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The Impact of Cadmium on Seed Germination, Seedling Growth and Antioxidant Enzymes in Pea (*Pisum sativum sp*.)

Shahida Hamid[1], Munna Singh[2]

[1]Research scholar, Department of Botany, Faculty of Science, University of Lucknow, Lucknow, U.P, INDIA

[2]Professor, Department of Botany, Faculty of Science, University of Lucknow, U.P, INDIA

ABSTRACT

In the present study, a noval approach has been made to evaluate the effect of cadmium in pea in terms of germination, seedling growth, pigment development and relevant enzymes activity. Pea (*Pisum sativum sp*.), an important pulse crop consumed by humans, was selected as a test plant. During the present investigation pea seeds were grown in petridishes on filter paper in triplicate containing different concentration of cadmium viz., 1.0, 2.0, 4.0, 8.0 and 16.0 ppm respectively. Changes in the physiological and biochemical activities were observed. At the high cadmium concentration, germination percentage was decreased as compared to control. There was also observed considerable reduction in shoot length, root length along with the number of lateral roots.The fresh weight, dry weight and moisture content were also found reduced with higher concentration of cadmium. Declined chlorophyll contents were noticed under the influence of higher concentration of cadmium. There was marked increase in peroxidase ,catalase and lipid peroxidase activity by the application of the test chemical was observed in different concentration of cadmium. The results suggest that the activities of peroxidase ,catalase of pea (*Pisum sativum sp*.), seedlings are inhibited under cd stress affecting their growth.

Keywords*-----** Cadmium, seedling growth, photosynthetic pigments, and antioxidative enzymes, ***Pisum sativum sp.

I. INTRODUCTION

Cadmium (Cd), a non-essential element, is among the most hazardous environmental pollutants for humans, animals and plants even at low concentrations (Benavides et al., 2005; Mobin and Khan, 2007; Wahid and Ghani, 2008). Cd is not an essential nutrient for plants, and it can accumulate at higher levels in aerial organs (Wang et al., 2007; Zou et al., 2008), inducing phytotoxicity manifested in leaf roll, chlorosis, growth reduction, and eventually death (Benavides et al., 2005; Zou et al., 2008, Goel, S. 2012.). It has been demonstrated that Cd affects a wide range of physiological and metabolic activities in plants: for example, Chl *a* and *b* content (Mobin and Khan, 2007), the activities of photosynthetic carbon reduction cycle enzymes (Burzyński and Zurek, 2007), mineral distribution (Wang et al., 2007; Zou et al., 2008), photosynthetic processes (Zou et al., 2009; Chen et al., 2010) and oxidative stress (Markovska et al., 2009). The intensity of the effects depends on the species, metal concentration and duration of exposure (Benavides et al., 2005; Zou et al., 2012).

II. MATERIALS AND METHODS

Seeds of pea were surface sterilized with 0.1% HgCl2 followed by three rinses in sterile distilled water and germinated in Whatman No. 1 filter paper for 15 days at 25±20C. Cd treatment in the form of CdCl2 was given with distilled water at 1.0-16.0 ppm concentrations. After 15 days of treatment, the plants were harvested for various physiological and biochemical estimations.

III. GROWTH AND PHOTOSYNTHETIC PIGMENTS ANALYSIS

Growth was studied by measuring the shoot length, root length leaf no and lateral root as shown in **Fig.1.** The pigment of leaves was estimated by following the procedure of (Arnon, 1949; Lichtenthaler and Wellburn, 1983). 100 mg of fresh leaf tissues was ground in cold pestle and mortar with 10 ml 80% acetone, and centrifuge at 10000×g for 10 min and blank serves as 80% acetone. Absorbance of supernatant was measured at 480,

510, 645 and 663 nm and using spectrophotometer (Systronic UV-Vis 118). The concentration of chlorophyll a and b along with total chlorophyll was calculated according to the following formula and expressed as mg / g fresh weight of leaves.

