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Article

Weed Control, Growth, Nodulation, Quality and Storability of Peas as Affected by Pre- and Postemergence Herbicides

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Abstract: Weeds represent a major constraint for successful pea cultivation, resulting in loss of green pea yield and quality. Two field experiments were carried out during the winter seasons of 2018/2019 and 2019/2020. The objectives of the current study were to evaluate the efficacy of pendimethalin, butralin, fluazifop butyl, bentazon, and hoeing on weed control, and their impact on pea growth, nodule formation, yield, quality, and storability. The results indicated that hoeing and fluazifop butyl were the most effective treatments for weed control in terms of the lowest values of total weed dry weight. The bentazon and fluazifop butyl herbicides didn't affect active nodules number, plant height, plant weight, root length, or number of leaves and branches. Additionally, bentazon and hoeing resulted in the highest total yield per plant and protein content. Butralin and pendimetalin achieved the lowest yield, and butralin also resulted in the lowest plant height and weight. Bentazon-treated peas had the highest chemical compositions in terms of chlorophyll, carotenoids, total sugar, and vitamin C contents in pea pods at harvest and during cold storage at 4 °C and 95 RH for 45 days. No detectable residues of the four herbicides under study were detected in green pods, suggesting that pods can be safely consumed at the time of harvesting. It can be concluded that nodules formation in pea was not affected significantly by the application of the tested herbicides, except pendimethalin and butralin. Furthermore, bentazon had a positive impact on nodules formation and pods quality and could be used effectively for controlling the broadleaf weeds, and it was simultaneously a selective and safe herbicide in pea cultivation.

Keywords: *Pisum sativum*; weed control efficacy; yield; chemical composition; nodule formation; fluazifop butyl; bentazon; pendimethalin; butralin

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1. Introduction:

Pea (*Pisum sativum L.*) is one of the most important legume crops in the world. It is mainly grown for its tender green pods, consumed as a fresh vegetable and as cooked green seeds. Pea is a good source for protein (27.8%), complex carbohydrates (42.65%), vitamins, minerals, dietary fibers, and antioxidants [1]. It is a lead export and cash crop representing about 40% of the total trading in legumes for both fresh use and processing [2]. Pea is also very useful for enhancing soil fertility, as it provides the soil with supplementary nitrogen due to a symbiotic relationship with N_2-fixing *Rhizobium* species [3]. However, the productivity and quality of peas are substantially affected by weed competition.

Peas compete poorly with weeds because of their slow growth at the early growth stages and limited canopy development, resulting in substantial yield reduction

Weeds pose a threat to the sustainability of pea cultivation, since pea is vulnerable to grass and broad-leaved infestations, with both weeds resulting in significant yield loss and quality. The most predominant and common weeds of pea are *Chenopodium album*, *Medicago polymorpha, Rumex dentatus*, *Sisymbrium irio, sonchus oleraceous, Rumex dentatus, Emex spinosus*, *Malva parviflora, Cichorium pamilum, Avena fatua, lolium sp.*, and *Cenchrus ciliaris.* Weeds can reduce pea yield up to 64% if left uncontrolled [4]. This loss is considerable during the critical early crop growth and development period.

This critical period for crop–weed competition in pea varies from 40 to 60 days after sowing [5]. Weeds compete with the crop for sunlight, water, and nutrients, and can also serve as harbors for insect pests and pathogens that can affect crop production. In addition, late season weeds can decrease harvest efficiency and reduce the quality of pea's grains [6]. In Egypt, pea's farmers typically use hand hoeing for controlling weeds, but this method is labor and time consuming, costly, and less efficient. Thus, chemical weed control became popular among the farmers because herbicides are a more effective, reliable, and cost-effective method for controlling different types of weeds. Currently, two registered herbicides are recommended for weed control in pea according to the Egyptian Ministry of Agriculture and Land Reclamation, namely butralin for pre-emergence and bentazon for postemergence. Pre-emergence herbicide application can help control weeds, especially during the vulnerable early crop growth stage [7]. Postemergence herbicides are also important and can protect pea crops throughout the season and during the different growth stages. Reports are available on the effect of herbicide on pea weeds. Post-emergence herbicide imazethapyr, used for controlling weeds in garden pea, led to optimum seed yield [8]. Postemergence application of quizalofop was also proved to reduce weed density and weed dry weight in peas [9]. Pendimethalin resulted in high weed control efficiency in pea cultivation [10].

However, little work has been reported on the effect of herbicides on the pea's growth, quality, or nodulation [11]. Although legumes are capable of fixing atmospheric nitrogen by means of root nodules, the amount of N_2 fixed can be affected by several factors. Among these important factors are the herbicides, which may shift and disrupt microbial communities in the soil and disturb the symbiotic relationship between N_2-fixers and legumes [12]. In addition, there was no previous study on the effect of herbicides on postharvest quality of green peas. Therefore, the present study was conducted to: (1) assess the efficacy of four commonly used herbicides against grass and broadleaf weeds in peas; (2) evaluate their impact on nodule formation, yield, chemical composition, and storability of peas; and (3) determine the herbicide residues in mature green pods at harvest time.

2. Materials and Methods

2.1. Experiment Layout and Treatments

Field experiments were conducted in the winter seasons of 2018/2019 and 2019/2020 at the Agricultural Research Station (30° 0′ 47.0016″ N and 31° 12′ 31.8708″ E), Cairo University, Faculty of Agriculture. The experiment was set up and repeated at the same location in the second consecutive year. The soil of the experimental field was loamy clay in texture and slightly alkaline in reaction (pH = 7.5). The experimental plot of pea cultivar "Entsar 2" was 8.4 m^2 and planted with 10 cm spacing between plants. Flood irrigation was applied uniformly throughout the study. The experiment was arranged in a randomized complete block design with three replications. The experiment comprised six treatments: untreated check (control), hand hoeing twice (after 20 and 40 days from sowing date), pendimethalin and butralin (as soil-applied and pre-emergence), and bentazon and fluazifop butyl as foliar post-emergence (applied 21 days after sowing date). Description of the tested herbicides is summarized in Table 1. All herbicides were sprayed once at the recommended rate with a hand-operated knapsack sprayer fitted with a flat fan nozzle using spray volume 500 L ha^{-1}. Water was applied for the untreated control in the same manner used for the other treatments.

Table 1. Characteristics of the herbicides used in the experiment.

Common Name	Trade Name	Recommended Rate (a.i ha^{-1})	Target Weed	Manufacturer
Pendimethalin	Stomp Extra 45.5%SC	1.8 kg	Broad leaf and grass	BASF
Butralin	Amex 48% EC	2.8 kg	Broad leaf and grass	Nufarm
Bentazon	Basagran 48% AS	570 g	Broad leaf	BASF
Fluazifop-P butyl	Fuzilade 12.5% EC	300 g	Grass	Syngenta

2.2. Weed Data Recording

Weed samples were collected randomly from 1 m^2 of each plot area 60 days after the sowing date. The annual weeds were divided into two groups, grasses and broad leaves. The fresh and dry weights of total weed in each group were calculated. The weed control efficiency was determined as described by Shehata et al. [13].

$$\text{Weed control (\%)} = ((\text{WDWC} - \text{WDWT})/(\text{DFWC})) \times 100 \quad (1)$$

where WDWC = weed dry weight in the unweeded check; WDWT = weed dry weight in treatment.

