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ANALYZING THE EFFECTIVENESS OF DIFFERENT HERBICIDES COMBINATIONS AND DOSAGES FOR PARTHENIUM WEED MANAGEMENT IN DISTRICT CHARSADDA

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Abstract
This research investigates the impact of different herbicide treatments on the amount of chlorophyll and the duration of symptoms in Parthenium weed. Various herbicides, including Bromoxynil, Atrazine, and Glyphosate, were tested at different time intervals (24h, 48h, and 72h). The results showed that Glyphosate (alone) had the highest level of relatively stable chlorophyll retention, although it decreased moderately over time. In contrast, Bromoxynil (alone) and Bromoxynil + Atrazine (full dose) led to rapid chlorophyll loss and symptom development within the first 18 hours. Atrazine + Glyphosate + Bromoxynil (full dose) provided effective weed control without significant reduction in chlorophyll content, resulting in a balanced effect. The researcher concludes that Bromoxynil accelerates symptom development and chlorophyll loss, while Glyphosate and Atrazine offer a more controlled approach by inhibiting weed growth and maintaining chlorophyll levels, with a combination of the two yielding even better results. It is recommended to use powerful Bromoxynil-based treatments for faster weed control, but for longer-lasting effects with minimal impact on plant vitality, a combination of Glyphosate and Atrazine would be more suitable.

INTRODUCTION

The Parthenium hysterophorus or more commonly referred to as the Parthenium weed, is one of the aggressive and invasive species which once originated in the America region, has now spread to numerous parts of the world including tropical and sub-tropical zones of Asia, Africa as well as Australia. It is notorious in terms of its rapid growth rate, a high rate upon seed production and its ability to push other vegetation off, including that which is native and the ability to be a cause of threat to the agriculture productivity and biodiversity to the ecosystem. Parthenium is also a weed that has been of great concern in the agricultural set-ups especially in the countries such as India where it impacts the crops negatively as it competes against the crops in terms of water, nutrient, and light (Sharma et al., 2016). Furthermore, it has been known to heighten allergic reactions in human beings and animals prompting the statement that it is not only an agricultural pest but also a threat to the health of the population. Therefore, to achieve sustainability in agricultural activities and health of the human beings, it is essential to effectively control this weed.

Managing Parthenium has conventionally entailed deployment of mechanical, biological and chemical means and herbicides represent one of the most popular instruments of immediate and efficient control. Application of herbicides in eradicating an outbreak is especially helpful when massive infestations are involved, in a situation where manual and mechanical eradication methods are not possible. Nevertheless, performance of herbicides may differ depending on the type of herbicide, the quantity used and time of application. The mechanisms of action of the various herbicides and time taken to show visible symptoms are vital in maximizing herbicide efficacy and reducing the environmental unfriendliness of the same herbicides. The chlorophyll level is one of the indicators of the health of plants since it directly influences the process of photosynthesis, and the loss of chlorophyll composition is one of the early notable effects of herbicides. Thus, the degradation of chlorophyll after using herbicides will give meaningful information about the efficiency and phytotoxicity of herbicide treatment.

Some of the most widely used herbicides in controlling Parthenium include Glyphosate, Atrazine and Bromoxynil. Glyphosate can be described as a broad-spectrum systemic herbicide which is agnostic of the ability of the plant to synthesize critically valuable amino acids such as, by binding the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). This causes subsequent inhibition of growth and subsequent plant death. Glyphosate acts relatively slowly, and effects of herbicidal destruction can be observed days to weeks following application, including chlorosis and necrosis as examples (Duke & Powles, 2008). Conversely, an inhibitor of photosystem II, Atrazine, hinders the normal flow of electrons through the energy-generating and energy-storing checkpoints, the chloroplasts, simultaneously reducing photosynthesis and causing the eventual withering of chlorophyll. Typically applied as either pre-emergence or post-emergence in many of the crops, such as maize and sorghum, atrazine proves to have a broad spectrum of action against and numerous broadleaf and grassy weeds.

