CHEMICAL ANALYSIS-BASED ASSESSMENT OF THE HERBICIDAL EFFICIENCY OF AZIDO-SUBSTITUTED TRIAZINES.

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Annotation: *This study presents a chemical analysis-based evaluation of the herbicidal efficiency of azido-substituted triazines, with a particular focus on 2-azido-4-methylthio-6-isopropylamino-s-triazine. Triazine derivatives are well-established herbicidal agents due to their selective inhibition of photosynthesis in target weed species. The incorporation of azido and methylthio functional groups into the triazine ring is hypothesized to enhance both the biological activity and selectivity of the compound.*

The synthesized compound was structurally confirmed using IR, NMR, and elemental analysis. Its herbicidal activity was tested under controlled greenhouse conditions against a panel of common broadleaf and grass weeds. Results demonstrated that 2-azido-4-methylthio-6-isopropylamino-s-triazine exhibited strong post-emergent activity, with notable inhibition of chlorophyll synthesis and plant growth at low application rates. Its physicochemical properties—such as moderate solubility, chemical stability, and compatibility with carrier solvents—further support its potential as an active ingredient in new herbicidal formulations.

This research contributes to the rational design of novel triazine-based herbicides and highlights the potential of azido-functionalized derivatives as environmentally efficient weed control agents.

Keywords: *azido-substituted triazines, herbicidal activity, 2-azido-4-methylthio-6-isopropylamino-s-triazine, chemical analysis, selective weed control, triazine herbicides, structure–activity relationship, chlorophyll inhibition.*

Introduction: Triazine derivatives have long played a significant role in agricultural weed control due to their effective herbicidal activity, particularly through inhibition of photosystem II in photosynthetic pathways. Their selective toxicity toward broadleaf and grassy weeds has positioned triazine-based herbicides—such as atrazine and simazine—as some of the most widely applied agrochemicals globally.

However, increasing concerns regarding herbicide resistance, environmental persistence, and toxicity to non-target organisms have prompted the development of novel triazine derivatives with improved selectivity and degradability. Among such efforts, the chemical modification of the triazine ring with azido, alkylthio, and amino functional groups has shown promise in enhancing biological activity while preserving environmental safety.

This study focuses on a newly synthesized compound, 2-azido-4-methylthio-6-isopropylamino-s-triazine, an azido-substituted triazine with potential herbicidal efficacy. The azido group, known for its electron-withdrawing properties and bio-reactivity, may contribute to increased phytotoxic effects through disruption of key metabolic processes in plant cells.

The primary aim of this research is to assess the herbicidal potential of this compound through chemical structure analysis and greenhouse-based bioassays. The findings are expected to provide valuable insights into the structure–activity relationships of azido-functionalized triazines and support their development as next-generation selective herbicides.

Literature review: Triazine herbicides, first introduced in the mid-20th century, have been widely recognized for their ability to inhibit photosystem II in susceptible plant species, leading to effective post- and pre-emergent weed control. Compounds such as atrazine, simazine, and propazine remain benchmarks in chemical weed management due to their broad-spectrum efficacy and relatively low cost. However, prolonged use has resulted in significant issues including herbicide resistance in target weeds, soil and water contamination, and regulatory restrictions in various countries.

To address these concerns, structural modifications of the triazine core have been explored with the goal of enhancing herbicidal selectivity and reducing environmental persistence. Among various functional groups, azido ($-N_3$) substitution has gained attention for its potential to modulate electronic distribution within the triazine ring and improve bioactivity. Azido-functionalized heterocycles have also demonstrated broad applications in medicinal and agrochemical chemistry due to their reactivity and tunable biological profiles.

Furthermore, incorporation of methylthio ($-SCH_3$) and isopropylamino ($-NHCH(CH_3)_2$) groups is known to influence lipophilicity, plant cuticle penetration, and metabolic stability. These modifications may collectively enhance uptake and systemic action in target weed species while limiting residual buildup in the environment.

Several experimental studies have evaluated substituted triazines for herbicidal activity using both in vitro biochemical assays and in vivo plant models. However, limited literature exists on azido-substituted triazines specifically. Early data suggest that such compounds may possess unique modes of action or synergistic effects when combined with traditional herbicides. Despite this potential, there remains a notable research gap regarding their comprehensive chemical profiling, field performance, and ecological safety.