2.3. Plant Growth, Nodulation, and Yield Determination

After 60 days from the sowing date, ten pea plants were selected randomly from each experimental unit to measure plant height, plant weight, number of leaves, number of branches, and root length. To measure dry matter, the plants were dried at 70 °C until constant weight and dry matter was calculated according to the following equation:

$$\text{Dry matter \%} = ((\text{dry weight})/(\text{fresh weight})) \times 100 \quad (2)$$

To measure the nodules numbers, pea plant roots were gently washed with water to remove adhering soil, and the nodules were manually removed from the roots and dried. The activity of nodules was examined by slicing nodules in half and considering only those with internal pink coloration, according to the method described by Somasegaran and Hoben (1985) [14]. White or green nodules and inactive nodules were not considered when nodulation was assessed. The photos of nodules were taken by Carl ZEISS microscopy (Gottingen, Germany). At harvest time, twenty plants from each plot were selected randomly to measure total yield per plant.

2.4. Chlorophyll, Carotenoid, Vitamin C, Protein, and Total Sugar Contents of Pea Pods

Chlorophyll and carotenoid contents in green seeds of peas were determined by using a spectrophotometer. Briefly, 1 g of the sample was extracted for 48 h in 10 mL of N, N-dimethylformamide. The extract was filtered by filter paper and measured at 470, 647, and 663 nm according to Moran [15], and the results were expressed as mg·g^{-1} (FW). To measure ascorbic acid in green pea seeds, titration methods were used in which 10 g of green pea seeds were homogenized with 90 mL of oxalic acid (6%) for 10 min. Then, 2,6 –dichlorophenol indophenol was used to titrate 25 mL of filtrated solution. The results were expressed as mg·100^{-1} g (FW), as described by El-Mogy et al. [16]. Protein content was determined as total nitrogen by micro-Kjeldhal method as described previously [17]. Total sugar content was measured by the phenol–sulfuric acid method, as described by Dubois et al. [18]. Briefly, 0.25 g of green seeds were homogenized in 20 mL of 80% ethanol for 5 min and then kept in a hot water bath (70 °C) for one hour. The extract was filtrated by filter paper, and then 5 mL of phenol and 5 mL of 98% sulfuric acid were added to 1 mL of the filtrated extract and left for one hour. The colored extract was measured at 490 nm using the spectrophotometer (UNICO S2100, Cole Parmer Instruments, Vernon Hills, IL, USA). A standard curve of glucose was used, and the results were expressed as mg·100^{-1} g fresh weight.

2.5. Storage Experiment

The storage experiment was carried out on the same day of harvest. Pea pods were harvested at 7:00 a.m. and transported immediately to the postharvest laboratory in one hour. Pea pods free from damages and defects, and uniform in size and shape were selected. The selected pods from every treatment were placed in polyethylene trays (about 250 g of pods), covered with stretch plastic film, and stored at 4 °C and 95% relative humidity for 45 days. The weight loss of pea pods was determined after 7, 15, 22, 30, 37, and 45 days of storage according to Ali et al. [19]. The chlorophyll, vitamin C, and total sugar contents were also determined during the storage experiment.

2.6. Determination of Herbicide Residues in Green Pods

2.6.1. Standards and Reagents

Reference standards of the four herbicides were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and their purity ranged from 94.8% to 98.8%. A stock solution was prepared in acetonitrile and kept in a refrigerator (0–5°C). All the reagents used in this study were purchased from Sigma-Aldrich (Oakville, ON, Canada), except for menthol, which was supplied by Supelco (Bellefonte, PA, USA). Acetonitrile was purchased from J.T. Baker (Phillipsburg, NJ, USA). Citrate-buffer-based QuEChERS (EN-QuEChERS) extraction kits, primary secondary amine bonded phase silica (PSA), and dispersive solid phase extraction (D-SPE) kits were supplied by Agilent (Santa Clara, CA, USA). Water was deionized in the laboratory using a Millipore (Burlington, MA, USA) MilliQ water purification system

2.6.2. Sample Extraction

Samples of green pea pods were collected randomly at the harvesting time from each plot of the treated and untreated plots. Samples were stored in a deep freezer at –20°C until further analysis. Pea samples were extracted according to the QuEChERS method [20]. A 10 g sample was weighed into a 50 mL centrifuge tube and extracted by adding 10 mL acetonitrile. Tubes were shaken by hand for 1 min and QuEChERS liquid extraction salt packet, containing magnesium sulfate anhydrous, sodium chloride, sodium citrate dibasic sesquihydrate, and sodium citrate tribasic dehydrate, was added to the tube. Sample tubes were capped tightly and shaken vigorously for 1 min by hand. Then the tubes were centrifuged at 4000 rpm for 5 min. The supernatants were filtered using syringe filter, transferred to a clean vial, and then injected into the GC or LC system for analysis.

The recovery tests were performed using herbicide-fortified samples, and the average recovery percentages were 78, 80.4, 88.9, and 94.3% for butralin, pendimethalin, bentazon, and fluazifop, respectively.

Analytical instrumentation: The herbicides bentazon, butralin, and fluazifop were analyzed by HPLC-DAD. The HPLC system was an Agilent 100 (Agilent Technologies) equipped with a quaternary pump, a variable wavelength, an autosampler, and an analytical column (150 mm × 4.6 mm id, ×5 μm ODS). The mobile phase used was as follows: acetonitrile/water (80%:20%) and flow rate 0.8 mL min^{-1} for bentazon; acetonitrile/water (65%:35%) and flow rate 0.7 mL min^{-1} for butralin; and methanol/water (85%:15%) and flow rate 0.7 mL min^{-1} for fluazifop. Injection volume was 20 μL, and the detection wavelength was 270 nm. The retention times under the above instrumental conditions for bentazon, butralin, and fluazifop were 2.1, 3.4, and 2.6 min, respectively. Pendimethalin was analyzed using GC-ECD as described by Abdallah et al. [21]. The gas chromatograph was an HP 6890 with auto sampler, equipped with double micro-electron capture detector (μECD) with capillary column (30 m × 0.32 mm × 0.25 μm). The injector temperature was 250 °C, detector temperature 300 °C, and flow rate of N_2 1 mL/min^{-1}. The temperature programs of GC were as follows: initial temperature 70 °C for 2 min, rise 5 °C/min up to 200 °C and held for 2 min, then 5 °C/min up to 260 °C and held for 5 min. The retention time of pendimethalin was 3.8 min. The evaluation of the calibration curves and linearity for all herbicides were carried out based on injections of the standard solutions prepared at different concentrations. The linearity (R^2 value) obtained was ~0.99 for all the standard herbicides analyzed.

