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EFFECT OF MICRONUTRIENTS AND BIOINOCULANTS ON PHYSIOLOGICAL AND YIELD PARAMETERS OF CHILLI (*CAPSIUM ANNUUM* L.)

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ABSTRACT

The present research aimed to investigate the impact of micronutrients, *Trichoderma viride* and Plant Growth Promoting Rhizobacteria (PGPR) on physiological parameters and yield of chili. The study took place at the Horticulture complex, Maharajpur, Department of Horticulture, J.N.K.V.V., Jabalpur (M.P.) during the *Rabi* season of 2017-2018 and 2018-2019, following a Randomized Complete Block Design (RCBD-Factorial) with three replications. The experimental setup included 20 treatment combinations, incorporating five levels of micronutrients as the first factor and four levels of bioinoculants (PGPR and *Trichoderma viride*) as the second factor.

Results indicated that foliar application of micronutrients significantly influenced growth parameters. The physiological developments across different treatments varied, with notable improvements observed in yield. The most favorable physiological developments were recorded for treatment M5 (foliar application of $ZnSO_4$ - 0.2%) and B3 (TV + PF + AC, 2.5 kg/ha + 2.5 kg/ha + 5.0 kg/ha). Regarding physiological parameters, the treatment combination M5B3 ($ZnSO_4$ - 0.2% + TV + PF + AC, 2.5 kg/ha + 2.5 kg/ha + 5.0 kg/ha) outperformed the control M1B0 (No micronutrient + No bioinoculant). Treatment M5B3 demonstrated superior morpho-physiological, yield and quality parameters for chili. This suggests that these treatment combinations hold promise for enhancing productivity and warrant further exploration.

Key words : Chilli, Photosynthetic rate (PR), Stomatal Conductance (SC), Transpiration Rate (TR), Quantum Efficiency (QE), Yield.

Introduction

Chilli (*Capsicum annuum* L., 2n=24) holds significant importance as both a vegetable and spice, serving as a vital cash crop in India. This plant belongs to the Solanaceae family. Within the Capsicum genus, five species are cultivated: *Capsicum annuum*, *Capsicum frutescens*, *Capsicum pubescens*, *Capsicum baccatum* and *Capsicum chinensis*. Chilli cultivation is widespread throughout the country, adapting to diverse agro-climatic zones. However, the cultivation of dry chilli is predominantly concentrated in Southern states, particularly Andhra Pradesh, Maharashtra, Karnataka, Tamil Nadu, Bihar, Rajasthan, Punjab, Haryana and Madhya Pradesh.

In Madhya Pradesh alone, chilli is cultivated across 33.64 thousand hectares, contributing to a total annual production of 574.80 thousand tonnes of green chilli (Anonymous, 2017). This underscores the agricultural significance and widespread cultivation of chilli, emphasizing its role as a major crop in India.

Micronutrients play a crucial role in facilitating nutrient absorption and maintaining a balance among various nutrients (Singh and Kalloo, 2000). Iron, in particular, is essential for chlorophyll synthesis and contributes significantly to carotenoid synthesis in red chillies, indirectly enhancing the quality of red chillies or paprika. A study by Natesh *et al*. (2005) demonstrated that foliar spray of ZnSO4 (0.1%) at the flowering stage for the Byadgi Kaddi chilli variety resulted in higher fruit yield and improved seed quality. Utilizing foliar fertilization alongside soil fertilization has been recognized as a strategy to optimize crop yield, as suggested by Fageria *et al*. (2009). In horticultural crops, Plant Growth Promoting Rhizobacteria (PGPR) has gained popularity for promoting plant growth, development and overall yield globally. Various studies have indicated that root inoculation or foliar spraying with PGPR can enhance germination, seedling emergence and modify the growth and yield of diverse horticultural crops. PGPR serves as a biofertilizer and bioenhancer, offering an alternative to chemical fertilizers for different crops.

Materials and Methods

The current investigation was conducted to assess the impact of micronutrients, *Trichoderma viride* and Plant Growth Promoting Rhizobacteria (PGPR) on physiological parameters and chili yield. The study took place at the Horticulture complex, Maharajpur, Department of Horticulture, J.N.K.V.V., Jabalpur (M.P.) during the *Rabi* season and data were pooled over the years 2017-2018 and 2018-2019. The experimental design employed a Randomized Complete Block Design (RCBD-Factorial) with three replications. The experiment comprised 20 treatment combinations, incorporating five levels of micronutrients as the primary factor and four levels of bioinoculants (PGPR and *Trichoderma viride*) as the secondary factor. Detailed information regarding the treatments is provided below.

