COMBINED EFFECTS OF BIOSTIMULANT AND HERBICIDE APPLICATION ON *MEDICAGO SATIVA* L.

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Abstract
Ambrosia artemisiifolia L. is an invasive weed species with major negative agronomic, economic and health impacts, reducing crop yield through intense competition for resources and producing highly allergenic pollen with harmful effects on human health. Alfalfa (Medicago sativa L.) is most vulnerable in the first developmental periods, when biomass accumulation is slow and seedlings cannot effectively compete for resources, leading to significant loss in stand density and productivity. The presence of A. artemisiifolia in M. sativa forage pastures is problematic, as it has no nutritional value and may even be harmful to livestock. The aim of this study is to evaluate the efficacy and selectivity of halauxifen-methyl and imazamox, applied individually and in combination with biostimulants, in a M. sativa forage field infested with A. artemisiifolia population. While halauxifen-methyl is not currently registered for use on this crop and no official data exists regarding its application with biostimulants, imazamox is approved and is the comparative standard for this trial. This study seeks to highlight the potential of halauxifen-methyl as a treatment for alfalfa, as well as the role of biostimulants in reducing herbicide-induced stress and enhancing M. sativa tolerance to herbicides during its early developmental stages. The mixture of halauxifen-methyl and biostimulants provided the highest control levels, ranging from 70 to 95% during the trial period. Control of A. artemisiifolia reached 90 - 95% at 28 days after application, compared to 85- 90% without a biostimulant. Alfalfa injury symptoms in the variant treated with halauxifen-methyl and biostimulants were transient, consisting mainly of slight to moderate chlorosis, with the plants fully recovering by the third assessment, at 42 days after the application. This study confirms that the post-emergence application of halauxifen-methyl at 1 L/ha combined with biostimulants, provided excellent control of A. artemisiifolia. These results demonstrate that the combination of herbicides and biostimulants offer a promising option for an efficient and sustainable chemical control.

Key words: *halauxifen methyl, biostimulant, alfalfa, chemical weed control, herbicide efficacy*

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the most important perennial forage legumes worldwide, valued for its high protein content, nitrogen-fixing capacity, and contribution to soil fertility and sustainable livestock systems. However, its productivity and persistence are often compromised by strong competition from weeds that reduce stand density, forage quality, and seed yield [1], [2]. Weed management in alfalfa remains particularly challenging due to the crop's morphological and physiological similarity to many broadleaf weeds, which limits the range of selective herbicides available for postemergence applications [3], [4].

Among the invasive species affecting forage systems, *Ambrosia artemisiifolia* L. (common ragweed) is particularly

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problematic due to its rapid growth, high seed production, and strong competitiveness. This species not only reduces yields but also significantly lowers feed quality and poses severe public health risks through its allergenic pollen [5]. Its inclusion on the European Union list of invasive alien species of concern under Regulation (EU) No 1143/2014 requires coordinated prevention and control actions at national and regional levels. In Romania, these measures are also reinforced by Law No. 62/2018, which mandates active management of ragweed across agricultural and non-agricultural lands.

Chemical control remains a cornerstone of *A. artemisiifolia* management. In recent years, halauxifen methyl has emerged as one of the most effective post-emergence herbicides against broadleaf weeds. Classified within HRAC Group O, synthetic auxins, halauxifen-methyl acts systemically after foliar absorption and is translocated to meristematic tissues, where it mimics the natural plant hormone indole-3-acetic acid (IAA). This process triggers overexpression of auxin responsive genes and stimulates the synthesis of ethylene and abscisic acid (ABA), leading to cell elongation imbalance, chlorosis, and ultimately necrosis of sensitive plants [6], [7]. These physiological effects translate into rapid visual symptoms, often visible within a few days after application, followed by complete desiccation of susceptible weeds.

Postemergence herbicides such as bentazon, imazamox, imazethapyr, and 2,4-DB have shown variable efficiency depending on the growth stage of both weeds and alfalfa, as well as environmental conditions [1], [4]. Glyphosate resistant cultivars have expanded management options, allowing broad spectrum weed control with minimal crop injury [3]. Compared with the traditional herbicides used in alfalfa, halauxifen methyl provides a broader spectrum of control against annual and perennial broadleaf weeds, including *A. artemisiifolia*, while exhibiting a distinct mechanism of action that reduces the risk of cross resistance.

