Original Research Article

Natural Mycorrhization, Mineral Uptake, Total Polyphenols and Total Flavonoids of Oat as Affected by Tillage Practices under Rainfed Conditions

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ABSTRACT

Comment [S2]: The Abstract should be modified to reperesent a ''Read Alone Summary'' of the investigation. The Abstract does not interprete the research findings with definite data.

Aims: As conservation agriculture have been proposed as an option to limit conventional agriculture impact and to ensure sustainability and food security. This study examined the effect of conventional tillage (CT) and no tillage (NT) on mycorrhization rate, mineral elements uptake, total phenolic content (TPC) and total flavonoid content (TFC) of oat.e
Study design: Split-plot design was applied for this study.
Place and Duration of Study: The experiment was conducted at the referential farm for direct drilling (Krib, Siliana) situated in northwestern Tunisia during 2015/2016 cultivation year.
Methodology: Mycorrhization rate (MR), mineral elements uptake, total phenolic content (TPC) and total flavonoid content (TFC) of oat were studied as affected by conventional tillage (CT) and no tillage (NT) during tillering stage.
Results: The results showed that tillage practices (T) had no significant effect on mycorrhization rate, mineral uptake, total phenolic content and total flavonoids content under rained conditions. Even if NT had no significant effect on MR, higher rates were noted for NT compared to CT.
Conclusion: This study extends our knowledge on oat mycorrhization, mineral elements uptake, TPC and TFC as affected by tillage practices to advance results helping decision makers for no tillage adoption upscaling in Tunisia under rainfed conditions.

Keywords: Tillage practices, oat, mycorrhization rate, mineral elements, TPC, TFC.

1. INTRODUCTION

Cultivated for over 5000 years, Oat (*Avena sativa* L.) is the fourth most important crop worldwide and a significant multi-purpose cereal crop grown for grain, feed, fodder and straw. This crop is one of cultivated cereal in Tunisia, Africa and World occupying respectively 3541 ha, 140927 ha and 9418493 ha and producing 1319 tonnes, 177921 tonnes and 23104147 tonnes [1]. Generally, oats are mostly cultivated in cool moist climates and they present sensitivity to dry weather and hot between head emergence and maturity. Consequently, world oat production is generally condensed in latitudes 35 and 65° N. Traditionally farmers devote for oats cropping areas that are unappreciated for wheat, maize or barley and they maintain these cultivated area stables over the years [2]. For the reason

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of its good adaptation to a large range of soil types, oats can show better production than other small-grain cereals. Then, an increasing interest to amplify oat cultivation to southern countries and subtropical areas has been reported. Expansion of oats cultivation in these rainfed rained Mediterranean environments will possibly encounter water constraints as well as disease attacks [3-4]. In Tunisia, oat is essentially cultivated in regions with arid and semiarid climates that present annually less than 400 mm of rainfall.

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Oat contains valuable nutrients such as proteins, fibers, minerals, vitamins, and phytochemicals. Antioxidants and phytochemicals react against cardiovascular disease [5], diabetes [6], skin disorders [7] and several types of cancer [8]. Furthermore, biochemical analysis revealed that oat bran contains carbohydrates (67.9%), proteins (17.1%), fat (8.6%), β-glucan (10.4%), minerals elements etc [9]. Several classes of compounds having antioxidants activity have been found in oat including phytic acid, vitamin E, flavonoids and phenolic compounds [10]. Phenolic compounds participate to essential functions in plant growth and reproduction, and to plant protection system encounter insects, fungi and nematodes [11].

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Nonetheless, during plant growth cycle, chemical and biochemical composition could vary according to environment~~s~~al factors. Agricultural practices as tillage altered nutrient content [12]. In addition, cereals antioxidants vary according to the environment, the genotype and probably genotype-environment interactions [13-14]. Moreover, oat production is threatened by climates changes, problems of cultivars adaptation to agro-ecological zones and unbalanced socio-economic conditions.

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Generally, farmers applied tillage before sowing cereals in conventional agriculture to limit weed effect, to prepare seedbed and to eschew crust formation. Nonetheless, these practices joined to climate change and monocultures induced soil moisture loss and soil organic matter reduction [15] and erosion [16].

Therefore, conservation agriculture arose as a substitution to conventional agriculture. Thus, no tillage was applied for the first time in 1999 under rainfed conditions in North West Tunisia [17]. About 260000 ha of agricultural area have been cited as a priority for conservation agriculture adoption in Tunisian semi-arid and sub-humid regions [18].

