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A Preliminary Assessment of the Combined Effects of a Novel Microbial Biostimulant Product, Fertilizers, and Herbicides on the Growth and Yield of Field Crops in Greece

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Abstract: Field trials were conducted (2022–2023) in a randomized complete block design to evaluate the combined use of a microbial biostimulant, P-K Stim, which contains phosphate and potassium solubilizing bacteria (10^9 Colony Forming Units mL^{-1}), various fertilization rates, and herbicide use on weed growth and the productivity of alfalfa (*Medicago sativa* L.), oilseed rape (*Brassica napus* L.), and durum wheat (*Triticum durum* Desf.). The following treatment list was the same on all trial fields: (1) 100% basal fertilization (100N), (2) 20% basal fertilization and application of microbial biostimulant P-K Stim (20N + PK), (3) 100% basal fertilization and post-emergence herbicide treatment (100N + H), (4) 20% basal fertilization together with the application of P-K Stim and a post-emergence herbicide treatment (20N + PK + H), and (5) 50% basal fertilization together with the application of P-K Stim and a post-emergence herbicide treatment (50N + PK + H). The combined use of fertilization, biostimulants, and herbicides significantly affected crop yield, its components and weed biomass ($p \leq 0.05$). The concentrations for potassium and phosphorus were higher in the 20N + PK + H and 50N + PK + H treatments for all crops compared to other treatments. Nutrient concentrations were remarkably high across all crops, closely approximating the values of the recommended nitrogen fertilization. Crop yield and its components were positively influenced by the 20N + PK + H and 50N + PK + H treatments. Weed biomass was significantly lower in these plots compared to other treatments.

Keywords: P-K Stim; *Medicago sativa* L.; *Brassica napus* L.; *Triticum Durum* Desf.; weeds

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1. Introduction

In agricultural production, practices such as the application of fertilizers and herbicides are pivotal, primarily aimed at enhancing crop productivity and addressing the escalating food requirements of the ever-growing human population. Yet, the extensive usage and inputs of agrochemicals into the soil have severely impacted the health of agroecosystems and humans [1]. Ensuring the maintenance of biodiversity and soil health is essential; therefore, there is a need for environmentally friendly practices and agricultural sustainability that can reduce agrochemical inputs through the proper use of natural resources with greater ecosystems services in full agreement with the EU Green Deal's goals [2].

Conventional fertilization practices may favor weed growth over crop growth, thereby conferring a competitive advantage to weeds during the early growth stages of crop establishment, since weeds tend to grow more vigorously and absorb nutrients more rapidly than the crops [3]. Biostimulants appear to be a viable component of a strategy to meet the urgent need for sustainable products and diminish fertilizer and herbicide usage, due to their bioactivity, lack of toxic effects on non-target organisms, and low ecological persistence [4]. Moreover, biostimulants are plant growth regulators, improving plant functions and physiology, resulting in better efficiency of nutrient utilization and tolerance to biotic and abiotic stress factors and thus having a positive effect on crop yield and product quality [5,6]. Furthermore, biostimulants may lead to reduced weed emergence when used in combination with herbicide mixtures, creating an effective and sustainable weed control system. Reduced weed weight and increased yields were observed in potato growth when a biostimulant was applied along with an herbicide [7].

The bacterial plant biostimulants that are based on plant growth-promoting rhizobacteria can stimulate crop growth through several mechanisms, such as the solubilization of insoluble minerals, like potassium (K) and phosphorus (P). Phosphorus is a crucial macronutrient for crop productivity and for various physiological and biochemical functions at the plant cellular level, such as photosynthesis, biological oxidation, and cell division [8,9]. It is mostly provided by fertilizers that also contain metal cations, which can easily be lost through leaching and become inaccessible to plants [10]. Instead, biostimulants containing plant growth-promoting rhizobacteria, from various bacterial genera including *Pseudomonas*, *Rhizobium*, *Bacillus*, and *Enterobacter*, are capable of solubilizing insoluble phosphates by secreting organic acids, phenolic compounds, siderophores and protons [11]. Consequently, phosphate solubilizing bacteria facilitate the access of crops to phosphorus, thereby enhancing soil fertility and increasing crop yields [12]. Potassium is an essential macronutrient for plant development and several physiological processes, such as enzyme activity regulation, gas exchanges, and protein formation [13]. When potassium is applied to soil in the form of an inorganic fertilizer, it frequently tends to form insoluble complexes; yet, biostimulants containing plant growth-promoting rhizobacteria (PGPR) are able to solubilize insoluble potassium by secreting inorganic acids, making it available from the potassium-bearing minerals in the soil [14,15]. Bacterial genera including *Bacillus edaphicus*, *Acidothiobacillus* sp., *Ferrooxidans* sp., *Pseudomonas* sp., *Bacillus mucilaginosus*, *Burkholderia* sp., and *Paenibacillus* sp. are well known for their ability to produce the available form of potassium [16].

The objective of the present study was to evaluate the effects of alternative fertilization practices based on biostimulants containing phosphate and potassium solubilizing bacteria, in combination with varying rates of fertilization and herbicide application, on weed growth and the productivity of alfalfa (*Medicago sativa* L.), oilseed rape (*Brassica napus* L.), wheat (*Triticum durum* Desf.), under real field conditions.

2. Materials and Methods

2.1. Site Description

Field trials were conducted during the 2022–2023 growing season in three different experimental fields. Alfalfa was tested on the first trial field located in the Agrinio region (38.364° N, 21.211° E). Oilseed rape and durum wheat were tested in the second and third trial fields in the Domokos region (39.063° N, 22.174° E).

In Agrinio, the soil texture (0–30 cm) was clay loam (CL), with the following characteristics: clay 296 g kg^{-1}, silt 337 g kg^{-1}, sand 367 g kg^{-1}, organic matter 14.4 g kg^{-1}, and pH (1:2 H_2O) 7.6. In Domokos, the soil texture (0–30 cm) was clay loam (CL), with the following characteristics: clay 344.5 g kg^{-1}, slit 260.3 g kg^{-1}, sand 395.2 g kg^{-1}, organic matter 71.8 g kg^{-1}, and pH (1:2 H_2O) 7.43.

Regarding climatic conditions, the average monthly air temperature in Domokos was lower than in Agrinio from 2022 to 2023. The lower air temperatures could be due to the higher altitude of the experimental fields in Domokos (560 m) compared to that of Agrinio (91 m). In Agrinio, precipitation was highest in January 2023 (211.6 mm) and remained high from March to June. Regarding the monthly rainfall heights in Domokos, September was the month with the highest precipitation (511.1 mm). Additionally, monthly precipitation was exceptionally high from April to June (Table 1).

Table 1. Climatic conditions in the two experimental fields during the 2022–2023 growing seasons.

