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# Bio-Management of *Fusarium oxysporum* in carnation by application of Bio Agents

**ANUJ PRASAD, VIJAY KUMAR*, KALPNA, GURUSIMRAN KAUR AND PANKAJ BAHUGUNA**
*Department of Plant Pathology, Veer Chandra Singh Garhwali Uttarakhand University of Horticulture and Forestry, Bharsar, Pauri Garhwal, Uttarakhand – 246123*

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Carnation (*Dianthus caryophyllus* L.) is one of the most important cut flowers cultivated worldwide. It belongs to the family Caryophyllaceae and has a diploid chromosome number of 2n = 30. In India annual production of carnation is 6 MT.Five bioagents were evaluated against *F. oxysporum* f. sp. *dianthi* under both *in vitro* and *in vivo* conditions. Among these, *Trichoderma viride* exhibited the highest inhibitory effect, reducing mycelial growth by 73.21%, followed by *T. harzianum*(56.43%) in dual culture assays. In field trials, application of *T. harzianum* @ 5 g/l resulted in the lowest disease incidence (17.03% and 28.07%) and the highest disease control (58.75% and 61.36%) at 45 and 90 days after treatment (DAT), respectively. Correspondingly, the maximum yield (386g/plot) was also recorded in the *T. harzianum*treated plots.

**Keywords :** *Dianthus caryophyllus,* dual culture, *Fusarium* wilt,*Trichoderma harzianum*

## INTRODUCTION

Carnation is a semi-hardy perennial herb characterized by thick, narrow and fleshy leaves, typically green to bluish or purple. The plant has strong stems with swollen nodes, each generally bearing a single terminal flower arranged in a cyme. Flowers are mostly bisexual and range from white to pink and purple, usually measuring 6-8.5 cm in diameter, although exhibition varieties may reach up to 10 cm(Ali *et al*.2008). Carnation (*Dianthus caryophyllus* L.), a dicotyledonous member of the family Caryophyllaceae, is one of the most commercially cultivated flowers worldwide. It is an important floriculture crop grown year-round in temperate climates. Its extended vase life exhausts its market value in both wholesale and retail sector (Aalifar*et al*. 2020).As a cut flower due to its excellent keeping quality, wide array ofcolour and forms,also ability to withstand long distance transportation and remarkable ability torehydrate after continuous shipping. Their significance has been widely highlighted in ancient Sanskrit literature such as the *Rig Veda*, *Ramayana* and *Mahabharata*, where flowers are frequently associated with beauty and sacredness.

Carnations are preferred to roses and chrysanthemums, in several exporting countries as cut flower can also become useful in gardening for bedding, edging, borders, pots, and rock gardens and commercially utilized for extraction of perfume in France and the Netherlands (Samatha, 2016).

*Fusarium* wilt symptoms include yellowing and stunted growth in older plants, initially affecting one side, which eventually leads to the wilting of the entire plant. The xylem tissue dries up and turns brown, causing the plant to die. In rooted cuttings, stem rot appears as a moist, greyish-black decay at the soil level, leading to wilting and death of the plant upper part. Brown mycelial strands are often visible on the surface of the affected tissues.

The pathogen infects the plant vascular system, disrupting water and nutrient flow, causing

*Correspondence : vijaykumar.india28@yahoo.in

chlorosis and wilting of the lower leaves and shoots, typically on one side of the plant. The remaining leaves may stay green for a while. *Fusarium* infection results in cell wall degradation, including lignin breakdown and vascular browning due to discoloration of the infected vessels and surrounding cells (Sharma and Sharma, 2008).*Fusarium* wilt of carnation, caused by *Fusarium oxysporum* f. sp. *dianthi,* is a significant threat to carnation (*Dianthus caryophyllus*) farming, causing major economic losses. Biological control agents (BCAs) provide an eco-friendly and sustainable alternative to chemical fungicides for controlling this disease. Different biocontrol agents, such as *Trichoderma* spp., *Pseudomonas* sp. and *Bacillus* sp., have been effective in suppression *Fusarium* wilt (Singh *et al*., 2022).

## MATERIALS AND METHODS

The present study was conducted at VCSG, Uttarakhand University of Horticulture and Forestry (UUHF), Bharsar, Pauri Garhwal, Uttarakhand. The investigation focused on assessing the efficacy of various bioagents against *Fusarium oxysporum* causing wilt disease in carnation under both *in-vitro* and *in-vivo* conditions.

