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RESEARCH ARTICLE

Effect of Excessive Nitrogen Fertilizer and Glyphosate Application in *Brassica rapa* var. *chinensis*

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ABSTRACT

Nitrogen fertilisers and herbicides are commonly used in agriculture to increase crop yields. However, their excessive use can lead to soil pollution and other adverse effects. Thus, an experiment was conducted to evaluate the physical growth and oxidative stress of *Brassica rapa* var. *chinensis* (Bok choy) when exposed to different rates of nitrogen fertiliser and herbicide (glyphosate). The peat moss was treated with urea fertiliser at 0.0 kg N ha^{-1} (C0), 70.0 kg N ha^{-1} (CT1), 80.0 kg N ha^{-1} (CT2), 90.0 kg N ha^{-1} (CT3), and 115.0 kg N ha^{-1} (CT4) as control treatments. Additionally, glyphosate at 0.5 kg ai ha^{-1} was applied to corresponding treatments with urea at 0.0 kg N ha^{-1} (CT0), 70.0 kg N ha^{-1} (T1), 80.0 kg N ha^{-1} (T2), 90.0 kg N ha^{-1} (T3), and 115.0 kg N ha^{-1} (T4). The height of *B. rapa* significantly reduces to 85%, 86%, and 86% with the application of glyphosate at N-treated peat moss at 80, 90, and 115 kg N ha^{-1}, respectively. Interestingly, the root length of *B. rapa* was significantly reduced at all treatments, with nitrogen alone and in combination with glyphosate. Nevertheless, shoot fresh weight (SFW) appeared to be less susceptible, with significant growth stimulation ($p<0.05$) observed in all single urea applications, resulting in weight doubled at CT1 (228%) and CT3 (227%), and tripled in CT4 (335%), except at 80.0 kg N ha^{-1} (CT2), where the shoot fresh weight was only 66% of the control. The leaf diameter of *B. rapa* was greatly promoted at all single urea applications but reduced to 62% with the application of glyphosate at T2. However, no significant effects on chlorophyll contents were detected across treatment combinations but increased to 119% compared to the control in the nitrogen-only treatment at both 70 (CT1) and 90 (CT3) kg N ha^{-1}. Glyphosate alone caused the highest injury level to the leaf membrane (667.8%), with the root (32.4%) recording the lowest injury level compared to the control. The root membrane leakage showed a higher injury level of 279.7% and 309.5% at T1 and T2, respectively. Therefore, this study suggests that *B. rapa* gave various responses to urea and glyphosate toxicity when applied to urea fertiliser alone or in combination with urea fertiliser and glyphosate. *B. rapa* was found to be more sensitive to glyphosate efficacy when urea was supplied at 80 kg N ha^{-1} than other fertiliser concentrations.

1. INTRODUCTION

Chemical fertilisers are commonly applied in the cultivation system of vegetables. However, the excessive use of chemical fertilisers in vegetable farms causes growth inhibition and accelerated leaf senescence (Pan et al., 2022). Although nitrogen fertiliser played a vital role as it stimulated robust vegetative growth, encouraging the flourishing development of lush green foliage and healthy stems, the excessive use of fertilisers may harm their growth (Putney and Maguire, 2020; Pardo-Aguilar et al., 2021; Wang et. al., 2023). This is because excessive nitrogen application leads to nutrient imbalance, which later inhibits the uptake of other essential nutrients such as P, K, and Mg, resulting in deficiency and poor plant health (Wang et al., 2023; Guo et al., 2019). A recent study reported that excess N application damaged the metabolism of the seedlings by decreasing the soil physicochemical properties, including soil pH, soil exchangeable base cations, soil total exchangeable bases, soil cation exchange capacity, and soil base saturation, which are critical for nutrient availability and uptake (Wang et al., 2023). Additionally, when soil pH decreases (soil acidification), the root system may be damaged by the solubilisation of toxic elements such as aluminium (Ofoe et al., 2023). Nitrogen fertilisers significantly enhanced the process of photosynthesis, ensuring efficient conversion of sunlight into energy and promoting the production of carbohydrates essential for plant vitality (Verma et al., 2023). Moreover, the application of nitrogen fertiliser led to bountiful yields, fostering the proliferation of vibrant flowers and fruits, both in terms of quantity and quality. In order to eliminate competition for the nutrients, herbicides are widely used to manage weed populations in vegetable gardens. However, in addition to their effects on weeds, these herbicides could also have had unintended consequences on non-target crops such as the vegetable crops that grew nearby (Tudi et al., 2021). The application rates for herbicides on vegetable farms are varied, depending on the target crop.

