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Original article

Soil enzymatic activities and available P and Zn as affected by tillage practices, canola (*Brassica napus* L.) cultivars and planting dates

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ABSTRACT

Due to high sensitivity and rapid response, soil biological properties including microbial enzymatic activities are appropriate indicators of soil quality, under different agricultural systems. Hence, a two-year field experiment was performed in 2002 and 2003 hypothesizing that soil microbial activities and P and Zn availability differ under different management practices. The objective was to evaluate the effects of different tillage (T) practices, canola (*Brassica napus* L.) cultivars (V's) and planting dates (PD's) on the soil enzymatic activities of alkaline and acid phosphatase and dehydrogenase and available P and Zn. Using a split plot design, different T practices (no (NT), minimum (MT) and conventional (CT)) and the combination of different V's (Hyola 401 and PF) and PD's (8th (PD1), 23rd September (PD2) and 7th October (PD3)) were assigned to the main and subplots, respectively. Soil enzymatic activities and P and Zn were measured. The actions and interactions of T, and PD significantly affected the activity of alkaline and acid phosphatase. Although, dehydrogenase activity at 0–10 cm was affected by T, V and PD and the interaction of T and PD, only T and the interaction of T and PD influenced the activity of this enzyme at 10–20 cm. Compared with other tillage practices, NT significantly increased enzymatic activities. The enzymatic activity at the 0–10 cm depth was in the order of PD1 > PD2 > PD3. However, at the 10–20 cm depth MT had a significant effect on dehydrogenase activity. NT reduced soil available P and Zn. NT can significantly influence soil biological properties and hence canola growth, resulting in a sustainable agricultural system.

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Abbreviations: P, phosphorous; Zn, zinc; N, nitrogen; C, carbon; NT, MT, CT, no-, minimum-, and conventional tillage, respectively; PD1, PD2 and PD3, 8th and 23rd of September, and 7th of October, respectively.

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1. Introduction

1.1. Tillage practices and soil enzymatic activities

Soil enzymes are used as biological indexes of soil fertility under different tillage practices [70]. With respect to their interaction with plants and nutrients and organic matter cycling, microorganisms are important fractions of the ecosystem [37,52]. In addition, they are also involved in the formation and stability of soil aggregates. All these microbial influences are very much affected by tillage practices. Of the total soil organic matter, 1–5% exists in the microbial biomass [24,30].

It has been known that the plant–microbe interactions are very much under the influence of tillage practices [34]. In addition to the soil texture influencing the distribution of soil microorganisms and enzymatic activities (e.g., alkaline phosphatase activity was found in the silt (37.9–43.0%) and clay (48.9–54.0%) fractions); different soil tillage practices also affected the amounts and distribution of soil enzymatic activities at different soil depths [25,64]. The nature and the method of soil fractioning also determine the distribution of soil enzymatic activities [26,32].

The significance of soil enzymatic amount and distribution in the soil is because of planning an appropriate tillage practice for the enhancement of soil organic N and C, and hence, long term productivity of soil [26].

Since the rate of physical and chemical processes in the soil is slow, soil biological parameters, including soil microbial population and enzymatic activities can be used as good indicators of soil quality, when soil is subjected to ecological changes such as different soil practices [14,25,26]. At different management practices, the microbial attributes, including the amount and activity of phosphatase and dehydrogenase enzymes determined soil biological quality more sensitively and more responsively, compared with total organic C and N [5,11,42].

MT affects soil properties, including soil temperature [12], soil physical properties [43], soil organic C [40], soil phosphatases [8] and soil microorganisms [21].

Green manure of crop residues can enhance microbial population and activities [5,13,15,38,42,]. Different tillage practices alter microbial composition and C utilization of substrates [2,6,13], and increase microbial variation [63].

