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# Employing Trichoderma To Alleviate Dimethoate Phytotoxicity In *Sorghum Bicolor*

**Archana Kumari[1], Krishna Sundari Sattiraju***

[1]Department of Biotechnology, Jaypee Institute of Information Technology,A-10, Sector 62, 201309, Noida, Uttar Pradesh, India.
*Corresponding Author: Krishna.sundari@jiit.ac.in

**Abstract**

Dimethoate is a broad spectrum organophosphate pesticide which is used to control pests of order hemiptera, diptera, orthoptera, and araneae. In *Sorghum bicolor* it is found to control green bugs, aphids, spider mites, and grasshoppers. The present study details the impact of dimethoate (30% EC) on *S. bicolor* through a short term (a fortnight study) *in-vivo* bioassay and an extended soil microcosm study (7 weeks study). Experiments were simultaneously carried out at low (10 ppm, 50 ppm) and high (300 ppm, 600 ppm) range of dimethoate. The *in-vivo* bioassay provided evidence of increasing dimethoate toxicity on *Sorghum bicolor* ranging from 19 % to 70 % at 10 ppm and 300 ppm respectively. *Trichoderma harzianum* T103, a plant growth promoting fungus (PGPF) with proven monocrotophos (organophosphate pesticide) tolerance, was engaged to verify its potential to support plant growth under dimethoate stress. Rhizospheric administration and seed biopriming with T103 improved growth of *Sorghum bicolor* by a factor of; root length (1.6, 1.8), shoot length (1.1, 1.2) and biomass (1.4, 1.4) at 10 ppm and 300ppm respectively. $LD_{50}$ of T103 value for dimethoate is ~ 300 ppm. T103 was observed to sporulate under dimethoate stress (at concentrations as high as 1000 ppm), however, the spore density was lesser as compared to unchallenged isolate growth. FTIR spectra indicated ongoing dimethoate degradation registering change in peaks at 1043 (-C-O stretching), 1235 (-OH,–NH deformation), and 1737 $cm^{-1}$ (C=O stretching). In addition, appearance of new peak at 3456 $cm^{-1}$ (–OH, -NH stretching) also suggest formation of intermediate and end product. Concentration of proline, a biochemical stress indicator, was greater in untreated plants than in treated plants suggesting signs of phytotoxicity in the absence of T103. The findings demonstrate that, at both low and high concentrations of dimethoate T103 can reduce dimethoate-induced growth retardation in *S .bicolor.*\KEYWORDS: DIMETHOATE, PGPF, *Sorghum bicolor,* phytotoxicity, proline, chlorophyll

## Introduction

Sorghum is the world's fifth-most important cereal crop after maize, rice, wheat, and barley. India is the second-largest producer of sorghum in the world, with a yield of more than 7.5 million metric tons recorded in 2021 [1]. Major insect pests of sorghum are stem and stalk borers, shoot flies, aphids, and shoot/ear head bugs. In India, infestation

of insect pests causes the loss of the sorghum crop more than 30% and even upto 84% under certain conditions. Losses linked to insects are estimated to reach more than $1 billion annually in semi-arid tropical regions [2, 3]. *Sorghum bicolor* is chosen in the study as a model plant, as it can be easily grown in a lab and greenhouse with low irrigation and fertilizer requirements, apart from being one of the most important cereal crops in India [4]. Insect pest populations that cause economic damage can be managed with a variety of organophosphate pesticides (OPPs) [5]. These pesticides accumulate in the soil when they are used in excess, causing a variety of stress responses in host plants. Under abiotic stress conditions, plants redistribute majority of the nutrients and energy towards overcoming stress, resulting in a much lower percentage of nutrients and energy going towards biomass accumulation. This would reflect as a significant decrease in yield due to compromised host metabolic activities [6-8]. Most common OPPs used in sorghum are dimethoate, chlorpyrifos, phorate, phosalone and so on [9, 10]. Research studies reported DM residues in soil (58.63 mg/kg), honey (1–10 mg/kg), spinach (2.54 mg/kg) and many other edible substances. As the maximum residue limit for DM is 0.04 mg/kg, the reported residue values in food materials are alarming. Table 1 represents the data on DM chemistry, rate of application, residues reported **[**11, 12]. The effects of DM on the human immunological, neurological, and reproductive systems were thoroughly reviewed by Eken, 2017 [13]. Kumari and John, 2019; Katsikantami et al., 2019 threw light on health risks associated with dietary exposure to