In vegetable farms, nitrogen fertilisers and herbicides are frequently used to promote and suppress the growth of weeds, respectively. Nitrogen fertilisers give plants the nutrients they need to develop and yield, while glyphosate functions by blocking a particular enzyme, EPSPS that is vital for plant growth and development. The excessive and careless use of glyphosate raises concerns about residues in food, wildlife, and potential health effects (Ruuskanen et al., 2023; Rivas-Garcia et al., 2022). To preserve the long-term production and well-being of vegetable farms, it is crucial to utilise this chemical prudently and sustainably. In agriculture, glyphosate was frequently used for controlling the weed in three main situations, such as during land preparation, between the crop rows and surrounding field edge bunds during crop growth, and directly over the top of the crop plants (usually GM crops tolerant to glyphosate) (Brookes, 2020). Australia, Indonesia, the Philippines, Thailand, and Vietnam do not have many vegetable farms that use glyphosate. However, in China, nine out of fifty vegetable crops use glyphosate as their primary herbicide for weed control, while five out of eleven crops in India require glyphosate (Brookes, 2020).

Brassica rapa var. chinensis (Bok choy), also known as white Chinese cabbage, is a widely grown and popular vegetable crop and is typically eaten as cooked vegetables like soups and stews. The cultivated area and production of Brassica crops have significantly increased, from 1.6 million hectares in 1990 to 2.4 million hectares in 2020 and from 39.3 million tonnes to 70.9 million tonnes, respectively (Coves et al., 2023). To meet the high demand, a high amount of chemical fertiliser is used to increase the yield of vegetables. B. rapa encompasses various subspecies that are grown all over the world for a variety of purposes, from oil-rich seeds (like canola) to culinary uses (like turnips and bok choy) and the production of biodiesel. Its adaptability, genetic diversity, and important role as a model organism in agriculture, culinary arts, and scientific research are all highlighted by this plant. In Malaysia, B. rapa is particularly significant due to its rich nutritional value, cultural importance, economic contributions, role in food security, and dietary diversity. Thus, this underscores the need to investigate the potential impact of herbicides and fertilisers to guarantee safe production and consumption of B. rapa. Based on a recent study, the recommended rate of urea application at vegetable gardens is between 70 to 115 kg N ha-1 (Katuwal et al., 2023).

The importance of examining the phytotoxic effects of glyphosate-based herbicides and nitrogen fertilisers on B. rapa is highlighted by the paucity of studies and information available about the effects of these substances on vegetables. While most ecotoxicological research has focused on the effects of different nitrogen levels on growth and yield, it has also examined the effects on non-target crops and traits like drought tolerance and resistance to common soil-borne diseases in the examined area. This consideration is necessary to ensure the sustainability of the dynamic agricultural problems faced by B. rapa (Katuwal et al., 2023).

Chemical residues present in the soil as a result of pesticides, herbicides, or excessive fertiliser use can have a detrimental impact on crop productivity by disrupting soil health and plant growth (Tallapragada and Lather, 2022). The use of chemical herbicides and nitrogen fertilisers can lead to a loss of soil fertility, pollution of air and water, soil compaction, and the emission of greenhouse gases (Baweja et al., 2020). The concurrent use of these chemicals can make the soil more acidic, negatively impacting surrounding vegetation. The study hypothesised that the growth of B. rapa decreases with treatment application as the level of injuries increases. The objectives of the study were to evaluate the growth of B. rapa when treated with various application rates of nitrogen fertiliser (urea) and glyphosate and to investigate the oxidative stress or cellular injuries of B. rapa after applying different nitrogen fertiliser (urea) rates and glyphosate.

2. MATERIALS AND METHODS

2.1. Study area

The experiment was conducted in a nursery at Universiti Malaysia Kelantan (UMK), Jeli Campus, Kelantan, Malaysia (5.74582° N, 101.86584° E). It was designed to stimulate real-world conditions of herbicide spraying on farms, focussing on the effects of excessive nitrogen fertiliser and glyphosate application on *Brassica rapa var. chinensis*. The relative humidity was 70–80%, and the temperature ranged between 25–34 °C.

2.2. Experimental materials and design

The study was designed to evaluate the effect of urea fertiliser and glyphosate on the growth response of *B. rapa*. A total of ten treatments were established, including one negative control (C0), five positive controls (CT0, CT1, CT2, CT3, CT4), and four combined treatments (T1, T2, T3, and T4) (Table 1). The negative control (C0) received neither urea nor glyphosate, serving as the baseline. The positive controls included a glyphosate-only treatment (CT0) with glyphosate applied at 0.5 kg ai ha^{-1} and urea-only treatments (CT1, CT2, CT3, and CT4) with urea applied at concentrations of 70.0, 80.0, 90.0, and 115.0 kg N ha^{-1}, respectively. The combined treatments (T1, T2, T3, and T4) involved the application of both urea and glyphosate, with the same urea rates as the positive controls and glyphosate at 0.5 kg ai ha^{-1}. For glyphosate treatment at CT0, T1, T2, T3, and T4, the glyphosate was applied a week after seedlings were transplanted into polybags.

