



## Mycostimulation in a glyphosate treated arable soil: implications on the yield and agronomic characters of *Talinum fruticosum* (L.) Juss

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**ABSTRACT:** The use of pesticide, although increases agricultural yield and improves public health is also fraught with a number of ecologic, agronomic and health concerns. This research investigated the impact of an *ex-situ* mycostimulation of one of the *in-situ* soil fungi on some agronomic characters and yield of *Talinum fruticosum* planted on a herbicide treated soil. Experimental site was laid out in a 3x8 Randomised Complete Block Design (RCBD). Amongst other fungal species found in the soil, *Paecilomyces variotii* was selected for multiplication and re-introduction to the field 96 hours after the application of glyphosate and 48 hours after the transfer of *T. fruticosum* from the nursery. Data on agronomic parameters were taken between the 1 – 6 weeks after planting (WAP) while data on the biomass yield (kg ha<sup>-1</sup>) was taken (on harvesting) at 6 WAP. Data were mean values from 8 replicates and analysed using the statistical package IBM SPSS version 20. Mean values were separated for statistical significance at 95% confidence interval, using the Least Significant Difference (LSD). The results showed that the Treatments had significant (P<0.05) effects on height of plants, density of plants, size of leaves and internode spacing at the different WAP as well as the biomass and yield of *Talinum* at 6 WAP. The results obtained from the present study thus further reaffirm the crucial role of fungi as nature's original recyclers. If properly managed and stimulated, fungi can contribute significantly to improving soil health, thus improving food security in a sustainable manner. © JASEM

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The most common use of pesticides is as Plant or Crop protection product. In general, pesticides are known to protect plants from damages caused by insects, molluscs, weeds, fungi, nematodes, birds, rodents etc (Ellegaard-Jensen, 2012). Through the use of pesticides, there has been a significant improvement in agricultural productivity (Kuo and Regan, 1999; Helweg *et al.*, 2003). The corollary to this improvement in yield is a corresponding reduction in the prices of food on a global scale. The use of pesticide is global and extensive in nature (Ellegaard-Jensen, 2012). Of this global usage, herbicides account for the largest part of the total pesticide use (Grube *et al.*, 2011).

According to Braschi *et al.* (2000), active substances found in many herbicides may hamper the rate of a series of biochemical processes and microbial growth in the soil. These modifications in the count and activity of soil microorganisms may lead to upsetting the biological equilibrium of the soil environment, thus precipitating a concomitant decrease in soil fertility and the biological productivity of the plants cultivated on such soils.

In view of all of these, this research sets out to investigate the impact of an *ex situ* stimulation of the population density of one of the resident fungal species in an arable land under the influence of glyphosate, on the biomass yield and some

agronomic characters of *T. fruticosum* planted on this soil.

### MATERIALS AND METHODS

*Isolation of fungi and preparation of pure culture from the experimental plot:* One gram of topsoil sample was aseptically taken from each of the 8 Blocks in the field and thoroughly mixed together to form a composite sample. This bulked sample was transferred under an aseptic condition to the laboratory, where serial dilution using sterile distilled water was then carried out on the sample to the 6th concentration. One or two drops of the solution from each concentration of the serially diluted soil sample was aseptically placed (using sterile pipettes) into a sterile plate to which freshly prepared sterile Potato Dextrose Agar-PDA (containing antibiotic and lactic acid) was added. The set up for each concentration was thereafter incubated for between 1- 2 weeks until growth containing mixed species was observed in each Plate. Distinct fungal colonies from each plate was thereafter repeatedly sub cultured in fresh sterile PDA plates until pure cultures containing only one type of growth in each plate was obtained. After this, the pure plate that showed the most aggressive growth during the isolation study in the laboratory was identified as *Paecilomyces variotii*. This organism – *P. Variotii* was thereafter mass cultured to obtain 400 pure plates of same. Spores from all the pure plates containing *P. variotii* were aseptically harvested by scrapping each pure plate soaked in 10

ml of sterile distilled water with pre sterilized spatulas until only the PDA was left in the plate.

*Re-introduction of harvested fungal spores (P. variotii) to the experimental site:* At the end of the harvesting of the spores of *P. variotii* in the laboratory, about 4000 ml of suspension of sterile distilled water containing the spores of this fungus was collected into a pre sterilized, fine nozzle watering can. The content of this watering can was thereafter applied to the different Treatment cells in each of the Blocks earmarked to receive the fungal spores. The portions marked out to not receive the spore of this fungus were equally sprayed with about 4000 ml of sterile distilled water using another pre sterilized, fine nozzle watering can.