pesticides [14, 15]. Therefore, it is required to study the change in morphological, biochemical, and stress parameters of plants in the presence of pesticides at both low and high concentrations. However, to date, research publications demonstrating the pesticide toxicity in plants is comparatively less, even though they form the principal routes of accidental entry of these toxic substances in food chain. Number of scientific records in PubMed, Web of Science, Google Scholar and Science Direct database is evidence. Though some researchers have demonstrated the presence of pesticide residues in various crops as well as their negative effects on plant growth; still, more research work needs to be done in order to understand the actual impact on various morphological and biochemical parameters in many staple food crops owing to their consumption in large quantities. Nine out of ten fractions of the pesticide volume sprayed drift towards non-target plants and eventually sink into the soil. The pesticide enters the plant from the soil and causes several alterations to its physiological, metabolic and mitotic functions [16, 17]. Contrary to occupational direct contact exposure, which is frequently experienced by farmers, dietary exposure is involuntary and cannot be prevented by using external safeguards like wearing masks, gloves, and other safety equipment. Plant growth promoting fungi (PGPF) are important partners for enhancing plant productivity in a sustainable and environment friendly manner. *Trichoderma harzianum,* a prominent PGPF, is extensively known for its biotic stress tolerance/ biocontrol potential. Moreover, reports also exist for its potential to mitigate drought and salt stresses faced by the plants [18, 19]. Employing plant growth promoting microorganism for pesticide bioremediation is a comparatively new trend. Dimethoate (organophosphate pesticide) remediation using *T.harzianum* is a step forward in this orientation. *T.harzianum* isolate T103 used in the present study has demonstrated potential to support the plant growth by providing a. better nutrient access, b. better resistance to the soil competitors, and c. breaking down complex substrate in order to provide plant ready nutrients [20]. This is the reason in choosing the isolate for the current investigation to mitigate DM stress via plant-microbe interaction. The manuscript attempts to provide a definitive experimental evidence for asserting the potential of PGPF-T103 as prob-

able soil remediator, particularly in agricultural soils that have a continuous buildup of residual pesticides reaching either as direct application residues or irrigation surplus. The purpose of this study was (i) to check the impact of dimethoate pesticide on the morphological (R/S ratio, biomass), biochemical (Chlorophyll a), and stress indicative parameters (phytotoxicity and proline content) of *Sorghum bicolor* plants, (ii) to explore the pesticide tolerance of PGPF T103 (mitotic activity, sporulation and pesticide bond breakage); and (iii) to validate the protective effect of T103 on *S. bicolor* subjected to low and high concentrations of dimethoate stress, and its role in restoring plant growth at the greenhouse level.

## Materials and Methods

### *Plant material and pesticide*

*Sorghum bicolor* CSH-16 seeds were procured from the Indian Institute of Millets Research (IIMR), Hyderabad. Seeds were surface sterilized with $HgCl_2$ (0.1%) for 5 minutes and thoroughly rinsed with double distilled water to remove $HgCl_2$ completely [23]. Dimethoate, 30% EC (Rogor) formulation was procured from Agriberi India. A fresh stock solution of 10,000 ppm was prepared in molecular grade double distilled water for every experiment and diluted to varying concentrations (10 to 600 ppm) as per the dose required.

### *Plant growth promoting fungi and plant material*

PGPF *Trichoderma harzianum* T103 isolate (GenBank ID- KP122935.1) was used in the present study. Culture was grown and maintained on malt dextrose agar (Himedia laboratories private limited, Mumbai). Stocks are maintained for longer periods of time at -80$^0$C in glycerol stock (30%). PGPF isolate T103 was grown on MDA plates for one week at 30$^0$C. Spores were harvested in sterilized saline (0.85% w/v) and further pooled into 1.5% CMC (carboxymethyl cellulose), post centrifugation, at 3000 RPM for 10 minutes. The spore dose was adjusted to $10^8$ spores /

**Table 1:** Dimethoate application and residues factsheet

| Pesticide | Field application levels | DM residue levels | Application frequency |
|---|---|---|---|
| Dimethoate (DM)<br><br>1. Molecular formula- $C_5H_{12}NO_3PS_2$<br><br>2. Hazardous substanceClass II<br><br>3. Readily available in the market and lead agrochemical produced by Indian agricultural companies<br><br>(Annual production in India for the year 2021 is 1391 MT ) | DM 30% E.C (100mL bottle dissolved in 100 to 500 liters of water)-<br>Concentration of applying solution ranges from 60 ppm to 300 ppm | Concentration range in ppmSoil (58 ppm)<br><br>Honey (1-10 ppm)<br><br>Spinach (2.54 ppm) MRL fixed by C.A is<br><br>0.02ppm-0.5 ppm in majority of the crops, and 2 to 5 ppm<br><br>in some citrus fruits andspices | One to 6 times in a crop season, depending on the type of crops andinfection. |

**Source-** INCHEM, Codex Alimentarius, Sun et al., 2015 [8, 9, 21, 22].

ml and used for *in-vivo* plant growth assays and greenhouse studies [24].

***Dimethoate tolerance study***

A 6 mm Agar plug was detached from sterile MDA plate (centrally) and replaced with a T103 culture plug of the same size. Plates were incubated at 30±2 $^0$C for 120 hours, and radial growth was recorded at the interval of 24 h. Average linear growth rate per day was estimated from the growth recorded at 24 and 72 hours. The $LD_{50}$ value for DM in T103 was estimated as previously described by Kumari and Sattiraju, 2022 [20]. In a separate assay, Malt dextrose broth was supplemented with different concentration of DM (0-2100 ppm) and incubated at 30±2$^0$C for 5 days at 120 RPM. On fifth day, mycelia were harvested, and biomass was recorded in triplicate after drying at 70$^0$ C overnight.

