Effects of effective microorganisms biochar-based fertilizer on photosynthetic characteristics and chlorophyll content of flue-cured tobacco under water-saving irrigation strategies

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ABSTRACT

Effective microorganism biochar-based fertilizer (EMBF) can improve the physiological properties of tobacco (Nicotiana tabacum L.) In this study, the irrigation and EMBF rates were applied as factors that influence the photosynthetic characteristics and chlorophyll content of tobacco. The experiment involved 12 treatments: CK1-CK3, T1-T3, T4-T6, and T7-T9; these four groups represented 0, 100, 300, and 600 g EMBF pot⁻¹, respectively. Each group was irrigated at rates of 40, 80, and 120 L pot⁻¹. When comparing with the control treatment CK, results showed that net photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO₂ concentration (Ci), transpiration rate (Tr), and soil plant analysis development (SPAD) increased by 8.21%-107.03%, 18.78%-118.27%, 18.78%-118.27%, 7.24%-104.15%, and 3.47%-69.09%, respectively, after EMBF application. The Pn, gs, Ci, and Tr at the growth and maturity stages were highly significant (P < 0.01) and positively correlated with EMBF application. The Pn, gs, and Tr were significant (P < 0.05) and positively correlated with irrigation, and Ci was less affected by irrigation (P > 0.05). The SPAD value of flue-cured tobacco at three growing stages was highly significant (P < 0.01) and positively correlated with the irrigation and fertilization rates. The SPAD values at the growth and maturity stages were higher than those at the rooting stage by 46.73%-79.2% and 2.21%-46.17%, respectively. Furthermore, the 80 L pot⁻¹ irrigation rate combined with 300 g EMBF pot⁻¹ is the best water and fertilizer combination. The results of this study might provide theoretical and practical guidance for growing flue-cured tobacco in production areas.

Key words: Chlorophyll content, EMBF, flue-cured tobacco, Nicotiana tabacum, photosynthetic characteristics.

INTRODUCTION

Tobacco (Nicotiana tabacum L.) is an important cash crop worldwide, and China is one of the largest producers (Kulik et al., 2017). Water and fertilizer are the two main factors affecting the growth, yield, and quality of flue-cured tobacco, and adjusting the water-fertilizer application rate is essential for controlling the yield and quality of flue-cured tobacco (Chen et al., 2017; Xue et al., 2019). In recent years, water resources have been scarce in China’s major tobacco-growing areas, and their spatial and temporal distribution has been uneven (Zhang et al., 2019a). In addition, most of the water-fertilizer management approaches were used to achieve the goal of high yielding flue-cured tobacco. The long-term application of chemical fertilizers deteriorates soil physical properties, decreases fertility, and causes soil compaction (Xie et al., 2019). Therefore, studying a new water retention fertilizer and exploring the effect of a water-fertilizer interaction mechanism is an effective method to improve the water and fertilizer use efficiency quality, and yield of flue-cured tobacco.
There are currently many reports on flue-cured tobacco base fertilizers. Biochar has a strong adsorption ability because of its great porosity and high specific surface area (Zhu et al., 2018). Biochar-based fertilizer prepared with biochar as a fertilizer carrier can promote tobacco growth and increase its quality (Qin et al., 2018; Chen et al., 2019; Li et al., 2019a). Compared with conventional fertilization, adding biochar-based fertilizer can significantly increase total sugar in tobacco leaves (Wang et al., 2019), chlorophyll content, fertilizer rate (Chen et al., 2019), and net photosynthetic rate (Pn) in leaves (Li et al., 2019b). Some studies have shown that applying large amounts of biochar-based fertilizer would not improve tobacco leaf quality (Zhang et al., 2019b). Ye et al. (2015) showed that the highest nicotine content and lowest sensory quality of tobacco leaf in treatment conventional fertilization and biochar 900 kg hm⁻² ha⁻¹ might be due to the increase of the N fertilizer rate after an excessive biochar application. Effective microorganisms (EM) are cultures of coexisting beneficial microorganisms, including up to 80 different species, which predominantly consist of photosynthetic and lactic acid bacteria, yeast, and actinomycete species (Daly and Stewart, 1999). They are a soil activator and improve soil structure, organic matter management, and nutrient cycling, which complement efforts to reduce the reliance on synthetic fertilizers and pesticides; they have excellent development prospects in agriculture, animal husbandry, breeding, and environmental protection (Talaat et al., 2015). Bokashi is the growth medium for microorganisms and provides a suitable micro-environment for EM in the soil; EM-bokashi is an anaerobic fermentation product from solid agricultural byproducts and EM (Higa and Parr, 1994; Shin et al., 2017). Hu and Qi (2013) showed that long-term EM application combined with compost enhanced wheat straw biomass, grain yields, and straw and grain nutrition. Dai et al. (2019) showed that the EM treatment markedly promoted Pₙ, stomatal conductance (gₛ), and transpiration rate (Tᵣ) of Quercus shumardii, and notably improved the leaf chlorophyll mass fraction.

