Hormonal and Enzymatic Responses and Seed Vigor of Coated Maize (*Zea Mays* L.) Seeds with Calcium Alginate in Diesel-Contaminated Soils

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Abstract

The most important problem of oil industries and refineries is the leakage and spraying of oil compounds and products through transportation systems on agricultural soils. Penetration of petroleum compounds in the soil causes the fertility of the agricultural soil to decrease drastically. The seeds of agricultural and garden plants either do not grow at all or do not grow optimally in soils contaminated with petroleum compounds. Seed coating is one of the ways to enhance the plants' seed germination in contaminated soils with diesel. The use of natural materials is preferable to chemicals to prevent the aggravation of environmental pollutants. The main aim of this study was to evaluate the effect of calcium alginate as a coating layer on maize seed for germination in contaminated soil with diesel. This experiment was organized in a two-way ANOVA (4 × 4) in which factor A consisted of four levels of diesel-contaminated soil (0, 2, 4, 6%), and factor B consisted of calcium alginate concentrations (0, 1, 2, 3%). Experimental treatments' effects were studied in a completely randomized design with four replications. Experimental variables included some components of vegetative growth, hormones, and enzymes. The most important results showed that seeds coated with calcium alginate concentrations (1, 2, 3%) under diesel-contaminated soils increased germination percentage, shoot and root lengths, and shoot fresh weight while decreasing the activity of antioxidant enzymes including catalase, superoxide dismutase, as well as malondialdehyde production. Also, the results showed that 2 and 3% of calcium alginate concentrations produced the highest amount of Gibberellin A3 hormone, while these concentrations produced the lowest amount of abscisic acid hormone in the coated seeds under diesel-contaminated soils.

Keywords: Maize, Enzymes, Diesel, Phytohormones, Seed Coating

Introduction

Oil industries and refineries in many countries and oil-rich regions of the world are responsible for the creation and development of oil pollutants in ecosystems. Fertile agricultural soils in oil-rich areas are at risk of oil product leakage and pollution due to the construction of refineries and oil industries, and in case of oil compound leakage and penetration into agricultural

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soils, they lose the ability to produce and grow plants. The relatively high hydrophobicity of petroleum hydrocarbons causes the accumulation of these pollutants in the soil and their deposition relative to the aquatic environment, Koshlaf and Ball (2017). Therefore, it is necessary to create appropriate solutions to eliminate or reduce the effects of soil oil pollutants.

Diesel is one of the reserves in nature with very poor solubility in water, the presence of which in the soil leads to pollution and toxicity. Under natural conditions, the downward movement of diesel is prevented due to adsorption by organic matter on the soil surface, so contamination remains superficially in the soil and root system of most plant species (Yakovleva et al., 2017).

The technique of seed coating dates back to 1928 and was first used by Allen and Stevenson to increase 1000-seed weight and uniform seed distribution in aerial seeding. However the general concept of the word seed coating was first used by Sweet Anion, and the technique developed very rapidly in Western countries, including the United States and Germany (Song et al., 2014).

The British company Germains was established in the 1930s to cover grain seeds and began commercial use in the 1960s for precision arable farming in Europe (Gorim, 2014). This technology involves the application of a very thin layer permeable to the seed, which increases seed yield, improves germination, and ultimately increases yield without harming the environment. This layer can contain fungicides, insecticides plant growth regulators, and fertilizers, Rocha, Ma et al. (2019). Then the use of bacteria in seed coats as biological coatings that were first used in China on rice, wheat, maize, and other grains was developed in other countries (Song et al., 2014; Kimmelshue et al., 2019).

The results of previous research show that if the seed coating is of hydrophilic polymers, it can protect the seed against drought stress and increase the rate of water absorption (Su et al., 2017).

The seeds of some plants are naturally round and large, which makes them easy to grow, but the seeds of some other plants are shapeless, very small, or have different sizes, which disrupts their cultivation in mechanized agriculture the use of seed coating technology solves this problem, while hydrophobic polymers reduce water absorption (Afzal et al., 2020).

On the other hand, several areas around most oil refineries have been left unused due to contamination with petroleum compounds, while these areas can be cultivated through biological treatment of contaminated soils and the use of resistant plants such as maize. Indeed, the economic importance of maize products and their physiological capabilities and resistance to environmental stresses such as stresses caused by oil pollutants can be used as a suitable candidate in soils contaminated with petroleum compounds (Ghalamboran et al., 2020).

One of the most important problems of planting plants in soils contaminated with petroleum compounds (such as diesel) is a sharp decrease in seed vigor rate and percentage. That is, due to the inhibitory effects of petroleum compounds such as toxicity, and hypoxia in the seed growth

medium, the seed vigor process is reduced and may be stopped (Dib and Sadoudi, (2020).

