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Effects of Continuous Cropping on Seed Germination and Seedling Growth, Physiological Characters of Alfalfa

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ABSTRACT

Background: Alfalfa crops are subject to intercalation when grown continuously on the same field, and autotoxicity appears to be another factor in this thinning. Alfalfa fields are often thinned out and have lower yields during consecutive production, and autotoxicity is increasingly found to be a major reason. Hence, Therefore, understanding the seed germination and seedling growth and physiological mechanisms of the alfalfa under continuous cropping system becomes important for sustainable the use of alfalfa grassland.

Methods: The leaves, stem, root and rhizosphere soil of alfalfa (*Medicago sativa* L. cv ZhongMu No.1) were collected to determine the seedling growth germination index (GI) and vigor index (VI), physiological characters malondialdehyde (MDA), proline, soluble sugar (SS), soluble protein (SP), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD).

Result: The germination rate, germination potential, germination index (GI) and vigor index decreased with the increasing extract contents, particularly in leaf extracts. In the extract of plant and soil, the concentration of malondialdehyde altered indistinctly, while the contents of osmo-regulatory substances and the activities of anti-oxidative enzymes generally decreased. Furthermore, the extract increased the seedling length of alfalfa and GI was negatively correlated with seedling growth characteristics.

Key words: Alfalfa, Autotoxicity, Germination, Physiological, Seedling growth.

INTRODUCTION

Alfalfa is an excellent perennial leguminous forage. Because of its high nutritional value, rich in protein, amino acid (Ayhan *et al*. 2016; Avci *et al.* 2018), this crop has already been as one of the important fodder crops in the herbivorous animal husbandry. However, alfalfa fields are often thinned out and have low yields in the consecutive production (Seguin *et al.* 2002). Previous studies have indicated the optimal cultivated years of alfalfa should be not more than 8 years (Li and Huang 2008; Wang *et al.* 2015). This might be caused by soil water depletion, plant diseases and autotoxicity (Seguin *et al*. 2002; Li and Huang 2008). And autotoxicity was increasingly found to be a major reason (Seguin *et al.* 2002; Zheng *et al.* 2019).

Alfalfa autotoxicity is the harmful allelopathy to its growth and development (Seguin *et al.* 2002; Zhang *et al.* 2020; Sun *et al*. 2020). It is caused by the released water-soluble compounds (*i.e.* saponins, canavanine and phenolic acid) from organs and litters of alfalfa (Abdul and Habib 1989; Hall and Henderlong 1989; Wyman-Simpson *et al.* 1991; Miersch and Jiihlke 1992; Yang *et al.* 2009). These water-soluble compounds often inhibit the seedling survival rate, yield and nutritional quality of alfalfa during continuous cropping (Jennings 2001; Chon *et al.* 2002; Rong *et al.* 2016; Liang *et al*. 2021). The inhibiting effect would increase with the increased content of the water-soluble compounds (Chon *et al.* 2002; Rong *et al.* 2016).

Plant functions is guaranteed by cell viability and structure integration which are controlled by plant physiological characteristics. Plants often change osmo-regulatory and anti-oxidative status in response to various stress conditions (Wang *et al.* 2019). Continuous cropping also leads to increase in leaf malondialdehyde content, decrease in leaf activity of peroxidase and catalase (Liang *et al.* 2021). These researches showed a few of clues in finding possible links of seed germination and seedling physiological characteristics, seedling growth conditions. However, there were few researches to describe their possible links in the continuous cropping. So understanding the seed germination and seedling growth and physiological mechanisms of alfalfa under continuous cropping system are important to sustainable use of alfalfa grassland.

This study is aimed to test the hypothesis that continuous cultivation would affect seed germination, seedling growth and physiological characteristics.

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The specific objectives have been to find out above-described parameters.

Materials and Methods

Experimental design

The experiment was conducted in an artificial climate chamber (PGX-380C, Shanghai, China), where the temperature was maintained at 25°C, the light/dark time was 16 hours/8 hours. The fresh samples, *i.e.* leaves, stem, root, and rhizosphere soil of alfalfa (*Medicago sativa* L. cv ZhongMu No.1) were collected randomly in the harvest period on 18 May, 2019. According to the ratio of 1 g fresh samples soaked in 10 ml sterile water, the extract was filtered by a quantitative filter paper and the extract content was 0.1 g/ml. Then the 0.1 g/ml extract were diluted to 0.05 g/ml and 0.01 g/ml by sterile water. The sterile water was used as the control treatment (0 g/ml).

