Research

Fertilizer management strategies for improved quality and yield in winter wheat

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Abstract
In order to study the effect of different soil amended and biological fertilizers on the accumulation of nitrogen and phosphorous in the wheat grain and some physiological and morphological characteristics of wheat, this experiment was performed as a split factorial arranged in randomized block design with 32 treatment and three replication for 2 years. The treatments included mycorrhiza inoculation as a main factor (without inoculation, bacterial inoculation with *Glomus. mosseae*, *Glomus. intraradices* and *G. mosseae* + *G. intraradices*), and sub factor was super absorbent polymer treatment (no application, 9 ton zeolite ha^{-1}, 3 kg stacosorb ha^{-1} and 9 ton zeolite ha^{-1} + 3 kg stacosorb ha^{-1}) and phosphorous in the form of nano chelated phosphorous (no application and application 200 mg L^{-1}). The use of *G. mosseae* + *G. intraradices* had a positive and significant effect on the biological and grain yield so that it caused an increase of 5.9% and 6.4%, respectively compared to the control. The results showed that zeolite + stacosorb resulted the most to grain yield (6903 kg ha^{-1}). The highest and lowest grain nitrogen content were related to the treatment of *G. mosseae* + *G. intraradices* inoculation and nano phosphorous, with 1.75% and 1.76%, respectively. Indeed, the results showed that the use of biological fertilizer and nano phosphorous together had better results than solo application, which could be helpful in attaining high grain yields while preventing excessive phosphorous chemical fertilization, reduce environmental pollution and moving towards sustainable agriculture.

Keywords Arbuscular mycorrhizal fungi · Nano particles · Photosynthesis pigments · Soil conditioners · Spike length · Sustainable agriculture

1 Introduction

Wheat (*Triticum aestivum* L.) is the main staple crop in the world because of its great components like carbohydrates, vitamins (especially B vitamins), gluten protein, and phytochemicals that are necessary for human health. Indeed, the exclusive characteristics of the gluten protein fraction allow the processing of wheat for bread, noodles, pastries, pasta and production of many ranges of functional ingredients. Currently, wheat production is not satisfied for human need and annual wheat grain yield needs to rise from the current level up to 1.6% [1]. Although, the optimum yield seems to have been increasing by new varieties and new management methods, but some field researches have documented that satisfactory and profitable quality and quantity of grain yield can be gained with suitable soil practices and availability of fertilizer such as nitrogen (N) and phosphorus (P) [2, 3]. Lack of sufficient plant nutrients in the root zoon not only decreases the production of the plant, but also decreases the nutritional quality of agricultural products and leads to various diseases and endangers public health [4, 5]. For this purpose, traditional

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chemical fertilizers are used as the easiest way to reached maximum yield, results to enhanced production costs and the destruction of soil, water and natural resources as well which is the most important agricultural concerns today. However, nano or bio fertilizers will not cause contamination in the soil compared to chemical fertilizers [6].

Foliar application of mineral nutrients is an effective method for reduction the natural resources from an excess of soil-applied nutrients particularly [7], when treated in the form of nanomaterials due to the smaller particle size (diameter and weight) compared to traditional ones, resulted to increased solubility, lower leaching and rapid absorption by plants in related to usual fertilizers. Nanoparticles are commonly less than 100 nm in size change physic-chemical properties of fertilizers. Therefore, nanofertilizers, especially for ions with low bioavailability in the soil, can be more effective than ordinary fertilizers to reduce the application and loss of mineral nutrients while improving crop growth [8]. Arbuscular mycorrhiza (AM) fungi (e.g., *Glomus mosseae* and *Glomus intraradices*), are well-known for advantageous effects on plant growth and crop production in exchange to carbon from host plants. AM has the capability to increase the rate of water and mineral nutrients uptake, enrich soil fertility, synthase of substances with auxin like activities which increased root system and solubilization of micronutrients (e.g., manganese, iron and zinc) and macronutrients (e.g., N and P). The researchers further documented that the AM can interplay with other soil microflora by supporting the growth of various biological systems, serving the best medium for microorganisms and mitigate biotic and abiotic stresses [9, 10]. AM improved the capacity of the root system by two ways, 1) increasing the root surface by extending external hyphae network for up taking minerals and/or by profusion of minerals transporters in symbiont membranes. 2) Enhance the absorbing of immobile phosphate ions in the soil by secreting phosphatases which hydrolyzes the organic phosphorous to minerals phosphorous [11].

Super absorbents can be considered a proper method to improve the sustainability of agricultural systems due to their high ion cation exchange capacity (CEC) and regulate nutrient availability. Zeolite and stacosorb as soil amended are known to lower the soil pH, increasing soil moisture conditions, ease the movement of elements to and from the mineral structure, improving ions sorption which can be released in a timely [12]. In this regard, these materials can be considered as a means of decreasing the use of chemical fertilizers while maintaining agricultural productivity. Zeolites and stacosorb made of an inorganic polymer built from crystalline aluminosilicates [$(AlO_4)^{5-}$ and $(SiO_4)^{4-}$] linked by oxygen atom [11]. When some of the Si_4^+ is replaced by Al_3^+ (in the silica framework), this makes negatively charged, which is facilitates exchanging cations, nutrient use efficiency and water availability. In any case, studies with stacosorbs are much less abundant than with zeolites, which enhance the worth of research and getting more data on this material, especially from field trials.

Therefore, in this study the effect of nano phosphorus (nano-P), arbuscular mycorrhizal and super absorbent on different parameters on wheat were evaluate. By doing this, we wanted to 1) clarify the role of *P* ion in yield components development and subsequent yield production, 2) quantify the *N* and *P* content in grain which is cooperated with soil nutrients availability and 3) determine the responses of flag leaf area (LA) and chlorophyll content to the nano-P, bio fertilizer, soil remediation and interaction between them that are associated with biological yield.

2 Materials and Methods

2.1 Study site

The study was carried out at one of the silt–clay field in Baye Kola Agricultural Research Station affiliate to Mazandaran province, Agriculture Research Center, Iran (53° 13′ E, 36° 41 ′ N, 4 m above sea level). With maximum (29.9 °C) and minimum (14.4 °C) average monthly temperature were happened in August and April, respectively (Fig. 1). The experiment had thirty-two treatments and replicated three times as a split factorial arranged in randomized block design for 2 years (2017–2019). For soil analyses, the samples (0–30 cm depth) were randomly take, were air-dried, ground to pass through a 2 mm sieve and analyzed for physicochemical properties. The experimental silt–clay soil possessed an EC of 0.8 dS m^{-1}, a pH of 7.7, and an organic carbon of 1.39%. Initial micronutrient including Cu, Mn, Zn and Fe values were 0.9, 76, 1.28, and 19.2 mg kg^{-1}, respectively. The available N, P, and K values were 0.115%, 9 kg ha^{-1}, and 353 kg ha^{-1}, respectively.

