**Journal of Medicinal Plants and By-Products (2025) 1: 9 – 19**

# Phytochemical Properties of Quinoa Seed by Using of Mycorrhizal and Phosphorus Fertilizer in Different Cropping Ratios

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**Article Info**

**Article Type**
Original Article

**Article History**
Received: 28 April 2024
Accepted: 02 July 2024


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**ABSTRACT**

Quinoa (*Chenopodium quinoa* Willd.), is currently attracting worldwide attention because of its substantial nutritional value, is currently attracting worldwide attention. This study was conducted to evaluate the use of mycorrhizal and different concentrations of phosphorus fertilizer on phytochemical traits of Quinoa seed intercropped with Maize. The study was done separately in two Locations (Shahrood and Mayamey, Iran). Use of mycorrhizal (AMF) in two levels (unused and use of mycorrhizal), Cropping ratios (CR) have three levels (100, 75, and 50%), and Phosphorus fertilizer (P), has three levels (0, 50, and 100 kg/ha). Results show the highest ash (4.43 %), Tartaric acid (13.39 %), Lactic acid (1.17 %), and Soluble carbohydrates (76.37%), were observed in Mayamey location by 50 % CR and use of 50 kg/ha P fertilizer and AMF inoculated in Mayamey location. The measured values were higher than the Shahrood location. Our findings showed that AMF display essential roles in optimizing plant strategies for phosphorus uptake and the efficient utilization of phosphorus in the intercropping system. Application of AMF in a 50 % ratio could be proposed to farmers as a friendly method to obtain favorable biochemical traits, mainly when using 50 kg/ha of P fertilizer. In general, the use of AMF inoculation and 50 kg p/ha in 50% CR has increased the biochemical traits of Quinoa seed.

**Keywords:** Bioactive compound, Cropping ratio, Phytochemical, Mycorrhiza, Phosphorus fertilizer

**How to cite this paper**
Rostami, T., Abbasdokht, H., Makarian, H., gholipoor, M., karimzadeh, K. Phytochemical Properties of Quinoa Seed by Using of Mycorrhizal and Phosphorus Fertilizer in Different Cropping Ratios. Journal of Medicinal plants and By-Products, 2025; (1):9-19. doi: 10.22034/jmpb.2024.365617.1683

## INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a pseudo-cereal plant and belongs to the Chenopodiaceae family, native to the Andean location in South America [1]. Abdelalem *et al,* [2] showed that quinoa seeds contain high phenolic, flavonoids and other secondary metabolites. Quinoa seeds with 2– 9.5 % fatty acids [3] are known as an alternative oilseed plant due to the qualitative and quantitative of fatty acids, such as Omega 3 and Omega 6 [4]. Tang, li *et al*, [5] reported the presence of β-Cyanidins in the seeds of the quinoa plant for the first time. Inventing a healthy and sustainable food system is one of the United Nations' plans for the growing world population until 2030. That means prioritizing products that guarantee improved efficiency by using natural resources and contribute to meeting the requirements of healthy diets. In this framework, the cultivation of quinoa as an alternative product is taken into significant consideration [6]. Today, farming and use of quinoa have attracted more attention because of its remarkable compatibility with various environmental conditions, high nutritional value, and high priority as an ingredient in functional meals. It is gluten-free and used as a source of amino acids, unsaturated fatty acids, tocopherol, phytosterols, phenols, and a low index of Glycaemic [7]. Quinoa is a functional component in products of meat because the fatty acids in seeds substitute traits [8]. The phosphorus fertilizer (P) is one of the most essential elements for plant growth and development of metabolite, and plays a crucial role in production. Today, soil deficiency of P has become a factor limiting production and productivity [9]. Due to their high quality and nutritional value, the seeds of quinoa are considered a new source of superfoods [10]. Additionally, the consumption of quinoa seeds is due to having fatty acids with high nutritional value and antioxidant compounds associated with anti-inflammatory and cardiovascular diseases [11]. The multi-cropping system is essential for smallholder farmers in all countries especially developing countries due to the more use of land and better control of pests and diseases, today [12]. The availability of P is positively correlated with biomass and bioactive compounds of plants [13]. Amani Machiani *et al,* [14] reported that AMF increased primary and secondary metabolites by increasing water and mineral uptake. Their study showed that intercropping of Thyme /Soybean and AMF inoculation improved the yield, essential oils and phenolic compounds in thyme. The use of intercropping agricultural system (growing more than one plant in the

same area in a year) is one of the most effective ways to increase the production of plants [15]. Li and Cai, [16] showed that using AMF, increased biomass, chlorophyll a, b, total chlorophyll, Glucose content and Total fatty acids, significantly. Due to their nutritional value and protein quality, quinoa seeds are considered a new source of functional nutrients such as low glycemic index, quinoa milk, and protein-enriched pasta [17]. Quinoa seed has natural antioxidants, such as acid ascorbic, phytosterols, phenolic compounds, and flavonoids, and is associated with anti-inflammatory and antifungal properties. In addition, these compounds serve as immunological adjuvants, preventing diabetes and cardiovascular disease [18, 19]. The objectives of this study to evaluate the use of mycorrhizal and phosphorus fertilizer on phytochemical characteristics of quinoa seed in intercropping systems at two different locations.

## MATERIALS AND METHODS

This experiment was performed in 2022 in Semnan province (Shahrood and Mayamey location), Iran. Geographical and climatic characteristics of these areas are shown in Table 1. Quinoa seed biochemical traits across four variables (Location, CR, AMF, and P fertilizer) were analyzed. This study was conducted as a factorial split-split plot in a randomized complete block design with three replications in two locations. Factors include the use of mycorrhizal (unuse and use of mycorrhizal), Cropping ratio (100, 75, 50 and 25%) as factorial in main plots, and Phosphorus fertilizer (0, 50 and 100 kg/ha) were placed in subplots.

**Land preparation and sowing seeds:** The physical and chemical properties of the soils were performed in the soil science laboratory of Shahrood Agricultural Research and Training Centre. The physical and chemical soils properties of two studied locations are shown in Tables 1 and 2. In this study, the maize seeds cultivar 704 and the quinoa variety Titicaca were used in this study, prepared from the seed breeding institute in Karaj, Iran. After plugging, the plots were arranged 6.09 × 8.53 m in size. The phosphorous fertilizer was placed at a distance of 5 cm under the seeds at the same time as planting weed control was done manually.