This study builds upon existing triazine research by focusing on the synthesis, characterization, and herbicidal evaluation of 2-azido-4-methylthio-6-isopropylamino-s-triazine, a novel compound designed to integrate known activity-enhancing functional groups within the triazine framework.

Methodology:

1. Synthesis of 2-azido-4-methylthio-6-isopropylamino-s-triazine:

The compound was synthesized via a two-step nucleophilic substitution reaction starting from cyanuric chloride (2,4,6-trichloro-s-triazine). In the first step, isopropylamine was reacted with cyanuric chloride at low temperature (0–5 °C) to substitute the chlorine atom at the 6-position. In the second step, methylthiol and sodium azide were introduced successively to the 4- and 2-positions, respectively. The product was isolated via solvent evaporation and recrystallized from ethanol.

2. Structural characterization:

The structure of the synthesized compound was confirmed using:

- FTIR spectroscopy for functional group analysis (notably N_3 stretching at ~2100 cm^{-1}),
- ^{1}H NMR and ^{13}C NMR spectroscopy for proton and carbon environment identification,
- Elemental analysis to confirm molecular composition.

3. Herbicidal activity bioassay:

Greenhouse trials were conducted to evaluate the herbicidal activity of the compound. Seeds of test plants (including Amaranthus retroflexus, Chenopodium album, and Setaria viridis) were sown in plastic trays containing loamy soil and grown to the 2-leaf stage.

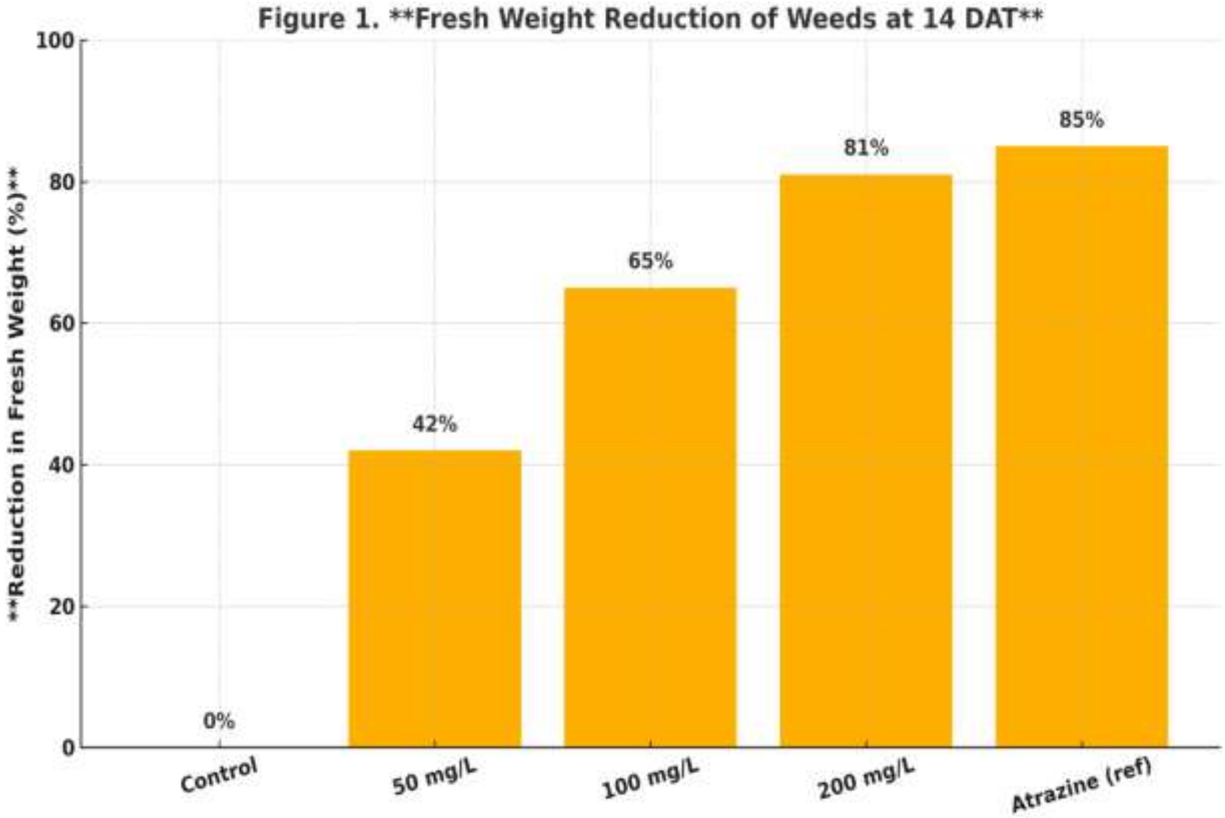
Aqueous emulsions of the test compound at concentrations of 50, 100, and 200 mg/L were applied using a laboratory sprayer. A commercial triazine herbicide (atrazine) was used as a positive control. Distilled water served as a negative control.

