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In Silico Evaluation of Herbicide Synergism to Identify Effective Mixtures for Weed Management in Indonesia

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ABSTRACT

This study presents a comprehensive approach to evaluating herbicide synergism through *in silico* molecular docking analysis combined with physical observations of herbicide mixtures. The research investigated nine commonly used herbicides in Indonesia, examining their potential synergistic and antagonistic interactions when mixed. Molecular docking analysis was performed using PyRx software to evaluate the interactions between herbicide active compounds and their target proteins. The analysis revealed twelve potentially synergistic combinations, with the clomazone-paraquat mixture emerging as the most promising based on both molecular docking results and compliance with Lipinski's rule of five. Physical observations in simulated tank mix conditions validated the computational predictions, showing consistent results with the *in silico* analysis. The study demonstrated that synergistic combinations maintained ligand interactions with their respective target proteins while showing favorable physicochemical properties for cellular penetration. The integration of computational methods with experimental validation provided valuable insights into the complex interactions between herbicide active compounds and their target proteins. This research establishes a robust framework for evaluating herbicide combinations, potentially leading to more effective and sustainable weed management strategies in agricultural practices.

Keywords: Herbicide synergism; *In silico* analysis; Ligand interactions; Molecular docking; Weed management

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1. Introduction

The currently proven effective and efficient weed control technique is the application of active chemical compounds in the form of herbicides [1]. However, the continuous use of herbicides leads to herbicide resistance in weed populations [2]. Therefore, farmers mix various types of herbicides in the hope of achieving more effective results [3]. Herbicide mixtures are commonly used in agricultural weed management to broaden the spectrum of weed control, reduce application costs, and delay the evolution of herbicide resistance in weed populations [4]. What is not well understood by farmers is that when two or more herbicides are combined, their interactions can result in additive, synergistic, or antagonistic effects on the efficacy of weed control [5]. Synergism occurs when the combined effect of two herbicides is greater than the sum of their individual effects, while antagonism refers to a reduced effect compared to what would be expected based on the herbicides' individual activities [6]. Understanding and identifying these interactions is crucial for optimizing herbicide use and developing effective weed management strategies [7].

The concept of herbicide synergism has intrigued weed scientists for decades, leading to numerous studies aimed at testing and evaluating the interactions within herbicide mixtures [8]. Despite these efforts, conclusive data on true synergism remain elusive due to the varying responses of different plant species and the occasional development of resistance when herbicide mixing is not done properly [9]. Several methods have been proposed to assess and characterize the synergistic, antagonistic, and additive effects of herbicide combinations, including direct plotting of dose-response curves, isobole analysis, and statistical modeling approaches [5]. In recent years, there has been growing interest in systematically screening herbicide combinations to identify novel synergistic pairs [10]. This strategy offers the potential to discover previously unknown interactions that could enhance weed control. For instance, a comprehensive study evaluated 276 pairwise combinations of 24 herbicides with different modes of action against the model plant *Arabidopsis thaliana* [5]. Their research uncovered several new synergistic herbicide pairs, highlighting the effectiveness of systematic screening approaches.

However, accurately detecting and quantifying herbicide synergism presents several challenges [2]. These include selecting appropriate experimental designs and dose levels for testing herbicide combinations, choosing suitable methods for data analysis and interpretation of results, accounting for variability in plant responses and potential confounding factors, and distinguishing true synergism from other forms of interaction or statistical artifacts [5]. To address these challenges, researchers have developed various statistical approaches for analyzing herbicide interaction data. These range from simple multiplicative models [1] to more complex nonlinear regression [2] and mixed-model techniques [3]. Each approach has its strengths and limitations, and the choice of method can significantly impact the conclusions drawn from experimental data [11].

This study aims to contribute to ongoing efforts in herbicide discovery and development by leveraging advancements in bioinformatics and reducing reliance on traditional wet lab methods. Our approach utilized an *in silico* approach through molecular docking evaluation to predict the synergistic and antagonistic properties of herbicide mixtures commonly used by farmers in Indonesia. By performing computational studies on the interactions between herbicide active compounds and their target proteins, we seek to provide a robust framework for evaluating possible herbicide combinations that can be used by the farmers.

The approaches employed here draw inspiration from multi-combination compound therapy for humans that have shown promising results related to synergistic effects [6, 12-14], such as a study on multi-combination

compounds of phytonutrients for treating joint pain [6]. By adapting these methodologies to herbicide studies, we introduce a novel *in silico* approach that addresses key challenges in herbicide interaction analysis. This method significantly reduces the time and costs associated with conventional synergism and antagonism research [4], making it particularly valuable in the context of Indonesia's agricultural landscape. Our work bridges the gap between laboratory conditions and real-world applications by moving beyond the limitations of traditional synergism tests, which often rely on analytical grade chemicals [5] that may not accurately represent field conditions. Furthermore, we examine physical changes in mixtures that mimic farmers' tank mixtures, providing crucial insights into potential chemical interactions between different herbicides. This comprehensive approach not only advances the field of herbicide research but also offers practical implications for agricultural practices in Indonesia, where such studies have been limited. By combining computational methods with practical considerations, our study paves the way for more efficient and effective herbicide use, potentially revolutionizing weed management strategies in the region.

