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Research Article

THE ROLE OF *Rhizobium gallicum* INOCULATION IN THE AGRONOMIC PERFORMANCE AND SOIL BIOCHEMICAL PROPERTIES OF THE BEAN (*Phaseolus vulgaris* L.) PLANT IN RELATION TO HERBICIDE APPLICATIONS

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ABSTRACT. Legume cultivation is a strategic agricultural sector that improves soil fertility through biological nitrogen fixation in sustainable farming systems. The symbiosis between legumes and *Rhizobium* species is central to this process. However, pesticides widely used in modern agriculture, particularly those applied to seeds and soil, are known to impair this symbiosis and the soil microbiota. Although soil microorganisms can sometimes degrade pesticides into harmless compounds, pesticides can also reduce the viability and activity of beneficial microorganisms. Few studies have addressed the effects of soil-applied pesticides on the survival of *Rhizobium* bacteria, nodule formation, and symbiotic nitrogen fixation. The aim of this study was to determine the effects of two commonly used herbicides on plant growth, nodulation, and soil biological properties in a common bean (*Phaseolus vulgaris* L.) cultivar. The experiment was conducted under controlled greenhouse conditions in pots containing 4 kg of soil (oven-dry basis). Two soil-applied herbicides widely used in agriculture, Bonaflan and Fist Gold, were tested. After basal fertilization and herbicide application, surface-sterilized dry bean seeds were sown either inoculated or non-inoculated with *Rhizobium gallicum*. Plants were harvested after flowering, and morphological, physiological, and microbiological parameters were evaluated. Shoot and root length, shoot and root fresh weight, and shoot and root dry weight were measured as growth parameters; nodule number and nodule weight were determined as nodulation parameters; and nitrogen content was analyzed in shoot and root tissues. Soil biological properties were assessed by measuring soil respiration and the activities of dehydrogenase, urease, alkaline phosphatase, and arylsulfatase. Herbicides applied at the recommended doses had statistically significant effects on some growth and nodulation parameters of the dry bean cultivar. The herbicides also significantly affected soil enzyme activities, which are biological indicators of soil microbial activity. These results indicate that agricultural pesticides affect not only target organisms but also the microbial balance of the soil ecosystem and plant–microorganism interactions, which should therefore be carefully evaluated.

Keywords: *Bacterial inoculation, Bonaflan, Fist Gold, Nodulation, Soil enzymes, Yield parameters*

INTRODUCTION

Legumes are often inoculated with *Rhizobium* bacteria at sowing to improve nodulation and nitrogen fixation. The effects of pesticides on *Rhizobium* bacteria, which form a symbiotic relationship with legumes, have important implications for agricultural productivity. Pesticides may not be compatible with microbial inoculants, because most of them also affect non-target organisms. In addition, pesticides can disrupt the metabolism of soil microorganisms. The accumulation of fungicides, insecticides, and herbicides in soil generally causes only minor changes in the microbial environment;

however, more pronounced changes may occur when pesticides are applied frequently and in large quantities [1, 2]. Bacteria of the genus *Rhizobium* play an important role in agriculture because they form symbioses with legumes, fix atmospheric nitrogen, and thereby enhance soil fertility [3]. This ability of nitrogen-fixing bacteria has been exploited in the field to meet crop nitrogen requirements, and it offers an alternative to nitrogen fertilizers, whose excessive use harms the environment [4].

Herbicides interfere with the biochemical and physiological processes of weeds, killing them or substantially delaying their growth [5, 6]. Their effects on soil microorganisms, including nitrifying bacteria, may be harmful, beneficial, or neutral, depending on numerous environmental factors [7]. Adverse effects are particularly likely in microorganisms that share biosynthetic pathways with the target plants [8]. Applying herbicides such as bromoxynil and paraquat at doses above the recommended rate has been reported to markedly inhibit plant growth, nodule formation, and symbiotic nitrogen fixation in *Vicia faba* [9]. Singh and Wright [10] reported that several pre- and post-emergence herbicides had dose-dependent inhibitory effects on nodulation, nitrogen fixation, photosynthetic capacity, and yield of *Pisum sativum* L. grown under nitrogen-deficient conditions. Abd-Alla et al. [11] reported that various pesticides adversely affected plant growth, nodule formation, and arbuscular mycorrhizal colonization in legumes. Dığrak and Kazanıcı [12] found that organophosphorus insecticides generally had no adverse effect on the soil microbiota, although isofenphos increased the total bacterial count. Araújo et al. [13] reported that glyphosate increased microbial activity in soil, with increases in actinomycete and fungal populations and a slight decrease in bacterial counts.

Taylor [14] evaluated the effects of various herbicides on nitrogen fixation in pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.) and showed that the reduction in nitrogen fixation resulted not from a direct effect of the herbicides on *Rhizobium* bacteria but from a temporary suppression of plant growth. Aurelia [15] investigated the effects of the pesticides Decis, Bavistin, and SDMA applied at recommended doses on soil microorganisms and concluded that Decis increased the total number of viable bacteria, whereas Bavistin and SDMA did not inhibit the microbial population. Datta et al. [16] reported that isoxaflutole generally had negative effects on plant growth and symbiotic nitrogen fixation in chickpea cultivars. Ahemad and Khan [17] reported that pyriproxyfen caused dose-dependent negative effects on growth, nodulation, and nutrient uptake in *Pisum sativum*, and that inoculation with strain MRP1 significantly alleviated these effects. Kunal and Sharma [18] reported that Captan was highly toxic to *Mesorhizobium ciceri*; however, pesticides used at recommended doses neither disrupted the symbiosis with chickpea nor reduced yield.

Adhikary et al. [19] investigated the effects of three herbicides commonly used in India (pendimethalin, oxyfluorfen, and propaquizafop) on soil microbial populations in transplanted chili and found that the herbicides significantly inhibited soil microbial populations, depending on the herbicide type. Kurrey et al. [20] noted that certain herbicide–insecticide combinations may increase yield and leaf area index in soybean but can also inhibit *Rhizobium* populations. Simarmata et al. [21] reported that residues of a herbicide mixture of atrazine and mesotrione applied at different ratios had long-term, dose-dependent negative effects on legumes and soil microorganisms, decreasing dry biomass and markedly reducing the number of colony-forming microorganisms. Sevilmiş and Sevilmiş [22] chronologically reviewed international studies on the effects of different herbicides and application rates on nodule formation, root development,

nitrogenase activity, and yield, and emphasized that herbicides generally reduce nitrogen fixation, although this effect varies with plant species, environmental conditions, and the tolerance of *Rhizobium* strains.