Seeds were sown in both areas on June. 2022. The first irrigation was done after sowing the seeds. Inoculation of mycorrhizal fungi was obtained from Green Biotechnology Company, Karaj, IRAN, for more adhesion seed with AMF inoculum, used of Arabic gum. Pour the coated seeds into a polythene bag and shake vigorously for 30 seconds until the surface of all the seeds is uniformly coated. After that, 50 grams of inoculum per kilogram was added to the sticky seeds and after 10 minutes and ensuring that the seeds were mixed with the fungus, the seeds were dried in the shade for one hour and then the planting operation was done quickly. 100 days after planting, harvesting was done, and traits were evaluated.

### Evaluated Traits

#### Oilseed Content

Oilseed content was prepared according to the Fletch method [20]. For oil extraction, 15 ml of a mixture of two solvents of chloroform and methanol in a ratio of 1:2 was added to1 gr of powdered seeds and shaken 10 to 15 times per min, maintained at 4˚C for 24 hours, add 5ml of distilled water and intensity shakes. Finally, the lower phase was separated by using a rotary evaporator and placing it in a heating bath and solvent nitrogen gas, the solvent was evaporated. Thus, the pure seed oil is extracted.

#### Fatty Acid Compositions

Fatty acid compositions were prepared according to the method reported by Metcalfe [21]. 1 g of the oil was added into 5 ml methanolic potassium (2%) and placed in boiling water for 10 minutes. After cooling at room temperature, 2.175 ml boron trifluoride ($BF_3$) was added and boiled again for 3 min after shaking. After that, the flask was cooled again and shook ten times. Finally, 1 ml n-hexane and saturated NaCl solution were added, and the flask was shaken vigorously 12 times. 1.5 ml of upper phase was collected and 0.2 µ L was injected for analysis by GC-MS.

#### Total Sugars Content

Total sugar measurement was performed based on Vundavalli *et al* method [22].

#### Organic Acid and Sugar Content

Free sugar and organic acid extractions were performed by the methods of Silva *et al*. [23].

**Table 1** Geographical and climatic characteristics of experiment Locations

| Location | Elevation above sea level (m) | Longitude | Latitude | Maximum temperature (ºC) | Minimum temperature (ºC) | Climate |
|---|---|---|---|---|---|---|
| Shahrood | 1420 | 35.36 | 58.54 | 41.68 | 20.80 | Temperate Warm |
| Mayamey | 1025 | 36.4108 | 55.6503 | 45.66 | 19.72 | Temperate |

**Table 2** Soil physical and chemical properties of experiment locations

| Location | C (%) | N (%) | K(ppm) | P(ppm) | EC (ds/m) | pH | Texture |
|---|---|---|---|---|---|---|---|
| Shahrood | 0.4 | 0.04 | 149 | 19 | 0.41 | 8.30 | Loam-silt |
| Mayamey | 0.189 | 0.08 | 339 | 33.16 | 3.25 | 7.75 | Silt- loam |

A definite mass and 50 ml of 0.01 N H2SO4 were mixed and stirred (300 rpm) for 30 min. The extracts were then filtered, evaporated to dryness (40 ºC), and re-dissolved in 1 ml of 0.01 N H2SO4, followed by filtration using a 0.22-lm membrane. Sugar and organic acid analyses were assessed by reversed-phase HPLC using a Shimadzu 10AVP HPLC system (Shimadzu Corp., Japan) equipped with a RID-10A refractive index detector. A 20-ll sample was injected onto an Aminex HPX-87H column (BioRad, CA, USA) and then eluted isocratically using 5 mM degassed and filtered $H_2SO_4$ for 25 min at a flow rate of 0.6 ml/min and a column temperature of 65 ºC. The free sugars and organic acids were quantified by comparing their absorbance in 214 nm with external standards (acid acetic). The following procedure was used according to Bates *el al*. [24]. 4 ml of sulfuric acid (3%) was added in 0.05 g of fresh leaf tissue. The upper phase was separated using a centrifuge and mixed with 2 ml of glacial acetic acid and 2 ml of ninhydrin solution. The mixture was heated at 90 °C for half an hour and 4 ml toluene was added into each tube after cooling and then shaking them variously. The supernatant was removed, and the samples were read inside the device at 520 nm. The individual sugars were identified and quantified by comparison with the retention times and peak areas of individual sugar standards (prepared to contain between 5 and 50 mg/l).

Preparation of standard dextrose solution for making a calibration curve: Exactly 1 g 1 g of dextrose, transfer to a one L volumetric flask, and dilute to volume with water.

Preparation of standard dextrose solution for standard addition:

Four g of dextrose, transfer to a one L volumetric flask, and dilute to volume with water for Preparation of standard invert sugar solution and other reagents 4.75 g of sucrose, transfer with 90 mL of distilled water to a 500 mL volumetric flask, and add 5 mL of hydrochloric acid (specific gravity, 1.18). After leaving to stand at 20–30 °C for three days, dilute the solution to volume with water and store in a cool dark place. Transfer a 50 mL of the solution above to a 200 mL volumetric flask, neutralize with 1 mol/L sodium hydroxide aqueous solution using phenolphthalein as an indicator, and dilute to volume with water. Used the solution as standard inverted sugar solution for to standardize of Fehling's Solution. To prepare 1% Methylene Blue solution, 1 g of methylene blue dissolved in distilled water to make 100 mL for Fehling's Solution dissolved 34.639 g of copper sulfate ($CuSO_4.5H_2O$) in distilled water to make JCAM No.114-R1 exactly 500 mL, leave it for two days, and then filter.

## Lycopene Assay

To lycopene contents of quinoa seed extracts were determined by the method of Nagata and Yamashita. [25]. 1 g of quinoa seed was homogenized with 10–20 ml of acetone–hexane (4:6) solvent. After homogenization, the supernatant was used for the lycopene analysis. Lycopene content was determined based on spectrophotometric analysis (503 nm wavelength) as mg/gr.

## Statistical Analysis

This experiment was analyzed by combined analysis; the basic design of this experiment is factorial. The experiment was carried out in three replicates **.**Analysis of variance and mean data comparisons based on the LSD test ($p\leq 05$) were performed by using of SAS software (Ver. 9.4). MS Excel was also used to plot the diagrams.