### *Isolation of pathogen*

An infected carnation leaf showing typical wilt symptoms was collected from the Floriculture and Landscaping Block, College of Horticulture, VCSG UUHF, Bharsar and brought to the laboratory for pathogen isolation. The sample was washed under running tap water, air-dried and small sections (2 cm) taken from the junction of healthy and infected tissues. These pieces were surface sterilized in 0.1% sodium hypochlorite for 30 seconds, rinsed thoroughly with sterile distilled water and aseptically plated on Potato Dextrose Agar (PDA). The plates were incubated at 25 ± 2 °C and monitored daily for fungal growth. After about one week, the emerging fungal colony was purified through repeated subculturing and maintained on PDA slants at 4 °C for further study. Identification was carried out based on microscopic examination of the mycelial characteristics.

### *In vitro evaluation of different biocontrol agents against Fusarium oxysporum* f. sp. *dianthi*

#### Dual culture technique

Bioagents collected from the College of Horticulture, Bharsar and maintained on PDA medium were evaluated for their effectiveness against the carnation wilt pathogen. The fungal and bacterial isolates were tested *in vitro* for their antagonistic activity against *Fusarium oxysporum* f. sp. *dianthi* using the dual culture method (Dennis and Webster, 1971) on PDA medium. 7 day old cultures of both the bioagents and the *Fusarium* pathogen were used for the experiment.

A 5 mm fungal disc of the antagonist and the test fungus were placed in opposite directions on the PDA medium. The plates were incubated at 25 °C for seven days, after which the interaction or inhibition zones were measured. The nature of the antagonism was classified as weak, medium or strong parasitism based on the extent of growth suppression of *Fusarium oxysporum* f. sp. *dianthi* by the bioagent.

Bacterial antagonists such as *Bacillus subtilis* and *Pseudomonas fluorescens* were streaked on one side of the PDA medium, while the carnation wilt pathogen was streaked on the opposite side. The inhibition zone was measured and the mode of antagonism was recorded. After incubation, the growth of the *Fusarium* colony was recorded and the percentage inhibition of the colony, compared to the control was calculated using the previously established method and formula.

The *in vitro* assessment of the plant extracts was conducted using a Completely Randomized Design (CRD) with three replications. The radial colony growth of the fungi was measured at 7 days of incubation. The efficacy of plant extracts was expressed as per cent growth inhibition of mycelial growth over control, calculated by using formula suggested by (Vincent, 1947).

Per cent growth inhibition

$$(PGI)=\frac{C-T}{C}\times 100$$

Where, PGI = Per cent inhibition; C = Radial growth of fungus in control; T = Radial growth of fungus in treatment.

***In vivo evaluation of different plant extracts againstFusarium oxysporum f. sp. dianthi***

The field experiment was conducted in 2024-2025 at Floriculture and Landscaping block,Bharsar, Uttarakhand using Randomized Complete Block Design with four replications. The field was properly ploughed and then pulverized discing in order to bring the soil in well pulverized condition. The stones, pebbles, weeds and residues of previous crops was removed manually. It was then levelled using a *pata* and divided into 24 plots with irrigation channels, maintaining proper spacing to distinguish different replications as shown in the layout. Each plot measured 90 cm × 60 cm. The cuttings were planted at a spacing of 15 cm × 15 cm per bed in the soil. After planting, the plots were watered to ensure adequate moisture for proper growth and establishment. Cuttings were planted in the second week of April 2024. The observations were recorded taking five randomly selected plants from each treatment in each replication and their average was worked out to record the data. The observations were recorded as Per cent disease incidence (PDI), Per cent disease control (PDC) from each plot and yield g/plot and q/h was also recorded from each plot separately.

