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AGRONOMIC POTENTIAL OF BIOFERTILIZERS ON GROWTH, YIELDS AND NUTRITIONAL QUALITY OF A VETCH-OAT-TRITICALE INTERCROPPING SYSTEM

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ABSTRACT

The existing issues of climate change and population growth have driven to a rising awareness of the need to innovate in sustainable agricultural practices, such as intercropping, Enhancing the efficiency of intercropping systems by applying beneficial microorganisms (rhizobacteria, mycorrhizae or both) is a valuable approach. Two experiments were conducted to assess the effect of inoculation with beneficial microorganisms: rhizobacteria (B), mycorrhizae (M), and a rhizobacteria-mycorrhizae consortium (B+M) compared to a control of uninoculated plants (C) on a vetch-oat-triticale intercropping system. Growth, physiological traits, forage yield and qualities were determined at two harvesting periods (74 and 142 days after seeding, das). Overall, the different studied intercrops varied in terms of inoculation effects. Mycorrhizal inoculation had a significant positive effect on SPAD, total nitrogen and leaf to stem ratio for vetch, oat and triticale, respectively. Bacteria inoculation promoted leaf area and plant height for vetch and oat crops, but only plant height for triticale. The dual bacteria and mycorrhizae inoculum significantly improved the yield dry matter (YDM) by 41.2 % ensuring an increase of 2.1 and 1.67 Mg ha^{-1} at 74 and 142 das respectively, compared to the control. Further data analysis revealed an improvement of calcium (Ca) and phosphorus (P) status after mycorrhizae inoculation. This study highlighted the potential applications of the dual biofertilizers on vetch-oat-triticale intercropping as a way to increase forage yield and qualities in semi-arid regions.

Keywords: rhizobacteria, mycorrhizae, forage yield, forage quality.

INTRODUCTION

Growing two or more species simultaneously in the same area is known as intercropping (Stomph *et al.*, 2020). This farming method boosts production, biodiversity, and agroecosystem stability while effectively utilizing the land, nutrients, and water resources (Kamalongo *et al*., 2020). Intercropping is based on eco-functional intensification and may enhance crop productivity, increasing the land utilization ratio (Agegnehu, 2008; Qin *et al*., 2013) while emitting significantly lower amounts of greenhouse gases compared to individual crops. In arid and semiarid environments, mixtures of certain annual legumes with winter cereals are extensively used in forage production (Papastylianou, 2004). In such regions, legume and cereal mixtures have shown great potential for increasing forage yield and soil conservation (Anil *et al*., 1998). Thus, extended studies on intercropping systems, particularly for cereals and legumes, become essential to promote and increase the quality of fodder suitable for silage and to adapt crop management to climate change (Iannucci *et al*., 2006).

Systems using legumes as main crop and cereals as intercrops provide significant advantages, including higher total yield and more efficient land use (Jayanta *et al*., 2018). This system enhances fodder yield relative to dry weight due to the grasses (Hashemi *et al*., 2013), and improves feed quality due to the higher crude protein (CP) concentration of legumes. Furthermore, increased fodder yields imply heavy fertilizer and pesticide use, which increases the risk of soil and water pollution (Dar *et al*., 2020). Besides, it also debilitates the plant roots, rendering them defenceless against certain pathogenic organisms. Therefore, advancement for biofertilizers in agricultural systems is required direly due to consumer demand for safe, residue-free food. In this case, the use of microbial inoculants is one of the potential tools of achieve this goal (Pertot *et al*., 2016). Some species of the genera *Sinorhizobium*, *Allorhizobium*, and *Bradyrhizobium* constitute a significant group of beneficial organisms in agriculture. By forming nodules in the roots of host legume species, they can increase nitrogen fixation efficiency of up to 450 kg ha^{-1} (Graham and Vance, 2003; Thomas and Singh, 2019).

Among these beneficial microbes, plant growth-promoting rhizobacteria (PGPR) are known to improve nutrient uptake and utilization in plants. PGPR are a group of rhizosphere bacteria that can colonize plant roots and provide beneficial effects on the host plant when available in large populations. The genera *Rhizobia*, *Bacillus*, *Azospirillum*, *Pseudomonas* and *Burkholderia* are among the most researched PGPR. They have been found to have many beneficial effects on plants (Saharan, 2011) and activate direct and indirect mechanisms for the benefit of the host plants (Glick, 2012). It has been reported that PGPB can improve soil nutrient availability, plant nutrition and growth, as well as the yields of a large diversity of crops around the world, including cereals (Youseif, 2018), legumes (Ferchichi *et al*., 2019), vegetables and spices (Kang *et al*., 2021). Furthermore, they have the ability to increase plant resilience against abiotic stress factors, such as alkalinity, salinity, drought and toxic metals (Abdelkrim *et al*., 2018). Rhizobia also contribute to secrete plant hormones which are necessary for plant growth (Rosenblueth *et al*., 2018), and may protect plants from phytopathogenic microorganisms (Compant *et al*., 2005).