Prior to the study, the seeds of *B. rapa* were initially sown in the seedling trays and allowed to grow for a week before being transplanted into polybags. A day before transplanting activity, each polybag, with a diameter of 7 cm x 7 cm, was filled with 700 g of peat moss, pre-treated with four urea rates (70.0, 80.0, 90.0, and 115.0 kg N ha^{-1}). One-week-old seedlings were then transferred into these polybags, which corresponded to the treatment group containing pre-treated nitrogen peat moss (CT1, CT2, CT3, CT4, T1, T2, T3, T4). At week two, glyphosate was applied at 0.5 kg ai ha^{-1} (RoundupBio®, glyphosate as an isopropylamine salt) for CT0, T1, T2, T3, and T4 treatments using a 10L knapsack sprayer (equipped with a medium-hole nozzle producing coarse droplets) in open-space conditions. To prevent cross-contamination, treatments CT0, T1, T2, T3, and T4 were placed at a distance from the non-glyphosate treatments after being sprayed with glyphosate. The study was designed with ten treatments, each replicated three times, resulting in a total of thirty experimental units. The growth performance and biochemical parameters of cell injury in *B. rapa* were determined upon harvesting, at day 3, after herbicide application. The polybags were watered twice a day to prevent water stress.

Table 1: The concentration of nitrogen and herbicides for the toxicity test on *B. rapa*

Treatment	Application rate
C0 (negative control)	Urea (0.0 kg N ha^{-1}) + glyphosate (0.0 kg ai ha^{-1})
CT0 (positive control; glyphosate)	Glyphosate (0.5 kg ai ha^{-1})
CT1 (positive control; urea)	Urea (70.0 kg N ha^{-1})
CT2 (positive control; urea)	Urea (80.0 kg N ha^{-1})
CT3 (positive control; urea)	Urea (90.0 kg N ha^{-1})
CT4 (positive control; urea)	Urea (115.0 kg N ha^{-1})
T1	Urea (70.0 kg N ha^{-1}) + Glyphosate (0.5 kg ai ha^{-1})
T2	Urea (80.0 kg N ha^{-1}) + Glyphosate (0.5kg ai ha^{-1})
T3	Urea (90.0 kg N ha^{-1}) + Glyphosate (0.5 kg ai ha^{-1})
T4	Urea (115.0 kg N ha^{-1}) + Glyphosate (0.5 kg ai ha^{-1})

Recommended rate of urea application at the vegetable garden: 70 -115 kg N ha^{-1} source: Katuwal et al., (2023); recommended rate of glyphosate: 0.5 kg ai ha^{-1} source: Alister and Kogan, (2004)

2.3. Plant growth characteristics

At day 3 post-herbicide application, the leaf chlorophyll content was measured using a SPAD meter. Later, the plants were harvested, and the growth performance of *B. rapa* was evaluated in terms of plant height (cm), root length (cm), leaf diameter (cm), and shoot fresh weight (g). Later, the data was presented as a percentage relative to the control. The plant height was measured from the above ground, while the root length was measured as the length of taproot. Leaf diameter was measured using a calliper. The shoot was weighed using a weighing scale for the shoot fresh weight parameter. Meanwhile, to determine the percentage of membrane leakage, the leaves and root tissues of *B. rapa* were separated and used in electrolyte leakage measurement. The first fully developed leaves were used for the electrolyte leakage experiment.

2.4. Membrane leakage

Membrane integrity was determined by measuring the amount of electrolyte leakage (Harun et al., 2014). The leaf or root was immersed in the test tube containing 10 mL of deionised water and left to stand at room temperature in the dark. After 24 hours, an EC meter was used to measure the electrical conductivity (EC1). Then, the tissue with bathing solution was heated in a water bath at 95 °C for 20 minutes and allowed to cool before the second electrical conductivity (EC2) was measured. The percentage of membrane leakage was determined as a percentage of EC1/EC2*100%.

2.5. Data analysis

All the treatments were carried out with three replicates, which are arranged in a completely randomised design (CRD). The results obtained were statistically elaborated further by using SPSS Statistics V27. The percentage data of plant growth parameters and cell injury were subjected to one-way analysis of variance (ANOVA), and the differences between means for a significance level of <0.05 were calculated. The post-hoc analysis of the Tukey test was used to compare the means of different treatment groups at a 5% significance level. The test results were presented in the form of a relative mean comparison to the control, with letters indicating significant differences. The post-hoc analysis is important to identify specific treatment differences after an ANOVA indicates a significant effect. This will allow for a more detailed understanding of how the treatments differ from each other.

3. RESULTS AND DISCUSSION

3.1. Effect of nitrogen fertiliser (urea) and glyphosate on the growth of *B. rapa*

Plant height

The application of urea fertiliser and glyphosate has a significant impact on the height of *B. rapa* (Figure 1). It was observed that urea treatment promotes the growth of *B. rapa,* with a significant increase in height recorded at concentrations 70 and 90 kg N ha^{-1}. Nevertheless, glyphosate spray inhibited the growth of *B. rapa* in the positive control treatment (CT0), with significant decreases of 76% from the control's height (C0). A similar inhibited growth trend was observed as glyphosate was applied to the N-treated soil at T2, T3, and T4. Although the application of urea was found to increase the height of *B. rapa* (CT1–CT4), glyphosate sprayed was found to effectively inhibit the growth (T1–T4). The highest inhibition was recorded at T2, where 80 kg N ha^{-1} of urea was applied before the herbicide was sprayed. Noteworthy, urea application at a lower concentration of CT1 (70 kg N ha^{-1}) significantly promotes the growth of *B. rapa* while minimising the impact of glyphosate at T1.