Although the above studies have achieved satisfactory results, the water retention performance of EM biochar-based fertilizer (EMBF) and the influence of EMBF on agronomic traits, photosynthetic characteristics, quality, and yield of flue-cured tobacco are still unclear. Therefore, the aim of this study was to evaluate the effect of different water and EMBF application rates on the physiological indices of flue-cured tobacco (photosynthetic characteristics and chlorophyll content) and evaluate the water retention ability of EMBF.

**MATERIALS AND METHODS**

**Experimental site**

The experiment was conducted in a plastic covered greenhouse in the Vegetables and Flowers Institute of Hohai University research base (31°43’ N, 118°46’ E), Nanjing, China, in 2019. The research area belongs to the north subtropical monsoon climate zone with an average annual temperature of 15.4 °C, average annual precipitation of 1106.5 mm, and an average frost-free period of 224 d. The soil in the test site was yellow-brown soil with a thick texture. The soil physicochemical properties were pH 5.87, 14.2 g kg⁻¹ organic matter content, 19.72 mg kg⁻¹ available P, 174.23 mg kg⁻¹ available K, 121.65 mg kg⁻¹ available N, and 0-60 cm depth soil density of 1.35 g cm⁻³.

**Experimental design**

The experiment was carried out in plastic flower pots (360 mm diameter, 300 mm height). ‘Yunyan 87’ was selected as the tobacco (Nicotiana tabacum L.) seedling, and soil was taken from the experimental greenhouse. The experiment divided the growth cycle of tobacco into three stages: rooting stage (35 d after seedling stage), growth stage (36-65 d after transplanting), and maturity stage (66 d after harvest). The experiment consisted of 12 treatments: CK1-CK3, T1-T3, T4-T6, and T7-T9; the four groups of treatments received 0, 100, 300, and 600 g pot⁻¹ of effective microorganism biochar-based fertilizer (EMBF), respectively, and each group was irrigated with 40, 80, and 120 L pot⁻¹. Each treatment was in triplicate. During the transplanting stage, 2 L of water were applied to each pot in all treatments to stabilize the roots. The amount of irrigation was distributed according to the rooting, growth, and maturity stages of flue-cured tobacco, which accounted for 30%, 40%, and 30% of the total irrigation rate, respectively, and watering occurred every 5 d. The fertilizer used is a special base fertilizer for flue-cured tobacco (N:P₂O₅:K₂O 9:13:22) and a biochar-based compound fertilizer (N:P₂O₅:K₂O 9:11:18) (SEEK Bio-Technology, Shanghai, China). The EM-bokashi was produced by diluting the EM stock solution 50 to 100 times and a mixed fermentation with rice hulls and distilled water for 5 to 7 d. The EM-Bokashi and EM stock solution were produced by EMRO Environmental Protection Biotechnology (Nanjing, China). One tonne of biochar-based compound fertilizer and 15 kg of EM-bokashi were mixed thoroughly. During mixing, the EM stock
solution, which was diluted 100 times, was sprayed on the surface of the compost at 20 kg dilution per tonne of compost. The compost was covered with a film and fermented for 3 to 5 d to produce EMBF. The hole fertilization method was used and the special base fertilizer rate for flue-cured tobacco was 100 g pot⁻¹. The fertilization and irrigation rates for each treatment are shown in Table 1.