***FTIR analysis***

The ethyl acetate extracts of DM control (300 ppm) and PGPF treated samples were analyzed with a FTIR spectrophotometer (Spectrum two FTIR, PerkinElmer, UK). Quantities of 10 µL of both samples were placed on a diamond window of the instrument separately at room temperature (25$^0$C) in the frequency range of 4,000–400 cm$^{-1}$. The procedure followed was as given by Kathuria et al., 2022 [25].

***In-vivo plant growth study with and without PGPF treatment***

Surface sterilized sorghum seeds were transferred to 0.25% water Agar and incubated in plant growth chamber for 72 h. Post emergence, seedlings were treated with PGPF inoculum (@ $10^8$ cells/ml for 60 minutes. After seedling treatment, seeds were transferred to sterilized ASURE boxes containing 300 ml of PMB media. Detailed information on ASURE assay is published previously [26]. After 15 days, plants were harvested and plant growth parameters were analyzed.

***Greenhouse study***

Surface sterilized sorghum seeds were sown in plant growth bags of 1 kg containing a sterile mixture of soil and peat moss in the ratio of (7:3). Post 1 week uneven seedlings were removed and 3 seedlings were maintained per pot. Healthy and uniformly grown seedlings were treated with PGPF inoculum For each treatment, 15 replicates were maintained. DM was added at 50 ppm, 150 ppm, 300 ppm, and 600 ppm, in the rhizospheric region of potted plants. Plants were kept in a greenhouse with a temperature of 28 ± 2$^0$C and a relative humidity of more than 70%, and watered every alternate day. Plants were harvested and analyzed to observe growth impact on pesticide stress as compared with unchallenged host plants [27].

***Statistical analysis***

Each experiment was conducted in triplicates, and results are presented as the mean ± standard deviation. GraphPad Prism version 9.5 (GraphPad, San Diego, CA, USA) was used for the statistical analyses through one-way ANOVA to identify significant differences amongst the treatments followed by Tukey's post hoc test. The values of $P<0.01$, $P<0.05$ for biochemical assay and plant based studies, respectively were considered statistically significant.

**Results and Discussion**

***Evaluation of dimethoate tolerance by selected PGPF***

To check the impact of pesticide on growth of chosen PGPF T103, DM plate assay was done at the concentration of 0 to 2000 ppm of DM. Average linear growth of PGPF was decreasing with increasing concentration of DM. The maximum ALGR (mm/Day) was recorded at 0 ppm and the minimum at 1000 ppm. Sporulation was observed to take place even at a concentration as high as 1000 ppm; however, spore count was reduced. Spores are the

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seeds for *Trichoderma,* which are required for their propagation [28]. Figure 1 shows that it can produce spores up to 1000 ppm of DM (which is almost 100 times more than the maximum residue reported in crops).

Spore production at 1000 ppm is indicative of the isolates' potential to sustain their growth at higher concentrations of pesticides. Pesticides are known to impact microorganisms, animals and plants at cellular level adversely. According to Ore et al., 2022, pesticide application can influence microbial diversity, which can be detrimental to plant growth and development by decreasing nutrient availability or disrupting the nutrient cycle [29]. Hage-Ahmed et al., 2014 showed that pesticides changed the sporulation and fungal community composition [30]. Therefore, employing microbial bioinoculants with high tolerance that can grow and divide at higher concentrations of pesticides is the need of the hour.

| DM (ppm) | $T_0$ (0 ppm) | $T_{500}$ (500 ppm) | $T_{1000}$ (1000 ppm) |
|---|---|---|---|
| ALGR (mm/Day) | 21±0.96 | 13.3±0.57 | 8.3±0.33 |
| Spore suspension |  | | |
| Spore count | 1x $10^{10}$ | 5.5x $10^{9}$ | 4.4x $10^{9}$ |

**Fig.1.** Average linear growth and sporulation of PGPF-T103 under dimethoate stress

The liquid broth assay showed a negative correlation between pesticide concentration and biomass. At 0 ppm, the biomass was around 3.95± g/L which reduced to 1.91±0.18 g/L at 300 ppm, and at 2000 ppm, the concentration was as low as 0.55±0.18 g/L. The estimated $LD_{50}$ value for DM was near 300 ppm. In both DM plate and broth assays, PGPF strain T103 showed striking pesticide tolerance potential.

***Comparative analysis of infrared spectra of control and PGPF treated samples***

To observe any structural alterations that occurred due to PGPF-T103 treatment in contrast to untreated DM control, samples were analyzed using FTIR, where spectral readings ranged from 600 to 4000 $cm^{-1}$. In the PGPF-T103 treated sample, a new peak was recorded at 3456 $cm^{-1}$ which was absent in the control sample. This peak corresponds to –OH and -NH stretching [31]. Significant variation in the peaks corresponding to 1043 $cm^{-1}$ and 1235 $cm^{-1}$ was observed in the treated sample, indicating C-O stretching. Characteristic peak variation at 1737 $cm^{-1}$ (C=O stretching) was observed, with considerable variation in peak reduction in the PGPF treatment (35%T) compared to the control (28%T) (Figure 2). In the current study, variations in FTIR readings were observed at 1043, 1235, and 1737 $cm^{-1}$, similar to what was observed in other studies [32, 33]. Prolonged treatment (15 days) with PGPF resulted in effective disappearances of these pesticide specific bonds.