A previous study suggested that the growth and development of *B. rapa* are greatly influenced by the available nutrients to be uptaken by the root or the leaf (Merta and Raksun, 2021). Fertilisers are also suggested to increase crop performance in terms of plant height, number of leaves, and leaf length, with the highest growth observed at day 30 after planting (Merta and Raksun, 2021). This is because fertiliser increases the nutrients needed by plants such as N, P, and K, while improving the physical, chemical, and biological properties of the soil.

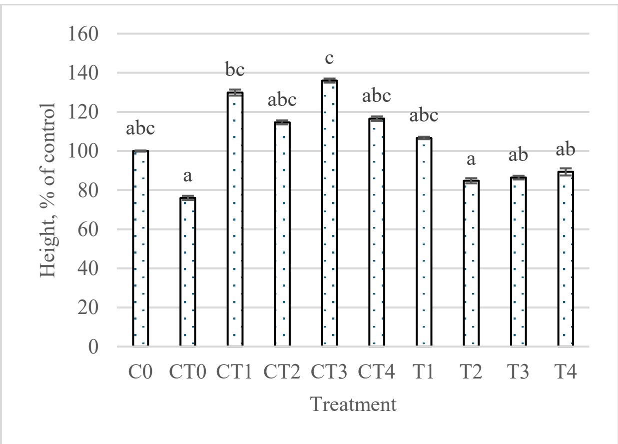

Figure 1: The effect of urea fertiliser and glyphosate on the plant height of B. rapa. The error bars represent the standard deviation (SD) of the mean. The treatment effects were evaluated relative to the control, with the control (C0) serving as the baseline for comparison.

A single application of herbicide was found to cause a great reduction in the height of *B. rapa*. However, it was found that urea application can act as an herbicide adjuvant to increase herbicide efficiency in controlling weeds (Samai and Muhidin, 2021). The study reported that a mixture of glyphosate and urea not only controls weeds in the cultivation area but also benefits crop production (Samai and Muhidin, 2021). This antagonistic effect of urea and glyphosate may benefit the cover crop in the plantation areas since the efficacy of glyphosate on this beneficial crop might be minimized. Additionally, the study also concluded that the mixture of urea and glyphosate may reduce the cost of controlling the weed population (Samai and Muhidin, 2021). Additionally, higher fertiliser levels (urea at 300 and 240 kg N ha^{-1}) were reported to affect *B. rapa* in terms of dry plant biomass (g), dry root biomass (g), plant height (cm), number of leaves per plant, maximum root length (cm), and leaf area index (LAI, calculated based on the number of plants in a square meter), which later contributes to the crop yield (Hamad et al., 2021).

Root length

The treatment significantly reduced the root length of *B. rapa* with a single glyphosate application (CT0), which was recorded as having the highest inhibition with a value of 27.6% from the control treatment (Figure 2). The antagonistic effect of glyphosate was not significant at N-treated treatments T1–T4, although the inhibition growth was less than CT0. This suggests that the efficiency of glyphosate in inhibiting root length was not affected by soil nutrients. The current study also suggested the root length of *B. rapa* is sensitive to available nitrogen, and the amount of nitrogen tested is in excess and able to inhibit the root growth. Additionally, further application of glyphosate was suggested to provide a synergistic effect on inhibition of root length. Kano et al. (2021) suggested suitable fertiliser application in terms of amount and frequency of application is needed to ensure successful practices in the organic hydroponics of *B. rapa* (Kano et al., 2021). Besides being an important structure and role for plants, roots help absorb the available nutrients from the soil and provide a place for beneficial microorganisms to form root biofilm. Root surface biofilm is important for promoting nutrient supply, enhancing plant growth, strengthening physical and chemical barriers against pathogens, and inducing plant resistance against environmental stress by promoting a synergistic relationship between roots and beneficial microbes (Fujiwara et al., 2016; Haque et al., 2020; Kano et al., 2021; Li et al., 2024; Patten and Glick, 2002).

This study's findings are consistent with earlier research in maize and cotton, which found that high N supplies, 400 kg N ha^{-1} and 480 kg N ha^{-1}, respectively, inhibited root elongation (Chen et al., 2020; Zheng-rui et al., 2008). Higher N supplies could potentially increase the production of reactive oxygen species, thus causing oxidative damage to the cell structures, including the root cells (Choudhary et al., 2020). A recent study summarises that high ammonium levels inhibit the roots from growing via increasing auxin inactivation, which lowers the ability of auxin to support root development (Di et al., 2021). Additionally, N fertilisers are shown to significantly enhance root elongation in cotton cultivars, with the highest elongation at a moderate amount of N supplied (240 kg N ha^{-1}), but start to deteriorate as the increased amount of N is supplied (480 kg N ha^{-1})(Chen et al., 2020). Another study in maize suggested root elongation is a way of plant adaptation in response to nitrogen deficiency (Sun et al., 2020). In nitrogen deficiency, auxin is transported from the shoot to the root, causing accumulation of the auxin in the root tips, which stimulates the production of nitric oxide and promotes the synthesis of strigolactones (Sun et al., 2022; Sun et al., 2020). Strigolactones responsible for cell division, in a synergistic role with auxin, regulate root elongation. Additionally, a study on wheat reported that the herbicide (chlorsulfuron) produces shorter roots and tends to decrease the ability of wheat to absorb micronutrients (Cu, Mn, and Zn) (Rengel and Wheal, 1997).