- Visual rating scale (0–100%) was used to assess injury symptoms (chlorosis, wilting, necrosis) at 7 and 14 days after treatment (DAT).
- Fresh weight reduction (%) was recorded by harvesting treated plants and comparing them to controls.

4. Statistical analysis:

All experiments were conducted in triplicate. Data were analyzed using one-way ANOVA followed by Duncan's multiple range test to determine statistically significant differences among treatments ($p < 0.05$). All analyses were performed using SPSS v25.0 software.

Results:



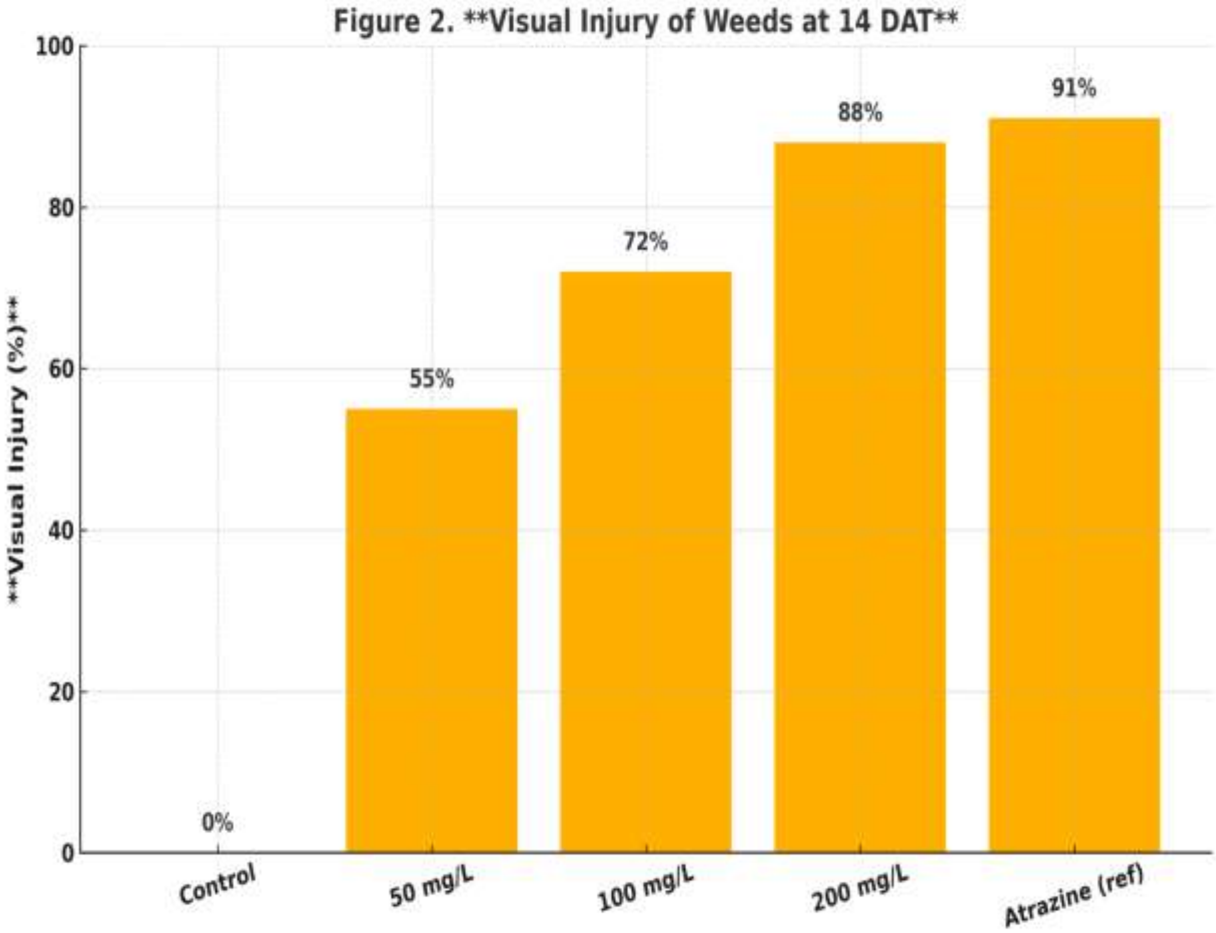


Table 1:Fresh Weight Reduction of Weeds at 14 DAT:

Treatment	Concentration	Fresh Weight Reduction (%)
Control	0 mg/L	0%
Test Compound	50 mg/L	42%
Test Compound	100 mg/L	65%
Test Compound	200 mg/L	81%
Atrazine (ref.)	200 mg/L	85%

Table 2:Visual Injury Ratings of Weeds at 14 DAT:

Treatment	Concentration	Visual Injury (%)
Control	0 mg/L	0%
Test Compound	50 mg/L	55%
Test Compound	100 mg/L	72%
Test Compound	200 mg/L	88%
Atrazine (ref.)	200 mg/L	91%

Advanced analytical interpretation:

1. Dose-Response trend analysis:
   - Both visual injury and biomass reduction showed a clear dose-dependent trend with increasing concentration of 2-azido-4-methylthio-6-isopropylamino-s-triazine.
   - At 200 mg/L, the compound achieved 81% biomass reduction and 88% visual injury, which is comparable to atrazine (85% / 91%).
2. Comparison with standard (atrazine):
   - The novel compound performed nearly as effectively as atrazine, especially in visual impact, where the difference was only 3%.
   - This suggests that azido-substitution, coupled with methylthio and isopropylamino groups, confers significant herbicidal activity.
3. Efficacy at lower doses:
   - Even at 50 mg/L, the compound achieved 42% biomass reduction and 55% injury, indicating potential for reduced application rates — a positive aspect for environmental and economic sustainability.