Year	Month	Average Temperature (°C)		Precipitation (mm)	
		Agrinio	Domokos	Agrinio	Domokos
2022	September	23.4	21.2	34.8	17.6
2022	October	19.4	16.4	28	22
2022	November	15	12.2	154.4	67.2
2022	December	13	9.6	68	28.6
2023	January	10.2	7.3	211.6	76.6
2023	February	9.1	6.4	7.2	12
2023	March	12.7	10.1	92.2	47.2
2023	April	14.3	12	75.8	98
2023	May	19	15	76	83.2
2023	June	24.1	21	81.4	100
2023	July	29.6	28.2	0	24.4
2023	August	27.6	25.7	23.6	27.6
2023	September	24.1	20.6	41.2	511.2

The composition of the weed flora was similar in all trial fields. In particular, *Capsella bursa-pastoris* Medik. and *Sinapis arvensis* L. were the predominant annual broad-leaved weeds, while the predominant annual grass weed species was *Avena sterilis* L., which is one of the most common and troublesome weeds in Greek agriculture, causing severe yield losses to economically important field crops [17].

2.2. Experimental Setup and Design

Regarding the experimental setup, all experimental runs were conducted in a Randomized Complete Block Design (RCBD) with five treatments repeated four times, resulting in a total of twenty experimental plots.

The following treatment list was the same on all the trial fields (Table 2).

Table 2. Experimental treatments applied in the field trials. ✓; applied, ×; not applied.

Treatment	Fertilization	Biostimulant	Herbicide
100N	100%	×	×
20N + PK	20%	✓	×
100N + H	100%	×	✓
20N + PK + H	20%	✓	✓
50N + PK + H	50%	✓	✓

In detail, the treatments were: (1) 100% basal fertilization (100N), (2) 20% basal fertilization and the application of microbial biostimulant P-K Stim (20N + PK), (3) 100% basal fertilization and post-emergence herbicide treatment (100N + H), (4) 20% basal fertilization together with the application of P-K Stim and a post-emergence herbicide treatment (20N + PK + H), and (5) 50% basal fertilization together with the application of P-K Stim and a post-emergence herbicide treatment (50N + PK + H).

In all trial fields, 1 m wide margins between adjacent plots were kept free of weeds and crop plants by hand-weeding to prevent the transfer of dissolved nutrients and bacteria from one plot to another.

Alfalfa cultivar Ypati 84 (K&N Efthymiadis S.A., Thessaloniki, Greece) was chosen due to its high productivity, vigorous growth, and excellent adaptability to the soil and climatic conditions in Greece. The field was disked, harrowed, and finally, a cultipacker (Agricultural Machinery—Stefanos Milonas 1983 O.E., Adendro Thessaloniki, Greece) was run across the field. Alfalfa was sown on 15 September 2022, with a sowing rate of 30 kg ha^{-1} and a sowing depth of 2 cm. A calibrated Pannon K1 hand seed drill (Pannon Machine and Equipment Manufacturer, Ltd. Liability Co., Vecsés, Hungary) was used for sowing. Row spacing was 20 cm. Before sowing, Clover and Alfalfa-Starter (Humofert S.A., Athens, Greece) was used as a microbial inoculant for alfalfa seed to promote the establishment of alfalfa-specific beneficial symbiotic bacteria. The inoculation rate was 1 kg of inoculant per 50 kg of alfalfa seed. The microbial inoculant product contains a beneficial microbial population of 2×10^9 CFU (Colony Forming Units) mL^{-1}. The plots were 8 m^2 (2 m long × 4 m wide), resulting in a total experimental area of 160 m^2. Fertilization and biostimulant treatments were carried out on 3 March 2023, when the plants had fully developed their leaf area. A complete synthetic fertilizer 15-15-15 (N-P-K) (YaraMila® Universe®, Yara Hellas S.A., Athens, Greece) was applied using a broadcast for top dressing. The recommended rate of 200 kg ha^{-1} was applied in plots of 100N and 100N + H treatments. For 20N + PK + H and 50 + PK + H treatments, the reduced rates were 40 and 100 kg ha^{-1}, respectively. P-K Stim (Humofert S.A., Athens, Greece) is a microbial solution containing genera of PGPR bacteria belonging to the plant microbiome at a concentration of 10^9 CFU ml^{-1}. The biostimulant was applied by using an Elettra Venus™ 5 pressure sprayer (Viopsec Kalimeris SMPC, Athens, Greece) calibrated to deliver 5 L ha^{-1} of spray solution. The selective herbicide imazamox was applied as a post-emergence treatment with an application rate of 50 g a.i. ha^{-1} (Pulsar 4 SL®, Basf Hellas S.A., Athens, Greece), at the five-leaf growth stage of alfalfa (BBCH: 15). The herbicide was sprayed using a Gloria® 405 T (Gloria Haus & Gartengeraete GMBH, Witten, Germany) pressurized sprayer equipped with a 2.4 m wide boom and six TeeJet® 8002 flat fan nozzles (TeeJet Technologies Northwest Europe, Schorndorf, Germany) calibrated to deliver 300 L ha^{-1} of spray solution at a constant pressure of 250 kPa (H). Herbicide was applied in the 100N + H, 20N +PK + H and 50N +PK + H treatments. The herbicide application date was 10 March 2023. The herbicide was applied in the plots of 100N + H, 20N + PK + H and 50N + PK + H treatments. The equipment and procedure for applying biostimulants and herbicides were the same in the other two trial fields.

Oilseed rape Clearfield® hybrid PT279CL (Corteva™ Agriscience Hellas S.A. Athens, Greece) was selected for its high seed yield, oil content and stiff straw. The preparation of the seedbed consisted of plowing the soil with a moldboard to a depth of 30 cm 3 weeks before sowing. After plowing, the soil was disked twice to a depth of 20 cm. Sowing was carried out on 15 October 2022, with a sowing rate of 60 seeds m^{-2} and a sowing depth of 2 cm. The seeds were sown using the same hand seeder as in the alfalfa experiment. The distance between the rows was 12 cm. Plots were 2 m long and 5 m wide and had an area of 10 m^2, resulting in a total experimental area of 200 m^2. A complete synthetic fertilizer, 18-6-12 (N-P-K) (YaraMila® Panther, Yara Hellas S.A., Athens, Greece) was incorporated into the soil. The recommended rate of 300 kg ha^{-1} was applied in plots of 100N and 100N + H treatments. For 20N + PK + H and 50 + PK + H treatments, the reduced rates were 60 and 150 kg ha^{-1}, respectively. Foliar applications of P-K Stim and selective post-emergence herbicide were carried out when the plants had developed from 6 to 8 true leaves (BBCH: 16–18). The biostimulant was applied on 4 December 2022. The mixture of selective herbicides, imazamox + metazachlor (Cleranda®, Basf Hellas S.A., Athens, Greece), was applied on 16 December 2022 as a post-emergence treatment with an application rate of 35 + 750 g ai ha^{-1}.