In recent decades, the seed coating technique has been used to increase seed resistance under stress conditions. However, according to reports from companies and seed industries, different coatings are used for maize seeds, that are not of natural origin (e.g., polyacrylamide + Carboxymethyl cellulose (CMC) for film former, ethylene glycol for antifreeze, gelatin for thickener, and Lauryl Alcohol Ethoxylate (LEA-9) as a nonionic surfactant in the composition of seed coatings) and the entry of these compounds into the soil, in the long run, can cause damage to the environment and shoot growth (Pedrini et al., 2017). Therefore, it is necessary to use biocompatible, degradable, available, and affordable compounds for seed coating. In addition, the material used in the seed coating must have high water absorption and retention power so that the increase in the thickness of the seed shell by the coating layer does not reduce the absorption and penetration of water inside the seed.

Calcium alginate is one of the biocompatible polymer compounds, which has the above advantages. The biggest advantage of alginates is their liquid–gel behavior in aqueous solutions. When monovalent ions (e.g., sodium in sodium alginate) are exchanged for divalent ions (particularly calcium), the reaction proceeds almost immediately, changing from a viscosity low-viscosity solution to a gel structure.

Previous results showed that the use of calcium alginate enhanced the activities of several enzymes beneficial for germination like proteases (cysteine proteases (CPs), followed by serine proteases (SPs) and aspartic proteases (APs)), and the germination process improves (Hu et al., 2004). Furthermore, the use of calcium alginate as a biodegradable compound to coat maize seeds, consequently, the coated seeds easily germinated, and if remaining residual layer in the soil would be used by microorganisms (de Castro et al., 2020).

The current research is based on the assumption that the use of calcium alginate biopolymer due to its advantages such as having high water absorption, the possibility of creating a moisture layer around and attached to the surface of the seed, the possibility of accelerating and facilitating the absorption of water by the seed in the germination process, preventing The direct contact of pollutants with the surface of the seed, the reduction of the concentration of pollutants and the possibility of their penetration into the seed, through increasing the thickness of the seed coat, which ultimately improves and increases the germination power of maize seeds under the conditions of soil stress contaminated with diesel substances. Also, the main goal of this research was whether the use of calcium alginate as a layer on the surface of maize seeds protects and improves the processes of maize seed germination and growth under the stress of soil-contaminated soils with diesel. Furthermore, other objectives such as the physiological and metabolic responses of coated and uncoated maize seeds under the stress of diesel pollution were investigated and evaluated.

Material and methods

The commercial diesel provided by the National Iranian Oil Company (NIOC) was used. The diesel components were determined in the lab of NIOC and the details are given in Tables 1 and 2.

Preparation of contaminated soil with diesel

The contaminated soil with diesel was prepared using a modified method according to Ghalamboran et al. (2020). First, the sandy loam (sand 26%, silt 68%, and clay 6% (Table 3)) was washed in a container with tap water and then immersed in 0.5% HCl for 1–2 days. The acid was drained off and then the soil was washed with at least 6–8 times of sterile water. If needed, 1 M of KOH was used to adjust the pH. To prevent the interference of the bacteria in reducing the adverse effects of oil pollutants in the growth environment of the tested plants, the soil was autoclaved for 4–6 hours at 121 °C and 131 kPa. After cooling, first, the soil was mixed with sterilized coco-peat at the rate of 5% by weight of the total soil used, and then, the soil was mixed with different concentrations of diesel (no diesel as the control and 2, 4, and 6 %). Next, 150 g of the contaminated soil was placed in each seedling tray (70 cells).

Coating method of the seeds

The seeds were coated according to the modified method of Kikowska et al. (2011).

Table 1. Details of analysis commercial diesel (light – medium) from National Iranian Oil Company

Description	Result	Method of standard
Color	3	
Density / Kg L^{-1}	0.820-0.860	ASTM D-1298
Distillation		ASTM D-86
Recovery at 375 °C/(minimum) vol%	90	
Final boiling point / (maximum) °C	385	
Flashpoint (minimum)/°C	54	ASTM D-93
Pour point (maximum)/°C	-4	ASTM D-97
Sulphur total (maximum)/mass %	1	ASTM D-1552
Viscosity kinematic at 37.8 °C/ mm^2/s	2.0 – 5.5	ASTM D-445
Cloud point (maximum)	2	ASTM D-2500
Cetan index (minimum)	50	ASTM D-976
Ash/mass%	0.01	ASTM D-482
Water and sediments/vol%	0.05	ASTM D-2709
Carbon residue/mass%	0.1	ASTM D-189
Aliphatic compounds total/%	45-55	
Aromatic compounds total/%	12-15	

The sodium alginic acid was used as a bio-polymer coating, along with calcium chloride (100 mM) with a purity of 95% and a molecular weight of 110.99. To prepare different concentrations of sodium alginic acid (1, 2, 3, 4, 5% by weight), first, different concentrations of sodium alginic powder were weighed (Figure 1). Each weighed sample was then dissolved in 100 ml of distilled water at 40-50 ° C and then the seeds (20 to 30) were mixed manually with this solution in a 500 ml beaker. Then, coated seeds were placed in 100 mM calcium chloride solution by drop-by-drop immersion method for 20 to 30 minutes to perform cation exchanges to harden the calcium alginate coating. Finally, the seeds coated with calcium alginate were placed in the open air for 2 days to dry (Kikowska and Thiem, 2011).