Main tested indices and methods
The photosynthetic characteristics of flue-cured tobacco were measured with a portable photosynthesis system (TPS-2, PP Systems, Amesbury, Massachusetts, USA). The fifth leaf from the top of the tobacco plant was selected between 09:00 and 11:00 h in fine weather. The measured leaf was the most recently fully expanded and its color was uniform. Light intensity was controlled at 800 μmol m⁻² s⁻¹. Measured characteristics included net photosynthetic rate (Pₙ), stomatal conductance (gₛ), intercellular CO₂ concentration (Cᵢ), and transpiration rate (Tᵣ). Leaves free of pests, physiological lesions, and mechanical damage were sampled. The first measurement was taken in the middle of the leaf and avoiding the veins; afterward, a measurement was taken at 3 cm on the left and right sides of the first sampling position. The error of the three soil plant analysis development (SPAD) values should be within 1 and the mean represented by the SPAD value of this leaf. The soil physicochemical properties were measured following the methods described by Li et al. (2008). A one-way ANOVA with Duncan’s multiple range test was used to assess significant differences in the photosynthetic characteristics and SPAD, and Pearson’s chi-squared test was used to analyze the correlation. All data in this experiment were processed with Origin 9.5 (OriginLab Corporation, Northampton, Massachusetts, USA), SPSS 17.0 (IBM, Armonk, New York, USA), and Excel 2019 software.

RESULTS

Net photosynthetic rate (Pₙ)
The photosynthetic characteristics of flue-cured tobacco were measured at the growth and maturity stages. The effects of different EMBF and irrigation rates on Pₙ of flue-cured tobacco are shown in Figure 1. The highest Pₙ was obtained in T9 at the growth stage with 19.57 μmol m⁻² s⁻¹, followed by T5 with 19.43 μmol m⁻² s⁻¹. The Pₙ at the maturity stage was the highest in T5, followed by T6 with values of 12.77 and 10.97 μmol m⁻² s⁻¹, respectively. The Pₙ of CK1 at the growth and maturity stages was 12.33 and 5.10 μmol m⁻² s⁻¹, respectively; these values were significantly (P < 0.05) lower than in other treatments. Compared with the control CK, Pₙ at the growth and maturity stages increased by 8.21%-40.28% and 36.6%-107.03%, respectively, after EMBF application. In the CK treatments with 100 g and 600 g fertilizer, Pₙ at the growth stage increased as irrigation increased. In the 300 g fertilizer treatment, Pₙ of the 80 L (medium) irrigation treatment T5 was 32.18% higher than the 40 L (low) irrigation treatment T4. However, it was nonsignificantly different from the 120 L (high) irrigation treatment T6 (P > 0.05). For the same irrigation rate, Pₙ at the growth stage increased as fertilization increased, and T9 had the highest value. There was a positive correlation between the irrigation and fertilization rates and Pₙ at the growth stage of flue-cured tobacco.