The durum wheat cultivar Maestà (CGS Sementi S.P.A., Acquasparta, Italy) was selected for its robust resistance to low temperatures and its ability to produce high grain yields on fertile or semi-fertile soils. The trial field was plowed to a depth of 30 cm and cultivated with a disk harrow to a depth of 20 cm a few days before sowing to prepare the seedbed. Durum wheat was sown on 22 November 2022, at a sowing rate of 200 kg ha^{-1} and a row spacing of 15 cm. Sowing was carried out with the same hand seed drill as in the other two field trials. The plots were 10 m^2 (2 m long × 5 m wide), resulting in a total experimental area of 200 m^2. A complete synthetic fertilizer, 18-23-0 (N-P-K) (Ωmega® fert, Hellagrolip S.A., Athens, Greece), was used at the recommended rate of 250 kg ha^{-1} in the plots of the 100N and 100N + H treatments. For the 20N + PK + H and 50N + PK + H treatments, the reduced rates were 50 and 125 kg ha^{-1}, respectively. Foliar treatments with P-K Stim and selective herbicide were applied when the durum wheat plants were in the growth stage of tillering (BBCH: 20–29). The biostimulant was applied on 17 January 2023. The mixture of selective herbicides mesosulfuron-methyl + iodosulfuron-methyl-sodium (Atlantis® WG, Bayer Hellas A.G., Athens, Greece) was applied on 18 December 2022 as a post-emergence treatment with an application rate of 15 + 3 g ai ha^{-1}.

2.3. Data Collection

In all the experimental fields, 1 kg of crop biomass was collected from each plot for plant tissue analysis. Vegetation samples were taken twice in the growing season from all trial fields and crops. The alfalfa samples were taken on 20 April 2023 and 7 June 2023, while oilseed rape and durum wheat samples were taken on 25 April 2023 and 30 May 2023. The samples were then taken to the AUA Soil Science Laboratory to determine the concentrations (%) of nitrogen (N), phosphorus (P) and potassium (K) in the plant tissues of each crop. The total nitrogen content was measured by applying the Kjeldahl procedure using a Kjeltec 8400 auto-analyzer (Foss Tecator AB, Höganäs, Sweden). Phosphorus concentration was determined colorimetrically by molybdenum method using a UV-1700 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Potassium concentration was determined by using a SpectrAA-300 atomic absorption spectrophotometer (Varian Inc., Palo Alto, CA, USA).

The yield and its components were assessed for each crop at harvest. Specifically, for alfalfa, the components of forage yield determined were the stand density, the number of shoots per plant, and the average shoot weight after oven-drying at 65 °C for 48 h (DHG-9025, Knowledge Research S.A., Athens, Greece). For oilseed rape, the components of seed yield measured included the stand density, the number of pods per plant, the number of seeds per pod, and the weight of 1000 seeds. For durum wheat, the components of grain yield per 1 m^2 measured included the density of spikes per unit area, the number of grains per spike, and the weight of 1000 grains. Crop yield was determined based on yield component determination. The alfalfa harvest dates were 12 May 2023, 7 June 2023, 15 July 2023, 8 August 2023, and 14 September 2023. The data of the second harvest (7 June 2023) are presented. The oilseed rape and durum harvest dates were 16 June 2023 and 21 June 2023, respectively.

In all the trial fields, four 0.25 m^2 metal quadrats were randomly placed near the center of each plot, away from the plot edges, in areas with uniform weed flora. Weed samples were taken twice from these areas to measure weed biomass, approximately 45 and 90 days after herbicide treatment (DAT) for each crop. Weeds were harvested by cutting the plants at a height of 3 to 5 cm and carefully storing them in numbered plastic bags. The harvested weed vegetation was taken to the Laboratory of Agronomy of the Agricultural University of Athens (AUA), where it was oven-dried at 65 °C for 48 h (DHG-9025, Knowledge Research S.A., Athens, Greece). The biomass of the weed samples was then measured using an electronic scale with three decimal places (KF-H2, Zenith S.A., Athens, Greece). For alfalfa, the weeds were harvested on 25 April 2023 and 9 June 2023. The exact dates of the weed samplings in the oilseed rape field were 29 January 2023 and 15 March 2023. In the durum wheat field, weeds were harvested on 2 February 2023 and 19 March 2023.

2.4. Statistical Analysis

Normal distribution of all data was confirmed with the Shapiro–Wilk test [18], while their homoscedasticity was tested by performing Levene's test [19]. All data were subjected to a one-way analysis of variance (ANOVA) with treatments as fixed effects and replications as random effects. All data analyses were performed at a significance level of $a = 0.05$. Means were then compared using Fischer's Least Significant Difference (LSD) procedure. Statgraphics Centurion XVI (version 16.2.04) (Statgraphics Technologies, Inc., P.O. Box 134, The Plains, VA, USA) was the statistical package used.

3. Results

3.1. Plant Tissue Analysis

During the middle of the growing season, the highest nitrogen concentrations for alfalfa were observed in the 50N + PK + H and 100N + H treatments. The 50N + PK + H treatment increased the N concentration by 23% compared to the 20N + PK + H treatment. Plant tissues from the control treatment (100N) and the 20N + PK treatment contained 57% and 60% less N, respectively, than those from the 50N + PK + H treatment. At the end of the growing season, the highest nitrogen values were observed in the 20N + PK + H treatment. This treatment increased the nitrogen concentration by 70% compared to the untreated control (100N) and by 68% compared to the 20N + PK treatment. For phosphorus concentration in the plant tissues, by the middle of the growing season, the 50N + PK + H treatment had increased P concentration by 24% compared to 20N + PK treatment. Moreover, this treatment increased P concentration by 35% and 54%, respectively, compared to the 100N + H and 100N treatments. At the end of the growing season, the highest phosphorus values were observed for 50N + PK + H and 20N + PK + H treatments. The 50N + PK + H treatment increased phosphorus concentration by 23% compared to the 20N + PK + H treatment. Additionally, plants from the 100N, 20N + PK and 100N + H treatments contained 72%, 55%, and 64% less phosphorus concentration, respectively, compared to the plants from the 50N + PK + H treatment. For potassium concentration, by the middle of the growing season, the 50N + PK + H treatment had increased potassium concentration in plant tissues by 17% and 46%, respectively, compared to the 100N + H and 20N + PK treatments. The percentage of K was 60% lower in plants from the untreated control (100N) compared to those from 50N + PK + H treatment. The effect from 20N + PK + H treatment was also encouraging. At the end of the growing season, 50N + PK + H and 20N + PK + H treatments had the highest values, and no significant differences were observed between these two treatments. The 50N + PK + H treatment increased the percentage of K in alfalfa plant tissues by 37% and 32%, respectively, compared to the 100N + H and 20N + PK treatments. Similarly, a 58% increase in potassium was observed in plants from the 50N + PK + H treatment compared to the untreated control (100N) plants (Table 3).

