii* and compared with an untreated control.

Similarly, the impact of 50% SC formulation of AITC formulation on microsclerotia bodies of *S. rolfsii* was carried out by exposing the freshly collected microsclerotia bodies from an old culture of *S. rolfsii* against each test concentration in a closed Petri plates for 24 hrs and then allowed them to grow on a PDA medium. Observation was made on their germination and growth at 24h intervals and compared the growth and germination with untreated microsclerotia bodies. Experiment was repeated thrice by keeping three replications for each treatment and 10 numbers of microsclerotia bodies for each replication.

To assess the effective dosage of 50% SC formulation of AITC in disease suppression under controlled condition an artificial sick soil was established by inoculating *S. rolfsii* culture in potted soil and allowed to grow healthy and surface sterilized seeds of Bengal gram in both AITC treated and untreated potted soil. The test formulation was diluted @ 1% in water and used to treat per 5kg of sick soil at low (10ml), medium (15ml) and high (20ml) dosage. Each dosage was considered as different treatment along with sick soil as control and sterilized soil as check. Ten number of seeds used for each treatment and observation was made on seed germination and disease incidences in different treatment.

The impact of 50% SC formulation of AITC on root knot nematodes (RKN) was evaluated by establishing nematode (*Meloidogyne incognita*) sick soil under net-house condition by growing tomato seedlings as a host plant in 5kg of sterilized pot mixture (soil: sand: FYM @1:1:1) per pot.

Once the symptom of nematode infestation became visible the pots were treated with low (10 ml), medium (15 ml) and high (20 ml) dosage of 1% AITC as a treatment along with an untreated control. Observation was made on RKN population in pre and post treated soil sample.

### Under field condition

Field performances of 50% SC formulation of AITC was carried out on a Capsicum crop (Variety: Indira) grown under protected playhouse condition in Naramangla area, 40 KM away from Bangalore city. For effective delivery different methods were adopted like point application, drenching but finally delivery through drip irrigation method under mulched condition was found to be a most convenient method because of high pungent and lacrymator nature of AITC and finally the trial was carried out in one-acre area keeping half of it as untreated control and rest half was mulched and bio-fumigated with 50% SC formulation of AITC @ 500ml/acre through a controlled drip irrigation pump system. Each bed was measured 78ft X 4 ft and four such bed was considered as a block or treatment. The total treatment was four comprising bio fumigated plot (T-2), bio fumigated plot with *Trichoderma viride* (T-3), bio fumigated plot with *T. viride* and *Pseudomonas fluorescens* (T-4) along with a Non-fumigated control plot (T-1). Transplantation of 30 days old capsicum seedling was done after 6 days of AITC treatment having 300 number in each treatment. To assess the impact of AITC on biocontrol agents *T. viride* ( $2 \times 10^5$  spore/ml) and *P. fluorescens* ( $2 \times 10^6$  spore/ml) was applied @ 25ml/plant after 7 days of transplantation while in untreated control plot both the BCA were applied with enriched FYM during land preparation @  $1 \times 10^8$  spores/acre. Observations were made on plant growth, nematodes population % disease incidences, populations of both BCA and finally yield in different treatments to assess the impact of AITC.

## RESULTS AND DISCUSSION

### Impact of biofumigation on growth of *Sclerotium rolfisii* under controlled condition

Assay using Petri plates showed that all the test concentrations could bring total suppression in growth of *S. rolfisii*. At 72h of incubation no growth of *S. rolfisii* was recorded whereas in untreated control normal growth of *S. rolfisii* was recorded as 10.5cm, 18.5cm and 32.5cm after 24, 48 and 72h of incubation, respectively. The results confirms an earlier study where 100% mycelial growth inhibition of *S. rolfisii* was reported by Harvey, *et al.* (2002) when compared with commercially available pure AITC.

