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THE EFFECT OF MOLASSES APPLICATION ON SOIL BIOLOGICAL INDICATORS AND MAIZE GROWTH OF DIFFERENT TILLAGE SOIL: A POT EXPERIMENT

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Abstract

Soil enzyme activity and labile carbon (LC) have long been used as soil health indicators. Soil health can be improved by molasses addition resulting in better plant growth and productivity. The effect of molasses on soil biological activity and plant growth under different tillage soil has not been discussed in many studies in Hungary. We assessed two soil types under different long-term tillage practices: conservation tillage (CT), which leaves 30% or more residue on the soil surface, and conventional-ploughing tillage (PT). A pot experiment with maize as the crop was carried out using the composite soil (0-20 cm) of CT and PT; a randomized block design with four replications was employed. Three levels of molasses concentration, 0 g L^{-1}, 0.05 g L^{-1}, and 0.2 g L^{-1} were applied. LC, dehydrogenase (DHA), β-glucosidase activity, plant height, and dry weight biomass were measured at the end of the experiment (after eight weeks). The results indicated that LC in CT increased by 7.61-21.23% over the increase in molasses concentration. LC concentration was significantly higher in the CT than in the PT soil. β-glucosidase activity increased along with the increase of molasses concentration by 11.42-30.43% in CT and 16.03-56.67% in PT; however, the significantly different appeared only in PT soil. The molasses application affected the DHA as well. The activity of dehydrogenase increases by 39.49-80.76% and 30.43-50.59%, respectively, in CT and PT. Nevertheless, no significance occurred in the tillage system or the molasses concentration. Our study also found that the different molasses concentrations did not affect the plant height and dry weight biomass in CT and PT. However, applying each molasses concentration in CT markedly escalated the plant height and dry weight biomass compared to PT. The enhancement of soil biological activity and plant growth by the molasses application allows a promising strategy for maintaining the soil health of agricultural land.

Keywords: conservation tillage, dehydrogenase, glucosidase, molasses, soil biology

INTRODUCTION

Tillage is a mechanical action providing a suitable environment for seed germination and root development. Ploughing tillage (PT) is a conventional intensive method applied primarily in agricultural land in Hungary since many decades. PT stimulates root penetration resulting in high root biomass (Shirani et al., 2002); however, many studies reported the deterioration effects such as soil compaction, decreased soil stability and structure, and increased soil erosion as a result of PT application (Zheng et al., 2018; Sokolowski et al., 2020). Conservation tillage (CT), reduced tillage and leaving ~30% of the crop residue on the soil surface after harvesting, was introduced in Hungary more than 20 years ago. Some investigations revealed that long-term CT implementation in Hungary brings a beneficial effect on soil structures and water content (Bogunović et al., 2019; Dekemati et al., 2019), soil organic carbon and soil nutrient, soil erosion and nutrient loss, and soil biological activity (Jakab et al., 2017; Madarász et al., 2021).

It is generally known that biological activities play an essential role in soil nutrient cycling and in forming and developing soil structure contributing to soil health (Fekete et al., 2022; Yang et al., 2021). Soil biological activity is affected by some anthropogenic factors like fertilization, crop residue management, and tillage activity (Reardon et al., 2022). Soil biological activity reflects the activity of microorganisms or microbiological processes in the soil. Many studies across the world have reported that the addition of soil organic amendment induces the soil's biological properties as well (Kotroczó et al., 2012; Koishi et al., 2020; Bartkowiak et al., 2022; Wydro et al., 2022)

Among the soil organic amendments, molasses, a byproduct of the sugarcane industry containing macro and micronutrients (likes N, P, K, Ca, Mg, and Fe), has been applied in arable land to improve soil fertility (Madejón et al., 2001; Srivastava et al., 2012; Pyakurel et al., 2019; Li et al., 2020). The increase in soil microbial activity due to the molasses application was also noted by Waguespack et al. (2022). Furthermore, another study by Omara et al. (2022) indicated that the addition of molasses incline dehydrogenase activity. In addition, the β-glucosidase activity increased as well by the presence of molasses (Yi et al., 2020).

The application of molasses in the soil of different tillage systems has not been widely investigated. On the other hand, molasses is potentially used to restore soil properties under long-term intensive tillage. This study aimed to determine the effect of molasses application on soil biological enzymatic activities and plant growth of conservation (CT) and conventional tillage system (PT).