2. Materials and Methods

This study provides a comprehensive approach to evaluating herbicide synergism, combining computational methods with physical observations on a mixture that only contains two herbicides diluted in distilled water at their recommended working dosage. The observation on the mixture was also used to validate the computational analysis results, identifying promising combinations for further study or practical application. A multi-faceted approach was employed for the investigation that can significantly help farmers in Indonesia and other developing nations in choosing herbicides that can be mixed for optimum weed control in their field. Indonesian farmers predominantly rely on nine brands accessible in the national market: Starlon, Ronstar, Sidaxone, Callisto, Scepter, Command, Sidaron, Roundup, and Ignite. For each herbicide, the active compounds and their target proteins were identified from the Herbicide Resistance Action Committee (HRAC) Global database (https://hracglobal.com/). Three-dimensional chemical structures of active compounds were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The structures of the target proteins were sourced from the Protein Data Bank Japan (https://pdbj.org/), focusing on proteins from the model plant *Arabidopsis thaliana* (except for glutamine synthetase's structure that originates from *Zea mays*). When the target protein is a highly complex protein, such as photosystem II, and the structural data file was too large for autodocking processes, the alternative protein structure was obtained from the AlphaFold database [15] based on the amino acid sequence of the subunit targeted by the herbicide active compound. This AlphaFold-derived structure was processed and aligned with the large and complex structure of the target protein from the PDBj using PyMOL Molecular Graphics System for Windows, Version 3.0 (Schrödinger, LLC). Truncating the target proteins into smaller specific segments can ensure efficient computational processing [16].

The *in silico* analysis began with the preparation of molecular structures where the herbicides active compound structures were converted to PDB format using PyMOL and processed to minimize kinetic energy using PyRx [17]. The protein structures were cleaned by removing water molecules and other bound compounds using Discovery Studio 2024 (BIOVIA, Dassault Systèmes). Initial validation was performed through re-docking of native ligands with target proteins using PyRx software, with grid box sizes determined for each protein [7, 18].

Molecular docking was then performed using the prepared individual active compound structures and their respective validated target proteins. Docking results were analyzed using

ProteinsPlus server (https://proteins.plus/) [19], considering parameters such as Root Mean Square Deviation (RMSD) values, binding energy, inhibition constant (K_i), and chemical bonds that included hydrogen bonds and van der Waals bonds [8]. Synergism and antagonism were analyzed by docking combinations of two active herbicide compounds with their respective target proteins. Synergism was indicated by ligand interactions occurring on both target proteins, whereas antagonism was when the ligand interactions did not occur or occurred only with one of the target proteins [9].

To further validate potential synergistic combinations, an ADME (Absorption, Distribution, Metabolism, and Excretion) analysis was conducted using Lipinski's Rule of Five. This analysis was performed using an online tool (http://www.scfbio-iitd.res.in/software/drug-design/lipinski.jsp) [20, 21], assessing parameters such as H-donor and H-acceptor counts, molar mass, molar refractivity, and log-P values [10]. Other than that, the synergistic herbicide mixtures forming ligand interactions and hydrogen bonds on target proteins were visualized using Discovery Studio 2024.

To complement the computational analysis, physical observations were conducted to simulate the tank mixing processes commonly performed by farmers. These simulations were carried out on a small scale using 96-well plates. To do this, the recommended dosage for each of the nine tested herbicides was prepared per the manufacturer's instructions by mixing the herbicidal liquid/powder with distilled water. From this prepared dosage, 50 µL of Roundup (Glyphosate) was mixed directly with 50 µL of Scepter (Imazaquin) in one well, simulating what farmers often do in the field. In total, there were 36 herbicide pairs. Observations were made in the first 5 minutes and 24 hours after mixing for any signs of precipitation, changes in solubility, color, and odor, along with foam formation. These physical changes were interpreted as indicators of chemical reactions between active compounds and compared with the *in silico* prediction results to validate the computational approach [22-24].

Finally, the results from the *in silico* analysis, ADME testing, and physical observations were compiled and compared. Synergistic and antagonistic combinations were identified based on the combined results of all analyses, and findings were interpreted in the context of existing literature on herbicide interactions and weed management practices [11].