2.2 Experimental setup and growth conditions

The main factor included mycorrhiza inoculation (without inoculation, bacterial inoculation with *Glomus. mosseae*, *Glomus. intraradices* and *G. mosseae* + *G. intraradices*), and sub factor was super absorbent polymer treatment (no application, 9 ton zeolite ha^{-1}, 3 kg stacosorb ha^{-1} and 9 ton zeolite ha^{-1} + 3 kg stacosorb ha^{-1}) and phosphorous in the form of nano chelated phosphorous (no application and application 200 mg L^{-1}). The sowing of wheat (cv. Ehsan) was done in first week of October (in both years) using drill methods with inter-row spacing of 4 cm, an intra-row distance of 20 cm (with six sowing rows) and a plot size of 1 m × 5 m (5 m^2). The irrigation schedule was applied as needed during the growing season.

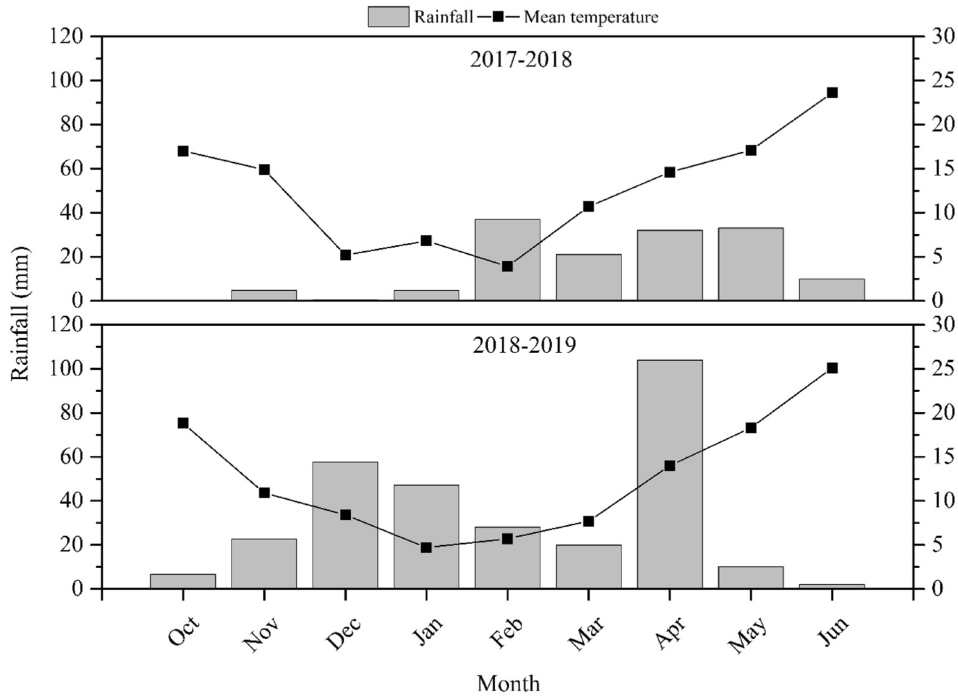

Fig. 1 Average monthly air temperature and rainfall during the period of October–June in 2017–2018 and 2018–2019

During planting, the recommended dose of 180 kg of N h^{-1} was applied as a base through urea. The first nano-P foliar application (an approximate of 400 L ha^{-1} of mixtures was applied) was laid out at the beginning of tillering stage at the morning. Second application were given 14 days later with the help of knapsack sprayer with flat fan nozzle. Distilled water was sprayed on the control treatments for uniformity.

The super absorbent polymer (Zeolite and stacosorb) were given in selected treatments, before sowing and after preparation of the land. The mycorrhizal inoculum contained the propagules of two different species of AM fungi (*Glomus mosseae* or *Glomus intraradices*) and a sand acting as a carrier. The rate of the commercial product applied was 680 kg ha^{-1} $year^{-1}$. The super absorbent polymer and mycorrhizal fungi were applied in the two years (2017–2018), recommended by the producer.

2.3 Harvest and nutrient analysis

On the spike heading, the flag leaf samples were manually harvested and measured for flag leaf area (Delta-T area meter; Delta-T Devices Ltd., Cambridge, UK) and total chlorophyll content based on Arnon methods [13]. Also, number of days until heading (50% appearance) was counted and registered. In order to study yield and yield components (at the stage of grain maturity), plants were harvested, from the net plot area leaving the border rows, weighted and dried at 70 °C for 48 h and registered as biological yield (kg ha^{-1}). Number of tillers, stem length, spike length, grain number in spike, spikelet density and grain yield (kg ha^{-1}) were calculated counted manually. Plant height was measured from the first reaching node to the base to the tip of the uppermost spikelet including the awns then, spike length was subtracted from plant height to calculate stem length. The spikelet per spike were counted then divided by spike length to calculate spikelet density. The concentrations of nitrogen (N) and phosphorous (P) in grain were measured by standard macro-Kjeldahl procedure [14] and colorimetric method at wavelength of 450 nm, respectively.

2.4 Data and statistical analyses

Data were analyzed using the general linear model (GLM) procedure of the SAS program and the critical difference were examined between studied treatments by Less Significant Difference (LSD) test at ($P \leq 0.05$). XLSTAT software (2018 version) was used to perform principal component analysis and cluster analysis.

3 Results

The results of principal component analysis showed that among all the obtained components, the first and second components had eigenvalues higher than one and were selected as effective components (Fig. 2A). The first and second components had the highest relative variance among all components with 72.21 and 12.06%,

