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Study the Effect of Mycorrizal Fungi¸Chitosan and Cycocel on Agronomic Characteristics of Rainfed Chickpea

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ABSTRACT

This experiment was conducted as split-split plot in a randomized complete block design in two consecutive years (2016-2017) in rainfed conditions of Iran. The main plot consisted of mycorrhiza inoculations (uninoculation: control and inoculation) by *Glomus intraradices* and sub plot involved chitosan application (0, 0.5 and 1gr/lit) and sub-subplots were foliar application of cycocel (0, 0.5 and 1 gr/lit). The results showed that the number of infertile pods was influenced by chitosan, cycocel, interactions of mycorrhiza × cycocel and chitosan × cycocel in the first year. Also in the first year, the number of fertilized pods and seed yield was also affected by the interaction of mycorrhiza × cycocel and increased by mycorrhiza × cyocel (1gr/l) compared to the control by 23.35 and 24.05%, respectively. The protein percentage in the second year was also significantly affected by the interaction of chitosan × cycocel.

Key words: Chickpea, Chitosan, Cycocel, Mycorrhizal fungi, Protein.

INTRODUCTION

Under semi-arid condition of Iran, where the farmers have reached 53% of chickpea (*Cicer arietinum* L.) potential yield (Soltani *et al.* 2016), alleviating or compensating the adverse and negative effect of drought stress that takes place on the reproductive growth stage of rainfed legume, is very important (Janmohammadi *et al.* 2014). In order to improve drought resistance and enhance water use efficiency of plant, closure or reduce the size of stomata openings introduced by Zeng and Luo (2012). Among different anti-transpiration compounds, Chitosan is biodegradable and sub-product of the marine industry that introduced as a plant growth bio-stimulant and lowering agent of drought effect (Janmohammadi *et al.* 2014). The antitranspirant effect of chitosan and consequently decreasing water needed for plant reported by some researchers. Bitelli *et al.* (2001) was found that chitosan could closure of the plant's stomata, following that decrease water consumption by 26-43% without reduction in biomass. Also, increasing growth under water deficit conditions by chitosan was found by Mahdavi *et al.* (2011) and Abu-Muriefah (2013). Improvement of root system, leaf water content of seedlings and boosting the capability of water absorption by chitosan were found by Zeng and Luo (2012).

Another way to overcome the harmful effects of water deficit in plants has been announced by application of growth retarding chemicals, including Cycocel. Cycocel were pointed to prevent the excessive vegetative growth (Bhattarai 2017; Koutroubas *et al.* 2014) and improving more translocation of photo-assimilates, results in increasing the yield component (Bhattarai 2017), increasing seed yield (Kumari 2017; Koutroubas *et al.* 2014; Sarkar *et al.* 2006) and protein content (Bora and Sarma 2006).

In order to improve the absorption and use of water available in water shortage conditions, the use of *Arbuscular mycorrhizal* fungi as a promising option has been introduced.

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Mycorrhizal inoculation is known as the coexistence of fungi and root plant (Temegne *et al.* 2018) which has a lot of favorable effects for the coexistence plant, such as: increasing availability and absorption of nutrients from the soils (Njeru *et al.* 2014), resistant to drought condition due to enhancing water uptake (Eulenstein *et al.* 2017) and finally enhance crop yield (Hazzoumi *et al.* 2017; Eulenstein *et al.* 2017).

Keeping this view, we assumed that increasing the seed yield of chickpea in dry conditions could take place by increasing the absorption of water and nutrient by mycorrhizal inoculations, along with decreasing water loss by reducing the transpiration caused by chitosan, and ultimately reducing vegetative growth and enhancing reproductive growth of the chickpea plant by cycocel.

MATERIALS AND METHODS

This experiment was conducted as split-split plot in a randomized complete block design at Kamyaran in northwest of Iran (34°47′ 16″ N and 46°53′ 41″ E and 1425 m above sea level) during two consecutive years (2016-2017) in rainfed conditions.

Meteorological parameters in two growing seasons in the experimental field pick up from synoptic meteorological

site of Varmahang next to field study (Table 1). In this study the main plot consisted of mycorrhiza inoculations (uninoculation: control and inoculation) by *Glomus intraradices* and sub plot involved the chitosan application (0, 0.5 and 1gr/lit) and sub-subplots were foliar application of cycocel (0, 0.5 and 1gr/lit).

In order to prepare the seedbed, the first plow was carried out in autumn and the second plowing and the leveling was carried out in the spring. To determine the physical-chemical characteristics of the soil, several soil samples were randomly picked up at several points in the experimental farm. Based on soil analysis recommendation, 55 kg ha^{-1} urea, 160 kg ha^{-1} triple super phosphate and potassium sulfate fertilizers were added to the soil prior to planting.