3. Results and Discussion

The screening to identify synergistic and antagonistic properties of herbicide mixtures was conducted using *in silico* approaches [25]. The screening data were then supported by observational data on physical changes in herbicide mixtures that mimics the tank mixing often performed by farmers. Nine herbicides accessible in the Indonesian agrochemical market, representing different modes of action, were employed in this study (Table 1) [26, 27].

Table 1. Target Protein Data with Herbicide Active Compounds.

Herbicide	Active Compound	Target Protein	PDB Code
Command	Clomazone	1-deoxy-D-xylulose-5-phosphate synthase	7BZX
Ronstar	Diuron	Photosystem II (specifically D1 subunit)	5MDX
Ignite	Glufosinate-am	Glutamine synthetase	2D3A
Roundup	Glyphosate	Enolpyruvyl shikimate phosphate synthase	7PXY
Scepter	Imazaquin	Acetolactate synthase	1Z8N
Callisto	Mesotrione	4-hydroxyphenylpyruvate dioxygenase	1SP9
Ronstar	Oxadiazon	Protoporphyrinogen oxidase, mitochondrial	1SEZ
Sidaxone	Paraquat	PS I Electron diversion (specifically ferredoxin)	3VO1
Starlon	Triclopyr	Auxin-responsive protein (specifically TIR1)	2P1O

The downloaded structures of the active herbicidal compounds were converted to *.pdb* format in PyMOL and processed to minimize its kinetic energy in PyRx [28], [29]. This step is important to obtain the compatibility of the ligand (active herbicidal compound) with its target protein when docked in molecular docking simulations [30]. The energy minimization process optimizes the ligand structure to achieve its most stable conformation, as demonstrated by Fu et al. [31] in their study of 4-Hydroxyphenylpyruvate Dioxygenase (HPPD) inhibitors using the Merck molecular force field (MMFF94) with conjugate gradient algorithm. This optimization evaluates various atomic interactions, including van der Waals forces, electrostatic interactions, and bond-stretching parameters, which Fu et al. [32] and Ndikuryayo et al. [33] have shown significantly improves docking results and binding affinity predictions in herbicide-target interaction studies.

Many of the acquired target protein structures from the database already have bound ligands (often referred to as native ligands). The bound compounds from this structure had to be cleaned in Discovery Studio 2024. Redundant water molecules and co-crystallized ligands can lead to incorrect binding poses and interfere with proper ligand placement [31, 34-36]. While some studies retain specific water molecules that play important roles in protein-ligand interactions, most traditional molecular docking methods often ignore water impacts to simplify the calculations [36]. However, recent research has shown that water molecules can significantly influence binding modes, particularly in cases where they mediate the binding of ligands with target proteins through hydrogen bond networks [32].

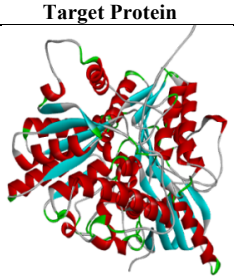
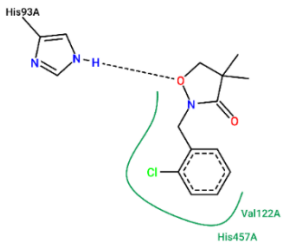
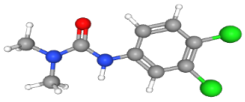
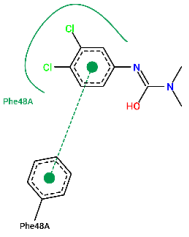
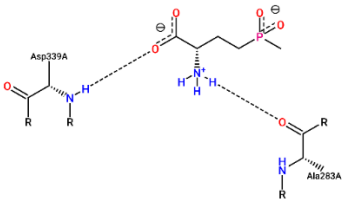
After cleaning, the protein structure requires additional preparation steps, including hydrogen addition and correction of incomplete residues, followed by assigning appropriate force field potentials for accurate molecular docking simulations. Protein conformation changes during preparation can potentially alter the initial structure and affect binding site geometry [36-38]. Therefore, initial validation of proper protein conformation was performed by re-docking the native ligand with the cleaned target protein using PyRx to determine the optimal grid box parameters [34, 39]. For instance, in the case of ferredoxin of the PS I Electron diversion targeted by paraquat, the optimal grid box parameters were established at x: 5.5299, y: -0.0171, and z: 58.6430 with dimensions of 25 Å. Significant deviations from these initial grid box coordinates would indicate inappropriate protein conformational changes, rendering the structure unsuitable for docking studies. The grid box size is crucial, as oversized boxes increase computational costs and may result in non-specific binding predictions, while undersized boxes may exclude portions of the binding site or restrict proper ligand positioning [8, 40]. This validation protocol was systematically applied to all herbicide target proteins in this study.