**Percent Disease Incidence (PDI)**

Percent Disease Incidence or Wilt incidence was calculated with the assistance of following formula and also the marking table given by (Wheeler, 1969).

$$\text{Percent disease incidence} = \frac{\text{No. of infected plant}}{\text{Total No. of plants}} \times 100$$

**Percent Disease reduction over control**

Percent Disease reduction over control was calculated by the following formula (Sahu *et al.* 2013).

$$\text{Percent disease reduction over control} = \frac{\text{PDI in control plot} - (\text{PDI in treatment plot})}{\text{PDI in control plot}} \times 100$$

***Statistical analysis***

Data from the laboratory experiments were analyzed using the standard procedures for a Completely Randomized Design (CRD), while data from the field trials were assessed following a Randomized Complete Block Design (RCBD). All measured parameters were evaluated through analysis of variance (ANOVA) based on the method described by Gomez and Gomez (1984). The statistical analyses were carried out using OPSTAT and Microsoft Excel.

**RESULTS AND DISCUSSION**

***Effect of different bioagents on mycelial growth inhibition against Fusarium oxysporumf.* sp*. dianthi***

The effectiveness of various bioagents in suppressing the growth of *Fusarium oxysporum* f. sp. *dianthi* was assessed under laboratory conditions using the dual culture technique. The bioagents tested included *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma asperellum*, *Pseudomonas fluorescens* and *Bacillus subtilis*.

After a 7days incubation period, the average mycelial growth (in mm) of the pathogen was measured and the percentage of mycelial growth inhibition was calculated. Among the bioagents tested, *Trichoderma viride* - showed the maximum inhibition of mycelial growth, with a mycelial growth of (23.02 mm) and (73.21 %) inhibition. This was followed by *T. harzianum* (37.43mm),(56.43%) inhibition and *T. asperellum* (44.89 mm), (47.47 %) inhibition. The minimum inhibition was recorded with *P. fluorescens* and *Bacillus subtilis* which showed a mycelial growth of (52.01 mm) and (39.45%) inhibition, (58.10) and (32.36%) inhibition ( Fig.1, Table 1). All treatments demonstrated significant effectiveness in reducing the pathogen's growth compared to the control.In alignment with these observations, Marquex*et al.* (2002) and Kishore

and Kulkarni (2008) demonstrated that both *T.viride* and *T. harzianum* effectively suppressed the pathogen. Negi *et al.* (2009) reported that among different isolates of *Trichoderma spp*., *T. viride* is the most effective with 67.4 per cent inhibition followed by *T. harzianum* with 63.7 per cent inhibition in mycelial growth of wilt pathogen *F. oxysporum*f.sp.*dianthi.*

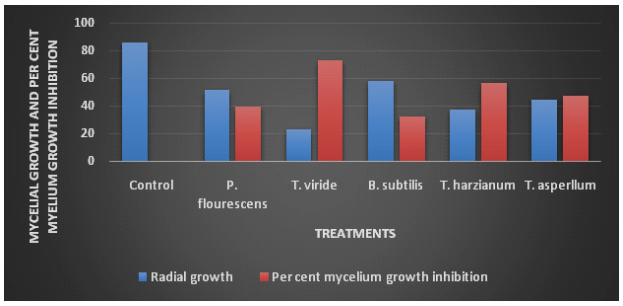


**Fig.1**: Effect of different bioagents on mycelial growth and per cent mycelium growth inhibition against *Fusarium oxysporum*f. sp.*dianthi*

### *Effect of bioagents on per cent disease incidence (PDI) 45 DAT and 90 DAT*

#### At 45 DAT

The data recorded in the table 4.5 showed significant differences between control and all the treatments for wilt incidence. The performance of the treatments ranged from (17.03% to 41.37%). Minimum per cent disease incidence was observed in $T_1$ (*Trichoderma harzianum)* (17.03%) followed by $T_3$ (*Trichoderma viride)* (19.29%), $T_2$ (*Pseudomonas flourescens)* (24.35%), $T_6$ *(Trichoderma aperellum)* (27.16%) whereas maximum per cent disease incidence was observed in $T_1$ (control) (41.37%) followed by $T_4$ (*Bacillus subtilis)* (34.48%).

#### At 90 DAT

The data showed significant differences between control and all the treatment. It ranged from (28.07%) (72.67%) at 90 days after transplanting. The minimum disease incidence was recorded in $T_5$ (*Trichoderma harzianum)* (28.07%), with $T_2$ (*Trichoderma viride)* (32.96%) and $T_6$ (*Trichoderma asperllum)* (43.52%). The maximum disease incidence was recorded in $T_1$(control) (72.67%). All the treatments were found effective than control for reducing wilt incidence.

