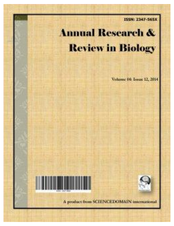

Annual Research & Review in Biology

26(1): 1-17, 2018; Article no.ARRB.41106
ISSN: 2347-565X, NLM ID: 101632869

Growth, Carbon Assimilation and Biochemical Changes of *Polygonum minus* Huds as Affected by Nitrogen Fertilization

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Authors' contributions

This work was carried out in collaboration between all authors. Author MHI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NNM managed the analyses of the study. Author NAMZ managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/41106
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Nusret Ozbay, University of Bingol, Turkey.
(2) Nono Carine, University of Yaoundé I, Cameroon.
Complete Peer review History: http://www.sciencedomain.org/review-history/24372

Original Research Article

Received 10th February 2018
Accepted 19th April 2018
Published 28th April 2018

ABSTRACT

Aims: To study the effect of different nitrogen fertilizer rates on growth, carbon assimilation and biochemical changes of *Polygonum minus*.
Study Design: *Polygonum minus* were treated with different nitrogen fertilizer (0 kg N ha^{-1}, 50 kg N ha^{-1}, 100 kg N ha^{-1} and 150 kg N ha^{-1}) using Urea (46%N) as a source of nitrogen. This experiment was arranged in Randomized Complete Block Design (RCBD) with three replications.
Place and Duration of Study: The experiment was conducted at Taman Pertanian Universiti (TPU), Universiti Putra Malaysia between April 2017 to July 2017.
Methodology: The growth parameters measured includes the leaf number, plant height, diameter of the stem, and the leaf area. The carbon assimilation parameters were measured using LICOR 6400XT Portable Photosynthesis System. Total phenolic and flavonoids contents from the leaves extracts of *Polygonum minus* were measured using Folin-Ciocalteu reagents.

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Results: The best rates of nitrogen in enhancing the best growth and quality of *Polygonum minus* was observed at 150 kg N ha^{-1} in most parameters of growth and carbon assimilation. At the final harvest, the parameters such as number of leaves, plant height, stem diameters, total chlorophyll contents, leaves area, total dry weight, net assimilation rate (NAR), water use efficiency (WUE), net photosynthesis rate (A) stomatal conductance (gs), and maximal efficiency of photosystem II (fv/fm) were highest at 150 kg N ha^{-1} treatments. In the terms of biochemical changes, the parameter such as total phenolics contents and total flavonoids gain the highest production of total phenolics and flavonoids at 50 kg N ha^{-1}.

Conclusion: This study indicated growth and carbon assimilation parameters were upregulated under higher nitrogen fertilization and production of secondary metabolites was decreased with high rates of nitrogen. The recommended nitrogen fertilization for *P. minus* was at 50 kg N Ha^{-1}, where it obtained the highest harvest index.

Keywords: Nitrogen; growth parameters; carbon assimilation parameters; biochemical changes parameters; Polygonum minus.

1. INTRODUCTION

Malaysia is one of the countries that have the potential to elevate the growth of herbal industries [1-6]. It has numerous variety of plant species that possess the properties of traditional medicinal which are more than 1300 species of medicinal plants in Peninsular Malaysia alone. In contrast, about 7411 of plant species which exclude bryophyte, algae and fungi have been recorded in Sabah that used by communities to ensure their good health [4]. A study conducted by Mansor [7] reported that there were 120 species which represented numerous families of traditional vegetables in Malaysia that locally is known as “ulam”. Generally, the “ulam” are eaten by people in raw forms just like salads or cooked while some can be boiled and eaten. In fact, it was predicted that both medical and aromatic plants contributed to Malaysia about RM 4.6 billion in the Malaysian herbal product market with the annual growth rate of 15-20% in 1999 [8]. Recently, people consumed vegetables which include herbs, “ulam” and fruits in conserving their healthy lifestyle [9,10] as these can provide human to oppose the oxidative stress in their body due to the activities of the plant-derived phenolic compounds with antioxidant activity [9,11]. In addition, according to Garodia, Ichikawa, Malani, Sethi and Aggarwal [12], herbs had been widely exploited by the human in maintaining their health since herbs are believed to act as a defence mechanism against cardiovascular diseases and certain types of cancer [13]. One of the herbs that gaining a lot of attention and potential in the market is *Polygonum minus* or known by Malaysians as “kesum”.

Polygonum minus comes from Polygonaceae family which generally known as knotweed in English, “daun kesum” or “laksa leaves” in Malaysia, “phakphai” in Thailand, “Kleiner Knoterich” in German and “Chakhong-machain Manipuri” in India is arises from countries of Southern Asia such as Malaysia, Thailand, Vietnam and Indonesia. *P. minus* is basically can be found grow extremely in humid areas near the ditches, river banks and lakes [14]. According to Vimala, Ilham, Rashih and Rohana [15], *P. minus* is believed to have a nice, sweet and pleasant aroma. For ages, the leaves of *P. minus* is prominently used to as ingredients in the food industries such as “laksa”, “kerabu”, “asam pedas” and “tomyam”. Due to its taste and aroma, *P. minus* is included by National Agrofood Policy (NAP) in eleven of the herbs that will be aimed by the Malaysian Government. In the aspect of food commodity in Malaysia, *P. minus* is regarded as vital plants for the purposes of local as well as international trades in Malaysia [16]. *Polygonum minus* had become quite attracted to the communities in Malaysia as it had been used in the treatment of the digestive disorder as well as a dandruff problem [17]. To treat the problems of the digestive disorder, the leaves of *P. minus* needs to be boiled with some water and drink, whereas to treat the dandruff problems, the extract from the leaves need to be mixed with some water and apply it into the scalp. In fact, *P. minus* is also been used in perfume industries due to its volatile oils [18].

