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Interaction Effects of Phosphorus and Zinc on their Uptake and ^{32}P Absorption and Translocation in Sweet Corn (*Zea mays* var. *Saccharata*) Grown in a Tropical Soil

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Abstract: Zinc (Zn) and Phosphorus (P) interact with each other and this interaction can result in impact on the yield of corn plants. This study was conducted to examine the effect of different levels of Zn and P on the yield, Zn and P concentration and uptake, chlorophyll content and percentage of P derived from fertilizer of corn plants in a tropical soil. Sweet corn grown in pot culture containing all combinations of Zn at levels of 0.0, 5.0 and 10.0 mg kg^{-1} soil and P at levels of 0.0, 50.0, 100.0 and 200.0 mg kg^{-1} soil as $ZnSO_4.7H_2O$ and KH_2PO_4, respectively and harvested at 28 days after transplanting. Dry matter yield increased with P supply, while Zn application did not show any significant effect on this parameter. The Zn and P uptake by shoots increased with increasing Zn and P application into the soil. The Zn concentration in shoots decreased with increasing P supply but P concentration and uptake enhanced. Phosphorus (P) induced Zn deficiency in this study mostly related to the dilution effect. Chlorophyll a/b ratio increased with P supply. The percentage of P derived from fertilizer reduced with increasing Zn application, although P uptake by shoots was unchanged.

Key words: Phosphorus, zinc, corn, radioisotope, tropical soil

INTRODUCTION

Phosphorus and zinc are two essential nutrients which are required for normal plant growth. These nutrients are mutually antagonistic in certain circumstances which can cause yield reductions in many crops due to either P or Zn deficiencies. Deficiencies typically happen when a nutrient is available in small amounts. In this phenomenon, the nutrient is present in marginal to normal levels but the antagonizing nutrient is available in such a large amount that it induces the deficiency of the other. The Zn induced P deficiency is a very rare phenomenon because growers commonly apply large amounts of P fertilizer as compared to Zn fertilizer. The P induced Zn deficiency is related to the application of phosphatic fertilizers at high dose to the soils that are low or marginal in available Zn. Four hypotheses have been suggested to explain this phenomenon: (1) P may interfere with the translocation of Zn from roots to top. Cakmak and Marschner (1987) stated that total Zn concentration in plant tissues was not changed with P supply but water-soluble Zn was decreased and hence visual Zn deficiency symptoms were observed in cotton, grown in nutrient solution. (2) High availability of P can accentuate Zn deficiency in plant tissues. (3) Zn concentration may reduce because of the dilution caused by growth response of P. Loneragan *et al.* (1979) and Singh *et al.* (1988) observed that P application increased the growth of upper plant parts enough to dilute the Zn concentration in bean plants. (4) An imbalance between Zn and P can cause metabolic disorder with plant cells (Wijebandara, 2007). Soltangheisi *et al.* (2013) stated that P/Zn ratio can be a better indicator of Zn nutritional status than Zn concentration alone when sweet corn plants were grown in nutrient solution. Soltanpour (1969) applied Zn and P in separate fertilizer bands in calcareous soil in which case they cannot contact each other and still, he found reduced Zn uptake by potato similar to the treatment where Zn and P were banded together. He suggested that P-Zn interaction was related more to plant physiology than to soil reactions, since in his work the Zn and P bands did not contact one another in the soil. Although it is known that main P-Zn interactions occur within the plant, the importance of this interaction in the soil has also been reported. Saeed and Fox (1979) showed that P fertilization increased Zn sorption in Hawaiian soil. They stated that sorption of P on the surface of iron and aluminum oxides enhanced negative charges on them resulting in an increased sorption of Zn and decreased absorption of Zn by plant roots. Similar results were observed by Stanton and Burger (1967), Chaudhry and Loneragan (1972) and Bolland *et al.* (1977).

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The aim of this study was to determine the effects of different levels of P and Zn on yield, chlorophyll content and Zn and P concentration and uptake in shoots of sweet corn plants in a tropical soil. The percentage of P derived from fertilizer (% Pdff) as affected by Zn rates was also evaluated. Furthermore, the correlation between Zn and P supply in soil and concentration and uptake in plant was investigated.

