Reviewer Comments:

Reviewer

Thank you very much for your careful review of our manuscripts and constructive suggestions. We use black text to mark your questions. The content of our answers to your questions is marked with blue text. The place where the article content is modified is marked with red text.

1. This paper requires extensive language and formatting improvements.

Response 1：

Dear Reviewer,

Thank you for your comments. According to your suggestions, our manuscripts have been handed over to professional institutions for English quality polishing. Manuscript formatting has also been revised in accordance with journal guidelines.

2. Objective should be elaborated on in the introduction section.

Response 2：

Dear Reviewer,

Thank you very much for your careful review of our manuscripts and constructive suggestions. According to your suggestion, we have stated the purpose in the manuscript. And in the manuscript made a corresponding modification. Our changes to the content of the article are marked in red text

Line:92-105

Tomato, being a vital vegetable crop with substantial economic and nutritional value, is known to rely heavily on nitrogen for its growth and development. It is crucial to maintain an appropriate nitrogen supply, as both excess and insufficient nitrogen can impose limitations on the growth and development of tomato plants [21]. Therefore, when providing a specific quantity of nitrogen, the nitrogen form becomes a critical factor influencing plant growth and development. The application method of nitrogen has long been a subject of investigation. While previous research has explored the mixed application of ammonium and nitrate nitrogen, there has been limited investigation into the role of amide nitrogen in tomato photosynthesis and its associated internal enzymes and molecular mechanisms. To delve deeper into the

potential mechanisms through which different nitrogen forms in nutrient solutions can enhance plant photosynthesis, further research is needed to validate how various nitrogen forms can promote plant photosynthesis. In light of these considerations, gaining in-sights into tomato's preference for different nitrogen fertilizer forms is essential when formulating effective fertilization strategies.

3. References need to be rechecked and formatted as per the journal's guidelines.

Response 3：

Dear Reviewer,

Thank you very much for your comments, according to your suggestion, we have carried out in accordance with the journal guide format modification.

4. The discussion section needs significant improvement. Currently, it is few diffuse, and provides no real insights.

Response 4：

Dear Reviewer,

Thank you very much for your careful review of our manuscripts and constructive suggestions. According to your suggestion, we have made substantial changes in the discussion section of the manuscript. Our changes to the content of the article are marked in red text.

Line:569-573

Kirkby et al. [40] discovered that when AN and UN were mixed, tomato plants exhibited increased lateral root length and lateral root numbers. In our study, 25%AN:75%NN treatment also significantly promoted root development in tomato plants. However, the 25%NN:75%UN treatment was more effective than the 25%AN:75%NN treatment.

Line:576-579

It indicated that the high proportion of UN and NN combined application could promote the absorption and utilization of nitrogen in tomato plants, and then improve the growth of tomato plants. The promotion effect of a high proportion of NN combined with UN on tomato growth was very weak.

Line:594-600

Zou et al. [48] studied tomato roots under different ammonium supply levels and found that when the NH_4^+ supply exceeded 0.5 mmol L^{-1}, the main root length, lateral root number and plant height decreased with the increase of NH_4^+, and 10 mmol L^{-1} NH_4^+ almost completely inhibited lateral root formation, which was consistent with our results. Therefore, the appropriate amount of NH_4^+ combined with NO_3^- can increase the absorption and utilization of nitrogen in tomato plants, reduce the toxicity of NH_4^+ to tomato, and promote the growth of tomato roots and plants.

Line:607-615

This may be due to the combined application of 25% AN and 75% NN, 25% NN and 75% UN significantly increased the flow of photosynthetic carbon to amino acids, which in turn led to an increase in photosynthetic enzyme content, while a high proportion of ammonium nitrogen inhibited the synthesis of photosynthetic products, resulting in a decrease in photosynthetic carbon content, which is consistent with the results of Golvano et al. [49]. Glaussen et al. [50]and Puritch et al. [51] also found the accumulation of ammonium nitrogen in plant leaves may lead to the uncoupling of electron transport in chloroplasts to form phosphorylation, which eventually leads to a decrease in photosynthetic rate.

Line:653-658

Singh et al. [65] found that nitrate nitrogen sources can protect photosynthetic pigments from ammonium nitrogen stress in tomato leaves, which competitively reduces the absorption of ammonium nitrogen by tomato plants. Moreover, the mixed application of nitrate nitrogen with urea or ammonium nitrogen had the best effect, and the high concentration of nitrogen source (15 mmol L^{-1}) was more conducive to the growth and development of tomato seedlings.