2.7. Statistical Analysis

The data from the two seasons were combined due to the non-significant interaction between treatments and years for all measured parameters. Data were statistically analyzed by SPSS software and treatment means were compared using Duncan's multiple range test at the 0.05 level. Correlation was statistically analyzed using SPSS program (14.0).

3. Results and Discussion

3.1. Weed Control Efficiency, Total Weed Dry Weights

The prevalent annual broadleaf and grass weed species associated with pea in the weedy check plot were identified, and the corresponding density percentages are shown in Table 2. Five annual weed species were recorded from the experimental fields over the two years. The majority was the grass weed *Cenchrus ciliaris*, which represented almost 80%, whereas broadleaf weeds density was about 20%. The major broadleaf weed species was *Malva parviflora*, one of the most common and troublesome weeds in pea, which alone represented about 13% out of 20%, while *Chenopodium album*, *Rumex dentatus*, and *Cichorium endivia* comprised only 7%.

Table 2. Dominant annual weed species and their density (% m^2).

The Name of Weed	Relative Frequency of Weeds (%)
Cenchrus ciliaris	79.2
Malva parviflora	12.8
Chenopodium album	6.5
Rumex dentatus	0.79
Cichorium endivia	0.56
Total	100

Data recorded at 60 days from the sowing date showed that all the weed control treatments were superior to weedy check in reducing the total weed dry weight (Figure 1A). Twice hand-weeding at 20 and 40 days after the sowing date achieved the lowest total weed dry weight of 5 g/m^2, while the highest weed dry weight (29 g/m^2) was recorded from unweeded plots. The grass weeds density percentages, after application of fluazifop (selective grass herbicide), butralin, pendimethalin, and bentazon (selective broadleaf herbicide) were 0, 100, 100, and 100%, respectively, whereas weed density in hoeing plots was 84% (Figure 1B). Bentazon, butralin, and pendimethalin effectively controlled broadleaf weeds, as the lowest infestation percentage of 0% was observed, while the greatest broadleaf weeds density of 100% was in the fluazifop plots. Data also revealed that hoeing was more effective for controlling broadleaf than grass weeds (Figure 1B,C). In terms of total weed control, combined data showed that the highest total weed control efficacy was 87, 76, and 69%, respectively, in the hoeing treatment, then the fluazifop and bentazon treatments, without statistical difference. A significant difference in weed control efficiency was shown among bentazon, butralin, pendimethalin, and hoeing (Figure 1D). Pre-emergence application of pendimethalin and butralin equally exhibited the lowest bioefficacy by 62% and 61%, respectively, with no significant difference from bentazon (Figure 1D). To our knowledge, there are no published data on the efficacy of the tested herbicides for controlling the grass weed *Cenchrus ciliaris* in pea. Poor weed control by pendimehalin and butralin against such grass weed could be due to higher infestation and weed density of the grass weeds compared to the broad-leaved. It is a troublesome and aggressive grass due to its root system and readily spreading mechanism. It thrives in conjunction with legumes because of the improved N status of the soil [22]. It seems that *Cenchrus* is difficult to control and less sensitive to pre-emergence herbicides. Pre-emergence herbicides also give poor control of *Cenchrus* in soybean [23]. S-metolachlor was inefficient against annual grass weeds *Bromus arvensis L.*, *Avena fatua L.*, and *Avena ludoviciana* in forage pea [24]. Fluazifop can provide season-long control of such annual grasses [25].

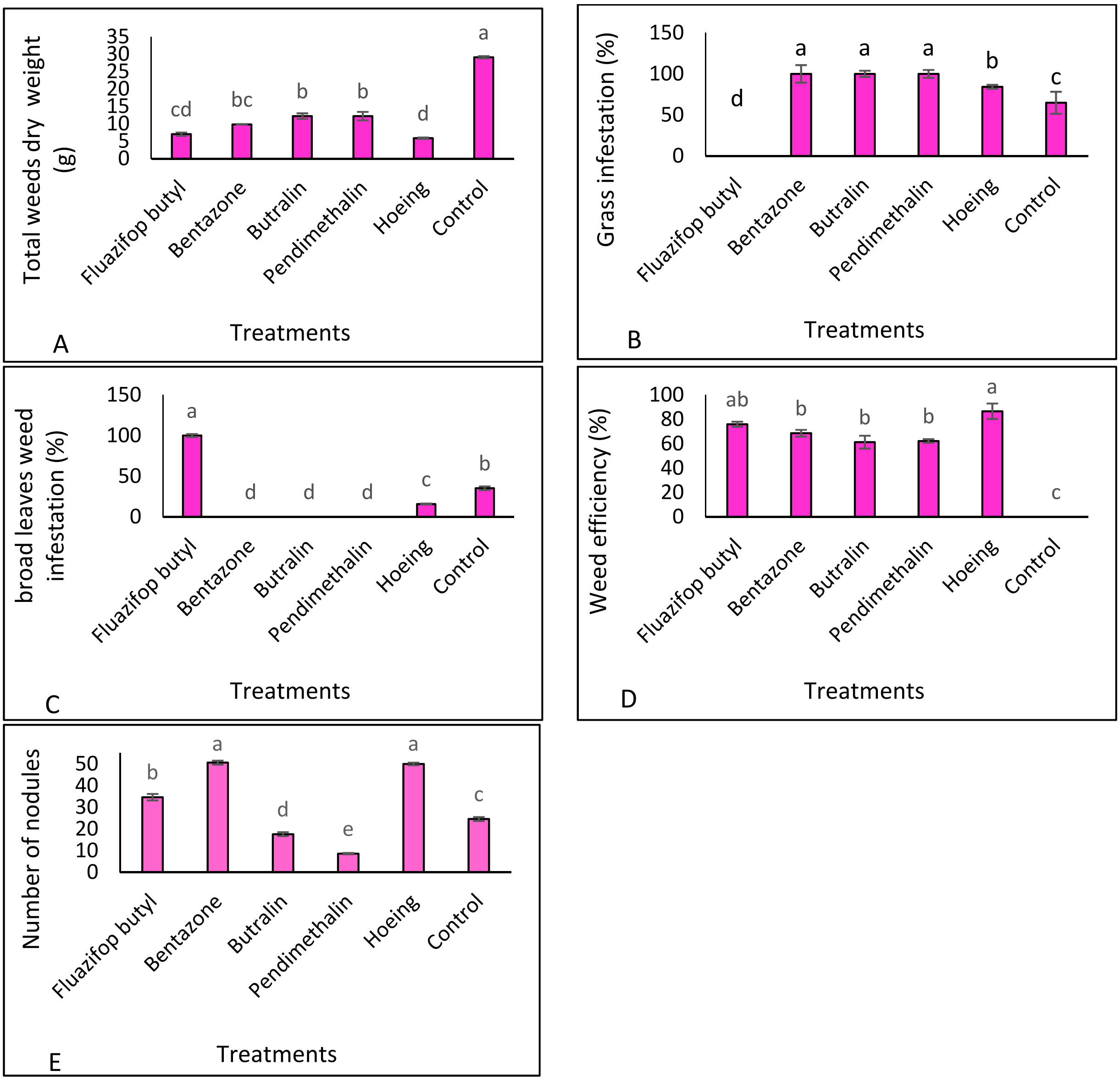

Figure 1. Effect of pre- and postemergence herbicides on total weed dry weight (**A**), grass infestation (**B**), broad leaf infestation (**C**), weed efficiency (**D**), and number of nodules (**E**). Different letters indicate significant differences between treatments (Duncan test at $p < 0.05$); vertical bars indicate standard error.