Arbuscular mycorrhizal fungi (AMF) as a source of biofertilizers to improve plant mineral nutrition may significantly minimize fertilizer input while maintaining adequate agricultural yields (Mohammad *et al*., 2004). AMF also improve plant uptake of other macronutrients such as nitrogen (N), potassium (K), and magnesium (Mg) (Labidi *et al*., 2012).

In order to answer two main questions, a research was conducted on vetch, oat, and triticale cultivated in a mixture in the presence or absence of mycorrhizae, bacteria, and a mixed consortium for two cuttings. Questions arisen are Does the presence of these inoculum have a different impact on agriculture´s morphological and physiological traits, as well as nutrient uptake, in the three species grown in mixture? and What role do these inoculums play in increasing yield and fodder quality?

MATERIALS AND METHODS

Plant material, treatments, and research site

The plant material used in this study consisted of two forage cereals: black oat (*Avena strigosa*) cv. Saia. and triticale (x *Triticosecale*) cv. Our., and one forage legume species, hairy vetch (*Vicia villosa*) cv. Sejnane. These three mixture components were chosen on the basis of a previous study in which intercropping advantage was evidenced using LER (Land Equivalent Ratio) (Beya, 2018).

Four different inoculation treatments were assigned to the mixture; 1) mycorrhizal inoculum M: commercial product from France (Symbivit); 2) bacterial inoculum B: a consortium of three strains (patent pending) isolated from white lupin nodules (*Lupinus albus*). These three strains belong to the species *Agrobacterium radiobacter*, are non-antagonistic and have PGPR effects (Hammami *et al*., 2019); 3) combined mycorrhizae and bacteria inoculum (M+B); and 4) the control treatment (C), without inoculation plus a reduced rate of 50 kg ha^{-1} of diammonium phosphate (DAP).

The experiment was conducted in the 2018–2019 growing seasons in the experimental station of the Higher School of Agriculture of Mateur, located in Northwest of Tunisia (37° 03' N, 9° 36' E). This region has a semi-arid climate with annual precipitations of 560 mm and average annual air temperatures of 17.07 °C; weather conditions during the experimental season are shown (Table 1). The soil type of the experimental site, classified as a vertisol, had a clay-silty texture. Physical and chemical properties, of soil at 0–40 cm depth, are shown (Table 2).

Experimental procedure

The experimental procedure was divided into two essays, the first was conducted in the field and the second in pots.

Field experiment implementation

The field experiment was carried out under rainfall conditions. A one-way factorial experiment (four treatments, four replicates per treatment) was distributed using a completely random design across 16 experimental plots of 12 m² (3 m × 4 m), containing 15 rows spaced 20 cm apart. Seedbed preparation was conventional, using a medium depth tillage followed by two discs harrowing in early autumn. On November 16th,

Table 1. Weather conditions during 2018-2019 growing season at the experimental station under the Higher School of Agriculture of Mateur, Tunisia.

Month	Rainfall (mm)	Tmax (°C)	Tmin (°C)
November	120	20	10
December	41	17	8
January	127	15	6
February	18	16	6
March	34	19	8
April	16	21	10
May	22	23	11
Total rainfall	378	-	-
Average	-	18.71	8.43

Table 2. Selected properties of soils used during greenhouse and open-field experiments at the experimental station under the Higher School of Agriculture of Mateur, Tunisia.

Properties	Value	Unit
pH	8.65	-
EC	0.14	dS m^{-1}
OM	2.06	%
C	1.19	%
TN	0.26	%
C/N ratio	4.57	-
$CaCO_3$	18.67	%
Sandy	18	%
Loam	55	%
Clay	23	%
Texture	Clay-silty	-

EC: electrical conductivity; OM: organic matter; TN: total nitrogen; $CaCO_3$: total calcium carbonate; P: phosphorus; C/N: carbon to nitrogen ratio. Data are presented as means of three replicates (n = 3).

2018, the crop combination was hand sown. The final plant density was 250 grains m^{-2}, considering 50, 50 and 150 seeds m^{-2} of oat, triticale and vetch, respectively.

A dosage of 1.4 kg of mycorrhizae was administered immediately after sowing along the rows at a rate of 3 g m^{-1}, or 180 g per unit. In terms of bacterial inoculum, fresh preparations of individual strains (109 bacteria mL^{-1}) were done and mixed equally to generate a bacteria consortium (2 L ha^{-1}), then diluted with well water (1/20) and finally sprayed manually along the seeding line using a portable hand spray. In order to reduce UV light interaction with bacterial viability, the injection of inoculum was done after seedling emergence (December 8th, 2018) in the late afternoon. The volume applied ensured enough soil moisture and an expected bacterial density 4 x 10^7 CFU m^{-2}.

The crop mixture was harvested 74 and 142 d after sowing, which corresponds to BBCH stages 31 and 55 for cereals, respectively (Lancashire *et al*., 1991).