Table 1. Irrigation and fertilization schemes for each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EMBF</th>
<th>Rooting</th>
<th>Growth</th>
<th>Maturity</th>
<th>Total irrigation rate</th>
</tr>
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<tr>
<td></td>
<td>g pot⁻¹</td>
<td>L pot⁻¹·time⁻¹</td>
<td>L pot⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK1</td>
<td>0</td>
<td>1.5</td>
<td>2.0</td>
<td>1.5</td>
<td>40</td>
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<tr>
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<td>4.0</td>
<td>3.0</td>
<td>80</td>
</tr>
<tr>
<td>CK3</td>
<td>0</td>
<td>4.5</td>
<td>6.0</td>
<td>4.5</td>
<td>120</td>
</tr>
<tr>
<td>T1</td>
<td>100</td>
<td>1.5</td>
<td>2.0</td>
<td>1.5</td>
<td>40</td>
</tr>
<tr>
<td>T2</td>
<td>100</td>
<td>3.0</td>
<td>4.0</td>
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<td>80</td>
</tr>
<tr>
<td>T3</td>
<td>100</td>
<td>4.5</td>
<td>6.0</td>
<td>4.5</td>
<td>120</td>
</tr>
<tr>
<td>T4</td>
<td>300</td>
<td>1.5</td>
<td>2.0</td>
<td>1.5</td>
<td>40</td>
</tr>
<tr>
<td>T5</td>
<td>300</td>
<td>3.0</td>
<td>4.0</td>
<td>3.0</td>
<td>80</td>
</tr>
<tr>
<td>T6</td>
<td>300</td>
<td>4.5</td>
<td>6.0</td>
<td>4.5</td>
<td>120</td>
</tr>
<tr>
<td>T7</td>
<td>600</td>
<td>1.5</td>
<td>2.0</td>
<td>1.5</td>
<td>40</td>
</tr>
<tr>
<td>T8</td>
<td>600</td>
<td>3.0</td>
<td>4.0</td>
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<td>80</td>
</tr>
<tr>
<td>T9</td>
<td>600</td>
<td>4.5</td>
<td>6.0</td>
<td>4.5</td>
<td>120</td>
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</tbody>
</table>

EMBF: Effective microorganism biochar-based fertilizer.
The Pn at the maturity stage decreased more than at the growth stage. The Pn of the CK, 100, 300, and 600 g fertilization treatments decreased by 55.97%-58.38%, 41.96%-48.77%, 34.31%-49.66%, and 39.10%-44.09%, respectively. Under the same fertilization application conditions, Pn of the CK and 100 g fertilization treatments increased as irrigation increased, whereas the 300 and 600 g fertilization treatments increased and then decreased. Treatment T5 had the highest Pn, and there was a significant difference with other treatments. Under the same irrigation rate, Pn at the growth stage with 40 L irrigation increased as the fertilizer application increased. Among treatments, T7 had the highest value. Under 80 and 120 L irrigation, Pn increased and then decreased as the fertilizer application increased, and the highest value was for treatment T5.

Stomatal conductance (gs)
The effects of different EMBF fertilization and irrigation rates on gs of flue-cured tobacco are shown in Figure 2. The highest gs was obtained in T9 at the growth stage with 538.67 mmol m⁻² s⁻¹, followed by T5 with a value of 496.67 mmol m⁻² s⁻¹. Treatment CK1 had the lowest value at only 305.33 mmol m⁻² s⁻¹. In contrast to the growth stage, gs at the maturity stage had the highest value in T5 with 380.33 mmol m⁻² s⁻¹. Treatment T9 was second with 350.33 mmol m⁻² s⁻¹ and CK1 had the lowest value with only 146.67 mmol m⁻² s⁻¹. Compared with the control CK, gs at the growth and maturity stages increased by 10.17%-48.12% and 29.91%-109.36%, respectively, after EMBF application. In the CK and 100 and 600 g fertilization treatments, gs at the growth stage increased as irrigation increased. For the 300 g fertilization rate, gs at the growth stage increased and then decreased as irrigation increased, and gs of T5 had the highest value. Under the same irrigation condition, gs at the growth stage of the 40 and 120 L irrigation treatments was positively correlated with the fertilization rate. However, gs in the 80 L irrigation treatment increased then decreased and T5 had the highest value.

