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Effect of AM Fungi on Growth Performance of *Capsicum annum* L. Raised in Heavy Metals Contaminated Soil

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Abstract
Heavy metal contamination in soil poses serious environmental and health problems due to their non-degradable nature and it requires biological technological solutions. In this context, AM fungi play an important role for alleviating metal toxicity in plants by providing nutritional and non-nutritional benefits. The present study has been undertaken to evaluate the effects of AM fungi on growth and physiological parameters in *Capsicum annum* raised in heavy metal contaminated soil. The results of the present study show that in control (non-amended or non-inoculated) plants, root and shoot dry weight, number of flowers and fruits, total chlorophyll content, total protein content and AM colonization were minimum, whereas maximum in plants where soil was amended with farm yard manure and inoculated with AM fungi and PSF. Thus, the inoculation of AM fungi improved the growth and physiology of *Capsicum annum* raised under heavy metal contaminated soil.

Keywords
Capsicum annum, AM fungi, Heavy metals.

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INTRODUCTION
Contamination of soils with heavy metals is a major problem all over the world and these metals are continuously being added to the soil by various activities including application of sewage and industrial effluents, atmospheric deposition, waste disposal and incineration and use of chemical fertilizers (Shen et al., 2002). According to Holleman and Wiberg, (1985) metals having molecular weight greater than 5cm^3 are considered as heavy metals. Heavy metals are broadly divided into two categories (i) essential metals such as Cu, Zn, Fe, Mn and Ni and (ii) non-essential metals such as Cd, As, Hg and Pb (Mertz, 1981). Existence of heavy metals for long period in soil is harmful to soil health due to their non-degradable nature. Heavy metals were also reported to affect the plant growth and biomass (Jamal et al., 2006) as they decline the growth of plants mainly by (i) generating reactive oxygen species (ii) causing ion imbalance and (iii) reducing the water potential.

Soil microorganisms play an important role in degraded land (Zoomi et al., 2017) including metal contaminated soil as it alters the availability of heavy metals to the plants (Birch and Bachofen, 1990). Among soil microorganisms, arbuscular mycorrhizal (AM) fungi are important components of soil biodiversity that establishes mutualistic symbiotic association with plants (Smith and Read, 2010). AM fungi occurs in most of the ecosystems including metal contaminated soils (Vallino et al., 2006; Zarei et al., 2010; Yang et al., 2015; Kehri et al., 2017; Akhtar et al., 2017; Pandey et al., 2019). AM colonization has been reported to improve the plant growth, particularly by increasing accessibility of water and mineral nutrients (Smith and Read, 2010), absorbing heavy metals in their fungal hyphae or immobilizing the heavy metals in the soil (Gaur and Adholeya, 2004; Wu et al., 2016). Besides this, there are evidence that AM fungi provide tolerance toward heavy metals by modulating various physiological process (Latef, 2013).

On the other hand, farm yard manure (FYM) is a chief source of organic resource for agronomic production (Ashiono et al., 2006). FYM reported to improve the soil structure, alter the pH of the soil and reduce the availability of metal (Aggarwal et al., 1997; Singh and Agarwal, 2010). Juwarkar and Jambhulkar (2007) reported that the inoculation of microbial inoculant along with FYM reduce the toxicity of heavy metals in the plant.

Therefore, the present study has been undertaken to understand the ameliorating effects of microbial inoculants and FYM alone and in combination on growth and physiology of *Capsicum annum* L. grown in heavy metal contaminated soil.

MATERIALS AND METHODS

Physico-Chemical Properties of Soil

Soil for the experiment was collected from the sewage water irrigated site of Naini, Allahabad (25° 23' N, 82° 11' E). Soil pH was 7.5, organic carbon 1.64 (%), total nitrogen 0.28 (%), electrical conductivity (EC) 3.0 (dscm $^{-1}$), Cd 6.86 mg/kg and Ni 78.3 mg/kg.

Experimental Plant Material

Seeds of *Capsicum annum* L. were procured from certified seed agency of Allahabad. Seeds were surface sterilized with 2% (v/v) sodium hypochlorite (NaClO) solution for 5 min, then rinsed with distilled water several times and sown in plastic tray for germination.