Generally, the variation in weed control efficiency might be due the differences in effectiveness of herbicides against different weeds in the field. The weed densities were influenced by weed management during all the growth seasons. Several reports have confirmed that hoeing twice is the most effective weed control practice for reducing weed dry matter [3,8,26–28]. Butralin and pendimethalin, sharing the same mode of action, also decreased weed dry weight and showed better weed efficiency compared to untreated check plots. Similar results were found by Hassanein [29] in peas and by El- Metwally and Saad El-Din [30] in soybean fields. Fluazifop, the selective grass weed herbicide, exhibited the greatest efficacy based on total weeds, with no significant difference with hoeing. This was attributed to the fact that the grass weed density/m^2 represented almost 80% of the total. The results on fluazifop are consistent with Vaghasia and Nadiyadhara [31], who showed the herbicidal treatment fluazifop was associated with a significantly lower number of grassy weeds and the lowest total dry weed matter in a groundnut field, with weed control efficiency (79.55%) close to the present data (76%).

Although hand weeding was found to be an effective method of weed control, labor unavailability and its high cost limit its practical use. Selective herbicide application is a better option for effective annual weed control and for reducing expenditure while boosting pea yield [30]. It may be inferred from the results that bentazon can be used effectively for controlling broadleaf weeds in pea with high selectivity, which was earlier supported by Delchev and Barakova [24].

3.2. Nodule Formation

Data in Figure 1E and photos shown in Figure 2 show the effect of weed control treatments on the number of active nodules in pea roots. Pea plants responded differently when different weed control treatments were applied concerning the active numbers of nodules per plant. Postemergence herbicides (bentazon and fluazifop) had no negative effect on the number of active nodules per plant (pink in color, Figure 2). However, pre-emergence herbicides (butrallin and pendimethalin) decreased active nodule numbers compared with the untreated control (Figure 1E). A significant increase in the number of active nodules was observed in the bentazon treatmentcompared to the unweeded check. In contrast, a significant reduction in nodules number was noted compared to the untreated plants, as 28.5 and 66% less nodules in butrallin and pendimethalin, respectively (Figure 1E). It is well known that legume symbionts, rhizobia, are essential microbial communities that catalyze many processes important for soil fertility and plant growth (e.g., cycling of nutrients from both soils and fertilizers and the direct transfer of nutrients to crops) [32]. Reports on herbicides' interaction with rhizobia and legume—*Rhizobium* symbiosis are conflicting. This may be due to differences in the nature and the level of these effects related to type and application rate of herbicides, crops species, crop varieties, *Rhizobium* species and strains, and soil environment, as well as the interactions amongst all of these factors [33–35]. Bentazon was found to have no adverse effects on pea nodule biomass when used at recommended doses [36]. Present results are supported by the studies of Santos et al. [37], in which no harmful effects on nodulation in cowpea were observed after the application of bentazon. However, the current data disagree with those from Singh and Wright [27], who indicated that bentazon was harmful to nodulation in two varieties of peas.

This could be due to the rate they used, which was three times higher than the recommended labeling rate used in the current study. Opposite results were obtained by the same authors in another in vitro study, as they found that bentazon was safe to rhizobia and nodulation in peas when they tested bentazon at the recommended rate [38]. Monteiro et al. [39] observed that bentazon showed low phytotoxicity to cowpea and had little influence on the nodulation of the crop. On the other hand, pendimethalin had inhibitory effects on the nodulation process and the number of nodules of soybean when applied at the recommended rates of 1.7 ai$\cdot$ha^{-1} [40], which is consistent with our findings. Pendimethalin also adversely affected the legume—*Rhizobium* symbiosis when applied at 1.0 kg ai$\cdot$ha$^{-1\cdot}$ [41]. On the contrary, pendimethalin did not inhibit the development of nodulation or nodule number of cowpea [42]. Moreover, pendimethalin at 0.9 kg ai$\cdot$ha^{-1} enhanced the number of nodules/plants in soybean, as reported by Rupareliya et al. [43]. In addition, nodulation diminished significantly when trifluralin (chemically and actively similar to pendimethalin) was applied to chickpea, as described by Zargar et al. [44].