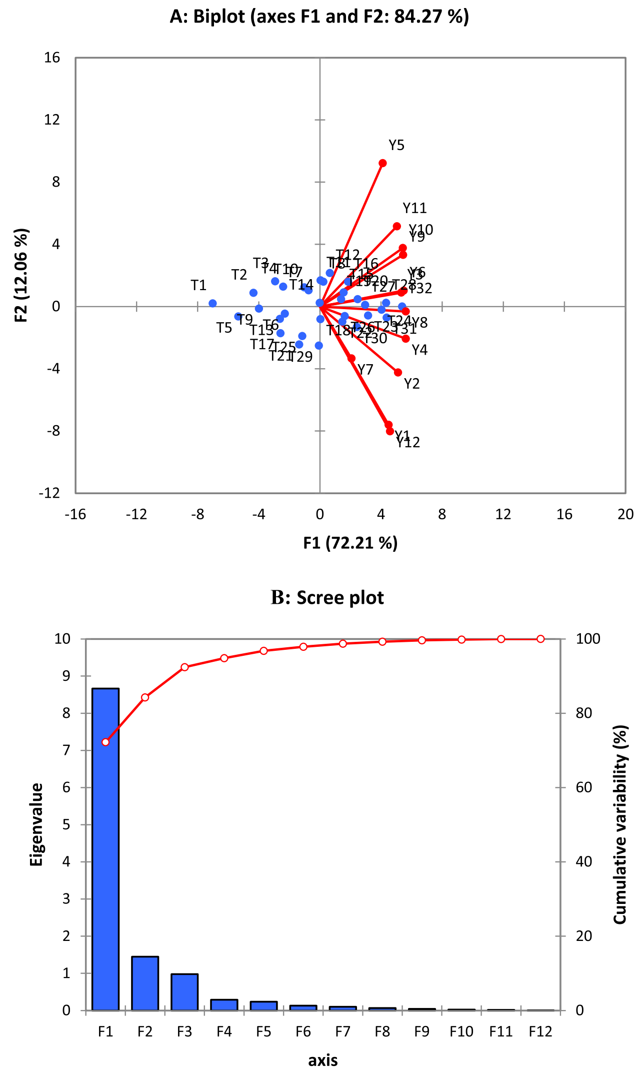

Fig. 2 The results of principal component analysis based on all ▸ studied traits. biplot based on first and second component **A** and scree plot derived from principal component analysis **B** the availability of the essential elements. Y_1: days to heading, Y_2: flag leaf area, Y_3: total chlorophyll content, Y_4: stem length, Y_5: number of tillers, Y_6: spike length, Y_7: spikelet density, Y_8: number of grain in tiller, Y_9: biological yield, Y_{10}: grain yield, Y_{11}: grain nitrogen content, Y_{12}: grain P content. T_1: no inoculation + no super absorbent + no nano phosphorous, T_2: no inoculation + no super absorbent + nano phosphorous application, T_3: no inoculation + zeolite + no nano phosphorous, T_4: no inoculation + zeolite + nano phosphorous application, T_5: no inoculation + stacosorb + no nano phosphorous, T_6: no inoculation + stacosorb + nano phosphorous application, T_7: no inoculation + zeolite and stacosorb + no nano phosphorous, T_8: no inoculation + zeolite and stacosorb + nano phosphorous application, T_9: *Glomus mosseae* + no super absorbent + no nano phosphorous, T_{10}: *Glomus mosseae* + no super absorbent + nano phosphorous application, T_{11}: *Glomus mosseae* + zeolite + no nano phosphorous, T_{12}: *Glomus mosseae* + zeolite + nano phosphorous application, T_{13}: *Glomus mosseae*+stacosorb + no nano phosphorous, T_{14}: *Glomus mosseae* + stacosorb + nano phosphorous application, T_{15}: *Glomus mosseae* + zeolite and stacosorb + no nano phosphorous, T_{16}: *Glomus mosseae* + zeolite and stacosorb + nano phosphorous application, T_{17}: *Glomus intraradices* + no super absorbent + no nano phosphorous;, T_{18}: *Glomus intraradices* + no super absorbent + nano phosphorous application, T_{19}: *Glomus intraradices* + zeolite + no nano phosphorous, T_{20}: *Glomus intraradices* + zeolite + nano phosphorous application, T_{21}: *Glomus intraradices* + stacosorb + no nano phosphorous, T_{22}: *Glomus intraradices* + stacosorb + nano phosphorous application, T_{23}: *Glomus intraradices* + zeolite and stacosorb + no nano phosphorous, T_{24}: *Glomus intraradices* + zeolite and stacosorb + nano phosphorous application, T_{25}: G. *mosseae* and *G. intraradices* + no super absorbent + no nano phosphorous, T_{26}: G. *mosseae* and *G. intraradices* + no super absorbent + nano phosphorous application, T_{27}: G. *mosseae* and *G. intraradices* + zeolite + no nano phosphorous, T_{28}: G. *mosseae* and *G. intraradices* + zeolite + nano phosphorous application, T_{29}: G. *mosseae* and *G. intraradices* + stacosorb + no nano phosphorous, T_{30}: G. *mosseae* and *G. intraradices* + stacosorb + nano- phosphorous application, T_{31}: G. *mosseae* and *G. intraradices* + zeolite and stacosorb + no nano phosphorous, T_{32}: G. *mosseae* and *G. intraradices* + zeolite and stacosorb + nano phosphorous application

respectively, and accounted for 84.27% of the total variance (Fig. 2B). The biplot obtained from the first and second components showed that the T11, T8, T12, T16, T15, T19, T20, T27, T28 and T32 treatments were placed in one group and they had a high correlation with total chlorophyll content, number of tillers, spike length, biological yield, grain yield and grain nitrogen traits. On the other hand, the T18, T26, T22, T30, T23, T24 and T31 treatments were placed in the same group due to the similarity in terms of the first and second components and showed a strong correlation with spikelet density, flag leaf area, stem length, spikelet density, number of grain in tillers and grain P content traits (Fig. 2B).

3.1 Days until heading

The number of days to heading was significantly affected by main effect of mycorrhiza, super absorbent, nano-P and interaction of mycorrhiza and nano-P which clearly noted in Table 1. The most umber of days to heading was recoded in all soil amended treatments except none application (zeolite, 180.9; stacosorb, 180.4 and zeolite + stacosorb, 180.7) (Table 2). Nonetheless, the foliar application of nano-P significantly amplified the number of days to heading in mycorrhiza inoculation as well as none inoculation. The highest number of days to heading (191.8) was recorded in plants exposed to 200 mg L^{-1} nano-P in mycorrhiza inoculation plants (*G. mosseae* + *G. intraradices*) while the lowest (169.01) was noted in none inoculated plants treated without nano-P. Although, there was no significant variance between application and without nano-P application but inoculated plants by *G. intraradices* had higher number of days

Table 1 Combined analysis of variance on some physiological and agronomic traits of wheat (*Triticum aestiuvm* L.) as affected by inoculation, super absorbent polymer and nano-phosphorous (Nano-P) foliar application treatments

