

Article

Meloidogyne graminicola's Effect on Growth Performance of Rice under Low Population Density

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Abstract: *Meloidogyne graminicola* is a destructive soil-borne pathogen that causes rice yield losses (*Oryza sativa* L.) in tropical and subtropical areas. This study investigated the effect of *M. graminicola* population densities on plant height, heading, and the photosynthetic parameters of rice in a greenhouse. Two-week-old rice plants were inoculated with different *M. graminicola* densities (250, 500, 750, 1000, 1500, and 2000 J2s/plant) and observations were recorded at 30, 60, and 90 days after inoculation (DAI). Reductions in growth and photosynthetic parameters caused by *M. graminicola* densities were calculated in relation to a control (non-inoculated rice). Results revealed that *M. graminicola* infection with low population densities (0–500 J2s/plant) did not influence the rice plant height during 30–60 DAI, but significantly lowered the plant height, panicle growth rate, and panicle length of rice at 90 DAI. The chlorophyll content of rice inoculated with 500–2000 J2s was significantly lower than that of the control. Furthermore, *M. graminicola* infection with 500 J2s/plant significantly lowered the transpiration rate and net photosynthetic rate by 21.21% and 21.81%, respectively, compared with the control ($p < 0.05$). *M. graminicola* with a low population density significantly reduced the net photosynthetic rate of rice, which affected organic matter accumulation, resulting in growth retardation and lower yields ($p < 0.05$).

Keywords: population density of *Meloidogyne graminicola*; growth performance; photosynthetic pigments; rice



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1. Introduction

Rice (*Oryza sativa* L.) is considered a staple cereal in the Asia–Pacific region, producing and consuming more than 90% of the world's rice. As the dominant rice-producing country, China cultivated 30.08 million hectares of rice in 2020 with an annual rice output of 21.86 million tons [1]. However, various biotic and abiotic factors are responsible for the lower productivity of rice under field conditions. Among the biotic factors, plant-parasitic nematodes (PPNs) play an important role and account for yield losses to the extent of 90% [2]. Moreover, root-knot nematodes (RKNs) are responsible for about 15% of the total economic losses in rice production in Asia [3]. The major RKNs associated with rice are *Meloidogyne graminicola*, *M. incognita*, *M. javanica*, *M. triticoryzae*, *M. arenaria*, *M. oryza*, *M. salasi*, *M. lini*, and *M. hannanensis* [4–10]. Among those species, *M. graminicola* is considered to be a major threat to rice agriculture, and it is widespread across Asian rice production systems [4]. In China, *M. graminicola* was first reported on *Allium fistulosum* in Sanya, Hainan Province [11]. Afterward, the damage of *M. graminicola* was successively identified in Fujian, Guangdong, Yunnan, Jiangxi, and Jiangsu [12,13]. During the past five years, *M. graminicola* spread to Zhejiang, Hubei, Hunan, Sichuan, Anhui, and Henan [14–19]. In Guangxi province, *M. graminicola* was detected in 32.04% of 206 rice fields in 67 counties [20]. In addition to upland and irrigated rice, several vegetables, fruit, and even weeds have also been attacked by *M. graminicola* [21]. Intermittent irrigation in paddy fields leaves the

soil in an anaerobic state for a period without affecting the development of *M. graminicola* in the soil. The reason is that rice roots develop here, and the ability of oxygen to diffuse to the root tip is enhanced due to a synergistically formed oxic rhizosphere in the anoxic environment [22]. Therefore, *M. graminicola* can continue their life cycle after invading rice roots, and the interference of plant physiological as well as biochemical processes is likely a result of nematodes feeding on giant cells, causing root growth to stop and tips to swell [23,24]. When the *M. graminicola* infects rice, hook-shaped or spiral galls form at the rice root terminal, which are characteristic symptoms caused by this nematode species. Moreover, the eggs are laid inside the root so the next generation J2s can remain within the maternal gall after hatching and migrate from these damaged roots to establish new galls on healthy tissue [25].

Due to the injury of the underground parts of the rice, the aerial parts show yellowing, stunting, reduction of tillering, and delayed maturation, which constitute important constraints in successful crop production [26]. In addition to the external appearance, the normal physiological metabolic processes, such as photosynthesis in the plant, is disrupted by RKNs [27]. Among the indicators of photosynthesis in plants, the chlorophyll content is crucial because it provides a primary medium through which plants obtain the energy for metabolism and growth [28,29]. In addition, the chlorophyll content is highly susceptible to biotic stress [30,31]. Previous studies have indicated that RKN infestation results in the decrease in chlorophyll content in hosts. At the *M. incognita* density of 1500 J2s/plant, the chlorophyll content of tomato cultivar Gailiang maofen 802 was significantly reduced by 2.4% [32]. Another study confirmed that the relationship between the relative leaf chlorophyll content of the cucumber plant and the population density of nematodes fitted the Seinhorst damage model, and chlorophyll content decreased with the increase in the nematode population density of *M. incognita* and *M. javanica* [33]. In addition, the growth parameters of cucumber, including shoot and root lengths, shoot and root weights, and yield, all decreased, but fell in the ranges of 2.82–14.26% after being inoculated with 500 *M. incognita* J2s for two weeks. Significant reductions in these parameters were noticed when the inoculum density of *M. incognita* was 8000 J2s/plant, with the decrease range between 26.85% and 68.06% [34].

The life cycle characteristics of *M. graminicola* mean that the next generation of J2s can continue to feed in the roots without migrating through the soil in search of new hosts. Therefore, the initial nematode population density in the paddy field is an important indicator of the tolerance threshold of rice to *M. graminicola*. In *M. graminicola*–rice interaction, the *M. graminicola*-resistant rice was not destroyed by 15000–6000 J2s per plant, while the susceptible rice suffered more in this range of inoculum density [35]. This population density occurred in the field as well [36]. As mentioned above, nematodes with different densities have different effects on rice. However, the performance of susceptible rice after low density *M. graminicola* infection and the internal relationship between *M. graminicola* infection and rice growth and photosynthesis remains unclear. Hence, this is the focus of the present study. This research aimed to clarify the impact of the *M. graminicola* population density (0–2000 J2s/plant) on susceptible rice cultivars, with an interest in the variation among the morphological index and photosynthetic parameters, which provide important information for understanding the incidence of rice root-knot nematode and for guiding the management strategies of the disease.