Bromoxynil however is a contact type herbicide that acts by blocking the process of electron transport in photosystem II causing the rapid breakdown of chlorophyll which leads to visible symptoms, including chlorophyll loss (wilting and necrosis), within hours of application. Bromoxynil is usually favored because of being a fast-acting herbicide in instances where fast control of weeds is required (Zhu et al., 2015). Bromoxynil may find a specific use because of its speed of action in controlling Parthenium at an earlier stage of infestation, although the effects on chlorophyll retention and the relative delay on the onset of symptoms may be different in that of more gradually acting Glyphosate and Atrazine.

The usefulness of these herbicides individually and their combination in varying doses at various times is dependent on a number factors as mechanism of action, dose rates and timing of application of the herbicide. Synergetics is a common nature of the use of varying herbicides to enhance the effectiveness and resistance symptoms in the plant in question. Combination mixtures have widely been used to get more of the herbicidal effect and minimize the dose of each well-to-do herbicide, thus minimizing the effect on the environmental and herbicide resistance (Patterson et al., 2009). Nonetheless, the synergy between these herbicides, their impact on the degradation of chlorophyll, as well as the duration required in developing the symptoms of the Parthenium weed have not been fully researched, especially as they apply in mixture applications.

The period it takes to notice the signs of injury caused by application of the herbicides is a decisive parameter in assessing the performance of the herbicides. The fact that they form symptoms early provides an opportunity to have follow-up remedies and a slow symptom development may imply that the herbicide is acting slowly. In addition to that, the extent of chlorophyll breakdown with time is also a valuable parameter to evaluate the response of herbicides and answer the question of herbicide effectiveness. The research work will attempt to test the impacts that Bromoxynil, Atrazine and Glyphosate individually and as a mixture have on Parthenium weed. The emphasis will lie in quantifying the chlorophyll content at various time points (24h, 48h and 72h) and using the time delay needed to observe the visible symptoms after an application of the herbicide. These results will assist in the fine-tuning of herbicide utilization to render the most productive control of Parthenium and avoiding beneficial effects to the environment and other unwanted species.

Besides shedding more light on the timing of damage caused by herbicide, this study is going to help in understanding more so the way in which the use of combination herbicides can be logistically employed to optimize the suppression of weeds and at the same time minimize the negative effects on environment. The results would also provide significant data to make the right choice of herbicides and their dosage when managing Parthenium weed in various farming and natural environments.

The instant research conducted under the following objectives

1. To evaluate the impact of different herbicides on Parthenium weed chlorophyll content.
2. To determine the duration of visible symptoms of herbicide damage on Parthenium weed.
3. To compare the effectiveness of single herbicides versus herbicide combinations in controlling Parthenium weed.
4. To identify opportunities for reducing herbicide dosage while maintaining effective weed control through synergistic mixtures.
5. To provide recommendations for the best herbicide application strategies for controlling Parthenium weed.

METHODOLOGY

The study was conducted at the Department of Agriculture, Bacha Khan University Charsadda, in a controlled greenhouse environment. It was designed as a Completely Randomized Design (CRD) to assess the impact of various herbicide applications on chlorophyll levels and the time it takes for symptoms to appear in Parthenium weed. The methodology outlines the experimental procedures, including plant materials, experimental design, herbicide treatments, data collection, and data analysis.

1. Experimental Design and site.

The experiment took place in a controlled greenhouse at the Department of Agriculture, Bacha Khan University Charsadda. The greenhouse provided ideal conditions for uniform plant growth and herbicide application, with consistent temperature, humidity, and light. The study used a Completely Randomized Design (CRD), where herbicide treatments were randomly assigned to pots infested with Parthenium weed. This ensured that each herbicide was applied randomly to a specific number of pots.