Plant cultivation

The seeds of Maize (*Zea mays* L.) cultivar KSC 260 were obtained from the Seed and Plant Improvement Institute, Iran. The germination seeds test was carried out based on the modified method of Lizárraga-Paulín et al. (2013). The planting of coated seeds and measuring of shoot length, root length, dry weight, and fresh weight of shoots and roots were according to the method of Ghalamboran (2011). The coated seeds were planted in the shoot trays (which were

Table 2. Characteristics of compounds in commercial diesel

Compound	Component	Carbone number	Structure
Aliphatic Hydrocarbons	n-Pentane	5	Straight chain
	Iso-Pentane	5	Branched chain
	n-Hexane	6	Straight chain
	n-Heptane	7	Straight chain
	n-Octane	8	Straight chain
	Iso-Octane	8	Branched chain
	n-Nonane	9	Straight chain
Aromatic Hydrocarbons	Benzene	6	Monocyclic
	Methyl cyclohexane	7	Monocyclic
	Toluene	7	Monocyclic
	Ethyl benzene	8	Monocyclic
	p-Xylene	8	Monocyclic
	o-Xylene	8	Monocyclic
	m-Xylene	8	Monocyclic

Table 3. Characteristics of the soil used in this experiment

Soil Texture	$CaCO_3$ (%)	Na (mg/kg)	K (mg/kg)	P (mg/kg)	N (mg/kg)	EC (µSiemens/cm)	pH	Organic matter
Sandy-Loam	7.43 ± 0.1	3.2 ± 1	63 ± 1	16.5 ± 2	0.19 ± 0.02	143.2	6.8±0.2	3.5 ± 0.5

filled with contaminated soils). Then they were placed in the greenhouse for 22–25 °C day/15–18 °C night cycles under ambient light. Each shoot tray was irrigated with 1500 ml of tap water every day. Seed vigor percentage was determined, 8 days after sowing. The vegetative components of the shoots grown in the greenhouse such as the root and shoot length were determined using a ruler (cm) at 21 days after the planting. Also fresh shoots were weighed.

Extraction of proteins

The sample preparation of protein extraction was carried out according to the modified method of Laing and Christeller et al. (2004). At 21 days after planting the maize in the greenhouse, 0.1 g of each shoot tissue was homogenized over an ice bath with 1500 µL Tris–HCl 10 nM, 0.0186 g of EDTA, and 5.47 g sucrose. The homogenized tissue was then centrifuged for 30 min at 4 °C (13,600 rpm), and the supernatant was used immediately to determine enzymatic activity (Laing and Christeller, 2004).

Quantification of enzyme activity

The activity of Catalase was determined following the adapted protocol by Shi (2005), using 50 mg of fresh shoot tissue grounded for 2 minutes on ice with 2 ml of sodium phosphate buffer (pH 6.8, 0.1 M). The resulting extracts were then centrifuged at 15000 rpm for 10 minutes at 4 °C and the supernatant was utilized to measure the enzymatic activity and the soluble protein content. Subsequently, 200 µl of the supernatant and 100 µl hydrogen peroxidase 0.2 M were added to 1.5 ml of phosphate buffer (pH 6.8, 50 mM). The absorption was determined at 240 nm to assess the activity change, and the enzyme activity was expressed as fresh weight in mg of protein/ min. Guaiacol peroxidase activity was evaluated following the method described by Ruley et al. (2004). Where 100 mg of fresh shoot sample was grounded for 4 minutes on ice with 1 ml of phosphate buffer at pH 7, 0.1M, containing 1 mM of EDTA and 1 mM of PVP 0.1%. The extract was