(A) Factor – I : Micronutrients (M)

- **M_1 :** Control
- **M_2 :** Ferrous sulphate ($FeSO_4$) (0.2%)
- **M_3 :** Calcium nitrate ($CaNO_3)_2$ (0.2%)
- **M_4 :** Borax ($Na_2B_2O_7.2H_2O$) (0.1%)
- **M_5 :** Zinc sulphate ($ZnSO_4$) (0.2%)

(B) Factor – II : Bio-inoculants (B)

- **B_0 :** Control
- **B_1 :** *Trichoderma viride* (TV) @ 2.5 kg/ha
- **B_2 :** *Trichoderma viride* (TV) @ 2.5 kg/ha + *Pseudomonas fluorescence* (PF) @ 2.5 kg/ha
- **B_3 :** *Trichoderma viride* (TV) @ 2.5 kg/ha + *Pseudomonas fluorescence* (PF) @ 2.5 kg/ha + *Azotobacter chroococcum* (AC) @ 5 kg/ha

Chlorophyll content index: (SPAD-502)

Chlorophyll content was quantified in terms of grams of chlorophyll per unit ground area, as per the methods outlined by Nishimura (1964) and Okubo *et al*. (1968). The assessment was conducted on the fourth leaf of five-week-old plants using a non-destructive approach, employing an optical instrument known as the chlorophyll meter (Model: CCM 200, Made in USA).

To analyze physiological mechanisms such as photosynthetic rate, stomatal conductance and transpiration rate, an Infra Red Gas Analyzer (IRGA, model LI-6400) was utilized. The quantum efficiency was determined in accordance with the specifications provided by Pandey *et al*. (2001). Additionally, water use, carboxylation efficiency, and mesophyll efficiency were calculated based on the guidelines outlined by Kannan and Vankataraman (2010).

Quantum efficiency (mol m^{-2} s^{-1} (mol mol^{-1})$^{-1}$

The quantum efficiency was determined as per the formula given by Pandey *et al*. (2001) as follows:

$$Q.E. = \frac{Pn}{Q}$$

Where,

Pn = Net photosynthesis

Q = PAR absorption.

Carboxylation efficiency {µmol m^{-2} s^{-1}(µmol mol^{-1})$^{-1}$}

The carboxylation efficiency was worked out as per specifications given by Kannan *et al.* (2010) as follows

$$C.E. = \frac{Pn}{Ci}$$

Where,

Pn = Net photosynthesis

Ci = Intercellular CO_2 concentration

Mesophyll Efficiency (µmol mol^{-1}(mol $m^{-2}s^{-1}$)$^{-1}$

The mesophyll efficiency was determined as per the method given by Kannan *et al*. (2010) as follows:

$$ME = \frac{\text{Intercellular } CO_2 \text{ concentration (Ci)}}{\text{Stomatal conductance (Cond)}}$$

Where,

Ci = Intercellular CO_2 concentration

Cond = Stomatal conductance

Fruit yield ha^{-1} in (q/ha)

Fruit yield was recorded in q/ha.

Results and Discussion

Chlorophyll content index

The data unveiled a trend, where the highest chlorophyll content index was noted in the young, fully expanded leaf and subsequently exhibited a gradual decline with maturity. Specifically, the maximum value was observed for treatment M5 at 44.85, as presented in Table No. 1 and this value was significantly higher compared to all other treatments. Conversely, the minimum chlorophyll content index was recorded for treatment M1 at 38.18. In terms of bio-inoculants application, the maximum chlorophyll content index was documented for B3 at 45.80, while the minimum was observed for B0, specifically at 40.64.

The application of Zn enhances the photochemical reaction occurring in the thylakoid membrane, electron transport through PSII and increase chlorophyll content. Similar findings were also reported by Verma *et al.* (2015) and Arough *et al.* (2016).

Regarding the interaction effect, the highest chlorophyll content index was documented for the treatment combination M5B3 at 49.56, significantly surpassing other interactions. Following closely were M4B3 and M3B3 combinations, while the lowest chlorophyll content index was observed for the treatment combination M1B0, registering at 35.05. This particular trait holds promise in the context of a breeding program aimed at enhancing the photosynthetic efficiency of crops. The rationale behind this lies in the positive correlation between chlorophyll concentration and the plant's photosynthetic capability, as highlighted by Bonner (1952) and Ziyad (2014).

Photosynthetic rate (µmol m^{-2} s^{-1})

The findings indicated that M5 had the highest photosynthetic rate (11.05), significantly outperforming all other treatments listed in Table 1. In contrast, M1 displayed the lowest rate (8.70). Combining data from bio-inoculants application, B3 showed the maximum photosynthetic rate (11.12), while the minimum rate was recorded for B0 (9.53).