Table 3. Concentration (%) of N, P, and K in plant tissues of alfalfa. Different letters indicate significant differences. ***; $p \leq 0.001$, E; Evaluation.

Treatment	N (%)		P (%)		K (%)	
	E1	E2	E1	E2	E1	E2
100N	1.89 c	1.49 b	0.17 c	0.27 d	0.84 c	1.20 c
20N + PK	1.77 c	1.60 b	0.28 b	0.44 c	1.15 c	1.92 b
100N + H	3.94 a	4.94 a	0.24 b	0.36 cd	1.75 b	1.78 b
20N + PK + H	3.39 b	5.02 a	0.36 a	0.76 b	1.89 ab	2.57 a
50N + PK + H	4.43 a	4.99 a	0.37 a	0.99 a	2.12 a	2.84 a
LSD	0.78	0.69	0.06	0.28	0.24	0.63
p	***	***	***	***	***	***

For oilseed rape, the highest nitrogen concentrations by the middle of the growing season were observed in the 50N + PK + H and 100N + H treatments, with no significant

differences between these two treatments. The 50N + PK + H treatment increased N concentration in oilseed rape plant tissues by 18% compared to the 20N + PK + H treatment. Additionally, untreated control (100N) and 20N + PK treatment contained 54% and 58% less concentration of N, respectively, compared to the 50N + PK + H treatment. At the end of the growing season, the highest nitrogen values were observed in plants from the 20N + PK + H, 50N + PK + H and 100N + H treatments. The 50N + PK + H treatment increased nitrogen concentration by 49% compared to the 20N + PK treatment. Finally, oilseed rape plants of the untreated control (100N) had 48% less N concentration compared to plants from the 50N + PK + H treatment. For phosphorus concentration, in the middle of the growing season, the highest values were observed for the 50N + PK + H treatment. Oilseed rape plants from the 20N + PK + H and 100N + H treatments contained 25% and 33% less phosphorus, respectively, compared to those from 50N + PK + H treatment. Moreover, 50N + PK + H treatment increased P concentration by 58% and 67% compared to the 20N + PK treatments and the untreated control (100N), respectively. The means of the treatments differed significantly ($p \leq 0.001$) at the end of the growing season. The highest value of P was observed for the 50N + PK + H treatment. The phosphorus content was 24% and 29% higher in the plants from the 50N + PK + H treatments compared to those from the 20N + PK + H and 100N + H treatments, respectively. Also, the 50N + PK + H treatment increased the P concentration by 63% and 78% compared to 20N + PK and 100N, respectively. During the middle of the growing season, the application of a microbial biostimulant combined with an herbicide and reduced nitrogen fertilization either by 50% (50N + PK + H) or 80% (20N + PK + H) and resulted in the highest values of potassium content in plant tissues. Specifically, the application of the microbial biostimulant, along with an herbicide and an 80% reduction in nitrogen fertilization (20N + PK + H), increased the K content in the tissues of oilseed rape by 31% and 24% compared to the 100N + H and 20N + PK treatments, respectively. Compared to the control plants (100N), a 49% increase was observed in the plants from the 20N + PK + H treatment. At the end of the growing season, the plants from the treatment where the recommended dose of nitrogen fertilization was applied along with an herbicide (100N + H) had a 34% lower potassium concentration compared to the 50N + PK + H treatment. Finally, the 50N + PK + H treatment increased the potassium concentration in the tissues of the oilseed rape by 22% and 66% compared to the 20N + PK and the control (100N), respectively (Table 4).

Table 4. Concentration (%) of N, P and K in plant tissues of oilseed rape. Different letters indicate significant differences. **; $p \leq 0.01$, ***; $p \leq 0.001$, E; Evaluation.

Treatment	N (%)		P (%)		K (%)	
	E1	E2	E1	E2	E1	E2
100N	1.74 c	1.71 b	0.17 d	0.09 d	1.37 c	0.69 c
20N + PK	1.91 c	1.70 b	0.22 c	0.15 c	2.04 b	1.56 b
100N + H	4.20 a	3.53 a	0.35 b	0.29 b	1.85 b	1.32 b
20N + PK + H	3.39 b	3.09 a	0.39 b	0.31 b	2.69 a	1.96 a
50N + PK +H	4.13 a	3.31 a	0.52 a	0.41 a	2.50 a	2.00 a
LSD	0.47	0.61	0.17	0.13	0.64	0.46
p	***	***	**	***	**	***

During the middle of the growing season, the highest nitrogen concentrations for wheat were observed in the 100N + H and 50N + PK + H treatments, while the lowest concentrations were noticed in the 100N and 20N + PK treatments. The 100N + H treatment increased the concentration of N by 31% and 32% compared to the 100N and 20N + PK treatments, respectively. Similar results were observed at the end of the growing season. The 100N + H, 20N + PK + H, and 50N + PK + H treatments contained the highest nitrogen concentrations, while the lowest was observed in the 20N + PK treatment. The application of 100% nitrogen fertilization along with an herbicide (100N + H) increased the nitrogen

concentration in wheat tissues by 55% compared to the application of 20% nitrogen fertilization with the PK biostimulant. The concentration of phosphorus (P) in wheat tissues, in the middle of the growing season, were highest in plants treated with 20N + PK + H and 50N + PK + H, while the lowest concentration was observed in plants from the untreated control. Specifically, the control plants that received only 100% nitrogen fertilization (100N) had 50% less phosphorus in their tissues compared to plants treated with 20N + PK + H. At the end of the growing season, the 20N + PK + H treatment, which exhibited the highest concentration, increased the phosphorus concentration by 65% and 35% compared to the 100N and 100N + H treatments, respectively. For potassium concentration, during the middle of the growing season, the highest potassium concentrations were observed in plants treated with 50N + PK + H and 20N + PK + H, while the lowest concentration was observed in the control plants (100N). The 50N + PK + H treatment increased the potassium concentration in the wheat tissues by 18%, 30%, and 49% compared to the 20N + PK, 100N + H, and 100N treatments, respectively. At the end of the growing season, the potassium concentration in wheat plants was significantly affected by the treatments ($p \leq 0.001$), with the highest value observed in plants treated with 20N + PK + H. Specifically, the 20N + PK + H treatment increased the potassium concentration in the plants by 18% and 60% compared to the 20N + PK and 100N treatments, respectively (Table 5).

Table 5. Concentration (%) of N, P, and K in durum wheat tissues. Different letters indicate significant differences. ***; $p \leq 0.001$, E; Evaluation.