The chickpea seed cultivar, ILC482, were planted manually with 10 cm distance and 5 cm depth in soil in the beginning of April.

Inoculant mycorrhiza (*Glomus intraradices*) were prepared by Organic-Iran Company representative of Utapan-Canada and placed in the sowing furrows, just before planting in mycorrhiza treatment plots, then a thin layer of soil was poured on them and the seeds were planted in the soil. Cycocel and chitosan were prepared by Sigma-Aldrich Company and they were sprayed onto the plant before flowering initiation stage at, 50 days after planting at intervals of one day. The spray application was carried out by hand sprayer in a cool hour of the day (17-20 h) and the spraying operation continued until the drip of soluble droplets began on the plant's air organs.

Weed control was carried out by hand several times during the growing season. For determining yield attributes and seed yield, at physiological ripening stage in the middle of July, a space equivalent to one square meter of the middle lines of each experimental plot was taken. Total biomass were placed in sunlight for 3 days to achievement seed dry weight. 5 single chickpea plant samples were selected to determine yield attributes characters. The amount of seed protein was achieved according to the Kejeldal method.

Finally, the combined Anova analysis of the two year data was carried out by $SAS_{9.4}$ software and the mean compared with the Duncan test at 5% probability level.

RESULTS AND DISCUSSION

Yield attributes

The fertilized pods increased by mycorrhiza, chitosan and cycocel by 11.93, 7.97 and 10.59%, respectively (Table 2). Since the number of fertilized pods in the chickpea plant is the most important determinant component of seed yield, its increase is very important. According to Fig 1, the interaction of mycorrhiza × cycocel has a significant difference and the maximum fertile pods (by 23.35%) has been obtained in the mycorrhizal and cycocel at 1gr/l. It seems that the synergistic effect of mycorrhiza has increased the number of pods by increasing the absorption of water and nutrients, as well as decreasing vegetative growth in order to increase reproductive growth by cycocel. Njeru *et al.* (2014) found improving nitrogen and phosphorus absorption in all maize genotypes with mycorrhizal colonization. Pourmohammad *et al.* (2014) revealed that cycocel increased the branch numbers, 1000 seed weight and seed yield. In another study, Sarkar *et al.* (2006)

Table 1: Meteorological parameter during two consecutive years (data from Varmahang Synoptic Meteorology station).

Parameters / Month	April 2016 (17)	May 2016 (17)	June 2016 (17)	July 2016 (17)
Monthly rainfall (mm)	73.7 (136.1)	6.2 (33.9)	0.4 (0.0)	0.0 (0.0)
Average temperature (°C)	10.2 (11.1)	16.6 (16.5)	23.1 (19.8)	28 (27)
Average humidity (%)	59.6 (63)	38.8 (52.3)	29.6 (36.5)	22.7 (25)
Rainy day numbers	9 (18)	5 (12)	2 (0)	0 (0)
Monthly sunshine hours	273 (141.3)	319.2 (303.6)	351.4 (403)	350.6 (359.6)
Monthly evaporation pan (mm)	109.6 (86)	237 (194.3)	310.7 (255.7)	399.3 (338.6)

Table 2: Mean comparison of mycorrhiza, chitosan and cycocel effects on rainfed chickpea.

Treatment\ characters	Fertile pod		Infertile pod		100S weight (gr)		Seed yield (kg/ha)		Protein (%)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Mycorrhiza										
Control	24.64^{a}	21.43^{a}	0.618^{a}	0.763^{a}	25.13^{a}	24.75^{a}	1734.6^{a}	1802.2^{a}	22.14^{a}	23.59^{a}
Inoculation	27.58^{a}	24.26^{a}	0.607^{a}	0.837^{a}	25.13^{a}	25.07^{a}	1910^{a}	2284.3^{a}	22.31^{a}	23.19^{a}
Chitosan (gr/l)										
0	24.94^{a}	24.03^{a}	0.5^{b}	0.72^{a}	25.4^{a}	24.97^{a}	1724.4^{a}	2048.6^{a}	22.36^{a}	23.46^{a}
0.5	26.93^{a}	22^{a}	0.78^{a}	0.74^{a}	25.08^{a}	24.82^{a}	1897.4^{a}	2065.6^{a}	22.25^{a}	23.32^{a}
1	26.46^{a}	22.51^{a}	0.55^{ab}	0.93^{a}	24.91^{a}	24.95^{a}	1845.1^{a}	2015.6^{a}	22.07^{a}	23.39^{a}
Cycocel (gr/l)										
0	24.53^{a}	24.03^{a}	0.72^{a}	0.72^{a}	25.48^{a}	24.88^{a}	1704.4^{a}	2005.3^{a}	22.13^{a}	23.35^{a}
0.5	27.13^{a}	21.7^{a}	0.64^{a}	0.88^{a}	24.83^{a}	24.96^{a}	1895.4^{a}	2048.6^{a}	22.17^{a}	23.57^{a}
1	26.67^{a}	22.81^{a}	0.46^{b}	0.78^{a}	25.9^{a}	24.08^{a}	1867.2^{a}	2075.9^{a}	22.38^{a}	23.25^{a}