MATERIALS AND METHODS

Experimental design: A pot experiment was conducted at the experimental glasshouse of the University Putra Malaysia (UPM). The soil samples used in the investigation were collected from the surface layer (0-15 cm) of field located in Serdang, Selangor, Malaysia. The soil samples were air-dried at room temperature, passed through a 2 mm polyethylene sieve and potted (2 kg) in plastic pots. The physico-chemical analysis of the soil was carried out before the commencement of the study following the methods described by AOAC (1984) and the analytical results are listed in Table 1.

All combinations of Zn treatments (in the form of $ZnSO_4.7H_2O$) at levels of 0.0, 5.0 and 10.0 mg Zn kg^{-1} soil and of P treatments (in the form of KH_2PO_4) at levels of 0.0, 50.0, 100.0 and 200.0 mg P kg^{-1} soil were included. Zn and P treatments were added to each pot and mixed thoroughly with the soil before transplanting. About 250 mg N as ammonium nitrate and 200 mg K as potassium sulphate per pot were applied in solution form at the time of P fertilizer addition. Soils were saturated with distilled water and allowed to air-dry.

The percentage of P derived from fertilizer was measured directly in sweet corn using a ^{32}P radioisotope tracer method ($t_{1/2}$ = 14.3 days). A 100 μCi of ^{32}P was mixed with 400 mL of distilled water and 1 mL of stock P solution (1000 mg L^{-1}) and added to each pot and mixed completely with the soil. The treated soil was allowed to incubate for 1 week.

Sweet corn seeds hybrid 926 from Green World Genetics in Malaysia were used as the indicator plant. The seeds were soaked in water for 24 h and then geminated in rolled paper towels saturated with deionized water in the laboratory at 24°C. The paper towels were kept saturated for 5 days and the sweet corn seedlings were transplanted into the pots containing 2 kg of the treated soil at the rate of 3 seedlings per pot. During the course of the experiment, the soil moisture was maintained at approximately 70% of the normal field capacity by watering plants every day with deionized water. The experimental design was a randomized complete block consisting of 3 blocks (replications). The plants were grown at ambient sunlight. The temperature and humidity were 24-33°C and 70-88%, respectively.

Table 1: Selected physico-chemical properties of the soil

Soil characteristics	Values
pH (H_2O)	4.65
pH (KCl)	3.84
Total nitrogen (%)	0.04
Available phosphorus (mg kg^{-1})	30.00
Organic carbon (%)	0.93
Sulfur (%)	0.04
CEC ($cmol_c$ kg^{-1})	5.00
Exch. Ca ($cmol_c$ kg^{-1})	1.25
Exch. K ($cmol_c$ kg^{-1})	0.11
Exch. Mg ($cmol_c$ kg^{-1})	0.08
DTPA-Fe (mg kg^{-1})	54.40
DTPA-Zn (mg kg^{-1})	3.00
DTPA-Cu (mg kg^{-1})	1.29
DTPA-Mn (mg kg^{-1})	0.86
Clay (%)	23.00
Silt (%)	4.00
Fine sand (%)	46.00
Coarse sand (%)	27.00
Texture (Sand clay loam)	

Measurements: Harvesting was done by cutting the stem immediately above ground at 28 (V8-plants had 8 leaves) days after transplanting. The plant samples were rinsed with distilled water, dried at 70±2°C for 48 h, weighed, ground to <2 mm and ashed at 300°C for 3 h followed by 500°C for 2 h in a muffle furnace. The ash was dissolved in 20 mL 2.0 M HCl solution. P was measured using an autoanalyzer (8000 series, Lachat QuickChem FIA+, USA) and Zn was determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Optima 8300, PerkinElmer, USA). The ^{32}P activities were measured using a Winspectral α/β Wallac Liquid Scintillation Counter (1414, PerkinElmer, USA). All counts were corrected for decay and dilution.

For chlorophyll content analysis, the fresh leaves (0.2 g) were put into the 20 mL glass vials. The 5 mL concentrated dimethyl sulfoxide (DMSO) was added and the glass vials placed in an oven at 70°C for 1 h. Under the wavelength of 645 and 663 nm, the content of chlorophyll b and chlorophyll a were measured, respectively, using spectrophotometer (1000 series, Cecil CE 1011, Auckland, New Zealand). Arnon's equation (Arnon, 1949) was used to convert absorbance measurements to milligram chlorophyll per gram in leaf tissue.

Calculations and statistical analysis: The dry weight of shoots was multiplied by the concentrations of Zn and P to measure Zn and P uptake by shoots.