1.2. Canola genotypes and soil biological activities

Since canola prices are competitively comparable with cereals price, its cultivation has extensively increased. Different plant genotypes may have different microorganisms combination in their rhizosphere [65], attributed to the production of their root exudates [39,44,60]. Usually brassica genotypes can efficiently utilize P, compared with other crop plants [17]. This has been attributed to their root architecture, which is delicately branched, and has long root hairs. They are also able to enhance P uptake by production of organic acid anions [39,44,49,60]. The amount of canola P uptake from the soil is usually 1% of its yield production [23]. It is worth mentioning high levels of P inhibit such mechanisms [59].

Plant roots release about 17% of plant photosynthate into the soil [37,50], resulting in enhanced microbial population and activity [28,53].

1.3. P and phosphatase

Although there is a high amount of P in the soil only soluble P, in small concentration (usually $<1\mu g$ [4]), is available to plants and soil microorganisms. P availability is very much affected by the formation of insoluble P compounds, soil fixation and microbial immobilization [39,44,60].

Microbial biomass and organic P are the most important sources of P in the soil, accounting for up to 10% [57] and 80% [62] of total soil P, respectively. The mineralization of organic P, and hence its availability, is dependent on the production of phosphatase enzymes by plant roots and microorganisms [39,44,60].

Soil microorganisms are able to increase plants available P by decreasing soil pH, and hence increasing phosphatase solubility, and also production of organic acid onions [39,71] and phosphatase enzymes (exocellular enzymes) [60,69].

1.4. Zn availability

The dynamic of Zn in the soil, like other nutrients, is subject to alteration of soil properties including the physical, chemical and biological ones. However, unlike P, Zn is not directly under the influence of soil microbial and enzymatic activities. Under different tillage practices soil properties such as the potential of oxidation–reduction are altered, affecting soil microorganisms activities, and eventually influencing Zn availability. Hence, the solubility of different nutrients, including Zn, in the rhizosphere of different plants, cropped at various planting dates, may differ depending upon their microbial combination and also soil properties, resulted by tillage practices.

Since to our knowledge there is very little documented data regarding the effects of different tillage practices, canola genotypes and planting dates on soil enzymatic activities and P and Zn, we performed these experiments.

2. Materials and methods

2.1. Experimental design

Two field experiments were conducted in 2002 and 2003 as split plots on the basis of completely randomized block design, in the Research Agricultural Center of Sari, Iran. The main plots were devoted to tillage practices including no- (planting at the previous cereal residues), minimum- (using chisel plow), and conventional tillage (using moldboard plow). The combination of canola cultivars, including Hyola 401 and PF, and planting dates (PD) of 8th (PD1) and 23rd (PD2) of September, and 7th of October (PD3) was assigned to subplots.

2.2. Experimental procedure

Each subplot was made of ten 7-m rows with a 20-cm spacing between the rows and 3-m spacing between the plots,

respectively where seeds were planted at a 5-cm spacing, resulting in a 14-m^2-plot area. For each of the three replications 18 experimental plots were used, producing a total of 2000 m^2 research area. Fertilization was applied according to soil analysis and the manual for canola fertilization. P (P_2O_5) at 59 kg, and K (K_2O) at 100 kg, were fertilized completely at seeding, while N at a total of 150 kg was fertilized, equally three times during the growing season, including seeding, stemming and flowering. Hand thinning was made at the 6-leaf stage.

Field preparation, fertilization and chemical controlling of weeds were conducted before seeding. The field was irrigated twice with a 7–9 day interval for the better germination of seeds. The field was also irrigated at stemming and flowering along with fertilization, and at poding and grain filling. Pests were chemically controlled at the period of flowering.

2.3. *Soil biochemical analyses*

Soil chemical and physical properties were determined including soil texture (silty clay loam, hydrometric method [22]), soil organic carbon (0.63%, wet oxidation [48]), pH (7.7) and electrical conductivity (0.6 dS/m [58]), total N (0.055% [47]), and K (emission spectrophotometry [31]). Soil P and Zn were measured by sodium bicarbonate extraction [51] method, using the colorimetric method (autoanalyzer), and diethylenetriaminepentaacetic acid (DTPA) method [3], using an atomic absorption spectrometer (Model Perkin Elmer 3110), respectively.