The cleaned target protein structure acquired from the previous re-docking step was subsequently docked again with the prepared active compound's structure. This was performed for each active herbicidal compound with its respective target protein. The docking results were then analyzed in ProteinsPlus to evaluate potential protein-ligand interactions [40]. The server's prediction accuracy improves when comprehensive information about both ligands and their protein targets is provided to the system [34, 41]. However, for reliable computational prediction of binding affinity, both binding affinity and specificity must be considered, and these predictions should ultimately be validated through experiments to confirm physiological effects [42-45]. The ProteinsPlus analysis serves as a valuable initial assessment of interaction quality, revealing the nature and number of interactions between herbicide active compounds and their respective target proteins [46].

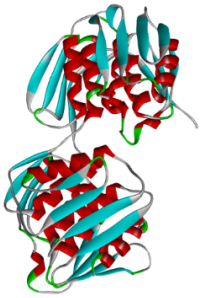
Other than the possible ligand interactions formed, other parameters chosen were RMSD values, binding energy, K_i values, hydrogen bonds, van der Waals interactions, and other types of chemical bonds. In this initial test, the chosen RMSD value was the lowest, and it had to be below 2 Å (RMSD ≤ 2 Å), as this threshold is widely accepted as a validation criterion for docking success [36]. This value indicates that the herbicide active compound has compatibility with its target protein structure, forming a good ligand interaction with minimal structural deviations [47]. The binding energy value chosen was the most negative value, indicating that the bond between the active herbicidal compound and its target protein can occur spontaneously, with lower values suggesting stronger binding affinity and better complex stability [48]. The inhibition constant (K_i) value chosen must also be as small as possible because a smaller K_i value indicates stronger bonds between the herbicide active compound and its target protein, reflecting the energy required for ligand-receptor interaction at the binding site [49, 50].

Among all chemical interactions, hydrogen bonds are particularly crucial as they play a dominant role in determining the specificity and strength of ligand-protein interactions. These hydrogen bonds, along with water-mediated interactions, significantly influence the binding mode and stability of the protein-ligand complex [51, 52]. The presence of specific water molecules can enhance these interactions by mediating hydrogen bonds between the protein and ligand through their dual ability to act as both donor and acceptor. The results of this comprehensive *in silico* analysis, including all evaluated parameters and possible ligand interactions generated by ProteinPlus, are summarized in Table 2 for each herbicide active compound with its respective target protein.

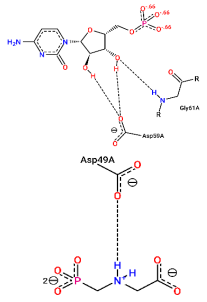
Table 2. Results of Docking Herbicide Active Compounds with Target Proteins.

Active Compound Structure	Target Protein	Ligand Interaction(s)
 Clomazone	 7BZX	 1 possible ligand interaction
 Diuron	 5MDX	 1 possible ligand interaction
 Glufosinate-ammonium	 2D3A	 1 possible ligand interaction