Kumar et al. [23] evaluated the effects of four organophosphate pesticides on soil microorganisms and plant growth-promoting rhizobacteria. The adverse effects of the pesticides applied in combination were greater than those of the individual pesticides, and the overall order of toxicity was phorate > acephate > monocrotophos > glyphosate. Rodrigues et al. [24] reported that the pesticide StandakTop® reduced the viability of a *Bradyrhizobium elkanii* strain and nitrogen accumulation under field conditions. Zargari et al. [25] reported that trifluralin and imazethapyr inhibited chickpea growth and nitrogen fixation, whereas pyridate supported growth but limited symbiotic activity. Sah [26] reported that pendimethalin increased chickpea growth and yield, controlled weeds effectively, and caused minimal harm to the soil microbiota, whereas imazethapyr markedly reduced *Rhizobium* populations and nodule numbers. Pertile et al. [27] reported that the combined application of flumioxazin and imazethapyr inhibited early nodulation, nitrogen accumulation, and biochemical parameters in soybean. Pesticides have also been reported to reduce the growth and survival of rhizobia, nodulation, and the symbiotic efficiency of legume–rhizobia associations [28, 29].

In this study, two commonly used herbicides, Bonaflan (pre-emergence) and Fist Gold (post-emergence), were applied to a clay loam soil sown with bean seeds inoculated or non-inoculated with *Rhizobium*. The effects of these soil-applied herbicides on yield components, shoot and root nitrogen contents, nodulation, and selected soil biological properties of the bean cultivar were investigated.

MATERIALS AND METHODS

The study was conducted in a computer-controlled greenhouse (temperature 25±3°C, solar radiation 1750±50 kcal m^{-2}, relative humidity 60±10%) in a randomized block design with four replications. Basal fertilization was applied according to the nutrient status of the soil [200 mg N/kg (urea), 80 mg P/kg (TSP), 150 mg K/kg (K_2SO_4), 100 mg Mg/kg ($MgSO_4$), 6 mg Fe/kg ($FeSO_4 \cdot 7H_2O$), 4 mg Zn/kg ($ZnSO_4 \cdot 7H_2O$), 4 mg Mn/kg ($MnSO_4$), 1.5 mg B/kg (H_3BO_3)]. The bacterial culture was grown under sterile conditions on Yeast Mannitol Agar (YMA) and in Yeast Mannitol Broth (YMB). Bean seeds were surface-sterilized with 0.5% sodium hypochlorite solution and sown in pots containing 4 kg of soil (oven-dry basis), either inoculated with *Rhizobium gallicum* (1×10^8 CFU/ml in YMB) or non-inoculated. The herbicides were applied to the soil at their recommended doses: Bonaflan (pre-emergence) at 250 g/ha before sowing and Fist Gold (post-emergence) at 300 ml/ha three days after sowing. Plants were harvested at the flowering stage, and shoot and root length, shoot and root fresh and dry weights, nodule number and weight, and shoot and root nitrogen contents were determined. After harvest, soil samples were collected from the pots to determine soil respiration [30] and the activities of dehydrogenase [31], urease [32], alkaline phosphatase [33], and arylsulfatase [34]. Selected physical and chemical properties of the experimental soil are presented in Table 1.

Table 1. *Physical and chemical properties of the soil used in the experiment*

Parameters	Units	Results	Method
Texture		Clay loam	Bouyoucos [35]
pH (1:2.5, soil:water)		7.75	Richards [36]
EC (1:5, soil:water)	µS/cm	130	Richards [36]
O.M.	%	0.75	Smith and Weldon [37]
Lime		30	Hızalan and Ünal [38]
N (NH_4-N + NO_3-N)	$mgkg^{-1}$	17	Bremner [39]
P (available)		9.6	Olsen et al. [40]
K		236	
Ca	$mgkg^{-1}$ (extractable)	7400	Soltanpour and Workman [41]
Mg		168	
Fe		1.78	
Zn	$mgkg^{-1}$ (available)	0.16	Lindsay and Norvell [42]
Mn		6.60	
Cu		1.3	
B	$mgkg^{-1}$	0.38	Richards [36]

Statistical Analyses

Data from the greenhouse experiment (randomized block design with four replications) were subjected to analysis of variance using MINITAB-19 statistical software, and the means of parameters with significant F-tests were compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

Plant Growth and Nodulation Parameters

Rhizobium gallicum inoculation under different herbicide treatments had no statistically significant effect on plant height, although some notable trends were observed. Plant height ranged from 25.75 to 28.75 cm across treatments. The tallest plants were recorded in pots inoculated with *Rhizobium gallicum* without herbicide application. Although this difference was not significant, it suggests that symbiotic nitrogen fixation provided by inoculation, together with the absence of chemical inputs, favored plant growth. Plant height was generally lower in herbicide-treated pots, suggesting that the herbicides may have inhibited plant growth. Herbicides are frequently reported to limit plant growth either directly, by affecting plant metabolism, or indirectly, by influencing microbial activity in the rhizosphere. Thus, although the interaction between herbicide application and *Rhizobium* inoculation was not significant for plant height, the observed trends indicate that herbicides may act as a stress factor for plant growth and that bacterial inoculation can partly counteract this effect. Kurrey et al. [20] reported that certain herbicide–insecticide combinations increased soybean yield and economic returns but

could suppress *Rhizobium* populations. Gawęda et al. [43] reported that the combined application of herbicides and a biostimulant increased soybean seed yield and quality, particularly under stressful environmental conditions.

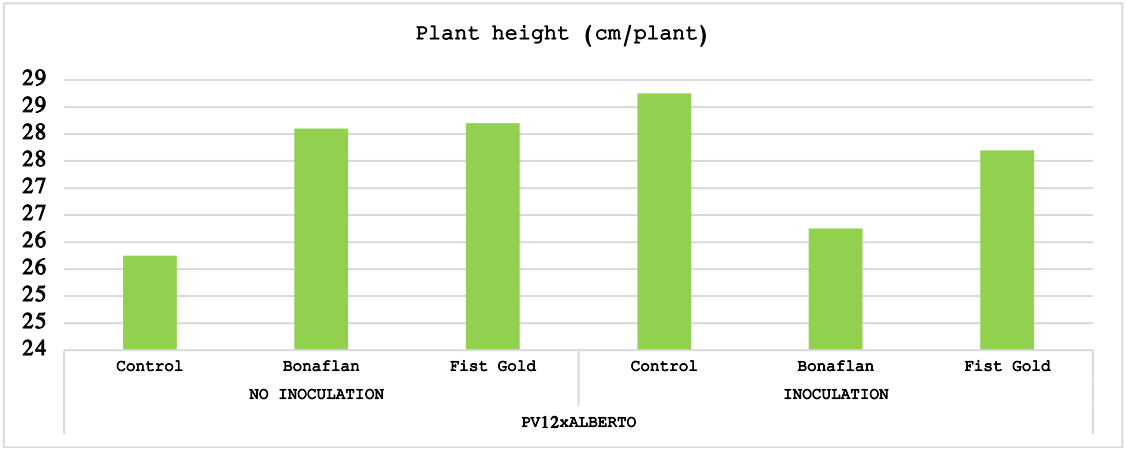


Fig. 1. *Effects of Rhizobium gallicum inoculation under herbicide applications on plant height of PV12 × Alberto bean (Phaseolus vulgaris L.)*

