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Effects of phosphorus on germination and seedling growth of *Cucumis sativus* L. in arsenic solution culture

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Abstract— *Arsenic (As) is a toxic metalloid which poses a serious threat to the environment and human health. Enrichment of soil with phosphorus (P) is believed to reduce the negative effects of As on plant growth. However, only a few attempts have been conducted towards finding the precise role of P. This study aimed to determine the efficacy of P in reducing the negative effects of As on Cucumis sativus with respect to seed germination rate, seedling growth and biomass production of seedling. The experiment was conducted in petridish under laboratory conditions using P and As solution cultures. Seeds of C. sativus were treated with different concentrations of As (2 ppm, 5 ppm and 10 ppm), with or without addition of P (10ppm). The results revealed that, As solutions reduce the germination rate, seedling growth and biomass production of seedling in different magnitudes, based on the level of concentrations (2ppm, 5 ppm and 10 ppm) in C. sativus. However, addition of P (10 ppm) in As solution cultures (2ppm, 5ppm and 10ppm respectively) increases those parameters significantly in comparison with their corresponding As solutions. In treatments T1 (2ppm As), T3 (5ppm As) and T5 (10ppm As), seedling dry biomasses were 21.1mg, 18.3mg and 13.4mg respectively, while they were 25.6mg, 27.6mg and 20.9mg respectively in T2 (2ppm As+10ppm P), T4 (5ppm As+10ppm P) and T6 (10ppm As+10ppm P). Similar trend was also recorded for germination rate, plumule growth, seedling fresh biomass production, seedling vigor, sturdiness etc. Therefore, it can be recommended to apply P in As solution culture to reduce the negative effects of As on C. sativus growth in the laboratory, that might also be applicable in the field.*

Keywords— Arsenic, Cucumis sativus L, germination, phosphorus, seedling growth, sturdiness, vigor index.

I. INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a widely cultivated plant in the gourd family, Cucurbitaceae. It is a creeping vine that bears cucumiform fruits that are used as vegetables [1, 2]. It contains water, minerals, carbohydrates, protein, lipid, ion and vitamin in human diet. The crop is originally from South Asia, but now grown in whole over the world including Bangladesh. Cucumber can be grown on a variety of soils, but loamy soil and temperature between 18^0C and 24^0C is preferred for good quality fruit [2, 3, 4].

Arsenic (As) is a toxic element widely distributed in the environment and in organisms [5 ,6, 7, 8]. Through both natural formation and anthropogenic activities, As can enter into terrestrial and aquatic environments [9]. In some parts of Bangladesh, people irrigate their crops with As-contaminated groundwater. Moreover, 33% of total arable land of this country is more or less contaminated with As [10]. As is a nonessential element for plants and inorganic As are highly phytotoxic. At high level of As concentrations, biomass production and yields of a variety of crops are reduced significantly [11, 12]. Crops grown in As-contaminated soil or irrigated with As-contaminated water accumulate As in the seeds or grain, and this is an increasingly important problem in many parts of the world [13] because of causing significant risk to animal and human health through soil-crop transfer [14, 15].

Phosphorus (P) is the chemical analogue of As and an essential element required for plant growth [16, 17, 18]. This plant nutrient interferes in cellular energy transfer, photosynthesis, and respiration [19]. Reduction in As uptake can be related to changes in the transport mechanisms of phosphate because P has a chemical similarity of As [20] and in plant uptake contest with As [21]. When P is used as a crop fertilizer the effect of P on the sorption/desorption of As in soil environments has received great attention [22]. Most of the previous hydroponic and soil studies on As and P interactions have shown variable results which included both increases and decreases of As effect under P addition. But very few studies have been conducted to find out the precise role of P in reducing negative effects of As specially on seed germination and seedling growth. Therefore, the objective of this study was to find out the efficacy of P in reducing the negative effects of As on *C. sativus* with respect to seed germination rate, seedling growth and biomass production of seedling.

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II. MATERIALS AND METHODS

2.1. Study site and period of study

The experiment was carried out in the environmental lab of the Institute of Forestry and Environmental sciences, University of Chittagong (IFESCU), Bangladesh (lies approximately at the intersection of $91^0 50´$E and $22^0 30´$N) (Fig. 1). The study was conducted during the month January to May, 2017, as the seeds of *C. sativus* are mostly available in this period in Bangladesh.