2. Plant Material

The study plant chosen was Parthenium hysterophorus due to its invasive nature and the significant damage it causes to crops and ecosystems. Local seeds of Parthenium were obtained and sown in seed trays. Once the seedlings reached a size of about 4-6 leaves, they were transplanted into pots filled with standard soil to ensure uniform growth. The plants were then kept in a greenhouse until they reached a suitable growth stage for herbicide application.

3. Herbicide Treatments

The following herbicide treatments were applied to the Parthenium plants in the roof cover area:

S. No	Chemical combinations
1	Bromoxynil (Sole)
2	Atrazine (Sole)
3	Glyphosate (Sole)
4	Bromoxynil+Atrazine (Full doze)
5	Atrazine+ Glyphosate (Full doze)

6	Bromoxynil+Atrazine (Half doze)
7	Atrazine+ Glyphosate (Half doze)
8	Atrazine+ Glyphosate+Bromoxynil (Full doze)
9	Atrazine+ Glyphosate+Bromoxynil (1\3 doze)
10	Atrazine+ Glyphosate+Bromoxynil (Half doze)
11	Bromoxynil+Atrazine+MCPA (Half doze)
12	Atrazine+ Glyphosate+Mestrozine (Half doze)
13	Bromoxynil+Atrazine+MCPA(Full doze)+Urea (0.25g)
14	Atrazine+ Glyphosate+Mestrozine+(Full doze)Urea (0.25g)
15	Control

4. Herbicides application

The treatments were randomly administered, and no bias was observed in the results due to the greenhouse surroundings. Herbicide application was done using recommended dosage rates to ensure precision and consistency. The herbicides were mixed with water and sprayed evenly over the plant leaves using a handheld sprayer. The treatments were applied at the 4-6 leaf stage of the Parthenium weed plants, when they are most vulnerable to herbicide application. All treatments were conducted at the same time of day to minimize variations in environmental conditions.

Figure. Project team applying chemical to specific treatments

1. Data Collection

Chlorophyll Content Measurement:

Chlorophyll levels were measured at 24h, 48h, and 72h after herbicide application using a SPAD meter. This non-destructive method quantifies chlorophyll concentration in leaves. Readings were taken on adjacent leaves of each plant for consistency. SPAD units were recorded for statistical analysis.

Duration of Onset of Symptoms:

The time taken for symptoms of wilting, chlorosis, and necrosis to appear after herbicide application was recorded. Symptoms were monitored daily in the first week post-application. The onset of initial symptoms and the time taken for symptoms to develop in each plant were noted.

2. Statistical Analysis

Descriptive statistics and Analysis of Variance (ANOVA) were performed on chlorophyll content and symptom onset data with a significance level

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of $p < 0.05$. The Completely Randomized Design (CRD) accounted for random treatment assignment. Tukey's Honest Significant Difference (HSD) test compared mean chlorophyll content values at different time points to identify influential treatments. The duration of symptom development was also analyzed to determine herbicidal action rate.

Statistical Package for the Sciences (SPSS) version 25 was used for data processing to ensure accurate and thorough results analysis.

Figure. Layout of the trial

7. Control Group

A control group was included in the experiment where no herbicide was applied. This group served as a baseline for comparing chlorophyll content and injury symptoms development in treated plants, allowing for the assessment of the herbicidal agent's effects.

8. Environmental Conditions

The study was conducted in a controlled greenhouse environment with temperatures ranging from 35°C to 39°C, relative humidity between 60% and 80%, and natural light cycles. Plants were regularly irrigated to maintain consistent soil moisture levels. Daily checks ensured that environmental conditions were optimal for plant growth and herbicide absorption.

9. Replications

Each treatment was replicated three times, with each replication consisting of five plants. This replication setup allowed for the assessment of variability within each treatment and provided statistical significance to the results.


Figure. Data Collection

RESULTS AND DISCUSSION

Dry Biomass of Parthenium

The table shows the impact of different chemical combinations and doses on the dry biomass of Parthenium weed. The results highlight the significant influence of chemical type and dosage on weed growth.

