SYNOPSIS ON

Pre-dissertation

(AGR 596)

"Role of polyamines and mycorrhiza for the mitigation of cadmium toxicity in pea (*Pisum sativum)*"

Submitted To

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UNDER GUIDANCE OF

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CERTIFICATE

This is to certified that this synopsis entitled **"Role of polyamines and mycorrhiza for the mitigation of cadmium toxicity in Pea (*Pisum sativum*)"** is submitted in partial fulfilment of requirements for degree – Masters of Science in Agronomy by **Nada Jyoti, Registration no. 11715464** to Department of Agronomy, School of Agriculture, Lovely Professional University, has been formulated and finalized by the student herself on the subject.

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DECLARATION

I hereby declare that the project work entitled --**"Role of polyamines and mycorrhiza for the mitigation of cadmium toxicity in pea (*Pisum sativum*)"** is an authentic record of my work carried at **Lovely Professional University** as requirements of Project work for the award of degree -Masters of Science in Agronomy, under the guidance of **Dr. Prasann Kumar,** Assistant Professor, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India.

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INTRODUCTION

Deprivation of accepted resources is perhaps one of the lethal lapses mankind has ever prepared in its voyage of progress and civilization. All the natural resources are contaminated with lethal lapses. Among them the land and water resources are worst affected and under continuous stress with both biotic and abiotic, due to anthropogenic interventions. If we talk about the soul of infinite life, i.e., soil then it seems to appear that the primary recipient by design or accident of a myriad of waste products and chemicals used in modern society. Soil contamination can be defined as the, addition of any substance to the soil that may exert adverse effects on its functioning and capacity to yield a crop. Contamination of heavy metal is one of the major concern due to well-known reports emanating both from India and abroad. Various diseases and disorders have been observed both in human and livestock due to metal toxicity. A scientist reported that, the greatest problems will arise due to the involvement of mercury, cadmium, lead, chromium, arsenic, nickel, etc in anthropogenic activities. To a greater or lower degree all of these elements are toxic to humans and any others animals. Cadmium is extremely toxic, causing heart and kidney disease, bone embrittleness; Cr, Ni, and Pb are moderately so which are responsible for mutagenic, lung cancer, convulsion and brain damage like some deadlier diseases.

Pisum sativum also known as the Garden pea belongs to the family Fabaceae. Pea is an annual herbaceous plant with a life cycle of one year. It is believed to have originated from the Mediterrean region, Central and Western Asia and Ethopia. It is the cool season crop which is cultivated throughout the world. Pea is a rich source of protein, fibre, carbohydrate, minerals like iron, magnesium, phosphorus, zinc and vitamin like A and C, etc.There are generally 3 types of pea that are commonly eaten. They are Garden pea or green pea- *Pisum sativum*, snow pea-*Pisumsativum var. macrocarpon*, snap pea-*Pisum sativum var. macrocarpon ser cv*. These peas have rounded pods that are usually slightly curved in shape with a smooth texture and vibrant green colour. Several peas are enclosed inside the pods. Usually snow peas are used as a vegetable in their immature stage. They have tender pods and are consumed fresh, frozen or canned whereas field pea are generally grown to produce dry pea like the split pea shelled from the mature pods. *Pisum sativum* cultivars are either low growing or vining type. The vining type of cultivar grows tendrils that coils around any available support and can climb upto 1-2m. Pea like many other legumes contains symbiotic bacteria called Rhizobia within their root nodules. These bacteria help in fixing the atmospheric nitrogen. Using the symbiotically fixed nitrogen instead of using chemical nitrogen fertilizer will help in the development of sustainable agriculture and also increase the yield production.