Since the worldwide adoption of conservation agriculture, research focused on its effect on soil organic matter, soil physical properties, soil moisture and yield [19]. This effect is related to weather conditions, rainfall, crop sequence, and the interaction between many other factors [20-21]. These interactions could result in stability, decrease or increase of grain yield [22]. As well, no tillage is recognized to ameliorate soil moisture, soil physico-chemical properties, soil organic matter, and soil biological processes [17,19]. In contrast, few research activities have treated the tillage effects on mycorrhyzation rate, grain mineral elements, total phenolic content, total flavonoids content and antioxidant capacity. This is despite of the tillage effects remarked on protein and gluten content [23], hormone activity [24] and sucrose content [19]

The main objective of this study was to determine the effect of tillage practices on natural mycorrhization, some minerals, elemental concentrations, total phenolic content, total flavonoid content and antioxidant capacity of oat during tillering stage in North West Tunisia under rainfed conditions.

2. MATERIAL AND METHODS

2.1 Trial Description

This study was conducted at the referential farm for direct drilling (Krib, Siliana) situated in northwestern Tunisia (36°22'24"N; 9°10'26"E; elevation = 460m). Krib present a specific microclimate fluctuated between superior Semiarid and sub-humid with an annual precipitation of about 450 mm. The annual mean of temperatures and rainfall for the cultivation year of the experimental site are presented in Figure 1. The soil was sandy clay and relatively poor in organic matter (2.1%) and slightly alkaline (pH=7.6). The trial was settled since 1999-2000 growing season and the sampling was achieved during the cultivation year 2015-2016. The biannual crop-rotation was faba bean (*Vicia faba* L. minor) and oat (*Avena sativa* L. 'méliane'). Two tillage practices: conventional tillage (CT) and no-tillage (NT) were tried. CT consisted of reversible moldboard ploughing to 30-40 cm depth followed by secondary tillage with offset 15-20 cm for seedbed preparation and NT plots were sown by a direct driller. For NT, weeds were treated with glyphosate at a rate of 3 l.ha^{-1}. The sowing rates were 160 kg.ha^{-1} for oat and 120 kg/ha for faba bean. Fertilizers were surface supplied; oat received 100 kg.ha^{-1} of Di-Ammonium Phosphate at early tillering and 70 kg.ha^{-1} of ammonium nitrate half at end of tillering.

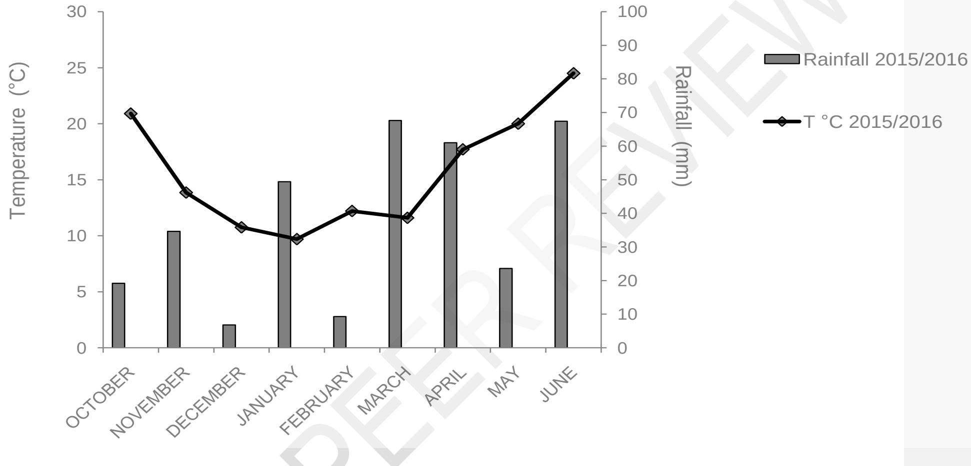

Fig.1. Temperatures (°C) and rainfalls (mm) recorded in the region of Krib from October to June during the cultivation year 2015-2016.

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Or it should have a ''reference'' if it was related data.

2.2. Sampling and measurements

To analyze mycorrhizal colonization, oat samples were collected during tillering stage for the cultivation year 2015-2016. Roots were washed then conserved in ethanol 50% till trypan blue coloration. The areal parts of oat were dried, ground, sieved and stored before mineral elements analysis.