Pot experiment

This experiment was carried out under controlled conditions, in order to assess the root development of cereals and vetch. Fresh aerial weight (FAW), fresh root weight (FRW) and root length (RL) were recorded. Nodulation variables such as the number (NodN) and weight (NodW) of vetch nodules were assessed. Thus, roots were separated from the shoots and washed with water to remove adhering soil particles. The nodules were recovered from the roots and counted.

Sixteen pots (25 cm diameter × 40 cm height) were placed in a semi-closed environment at Higher School of Agriculture of Mateur during the 2018-2019 growing season. The same protocol was used in order to simulate the same conditions of the field experiment. Soil was collected from the surface layer (0-40 cm) of a single field, sieved (2 mm mesh size) and mixed with perlite. Each plastic pot was filled with 10 kg of substrate composed by 30 % in volume of perlite and 70 % of agricultural soil. For each pot, 15 seeds (five seeds per species) were sown on November 16th, 2018 and thinned to three plants per species after emergence. Irrigation was applied with tap water to reach soil moisture with 100 % water-holding capacity.

The control treatment (C) consisted of seeds without inoculation with a rate of 50 kg ha^{-1} of DAP, equivalent to 1 g DAP per pot. The inoculation treatments were as follows: 2 g of mycorrhizal inoculum (M), 30 mL of bacterial inoculum (B) adding 15 mL at sowing and 15 d after emergence, and the mixture of mycorrhizae and bacterial (B+M) by adding 1 g of mycorrhizal inoculum and 30 mL of bacterial inoculum containing about 109 bacteria per mL.

For cereals and legumes, root characteristics were determined 14 d after sowing at stage 11 and 13 on the BBCH scale (Meier *et al*., 2009), respectively.

Traits measurement

Measurement of traits in this study included morphological, physiological and forage qualities, which were determined in the field experiment, while the root characteristics were measured under greenhouse conditions. The height measurements were performed using a marked ruler on 10 plants from each experimental unit. Three measurements of height growth at the two harvesting stages (74 and 142) were taken. The leaf:stem ratio was measured after drying leaves and stems in an oven at 65 °C for 72 h for dry matter content determination.

Indirect chlorophyll content in leaf tissues was measured on six plants per crop species randomly taken from each plot at the two harvesting dates. Measurements were performed using a portable meter (Minolta SPAD 502m; Plainfield, IL, USA). For

Leaf area, data were collected from 20 plants at two central rows of each plot 74 and 142 d after sowing for the three species. Single leaf area was measured using an LI-3100 leaf area meter device (LI-COR; Lincoln, NE, USA).

To determine the effect of the inoculation treatments on N, P and Ca uptake, the content of these nutrients in the three species aboveground tissues was measured as it is described. Total nitrogen was measured using the Kjeldahl method (Nelson and Sommers, 1980); the amount of crude protein was calculated by multiplying the percentage of N by 6.25; the SAAF method (ISO6869:2000) was used to assess the calcium content of plants (Ca), and plant phosphorus concentration (P) was assessed using a spectrophotometric approach (ISO 6491:1998). At the first harvest date, the leaf nitrate (NO_3^-) content was determined on 20 g of fresh leaves cut into thin strips and crushed until juice was extracted, which was then analysed with nitrate-check NO_3^- meter LAQUA twin (HORIBA; Irvine, CA, USA).

For each crop, the net area of individual experimental plot was hand harvested separately, discarding the borders rows. Dry matter (DM) content was assessed through oven drying a known amount of representative fresh samples from each plot just after harvest, at 70 ± 2 °C for 48 h. The yield as dry matter (YDM) was then calculated by multiplying DM content by the green fodder yield. At harvest, plant samples from each plot were collected, oven-dried, mixed, powdered, and kept for further quality variables analyses. The crude fibre (CF) was determined using FIBERSAC procedures outlined by ANKOM method (AOCS procedures Ba 6a-05). Ash (Ash) content was determined after burning the samples in muffle furnace at 550 °C for 10 h.

Statistical analyses

All collected data were tested for normality using Kolmogorov-Smirnov test. Analysis of variance was then performed using IBM SPSS Advanced Statistics, version 22. The inoculum application was considered as fixed factor. The Tukey's test at $p < 0.05$ was used for means comparison.

RESULTS AND DISCUSSION

Choosing the appropriate combination among forage species is of prime importance to enhance either forage yield, or forage quality traits of a crop mixture (Iannucci *et al*., 2006). The introduction of beneficial bio-fertilizers is recognized as a powerful natural way of providing nutrients to plants and preserving the environment in a natural and environment-friendly way (Yadav and Smritikana, 2019). Accordingly, this research aims to contribute to our knowledge on the positive effects of combining inoculation sources and intercropping, as well as to assess at how much extent this strategy would improve morphological and physiological traits, yield and quality of a multi-specific crop mixture.

Agro-morphological variables of vetch and cereals under controlled conditions

The analysis of variance showed that vetch and cereals agro-morphological variables (FAW, FRW, RL, NodN, NodW) were significantly ($p < 0.001$) dependent on inoculation treatments, except vetch for which AFW was unaffected (Table 3). Overall, for either vetch or cereals, means comparison showed that the highest AFW and RFW were obtained on the plants treated with B and the combination B+M. Our findings are in full agreement with those reported by Ertekin and Cakmakçi (2020). Compared to the control, vetch plants treated with B provided 15 and 30 % more AFW and RFW, respectively.

