I. INTRODUCTION

The critical issue of weed infestation remains a significant concern in modern agriculture, particularly when adequate regulation and proper management are not implemented, posing a substantial threat to global food security (Kumar et al., 2024). When left uncontrolled, weed growth aggressively outcompetes surrounding farmers' crops, reducing the availability of essential resources, including water, soil nutrients, light, carbon dioxide, and growing space (Horvath et al., 2023). The occurrence of intensified competition in living environments leads to substantial decreases in crop productivity, resulting in yield losses of up to 20% and exceeding 50% of the expected yield, and is influenced by the composition of weed species and the crops grown (Singh et al., 2022). In addition, many farmers across various agricultural systems report constraints in effectively managing weed infestations, as this requires considerable financial investment and labor-intensive approaches. As a consequence of this challenge, the investigation of an enhanced weed management system remains the primary focus to help minimize environmental impacts and strengthen global food production (Monteiro & Santos, 2022).

For decades, herbicides have been used to control weed populations across various agricultural systems (Baćmaga et al., 2024). Their use has contributed to the development of farmers' crop protection and productivity strategies, as optimal herbicide application reduces weed growth, thereby suppressing competition for resources, ultimately leading to higher crop productivity (Paul et al., 2024). Despite its well-established capacity to manage weed spread in agriculture, the continuous overreliance on herbicide applications has led to the emergence of herbicide resistance, driven by the evolving abilities and genetic diversity of weed populations (Damalas & Koutroubas, 2024). Given this condition, the intensifying prevalence of herbicide-resistant weeds poses a more complex challenge, as an escalation in resistance cases will lead to greater herbicide use by farmers due to the significantly reduced herbicide efficacy from the genetically evolved weed populations, which will eventually accelerate the rate of weed evolution, worsening the problem of weed resistance (Ofosu et al., 2023).

Several data values compiled by the Herbicide Resistance Action Committee (HRAC) provide evidence of an increase in herbicide resistance worldwide across multiple weed species and herbicide groups. Conventionally, each herbicide group is classified by its mode of action (MoA), which is defined by the target site or biochemical pathway affected in plants. Despite the widely used classification framework for herbicides developed by the HRAC, the agricultural industry has been heavily influenced by overreliance on single-mode-of-action herbicides over the past four decades, as noted by Hulme (2023) and Parven et al. (2024). According to the International Herbicide-Resistance Weed Database of HRAC, as of 2026, there are approximately 541 cases of herbicide resistance across 75 countries, involving 273 species in 156 dicot and 117 monocot families, and resistance to 168 herbicide types with 21 different sites of action (Heap, 2026). The reliance on single active ingredients in common herbicide practices has further accelerated the development of herbicide resistance (Perotti et al., 2020). Therefore, at this stage of research, many scientists have underscored the importance of understanding the prevalence of herbicide resistance to establish more sustainable weed management practices (Nath et al., 2024).

One promising approach to respond to this rising resistance is to use synergistic combinations of two or more herbicide compounds, in which interactions are expected to enhance their overall herbicidal activity (Beckie et al., 2019). According to Barbieri et al. (2022), combining herbicides with different modes of action can reduce the likelihood that resistant weed populations survive and reproduce, thereby making this approach a suitable alternative to single-mode chemical control. In addition, the use of herbicide combinations in agricultural systems is expected to further reduce the rate of evolution in weed populations compared with known herbicide rotations (Renton et al., 2024). However, the adoption of herbicide combinations may also lead to various physicochemical and physiological interactions, ranging from synergistic to antagonistic effects on weed management, depending on applied herbicide doses and weed growth stages, and are highly complex and challenging to predict (Ndikuryayo & Yang, 2023).

As a result, researchers have shifted their investigations toward more detailed mechanistic studies of herbicide synergies, including the use of *in silico* approaches. An effective combination promotes more controlled weed population growth while also reducing the risk of resistance and environmental impacts on surrounding crops (Pingarron-Cardenas et al., 2024). In earlier stages of *in silico* analysis (Sulaiman et al., 2024), the promising synergistic effects of Glufosinate (Glu) and Paraquat (Par), as well as Glyphosate (Gly) and Paraquat (Par), have been established. Preliminary findings on these two herbicide combinations highlight the potential for synergistic effects to enhance herbicidal activity (Sulaiman et al., 2024). To further understand the synergistic effects of glyphosate and paraquat, as well as of glufosinate and paraquat, the need to manage the alarming issue of weed resistance is emphasized. This research aims to address this issue.

I.I Objective

This research aims to evaluate the synergistic effects of the selected herbicide combinations between Glyphosate (Gly) and Paraquat (Par), as well as Glufosinate (Glu) and Paraquat (Par), on targeted plants of *Nicotiana tabacum* (representing dicotyledon) and *Cyperus rotundus* (representing monocotyledon).

I.II Scope of Work

The scope of work of this research is:

- Prepare and conduct the germination, along with the cultivation procedure, for both target plants, *N. tabacum* and *C. rotundus.*
- Prepare and combine the herbicide combinations of Glufosinate (Glu) and Paraquat (Par) and Glyphosate (Gly) and Paraquat (Par).
- Conduct an in vivo assessment of the expected synergistic effects of using two target plants, which is validated using the application of green pixel analysis using ImageJ software, fresh weight and dry weight measurement, and chlorophyll extraction and quantification.

I.III Hypothesis

The hypotheses of this research include:

- H_0 (Null hypothesis): The application of herbicide type combinations between Glyphosate-Paraquat and Glufosinate-Paraquat does not establish a significant phytotoxic effect in comparison to the expected effects of individual herbicides, indicating the absence of a synergistic interaction between the two herbicide combinations.
- H_1 (Alternative hypothesis): The application of herbicide type combinations between Glyphosate-Paraquat and Glufosinate-Paraquat does establish a significant phytotoxic effect in comparison to the expected effects of individual herbicides, indicating the presence of a synergistic interaction between the two herbicides.