Microsclerotia produced by *S. rolfisii* are made up of compact masses of hardened mycelia and it helps to overcome environmental stresses. Low concentration (100

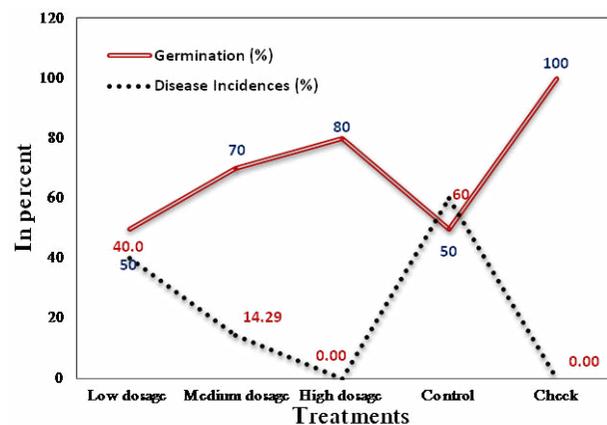
ppm) of AITC had no effect on germination and growth of microsclerotia but medium (150 ppm) and high concentration (200 ppm) had shown 40% and 100% nongermination/delayed germination of microsclerotia bodies respectively. In addition, an impaired vegetative growth was also noticed till 72h of incubation whereas in untreated control and low concentration normal growth of *S. rolfisii* was recorded (Table 1). Similar observation was made by Onkar *et al.* (2013) in suppression of germination of *S. rolfisii*.

**Table 1. Impact of AITC formulation on growth and germination of microsclerotia body of *Sclerotium rolfisii***

Treatments	Germination (%) at 24h	Growth	
		At 48h	At 72h
Control	100	Normal	Normal
Low: 100 ppm	100	Normal	Normal
Medium: 150 ppm	40	Impaired	Impaired
High: 200 ppm	100	Impaired	Impaired

A strong correlation was seen between dosage of AITC used and disease incidences (Fig. 1). Seed germination was recorded lowest in untreated control (50%) and highest in check (100%) where seeds were allowed to grow under sterilized soil media. In biofumigated sick soil, germination was 80% and no disease incidences were noticed, this was followed by medium (70% germination and 14.5% DI) and low concentration (40% germination and 50% DI) respectively. Maximum disease incidences were recorded in untreated control (60%) and lowest in both high dosage of soil fumigation and check.

A strong correlation was seen between dosage of AITC used and disease incidences (Fig. 1). Seed germination was



**Fig 1. Impact of AITC on germination and disease incidences of *Sclerotium rolfisii* in Bengal gram under controlled condition**

recorded lowest in untreated control (50%) and highest in check (100%) where seeds were allowed to grow under sterilized soil media. In biofumigated sick soil, germination was 80% and no disease incidences were noticed, this was followed by medium (70% germination and 14.5% DI) and low concentration (40% germination and 50% DI) respectively. Maximum disease incidences were recorded in untreated control (60%) and lowest in both high dosage of soil fumigation and check.

**Table 2. Bio-suppression of root knot nematode population<sup>1</sup> by AITC under controlled condition**

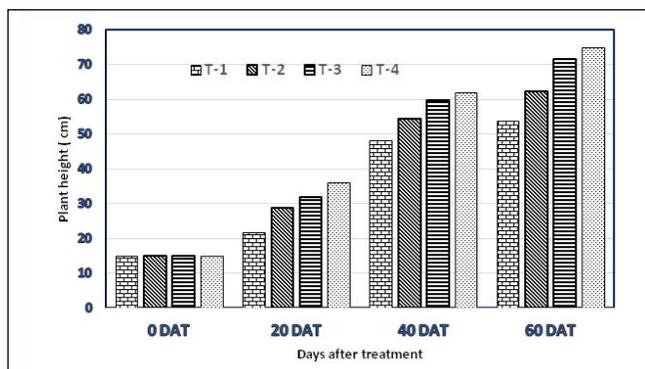
Treatments	Population of nematode/g of soil sample		
	Pre-treatment	7 DAT	15 DAT
Low Dosage	20.2a	22.2b	28.5b
Medium Dosage	22.0a	10.6c	15.5c
High Dosage	21.5a	5.3c	7.8c
Untreated control	20.8a	35.8a	48.8a
CD ( $P < .05$ )	2.8	11.88	9.2