2. Materials and Methods

2.1. Rice Seedling Nursery

Rice seeds (susceptible cultivar, Yexiangyou 9) were soaked in water for 48 h after surface sterilization with 5.25% NaOCl for 5–10 min and put in Petri dishes at 30 °C for 2 days for germination. The germinating seeds were sown in a tray containing autoclaved sandy soil (sand: soil = 3:1) and kept in an incubator (CONVIRON, Winnipeg, MB, Canada) for 5 days with constant moisture. Well-growing seedlings with a uniform height were transplanted into a plastic pot containing 1000 cm³ autoclaved sandy soil, with one plant

in each pot. The seedlings were watered with 20 mL Hoagland nutrient solution twice a week [37].

2.2. Nematode Inoculum

The *M. graminicola* population was established in rice (cv. Teyou 09 × 103) in the greenhouse at the Agricultural College of Guangxi University Nanning, Guangxi, China. Then, 3–4 weeks after inoculation, eggs were extracted from infected rice roots by dissecting galls, and the egg suspension was incubated in darkness at 28 °C for 2–3 days to obtain *M. graminicola* J2s [38]. The J2 inoculum was quantified by concentrating or diluting the initial suspension or the counted suspension to the desired concentration in the new dilution.

2.3. Experimental Design

In order to investigate the response of susceptible rice to *M. graminicola* infestation at different population densities, especially when the nematode density was less than 1000 J2s/plant (low inoculum densities), two greenhouse experiments were conducted at the ambient temperature of 28 °C and 85% relative humidity from August to October and from September to November in 2020. The inoculum densities of *M. graminicola* J2 in the first experiment were 500, 1000, 1500, and 2000 J2s/plant and, in the second experiment, they were 250, 500, 750, and 1000 J2s/plant. Each experiment was laid out in a completely randomized design with ten replicates, each independently repeated twice. Two-week-old plants were inoculated with freshly hatched J2 of *M. graminicola* by pipetting four aliquots of 1 mL in one 2 cm-deep hole around the seedling's base, and uninoculated plants served as a control.

For each rice plant in all treatments, plant height (from the soil surface to the tip of the tallest panicle) was measured at 30, 60, and 90 days after inoculation (DAI), and panicle length was measured at 90 DAI (and rice heading from 75 DAI). The panicle presence rate (PR) at 90 DAI and the plant height growth rate (PHR) in each treatment at each measured time were calculated by following the formula given by:

$$PHR (\%) = \frac{h' - h^0}{h^0} \times 100\%$$

$$PR (\%) = \frac{n}{N} \times 100\%$$

where h' refers to the plant height at each measured time, h^0 stands for initial plant height, n is the number of rice plants with panicles, and N is the number of rice samples.

In addition, the chlorophyll content in the flag leaves of rice was determined three times using a non-destructive portable soil–plant analysis development meter (SPAD-502, Konica-Minolta, Japan). Here, we take the average of the three SPAD values of the same flag leaf recorded at 30, 60, and 90 DAI. Photosynthetic parameters, including transpiration rate (Tr), net photosynthetic rate (Pn), total conductance of CO₂ (GTC), total conductance of water (GTW), partial pressure of intercellular CO₂ (PCI), and intracellular CO₂ concentration (Ci), were measured using the portable infrared gas analyzer photosynthetic system LI-6800XT (LI-COR, Biosciences, Lincoln, CA, USA). Photosynthetic gas exchange was measured on each flag leaf from 9:00 to 12:00 on bright clear sunny days.

2.4. Statistical Analyses

The growth and photosynthetic parameters were calculated before statistical analysis. Data were analyzed by using SPSS statistical software (SPSS version 19.0, Chicago, IL, USA). A one-way analysis of variance (ANOVA) was carried out to determine the significant differences among the treatment means at $p < 0.05$.

3. Results

3.1. Effect of Inoculum Density on Rice Plant Growth

The growth of inoculated rice was inhibited when the nematode inoculum was denser (500–2000 J2s/plant). The *PHR* of rice with different inoculum densities at each measurement time are shown in Tables 1 and 2. The rice with the 500-nematode inoculation showed no significant differences in *PHR* at 30, 60, or 90 DAI. Significant differences were observed for the *PHR* between rice inoculated with 1000–2000 J2s and the 0–500 J2-inoculated rice, and the difference was even greater at 90 DAI ($p < 0.05$); here, the *PHR* values of the nematode-free control were 1.24, 1.87, 1.66, and 1.70 times higher than the 500, 1000, 1500, and 2000 J2-inoculated rice, respectively.

Table 1. Plant height growth rate of rice in the first experiment (%).

J2 Population	Inoculation Time (Day)		
	30	60	90
0	106.4 ± 4.91 a	138.0 ± 8.83 a	184.6 ± 12.89 a
500	116.4 ± 5.36 a	131.8 ± 16.18 a	149.4 ± 9.95 b
1000	80.4 ± 2.13 b	89.2 ± 5.67 b	98.6 ± 6.68 c
1500	80.2 ± 1.24 b	86.4 ± 5.30 b	111.0 ± 6.30 c
2000	74.2 ± 3.18 b	100.8 ± 6.71 b	108.6 ± 2.69 c

Note: Data are presented as mean ± SE. Values followed by the different letters in the same columns are significantly different according to LSD at the $p < 0.05$ level.

Table 2. Plant height growth rate of rice in the second experiment (%).

J2 Population	Inoculation Time(Days)		
	30	60	90
0	93.89 ± 6.87 a	131.00 ± 10.05 a	169.71 ± 13.09 a
250	103.00 ± 11.43 a	126.67 ± 19.00 ab	155.71 ± 25.04 a
500	79.89 ± 11.26 ab	115.57 ± 15.31 abc	130.71 ± 13.99 ab
750	62.50 ± 6.95 b	80.87 ± 8.18 c	83.14 ± 7.04 c
1000	65.50 ± 5.51 b	93.17 ± 6.10 bc	93.50 ± 7.46 bc

Note: Data are presented as mean ± SE. Values followed by the different letters in the same columns are significantly different according to LSD at the $p < 0.05$ level.

In the condition with low inoculum densities of 250–1000 J2s/plant, the mean *PHR* did not significantly decrease from the control in rice inoculated with 250 and 500 J2s at the time of measurement ($p < 0.05$). However, the *PHR* of 750 J2-inoculated rice was significantly lower than the control, but was statistically similar to 1000 J2-inoculated rice ($p < 0.05$).