The gs value at the maturity stage decreased more than at the growth stage. The gs values of CK, 100, 300, and 600 g fertilization rates decreased by 36.57%-51.97%, 25.21%-44.48%, 23.42%-29.05%, and 20.79%-34.96%, respectively. Under the same fertilizer application conditions, gs at the maturity stage of CK, 100, and 600 g fertilization rates increased as irrigation increased. At the 300 g fertilization rate, gs at the maturity stage increased and then decreased; T5 had the highest value and there was a significant difference with other treatments (P < 0.05). Under the same irrigation condition, gs treated with 40 and 120 L irrigation was positively correlated with the fertilization rate, while the 80 L irrigation treatment increased and then decreased.
The effects of different EMBF and irrigation rates on $\text{Ci}$ of flue-cured tobacco are shown in Figure 3. The highest $\text{Ci}$ value was obtained in T5 at the growth stage with 416.33 μmol mol$^{-1}$, followed by T8 with 389.67 μmol mol$^{-1}$. Treatment CK1 had the lowest value with only 229 μmol mol$^{-1}$. The same occurred for the growth stage, $\text{Ci}$ at the maturity stage had the highest value in T5 with 302.67 μmol mol$^{-1}$, followed by T8 with 285.33 μmol mol$^{-1}$. The CK1 treatment had the lowest value with only 125.33 μmol mol$^{-1}$. Compared with the control CK, $\text{Ci}$ at the growth and maturity stages increased by 18.78%-70.16% and 28.19%-118.27%, respectively, after EMBF application. For the same fertilization rate, $\text{Ci}$ at the growth stage of the CK treatment increased as the irrigation rate increased. Under the 100, 300, and 600 g fertilization conditions, $\text{Ci}$ increased and then decreased, and T5 had the highest value. The difference between 100 g fertilization treatment was nonsignificant ($P > 0.05$). For the 40 and 80 L irrigation rates, $\text{Ci}$ increased and then decreased as the fertilization rate increased; T1 and T5 had the highest values, respectively. For the 120 L irrigation rate, $\text{Ci}$ increased as fertilization increased. The difference between T6 and T9 was nonsignificant ($P > 0.05$).

The $\text{Ci}$ at the maturity stage decreased more than at the growth stage. The $\text{Ci}$ of fertilization treatments at CK, 100, 300, and 600 g decreased by 43.32%-45.27%, 32.93%-33.89%, 27.3%-33.21%, and 26.78%-45.35%, respectively. For the same fertilization rate, $\text{Ci}$ at the maturity stage increased and then decreased as the irrigation rate increased, and it reached the maximum value under medium irrigation conditions. For the same irrigation rate, $\text{Ci}$ treated with the 40 and 80 L irrigation rates increased and then decreased, and the highest values occurred in T4 and T5, respectively. For the 120 L irrigation rate, $\text{Ci}$ increased as fertilization increased. The difference between T6 and T9 was nonsignificant ($P > 0.05$).
Transpiration rate (T) 

The effects of different EMBF and irrigation rates on T, of flue-cured tobacco are shown in Figure 4. The highest T value was obtained in T5 at the growth stage with 6.18 mmol m⁻² s⁻¹, followed by T9 with 5.963 mmol m⁻² s⁻¹. Treatment CK1 had the lowest value with only 3.34 mmol m⁻² s⁻¹. The same occurred at the growth stage, T at the maturity stage had the highest value in T5 with 4.10 mmol m⁻² s⁻¹, followed by T9 with 3.97 mmol m⁻² s⁻¹ and CK1 had the lowest value with only 1.89 mmol m⁻² s⁻¹. Compared with the control CK, T at the growth and maturity stages increased by 12.66%-62.15% and 7.24%-104.15%, respectively, after EMBF application. Under the CK, 100, and 600 g fertilization conditions, T at the growth stage increased as the irrigation rate increased and these values were significantly different (P < 0.05). For the 300 g fertilization rate, T increased and then decreased, and T5 had the highest value. Under the same irrigation conditions, T for the 40 and 120 L irrigation rates was positively correlated with the fertilization rate, but increased and then decreased for the 80 L irrigation rate.