MATERIALS AND METHODS

Soil preparation and treatment

A pot experiment study was conducted at MATE University, Budai campus. In the spring, a Luvisol material soil was taken at 0-20 cm depth of two different long-term tillage systems, conservation tillage (CT) and conventional tillage (PT). CT was treated by non-inversion minimum tillage at 8–12 cm depth. Crop residue of ~30 % was left and covered the soil surface. The weed was controlled mechanically by the cultivator at 8–10 cm depth. PT was treated by Mouldboard ploughing (up to 25–30 cm depth). The soil pH was slightly acidic to neutral, 6.25 (pH-H_2O) and 4.69 (pH-KCl). Soil texture is moderately fine, with 37% sand, 58% silt, and 5% clay.
A randomized block design was employed with two factors (tillage system and molasses concentration). There were six treatment combinations with four replications (24 experimental units/pot total). One kg soil was packed in the plastic pot, and three maize seeds were sown and watered regularly. A week after sowing and the young plant had emerged, culling was done, remaining plant with superior growth was then put on the building terrace. Three levels of molasses concentration, 0, 0.05, and 0.2 g L^{-1} of water, were applied during the experiment. The amount of molasses application was 100-150 ml per pot in every 7-8 days.

Plant measurement

Plant height was measured together with the molasses application. After eight weeks, the plants were harvested and dried in the oven at 80 °C temperature for 72 hours. The chlorophyll content was measured using a SPAD-502 chlorophyll meter at the same time as the molasses application.

Soil analysis

Soil samples were taken during harvesting and preserved at 4°C temperature to keep the soil fresh. This treatment was essential for the analysis of soil enzyme activity. On the other hand, the other part of the soil samples was dried at room temperature (20°C) to analyze labile carbon. Dehydrogenase activity (DHA) was determined by the triphenyl tetrazolium chloride (TTC) method proposed by Veres et al. (2013). 1 ml of soil-water solution (ratio 1:10) was reacted by the TTC in a test tube followed by the incubation at 30°C for 24 hours. The Methanol solution then suspends the enzymatic reaction. The supernatant was obtained by centrifuging the mixture. The DHA was determined using a spectrophotometer at 546 nm wavelength.

β-glucosidase activity was assessed by Sinsabaugh et al. (1999). 1 ml of soil-water solution (1:20) in the three test tubes was prepared. Soil water solution in one tube reacted to PNP-β, while the other two tubes reacted with the Na-acetate buffer. The tubes were kept in the incubator for 2 hours at 30°C, offering the optimum condition for microorganism growth and development. Then, a Tris-hydroxymethyl (aminomethane) (pH 12) and $CaCl_2$ solution were added to the three tubes to eliminate the enzymatic reaction. The supernatant was obtained by centrifuging the tubes. The activity of β-glucosidase was measured spectrophotometrically at 410 nm wavelength.

Labile carbon (LC) was determined by the $KMnO_4$ oxidation method (Weil et al., 2003). First, 1g of air-dried soil was reacted in $KMnO_4$ and shaken. Samples were then measured spectrophotometrically at 565 nm wavelength.

All soil enzyme activity and LC measurements in this study employed Libra S22, UV/Visible spectrophotometer, Biochrom. Ltd, UK.

Data analysis

Two-way ANOVA, Boxplot, and Pearson correlations were performed by IBM SPSS Statistics for Windows software version 27.0 (IBM Corp., 2019). The significant level of the *p-value* of the analysis was <0.05. Before running the analysis, the assumption test was checked for the whole data set, including normality (by Kolmogorov-Smirnov or Shapiro-Wilk test) and the variance heterogeneity. The result suggested that the whole data set was normal, and the variance heterogeneity was "not-violated" with a *p-value* > 0.05, indicating that Tukey's method performed the post hoc test.

RESULTS AND DISCUSSION

The results indicate that the soil biological activity and plant growth response depended on the molasses concentration and soil tillage (Table 1).