Regarding the cereals, B+M treatments improved AFW and RFW by 50 and 30 % (Table 3). The observations described above are in line with those reported by Xin *et al*. (2014), who demonstrated that an optimal combination of bacterial and mycorrhizal is able to improve dry biomass weight in different plant species. In addition, the highest RL was obtained for all species treated with bacteria, reaching 23 cm and 25 cm for vetch and cereals, respectively. The lowest values were recorded in the control treatment, averaging 11 cm and 17.67 cm for vetch and cereal plants, respectively (Table 3). These results might be related to the indigenous nature of the bacteria used, which makes them more suited to the environment. Furthermore, mycorrhizae would take more time to grow and to colonize their surroundings; therefore, their effect is likely to be delayed. For NodN, the highest value was obtained with M treatment (30 % higher than the control). However, maximum value of NodW was recorded in untreated plants (10.53 mg).

The beneficial effect of bacteria on nodulation has been widely documented (Kidaj *et al*., 2012). Nonetheless, the decreasing NodN obtained with the dual inoculation by rhizobia bacteria and AMF could be attributed to the competition among the different AMF applied and those already naturally present in the soil, which would affect the final extent of plant colonization.

Morphological variables under field conditions

In the field, the differences among treatments were more pronounced at the second harvest (142 d after sowing), mainly due to the to the adapting duration of

Table 3. Effect of inoculation treatments on agro-morphological variables of vetch and cereal plants.

Treatments	FAW (g)		FRW (g)		RL (cm)		NodN	NodW
	Intercropping species							
	Vetch	Cereals	Vetch	Cereals	Vetch	Cereals	Vetch	Vetch
C	0.21a	0.11c	0.20c	0.12d	11.00d	17.67b	12.67c	10.53a
M	0.19a	0.18b	0.27ab	0.18c	17.00c	23.33a	18.17a	7.87c
B	0.23a	0.16b	0.28a	0.27b	23.00a	25.00a	15.00b	8.77b
M+B	0.18a	0.22a	0.25b	0.45a	20.00b	23.00a	14.67b	9.07b
ANOVA	ns	***	***	***	***	***	***	***

FAW: fresh aerial weight; FRW: fresh root weight; RL: root length; NodN: nodules number; NodW: nodules weight. Significant at ** $p < 0.01$ and *** $p < 0.001$; ns: non-significant. Means followed by different letters show statistical difference (Tukey, $p < 0.05$).

biofertilizers. In most cases, significant variation among inoculation treatments for the tested morphological traits (height and leaf:stem ratio) were observed, at both harvest dates for individual species. However, no significant differences were recorded for height among the treated plants 74 d after sowing (Figure 1). At the first harvest (74 d after sowing), among the four treatments, the mean leaf:stem ratio was observed on the plants subjected to M+B, C and M+B for vetch, oat, and triticale respectively. At the second harvest, application of the combined M+B resulted in 7 % extra height compared to the control for oat and vetch, respectively. For triticale, plants treated with B recorded the tallest plants (Figure 1A). Regarding leaf:stem ratio, mycorrhiza and bacteria applications increased leaf:stem ratio values for oat and vetch with 38 and 19 %, respectively, compared to the corresponding controls. Whereas, for triticale plants, very slight differences were obtained after inoculum applications (Figure 1B).