A polyamine is an organic compound having two or more primary amino groups-NH2. They are also known as a group of natural compounds with an aliphatic nitrogen structure present in almost all living organisms. It plays an important role in many physiological processes such as cell growth and development. Polyamines which are commonly found are putrescine, spermidine, spermine etc. the diamine putrescine, the triamine spermidine and the tetramine spermine are ubiquitously found in plant cells while other polyamines are of more limited occurrence. In animals, their levels are maintained from both the diet and denovo synthesis. Polyamine metabolism is regulated by the activity of the enzyme ornithine decarboxylase. Polyamine can also be synthesized from the aminoacid arginine and methionine. The first step in the pathway is the production of ornithine from arginine by the mitochondrial enzyme arginase. Ornithine is then decarboxylated by ornithine decarboxylase to produce putrescine. Polyamine is found in high concentration in the mammalian brain. The polyamines declines with the ages in the organism. Polyamines including putrescine are small ubiquitous nitrogenous compounds which are involved in several plant growth and developmental processes (Farooq *et al*., 2009). They are the recent additions to the class of plant growth regulators, and also considered as a secondary messenger in signaling pathways (Kusano *et al*., 2008). Polyamines are involved in abiotic stress tolerance in plants (Nayyer *et al*., 2005). Increased polyamines level in stressed plants are of adaptive significance because of their involvement in regulation of cellular ionic environment, maintenance of membrane integrity, prevention of chlorophyll loss, and stimulation of protein, nucleic acid and protective alkaloids (Sharma, 1999). Interaction of polyamines with membrane phospholipids implicates membrane stability under stress conditions (Roberts *et al*., 1986). Polyamines also protect membranes from oxidative damages as they as free radical scavengers (Besford *et al*., 1993).

Mycorrhiza is a fungus having a symbiotic or mutualistic relationship with the rhizosphere or roots of the plants. They share a mutualistic relationship in which the mycorrhiza forms a network of filaments that associates with the plant roots which helps them in uptaking the mineral nutrients or water while the mycorrhiza are benefitted by getting access to carbohydrates such as glucose and sucrose. The carbohydrates are translocated from their source to root tissues and onto the plant fungal partners. Some plant roots may be unable to uptake nutrients that are chemically or physically immobilized. For example, phosphate ions and micronutrients such as ions. . The mycelium of mycorrhiza fungus can however, access many nutrient sources and make them available to the plants they colonize. Thus many plants are able to obtain phosphate without using soil as a source. Some other form of immobilization is when nutrients are locked up in organic matter that is slow to decay. In such cases, some mycorrhizal fungus acts as a decay organisms and mobilize the nutrients. Some of the fungus has shown resistance to toxicity in plants. Those fungus is found to play protective role for plants rooted in soils with high metal concentration, such as acidic and contaminated soils.

HYPOTHESIS

For the present study, I select pea plant, because

[A] the research of the poisoning effect of the heavy metal Cd on plant mainly focuses on food crops such as rice, wheat and maize, but less on pea plants;

[B] Pea which is often as protein feed sources and quality assurance particular in concern with cadmium is not known that's why evidence of presence of heavy metal in pea is still a point of research;

[C] Level of sensitivity of pea for heavy metal is not known for cadmium; by conducting this type of experiment, I will be able to detect level of sensitivity which will be beneficial for plant breeders for development of cadmium tolerant Pea.

OBJECTIVE

The objectives of my work are to study:

1. The effect of cadmium stress on various morphological, biochemical and yield attributes in pea.

2. The effect of polyamines (putrescine) and mycorrhiza in ameliorating cadmium induced stress in pea.

3. The scavenging capacity of pea plant for cadmium present in the soil.

REVIEW OF LITERATURE

Heavy metal is a naturally occurring element which has a huge deterioration effect on the health of the environment and it does not degrade naturally and heavy metals cannot be destroyed biologically, they can only be transformed from one oxidation state or organic compound to another. Remediation of soil contaminated with heavy metals is more difficult than the remediation of other contaminants [De Jing et al. 2007].

Abedin *et al.* (2002) studied the uptake kinetics of arsenic species, arsenite and arsenate, in rice plants and found that arsenate uptake was strongly suppressed in the presence of arsenite.

Al-Enezi *et al.* (2005) reported that the propagation of heavy metals in biological food chain is one of the important issues of this behavior, as increasing the amount of several heavy metals in higher stages of the food chain is many times more than initial levels

Al-Jaloud *et al.* (1995) reported that, sorghum crop is important to provide livestock forage, and forage health has a direct effect on human health.

Arduini *et al.* (2004) found that changes in root morphology did not affect cadmium translocation to the above ground parts of the plant. The distribution of the Cd in leaves, bulbs and roots of plants differed with the increasing concentration of the cadmium concentration.