3.2. Effect of Inoculum Density on Rice Panicle Development

A significant reduction both in the *PR* and the panicle length among the inoculated rice was observed at 90 DAI ($p < 0.05$). The *PR* of the control reached 80%, while that of the inoculated rice was only 10–20% (Figure 1B). Likewise, the average panicle length of the control was 9.15 cm, while for the rice with 500, 1000, 1500, and 2000 J2 inoculation, these values were 0.90, 1.41, 1.75, and 0.52 cm, respectively (Figure 1A).

Similar results were obtained in the range of low inoculum densities. The *PR* of rice inoculated with 250 J2s was significantly lower than the control ($p < 0.05$), with values at 20% and 80%, respectively. Meanwhile, the average panicle length of this rice was 2.12 cm, which was only 0.25 times that of the control (Figure 1B).

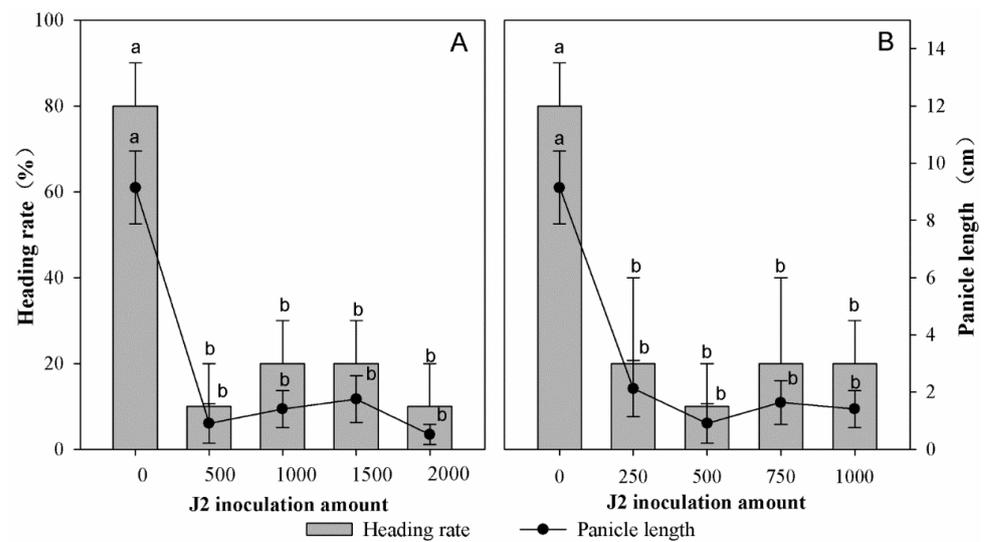


Figure 1. Effect of different inoculum densities of *M. graminicola* J2 on panicle development of rice. (A): heading rates and panicle lengths of rice in the first experiment; (B): heading rates and panicle lengths of rice in the second experiment. Note: different lowercase letters on the same date indicate significant difference at $p < 0.05$ level according to LSD.

3.3. Effect of Inoculum Density on Chlorophyll Content in Rice

In the first experiment, the infected rice showed a drastic decline at each measurement time in chlorophyll content compared to the nematode-free control ($p < 0.05$). Specifically, the chlorophyll content of all treatments was lower at 60 DAI than at 30 DAI, but it showed an increasing trend from 60 DAI to 90 DAI, which the chlorophyll content at 90 DAI was higher than that at 30 DAI, but no significant differences were observed between 30 DAI and 90 DAI. On the contrary, the chlorophyll content of the control showed an upward trend from 30 DAI to 90 DAI, kept the highest at each measurement time, and was significantly higher than the treatments ($p < 0.05$). The chlorophyll content of rice treated with 500–2000 J2s/plant were decreased by 14.59%, 31.09%, 36.62%, and 38.66% at 90 DAI, respectively, compared with the control (Figure 2A).

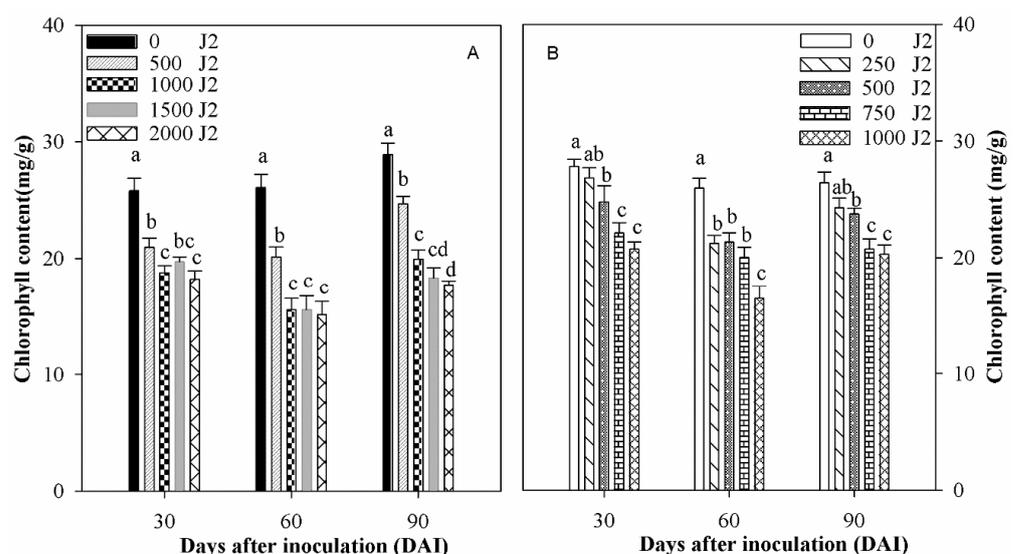


Figure 2. Chlorophyll content of rice leaf during 30–90 DAI with different inoculum densities of *M. graminicola* J2. (A): chlorophyll contents of rice leaf in the first experiment; (B): chlorophyll contents of rice leaf in the second experiment. Note: different lowercase letters on the same date indicate significant difference at $p < 0.05$ level according to LSD.

Similar dynamics of the chlorophyll content in infected rice were evident in second experiment. In addition, by comparing the differences in chlorophyll content between inoculated rice and the control, we found that the degree of decreasing chlorophyll content in rice with the increasing nematode inoculum density (≥ 500 J2s) was higher than rice inoculated with fewer nematodes (250 J2s) (Figure 2B).