Soil enzymes including, alkaline and acid phosphatase, and dehydrogenase at the depths of 0–10 and 10–20 cm were determined according to Tabatabai [67]. To measure dehydrogenase soil samples (5 g) were incubated at 30 °C for 24 h using triphenyltetrazoliumchloride (an electron receiver, 2 ml of 0.6% [33]). Using acetone the produced compound, triphenylformazan, was extracted and measured by a spectrophotometer at 546 nm [16].

To measure alkaline and acid phosphatase enzymes [37] *p*-nitrophenyl phosphate disodium (0.115 M) was used as the substrate. Soil samples (1 g) were treated with 2 ml of 0.5 M sodium acetate buffer with a pH of 5.5 (using acetic acid) [46] and 0.5 ml of substrate and were incubated at 37 °C for 90 min. Cooling at 2 °C for 15 min inhibited the reaction. The treated samples were then mixed with 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH (to inhibit the enzyme reaction) and centrifuged at 4000 rpm for 5 min. Using spectrometry at 398 nm the produced *p*-nitrophenol was measured [68,70]. Although controls were made similarly, $CaCl_2$ and NaOH were mixed after substrate addition [29].

2.4. *Statistical analysis*

Using SAS (SAS Institute Inc. [61]) data were subjected to analysis of variance including combined analysis. The main effects and their interaction effects were compared using Duncan's multiple range tests. In addition correlation coefficients among soil enzymes were also determined [66].

3. Results

The climatic parameters are presented in Table 1. In addition to the significant effects of tillage, planting date, and the interaction effects of tillage and planting date on the activities of alkaline and acid phosphatase, the interaction effects of planting date with year and cultivar, significantly affected the activity of alkaline and acid phosphatase enzymes, respectively. Although the effects of tillage, planting date, cultivar (Fig. 1), and the interaction effect of tillage and planting date were significant on the activity of dehydrogenase at the 0–10 cm depth, only tillage and the interaction effect of tillage and planting date significantly affected the activity of dehydrogenase at the 10–20 cm depth (Table 2).

The activity of alkaline, acid phosphatase and dehydrogenase enzymes under NT was significantly higher than those of MT and CT. In addition, under CT the activities of these enzymes were at the lowest. The activity of alkaline phosphatase and dehydrogenase enzymes at the 10–20 cm depth under MT and CT was not significantly different (Table 3).

The effects of PD1 and PD2 on the activity of alkaline phosphatase were not significantly different. However, there was a significant reduction in the activity of the enzyme at PD3, compared with PD1 and PD2. The activity of acid phosphatase and dehydrogenase at the 0–10 cm at different planting dates was in the order of PD1 > PD2 > PD3, being significantly different from each other (Table 3).

Although tillage, cultivar and the interaction effect of year and cultivar had significant effects on the amount of soil P, only the interaction effect of tillage and planting date significantly affected the amount of Zn (Table 3).

There were no significant differences between Zn levels at different PD's and for different cultivars. At PD1 under Hyola 401 and PF cultivars amounts of 25.43 and 25.78 ppm resulted, respectively. At PD2 the corresponding values were 25.08 and 26.12 ppm, and at PD3 were 25.44 and 25.17, respectively.

Table 1 – Climatic parameters

	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Annual
Parameter													
Temperature (°C)	7	7.3	9.5	14.9	19.5	23.5	25.5	25.7	22.8	18.1	13	8.6	16.3
Humidity (%)	84	83	84	81	78	79	81	82	83	84	85	86	82
Precipitation (mm)	66.2	62.6	63.6	36.4	31.2	31.1	31.4	48.2	80.4	103.6	99.9	77	731.6
Wind (m/s)	2.9	3	3.3	3.4	3.1	2.7	2.4	2.4	2.3	2.4	2.4	2.5	2.8
Degree-days	135.2	120.7	125.1	161	205.7	215.8	210.8	171.5	157.7	170.3	145.4	130.4	1949.6