Bacterial inoculation did not significantly affect root length, which generally ranged from 20.10 to 22.40 cm. Thus, inoculation alone or in combination with herbicides did not significantly affect root development. The longest roots were measured in non-inoculated plants treated with Fist Gold, suggesting that some herbicides may not impair, and may even stimulate, root development at low doses or under specific conditions. In contrast, the shortest roots were observed in the control (neither inoculation nor herbicide), indicating that root development may remain limited when neither herbicide nor inoculant is applied. Although the effects of inoculation and herbicides on root length were not significant, certain combinations appear to have the potential to support root development; therefore, further studies are needed to evaluate the long-term effects of these applications and their performance under different environmental conditions. Ahemad and Khan [44] reported that *Bradyrhizobium* sp. strain MRM6 retained its plant growth-promoting traits under herbicide stress and could be used as a biofertilizer and bioremediation agent. Ahemad and Khan [17] reported that the insecticides fipronil and pyriproxyfen were toxic to the growth, symbiotic activity, and yield of pea, but that inoculation with the plant growth-promoting strain MRP1 significantly alleviated these adverse effects.

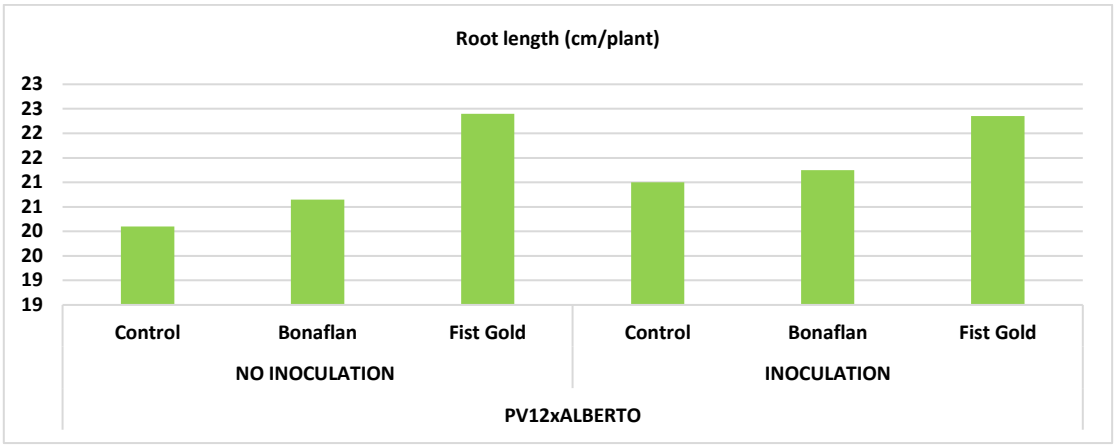


Fig. 2. *Effects of Rhizobium gallicum inoculation under herbicide applications on root length of PV12 × Alberto bean (Phaseolus vulgaris L.)*

The effects of Bonaflan and Fist Gold applications and bacterial inoculation on the shoot fresh weight of bean were significant (p<0.01). The highest shoot fresh weight (30.52 g) was recorded in plants that were inoculated but not treated with herbicide, indicating that inoculation promoted plant growth and that this effect was more pronounced in the absence of herbicides. The lowest value (21.65 g) was measured in the control, which received neither inoculation nor herbicide. Overall, the findings show that bacterial inoculation had a positive effect on plant biomass, particularly when no herbicide was applied.

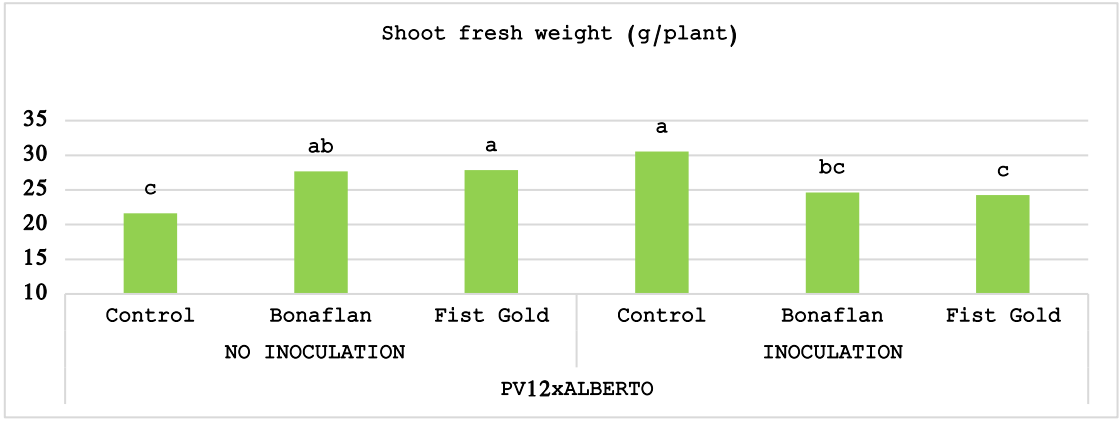


Fig. 3. *Effects of Rhizobium gallicum inoculation under herbicide applications on shoot fresh weight of PV12 × Alberto bean (Phaseolus vulgaris L.)*

Herbicide applications and *Rhizobium* inoculation had highly significant effects on shoot dry weight (p<0.01), indicating that both factors influenced plant biomass. The highest shoot dry weight (4.53 g) was obtained from plants inoculated with *Rhizobium* without herbicide application, suggesting that *Rhizobium* promoted plant growth through nitrogen fixation and that this effect was more pronounced in the absence of herbicide stress. The lowest shoot dry weight was observed in the complete control, which received neither herbicide nor inoculation.