AM Inoculum Production

AM inoculum was produced in trap cultures using *Sorghum bicolor* and *Trifolium* sp. as host plants. The collected soil sample from the survey site was mixed with sterilized sand in 1:1 ratio (v/v) and this mixture was then transferred to the earthen pots. Seeds of *Sorghum bicolor* and *Trifolium* sp. were sown and the cultures were maintained under greenhouse conditions for eight months (four months for each plant). AM inoculum consisted of soil having spores (138 AM spores/g air dried soil) and infected root fragments (86%). *Glomus intraradices, Glomus mosseae* and *Acaulospora scrobiculata* were the dominant AM fungi.

Experimental Design

A pot experiment was conducted under greenhouse conditions to access the effect of AM fungi on *Capsicum annum* L. in heavy metal contaminated soil of Naini, Allahabad. Eight different treatment series in five replicates and the treatment series were

1. Control (heavy metal contaminated soil)
2. +FYM (heavy metal contaminated soil amended with FYM)
3. AM (heavy metal contaminated soil inoculated with AM fungi)
4. +PSF (heavy metal contaminated soil inoculated with phosphate solubilizing fungi)
5. +FYM+PSF (heavy metal contaminated soil amended with FYM and inoculated with phosphate solubilizing fungi)
6. +AM+FYM (heavy metal contaminated soil amended with FYM and inoculated with AM fungi)
7. +AM+PSF (heavy metal contaminated soil inoculated with AM fungi and phosphate solubilizing fungi)
8. +AM+FYM+PSF (heavy metal contaminated soil amended with FYM and inoculated with AM fungi and PSF).

Earthen pots were filled with 5.0 kg soil contaminated with heavy metal. FYM (2% w/w) was incorporated 15 days before transplanting the seedlings of *Capsicum annum* in their respective pots and watered regularly. On the basis of phosphate solubilizing activity test *Trichoderma* sp. was selected as phosphate solubilizing fungi (PSF). PSF was inoculated at the time of seedling transplantation. The seedlings were transferred to the pot (one seedling per pot) after 30 days of sowing.

Determination of AM Colonization

For determination of AM colonization, root bits were stained in 0.05% trypan blue (Phillips and Hayman, 1970) and AM colonization (%) was determined by gridline intersect method given by Giovannetti and Mosse (1980).

Determination of Total Chlorophyll Content and Protein Content

Photosynthetic pigments were extracted in 80% acetone and was determined by method given by Lichtenthaler and Welburn (1983). Protein content was measured by the method given by Lowry et al. (1951).

Statistical Analysis

Data on each parameter studied were subjected to one-way analysis of variance (ANOVA) and means were compared by Duncan's multiple range test ($p<0.05$) using SPSS 16 software.

RESULTS AND DISCUSSION

Biomass

Minimum dry weight of roots (1.21 ± 0.02 g plant^{-1}) and shoots (4.12 ± 0.09 g plant^{-1}) were recorded in control series (Table 1). Similar to the present study Garg and Aggarwal (2011) also observed significant inhibition of plant growth under metal stress. Amendment of FYM increased the biomass of *Capsicum annum* as it improves the physico-chemical properties of soil and reduces the metal availability (Singh et al., 2010). A significant increase in dry weight of root (2.69 ± 0.08 g plant^{-1}) and shoot (7.55 ± 0.17 g plant^{-1}) were recorded in series where soil was inoculated with AM and PSF and amended with FYM over the other series (Table 1) and 122.3 % increase in root dry weight and 83.3 % increase in shoot dry weight over control was recorded over control (Fig. 1&2). PSF solubilize the insoluble phosphate and make it available to the plant. Inoculation of AM fungi improve the growth as they enhance the nutrient availability, maintain the cell water content of the plant (Ling-Zhi et al., 2011) and reduce the heavy metal content in the plant.