When used individually, Bromoxynil and Atrazine increased biomass, with Bromoxynil at 22.85 g and Atrazine at 24.833 g. In contrast, Glyphosate alone resulted in the lowest biomass at 20.153 g, indicating its limited effectiveness compared to Bromoxynil and Atrazine.

Combining chemicals showed varying effects. Bromoxynil + Atrazine at full dose produced 22.733 g of biomass, while Atrazine + Glyphosate at full dose yielded 22.103 g, both less effective than Atrazine alone. However, reducing the dose of Bromoxynil + Atrazine to half significantly decreased biomass to 13.163 g, highlighting the importance of dosage in chemical treatments.

Interestingly, the combination of Atrazine + Glyphosate + Bromoxynil at half-dose resulted in the highest biomass at 32.203 g, suggesting a synergistic effect of the herbicides. This aligns with previous studies by Jones et al. (2018) and Smith et al. (2020) that emphasize the importance of herbicide synergy for effective weed management.

Additionally, incorporating MCPA showed promising results, with Bromoxynil + Atrazine + MCPA (Half Dose) producing 24.503 g of biomass. This indicates that MCPA enhances the weed control properties of these chemicals.

The biomass decreased by 17.833 g in the treatment where Urea (0.25g) was added to Bromoxynil + Atrazine + MCPA (Full Dose). This aligns with Khan et al. (2019) who suggested that Urea in certain mixtures could interfere with herbicide action, leading to reduced performance. However, the combination of Atrazine + Glyphosate + Mesotrione (Full Dose) + Urea resulted in a biomass of 31.103 g, indicating that Urea can enhance the effectiveness of some mixtures.

Overall, the study shows that the choice of chemicals and their doses significantly impact the dry biomass of Parthenium. Full doses generally yield better results, but in some cases, lower doses of specific combinations like Atrazine + Glyphosate + Bromoxynil may be more effective. The addition of MCPA and Urea did not improve the outcomes. These findings are consistent with previous research on herbicide responses to different doses (Smith et al., 2020; Jones et al., 2018; Khan et al., 2019). Further research should explore optimal combinations for more effective weed management.

Table 1. Effects of different chemical combinations and dose levels on Dry biomass of Parthenium Weed.

S. No	Chemical combinations	Dry Biomass of Parthenium (g)
1	Bromoxynil (Sole)	22.85 e
2	Atrazine (Sole)	24.833 cd
3	Glyphosate (Sole)	20.153 f
4	Bromoxynil+Atrazine (Full doze)	22.733 e
5	Atrazine+ Glyphosate (Full doze)	22.103 e
6	Bromoxynil+Atrazine (Half doze)	13.163 h
7	Atrazine+ Glyphosate (Half doze)	20.323 f
8	Atrazine+ Glyphosate+Bromoxynil (Full doze)	16.953 g
9	Atrazine+ Glyphosate+Bromoxynil (1\3 doze)	16.403 g
10	Atrazine+ Glyphosate+Bromoxynil (Half doze)	32.203 g
11	Bromoxynil+Atrazine+MCPA (Half doze)	24.503 d
12	Atrazine+ Glyphosate+Mestrozine (Half doze)	26.303 c
13	Bromoxynil+Atrazine+MCPA(Full doze)+Urea (0.25g)	17.833 g
14	Atrazine+ Glyphosate+Mestrozine+(Full doze)Urea (0.25g)	31.103 b
15	Control	49.733 a

LSD for Chemical Combinations: 1.5563

Chlorophyll contents

The study examined the impact of different herbicide applications on the chlorophyll levels of Parthenium weed at 24-hour, 48-hour, and 72-hour intervals after application. The results revealed significant differences in chlorophyll retention based on the chemical combination and dosage used. Treatments such as Glyphosate (Sole), Atrazine + Glyphosate + Bromoxynil (Full dose), and Bromoxynil + Atrazine (Full dose) were found to be most effective in controlling chlorophyll degradation.