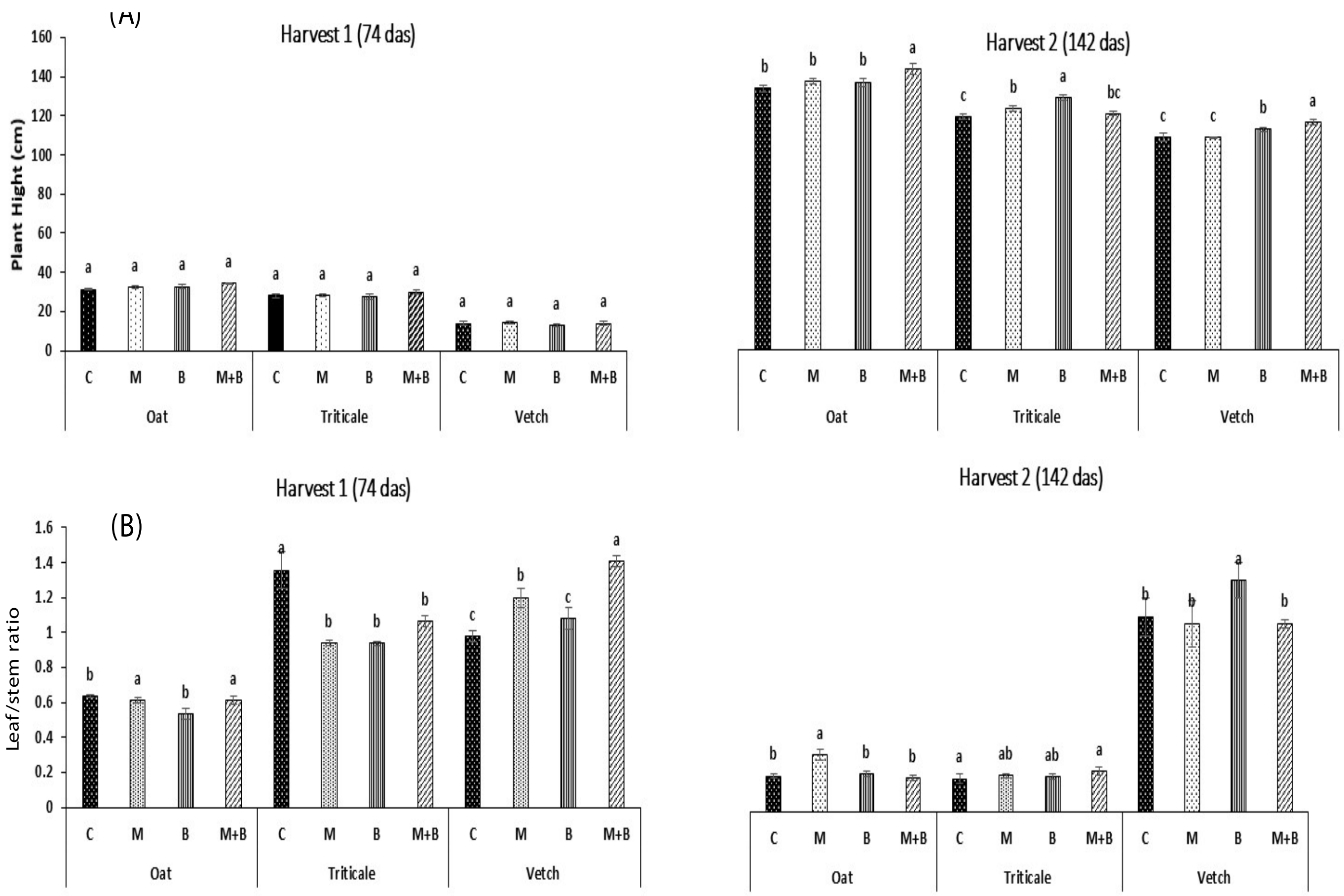

Figure 1. (A) plant height and (B) leaf:stem ratio at two harvesting dates; harvest 1 (74 d after sowing, das) and harvest 2 (142 das), under four inoculation treatments for oat, triticale and vetch. C: control; M: mycorrhizae inoculum; B: bacterial inoculum; M+B: dual mycorrhizae and bacterial inoculum. For each crop, means followed by different letters indicate statistical difference (Tukey, $p < 0.05$).

Changes of physiological variables under field conditions

Among different inoculation treatments, significant differences in SPAD and LA values were recorded (Figure 2). At the first harvest, the bacterial inoculum application enhanced SPAD values by 11 % for the two crops oat and vetch. For triticale, SDAP values were improved in plants treated with M+B. Only triticale plants showed substantial variation during the second harvest, with a preponderance of bacteria-inoculated plants showing 16 % higher values than the control. (Figure 2A). Overall, LA varied significantly from 206 to 536 cm^2.per plant. In particular, plants treated with M+B showed an improvement of LA compared to the control with 29, 20 and 15 % for oat, vetch and triticale crops, respectively. At the second harvest, the prevalence of the combined M+B treatments plants were detected only for vetch and triticale. In contrast, LA values for oat declined with inoculum applications (Figure 2B).

Changes in nutrient values under field conditions

N, P and Ca uptake by crops was significantly affected ($p < 0.01$) by inoculations. Specifically, all of them significantly improved their nutritional status when compared