The current research findings ( Fig.2, Table 2) are consistent with those of Sharma et al. (2019), who reported that all the tested bioagents demonstrated antagonistic activity against the wilt pathogen *Fusarium oxysporum* f. sp. *dianthi*under field conditions. Among the treatments, *Trichoderma viride* showed the maximum effectiveness, reducing the incidence of carnation wilt to 14.6% compared to 45.3% in the untreated control plots. Mahmoud and Abdalla (2018) demonstrated that *T. viride* and *T. harzianum* reduced sesame wilt severity by 77% and 74%, respectively, under greenhouse conditions. These results highlight the strong biocontrol potential of *Trichoderma* species against *Fusarium* wilt.

### *Effect on Bioagents on per cent disease control 45 DAT and 90 DAT*

#### At 45 DAT

The data ( Table 3) shows that all tested plant extracts effectively reduced the disease incidence compared to the untreated control. However, their effectiveness varied, with disease reduction ranging from 16.54% to 58.75%. The maximum reduction in disease severity (58.75%) was observed in treatment $T_5$ with *Trichoderma harzianum*, while the minimum reduction (16.54%) occurred in treatment $T_4$ with *Bacillus subtilis* ( Fig.3).

#### At 90 DAT

At 90 days after transplanting (DAT), significant differences in disease control were observed among the various bioagent treatments (Fig.3, Table 3). The maximum disease control (61.36%) was achieved with treatment $T_5$ (*Trichoderma harzianum*), showing its strong effectiveness in reducing disease incidence. This was followed by $T_3$ (*T. viride*, 54.63%), $T_2$ (*Pseudomonas fluorescens*, 40.07%) and $T_6$ (*T. asperellum*, 34.73%), which showed moderate control. The minimum disease control (16.54%) was recorded in $T_4$ (*Bacillus subtilis*), indicating its relatively lower performance under the study conditions.

The current research findings are consistent with those of Sharma *et al*.(2019), who reported that

**Table 1:** Effect of different bioagents on on mycelial growth and per cent mycelium growth inhibition against *Fusarium oxysporum*f. sp. *dianthi*

| T. No. | Treatment detail | Dose g/lit. | Percent disease incidence (%) | |
|---|---|---|---|---|
| | | | 45 DAT ± S.E.(m) | 90 DAT ± S.E.(m) |
| **$T_1$** | Control | ---- | 41.37 ± 0.76 (40.01) | 72.67 ± 0.81 (58.46) |
| **$T_2$** | *Pseudomonas fluorescens* | 5 | 24.35* ± 0.31 (29.55) | 43.52* ± 0.56 (41.26) |
| **$T_3$** | *Trichoderma viride* | 5 | 19.29* ± 0.87 (26.02) | 32.96* ± 0.77 (35.02) |
| **$T_4$** | *Bacillus subtilis* | 5 | 34.48* ± 0.43 (35.94) | 56.80* ± 0.36 (48.89) |
| **$T_5$** | *Trichoderma harzianum* | 5 | 17.03* ± 0.58 (24.35) | 28.07* ± 0.12 (31.98) |
| **$T_6$** | *Trichoderma asperellum* | 5 | 27.16* ± 0.39 (31.39) | 47.42* ± 0.28 (43.50) |
| **SE (d)** | | | **0.74** | **0.78** |
| **C.D (0.05)** | | | **1.59** | **1.67** |

*Significant at 5% level of significance compared with $T_1$ (Control); () Angular values