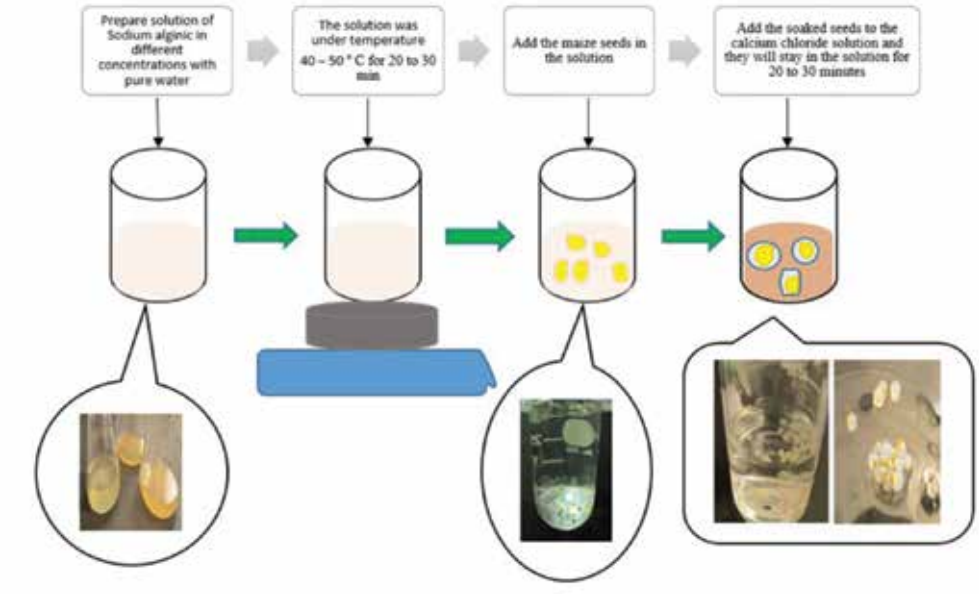

Fig. 1. Schematic of the coating process of maize seeds

then centrifuged at 4000 rpm for 10 minutes at 4 °C, then the supernatant was used to measure enzymatic activity. 100 µl of the supernatant with 450 µl of H_2O_2 (20 mM) and 450 µl of guaiacol (2%) were mixed and the adsorption changes were recorded for 3 min at 510 nm (Ruley, Sharma et al. 2004). Ascorbate peroxidase enzyme activity was measured following the protocol described by Koushesh Saba et al. (2012) with minor adjustments. The procedure were similar to the determination of Guaiacol peroxidase activity, with the exception that 100 µl of the supernatant with 60 µl of H_2O_2 (20 mM) and 850 µl of phosphate buffer at pH 7, 0.1M, containing 1 mM of EDTA and 1 mM of PVP 0.1% were mixed and the adsorption rates recorded for 1 min at 290 nm (Arzani et al., 2012). The SOD activity was measured as recommended by Du et al. (2015) with slight modification using 0.2 g of frozen sample in 3 ml HEPES–KOH at pH 7.8 containing 0.15 EDTA for absorbance at 560 nm. The SOD activity was defined as enzyme content that resulted in 50% nitroblue tetrazolium in 560 nm Du, Liu et al. (2015).

Determination of malondialdehyde content

The malondialdehyde (MDA) concentration as a biomarker for lipid peroxidation was measured according to the modified method of Du et al. (2015). 50 mg of the shoot tissue was frozen with 2.5 ml of 10 % thiobarbituric acid, and the homogenate was centrifuged at 10,000 rpm for 10 minutes. Then, 1000 µl of the extracted supernatant was mixed with 2000 µl of 10% trichloroacetic acid, and it was placed in a Bain-marie with a temperature of 80-90 °C for 30 minutes. Next, the reaction mixture was quickly cooled in an iced bath, and after, it was re-centrifuged at 1000 rpm for 10 minutes. The absorbance of the supernatant was recorded at 450 nm, 532 nm, and 600 nm. The MDA content was calculated using a constant extinction coefficient (ε = 155 mM^{-1} cm^{-1}).

Quantification of Gibberellin A3 (GA3) and abscisic acid (ABA)

The phytohormones GA3 and ABA were determined following the modified method of Delavari, et al. (2017) and Razifah et al. (2014). The coated seeds were germinated up to the emergence of the cotyledonary leaf, and then from each treatment, the emerging seeds were used to measure the hormones at 4 days after planting. This time (4 days after planting) was selected as a stage of the seed vigor duration, the emerged seeds' hormones reached maximum. First, 0.5 g of the seed tissue was homogenized on ice with 10 ml of pure methanol (99%) for 3 minutes. Next, the homogenate was placed in a sonication bath for 2 hours and then was left overnight at 0 °C. The resulting extract was centrifuged at 16,000×g and then filtered through Whatman filter paper No. 42, concentrated by evaporation under dark conditions. Subsequently, it was filtered through 0.22-mM BioFil filters, and 20 mL of the filtrate was analyzed by high-performance liquid chromatography (HPLC) (Knauer, Germany). The system was equipped with an HPLC pump K1001 and a C18 column (5 mm, 250 × 4.6 mm). Phytohormones were eluted at a flow rate of 0.6 mL min¡1 with a concave gradient of methanol acidic water (deionized water containing 0.67% acetic acid, pH 3.0). The gradient was as follows: starting from

80:20 is shifted to 70:30 within 6 minutes, and then further to 60:40 after 15 minutes. Gibberellic acid (GA3) and abscisic acid (ABA) were detected at 220 nm using an ultraviolet detector (PDA, Berlin, Germany) and were quantified by comparison of their retention times and peak area with genuine standards (Sigma) (Razifah et al., 2014; Delavar et al., 2017).