Beyond chemical control, new approaches emphasize integration with biostimulants, which can mitigate transient phytotoxic effects and enhance crop recovery after herbicide application. Biostimulants based on amino acids, humic substances, and micronutrients improve stress tolerance and metabolic activity, supporting plant resilience under post-treatment stress conditions [8], [9]. Such integrated strategies combining herbicides with physiological enhancers represent a promising step toward sustainable weed management in alfalfa, where crop selectivity and long-term productivity are critical.

In this context, the present study aimed to evaluate the efficacy and selectivity of halauxifen-methyl and imazamox, applied alone or in combination with biostimulants, for the post-emergence control of *Ambrosia artemisiifolia* in alfalfa. The objective was to identify combinations between herbicide and biostimulant capable of maintaining high weed control efficacy while reducing crop stress.

MATERIALS AND METHODS

A one-year field experiment was conducted during the 2025 growing season in a second year *M. sativa* crop naturally infested with *A. artemisiifolia*, located near Hodoș, Timiș County, western Romania (45°39′26″ N, 21°48′40″ E). The soil type at the experimental site was classified as cambic chernozem, with a neutral pH 7.2, 3.2% organic matter, and good drainage capacity, representing favorable conditions for both alfalfa development and *A. artemisiifolia* infestation.

The experiment followed a randomized complete block design (RCBD) with four replications and six variants. Each experimental plot measured 21 m² (3 m × 7 m), with 1 m buffer zones to prevent spray drift.

The experimental variants included E0_B0, untreated control; E0_B12, biostimulant only; E1_B0, imazamox (1.0 L ha^{-1}); E1_B12, imazamox (1.0 L ha^{-1}) + biostimulant (1.2 L ha^{-1}); E2_B0, halauxifen-methyl (1.0 L ha^{-1}); and E2_B12, halauxifen-methyl (1.0 L ha^{-1}) + biostimulant (1.2 L ha^{-1}), as shown in Table 1.

Treatments were applied to *A. artemisiifolia* plants at the 2-6 leaf stage.

Herbicide-containing treatments were applied using a portable manual compressed-air boom sprayer equipped with flat-fan Lechler 03 nozzles. The sprayer was calibrated to deliver 250 L ha^{-1} at a pressure of 500 kPa. Applications were made at a constant walking speed of approximately 0.9 m s^{-1}. During spraying, ambient conditions were favorable, with air temperatures of 23.4 °C, relative humidity of 61.2%, and wind speed under 2 m s^{-1}.

Herbicide treatments were applied post-emergence, in a single pass, at the 2-6 leaf stage of *A. artemisiifolia*, with the first application (A) on 6 June 2025, including herbicides and biostimulants, followed by a second biostimulant application (B) on 12 June 2025.

The evaluations were performed visually and quantitatively according to EPPO standards [10], [11]. Weed control efficacy was assessed focusing on the target species *A. artemisiifolia* at 7, 14, 28, and 42 days after application (DAA). Visual assessments were based on a percentage scale, where 0% represented no visible effect and 100% represented complete weed control.

In addition to efficacy, crop phytotoxicity and vigor reduction were visually estimated on a 0-100% scale, where 0% indicated a healthy, unaffected crop and 100% represented complete plant death. Morphological abnormalities: leaf

deformation, stem curvature, and chlorosis were recorded and expressed as a percentage of affected plants per plot.

Data were collected from the central area of each plot to avoid border effects and averaged across four replications. Photographic documentation was carried out at each evaluation stage to ensure consistency and accuracy in visual scoring.

All experimental data were processed using the Agricultural Research Manager (ARM) software to calculate mean values, standard deviations, and treatment comparisons. Graphical representations of weed control and crop tolerance dynamics were prepared in Microsoft Excel 2021.

RESULTS

At 14 days after application, all herbicide treatments produced a rapid visual response, with control values ranging between 67.5% and 73.0% as presented in Table 2. The highest efficacy at this stage was recorded for the halauxifen-methyl + biostimulant treatment (E2_B12), which achieved 73.0% followed closely by the imazamox + biostimulant treatment (E1_B12), which achieved 71.8%. The treatments applied without biostimulants (E1_B0 and E2_B0) showed slightly lower control, ranging from 67% to 69% , confirming that the biostimulant addition improved early herbicide activity. The initial differences can be attributed to enhanced foliar penetration and systemic distribution facilitated by the amino acid - humic complex.

By 28 DAA, the overall control increased considerably across all variants, ranging between 85.0% and 92.5%. The most effective treatment remained E2_B12, achieving 92.5% weed suppression, while E1_B12 reached 88.0%, and E2_B0, in which halauxifen-methyl was applied alone, maintained 87.5% control. The consistent improvement observed in both halauxifen-methyl and imazamox based treatments highlights the sustained post-emergence activity of the herbicides and the beneficial physiological support provided by the biostimulant under field conditions.