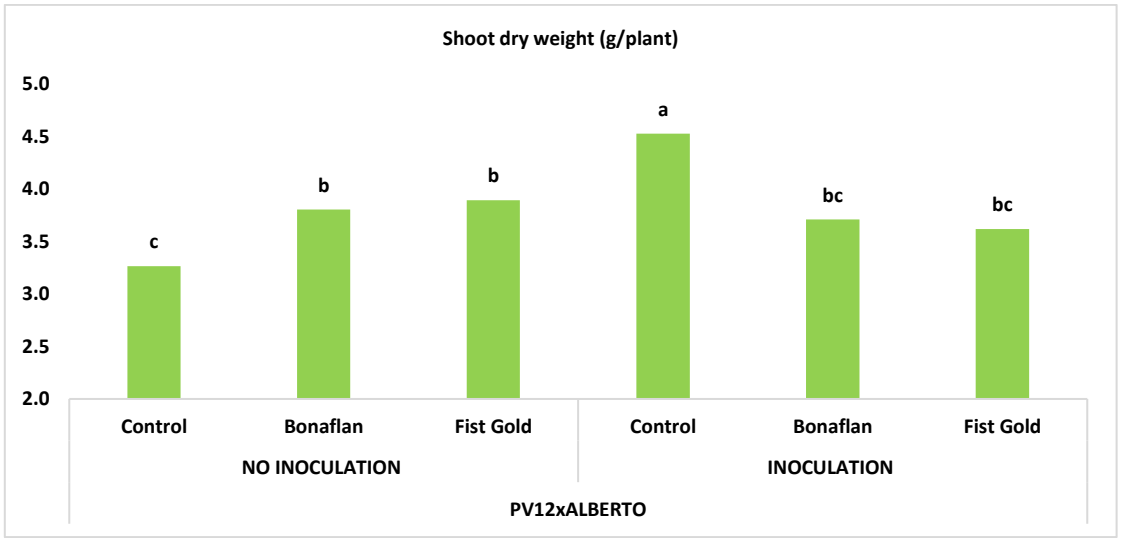


Fig. 4. *Effects of Rhizobium gallicum inoculation under herbicide applications on shoot dry weight of PV12 × Alberto bean (Phaseolus vulgaris L.)*

Bacterial inoculation and herbicide applications, alone and in combination, had highly significant effects on root fresh weight (p<0.01). The lowest root fresh weight was recorded in the control plants, which received neither inoculation nor herbicide, whereas the highest value was found in plants that were inoculated but not treated with herbicide. These data indicate that bacterial inoculation increased the root fresh weight of bean, whereas the effect of herbicides may vary with the herbicide type and its interaction with the bacteria.

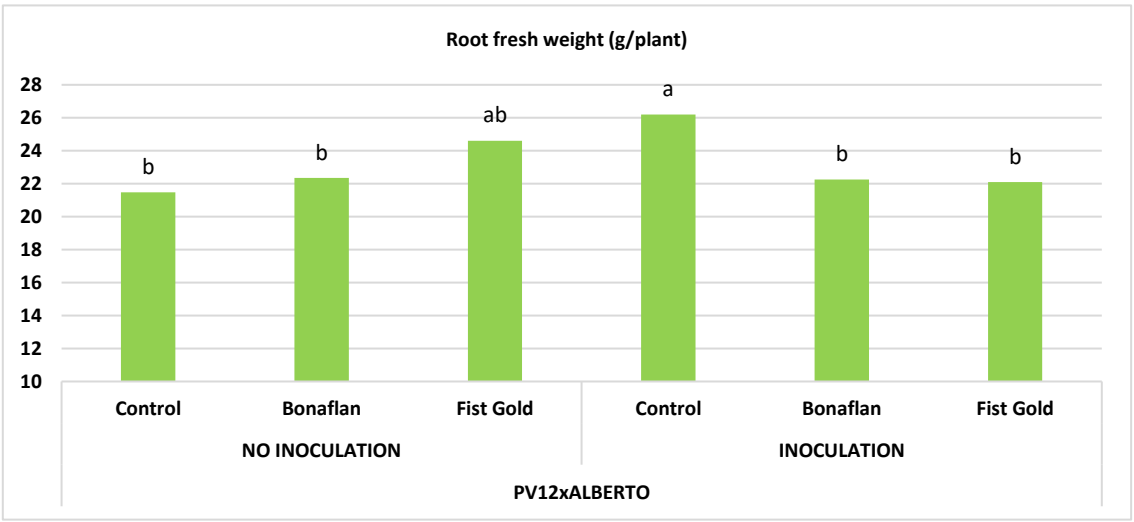


Fig. 5. *Effects of Rhizobium gallicum inoculation under herbicide applications on root fresh weight of PV12 × Alberto bean (Phaseolus vulgaris L.)*

Bacterial inoculation and herbicide applications, alone and in combination, significantly affected root dry weight (p<0.01), which ranged from 1.22 to 1.61 g depending on the treatment. The lowest root dry weight was recorded in plants receiving both bacterial inoculation and Fist Gold. This suggests that combining this herbicide with inoculation may reduce root dry matter accumulation, probably because of the inhibitory effect of the herbicide on microbial populations. These findings show that both biotic

(bacterial) and abiotic (herbicide) factors play important roles in root development and interact to shape plant performance. In sustainable agriculture, potential adverse interactions should be considered when beneficial microorganisms are applied together with certain herbicides.

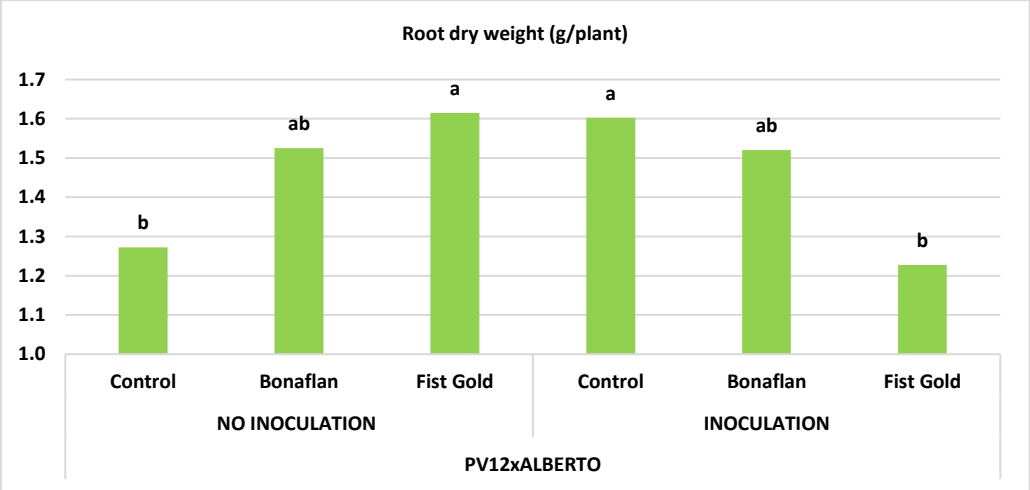


Fig. 6. *Effects of Rhizobium gallicum inoculation under herbicide applications on root dry weight of PV12 × Alberto bean (Phaseolus vulgaris L.)*

Herbicide applications and bacterial inoculation, alone and in combination, significantly affected nodule number ($p<0.01$), indicating that both *Rhizobium* inoculation and herbicides had a decisive effect on symbiotic nodule formation. Fist Gold is a commercial herbicide of the acetolactate synthase (ALS) inhibitor class, which disrupts the synthesis of branched-chain amino acids in plants by inhibiting the ALS enzyme. Importantly, this enzyme is also present in many *Rhizobium* strains. Because the herbicide targets a shared metabolic pathway, its application raises concerns about potential adverse effects on both the growth of the host plant and the symbiotic bacteria responsible for nodulation and nitrogen fixation [14].