## Results and Discussion

### Linoleic Acid

As can be seen in Table 3, all treatments had a significant effect on the linoleic acid in quinoa seeds. Based on mean comparisons (Fig. 1), the highest linoleic acid content (53.60 %) was obtained in the cultivation treatment of 100% quinoa by use of mycorrhiza inoculated and 50 kg/ha p fertilizer in the Mayamey location. The lowest Linoleic acid content (38.2 %) was obtained in the planting ratio of 50 % quinoa by useless of mycorrhiza and p fertilizer (control) in Shahrood location. All mycorrhiza treatments were observed to be more effective than those without mycorrhiza inoculation.

**Ash Content**

The influence of varying phosphorus fertilizer concentration, locations, and intercropping on ash content was a notable significance at a 1 % level. Furthermore, all main effects and the interactive effects of cropping ratio, locations, phosphorus fertilizer, and use of mycorrhizal were significant at the 5 % level (Table 3). Examination of mean comparisons unveiled that the maximum amount of ash (4.43 %) was identified in the intercropping of 50% quinoa + 50% maize, accompanied by the application of 50 kg/ha phosphorus and mycorrhiza, at the Mayamey location. On the contrary, the minimum ash content (3.85 %) was documented under the 50% quinoa in the Shahrood locale, without of mycorrhiza utilization (Fig. 3).

### Moisture

The moisture content in quinoa was significantly impacted by all individual treatments ($p<0.001$). However, most interactive treatments did not show statistically significant effects on moisture percent (Table 3). The treatment with the highest moisture content (11.83%) was observed in the intercropping of 50% quinoa and 50% maize with a phosphorus application of 50 kg/ha in the Shahrood location.

**Table 3** Results of analysis of variance (mean squares) of phytochemical characteristics of quinoa seed in two location

| Sours | DF | Linoleic acid | Lycopene | Fibre | Ash | Moisture | Tartaric | Lactic | Carbohydrate | Mannitol | Galactose | Maltose | Glucose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location (L) | 1 | 1266.76** | 276.667** | 0.419916** | 24.2474** | 8.1225** | 1.46006** | 0.2554** | 1.0254** | 0.3458** | 1236.694** | 1266.76** | 1432.634** |
| $Error_1$ | 4 | 5.0238 ns | 9.18444 ns | 0.000294ns | 5.79652 ns | 1.69854 ns | 0.011597 ns | 0.01142 ns | 1.03546 ns | 4.2584 ns | 23.3819 ns | 5.0238 ns | 31.142 ns |
| Cropping Ratio (C) | 2 | 354.216** | 2827.16** | 0.32129** | 3252.126** | 24.8264** | 4.2531** | 75.3264* | 22.370 * | 79.716 * | 225.114** | 59.421 * | 348.154** |
| Phosphorus (P) | 2 | 202.428** | 274.191** | 0.017218** | 163.2641** | 28.2893** | 1.85442** | 150.103** | 176.213** | 181.226 ** | 98.1642** | 92.316 ** | 105.1692** |
| Mycorrhizal (M) | 1 | 267.381** | 281.229** | 0.000179ns | 82.3917** | 6.2133** | 1.3296** | 96.538 ** | 20.186** | 32.9514** | 102.415** | 26.904** | 154.211** |
| C* P | 6 | 16.131* | 38.2891* | 0.000386ns | 77.412** | 2.1345** | 0.15384 * | 77.1054** | 82.5412** | 0.19847 * | 46.194** | 1.317 * | 97.28** |
| C* M | 3 | 73.126** | 62.8471** | 0.006531** | 134.321** | 0.35241* | 0.925118** | 146.347** | 17.6541* | 0.67142** | 22.0168* | 2.442** | 75.224* |
| P* M | 2 | 1.382 ns | 0.9863 ns | 0.003415** | 57.642** | 0.2561* | 0.86573** | 75.251** | 18.4125 * | 0.98255** | 22.181 * | 3.229** | 27.19 * |
| C* P * M | 6 | 17.657** | 56.268** | 0.00351** | 83.1582** | 0.03983 ns | 0.020374 ns | 90.0012** | 65.2893 ** | 0.11287 ns | 51.761** | 0.54741* | 84.63** |
| L*C | 3 | 325.211** | 411.226** | 0.009454** | 1.4127 ns | 0.9245** | 0.090128 * | 9.0014 ** | 10.46324* | 0.14254 ns | 10.2196** | 13.4364 ** | 17.967** |
| L*P | 2 | 99.226** | 58.483** | 0.001123ns | 20.16806 ** | 0.01425 ns | 0.01546 ns | 1.9850 ** | 12.01425 ns | 1.11540 * | 35.96313* | 17.2250 * | 71.543* |
| L*M | 1 | 15.604 ns | 1.2135 ns | 0.009318** | 10.2154 ** | 0.03894 ns | 0.23416** | 4.21500 ** | 0.03240 ns | 4.1225** | 101.4251** | 6.6237** | 126.451** |
| L*C*P | 6 | 56.167** | 66.371** | 0.0042613** | 14.32174 ** | 0.6358* | 0.097415* | 1.3356 * | 1.314520* | 0.46350* | 23.05324 * | 2.3741* | 37.524 * |
| L*C*M | 3 | 46.715** | 47.347** | 0.005066** | 9.1029 * | 0.4152 * | 0.24165** | 1.52143* | 2.1525 * | 11.1084** | 114.4281** | 5.1142** | 126.681** |
| L*P*M | 2 | 23.227** | 38.12* | 0.00532** | 18.167* | 0.12457 * | 0.029731 * | 0.02548 ** | 19.1006 ** | 3.1635* | 40.7316** | 6.5831* | 57.636** |
| L*C*P*M | 6 | 16.411** | 5.0136 ns | 0.003411** | 12.103674 * | 0.16478 * | 0.12114 * | 28.1156 ** | 24.1427 ** | 0.1811 ns | 38.1842** | 1.3412 * | 47.762** |
| Error | 70 | 2.95764 | 6.25411 | 0.000696 | 7.11542 | 0.09271 | 0.023658 | 0.009545 | 0.35412 | 0.18416 | 7.09934 | 0.23654 | 8.0247 |
| CV% | | 4.18 | 7.9 | 10.21 | 5.38 | 3.74 | 5.86 | 8.32 | 7.18 | 6.52 | 7.15 | 8.12 | 9.12 |

* And ** significantly at the probability level of %5 and %1, respectively.