Nitrogen is a type of macronutrients that typically complies numerous roles in plant metabolic processes. Macronutrient such as N plays the important roles in the metabolism of plants, likewise can give shelters to the plants by averting plants from diverse biotic and abiotic stresses such as heavy metals stresses, drought, ultraviolet and pest attack [19]. Nitrogen is needed by the plant in the huge amount, which contains about 2% of plant dry matter and 16% of total plant protein. [20] suggested that the application of N fertilizer on the plants may double the crops production. The requisite amount of N supply in plants is one of the key factors in sustaining the production of crops since N is regarded as the indispensable element in the components of enzymes and proteins. Moreover, N is claimed to facilitate the production of fruits and seeds, quality of the forage crops and leaf maturity [21]. The uptake of inorganic N by the plants is predominantly by nitrate or ammonium absorption in the plant's roots [19]. Inorganic N compounds such as ammonium ion (NH^{4+}), nitrite ion (NO^{2-}) and nitrate ion (NO^{3} $^{-}$) are the main form of N elements that are absorbed by plants even though they only accounted about 5% of the total N in the soils [22]. Plants can get ammonia (NH_3) through the absorption from their roots or via the assimilation of NO^{3-} whereby the NH_3 itself will be converted to glutamine and glutamate. As assimilation process of NO^{3-} by plants was done, N will be converted through various reactions into the other organic compounds such as amino acid. Generally, NO^{3-} is absorbed by plant roots will be assimilated in either roots or shoots which depend on the plant's species and NO^{3} $^{-}$ availability [23-25].

Secondary metabolites such as flavonoids and phenolics are the most valuable compounds in plants which have the competency to scavenge free superoxide radicals, anti-ageing and lower the risk of cancer [26,27]. Higher concentration of phenolics can be explained by organic fertilizers such as N which play the important roles in biosynthesis process which catalyzed the acetate shikimate pathway, thus out coming in greater production of phenolics and flavonoids [19]. *Polygonum minus* is believed as one of the medicinal plants that can be used by humans to treat and cure their sickness. Treatment from the medicinal plants is believed as save with minimal or sometimes no side effects. However, most of the earlier studies on this plant were less interested to show how the user of single fertilizer especially N which may affect the growth, carbon assimilation and biochemical changes of *P. minus*. Previous study has shown that high application rates of nitrogen would reduce the production of secondary metabolites although the plant total biomass production was enhanced under high N [28]. Hence, this experiment was implemented to investigate the effects of different N fertilization rate (0, 50, 100, 150 kg N ha^{-1}) on the growth, carbon assimilation and biochemical changes of *P. minus* and to determine the best rates of N to promote the best growth and quality of *P. minus.*

2. MATERIALS AND METHODS

2.1 Plant Materials

Polygonum minus was purchased at Bukit Kerayong, Selangor. The shoot stem cuttings were cut on the first internodes at the bottom of the shoots with 10 cm in height and five numbers of leaves. These shoots stem cutting were soaked overnight in the container filled with tap water to make them turgid in order to fasten their germination before proceeding with propagation step [29]. The shoot stem cuttings were then propagated in trays which contained peat moss as the medium and were left in the Taman Pertanian Universiti (TPU) for about one month and waited until the roots of these plants developed. Foliar treatment by using WELGRO fertilizer was applied to promote the rooting process as the rooting process may consume a few days. After about one month, the plants that were already developed their roots were transferred into a standard polybag with one plant per one polybag. They were placed at the main project site (TPU) and left to grow under the sunlight for about 3 months (12 weeks). Net shading and also some black plastic to cover the soil were set up at the research site in order to reduce the amount of water that can be absorb by the sunlight since this plants are very sensitive to water and to minimize the competition between the grasses and also plants respectively [30]. The medium used in this study was a top soil. This medium was prepared and transferred into each of the polybag until it filled three-quarter of the polybag size (16 x 30 cm).

2.2 Experimental Design and Treatments

The experiment was arranged by using Randomized Complete Block Design (RCBD) as it was randomly designated to each experimental unit which also known as plot [31]. The treatments comprised of three blocks with four treatments that were arranged randomly in each block. Each treatment consisted of eight plants making the total plants used in this study are 96 plants. The N-fertilizers used in this study was single fertilizer, Urea (46% N). This fertilizer was applied once in one month for every polybag which was the first week of each month.

2.3 Data Collection

2.3.1 Number of leaves

The number of leaves from each plant inside the polybags was counted manually including the tips of the new leaves which just started to emerge from the stem.

2.3.2 Height of plant (cm)

The plant height was measured from the soil surface to the tallest of the shoot tip using a standard meter ruler.

2.3.3 Diameter of stem (mm)

The diameter of stem at the first node from the soil surface was measure by using manual Vernier calipers and recorded in millimeter (mm).

2.3.4 Total Chlorophyll content

The chlorophyll content from the leaves of *P. minus* were measured using Chlorophyll Meter SPAD 502 Minolta. The meter was calibrated first by clipping the meter on the surface of the leaves before taking the readings. Three readings were randomly taken on the three different leaves (first of three leaves from the shoots) on the middle of leaves surface for every treatment in each replicate. The average readings were then calculated and recorded.

2.3.5 Total leaves area (cm^2)

The leaves of each plant were harvested by detaching them from the stem. The total leaves area of *P. minus* was measured using Automatic Leaf Area Meter (Model LI-3100) that calibrated by using a steel plate. The measurement of each leaf was recorded by placing them on the meter and expressed as cm^2 per plant.

2.3.6 Total biomass (g)

Total plant biomass was taken by calculating the dry weight of root, stem, and leaf per seedling. Plant parts were separated and placed in paper bags and oven dried at 80°C until constant weight was reached before dry weights were recorded using the electronic weighing scale that have precision range of 0 g – 500 g.

2.3.7 Leaf gas exchange measurement

This measurement was conducted in accordance with Matimati et al. [32]. These parameters were measured using a close infra-red gas analyzer (IRGA) of LICOR 6400 Potable Photosynthesis System which functioned in calculating the photosynthesis and cellular respiration of a plant by measuring the uptake and release of carbon dioxide. Apart from that, the IRGA also measured the stomatal conductance (g_s) and water use efficiency (WUE). The instrument was calibrated with ZERO IRGA mode and warmed for 30 minutes before used. The measurement involved the optimal conditions sets of 40 µmol m^{-2} s^{-1} CO_2 30°C cuvette temperature, 60% relatively humidity with air flow rate set at 500 cm^3 min^{-1} and modified cuvette condition of 800 µmol m^{-2} s^{-1} photosynthetically photon flux density (PPFD). The measurements of gas exchange were conducted between 9.00 am to 11.00 am using fully expanded young leaves numbered three and four from plant apex to record net photosynthesis rate (A). The operation was automatic and the data was stored in the LI-6400 console and analyzed by the Photosyn Assistant Software. Besides, the efficiency of photosystem II was also measured by the emission of leaf chlorophyll fluorescence using Fluorescence Monitoring System by placing the leaves surface of *P. minus* about 7 mm away from the fluorometer probe.