Treatment	N (%)		P (%)		K (%)	
	E1	E2	E1	E2	E1	E2
100N	2.31 c	1.33 b	0.24 b	0.11 c	0.99 d	0.57 c
20N + PK	2.28 c	1.16 b	0.31 b	0.18 b	1.59 b	1.17 b
100N + H	3.36 a	2.59 a	0.30 b	0.20 b	1.36 c	1.09 b
20N + PK + H	3.12 b	2.42 a	0.48 a	0.31 a	1.93 a	1.43 a
50N + PK + H	3.30 a	2.51 a	0.48 a	0.30 a	1.95 a	1.41 a
LSD	0.53	0.45	0.1	0.056	0.36	0.26
p	***	***	***	***	***	***

3.2. Crop Yield Components and Final Yield

During the second harvest, the treatments did not significantly affect ($p \geq 0.05$) the density of alfalfa and the number of shoots per plant (Figure 1a,b). However, the average shoot weight was significantly affected by the different treatments ($p \leq 0.001$). Specifically, the application of the microbial biostimulant combined with an herbicide and a 50% reduced dose of nitrogen fertilizer (50N + PK + H) increased the weight per shoot by 15%, 25%, and 30% compared to the 100N + H, 20N + PK, and 100N treatments, respectively. Also, the 20N + PK + H treatment led to high values, while the lowest values were observed in the untreated control and the 20N + PK treatments (Figure 1c). Forage yield was significantly affected by the treatments during the second harvest ($p \leq 0.01$). The 50N + PK + H treatment increased forage yield by 25%, 38%, and 39% compared to the 100N + H, 100N and 20N + PK treatments, respectively. Moreover, the aforementioned treatment increased the forage yield to statistically significant levels compared with the 20N + PK + H treatment (Figure 1d).

From the analysis of variance (ANOVA), it was observed that the treatments significantly affected the number of oilseed rape pods per plant ($p \leq 0.001$). The highest values were observed in plants treated with 50N + PK + H and 20N + PK + H, while the lowest were observed in the control plants (100N). Specifically, the 50N + PK + H and 20N + PK + H treatments increased the number of pods per plant by 29% and 25%, respectively, compared to the 100N treatment (Figure 2a). The number of seeds per pod in the oilseed rape crop was significantly affected by the treatments ($p \leq 0.001$). Plants with a higher number of seeds per pod were associated with the 50N + PK + H and 20N + PK

+ H treatments, while no statistically significant differences were observed between the 100N + H and 20N + PK treatments. Control (100N) plants had 25% and 21% fewer seeds per pod compared to the plants treated with 50N + PK + H and 20N + PK + H, respectively (Figure 2b). Treatments significantly affected the weight of 1000 seeds ($p \leq 0.001$). The seeds from the treatments 50N + PK + H and 100N + H were observed to have the highest 1000-seed weight (Figure 2c). On the other hand, the lowest 1000-seed weight was observed in the treatments with 100% nitrogen fertilization (100N) and in the treatment with 20% nitrogen fertilization and the application of the PK biostimulant (20N + PK). Specifically, the 50N + PK + H treatment increased the 1000-seed weight by 14 and 15% compared to the 100N and 20N + PK treatments, respectively. For seed yield of oilseed rape, the highest seed yield was observed in the plots treated with 50N + PK + H, which increased yield by 13%, 27%, 43%, and 54% compared to the 20N + PK + H, 100N + H, 20N + PK, and 100N treatments, respectively (Figure 2d).

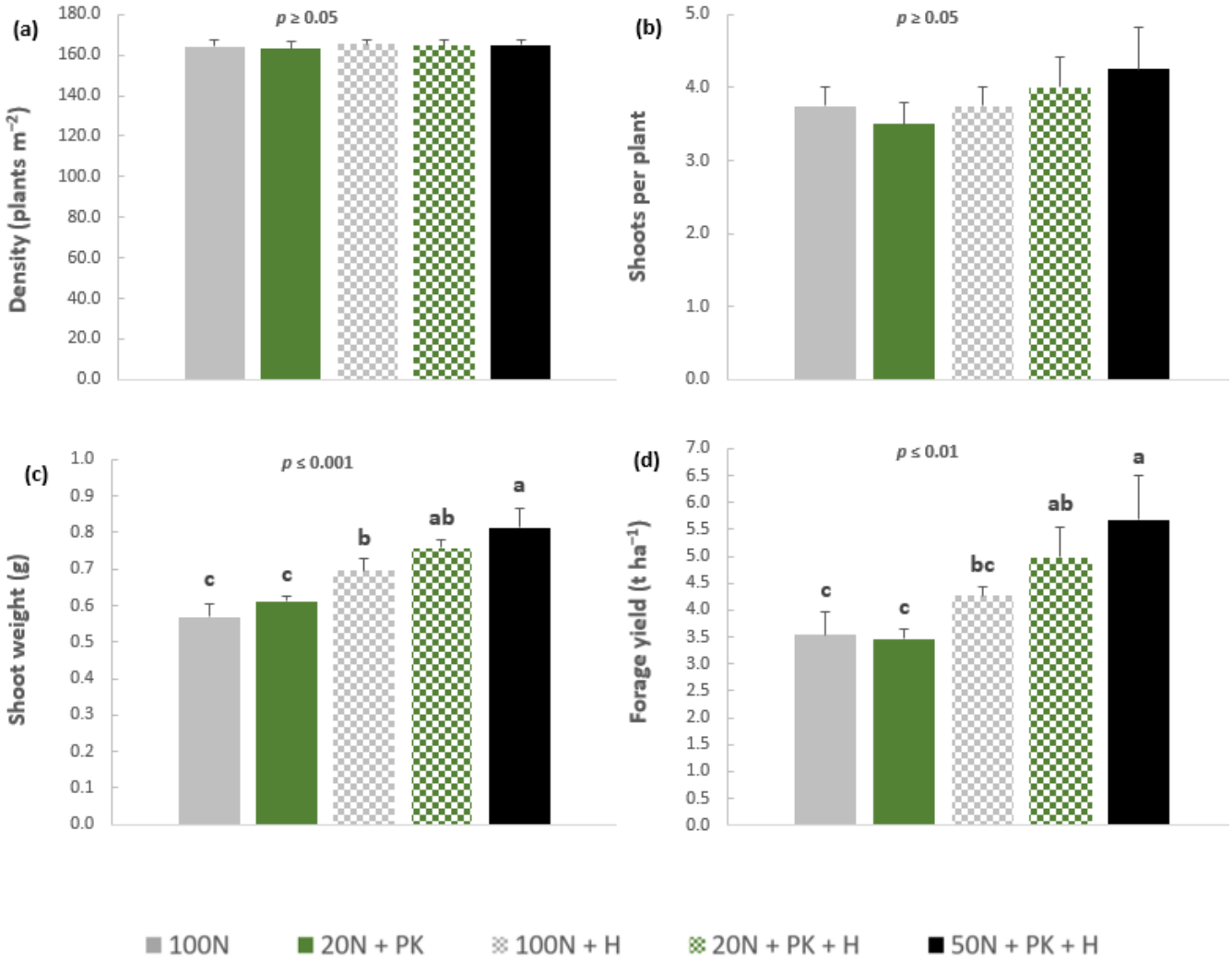


Figure 1. (**a**) Density of alfalfa; (**b**) Number of shoots per plant; (**c**) Mean shoot weight; (**d**) Forage yield during the second harvest, as influenced by the different treatments. Different letters indicate significant differences. Vertical bars indicate standard errors.