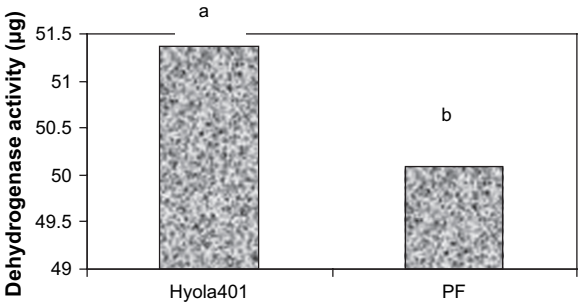

Fig. 1 – Effects of different cultivars on soil dehydrogenase (µg) activity at the 0–10 cm depth averaged at different tillage practices and for different planting dates.

However, the highest amounts of Zn resulted under CT and MT at different PD's, compared with NT (Table 3). There was a significant but not high correlation between the soil P percentage and Zn (0.073*).

Compared with NT, MT and CT increased soil P significantly. The same trend was observed for Zn but not significantly. The effect of cultivar on soil P (0.547% and 0.543% for Hyola 401 and PF, respectively) and Zn (25.316 and 25.690 ppm for Hyola 401 and PF, respectively) was not significant. Although the effect of planting dates on soil P was significant and, PD2 and PD3 significantly increased soil P, relative to PD1, planting date numerically and not significantly decreased Zn (Table 3).

NT resulted in the highest activity of alkaline and acid phosphatase and dehydrogenase enzymes at a depth of 0–10 cm in PD1 and PD2, being significantly different from PD3 and MT and CT. Compared with CT, the activity of alkaline phosphatase under MT and at PD1 was the highest and significantly different form other PD's. However, there was interestingly a completely different scenario for dehydrogenase at the depth of 10–20 cm. The enzymatic activity of dehydrogenase under MT was the highest at all PD's and significantly different from NT and CT. There were no significant differences between NT and CT with respect to the activity of dehydrogenase enzyme at a 10–20 cm depth (Table 4).

Comparisons of coefficients of correlation indicated that there were significant correlations between the dehydrogenase enzymes at different depths and acidic and alkaline phosphatase, being the highest for alkaline phosphatase and dehydrogenase at 0–10 cm, and acid phosphatase, respectively (Table 5).

4. Discussion

Our results indicate that NT significantly affected the enzymatic activities of alkaline and acid phosphatase and dehydrogenase at the 0–10 cm depth. However, at the 10–20 cm depth MT resulted in the highest activity of dehydrogenase. This is in agreement with the work of other scientists who found that under NT the amount of microbial enzymatic activities on the soil surface was significantly higher than that of CT, while at a deeper soil depth the reverse was observed [8–10]. Although the tillage method substantially affected the activity of phosphatase enzymes, sampling time (planting date) had less pronounced effects [25], which is similar to our results. According to Mullen et al. [45] NT and distribution of crop residues on the soil resulted in enhanced activities of soil enzymes including acid phosphatase and dehydrogenase.

Table 2 – Combined analysis of variance for the effects of different parameters and their interaction effects on the activity of dehydrogenase and phosphatase enzymes, and P and Zn contents of soil