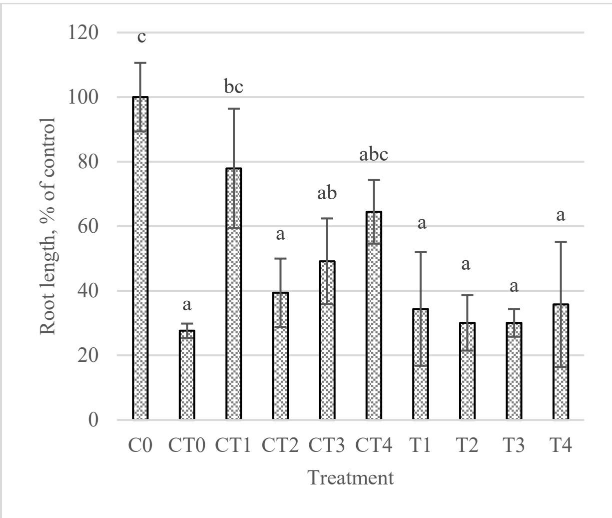

Figure 2: The effect of urea fertiliser and glyphosate on the percentage root length of *B. rapa* compared to the control (C0). The error bars represent the standard deviation (SD) of the mean. The treatment effects were evaluated relative to the control, with the control (C0) serving as the baseline for comparison.

Shoot fresh weight

The amount of nitrogen fertiliser significantly stimulates the growth of B. rapa by increasing the shoot fresh weight at CT1, CT3, and CT4 (Figure 3). This is in line with the role of nitrogen in promoting the growth of crops by stimulating the rate of photosynthesis, thus promoting the synthesis of amino acids and proteins, chlorophyll production, enzyme activity, osmotic regulation, and stress tolerance in plants (Mu and Chen, 2021). Furthermore, a study on B. rapa showed a significant increase in the fresh weight of B. rapa with different forms of nitrogen fertiliser (chemical and biochar-coated) applied (Bi et al., 2024). However, at CT2 (80 kg N ha-1), the effect of fertiliser was not significant. This drew many possibilities, such as other possible environmental factors such as light intensity, soil pH, temperature in the root environment, seed quality, and the nitrogen concentration itself (Fallovo et al., 2009; Guo et al., 2007).

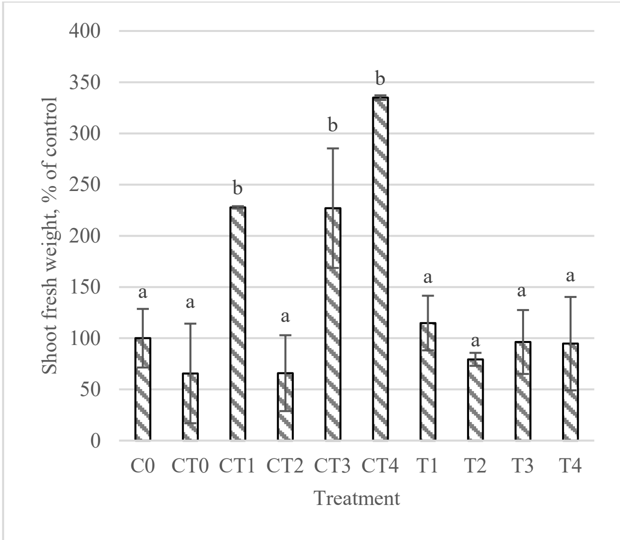

Figure 3: The effect of urea fertiliser and glyphosate on the shoot fresh weight of *B. rapa.* The error bars represent the standard deviation (SD) of the mean. The treatment effects were evaluated relative to the control, with the control (C0) serving as the baseline for comparison.