2.2. Collection of seeds

Ripe, healthy and disease free seeds were extracted from the fruits of *C. sativus*, dried in the sunlight and stored in airtight polybags and kept in refrigerator until use. Seeds of uniform size and color were selected to avoid non-treatment variation [23]. Before using, viability test of the seeds were also performed by using water floating method.

2.3. Preparation of Hoagland's and other solutions

The Hoagland's nutrient solution was comprised of KNO_3, 0.5 g L^{-1}; $Ca(NO_3).4H_2O$, 1.2 g L^{-1}; $MgSO_4.7H_2O$, 0.5 g L^{-1}; H_3BO_3, 2.8 mg L^{-1}; $ZnSO_4$, 0.2 mg L^{-1}; $CuSO_4$, 0.05 mg L^{-1}; NH_4NO_3, 0.08 mg L^{-1}; $MnCl_2.4H_2O$, 1.8 mg L^{-1}; $Na_2MoO_4.2H_2O$, 0.12 mg L^{-1}; FeEDTA, 0.02 g L^{-1}; in a volume of 1 L. Moreover, the P was added as KH_2PO_4, 0.07 g L^{-1} for P treatment while the As was added as $Na_2HAsO_4.7H_2O$, 0.042 g L^{-1} for As treatment. The stock solutions were diluted as requirement for various treatments. The pH (6.0) of the stock and diluted solutions was adjusted with 1M HCl and 1M NaOH for all the treatments. Hoagland's solution without P was common for all the treatments including control.

2.4. Experimental design and treatment combinations

Petridishes were sterilized in oven at 200^0 C overnight. In each petridish three layers of moist sterilized filter paper were placed. A Randomized Complete Block Design (RCBD) with 7 treatments and 4 replications for each treatment was adopted for this experiment. For this experiment, 28 petridishes were needed. 25 seeds of *C. sativus* were sown in each petridish and a total seven hundred seeds were subjected to 7 different treatments. The treatment combinations used in the experiment were: **T0** = Control (Hoagland's solution without P, which was also common for all other treatments); **T1** = 2ppm As; **T2** = 2ppm As + 10ppm P; **T3** = 5ppm As; **T4** = 5ppm As + 10ppm P; **T5** = 10ppm As; **T6** = 10ppm As + 10ppm P.

Seeds were sterilized by soaking them in 0.05% Mercuric chloride solution for 1 minute followed by washing with distilled water and then drying before placement on petridishes. After sowing the seeds, all the petridishes were placed in the incubator at 25 ± 2^0C temperature. The filter papers of the petridishes were kept wet always at the same level by applying the specific solution of As and P to the specific petridishes.

2.5. Data recording

Germination was recorded daily from the date of seed sowing to the last of germination. The seedlings were allowed to grow altogether for ten days from the time of seed sowing. After ten days, five representative seedlings from each treatment were selected for measuring growth parameters. The recorded parameters were plumule and radical lengths, collar diameter, fresh weights of plumule and radicle, dry weights of plumule and radicle, number of lateral roots. For recording dry weights, plumule and radical were oven dried at 70ºC for 48 hr. To assess the seedling vigor, total height (from the collar area to seedling tip) of each seedling in each petridish was measured using a ruler to the nearest 0.1 cm. Vigor index was calculated according to [24] as germination percent X seedling total length i.e. total shoot and root length. Volume index was obtained by multiplying shoot height or shoot length (cm) with the square of collar diameter $(mm)^2$ of the seedling [25]. Quality index was developed following [26] to quantify seedlings morphological quality. The formula for calculating quality index is as follow:

$$QI = T_{dw} / \left(\frac{H}{D_c} + \frac{S_{dw}}{R_{dw}} \right)$$

where, QI is quality index, T_{dw} is total dry weight (g), H is seedling height or plumule length (cm), D_c is collar diameter (mm), S_{dw} is shoot/plumule dry weight (g), R_{dw} is root/radicle dry weight (g). Sturdiness was obtained by dividing or shoot/plumule length (cm) with collar diameter (cm) of the seedling.

2.6. Statistical analysis

Data related to seed germination and seedling growth attributes were analyzed statistically by using computer software SPSS ver.23. Duncan's multiple range test (DMRT) was employed to determine the statistical significance ($P< 0.05$) of the differences among the mean values. Significant differences were indicated by different letters in the table.