IV. LIPID PEROXIDATION ANALYSIS

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation by the method described by Heath and Packer (1968). Plant tissue was homogenized in TCA. The homogenate was centrifuged at 15,000 g for 5 min. To the Supernatant, 0.5% of TBA was added. The mixture was heated at 95°C for 30 min. and then quickly cooled in ice bath. After centrifugation at 10,000 g for 10 min. The absorbance of the supernatant was recorded at 532 nm. Lipid peroxidase activity was expressed in terms of µmol MDA g^{-1} Fresh tissue.

V. CATALASE ANALYSIS

Catalase activity was determined by the Euller and Josephson (1927) method. 2 ml of potassium phosphate buffer (pH = 7.0), 1 ml distilled water, 1 ml enzyme extract (2.5%) and 1ml H_2O_2 (0.5%) in test tubes were added and incubated for 10 minutes. After 10 min 2 ml of 4 N H_2SO_4 was added to stop the reaction. For blank 2 ml of 4 N $H_2SO_4$was added prior to the addition of 1 ml H_2O_2. After ten minutes the final volume of both blank and sample was titrated against 0.01 N $KMnO_4$ with the help of burette and 100 ml conical flask. Catalase activity was expressed in terms µmol decomposed H_2O_2 g^{-1} fresh weight of tissue.

VI. PEROXIDASE ANALYSIS

Peroxidase activity was determined by the method of Luck (1963). 2 ml of Potassium phosphate buffer (pH = 6.0), 1 ml distilled water, 1 ml p-phenyl diamine, 1ml H_2O_2 (0.5%) and 1 ml enzyme extract(2.5%) in test tubes were added and incubated for 10 minutes. After 10 min 2 ml of 4 N H_2SO_4 was added to stop the reaction. For blank 2 ml of 4 N $H_2SO_4$was added before adding enzyme extract. The final volume of both blank and sample was centrifuged at 4^0C at 5000 rpm for 10 minutes and optical density was read at 485 nm wavelength. The peroxidase activity was expressed in terms of ΔOD/gm fresh weight of tissue.

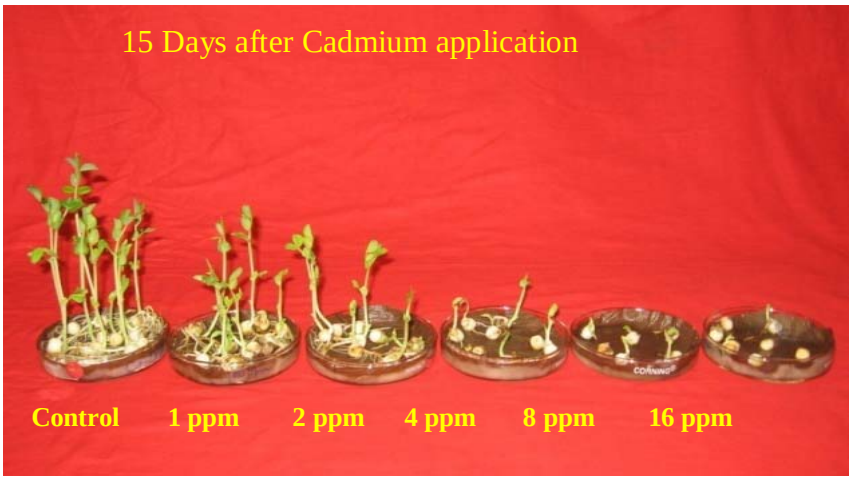

Fig.1. Influence of differential cadmium levels on shoots and root biodynamic in *Pisumsatvum* var. Arkel seedlings. A and B indicate acquisition of root and shoot canopies as affected by cadmium treatment levels (1, 2, 4, 8 and 16 ppm) coupled with cadmium treatment durations as indicated.