Statistical analysis

The experiment was organized in a two-way ANOVA (4 × 4) in which factor A consisted of four levels of diesel-contaminated soil (0, 2, 4, 6% diesel concentrations), and factor B consisted of sodium alginate concentrations (0, 1, 2, 3%). Experimental treatments' effects were studied in a completely randomized design with ten replications. Experimental variables included some indices of physiological growth such as germination percentage, shoot length, root length, fresh weight of shoots and roots, hormones such as gibberellic acid and abscisic acid, enzymes such as catalase, superoxide dismutase, as well as malondialdehyde activity. Experimental data were first normalized by the Kolmogorov-Smirnov test; the data were then analyzed statistically by using SPSS software version 20. Comparisons of treatment averages were performed with Duncan's test at 99% and 95% probability levels, and also the data were plotted using Excel.

Results

The seed vigor response

The results of the analysis of variance (Table 4) showed that the use of calcium alginate in the production of seed coating could be effective in preventing the negative effects of diesel compound stress on seed vigor percentage and increasing germination percentage in coated seeds. The results showed that firstly, the coating factor had no inhibitory effect on seed vigor compared to control treatment. Secondly, under contaminated soil with diesel, the germination percentage of coated seeds increased compared to the control (uncoated seeds). Among the concentrations (calcium alginate) used in this study, the highest germination percentage was obtained at concentrations of 2 and 3% calcium alginate (Figure 2-A).

The vegetative components of shoot responses

The vegetative components of the shoots, including shoot length, root length, and fresh weight of shoots, were affected by the seed coating factor (Table 4). The vegetative

Table 4. Mean squares of CAT, APX, GPX, SOD, MDA; phytohormones (GA3 and ABA); seed vigor (S.V.), and components of vegetative shoot growth under experimental treatments

Treatments	df	Mean squares										
		CAT	APX	GPX	SOD	MDA	GA3	ABA	S.V.	Shoot Weight	Shoot length	Root length
Coating (C)	3	0.205**	0.639*	0.04308**	0.0801**	64098**	98844.5**	1359264**	2970.2**	8.8**	1633.6**	167**
Diesel (D)	3	0.075**	0.555*	0.05080**	0.03031*	5992.1*	55688.45**	976766.1**	1735.1**	10.5**	878.2**	354.3**
C×D	9	0.078**	0.501 n.s	0.04778**	0.0351**	5822.2*	27683**	805331.8**	1182.5**	6.1**	301.6**	92.5**
Error	64	0.003	0.2	0.00454	0.0075	2095.2	9886.77	130999.4	411.03	1.99	56.45	20.22

* and ** *F* test significance at $P < 0.05$ and $P < 0.01$, respectively; n.s. nonsignificant result

components of the shoot increased compared to the control treatment under diesel-contaminated soil. The highest growth of vegetative components of shoots was related to coated seeds 2 and 3% calcium alginate concentration was used in the production of seed coat (Figures 2b, c and d).

The enzymatic responses to diesel-contaminated soil

According to variance analysis (Table 3), the effect of seed coating could significantly prevent the increase of enzyme activity in the coated seeds (Figure 3a, b, c, and d). By covering the seeds with calcium alginate, the negative effects of contaminants in diesel-contaminated soils on seed growth and consequently shoot growth were prevented. It means that the activity of enzymes such as the CAT, the GPX, the APX, and the SOD did not increase compared to the control under the contamination levels. Also, based on the results, it seems that the coating layer added to the surface of the maize seeds probably prevents the penetration of pollutants into the seed, because the activity of enzymes in the coated seeds was not stimulated and this response is opposite to the reaction of the control treatment (seeds without coating), therefore the difference in enzyme activity in coated and uncoated seeds can be a reason for the possibility of no penetration of pollutants. Among the concentrations of