III. RESULTS

3.1. Germination percentage

Germination were 68% in T1 (2ppm As), 66% in T3 (5ppm As) and 54% in T5 (10ppm As), while they were 72% in T2 (2ppm As+10ppm P), 78% in T4 (5ppm As+10ppm P) and 60% in T6 (10ppm As+10ppm P), meaning that germination percent increased with the addition of P in corresponding As solution cultures (Table 1).

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3.2. Mean daily and cumulative germination percentages

Mean daily germination percent was highest (51%) in T4 (5 ppm As + 10 ppm P) at the 2nd day followed by 37% in T5 (10 ppm P) at the same day (Figure 2). From 1st day up to the 3rd day, cumulative germination percent was highest in T4 (5ppm As +10 ppm P) while it was highest in T0 (Control) from 4thday up to the 8th day. The lowest cumulative germination was recorded in T5 (10 ppm As) (Fig. 3).

3.3. Growth performance

In treatments T1 (2ppm As), T3 (5ppm As) and T5 (10ppm As), plumule lengths were 6.4cm, 5.4cm and 3.9cm respectively, while they were 6.7cm, 7.7cm and 5.6cm respectively in T2 (2ppm As+10ppm P), T4 (5ppm As+10ppm P) and T6 (10ppm As+10ppm P) also meaning that plumule length increased with the addition of P in corresponding As solution cultures (Table 1). Significantly (P<0.05) highest radical growth (6.5cm) was recorded in T0 (Control) while lowest (1.2cm) in T5 (10ppm As). In treatments T1 (2ppm As), T3 (5ppm As) and T5 (10ppm As), number of lateral roots were 16, 14 and 10 respectively, while they were 19, 17 and 12 respectively in T2 (2ppm As+10ppm P), T4 (5ppm As+10ppm P) and T6 (10ppm As+10ppm P). Similar trend was also observed in collar diameter (Table 1).

3.4. Biomass production

In treatments T1 (2ppm As), T3 (5ppm As) and T5 (10ppm As), seedling dry biomasses were 21.1mg, 18.3mg and 13.4mg respectively, while they were 25.6mg, 27.6mg and 20.9mg respectively in T2 (2ppm As+10ppm P), T4 (5ppm As+10ppm P) and T6 (10ppm As+10ppm P). Similar trend was also recorded for fresh biomasses of seedling (Table 2).

3.5. Vigor, volume and quality indices, and sturdiness

Vigor index were 843 in T1 (2ppm As), 482 in T3 (5ppm As) and 275 in T5 (10ppm As), while they were 821, 811 and 426 respectively in T2 (2ppm As+10ppm P), T4 (5ppm As+10ppm P) and T6 (10ppm As+10ppm P). In treatments T1 (2ppm As), T3 (5ppm As) and T5 (10ppm As), volume index were 16.4,12.2 and 6.6 in T1 (2ppm As), T3 (5ppm As) and T5 (10ppm As) respectively while they were 19.4 in T2 (2ppm As+10ppm P), 19.7 in T4 (5ppm As+10ppm P) and 11.0 in T6 (10ppm As+10ppm P). Quality index were 0.003 in T1 (2ppm As), 0.0025 in T3 (5ppm As) and 0.0016 in T5 (10ppm As), while they were 0.0038, 0.0035 and 0.0024 respectively in T2 (2ppm As+10ppm P), T4 (5ppm As+10ppm P) and T6 (10ppm As+10ppm P) (Fig. 4). In treatments T1 (2ppm As), T3 (5ppm As) and T5 (10ppm As), sturdiness were 40.1, 34.8 and 26.9 respectively while they were 43.3 in T2 (2ppm As+10ppm P), 44.2 in T4 (5ppm As+10ppm P) and37.6 in T6 (10ppm As+10ppm P) (Fig. 5).

IV. DISCUSSION

In plants, As accumulates mainly in the root system, and to a lesser degree in the aboveground organs. It causes physiological changes in plants and reduces crop productivity [7, 17, 18, 27]. As interrupt the biochemical function of cells and severely impedes different plant metabolic processes viz. photosynthesis, transpiration, respiration and other physiological functions through reacting with proteins and enzymes, and stop plant growth [28]. To alleviate As toxicity, plants must take up sufficient amount of P to balance excessive As. The plants react by increasing P accumulation as plant As increases [7, 17, 29].