At 42 DAA, the control values stabilized, confirming the residual effectiveness of halauxifen-methyl under field conditions.

The E2_B12 variant retained the highest level of efficacy (93.0%), followed by E2_B0 (90.0%) and E1_B12 (89.0%). The untreated control plots remained fully infested, emphasizing the aggressiveness and competitive dominance of A. artemisiifolia in alfalfa stands.

Visual observations throughout the experiment revealed that halauxifen-methyl treatments induced mild chlorosis and transient growth delay within the first 10-14 days after spraying. These symptoms were more pronounced in the herbicide-only variant (E2_B0), but plants exhibited full recovery by 42 DAA. In contrast, the addition of the biostimulant clearly reduced the intensity and duration of these effects, accelerating chlorophyll restoration and regrowth. No leaf malformations or irreversible phytotoxic symptoms were observed in any variant, confirming a high level of crop selectivity and safety.

The vigor index followed a similar pattern, showing moderate initial reductions after herbicide application, followed by recovery in all variants. The combination treatments E1_B12 and E2_B12 maintained higher vigor values than herbicide-only variants, suggesting that biostimulants mitigated herbicide-induced stress and supported faster metabolic compensation.

The E0_B12 variant, which received only the biostimulant without herbicide application, was included as a reference treatment to assess the physiological effect of the biostimulant alone.

DISCUSSIONS

The results of this study confirm that *A. artemisiifolia* remains a highly competitive and persistent weed in perennial forage systems, capable of rapidly depleting resources and suppressing alfalfa growth. The observed control dynamics and crop tolerance demonstrate that both tested herbicides based on halauxifen-methyl and imazamox provided effective suppression of the target species, although with distinct differences in performance and physiological response.

At 14 DAA, all herbicide variants produced rapid initial effects, but control levels were moderate and similar to the early post application responses reported by Mesbah and Miller (2005) for broadleaf weeds in alfalfa treated with imazamox [4]. The slightly higher performance of halauxifen-methyl (1.0 L ha^{-1}) with biostimulant (1.2 L ha^{-1}) at this stage suggests improved foliar absorption and translocation facilitated by the amino acid humic complex. Comparable results were described by Katsenios et al. (2023), who observed accelerated uptake and translocation of systemic herbicides in the presence of biostimulants, attributed to enhanced membrane permeability and stress-mitigation effects [9].

As shown in Figure 1, at 28 and 42 DAA, control efficacy exceeded 90% in the halauxifen-methyl with biostimulant treatment, indicating both high intrinsic herbicidal activity and sustained post-emergence persistence. These results align with Grossmann (2010), who described the systemic translocation and rapid hormonal disruption induced by synthetic auxins [6].

The present study, however, extends these observations to a new context for alfalfa where selective control of broadleaf weeds has traditionally been more difficult due to crop sensitivity. The achieved selectivity in this experiment is therefore of particular importance, suggesting that halauxifen-methyl may have a safer application window in perennial legumes than previously assumed.

The consistent improvement observed in both halauxifen-methyl and imazamox treatments highlights the sustained post-emergence activity of the herbicides and the beneficial physiological support provided by the biostimulant under field conditions. The integration of biostimulants not only improved control uniformity but also reduced variability among replicates, implying stabilization of plant physiological responses under mild abiotic stress. This effect supports the working hypothesis that biostimulants can act as metabolic modulators, buffering oxidative stress and promoting chlorophyll regeneration following herbicide exposure [8], [9].

Phytotoxicity symptoms observed in the first 10-14 days, mainly chlorosis and transient growth delay, are consistent with the auxinic response described by Grossmann (2010) and Xu et al. (2022) [6], [7]. However, the rapid recovery recorded in treatments combined with biostimulants differs from results reported by Mitkov (2021) for similar halauxifen-based treatments in annual crops, where recovery occurred later and mild deformation persisted. This difference could be attributed to the perennial growth habit and high regenerative capacity of alfalfa, which enable more efficient redistribution of resources and hormonal compensation after chemical stress. The improved recovery in biostimulant-enhanced variants further indicates stimulation of antioxidant enzyme systems and increased nutrient uptake efficiency-mechanisms widely documented in the literature (Kanatas et al., 2022).

Interestingly, while both herbicides maintained high efficacy, halauxifen-methyl produced slightly more stable results over time, suggesting longer residual activity or stronger systemic translocation compared to imazamox. Similar patterns have been described by Arregui et al. (2001), who reported variable imazamox performance under fluctuating temperature and humidity conditions. The superior persistence of halauxifen-methyl in this study may also relate to its mode of action, which involves irreversible hormonal deregulation rather than enzymatic inhibition, thus leading to more complete tissue necrosis.