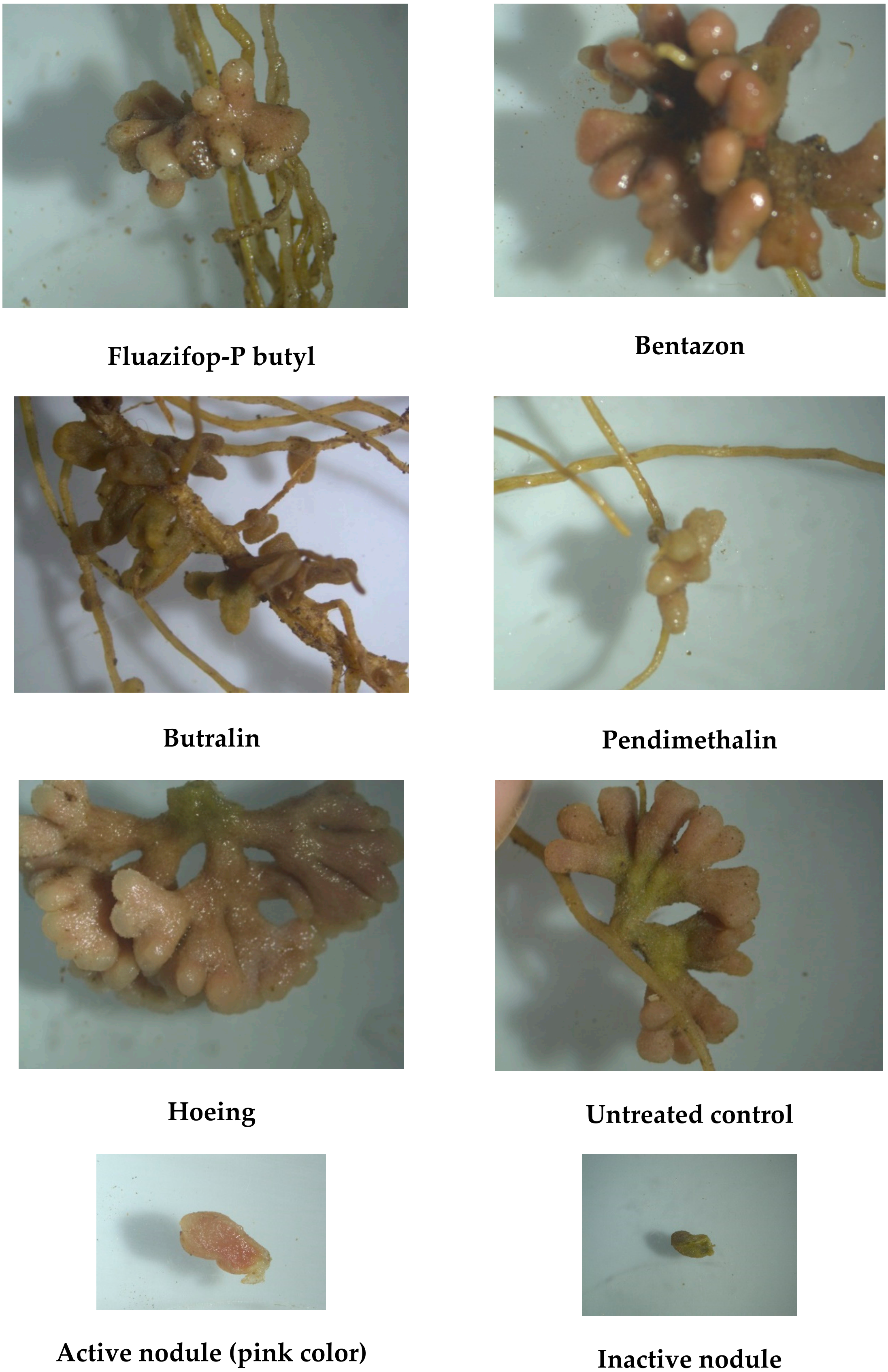

Figure 2. Effect of pre- and postemergence herbicides on nodule formation.

Regarding fluazifop, current results are in agreement with the studies of Zaid et al. [45], which showed no significant effects on the number of nodules per plant. Raman and Krishnamoorthy [46] and Das [8] also found that the nodulation in pea was not affected significantly by fenoxaprop (chemically and actively similar herbicide to fluazifop). The present data also indicated that nodulation of pea plants is sensitive to the recommended rates of butralin. This result was confirmed by González et al. and Abdelhamid and El-Metwally [28,47], as butralin at the recommended rate markedly decreased the number of nodules in soybean. Based on the obtained results, Procopio et al. [48] reported that herbicides can affect the formation and growth of root hairs, which in turn affects the process of infection by nitrifying bacteria. The herbicide applied at the recommended rate for a legume crop would be fairly nontoxic to the plant, but possible toxic effects on the rhizobium bacteria or the nodulation process may occur [49]. The lack of inhibitory effect of an herbicide on nodulation could be due to its rapid inactivation in soil or its quick translocation, along with photosynthate, to a distant metabolic sink [45].

3.3. Plant Growth and Dry Matter

Peas treated with bentazon were taller than plants in the weedy check by 29.3%, followed by fluazifop butyl and hoeing treatments (24.7% and 20.5%, respectively) (Figure 3A). There was no significant difference in root length among fluazifop butyl, bentazon, hoeing, and untreated control treatments (Figure 3B). The heavier plants were recorded from manual hoeing plots, followed by bentazon and pendimethalin, compared with the untreated control, by 41.6, 30.2, and 25.9%, respectively (Figure 3Cc). Number of pea leaves was decreased by fluazifop, butralin, and pendimethalin compared to untreated plants (Figure 3D). However, bentazon and hoeing showed the highest number of leaves per plant. The effect of the different treatments had little or no effect on the number of branches (Figure 3E). The highest dry matter content of pea plants was observed in fluazifop and unweeded treatments, while the lowest dry matter was noted in the pendimethalin treatment (Figure 3F). The current results correspond to those of Eldabaa et al. [50], who found that application of bentazon at 0.5 and 0.75 L·fed^{-1} for weed control in soybean significantly increased the plant height by 12.6 and 16.2%, respectively, compared to control check. Singh and Wright [27] found that shoot and pod dry weights in pea were decreased by bentazon applied at the recommend rate. Two hand-hoeings were the most superior treatment in increasing plant height and shoot dry weight of faba bean at 90 days from sowing [51]. The growth enhancement might be due to the effective control of weeds, which offers less competition between crop and weeds during the critical period of crop growth, as reported by Singh et al. [52], Singh and Jolly [53], Mishra and Singh [54], and Meena et al. [55]. In contrast to our results, shoot and root growth of lentil was reduced in fluazifop-treated groups [56].

3.4. Protein Content and Total Yield

Protein content of pea pods was increased by 32.9% and 15.7% in bentazon and hoeing treatments, respectively, compared to the non-treated control. In contrast, fluazifop and pendimethalin reduced protein content by 12.1 and 27.9%, respectively. Butralin had no significant effect on protein content compared to the untreated check (Figure 4A). All the tested herbicides (except butralin) and hoeing resulted in higher total yield per plant compared to non-treated control by 122%, 87.7%, 41.1%, and 25.2% for bentazon, hoeing, fluazifop butyl, and pendimethalin, respectively (Figure 4B). Bentazon at 0.5 and 1.0 L also increased the protein and carbohydrate contents in soybean seeds [57]. Nitrogen percent was also increased from 16.4 to 23.6% by application of bentazon compared to unweeded check treatment [50].