The highest nodule number (84.25 nodules) was recorded in plants that were inoculated but not treated with herbicide. This result demonstrates the symbiotic effectiveness of the inoculant and indicates that the plant–*Rhizobium* interaction was greatest in the absence of herbicides. Nodule numbers decreased markedly when inoculation was combined with herbicide application. The lowest nodule number (mean 19.75) was found in inoculated plants treated with Fist Gold, followed by inoculated plants treated with Bonaflan (mean 41.75). These results indicate that both herbicides impaired symbiotic nodulation, with Fist Gold having the stronger effect.

Bacterial inoculation was essential for nodule formation, whereas herbicide applications inhibited the symbiotic interaction. In many cases, herbicide-induced inhibition of nodulation is linked indirectly to a reduced allocation of photosynthates to the roots rather than to a direct toxic effect on *Rhizobium* [14]. Nodule weight also differed significantly among treatments ($p<0.01$), showing that both herbicides and inoculation significantly affected nodule development.

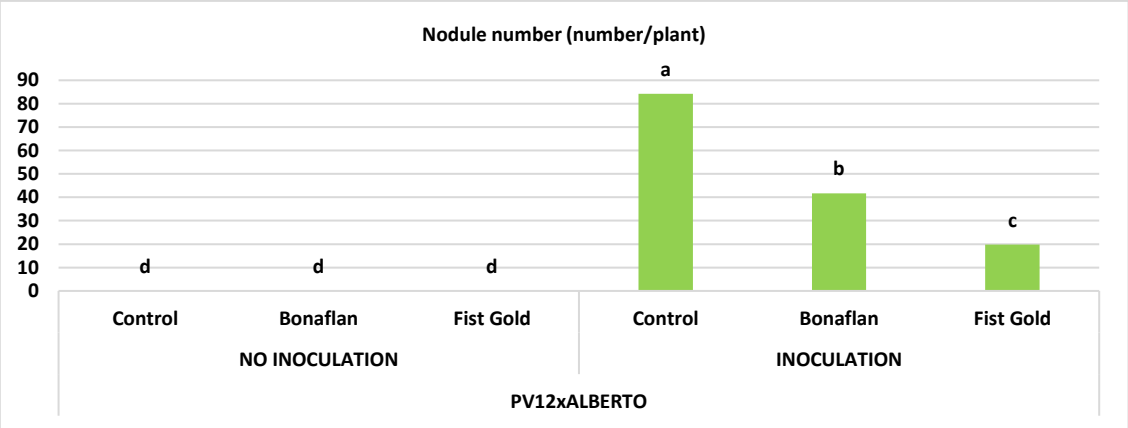


Fig. 7. *Effects of Rhizobium gallicum inoculation under herbicide applications on nodule number of PV12 × Alberto bean (Phaseolus vulgaris L.)*

The highest nodule weight (0.91 g) was observed in plants that were inoculated but not treated with herbicide, whereas nodule weight decreased markedly when inoculation was combined with herbicide application. The lowest mean nodule weight (0.24 g) was recorded in inoculated plants treated with Fist Gold, followed by those treated with Bonaflan (0.48 g). These data indicate that both herbicides impaired nodule development, with Fist Gold having the more pronounced inhibitory effect. Paniagua-López et al. [45] showed that herbicides such as pendimethalin can severely inhibit nodulation and impair nitrogen fixation in bean, with pendimethalin drastically inhibiting nodulation and reducing bacterial motility.

Overall, bacterial inoculation increased nodule weight, whereas herbicide applications markedly reduced this positive effect, with Fist Gold having the more negative impact on symbiotic nitrogen fixation. Pertile et al. [27] investigated single and combined applications of flumioxazin and imazethapyr on early nodulation and biological nitrogen fixation (BNF) in the soybean rhizosphere in different soils and found that the herbicides suppressed glutamine synthetase (GS) activity and leghemoglobin content only when applied as a mixture, which could impair the early stages of the symbiosis with native rhizobia. Singh and Wright [10] demonstrated adverse effects of pre-emergence (terbutryn/terbuthylazine, trietazine/simazine, prometryn) and post-emergence (bentazon) herbicides on nodulation, symbiotic nitrogen fixation, growth, and yield of pea, with the terbutryn/terbuthylazine and trietazine/simazine mixtures being the most toxic.

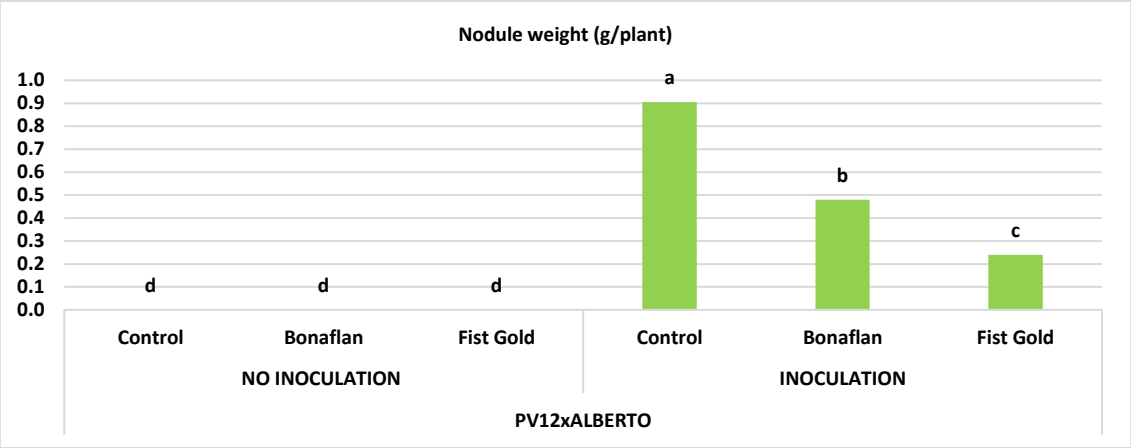


Fig. 8. *Effects of Rhizobium gallicum inoculation under herbicide applications on nodule weight of PV12 × Alberto bean (Phaseolus vulgaris L.)*

The interaction between bacterial inoculation and herbicide application had a significant effect on shoot nitrogen content (p<0.01), which ranged from 3.62% to 4.75% across treatments. The highest value (4.75%) was found in plants that were inoculated but not treated with herbicide, indicating that inoculation without herbicide pressure improved nitrogen uptake and metabolism. In contrast, the lowest value (3.62%) was found in non-inoculated plants treated with Bonaflan. Rodrigues et al. [24] reported that the pesticide mixture StandakTop® applied to soybean seeds significantly reduced the survival of a *Bradyrhizobium elkanii* strain and impaired nitrogen accumulation and remobilization under field conditions. Herbicide application therefore raises concerns about potential adverse effects on both host plant growth and the symbiotic bacteria responsible for nodulation and nitrogen fixation [14].

