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RESEARCH ARTICLE

Effect of Different Plant Nutrients on Population Dynamics of *Trichoderma* sp. in Soil

Abisag Lalremruatpuii and Susanta Banik*

Dept. of Plant Pathology, SASRD, Nagaland University, Medziphema, Nagaland-797106

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ABSTRACT

A pot culture study was conducted to see the effect of organic and inorganic plant nutrients on the survival of *Trichoderma* in soil with tomato plants. How *Trichoderma* influences tomato plant growth is also assessed under different regimes of plant nutrients. Farmyard manure (FYM), vermicompost, and NPK were used in different combinations to assess their effect. Among the treatments applied with plant nutrients, the population of *Trichoderma* was maximum at 15 DAS with NPK (39.31 × 10^4 cfu/g of soil) followed by FYM (26.41 × 10^4 cfu/g of soil) and vermicompost (23.33 × 10^4 cfu/g of soil). The highest mean plant height and the number of leaves at 60 DAS were observed in 50% NPK + 50% Vermicompost (50.50 cm and 47.83 respectively) followed by 50% NPK + 50% FYM (46.33 cm and 45.41 respectively). The highest mean number of branches and leaf area of tomato plants were observed with 50% NPK + 50% Vermicompost followed by 50% NPK + 50% FYM. The treatments incorporated with plant nutrients (FYM, Vermicompost, and NPK) showed a positive result on the growth parameters of the tomato plant.

Keywords: *Trichoderma; NPK; FYM; Vermicompost*

INTRODUCTION

Unrelenting research on *Trichoderma* spp. has made them one of the most promising bio-control agents against plant diseases (Elad *et al.,* 1984; Lui and Baker, 1980) and now they are the most widely used biopesticides against plant diseases. *Trichoderma* spp. has provided one of the first economical antagonistic control methods against soil-borne pathogens like *Fusarium, Sclerotium, Phytophthora,* and *Pythium* etc. (Backman and Kabana, 1975). *Trichoderma* species possess various mechanisms to affect the growth of plant pathogens, control diseases, and improve overall plant health (Singh *et al*., 2004). Biological control has established itself as a safe and eco-friendly alternative to chemical management. *Trichoderma* spp. is among the most studied fungal bio-control agents and is commercially marketed as biopesticides (Kamala and Devi, 2012).

Trichoderma has several beneficial attributes that include antifungal, insecticidal, soil remedial, plant growth-promoting, and rhizosphere colonizing attributes (Singh *et al*., 2004). It also produces antibiotics, vitamins, and hormones that contribute to its bio-control performance (Rojan *et al*., 2010). It enhances seed germination of flowering plants (Celar and Valic, 2005) and increases phosphorus uptake by plants (Rudresh *et al*., 2005).

Trichoderma spp. is better rhizosphere colonizers than plant pathogenic fungi. *Trichoderma* colonizes and penetrates plant root tissues inducing a series of changes in the plant leading to induced systemic resistance (ISR) in the entire plant (De Meyer *et al*., 1998). *Trichoderma* enhances plant growth by improving stress tolerance and making nutrients available to plants (Sathiyaseelan *et al*., 2009). It is necessary to study ecology and many other factors that could affect the performance of the biocontrol agent keeping in view the variability in soil and atmospheric environment in the field situations (Cook and Baker, 1983; Papavizas, 1985; Lewis and Papavizas, 1991).

Agricultural inputs are mostly added to soil including *Trichoderma* a living fungus. So, for effective performance in bio-control of soil-borne plant pathogens, the externally applied *Trichoderma* must survive and grow in the soil and rhizosphere (Garrett, 1956). Understanding the effect of soil factors, especially nutritional aspects on *Trichoderma* spp. may provide clues about their

*Corresponding author's e-mail: susanta@nagalanduniversity.ac.in

in-situ performance on biological control of plant pathogenic fungi and their effect on crop plants. Therefore, the present investigation was carried out to study the effect of some plant nutrients on the survival and proliferation of *Trichoderma* spp. in soil and the effect on tomato plants.

MATRIALS AND METHODS

The investigation was carried out in the laboratory and experimental greenhouse of the Department of Plant Pathology, School of Agricultural Sciences and Rural Development, Nagaland University, Medziphema Campus, Nagaland.