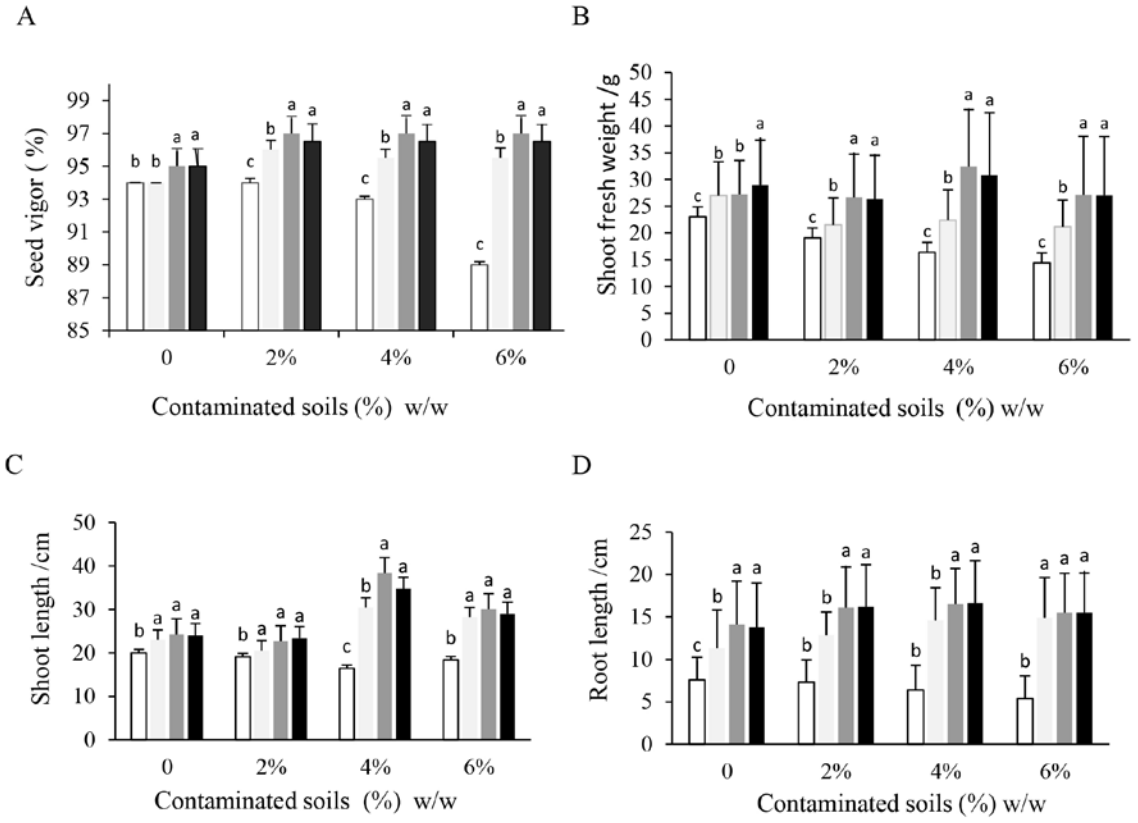

Fig. 2. Response of average seed vigor(A), shoot fresh weight (B), shoot length (C) and root length, and (D) by contamination levels and alginate calcium concentrations (white as uncoated seed (control), light gray as 1%, dark gray as 2% and black as 3% of alginate calcium). Lowercase letters denote a significant difference at $p < 0.01$. Error bars denote standard error

calcium alginate used for the production of the coating layer, 2 and 3% calcium alginate concentrations had the greatest effect in preventing the increase in the activity of antioxidant enzymes.

The malondialdehyde response to diesel-contaminated soil

At each level of gasoline pollution in this experiment, the production of malondialdehyde (MDA) was significantly increased only in the control treatment compared to other treatments (Table 4), and also the results showed that the effect of using calcium alginate as a layer on maize seeds could reduce MDA levels in coated seeds. In other words, by covering the seeds, lipid peroxidation can be reduced under diesel contamination (Figure 4a).

The phytohormone's responses to diesel-contaminated soil

Analysis of variance (ANOVA: Table 4) was used to determine the influence of seed coating on the produced phytohormones in germinated maize seeds under diesel-contaminated soil. The production of GA3 in the coated seeds increased compared to the control treatment in the diesel-contaminated soils (Figure 4b). Also, the use of 2% and 3% calcium alginate in the preparation of a coating on maize seeds, increased the production of GA3 in seeds compared to other treatments. Also, the results showed that the amount of produced ABA in the coated seeds was less than the control