2.3.8 Total phenolics and flavonoids quantifications

Both methods of quantifications for total phenolics and flavonoids contents were followed by the methods that were proposed by Ibrahim et al. [33]. During the process of leaves extractions from *P. minus*, 0.2 g of dried ground samples were weighed and extracted with 5 mL of 70% ethanol for an hour at 40°C using a sonicator. The extracts were then placed in an orbital shaker for two hours at 50°C. Next, the extracts were filtered with Whatman No 5 filter paper. The filtrates were then used in identifying the quantification of the total phenolic and flavonoids contents

2.3.8.1 Total phenolics

The phenolic contents from the leaves extracts of *Polygonum minus* were measured using some modifications from Folin-Ciocalteu calorimetric method [34]. A mixture was formed by combining about 200 µl of the leaves extracts, 1.5 ml Folin-Ciocalteu reagent and 1.5 ml sodium carbonate (Na_2CO_3) together. At the same time, a blank was also prepared by replacing 200 µl of the extracts with 70% ethanol. The mixture was then incubated in a dark condition for about two hours at ambient temperature before measuring the absorbance against the blank using spectrophotometer at a wavelength of 725 nm. The data were expressed in the unit of milligram of gallic acid equivalent to per gram of sample (mg g^{-1}).

2.3.8.2 Total flavonoids quantifications

The determination of total flavonoids contents from the leaves extracts was measured by calorimetric assay. A mixture of the solution was formed by combining about 150 µl leaves extracts, 600 µl water (H_2O) and 45 µl 5% $NaNO_2$ together. The mixture was then incubated at room temperature for 5 minutes followed by the addition of 45 µl $AlCl_3$ and incubated back for 1 minute. Next, the mixture was then measured using the same spectrophotometer against the blank at a wavelength of 510 nm. The data were expressed in the unit of milligram of rutin equivalents per gram of sample (mg g^{-1}).

2.4 Harvest Index

The plant harvest index was calculated by multiplying the total plant biomass with total phenolics and flavonoids content.

2.5 Statistical Analysis

All the data were statistically analyzed by using Statistical Package for Social Sciences (SPSS) version 2.1. All the data were subjected and analyzed using 2-ways Analysis of Variance (ANOVA). The mean for each data among the treatments was compared using Duncan Multiple Range Test (DMRT). The standard error of differences between means was calculated with the assumption that the data were normally distributed and equally replicated. The data which showed $p \leq 0.05$ was considered to have statistically significant.

3. RESULTS AND DISCUSSION

3.1 Growth, Carbon Assimilation and Biochemical Changes Parameters

3.1.1 Number of leaves

Fig. 1 shows the effect of different N fertilization rates on the plant height of *P. minus* after twelfth weeks of harvesting. The result from the analysis of variance showed that the number of leaves in *Polygonum minus* was affected by N treatments ($P \leq 0.05$). It was found that 150 kg N ha^{-1} to have the tallest plant height as compared to the other treatment while 0 kg N/ha (control) was observed to have the shortest plant height among the other treatments by the end of this experiment. This can be justified due to the fact that N generally enhances plant growth and plant height, thus caused the formation of nodes and internodes and finally increased the formation of leaves [35]. Similarly, Marschner [36] specified that the application of N stimulated the process of cell division and elongation as well as vegetative growth such as the number of leaves since N is very crucial of plant nutrients that may devote to nucleic acids and proteins synthesis. On the contrary, low N supply caused undeveloped leaves, reduction of the stems length and hence, produce little leaves number [37]. Hence, it can be concluded that the number of leaves was increased with the additive in N fertilization rates in *P. minus.*

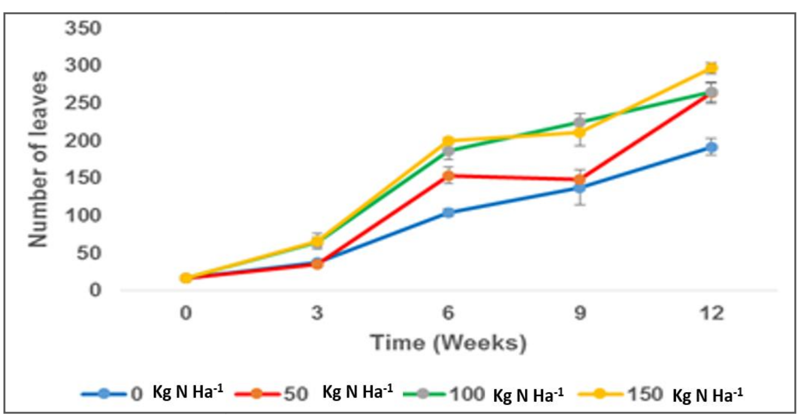

Fig. 1. The effect of different N fertilization rates on the leaves number of *Polygonum minus*.
Data are mean ± standard error of mean (SEM). N= 6

3.1.2 Plant height

Fig. 2 depicted the effect of different N fertilization rates on the plant height of *P. minus* after twelfth weeks of harvesting. It was found that 150 kg N ha^{-1} conquered to have the tallest plant height as compared to the other treatment while 0 kg N/ha (control) was observed to have the shortest plant height among the other treatments by the end of this experiment (12^{th} weeks) in *Polygonum minus*. This might be due to the fact that N promotes the activity of meristematic tissue and cell growth in plants [38]. This result was in a good compatible with the findings from Hassan Amin [35] and Namvar et al. [39] who claimed that plant height increased with the increment of N application since N endorsed plant growth by raising the number and length of the internodes [39] and speeding the process of cell division [40,41]. According to Omotoso and Akinrinde [42], plants height is one of the crucial attributes in plant growth that directly related to their potential in plant productivity in the aspect of fodder, grains and food yields. Therefore, it can be concluded that plant height will be improved by the increment of N application. Ordinarily, a surplus of N supply will lead to the extreme succulent growth and weakness to the pest [43] since N can contribute to the soil fertility which will contribute for the plants to increase in height [42]. Nonetheless, the untreated plant (0 kg/ha) was mostly having the shortest plant height since it needs to depend on the native soil fertility [39]. Thus, it can be said that the availability of N will lead to the increment of plant height in *P. minus*.