The Specific Activity (SA) of P in plant was calculated as:

$$SA\ (kBq\,mg\,P^{-1}) = \frac{plant\ activity\ (kBq\,plant^{-1})}{P\,uptake\ (mg\,plant^{-1})}$$

The percentage of P derived from fertilizer in plants (%Pdff) was calculated by the following equation:

$$pdff(\%) = \left(1 - \frac{SA\ of\ P_0\ treatments\ (kBq\,mg\,P^{-1})}{SA\,of\ added\,P\,treatments\ (kBq\,mg\,P^{-1})}\right) \times 100$$

Data was analyzed statistically by using SAS 9.2 software (SAS institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Both dry matter yield (Fig. 1) and Zn uptake by shoots (Fig. 2a) showed a highly positive quadratic correlation with P application. Plants which did not receive P, had the lowest yields of shoots which was 9.8 folds lower than the treatments supplied with P. These plants suffered from P deficiency. Plants supplied with 200 mg P kg^{-1} soil showed the highest dry weight which became slightly lower with Zn application (Table 2). Mawardi *et al.* (1975) showed that dry matter yield and Zn content were doubled in a calcareous soil under corn cultivation at high rates of P application. Orabi and Abuleenane (1980) observed the same results in rice plants.

Phosphorus (P) application increased plant growth (Fig. 1) and P uptake by shoots (Fig. 3) but decreased Zn concentration in upper plant parts with a very high non-linear correlation (Fig. 2b). Increased shoot biomass and P uptake by shoots with P supply indicate that the soil used is P deficient. As Zn uptake increased with P application (Fig. 2a), the decrease in Zn concentration can be explained by a dilution effect rather than by reduces Zn uptake by roots. P and Zn were limiting in this soil and P application promoted plant growth and caused dilution in tissue Zn which might lead to Zn deficiency. Oseni (2009) reported the decrease of Zn concentration in cowpea grains with P application. Gianquinto *et al.* (2000) observed a reduction in leaf Zn concentration of dwarf bean by the addition of P to plants grown at low Zn supply. Loneragan *et al.* (1979) and Singh *et al.* (1988) observed the same results in bean plants. Khorgamy *et al.* (2009) demonstrated that P fertilizer reduced Zn absorption by roots of chick pea. The immobilization of Zn by P within the roots and at the root surfaces as well was recorded in fiber flax (Burleson and Page, 1967), corn (Langin *et al.*, 1962) and sorghum (Ellis *et al.*, 1964). Contrary results were reported by Mandal and Mandal (1990) in rice plants. They observed that although the dry matter yield of both shoot and root increased with P supply, the uptake of Zn by the shoot declined, while increased in roots. They suggested that the decrease in shoot Zn concentration was not due to a dilution effect and might be attributed to retardation of its translocation

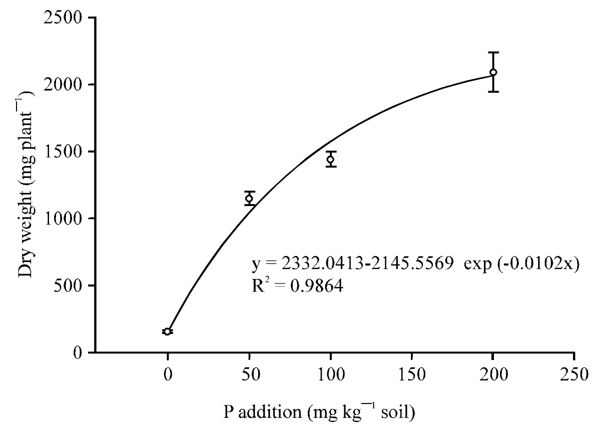

Fig. 1: Relationship between P supplies and dry weight in shoots of sweet corn plants (vertical bars represent standard error)