Bavi *et al.* (2006) studied that isolated chloroplast of 21 days old plants grown in nutrient solution containing different concentrations of cadmium did not show a significant changes in the rate of Hill reaction.

Bhardwaj *et al.* (2009) studies that higher concentration of Cd is responsible for the complete inhibition of germination and growth also decreased as concentration of heavy metals was increased as compared to control plants.

Bhardwaj *et al.* (2009) reported that along with the growth of plant under increasing concentration of heavy metals, the entire metabolic activity of the plants under metal stress was affected resulting in recued metabolic activity.

Bi *et al.* (2003) reported that, the metal concentration in plants has been shown to be higher or lower compared to the control (non-bioaugmented soil), depending on the metal (Bi *et al.,* 2003 and/or its concentration in the soil. Nevertheless bio-augmentation almost always increases the rate of metal extracted from plants.

Bjerrum's (1959) definition of heavy metals is based upon the density of the elemental form of the metal, and he classifies heavy metals as those metals with elemental densities above 7 g cm^{-3}

Brooks (1998) reported that the hyperaccumulator can accumulate large amounts of element in their above ground biomass from the surrounding soils and tolerate high soil concentration. Therefore, Hyperaccumulator usually contain over 1000 mg kg^{-1} of elements in their above ground biomass, or the enrichment factor (EF) (the ratio of element concentrations in Hyperaccumulator to those in the surrounding soil) should be above one. The most common method to identify hyperaccumlating plants is to collect plant species that are growing in contaminated sites, and determine the concentrations of elements in the biomass.

Caddel and Weibel (1971) reported that under long day condition the number of leaves increased with the increase in temperature. This happening is not common in all but reported in some genotypes.

Cao *et al.* (2007) reported that microbial performances cannot be compared with each other because the methods used to estimate the bioavailable fractions of metals vary from one experiment to other experiment. Indeed several extractants are used such as water, $MgCl_2$, NH_4NO_3, DTPA, KNO_3 and HCl. Bioavailability of metals is even divided into three different pools: immediately soluble metals (with water), exchangeable metal forms (with KNO_3) and complexes or adsorbed metal form (with EDTA).

Cho and Kim (2003) Noticed that cadmium has a great mobility in soil as compared with other heavy metals and is easily taken up by roots and is translocated to different pats of the plants.

Clark *et al*, (1968) reported that seed pericarp and tests may contain the germination inhibitor and which lead to the dormancy in the sorghum and when this pericarp was removed from the seed surface then it is observed that germination rate will enhance. Dormancy in seed is associated with ABA because the hormone can be detected in both developing and mature seeds and is proved to be inhibited when applied exogenously. In general dormancy is defined in a functional sense.

Seeds that do not germinate given optimal conditions of temperature, water and oxygen are considered dormant, whereas those that do germinate are considered non-dormant.

Cramer and Quarric (2002) reported that excessive concentrations of the various essential micronutrients had reported to affect initiation of the leaf primordial but did not depict any decline in the leaf number of concerned plants.

Da Lin *et al.* (2011) reported some important changes in growth and physiological characteristics of the sorghum plant under cadmium stress, such as height, chlorophyll content, root activities and MDA content. They concluded that Cd effect was the manifestation of oxidative stress. Low concentration of cadmium can promote the growth of hard wheat, while under a relatively high concentration, the growth of wheat and tillering were both inhibited and the

Elobeid et al. (2012) elucidated that Cd treatment caused significantly growth reductions in leaf formation, shoot height growth, stem radial growth and root elongation compared with controls, which significant decrease in plant biomass. However, the growth reductions differed between the different tissues; shoot elongation and leaf formation almost stopped after one week of Cd exposure, whereas stem radial growth was reduced from two weeks of Cd exposure onwards from the beginning.

Falkowski and Raven (2007) observed that chloroplast contains many different parts that respond to heavy metal stress, therefore any changes in chlorophyll synthesis and activity used as the index of the direct toxic effects of heavy metals.

Galavi et al. (2010) conducted an experiment in order to investigate the effect of treated municipal wastewater on soil chemical properties and heavy metal uptake by sorghum.

Galvez and Clark, (1991) reported that silicon mitigates the aluminum toxicity produced in the plants.