2.3. Trypan blue coloration

For root colonization estimation, 3 plants/plot were collected at tillering stage during the cultivation year 2015-2016. Oat roots were placed in a solution of KOH (5%) to be clarified at 90°C during 20 minutes. To facilitate colorant fixation, roots were then emerged in HCl (2%) solution during 5 min. After filtration, roots were dyed with Trypan blue as explained by Phillips and Hayman [25]. Mycorrhization rates were estimated using the method of Mc Gonigle et al. [26].

2.4. Mineral elements determination

For mineral elements analysis, 1 g of dry sample were ashed in a muffle oven at 600 °C for 6h, and mineralized with HCl. Mineral elements measurements were made in quadruplicate. Potassium, calcium and sodium concentrations were estimated using the flame photometry. Spectrophotometery was used to determine phosphorus concentrations [27].

2.5. Extraction

Ground plant material (0.5 g) were put in 25 ml of methanol (80%) then shaken during 2 h and the solid phase was discarded using a Whatman filter paper. For each treatment, four extracts were prepared and stored until analysis.

2.6. Determination of total phenolic content (TPC)

A method based on Folin–Ciocalteu reagent, proposed by Singleton and Rossi [28] was used for TPC quantification. At 720 nm, the spectrophotometer was used to measure absorbance of different extract and a blank after 1 h. Gallic acid (GA) was used for the standard curve (0–1000 ppm) and TPC was expressed as milligram of gallic acid equivalent (GAE) per gram of dry weight.

2.7. Estimation of total flavonoid content (TFC)

The colorimetric method proposed by Zhishen et al. [29] and modified by Chaieb et al. [30] was used to determine TFC of oat samples at 510 nm against a blank. Rutin was used for the calibration curve and TFC was expressed as milligram of rutin equivalents (RE) per gram of dry weight.

2.8. DPPH scavenging effect

Samples antiradical capacity estimation is based on the DPPH reduction. As DPPH is stable, it is generally used for samples free radical-scavenging ability evaluation. Thus, the method of Chen et al [31] with some modifications was used for DPPH determination. For each sample, the methanolic extract (10 µL) was mixed with 3 mL of 0.06 mM DPPH in methanol. After the incubation step in darkness (30 min), the absorbance was measured at 517 nm against methanol blank. The DPPH radical inhibition percentage was calculated based on the expression of Maisuthisakul et al. [32], that below:

DPPH radical scavenging capacity (%) = $[A0-(A1-As)]/A0*100$

2.9. Statistical Analysis

The Statistical Package for the Social Sciences software (SPSS 20.0, SPSS Inc., Chicago, IL, USA) was used for all statistical analysis to identify treatment effects and interactions (Two-way MANOVAand PEARSON correlation). If significant effects were noted ($p < 0.05$), DUNCAN post hoc test was used to check differences between variables.

3. RESULTS AND DISCUSSION

3.1 Mycorrhization Rate

As mycorrhizal symbiosis is recognized to improve plant water and nutrients supply, this study aims to elucidate tillage effects on the root colonization by AMF. Results of mycorrhizal colonization are presented in Figure1. Tillage practices (T) did not show significant effects on mycorrhization rate (MR). Even if NT had no significant effect on MR, higher rates were noted for NT compared to CT.

Comment [S8]: The factors present are always relational thus there should be an effect either positive or negative.

Similarly, Curaqueo et al (2010) [33] studied the effect of different tillage systems on spring durum wheat mycorrhization rate and noted that MR% under NT presented higher value compared to CT even if this difference was statically no significant. Kabir (2004) [34] reported that soil disturbance result in reduction of MR%. Furthermore, maize and bean showed higher mycorrhization rate for no tilled soil compared to tilled soil [35]. After three years of NT adoption in Algeria, an increase of MR was detected compared of CT [36]. Similarly in Tunisia, durum wheat presented higher MR% for NT compared to CT and its statistically significance is related to cultivation year [37]. As tillage disturbs soils and decelerates their biological processes, higher MR% was noted under NT compared to CT.