Source of Trichoderma:

Talc formulation of *Trichoderma* having an average cfu count of 13 × 10^4 was obtained from the Department of Plant Pathology, School of Agricultural Sciences and Rural Development, Nagaland University, Medziphema Campus.

Culture media:

Trichoderma selective media (TSM) with the below-mentioned composition was used for estimating the *Trichoderma* population in the soil.

Composition of TSM

Magnesium sulphate ($MgSO_4$) : 0.2g

Dipotassium hydrogen phosphate (K_2HPO_4) : 0.9g

Potassium chloride (KCl) : 0.15g

Ammonium nitrate (NH_4NO_3) : 1.0g

Glucose : 3.0g

Agar agar : 20g

Chloramphenicol : 0.25g

Metalaxyl : 0.3g

Rose Bengal : 0.15g

Captan : 0.02g

Water : 1000 ml.

Evaluation of organic and inorganic sources of plant nutrients on Trichoderma

The *in vivo* pot culture experiment was conducted using pots of 22 cm diameter and 19 cm height approximately containing 6 kg of soil. The experiment was laid out in Completely Randomized Design (CRD) with seven treatments and four replications each. Tomato crop of variety Samruhi F1 hybrid was sown @ ten seeds/pot. Organic nutrients *viz*. FYM (Farm Yard Manure, @5 t ha^{-1}), vermicompost (1 t ha^{-1}), and inorganic nutrients *viz*. urea (150 kg ha^{-1}), SSP (90 kg ha^{-1}), and MOP (90 kg ha^{-1}) were used to assess the effect of their application on the test isolate of *Trichoderma*. The total number of treatments is seven and the number of plants is three/pot. The treatments were T_1- NPK, T_2-FYM, T_3-Vermicompost, T_4-50% NPK+ 50% FYM, T_5-50% NPK+ 50% Vermicompost, T_6-50% FYM+ 50% Vermicompost and T_0-control-only *Trichoderma* without any plant nutrient**.** FYM and vermicompost were applied at the recommended dose. For NPK, nitrogen was applied in two split doses, the first dose was given at pot filling and the remaining dose was applied at 45 days after sowing of the crop. The required amount of these nutrients was incorporated at the time of pot filling.

Application of Trichoderma

Talc formulation of *Trichoderma* was applied at 10g/pot at the time of pot filling with a pot size of 22 cm diameter, 19 cm height, and approximately containing 6 kg of soil.

Estimation of Trichoderma population from treated soil

The estimation of *Trichoderma* population was done at 15 days interval by collecting the soil sample from the pots under study using the dilution plating technique. One gram of air-dried representative rhizosphere soil sample from each treatment having tomato crop was separately mixed in nine ml of sterile distilled water in the test tube to obtain 10^{-1} dilution. This soil suspension was serially diluted to 10^{-2}, 10^{-3}, and 10^{-3}. Dilution of 10^{-4} was used for monitoring *Trichoderma* from inoculated treatments at 15, 30, 45, and 60 DAS.

One hundred µL of each diluted soil suspension was pipetted out with the help of a micropipette onto Petri plates containing TSM medium under aseptic conditions in a laminar chamber and spread with a sterile spreader. The inoculated plates were incubated at 28 ± 1 °C for 4 days. Observations on the number of colony-forming units (cfu) of *Trichoderma* were recorded.

Observations taken

The percent germination of tomato seeds was calculated using the formula-

$$\text{Percent germination} = \frac{\text{Number of seed germinated}}{\text{Number of seeds planted}} \times 100$$

The observation was recorded on the 15th day after sowing. The observations of number of leaves per plant, number of branches per plant, and leaf area (cm^2) were recorded at 15 DAS and it was continued for a period of two months at 15 days

intervals. The plant heights in (cm) were measured from the ground level to the base of the terminal leaf, commencing from 15 days after sowing, with the help of a linear scale at 15 days interval for a period of 2 months. Freshly uprooted tomato plants were rinsed in running water and weighed (g) 60 DAS of the crop. The mean weight of three tomato plants/pot was taken for the fresh weight.