Gisbert et al. (2003) reported that the heavy metal stress is one of the major abiotic stresses that cause environmental pollution in recent decades. These metals unlike other inorganic pollutants are not degraded and converted into harmless compounds via biological processes.

Glass (2000) reported that phytoremediation is eco-friendly approach for the removal of contamination of agricultural soils. After the treatment the soil health going to improve and it allows sustaining crop production. If you have considering the economics incurred in the treatment, then these are supposed to be generally cheaper than physical ones.

Haussmann *et al*. (2000) showed that heterosis in yield is well expressed under a wide range of the stress condition. Scientist after exhaustive research reaches to the conclusion that heterosis in grain sorghum productivity is based on unique development events in the formation of the panicle structure and most likely involved in the stability of some important physiological parameters like photosynthesis, and other under diverse environmental conditions.

Kennedy and Filippis (1999) reported that the anthocynin pigments increased with salt stress in both salt tolerant and salt sensitive plant.

Krantev *et al.* (2008) reported that the net photosynthesis is also sensitive to Cd because it directly affects chloroplast biosynthesis and the proper development of the chloroplast ultrastructure.

Kupier *et al.* (2004) reported that a potential alternative option consists of optimizing the synergistic effect of plants and microorganism by coupling phytoextraction with soil bio-augmentation, also called rhizoremediation.

Kusano *et al.* (2008) polyamines including putrescine are small ubiquitous nitrogenous compounds which are involved in several plant growth and development processes. This is considered as the recent additions to the class of plant growth regulators, and as a secondary messenger in signaling pathways.

Lewis (1993) suggested that forming soap with fatty acids is an important criterion of "heaviness". Thus, again we have no consistent basis for defining the term. Another group of definitions is based on atomic number. A fourth group of definitions is based on other chemical properties, with little in common, density for radiation screening, density of crystals, and reaction with dithizone.

LIU Da-lin *et al.* (2010) reported that the regulatory mechanism of the soil cadmium (Cd) on antioxidants in sorghum was studied using three sorghum species viz., sweet sorghum and sorghum hybrid sudangrass.

Liu *et al.* (2003) reported that the cadmioum (Cd) pollution in agricultural farmland has become a serious problem in many parts of the world, due to industrial development and the heavy use of the chemical fertilizers, pesticides and herbicides, which produce serious thrust to human health through the food chain.

Premachandra *et al.* (1995) reported that the main solutes accounting for osmotic adjustment are due to sugars and potassium.

Rainbow (1985), reported that the various industries of electroplating, pigments, paints, stainless steel, mine tailing, battery, coal combustion, etc. are continuously causing soil pollution by adding different heavy metals viz. Cd, Cr, Pb, Ni, Zn, Cu, etc.

Tomsett and Thurman (1986) reported that the hyper-accumulation is an eco-physiological adaptation of plants to metalliferous soils. The mechanism of metal accumulation, which involves extra-cellular and intracellular metal chelation, precipitation, compartmentalization and translocation in the vascular system, are poorly understood.

Wilson (1988) reported that the effect of the most common environmental factors, such as water, available nutrients, perception of light and ambient CO_2 concentration, root growth can be explained reasonably well by its relationship to shoot growth

Yang *et al.* (2005) researched the effect of sewage irrigation accelerated the decline of wheat seedlings and root, reducing the root number and the root activities significantly.

METHODOLOGY

The pot experiment will be conducted in the poly house of the School of Agriculture, Lovely Professional University, Jalandhar, Punjab with one genotype of pea Arkel. Pea genotype will be taken from Punjab Agriculture University, Punjab. Pot size for experiment will be diameter: 30 cm and height 25 cm. Heavy metal stresses will be created in plant by exogenous application of cadmium nitrate in soil. One best concentration after initial screening within the range of 1-100 ppm of Cadmium will be finally selected. There is one concentration of heavy metals (after screening), will be applied in soil for creating stress in pea plant. Putrescine will be applied at the rate of 1.50 mM and 3.0 mM through foliar application. The various measurements will be made at three stages such as 30 day, 60 days and 90 days.