The density of wheat spikes was affected by the different nitrogen treatments combined with a biostimulant and herbicide ($p \leq 0.001$). Specifically, plots treated with 50N + PK + H, 20N + PK + H, and 100N + H had the highest number of spikes. Plots treated with 20N + PK and 100N were observed to have 30% and 31% fewer spikes, respectively, compared to those treated with the 20N + PK + H (Figure 3a). The number of grains per spike was

significantly affected by the treatments ($p \leq 0.001$), with the highest number observed in plants treated with 50N + PK + H. The treatments 20N + PK + H and 100N + H showed no statistically significant differences between them. Similarly, no significant differences were observed between the 100N and 20N + PK treatments. In addition, the 50N + PK + H treatment increased the number of grains per spike by 25% and 26% compared to the 100N and 20N + PK treatments, respectively (Figure 3b). The treatments had also affected the 1000-grain weight of wheat ($p \leq 0.001$). The highest values were observed in the 50N + PK + H and 20N + PK + H treatments (Figure 3c). The control (100N) plants had 18% lower 1000-grain weight compared to the 50N + PK + H plants (Figure 3c). Wheat grain yield was significantly affected by the treatments ($p \leq 0.001$). The highest yield was observed in 50N + PK + H plots, while the lowest yields were noted in the 20N + PK and 100N plots (Figure 3d). In particular, the 50N + PK + H treatment increased the grain yield by 53% and 57% compared to the 20N + PK and 100N treatments, respectively (Figure 3d).

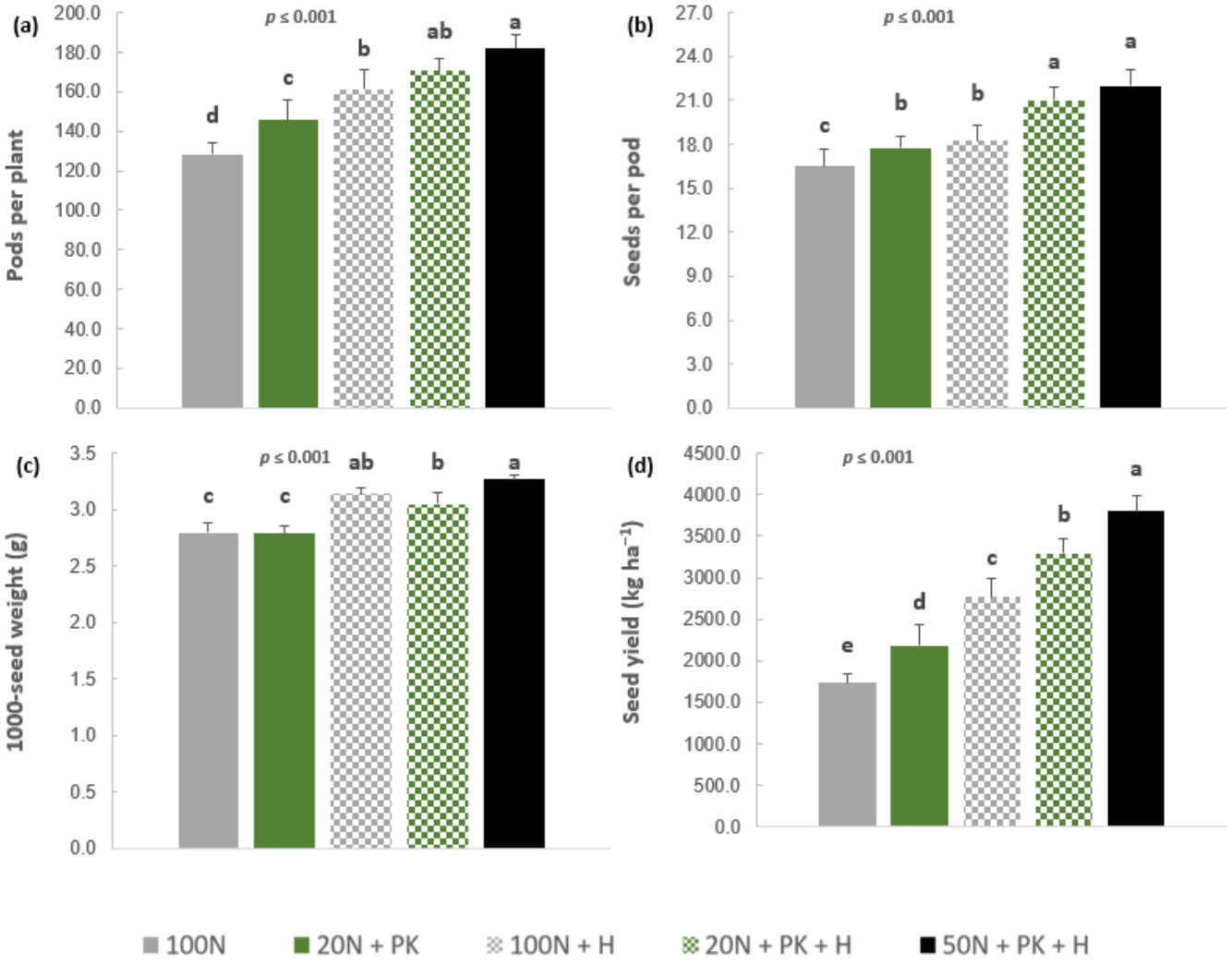


Figure 2. (**a**) Pods per plant; (**b**) Seeds per pod; (**c**) 1000-seed weight; (**d**) Seed yield of oilseed rape as influenced by the different treatments. Different letters indicate significant differences between means using Fischer's Least Significant Difference (LSD) procedure. Vertical bars indicate standard errors.