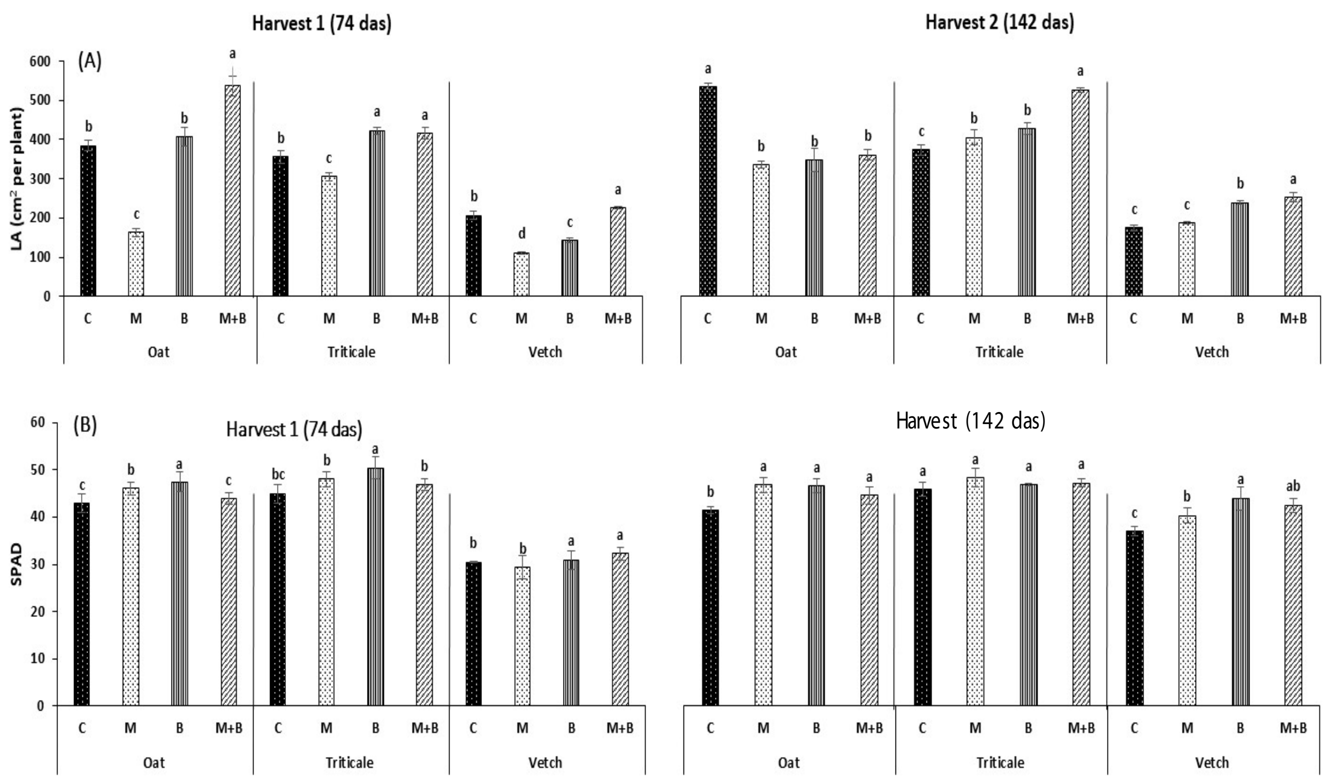

Figure 2. (A) leaf area and (B) SPAD units in oat, triticale and vetch under four inoculation treatments. C: control; M: mycorrhizae inoculum; B: bacterial inoculum; M+B: dual mycorrhizae and bacterial inoculum. For each crop, means followed by different letters indicate statistical difference (Tukey, $p < 0.05$).

to the corresponding controls (Figure 3). For CP, we recorded the maximum values for plants treated with M+B (16.9 %) and B (16.83 %) at the first and second harvest, respectively. The lowest values were found for untreated and mycorrhizae inoculated plants (Figure 3A). At the first harvest, inoculated plants with mycorrhizae surpassed the other treated plants for phosphorus uptake. This can be attributed to the ability of AMF to improve the efficiency of soil P uptake, which is otherwise difficult to be absorbed by plants in alkaline soils (Hou *et al*., 2012). At the second harvest, the highest P uptake was recorded in plants inoculated with bacteria (Figure 3B). The differences among the three species could be due to changes in root morphology, which is proven to alter mycorrhizal colonization of the rhizosphere.

In regard to Ca uptake, we recorded a 23 % improvement with the three inoculum treatments at 42 das. Nonetheless, at 142 das, there was no significant effect of inoculation on Ca uptake (Figure 3C). Additionally, the gain in leaf nitrate (NO_3^-) content was only noticeable in the plants inoculated with the bacteria and mycorrhizae combination with 40.52, 53.11 and 54.54 % higher values compared to the control plants for oat, triticale and vetch, respectively (Table 4). It can be stated that the inoculation