The rate of photosynthesis assessed as the carbon exchange rate was the important component that has direct relevance with yield components (Camussi and Attaviano, 1987). Similar findings were also reported by Verma *et al.* (2015).Regarding interaction effects, the combination M5B3 exhibited the highest recorded photosynthetic rate at 12.74, whereas the lowest rate was observed for the combination M1B0 at 7.61. This data has implications for incorporation into breeding programs aimed at improving crop photosynthetic efficiency, given the established positive correlation between plant photosynthetic capability and chlorophyll concentration, as documented by Bonner in 1952 and Ziyad in 2014.

Stomatal conductance (mol $m^{-2}sec^{-1}$)

In this study, the results in Table 1 revealed that M5 exhibited the highest stomatal conductance (0.132), significantly surpassing all other treatments. Conversely, M1 recorded the minimum conductance (0.052). Regarding bio-inoculants application, the data indicated the maximum stomatal conductance for B3 (0.003) and the minimum for B0, which was 0.066.

Zinc (Zn) plays a crucial role in regulating stomatal aperture, possibly by influencing potassium (K) content in guard cells. The control of stomatal opening involves carbonic anhydrase (CA), which helps maintain sufficient bicarbonate (HCO_3^-) levels in guard cells, with Zn playing a vital role in stomatal conductance. The inoculation of Plant Growth-Promoting Rhizobacteria (PGPR) has been shown to enhance stomatal conductance, thereby improving leaf water potential, especially under adverse conditions (Mia *et al*., 2010).

The present findings collaborated with earlier observations recorded by Anitha *et al.* (2009), Wang *et al*. (2009) and Verma *et al.* (2015).

The interplay of micronutrients and bio-inoculants, specifically *Trichoderma viride* and Plant Growth-Promoting Rhizobacteria (PGPR), demonstrated a discernible impact on stomatal conductance. The treatment combination M5B3 yielded the highest recorded stomatal conductance at 0.235, whereas the lowest was observed for the combination M1B0, measuring 0.035.

Transpiration rate (µmol m^{-2} sec^{-1})

Table 1 discloses that M5 exhibited a higher transpiration rate (4.42), significantly surpassing all other treatments, while the minimum was recorded for M1 (3.04). Conversely, pooled data from bio-inoculants application indicated a higher transpiration rate for B3 (4.55) and a lower rate for B0 (3.56).

It is necessary to have higher plant conductance to achieve higher canopy photosynthesis which not only enhances the CO_2 exchange rate but also results in a higher transpiration rate (Farquhar and Sharkey 1982). The present findings are similar to Anitha *et al.* (2009), Gupta *et al*. (2012) and Verma *et al.* (2015).

Regarding interaction effects, the combination of micronutrients and bio-inoculants (*Trichoderma viride* and PGPR) demonstrated a discernible impact on the rate of transpiration. The highest transpiration rate was recorded for the treatment combination M5B3 (5.27), while the lowest was observed for M1B0. Specifically, the combination M1B0 exhibited a lower transpiration

Table 1 : Individual and interaction effect of different micronutrients and bio-inoculants on chlorophyll content index, Photosynthetic rate, Stomatal conductance and Transpiration rate (pooled).