The matured seeds of *Medicago sativa* were picked up and sterilized with 0.1% $KMnO_4$ for 20 minutes, then washed with sterile water. The seeds were soaked in sterile water for 12 hours before processing. Two layers of wet filter paper were laid in the petri dishes (10 cm diameter) and 100 seeds and 10 ml extract were placed slowly in each dish. Subsequently germination, seedling growth and physiological characters were recorded as described.

Measurements

Germination rate (GR) and germination potential (GP) were estimated at day 7 and day 4 by radicle protrusion (*i.e.* appearance of a radicle ≥ 2 mm in length) as the criterion, then the germination index (GI) and vigor index (VI) were calculated (Li *et al.* 2020).

The malondialdehyde (MDA) was measured using a modified thiobarbituric acid method (Puckette *et al.* 2007). The proline was measured using an acid-ninhydrin colorimetric method (Zhang *et al.* 2014). Soluble sugar (SS) was measured using an anthrone method (Sánchez and others 1998). Soluble protein (SP) was measured using a coomassie brilliant blue method (Georgiou *et al.* 2008). Superoxide dismutase (SOD) was measured on the basis of its ability to inhibit the photochemical reduction of nitro blue tetrazolium (Stewart and Bewley 1980). The catalase (CAT) was measured by the method of Iodin titration (Kenten and Mann 1952). The peroxidase (POD) was assayed as the recorded absorbance increased at 470 nm by guaiacol method (Castillo *et al.* 1984).

Statistical analysis

The differences in germination parameters of alfalfa seed, and the seedling growth and physiological characteristics were analysed using One-Way ANOVA. The effects of extract content (Co), extract part (Pt) and their interactions (Co×Pt) were analysed using Two-Way ANOVAs. The linear correlations were analysed with the model y = a*x* + b using SPSS 17.0.

Results and Discussion

Changes in GR, GP, GI and VI

The GR, GP, GI and VI of alfalfa seeds were all significantly affected by extract content, part and their interaction ($P<0.01$, Fig 1). Generally, GR, GP, GI and VI decreased with the increased extract contents, which were also found in the previous studies (Rong *et al.* 2016; Tanha *et al.* 2017; Ghimire *et al.* 2019). But they were shown differently on the extract parts of alfalfa. Overall, the negative effects of extract parts showed leaf > stem > root > rhizosphere soil on seed germination. This finding has been consistent with the finding of (Rong *et al.* (2016). These indicated alfalfa continuous cropping induced the autotoxicity of alfalfa, and firstly and negatively affected the seed germination of subsequent alfalfa. The seed germination has also been found to be affected most adversely in the leaf extract among the four extract parts.

Changes in MDA content

The MDA content in alfalfa seedling was significantly affected by extract content in using two-way analysis of variance at $P<0.05$ (Fig 2). But it was not affect by extract content or parts in using one-way ANOVA at $P<0.05$. This might be that the extract inhibit the germination of weak seeds, and the remaining germinated seeds have high resistance to autotoxicity.

Changes in osmotic adjustment substances (Proline, SS and SP) and enzyme activities (POD, CAT and SOD)

The proline and SP contents in alfalfa seedling were all significantly affected by extract content, part and their interaction ($P<0.01$, Fig 3), while the SS content in alfalfa seedling was only significantly affected by extract content ($P<0.01$).

The SOD activity of alfalfa seedling was significantly affect by extract content, part and their interaction, POD activity was significantly affect by extract content and part, and CAT activity was significantly affect by extract part and Co×Pt ($P<0.01$, Fig 4). Generally, the extract decreased the enzyme activities and the reduced conditions were different in the four parts, *i.e.* the POD and CAT activities were higher in the extracts of root than other parts.

Generally, MDA content is known to increase when a plant encountered an adverse condition (Cavalcanti *et al.* 2004; He *et al.* 2012), while there were no significant changes in the extract treatments of this study. But the reductions of osmotic adjustment substances (Proline, SS and SP) and enzyme activities (POD, CAT and SOD), which would weaken the abilities of scavenging radical oxygen species (ROS) (He *et al.* 2012; Usha and Dadlani 2016). Furthermore, the osmotic adjustment substances, POD and CAT were highest in the extract of root in the study, suggesting the seedling was affected less in the extract of root than in other extract parts of alfalfa.

Changes in the lengths of root, bud and whole seedling

The lengths of alfalfa root, bud and whole plant were significantly affect by extract content, part and their interaction (P<0.01, Fig 5). Generally, the extract enhance the lengths of root, bud and whole plant. During earlier studies (Rong *et al.* 2016; Tanha *et al.* 2017), a reduction in root length, bud length and in the soil extract was found (Yang *et al.* 2009) was noticed but during present studies enhancement was noticed in each aspect. These might be caused by result of the promoting effect of allelopathy in the extract (Li *et al.* 2021) and the effect was shown the most in the root extract in this study.