VII. RESULT

The root and shoot lengths were recorded in pea as influenced by cadmium contaminated irrigation water (1, 2, 4, 8 and 16 ppm). Both shoot and root have been found to be down-regulated (31-89 and 58-89% in relation to levels of cadmium. The lower level of cadmium application (1 ppm) cause effectively to down-regulate root and shoot growth. Upon, increase in cadmium levels i.e., 1, 2, 4, 8 and 16 ppm, the root and shoot both have been found severely down-regulated in case measurements were made 15 days after the application of cadmium as compared to control. The lower level of treatment (1 ppm) followed parallel trends with much accuracy. Nearly, 31% loss in shoot length could occurred in case seeds were treated with 1 ppm of cadmium till the termination of the observation (15 days). The 2, 4, 8 and 16 ppm cadmium

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application could down-regulate the shoot length ca. 43, 56 and 78and 89% as compared to control **(Fig.2A and B).** Similarly, the down-regulation in root length was found ca. 58, 70, 74, 82 and 89% (1, 2, 4, 8 and 16 ppm) cadmium treated respectively in case cadmium treatment was allowed for 15 days. The number of leaves and lateral roots shown similar trends as influenced by treatment levels and durations both. The loss in leaf number and lateral roots (21-79 and 42-84%) occurred in case treated with 1, 2, 4 and 16 ppm cadmium for 15 days, in abled seedlings for growth. The effect of cadmium treatment of total number of leaves on per seedlings basis. The higher levels of cadmium (16 ppm) could cause loss in leaf reduction to the tune of ca. 79% as compared to control as shown in **Fig. 2C,D.**

The photosynthetic pigments i.e., chlorophylls a and b was found to be affected. The chl b was found more significantly affected as compared to chl a. About 31% chlorophyll a and 46% chlorophyll b (1 ppm) were found down-regulated in pea seedlings, which could reach about 76% and 93% (16 ppm) after 15 days of cadmium. Similarly total chlorophyll was also found to be down-regulated in pea, which could also reach about 79% (16 ppm) after 15 days of cadmium. The loss in carotenoids was also observed. The values of carotenoids have shown down regulation ca. 29-65% (1, 2, 4, 8 and 16 ppm) depending upon treatment levels within 15 days as shown by pea seedlings was recorded **(Fig. 3A-D).**

