

Original Research Article

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Compatibility of *Trichoderma harzianum* (KBN-29) with Selected inorganic Fertilizers: An *In vitro* Study

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ABSTRACT

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The compatibility of indigenous *Trichoderma harzianum* (KBN-29) with a few foliar fertilizers *i.e.* muriate of potash (MoP), urea and zinc sulphate was studied under *in vitro* conditions. The recommended doses of fertilizers (1 and 2% w/v) were tested using solid (PDA) as well as liquid (PDB) media. When these fertilizers were tested individually on solid medium, the antagonist could produce 8.5, 6.9 and 2.6 cm diameter colony in presence of MoP, urea and zinc sulphate, respectively, which indicated, that zinc sulphate as the least compatible when compared to fertilizers. Similarly, in liquid medium, the antagonist could produce 0.76, 0.63 and 0.53 g biomass as compared to untreated control (0.85 g) that showed the similar trend. When the combination of all three fertilizers was tested in solid medium, *T. harzianum* could develop the colony of 1.4 to 3.4 cm diameter which is significantly less as compared to control (8.7 cm). In case of liquid medium the combination of fertilizers exhibited the similar pattern where the antagonist yielded lesser biomass (0.20 to 0.21 g) compared to control (0.89 g). The study revealed that MoP and urea are compatible with the antagonist, however zinc sulphate was found to be the least compatible.

Introduction

Genus *Trichoderma* is one of the most important beneficial fungi, amongst the known fungal flora. Different formulations of *Trichoderma* spp have been applied in the tea crop to protect the tea plantation from several fungal phytopathogens. It is well established that, this fungus has the ability to control a wide array of fungal phytopathogens, such as *Fusarium* sp, *Pythium* sp., *Rhizoctonia solani* Kühn, *Sclerotium rolfsii* Sacc, *Macrophomina phaseolina* etc (Muhammad and Amusa,

2003; Sivan *et al.*, 1987, Adekunle *et al.*, 2006, Shalini *et al.*, 2006; Elad *et al.*, 1980, Upadhyay and Mukhopadhyay, 1986; Sivan *et al.*, 1984, Howell 1982).

In general, certain inorganic fertilizers have also been applied as foliar spray on tea crop (*Camellia* sp) for better yield (Fan *et al.*, 2005; Drinnan, 2008). The application of such fertilizers ensures the adequate nutrient availability to the crop which ultimately

influences both yield and its quality (Anonymous, 2002). Often, these fertilizers are applied to the soil, however, under certain circumstances especially during stress conditions, fertilizers such as, urea, DAP, muriate of potash (MoP), zinc sulphate (ZnSO₄), manganese sulphate (MgSO₄) etc. have also been applied as foliar spray to fulfill the immediate nutritional requirement of the tea plant. The combined application of urea, zinc sulphate and boric acid as foliar spray enhances the growth and development of crop plants (Amin and Shahsavari, 2012).

The performance of *Trichoderma* spp. under the lab as well as field condition is dependent on its growth and sporulation potential, which is affected by numerous factors such as pH, temperature, RH etc (Reetha *et al.*, 2014). It has been reported that certain inorganic fertilizers also have adverse effect on the growth and development of this antagonist to variable extent (Shylaja and Rao, 2012; Mondal, 2012; Gampala and Pinnamaneni, 2010, Bhai and Thomas, 2010).

The present study was carried out to determine the compatibility of *T. harzianum* with selected foliar fertilizers to find out whether they have any adverse effect on its performance with an ultimate aim to incorporate both antagonist and fertilizers in integrated crop improvement strategies in tea plantation.

Materials and Methods

Procurement of *Trichoderma harzianum* and fertilizers

The strain of antagonist, *T. harzianum* (KBN-29) has been isolated and developed at the Department of Mycology & Microbiology, Tea Research Association, North Bengal Regional R & D centre, Nagrakata, West Bengal, India. The required fertilizers were purchased from the local market.

Determination of effect of fertilizers on *T. harzianum*

Effect of individual fertilizer

The poisoned food technique (Nene and Thapliyal, 1993) was employed to determine the effect of selected fertilizers on growth and development of local antagonistic strain of *T. harzianum*. All the fertilizers were assessed at two different doses *i.e.* 1 and 2% (w/v). The required quantities of MoP, urea and zinc sulphate were measured using the weighing balance (Mettler-ME204), and used for the laboratory assays.

Preparation of media to study the effect of individual fertilizer

Solid medium

In case of solid medium culture, the potato dextrose agar (PDA) medium was sterilized in the autoclave and required quantities of fertilizers were added separately in to it at its lukewarm state followed by proper mixing.