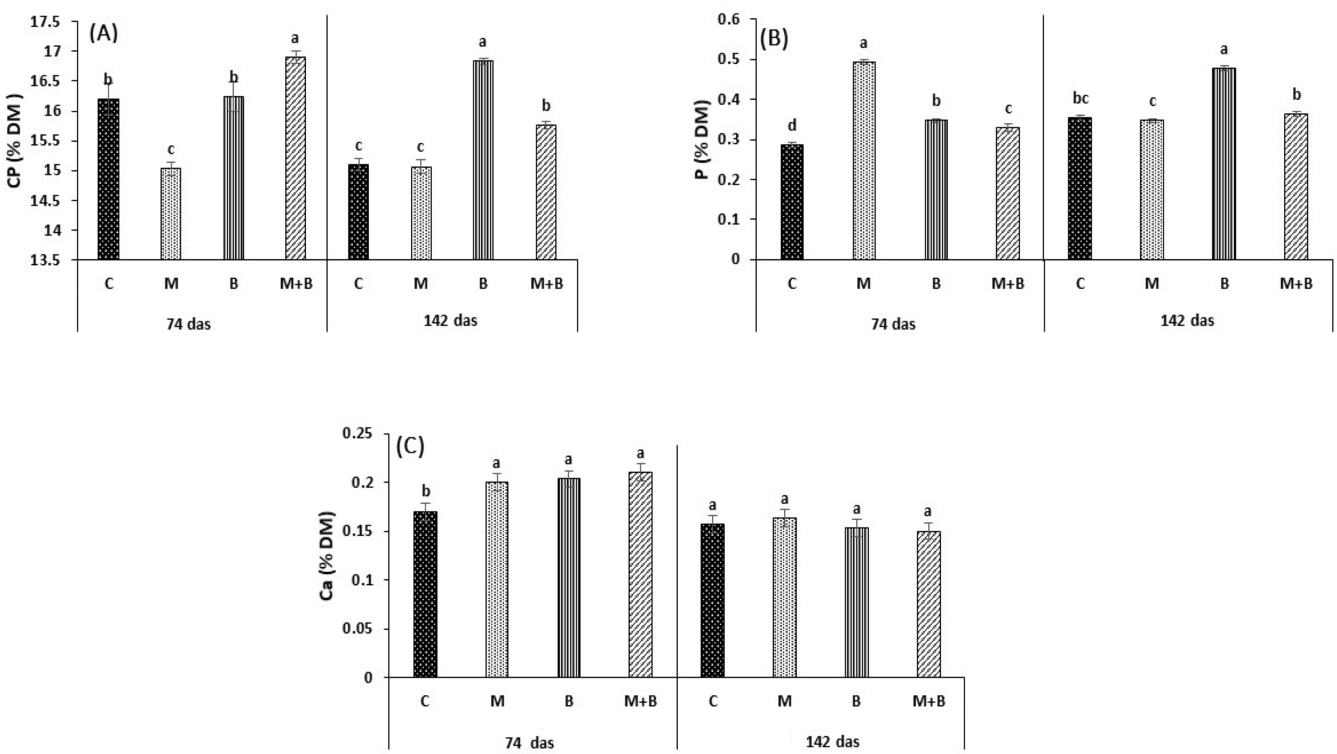

Figure 3. Contents in dry matter of crude protein (A), phosphorus (B) and calcium (C) leaf tissues of oat, triticale and vetch plants under four inoculation treatments. CP: crude protein; C: control; M: mycorrhizae inoculum; B: bacterial inoculum; M+B: dual mycorrhizae and bacterial inoculum. For each crop, means followed by different letters indicate statistical difference (Tukey, $p < 0.05$).

Table 4. Nitrate (NO^-_3) content in leaf tissues of oat, triticale and vetch plants under four inoculation treatments. C: control; M: mycorrhizae inoculum; B: bacterial inoculum; M+B: dual mycorrhizae and bacterial inoculum

Species Treatments	Oat	Triticale	Vetch
C	451.67d±17.5	578.33c±16.0	583.33d±25.1
M	630.00b±10.	573.33c±15.0	1213.33b±104.0
B	567.00c±7.0	703.33b±15.0	1050.00c±50.0
M+B	714.00a±15.0	1233.33a±58.0	1283.33a±28.0
ANOVA	***	***	***

Analysis of variance; significant at *** $p < 0.001$. For each crop, means followed by different letters indicate statistical difference (Tukey, $p < 0.05$).

of rhizobacteria led to an improvement in microbial biomass due to the mutually beneficial relationship which improved the uptake of soil nutrients (Zaidi *et al*., 2017). Mpairwe *et al*. (2002) reported similar results, they found that intercropping cereals and forage legumes increased plant protein content. Mostly due to the hyphal network and nodules, which facilitate the movement of nutrients and carbohydrates from legume to cereal crops (Megueni *et al*., 2011). Rodriguez *et al*. (2020) demonstrated a good structuring effect of the legume root system increased soil biodiversity and improved availability of nutrients from compounds that would otherwise be inaccessible to the cereal root systems.

Forage quality and yield

Variance analysis showed a significant variation of the quality indicators with inoculation treatments. Overall, a slight difference was obtained for crude protein at the two harvest dates. Particularly, the highest CP was found at 142 das for control and treated plants (Figure 4A).

For the ash property, the highest values were recorded at the first harvest (74 das). Compared to the control plants, all treatments gained ash values with a maximum for mycorhizal plants (7.7 %). At 142 das, plants inoculated with B and B+M produced more ash than other treated or untreated plants (Figure 4B). These results are similar to Sibi *et al*. (2015) and Heydarzadeh *et al* (2022), who demonstrated the positive effect of these inoculum on the ash content for corn intercropping with green beans. It has been also published that inoculation with mycorrhizae increased ash content, given its beneficial role in promoting nutrient uptake, by adjusting pH or enzyme release (Puzynska *et al*., 2021).