Dehydrogenase activity (DHA)

The application of molasses increases DHA in both CT and PT (Fig. 1). The highest DHA has recorded in 0.05 g L^{-1} of molasses application by 0.098 ± 0.014 TPF μg g^{-1} dry soil in CT (80.76% higher than control) and 0.083 ±0.031 TPF μg g^{-1} dry soil in PT (50.59% higher than control). This finding corroborates the previous study by Trevors (1984), who reported that substrate is the important factor driving dehydrogenase activity. The addition of 0.2 g L^{-1} molasses concentration tended to decrease DHA in this experiment despite the fact that it was still higher than the control. Therefore, we inferred that applying high molasses (M2) concentrations were potentially inhibiting DHA. A similar situation was reported by Li et al. (2020) in which the activities of peroxidase (POD) and catalase (CAT) decreased because of the high molasses concentration addition.

Apart from substrate availability, DHA is much affected as well by the soil redox system associated with the soil water content (r=-0.57, *p-value*<0.01), confirming the prior result by (Wolińska and Bennicelli, 2010). Our previous investigation revealed that the minimum soil disturbing in the CT resulted in higher soil water stable aggregates (WSA) than in PT, affecting the soil enzyme activity (Madarász et al., 2021). WSA positively affects the soil's water-holding capacity, WHC (Hallam and Hodson, 2020). Soil with low WHC tends to dry out quickly and induces soil water potential (ψ). The microbial activity will slow down in high soil water potential (Geisseler et al., 2011). In CT application, the water losses with respect to volatilization and infiltration

Table 1 The two-way ANOVA table for means of assessed soil biological activity in the model of pot experiment with tillage system and various molasses concentrations.

Dependent	Independent	df	Mean Square	F	Sig.
Dehydrogenase activity	Tillage system	1	0.001	0.410	0.530
	Molasses concentration	2	0.001	0.572	0.574
β-glucosidase activity	Tillage system	1	0.000	0.001	0.981
	Molasses concentration	2	1.447	4.568	0.025
Labile carbon	Tillage system	1	25255.513	12.725	0.002
	Molasses concentration	2	2758.776	1.390	0.275
Plant height	Tillage system	1	297.510	16.336	0.001
	Molasses concentration	2	15.135	0.831	0.452
Plant biomass	Tillage system	1	8.120	90.679	0.000
	Molasses concentration	2	0.004	0.042	0.959

can be eliminated. Henceforth, this condition offers a conducive environment for soil biological activity.

β-glucosidase activity

The activity of β-glucosidase in CT and PT increases concurrent with the increase of molasses concentration (Fig. 2); however, the significant difference only appeared in PT soil. The application of 0.2 g L^{-1} molasses increased the β-glucosidase activity by 30.43% and 56.57% higher than the control, respectively, in CT and PT, suggesting PT soil was more responsive to the additional molasses. β-glucosidase activity is strongly associated with the quantity and quality of soil organic matter (Ferraz De Almeida et al., 2015). García-Gil et al. (2000) reported that β-glucosidase activity could increase by 100% compared to the control due to adding simple organic substrates.

The higher increase in β-glucosidase activity in PT was probably caused by a lack of energy for the activity of microorganisms, that it amplified by the lower content of total organic carbon in PT of our previous study (Madarász et al., 2021) and the lower LC concentration in the recent study (Fig. 3). Accordingly, the addition of available substrate by molasses directly stimulated the activity of β-glucosidase (Hernández and Hobbie, 2010). This phenomenon also indicated that tillage activity can lead to significant reductions in soil organic carbon. Therefore, in the disrupted soil with low organic matter content (like in PT soil), there is less simple sugar for the microbial population (Stott et al., 2010; Zhou et al., 2021), reducing the β-glucosidase activity.

LC concentration

LC concentration was significantly higher in the CT than in the PT soil. The application of molasses only affected the LC in the CT. The LC concentration increases together with the increase of molasses concentration application. LC concentration in CT increased by 7.61% and 21.23% in applying 0.05 and 0.2 g L^{-1} molasses, respectively.