Liner correlations of germination parameters with seedling growth, physiological characters of alfalfa

Few correlations were found between germination parameters and seedling physiological characters of alfalfa.

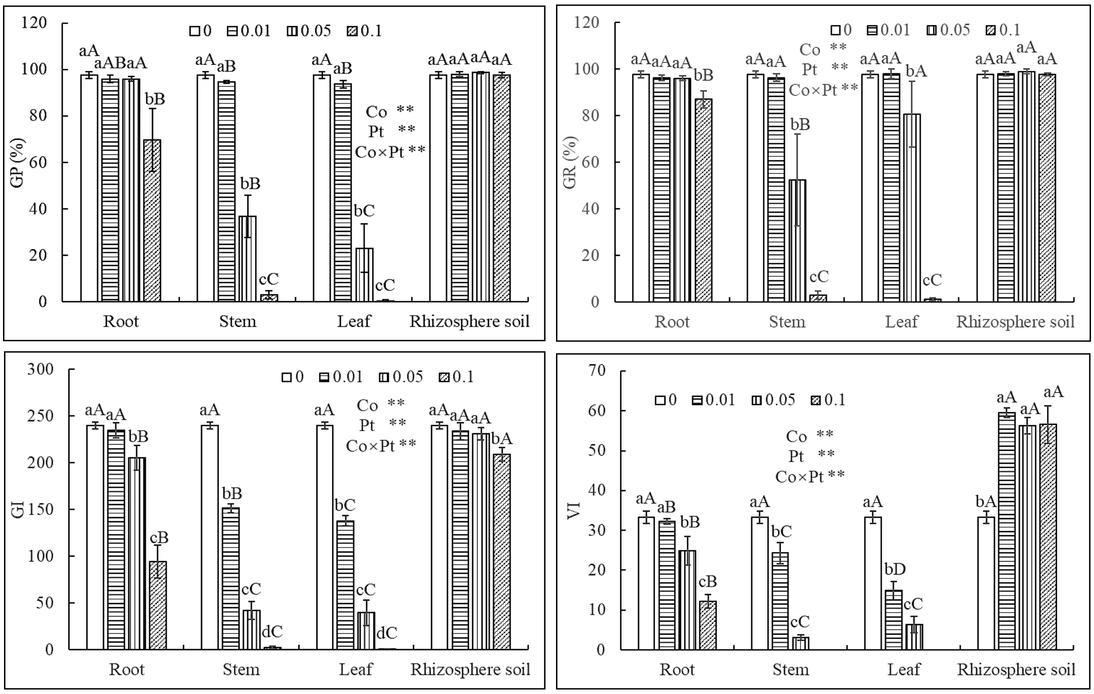

Fig 1: The GP, GR, GI and VI of alfalfa seeds under the four extract contents of root, stem, leaf and rhizosphere soil of ZhongMu No.1. The Co, Pt and Co × Pt refer to the effects of extract content, extract part and their interaction. And the asterisk (*) and the double asterisks (**) represent the significant differences in using two-way analysis of variance at P<0.05 and P<0.01. Different lowercase letters represent significant differences in four extract contents at P<0.05. Different capital letters show significances in four extract parts at P<0.05.

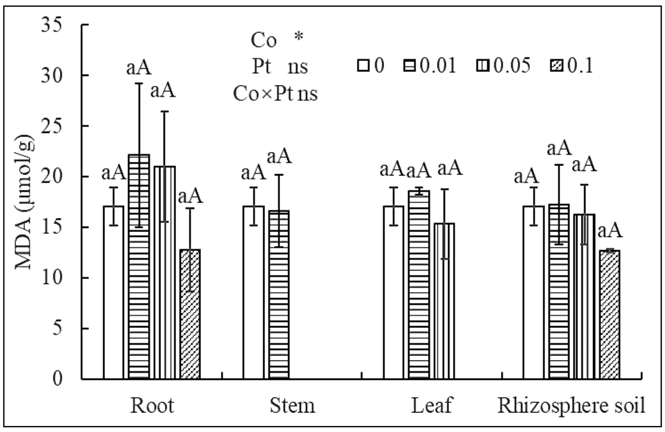

Fig 2: The MDA contents of alfalfa seedlings under the four extract contents of root, stem, leaf and rhizosphere soil of ZhongMu No.1. The Co, Pt and Co × Pt refer to the effects of extract content, extract part and their interaction. And the asterisk (*) represent the significant differences in using two-way analysis of variance at P<0.05. "ns" means no significant difference. Different lowercase letters represent significant differences in four extract contents at P<0.05. Different capital letters show significances in four extract parts at P<0.05.