The T value of flue-cured tobacco at the maturity stage decreased more than at the growth stage. The T values of the 0, 100, 300, and 600 g fertilization rates decreased by 43.57%-47.29%, 40.87%-46.28%, 31.94%-34.02%, and 30.82%-35.98%, respectively. For the 0, 100, and 600 g fertilization rates, T of flue-cured tobacco at the maturity stage increased as irrigation increased. For the 300 g fertilization rate, T of flue-cured tobacco at the maturity stage increased and then decreased, and T5 had the highest value, which was not significantly different from T6 (P > 0.05). The T value for the 40 and 120 L irrigation rates was positively correlated with the fertilization rate, while with the 80 L rate increased and then decreased.

Tables 2 and 3 show the correlation analysis results between the main photosynthetic characteristic indicators of flue-cured tobacco at the growth and maturity stages and irrigation and fertilization rates. Table 2 shows that Pₑ, gₑ, Cₑ, and Tₑ were significantly and positively correlated with the EMBF rate at the growth stage (P < 0.01, R = 0.567, 0.673, 0.615, and 0.650, respectively). The Pₑ, gₑ, and Tₑ were all highly significant and positively correlated with the irrigation rate (P < 0.01, R = 0.543, 0.519, and 0.517, respectively). Table 3 shows that Pₑ, gₑ, Cₑ, and Tₑ were highly significant and positively correlated with the EMBF application rate at the maturity stage (P < 0.01, R = 0.624, 0.727, 0.573, and 0.729, respectively). The Pₑ, gₑ, and Tₑ were significantly and positively correlated with the irrigation rate (P < 0.05, R = 0.372, 0.410, and 0.401, respectively).
Effects on chlorophyll content
Figure 5 shows the SPAD values of flue-cured tobacco at different growth stages for the 0, 100, 300, and 600 g EMBF application rates. Different treatments had higher SPAD values at the growth stage, and there was a slight difference between the rooting and maturity stages. Compared with the control CK, the SPAD values at the growth and maturity stages increased by 3.47%-26.36% and 5.25%-69.09%, respectively, after EMBF application. For the 0 g fertilization application rate (Figure 5a), the ranges of SPAD values at the rooting, growth, and maturity stages were 16.57-20.63,
24.85-34.62, and 16.93-25.3, respectively, which all increased as irrigation increased. The SPAD values for CK1, CK2, and CK3 at the maturity stage decreased by 31.86%, 30.83%, and 26.91%, respectively, compared with the growth stage. For the 100 g fertilization rate (Figure 5b), the ranges of SPAD values at the rooting, growth, and maturity stages were 18.9-22.58, 27.98-35.82, and 26.1-31.52, respectively, which all increased as irrigation increased. The SPAD values for T1, T2, and T3 at the maturity stage decreased by 6.73%, 19.54%, and 11.99%, respectively, compared with the growth stage, and T1 had a slight decrease. For the 300 g fertilization rate (Figure 5c), the ranges of SPAD values at the rooting, growth, and maturity stages were 19.59-26.51, 31.4-41.5, and 28.63-36.09, respectively, which all increased as irrigation increased. The SPAD values of T4, T5, and T6 at the maturity stage decreased by 8.81%, 14.30%, and 13.04%, respectively, compared with the growth stage, which was slightly lower than in other fertilization treatments. For the 600 g fertilization rate (Figure 5d), the ranges of SPAD values at the rooting, growth, and maturity stages were 17.01-29.06, 30.48-42.63, and 21.82-36.22, respectively, which all increased as irrigation increased. Treatment T9 had the highest SPAD value at all three growth stages and was significantly different from the other treatments. The SPAD values for T7, T8, and T9 at the maturity stage decreased by 28.41%, 16.63%, and 15.04%, respectively, compared with the growth stage and T7 had a higher decrease.