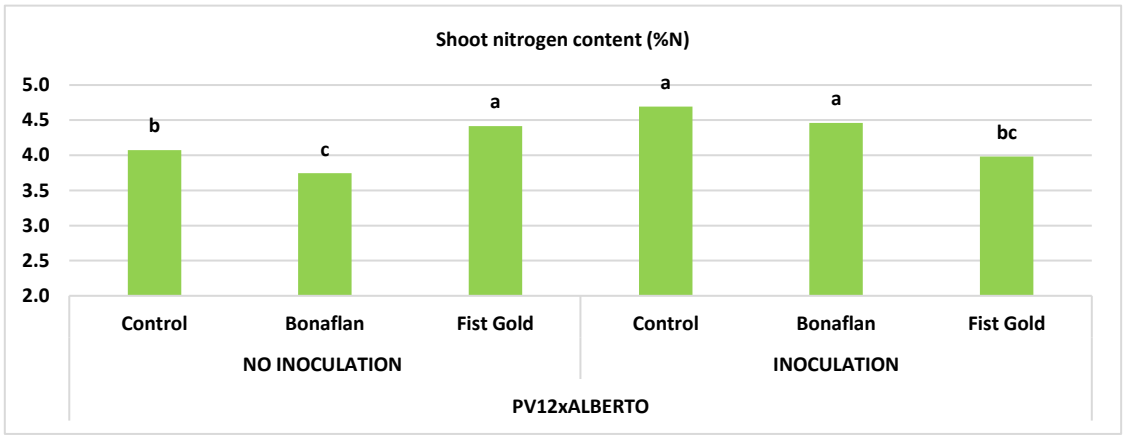


Fig. 9. *Effects of Rhizobium gallicum inoculation under herbicide applications on shoot nitrogen content of PV12 × Alberto bean (Phaseolus vulgaris L.)*

Bacterial inoculation and herbicide applications, alone and in combination, significantly affected root nitrogen content (p<0.01). The highest root nitrogen content (1.44%) was measured in plants that were inoculated but not treated with herbicide, whereas the lowest values were observed in the control plants, which received neither inoculation nor herbicide. Overall, the results indicate that bacterial inoculation can

substantially increase nitrogen accumulation in roots, although this effect may vary depending on herbicide application.

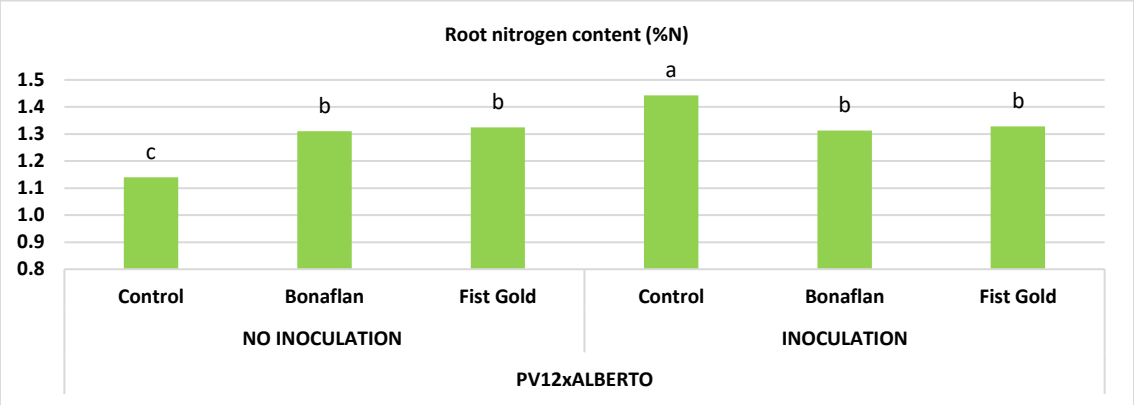


Fig. 10. *Effects of Rhizobium gallicum inoculation under herbicide applications on root nitrogen content of PV12 × Alberto bean (Phaseolus vulgaris L.)*

Simarmata et al. [21] reported that herbicides containing atrazine and mesotrione applied at different doses had long-term, dose-dependent adverse effects on legumes and soil microbial populations. Ahemad and Khan [17] demonstrated that the insecticides fipronil and pyriproxyfen had dose-dependent toxic effects on pea, which were significantly alleviated by inoculation with strain MRP1. Datta et al. [16] reported that isoxaflutole markedly impaired growth, nodulation, and nitrogen fixation in chickpea, particularly in herbicide-sensitive cultivars, reducing nitrogen fixation by up to 51%. Zaidi et al. [46] reported that several herbicides applied to green gram (*Vigna radiata*) inoculated with *Bradyrhizobium* sp. had varying effects on physiological and yield parameters, and that metribuzin caused considerable yield losses owing to its high phytotoxicity. Anderson et al. [47] reported that chlorsulfuron in soil reduced nodulation and nitrogen fixation in chickpea. Abd-Alla et al. [11] reported that various pesticides adversely affected growth, nodulation, and mycorrhizal colonization in legumes; in particular, a delayed increase in spore formation was observed in faba bean.

Biological Properties of the Experimental Soils

Bacterial inoculation under Bonaflan and Fist Gold applications significantly affected soil respiration ($p<0.01$), which ranged from 19.57 to 29.27 mg CO_2 100 g^{-1} soil 24 h^{-1}. The highest soil respiration was measured in the treatment that received bacterial inoculation without herbicide, indicating that inoculation increased microbial activity and thus CO_2 production in the soil. In contrast, the lowest values were recorded in non-inoculated soils treated with Fist Gold alone. Araújo et al. [13] found significant increases in soil respiration and microbial enzyme activity during a 32-day laboratory incubation of two Brazilian soils treated with glyphosate, and reported increases in actinomycete and fungal populations but a slight decrease in bacterial counts in soils with a long history of glyphosate exposure.