Number of Flowers and Fruits

In the present study, minimum number of flowers (23 ± 2.9 flowers plant^{-1}) and fruits (11 ± 2.3 fruits plant^{-1}) were recorded in control series (Table 2) and it is correlated with the reduced biomass and photosynthetic pigment (Table 1 & 4). Amendment of FYM increased the number of flowers and fruits alone and in combinations. However, maximum numbers of flowers (36 ± 2.3 flowers plant^{-1}) and fruits (29 ± 2.9 fruits plant^{-1}) were recorded in a series where soil was amended with FYM and inoculated with the microbial inoculants (Table 2) and 56.5 % increase in number of flowers and 163.6 % increase in number of fruits over control was recorded (Fig. 3&4). The inoculation of AM fungi improved the number of flowers as they improve the 'P' uptake and improve the hormones (Perner et al., 2007) thereby enhanced the yield.

AM Colonization

In the present study, minimum AM colonization (43 ± 2.5 %) was recorded in control series (Table 4). It has been reported that the heavy metal contamination reduces the spore germination, inhibits the hyphal growth and decreases the spread of mycorrhizal hyphal network (Griffioen et al., 1994; del Val et al., 1999; Pawlowska and Charvat, 2004). However, maximum AM colonization (74 ± 3.2 %) was recorded in a series where soil was amended with FYM and inoculated with AM fungi and PSF (Table 3) and 72.1 % increase over control at fruiting was recorded (Fig. 5). Addition of organic matter improved the mycorrhization in plants by the release of nutrients, it also forms complexes with heavy metals (Mahmood, 2010) and it positively influenced the community structure of AM fungi (Alguacil et al., 2011). On the other hand, PSF enhanced the AM colonization and spore population by producing the growth stimulating compounds (Balota et al., 1995) and by adsorption or chelation of heavy metals (Zhang et al., 2015).

Chlorophyll Content

In the present study, minimum total chlorophyll content (1.63 ± 0.040 mg g^{-1} FM) was observed in control series (Table 4) and it was corroborated with the study of Mangal et al. (2013) for *Abelmoschus esculentus* and *Cyamopsis tetragonoloba* and Abd_Allah et al. (2015) for *Helianthus annuus*. Heavy metals generally reduce the chlorophyll content due to reduction in mineral uptake such as Mg which is required for chlorophyll biosynthesis (Ewais, 1997). However, maximum total chlorophyll content (1.98 ± 0.058 mg g^{-1} FM) was noticed in the plants where soil was amended with FYM along with both the microbial inoculants (Table 4) and 21.5 % increase over control was recorded (Fig. 6). Similar to the present study, Singh et al. (2010) reported the application of FYM increased the photosynthetic pigments in cow pea. On the other hand, AM fungi alone and in combinations significantly improved the total chlorophyll content and this stimulatory effect might be due to the increased plant hormones (Allen et al., 1980) and enhanced the nutrient uptake in plants (Andrade et al., 2009; Gómez-Bellot et al., 2015).

Protein Content

Apart from photosynthetic pigments, protein content in plants are also important indicators to assess the growth performance of plants raised in metal contaminated soil. The results show minimum total protein content (13.10 ± 0.89 mg g^{-1} FM) in control series (Table 4). Minimum total protein content in plants could be attributed due to the

generation of reactive oxygen species (ROS) that damage the proteins (Gajewska et al., 2006) thereby inhibit the proteins synthesis. Inoculation of AM fungi reduced the metal accumulation in *Capsicum annum* and improve the total protein content (20.90 ± 1.21 mg g^{-1} FM) (Table 3). 59.5 % increase in the protein content over control was recorded (Fig. 7). Our results are in conformity with the reports of Singh et al. (2010) who reported that the application of AM fungi improves the protein synthesis.

Table 1: Effect of AM fungi alone and in combination with PSF and FYM on dry weight of shoot and root of *Capsicum annum* grown in heavy metal contaminated soil.