3.4. Effect of Inoculum Density on Photosynthetic Parameters of Rice

One of the prime impacts of nematode infection is on the physiological process of photosynthesis. The results showed that nematode inoculation caused a severe decrease in the transpiration rate (Tr) and net photosynthetic rate (Pn), as well as total conductance of CO₂ (GTC) and water (GTW), in the flag leaves of all treatments compared with the control (Figure 3A–D).

3.4.1. Effects of Inoculum Density on Transpiration Rate and Net Photosynthetic Rate of Rice

At 30 DAI, the transpiration rates (TR values) of rice treated with 1000–2000 J2 inoculum was significantly reduced by 57.58%, 60.61%, and 27.27%, respectively, while the 500 J2-inoculated rice was reduced by 21.21%, a value statistically similar to the control. In contrast, the Tr value of all treated rice showed a remarkable reduction at 60 DAI; significantly, the rice inoculated with 500 and 2000 J2s was severely reduced by 80.88% ($p < 0.05$) (Figure 3A).

Similar results were obtained for the rice Pn value measurements. At 30 DAI, the Pn value of the control was significantly higher than that for the rice treated with the inoculum densities of 1000–1500 J2s/plant, representing a reduction of 45.45% and 42.98%, respectively, and non-significant differences were observed in the Pn values of both the control and the 500 J2-inoculated rice. Although the Pn values of rice inoculated with 500, 1000, 1500, and 2000 J2s increased by 33.33%, 64.96%, 70.64%, and 20.13%, respectively, from 30 DAI to 60 DAI, the control was increased by 74.22%. Therefore, the Pn value of the control was also considerably higher than all treated rice (Figure 3B).

3.4.2. Effects of Inoculum Density on Total Conductance of CO₂ and Water of Rice

The different *M. graminicola* inoculum density treatments showed a reduction in GTC and GTW values compared to the nematode-free control (Figure 3C,D). At 30 DAI, the GTC and GTW values of the control were statistically similar to 500 J2-inoculated rice, but were significantly different from rice treated with the inoculum densities of 1000 J2s/plant and above ($p < 0.05$). Here, 500, 1000, 1500, and 2000 J2-inoculated rice recorded 20.71%, 58.95%, 63.19%, and 25.51% higher GTC values, as well as 20.61%, 58.81%, 63.05%, and 25.40% higher GTW values than non-inoculated rice, respectively. Furthermore, a significant variation in the GTC and GTW values between nematode-free control and all treatments was observed at 60 DAI ($p < 0.05$). The GTC and GTW values of the control were increased by 50.00% and 50.98% during 30 DAI to 60 DAI, respectively. On the contrary, the 500 J2-inoculated rice decreased by 61.54% and 60.00%, respectively, and the 2000 J2-inoculated rice decreased by 50.00% and 52.63%, respectively.

3.4.3. Effects of Inoculum Density on Partial Pressure of Intercellular CO₂ and Intracellular CO₂ Concentration of Rice

The partial pressure of intercellular CO₂ (P_{ci}) and intracellular CO₂ concentration (C_i) values reflect the rate of photosynthesis. After the rice was treated with the *M. graminicola* inoculum densities of 500–2000 J2s/plant, the P_{ci} and C_i values did not decrease for all treatments (Figure 3E,F). For instance, at 30 DAI, both the P_{ci} and C_i values of 2000 J2-inoculated rice were 1.69% and 0.65% higher than the control, but this was not significant statistically. On the contrary, the P_{ci} values of 500 and 1500 J2-inoculated rice significantly decreased by 2.51% and 3.41%, respectively, and C_i values showed non-significant differences between treatments and control ($p < 0.05$). At 60 DAI, the P_{ci} values of rice inoculated with 1000 and 1500 J2s were 1.60% and 4.22% higher than the control, respectively. In contrast, the 2000

J2-inoculated rice was 5.55% lower than the control, and even the 500 J2-inoculated rice was significantly lower than the control by 24.08% ($p < 0.05$) (Figure 3E). Meanwhile, not only were the Ci values of rice inoculated with 1000 and 2000 J2s 1.02% and 8.92% lower than the control, respectively, but the 500 J2-inoculated rice was also significantly decreased by 21.55% ($p < 0.05$). In addition, the Ci value of 1500 J2-inoculated rice was 1.96% higher than the control, but both the Pci and Ci values of all treatments at 60 DAI were lower than at 30 DAI (Figure 3F).