EXPERIMENTAL DETAILS

1. Genotype: Arkel

2. No. of treatment

a. Control

b. Cadmium concentration 1

c. Endomycorrhizal fungi (AMF), *Glomus* species

d. Putrescine 1(1.50 mM)

e. Putrescine 2 (3.0 mM)

f. Cadmium concentration 1 + Putrescine 1

g. Cadmium concentration 1 + Putrescine 2

h. Cadmium concentration1 + Endomycorrhizal fungi (AMF), *Glomus* species

i. Cadmium concentration 1 + Putrescine 1(1.50 mM) + Endomycorrhizal fungi (AMF), Glomus species

j. Cadmium concentration 1 + Putrescine 2 (3.00 mM) + Endomycorrhizal fungi (AMF), *Glomus* species

3. Replication: Three

4. Treatment: 10

5. Total number of pots: 10 x 3= 30

6. Design: CRD

OBSERVATIONS TO BE RECORDED

The various measurements will be made at three stages such as 30 DAS, 60 DAS and 90 DAS

1. Morpho-physiological parameters

a. Germination percentage (for screening)

b. Plant Height (cm)

c. Leaf area (cm x cm)

d. Leaf number

e. Days to 50% anthesis

f. Days to maturity

g. Internodal length (cm)

h. Stem girth (cm)

2. Biochemical parameters

a. Chlorophyll content (Arnon, 1949)

b. Total soluble sugar (Anthrone method) (Hansen J. and Moller I.B., 1975)

c. Total soluble protein (Bradford method, 1976)

d. Lipid peroxidation (Malondialdehyde-MDA) (Hodges *et al*., 1991)

e. Catalase (EC. 1.11.1.6) (Teranish *et al*., 1974)

f. Membrane stability (Sullivan method, 1972)

g. Phenylalanine ammonia lyase (EC. 4.3.1.5) (Brueske, 1980)

h. Superoxide dismutase (SOD) (EC. 1.15.1.1) (Dhindsa *et al*., 1981)

i. Transportable ratio (Using Atomic absorption spectrophotometer in different parts of plant including developing seeds).

3. Anatomical features

Transverse section of root to study mycorrhizal colonization (Microscopic conventional method)

4. Yield and quality attributes

1. Number of pods

2. Number of seeds per pod

3. Test weight

4. Seed soluble sugar (Anthrone method) (Hansen J. and Moller I.B., 1975)

5. Seed total protein content (Bradford method, 1976)

PLAN OF WORK

1st Year

(a) Morpho-physiological parameters

(b) Determination of biochemical parameters

(c) Anatomical features

(d) Yield and quality attributes

2nd Year

The experiment conducted in 1st year will be repeated during the IInd year.

EXPERIMENT CONDUCTED TILL DATE

BIOCHEMICAL OBSERVATIONS

1. **ESTIMATION OF TOTAL SOLUBLE PROTEIN (BRADFORD METHOD)**

Protein content was estimated from 0.5g of pea powder and the Optical Density was measured at 660nm.

READINGS:

Reference: -0.000

Sample: 0.621

Sample: 0.573

2. **ESTIMATION OF TOTAL SOLUBLE SUGAR (ANTHRONE METHOD)**

Carbohydrate was estimated from 0.2g of dry pea powder each in 3 test tubes and the Optical Density was measured at 620nm.

READINGS:

Reference: -0.001

Sample 1: 0.015

Sample 2: 0.002

Sample 3: 0.004

3. **ESTIMATION OF TOTAL LIPIDS (SADASHIVAM AND MANICKYA METHOD)**

Fatty acid was estimated from 1g of dried sample of pea powder.

READINGS:

1g of dried pea powder contains 4.56g of fatty acid.

4. **TOTAL CHLOROPHYLL CONTENT (mg/g fresh weight) IN LEAVES (ARNON DI METHOD)**

Chlorophyll content in leaves was estimated from100mg of leaves for each cups and the Optical Density was measured at 664nm, 665nm, 410nm, 480nm, 510nm, 590nm respectively (Table 1).