Treatment / Series	Shoot dry weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)
Control	4.12 ± 0.09^{a}	1.21 ± 0.02^{a}
+ FYM	4.72 ± 0.12^{b}	1.38 ± 0.04^{b}
+ AM	5.96 ± 0.14^{e}	2.02 ± 0.03^{e}
+ PSF	5.08 ± 0.12^{c}	1.57 ± 0.03^{c}
+ FYM + PSF	5.34 ± 0.12^{d}	1.75 ± 0.03^{d}
+ AM + FYM	6.17 ± 0.15^{e}	2.23 ± 0.07^{f}
+ AM + PSF	6.45 ± 0.14^{f}	2.49 ± 0.08^{g}
+ AM + PSF + FYM	7.55 ± 0.17^{g}	2.69 ± 0.08^{h}

Data in each column is mean ± standard deviation in three replicates (n = 3) and value within each column followed by the same letter are not significantly different (p<0.05) using Duncan's multiple range test. FYM: Farmyard manure; PSF: Phosphate solubilizing fungi; AM: AM fungi.

Table 2: Effect of AM fungi alone and in combination with PSF and FYM on number of flowers and fruits of *Capsicum annum* grown in heavy metal contaminated soil.

Treatment/Series	Conversion of flowers into fruits	
	No. of flowers	No. of fruits
Control	23 ± 2.9^{a}	11 ± 2.3^{a}
+ FYM	25 ± 2.1^{a}	13 ± 3.0^{ab}
+ AM	30 ± 2.0^{cd}	21 ± 2.5^{c}
+ PSF	26 ± 3.2^{ab}	14 ± 3.6^{ab}
+ FYM + PSF	26 ± 2.0^{abc}	16 ± 2.1^{b}
+ AM + FYM	30 ± 2.3^{bcd}	22 ± 2.3^{c}
+ AM + PSF	31 ± 1.7^{d}	23 ± 2.1^{c}
+ AM + PSF + FYM	36 ± 2.3^{e}	29 ± 2.9^{d}

Data in each column is mean ± standard deviation in three replicates (n = 3) and value within each column followed by the same letter are not significantly different (p<0.05) using Duncan's multiple range test. FYM: Farmyard manure; PSF: Phosphate solubilizing fungi; AM: AM fungi and FW: Fresh weight

Table 3: Effect of AM fungi alone and in combination with PSF and FYM on AM colonization (%) in the root of *Capsicum annum* grown in heavy metal contaminated soil.

Treatment/Series	AM colonization (%)
Control	43 ± 2.5^{a}
+ FYM	46 ± 2.1^{ab}
+ AM	61 ± 3.0^{d}
+ PSF	49 ±1.7^{b}
+ FYM + PSF	54 ± 2.5^{c}
+ AM + FYM	64 ± 3.1^{de}
+ AM + PSF	67 ± 2.1^{e}
+ AM + PSF + FYM	74 ± 3.0^{f}

Data in each column is mean ± standard deviation in three replicates (n = 3) and value within each column followed by the same letter are not significantly different (p<0.05) using Duncan's. FYM: Farmyard manure; PSF: Phosphate solubilizing fungi; AM: AM fungi.

Table 4: Effect of AM fungi alone and in combination with PSF and FYM on total chlorophyll and protein content in *Capsicum annum* grown in heavy metal contaminated soil.

Treatment/Series	Total chlorophyll content (mg g^{-1} FM)	Total protein content (mg g^{-1} FM)
Control	1.63 ± 0.040[a]	13.10 ± 0.89[a]
+ FYM	1.73 ± 0.032[b]	14.10 ± 1.00[ab]
+ AM	1.85 ± 0.031[c]	16.90 ± 1.05[c]
+ PSF	1.75 ± 0.040[b]	14.30 ± 0.83[ab]
+ FYM + PSF	1.77 ± 0.038[b]	15.05 ± 1.01[b]
+ AM + FYM	1.86 ± 0.030[b]	17.07 ± 1.03[c]
+ AM + PSF	1.91 ± 0.021[c]	17.92 ± 1.09[c]
+ AM + PSF + FYM	1.98 ± 0.058[d]	20.90 ± 1.21[d]

Data in each column is mean ± standard deviation in three replicates (n = 3) and value within each column followed by the same letter are not significantly different (p<0.05) using Duncan's multiple range test. FYM: Farmyard manure; PSF: Phosphate solubilizing fungi; AM: AM fungi.