3.1.3 Diameter of stem

Fig. 3 portrayed the diameter of the stem of *P. minus* after treated with different N fertilization rates for twelfth weeks of planting. The result of the analysis of variance showed that the diameter of the stem in *Polygonum minus* was affected by N treatments ($P \le 0.05$). By the 12^{th} weeks (final harvest), it was noticed that diameter of the stem which was treated with 50 kg N ha^{-1}, 100 kg N ha^{-1} and 150 kg N ha^{-1} were larger by 0.97%, 21.31% and 38% respectively over the control (0 kg N ha^{-1}). This can be explained by the fact that N improved on the growth properties of the plants [44]. This finding was also similar to those of Bakhshian and Sharifabad [45], who addressed that the uptake of N by plants may promote fertility of the soil since the root will absorb this nutrient from the soil. The elevation of soil fertility may lead to the photosynthetic efficiency and well-functioned of light photosystem, thus increasing the growth of the plant such as stem diameter. Indeed, a satisfactory amount of N supply to plants can encourage the activity of the meristematic tissues which eventually enhance the activity of cell division, and consequently increase the diameter of the stem. However, insufficient of N supply may cause the plants to develop spindly and thin stem diameter [46]. Therefore, it can be inferred that 150 kg N ha^{-1} was the best rate in promoting the larger stem diameter in *Polygonum minus* for this study.

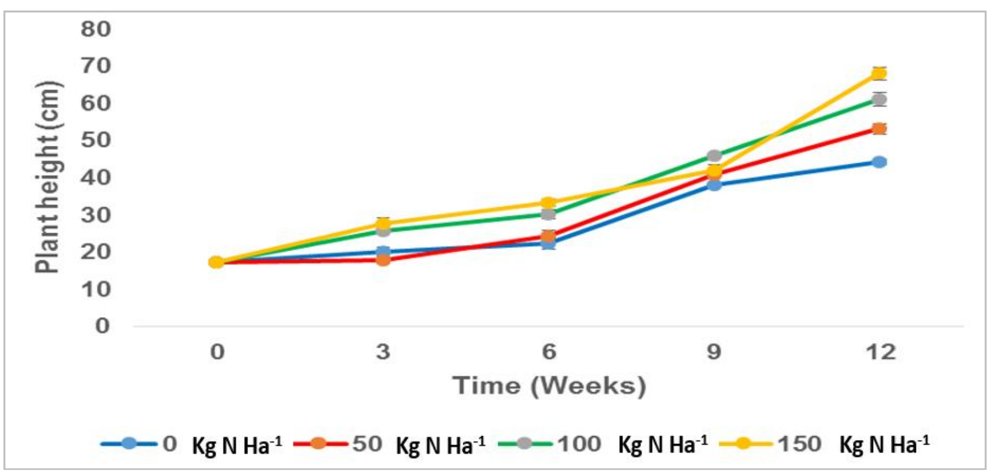

Fig. 2. The effect of different N fertilization rates on the plant height of *Polygonum minus*

Data are mean ± standard error of mean (SEM). N= 6

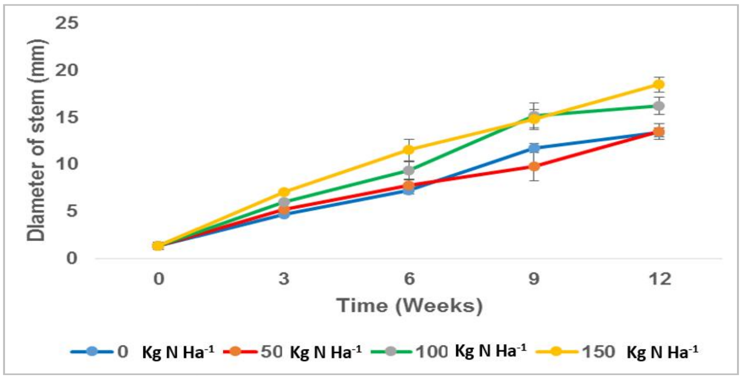

Fig. 3. The effect of different N fertilization rates on the stem diameter of *Polygonum minus*

Data are mean ± standard error of mean (SEM) N= 6

3.1.4 Total chlorophyll content

Fig. 4 highlighted the chlorophyll content of *Polygonum minus* after treated with different N fertilization rates for twelfth weeks of planting. The result from the analysis of variance showed that the chlorophyll content in *P. minus* was affected by N treatments ($p \le 0.05$). The trend indicated that 150 kg N ha^{-1} increased significantly over time as compared to the other treatments. By the final harvest (12^{th} weeks), the data showed that the maximum chlorophyll content was obtained by the 150 kg N ha^{-1} (57.82), followed by 100 kg N ha^{-1} (45.25) and 50 kg N ha^{-1} (45.03). Whereas, the minimum chlorophyll content was found by the control at 0 kg N/ha (43.73). Singh et al. [44] pointed out that the application of N stimulated the formation of active photosynthetic pigments in leaves by raising the amounts of thylakoid and stromal proteins. This formation of chloroplast contributes to the raised of lipid contents in leaves and chloroplast content such as chlorophyll a and carotene [44]. The similar finding was obtained by Bhuvaneswari et al. [43] who announced that N application on plants can subsidize to the strength of the chloroplast as N is the major constituent in of all amino acids in proteins and lipids. In summary, it can be concluded that the elevation of N fertilization rates will boost up the chlorophyll content in *Polygonum minus*.