The data were subjected to analysis by Fisher's method of analysis of variance. The significance of variance among the data was calculated out by calculating the 'F' value and comparing it with the tabulated value of 'F' (Senedecor and Cochran, 1967). The treatment means were also compared among themselves by calculating Critical differences (CD) as follows:

$CD_{0.05}$ = S.Ed × $t_{0.05}$ for error degrees of freedom

The standard error of difference of mean (S.Ed) was calculated by using the formula:

$$S.Ed\pm=\sqrt{\frac{2\times error\ mean\ square}{number\ of\ replication}}$$

Where S.Ed = standard error difference.

$T_{0.05}$ = Table value of student's 't' obtained at 5 % probability test.

The percentage value and population numbers were transformed into corresponding values by Arc sine transformation and square root transformation.

RESULTS AND DISCUSSION

Effect of Trichoderma in presence of organic and inorganic sources of plant nutrients on tomato

Germination percentage

The results of different treatments recorded at 15 DAS is depicted in Table 1. The highest germination % was recorded in *Trichoderma* + NPK (97.50 %) followed by *Trichoderma* + 50 % FYM + 50 % Vermicompost (92.50 %), however all the treatments are statistically at par.

Trichoderma spp. employ several mechanisms in influencing seed germination and seedling vigour (Zheng and Shetty, 2000; Celar and Valic, 2005). Rehman *et al*. (2013) reported that maximum germination per cent was obtained from seedbed treatment with *T. harzianum* + FYM (91.60%) and seed treatment with *T. harzianum* (89.99%) as compared to control (73.80%).

Khan *et al*. (2014) reported application of *Trichoderma* spp. reduced the inhibitory effect of the pathogens *viz*. *Fusarium oxysporum* and *R. solani* on seed germination resulted in a significant increase in seed germination, plant growth, and yield.

Table 1. Effect of *Trichoderma* in presence of organic and inorganic sources of plant nutrients on germination % of tomato

TREATMENTS	GERMINATION % (15 DAS)
T_0 – *Trichoderma* (Control)	87.50
T_1 – *Trichoderma* + NPK	97.50
T_2 – *Trichoderma* + FYM	85.00
T_3 – *Trichoderma* + Vermicompost	90.00
T_4 – *Trichoderma* + 50% NPK + 50% FYM	85.00
T_5 – *Trichoderma* + 50% NPK + 50% Vermicompost	85.00
T_6 – *Trichoderma*+ 50% FYM + 50% Vermicompost	92.50
SEM ±	6.65
CD (*p*=0.05)	9.58(NS)

Plant height

The data pertaining to the effect of various treatments in relation to plant height at 15, 30, 45, and 60 DAS is presented in Table 2. The maximum plant height at 60 DAS was recorded at *Trichoderma* + 50% NPK + 50% Vermicompost (50.50). The two treatments gave statistically significant results compared to the last mean control (*Trichoderma*) that recorded a plant height of 42.66 cm at 60 DAS followed by *Trichoderma* + 50 % NPK + 50 % FYM (46.33) and *Trichoderma* + 50 % FYM + 50 % Vermicompost (43.08). It is evident from the result that a reduced dose of NPK and the addition of Vermicompost is having a positive role in the increase in plant height. This is true in the case of the addition of FYM too.

The results of the present study are also in close agreement with the findings of Sundaramoorthy and Balabaskar (2013) who reported that tomato plants treated with *T. harzianum* (ANR-1) isolate showed a significant increase in plant height and dry weight.

Similar findings to the present investigation were reported by Ganesan *et al*. (2007) that stem rot of groundnut caused by *Sclerotium rolfsii* is controlled significantly by *Trichoderma harzianum* which also increases the plant growth. Yaqub and Shahzad (2008) reported that, maximum plant height was obtained with seed-pelleting with *T. harzianum*. There are many reports of *Trichoderma* spp.

effectively increasing plant growth and controlling plant diseases (Rudresh *et al*., 2005; Kleifeld & Chet, 1992; Elad *et al*., 1980).