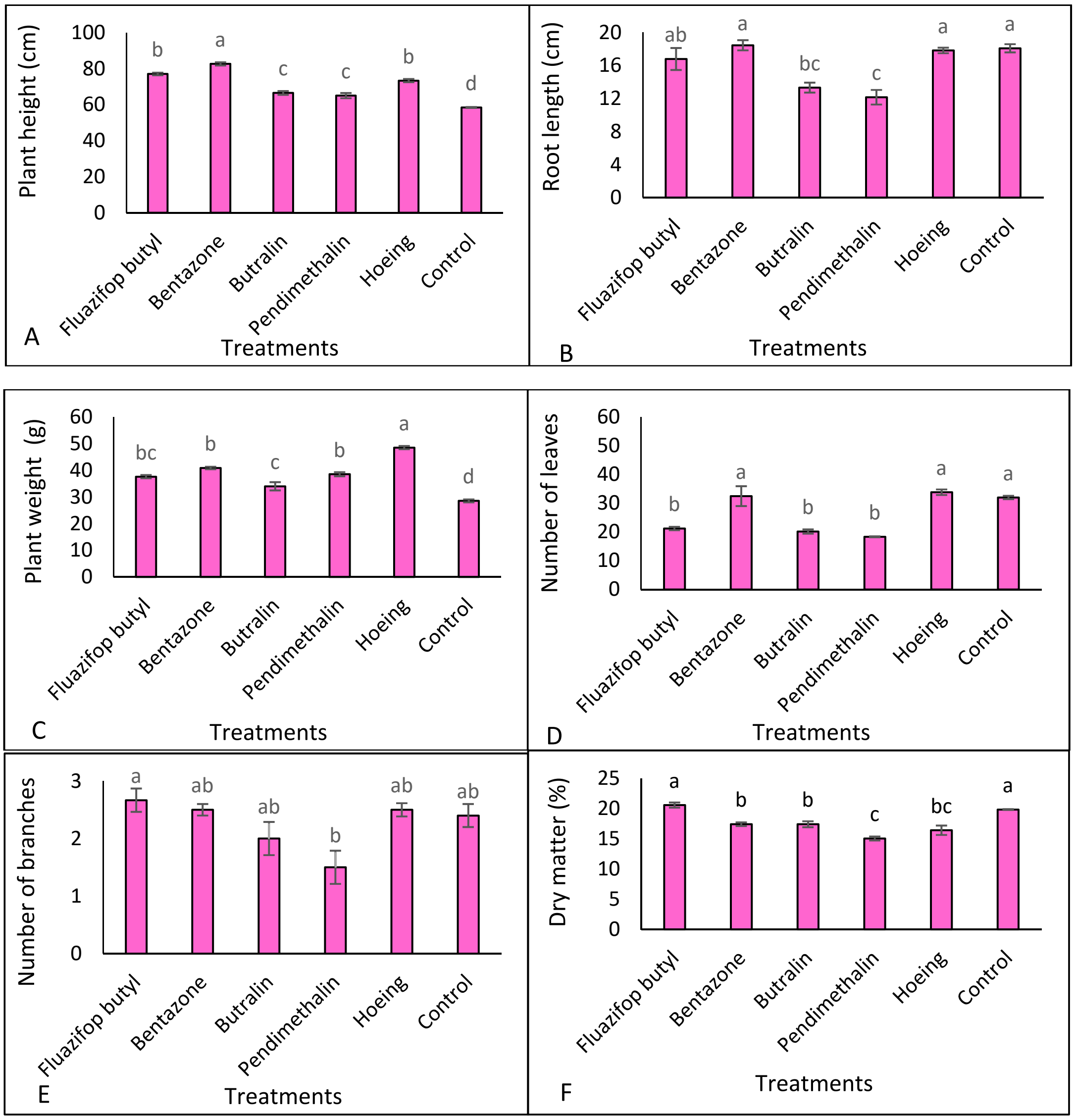

Figure 3. Effect of pre- and postemergence herbicides on plant height (**A**), root length (**B**), plant fresh weight (**C**), number of leaves (**D**), number of branches (**E**), and dry matter % (**F**). Different letters indicate significant differences between treatments (Duncan test at $p < 0.05$); vertical bars indicate standard error.

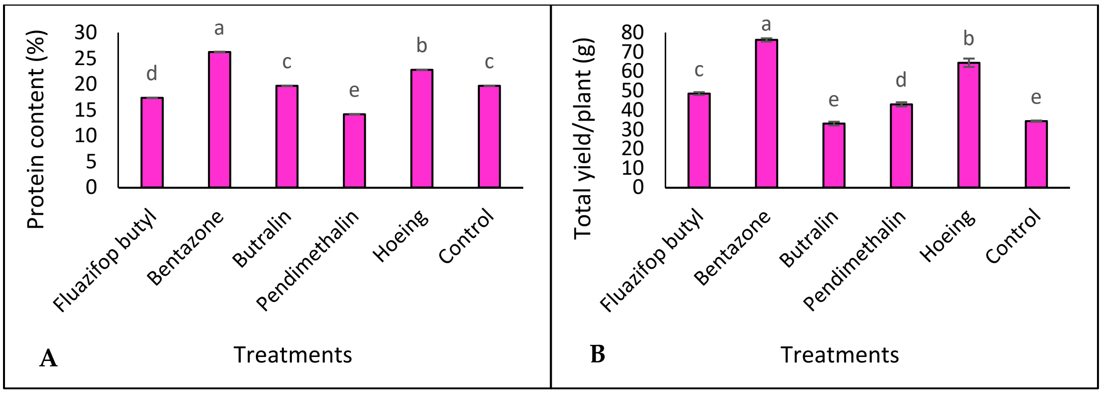

Figure 4. Effect of pre- and postemergence herbicides on protein content (**A**), yield/plant (**B**). Different letters indicate scheme 0. vertical bars indicate standard error.

The higher protein content in the bentazon and hoeing treatments could be attributed to the greater active nodule numbers (Figure 1F). The inhibitory effect of herbicides on proteins has been reported by other investigators [58,59]. This inhibition could be explained on the basis of N_2 assimilation or inhibition in the activity of nitrate reductase that is caused by herbicides [60].

The toxicological effects of various herbicides on legumes have been reported [34]. Chhokar et al. [61] demonstrated that weed interference caused decrease in pods number per plant and grain yield. Bentazon, also at 0.75 L·fed^{-1} (one hectare = 2.4 feddan), showed the highest pod weight and seed number per plant compared to the other two tested herbicides (butralin and dinitramine) [50]. However, Al- Khatib et al. [62] described injury to peas and reduction in yield with bentazon applied at 1.68 kg/ha (three times higher than that use rate). Pendimethalin significantly affected the physiological parameters, growth determinants, yield traits, and grain yield in mung bean [63]. Pendimethalin also decreased peanut growth, mature pods, and pods yield [55]. Butralin recorded the lowest seed number/soybean plant compared to the unweeded treatment [50]. However, Abdelhamid and El-Metwally [28] pointed out that butralin at 1.2 or 2.4 kg·ha^{-1} significantly increased the seeds number/plant compared to unweeded check in soybean. Post-emergence application of fluazifop-p-butyl at 167 g·ha^{-1} also showed significantly higher pod yield in groundnut, as pointed out by Vaghasia and Nadiyadhara [31].

3.5. Green Pea Pods Weight Loss % and Chemical Composition during Storage

The effect of herbicides treatments on weight loss of pea pods during refrigerated storage is presented in Figure 5. Weight loss of pea pods was not affected by any of the herbicide treatments at the beginning of storage (after 15 days). After 22 days from storage, pendimethalin showed the highest weight loss value. However, bentazon treatment significantly showed the lowest weight loss after 30 days of storage at 4 °C until the end of the storage period compared to the weedy check and all other treatments.