3.1.5 Total leaves area

Fig. 5 described the effect of different N fertilization rates on total leaves area of *P. minus* after twelfth weeks of planting. The result from the analysis of variance showed that the total leaves area in *Polygonum minus* was influenced by N treatments ($P \le 0.05$). The trend stipulated

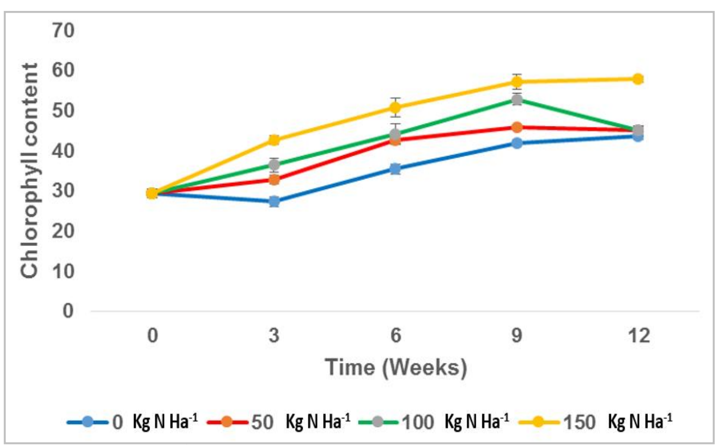

Fig. 4. The effect of different N fertilization rates on the chlorophyll content of *Polygonum minus*

Data are mean ± standard error of mean (SEM). N= 6

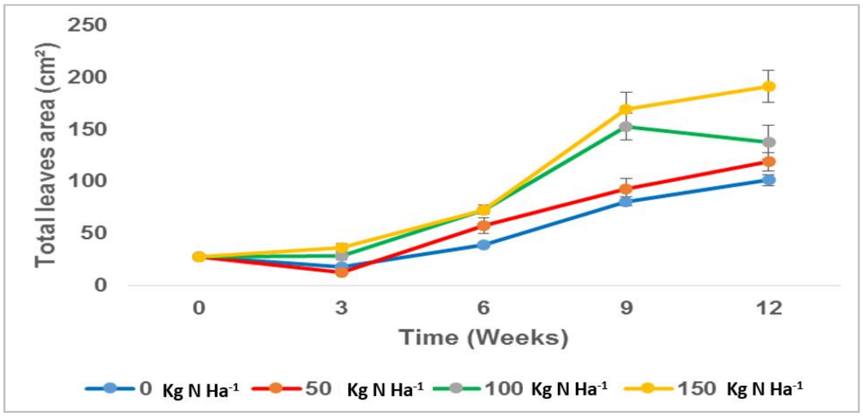

Fig. 5. The effect of different N fertilization rates on the total leaves area of *Polygonum minus*
Data are mean ± standard error of mean (SEM). N= 6

that the total leaves area was increased moderately with the moderate increased of N treatment at the most of the weeks. Omotoso and Akinrinde [42] demonstrated that the investment of the N caused the development of leaves area due to the stimulation of a direct increase in photosynthetic leaf area. Similarly, Sayed [47] and Watson [48] suggested that the N treatment caused the increased of the leaves area through the elongation process as a result of cell production and cell expansion. This finding was coincided with those of Parsons et al. [49] who revealed that the increment of N fertilizer will lead to the increased in the leaf area and hence resulted in the higher of photosynthetic rates. Leaf area is very critical in capturing sunlight; thus larger leaf area facilitates higher exposition to sunlight. Nonetheless, shortage in N supply caused the reduction of functional leaves and latterly photosynthetic efficiency [42]. Thus, it can be concluded, that the raising of N treatment promotes to the greater total leaves area in *P. minus*.

3.1.6 Total biomass

Fig. 6 illustrated the impact of total biomass as treated with different N fertilization rates for twelfth weeks of planting in *P. minus*. From this study, the result from the analysis of variance revealed that the total biomass in *P. minus* was influenced by N treatments. A significant difference (P<0.05) was observed for the entire week (3, 6, 9 and 12) after N treatments being implemented. Overall, the trend was depicted that the investment of N treatments raised the dry weight (leaves, stems and roots) progressively as compared to the control. By the 12th weeks, the data showed that the maximum total biomass was accomplished by 150 kg N ha^{-1} (44.09 g), followed by 50 kg N ha^{-1} (41.05 g) and 100 kg N ha-^{1} (37.7 g). Whereas, the minimum total biomass was found by the control at 0 (25.12 g). Even though 100 kg N ha^{-1} (the second highest of N treatment implemented in this stud kg N ha^{-1}) resulted in the third highest in total biomass which is after 50 kg N ha^{-1} during the final harvest (12th weeks), but 100 kg N ha^{-1} conquered consistently to be the second highest in total biomass in three consecutive weeks (week 3, week 6 and week 9).Total biomass raised significantly with the implementation of N fertilizer due to the fact N stimulated better crop growth rate, leaf area index, hence allowed better photosynthesis and resulted in more biological yields [50]. This finding corroborated with those of [51] who stated that a great of N supply can trigger plants height as it can boost greater photosynthetic rates, higher leaf chlorophyll concentrations, wider leaves and greater leaves area, and thus result in high total biomass. In contrast, according [52], N shortage was related to the decreased in the leaf area and leaf photosynthetic capacity, hence caused the reduction of plants biomass. Therefore, it can be concluded that as the N level was raised in the ascending order (0 kg N ha^{-1}, 50 kg N ha^{-1}, 100 kg N ha^{-1}, 150 kg N ha^{-1}) the total biomass in *P. minus* was improved steadily.

3.1.7 Net assimilation rate (NAR)

From this study, the result from the analysis of variance revealed that the NAR in *P. minus* was affected by N treatments (P≤0.05; Fig. 7). From the graph below, it was revealed that

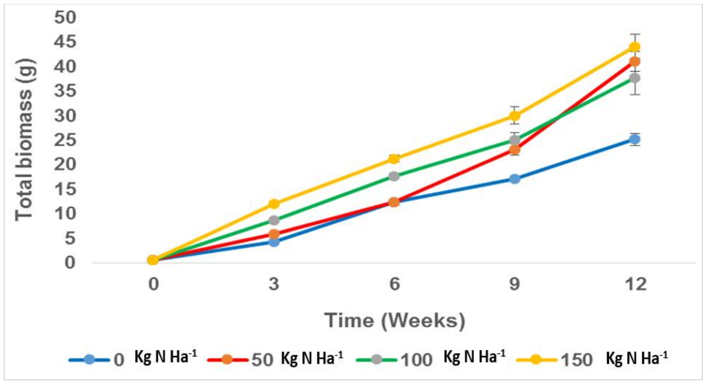

Fig. 6. The effect of different N fertilization rates on the total biomass of *Polygonum minus*

Data are mean ± standard error of mean (SEM). N= 6

the NAR in *P. minus* was increased with increasing N supply, achieving the maximum when the N supply was in 150 kg ha^{-1}. This might be due to the investment of N fertilizer which will raise up the NAR since N aided in the process of leaves proliferation which eventually raised up the efficiency of photosynthesis in the presence of solar radiation [53]. In addition, this finding was supported by Lopes et al. [54] who proposed that the presence of N supply caused the increment in the NAR as it allowed the raising of the number of cells which presiding to a higher leaves development with higher photosynthetic potential. According to Lopes et al. [54], NAR represents the production of dry matters by photosynthesis and use up by the respiration and photorespiration. However, a scarce of N supply in plants will lead to the reduction in sunlight radiation by leaves in a plant, thus reduced the NAR [55]. In conclusion, the NAR of *P. minus* was promoted by the application of a higher N treatment.