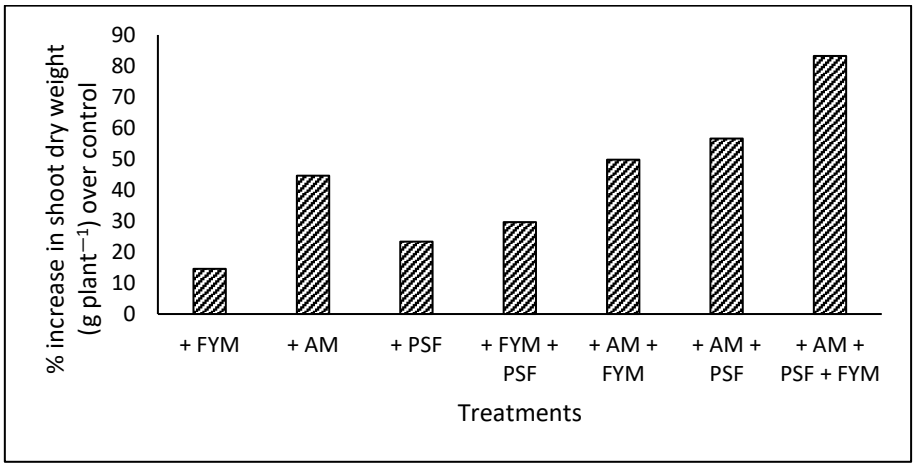

Fig. 1: Showing % increase in shoot dry weight over control of *Capsicum annum* plant raised in heavy metal contaminated soil amended with FYM (farm yard manure), PSF (phosphate solubilizing fungi) and AM (AM fungi).

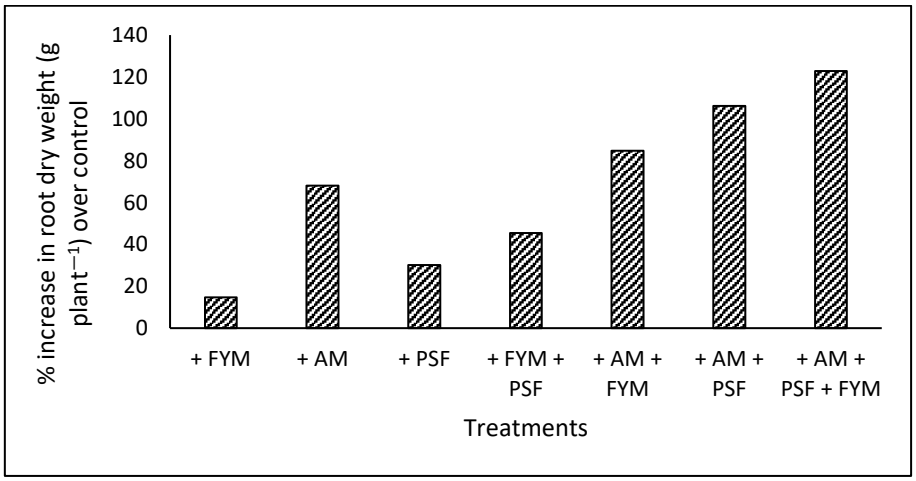

Fig. 2: Showing % increase in root dry weight over control of *Capsicum annum* plant raised in heavy metal contaminated soil amended with FYM (farm yard manure), PSF (phosphate solubilizing fungi) and AM (AM fungi).