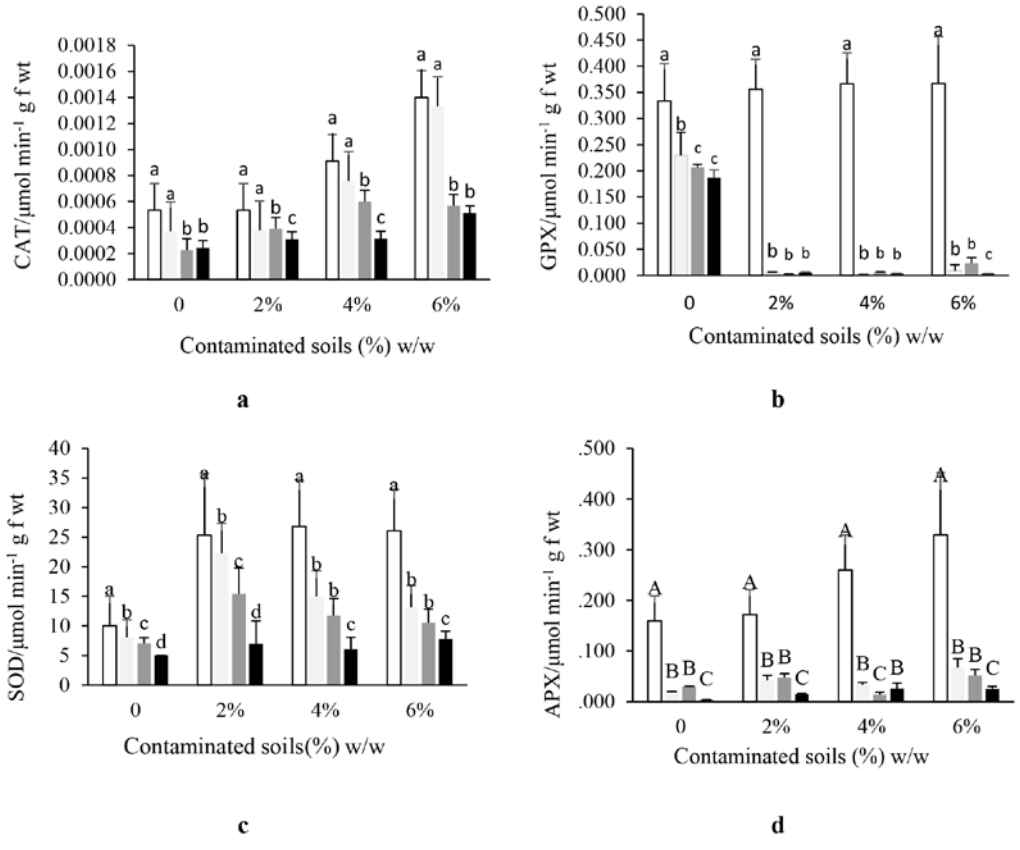

Fig. 3. Response of average CAT (a), GPX (b), SOD (c), and APX (d) by contamination levels and alginate calcium concentrations (white as uncoated seed (control), light gray as 1%, dark gray as 2% and black as 3% of alginate calcium). Capital letters denote a significant difference at $p < 0.05$, and lowercase letters denote a significant difference at $p < 0.01$. Error bars denote standard error

treatment (Figure 4c).

Discussion

Based on the results of the present study, the seed coating technique could create many benefits for the development of plant cultivation in contaminated soils with diesel. However, the results of previous research have mentioned the benefits of coated seeds,

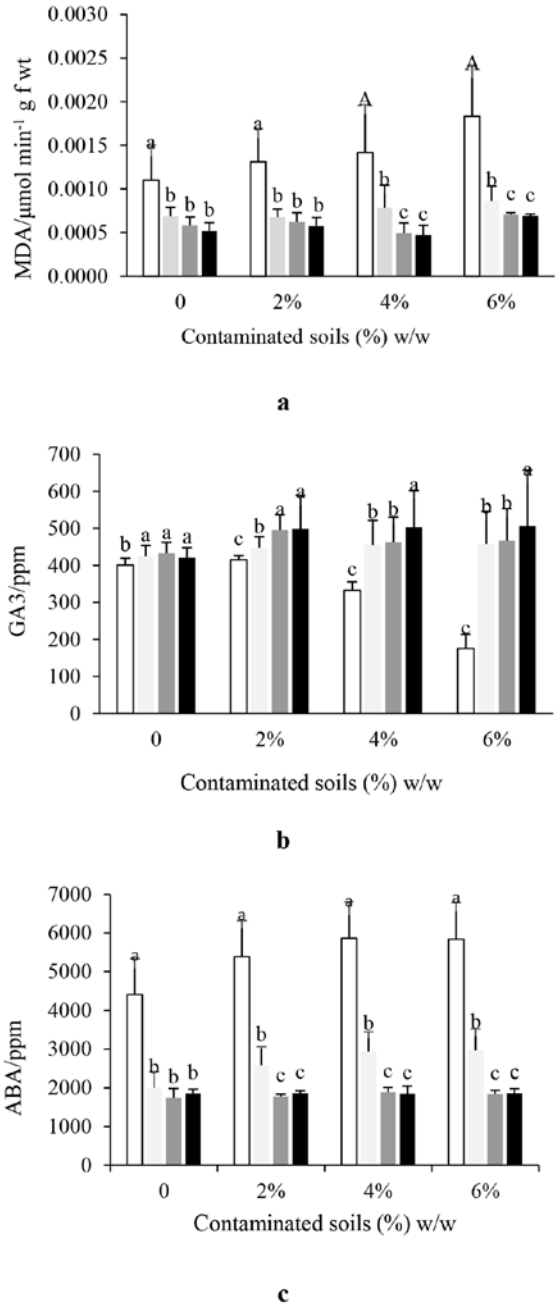

Fig. 4. Response of average MDA (a), GA3 (b) and ABA (c) by contamination levels and alginate calcium concentrations (white as uncoated seeds (control), light gray as 1%, dark gray as 2% and black as 3% of alginate calcium). Capital letters denote a significant difference at $p < 0.05$, and lowercase letters denote a significant difference at $p < 0.01$. Error bars denote standard error.