3.1.8 Net photosynthesis rate (A)

From this study, the result from the analysis of variance clarified that A in *P. minus* was influenced by N treatments (P≤0.05; Fig. 8) which is at P=0.001. From the graph, it can be explained that as the rate of N supply gained, the A in *P. minus* was gained as well. This might be due to the fact that a high dose of N supply promotes the activity of Rubisco enzyme which resulted in higher photosynthesis to take place at the fast rate [56]. This finding was in agreement with those of Kaufman et al. [56] who convinced that the activity of the Rubisco was depended on the existence of N. As N level increase, it contributes to a greater photosynthetic activity [57,58] as N promote the size and morphology of

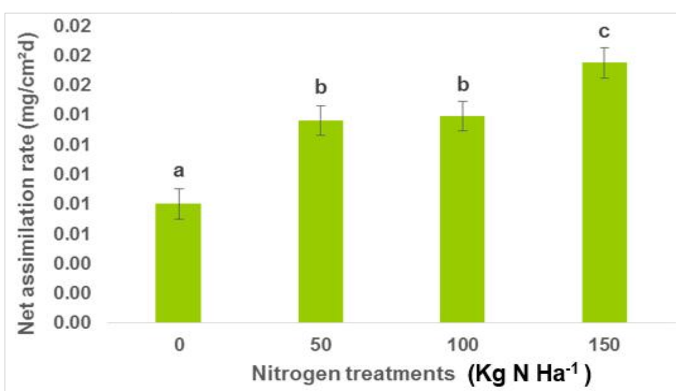

Fig. 7. The effect of different N fertilization rates on the net assimilation rate of *Polygonum minus*

Data are mean ± standard error of mean (SEM). N= 6. Mean with different superscript showed the significantly different at P<0.05 using DMRT at 95% of confidence levels the chloroplast as well as the activity of the chlorophyll [59]. In addition, according to Hidayati et al. [60], the higher amount of chlorophyll in the plant's leaves can maintain the greater photosynthetic rate in plants. Being a crucial component for building blocks of proteins, N helps in allowing the activity of chlorophyll as well as Rubisco enzyme for the process of carbon dioxide fixation during photosynthesis [58]. Hence, it can be concluded that the high dose of N supply promoted the NPR in *P. minus*. WUE is defined as the performances of the crops which are grown under any environmental restrictions.

3.1.9 Water use efficiency (WUE)

From this study, the result from the analysis of variance showed that the WUE in *Polygonum minus* was influenced by N treatments ($P \le 0.05$; Fig. 9) which is at $P=0.001$. Overall, it was observed that WUE in *P. minus* was greater with the highest availability of N. The previous study clarified that the increment of N fertilization rates on plants led to the increased of WUE since N triggered the photosynthetic activity and reduced the transpiration [61]. This similar finding was also derived from who demonstrated that the N application triggered the root surface area and depth together with the root biomass, hence resulted in reduced the drought effects in switch grass [62].

3.1.10 Stomatal conductance (g_s)

From this study, the result from the analysis of variance revealed that the stomatal conductance in *P. minus* was affected by N treatments ($P \le 0.05$; Fig. 10). The trend indicated that, as the N supply was raised, stomatal conductance was increased and the highest value was examined under 150 kg N ha^{-1} (31.4 mmol m^{-2}s^{-1}), followed by 100 kg N ha^{-1} (23.55 mmol m^{-2}s^{-1}) and 50 kg N ha^{-1} (14.574 mmol m^{-2}s^{-1}). While the smallest stomatal conductance was conquered by the control at 0 kg N ha^{-1} (9.384mmo m^{-2}s^{-1}). According to Broadley et al. [63], this might be due to the N supply which may alter the distribution or the frequency of the stomata which eventually lead to the increment of stomatal conductance. This finding was in agreement with Sun et al. [64] findings who found that N fertilizer may contribute the increment of stomatal conductance since higher N concentration was assembled into the leaves. On the contrary, failure of N supply in plants may cause stomata to respond directly to this signal by reducing the stomatal aperture which promoting their sensitivity towards the declined of stomatal size and decreased of plant water status through disrupting the aquaporin expression [61]. Reduction in stomatal aperture may restrain the maximal passage of carbon dioxide invading and water vapor expelling out through the stomata which eventually lead to the reduction in stomatal conductance [64]. In conclusion, the N treatment at 150 kg/ha was the favorable condition to enhance the stomatal conductance followed by 100 kg N ha^{-1}, 50 kg N ha^{-1} and 0 kg N ha^{-1} in *P. minus*.

3.1.11 Maximum efficiency of photosystem II (f_v/f_m)

From this study, the result from the analysis of variance presented that the maximum efficiency of photosystem II in *P. minus* was affected by N treatments ($P \le 0.05$). The trend showed that the maximum efficiency of photosystem II tended to be highest at N rates of 150 kg N ha^{-1} followed by 100 kg N ha^{-1} and 50 kg N ha^{-1} which is 43.1%, 31% and 15% respectively over the 0 kg N ha^{-1} in *Polygonum minus*. To explain this trend, it was believed that the high dosage of N fertilizer on a plant caused enhancement of photosynthetic performance and nitrogen use efficiency, doubtlessly via redistribution of N towards Rubisco enzyme [65]. Moreover, this finding was corroborated with those of Zhou, et al. [66] who pointed out that the implementation of N can boost the activity of electron transport chain, hence, promoted the quantity of reaction center of photosystem II since these components are firmly affected by the N fertilizer due to the increment in the portion of active reaction center in chlorophyll molecules. In addition, an appropriate amount of N supply accounts the activity of the Rubisco protein, hence helping the process of electron transport chain in the thylakoid [67]. In contrast, insufficient of N may lead to the decline in the photosynthetic chain reaction rate. Thus, it can be deduced that with the enhancement of N fertilization rates, the maximum efficiency of photosystem II in the *P. minus* will be raised.