The combination of halauxifen-methyl with a biostimulant complex provided the most favorable balance between efficacy and crop tolerance. From an ecological perspective, this synergy supports sustainable weed management by potentially reducing the need for higher herbicide doses. The observed results are consistent with the integrated control principles proposed by the European Green Deal, which emphasize reduced chemical dependency through optimized formulations and complementary biological inputs. The introduction of halauxifen-methyl into alfalfa weed management could therefore represent a significant advancement, provided that its safety profile is validated under multi-seasonal conditions.

Table 1 Experimental treatment variants, active ingredients, biostimulant composition, and application scheme used in the alfalfa field trial

Treatment cod	Herbicide (Dose/ha)	Biostimulant	Application
E0_B0	-	-	-
E0_B12	-	AminoQuelant + Foliq Ascovigor	A + B
E1_B0	Imazamox 1.0 L/ha^{-1}	-	A
E1_B12	Imazamox 1.0 L/ha^{-1}	AminoQuelant + Foliq Ascovigor	A + B
E2_B12	Halauxifen-methyl 1.0 L/ha^{-1}	AminoQuelant + Foliq Ascovigor	A + B
E2_B0	Halauxifen-methyl 1.0 L/ha^{-1}	-	A

Note: A – first application on 6 June 2025; B – second biostimulant application on 12 June 2025; Doses are expressed in liters per hectare (L/ha^{-1})

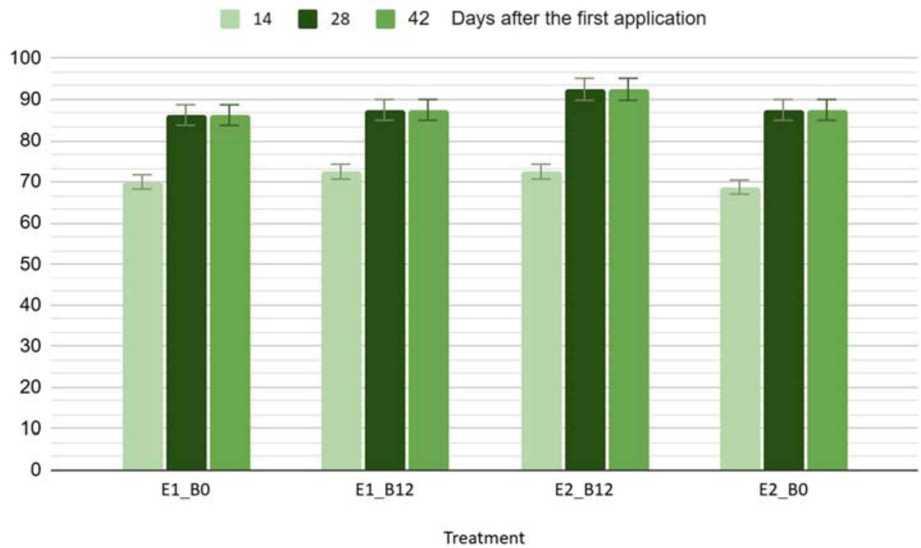

Fig. 1 Average weed control (%) of selected treatments at 14, 28 and 42 days after application (DAA), representing mean control percentages for *A. artemisiifolia* under field conditions.

CONCLUSIONS

The results of this study demonstrate that *A. artemisiifolia* can be effectively controlled in alfalfa using post-emergence applications of halauxifen-methyl and imazamox, with the auxinic herbicide providing superior and more persistent efficacy. Among all tested treatments, the combination of halauxifen-methyl 1.0 L/ha^{-1} with the biostimulant complex achieved the highest and most consistent weed control, exceeding 90% efficacy while maintaining full crop tolerance.

The inclusion of biostimulants not only enhanced early herbicide performance but also reduced transient phytotoxicity and accelerated alfalfa recovery, confirming their role in improving physiological stability under chemical and environmental stress. These findings validate the working hypothesis that integrating biostimulants with postemergence herbicides can improve both selectivity and sustainability in weed management.

From a broader agronomic perspective, this study provides the first experimental evidence supporting the potential selectivity of halauxifen-methyl in perennial forage systems. Future research should focus on optimizing application rates, exploring multi-seasonal effects, and evaluating the influence on yield quality and soil microbiota to support the possible registration of halauxifen-methyl for alfalfa cultivation.

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