Table 2: Effect of *Trichoderma* in presence of organic and inorganic sources of plant nutrients on plant height of tomato

TREATMENTS	Plant height (cm) at different DAS			
	15	30	45	60
T_0 – *Trichoderma* (Control)	6.33	15.83	34.58	42.66
T_1 – *Trichoderma* + NPK	6.65	15.90	34.247	39.99
T_2 - *Trichoderma* + FYM	5.37	12.83	26.25	36.91
T_3–*Trichoderma* + Vermicompost	5.91	14.75	36.33	42.41
T_4 – *Trichoderma* + 50% NPK + 50% FYM	6.36	16.99	34.58	46.33
T_5 – *Trichoderma* + 50% NPK + 50% Vermicompost	6.10	15.75	38.58	50.50
T_6 – *Trichoderma* + 50% FYM + 50% Vermicompost	5.64	14.91	35.75	43.08
SEM±	0.39	1.29	3.00	1.70
CD (*p*=0.05)	1.17(NS)	3.81(NS)	8.82(NS)	5.00(S)

Number of leaves

The result of different treatments on the number of leaves at 15, 30, 45 and 60 DAS is presented in Table 3. The maximum number of leaves was recorded at 60 DAS in *Trichoderma* + 50 % NPK + 50 % Vermicompost treatment (47.83) which is statistically different from the other treatments when compared to 37.91 number of leaves in control, followed by *Trichoderma* + 50 % NPK + 50 % FYM (45.41).

Naznin *et al.* (2015) reported a combination of organic and biocontrol agents *Trichoderma* increases the plant height, number of leaves per plant, and other plant growth parameters leading to higher yield.

Table 3. Effect of *Trichoderma* in presence of organic and inorganic sources of plant nutrients on number of leaves of tomato

TREATMENTS	Number of leaves at different DAS			
	15	30	45	60
T_0 – *Trichoderma* (Control)	3.91 (2.09)	12.91 (3.65)	29.08 (5.41)	37.91 (6.18)
T_1 – *Trichoderma* + NPK	3.75 (2.00)	11.82 (3.48)	27.08 (5.21)	40.25 (6.33)
T_2 - *Trichoderma* + FYM	3.25 (1.931)	9.50 (3.15)	20.33 (4.55)	32.33 (5.72)
T_3 – *Trichoderma* + Vermicompost	2.72 (1.78)	10.47 (3.30)	32.16 (5.70)	35.91 (6.02)
T_4 – *Trichoderma* + 50% NPK + 50% FYM	3.65 (2.03)	13.16 (3.67)	28.66 (5.37)	45.41 (6.77)
T_5 – *Trichoderma* + 50% NPK + 50% Vermicompost	3.24 (1.93)	10.91 (3.37)	33.00 (5.75)	47.83 (6.95)
T_6 – *T* + 50% FYM + 50% Vermicompost	3.08 (1.88)	10.83 (3.34)	27.74 (5.29)	39.24 (6.29)
SEM ±	0.13	0.18	0.28	0.23
CD (*p*=0.05)	0.39(NS)	0.54(NS)	0.82(NS)	0.67(S)

N.B.- Values in parenthesis are square root transformed values

Number of branches

The result of the effect of *Trichoderma* in presence of organic and inorganic sources of plant nutrients on number of branches at 15, 30, 45, and 60 DAS is presented in the Table 4. The highest number of branches at 60 DAS was recorded at *Trichoderma* + 50 % NPK + 50 % Vermicompost (8.07) followed by *Trichoderma* + 50 % NPK + 50 % FYM (7.91).

The results of the present study also are in close agreement with the findings of Gomaa and Mohamed (2000), that *T. harzianum* significantly increases plant height, number of leaves, fresh and dry weights of leaves, number of branches, and fresh and dry weights of branches per plant in dahlia

The present findings are also in line with Sharf *et al*. (2014) who reported that the combined application of *Trichoderma* and nitrogenous fertilizer in their treatment T-8 improved all the growth parameters as well as the biochemical parameters of a red kidney bean. This confirms the findings of Khair *et al*. (2010) who reported a significant increase in the number of branches in all

Trichoderma treatments. Better and fast growth in plants can result in the enhanced development of various plant parts and higher growth leads to more branching. This may be due to the action of *Trichoderma* in growth enhancements which also involves the decomposition of organic materials and solubilization of insoluble compounds to make more minerals available to plants in forms that can be utilized by them as was reported by Inbar *et al.* (1994); Manoranjitham *et al.* (2001) and Uddin *et al*. (2011).