Source of variation	df	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11	Y12
Year	1	218.5 ns	0.16 ns	177.6*	8.35 ns	0.97 ns	1.8 ns	0.0002 ns	31.08 ns	2046828 ns	4,900,906**	0.051 ns	0.001**
Block (Year)	4	401.4	0.069	5.8	1.73	0.83	0.47	0.033	24.8	786,229	147,504	0.016	0.000001
Mycorrhiza (M)	3	735.1*	25.7**	65.9**	161.5**	2.83 ns	12.3**	0.6 ns	184.7*	4,561,151**	2,485,473**	0.052**	0.0004*
Year × M	3	0.02 ns	0.11 ns	0.08 ns	0.014 ns	0.00075 ns	0.006 ns	0.00031 ns	0.015 ns	8458 ns	1512 ns	0.00057 ns	0.0000001 ns
M × Block (Year)	12	14.1	0.33	1.15	11.3	2.83	0.44	0.21	45.6	554,988	154,270	0.0055	0.00001
Super absorbent (S)	1	160.7**	6.57**	59.5**	280.3**	206.9**	19.8**	0.13 ns	539.01**	18,874,907**	8,572,806**	0.21**	0.00005**
Nano-P (n-P)	3	7698.4**	69.4**	77.05**	1572.08**	0.63 ns	46.1**	0.55 ns	1370.8**	19,706,138**	7,462,324**	0.57**	0.009**
M × S	3	30.1 ns	0.66*	0.44 ns	4.01 ns	1.37 ns	0.27 ns	0.06 ns	9.44 ns	123512 ns	42965 ns	0.0068 ns	0.000001 ns
M × n-P	3	147.8**	0.33 ns	8.59**	53.4**	0.43 ns	4.74**	0.029 ns	71.2*	2051364 ns	163632 ns	0.012 ns	0.00002**
S × n-P	9	8.81 ns	1.39**	1.83 ns	5.9 ns	1.36 ns	1.01 ns	0.15 ns	0.84 ns	885032 ns	90012 ns	0.011 ns	0.000003 ns
M × S × n-P	9	5.64 ns	0.38 ns	2.13 ns	2.03 ns	0.84 ns	0.28 ns	0.071 ns	2.9 ns	173944 ns	62426 ns	0.0068 ns	0.000002 ns
S × Year	1	0.003 ns	0.043 ns	0.071 ns	0.029 ns	0.022 ns	0.014 ns	0.0004 ns	0.056 ns	17936 ns	5221 ns	0.00078 ns	0.00 ns
n-P × Year	3	0.19 ns	0.29 ns	0.08 ns	0.15 ns	0.00002 ns	0.026 ns	0.001 ns	0.13 ns	9987 ns	4542 ns	0.000075 ns	0.000001 ns
M × S × Year	3	0.0017 ns	0.12 ns	0.00082 ns	0.00048 ns	0.001 ns	0.001 ns	0.00007 ns	0.001 ns	12719 ns	25.9 ns	0.0012 ns	0.0000001 ns
M × n-P × Year	3	0.0023 ns	0.17 ns	0.01 ns	0.0047 ns	0.001 ns	0.002 ns	0.0001 ns	0.0045 ns	17349 ns	100.05 ns	0.0019 ns	0.0000002 ns
S × n-P × Year	9	0.00078 ns	0.02 ns	0.003 ns	0.0018 ns	0.0007 ns	0.0017 ns	0.00016 ns	0.001 ns	16862 ns	54.47 ns	0.0011 ns	0.0000001 ns
M × S × n-P × Year	9	0.0013 ns	0.35 ns	0.0026 ns	0.0011 ns	0.0007 ns	0.0015 ns	0.0001 ns	0.0005 ns	10449 ns	37.88 ns	0.00098 ns	0.0000001 ns
Error	112	28.6	0.31	1.79	10.04	1.06	0.8	0.21	22.8	802,257	142,392	0.008	0.000004
CV (%)		2.9	6.05	4.78	2.51	20.1	6.97	11.3	9.02	8.19	5.82	5.19	3.30

Y_1 days to heading, Y_2 flag leaf area, Y_3 total chlorophyll content, Y_4 stem length, Y_5: number of tillers, Y_6: spike length, Y_7: spikelet density, Y_8: number of grain in tiller, Y_9: biological yield, Y_{10}: grain yield, Y_{11}: grain nitrogen content, Y_{12}: grain phosphorous content. **, * and ns indicate no significance and significance at *P* levels of 0.01 and 0.05, respectively

Table 2 Main effect comparison of super absorbent polymer on studied traits

Main effect of Super absorbent	Days until heading	Flag leaf area (cm)	Stem length (cmm)	No. of tillers	Spike length (cm)	Number of grain per spike	Biological yield (kg ha^{-1})	Grain yield (kg ha^{-1})	Grain nitrogen content (%)	Grain phosphorous content (%)
No application	177.1b	26.5c	122.6c	2.21d	12.01c	48.39c	10131c	5928d	1.58d	0.0615c
Zeolite	180.9a	27.9b	125.5b	5.09c	12.83b	52.64b	10745b	6384c	1.70c	0.0628b
Stacosorb	180.4a	28.6a	127.01a	6.31b	13.26a	54.58a	11307a	6686b	1.78b	0.0637ab
Zeolite + Stacosorb	180.7a	29.04a	128.2a	6.86a	13.46a	56.14a	11533a	6903a	1.83a	0.0638a

Each parameter with the same letter is not significantly different according to LSD test at the 5% level of probability.

to heading when treated by nano-P with respect to no application (Table 3).

3.2 Total chlorophyll content

There were observed significant variances in to total chlorophyll content due to the effects of mycorrhiza, super absorbent, nano-P and their interactions (Table 1). In case of interaction of mycorrhiza and super absorbent, the maximum total chlorophyll content (10.71 mg g^{-1} FW) was noted in plants inoculated *G. mosseae* + *G. intraradices* and stacosorb soil amended while the lowest was observed in none inoculated plants without soil amended application shown in Fig. 3. Overall, stacosorb in inoculated and none inoculated plants produced the highest total chlorophyll content with respect to treatments where zeolite was added (Fig. 4). Averaged by soil amended treatments, the results showed that nano-P application significantly enhanced total chlorophyll content as compared with no nano-P application treatment. In addition, the results revealed that the maximum total chlorophyll content was observed in nano-P application and zeolite + stacosorb treatment (10.35 mg g^{-1} FW) followed by stacosorb treatment (10.12 mg g^{-1} FW) which was in a same statistical level (Fig. 4). On the contrary, plants without soil amended and without nano-P application were the latest to produce total chlorophyll content (8.25 mg g^{-1} FW).

3.3 Flag leaf area

Year, mycorrhiza, super absorbent, nano-P and interaction of mycorrhiza and nano-P significantly affected the flag leaf area (Table 1). The highest flag leaf area (28.99 cm^2) was observed in second year (2020) which could be attributed to better growing conditions (Table 4). The flag leaf plant was increased with application of soil amended especially in wheat plants exposed to zeoilite + stacosorb (29.04) (Table 2). In fact, flag leaf plant increased in wheat under soil amended treatments performing better flag leaf area with respect to none application (26.5 cm^2).