Treatments	Chlorophyll content index (SPAD)	Photosynthetic rate (µmol m^{-2} s^{-1})	Stomatal conductance (mol $m^{-2}sec^{-1}$)	Transpiration rate (µmol m^{-2} sec^{-1})
Micronutrients				
M_1 No micronutrient	38.18	8.70	0.052	3.04
M_2 $FeSO_4$ (0.2%)	43.07	10.33	0.096	4.11
M_3 $(CANO_3)2$ (0.2%)	43.59	10.44	0.101	4.18
M_4 Borax (0.1%)	44.09	10.61	0.105	4.26
M_5 $ZnSO_4$ (0.2%)	44.85	11.05	0.132	4.42
S.Em±	0.32	0.09	0.004	0.03
C.D.5% level	0.93	0.25	0.011	0.08
Bioinoculants				
B_0 No bioinoculant	40.64	9.53	0.066	3.56
B_1 TV (2.5 kg/ha)	41.57	9.80	0.082	3.76
B_2 TV + PF (2.5 kg/ha +2.5 kg/ha)	43.02	10.46	0.099	4.13
B_3 TV + PF+AC (2.5 kg/ha +2.5 kg/ha +5.0kg/ha)	45.80	11.12	0.142	4.55
S.Em±	0.29	0.08	0.003	0.03
C.D.5% level	0.83	0.22	0.010	0.07
Interaction				
M_1 B_0	35.05	7.61	0.035	2.67
M_1 B_1	36.46	8.73	0.050	3.00
M_1 B_2	40.32	9.16	0.060	3.14
M_1 B_3	40.88	9.32	0.060	3.36
M_2 B_0	41.72	9.82	0.065	3.73
M_2 B_1	42.58	9.96	0.085	3.84
M_2 B_2	43.22	10.61	0.100	4.21
M_2 B_3	44.78	10.91	0.135	4.65
M_3 B_0	41.87	9.87	0.070	3.75
M_3 B_1	42.79	10.05	0.090	3.92
M_3 B_2	43.33	10.77	0.105	4.37
M_3 B_3	46.37	11.07	0.140	4.67
M_4 B_0	42.21	9.91	0.075	3.80
M_4 B_1	42.94	10.08	0.090	3.98
M_4 B_2	43.80	10.92	0.115	4.46
M_4 B_3	47.41	11.54	0.140	4.79
M_5 B_0	42.35	10.43	0.085	3.83
M_5 B_1	43.06	10.20	0.093	4.07
M_5 B_2	44.45	10.85	0.116	4.49
M_5 B_3	49.56	12.74	0.235	5.27
S.Em±	0.64	0.17	0.007	0.06
C.D.5% level	1.85	0.50	0.021	0.16

rate at 2.67.

Quantum efficiency (µmol/m^2/s^{-1}(µmol mol^{-1})$^{-1}$

The analysis of data from Table 2 revealed that M5 exhibited significantly higher quantum efficiency at 0.031, surpassing all other treatments, while M1 demonstrated a lower efficiency at 0.020. In the context of bio-inoculants application, B3 showed higher quantum efficiency at 0.033, whereas B0 displayed a lower efficiency at 0.023.

Quantum efficiency serves as a measure of crop plants' effectiveness in converting absorbed solar energy into chemical energy. These findings align with the results reported by Verma *et al.* (2015).

Table 2 : Individual and interaction effect of different micronutrients and bio-inoculants on Quantum efficiency, Carboxylation efficiency, Mesophyll efficiency and Fruit yield (Pooled).

Treatments	Quantum efficiency [µmol/m^2/s^{-1} (µmol mol^{-1})$^{-1}$]	Carboxylation efficiency [µmol m^{-2} s^{-1} (µmol mol^{-1})$^{-1}$]	Mesophyll efficiency [µmol mol^{-1} (mol m^{-2}s^{-1})$^{-1}$]	Fruit yield per ha (q)
Micronutrients				
M_1 No micronutrient	0.020	0.080	1277.99	56.60
M_2 $FeSO_4$ (0.2%)	0.028	0.110	1096.32	73.79
M_3 $(CANO_3)2$ (0.2%)	0.029	0.117	1075.94	74.89
M_4 Borax (0.1%)	0.030	0.128	1057.61	76.38
M_5 $ZnSO_4$ (0.2%)	0.031	0.132	1031.90	78.83
S.Em±	0.001	0.004	5.22	1.06
C.D.5% level	0.003	0.010	15.00	3.05
Bioinoculants				
B_0 No bioinoculant	0.023	0.088	1218.47	61.80
B_1 TV (2.5 kg/ha)	0.026	0.095	1142.53	67.57
B_2 TV + PF (2.5 kg/ha +2.5 kg/ha)	0.029	0.112	1079.35	72.70
B_3 TV + PF+AC (2.5 kg/ha +2.5 kg/ha +5.0kg/ha)	0.033	0.158	991.44	86.31
S.Em±	0.001	0.003	4.67	0.95
C.D.5% level	0.003	0.009	13.42	2.73
Interactions				
M_1 B_0	0.0165	0.072	1300.58	55.38
M_1 B_1	0.0199	0.076	1288.44	58.88
M_1 B_2	0.0217	0.085	1275.64	59.68
M_1 B_3	0.0217	0.086	1247.28	52.45
M_2 B_0	0.0232	0.090	1224.28	62.72
M_2 B_1	0.0263	0.097	1136.71	67.71
M_2 B_2	0.0293	0.113	1069.97	72.91
M_2 B_3	0.0336	0.142	954.30	91.80
M_3 B_0	0.0244	0.091	1202.40	63.70
M_3 B_1	0.0270	0.099	1113.51	68.70
M_3 B_2	0.0313	0.118	1043.99	75.02
M_3 B_3	0.0339	0.161	943.84	92.12
M_4 B_0	0.0253	0.093	1198.51	64.89
M_4 B_1	0.0275	0.102	1094.93	70.69
M_4 B_2	0.0318	0.120	1012.50	76.95
M_4 B_3	0.0369	0.197	924.50	92.97
M_5 B_0	0.0256	0.094	1166.60	62.31
M_5 B_1	0.0276	0.103	1079.06	71.84
M_5 B_2	0.0322	0.125	994.64	78.96
M_5 B_3	0.0389	0.206	887.29	102.2
S.Em ±	0.002	0.007	10.44	2.12
C.D.5% level	N.S.	0.020	30.00	6.11