Then the medium was poured in to Petri plates (15-20 mL medium per plate). After solidification, five millimeter discs of actively growing *T. harzianum* were inoculated in to the centre of plates and incubated at 26±2 °C in BOD incubator. Three replications per treatment were maintained and completely randomized design (CRD) was followed. When the control plates were fully covered with mycelial growth of antagonist, the colony diameter was measured from all other treatments. Finally, per cent growth inhibition over control was calculated by applying the formula of Vincent (1947).

Liquid medium

In second method, *i.e.* liquid medium culture, one hundred milliliter of potato dextrose broth (PDB) was taken in to 250 mL capacity

conical flask and sterilized using autoclave. After sterilization, the required quantities of fertilizers were added in to the flasks, separately when the medium was in lukewarm state. The flasks were inoculated with 5 mm mycelial discs of *T. harzianum* followed by incubation for 7 days.

To assess the quantity of biomass produced, the whole culture along with medium was filtered through Whatman filter paper number 1 and the retained biomass on filter paper was subjected to dry in an oven for a period of 6 hr at 60 °C and finally the biomass was determined using electric weighing balance.

Preparation of media to study the effect of fertilizer combinations

For required concentrations (1 and 2%), the quantities of the fertilizers were measured and simultaneously mixed with the pre-sterilized medium, homogeneously. The remaining procedure was same as described in the above experiments. All the experiments were replicated thrice.

Statistical analysis

To find out critical difference among the treatments, data were statistically analyzed through online package OPSTAT of Chaudhary Charan Singh, Haryana Agricultural University, Hisar, Haryana, India (www.hua.ernet.in).

Results and Discussion

The results of the experiments on individual fertilizer on solid medium indicated that, *T. harzianum* started growing within 24 hr of inoculation in each treatment except that of plates treated with zinc sulphate (Fig. 1). After 120 hr, the antagonist achieved its full growth in all the treatments except zinc sulphate treatment, which revealed that zinc

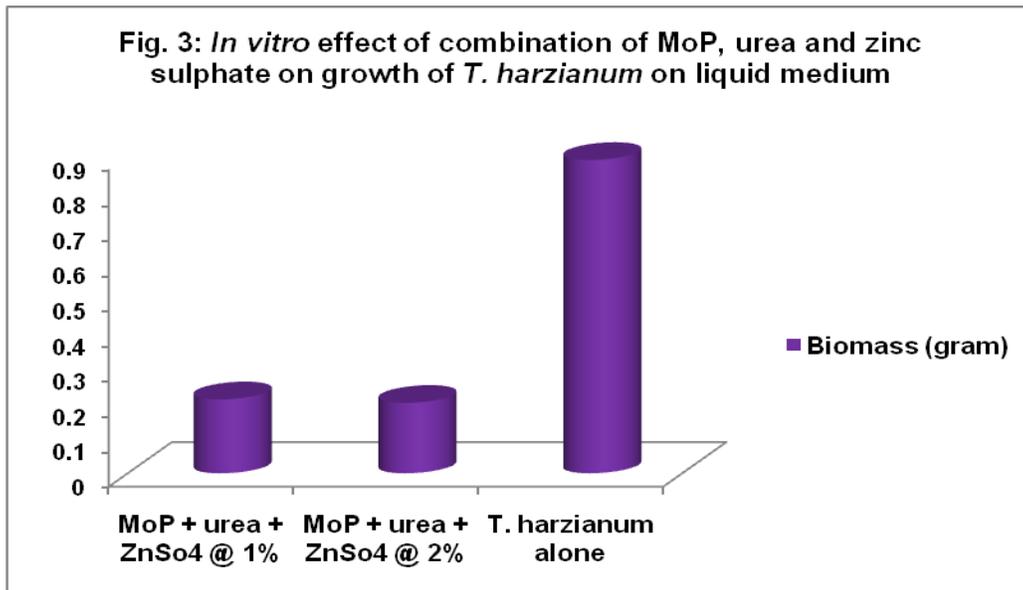
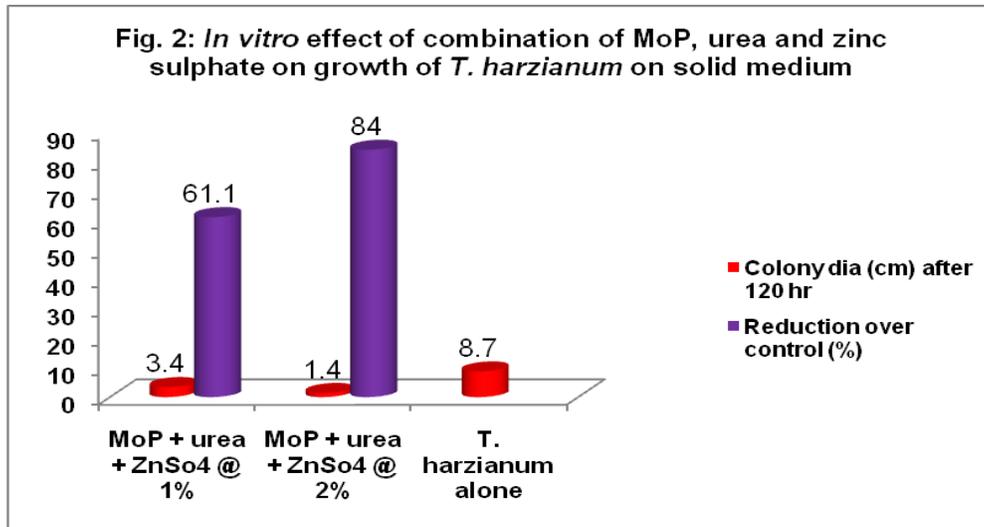
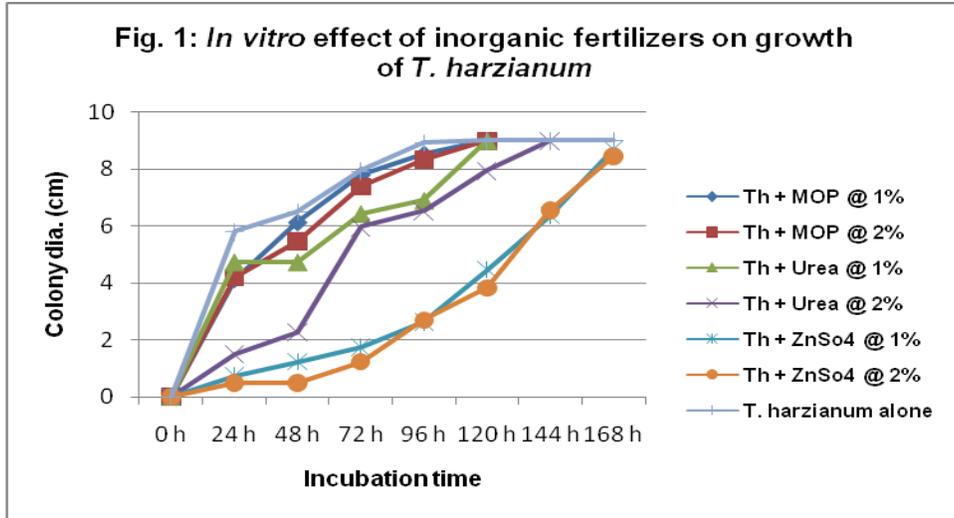
sulphate had adverse effect on the antagonist (plate 1 A). However, in case of other two fertilizers i.e. MoP and urea, both were found to be compatible and comparatively safer to this antagonist (Table 1).