3.4 Stem length

Mycorrhiza, super absorbent, nano-P and interaction of mycorrhiza and nano-P significantly effects the stem length of the plants as compared to untreated plants (Table 1). Zeolite + stacosorb application of 5 t.ha^{-1} for each (128.2 cm) had better stem length relative to 10 t.ha^{-1} of zeolite (125.5 cm) or stacosorb (127.01 cm) but no significant difference was recorded at zeolite + stacosorb application and stacosorb solo application (Table 2). In case of mycorrhiza and nano-P application (Table 3), the effect of nano-P on stem length in inoculated plants (*G.*

Table 3 Two-way interaction between mycorrhiza inoculation and nano- phosphorous (nano-P) application on studied traits

Mycorrhiza inoculation	Nano-phosphorous	Days until heading	Flag leaf area (cm)	Stem length (cm)	Spike length (cm)	Number of grain per spike	Grain phosphorous content (%)
No application	No application	169.01f	25.9d	119.7f	11.2e	46.6c	0.050f
	200 mg L^{-1} nano-P	177.1d	26.7c	121.7e	12.3d	50.4b	0.056e
Glomus mosseae	No application	173.9e	27.8b	124.07d	12.8c	49.8b	0.057e
	200 mg L^{-1} nano-P	173.9e	29.1a	126.4c	13.09bc	54.1a	0.059d
Glomus intraradices	No application	179.9d	27.1bc	127.5bc	13.05bc	54.4a	0.066c
	200 mg L^{-1} nano-P	188.01b	28.9a	128.9ab	13.4ab	56.6a	0.069b
Glomus mosseae + Glomus intraradices	No application	184.8c	29.2a	128.8ab	13.6a	55.01a	0.071a
	200 mg L^{-1} nano-P	191.8a	29.3a	129.6a	13.3abc	56.2a	0.072a

Each parameter with the same letter is not significantly different according to LSD test at the 5% level of probability.

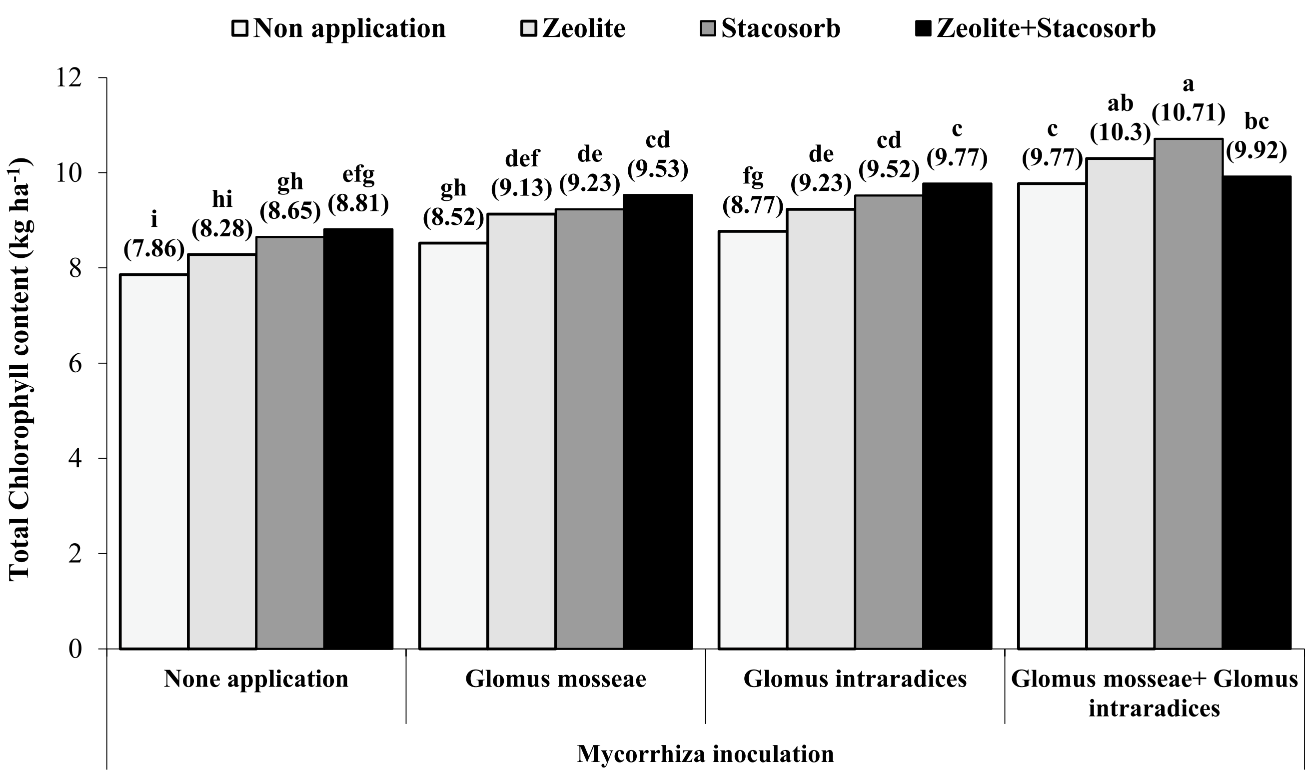

Fig. 3 Two-way interaction between mycorrhiza inoculation and super absorbent polymer application on total chlorophyll content. Means having similar letters have no significant difference at 5% probability level by LSD test

mosseae + *G. intraradices*) was not significant and they were in a same statistical level. Treatments without nano-P in uninoculated recorded lowest stem length. Maximum stem length (126.6 cm) was observed in *G. mosseae* + *G. intraradices* treatment and application of 200 mg L^{-1} nano-P followed by interaction of *G. mosseae* + *G. intraradices* and no nano-P treatment (128.8 cm) and *G. intraradices* inoculated plants and 200 mg L^{-1} nano-P application (128.9 cm) which were in a same statistical level (Table 3).

3.5 Number of tillers

As presented in Table 1, the number of tillers was significantly affected by super absorbent. The greatest number of tillers (6.86) was recorded in zeolite + stacosorb treatment while the minimum was observed (2.21) in plants treated without soil amended. In fact, the number of tillers was increased with application of soil amended especially in wheat plants exposed to zeolite + stacosorb treatment.