and these benefits are mainly to create uniformity of seed dimensions for ease of use in seeders' machines, and as fungicides, soil pesticides, and nutrients, all of which increase germination percentage and seed vigor, especially for small seeds Ehsanfar and Modarres-Sanavy (2005).

However, the point to consider in this study was the use of seed-coating techniques to counteract the inhibitory effects of diesel compounds on seed growth in contaminated soils. In addition, one of the most important reasons for choosing calcium alginate compound to use as a coating on the maize seeds surface was, first, the ability to store and absorb water for seed use in the germination process, to avoid contact with pollutants on the surface of maize seeds, and third, firming up the oxygen needed to continue metabolic and hormonal activities (He et al., 2018; de Castro et al., 2020). Because the molecular structure of calcium alginate can absorb water, it can play a positive role in solving the lack of water and oxygen needed for germination and metabolic processes of seeds under the stress of diesel pollution, and as a result, the coated seeds will have a better growth and germination process (Khan et al., 2011). Therefore, two important roles can be considered for the coating layer of calcium alginate: firstly, it prevents the penetration of pollutants into the seed, secondly, it delays the entry of pollutants, and these roles depend on the molecular structure of the pollutants.

The entry of diesel compounds into the soil and then into the seeds and/or roots of plants causes a series of problems in an environment of plant growth. For example, the penetration of diesel compounds into the soil, via sticking and covering the surface of soil particles and penetrating the soil pores, causes dehydration and decreases the flow of water and oxygen in the seed bed and root growth environment. In other words, a decrease in soil aeration, a decrease in the percentage of soil pores, an increase in the apparent density of the soil, a decrease in the flow of oxygen in the rhizosphere, the process of seed germination and seed vigor disturbed. In addition, when the diesel pollutant comes into contact with the surface of the seeds, the possibility of absorption and penetration of the pollutant inside the seed increases, which causes toxicity in the seeds and aerial organs (Han et al., 2016).

However, there are several ways to overcome the problems caused by petroleum contaminants in soils: first, cleaning and removal of contaminants in the environment through chemical and biological methods, second, the use of resistant cultivars to stresses caused by oil pollutants, and third, the use of seed coating technique for planting in contaminated soils. The results obtained from this study could confirm the third strategy that the use of calcium alginate, reduced part of the negative effects of diesel contaminants in the soil on the seed vigor process and shoot growth of maize. Therefore, according to the obtained results, coating maize seeds and then planting them in diesel-contaminated soils was an effective action to prevent the penetration of contaminants into the seeds and shoots. As the results showed, the reduction of seed germination in the control treatment compared to the coated seeds indicated

the ability of the calcium alginate layer to protect the seeds and reduce the inhibitory effects of contaminated soil on the growth and germination of maize seeds.

Among other noteworthy points based on the results of this research was the difference in the effect of calcium alginate used in making the coating layer of the seeds. In fact, the use of optimal concentration is an important factor in the emergence of the ability to protect corn seeds against factors that prevent germination and plant growth in soils contaminated with diesel compounds. Furthermore, an effective concentration of calcium alginate in the construction of the coating layer can have a positive effect on the ability of the coating layer against factors that inhibit seed germination and vigor. In fact, the optimal concentration means creating a sufficient thickness around the seed so that the amount of water retention meets the water requirement of the seed in the condition of soil pollution and also prevents the possible penetration of diesel pollutants by preventing the direct contact of pollutants with the surface of the seed. Therefore, the results obtained in this study showed that almost the most positive results were obtained using concentrations of 2 and 3%, and these coating layers did not allow for an increase in the activity of antioxidant enzymes as well as the production of ABA hormone. On the contrary, the use of these coatings (2 and 3% of alginate calcium) could increase the production of GA3 as an effective factor in seed growth and seed vigor under diesel-contaminated soil.

In conclusion, the use of calcium alginate for coating maize seeds was able to improve the germination percentage and vigor potential under diesel contamination soils. Thus, the approach of coating seeds can be used for other pollutants in contaminated soils. Of course, before using this approach there needs to survey of interaction responses between the type of pollutant and soil, and also properties of morphological plant seed and desired coating materials.

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