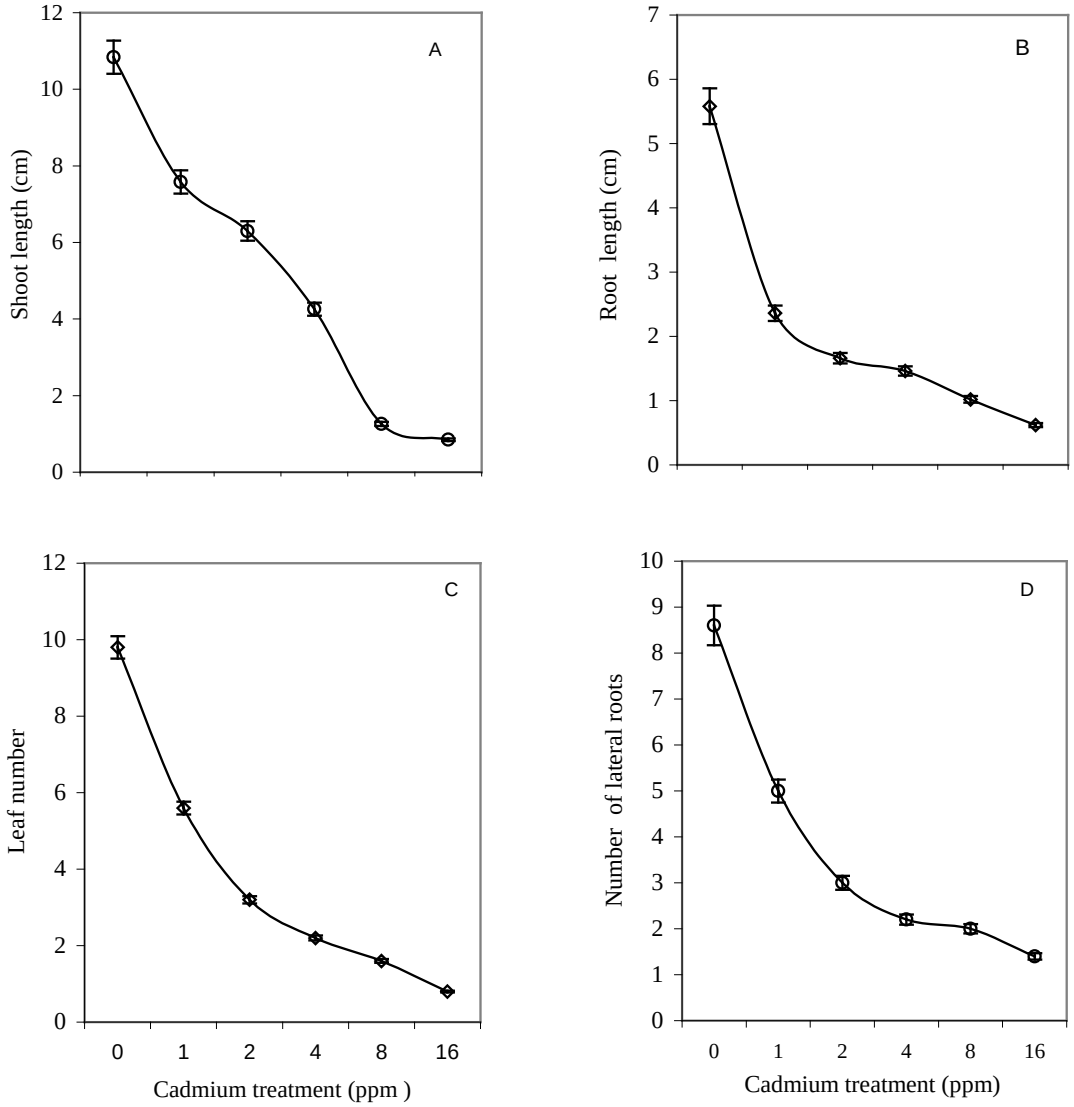

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Fig.2. Effect of cadmium on shoot length (A), root length (B), number of leaves (C) and number of lateral roots (D) in *Pisum sativum* var. Arkel. The seedlings were allow to grow till 15 days after application of various concentrations (1, 2, 4, 8 and 16 ppm). Values are mean (n=3) with S.E. (±).