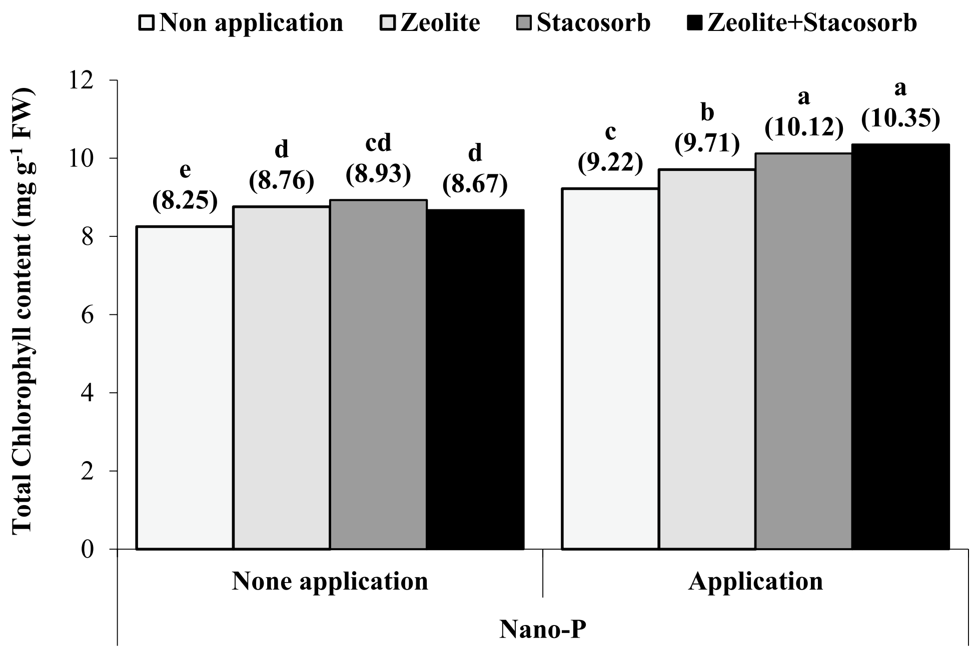

Fig. 4 Two-way interaction between super absorbent polymer and nano-phosphorous (nano-P) application on total chlorophyll content. Means having similar letters have no significant difference at 5% probability level by LSD test

Table 4 Main effect comparison of year on studied traits

Year	Flag leaf area	Grain yield	Grain phosphorous content
	(cm)	(kg ha^{-1})	(%)
2019	27.07b	6316b	0.060b
2020	28.99a	6635a	0.065a

Each parameter with the same letter is not significantly different according to LSD test at the 5% level of probability

3.6 Spike length

Results indicated that mycorrhiza, super absorbent, nano-P and interaction of mycorrhiza and nano-P had a significant effect on spike length (Table 1). Compared to control, when the plant treated by soil amended (Table 2), there appeared to be an enhance in spike length, while there was no significant variation in spike length exposed to stacosorb (13.26 cm) and zeolite + stacosorb (13.46 cm). the interaction between the mycorrhiza inoculation and nano-P application was also significant with regardless to spike that was high plants exposed to mycorrhiza and nano-P application (Table 3). The lowest was recorded for control plants (11.2 cm) while the highest one belongs to *G. mosseae* + *G. intraradices* in both with and without nano-P application (13.6 cm and 13.3 cm, respectively). Plant inoculating by *G. mosseae* were more affected by nano-P rather than plants inoculating by *G. intraradices* or *G. mosseae* + *G. intraradices* (Table 3) and there appeared to be improvement in spike length.

3.7 Number of grain per spike

Similar results as for spike length, occurred in number of grain p the availability of the essential elements per spike (Table 2). Application of super absorbent increased number of grain per spike. High number of grain in spike (56.14) was recorded in plants treated to zeolite + stacosorb, while the lowest (48.39) noted in plants treated without super absorbent (Table 2). Similarly, in case of interaction, of mycorrhiza inoculation and nano-P application, the maximum number of grain per spike (56.2) was achieved in *G. mosseae* + *G. intraradices* inoculation plants treated by 200 mg L^{-1} nano-P (Table 3). Averaged by nano-P application, there was no significant difference between *G. intraradices* and *G. mosseae* + *G. intraradices*.

3.8 Biological yield

The results of biological yield of wheat affected by treatment are presented in Table 1. The results showed that biological yield significantly affected by main effect of mycorrhiza, super absorbent and nano-P. The wheat plants produced the least biological yield (10,131 kg ha^{-1}) when planted without super absorbent (Table 2). Contrary, the high biological yield belongs to the plants treated by stacosorb treatments (11,533 kg ha^{-1}). Regarding biological yield affected by mycorrhiza inoculation (Table 5), inoculated plants by *G. mosseae* + *G. intraradices* produced the highest biological yield (11,275 kg ha^{-1}). Moreover, no inoculated plants produced the least weight of biological yield (10,605 kg ha^{-1}) and the results were not significantly differed from that was observed for *G. mosseae* treatments

Table 5 Main effect comparison of mycorrhiza inoculation and nano-phosphorous (nano-P) on studied traits

Main effect of mycorrhiza inoculation	Biological yield (kg ha^{-1})	Grain yield (kg ha^{-1})	Grain nitrogen content (%)
No application	10605b	6207b	1.68c
Glomus mosseae	10744b	6369b	1.72b
Glomus intraradices	11091a	6692a	1.74ab
Glomus mosseae + Glomus intraradices	11275a	6633a	1.75a
Main effect of nano-phosphorous			
No application	10608b	6278b	1.69b
200 mg L^{-1} nano-P	11249a	6672a	1.76a

Each parameter with the same letter is not significantly different according to LSD test at the 5% level of probability

(10,744 kg ha^{-1}). Biological yield however, increased in wheat under nano-P treatments (Table 5). In this regard plants treated by nano-P treatments had the higher biological (11,249 kg ha^{-1}) that was increased by 5.69% with respect to untreated plants.

3.9 Grain yield

The grain yield (kg ha^{-1}) was significantly differed due to studied years, mycorrhiza, super absorbent and nano-P application (Table 1). Grain yield significantly increased in second year (with an average of 6635 kg ha^{-1}) which was higher that first year by 4.8% (Table 4). In case of inoculation treatment (Table 5), untreated plants had the lowest grain yield (6207 kg ha^{-1}), but inoculation of plant by *G. intraradices* or *G. mosseae + G. intraradices* treatment enhanced grain yield production. Hence, there was no significant variation between no-application (6207 kg ha^{-1}) and *G. mosseae* (6369 kg ha^{-1}) as well as *G. intraradices* (6692 kg ha^{-1}) or *G. mosseae + G. intraradices* (6633 kg ha^{-1}) (Table 5). Similar to biological yield, there was a significant enhancement in grain yield (Table 5) and nano-P treatment (6672 kg ha^{-1}) enhanced grain yield production by 5.9% rather than no application (6278 kg ha^{-1}).

3.9.1 Grain nitrogen content

Main effect of mycorrhiza, super absorbent and nano-P application significantly affected the grain nitrogen content (Table 1) as compared to no application treatments. Zeolite + stacosorb treatments had the highest grain nitrogen content (1.83%) in both years, being 13.6%, 7.1% and 2.7% higher than no application, zeolite and stacosorb treatment, respectively (Table 2). Regardless of super absorbent and nano-P treatments, inoculation of plants by *G. mosseae + G. intraradices* enhanced the grain nitrogen content by 4%, 1.71% and 0.57% relative to the no inoculated, inoculated with *G. mosseae* and *G. intraradices* treatments, respectively (Table 5). However, there was no significant variation between *G. intraradices* and *G. mosseae + G. intraradices* treatments. Grain nitrogen content however, increased under nano-P treatments (Table 5). In this regard, plant treated by nano-P had the higher grain nitrogen content (1.76%) that was increased by 3.9% with respected to untreated plants.