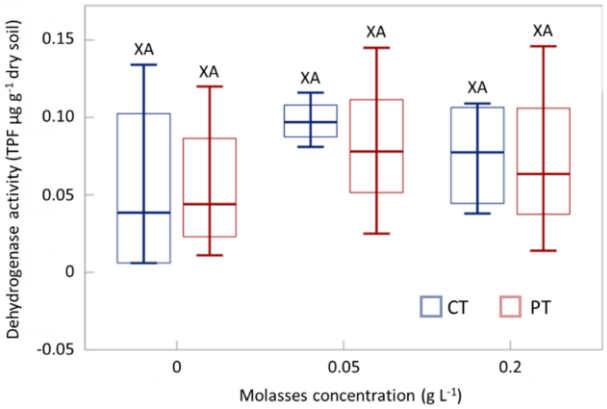

Fig.1 Dehydrogenase activity of soil under different molasses concentrations and tillage systems. Different capital letters (X and Y) indicate significant differences among the molasses concentration (*p-value*<0.05). Different capital letters (A and B) indicate significant differences between the tillage systems (*p-value*<0.05).

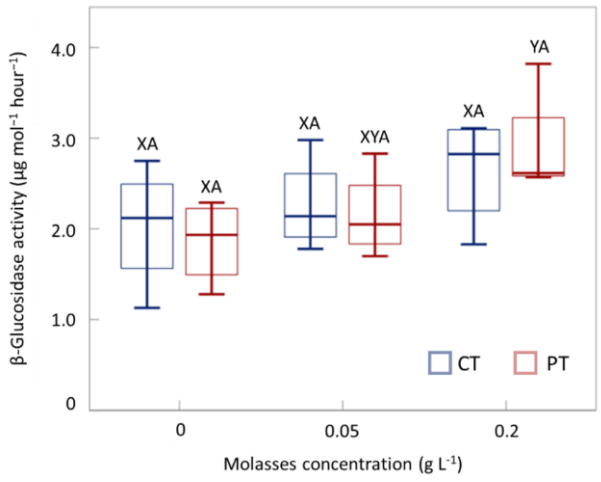

Fig.2 β-glucosidase activity of soil under different molasses concentrations and tillage system. Different capital letters (X and Y) indicate significant differences among the molasses concentration (*p-value*<0.05). Different capital letters (A and B) indicate significant differences between the tillage systems (*p-value*<0.05).

Whereas in the PT, the molasses application did not increase the LC concentration.

LC concentration positively correlates with the soil's organic carbon content (Zhang and Zhou, 2018). In the PT soil, low total organic carbon could not provide enough energy for microbial activity (Madarász et al., 2021). Molasses is a type of simple sugar available rapidly for microorganism activity. As an available substrate, molasses was preferred to be used by the soil microbes. Consequently, the LC concentration was fast depleted, generating insignificant differences between control and soil treated by molasses (Fig. 3). On the contrary, in CT, the source of the available substrate (LC) was not only contributed by the molasses but also by soil organic carbon, resulting in the LC concentration being significantly higher than in PT.

Plant height and dry biomass

Plant height and dry biomass showed a similar trend (r=0.98, *p-value*<0.05), which tended to decline along with the incline of molasses concentration. Plant height and dry biomass in CT were notably larger than in PT (Fig. 4). Molasses application suggests a positive effect on the soil enzymes activity inducing nutrient and carbon cycling in this study; however, the sole application of molasses was not yet able to contribute sufficient nutrition for the plant growth. This result aligns with the result of Waguespack et al. (2022), in which molasses application increased soil microbial activity but not plant nutrition. Another investigation by Expósito et al. (2022) suggested that increasing molasses concentration reduced the weight of dry shoots and fresh roots of tomatoes.

Phytotoxicity and overproduction of reactive oxygen species (ROS) are two possible factors inhibiting plant growth under high molasses concentrations. The critical concentration of inhibition by molasses is different depending on the crop and environment (Li et al., 2020; Expósito et al., 2022).

In this study, we used the chlorophyll content to describe the greenness of the leaf and reflect the nitrogen leaf status (Ali et al., 2017). Our measurement using a chlorophyll meter (SPAD) indicated that chlorophyll content decreased continuously with plant age (Table 2). In addition, the absence of fertilization during the experiment contributed to the low-nitrogen stress, decreasing chlorophyll content and chlorophyll fluorescence (WU et al., 2019).