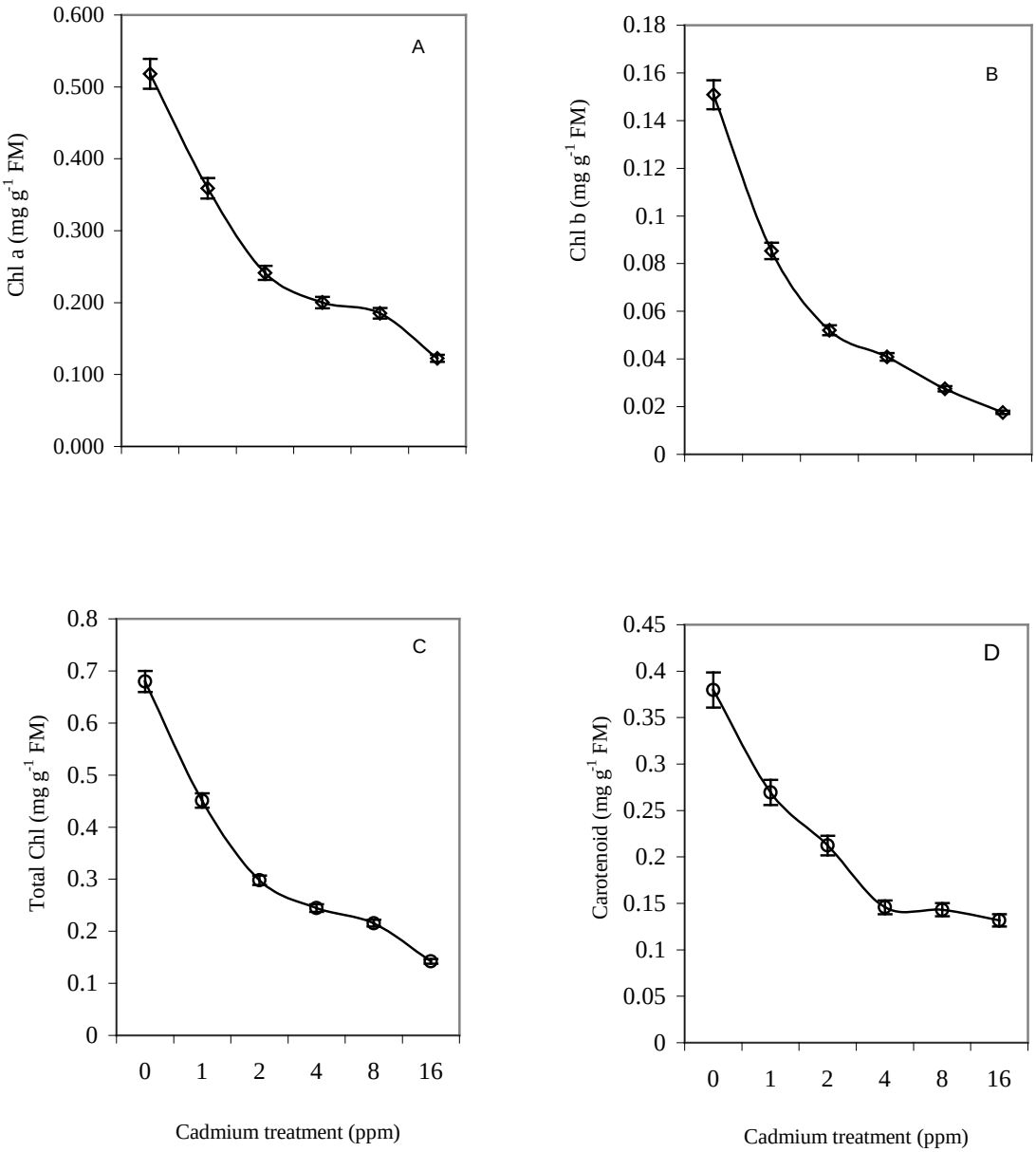

Fig.3. Effect of different cadmium concentrations (1, 2, 4, 8 and 16 ppm) on photosynthetic pigments in *Pisum sativum* var. Arkel. Chl a (A), chl b (B), total chl (C) and carotenoid (D). Cadmium treatment maintained for a period of 15 days. Values are mean (n=3) with S.E. (±).

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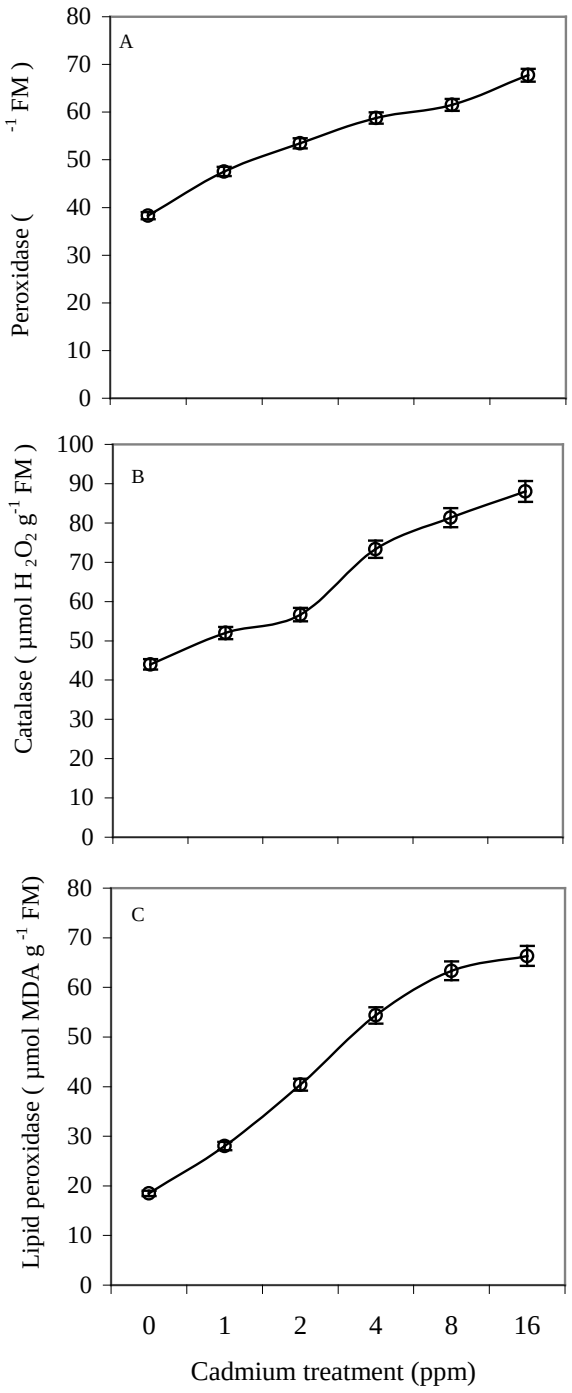