Table 1: Optical density at different wavelength(s)

TREATMENTS ($CdCl_2$) (ppm)	**OPTICAL DENSITY**					
	663nm	**664nm**	**410nm**	**480nm**	**510nm**	**590nm**
Reference	0.000	0.001	0.001	0.000	0.000	0.000
Control	0.539	0.523	0.750	0.432	0.054	0.085
10	0.376	0.362	0.565	0.314	0.039	0.059
20	0.506	0.491	0.712	0.383	0.064	0.088
30	0.610	0.593	0.893	0.524	0.073	0.105
40	0.511	0.547	0.784	0.441	0.052	0.092
50	0.374	0.365	0.614	0.350	0.044	0.065
60	0.727	0.723	1.055	0.671	0.137	0.175
70	0.419	0.407	0.662	0.323	0.048	0.070
80	0.832	0.928	1.184	0.683	0.104	0.157
90	0.923	0.951	1.245	0.813	0.133	0.197
100	0.379	0.366	0.569	0.322	0.038	0.061

(Source: Nada, J. 2018, Unpublished)

MORPHOLOGICAL OBSERVATIONS

SEED GERMINATION TRIAL

- 11 cups of 250ml volume is used
- Fill ¾ of the cup with soil
- A stock solution of 200 ppm $CdCl_2$ is prepared.
- Each of the cups were filled with 100ml of $CdCl_2$ of Control, 10, 20, 30, 40, 50, 60,70, 80, 90, 100ppm respectively.
- The soil is then left for saturation.
- After the $CdCl_2$ solution in soil got completely saturated, Pea seeds were sown.
- In each cups 10 seeds were sown
- Seeds started germinating in 2 days after sowing

Table 2: NUMBER OF SEEDS GERMINATED

SL. NO	$CdCl_2$ (ppm)	DAS-4	DAS-5	DAS-6	DAS-7
1.	Control	4	8	8	8
2.	10	10	10	10	10
3.	20	8	10	10	10
4.	30	8	8	8	8
5.	40	10	10	10	10
6.	50	8	9	9	9
7.	60	7	10	10	10
8.	70	8	8	8	8
9.	80	8	9	9	9
10.	90	10	10	10	10
11.	100	7	7	7	7

Table 3: PLANT HEIGHT (cm)

SL. NO	$CdCl_2$ (ppm)	PLANT HEIGHT (cm)	AVERAGE HEIGHT (cm)
1.	Control	6.9, 8, 9, 10, 9.1, 7.6	8.4
2.	10	6.9, 8.1, 8.5, 7, 9.8, 8.4, 10, 8.8, 5.9	8.1
3.	20	9.4, 10.2, 9, 7.2, 5, 8.3, 7.9, 8.9	8.2
4.	30	8.8, 8.9, 9, 7.2, 3.1, 7.3, 5.2, 5.3, 7.3	7.1
5.	40	7.7, 6. 8.5, 10.2, 5.5, 9.3, 8.2, 8.5, 10.2	8.2
6.	50	6.4, 7.6, 8.2, 7.5, 6.4, 9.3, 5.4, 5.7	6.9
7.	60	9, 5.8, 8, 5.5, 7.9	7.2
8.	70	9.8, 4.5, 8.4, 6 8.3, 9.6, 10.8, 4, 8.3	7.7
9.	80	5.2, 6.6, 6.3, 7.1, 7.3	6.5
10.	90	3.8, 5.8, 7.7, 5.9, 7.1, 6.4	6.1
11.	100	8, 6.5, 7.2, 6, 7.3, 6, 4, 7	6.5

Table 4: NUMBER OF LEAVES

SL. NO	$CdCl_2$ (ppm)	Average
1.	Control	9.1
2.	10	8.3
3.	20	8.3
4.	30	8.6
5.	40	8
6.	50	7.1
7.	60	7.3
8.	70	8
9.	80	6.8
10.	90	6.8
11.	100	6.5

CONCLUSION

Higher the concentration of the Cadmium more is the deteriorating effect it has shown. At 100ppm concentration of $CdCl_2$ the plant showed less germination as well as the cholorophyll content measured were seen to be low in 90ppm and 100ppm compared to other $CdCl_2$ concentration. So, the experiment will be conducted using 100ppm in pot cultivation.

ACKNOWLEDGEMENT

I am very thankful to my supervisor Dr. Prasann Kumar, for his consistent moral encouragement and support during the experimental work. I am also thankful to all the lab technician for their supports. I am highly thankful to Department of Agronomy, School of Agriculture, Lovely Professional University for providing each and every supports during the work.

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