CONCLUSIONS

In a pot experiment, we investigated the effect of molasses application on soil enzyme activity, LC, and plant growth in the soil under conservation tillage (CT) and conventional tillage (PT). Molasses application stimulated the activity of dehydrogenase and β-glucosidase activity in both CT and PT. PT was more responsive by molasses addition, implying the lack of energy in this soil, confirmed by the constant of LC concentration between molasses application and control. This result infers that molasses application can improve the soil health of disrupted soil, like PT. Molasses application only affected the soil enzymes activity

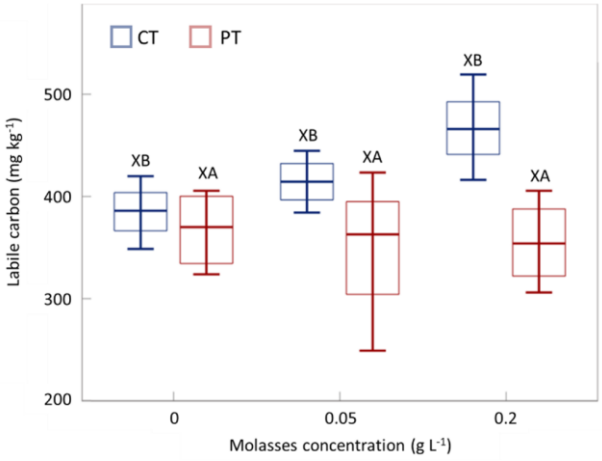

Fig.3 LC concentration of soil under different molasses concentrations and tillage systems. Different capital letters (X and Y) indicate significant differences among the molasses concentration (*p-value*<0.05). Different capital letters (A and B) indicate significant differences between the tillage systems (*p-value*<0.05).

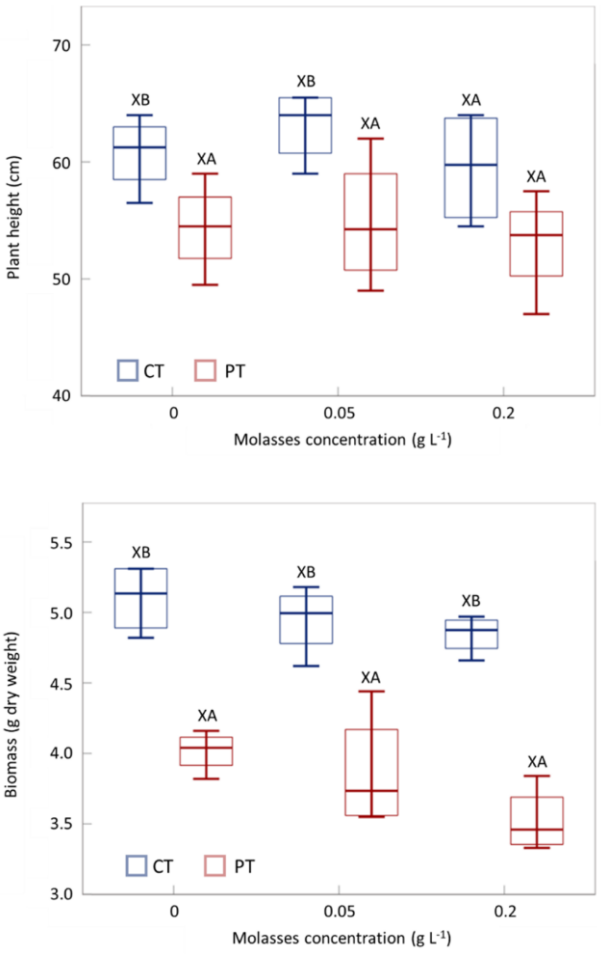

Fig.4 Plant height (up) and dry biomass weight (down) of maize under different molasses concentrations and tillage systems. Different capital letters (X and Y) indicate significant differences among the molasses concentration (*p-value*<0.05). Different capital letters (A and B) indicate significant differences between the tillage systems (*p-value*<0.05).

Table 2 The measurement result of the chlorophyll meter (SPAD) at the initial and the end of the experiment

Molasses concentration (g L^{-1})	Chlorophyll content (SPAD value)			
	CT		PT	
	Initial	End	Initial	End
0	35.1±2.6	23.6±3.3	35.2±0.7	23.4±1.2
0.05	34.5±2.5	23.6±1.6	35.1±0.6	23.5±1.4
0.2	33.1±0.5	23.2±1.0	34.7±0.8	22.1±0.4

associated with the carbon and nutrient cycle. However, it could not improve the plant growth. It infers that the other nutrient source, chemical or organic fertilizer, is still required to supply nutrients for plants. The excess level of molasses application can lead to contra productivity for crops, so it is essential to determine the critical point of molasses application.

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