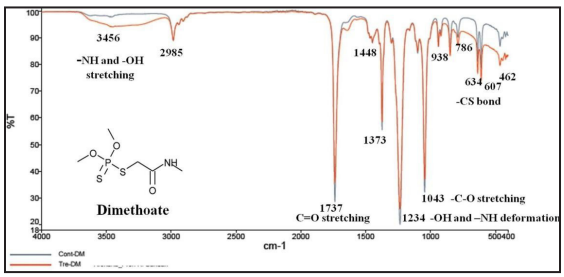


**Fig.2.** FTIR analysis of the PGPF-T103 treated sample as compared to the DM Control (300 ppm)

***Comparative phytotoxicity analysis at low and high concentrations of dimethoate***

In comparison to the control (No pesticide) drastic reduction in shoot length, root lengths, biomass and chlorophyll contents were recorded in presence of DM (at both 10 ppm and 300 ppm) (Table 2). Similar to our results Pandey et al, 2022, demonstrated that DM administration on wheat plants for

prolonged periods of time (>10 days) showed an inhibitory effect on plant growth parameters such as root length, shoot length, fresh weight, dry weight and chlorophyll content. DM is water soluble, so it is quickly absorbed by plant roots and accumulates in leaves, where the majority of plant metabolic activities occur. The buildup of DM in plant may impede cell division and elongation, resulting in a reduction in root and shoot length. Lower dosages that had a positive response for a short period of time become harmful if treatment periods are extended beyond 10 days [34]. Even though some scientific groups present an argument that OPPs are water soluble, have shorter half life and thus may not hold greater research emphasis due to lesser retention times in the soil ecosystems. This observation on impact at concentrations that are three times less to field application rate, as evidenced in this study, calls for a concentrated thought on possible future yield impact. Range of DM pesticide applied by farmers and reported residues in soil and plant sample is detailed in Table 1. The exposure to very high pesticide dose may be transient but the impact can be long lasting (Table 2). At higher concentrations there is stark indication of pesticide toxicity on photosynthesis. In this study almost half reduction in chlorophyll a content was recorded at 300 ppm. Chlorophyll is a necessary component of the plant photosystem that is required for photosynthesis. Gitelson et al. (2006) argued that estimating chlorophyll content is critical in determining output. As variations in canopy Chl are connected to crop phenology, canopy stresses, and vegetation photosynthetic capability, it can also be related to gross primary production (GPP) [35]. The amount of chlorophyll in the leaves determines photosynthetic capacity and is thus one of the most significant physiological factors influencing crop yield [36]. Sharma et al., 2019 proposed that pesticide stress affects photosynthesis for a variety of reasons, including the down regulation of photosynthetic enzymes, which reduces the production of photosynthetic pigments, the decreased stability of protein-pigment complexes, which causes an increase in pigment degradation, the restriction of QA oxidation, which prevents the electron transfer from PSII to PSI, and the production of free radicals [37]. Mishra et al, 2008, demonstrates that DM has an immediate effect on the different locations of the photosynthetic electron transport chain. At all DM concentrations, PS II reduction and chain activity were observed. With rising dimethoate concentrations, there was a considerable decrease in PS II and overall chain activity, which is linked to damage to the oxygen-evolving complex, which eventually gets transferred to the plastoquinone site and the PS II reaction centre [38]. Impacts of pesticides on animal systems are well studied [39-42]. On the contrary, phytotoxicity data is very scarce, which necessitates the studies being conducted on multiple crops. Parween et al, 2014 reviewed the effect of pesticide on plants and concluded that it leads to alteration in biochemical, physiologi-

Table 2: Evaluation of plant growth parameters at field application and at residue levels

| Pesticide concentration | Reduction inRL | Reduction in SL | Reductionin DW | Reduction in Chlorophyll content | Percent phytotoxicity |
|---|---|---|---|---|---|
| At field residue level | 14.6 % | 11.7 % | 22% | 6.8% | 15% |
| At field application level | 69.7 % | 37.6% | 39% | 48.8% | 70% |

cal parameters of plants which might be visible as decrease in yield [43]. The majority of research studies on plants, have focused on pesticide residue detection rather than investigating the physiological phytotoxicity caused by pesticides. The findings of this study will help researchers better understand the influence of DM on numerous plant development indices, notably in *Sorghum bicolor*, at both the residue and field application levels.**Table 2:** Evaluation of plant growth parameters at field application and at residue levels