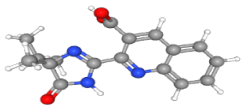
Glyphosate



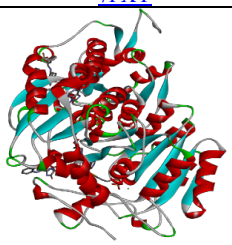
7PXY



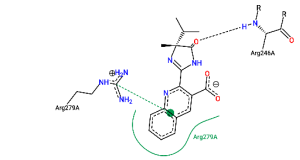
2 possible ligand interactions

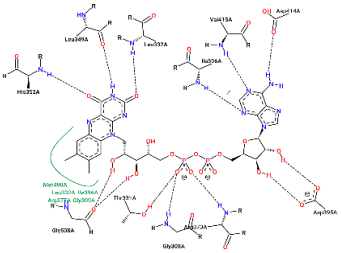


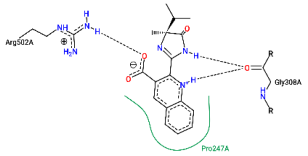
Imazaquin

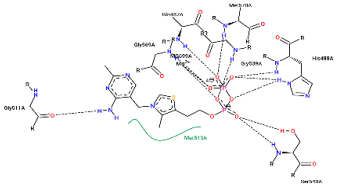


1Z8N

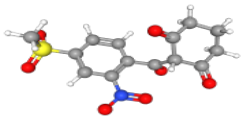




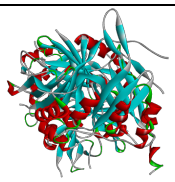




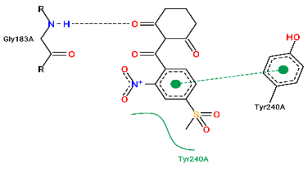
4 possible ligand interactions



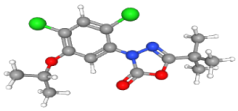
Mesotrione



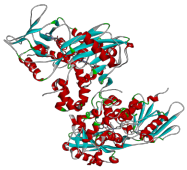
1SP9



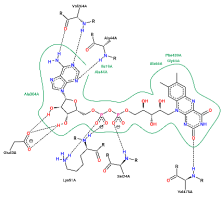
1 possible ligand interaction

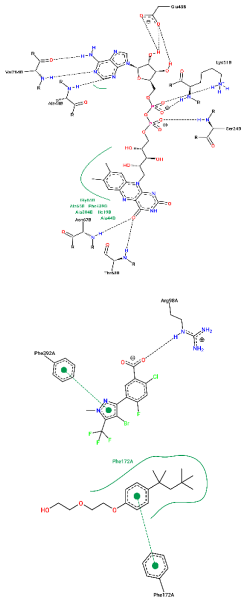


Oxadiazon

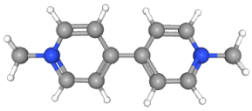


1SEZ

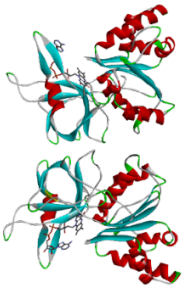




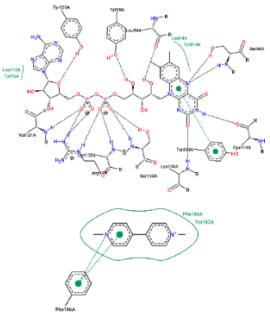
4 possible ligand interactions



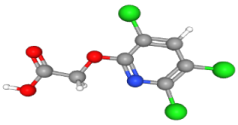
Paraquat



3VO1

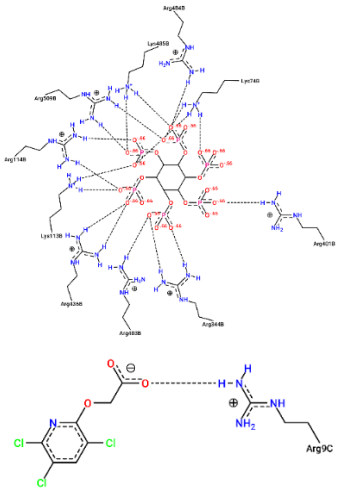


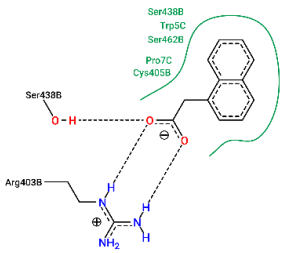
2 possible ligand interactions



Triclopyr

2P1O





3 possible ligand interactions

Legends:

hydrogen bond — metal interaction — pi-pi interaction
ionic interaction — cation-pi interaction — hydrophobic contact

Note: Column 4 (Ligand Interaction(s)) in Table 2 shows how many possible ligand interactions occur between the active herbicide compound and its target protein.

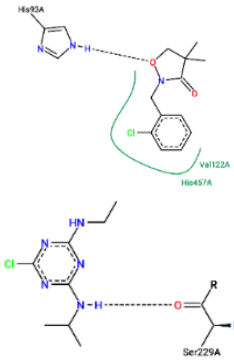
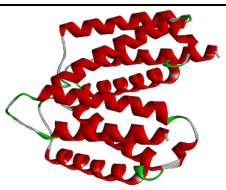
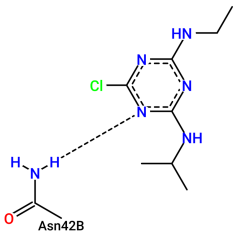
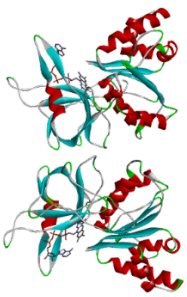
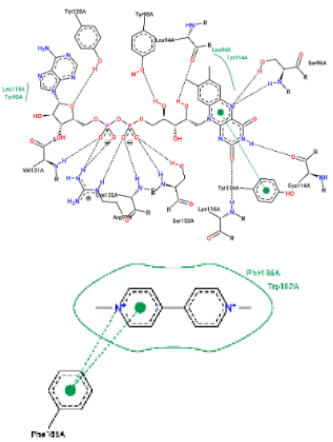
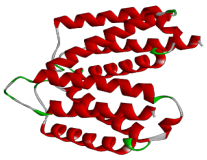
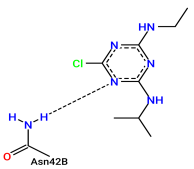
Drastic changes in RMSD values, binding energy, and K_i most likely indicate that mixing two herbicide active compounds is antagonistic [53]. However, false positives may be acquired from early assumptions based on the changes in parameter values alone, especially if the changes are still within the range of minimum values for each parameter [54]. Therefore, in this study, the number and the forms of the possible ligand interactions resulting from docking between mixtures of two different active compounds with their respective target proteins were used as additional validation criteria.