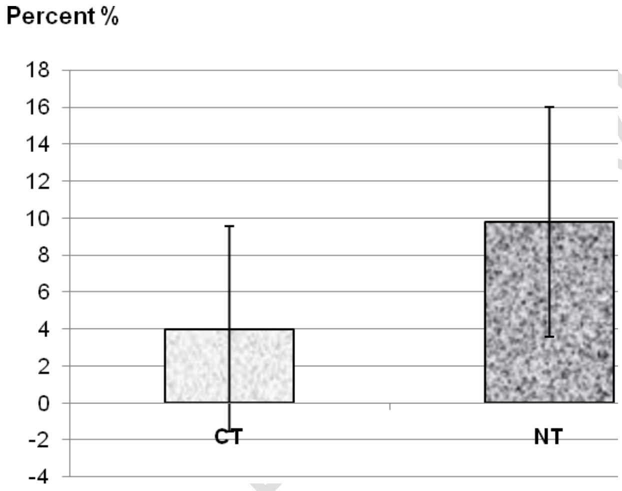

Fig.2. Effect of Conventional tillage (CT) and No tillage (NT) on mycorrhization rate of oat in Krib during 2016-2017 cultivation year.

3.2 Mineral Elements Contents

Minerals in plant are essential for many phytochemical processes. As macronutrients, phosphorus, calcium and potassium are components of nucleic acids, proteins, hormones, phospholipids, coenzymes, adenosine triphosphate (ATP) and chlorophylls etc. The plant contents of phosphorus, potassium, calcium and sodium are presented in Figure 3. Analysis of variance showed that tillage (T) had no significant effect on plant mineral composition.

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In Similar, Chaieb et al. [37] investigated tillage practices effect on nutrient uptake and proved that P, K, Ca and Na contents in durum wheat and barley did not depend on tillage practices. Besides, tillage practices did not affect significantly P and K in wheat (Ishaq et al. [38]) and corn Singer et al. 2007 [39]).

In contrast, Chaieb et al [40] remarked a significant effect of tillage practices on durum wheat mineral contents at maturity stage under semi-arid conditions. In addition, corn mineral contents were changed by tillage and lower amounts were noticed under NT compared to CT [41]. Guan et al. [42] investigated maize mineral contents and obtained lower values for NT compared to CT. Therefore fertilizer management, nutrients weak movement and crop

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residue, NT generates nutrients accumulation in the top of soil and for that cause, a decline of the plant nutrients availability [43].

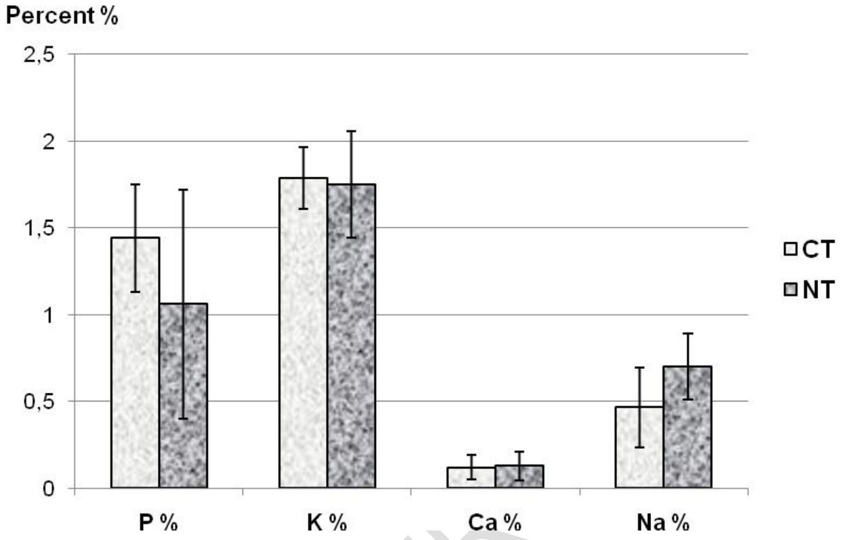

Fig.3. Effect of Conventional tillage (CT) and No tillage (NT) on phosphorus (P), potassium (K), calcium (Ca) and sodium (Na) contents of oat in Krib during 2016-2017 cultivation year.

3.3 Total Phenolic Content (TPC)

As phenolic compounds are important in abiotic and biotic stress, they react as defense system against fungi, nematodes and insects attacks. As shown in figure 4, analysis of variance showed that tillage did not affect significantly TPC.