When the same concentrations of these fertilizers were evaluated using broth culture medium, similar trend of growth pattern and conidial production was observed (plate 1B). The *T. harzianum* could produce the highest biomass (0.85 g) in control. Amongst the other treatments, MoP was observed to be the safest one followed by urea, and hence the antagonist could produce 0.76 and 0.63 g biomass at 1% concentration of MoP and urea, respectively. The biomass production was slightly lesser at their higher concentrations and all the treatments were statistically non-significant with each other (Table 2).

The combination of these three fertilizers showed the adverse effect on growth and sporulation of *T. harzianum* in both media. In case of PDA, the antagonist could produce only 3.4 and 1.4 cm diameter colonies at 1 and 2% concentrations, respectively, which were smaller as compared to that of the control (8.7 cm) as indicated in figure 2 and plate 1C.

In this experiment the per cent growth inhibition of *T. harzianum* due to combination of fertilizers was 61.1 and 84.0 at lower (1%) and higher (25%) concentrations, respectively.

Similar growth inhibitory effect was also exhibited by the antagonist when it was tested in liquid culture medium admixed with all three fertilizers (plate 1D). The antagonist produced very less amount of biomass (0.20 to 0.21 g) as compared to untreated control (0.89 g), resulting in 76.7 to 77.3 per cent reduction in biomass production over control.