*Treatments Applied and the Layout of experimental site:* The experimental field was laid out in a 3 × 8 Randomized Complete Block Design (RCBD) containing 3 Treatments each in a total of 8 Blocks. The size for each Treatment cell in each Block was 1m<sup>2</sup>. Each of these 3 Treatments were randomly assigned on each of the 1m<sup>2</sup> cell within the 8 Blocks. Treatments assigned were as follow: Treatment 1 (T1) = *P. variotii* - glyphosate +*T. fruticosum*; Treatment 2 (T2) = - *P. variotii* + glyphosate +*T. fruticosum* and Treatment 3 (T3) = *P. variotii* + glyphosate +*T. fruticosum*.

Where+ indicate presence or addition and - indicate not added or not present.

*Planting of T. fruticosum and application of glyphosate on the experimental site:* After the layout of the experimental site, and prior to the re-introduction of *P. variotii* to the site, glyphosate was applied at the recommended rate to the required portions on each Block. Two days after the application of this herbicide, *T. fruticosum* seedlings were transplanted from the nursery into the experimental site, while the fungus (*P. variotii*) was re-introduced into the required portions on the field 4 days and 2 days after the application of glyphosate and the transplanting of *T. fruticosum* seedlings respectively.

Data were taken on the *T. fruticosum* at between 1-6 weeks after planting (WAP) on such parameters as plant height, plant density, leaf size and internode space by measuring with a ruler while data on the biomass yield (kg ha<sup>-1</sup>) were taken on harvesting at 6 WAP by carefully uprooting each plant, and their roots washed under a running tap before weighing on the Mettler top loading weighing balance.

*Processing and analyses of data:* Data on the mean value of biomass yield was thereafter converted from gram per square metre to kilogram per hectare. Data reported for each Treatment was mean values from 8 replicates. These data were analysed using the IBM SPSS Statistics version 20 software statistical package. Using the same software package, mean values were separated for statistical significance at 95% confidence interval, using the Least Significant Difference (LSD).

## RESULTS AND DISCUSSION

The results for plant height show that the *ex situ* stimulation of *P. variotii* and the use of glyphosate had a significant (P<0.05) effect on the height of *T. fruticosum* at each WAP (Table 1), where plants in T1 consistently (in each of the WAP) had the least mean height while plants in T3 had the highest mean height.

The results in Table 2 show that the Treatments had a significant (P<0.05) effect on the density of *T. fruticosum* at the different WAP with plants in T3 having the highest mean density.

Following a similar trend, Table 3 show that there were significant (P<0.05) differences in the mean leaf size among the 3 Treatments at each of the WAP, with the lowest and highest mean values at each WAP belonging to T1 and T3 respectively.

Presenting a dissimilar picture from the already established trend, results from Table 4 show that T1 had the highest mean internode length at each of the WAP. The mean values recorded for T1 for this parameter at each of the WAP were significantly (P<0.05) higher than those of T2, while those for T2 were also significantly higher (P<0.05) than the mean values recorded for T3.

The results as shown in Figure 5 reveal that the Treatments had a significant (P<0.05) effect on biomass yield. The highest mean yield was obtained for T3 while the lowest was for T1.

**Table 1:** Mean Plant Height at 1-6 WAP

Weeks After Planting (WAP)	TREATMENTS		
	T1	T2	T3
1	4.71 <sup>a</sup>	5.61 <sup>b</sup>	10.16 <sup>c</sup>
2	5.21 <sup>a</sup>	6.7 <sup>b</sup>	15.28 <sup>c</sup>
3	5.56 <sup>a</sup>	9.59 <sup>b</sup>	15.8 <sup>c</sup>
4	5.75 <sup>a</sup>	10.28 <sup>b</sup>	16.15 <sup>c</sup>
5	6.13 <sup>a</sup>	11.69 <sup>b</sup>	16.4 <sup>c</sup>
6	6.59 <sup>a</sup>	12.54 <sup>b</sup>	17.85 <sup>c</sup>

Values carrying different superscripts along the column are significantly different at p<0.05

**Table 2:** Mean Plant density at 1-6 WAP

Weeks After Planting (WAP)	TREATMENTS		
	T1	T2	T3
1	15 <sup>a</sup>	21.38 <sup>b</sup>	32.25 <sup>c</sup>
2	12.25 <sup>a</sup>	18.62 <sup>b</sup>	32.25 <sup>c</sup>
3	4.5 <sup>a</sup>	18.67 <sup>b</sup>	32.25 <sup>c</sup>
4	4.5 <sup>a</sup>	18.67 <sup>b</sup>	32.25 <sup>c</sup>
5	4.1 <sup>a</sup>	18.67 <sup>b</sup>	32.25 <sup>c</sup>
6	3.7 <sup>a</sup>	18.67 <sup>b</sup>	32.25 <sup>c</sup>