It is observed that the application of glyphosate alone (CT0) exhibits more toxicity than treatments combined with urea fertiliser in the range of 70 kg N ha-1 to 115 kg N ha-1 (T1, T2, T3, T4). The addition of urea fertiliser in treatments T1, T2, T3, and T4 appears to mitigate the negative effect of glyphosate on shoot fresh weight, resulting in higher biomass compared to the treatment with glyphosate alone. The antagonistic effect of herbicide treatment and nitrogen stimulation on the shoot fresh weight suggested that the efficacy of glyphosate decreased with the application of urea. In rapeseed (Brassica napus L.) seedlings, the metabolic effects observed after exposure to glyphosate showed a complex pattern (Petersen et al., 2007). Although no visual effect was observed, such as in the shoot dry weight, the shikimate biomarkers were found to be sensitive to the glyphosate exposure, with a linear response to the increased dose applied. The decreases in shoot fresh weight in the combined treatment of N and glyphosate when compared to single nitrogen application showed the inhibition of shikimate pathways, which disrupt the synthesis of aromatic amino acids. Furthermore, it is reported that approximately 60% of a plant's dry weight consists of chemicals generated by the shikimate pathway (Jensen, 1986; Kashyap et al., 2023). In addition, wide glyphosate application can harm the plants since it is easily translocated from shoots to roots and released into the rhizosphere (Fernandes et al., 2020; Jang et al., 2020).

Leaf diameter

Glyphosate application at 0.5 kg ai ha^{-1} without urea application (CT0) significantly reduces the leaf diameter (Figure 4). This is in line with a previous result on the inhibition of the leaf area parameter of soybean by herbicide at a dosage of 1800 g ha^{-1}, which decreased from an average of 20 cm^2 to 5 cm^2 (Johnson et al., 2002). Meanwhile, fertiliser application stimulates the growth of the leaf by significantly increasing the leaf diameter, despite the dose applied (CT1 to CT4). In concordance with a previous study on spinach (*Spinacia oleracea L.*), nitrogen fertiliser was revealed to increase the leaf area of the crop to 119.1% after 224 kg N ha^{-1} was applied (Abdelraouf, 2016). The antagonistic effect of the treatments can be seen in T1 to T4, as the glyphosate significantly decreases the leaf diameter of nitrogen-treated *B. rapa* to be on par with control (C0). The efficacy of glyphosate at T2 was highest, as the leaf diameter was observed to be similar to the leaf of *B. rapa* treated with no urea (CT0). The result on leaf diameter suggested that with adequate nutrient availability, the detrimental effect of glyphosate can be reduced.

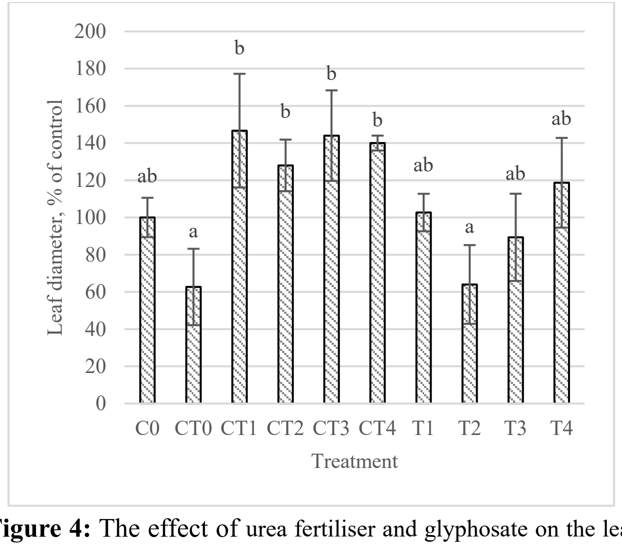

Figure 4: The effect of urea fertiliser and glyphosate on the leaf diameter of *B. rapa.* The error bars represent the standard deviation (SD) of the mean. The treatment effects were evaluated relative to the control, with the control (C0) serving as the baseline for comparison

Although limited studies have reported the effect of fertilisers on leaf diameter, other plant morphologies can be related to this, such as leaf size and leaf area. For example, in Hongyang Kiwifruit (*Actinidia chinesis*), the average area of leaves per plant (cm^2) was not significantly affected a year after the fertilisers were applied to the plant. Nevertheless, the total area of leaves per plant (m^2) was significantly higher in the treatment in the presence of N, P, and K (Zhang et al., 2020). According to the studies, the size and number of leaves were reduced as nutrients were deficient, especially for herbaceous and perennial plants (Kropat et al., 2011; Zhang et al., 2020). It has been observed that the application of fertiliser increased the amount of resources needed for the growth and development of leaves, such as area, thickness, and chlorophyll content (Kropat et al., 2011). Zhang et al. (2020) explained the different roles of N and P elements in the NPK fertiliser for the increase in total leaf area. N was responsible for increasing the total leaf area by promoting the growth of individual leaves, while P increased by stimulating the growth of more leaves. N fertilisers are also responsible for increasing the leaf area by stimulating the cell sizes, cell numbers, and cell expansion rate (Chandler and Dale, 1995). However, N concentrations (low and high) were shown to have no significant effect on the total leaf area of Kiwifruit, but relatively high nutrient supply had little effect on leaf expansion (Zhang et al., 2020). Furthermore, environmental factors were limiting factors that affected the leaf expansion of Kiwifruit when an adequate supply of nutrients was given. In addition, the increase in nutrients contained in the soil was shown to increase the number of leaves in *B. rapa* compared to *B. rapa* without nutrients supplied through organic fertiliser (Merta and Raksun, 2021).