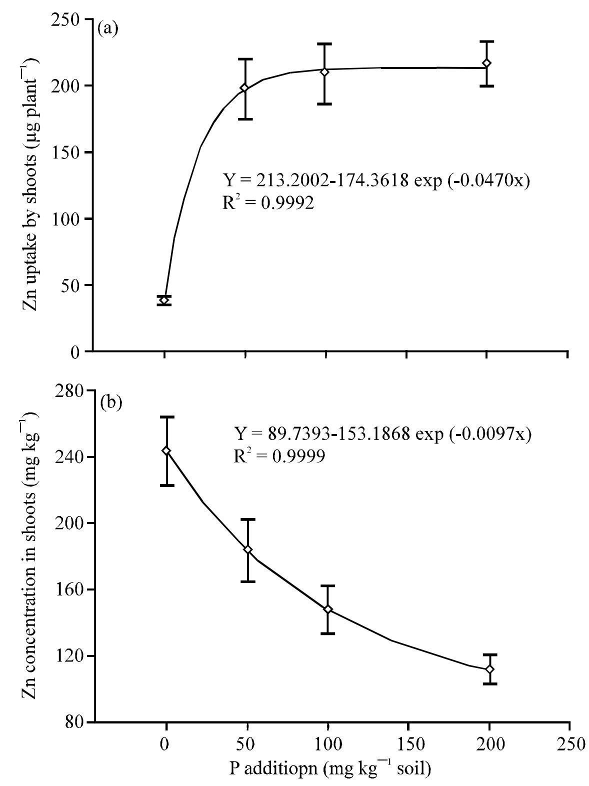

Fig. 2(a-b): Relationship between P supplies, (a) Zn uptake and (b) Zn concentration in shoots of sweet corn plants (vertical bars represent standard error)

Table 2: Dry weight, chlorophyll a/b ratio, P and Zn concentration in leaves and Zn uptake of sweet corn plant in soil with different P and Zn levels

Zn	P	Dry weight (mg plant^{-1})	Chlorophyll a/b ratio	P concentration in leaves (%)	Zn concentration in leaves (μg g^{-1})	P uptake shoots (mg plant^{-1})	Zn uptake by shoots (μg plant^{-1})
Treatments (mg kg^{-1} soil)							
0	0	142.3^{e}	3.99^{bc}	1.86^{a}	108.7^{g}	2.56^{fg}	15.5^{g}
	50	1350^{cd}	3.99^{bc}	0.32^{c}	59.7^{h}	4.17^{bcde}	79.2^{ef}
	100	1595.7^{bc}	4.37^{ab}	0.30^{c}	56.7^{h}	4.71^{abcd}	88.4^{ef}
	200	2320^{a}	4.23^{b}	0.23^{c}	54.3^{h}	4.83^{ab}	119.6^{e}
5	0	168.7^{e}	3.35^{d}	0.95^{b}	239.3^{c}	1.67^{g}	38.2^{fg}
	50	1032.3^{d}	3.75^{cd}	0.34^{c}	180.7^{d}	3.24^{ef}	184.7^{d}
	100	1464.3^{bc}	4.26^{b}	0.30^{c}	142.3^{ef}	4.29^{bcde}	212.6^{cd}
	200	2155.7^{a}	4.46^{ab}	0.24^{c}	118.3^{fg}	5.40^{a}	252.2^{bc}
10	0	165.7^{e}	3.43^{d}	2.03^{a}	381.0^{a}	3.44^{ef}	62.8^{fg}
	50	1065.7^{d}	3.62^{cd}	0.35^{c}	311.0^{b}	3.61^{def}	327.6^{a}
	100	1269^{cd}	4.80^{a}	0.32^{c}	245.3^{c}	3.69^{cde}	324.2^{a}
	200	1784.3^{b}	4.29^{b}	0.28^{c}	162.3^{de}	4.73^{abc}	275.6^{ab}

Different superscipt letters in colums means results are significant

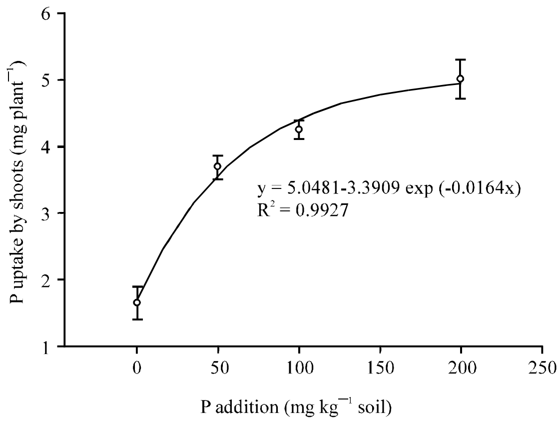

Fig. 3: Relationship between P supplies and P uptake by shoots of sweet corn plants (vertical bars represent standard error)