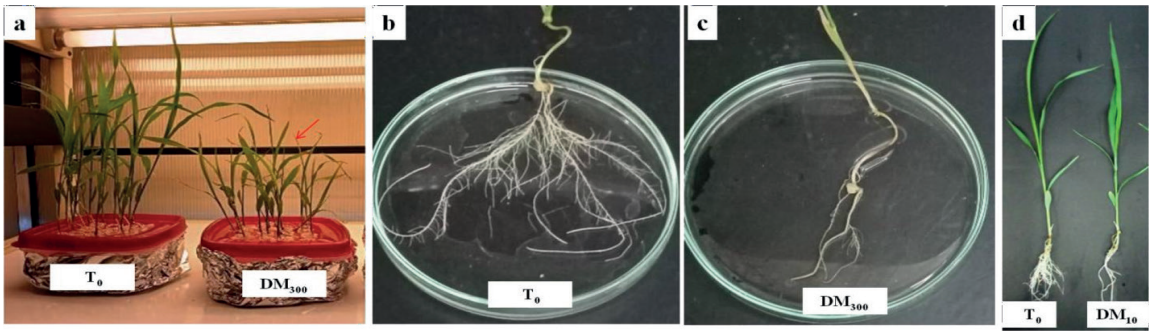


**Fig.3.a.** Phytotoxic impact of DM on aerial plant parts at field application level; $\mathbf{T_0}$ (No pesticide), $\mathbf{DM_{300}}$ (300 ppm of DM), **b.** Phytotoxic impact of DM on root system architecture at field application level **c.** plant phytotoxicity at residue level (10ppm); $\mathbf{T_0}$ (No pesticide), $\mathbf{DM_{10}}$ (10 ppm of DM).

Comparative analysis of RSA was done both visibly and by using RhizoVision Explorer version 2.03 software and results are detailed in Table 3. Reductions in root branching and root hairs were clearly visible in the presence of DM (Figure 3). Profuse primary and secondary root branching and extended root system architecture was observed in the absence of pesticide stress. Upon challenging with DM the plant exhibited a severely constricted root architecture, where there is a predominant decrease in the number of primary, secondary and tertiary roots were recorded. The overall root surface area was reduced by >1000 fold in DM challenged plants. As the root hairs take nutrients and water from the soil, a healthy and robust root system is essential for plant growth. [44]. These morphological changes may have an influence on yield, since biomass was reduced by 22% and 39% at 10 ppm and 300 ppm, respectively. The findings of this study reveal that DM has a detrimental impact on plant development and that the plant cannot overcome it on its own. Therefore, utilizing microorganisms with the ability to protect plants under pesticide stress (DM) would help in alleviation of phytotoxicity due to pesticides. In this study, PGPF-T103 was selected for further *in-vivo* plant growth assays and greenhouse studies.

Table3. Comparative analysis of RSA under no pesticide condition and DM stress

| DM (ppm) | R.O.I | No. of roottips | No. of branch points | Total root length. px | Network area.px2 | Volume. px3 | Surface area.px2 |
|---|---|---|---|---|---|---|---|
| **0 ppm** | Full | 38 | 40 | 1956 | 42701 | 17315259 | 476353 |
| **300 ppm** | Full | 2 | 4 | 31 | 247 | 0.96 | 427 |

* Pixel to millimeter conversion factor-25.4, px-pixel, RSA-root system architecture, R.O.I-Region of interest

***Plant growth support by PGPF under dimethoate stress in in-vivo bioassay***

The effects of inoculation with PGPF on plant growth were visible on shoot length, root length, plant biomass, and the root system architecture of sorghum plants at 15 days after germination (Figure 4). Seed priming with PGPF exhibited a significant ($p < 0.05$) improvement in the growth of sorghum plants under normal (no pesticide) and pesticide stress (10 to 300 ppm DM) conditions. It resulted in an increase in root length, of 29%, 53%, 233%, and 119% at 10 ppm, 50 ppm, 100 ppm, and 200 ppm, respectively. However, the increases in shoot length were 15% at 10 ppm and around 3% at 200 ppm. *Trichoderma* was also earlier known to increase root length under drought and salinity stress [45, 46]. Biomass increased by 36%, 10% was recorded at 10 ppm and 200 ppm, respectively (Figure 4). Chlorophyll has direct functions in photosynthesis and is thus strongly related to crop photosynthetic capacity, development, and yield. Ghimire et al, (2015) [47] demonstrated that chlorophyll concentration has a favorable and substantial influence on maize grain production. A significant increase in chlorophyll content was observed in PGPF treatments up to 100 ppm of DM under *in-vivo* conditions. PGPF helps the plants under stress in two main ways, firstly, by giving growth support (increase in root length, shoot length, biomass); and secondly, by alleviating the phytotoxicity and by producing molecules like proline that help them cope with the stressed condition.