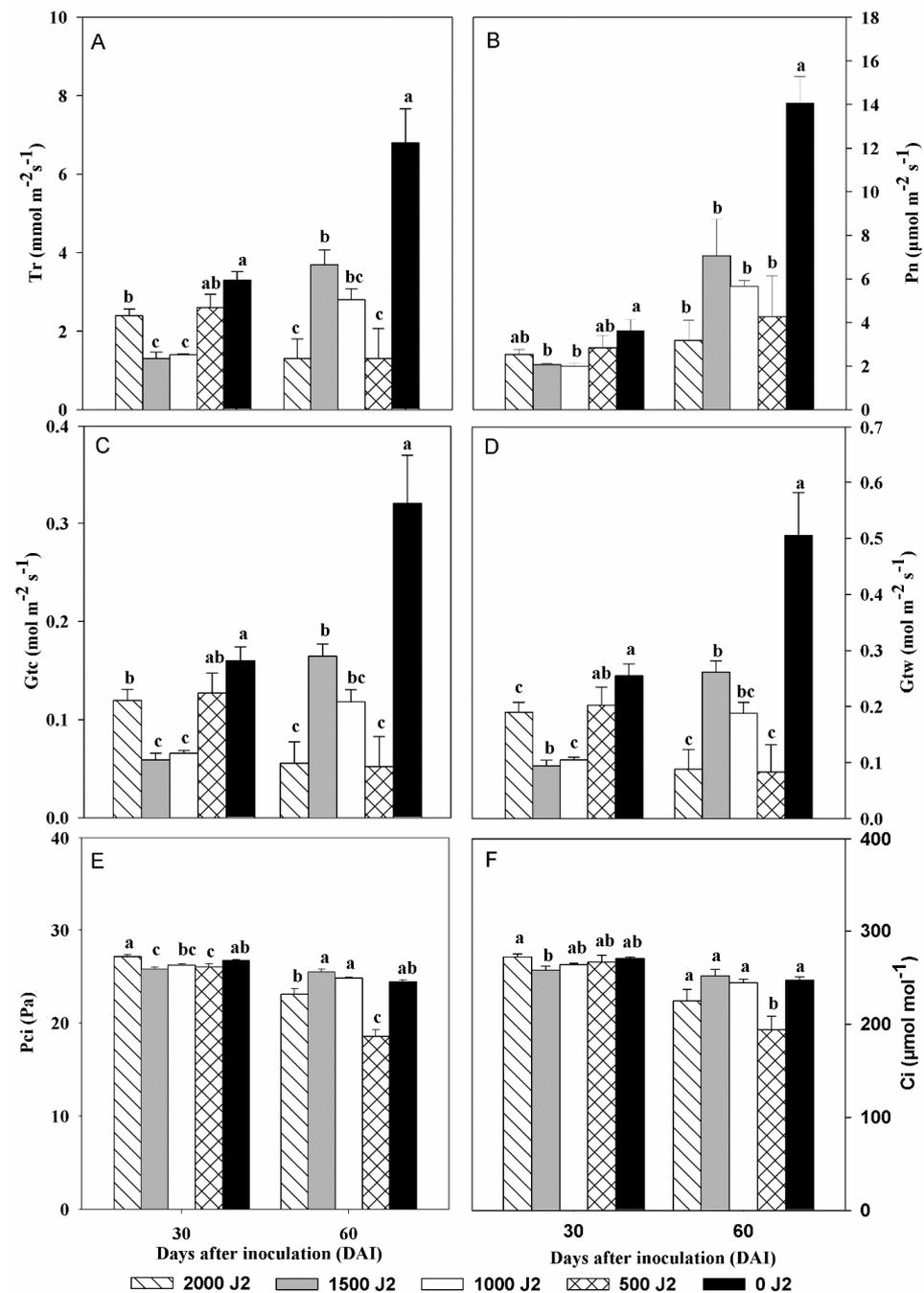


Figure 3. Effects of different inoculum densities of *M. graminicola* J2 on photosynthetic parameters of rice plant. (A): Transpiration rate (Tr); (B): net photosynthetic rate (Pn); (C): total conductance of CO₂(Gtc); (D): total conductance of water (Gtw); (E): partial pressure of intercellular CO₂ (Pci); (F): intracellular CO₂ concentration (Ci). Note: The different letters in the same group are significantly different according to LSD at $p < 0.05$.

4. Discussion

The accurate estimation of their population density and incidence in the field is critical to developing effective and sustainable management strategies for rice-infecting nematodes. The reports have demonstrated that nematode density in the soil directly impacts host physiological response, and the impact would be amplified by the increase in nematode density [39]. For instance, when the rice was planted in soil containing 1–6 J2s of *M. graminicola* per cm³, the yield of rice with very high levels of *M. graminicola* infestation was 44.5–50.7% lower than that of rice in paddies without *M. graminicola*, while the protein contents and amylose in rice grains decreased significantly. Additionally, the germination rate of rice seeds decreased with the increase in *M. graminicola* J2 density in soil [40]. In the greenhouse, a significant reduction in plant height, fresh weight of the plant, dry weight of the stem and root, chlorophyll content, and protein and starch content of rice grain was observed after rice (cv. Uma) was treated with an inoculum density of 500 *M. graminicola* J2s [41]. These findings have confirmed that the accumulation of organic matter in plants gradually decreased with the increase in the RKN density in soil. However, the ability of plants to accumulate organic matter during the growth period is related to the intensity of photosynthesis [42], which was significantly affected with *M. graminicola* inoculation, ultimately leading to reduced plant growth and a lower yield. In these pot experiments, representing irrigation conditions, the *M. graminicola* inoculum densities of 250–2000 J2s/plant simulated what existed in the field. A rice field surveyed in Hunan, China, showed that the density of *M. graminicola* in rice transplanting fields with sandy loam was 2.83 J2s/100g soil at seedling stage and 86.67 J2s/100g soil at tillering stage [13]. Additionally, these experiments were carried out from August to November, which is in line with the planting period of late rice in China. The same inoculation density (500 and 1000 J2s/plant) existed in both trials. When the inoculum density was 500 *M. graminicola* J2s/plant, the *PHR* of inoculated rice at 90 DAI in the first experiment was significantly lower than that of its control, but there was no significant difference between the inoculated rice and its control in the second experiment. However, the *PHRs* of the control at each measurement time in the first experiment were higher than those in the second experiment. Furthermore, this study showed that the *PHR* of the nematode-free control was not significantly different from the 250 J2-inoculated plants, but was significantly higher than that of rice inoculated with 750 J2s. This suggests that an *M. graminicola* density of 0–500 J2s per plant had no significant effect on rice plant height, and that the density of 750 J2s per plant and above hindered rice growth. On the other hand, the *PR* and panicle length of inoculated rice were significantly lower than that of the control, whether the inoculum density was 250 or 500 J2s/plant. This indicated that even the lower nematode inoculation density would cause rice yield loss because the rice heading growth was delayed or inhibited.

Leaf chlorophyll content drops depending on the interaction between the intensity of the inoculum densities and the length of the cropping cycle [43,44]. In the present study, the total chlorophyll content of the control increased gradually within 90 days, while that of the inoculated rice decreased. The chlorophyll content of plants inoculated 2000 J2s decreased by 38.66% at 90 DAI compared with the control, which are similar results to the previous report of a 62.30% reduction in the chlorophyll content of Patchouli inoculated with 1500 *M. graminicola* J2s compared with the control after seven months [45].

Our results showed that an inoculum density of 500 J2s/plant or more caused significant differences between the chlorophyll content of inoculated plants and the control. Therefore, the *M. graminicola* inoculum densities of 500–2000 J2s/plant would affect the photosynthetic gas exchange of rice. In the present study, the *Tr* value of the control was the highest at each measurement time, and the *Tr* value of the control at 60 DAI was 6.80 mmol·m⁻² s⁻¹, which was significantly higher than the inoculated plants ($p < 0.05$). These results are similar to a previous study, which found a significant reduction in the chlorophyll content and transpiration rate of *Triadica sebifera* after infection with *M. incognita* [46]. We hypothesized that the declined in the *Tr* value was caused by the nematode

infection, which reduced the water absorption capacity of the root. Meanwhile, the Pn value of the control was $14.08 \text{ mmol}\cdot\text{m}^{-2} \text{ s}^{-1}$, 3.88 times higher than the inoculated rice at 30 DAI. As for the relationship between the Tr value and the Pn value, the stomatal width was positively correlated with the transpiration rate, and reduced transpiration leads to a decrease in stomatal conductance, which reduces the absorption of CO_2 by leaves, while the transport of nutrients slows down, leading to a decrease in the Pn value of leaves [47]. We assumed that the decrease in the Pn value of the inoculated plants might be related to the Tr value reduction caused by *M. graminicola* infection.