Chlorophyll content

The greatest inhibitory effect of glyphosate on chlorophyll content was observed at CT0 (72%). This is following the role of glyphosate in inhibiting specific enzyme EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), leading to the disruption of aromatic amino acid synthesis, crucial for proteins, hormones, lignin, and photosynthesis-related compounds in plants (Kashyap et al., 2023). Although the synthesis of chlorophyll is not directly related to the shikimate pathway, chlorophyll production may be affected by the disruption of plant metabolism. The treatment combination of urea and glyphosate was shown to have no significant effect on the chlorophyll content of *B. rapa* (Figure 5). However, an increase in the chlorophyll content was observed at a single application of urea, and glyphosate was applied at higher urea concentrations (T3 and T4). This implied that glyphosate and urea had an antagonistic effect on the synthesis of chlorophyll. Research shows that an adequate supply of nitrogen fertilisers increases leaf nitrogen nutrition and chlorophyll content by enhancing chlorophyll synthesis, which leads to an increase in the yield and quality of *B. rapa* (Bi et al., 2024; Yeboah et al., 2017). Furthermore, a study reported that N fertiliser at 5 g m^{-2} had a favourable impact on the chlorophyll index of emerald grass (Dinalli et al., 2022).

Glyphosate is more efficient in disrupting chlorophyll synthesis in the nutrition deficiency state, as observed in CT0, T1, and T2 (Figure 5). A study proposed that the efficacy of glyphosate decreased as the supply of nitrogen fertiliser increased. In a previous study, it was concluded that high usage of herbicides inhibited the photosynthetic rate and nitrogen assimilation in rapeseed seedlings (Cui et al., 2020). However, compared to the other Brassicaceae family, *B. rapa* had the highest tolerance to glyphosate exposure (Kashyap et al., 2023). Another broad-spectrum herbicide, WeedLock, was shown to have a significant inhibition effect on the chlorophyll pigment content (chlorophyll a, chlorophyll b, and carotenoid), thus disrupting the rate of photosynthesis in several tested crops: *Ageratum conyzoides* L., *Eleusine indica* (L.) Gaertn, *Zea mays* L., and *Amaranthus gangeticus* L. (Hasan et al., 2022). Furthermore, a recent study reported a negative correlation between the total chloroplast amount and the days after glyphosate application on durum wheat (*Triticum durum* L.) (Benkadja et al., 2023). Besides that, the possibility of decreases in chlorophyll content is related to the degradation of chlorophyll as a result of an increase in ROS content (Gomes et al., 2017). A similar study on wheat and soybean reported that the inhibition effect on chlorophyll content was observed in glyphosate-treated compared with the non-glyphosate control (Benkadja et al., 2023; Zobiole et al., 2011). Moreover, glyphosate remnants in the soil were found to have significant damage to growth characteristics, nodulation, and chlorophyll content in maize (*Zea mays L.*) and bean (*Phaseolus vulgaris L.*) (Kamdem et al., 2016).

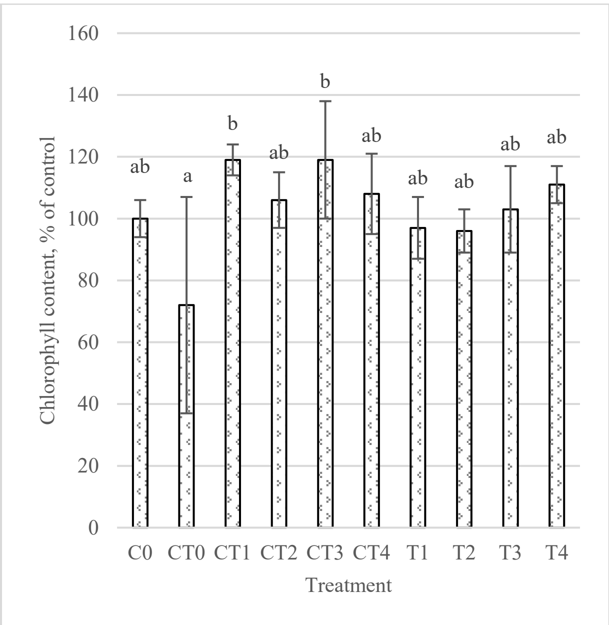

Figure 5: The effects of urea fertiliser and glyphosate on the chlorophyll content of *B. rapa.* The error bars represent the standard deviation (SD) of the mean. The treatment effects were evaluated relative to the control, with the control (C0) serving as the baseline for comparison

3.2 Antimicrobial susceptibility testing

Leaf and root membrane leakage

Leaf membrane leakage (LML) in *B. rapa* increased significantly in all treatments, with the highest leakage observed at the positive control (CT0), almost seven times higher than the negative control (C0), followed by glyphosate sprayed at the highest urea supply (T4) (Figure 6). In contrast, a significant decrease in LML was observed at the highest urea concentration with no glyphosate applied (CT4). This portrays the antagonistic effect of fertiliser and glyphosate on the permeability of the leaf membrane. The result proposed that the efficacy of glyphosate on *B. rapa* was dependent on its nutritional availability. The increase of urea from 70 to 80 and 115 kg N ha^{-1} was observed to increase the efficacy of glyphosate. In contrast, at T3 (90 kg N ha^{-1}), the efficacy and toxicity effects of glyphosate on the leaf of *B. rapa* were at their lowest. The antagonistic effect of N and glyphosate might explain the trend observed at CT4 and T4 and other treatment combinations with and without glyphosate. It also suggested that the N supplied promotes the cell membrane to be more tolerant to glyphosate susceptibility. The study suggested that at 90 kg N ha^{-1}, the leaf membrane was less susceptible to glyphosate application.