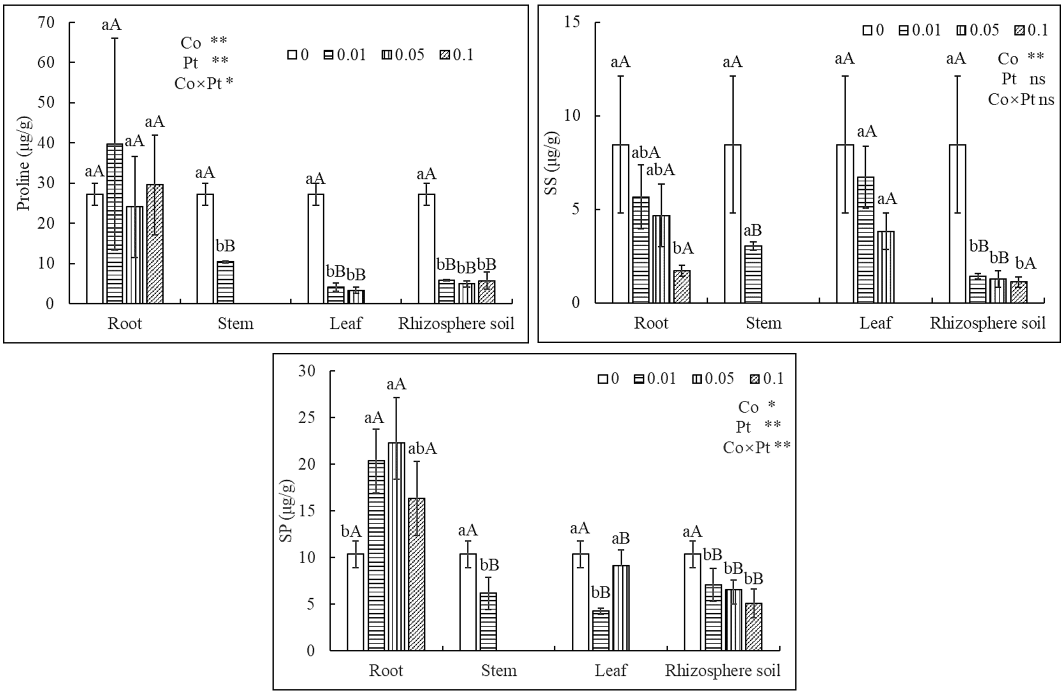

Fig 3: The proline, SS and SP contents of alfalfa seeds under the four extract contents of root, stem, leaf and rhizosphere soil of ZhongMu No.1. The Co, Pt and Co × Pt refer to the effects of extract content, extract part and their interaction. And the asterisk (*) and the double asterisks (**) represent the significant differences in using two-way analysis of variance at P<0.05 and P<0.01. "ns" means no significant difference. Different lowercase letters represent significant differences in four extract contents at P<0.05. Different capital letters show significances in four extract parts at P<0.05.