3.9.2 Grain P content

In line with grain yield, second year (2020) produced more grain P content (by 8.3%) than first year (Table 4). Mycorrhiza and nano-P application increased grain P content in both years (Table 3). Averaged by nano-P application, the highest grain P content was observed in *G. mosseae + G. intraradices* with application and no application of nano-P (0.071% and 0.072%, respectively) which were in a same statistical level (Table 3). However, in each level of mycorrhiza inoculation, nano-P treatments had higher grain P content. Moreover, increased grain P content induced by super absorbent which were greater than no super absorbent treatments (Table 2). Zeolite + stacosorb treatments had the highest (0.0638%) grain P content being 3.6%, 1.5% and 0.15% higher than no super absorbent, zeolite and stacosorb treatments, respectively (Table 2). The results were not significantly differed from that observed for stacosorb and zeolite + stacosorb as well as zeolite and stacosorb treatments (Table 2).

4 Discussion

The soil of the experiment site was poor in available P and N, thus the response of each of the plant growth parameters (i.e. flag leaf area, total chlorophyll content, stem length, grain nutrients content, yield and yield components) to the various treatments were significant and nearly identical exhibiting improves due to nano-P, super absorbent polymer and bio fertilization.

The results of present study showed that combinations of mycorrhiza and nano-P had more beneficial effects on days until heading than solo application (Table 3). Improvement of plant growth period by mycorrhiza, particularly in low P soils is largely attributed to extraradical hyphae making to available inaccessible water and nutrient to the root system. It should be mentioned that the increase growth periods and accumulation of small increases in photosynthesis rate and higher leaf area, might resulted to greater biological and economical yield [15]. Treatments with stacosorb + zeolite significantly enhanced days until heading compared to none treatments (Table 2), because super absorbent has the capacity to reduce nitrate and ammonium from leaching and improved P, K and Ca absorption in the soil and release them slowly [16]. By this technique stacosorb and/or zeolite play an important role in establishment and also enhances the growth of wheat.

The positive effects of zeolite treatment on total chlorophyll content is considered as the benefits of the super absorbent addition which was greater with nano-P treatment [17]. However, without nano-P fertilizers, there were lower, albeit still positive leaf chlorophyll responses to soil amended application (Fig. 4). These smaller reactions may be attributed to the insufficient value of P in root zoon, which do not allowed plants to process their physiological metabolites and nutrients uptake [18]. It should be mentioned that P had little effect on tap root extension and branching of primary laterals initiated suggest that root systems growing in optimum fertility would be more branched [19]. Increase in chlorophyll content with nano-P application might be explained by correlation and complex formations between Ribulose 1,5-bisphosphate (RuBP) carboxylase/oxygenase and HPO_4^{2-} in plant as proposed by Siebers et al. [20] in rice plants, means that P elements might be one of the primary factors for chlorophyll biosynthesis pathway. Also, P being a component of the protoplasm membrane, involved in a number of proteins, all nucleic acids and nucleotides, as well as main key in controlling the enzyme reactions and the regulation of metabolic pathways therefore, helps in generation of chlorophyll, translocation of photosynthates and in turn increases sink capacity and induced growth improvement in wheat [3]. It has been suggested by many researchers that the biosynthesis of the pigment molecules was dependent on the availability of P [21]. It could be concluded that at a low level of P, the rate of photophosphorylation and transport of electrons in the photosynthetic electron transport chain were decreased.

The results of present research confirmed our hypothesis that stacosorb and zeolite soil-amended co-addition with mycorrhizal colonization promotes leaf chlorophyll content. It has been reported that suitable use of bio fertilizers caused improvement in physic-chemical soil properties, soil fertility and plant performance through beneficial effects on the availability of the essential elements [22], particularly when the soil situations are improved by super absorbent materials due to improved nutrient and water uptake through the host plant. Zeolite as soil amended improves useful microorganisms' activities in root zone, increase bacteria survival that stimulating plant growth and increase soil enzyme activities such as the phosphatase and catalase [23]. Furthermore, using mycorrhiza is a proper method to increase soil fertility through providing of required mineral nutrients via two mechanisms: 1) Due to the extension of the hyphae network and root surface. 2) The increased total canopy transpiration, which accelerates the mass flow of these nutrients through the soil to the roots of inoculated plants [24].

However, super absorbents such as stacosorb and zeolites contain Ca^{+2} and Mg^{+2} in their own silica framework, negative charges and high ion exchange capacity as a result of the isomorphic substitutions of Si^{4+} by Al^{3+} in the structure, which may also have helped to regulate the supply of Ca and Mg to plants. Possibly, stacosorb and/or zeolites provide the nutrients in rhizosphere, which facilitates access to the roots. Improvement nutrient status has also been resulted to higher photosynthesis and net assimilation production. In dead, stacosorb and/or zeolites application increase photosynthesis capability due to higher chlorophyll content and leaf area which lead to better plant growth and dry weight production. In accordance with previews findings [25], Ca plays a significant role in photosynthesis, regulation of photoprotection, stomatal and chloroplast movements, the pathways of photochemical reactions and regulating the photosynthetic enzyme activities biosynthesis of assimilate. On the other hand, as described by Keshavarz-Mirzamohammadi [26], Mg playing a key role in the structure and photochemical activities of photosystems, involved for chlorophyll synthesis, activation of RUBPcar/oxy enzymes, photosynthetic electron transport and facilitated assimilate transition from source to sink which affects photosynthetic capacity as well. Thus, mycorrhized plants with zeolite would have expended more chlorophyll in their leaves than the plants of the control treatment.

Inoculation plants with mycorrhiza increased significantly the flag leaf area and stem length during two growing seasons. This might be due to the concurrent enhances that happened in the higher root area via the extended mycorrhizal hyphae [22], beside of the increases that occurred in P availability by nano-P application. Another mechanism of mycorrhiza inoculation probably is utilization of N element which decreased mineral N loses from soil, resulted to higher growth parameters. These micro-organisms have great role in improving the symbiotic relationship between soil and plant and contribute effectively to increasing the absorption of essential plant nutrients. Also, they enhance the activity of other microorganisms present in the soil and spread fungal hyphae to improve the soil physical and chemical properties [4], which improved the growth and development of wheat. Mycorrhiza is responsible for optimistic adjustment in the promoter hormones (i.e., auxins, cytokinins and gibberellins). However, negative effects of mycorrhizal inoculation on plant growth were also reported [21, 27] due to competition for photosynthesis assimilate. Indeed, competition for photosynthetic energy between plant (host) and fungus is the main item responsible for negative or positive effect of inoculation on plant growth. In our study, combination of *G. mosseae* + *G. intraradices* and stacosorb + zeolite treatment decreased total chlorophyll content (Fig. 3). It was documented that the fungus receives between 15 and 30% of photosynthetic production [22]. The fungus extends their hyphal network to access water and nutrients. The development of the hyphae network and structures requires assimilate. Thus, inoculated plants would have spent more assimilate than un-inoculated plants, which probably was the main reason for their reduced chlorophyll content.