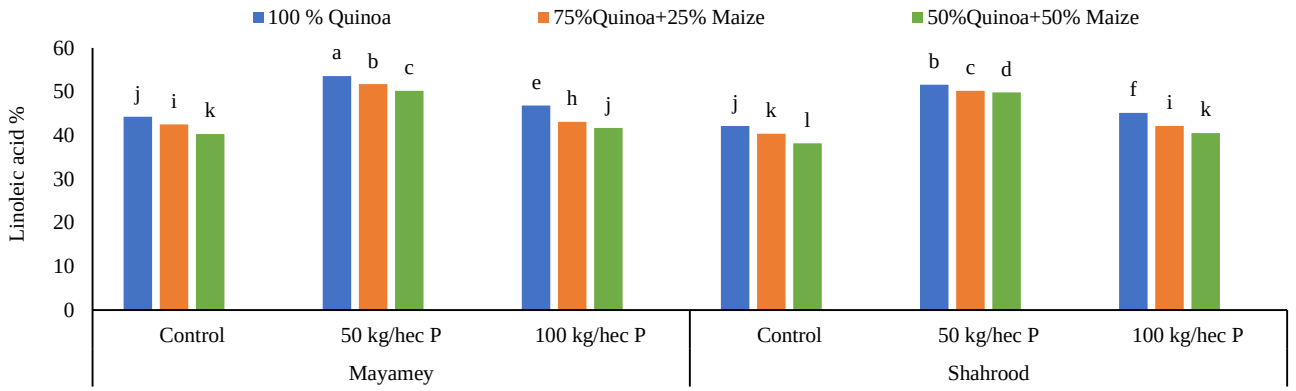


**Fig. 1** The interaction effect of cropping ratio, P fertilizer, and location on linoleic acid content. Columns sharing identical letters exhibited no significant distinctions.