3.1.12 Total phenolics content

In this study, the result from the analysis of variance revealed that the total phenolics content (TPC) in *P. minus* was affected by N treatments ($P \le 0.05$; Fig. 12). The greater TPC was found by 50 kg kg N ha^{-1} (2.644 mg gallic acid/g dry weight), followed by 100 kg N ha^{-1} (1.422 mg gallic acid/g dry weight), 0 kg N ha^{-1}

(1.274 mg gallic acid/g dry weight and finally 150 kg N ha^{-1} (0.49 mg gallic acid/g dry weight). From the graph below, TPC was observed to be increased from 0 kg N/ha to 50 kg N/ha and from there, a declined was spotted at 100 kg N ha-1 and 150 kg N ha-1. Overall, the TPC in *Polygonum minus* can be improved by lowering the N supply. The improvement of plant secondary metabolites in a restricted amount of N supply might be due to the elevated in the production of total nonstructural carbohydrates [68]. This finding was similarly derived by Ibrahim et al. [69] who proposed that the TPC of *Labisia pumila* was decreased as the rates of N raised from 0 to 270 kg N ha^{-1} because of the soluble sugar accumulation which might be more valuable in the up regulation in the production of plant secondary metabolites. This concluded that 50 kg N/ha was the excellent rates in triggering the production of phenolics in *P. minus.*

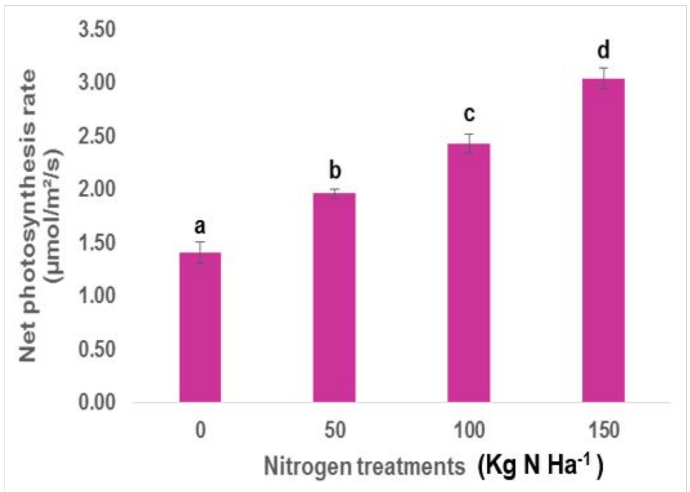

Fig. 8. The effect of different N fertilization rates on the net photosynthesis rate of *Polygonum minus* at twelfth weeks of planting

Data are mean ± standard error of mean (SEM). N= 6. Means with different superscript showed the significantly different at P<0.05 using DMRT at 95% of confidence levels

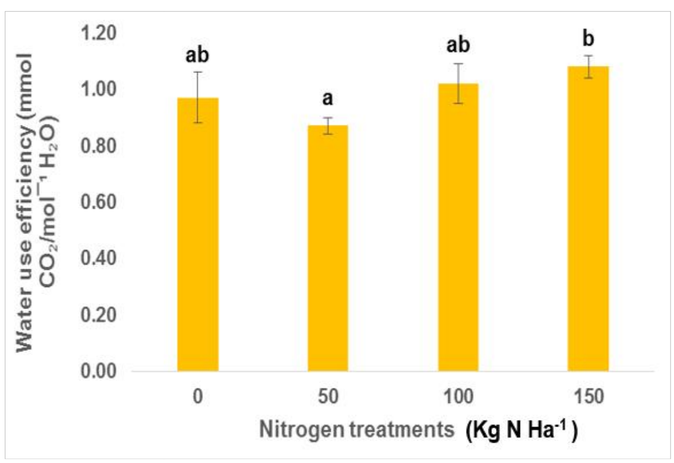

Fig. 9. The effect of different N fertilization rates on the water use efficiency of *Polygonum minus* after twelfth weeks of planting

Data are mean ± standard error of mean (SEM). N= 6. Means with different superscript showed the significantly different at P<0.05 using DMRT at 95% ofconfidence levels

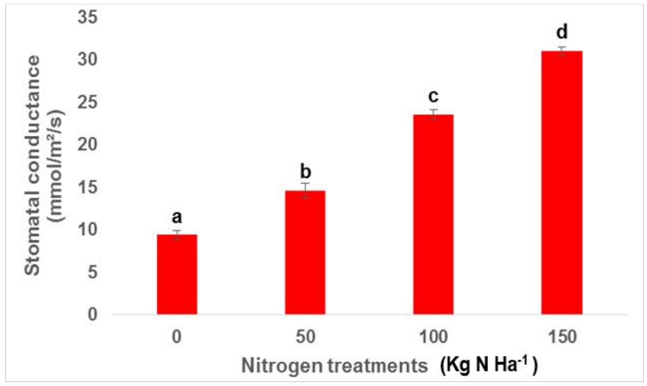

Fig. 10. The effect of different N fertilization rates on the stomatal conductance of *Polygonum minus* after twelfth weeks of planting

Data are mean ± standard error of mean (SEM). N= 6. Means with different superscript showed the significantly different at P<0.05 using DMRT at 95% of confidence levels

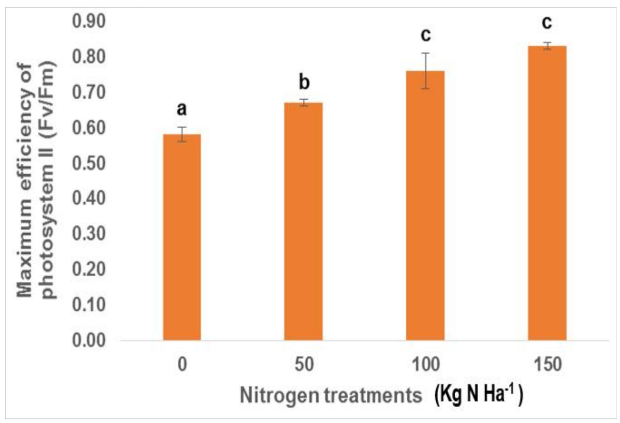

Fig. 11. The effect of different N fertilization rates on the maximum efficiency ofphotosystem II of *Polygonum minus* after twelfth weeks of planting