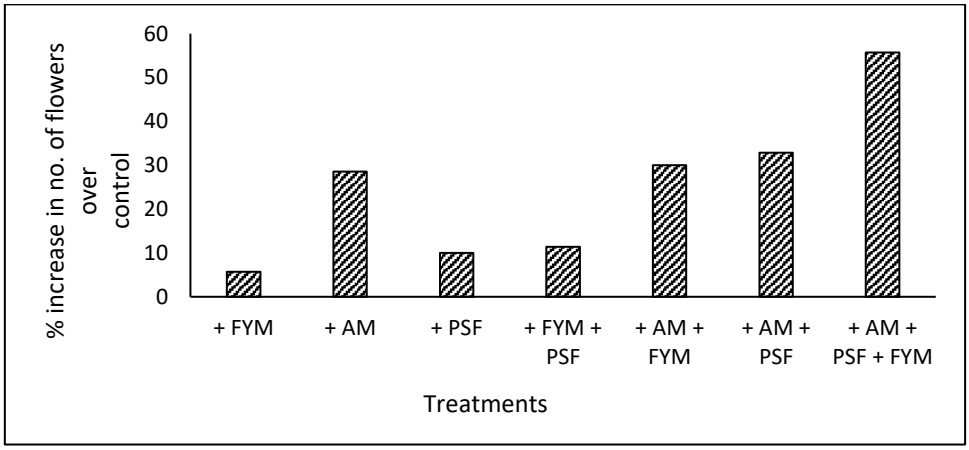

Fig. 3: Showing % increase in number of flower over control of *Capsicum annum* plant raised in heavy metal contaminated soil amended with FYM (farm yard manure), PSF (phosphate solubilizing fungi) and AM (AM fungi).

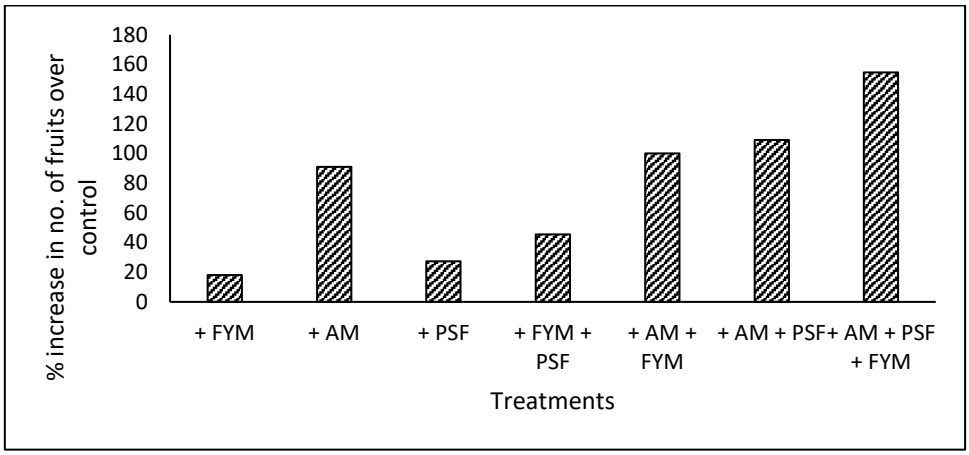

Fig. 4: Showing % increase in number of fruits over control of *Capsicum annum* plant raised in heavy metal contaminated soil amended with FYM (farm yard manure), PSF (phosphate solubilizing fungi) and AM (AM fungi).

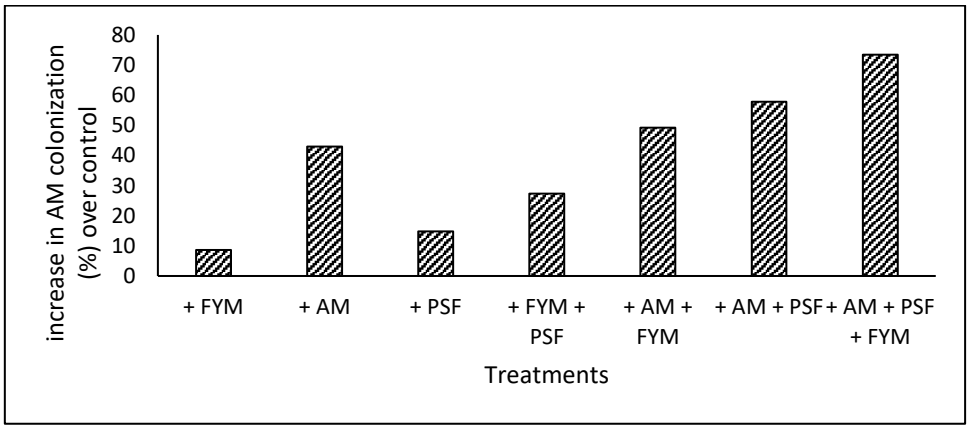

Fig. 5: Showing % increase in AM colonization over control of *Capsicum annum* plant raised in heavy metal contaminated soil amended with FYM (farm yard manure), PSF (phosphate solubilizing fungi) and AM (AM fungi).