Fig. 4: Foliar symptoms of Zn deficiency. $Zn_{10}P_{100}$ and $Zn_{10}P_{200}$ are normal leaves whereas Zn_0P_{100} and Zn_0P_{200} treatments are Zn deficient leaves. From the comparison of Zn_0P_{100} and Zn_0P_{200} treatments, it can be stated that P supply accentuated Zn deficiency symptoms

from root to shoot or to the decrease in its absorption by plants owing to its decreased availability in soil resulting from P application. In Zn_0 treatments, the necrotic symptoms developed in addition to Zn deficiency symptoms of bronzing and chlorosis of old leaves and distinct narrow, white band on old leaves (Fig. 4), although the Zn concentration in these treatments were higher than the critical range (>20 μg g^{-1}) according to Kuldeep (2009) (Table 2) and applications of P tended to accentuate Zn deficiency symptoms. Physiological requirement of Zn increased with increasing P concentration in plant tissues due to the inactivation of the Zn in some way. Leaf cell walls also could bind considerable amounts of Zn leading to cytoplasmic Zn deficiency. As the P concentrations in Zn_0P_{50}, Zn_0P_{100} and Zn_0P_{200} treatments were not toxic (<1%) according to Tyner (1946) (Table 2), thereby the aforementioned symptoms caused by Zn deficiency not by P toxicity. In some cases P supply decreased the total uptake of Zn in plants (Loneragan, 1951; Stukenholtz *et al*., 1966), while in others P had no effect on Zn uptake or increased it (Millikan, 1963; Watanabe *et al*., 1965; Jackson *et al*., 1967). In this study, Zn uptake by shoots increased with increasing P application (Fig. 2a). Friesen *et al*. (1980) reached similar conclusion in maize. This was possibly caused by the increased root growth due to improved P nutrition. Increase in shoot P uptake due to P supply ranged between 44-95% over P_0 treatments.

As expected, the concentration and the uptake of Zn in shoots increased with Zn application with a very high linear correlation (Fig. 5a, b). Zn concentration and Zn uptake in shoots were 3.2 and 2.8 folds higher in Zn-supplied plants as compared to non-applied ones, respectively. Soltangheisi *et al*. (2014) demonstrated that Zn concentration in roots and shoots of sweet corn plants

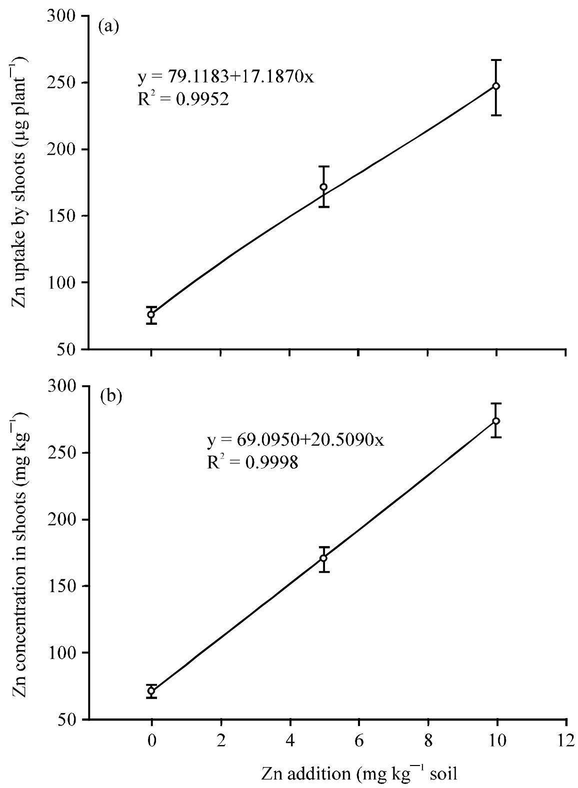

Fig. 5(a-b): Relationship between Zn supplies, (a) Zn uptake and (b) Zn concentration in shoots of sweet corn plants (vertical bars represent standard error)