Research has consistently shown that antagonistic interactions occur more frequently than synergistic ones, with approximately 67% of herbicide combinations showing antagonism and only 33% showing synergism [1]. These interactions can occur at various stages: within the spray tank, on the leaf surface, and/or inside the plant, resulting in both physicochemical and physiological interactions that affect herbicide efficacy [55]. When chemical interactions occur between mixed herbicides, the combined effect often results in reduced compatibility with their respective target proteins, particularly when the herbicides share the same mode of action. The antagonistic effects can manifest through decreased uptake/translocation or physiological changes in the plant, while physicochemical incompatibility in the spray tank usually leads to visible physical changes in the mixture, such as precipitation, foam formation, or color changes [56, 57].

To validate this *in silico* methodology's ability to predict herbicide mixture's interaction, testing was carried out using known synergistic combinations as positive controls. Literature studies have demonstrated synergism between clomazone-atrazine and paraquat-atrazine combinations (see Table 3) [5]. The clomazone-atrazine combination showed moderate synergism with enhanced effects of 5.9% to 19.3% depending on application timing, while the clomazone-paraquat mixture exhibited synergistic effects ranging from 14% to 21.8% under specific conditions [5]. Our *in silico* trials on these known synergistic mixtures demonstrated that both compounds in each mixture maintained their original ligand interactions with their respective target proteins, as shown in Table 3. Specifically, for the clomazone-atrazine combination, the computational analysis revealed two possible ligand interactions between clomazone and its target protein (7BZX), and one possible ligand interaction between atrazine and its target protein (5X56) (Table 3, column 4). The preservation of these ligand interactions *in silico* aligns with experimental findings, suggesting that when chemical properties remain unchanged in mixture, each compound can still effectively bind to its target protein, potentially leading to synergistic effects. This validation using known synergistic combinations provides confidence in the methodology's ability to

predict potential interactions between herbicide mixtures. The ability to predict these interactions computationally before experimental testing could significantly streamline the process of identifying effective herbicide combinations.

Table 3. Results of Clomazone-Atrazine and Paraquat-Atrazine Docking.

No.	Molecule	Target Protein	Ligand Interaction(s)
1.	Clomazone-atrazine	 7BZX (target protein of Clomazone)	 2 possible ligand interactions
		 5X56 (target protein of Atrazine)	 1 possible ligand interaction
2.	Paraquat-atrazine	 3VO1 (target protein of Paraquat)	 2 possible ligand interactions
		 5X56 (target protein of Atrazine)	 1 possible ligand interaction

Legends:

hydrogen bond
ionic interaction
metal interaction
cation-pi interaction
pi-pi interaction
hydrophobic contact

Building upon the validated methodology using known synergistic combinations, we extended our *in silico* screening to evaluate novel herbicide mixtures. Mixing herbicides with different modes of action is a crucial strategy, not only to broaden the spectrum of weed control but also to decrease the development of herbicide resistance [1, 5]. Recent research has emphasized that mixing herbicides is more effective than rotating between them for resistance management [1, 5], as weeds find it more difficult to evolve resistance to complex, unpredictable control methods. In our initial *in silico* screening, we observed the formation of ligand interactions when two active compounds were docked on their respective target proteins. The absence of initial ligand interaction or changes in the ligand interaction mechanism was interpreted as an indicator of potential antagonistic reactions between the mixed active compounds.

The docking analysis results of herbicide active compound mixtures on each target protein are shown in Figure 1. Our analysis defined synergism as the occurrence of ligand interactions on each target protein, while the presence of interaction with only one target protein or complete absence of ligand interactions was classified as antagonism. This approach aligns with previous research showing that herbicide mixtures are rarely synergistic, with most combinations being either antagonistic or additive [1, 5]. Among the tested combinations, synergistic interactions were observed in twelve pairs: clomazone-imazaquin, clomazone-oxadiazon, clomazone-paraquat, diuron-glyphosate, glufosinate-imazaquin, glufosinate-paraquat, glyphosate-imazaquin, imazaquin-oxadiazon, imazaquin-paraquat, mesotrione-oxadiazon, oxadiazon-paraquat, and paraquat-triclopyr.