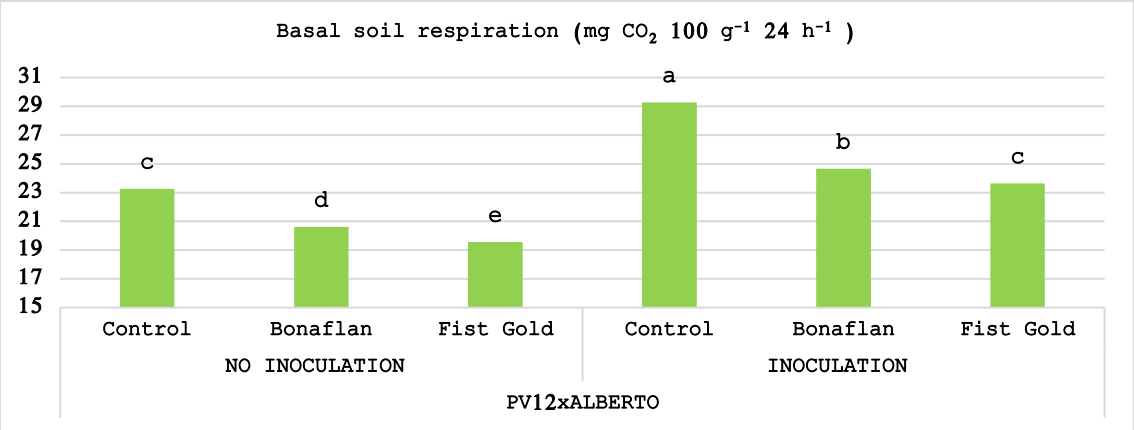


Fig. 11. *Effects of Rhizobium gallicum inoculation under herbicide applications on soil respiration (CO_2 evolution) in soil planted with PV12 × Alberto bean (Phaseolus vulgaris L.)*

Dehydrogenase activity ranged from 6.11 to 11.36 µg TPF g^{-1} dry soil 24 h^{-1}. The highest activity was found in soils that received bacterial inoculation without herbicide, whereas the lowest values were observed in soils treated with Fist Gold despite inoculation. This suggests that the active ingredients of Fist Gold may inhibit microbial activity and, in particular, limit the effectiveness of bacterial inoculants. These results, consistent with previous studies, show that herbicides can reduce the enzyme production capacity of soil microorganisms.

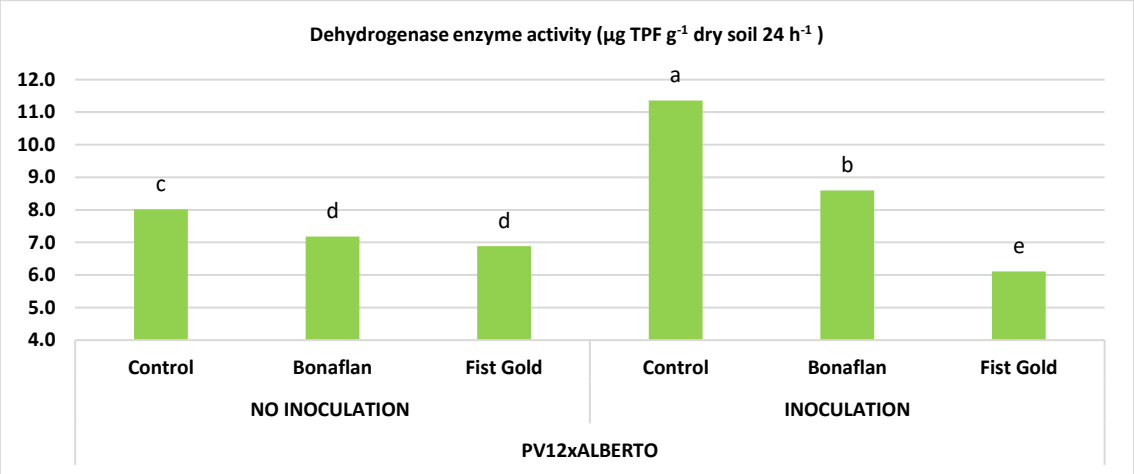


Fig. 12. *Effects of Rhizobium gallicum inoculation under herbicide applications on soil dehydrogenase activity in soil planted with PV12 × Alberto bean (Phaseolus vulgaris L.)*

Bacterial inoculation combined with herbicide application had a significant effect on soil urease activity (p<0.01). The highest urease activity was observed in soils that received bacterial inoculation without herbicide, indicating that inoculation enriched the soil microflora and thereby stimulated enzymes involved in the microbial nitrogen cycle. In contrast, the lowest urease activity was found in non-inoculated soils treated with Fist Gold alone, pointing to negative effects of this herbicide on the soil microbial community and suggesting that it may suppress enzymes involved in nitrogen transformations. These

findings, which suggest that herbicides can inhibit the metabolic activity of soil microorganisms, are consistent with the literature.

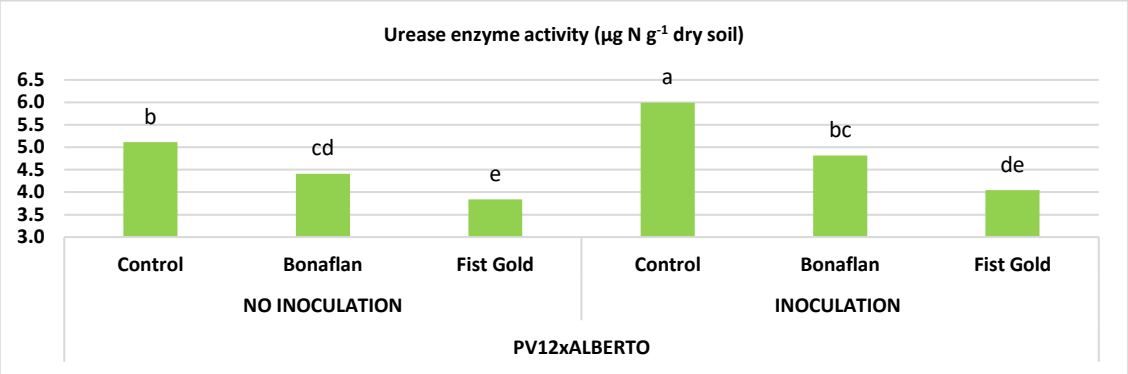


Fig. 13. *Effects of Rhizobium gallicum inoculation under herbicide applications on soil urease activity in soil planted with PV12 × Alberto bean (Phaseolus vulgaris L.)*

Bacterial inoculation under different herbicide treatments significantly affected alkaline phosphatase activity in the experimental soil (p<0.01), indicating that herbicides applied together with inoculation markedly influenced soil biochemical processes, particularly enzymatic activities involved in phosphorus transformations. The highest activity was observed in soils that received bacterial inoculation without herbicide, whereas the lowest alkaline phosphatase activity was found in soils treated with Bonaflan despite inoculation. This suggests that Bonaflan may reduce soil microbial activity and enzyme production.