Data are mean ± standard error of mean (SEM). N= 6. Means with different superscript showed the significantly different at P<0.05 using DMRT at 95% of confidence levels

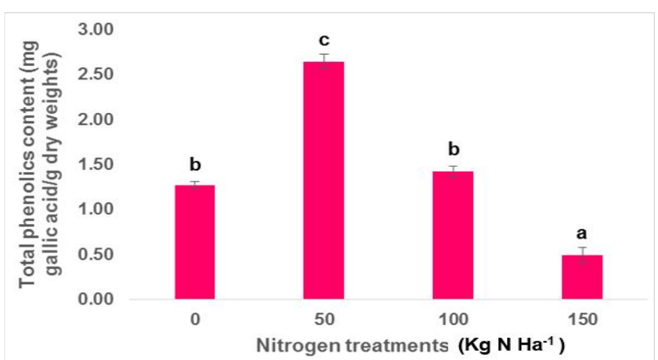

Fig. 12. The effect of different N fertilization rates on the total phenolics contents of *Polygonum minus* after twelfth weeks of planting

Data are mean ± standard error of mean (SEM). N= 6. Means with different superscript showed the significantly different at P<0.05 using DMRT at 95% of confidence levels

3.1.13 Total flavonoids content

In this study, the result from the analysis of variance highlighted that N treatments gave significant impact on the total flavonoids content (TFC) in *P. minus* ($P \leq 0.05$; Fig. 13). Similar to the result in TPC, TFC in *P. minus* was the highest by 50 kg N ha^{-1} (1.196 mg rutin/g dry weight) as well. Then, it was followed by 0 kg N ha^{-1} (0.75 mg rutin /g dry weight), 100 kg N ha^{-1} (0.602 mg rutin /g dry weight) and finally 150 kg N ha^{-1} (0.398 mg rutin /g dry weight). Altogether, the TFC in *Polygonum minus* can be enhanced with a scarce of N supply. The establishment of the TFC in scarce amount of N supply might be due to the elevated in the production of total nonstructural carbohydrates [69,70]. This finding was in a good compliance with the field study that was carried out by Liu et al. [71], who proved that the high supply of N rates may conceal the production of flavonoids in *Chrysanthemum morifolium* due to the presence of L-phenylalanine [20]. Since 50 kg N/ha gave the highest synthesis of flavonoids contents, hence, it can be deduced that 50 kg N/ha was the optimum rate in promoting the synthesis of flavonoids in *P. minus*. The result for Harvest index for total phenolics and flavonoids are depicted in Fig. 14. As can be seen harvest index (HI) for total phenolics and flavonoids was significantly influenced by nitrogen rates ($P \leq 0.05$). The highest HI for total phenolics and flavonoids production was observed in 50 kg N ha^{-1}, 100 kg N Ha^{-1}, 0 kg N Ha^{-1} and lowest at 150 kg N Ha^{-1}. This shows that the recommended fertilization for this plant was at 50 kg N Ha-1 due to higher HI in total phenolics and flavonoid. Application at high nitrogen rates 150 kg N Ha-1 would produced lower HI.

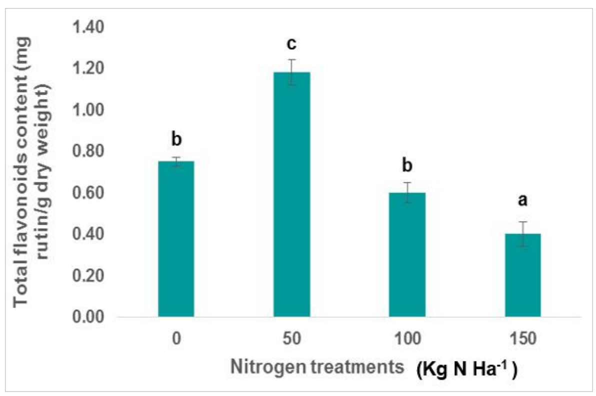

Fig. 13. The effect of different N fertilization rates on the total flavonoids content of *Polygonum minus* after twelfth weeks of planting

Data are mean ± standard error of mean (SEM). N= 6. Means with different superscript showed the significantly different at P<0.05 using DMRT at 95% of confidence levels

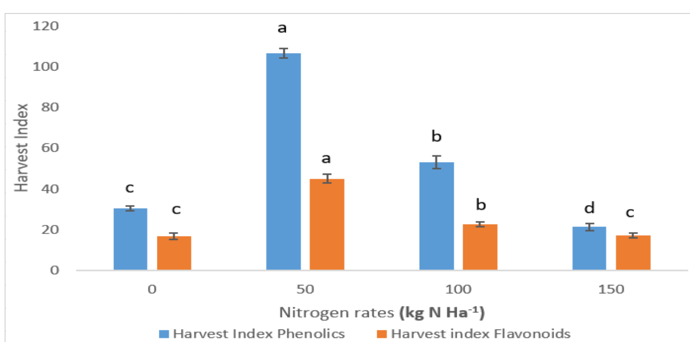

Fig. 14. The effect of different N fertilization rates on the Harvest Index (HI) of total phenolics and flavonoid of *Polygonum minus* after twelfth weeks of planting

Data are mean ± standard error of the mean (SEM). N= 6. Means with different superscript showed the significantly different at P<0.05 using DMRT at 95% of confidence levels

4. CONCLUSION

It was noticed that most of the parameters in growth and carbon assimilation in *P. minus* were increased with the increment of the nitrogen fertilization rates throughout the weeks. At the final harvest, the parameters such as the number of leaves, plant height, stem diameters, total chlorophyll contents, leaves area, total dry weight, NAR, WUE, net photosynthesis rate, stomatal conductance, and maximal efficiency of photosystem II were highest at 150 kg/ha treatments. In the terms of biochemical changes, the parameter such as total phenolics contents and total flavonoids gain the highest production of total phenolics and flavonoids at 50 kg N ha^{-1}. This showed that under a high level of nitrogen rate the growth and photosynthesis was enhanced and the production of secondary metabolites was reduced in *P. minus*. The recommended nitrogen fertilization rate for *P. minus* was observed at 50 kg N ha-*1*, where the highest HI was obtained.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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