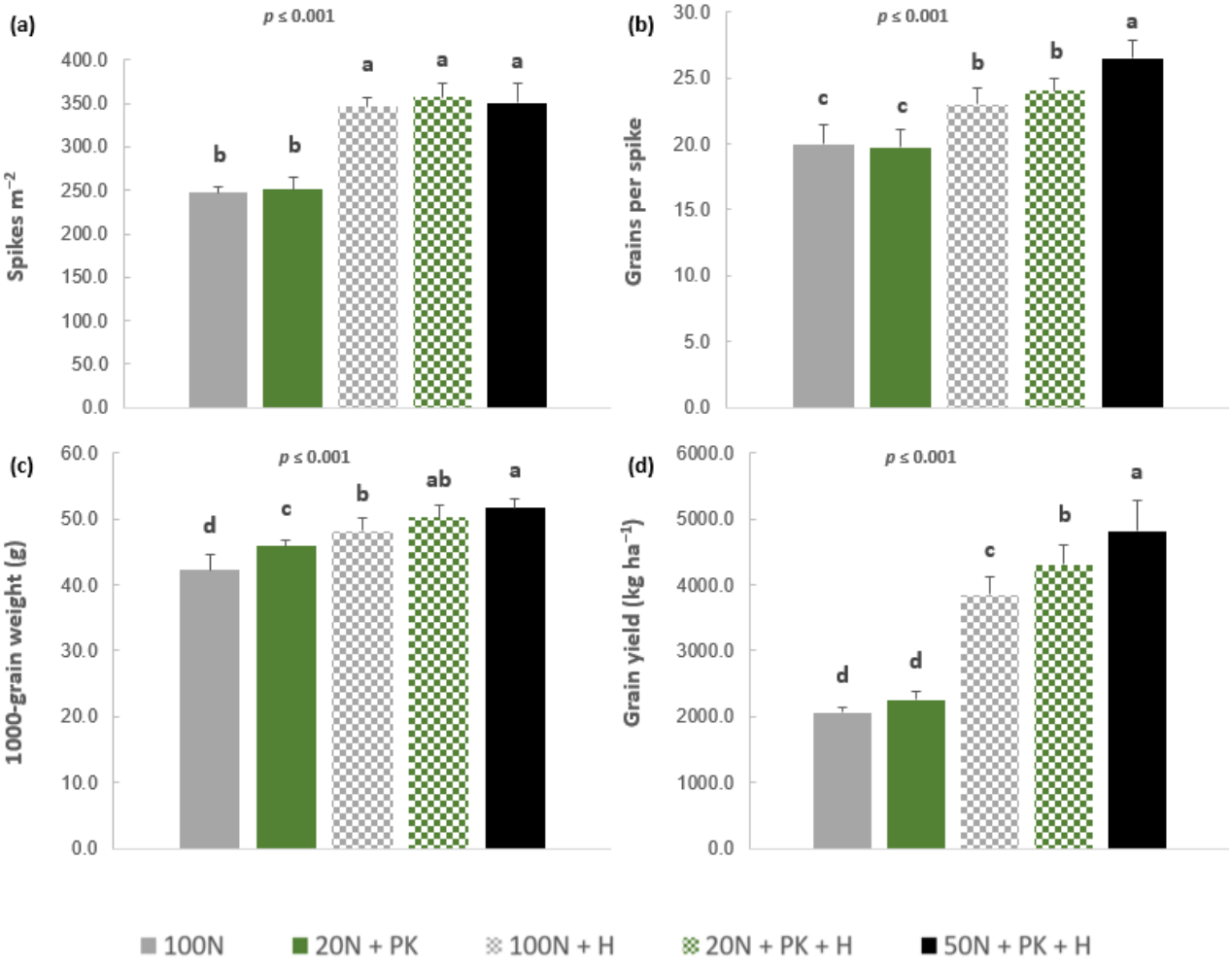


Figure 3. (**a**) Spikes; (**b**) Number of grains per spike; (**c**) 1000-grain weight; (**d**) Grain yield of wheat as influenced by the different treatments. Different letters indicate significant differences between means using Fischer's Least Significant Difference (LSD) procedure. Vertical bars indicate standard errors.

3.3. Weed Biomass

For the alfalfa crop, weed biomass was significantly lower in plots treated with 20N + PK + H and 50N + PK + H compared to other treatments in both evaluations. Specifically, for the first evaluation, taken 45 days after treatment, the weed biomass in the 20N + PK + H and 50N + PK + H plots was below 10 g m^{-2}, while it was higher than 60 g m^{-2} compared to the untreated control (100N) plots. The 20N + PK treatment resulted in higher weed biomass than the 20N + PK + H and 50N + PK + H treatments. In addition, the 100N + H treatment was less effective for weed control compared to the 20N + PK + H and 50N + PK + H treatments. Finally, the 20N + PK treatment resulted in 52% higher weed biomass in comparison to the 100N + H treatment. There were no significant differences between the 20N + PK and 100N treatments for both measurements. Similar results were observed in the evaluation of weed biomass 90 days after treatment. The 20N + PK + H and 50N + PK + H treatments caused substantial reductions in weed biomass compared to the 100N treatment. Weed biomass in 20N + PK + H and 50N + PK + H plots was by far lower than in 20N + PK plots. Moreover, the 20N + PK + H and 50N + PK + H treatments reduced weed biomass by 79% and 63% compared to 100N + H treatment, respectively. Finally, the 20N + PK treatment resulted in 66% lower weed biomass accumulation compared to the 100N + H treatment (Table 6).

Table 6. Weed biomass (g m^{-2}) in all experimental fields and crops at 45 and 90 days after treatment (DAT). Different letters indicate significant differences. *** $p \leq 0.001$.

Treatment	Alfalfa		Oilseed Rape		Wheat	
	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT
100N	62.2 a	167.7 a	85.6 a	202.2 a	82.9 a	170.0 a
20N + PK	58.5 a	143.3 a	72.1 a	166.7 b	90.7 a	158.4 a
100N + H	28.3 b	49.4 b	48.8 b	70.5 c	41.1 b	68.6 b
20N + PK + H	4.4 c	10.2 c	14.2 c	20.4 d	6.8 c	19.9 c
50N + PK + H	9.8 c	18.5 c	10.8 c	35.3 d	13.3 c	30.6 c
LSD	11.3	19.6	14.3	32.1	12.8	23.6
p	***	***	***	***	***	***

For the oilseed rape crop, weed biomass was significantly lower in plots treated with 20N + PK + H and 50N + PK + H compared to other treatments in both evaluations. Specifically, in the first evaluation, weed biomass was lower than 15 g m^{-2} in 20N + PK + H and 50N + PK + H plots and higher than 80 g m^{-2} in 100N plots. The 20N + PK + H and 50N + PK + H treatments also caused significant reduction in weed biomass compared to 20N + PK. Furthermore, the 100N + H treatment resulted in a far higher weed biomass than the 20N + PK + H and 50N + PK + H treatments. Finally, the 100N + H treatment suppressed weed biomass by 32% in comparison to the 20N + PK treatment. There were no significant differences between the 20N + PK and 100N treatments in the first measurement. Similar results were observed in the second evaluation of weed biomass. Untreated control (100N) plots reached weed biomass values above 200 g m^{-2}, which was the highest value. Weed biomass was lowest in 20N + PK + H and 50N + PK + H plots. Both these treatments had lower values of weed biomass than the 20N + PK treatment. Moreover, weed biomass in the 100N + H plots was significantly higher than that of the 20N + PK + H and 50N + PK + H plots. Another observation was that weed biomass decreased by 58% in the 100N + H plots than in the 20N + PK plots (Table 6).