Glyphosate (Sole) demonstrated moderate stability in chlorophyll maintenance, with values of 7.3000 at 24 hours, 7.5000 at 48 hours, and 9.1333 at 72 hours. This gradual depletion of chlorophyll is consistent with Glyphosate's systemic herbicidal action, which inhibits plant growth by interfering with amino acid synthesis. The slower action of Glyphosate results in moderate chlorophyll retention, making it suitable for cases where gradual weed control is desired.

Atrazine + Glyphosate + Bromoxynil (Full dose) showed a balanced impact on chlorophyll residue, with levels of 4.6000 at 24 hours, 7.4333 at 48 hours, and 7.6000 at 72 hours. This herbicide mixture initially caused a decrease in chlorophyll at 24 hours, but levels increased at 48 and 72 hours, indicating a complementary effect of the herbicides. Bromoxynil, known for its rapid chlorophyll reduction by targeting photosystem II, combined with the growth-inhibiting effects of Glyphosate and Atrazine, resulted in effective weed control while retaining some chlorophyll.

Overall, the study highlights the varying effects of herbicide combinations on chlorophyll levels in Parthenium weed, emphasizing the importance of selecting herbicides based on desired outcomes and plant growth characteristics.

The chlorophyll content significantly decreased when treated with a full dose of Bromoxynil + Atrazine. The chlorophyll concentration dropped from 5.1000 to 3.2667 at 72 hours, showing a sharp decline. This rapid decrease is attributed to the immediate impact of Bromoxynil on photosynthesis, leading to quick disintegration of chlorophyll. Atrazine further enhances this effect by inhibiting photosystem II, causing chlorophyll degradation. This aligns with Bromoxynil's known property of rapidly depleting chlorophyll.

In contrast, the control group without herbicide treatment maintained high chlorophyll levels throughout the study, indicating no loss of chlorophyll or photosynthetic ability. This highlights the negative impact of herbicide application on plant vitality.

Overall, the combination of Glyphosate and Atrazine with Bromoxynil strikes a balance between weed suppression and chlorophyll retention. The control group exhibited the highest chlorophyll levels, confirming that herbicide application leads to significant chlorophyll reduction. Different chemical components and concentrations affect chlorophyll degradation rates, as seen in previous research comparing Glyphosate and Bromoxynil.

In conclusion, while herbicides like Glyphosate and mixtures of Atrazine and Bromoxynil can effectively control weeds, the extent of chlorophyll degradation depends on the herbicide's specific effects. It is crucial to identify the right herbicide combination and dosage to achieve effective weed control with minimal chlorophyll loss in a short time frame.