Different letters above the bars indicate significant differences (P < 0.05) between biochar rates. R: Rooting stage; G: growth stage; M: maturity stage.

Treatments CK1-CK3, T1-T3, T4-T6, and T7-T9: Each group received 0, 100, 300, and 600 g effective microorganism biochar-based fertilizer pot⁻¹, respectively, and 40, 80, and 120 L irrigation pot⁻¹, respectively.
Table 4 shows the correlation analysis results between SPAD values, EMBF application and irrigation rates of flue-cured tobacco at different growth stages. The irrigation rate was the main factor influencing the SPAD value at the three growth stages. The SPAD value at the rooting stage was more affected by the fertilization and irrigation rates than at the maturity stage, with correlation coefficients of 0.492 and 0.637, respectively (P < 0.01). The fertilization and irrigation rates had a significant influence on the SPAD value at the growth stage, with correlation coefficients of 0.495 and 0.718, respectively (P < 0.01), in which the effect of irrigation was greater.

**DISCUSSION**

Photosynthesis plays a decisive role in crop yield (Zhao et al., 2019). In the present study, it was found that appropriate EMBF application and irrigation rates could significantly increase the $P_n$, $g_s$, $C_i$, and $T_r$ values of flue-cured tobacco. This indicates that EMBF could enhance the photosynthetic capacity of tobacco leaves and maintain a strong physiological metabolism, with results similar to those reported by Yang et al. (2019). Zhao et al. (2010) considered that this might be related to the water retention effect of EMBF. The EMBF can effectively control the water in the soil and release it slowly, increase soil water content around the roots, and show a lower water pressure deficit and stronger photosynthesis ability in the tobacco leaves. In the present study, the values of $P_n$, $g_s$, and $T_r$ increased as the irrigation rate increased for the 0, 100, and 600 g fertilization rates, and increased then decreased for the 300 g fertilization rate, indicating that under moderate fertilization conditions, a higher irrigation rate would limit the increase of $P_n$, $g_s$, and $T_r$ values of flue-cured tobacco. This can be due to fertilizer leaching caused by excessive irrigation (Zhang et al., 2019a), which exceeds the water-holding capacity of the applied EMBF. The excess water occupies the soil voids, squeezing out soil air, which affects the respiratory metabolism of the root system and affects root vitality of flue-cured tobacco, resulting in the photosynthetic decline of flue-cured tobacco leaves.

Studies by Li et al. (2019b) showed that both $P_n$ and $g_s$ of flue-cured tobacco seedling leaves significantly decreased, while $C_i$ increased significantly under drought stress. Hou et al. (2016) indicated that $C_i$ of flue-cured tobacco treated with EM water-retaining agents during the growth period was significantly higher than in the control treatment. In the present study, under the conditions of proper EMBF application and irrigation rates, $P_n$, $g_s$, and $T_r$ increased significantly, and differences in the values of $T_6$, $T_8$, and $T_9$ were small. This was probably because $g_s$ is the limiting factor of $P_n$ (Zhao et al., 2010), and $g_s$ and $T_r$ are positively correlated, while EMBF application promotes moisture absorption of flue-cured tobacco, improves nutrient effectiveness in fertilizers, and enhances transpiration. When the irrigation and fertilization rates reach a certain level, the photosynthetic characteristics of flue-cured tobacco are no longer affected.