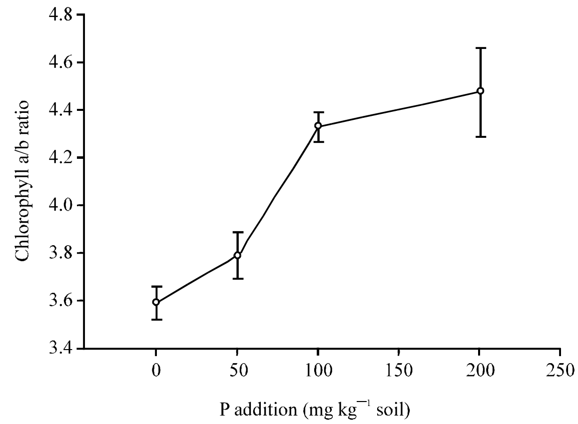

Fig. 6: Relationship between P supplies and chlorophyll a/b ratio of sweet corn plants (vertical bars represent standard error)

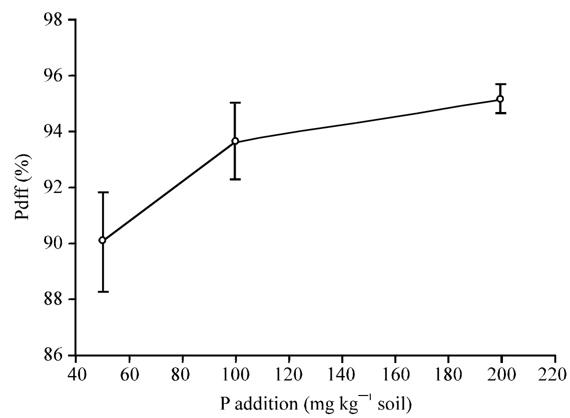

Fig. 7: Relationship between P supplies and Pdff% in shoots of sweet corn plants (vertical bars represent standard error)

increased with increasing Zn concentration in nutrient solution with a very high quadratic correlation.

Zinc deficiency increased total P content in potato (Christensen and Jackson, 1981), in okra (Loneragan *et al.*, 1982) and in cotton (Cakmak and Marschner, 1987). By contrast, Zn application did not have any effect on P concentration and uptake in shoots of corn plants in the present experiment. The Zn concentrations were not below the sufficiency range in treatments receiving different levels of Zn, so Zn addition had marginal effects on leaf P concentrations and uptake. Similar results were reported by Christensen and Jackson (1981) in corn and Webb and Loneragan (1988) in wheat.

Different levels of Zn and P application did not have any effect on chlorophyll a, chlorophyll b and total chlorophyll content but chlorophyll a/b ratio increased with increasing P application (Fig. 6), whereas Zn supply did not show any significant effect on this ratio. Enhancement of chlorophyll a was relatively higher than of chlorophyll b with P application.

The percentage of P derived from fertilizer (%Pdff) rose with increasing P application (Fig. 7). Zn rates reduced the percentage of P derived from fertilizer (%Pdff) (Fig. 8), although all the treatments were not Zn deficient which suggests antagonistic effects between these nutrients on %Pdff. On average, the %Pdff reduced from 96.6-86.4% with Zn rates and increased from 90.1-95.2% with P rates. It seems that low Zn supply enhanced P absorption rate. As Zn supply did not show any significant effect on P uptake and concentration in shoots, it could be stated that Zn application affected the part of P which is derived from fertilizer not available P in the soil. Zn supply can cause the formation of chemical bonds with phosphate anions ($H_2PO_4^-$ or HPO_4^{2-}) within the soil and make the phosphorus unavailable for transport to the roots. The relative strength of the P-Zn

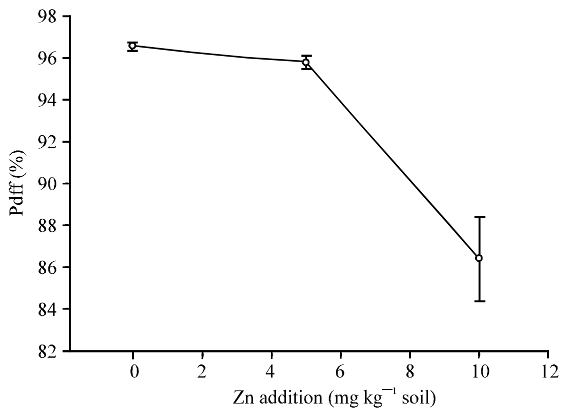

Fig. 8: Relationship between Zn supplies and Pdff% in shoots of sweet corn plants (vertical bars represent standard error)

bond is robust and does not easily separate without dramatic changes in the physical or chemical environment.

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