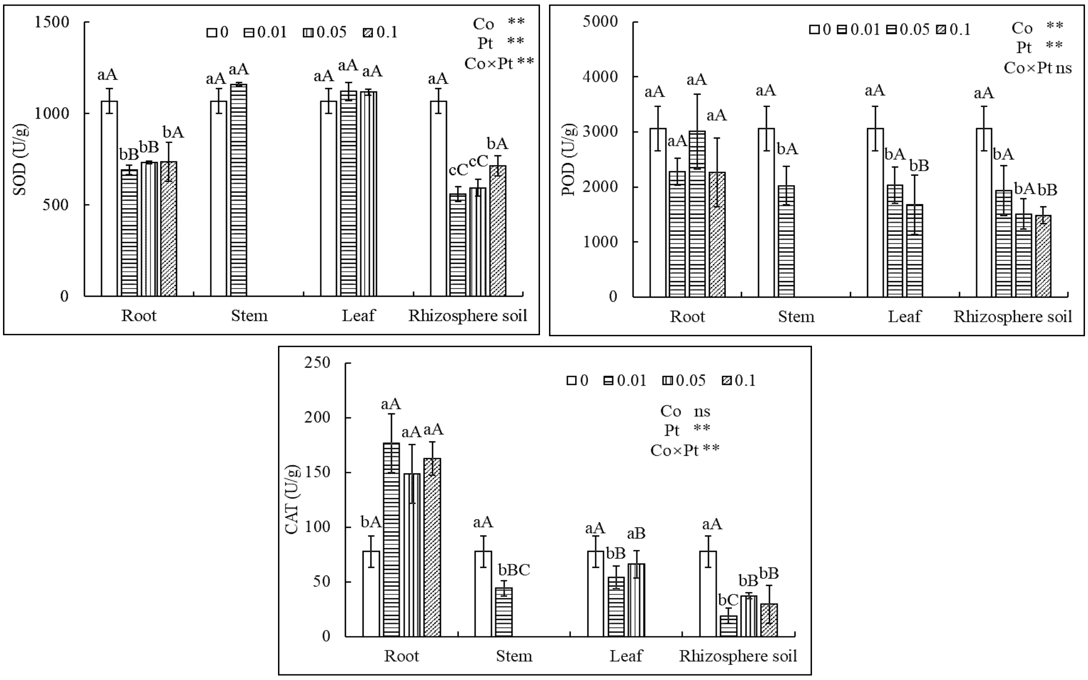

Fig 4: The POD, SOD and CAT activities of alfalfa seeds under the four extract contents of root, stem, leaf and rhizosphere soil of ZhongMu No.1. The Co, Pt and Co × Pt refer to the effects of extract content, extract part and their interaction. And the double asterisks (**) represent the significant differences in using two-way analysis of variance at P<0.01. "ns" means no significant difference. Different lowercase letters represent significant differences in four extract contents at P<0.05. Different capital letters show significances in four extract parts at P<0.05.

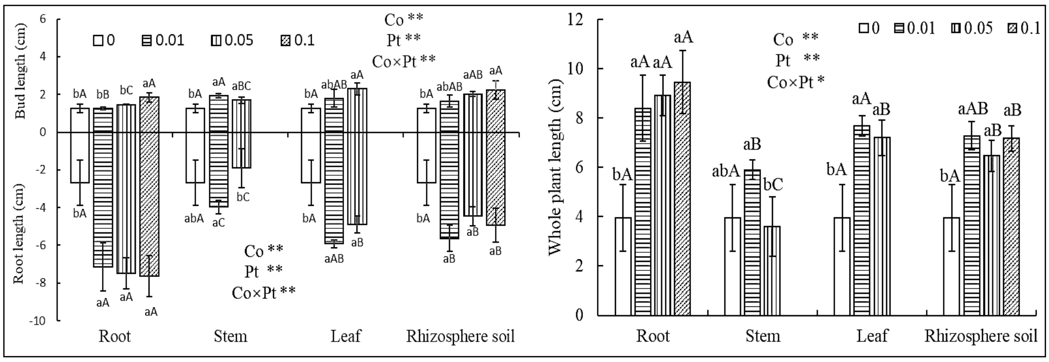

Fig 5: The bud, root and whole plant lengths of alfalfa seeds under the four extract contents of root, stem, leaf and rhizosphere soil of ZhongMu No.1. The Co, Pt and Co × Pt refer to the effects of extract content, extract part and their interaction. And the double asterisks (**) represent the significant differences in using two-way analysis of variance at P<0.01. Different lowercase letters represent significant differences in four extract contents at P<0.05. Different capital letters show significances in four extract parts at P<0.05.

Table 1: Linear correlation coefficients between germination parameters and seedling growth, physiological characters of alfalfa under the extract of four parts.

Parameters	Proline	MDA	CAT	POD	SOD	SS	SP	Length		
								Root	Bud	Whole
GP	0.230	0.251	-0.087	0.294	-0.218	0.196	-0.024	-0.183	-0.461**	-0.266
GR	0.087	0.212	-0.232	0.140	-0.137	0.267	-0.165	-0.208	-0.283	-0.252
GI	0.333*	0.256	-0.088	0.385*	-0.256	0.286	0.063	-0.353*	-0.566**	-0.445**
VI	-0.170	-0.039	-0.477**	-0.189	-0.579**	-0.273	-0.287	-0.213	-0.028	-0.204

The asterisk (*) represents a significant correlation at P<0.05, the double asterisks (**) represent a significant correlation at P<0.01.

And there were negative correlations of germination parameters with seedling physiological characters of alfalfa, expecially the correlations were significant between GI and the length of root, bud and whole plant (P<0.05, Table 1). These indicated that the extracts limited the germination and promoted the seedling growth and seed germination would negatively affect the seedling growth.

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Conflict of interest: None.

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