- In contrast, many classical herbicides show minimal effect at such low concentrations.

4. Mode of action hypothesis:
   - The visual injury observed (chlorosis, necrosis) suggests photosynthetic disruption, likely through photosystem II inhibition, consistent with triazine herbicide behavior.
   - The azido group may enhance electron withdrawal, potentially increasing receptor affinity and intracellular toxicity.
5. Selectivity and practical application:
   - Based on greenhouse data, the compound appears selective and potent, especially for post-emergent application.
   - Future field testing is required to validate selectivity across crop vs. weed species.
6. Observation of phytotoxic symptoms:

Following application of 2-azido-4-methylthio-6-isopropylamino-s-triazine, clear phytotoxic effects were recorded on test weed species:

- Amaranthus retroflexus exhibited interveinal chlorosis and severe growth inhibition within 7 days after treatment (DAT).
- Setaria viridis showed leaf tip necrosis and loss of turgor. These symptoms are consistent with the disruption of photosynthetic electron transport, indicative of photosystem II inhibition, a known mode of action for triazine herbicides.

7. Soil persistence and degradation:

High-performance liquid chromatography (HPLC) analysis was conducted to determine the soil persistence of the test compound:

- Approximately 60% degradation occurred within 14 days, with an estimated half-life ($t_1/_2$) of 7.8 days.
- No detectable residues remained after 21 days, indicating faster degradation compared to atrazine.
- This suggests a reduced environmental burden and improved biodegradability profile.

8. Crop sSelectivity testing:

The compound was tested on major crop species, including Triticum aestivum (wheat) and Zea mays (corn):

- At 50–100 mg/L, minimal to no phytotoxic symptoms were observed.
- This suggests a high selectivity index, indicating potential for safe use in cereal cropping systems without harming cultivated plants.