From this study, it was found that, As solution reduces the rate of seed germination, seedling growth and biomass production of seedling in different magnitudes, based on the level of concentrations (2ppm, 5 ppm and 10 ppm) in *C. sativus.* However, addition of P (10 ppm) in As solution cultures (2ppm, 5ppm and 10ppm respectively) increases those parameters significantly in comparison with their corresponding As solution. Research also reported that phosphate protects As toxicity of a rice variety- Bala at a lower concentration (13.3 µM) of As in the solution culture [30]. Being a phosphate analogue, phosphate transporters act as arsenate transporters and phosphate competes with arsenate for uptake at the level of membrane transport, hence phosphate would be expected to reduce arsenate influx and this would translate into an observed increase in arsenate tolerance under phosphate addition [17, 18, 31, 32]. Studies, especially in hydroponic environments have demonstrated that non-resistant plants can be made more resistant to arsenate by raising their P status, as the P is taken more effectively compared to arsenate [17, 33, 34]. Study also reported the role of phosphate in against As toxicity by increasing cytoplasmic phosphate concentration in wheat [35]. Higher phosphate concentration plays an important role to down-regulation of the arsenate-phosphate plasma membrane transporter and competes with arsenate for biochemical processes where arsenate substitutes for phosphate [36].

V. CONCLUSION

Our findings show that, As solution reduces the rate of seed germination, seedling growth and biomass production of seedling in different magnitudes, based on the level of concentrations in *C. sativus.* However, addition of P in As solution increases those parameters in comparison with their corresponding As solution. Hence, it can be recommended to apply P in As solution culture to reduce the negative effects of As on *C. sativus* growth in the laboratory that might also be applicable in the field.

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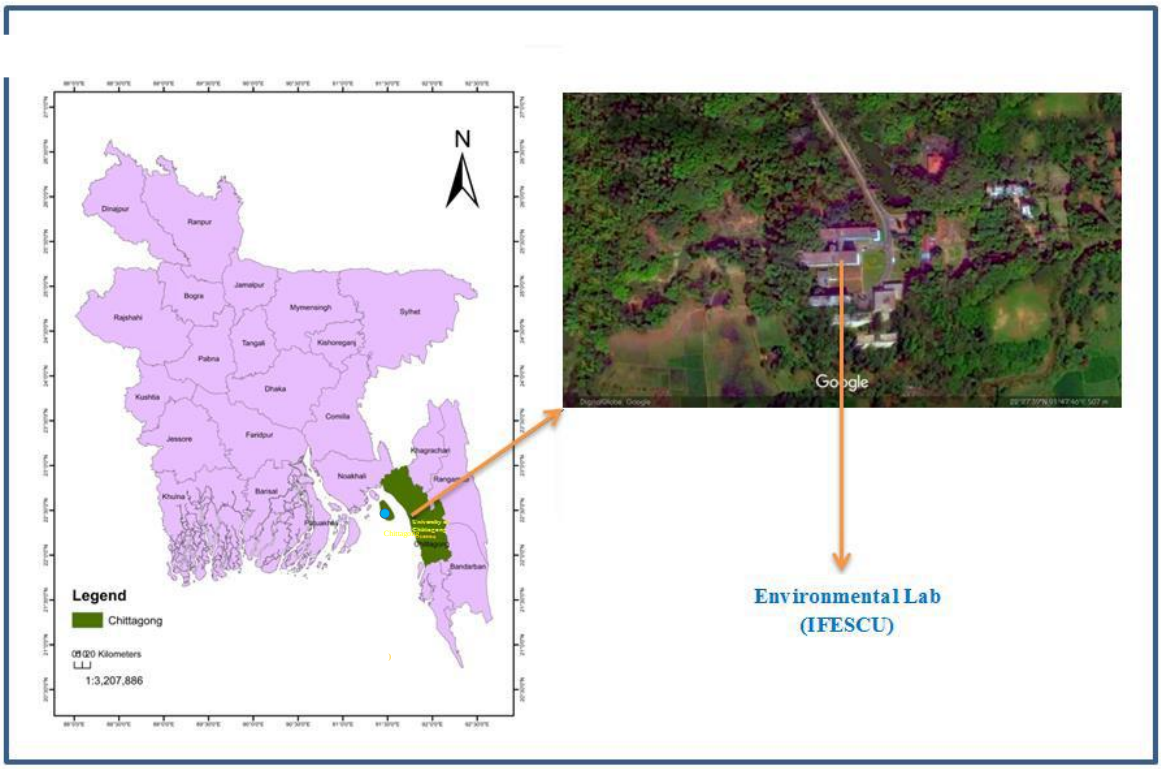

Fig.1: Map showing the location of environmental lab of the Institute of Forestry and Environmental Sciences, University of Chittagong (IFESCU), Bangladesh where the experiment was conducted.