Mean with the common letters in each column have not significant differences at 0.05 probability.

declared that cycocel enhanced number of capsules, number of seeds and seed weight in sesame plant. Also, enhancing the pod numbers of pea plant by cycocel was observed by Bora and Sarma (2006).

In this experiment, the number of infertile pods was also increased under the influence of chitosan, so that there was a significant difference in treatments with 0.5g/l in compared to other treatments. Chitosan application seems to have caused a significant increase in the number of pods, which some of them has not been formed and aborted due to the shorter vegetative season as well as the limited availability of water for such pods.

In addition to increasing the number of fertilized pods, the use of cycocel reduced the infertile pods. Possibly, the role of cycocel in reducing vegetative growth and promoting reproductive growth has probably caused this.

As shown in Fig 2, the maximum number of infertile pods was obtained in the control and the lowest in the cycocel at 1g/l. Also in Fig 3, the maximum infertile pod was obtained in chitosan at 0.5gr/l and the least in cycocel at 1gr/l. Considering the greater usefulness of the use of chitosan in the reproductive stage on the number of pods indicated by Jonmohammadi *et al.* (2014), it was probable that more pods would be fertilized, if multiple foliar application of chitosan was used in this experiment. On the other hand, the limitation of the nutritional elements may cause the pods to be not filled, as announced by Boonlertnirun *et al.* (2012) who stated that the maximum numbers of panicul, tiller per plant and grain yield in rice were achieved by chitosan in combination with chemical fertilizer. They found that the positive effects of chitosan alone, were scant on growth, yield and yield component.

Janmohammadi *et al.* (2014) concluded that chitosan could alleviate the adverse effect of unfavorable condition, such as terminal drought that takes place at the reproductive stage and consequently enhanced pods formation through partitioning of assimilates to reproductive organs. Mondal *et al.* (2013) concluded that the maximum yield obtained by chitosan at 50 ppm was due to increased production of pods per plant as 25-27%. However, all yield attributes lowered down at 100 ppm chitosan indicating its toxic effect at higher concentration.

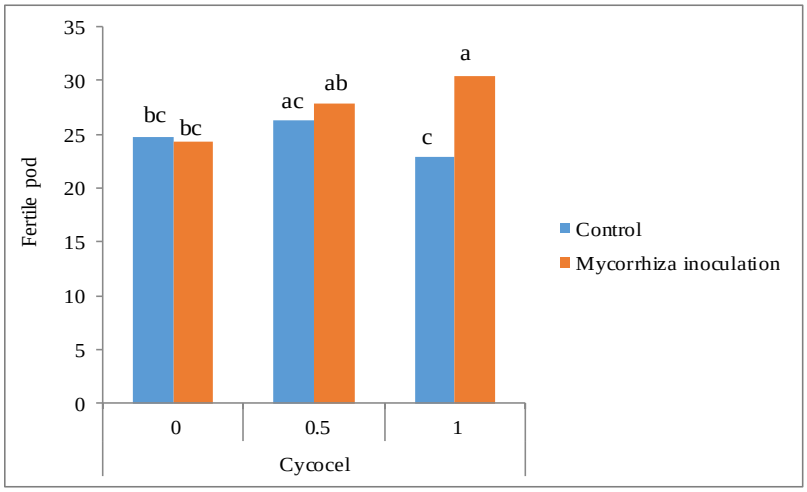


Fig 1: The fertile pod numbers influenced by mycorrhiza and cycocel.