Table 4. Effect of *Trichoderma* in presence of organic and inorganic sources of plant nutrients on number of branches of tomato

TREATMENTS	Number of branches at different DAS		
	30	45	60
T_0 – *Trichoderma* (Control)	3.08 (1.89)	5.83 (2.51)	7.08 (2.75)
T_1 – *Trichoderma* + NPK	2.91 (1.83)	6.04 (2.54)	7.58 (2.83)
T_2 – *Trichoderma* + FYM	2.3 (1.67)	4.66 (2.27)	7.08 (2.75)
T_3 – *Trichoderma* + Vermicompost	2.25 (1.65)	5.91 (2.44)	6.66 (2.67)
T_4 – *Trichoderma* + 50% NPK + 50% FYM	2.81 (1.81)	5.75 (2.49)	7.91 (2.90)
T_5 – *Trichoderma* + 50% NPK + 50% Vermicompost	2.16 (1.63)	5.99 (2.54)	8.07 (2.92)
T_6 – *Trichoderma* + 50% FYM + 50% Vermicompost	2.41 (1.69)	5.50 (2.44)	7.40 (2.80)
SEM ±	0.08	0.08	0.04
CD (*p*=0.05)	0.24(NS)	0.24(NS)	0.14(S)

N.B.-Values in parenthesis are square root transformed values

Leaf area

The data pertaining to effect of various treatments in relation to leaf area in cm^2 at 15, 30, 45 and 60 DAS is presented in Table 5. The maximum leaf area at 60 DAS was recorded at *Trichoderma* + 50 % NPK + 50 % Vermicompost (30.73 cm^2) which is statistically at par with *Trichoderma* + Vermicompost (27.43 cm^2) and *Trichoderma* + 50 % NPK + 50 % FYM (28.35) which is significantly different from *Trichoderma* + 50 % FYM + 50 % Vermicompost (20.85 cm^2).

The present findings are also in line with Rawat *et al*. (2011) and Dominguez *et al*. (2016) who reported that treatment of rice, wheat, and tomato seeds with *T. harzianum* enhances the rate of photosynthesis, plant biomass, plant height, and leaf area. The fungus *T. harzianum* when applied to pathogen-free soil, induced an increase in the emergence of seedlings, plant height, leaf area, and dry weight. *Trichoderma* induced growth response has been reported for various plant species including bean, cucumber, periwinkle and petunia (Kleifeld and Chet, 1992). Mechanisms of enhanced growth in the plant by *Trichoderma* have been explained by many authors (Inbar *et al*., 1994, Srivastava (2004) and Shabir *et al*. (2012).

Table 5.Effect of *Trichoderma* in presence of organic and inorganic sources of plant nutrients on leaf area of tomato

	Leaf area (cm^2) at different DAS			
TREATMENTS	15	30	45	60
T_0 – *Trichoderma* (Control)	2.58	8.38	8.91	21.62
T_1 – *Trichoderma* + NPK	2.30	8.26	12.76	25.02
T_2 - *Trichoderma* + FYM	2.43	8.11	11.06	24.71
T_3- *Trichoderma*+Vermicompost	2.19	8.02	9.71	27.43
T_4 – *Trichoderma* + 50% NPK + 50% FYM	2.54	6.86	10.65	28.35
T_5 – *Trichoderma* + 50% NPK + 50% Vermicompost	1.77	8.27	13.88	30.73
T_6 – *Trichoderma* + 50% FYM + 50% Vermicompost	2.39	7.88	7.601	20.85
SEM±	0.12	0.77	1.06	2.04
CD (*p*=0.05)	0.37(S)	2.27(NS)	3.13(S)	6.00(S)

Trichoderma population

The data pertaining to the effect of organic and inorganic sources of plant nutrients on the survival of *Trichoderma* in the soil at 15, 30 45, and 60 DAS is depicted on Table 6.

The *Trichoderma* population increases significantly at 15 DAS in all the treatments thereafter the population decline to 30 DAS.

Papavizas *et al*. (1982) reported that *Trichoderma* and *Gliocladium* multiply greatly after addition to soil as dry formulation from an initial amount of 5×10^3 to a maximum of $6\text{-}7 \times 10^6$ conidia/g of soil of various organic matter content.