Line:673-685

Nasraoui-Hajaji et al. [70] observed that tomato plants receiving ammonium nitrogen or amide nitrogen exhibited lower rates of CO_2 assimilation, stomatal conductance, and transpiration compared to those supplied with exclusively NO_3^--N as the nitrogen source. Furthermore, the inclusion of nitrate in the nitrogen supply mitigated the

reduction in these parameters. These findings suggest that a combination of low ammonium and high NO_3^--N supply is conducive to promoting the growth and development of tomato plants [71]. In our study, it was found that the application of 75% UN: 25% NN (T12) ratio significantly increased the gas exchange parameters, chlorophyll fluorescence parameters and net photosynthetic rate of tomato plants, and the effect was better than that of 75% NN: 25% AN (T4) ratio. Therefore, the effects of different nitrogen forms on photosynthesis of tomato were different.

Line:706-713

This phenomenon may be attributed to ammonium toxicity limiting the activity of Rubisco, which, in turn, results in decreased photosynthetic capacity and subsequently impacts the light utilization and overall performance of tomato plants [78,79]. In this study, single NN, AN and UN treatments reduced the expression of *SlRbcL* and *SlRbcS* genes. The addition of UN in-creased the expression of *SlRbcL* and *SlRbcS* genes. Therefore, we believe that UN can regulate the effect of a single nitrogen form on the activity of photosynthesis-related enzymes, thereby enhancing the photosynthetic capacity of tomato plants [80].

Line:718-724

However, we found that urea could not only reduce the effect of ammonium toxicity on tomato plants, but also significantly increase the enzyme activity and relative gene expression of Calvin cycle involved in photosynthesis of tomato plants through the study of NN combined with UN. In particular, the expression of Rubisco size subunit was elevated, which promoted the photosynthetic carbon cycle and organic matter synthesis. This provided a material basis for the plant to develop a larger leaf area and biomass, which ultimately promoted the growth of tomato.

Line:742-745

However, our findings were different from previous studies. We found that 25% NN: 75% UN treatment had better effect on the synthesis of tomato photosynthetic products than 25% AN: 75% NN treatment. This may be related to the sensitivity of different plants to nitrogen forms.

Line:759-764

The size of leaf area can directly reflect the nitrogen supply and can be used to evaluate any species [75]. The leaf area of tomato plants treated with 25% NN: 75% UN was larger than that of other nitrogen forms. The larger the leaf area, the more light energy absorbed by the leaves. This effect was consistent with the increase in the accumulation of photosynthetic product content after mixed application of 25% NN: 75% UN treatment.

Line:792-794

However, we found that the promotion effect of 25% NN: 75% UN treatment on NH_4^+ assimilation of tomato plants was better than that of 50% NN: 50% UN treatment, which may be caused by different varieties.

Line:826-829

However, interestingly, in our study, 75% UN: 25% NN treatment was more effective than 75% NN: 25 % AN treatment in improving nitrogen metabolism-related enzyme activity and gene expression.

Line:841-852

Horchani F et al. [93] found that with the increase of external NH_4^+ concentration, the GDH activity showed an upward trend and *SlGDH* also up-regulated expressed. In our study, the enzyme activity and gene expression of GDH were the highest after single AN treatment, and the enzyme activity of GDH decreased with the decrease of AN application ratio. However, under the condition of 75% UN: 25% NN treatment, the GS activity of tomato leaves increased significantly, and the gene expression levels also increased. This indicated that the metabolism of NH_4^+ in tomato plants was mainly dependent on the GS-GOGAT pathway at normal levels. GDH may play an important role in metabolizing excessive ammonium and reducing ammonium toxicity in plants [94,95].

5. Please add these references

Synergistic dose permutation of isolated alkaloid and sterol for anticancer effect on young swiss albino mice. DOI https://doi.org/10.2147/DDDT.S322769

Isolation and evaluation of anticancer efficacy of stigma sterol in a mouse model of DMBA-induced skin carcinoma. DOI https://doi.org/10.2147/DDDT.S83514

Response 5：

Dear Reviewer,

Thank you very much for your comments. We have added the two references you provided to the manuscript.

Line: 214. References:26 and 27.