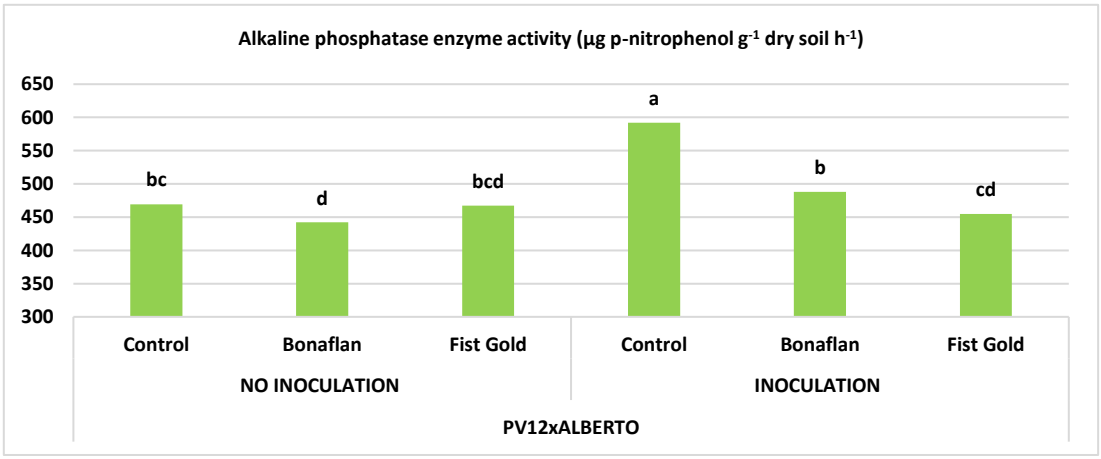


Fig. 14. *Effects of Rhizobium gallicum inoculation under herbicide applications on soil alkaline phosphatase activity in soil planted with PV12 × Alberto bean (Phaseolus vulgaris L.)*

Arylsulfatase activity, used as an indicator of microbial activity in soils receiving herbicides and bacterial inoculation alone or in combination, ranged from 92 to 235 µg p-nitrophenol g^{-1} dry soil h^{-1}. The lowest value (92 µg p-nitrophenol g^{-1} dry soil h^{-1}) was found in non-inoculated soils treated with Fist Gold, indicating that this herbicide may inhibit microbial enzyme activity.

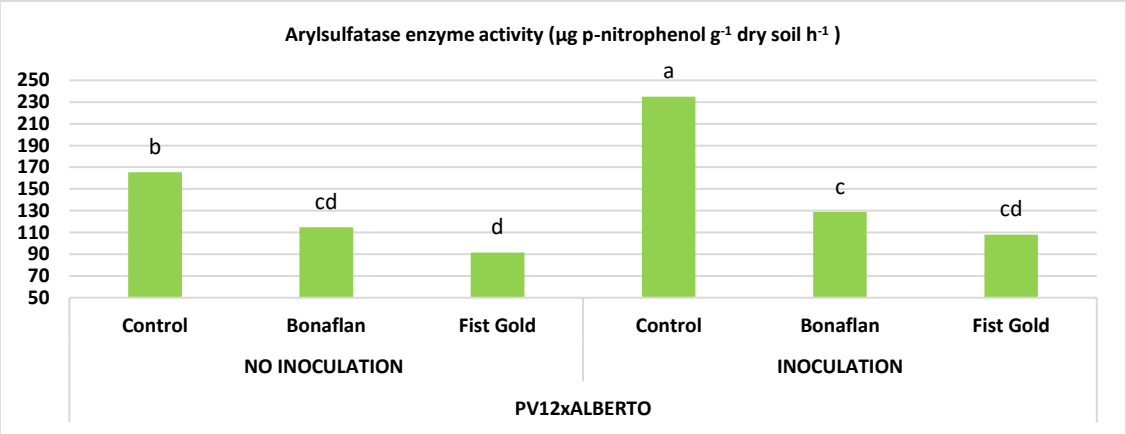


Fig. 15. *Effects of Rhizobium gallicum inoculation under herbicide applications on soil arylsulfatase activity in soil planted with PV12 × Alberto bean (Phaseolus vulgaris L.)*

Sarmamy and Khidir [48] reported that Treflan and glyphosate completely inhibited nodule formation and adversely affected growth parameters and micronutrient contents, and that residues of some pesticides could be detected in plant tissues after harvest, indicating that these compounds may have lasting effects on plant nutrition and soil microbiology. Aurelia [15] reported that the insecticide Decis could increase the total number of viable bacteria, whereas Bavistin and SDMA did not markedly inhibit the microbial population. Kumar et al. [23] demonstrated that organophosphate pesticides, particularly in combination, were toxic to soil microorganisms and plant growth-promoting rhizobacteria, with toxicity decreasing in the order phorate > acephate > monocrotophos > glyphosate. Yu et al. [49] reported that chlorothalonil markedly reduced soil bacterial and actinomycete populations after the first application, but that microorganisms adapted to repeated applications and the adverse effects diminished by the third or fourth application. Dığrak and Kazanıcı [12] investigated the effects of the organophosphorus insecticides isofenphos, phorate, and fonofos on soil microorganisms and reported that isofenphos increased the total bacterial count without inhibiting other microbial groups, whereas fonofos and phorate did not inhibit microbial growth.

CONCLUSION

This study examined the effects of two herbicides [Bonaflan (pre-emergence) and Fist Gold (post-emergence)] and inoculation with *Rhizobium gallicum* on morphological and physiological growth parameters, nodulation, and soil biological activity in the PV12 × Alberto bean cultivar. Under herbicide treatments, *Rhizobium gallicum* inoculation significantly (p<0.01) affected several yield parameters. Bacterial inoculation increased nodule weight, whereas the herbicides suppressed this positive effect. Because Fist Gold in particular markedly reduced nodule weight, the potential negative effects of herbicides on symbiotic nitrogen fixation and plant–*Rhizobium* interactions should be taken into account in crop production, and application timing and rates should be planned carefully. Bacterial inoculation also increased soil biological activity, including microbial enzyme activities, whereas certain herbicides limited these beneficial effects. Therefore, interactions between biological and chemical inputs should be evaluated carefully in sustainable agricultural systems; such studies can contribute to strategies that maintain both productivity and soil health.

In conclusion, inoculation with *Rhizobium gallicum* supported both plant growth and soil biological functions, whereas herbicide applications, particularly Fist Gold, adversely affected symbiotic nodulation and microbial enzyme activities. The present data indicate that Bonaflan and Fist Gold may have adverse effects on soil microorganisms. However, these effects are species- and dose-dependent and may vary with environmental conditions. Therefore, herbicides should be used with caution, recommended doses should not be exceeded, and soil health should be monitored regularly after application.

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