These results are similar to those of Chaieb et al [40], Chaieb et al. [44] who reported that under no tillage durum wheat and barley TPC at maturity stage and tillering stage did not show significant variability. Furthermore, Stake et al. [45] found that management operations did not alter wheat phytochemicals concentrations. In contrast, some studies revealed that management practices affected wheat TPC [46] and maize TPC [47].

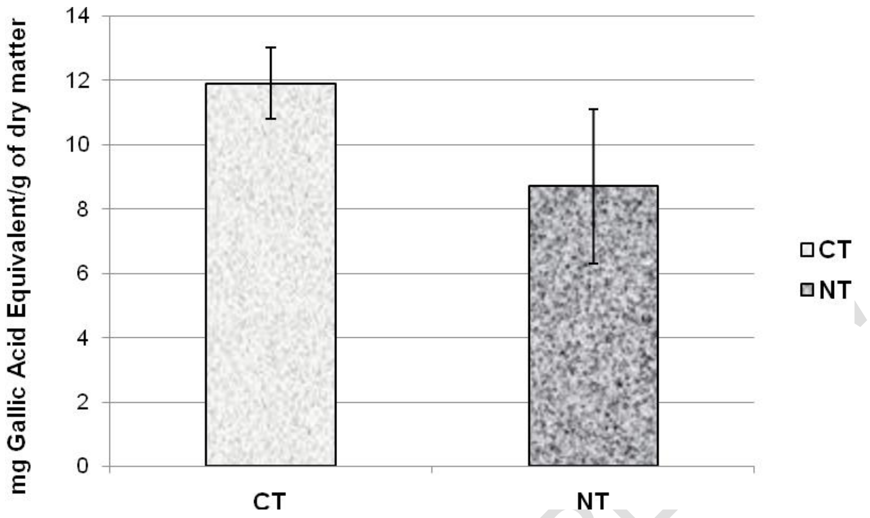

Fig.4. Effect of conventionnal tillage and No tillage on TPC of barley in Boulifa during 2016-2017 cultivation year.

3.4 Total Flavonoids Content (TFC)

In cereal, flavonoids are one of the major groups of phenolic compounds [39]. Thus, analysis of variance of TFC showed that the effect of tillage is no significant. These results are similar to those of Chaieb et al. [40] and Chaieb et al. [44] who found that tillage system did not affect significantly durum wheat and barley TFC at maturity satge and tillering stage as affected. As well, Stracke et al. [45] reported that mangement operations did not affect wheat phytochemicals concentrations. In contrast, Asami et al. [46] noted that wheat TFC could vary according to management practices.

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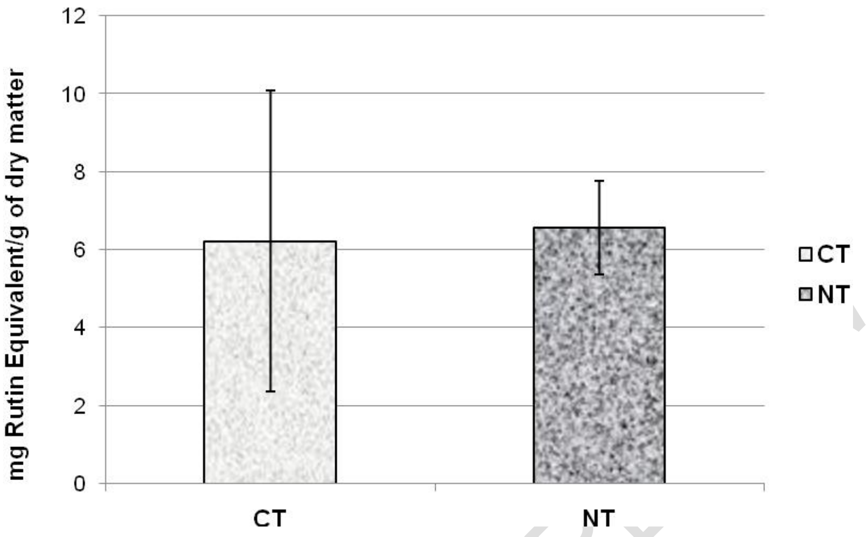

Fig.5. Effect of conventionnal tillage and No tillage on TFC of barley in Boulifa during 2016-2017 cultivation year.