Proline is required by plants in a number of stress scenarios. It is a good osmolyte, metal chelator, antioxidant defense molecule, and signaling chemical [48]. In this study, increasing DM concentrations resulted in a steady rise in proline content in untreated plants. Proline is a stress marker and produced under abiotic stress conditions to stabilize proteins, enzymes, to maintain membrane integrity, to detoxify ROS [49]. Pesticide leads to drying of plant which leads to cellular dehydration in such conditions plants produce proline which act as an osmoprotectant [50]. Shakir et al, 2018, also reported similar results, where imidacloprid pesticide significantly increased proline levels (65% to 138%) in the shoot tissues at all applied concentrations [51]. In this study, PGPF treatment resulted in lower proline levels than the untreated control at each concentration (Figure 5). The values are statistically significant; implicating that PGPF-T103 treatment was effective in easing stress response in plants. Lower proline concentration after PGPF treatment demonstrates the efficacy of PGPF to rescue the plants from DM stress, as greater levels of proline are recorded under various abiotic stresses.

**Greenhouse study**

A long-term greenhouse assay (45 days) was performed to evaluate the ability of PGPF-T103 to alleviate the DM stress symptoms in *S. bicolor* and support plant growth. Figure 6 shows the phytotoxicity percentage based on RL and SL recorded under varying concentrations of DM. Other investigations also noted a reduction in the length of the shoots and roots under pesticide stress [52, 53]. Phytotoxicity was more visible in roots than shoots. For instance, at 50 ppm DM, phytotoxicity was 29% in roots whereas in shoot phytotoxicity was 9%. A similar pattern was observed at 300 ppm DM, where the phytotoxicity in the root was 76% and that in the shoot was 48%. This could because roots are highly sensitive in recognizing the physicochemical variations in the soil, and also because they are the principal structures that can influence sustained nutrient and signaling functions of the plants that are challenged with a multitude of abiotic stresses [54]. Thus till a threshold concentration, the pesticide stress might be affecting the plant root system visibly more as compared to its aerial parts. However, once pesticide tolerance is breached the impact is prominent even in the aerial parts of the plant. For instance, browning of shoot tips, necrosis, chlorosis, burning, stunted growth, decrease in number of leaves as well as their width was recorded at higher concentration of DM (300, 600 ppm).

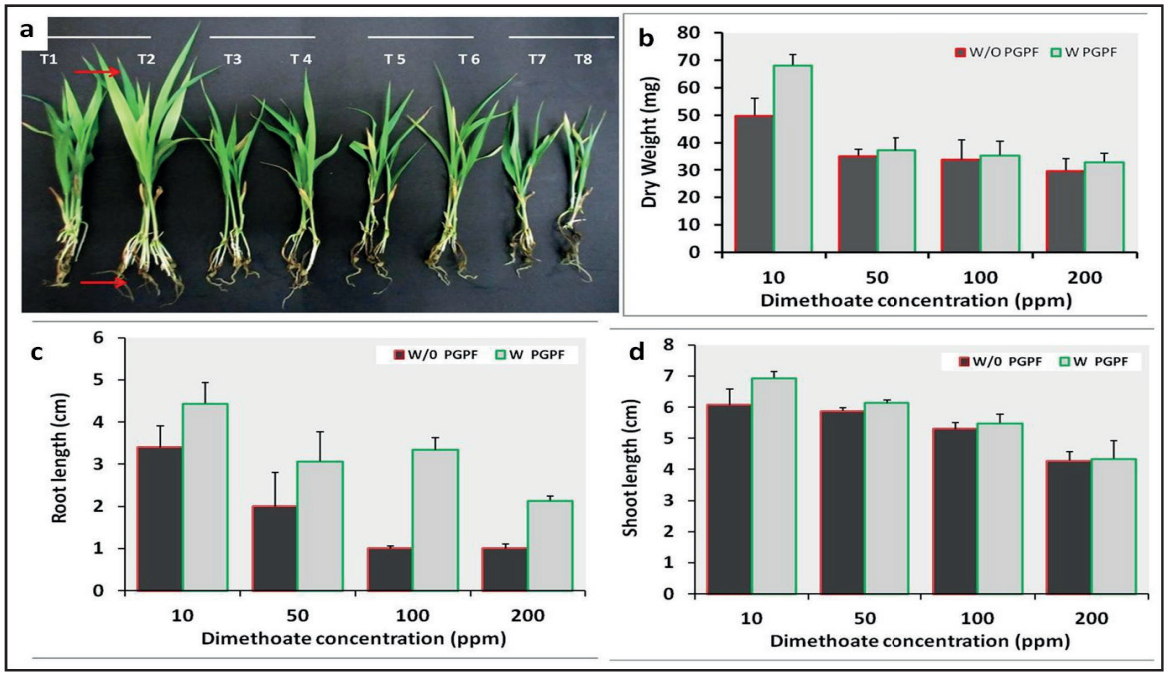


**Fig.4.** Plant growth support by PGPF-T103 under dimethoate stress **a.** Plant growth under DM stress (10-200 ppm), **T1** (10 ppm DM), **T2** (10 ppm DM+ T103), **T3** (50 ppm DM), **T4** (50 ppm DM+T103), **T5** (100 ppm DM), **T6** (100 ppm+ T103), **T7** (200 ppm DM), **T8** (200 ppm+ T103), **b.** Chlorophyll content, **c**. Root length, **d.** Shoot length and **e.** Dry weight, under DM stress 10 ppm with and without PGPF-T103 treatment. The comparison of treatments was performed by ANOVA single factor, P value <0.05 was considered as statistically significant. Phytotoxicity symptoms were recorded in plants under DM stress, where PGPF treatment is not done. At 10 ppm of DM, phytotoxicity was 10%, and it was around 65% at 300 ppm of DM.