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Table.1: Effects of phosphorus on seed germination, plumule and radical growth, collar diameter and number of lateral root of Cucumis sativus in arsenic solution culture.

Treatment	Germination (%)	Length (cm)		Total length (cm)	Collar diameter (mm)	Number of lateral root	Increased/decreased (%)	
		Plumule	Radicle				Germination	Total length
T0	76^{a}	8^{a}	6.5^{a}	14.5^{a}	1.8^{a}	20^{a}	00.00	00.00
T1	68^{b}	6.4^{bc}	6^{a}	12.4^{b}	1.6^{ab}	16^{abc}	- 10.53	- 14.48
T2	72^{ab}	6.7^{abc}	4.7^{b}	11.4^{b}	1.7^{ab}	19^{ab}	- 5.26	- 21.38
T3	66^{bc}	5.4^{c}	1.9^{d}	7.3^{c}	1.5^{ab}	14^{bcd}	- 13.16	- 49.66
T4	78^{a}	7.7^{ab}	2.7^{c}	10.4^{b}	1.6^{ab}	17^{abc}	+ 2.63	- 28.28
T5	54^{d}	3.9^{d}	1.2^{e}	5.1^{d}	1.3^{b}	10^{d}	- 28.95	- 64.83
T6	60^{cd}	5.6^{c}	1.5^{de}	7.1^{c}	1.4^{ab}	12^{cd}	- 21.05	- 51.03
***P* value**	<0.001	<0.001	<0.001	<0.001	0.144	0.011	---	---
***F* value**	16.337	9.214	134.382	28.141	1.938	4.359	---	---

Note: a-e = Mean values with different lowercase superscripts in a column are significantly different at $P<0.05$, according to Duncan's Multiple Range Test
(DMRT). T0= Control, T1=2ppm As, T2= 2ppm As+10ppm P, T3= 5ppm As, T4= 5ppm As+10ppm, P, T5= 10ppm As, T6= 10ppm As+10ppm P.

Table.2: Effects of phosphorus on the fresh and dry biomasses, vigor index and volume index of Cucumis sativus in arsenic solution culture.

Treatment	Seedling biomass (mg)		Dry biomass decreased (%)	Index	
	Fresh	Dry		Vigor	Volume
T0	240^{a}	30.3^{a}	00.00	1102^{a}	25.9^{a}
T1	169^{d}	21.1^{c}	- 30.29	843^{b}	16.4^{abc}
T2	205^{c}	25.6^{b}	- 15.44	821^{b}	19.4^{ab}
T3	146^{e}	18.3^{c}	- 39.79	482^{c}	12.2^{bc}
T4	218^{a}	27.6^{ab}	- 8.97	811^{b}	19.7^{ab}
T5	107^{g}	13.4^{d}	- 55.86	275^{c}	6.6^{c}
T6	139^{f}	20.9^{c}	- 31.21	426^{c}	11.0^{bc}
***P* value**	<0.001	<0.001	….	<0.001	0.034
***F* value**	446.278	15.967	….	18.896	3.193

Note: a-g = Mean values with different lowercase superscripts in a column are significantly different at $P<0.05$, according to Duncan's Multiple Range Test (DMRT). T0= Control, T1=2ppm As, T2= 2ppm As+10ppm P, T3= 5ppm As, T4= 5ppm As+10ppm, P, T5= 10ppm As, T6= 10ppm As+10ppm P.