<sup>1</sup>Means in a row followed by the same letter are not significantly different by DMRT

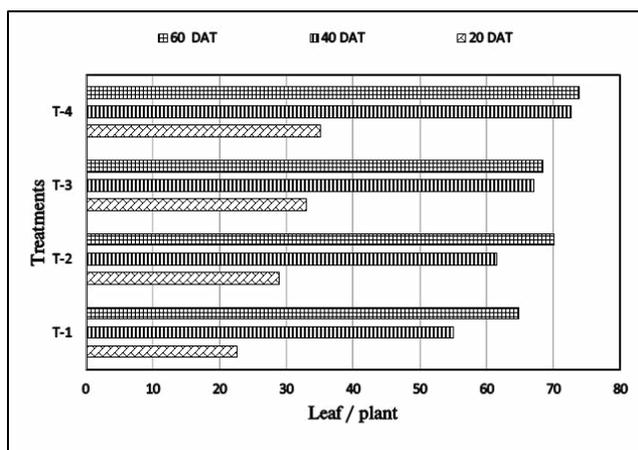
Before imposing AITC treatment the population of RKN was recorded 20.2 -22 per gram of sick soil with an insignificant difference in between treatments (Table 2). But after 7 days of AITC treatment a significant reduction in RKN population was recorded and lowest population was recorded in high dose of AITC treatment (5.3/gm) followed by medium and low dosage, 10.6/gm and 22.2/gm respectively whereas significantly highest population was recorded in untreated control pots with an increase rate of 72.11% and 134.61% after 7 days and 15 days respectively. In an earlier study, the  $LC_{50}$  and  $LC_{90}$  value for a commercial AITC formulation against *Meloidogyne javanica* was estimated at 0.10 and 0.29  $\mu\text{M}\cdot\text{mL}^{-1}$ , respectively (Zasada and Ferris, 2003).

**Under field condition**

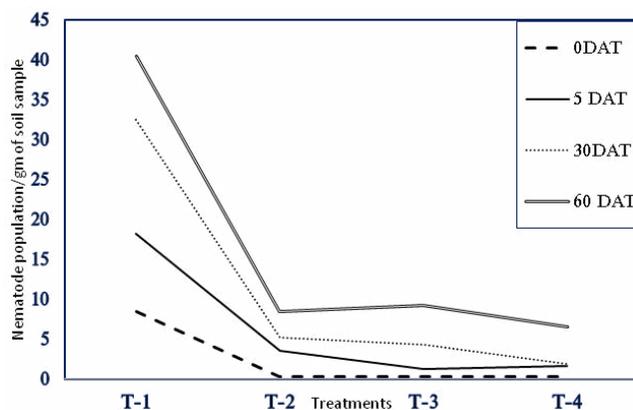
A significantly better plant growth was recorded after 20, 40 and 60 days in those plots where both the BCA's (*T. viride* and *P. fluorescens*) were applied after biofumigation followed by only *T. viride*, only bio-fumigated plots and control (Figure 2). A similar trend was recorded while number of leaf/plants was assessed (Figure 3). Highest number of leaf/plants was recorded in bio fumigated + combined treatment of BCA's (73.8/plant) followed by bio fumigated + *Trichoderma*, only bio fumigated plots and lowest number of leaves/plant was recorded in unfumigated control plots (64.8/plant) where both the BCA was applied in a recommended practice of pre-sowing soil treatment along with farm yard manure.