Regarding interaction effects, the combination M5B3 demonstrated a higher quantum efficiency at 0.0389, whereas the combination M1B0 recorded a lower efficiency at 0.0165.

Carboxylation efficiency [µmol m^{-2} s^{-1}(µmol mol^{-1})$^{-1}$]

The results from Table 2 revealed that M5 exhibited the maximum carboxylation efficiency at 0.132, significantly surpassing all other treatments, while M1 recorded the minimum efficiency at 0.080. In the context of bio-inoculants application, the data indicated the highest carboxylation efficiency for B3 at 0.158, and the lowest efficiency for B0, which was 0.088.

The intrinsic carboxylation efficiency, defined as the ratio of net photosynthesis rate to intercellular CO_2 concentration, signifies that a higher ratio indicates better efficiency for carboxylation. These findings are consistent with those reported by Verma *et al*. (2015).

Regarding interaction effects, the combination M5B3 demonstrated the highest carboxylation efficiency at 0.206, whereas the combination M1B0 recorded the lowest efficiency at 0.072.

Mesophyll efficiency [µmol mol^{-1}(mol $m^{-2}s^{-1}$)$]^{-1}$

Table 2 reveals that M1 exhibited the maximum mesophyll efficiency at 1277.99, significantly surpassing all other treatments, while M5 recorded the minimum efficiency at 1031.90. In the context of bio-inoculants application, the data indicated that the maximum mesophyll efficiency was recorded for B0 at 1218.47 and the minimum efficiency for B3, which was 991.44.

Significantly, at a given stomatal conductance, a lower Ci (intercellular CO_2 concentration) signifies superior mesophyll efficiency and a more effective drawdown rate of the substrate CO_2, as documented by Ramanjulu *et al.* (1968). These findings are in accordance with the results reported by Verma *et al.* (2015).

In terms of interaction effects, the combination of micronutrients and bio-inoculants (*Trichoderma viride* and PGPR) demonstrated a noteworthy impact on mesophyll efficiency. The highest mesophyll efficiency was recorded for the treatment combination M1B0 at 1300.58, while the lowest efficiency was observed for M5B3 at 887.29.

Fruit yield per hectare (q)

The current investigation, as indicated in Table 2, unveiled a significant impact of foliar micronutrient application on fruit yield per hectare. The results demonstrated that M5 exhibited the highest fruit yield per hectare at 78.83 q, significantly followed by treatment M4 at 76.38 q. Conversely, the minimum yield was recorded for the control treatment, M1, at 56.60 q.

Concerning fruit yield, the observed increase, possibly attributed to the application of zinc sulfate and boron (micronutrients), may be linked to enhanced photosynthetic activity. This enhancement results in increased production and accumulation of carbohydrates, contributing to a heightened yield of chili.

These findings align with the results reported by Barche *et al*. (2011), Patil *et al*. (2013), Saravaiya *et al*. (2014) and Ali *et al*. (2013).

Conclusion

The impact of physiological developments varied across different treatments and noteworthy physiological improvements significantly enhanced crop yield. The most favorable physiological developments were observed in treatment M5, involving foliar application of $ZnSO_4$ at 0.2%.

Regarding physiological parameters, the treatment combination M5 B3, consisting of $ZnSO_4$ (0.2%) + *Trichoderma Viride* (TV) + *Pseudomonas fluorescence* (PF) + *Azotobacter chroococcum* (AC) (2.5 kg/ha + 2.5 kg/ha + 5.0 kg/ha), demonstrated superiority. The findings indicate that treatment M5B3, incorporating zinc sulfate and boron (micronutrient) application, contributed to enhanced photosynthetic activity, leading to increased production.

Simultaneously, the inoculation of bio-inoculants facilitated nutrient uptake, including phosphorous, nitrogen, and potassium, through the synergistic effects of *Trichoderma viride* and Pseudomonas fluorescence. The interaction between *Trichoderma viride* and the plant potentially produced secondary metabolites, such as auxin, contributing to improved yield.

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