Fig.4. Effect of cadmium contaminated water on enzymes activities of peroxidase (A) catalase (B) and lipid peroxidase (C) in seedlings growth. *Pisum sativum* var. Arkel seedlings were exposed to 0, 1, 2, 4, 8 and 16 ppm Cd for a period of 15 days. Values are mean (n=3) with S.E.(±)

The effect of cadmium treatment in pea seedlings was also correlated with certain stress inducible enzymes such as peroxidase and catalase. These enzymes genes generally gets switched on during adverse experiences by plants as shown in **Fig. 4 A and B.** Almost there intrinsic abilities in relation to increase in peroxidase (24-76%) and (18-100%) catalase are correlated with cadmium (1 ppm-16 ppm) levels. Both these enzymes are stress mitigating biomolecules therefore; biologically both of them have

behaved as per biological rule in supporting the biological system. Similarly lipid peroxidase activity could enhanced ca. 258% higher in the case of leave in case treated with 16 ppm cadmium contaminated water for a period of 15 days **(Fig. 4C).**

VIII. DISCUSSION

Cadmium (Cd) is one such environmental toxicant, which persists and prevails as toxic heavy metal among animals and plants (Sanita di Toppi and Gabbrielli, 1999; Raza and Shafiq, 2013).

Increasing the concentration of $CdCl_2$ during the germination stage had suppressed the seed germination of *B. rapa* as found also by Asgharipour *et al*. (2011), Shaimma *et al*. (2012), Heidari and Sarani (2011).

Shan *et al.* (2012) reported that high Cd concentrations resulted in a reduction in seedlings growth, expressed as shoot and root length, induced root browning in peanut.

Hou *et al*. (2007), Katazyna and Smolik (2011) obtained a decreased amount of chlorophyll a, chlorophyll b and carotenoids in *Lemna minor* after the application of a Cd treatment. Erdei *et al*. (2002) recorded a higher decrease in the content of chlorophyll in barley after the application of cadmium as the shown in **Fig. 3 A-D**.

The peroxides produced in response to oxidative stress are converted to water by the antioxidant enzyme catalase, thus preventing membrane damage (Polidoros and Scandalios, 1999; Gill *et al*., 2011). The increase in peroxidase can develop a physical barrier in the cell by increasing lignin biosynthesis resulting in thickening of tissues, hence protecting the cell from ROS damage (Hegedus et al., 2001; Shanmugaraj *et al*., 2013). Our result clearly correlated with the results reported in *Brassica juncea* (Gill *et al*., 2011; Shanmugaraj *et al*., 2013), sunflower cotyledons (Gallego *et al*., 1996) and radish (El-Beltagi *et al*., 2010) as the shown in **Fig. 4 A**.

Cadmium chloride treatments effectively increased the activity of the peroxidase enzyme (POD) in *B. rapa* leaves (Shaimma *et al*., 2012).The increase in CAT activity after Cd treatments may be due to the scavenging role of CAT to H_2O_2, which could be quenched by the induction of specific enzymes like CAT (Elstner *et al*., 1988) as shown in **Fig.4B**.

Shafi *et al*. (2010) reported that MDA content was increased under salt and Cd stress in wheat plants *Soybean* (Aksoy and Dinler, 2012) as the shown in **Fig. 4C**. Although Cd does not generate ROS directly, it generates oxidative stress via interference with the antioxidant defense system (Shi *et al*., 2010) and increases MDA content in plants due to increased lipid peroxidation.

IX. CONCLUSION

Consequently our findings as reported have extended an overview about pea cultivation under the influence of differential levels of the cadmiu . It is found that pea may be preferred to be cultivated in agro-climate areas either free from cadmium or may be less affected to ensure crop productivity in relation to national economy (socio-economy) and food safety security for the masses.

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