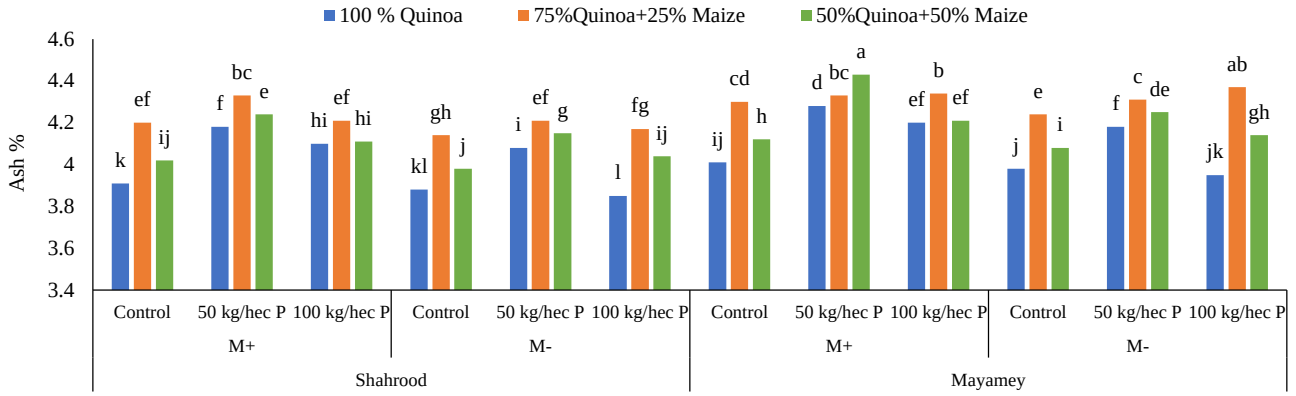


**Fig. 2** The interaction effect of cropping ratio, P fertilizer, and location on ash content.

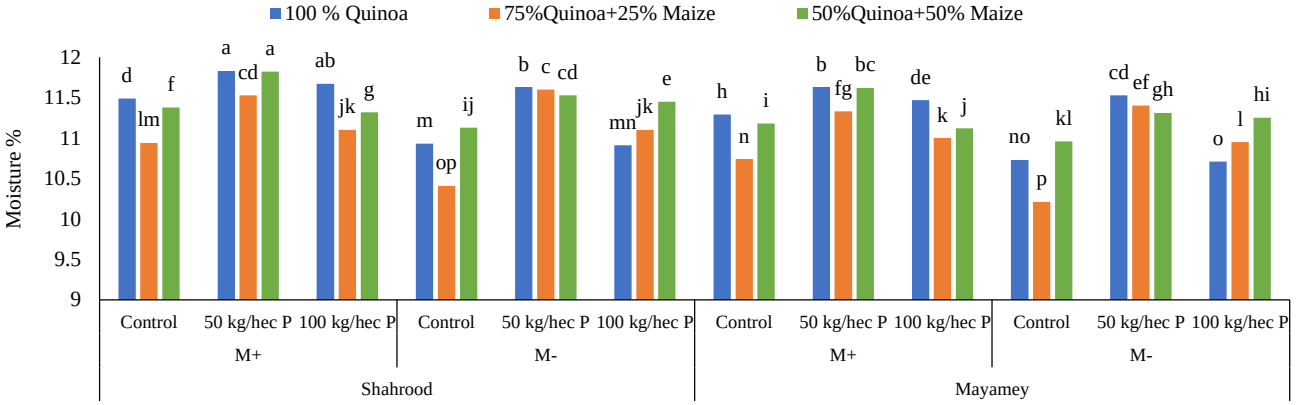


**Fig. 3** The interaction effect of cropping ratio, P fertilizer, and location on moisture content

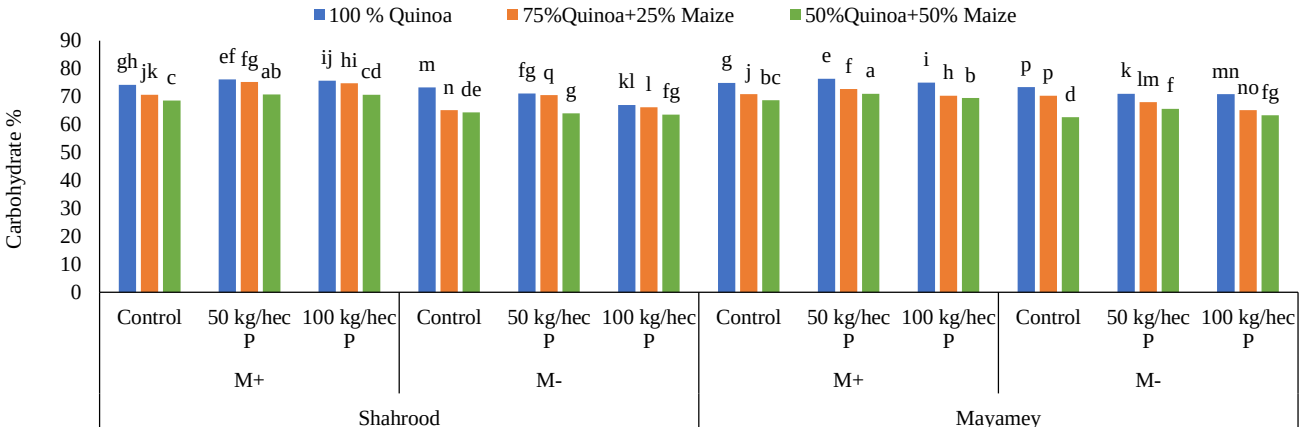


**Fig. 4** The interaction effect of cropping ratio, and application of phosphorus on carbohydrate content.

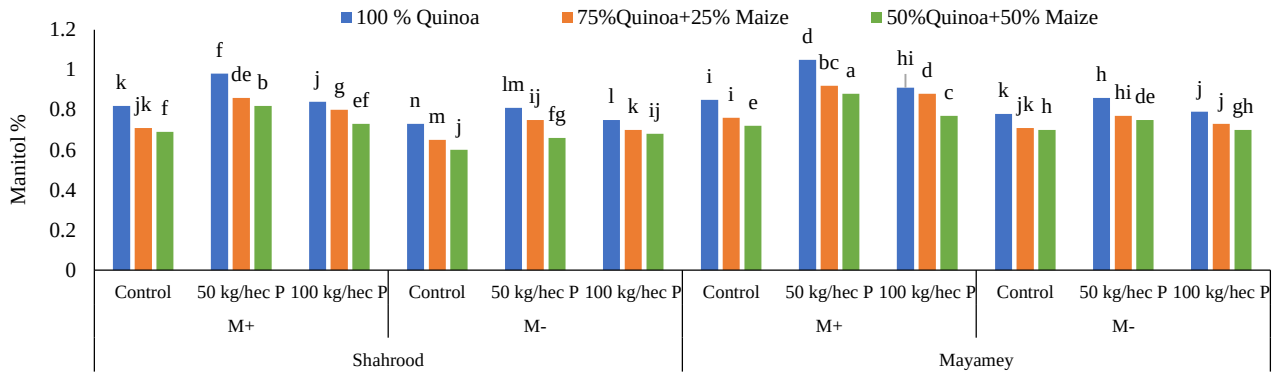


**Fig. 5** The interaction effect of cropping ratio, and application of phosphorus on mannitol content.

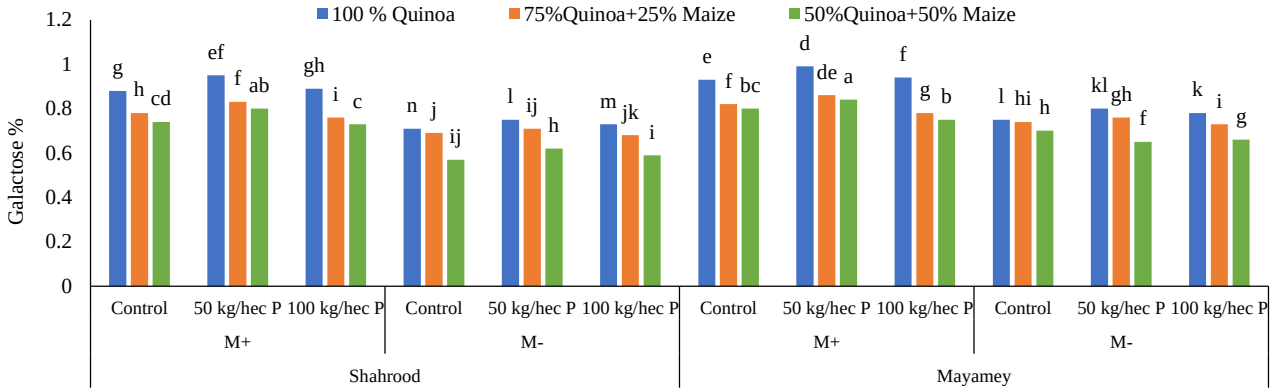


**Fig. 6** The interaction effect of cropping ratio, and application of phosphorus on galactose content.

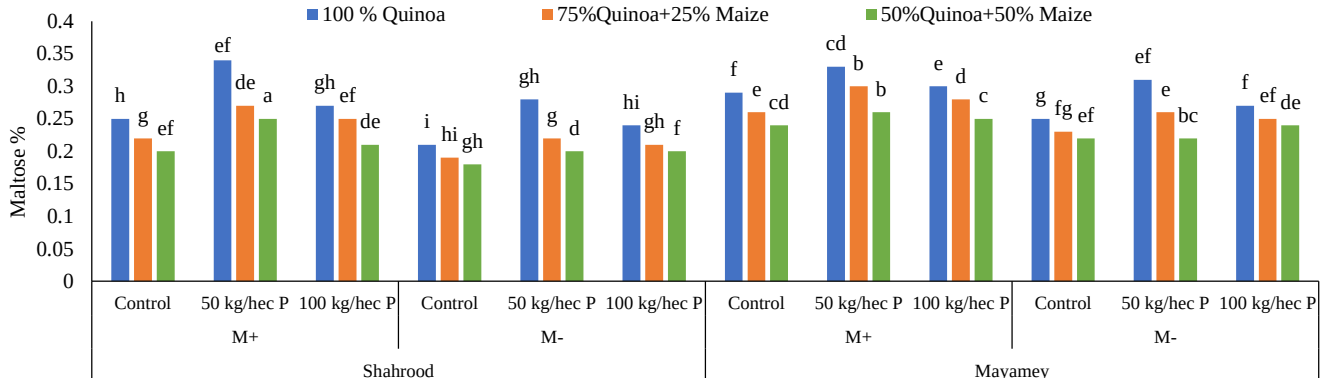


**Fig. 7** The interaction effect of cropping ratio, and application of phosphorus on maltose content.

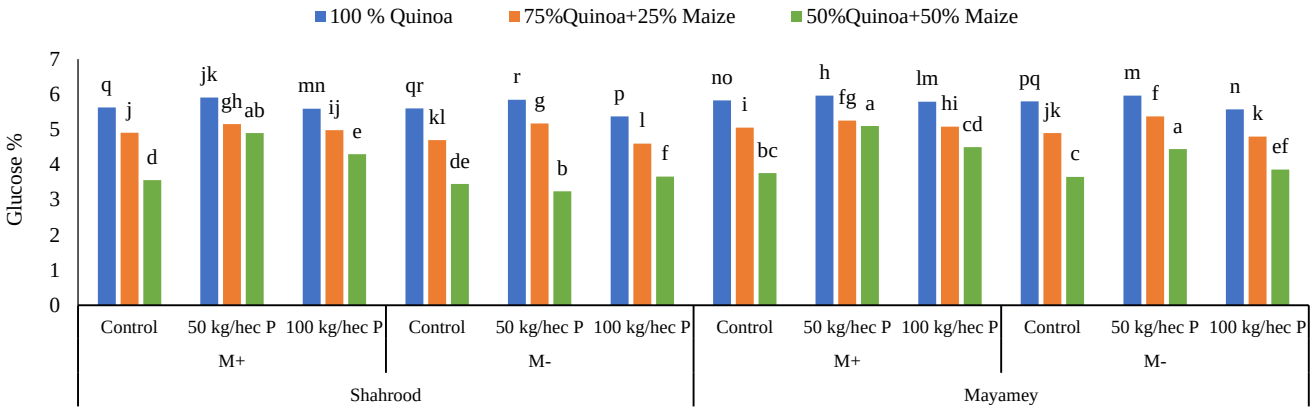


**Fig. 8** The interaction effect of cropping ratio, and application of phosphorus on glucose content.

Conversely, the lowest moisture content (10.21%) was found in the intercropping of 75% quinoa in combination without phosphorus application in the Mayamey location (Fig. 3).

## Carbohydrate and Sugar Contents

The cropping ratio, phosphorus fertilizer application, mycorrhiza inoculation, and locations had a significant impact on the carbohydrates and sugar content in quinoa seeds (Table 3). As can be seen in means comparison, intercropping system reduced the carbohydrates and sugar content in quinoa seeds compared to monoculture, significantly. The content of sugar in quinoa seeds were decreased in all cropping ratio pattern. The carbohydrate and sugar contents in 100 CR were significantly higher than that of 75% CR, and 50% CR. Upon examining the interactive effects of phosphorus levels with mycorrhiza inoculation, it was found that the highest carbohydrate (76.60%) was obtained with the application of 50 kg/ha of phosphorus, mycorrhiza treatment, and a cropping ratio of 100 % quinoa. Conversely, the lowest carbohydrates content (63.50%) was recorded in the cropped of 100 % quinoa, unuse phosphorus fertilizer, and the absence of mycorrhiza (Fig. 4). Among these, the highest amount was related to glucose, followed by arabinose, cellobiose, galactose, mannitol, and maltose. Their levels were differed among treatments significantly. The highest contents of glucose (5.96%), cellobiose (2.05%), galactose (0.99 %), mannitol (1.05 %), and maltose (0.35%) were obtained in cropping ratio of 100% quinoa, use of 50 kg P/ha, mycorrhizal inoculated at Mayamey location (Figs. 4-9).

## Organic Acids

### Acid Lactic and Tartaric Acid

The analysis of variance (Table 3), showed that the simple effects of phosphorus, mycorrhizal, and the interaction effects of phosphorus with mycorrhizal on acid lactic were significant at the 1% level. The mean comparison test results showed that the highest acid lactic (1.17%) was obtained in treatment with the use of mycorrhiza and 50 kg/ha phosphorus in the Mayamey location. The lowest content of acid lactic (0.3%) was obtained in the control treatment and unuse of mycorrhizal in the Shahrood location (Fig. 9). The tartaric acid contents were significantly impacted by the cropping ratio, phosphorus fertilizer, and mycorrhiza inoculation ($P \leq 0.05$) (Table 3). Elevated levels tartaric acid was evident with mycorrhizal and phosphorus applications when compared to the control conditions (Fig .10). The highest contents of tartaric acid were observed in cropping ratio 50%, utilization of mycorrhizal and 50 kg P/ha in Mayamey location (Fig. 10).

## Lycopene

The p fertilizer, mycorrhiza inoculation, and locations had a significant impact on the lycopene contents in quinoa seeds (Table 3).