Most of the organic and inorganic sources of plant nutrients showed compatibility with *Trichoderma*. The findings of the present

investigation are in line with the work done by Bhai and Thomas (2010) who reported that NPK (75:75:150) and *Trichoderma* were compatible.

The highest population at 15 DAS was recorded in *Trichoderma* + NPK (39.31 × 10^4 cfu/g) followed by *Trichoderma* + FYM (26.41 × 10^4 cfu/g). This finding is also in line with the work done by Gangwar *et al*. (2013) who reported that soil + FYM (1:1) was found to support maximum population growth of *T. harzianum* (78.33 × 10^4 cfu/g).

Table 6.Effect of organic and inorganic sources of plant nutrients on survival of *Trichoderma* in soil

TREATMENTS	*Trichoderma* population at different DAS [Average no. of cfu/g ×(10^4)]			
	15	30	45	60
T_0 – *Trichoderma* (Control)	14.58	7.92	19.00	19.25
T_1 – *Trichoderma* + NPK	39.31	7.00	6.83	15.75
T_2 – *Trichoderma* + FYM	26.41	12.16	8.00	17.75
T_3– *Trichoderma* +Vermicompost	23.33	13.58	11.58	12.66
T_4 – *Trichoderma* + 50% NPK + 50% FYM	11.66	11.41	18.00	16.08
T_5 – *Trichoderma* + 50% NPK + 50% Vermicompost	14.08	12.58	9.16	14.91
T_6 – *Trichoderma* + 50% FYM + 50% Vermicompost	12.25	11.91	6.91	12.08
SEM ±	4.32	2.34	1.36	1.66
CD (*p*=0.05)	12.72(S)	6.88(NS)	4.00(S)	4.88(NS)

Fresh weight

The effect of different treatments on the fresh weight of the tomato plant at 60 DAS is presented in Table 7. The highest fresh weight was recorded in *Trichoderma* + FYM (14.09) followed by *Trichoderma* + 50% FYM + 50% Vermicompost (13.92) and *Trichoderma* + Vermicompost (13.34). There is no significant difference among the treatments including control in terms of fresh weight of the tomato plant.

The present findings are in close agreement with the findings of Azarmi *et al*. (2011) who reported that the fresh weight of plant increased significantly when treated with *Trichoderma.* Khair *et al*. (2010) reported that *Trichoderma* significantly increased the fresh weight of bean plants. Similar results were reported by Uddin *et al*. (2011) where higher fresh shoot and root weight as well as fresh seedling weight in egg plant and tomato plants when treated with *Trichoderma*. This confirms the findings of the present study. This stimulatory effect of *Trichoderma* may be due to its interaction with plants in the root zones forming symbiotic associations thereby increasing plant nutrient content in the soil by breaking down complex organic matter; and nutrient exchange (Howell, 2003; Harman, 2006).

Table 7. Effect of *Trichoderma* in presence of organic and inorganic sources of plant nutrients on fresh weight of tomato plant

TREATMENTS	FRESH WEIGHT (g) * (60 DAS)
T_0 – *Trichoderma* (Control)	13.54
T_1 – *Trichoderma* + NPK	12.84
T_2 - *Trichoderma* + FYM	14.09
T_3 – *Trichoderma* + Vermicompost	13.28
T_4 – *Trichoderma* + 50% NPK + 50% FYM	12.98
T_5- *Trichoderm*+50%NPK+50% Vermicompost	13.34
T_6 – *Trichoderma* + 50% FYM + 50% Vermicompost	13.92
SEM±	0.62
CD (*p*=0.05)	1.83(NS)

(* Mean weight of three plants/pot)

CONCLUSION

It may be concluded from the present findings that the effect of *Trichoderma* in presence of plant nutrients showed a positive result on the growth parameters of tomatoes such as plant height, number of leaves, number of branches, and leaf area. *Trichoderma* could be integrated with different plant nutrients especially organic manure like FYM and vermicompost for better crop production. However, field investigation is imperative to clarify the effective utilization of *Trichoderma* under different biotic and abiotic conditions and further studies should be conducted to see the performance and survivability of *Trichoderma* in field conditions.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Author contributions

Conceptualization-AL, SB, Experiments- AL, Guidance -SB, Writing original draft – AL, Writing-reviewing &editing -SB

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