3.5 Antioxidants Capacity (DPPH)

Plant antioxidants such as phenolic compounds interact to deal with biotic and abiotic stresses. Thus, an estimation of the antiradical capacity based on the reduction of DPPH was achieved. Tillage system had no presented significant effect on DPPH.
Likewise, Costanzo et al. 2019 [48] and Chaieb et al. 2020 [40] revealed that tillage system had no significant effect on wheat antioxidants capacity. Contrary to, many results noted that antioxidants capacity is related to many factors ~~such~~ affecting genotype, environment, management practices and their interactions, added to the analysis test and the extraction solvents Mpfou 2006 Zrckova 2018 [49-50]. Huseynova 2012 [51] revealed that under stress conditions, antioxidants concentration increased and antioxidants act to protect plant against attacks.

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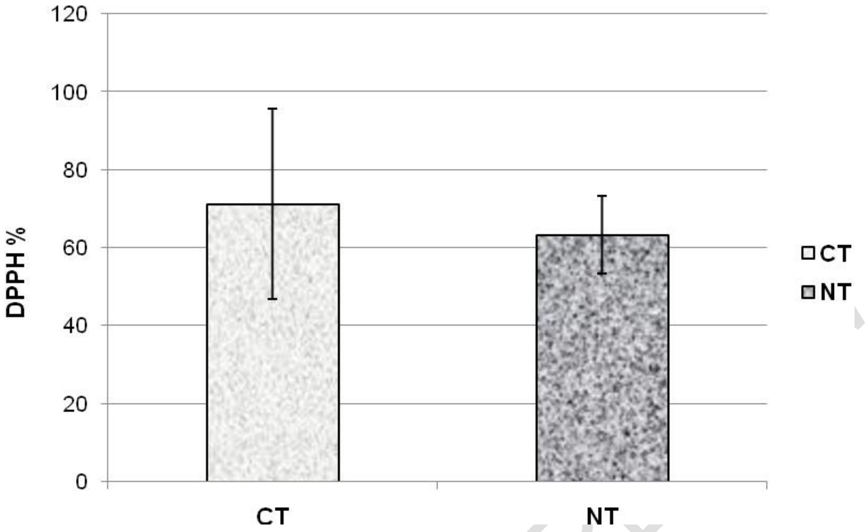

3.6 Correlation among mineral contents, TPC and TFC

As shown in Table 1, no significant correlations were noted among studied parameters. Even if, MR% presented negative correlations with phosphorous (P%), calcium(Ca%) and with TPC. Calcium (Ca%) showed significant negative correlation with TFC (r=-0.812).
In contrast, Chaieb et al. [29] found that P% had positive correlation with K% and that based on tillage practices P% presented positive significant correlations with Ca% and Na% for durum wheat during tillering stage. However, durum wheat grain had not presented any significant correlations for these parameters [40]. Furthermore, significant negative correlations were reported among MR% and mineral elements [36].

Table 1. Correlation coefficients among mineral concentrations, total phenolic content and total flavonoids content of barley conventional tillage versus no tillage in Boulifa for 2016-2017 cultivation year.

	MR%[a]	**P%**	**K%**	**Ca%**	**Na%**	**TPC**	**TFC**	**DPPH%**
MR%	1							
P%	-.641	1						
K%	.379	.027	1					
Ca%	-.300	.425	.346	1				
Na%	-.023	-.295	-.255	.325	1			
TPC	-.511	-.155	-.362	-.322	-.271	1		
TFC	.126	.007	-.572	-.812[*]	-.371	.079	1	
DPPH%	-.229	.010	-.013	.449	-.218	.423	-.396	1

** Significant correlation p=.05.*
*** High Significant correlation p=.01.*
[a] P%, phosphorus; K%, potassium; Ca%, calcium; Na%, Sodium; TPC, Total Phenolic Content; TFC, Total Flavonoids Content

4. CONCLUSION

This work is a contribution to discern the effect of tillage on oat mycorrhization rate, mineral elements uptake, total phenolic content and total flavonoid content during tillering stage. The results revealed that under rainfed conditions in North West Tunisia, tillage had no significant effect on mycorrhization rate, mineral element uptake, total phenolic content, total flavonoids content and DPPH. These results should encourage farmers in North West Tunisia to adopt No tillage for oat cultivation. These practices permit to limit soil erosion in this region and enhance the sustainability of cereal production.

CONSENT (WHERE EVER APPLICABLE)

No applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

No applicable

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