**Figure 2.** Plant height (in cm) after different days of bio fumigation in different treatments like T-1=Non-fumigated, T-2= Bio-fumigated, T-3=Bio-fumigated + TV, T-4=Bio-fumigated + TV+PF



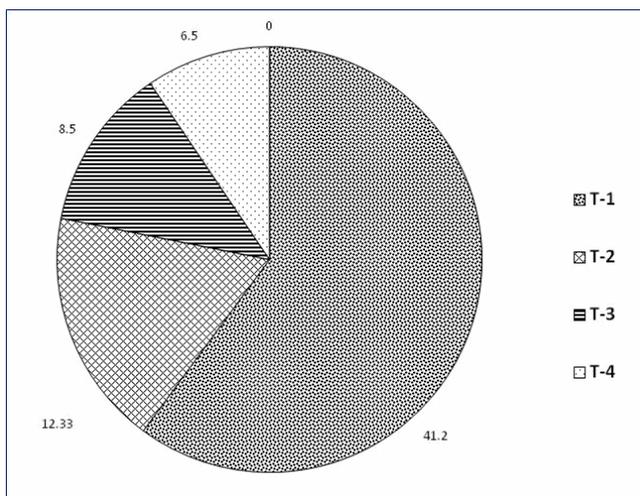
**Fig. 3.** Mean number of leaf/plants after different days of bio fumigation in different treated plots like T-1=Non-fumigated, T-2= Bio-fumigated, T-3=Bio-fumigated + TV, T-4=Bio-fumigated + TV+PF



**Fig. 4.** A population trend of nematodes in the soil at different days after treatment (0,5, 30,60) in different treated plots like T-1=Non-fumigated, T-2= Bio-fumigated, T-3=Bio-fumigated + TV, T-4=Bio-fumigated + TV+PF

After 60 days of treatment maximum RKN population was recorded in non-fumigated control plots (40.5 /gm) and lowest was recorded in all the bio fumigated plots, ranged from 6.5 to 9.25/plant. Immediately after biofumigation significant reduction was noticed in all treated plots however 5 days of treatment the population of nematodes was started building up in a slow rate in all the bio fumigated plots (Fig. 4).

A similar trend was recorded in disease incidences also (Figure 5). per cent disease incidences recorded were highest in non-fumigated crops (41.2%) whereas lowest was recorded in bio-fumigated + both BCA treatment (6.50%) followed by bio-fumigated + Trichoderma treatment (8.5%) and only bio-fumigated plots (12.33%).



**Fig. 5. % Disease incidences in different treated plots like T-1=Non-fumigated, T-2= Bio-fumigated, T-3=Bio-fumigated + TV, T-4=Bio-fumigated + TV+PF**

Similarly, the highest yield was recorded in the plot where both the BCA were treated after bio fumigation (47.15 tons/acre) whereas in bio fumigated + only Trichoderma treated plot the yield was recorded 43.12 tons / acre followed by only bio-fumigated plots (Table 3). The lowest yield was recorded in untreated control plots (28.75 tons/acre).

Highest population of *Trichoderma viride* (Figure 6) was recorded when both the BCA was applied combined after biofumigation of the soil followed by individual application of *Trichoderma* and non-fumigated control plot. Similarly, *P. fluorescens* was also recorded in biofumigated + combined treatments followed by non-fumigated control treatment (Figure 7). The lowest population of both *T. viride* and *P. fluorescens* was recorded in only biofumigated plots as there was no additional incorporation of BCA's were made except the possibility of cross contamination through irrigation

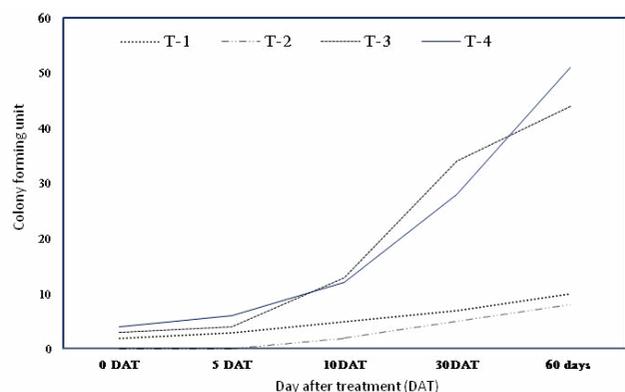
**Table 3. Impact of biofumigation in enhancing the yield<sup>1</sup> of capsicum under protected cultivation**