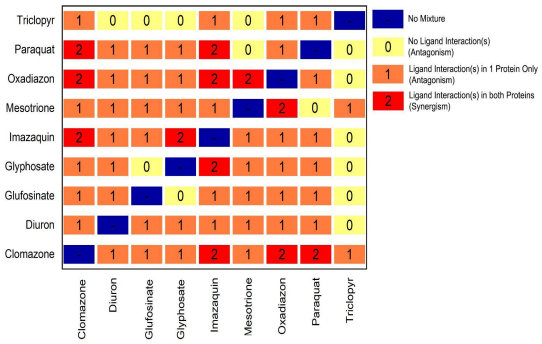


Fig. 1. Results Analysis of Active Herbicidal Compound Mixture Interactions on Each Target Protein.

Analysis of herbicide synergism and antagonism was then continued with the simulation of tank mixture tests commonly practiced by farmers in the field. This practical validation complements the *in silico* docking method by providing physical evidence of compatibility. The analysis evaluated multiple parameters including precipitation, solubility changes (sediment formation), color changes, pH alterations, odor changes, foam formation, and other changes in the physical appearance of the mixtures. According to Bianchi et al. [58], physicochemical incompatibility in the spray tank usually leads to herbicide antagonism, as interactions between herbicides can occur at various stages: within the spray tank, on the leaf surface, and/or inside the plant. When physical changes occur in the mixture, it indicates that the herbicide active compounds have undergone chemical reactions forming new compounds, thereby altering their original chemical and physical properties. These alterations typically result in the loss or decrease of ligand interactions with the target proteins, as the active compounds no longer maintain their original molecular structure and binding capabilities. Research has shown that such physicochemical interactions in tank mixtures can significantly impact herbicide efficacy, potentially leading to reduced uptake/translocation and physiological changes in the plant [1, 55]. The results of our

tank mixture analysis, presented in Fig. 2, provide crucial information about the physical compatibility of different herbicide combinations, which serve as an important predictor of their potential field performance.

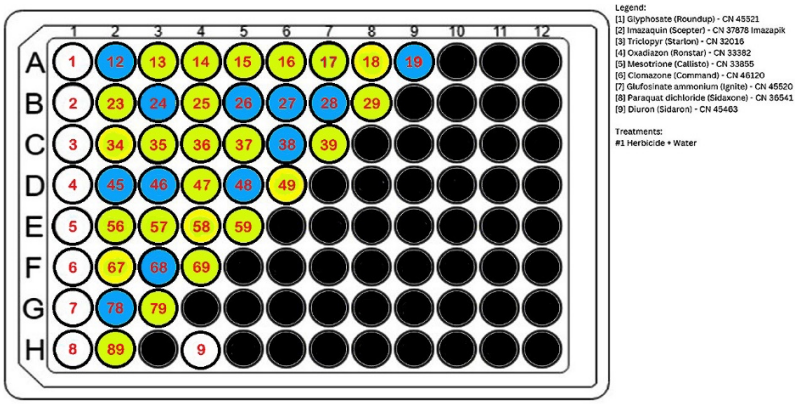


Fig. 2. Observation Data of Physical Changes in Herbicide Mixtures.

The results of *in vitro* validation testing using mixing in a 96-well plate demonstrated synergism in twelve herbicide combinations: clomazone-imazaquin (26), (46), clomazone-paraquat (68), diuron-glyphosate (19), glufosinate-imazaquin (27), glufosinate-paraquat (78), glyphosate-imazaquin (12), imazaquin-oxadiazon, imazaquin-paraquat (28), mesotrione-oxadiazon (45), oxadiazon-paraquat (48), and paraquat-triclopyr (38); each number in the bracket represents one active herbicidal compound. These results strongly correlate with our *in silico* predictions, validating the computational approach for assessing herbicide mixture interactions. This alignment between *in silico* and *in vitro* results is particularly significant as it demonstrates the reliability of computational methods in predicting herbicide interactions.

The penetration pattern of herbicide active compounds into plant cells shares similarities with drug compounds reaching their target proteins, making ADME considerations important [5, 23]. Traditional Lipinski's five rules specify that compounds should have: H-donor value ≤ 5, H-acceptor ≤ 10, molar mass ≤ 500, molar refractivity 40-130, and log-P ≤ 5. However, recent research has shown that herbicides often deviate from these parameters. Herbicide molecules typically have higher molecular weights and more hydrogen bond acceptor atoms, while also having a lower range of hydrogen bond donor atoms compared to the parameters for human oral drugs [5, 59].