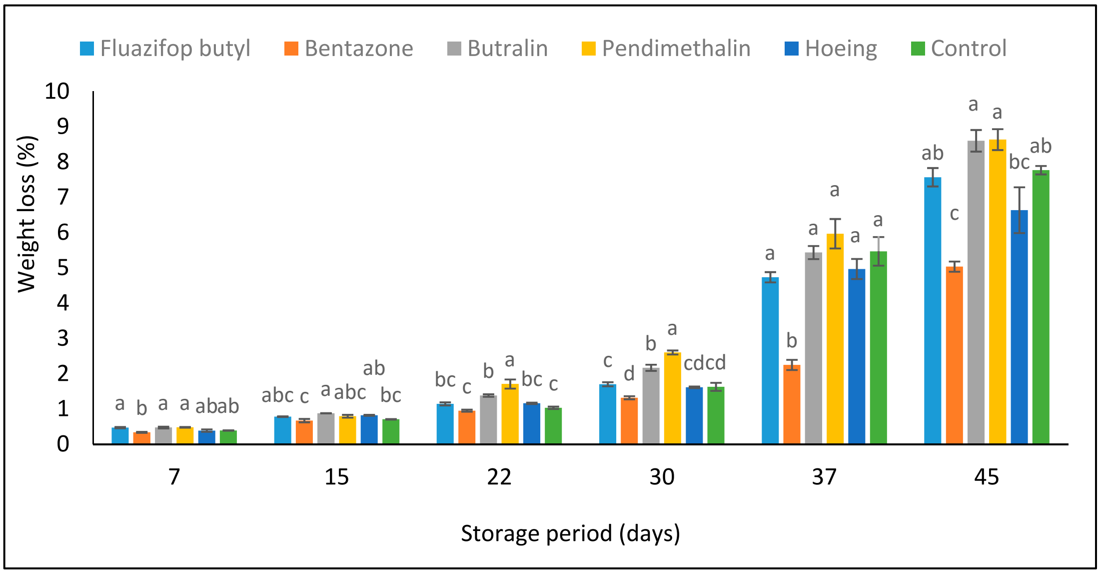

Figure 5. Effect of pre- and postemergence herbicides on pod weight loss % during cold storage at 4 °C for 45 days. Different letters indicate significant differences between treatments (Duncan test at $p < 0.05$); vertical bars indicate standard error.

There were very few previous reports on the effect of herbicides on quality and shelf-life of pea pods during refrigerated storage. At harvest time, chlorophyll content was higher in pods treated with pendimethalin and bentazon than other treatments or the non-treated control. During the storage period, chlorophyll content decreased with increasing storage period (Figure 6A). After 3 weeks of storage, bentazon treatment showed the highest chlorophyll content in pea pods compared to other treatments or the control.

At the end point of the storage, bentazon and butralin treatments maintained chlorophyll content compared to other treatments.

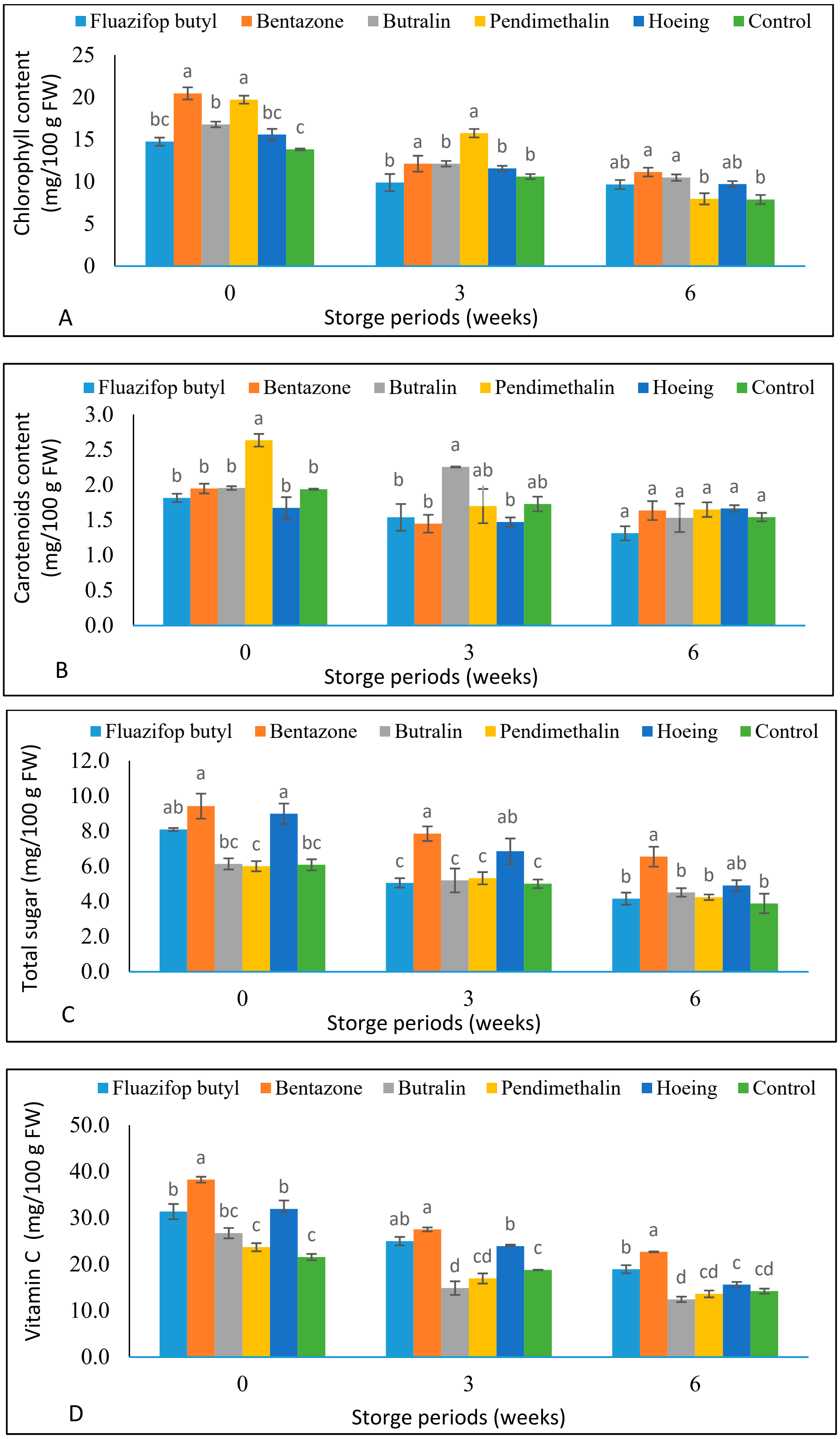

Figure 6. Effect of pre- and postemergence herbicides on chlorophyll content (**A**), carotenoid (**B**), total sugar (**C**), and vitamin C (**D**). Different letters indicate significant differences between treatments (Duncan test at $p < 0.05$); vertical bars indicate standard error.

Results shown in Figure 6B indicated that, at harvest time, carotenoids content in pea pods was increased in pods from pendimethalin plots compared to all other treatments. Moreover, after 3 and 6 weeks of storage, all treatments had no significant effect on carotenoids content in pea pods.

Additionally, total sugar content in pea pods decreased with increasing storage period (Figure 6C). At all measured points, the highest total sugar content in pea pods was recorded from plots treated either by bentazon or hand hoeing.