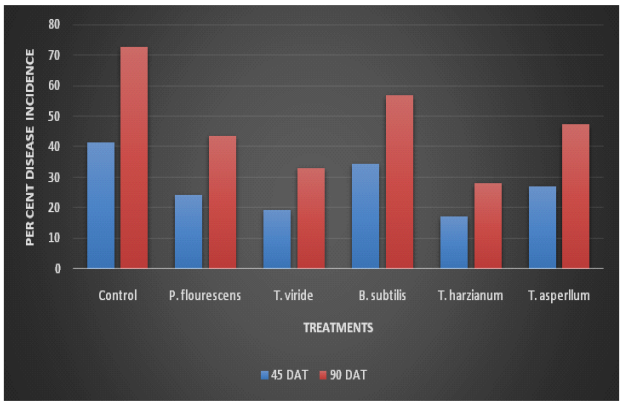


**Fig. 2** : Effect of bioagents on per cent disease index (PDI) on 45 and 90 DAT

all the tested bioagents demonstrated antagonistic activity against the wilt pathogen *Fusarium oxysporum* f. sp. *dianthi*under field conditions. Among the treatments, *Trichoderma viride* showed the maximum effectiveness, reducing the incidence of carnation wilt to 14.6% compared to 45.3% in the untreated control plots. Mahmoud and Abdalla (2018) demonstrated that *T. viride* and *T. harzianum* reduced sesame wilt severity by 77% and 74%, respectively, under greenhouse conditions. These results highlight the strong biocontrol potential of *Trichoderma* species against *Fusarium* wilt.

**Table 2 :** Effect of bioagents on PDI on 45 and 90 DAT

| T. No. | Treatment detail | Dose g/lit. | Percent disease incidence (%) | |
|---|---|---|---|---|
| | | | 45 DAT ± S.E.(m) | 90 DAT ± S.E.(m) |
| $T_1$ | Control | ---- | 41.37 ± 0.76 (40.01) | 72.67 ± 0.81 (58.46) |
| $T_2$ | *Pseudomonas fluorescens* | 5 | 24.35* ± 0.31 (29.55) | 43.52* ± 0.56 (41.26) |
| $T_3$ | *Trichoderma viride* | 5 | 19.29* ± 0.87 (26.02) | 32.96* ± 0.77 (35.02) |
| $T_4$ | *Bacillus subtilis* | 5 | 34.48* ± 0.43 (35.94) | 56.80* ± 0.36 (48.89) |
| $T_5$ | *Trichoderma harzianum* | 5 | 17.03* ± 0.58 (24.35) | 28.07* ± 0.12 (31.98) |
| $T_6$ | *Trichoderma asperellum* | 5 | 27.16* ± 0.39 (31.39) | 47.42* ± 0.28 (43.50) |
| | SE $_{(d)}$ | | 0.74 | 0.78 |
| | C.D $_{(0.05)}$ | | 1.59 | 1.67 |

*Significant at 5% level of significance compared with $T_1$ (Control); () Angular values