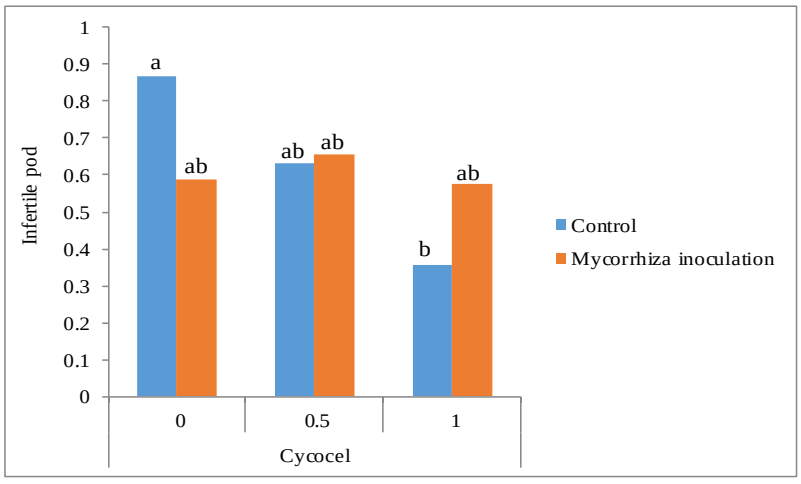


Fig 2: The infertile pod numbers influenced by mycorrhiza and cycocel.

The increase in bean yield due to chitosan may be due to its effects in stimulating physiological processes, improving vegetative growth, followed by active translocation of photoassimilates from source to sink tissues (Abu-Muriefah 2013).

Seed yield

The seed yield had a significant difference at the 5% level, under the influence of mycorrhizal × cycocel interaction (Table 2). As seen in Fig 4, the maximum seed yield was obtained in mycorrhizal × cycocel (1gr/l), which was superior to 24.05% in control. Based on the previous results of this experiment, the number of fertile pods increased by 23.35% in this treatment and since the number of pods is the most important component of the yield of chickpea, the occurrence of such a result seems logical. Özveren Yücel *et al.* (2006) studied 15 chickpea genotypes for 2 years in Turkey and found a positive and significant correlation between seed yield and fertile pod numbers (88.6%).

Increasing the seed yield in this experiment can be associated with increased absorption of water and nutrients by mycorrhiza as shown by other researchers.

Hazzoumi *et al.* (2017) declared that mycorrhizal counteracts the water deficit in stressed plants and increases the seed yield. Farzaneh *et al.* (2011) also demonstrated the increasing nutrient uptake, such as P, Mn, K, Cu and Fe in chickpea inoculated with mycorrhiza. On the other hand, the yield increases in this experiment can be related to increasing the activity of nitrogen fixation nodes on chickpea roots under dry conditions by inoculation with mycorrhiza. Erman *et al.* (2011) reported that mycorrhiza could increase the root colonization and nodulation, the phosphorus content of the seed and shoot and finally seed yield.

The positive efficacy of cycocel on the seed yield in this experiment was mainly due to the increase in the number of fertilized pods. Improving rapeseed yield by cycocel were found by Pourmohammad *et al.* (2014) who declared that cycocel improved the cytokinin translocation from roots to shoots and development of vegetative above ground organs and finally increased the seed yield. In another study increasing number of capsules, number of seeds, seed weight and also improving photosynthetic activities in sesame by cycocel application were reported by Sarkar *et al.* (2006) who found that cycocel could counteract 28 to 37% of yield loss under water stress condition. Bora and Sarma (2006) found that cycocel increased the numbers of branches, pod per plant, chlorophyll and seed yield. In similar study, increasing yield of pearl millet under moisture stress by cycocel, attributed to increasing root system and water

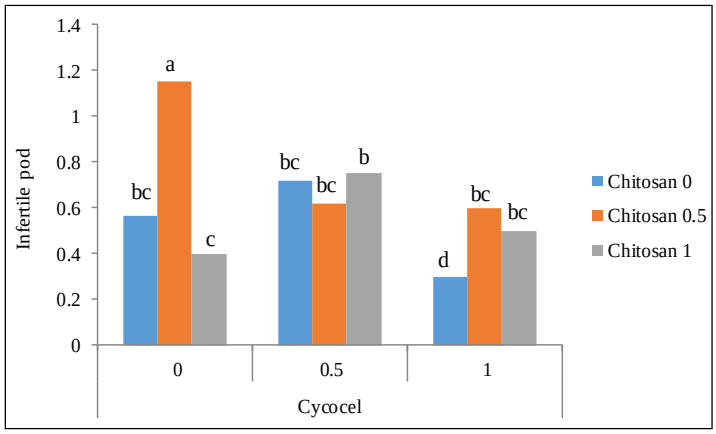


Fig 3: The infertile pod numbers influenced by chitosan and cycocel.