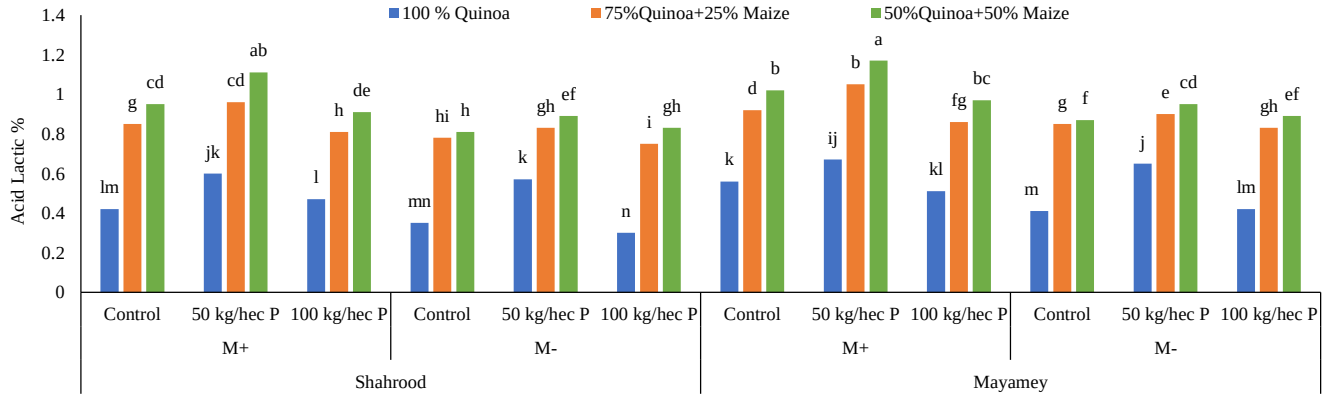


**Fig. 9** The interaction effect of cropping ratio, phosphorus mycorrhiza, and location on acid lactic content.

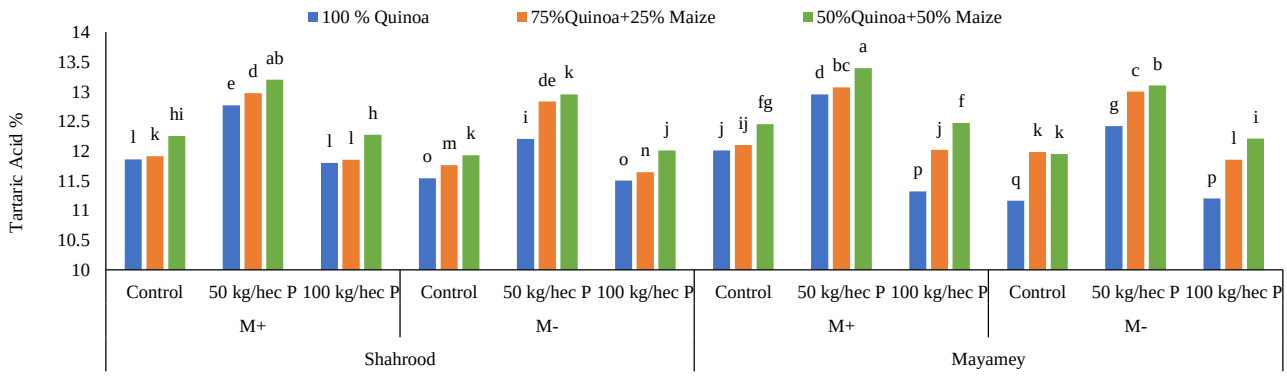


**Fig. 10** The interaction effect of cropping ratio, phosphorus mycorrhiza, and location on tartaric acid content.

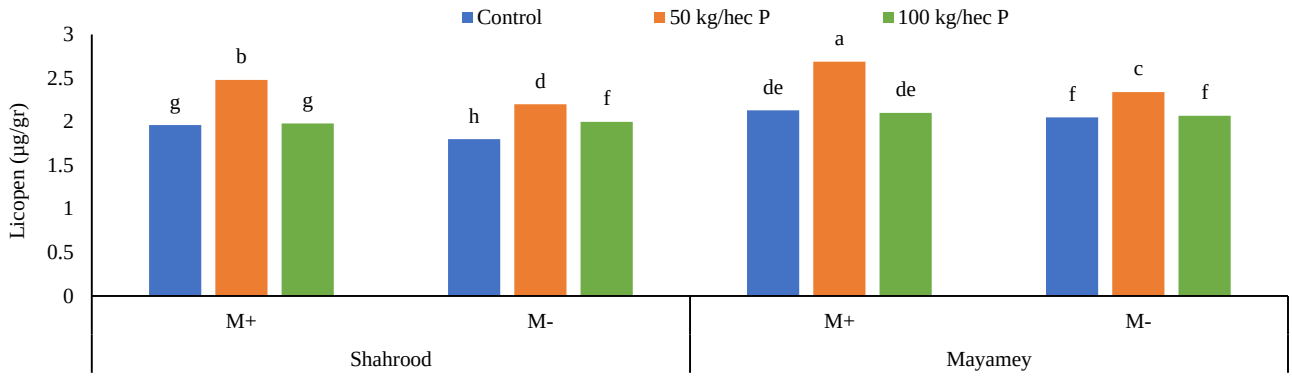


**Fig. 11** The interaction effect of phosphorus, mycorrhiza, and location on lycopene

Interactive effects of different p levels with mycorrhiza inoculation, showed that the highest lycopene (2.69 µg/gr) was attained in 50 kg P/ha mycorrhiza treatment, in Mayamey location. Conversely, the lowest lycopene content (1.8 µg/gr) was recorded in control treatment, and the absence of mycorrhizal in Shahrood location (Fig. 11).

## DISCUSSION

In this study, plants treatment with AMF increased their root system, due to enhancing the availability of water and essential nutrients, such as nitrogen, phosphorus, and potassium, crucial for plant growth and development [26]. The low amount of linoleic acid in the Shahrood location in the cultivation ratio of 100% of quinoa can be due to the climatic and soil conditions of this location (Tables 1 & 2) and the lack of sufficient light due to the predominance of maize over quinoa. Mycorrhizal hyphae increase magnesium absorption and, as a result, increase the amount of total chlorophyll and behenic in quinoa and corn plants [27]. Phosphorus has an essential physiological role in the phytochemical biosynthesis pathway because it has a synergizes effect with nitrogen. Our results determined that using AMF increased the amount of secondary metabolite in quinoa compared to control treatments. This increase can be due to improved biochemical composition [27]. Based on the results of this research the contents of carbohydrate and sugar contents (mannitol, galactose, maltose, and glucose) in the quinoa seeds were significantly lower under intercropping than those under monocropping, which was consistent with the other previous studies, [28, 29]. Studies have shown that under the shade condition, the stem of quinoa plant significantly increased the activity of sucrose synthase and sucrose phosphate synthase, while reducing the activities of acid invertase and neutral invertase. [30]. Considering the higher height of the maize plant compared to the quinoa plant and more shading, the lower cropping ratio % of quinoa to maize, the less shading will occur. As a result, it increased the amount of sugar content. Therefore, 75 % and 50 % cropping ratios were less affected by shading as their sucrose content compared to 100 %CR. On the other hand, the increased activity of sucrose synthase and sucrose phosphate synthase can more effectively decompose sucrose to produce diphosphouridine glucose [28]. Secondary metabolites can maintain cell structural and functional integrity and reduces damage of oxidative that caused by reactive oxygen species (ROS), thus protecting plants against drought stress [31]. Mycorrhizae can systemically alter plant primary and secondary metabolism [32]. For example, AMF markedly increased anthocyanin and total behenic concentrations in *Medicago truncatula* leaves [33]. However, these mycorrhizal effects can be partially attributed to the improved phosphorus (P) status [34]. Xia *et al*. [32] demonstrated the common specialized root metabolites accumulated after mycorrhizae, with an increase in flavan-3-ols and a decrease in flavanols regardless of mycorrhizal lifestyles. However, whether and how these secondary metabolites induced by AM symbiosis play roles in improving plant drought tolerance remains largely unknown [35]. The mechanisms by which mycorrhizae modulate plant metabolism can be divided into general and specific aspects. As one of the known benefits of AM symbiosis to the host plant, the substantial improvement of water and nutrient status by AMF can largely explain mycorrhiza-caused changes in carbohydrates and parts of secondary metabolism, which are defined as general mycorrhizal effects [35]. Although mycorrhizal effects on plants are often noticed as highly specific to host plants or fungal species, the common traits of mycorrhizal-associated metabolite alterations are present in plant roots [32].