In a study carried out with cucumber plants inoculated with *M. graminicola* eggs, the nematode inoculation (5000, 10,000, and 15,000 eggs of *M. incognita*) significantly decreased the photosynthetic intensity at 70 DAI by 63.6%, 70.4%, and 80.0%, respectively, compared to the control [48]. In other words, the photosynthetic rate of plants was negatively correlated with nematode inoculation density. In addition, Ci has a negative correlation with the photosynthetic rate [49]. This implies that Ci values might increase due to the infection with nematodes. In this study, the Ci value of 2000 J2-inoculated rice was 0.65% higher than the control at 30 DAI, which was consistent with the above, and also showed that the photosynthetic intensity of rice plants decreased when the inoculum density of *M. graminicola* was 2000 J2s/plant. In addition, the Ci value of each treatment at 60 DAI was lower than that at 30 DAI, and the results reveal that the net photosynthetic rate of leaves was decreased with lower intercellular CO_2 concentration.

In general, the stomatal conductance of leaves was decreased under adverse conditions, which led to the decrease in the photosynthetic rate. This reflects the resistance of plants to adversity. Previous studies have shown that the Pn and stomatal conductance of oats (*Avena Sativa* L.) decreased significantly under salt stress, which may be due to the change in osmotic potential and the inhibition of chlorophyll synthesis in the oats, speeding up chlorophyll degradation [50]. Nematode infection is a biotic stress to plants, damaging the root system and affecting the water and nutrient transport in rice, thus affecting photosynthesis and the transpiration of plants, leading to abnormal growth [51,52]. Therefore, the decline in photosynthetic characteristics in rice plants is attributed to the inhibition of chlorophyll synthesis by *M. graminicola*, and the destruction of the metabolic pathway of photosynthesis, for example, decreased the activity of some enzymes responsible for photosynthesis (such as RuBP and Rubisco) or ATP regeneration [53].

5. Conclusions

In conclusion, our study demonstrated that *M. graminicola* infection inhibits chlorophyll synthesis in rice plants and reduces the transpiration rate (which varies with inoculum densities of 500–2000 J2s/plant). It also reduces leaf stomatal conductance and further inhibits the ability of plants to absorb CO_2 , which was manifested in the reduction of intercellular CO_2 concentration and partial pressure, thus lowering the net photosynthetic rate of rice plants, signaling a reduction in the synthesis and accumulation of organic matter in rice plants. Therefore, the morphological indexes of plants, including plant height, panicle growth rate, and panicle length, decreased significantly, which will affect the rice physiological growth and yield. The selected rice variety in this experiment is Yexiangyou 9, which is susceptible to *M. graminicola*. Its yield was greatly affected, even when the population density of nematodes was low. At present, the large-scale cultivation of *M. graminicola*-resistant varieties of rice can reduce this effect. Nevertheless, *M. graminicola* can complete their life cycle in a short time, and they have overlapping generations in the rice growth period. Therefore, even if the population density of nematodes in the field is low, they cannot be ignored. In our study, the rice panicle growth rate and panicle length were inhibited obviously at the lower inoculum density (250 J2/plant); further study is needed to examine the reasons for this phenomenon.

Author Contributions: H.W. and M.L. conceived the ideas and designed the experiment. M.L. carried out the experiments and generated the data. B.C. help in data collection. B.C. analyzed the data and wrote the manuscript. A.S. and S.A. reviewed the manuscript. H.W. supervised and financed the research. All authors have read and agreed to the published version of the manuscript.