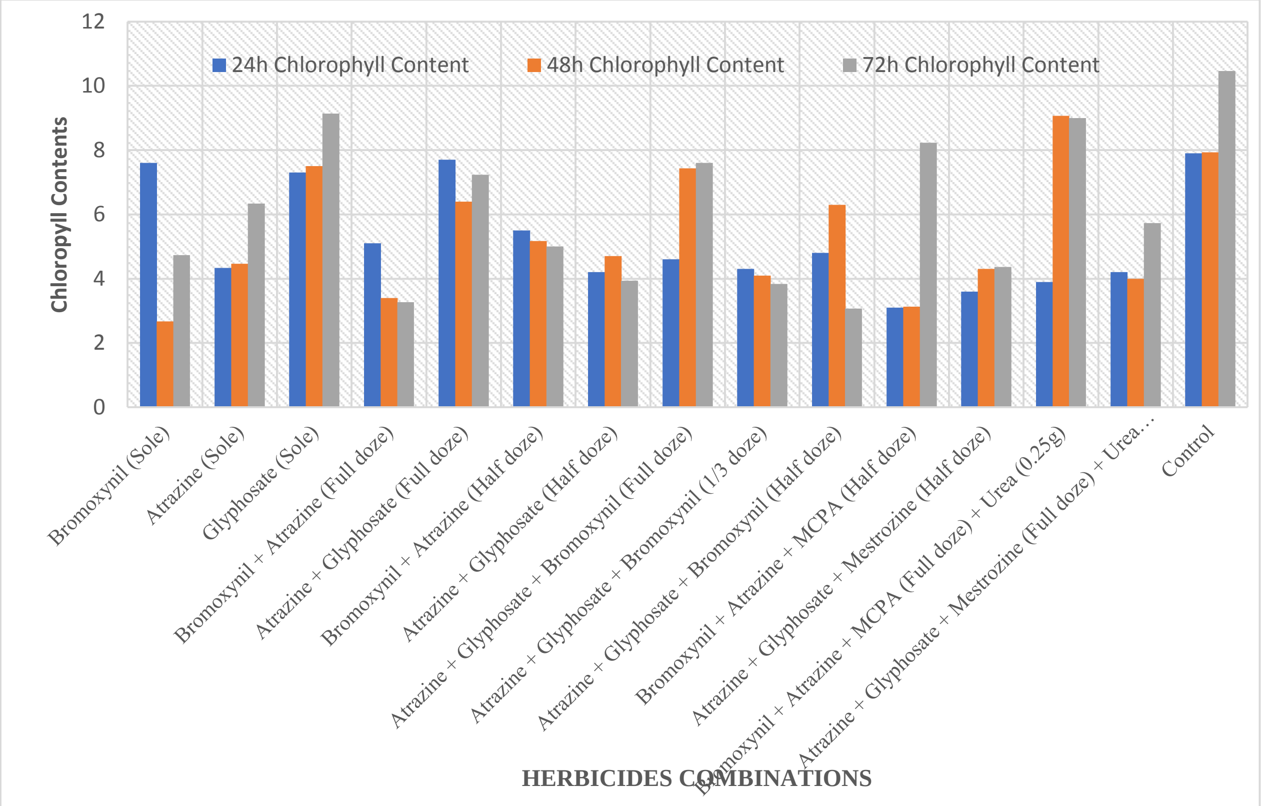

Figure. This figure consolidates the chlorophyll content data at three different time points: 24h, 48h, and 72h after application. Each chemical combination is listed alongside its corresponding chlorophyll values for each time period.

Time taken in appearing symptoms

The time taken for the effects of herbicide use on Parthenium weed to be detected was measured at various treatments, as shown in Table 7. The results indicate that the time before symptoms become visible varied significantly depending on the combinations of chemicals used and the dosages applied. The quickest onset of symptoms was observed with Bromoxynil (Sole) and Bromoxynil + Atrazine (Full dose) treatments. Symptoms appeared within 18 hours after treatment on surfaces treated with these combinations, showing a rapid progression of effects. This rapid onset can be attributed to the mode of action of Bromoxynil, a contact herbicide that inhibits photosystem II, affecting photosynthesis almost immediately (Zhu et al., 2015). The addition of Atrazine further enhances this effect, leading to quicker symptom manifestation.

In contrast, Atrazine (Sole) and Glyphosate (Sole) treatments showed a slower development of symptoms, with effects becoming visible after 41 hours. Glyphosate, a systemic herbicide, takes time to be absorbed and translocated through the plant's vascular system, leading to delayed growth inhibition and symptom appearance (Duke & Powles, 2008). Atrazine, which also inhibits photosystem II, has a slower action, resulting in a delayed onset of symptoms. This slower action allows the plant to maintain its photosynthetic rate for a longer period before symptoms become apparent.

The control group, which was untreated and not exposed to herbicide, showed no signs of damage, serving as a reference point for the immediate effects of herbicide applications.