For the durum wheat crop, weed biomass was significantly lower in plots treated with 20N + PK + H and 50N + PK + H compared to other treatments in both evaluations. In particular, the first assessment revealed that the weed biomass in the 20N + PK + H and 50N + PK + H plots was 84% and 92% lower, respectively, than in the 100N plots. The 20N + PK treatment resulted in significantly higher weed biomass than the 20N + PK + H and 50N + PK + H treatments. In addition, the 100N + H treatment resulted in much higher weed infestation than the 20N + PK + H and 50N + PK + H treatments. The 20N + PK + H and 50N + PK + H treatments did not differ from each other. Finally, 100N + H treatment reduced weed dry weight per unit area by 55% compared to the 20N + PK treatment. There were no significant differences between the 20N + PK and 100N. Similar results were observed in the second assessment of weed biomass. Untreated control (100N) plots had a weed dry weight value of 170 g m^{-2}, which was significantly higher than in the plots that received the 20N + PK + H and 50N + PK + H treatments. Weed biomass received its lowest values in the 20N + PK + H and 50N + PK + H plots. These were the most weed-suppressive treatments, and they were more effective for weed control than the 20N + PK and 100N + H treatments. Finally, the 100N + H treatment reduced weed biomass by 57% in comparison to the 20N + PK treatment (Table 6).

4. Discussion

This study demonstrated that the foliar application of P-K Stim significantly increased the concentrations of potassium and phosphorus in the tissues of oilseed rape, alfalfa, and wheat. This aligns with the results of the field trials conducted by Krey et al. [20] in winter oilseed rape and maize (*Zea mays* L.). Such findings indicate that PGPR solubilizes P and K and improves their concentrations in plant tissues. Since P and K solubilization occurs through microbial processes such as organic acid production and proton extrusion, the

ability of PGRP to solubilize P can also improve the availability of K in the soil. Thus, phosphorus solubilizing PGPR can not only improve the P concentration in plant tissue, but also the availability and concentration of K in plant tissue. The mechanisms of P solubilization by phosphorus solubilizing PGPR can be found in the paper by Ahemad and Kibret [21], while K solubilization by potassium solubilizing PGPR is explained by Shanware et al. [22]. As a result, the use of PGPR as alternative natural fertilizers should be considered as an effective way to improve nutrient uptake by crops [23].

The beneficial effects of biostimulant application were maximized when the weeds were adequately controlled with post-emergence selective herbicide treatment and the fertilization ratio was lower. These treatments led to a reduced weed biomass compared to others, likely due to the lower nitrogen fertilizer levels available to the weeds, which led to reduced weed emergence and growth. This observation is supported by the research of Blackshaw et al. [24], where redroot pigweed (*Amaranthus retroflexus* L.) demonstrated a competitive ability compared to wheat for N uptake and shoot biomass by increasing the nitrogen application rate from 60 to 240 mg N kg^{-1} of soil. In another study, a two-year field experiment with wheat showed that wild mustard (*Sinapis arvensis* L.) and sterile oat (*Avena ludoviciana* L.) competition reduced wheat grain yield under nitrogen fertilization rates of 90 and 210 kg ha^{-1} [25]. Another potential reason for the observed outcomes could be attributed to the application of a biostimulant directly to the leaves of the crop. This method may have considerably enhanced the growth of the crop, which, in turn, could have boosted its ability to compete more effectively against weeds. Evidence indicates that incorporating *Bacillus* spp. bacteria into wheat seeds can lead to a significant increase in shoot weight compared to wild oat (*Avena fatua* L.) [26]. It is important to note that biostimulants derived from plant growth-promoting rhizobacteria can produce antibiotics that also exhibit phytotoxic properties against weeds [27,28].

Another encouraging observation in our field trials was that the reduction in broadcast fertilization in the plots where the new PGRR biostimulant was applied resulted in lower weed biomass, as in previous studies [29]. This could be due to the fact that less fertilizer was available for weeds, resulting in lower weed emergence and growth. These results are consistent with those of Gholamhoseini et al. [30], who also observed a significant decrease in weed biomass in their two-year field trials by reducing the basal fertilizer rate by 33%. In another study, weed biomass was consistently lower in plots receiving a lower basal fertilizer rate [31].

In addition, the application of the biostimulant had a positive effect on the yield components of alfalfa, oilseed rape, and wheat, particularly when used in conjunction with chemical weed control. There is some evidence to suggest the potential of integrating PRGP-based biostimulants and herbicides. For example, in a study by Gazoulis et al. [29], the combined use of biostimulants and herbicides in maize resulted in improved grain yield compared to the untreated control. Similar positive results were also demonstrated by Soltani et al. [32] in their field trials with spring wheat (*Triticum aestivum* L.). However, there are very few studies reporting the beneficial effects of such combination on weed suppression and crop yield performance. Therefore, further research is required to evaluate the potential of PRGP-based biostimulants along with sustainable herbicide use under real-field conditions.

Overall, the results of our field trials showed that in the plots treated with the biostimulant P-K Stim, which received 50% or 80% less basal fertilization, there was no reduction in yield and nutrient uptake in any of the crops tested; in fact, these plots had the highest yields and nutrient uptake. The reason for using biostimulants in conjunction with reduced application of synthetic fertilizers is the need to reduce farmers' over-reliance on synthetic fertilizers for crop nutrition, thereby freeing agriculture from the environmental disadvantages of their over-use on agricultural land [33–36]. New PGPR-based biostimulants offer farmers this opportunity while minimizing the risk of yield loss or even leading to significant yield increases compared to conventional fertilization methods. Another reason for combining biostimulants with a 50% or 80% reduction in fertilizer use is the associated

reduction in weed growth. As already mentioned, the fewer nutrients are available to the weeds through the application of basal fertilizers, the lower the weed infestation and the weed pressure on the crops [29]. This indirect effect of such biostimulant–fertilizer combinations, as tested in this study, on weed growth is another reason for their use in modern agriculture.

However, if no weed control is applied in the field, the weeds will continue to emerge. Even if the weeds are present at lower densities and accumulate less biomass, they will continue to compete with the crops for nutrients and resources, and weed interference can lead to significant yield losses in all agronomic rotational crops [37]. Therefore, to achieve higher yields, the combined application of biostimulants together with reduced fertilizer applications should always be accompanied by efficient weed control measures such as herbicide treatment, i.e., as in the present study, mechanical weed control, false and stale seedbeds, and the application of weed-suppressive cultural and agronomic practices [38,39]. This integrated approach, including the appropriate weed management practices, can maximize the benefits of biostimulants in agricultural production.

5. Conclusions

This study highlights the potential benefits of biostimulants in crop production, especially when they are combined with herbicides and reduced fertilization rates. Our outcomes suggest that the strategic use of such combined practices can effectively reduce chemical input while simultaneously boosting crop performance. However, additional research is required to assess the potential and the long-term impacts of biostimulants, combined with other agronomic practices, on crop production.

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