Furthermore, various inoculations resulted in a considerable increase in DM yield. On average, at both harvest dates, plants treated with M+B had the highest yield values, followed by plants treated with B (Figure 5). In particular, compared to control, the

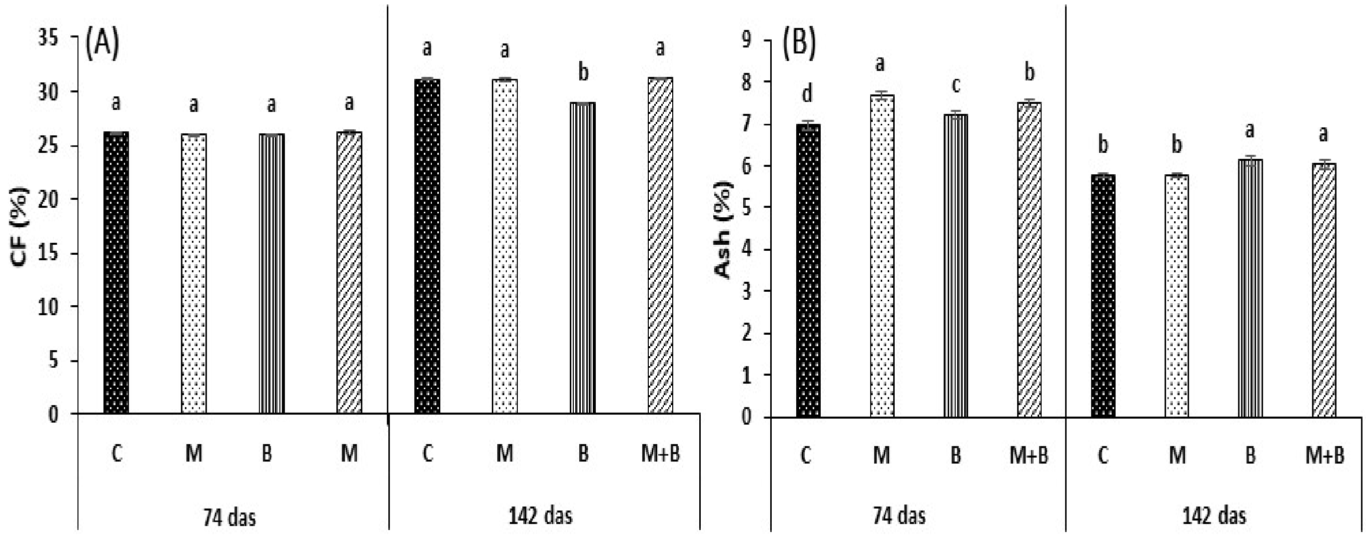

Figure 4. Variation of crude fibre (A) and ash (B) under four inoculation treatments for vetch, oat and triticale. C: control; M: mycorrhizae inoculum; B bacterial inoculum; M+B: dual mycorrhizae and bacterial inoculum.

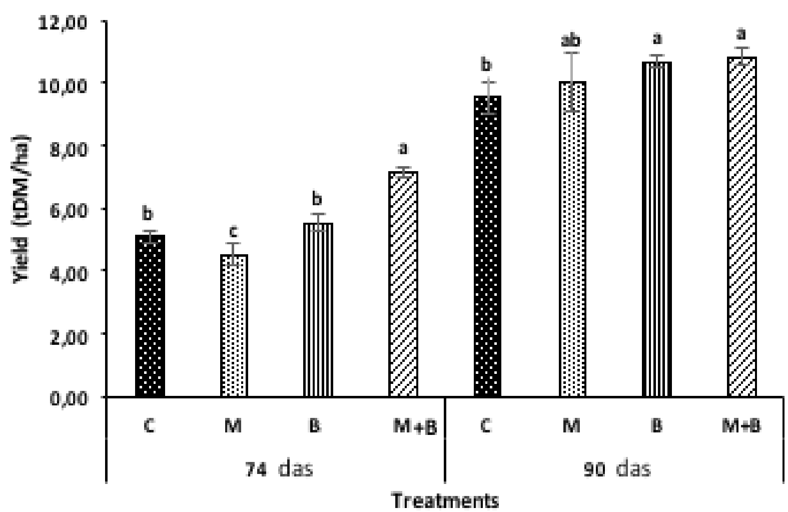

Figure 5. Dry matter yield affected by different inoculation treatments; C: control, M: mycorrhizae inoculum, B: bacterial inoculum and M+B: the dual inoculation mycorrhizae and bacterial inoculum, at the two harvested dates (74 and 142 d after sowing).

dual M+B inoculum improved significantly the DM yield by 41.2 %, corresponding to 2.1 and 1.67 Mg at 74 and 142 das, respectively (Figure 5). The increase in yield dry matter for inoculated plants is attributed to the positive effect of PGPR and mycorrhizal on root growth, which helps in more absorption of water and nutrients. According to

Sahoo *et al*. (2013), these microbial associations include many natural microorganisms which improve soil physicochemical properties, crops yield, soil health and plants growth.

CONCLUSIONS

The use of vetch-oat-triticale intercropping combined to mycorrhizae and bacterial biofertilizers provides an effective way to increase land use efficiency for smallholder farmers. More interestingly, this study highlighted how using a combination of biofertilizers could assist farmers to achieve higher fodder yields in terms of quantity and quality. Further research into the exact mechanisms of these biofertilizers, as well as their interactions with specific crop species, is needed.

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