		Mean of sum squares					
Source of variation	Degree of freedom	Alkaline phosphatase	Acid phosphatase	Dehydrogenase (0–10 cm)	Dehydrogenase (10–20 cm)	P	Zn
Year, Y	1	35606.676	32.67	7008.2189	0.00724	0.0972	4.898
Error, R(Y)	4	496.87	0.02352	21.7617	1.997	0.0024	27.68
Tillage, T	2	6346.009**	8.0226**	2984.3353**	253.433**	0.00243**	37.278
Y*T	2	90.3981	0.0001	0.00028	0.1378	0.00022ns	3.728ns
Plot error, R*T(Y)	8	244.328	0.01410	4.2328	3.4017	0.00028	22.022
Planting date, PD	2	6512.788**	0.20481**	11.2953**	2.745	0.00072	1.031ns
Variety, V	1	7.787ns	0.02373ns	3.7408**	1.021ns	0.00045*	3.778ns
Y*PD	2	569.287*	0.010ns	0.00028ns	0.6103ns	0.00031ns	0.0117ns
Y*V	1	83.565ns	0.020ns	0.00083ns	0.3445ns	0.00053*	0.213ns
T*PD	4	2642.523**	0.11925**	1.9664**	6.7940**	0.00026ns	7.735*
T*V	2	277.843ns	0.03370ns	1.5586ns	0.4444ns	0.00004ns	0.752ns
PD*V	2	459.898ns	0.05148**	0.4519ns	3.2336ns	0.00014	3.870ns
Y*T*V	2	214.5648ns	0.00010ns	0.00028ns	0.6504ns	0.00008ns	0.680ns
Y*PD*V	2	347.843ns	0.00010ns	0.00028ns	0.2089ns	0.0003ns	3.377ns
Y*PD*T	4	275.134ns	0.00010ns	0.00056ns	0.3005ns	0.00008ns	3.1717ns
Y*T*PD*V	4	64.8842ns	0.00010ns	0.00056ns	0.4223ns	0.00019ns	0.8175ns
V*PD*T	4	226.828ns	0.02148ns	1.1314ns	1.987ns	0.00014ns	1.8011ns
Error	60	186.698	0.01522	0.6146	1.8343	0.0001	3.082

ns, *, **: not significant, significant at 5% and 1% of probability, respectively. R: number of replicates, 3.

Table 3 – Effects of different tillage practices and planting dates on the activity of phosphatase and dehydrogenase enzymes, and soil P and Zn contents averaged at different planting dates and for different years and cultivars

	Phosphatase (μg)		Dehydrogenase (μg)			
Tillage	Alkaline	Acidic	0–10 cm	10–20 cm	P (%)	Zn (ppm)
NT	3020.47a	3.83a	58.26a	49.83a	0.536b	24.64a
MT	3000.6b	3.44b	54.38b	45.42b	0.547a	25.25a
CT	2995.3b	2.89c	40.91c	44.95b	0.552a	26.63a
Planting date						
8 Sep	3015.47a	3.47a	51.67a	ND	0.540b	25.605a
23 Sep	3010.72a	3.38b	51.29b	ND	0.548a	25.597a
7 Oct	2990.17b	3.32c	50.57c	ND	0.547a	25.308a

NT, MT and CT: no-, minimum-, and conventional tillage, respectively. ND: not determined. Values in the same column, followed by different letters are statistically different using Duncan's multiple range test at 5% of probability.

The results indicate that though no significant differences were found between the activities of dehydrogenase enzyme under NT and conventional tillage, acid phosphatase activity was significantly higher under NT. Hence, this can be very advantageous under limited P conditions [6]. Accordingly, for crop plants such as canola, which are not hosts for arbuscular mycorrhizal fungi [72] using NT can be very recommendable.

It is also worth mentioning that the significance of phosphatase enzymes for canola production is higher than dehydrogenase since the latter is a necessary enzyme for arbuscular mycorrhizal symbiosis [7] and quantitatively is much less available in the soil, compared with alkaline phosphatase, which is required for plant P uptake.

Some scientists have stated that the amount of P under NT on the surface soil is higher compared with conventional tillage [55,56], and have attributed this to the increased contact between fertilizer P and soil particles under plowed soils and hence the enhanced production of insoluble P [54] and P fixation by soil particles. However, according to our results the amount of soil P under NT decreased, compared with MT and CT. The reason may be the increased immobilization of P by soil microorganisms under NT, where their activity increases because of higher rate of organic matter.

Madejon et al. [35] observed that conservation tillage increased microbial population and activities including phosphatase and dehydrogenase production compared with traditional tillage. The two different tillage practices were significantly different with respect to their biochemical parameters, which were very much correlated at the deeper depth (10–25 cm). In addition, conservation tillage improves soil structure and stability compared with traditional tillage.