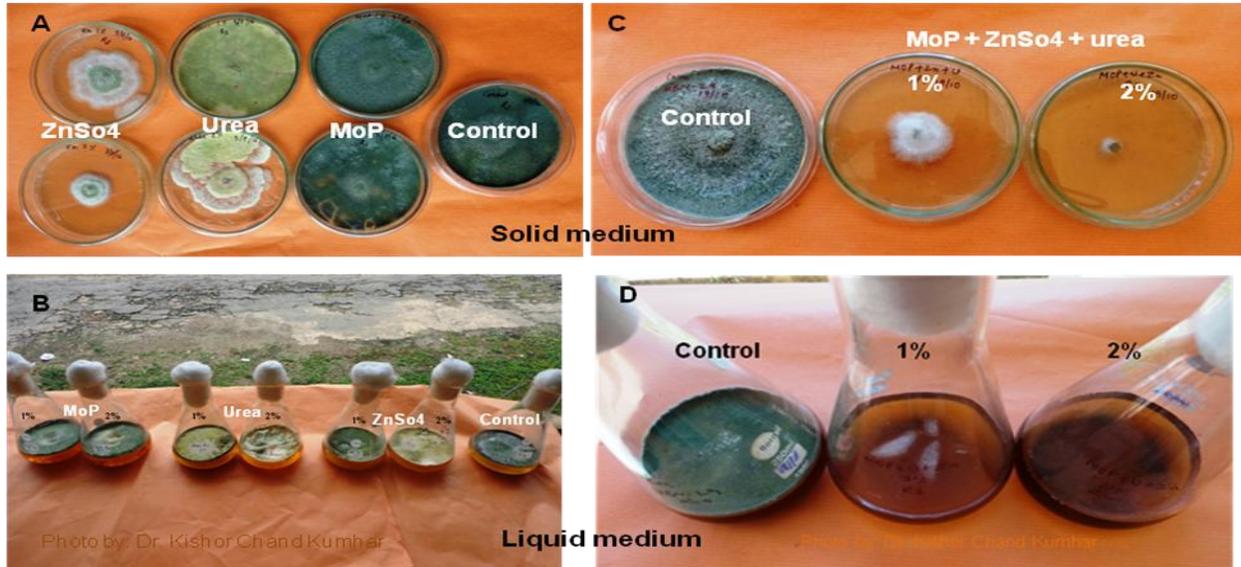


Plate 1: In vitro effect of individual and combination of fertilizers on growth and sporulation of *T. harzianum* (A & B: individual fertilizers and C & D: combination of MoP + urea + zinc sulphate)

Table.1 Effect of fertilizers on growth and sporulation of *T. harzianum*

<i>T. harzianum</i> + fertilizer combination	Mean colony diameter ± S.E.*		Sporulation status after 15 days
	after 120 hr	After 168 Hr	
Th + MOP @ 1%	8.5 ± 0.3	9.0 ± 0.0	+++
Th + MOP @ 2%	8.3 ± 0.1	9.0 ± 0.0	+++
Th + Urea @ 1%	6.9 ± 0.1	9.0 ± 0.0	+++
Th + Urea @ 2%	6.5 ± 0.1	9.0 ± 0.0	+++
Th + ZnSo4 @ 1%	2.6 ± 0.3	8.7 ± 0.2	++
Th + ZnSo4 @ 2%	2.7 ± 0.2	8.5 ± 0.3	++
<i>T. harzianum</i> alone	8.9 ± 0.1	9.0 ± 0.0	+++
CD	0.5	0.4	
SE(d)	0.2	0.2	
SE(m)	0.2	0.1	
CV	4.6	2.4	

*Mean of 3 replications ± SE, +++ = excellent, and ++ = good

Table.2 Effect of fertilizers on *T. harzianum* in liquid culture medium

<i>T. harzianum</i> and fertilizer combination	Dry biomass weight (g) produced in PDB ± SE*
Th + MOP @ 1%	0.76 ± 0.11
Th + MOP @ 2%	0.70 ± 0.17
Th + Urea @ 1%	0.63 ± 0.12
Th + Urea @ 2%	0.59 ± 0.06
Th + ZnSo4 @ 1%	0.53 ± 0.16
Th + ZnSo4 @ 2%	0.43 ± 0.09
<i>T. harzianum</i> alone	0.85 ± 0.19
CD	NS
SE(m)	0.14
SE(d)	0.19

*Mean of 3 replications ± SE

Gampala and Pinnamaneni (2010) investigated the effect of certain inorganic fertilizers such as urea (10g/L), muriate of potash (20g/ L) and super phosphate (20 g/L) using solid as well as liquid media and reported that muriate of potash and super phosphate were more compatible than that of urea. Shylaja and Rao (2012) tested the compatibility of *T. harzianum* with urea, single super phosphate (SSP), muriate of potash (MoP) and calcium ammonium nitrate (CAN) at different concentrations under lab conditions, and they found that the urea and MoP were compatible to the antagonist. However, both SSP and calcium ammonium nitrate were reported to inhibit the growth. These findings are in tune with our present findings. Similarly, Mondal (2012) assessed the compatibility of *T. harzianum* with zinc sulphate heptahydrate and observed that *T. harzianum* could exhibit significantly higher survival in the presence of zinc sulphate up to concentration of 150 ppm beyond this concentration there was some gradual decline in its viability. Our present findings would certainly be useful to the planters for taking decisions about the combined application of these fertilizers with the antagonist under field conditions.

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