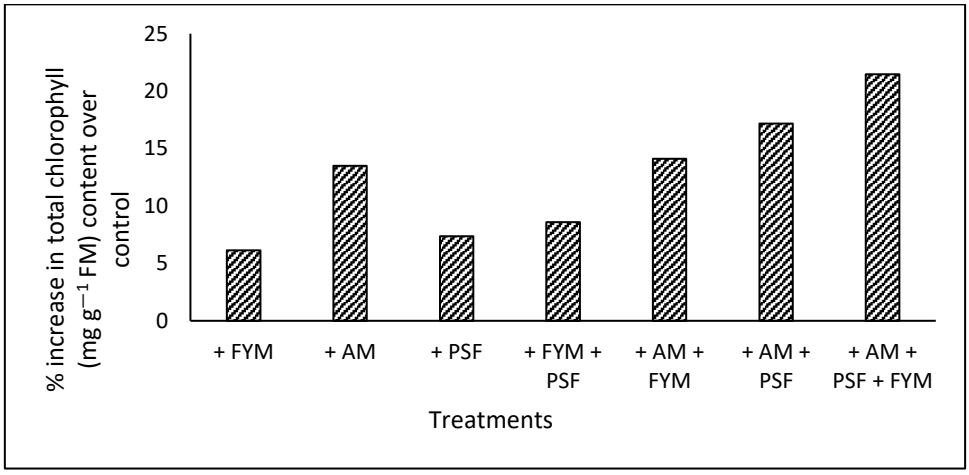

Fig. 6: Showing % increase in total chlorophyll content over control in the leaf of *Capsicum annum* plant raised in heavy metal contaminated soil amended with FYM (farm yard manure), PSF (phosphate solubilizing fungi) and AM (AM fungi).

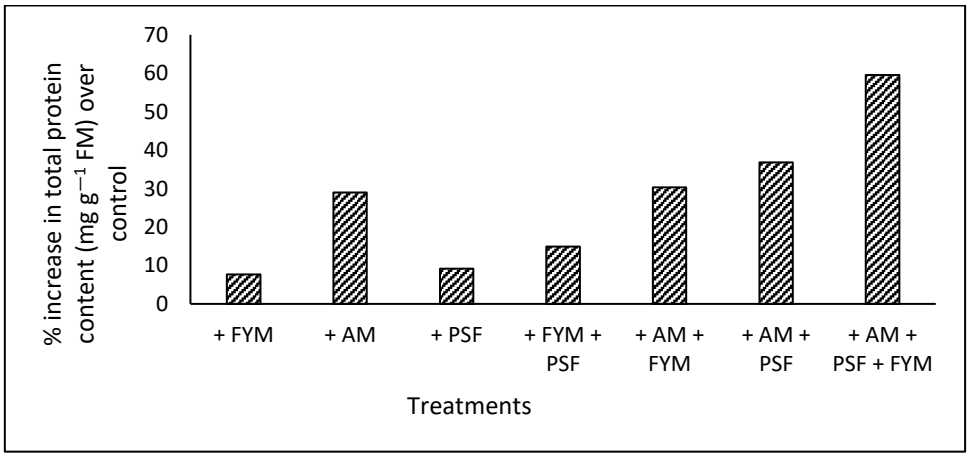

Fig. 7: Showing % increase in total protein content over control in *Capsicum annum* plant raised in heavy metal contaminated soil amended with FYM (farm yard manure), PSF (phosphate solubilizing fungi) and AM (AM fungi).

CONCLUSIONS

The present study concluded that soil amended with FYM slightly increased the growth and physiological parameters in terms of mycorrhization, root/shoot biomass, number of flowers and fruits, photosynthetic pigments and protein content. However, addition of microbial inoculants i.e. AM fungi and PSF (*Trichoderma* sp.) native to heavy metal contaminated soil along with FYM improved the overall performance of the plants by providing nutritional as well as non-nutritional benefits.

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