Values carrying different superscripts along the same column are significantly different at  $p < 0.05$

**Table 3:** Mean leaf size at 1-6 WAP

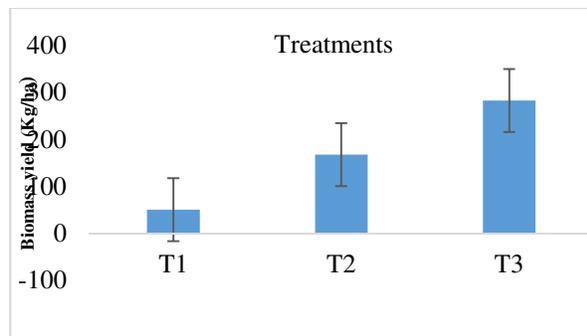
Weeks After Planting (WAP)	TREATMENTS		
	T1	T2	T3
1	2.46 <sup>a</sup>	2.95 <sup>b</sup>	3.78 <sup>c</sup>
2	2.85 <sup>a</sup>	3.17 <sup>b</sup>	3.99 <sup>c</sup>
3	2.98 <sup>a</sup>	3.66 <sup>b</sup>	4.4 <sup>c</sup>
4	3.11 <sup>a</sup>	4.2 <sup>b</sup>	4.67 <sup>c</sup>
5	3.37 <sup>a</sup>	4.36 <sup>b</sup>	4.77 <sup>c</sup>
6	3.39 <sup>a</sup>	4.54 <sup>b</sup>	4.98 <sup>c</sup>

Values carrying different superscripts along the same column are significantly different at  $p < 0.05$

**Table 4:** Mean Internode length at 1-6 WAP

Weeks After Planting (WAP)	TREATMENTS		
	T1	T2	T3
1	1.83 <sup>a</sup>	1.48 <sup>b</sup>	1.46 <sup>c</sup>
2	1.9 <sup>a</sup>	1.67 <sup>b</sup>	1.69 <sup>c</sup>
3	2.23 <sup>a</sup>	1.71 <sup>b</sup>	1.7 <sup>c</sup>
4	2.41 <sup>a</sup>	1.71 <sup>b</sup>	1.71 <sup>c</sup>
5	2.44 <sup>a</sup>	1.74 <sup>b</sup>	1.72 <sup>c</sup>
6	2.44 <sup>a</sup>	1.77 <sup>b</sup>	1.75 <sup>c</sup>

Values carrying different superscripts along the same column are significantly different at  $p < 0.05$

**Fig 1:** Mean Biomass yield for Treatments at harvest

Many of the chemicals used in pesticides are persistent soil contaminants, whose impact may endure for decades and adversely affect soil conservation (Yadav *et al.*, 2015). This notwithstanding, the uncontrolled growth of weeds in competition with cultivated species has been reported to have caused a significant loss in the growth and yield of these cultivated plants, sometimes, with

losses as high as between over 57 to over 73% being reported (Amare, 2014). The results obtained in this work corroborates the adverse effect that competition by weeds have on the growth indices and yield of cultivated species, where for all the parameters evaluated (except internode length), plants from T1 showed significantly lower values on account of competition by weeds, as opposed to the two other Treatments where weeds were controlled with the use of the glyphosate.

Results obtained in present study (with respect to T3) agree with the reports of earlier workers with respect to the role of saprophytic fungi in soil mineralization process and plant productivity. Saprotrophic fungi (such as *P. variotii*) are known to produce an impressive range of extracellular enzymes (Kjøller and Struwe, 2002) that are vital for degradation of organic pollutants (Ellegaard-Jensen, 2012). In this regard, the role of saprophytic fungi in soil mineralization process has been extensively documented (Ekundayo and Obire, 1987; Vwioko *et al.*, 2006; Covino *et al.*, 2010). By this mineralization process, elevation in soil nutrient status, which resulted in better growth of plants, has severally been reported by earlier workers (Adams and Ellis, 1960; Udo and Fayemi, 1975; Egunjobi and Onweluzor, 1979; Gadd, 2007).

**Conclusion:** It will therefore be rational to conclude that results obtained from this work further reaffirm the crucial role of fungi as nature's original recyclers. If properly managed and stimulated, fungi can contribute significantly to improving soil health, thus improving food security in a sustainable manner.

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