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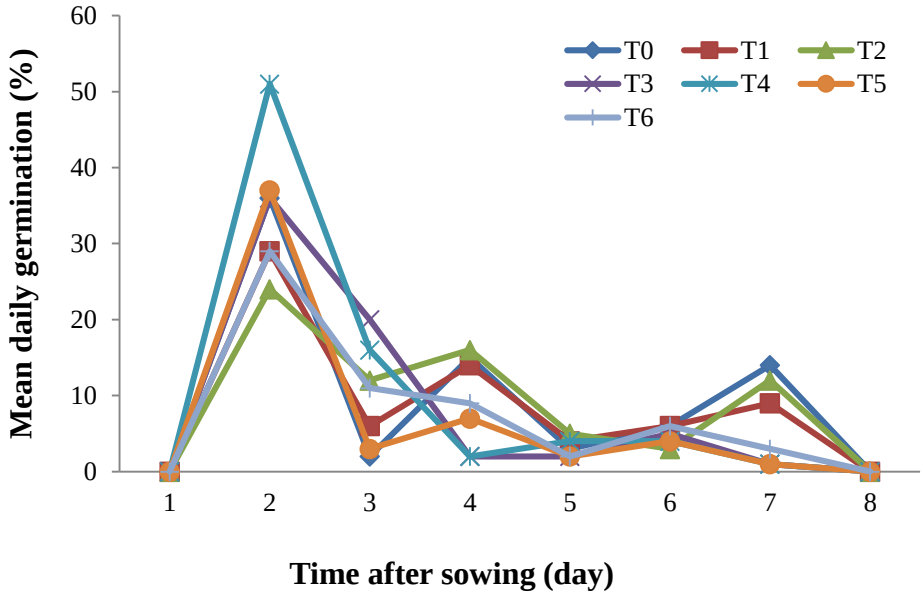

Fig.2: Effects of phosphorus on mean daily germination (%) of Cucumis sativus seeds in arsenic solution culture. T0= Control, T1=2ppm As, T2= 2ppm As+10ppm P, T3= 5ppm As, T4= 5ppm As+10ppm, P, T5= 10ppm As, T6= 10ppm As+10ppm P.

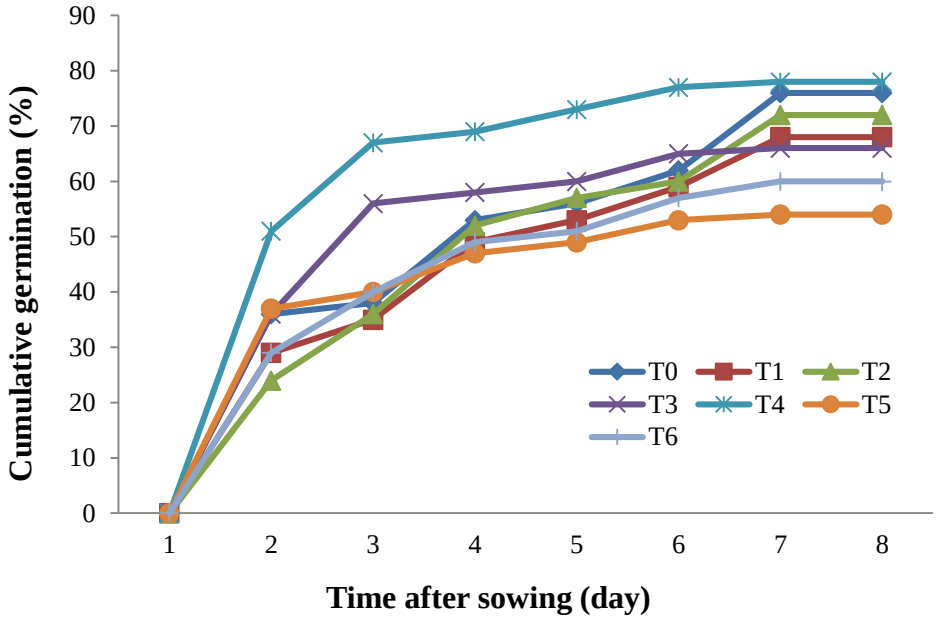

Fig.3: Effects of phosphorus on mean cumulative germination (%) of Cucumis sativus seeds in arsenic solution culture. T0= Control, T1=2ppm As, T2= 2ppm As+10ppm P, T3= 5ppm As, T4= 5ppm As+10ppm, P, T5= 10ppm As, T6= 10ppm As+10ppm P.