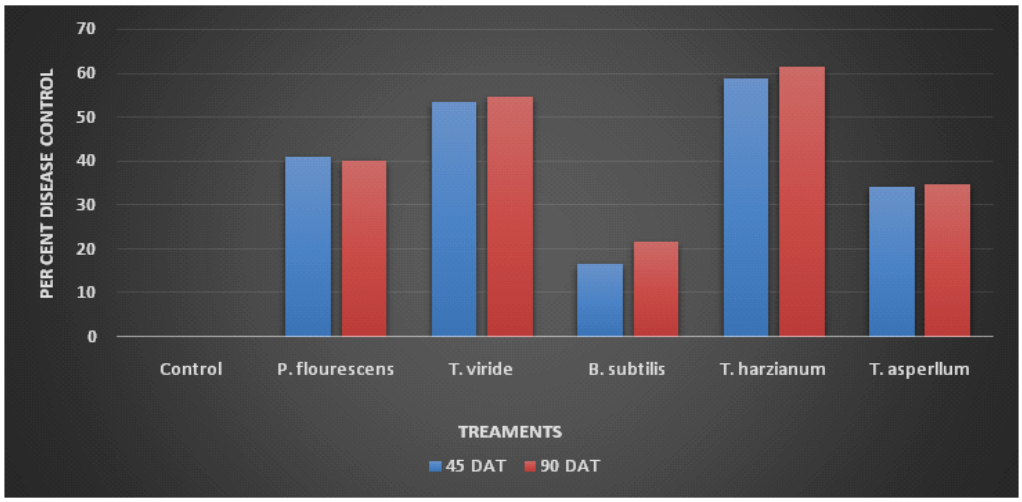


**Fig 3 :** Effect of bioagents on per cent disease control (PDC) at 45 and 90 DAT

**Table 3 :** Effect of bioagents on per cent disease control (PDC) at 45 DAT and 90 DAT

| T. No. | Treatment detail | Dose ( g/lit.) | Percent disease control | |
|---|---|---|---|---|
| | | | 45 DAT ± S.E.(m) | 90 DAT ± S.E.(m) |
| $T_1$ | Control | ---- | 0.00 ± 0.00 | 0.00 ± 0.00 |
| $T_2$ | *Pseudomonas fluorescens* | 5 | 41.10* ± 1.04 (46.94) | 40.07* ± 1.36 (39.25) |
| $T_3$ | *Trichoderma viride* | 5 | 53.42* ± 1.43 50.23 | 54.63* ± 1.13 (47.64) |
| $T_4$ | *Bacillus subtilis* | 5 | 16.54* ± 2.22 (35.81) | 21.78* ± 1.30 (27.78) |
| $T_5$ | *Trichoderma harzianum* | 5 | 58.75* ± 1.82 (23.85) | 61.36* ± 0.39 (51.55) |
| $T_6$ | *Trichoderma asperellum* | 5 | 34.28* ± 1.46 (39.85) | 34.73* ± 0.43 (36.09) |
| | SE(d) | | 1.75 | 2.10 |
| | C.D (0.05) | | 3.76 | 0.98 |

*Significant at 5% level of significance compared with $T_1$ (Control) () Angular values

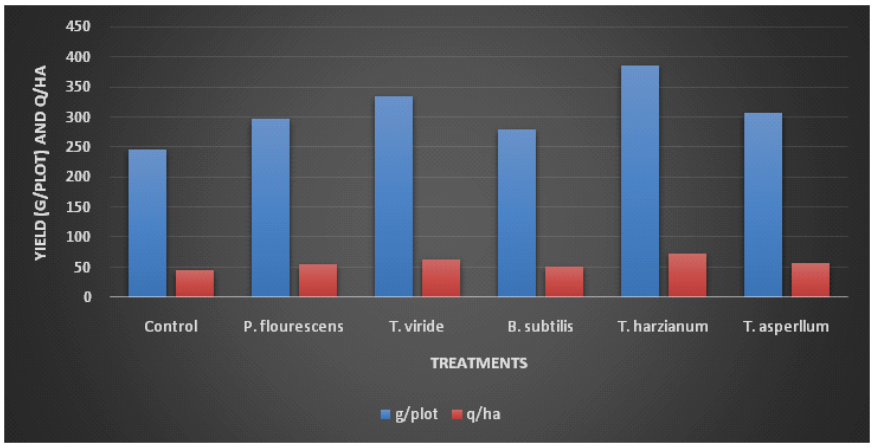


**Fig 4** : Effect of different bioagents @5 g/lit. on total yield (g/plot) and (q/ha)

**Table 4** : Effect of different bioagents on total yield (g/plot) and (q/ha)

| Treatments | Treatments detail | Yield (g/plot) | Yield (q/ha) |
|---|---|---|---|
| $T_1$ | Control | 245.48 ± 0.96 | 45.37 ± 0.53 |
| $T_2$ | *Pseudomonas flourescens* | 296.61* ± 2.57 | 55.09* ± 0.46 |
| $T_3$ | *Trichoderma viride* | 333.62* ± 1.29 | 62.96* ± 0.76 |
| $T_4$ | *Bacillus subtilis* | 278.45* ± 1.04 | 51.85* ± 0.00 |
| $T_5$ | *Trichoderma harzianum* | 386.65* ± 0.94 | 71.76* ± 0.46 |
| $T_6$ | *Trichoderma asperelum* | 306.30* ± 2.29 | 56.95* ± 0.47 |
| | $S.E_{(d)}$ | 2.31 | |
| | $C.D._{(0.05)}$ | 4.97 | |

* Significant at 5% level of significance compared with $T_1$ (Control)

### *Effect of different bioagents on yield (g/plot) and (q/ha)*

The yield varied significantly between the different biocontrol agent treatments ( Fig.4, Table 4). The maximum yield was found in $T_5$ (*Trichoderma harzianum*), producing 386.65g per plot (71.76 q/ha). This was followed by $T_3$ (*T. viride*) with 333.62 g/plot (62.96 q/ha), $T_6$ (*T. asperellum*) with 306.30 g/plot (56.95 q/ha) and $T_2$ (*Pseudomonas fluorescens*) with 296.61 g/plot (55.09q/ha). The minimum yield was obtained from $T_4$ (*Bacillus subtilis*), which produced 278.45 g/plot (51.85 q/ha).

## DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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