It worthy to mention that flag leaf area in inoculating plants with mycorrhiza species (individually or collectively) was higher in nano-P treatment rather than not application. Probably, the small size and large surface of nano particles account for increasing the P-utilization rather than ordinary fertilizers [28]. Nano technique provide the ease of penetration of nutrients into the walls of the plant cells, which facilitates access to vascular bundles [29]. Consequently, nano fertilizers provide sensible nutrition to various center reactions such as respiration, photosynthesis, cell division and elongation. Improvement nutrient status (due to mycorrhiza and nano fertilizer treatment) has also been resulted to higher photosynthesis and net assimilation production [22, 29]. In dead, combination of mycorrhiza and nano fertilizers increase photosynthesis capability due to higher chlorophyll content and leaf area which lead to better plant growth and dry weight production.

It was significant difference between application and no application of nano-P in term of biological yield (Table 5) which could be due to the fact that nano fertilizer has a higher chemical and physical activity than traditional ones because of the higher surface area of the nano particles which accelerate the enzymatic activities of the photosynthesis and other metabolic activities. Therefore, nano fertilizers directly reflected on increase the vegetative growth parameters such as stem length, leaves area and total dry weight. The current results showed that colonization can increase biological yield (Table 5) as well as yield components due to better plant N and P nutrition, especially after the plant was amended with nano-P.

The availability of P from bio fertilizers help the wheat plant to get higher leaf area and chlorophyll content, trap more photosynthetically active radiation which increased plant height, number of fertile tillers, number of grain per spike, grain weight, grain yield and biological yield. It has reported that emergence of cereals tillers enhanced positively with zeolite treatment, due to P and silicon availability which enhance the emergence of secondary tillers [16, 30]. The data of present research shows that zeolite as soil amended has a significant role in generating fertile wheat tillers (Table 2). It could be explained that enhancement of yield component values of wheat in our study is due to use of super absorbent and bio fertilizer which lead to better allocation assimilates to spikes and grain through increasing the cell division and better use of environmental conditions. In addition, soil treated by zeolite had higher soil water content due to its high porosity crystal structure which increase water holding capacity [11]. It has reported that zeolite treatments compared to the unamended, increased the yield of rice [16, 30] and this yield improvement was related to the increase in spikelets per spike, effective spikes, and 1000-grain weight.

Also, the increase in yield components could be attributed to improvement the absorption area through the activity of root hairs and fungal hyphaes deep permeation. Bio fertilizer produces the siderophores compound that works nutrients availability which increase their role in physiologic activity leads to raise assimilate production. In other hand, the ability of bio fertilizer in synthesis of phosphatase enzyme can be contribute in which positively improve yield and yield components of wheat. In addition, biofertilizers by nitrogen fixation can increased chlorophyll production and the leaves area which affected on the accumulation of photosynthesis activities and outputs leaded to increase the yield. In fact, there was a positive correlation between grain nitrogen content and grain yield (r = 0.885**) which approve our hypothesis (some data not shown). Usually, NH_4^+ (ammonium) hydrolysis and nitrified quickly before being uptake by plants. The high CEC level of super absorbent [12] indicating the beneficial effects of zeolite and stacosorb as a slow release fertilizer, to desorbed and uptake by plants to inhibit nitrification of NH_4^+ to NO_3^- which could be the reason why plant growth and grain yield was improved in super absorbent treatment.

In addition, there was a linear and significant correlation (r = 0.924**) between biological yield and grain yield (some data not shown). It was reported that the grain yield of wheat mainly was derived from shoot dry matter that was produced before anthesis and translocated to the grain during the grain filling stage [4]. This suggests that increasing total shoot biomass, is necessary for high yield production. It worthy to notice that higher grain yield in 2019 than 2020 were positively linked to the enhance in flag leaf area (r = 0.706**) and 1000-grain weight (r = 0.647**) (some data not shown).

The increased N levels of grain in super absorbent (stacosob + zeolite) treatment could be attributed to enhanced rates of mineralization and absorption of ammonium because of the high CEC and affinity of zeolite and stacosorb for ammonium. In term of NH_4^+ in particular, it is possible that the affinity of zeolite and stacosorb for NH_4^+ might have not only reduced nitrification but it also has facilitated slow release of NH_4^+ to prevent it from leaching. Furthermore, the presence of zeolite and stacosorb reduced microsite pH by preventing ureolytic activity of microorganisms to minimize ammonia volatilization from NH_4^+ [31]. Treatments with zeolite significantly increased soil nutrient levels compared to unamended soil, because when these materials are mixed with soil and chemical fertilizers, they help to retain plant nutrients in soil and, improving the long-term soil quality and as well reducing leaching of ammonium and nitrate in the soil [32–35].

The trend of P uptake was similar in inoculated plants regardless of the nano-P treatment. The content of P shows that the use of mycorrhiza resulted in significant improvement of P uptake for treatments with *G. mosseae* + *G. intraradices* whereas the no inoculated plants showed the lowest P content. The higher grain P content in the *G. mosseae* + *G. intraradices* and nano-P treatments was due to the higher solubility of the P compared non inoculated plant. Increase in soil pH due to zeolite application may have also contributed to these nutrients' availability.

5 Conclusions

Our results confirm that the application of nano-P according to crop demand and soil analyze status are appropriate without decreasing in grain yield. Further improvement in others mineral nutrients rates could be achieved, if the mineralization potential of AM treatments were taken into account. Further, this study shows that the super absorbent materials, either alone or through the use of other treatments could enhance yields and grain P and N content in this agricultural system. Therefore, AM are capable to increase crop plants production, particularly when the soil conditions are poor in terms of mineral nutrients and organic matter. And finally, improvement in grain yield (where soil amended and bio fertilizer treatment are used) mainly results from lengthening of the plants' lifetime, enlargement of the photosynthetic apparatus and yield components. These contradictory pieces of data suggest that mycorrhiza inoculation need to be carefully selected based on weather condition, soil and water ability status, and the availability of mineral nutrients. This study can provide guidance for the selection fertilizer management strategies of winter wheat in arid and semi arid conditions. Also, in future, studies may focus on investigating the effect of the super absorbance under drought stress conditions, because they help the soil to retain/hold moisture to prevent the plant from going to desiccation under drought condition.

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Declarations

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