The hyphae of mycorrhizal played an essential role in increasing magnesium absorption, consequently elevating the levels of plant pigments such as lycopene in quinoa seeds [27]. The synthesis of total plant pigments is depending on the sufficient light and vital elements, especially nitrogen and magnesium elements [36]. Phosphorus fertilizer play a crucial role in the biosynthesis pathway of phytochemical compound. The results of our studies showed that treatments inoculated with AMF, resulted in an increment of primary and secondary metabolites in quinoa seeds compared to the control treatment. This augmentation may be attributed to the increased phytochemical compound [27].

In general, it has been observed that increased biochemical traits in Mayamey location with low altitudes and more

warm temperatures. The maximum temperature was high in Mayamey location (Table 1). According to the results of other studies [37], the average temperature and the maximum temperature influenced the growth of plants such as asparagus [31]. Water and nutrient absorption, climate, and ecological conditions are physiological mechanisms for growth, development, and establishment in flowering plants [38]. Total soluble carbohydrates increase with colder and drier conditions in the tissues of plants, indicating they can plastically increase a plants' tolerance to cold and arid conditions [39].

In this study, it was found that Quinoa seed had organic and fatty acid contents. It was observed that the highest amount of organic acid was related to tartaric acid, that was vary between 11.16-13.39% in Mayamey location and 11.50-13.2% Shahrood Location. The carbohydrate content of the seed extracts, were in the range of 62.38 to 74.87 %. The amounts of fatty acids % and carbohydrates% in decreased Shahrood and Mayamey locations under 100% cropping ratio by 0 and 100 kg/ha of P fertilizer with unused of AMF respectively (Fig. 4). P fertilizer reduces water and food losses and increases water and food efficiency during the growing stage. As a result, it increases the durability of leaf area and the length of the photosynthesis period, and as a result, increases the metabolic content in plants [40]. Non-biotic stress decreased the production of secondary metabolites in lavender [41]. On the other hand, the reduction of some metabolites, such as fatty acids production, can be due to spending more energy on the plant to uptake water under stress, increase in protoplast concentration, and change in airways and pentose phosphate pathways that are related to the synthesis of fatty acid-producing enzymes in plants and thus reduce the production of fatty acid [42]. Using P fertilizer, it was found that Phosphorus fertilizer prevents a significant reduction in energy reduction [43]. In this study, phytochemical traits increased with increasing temperature in the Mayamey location, which was consistent with the results of other researchers [44]. Secondary metabolites can maintain cell structural and functional integrity as a result, reduces of damage caused by abiotic stress, thus protecting plants against different stress [45].

Plants cope with the different conditions through changes to cellular osmotic potential, the potential of water uptake, and activation of natural systems in the form of antioxidant enzymes and accumulation of metabolites such as phenolic, fatty acids compounds, and soluble sugars [46]. As the altitude increases, the difference between night and day temperatures rises, and therefore increasing some bioactive compounds [47].

Location, followed by CR, contributed most to discrimination in organic acids, fatty acids, and lycopene content, which had a coefficient significantly different from without AMF inoculation and P fertilizer. There is a significant increase in the contents of pigments as well as a decrease in growth, a decrease in chlorophyll content and a decrease in the activity of *RuBPC* enzyme [48]. Li *et al*, [49] reported that the application of P fertilizer increased soluble carbohydrates amounts by improving sucrose metabolism sink strength in crop conferred by the upregulations of the activities of sucrose-degrading and sucrose-synthesizing enzymes. Primary metabolites such as soluble carbohydrates can affect the solubility of various elements [50]. Increased organic acid concentration by use of AMF has been reported on clover [51]. Accumulations of secondary metabolites reported in plants during adaptation to various climate conditions such as drought, salinity, high temperature, nutrient deficiency and in response to heavy metals [82]. Soluble carbohydrates are the primary compounds found in various plant species, especially in growing leaves, they participate under high temperatures [53]. AMF stimulated traits compared with unuse of AMF. Inoculation AMF increased soluble carbohydrate content and accelerated the accumulation of glucose and fructose in *Trifolium repens* roots [54]. The Use of AMF can lead to changes biochemically and as an induction agent to increase photosynthetic pigments in the plant. AMF increased significantly the amount of chlorophyll in *Rosa damascene* [55]. As well as, the use of AMF in *Aloe vera* plant increased polysaccharide. The reason could be the effect of AMF on the exchanges of ions in the membrane or the effect of enzymes involved in the metabolic process [56]. Grown under organic acids increases the amounts of lycopene in tomatoes [57]. In the studies of Khalil and Yusuf 2018, the lycopene contents of the seed extracts were variable between (1.48-2.40 /µg dry weight) in different genotypes [58]. Quinoa seed are rich source of flavonoids, fatty acids and vitamin which are affected by various genetic and nutritional factors [59]. The estimated discriminant functions were used to calculate discriminant scores, which can be applied as the scale of proficiency of the Quinoa seed characters and to summarize variability as a function of variables [60].

## CONCLUSION

Our study showed the different effects of AMF inoculation and P fertilizer on the biochemical traits of quinoa plants in intercropping with maize. Biochemical traits significantly affected the cropping ratio, AMF inoculation, and 50 kg P/ha fertilizer. Phosphorus fertilizer optimized the symbiotic relationship between plants and AMF and improved the inoculation rate of AMF. This is closely related to the function of improving plant biological traits. AMF can significantly increase rhizosphere microbial and phosphatase activity, thus, enabling plants to activate and absorb more significant amounts of phosphorus. Our study showed that AMF plays different roles in optimizing plant strategies for phosphorus uptake and the efficient utilization of phosphorus in intercropping of quinoa and maize. According to the results, intercropping culture and

the use of mycorrhiza and phosphorus can affect the content of biochemical compounds. Based on the results obtained in our study, in different climate conditions, intercropping quinoa with maize with inoculation of AMF as biofertilizer is recommended as a feasible and eco-friendly alternative strategy to improve the biochemical characteristics compared with the monoculture. It is suggested to investigate different AM species in the intercropping system of quinoa/ maize and other phosphorus concentrations. Also, the contents and compositions of other biochemical traits should be investigated.

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