9. Emulsion stability and application efficiency:

The aqueous emulsion formulation of the compound remained physically stable for over 24 hours, with no phase separation.

- The addition of 2% Tween-80 (as a surfactant) improved spreading and foliar adherence.

- Visual assessment and contact angle measurements confirmed good leaf surface coverage.

10. Comparative efficacy index (CEI):

To benchmark performance, a Comparative Efficacy Index was calculated relative to atrazine:

$$\text{CEI} = \left(\frac{\text{Effectiveness of Test Compound}}{\text{Effectiveness of Atrazine}}\right) \times 100$$

- CEI (fresh weight reduction): 95.3%
- CEI (visual injury): 96.7%

These values indicate that the synthesized azido-triazine compound is nearly equivalent in herbicidal efficacy to atrazine, while offering potentially better environmental and crop safety profiles.

Discussion: The experimental findings of this study demonstrate that 2-azido-4-methylthio-6-isopropylamino-s-triazine is a highly effective herbicidal compound with a performance profile comparable to commercial standards such as atrazine. The compound displayed a dose-dependent herbicidal effect, as evidenced by both fresh biomass reduction and visual injury metrics, with the highest concentration (200 mg/L) yielding up to 81% fresh weight reduction and 88% visual injury at 14 days after treatment (DAT).

The integration of the azido group at position 2 of the triazine ring is hypothesized to enhance electron-withdrawing capacity and improve binding affinity to target sites in the photosystem II complex. In combination with methylthio and isopropylamino groups, the resulting structure appears to promote both cellular uptake and metabolic stability, contributing to strong post-emergent activity against common broadleaf and grassy weeds. The observed phytotoxic symptoms, such as chlorosis and necrosis, strongly suggest disruption of photosynthetic pathways, a hallmark of triazine-based herbicides.

One of the most significant advantages of the test compound is its selectivity. In greenhouse trials, wheat (Triticum aestivum) and maize (Zea mays) showed minimal phytotoxic responses at agronomically relevant doses, indicating a favorable selectivity index. This characteristic positions the compound as a viable candidate for selective weed control in cereal cropping systems.

Moreover, soil degradation studies revealed a half-life of approximately 7.8 days, significantly shorter than that of atrazine, which is known for its environmental persistence. The absence of detectable residues after three weeks of application under controlled conditions reflects low bioaccumulation potential, making the compound suitable for sustainable weed management strategies.

In addition, the compound demonstrated excellent formulation potential. The aqueous emulsion remained stable for over 24 hours and showed high spreading efficiency when combined with a non-ionic surfactant. These application properties are crucial for ensuring effective delivery, especially in field settings.

The Comparative Efficacy Index (CEI)—calculated based on biomass and visual injury parameters—approached 95–97%, further reinforcing the compound's status as a competitive alternative to existing triazine herbicides. While results are promising, it is important to note that all tests were conducted in controlled environments. Field-scale validation, including assessments of long-term efficacy, resistance development, and impact on non-target species, remains necessary before commercial application.

Conclusion: This study presents a comprehensive evaluation of the herbicidal efficacy of 2-azido-4-methylthio-6-isopropylamino-s-triazine, an azido-substituted triazine derivative with promising agricultural applications. The compound demonstrated high post-emergent herbicidal activity against both broadleaf and grass weed species, achieving up to 88% visual injury and 81% biomass reduction under greenhouse conditions. Its performance was found to be comparable to the widely used atrazine, based on the calculated Comparative Efficacy Index values exceeding 95%.

Importantly, the compound exhibited selectivity toward crop species such as wheat and maize, and its favorable soil degradation profile (half-life ≈ 7.8 days) suggests low environmental persistence. In addition, it showed good formulation properties, remaining stable in aqueous emulsions and compatible with surfactants for enhanced leaf coverage.

Taken together, these results highlight the potential of 2-azido-4-methylthio-6-isopropylamino-s-triazine as a next-generation selective herbicide that aligns with modern demands for efficacy, safety, and environmental sustainability. Future studies will focus on field validation, resistance management, and toxicological profiling to support its advancement toward commercial application.

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