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References

1. FAOSTAT. Food and Agriculture Organization of the United Nations. FAO Statistics. Available online: <http://www.fao.org/faostat/zh/#data/QCL/visualize> (accessed on 18 March 2021).
2. Jain, R.K.; Khan, M.R.; Kumar, V. Rice root-knot nematode (*Meloidogyne graminicola*) infestation in rice. *Arch. Phytopathol. Plant Prot.* **2012**, *45*, 635–645. [[CrossRef](#)]
3. Besnard, G.; Thi-Phan, N.; Ho-Bich, H.; Dereeper, A.; Nguyen, H.T.; Queneherve, P.; Aribi, J.; Bellafiore, S. On the close relatedness of two rice-parasitic root-knot nematode species and the recent expansion of *Meloidogyne graminicola* in Southeast Asia. *Genes* **2019**, *10*, 175. [[CrossRef](#)]
4. Mantelin, S.; Bellafiore, S.; Kyndt, T. *Meloidogyne graminicola*: A major threat to rice agriculture. *Mol. Plant Pathol.* **2017**, *18*, 3–15. [[CrossRef](#)]
5. Ruanpanun, P.; Khun-In, A. First report of *Meloidogyne incognita* caused root knot disease of upland rice in Thailand. *J. Int. Soc. Southeast Asian Agric. Sci.* **2015**, *21*, 68–77.
6. Negretti, R.R.R.D.; Gomes, C.B.; Mattos, V.S.; Somavilla, L.; Manica-Berto, R.; Agostinetto, D.; Castagnone-Sereno, P.; Carneiro, R.M.D.G. Characterisation of a *Meloidogyne* species complex parasitising rice in southern Brazil. *Nematology* **2017**, *19*, 403–412. [[CrossRef](#)]
7. Khan, M.R.; Haque, Z.; Kausar, N. Management of the root-knot nematode *Meloidogyne graminicola* infesting rice in the nursery and crop field by integrating seed priming and soil application treatments of pesticides. *Crops Prot.* **2014**, *63*, 15–25. [[CrossRef](#)]
8. Xie, J.; Fang, Y.; Huang, W.; Peng, D.; Peng, Y.; Ji, H. Advances in major rice parasitic nematodes in recent years. *J. Plant Prot.* **2017**, *44*, 940–949.
9. Baojun, Y. A new species of root—knot nematode—*Meloidogyne lini* n. sp. parasitizing rice. *J. Yunnan Agric. Univ.* **1988**, *1*, 11–17.
10. Liao, J.; Feng, Z. A new species of the genus *Meloidogyne*—*Meloidogyne hainanensis*. *J. South China Agric. Univ.* **1995**, *3*, 34–39.
11. Zhao, H.; Liu, W.; Chen, L.; Yu, D. *Meloidogyne graminicola*, a new record species from China. *Acta Phytopathol. Sin.* **2001**, *31*, 184–188.
12. Liu, G.; Xiao, S.; Zhang, S.; Zhang, D.; Wang, Y. Infection characteristic and life cycle of rice root-knot nematode, *Meloidogyne graminicola* in rice root. *Chin. J. Trop. Crops* **2011**, *32*, 743–748.
13. Tang, B.; Wang, D.; Wang, J.; He, Q.; Liu, Y.; Zhang, D.; Cheng, F. Effects of different planting modes on occurrence of rice root-knot nematode disease. *Plant Prot.* **2021**, *47*, 188–191.
14. Tian, Z.L.; Barsalote, E.M.; Li, X.L.; Cai, R.H.; Zheng, J.W. First report of root-knot nematode, *Meloidogyne graminicola*, on rice in Zhejiang, Eastern China. *Plant Dis.* **2017**, *101*, 2152–2153.
15. Wang, G.F.; Xiao, L.Y.; Luo, H.G.; Peng, D.L.; Xiao, Y.N. First report of *Meloidogyne graminicola* on rice in Hubei Province of China. *Plant Dis.* **2017**, *101*, 1056–1057. [[CrossRef](#)]
16. Song, Z.Q.; Zhang, D.Y.; Liu, Y.; Cheng, F.X. First report of *Meloidogyne graminicola* on rice (*Oryza sativa*) in Hunan Province, China. *Plant Dis.* **2017**, *101*, 2153. [[CrossRef](#)]
17. Xie, J.L.; Xu, X.; Yang, F.; Xue, Q.; Peng, Y.L.; Ji, H.L. First report of root-knot nematode, *Meloidogyne graminicola*, on rice in Sichuan Province, Southwest China. *Plant Dis.* **2019**, *103*, 2142.
18. Ju, Y.L.; Wu, X.; Tan, G.J.; Peng, D.L.; Xu, J.F.; Qiu, K.; Wu, H.P. First report of *Meloidogyne graminicola* on rice in Anhui Province, China. *Plant Dis.* **2021**, *105*, 512. [[CrossRef](#)]
19. Liu, M.Y.; Liu, J.; Huang, W.; Peng, D. First report of *Meloidogyne graminicola* on rice in Henan Province, China. *Plant Dis.* **2021**. *online ahead of print.* [[CrossRef](#)]
20. Luo, M.; Li, B.X.; Wu, H.Y. Incidence of the rice root-knot nematode, *Meloidogyne graminicola*, in Guangxi, China. *Plant Pathol. J.* **2020**, *36*, 384. [[CrossRef](#)]
21. Huang, W.; Xiang, C.; Liu, Y.; Ding, Z.; Peng, D. Research progress on the occurrence and controlling of root-knot nematode *Meloidogyne graminicola* in rice. *Acta Phytopathol. Sin.* **2018**, *48*, 289–296.
22. Eickhorst, T.; Tippkötter, R. Management-induced structural dynamics in paddy soils of south east China simulated in microcosms. *Soil Tillage Res.* **2009**, *102*, 168–178. [[CrossRef](#)]
23. Abad, P.; Favery, B.; Rosso, M.N.; Castagnone-Sereno, P. Root-knot nematode parasitism and host response: Molecular basis of a sophisticated interaction. *Mol. Plant Pathol.* **2003**, *4*, 217–224. [[CrossRef](#)] [[PubMed](#)]