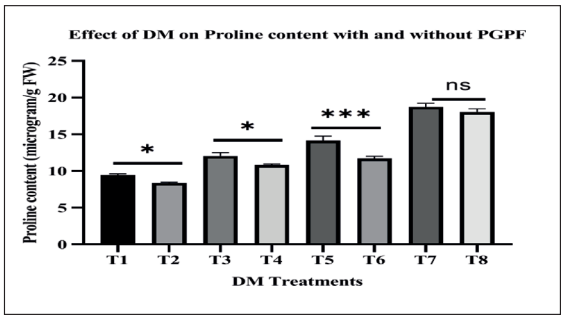


**Fig.5.** Proline content under DM stress in presence and absence of PGPF-T103, **T1** (10 ppm DM), **T2** (10 ppm DM+ T103), **T3** (50 ppm DM), **T4** (50 ppm DM+T103), **T5** (100 ppm DM), **T6** (100 ppm+ T103), **T7** (200 ppm DM), **T8** (200 ppm+ T103), The comparison of treatments was performed by ANOVA single factor, P value <0.05 was considered as statistically significant. ns- Not significant, *-P<0.05, **-P<0.01, ***-P<0.001

Similar symptoms due to pesticide stress were reviewed by Sharma et al, 2017 [55]. A close observation of Figure 6 reveals this fact, where up to 300 ppm the impact on shoot phytotoxicity was much lesser as compared to impact on root. However, beyond threshold (300 ppm), the adverse impact on the shoot is as strong as the adverse impact on root. PGPF-T103 application resulted in better root systems in *S. bicolor*. Growth parameters, such as the RL, SL and DW recorded from 45 days old plants, are shown in Table 3. Inthama et al, 2021 also observed that application plant growth promoting microbe *B. aryabhattai* in paraquat pesticide contaminated soil, resulted in longer root and shoot lengths in cowpea [55]. In another study *Trichoderma asperellum* increased the phoxim tolerance in *Solanum lycopersicum* by promoting plant detoxification potential [56].

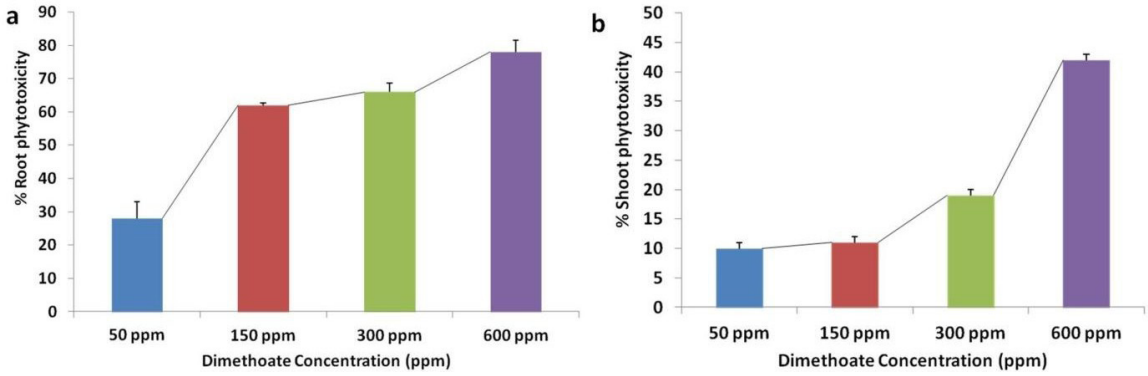


**Fig.6.** Phytotoxicity based on **a.** root length and **b.** shoot length under DM stress (50 ppm, 150 ppm, 300 ppm and 600 ppm)

Rhizospheric application of PGPF T103 increased the plant growth up to 300 ppm of DM (Figure 7). Findings of our study corroborate the previous findings where application of microbes helped the plants to cope up with phoxim pesticide stress [57]. PGPF-T103 enhances the R/S ratio at each concentration of DM. For instance, without PGPF treatments, the R/S ratio decreased from 5.6 (at 0 ppm), to 4.3 (at 50 ppm), 0.23 (at 150 ppm), and 0.21 (at 300 ppm). Upon PGPF treatment, the R/S ratio was 0.66, 0.35, and 0.34 at 0 ppm, 150 ppm, and 300 ppm, respectively. This could be because PGPF improves the architecture of root systems, which is evident from the increased root lateral branching and root hair development. Plants with a higher value of R/S indicate their enhanced ability to absorb water and nutrients. The percentage increase in root length, shoot length, and biomass at con

Values are the mean value ± S.D of three independent replicate. The comparison of treatments was performed by one-way ANOVA followed by Tukey's post hoc test, the values of P ≤0.05 were considered statistically significant*