At harvest time, 3 and 6 weeks after storage, higher levels of ascorbic acid (vitamin C) were observed in the fluazifop butyl, bentazon, and hand hoeing plots than in other treatments and non-treated plots (Figure 6D).

Bentazon did not significantly change either the content of chlorophyll a + b or the carotenoids content in soybean [64,65]. Moreover, the application of bentazon to potato fields caused an increase in the content of ascorbic acid in comparison with the control [66]. Ascorbic acid is found mainly in green leaf and fruits as antioxidant factor. It participates in a variety of processes, including photosynthesis, cell wall growth and cell expansion, and resistance to environmental stresses [67]. It was found that fenoxaprop (actively and chemically similar to fluazifop) increased ascorbic acid levels in wheat [68]. Although herbicides affected ascorbic acid level, different responses to the same herbicide can be negative in another cultivar of wheat [69]. It was reported that fenoxaprop also showed negative effects on levels of carotenoids and total chlorophyll in wheat [70]. After a certain period of growth in the presence of herbicide, pigment content can be reduced either by initiation of pigment degradation or by inhibition of biosynthesis of either chlorophylls or carotenoids [71].

3.6. Determination of Herbicide Residues in Green Pods

Results showed that no detectable levels of any of the four herbicides were found in the analyzed green pods. The herbicide residues determined were below the detection limit (LOD), which ranged between 0.02 to 0.1 mg·kg^{-1}. Limited data are available on the terminal residues of herbicides, particularly butralin, bentazon, and fluazifop, in pea pods or even in legumes. These findings imply that the application of these herbicides in peas at the recommended rates is harmless from a residue standpoint, and the green pods could be considered safe for human consumption. However, further studies might be useful to confirm the present findings. Kaur and Bhullar [72] pointed out that the residue level of pendimethalin analyzed by HPLC in mature peas was below the limit of quantification, which was 0.05 mg·kg^{-1}. In the studies of Saha et al. [73], no pendimethalin residues were detected in peanut kernel at harvest time, which supports our results. Pendimethalin was not detected in analyzed sweet potato tubers [74]. The residues of bentazon in maize plants were below the maximum residual level (MRL) value [75]. Residues of fluazifop analyzed by GC in soybean grains and harvested 90 days after application were not detected [76]. The final residue concentrations of butralin in peanut were below the limit of quantification at the normal harvest time [77]. No butralin residues were found in pea seed yield produced under subsurface drip irrigation systems in new reclaimed soil [78]. The residual effect of fluazifop applied to groundnut was safe on the plant and the succeeding crops of wheat and gram [31]. On the contrary, residues of fluazifop in soybean grains analyzed by HPLC were found to be 0.297, 0.300, and 0.312 µg·g^{-1} at 125, 250, and 500 g·ai·ha^{-1}, respectively [79].

4. Correlation Study

The Pearson's correlation analysis in Table 3 shows that the weed control efficacy significantly and positively correlated with pea plant length, yield per plant, and chlorophyll content. A positive correlation between active nodules number and protein content was obtained by the value of 0.831, which supports the present results.

Table 3. Correlation coefficients among measured growth and yield parameters.

	Weed Dry Weight	**Weed Efficacy %**	**N. of Active Nodules**	**Plant Length**	**Root Length**	**Plant Fresh Weight**	**Number of Leaves**	**Number of Branches**	**Dry Matter**	**Protein**	**Total Yield/Plant**	**Ch**	**Ca**	**T. Sugar**
Weed Efficacy %	−0.97**													
N. of Active Nodule	−0.398	0.392												
Plant length	−0.75 **	0.710 **	0.736 **											
Root length	0.129	0.135	0.752 **	0.368										
Plant Fresh weight	−0.79 **	0.798 **	0.606 **	0.615 **	0.125									
Number of leaves	0.186	−0.167	0.721 **	0.200	0.787 **	0.242								
Number of branches	−0.043	0.015	0.627 **	0.378	0.672 **	0.156	0.492 **							
Dry matter	0.298	−0.343	0.151	0.027	0.445	−0.478 *	0.101	0.482 *						
Protein	−0.138	0.128	0.831 **	0.556 *	0.671 **	0.350	0.779 **	0.491 *	0.049					
Total yield/plant	−0.553 *	0.556 *	0.847 **	0.828 **	0.506 *	0.748 **	0.533 *	0.374	-0.233	0.710 **				
Chlorophyll	−0.517 *	0.509 *	0.535 *	0.663 **	0.215	0.321	0.151	0.113	0.003	0.601 **	0.488 *			
carotenoid	0.006	−0.068	0.047	−0.066	−0.186	0.333	0.135	−0.037	−0.474 *	0.202	0.219	0.104		
T. sugar	−0.338	0.375	0.570 *	0.614 **	0.306	0.383	0.465	0.130	−0.262	0.696 **	0.728 **	0.583 *	0.081	
Vit. C	−0.359	0.310	0.719 **	0.854 **	0.584 *	0.312	0.366	0.442	0.231	0.557 *	0.780 **	0.449	−0.129	0.629 **

** Correlation is significant at the 0.01 level (2-tailed); * correlation is significant at the 0.05 level (2-tailed).

These results indicate that the reduction of weed density using herbicidal treatments and hand hoeing had a significant impact on plant growth and productivity of pea. The number of active nodules significantly correlated with plant length, root length, number of leaves and branches, total yield/plant, chlorophyll content, total sugar content, and vitamin C.

5. Conclusions

Not much work has been done on the effect of these herbicides on nodulation efficiency, growth, and quality of peas. On the basis of these two-year results, it could be concluded that the application of fluazifop was effective against *Cenchrus ciliaris,* while bentazon effectively controlled *Malva parviflora, Chenopodium album, Rumex dentatus*, and *Cichorium endivia* in peas. No inhibitory effects were observed on the root nodulation in peas, except for the pre-emergence herbicides (pendimethalin and butralin). Moreover, the postemergence herbicide bentazone had a positive effect on nodulation efficiency and also improved pea's quality and the chemical constituents of the pods. During cold storage, bentazone maintained the contents of chlorophyll, carotenoids, vitamin C, total sugars, and protein. Herbicide residues in pods were found to be within safe limits, suggesting that pods can be safely consumed at harvest. As a lower weed density of broad-leaved species (20.8%) was found in the experiment, bentazon should also be tested in cases with higher broad-leaved weed density in pea cultivation to ensure its contribution in controlling weeds and increasing yield. Despite the effectiveness of hand weeding twice, scarcity of labor and higher cost make herbicides a more cost-effective method favored for farmers. According to the overall study, bentazon showed higher pea crop selectivity and safety. However, future research is needed on the response of pea and its associated weeds to the combined, sequential, and or tank mixing of the pre- and postemergence herbicides (e.g., mixture of bentazon + fluazifop). In addition, the response of other pea varieties to common legume herbicides under different agro-climatic and ecological conditions should be investigated.

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