24. Strajnar, P.; Sirca, S.; Urek, G.; Sircelj, H.; Zeleznik, P.; Vodnik, D. Effect of *Meloidogyne ethiopica* parasitism on water management and physiological stress in tomato. *Eur. J. Plant Pathol.* **2012**, *132*, 49–57. [[CrossRef](#)]
25. Kyndt, T.; Fernandez, D.; Gheysen, G. Plant-parasitic nematode infections in rice: Molecular and cellular insights. *Annu. Rev. Phytopathol.* **2014**, *52*, 135–153. [[CrossRef](#)] [[PubMed](#)]
26. Soriano, I.R.; Reversat, G. Management of *Meloidogyne graminicola* and yield of upland rice in South-Luzon, Philippines. *Nematology* **2003**, *5*, 879–884. [[CrossRef](#)]
27. Kumari, C.; Dutta, T.K.; Banakar, P.; Rao, U. Comparing the defence-related gene expression changes upon root-knot nematode attack in susceptible versus resistant cultivars of rice. *Sci. Rep.* **2016**, *6*, 22846. [[CrossRef](#)]
28. Barna, B.; Dám, A.L.; Király, Z. Juvenility and resistance of a superoxide tolerant plant to diseases and other stresses. *Naturwissenschaften* **1993**, *80*, 420–422. [[CrossRef](#)]
29. Ort, D.R.; Merchant, S.S.; Alric, J.; Barkan, A.; Blankenship, R.E.; Bock, R.; Croce, R.; Hanson, M.R.; Hibberd, J.M.; Long, S.P.; et al. Redesigning photosynthesis to sustainably meet global food and bioenergy demand. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 8529–8536. [[CrossRef](#)]
30. Roca, L.F.; Romero, J.; Bohorquez, J.M.; Alcantara, E.; Fernandez-Escobar, R.; Trapero, A. Nitrogen status affects growth, chlorophyll content and infection by *Fusicladium oleagineum* in olive. *Crop Prot.* **2018**, *109*, 80–85. [[CrossRef](#)]
31. Alizade, S.; Keshtkar, E.; Mokhtasi-Bidgoli, A.; Sasanfar, H.; Streibig, J.C. Effect of water deficit stress on benzoylprop-ethyl performance and physiological traits of winter wild oat (*Avena sterilis* subsp. *ludoviciana*). *Crop Prot.* **2020**, *137*, 105292. [[CrossRef](#)]
32. Maqsood, A.; Wu, H.; Kamran, M.; Altaf, H.; Mustafa, A.; Ahmar, S.; Nguyen Thi Thang, H.; Tariq, K.; He, Q.; Chen, J.T. Variations in growth, physiology, and antioxidative defense responses of two tomato (*Solanum lycopersicum* L.) cultivars after co-infection of *Fusarium oxysporum* and *Meloidogyne incognita*. *Agronomy* **2020**, *10*, 159.
33. Gine, A.; Lopez-Gomez, M.; Vela, M.D.; Ornat, C.; Talavera, M.; Verdejo-Lucas, S.; Sorribas, F.J. Thermal requirements and population dynamics of root-knot nematodes on cucumber and yield losses under protected cultivation. *Plant Pathol.* **2014**, *63*, 1446–1453. [[CrossRef](#)]
34. Kayani, M.Z.; Mukhtar, T.; Hussain, M.A. Effects of southern root knot nematode population densities and plant age on growth and yield parameters of cucumber. *Crop Prot.* **2017**, *92*, 207–212. [[CrossRef](#)]
35. Cabasan, M.T.N.; Kumar, A.; De Waele, D. Effects of initial nematode population density and water regime on resistance and tolerance to the rice root-knot nematode *Meloidogyne graminicola* in African and Asian rice genotypes. *Int. J. Pest Manag.* **2018**, *64*, 252–261. [[CrossRef](#)]
36. Win, P.P.; Kyi, P.P.; De Waele, D. Effect of agro-ecosystem on the occurrence of the rice root-knot nematode *Meloidogyne graminicola* on rice in Myanmar. *Australas. Plant Pathol.* **2011**, *40*, 187–196. [[CrossRef](#)]
37. Ling, F.; Su, Q.; Jiang, H.; Cui, J.; He, X.; Wu, Z.; Zhang, Z.; Liu, J.; Zhao, Y. Effects of strigolactone on photosynthetic and physiological characteristics in salt-stressed rice seedlings. *Sci. Rep.* **2020**, *10*, 6183.
38. Salalia, R.; Walia, R.K.; Somvanshi, V.S.; Kumar, P.; Kumar, A. Morphological, morphometric, and molecular characterization of intraspecific variations within Indian populations of *Meloidogyne graminicola*. *J. Nematol.* **2017**, *49*, 254–267. [[CrossRef](#)]
39. Haseeb, A.; Pandey, R.J.P.P.A.; Summaries, N. Root-knot nematodes—A constraint to cultivation of *Davana*, *Artemisia pallens*. *Trop. Pest Manag.* **1990**, *36*, 317–319. [[CrossRef](#)]
40. Patil, J.; Gaur, H.S. The effect of root-knot nematode, *Meloidogyne graminicola*, on the quality and vigour of rice seed. *Nematology* **2014**, *16*, 555–564. [[CrossRef](#)]
41. Narayana, R.; Sheela, M.; Lal, D.S. Effect of different inoculum levels of *Meloidogyne graminicola* golden and birchfield on growth and biochemical parameters of rice (*Oryza sativa* L.). *Entomon* **2015**, *40*, 163–168.
42. Rampazzo, P.E.; Marcos, F.C.C.; Cipriano, M.A.P.; Marchiori, P.E.R.; Freitas, S.S.; Machado, E.C.; Nascimento, L.C.; Brocchi, M.; Ribeiro, R.V. Rhizobacteria improve sugarcane growth and photosynthesis under well-watered conditions. *Ann. Appl. Biol.* **2018**, *172*, 309–320. [[CrossRef](#)]
43. Ahmad, S.; Kumar, R. Effect of root-knot nematode on chlorophyll content and plant growth of chickpea. *Acta Bot. Indica* **1990**, *18*, 314–315.
44. Hisamuddin, S.S.; Azam, T. Pathogenicity of root-knot nematode, *Meloidogyne incognita* on *Lens culinaris* (Medik.). *Arch. Phytopathol. Plant Prot.* **2010**, *43*, 1504–1511. [[CrossRef](#)]
45. Bhau, B.S.; Bitupon, B.; Reshma, A.; Phukon, P.; Barbi, G.; Sarmah, D.K.; Lal, M.; Wann, S.B. Influence of root-knot nematode infestation on antioxidant enzymes, chlorophyll content and growth in *Pogostemon cablin* (Blanco) Benth. *Indian J. Exp. Biol.* **2016**, *54*, 254–261.
46. Liu, Y.; Xiang, Y.; Guo, W.; Gao, X.; Li, X. Photosynthetic physiology responses of *Sapium sebiferum* to the interactions between *Meloidogyne incognita* and aboveground herbivores with different diet breadths. *Plant Prot.* **2019**, *45*, 222–228.
47. Wu, L.; Sun, L.; Peng, Y.; Zhang, Y.; Ma, X. Study on the effect of photovoltaic modules on greenhouse microenvironment and net photosynthetic rate of crops in greenhouse—a case study of strawberry greenhouse in Kunming. *J. Yunnan Norm. Univ.* **2021**, *41*, 10–15.
48. Li, W. Effects of root-knot nematode on physiological and biochemical characteristics of cucumber in greenhouse. *Shandong Agric. Univ.* **2005**, *7*, 1–64.
49. Huan-Zhong, L.I.; Zhang, J.L. Effect of spray application on *Cotinus coggygria* atropurpureus leaf changes of transpiration rate and intercellular carbon dioxide concentration. *North. Hortic.* **2011**, *5*, 94–97.

50. Ma, X.; Jia, Z.; Liu, Y. Study on photosynthesis and chlorophyll metabolism of *Avena sativa* L. under salt stress. *Chin. Qinghai J. Anim. Vet. Sci.* **2021**, *51*, 7–14.
51. Carneiro, R.G.; Mazzafera, P.; Ferraz, L.C.C.B.; Muraoka, T.; Trivelin, P.C.O. Uptake and translocation of nitrogen, phosphorus and calcium in soybean infected with *Meloidogyne incognita* and *M. javanica*. *Trop. Plant Pathol.* **2002**, *27*, 141–150. [[CrossRef](#)]
52. Wenting, H.; Deliang, P. Physiological and biochemical response of plant to nematode stress. *Plant Prot.* **2007**, *33*, 11–15.
53. Saeed, I.; Macguidwin, A.E.; Rouse, D.I.; Sharkey, T.D. Limitation to photosynthesis in *Pratylenchus penetrans*- and *Verticillium dahliae*-infected potato. *Crop Sci.* **1999**, *39*, 1340–1346.