**Table 4.** Effect of DM stress on growth parameters of S. bicolor in presence and absence of PGPF-T103

| Treatments | RL (cm) | % Increase RL upon PGPF treatment | SL (cm) | % Increase in SLupon PGPF treatment | DW(mg) | % Increase in DWupon PGPF treatment |
|---|---|---|---|---|---|---|
| **T1 (0ppm)** | 5.6±0.5 | Control (0ppm) | 10±0.1 | Control (0ppm) | 202±2 | Control (0ppm) |
| **T2 (0ppm+PGPF)** | *7±0.5 | 25% | 12±9.1 | 20% | *227±6.7 | 12% |
| **T3 (50 ppm)** | 4±0.9 | Control (50ppm) | 9.1±0.5 | Control (50ppm) | 160±11 | Control (50ppm) |
| **T4(50ppm+PGPF)** | ****7.3±0.6 | 82% | 11±0.4 | 17% | ****204±11 | 27% |
| **T5 (150ppm)** | 2.1±0.1 | Control(150 ppm) | 9.1±0.1 | . | 89±2.5 | Control(150 ppm) |
| **T6 (150ppm+PGPF)** | *3.8±0.6 | 80% | 11±0.6 | 17% | 107±4.2 | 20% |
| **T7 (300ppm)** | 1.9±0.3 | Control(300 ppm) | 8.1±0.7 | Control(300 ppm) | 67±9.3 | Control(300 ppm) |
| **T8 (300ppm+PGPF)** | * 3.4±0.1 | 79% | *10±0.8 | 23% | **96±8.7 | 43% |
| **T9 (600ppm)** | 1.2±0.3 | Control(600 ppm) | 5.2±0.2 | Control(600 ppm) | 33±5.8 | Control(600 ppm) |
| **T10 (600ppm+PGPF)** | 2.3±0.5 | 92% | 5.9±1 | 14% | 50±6.8 | 52% |

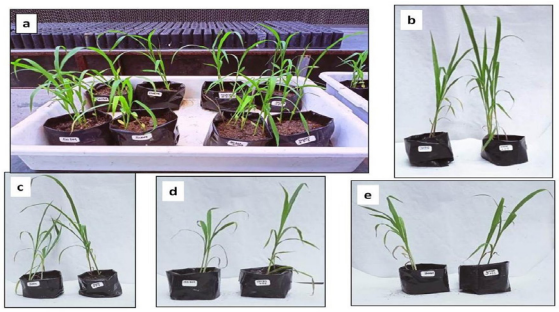


**Fig.7.** Effect of DM stress on *S.bicolor* grown in the greenhouse at 45 Days; **(a)** Plants grown in greenhouse **(b)** untreated control pesticide (0 ppm), treated 0 ppm+ PGPF; **(c)** 50 ppm, 50 ppm+ PGPF; **(d)** 150 ppm, 150 ppm+ PGPF; **(e)** 300 ppm, 300 ppm+ PGPF. In b, c, d and e- Plants on the left side are untreated and on the right side are treated with PGPF-T103 centrations of 0 ppm, 50 ppm, 150 ppm, and 300 ppm is presented in Table 3. PGPF was able to support plant growth both at 15 days and at 45 days of experiments. Plant growth support by microbes under xenobiotic stress was established by few other researchers [58, 59]

## Conclusion

DM influences plant growth and development in terms of both qualitative and quantitative parameters. The deleterious effects of DM are evident right from the concentrations as low as 10 ppm which escalated further as the concentration of DM has increased. PGPF-T103 primed seedlings were successful in overcoming stress due to DM more effectively till a concentration of 100 ppm, beyond 100 ppm and till 300 ppm even though they performed better than PGPF- T103 untreated host they were not completely successful in reversing the impact of DM induced stress. Nevertheless, irrespective of the concentration of pesticide PGPF-T103 treated seedlings invariably suffered less from the DM exposure as compared to those plants that were left untreated. Treated plants were supported by the increased root length; shoot length, Chlorophyll a and drop in the proline content and phytotoxicity. The protective impact and growth support of sorghum were observed both in *in-vivo* (15 days) and greenhouse (45 days) studies. These results, linked to the PGPF-T103 application, are definitive evidence that *Trichoderma harzianum* isolate T103 can protect the plants under pesticide stress.

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**Abbreviations:**

**PGPF**-Plant growth promoting fungi
**DM**-Dimethoate
**$LD_{50}$**-Lethal dose 50
**FTIR**-Fourier Transform Infrared Spectroscopy
**MRL**-Maximum Residue Limits
**OPP**-Organophosphate pesticides
**EC**- Emulsifiable concentrate,
**MDB**-Malt dextrose broth, PMB-Plant microbe bioassay
**CMC**-Carboxymethyl cellulose
**ALGR**-Average linear growth rate
**RPM**-Revolutions per minute
**R/S ratio**- Root/Shoot ratio
**RL**-Root length
**SL**-Shoot length
**DW**-Dry weight
**ChI**- Chlorophyll
**PMB**-Plant microbe bioassay
**PPM**-Parts per million
**PP%**-Phytotoxicity %