The value of $C_i$ was less affected by the irrigation rate, and the differences between the $T_1$, $T_2$, and $T_3$ treatments were nonsignificant. For the same fertilizer application rate, $C_i$ was higher in the treatment with the 40 L irrigation rate, indicating that the low fertilizer rate had nonsignificant effect on the $C_i$ value of flue-cured tobacco and the medium irrigation rate could maintain $C_i$ at a high level. However, Ma et al. (2018) showed that the $C_i$ value was positively related to the irrigation and fertilization rates, which could be because the temperature in the greenhouse was too high, the irrigation rate was excessive, soil water evaporation increased, and the soil salt return rate was accelerated (Shi et al., 2017). Leaf surface stomata were closed to reduce water evaporation, $C_i$ increased, and transpiration of flue-cured tobacco was weakened; this resulted in decreased water absorption capacity of the root system, which reduced the photosynthetic rate.

<table>
<thead>
<tr>
<th>Table 4. Correlation analysis of SPAD values, EMBF application and irrigation rates of flue-cured tobacco at different growth stages.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td>EMBF</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>R</td>
</tr>
<tr>
<td>G</td>
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<tr>
<td>M</td>
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</tbody>
</table>

a: Significant at the 0.05 level; b: significant at the 0.01 level.
SPAD: Soil plant analysis development; EMBF: effective microorganism biochar-based fertilizer; I: irrigation; R: rooting stage; G: growth stage; M: maturity stage.
Chlorophyll is the basis of photosynthesis, which can be used as a guide for timely fertilization to meet the needs of tobacco growth, improve fertilizer use efficiency, and minimize fertilizer waste and environmental pollution caused by excessive fertilization (Wei et al., 2012). It can also be used as a diagnostic indicator of the quality of flue-cured tobacco leaves (Fracchiolla et al., 2020). For the same irrigation rate, the SPAD value increased as fertilization increased; this might be because the absorption of N in the base fertilizer by flue-cured tobacco was promoted after EMBF application (Bai et al., 2013), thereby increasing the SPAD value. For the same fertilization rate, the SPAD value increased as the irrigation rate increased, which is consistent with the results of the study by Neupane et al. (2020). Among treatments, the treatment with high irrigation and high fertilizer application rates (T9) had the highest SPAD value during the three growth stages. This can be due to the good water retention performance of EMBF, which can ensure that the root system of flue-cured tobacco absorbs sufficient water; the mesophyll cells promote chlorophyll synthesis when there is sufficient water, which increases the total amount of chlorophyll inside the leaves. The experimental results also complement the research conducted by Du et al. (2019).

CONCLUSIONS

It could be concluded from the results that the water-fertilizer management approach with the appropriate irrigation rate and effective microorganism biochar-based fertilizer (EMBF) application rate could significantly improve the photosynthetic characteristics and chlorophyll content of flue-cured tobacco. Compared with the control CK, the net photosynthetic rate ($P_n$), stomatal conductance ($g_s$), intercellular CO$_2$ concentration ($C_i$), and transpiration rate ($T_r$) increased by 8.21%-107.03%, 18.78%-118.27%, 18.78%-118.27%, and 7.24%-104.15%, respectively, after the EMBF application. The $P_n$, $g_s$, $C_i$, and $T_r$ at the growth and maturity stages were highly significant and positively correlated with the EMBF application rate. The $P_n$, $g_s$, and $T_r$ were significant and positively correlated with the irrigation rate, and $C_i$ was less affected by irrigation.

The soil plant analysis development (SPAD) value of flue-cured tobacco in three plant growth stages was highly significant and positively correlated with the irrigation and fertilization rates. Compared with the control, the SPAD values at the growth and maturity stages increased by 3.47%-26.36% and 5.25%-69.09%, respectively, after the EMBF application. The SPAD values at the growth and maturity stages were higher than at the rooting stage by 46.73%-79.2% and 2.21%-46.17%, respectively. Higher EMBF application and irrigation rates were beneficial to maintain a high SPAD value. Furthermore, the 80 L pot$^{-1}$ irrigation rate and 300 g pot$^{-1}$ EMBF application rate is the best water and fertilizer combination. The results of this study might provide theoretical and practical guidance for the growth of flue-cured tobacco in production areas. Different varieties of flue-cured tobacco need to be studied in the future to improve the universality of the research results.

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