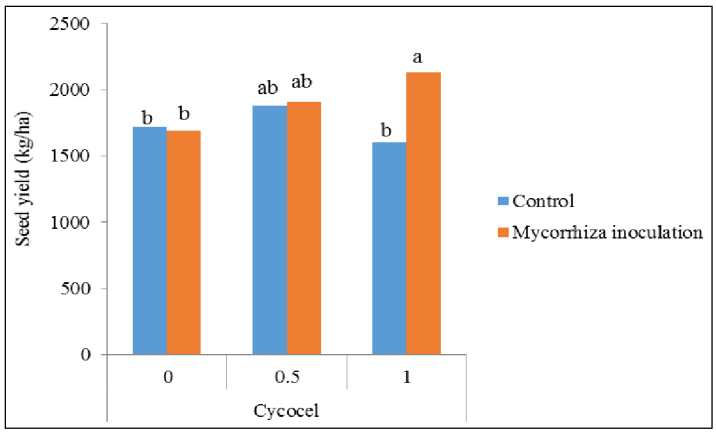


Fig 4: The seed yield influenced by mycorrhiza and cycocel.

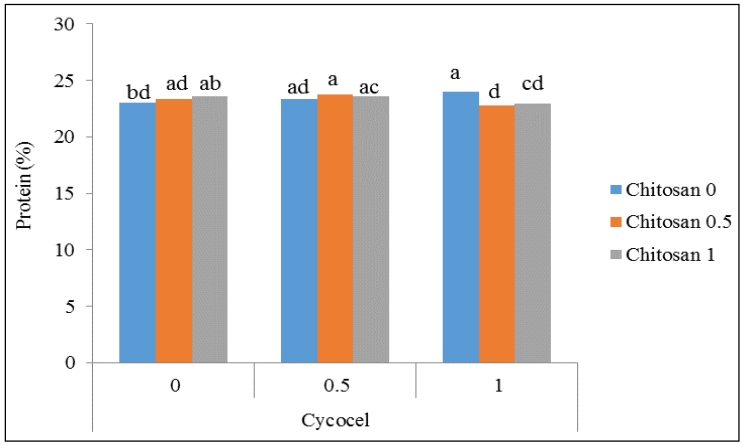


Fig 5: The seed protein influenced by chitosan and cycocel.

availability, increasing leaf area and therefore higher rate of light interception and photosynthesis and final results in higher yield (Kumari 2017).

Seed protein

The percentage of seed protein showed a significant difference at the 1% by the interaction of chitosan × cycocel in the second year. By comparing the means in Fig 5, it was observed that the maximum protein was achieved by 1g/l cycocel and also by 0.5g/l chitosan and cycocel. Soluble sugars and proline known as a popular compound in osmotic adjustment they can ameliorative effect on membranes in stress condition and save protein and enzyme activities (Karimi *et al.* 2012). The ameliorating effect of chitosan in reducing the undesirable effects of water stress conditions on dry matter and oil yield related to excitation of osmotic adjustment such as proline accumulation and reduction of lipid peroxidase level, which increased the integrity of cell membranes (Emami Bistgani *et al.* 2017).

Since the accumulation of various elements, including nitrogen, is one of the main elements in the protein formation in the plant, it appears that such an outcome appears logical, based on studies that the use of chitosan and cycocel increases the absorption of elements, especially nitrogen. Van *et al.* (2013) announced the growth intensification in coffee seedlings by chitosan which was attributed to increasing the chlorophyll content (30-50%), uptake of necessary nutrients, contains nitrogen (9.8-27.4%), phosphors (17.3-30.4%) and potassium (30-45%). Increasing seed quality of the bean by chitosan at 200mg/l under stressed or non-stressed conditions demonstrated by Abu-Muriefah (2013) who found increasing uptake of nutrient elements, protein and carbohydrate contents of the seeds in response to chitosan application. The application of low chitosan concentrations on safflower improved the soluble protein concentration (Mahdavi *et al.* 2011).

CONCLUSION

Based on the findings of this research, the agronomic traits such as fertile and infertile pods, seed yield and protein percentage were affected by treatments. As the most important traits, fertile pod numbers and seed yield in mycorrhiza inoculation × chitosan (1gr/l) increased by 23.35 and 24.05%, respectively in the first year study. Also the maximum seed protein content was obtained in 1gr/l cycocel and 0.5gr/l chitosan and cycocel treatments as increased by 1 and 0.73%, respectively in the second year of study. It seems that in this experiment, reducing the amount of rainfall and reducing the percentage of humidity in the first year with increasing air temperature, increasing the number of sunny hours and increasing evaporation caused significant effects of experimental treatments. These effects were not apparent in the second year of the experiment due to increased rainfall and the change of other meteorological parameters that reduced evaporation and transpiration.

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