Treatments	Yield/ plant (kg)	No. of plants/ acre	Yield (tons)/ acre
T-1= Non fumigated control plot	5	5750	28.75 <sup>d</sup>
T-2= Bio fumigated plot	6.4	5750	36.8 <sup>c</sup>
T-3= Bio fumigated plot treated with Trichoderma	7.5	5750	43.125 <sup>b</sup>
T-4= Bio fumigated plot treated with both Trichoderma + Pseudomonas	8.2	5750	47.15 <sup>a</sup>
<b>CD (p &lt; 0.05)</b>			<b>3.26</b>

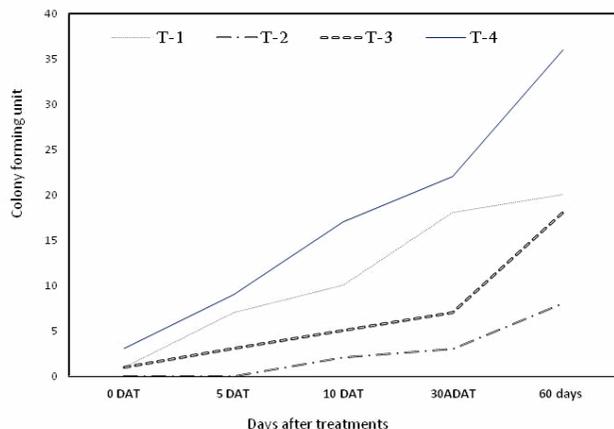
<sup>1</sup>Means in a row followed by the same letter are not significantly different by DMRT

system. Similar observations were made by Galletti *et al.* (2008) wherein the low sensitivity of some antagonistic fungi like *Trichoderma* to AITC makes it possible to maximize the colonization and effectiveness in disease suppression. In addition to low sensitivity the low competition in between BCA's and pathogen population may also play a role for better augmentation of BCA's in biofumigated soil.

The present results are based on one-year field experiment under protected cultivation of capsicum where biofumigation was made by means of drip irrigation under mulched condition but further studies to be conducted to assess the feasibility of its effective delivery method in field condition where drip -irrigation system is not available. In present study the scope to assess the potential of AITC as bactericide was limited and this needs further studies.



**Fig. 6. A population trend (-x10<sup>4</sup>/gm soil) of *Trichoderma viride* after different days of treatment in different treated plot like T-1=Non-fumigated, T-2= Bio-fumigated, T-3=Bio-fumigated + TV, T-4=Bio-fumigated + TV+PF**



**Fig. 7.** A population trend ( $\times 10^5$  cfu/gm soil) of *Pseudomonas fluorescens* after different days of treatment of bio-fumigation in different treated plot like T-1=Non-fumigated, T-2= Bio-fumigated, T-3=Bio-fumigated + TV, T-4=Bio-fumigated + TV+PF

## CONCLUSION

The results from the above studies indicate that suspension concentrate formulations of AITC and DSM could inhibit the growth of pathogens and nematodes significantly. Suppression of harmful pathogens and nematodes in biofumigated soil resulted into better colonization of BCA due to less competition, low disease incidences, better growth of plant and finally higher yield. In field situation it has been experienced that the efficacy of BCA's are erratic and inconsistent which might be due to variable population load of harmful pathogens when BCA'S are getting introduced. Pre-sowing biofumigation practice could be a viable solution to sterilize the soil by bringing down the population load of all harmful pathogens below threshold level and consequently will encourage better augmentation of BCA and higher yield. Bio fumigation could be environmentally sound and economically feasible alternatives for chemical fumigation but the difficulties in replacing such a widely used chemical with bio fumigation system is that available research data is insufficient to quantify the effects of AITC as biofumigants on crop yield and the soil microbial community. The methods developed in the present studies could be an initiation to consider biofumigation as a part of IPM programme to control soil borne pathogens and promoting microbial biocontrol agents for the better and safer future of agriculture.

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