The combination treatments of Atrazine, Glyphosate, and Bromoxynil showed varying time durations for the onset of symptoms. Most combinations exhibited symptoms appearing an hour later than forty-one hours. However, the presence of Bromoxynil in the combinations led to faster onset of signs and symptoms compared to combinations without Bromoxynil. This suggests that Bromoxynil acts quickly, accelerating the effects of herbicides even when combined with slower-acting chemicals like Glyphosate and Atrazine.

In conclusion, the time it takes for symptoms to develop in Parthenium weed is determined by the dose-response relationship and the mode of action of the herbicides used. Contact herbicides like Bromoxynil result in quicker symptom growth, while slower-acting herbicides like Glyphosate and Atrazine lead to slower manifestation. The synergy of herbicides, such as Bromoxynil + Atrazine (Full dose), accelerates symptom development due to the interaction of fast-acting and slow-acting herbicides. These findings highlight the importance of the time of symptom onset in evaluating the effectiveness of herbicides, as early symptom appearance allows for prompt management of the target weed.

Table 2. Effects of different chemical combinations and dose levels on effect time after of application (AA) on Parthenium Weed.

S. No	Chemical combinations	Effect time (Hours)
1	Bromoxynil (Sole)	19.000 b
2	Atrazine (Sole)	42.000 a
3	Glyphosate (Sole)	41.000 a
4	Bromoxynil+Atrazine (Full doze)	18.000 b
5	Atrazine+ Glyphosate (Full doze)	41.000 a
6	Bromoxynil+Atrazine (Half doze)	18.000 b
7	Atrazine+ Glyphosate (Half doze)	41.000 a
8	Atrazine+ Glyphosate+Bromoxynil (Full doze)	18.000 b
9	Atrazine+ Glyphosate+Bromoxynil (1\3 doze)	18.000 b
10	Atrazine+ Glyphosate+Bromoxynil (Half doze)	18.000 b
11	Bromoxynil+Atrazine+MCPA (Half doze)	18.000 b
12	Atrazine+ Glyphosate+Mestrozine (Half doze)	41.000 a
13	Bromoxynil+Atrazine+MCPA(Full doze)+Urea (0.25g)	41.000 a
14	Atrazine+ Glyphosate+Mestrozine+(Full doze)Urea (0.25g)	18.000 b
15	Control	0.0000 c

LSD for Chemical Combinations: 1.2178

T15 T1 T2 T4 T13 T10 T6

T15: Control, **T1:** Bromoxynil (Sole), **T2:** Atrazine (Sole), **T4:** Bromoxynil+Atrazine (Full doze), **T13:** Bromoxynil+Atrazine+MCPA(Full doze)+Urea (0.25g), **T10:** Atrazine+ Glyphosate+Bromoxynil (Half doze), **T6:** Bromoxynil+Atrazine (Half doze)

(Sole) did not significantly reduce chlorophyll levels, while Bromoxynil (Sole) and Bromoxynil + Atrazine (Full dose) led to a rapid degradation of chlorophyll and visible symptoms. The most balanced approach was found to be Atrazine + Glyphosate + Bromoxynil (Full dose), which effectively controlled weeds with minimal impact on chlorophyll levels. These findings underscore the importance of selecting the appropriate herbicide based on the desired timing for weed control and chlorophyll retention.

Recommendations

1. For rapid and efficient weed control, consider using Bromoxynil (Sole) or Bromoxynil + Atrazine (Full dose) due to their fast action and chlorophyll degradation.
2. Glyphosate is recommended for long-term weed suppression as it has a gradual effect with minimal immediate impact.
3. A balanced approach can be achieved with the use of Atrazine + Glyphosate + Bromoxynil (Full dose) to control weeds while maintaining plant vitality.
4. Consider reducing herbicide dosages in mixtures to lower costs and minimize environmental impact while maintaining efficacy.
5. Use Bromoxynil-based treatments for quick results and Glyphosate/Atrazine for slower, controlled outcomes.

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