In the root, the percentage of membrane leakage was highest at the two lowest urea doses applied (T1 and T2), followed by T4 and T3 (Figure 6). The study deduces that the synergistic effect of urea and glyphosate application on the root leakage is higher when the nutrient available is at a lower dose, between 70 and 80 kg ai ha^{-1}, and at a higher urea rate of 115 kg ai ha-1 (T4). However, the concentration of urea showed no significant effect on the root membrane leakage from CT1 to CT4, with a percentage range of 75% to 115% from the control. In line with the recent study, the recommended rate of urea application in vegetable gardens is in the range of 70–115 kg N ha^{-1} (Katuwal et al., 2023).

The permeability and integrity of plant membranes are associated with environmental stress and can be used as indicators for damage caused by the stress, such as by measuring the electrolyte leakage (Masoumi et al., 2010; Silva et al., 2016). Oxidative stress caused by environmental stress will increase the production of ROS, leading to oxidation of membrane lipids and thus leading to ion leakage due to increasing membrane permeability (Sakya et al., 2018). A previous study reported the sensitivity of fodder radish plants (*Raphanus sativus* L.) to glyphosate at every dose applied by increasing cell membrane damage, thus reducing chloroplast pigment content and leading to a decrease in photosynthetic efficiency (Silva et al., 2014).

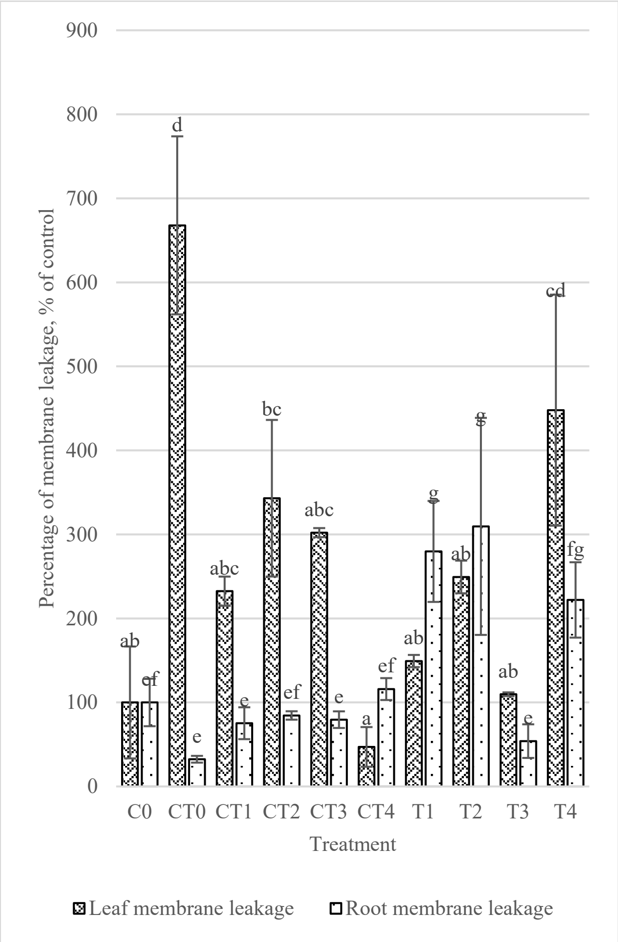

Figure 6: The percentage of membrane leakage that occurred in the leaves and roots of B. rapa when urea fertiliser and glyphosate were applied. The error bars represent the standard deviation (SD) of the mean. The treatment effects were evaluated relative to the control, with the control (C0) serving as the baseline for comparison

4. CONCLUSION

Oxidative damage induced by N fertilisers and glyphosate herbicides affects the physical parameters of *B. rapa*. Hence, these parameters can be used as a criterion to select an effective dosage of nitrogen fertiliser and glyphosate to reduce oxidative stress while contributing to high yield. The increase in N supplied does promote variations in plant response. However, once glyphosate was applied, the plant responses became consistent, especially in root length, shoot fresh weight, and chlorophyll content. The study suggests the interaction between nitrogen levels and glyphosate application might not be significant, or the efficacy of glyphosate is not strongly influenced by nitrogen levels. The glyphosate's specific action on the shikimic acid pathway may be the dominant factor, overriding any variations in nitrogen levels. The study also concludes that both nitrogen concentration and glyphosate application can cause oxidative stress in *B. rapa*, either in leaf, root, or other plant physiology.

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