Because of the specificity of the enzyme substrates, they are affected by soil-depth dependent parameters such as pH and temperature, ionic balance, and inhibitory parameters [70].

Unlike CT where the distribution of microbial activities is homogenous [18], soil enzymatic activities decreased with depth under NT and MT [1,25,36], which is in agreement with our results (Table 4).

Regular cultivation decreases soil organic matter and soil structure. Wheat intensive cropping and inclusion of green manure in the cropping system significantly increased soil microbial biomass and activities including the production of phosphatase and dehydrogenase enzymes [3,5,11,42].

The differences between microbial enzymatic activities under different tillage practices are due to the reduction and eventually depletion of substrates. Hence, addition of organic C can rapidly influence the balance of microbial activity and enzyme regulation (production and activity) [27]. As a result of microclimate formation under NT the soil is cooler and wetter, resulting in the shallow distribution of organic N and C.

Table 4 – The interaction effects of different tillage practices and planting dates on the activity of phosphatase and dehydrogenase enzymes, averaged for different years and cultivars

Tillage	Planting date	Alkaline phosphatase (μg)	Acid phosphatase (μg)	Dehydrogenase (0–10 cm, μg)	Dehydrogenase (10–20 cm, μg)
NT	8 Sep	3041.67a	4000a	58.87a	45.12b
	23 Sep	3033.33a	3800b	58.45a	45.77b
	7 Oct	2986.42d	3680c	57.45b	45.98c
MT	8 Sep	3010b	3400d	55.03c	48.99a
	23 Sep	2998.50bcd	3480d	54.67c	50.15a
	7 Oct	2993.33cd	3450d	53.43d	50.15a
CT	8 Sep	2994.75cd	3000e	41.13e	45.36b
	23 Sep	3000.33bc	3850f	40.83e	45.15b
	7 Oct	2990.75cd	2820f	40.77e	43.98c

NT, MT and CT: no-, minimum-, and conventional tillage, respectively. Values in the same column, followed by different letters are statistically different using Duncan's multiple range test at 5% of probability.

Table 5 – Correlation coefficients between phosphatase and dehydrogenase enzymes

	DH 0–10	DH 10–20	ACP
DH 0–10	1		
DH 10–20	0.489**	1	
ACP	0.881**	0.619**	1
AKP	0.598**	0.425**	0.733**

ACP, AKP and DH: acidic and alkaline phosphatase, and dehydrogenase enzymes, respectively. ** Significant at 1% of probability.

The effects of different tillage practices on soil microorganisms' population and activities are through influencing the quality, amount and distribution of plant residues applied to the soil, plant growth of roots and shoots, and nutrient cycling. Under Mediterranean conditions determination of soil biological functionalities may be more precise indicators of soil quality, compared with only soil organic matter measurement. Soil depth has important impacts on soil organic matter distribution and, hence soil biochemical properties [19,35].

It is obvious from our results that the later planting dates resulted in significantly less enzymatic activities. The reason is that under cooler temperatures the microorganism activities including bacterial activities decrease [41,73]. However, under warm and humid conditions the reverse may be resulted [20]. The effect of cultivar was not significant on the activity of phosphatase enzymes; however, it significantly affected the activity of dehydrogenase, which is available at a much less quantity in the soil, similar to the results of Soederberg et al. [65]. Additionally, the most likely explanation for higher P and Zn in soil at later planting dates is that plants are smaller when they were planted later and have therefore taken up less P and Zn.

5. Conclusion

Our results indicate the significance of tillage practices, planting dates and cultivar on soil biological activities. Biological indicators such as the activity of alkaline and acid phosphatase and dehydrogenase enzymes and P and Zn availability can be used as very good indexes of soil quality. Accordingly, appropriate management practices such as development of fertilizer strategies (Miransari and Mackenzie, unpublished data) and crop rotations can be planned. It is clear that use of NT for canola production can be very important affecting soil biological properties, which are very essential components of sustainable agriculture.

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