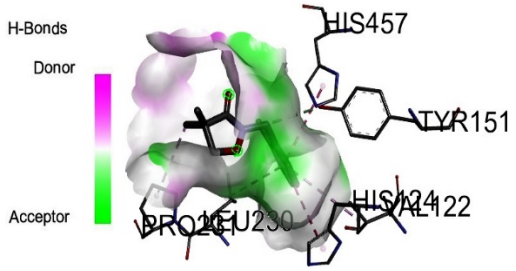
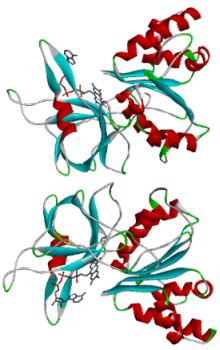
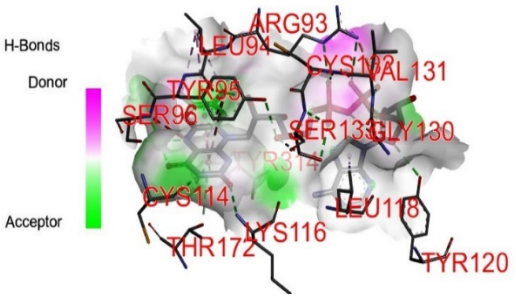
For our analysis, the structures of two different active compounds were combined using PyMOL while maintaining their chemical independence. When evaluated against Lipinski's five rules, only the clomazone-paraquat mixture met all the criteria for optimal cellular penetration and functionality. This finding aligns with previous studies that have demonstrated the effectiveness of this combination using different methodological approaches [5, 60]. The unique compatibility of the clomazone-paraquat mixture suggests that while Lipinski's rules may not be universally applicable to herbicides [23, 60], they can still provide valuable insights for specific combinations.

Table 4. Results of Lipinski's Five Rules Validation on Synergistic Herbicide Mixtures.

No.	Herbicide Mixtures	H-Donor	H-Acceptor	Molar Mass	Molar Refractivity	Log-P	Results
1	Clomazone-imazaquin	4	9	523.5	121.745872	-0.188080	No
2	Clomazone-oxadiazon	1	7	553.5	120.048660	0.968580	No
3	**Clomazone-paraquat**	**0**	**3**	**397.5**	**99.122002**	**0.524680**	**Yes**
4	Diuron-glyphosate	1	6	402.0	0.000000	0.764560	No
5	Glufosinate-imazaquin	3	7	492.0	0.000000	0.746320	No
6	Glufosinate-paraquat	**2**	**4**	**367**.0	0.000000	0.76640	No
7	Glyphosate-imazaquin	4	9	463.0	30.980600	-0.649900	No
8	Imazaquin-oxadiazon	3	6	626.0	0.000000	0.000000	No
9	Imazaquin-paraquat	3	3	470.0	0.000000	0.000000	No
10	Mesotrione-oxadiazon	1	11	654.0	0.000000	0.000000	No
11	Oxadiazon-paraquat	0	3	531.0	131.259491	2.081620	No
12	Paraquat-triclopyr	0	3	442.5	91.436874	0.865170	No

The synergistic interactions were visualized using Discovery Studio 2024, revealing the formation of hydrogen bonds between the active compounds and their target proteins. The visualization results, presented in Table 5, show distinctive surface layers wrapping the ligands within their target proteins, indicating maintained chemical activity. Recent research has emphasized that the preservation of hydrogen bond donation capability in herbicide mixtures is crucial for maintaining their individual efficacy [45]. This independent reactivity of each active compound, evidenced by the maintained hydrogen bonding patterns, supports their ability to form specific ligand interactions with their respective target proteins.

Table 5. Synergistic Interactions of Active Compounds and Target Proteins.

No.	Protein Target	Interaksi
1.	 7BZX (target protein of Clomazone)	
2.	 3VO1 (protein target Paraquat)	

4. Conclusion

This study presents a comprehensive approach to evaluating herbicide synergism, combining *in silico* molecular docking analysis with physical observations of herbicide mixtures. Our findings demonstrate that the integration of computational methods and experimental validation can provide valuable insights into the complex interactions between herbicide active compounds and their target proteins. The *in silico* screening successfully identified several potentially synergistic herbicide combinations, with the clomazone-paraquat mixture emerging as the most promising candidate based on both molecular docking results and compliance with Lipinski's rule of five. This combination showed consistent ligand interactions with target proteins and favorable physicochemical properties for cellular penetration and efficacy.

However, it is important to note that while computational methods offer a powerful tool for initial screening, they must be complemented by experimental validation and field trials. The physical observations of herbicide mixtures in simulated tank mix conditions provided crucial information on potential chemical interactions and physical changes that could affect herbicide efficacy. This study underscores the importance of a multi-faceted approach in herbicide research, combining advanced computational techniques with traditional experimental methods. Future work should focus on validating these findings through greenhouse and field trials, as well as investigating the underlying mechanisms of synergism in the identified herbicide combinations. This research contributes to the ongoing efforts to optimize herbicide use in agriculture, potentially leading to more effective and sustainable weed management strategies.

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