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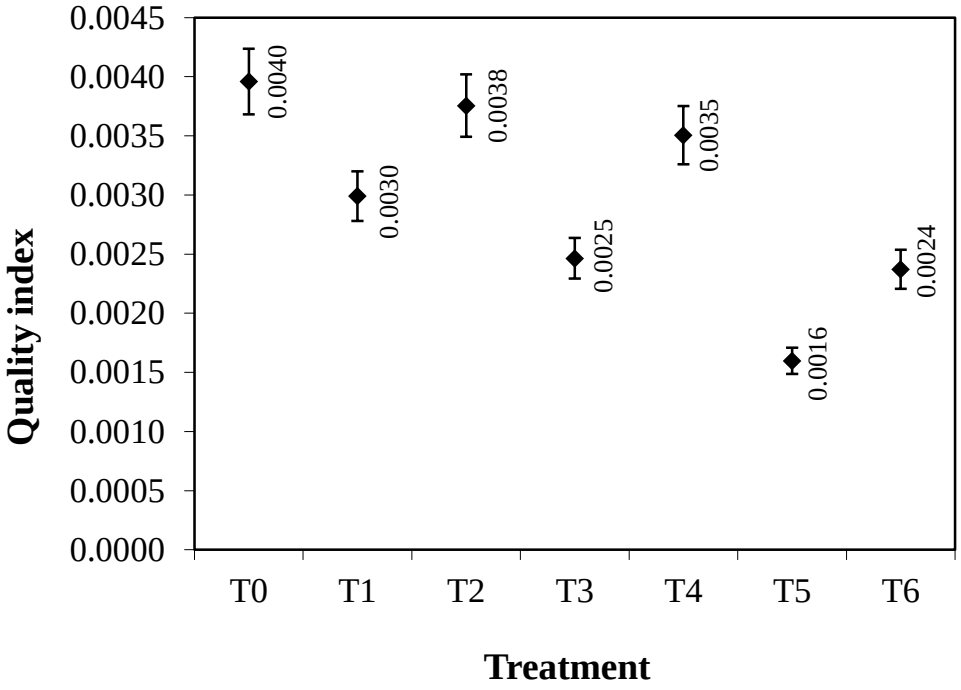

Fig.4: Effects of phosphorus on quality index of Cucumis sativus in arsenic solution culture. T0= Control, T1=2ppm As, T2= 2ppm As+10ppm P, T3= 5ppm As, T4= 5ppm As+10ppm, P, T5= 10ppm As, T6= 10ppm As+10ppm P.

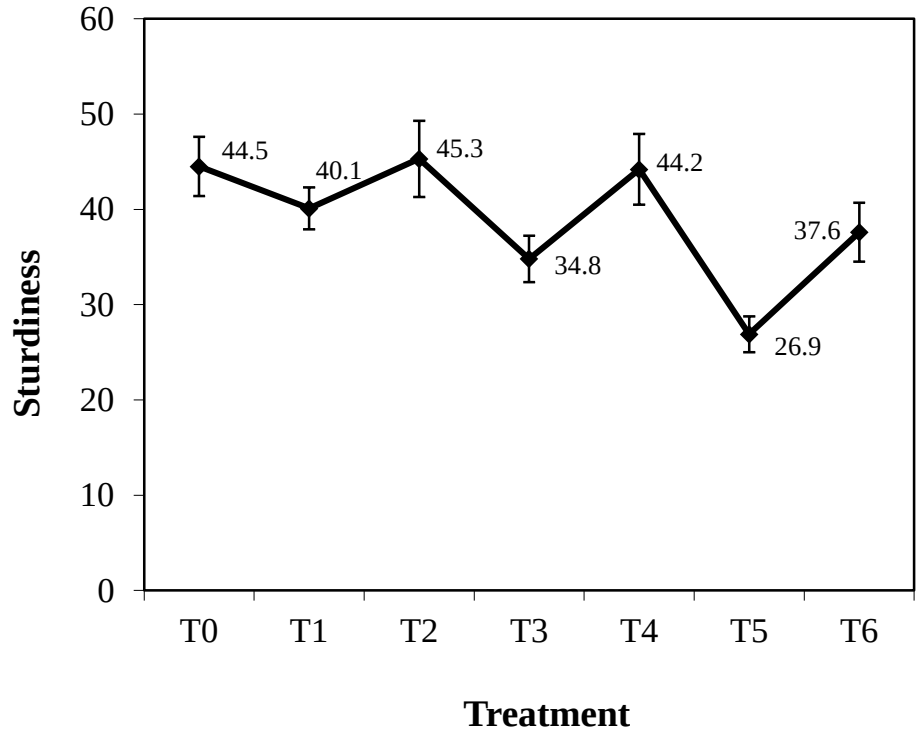

Fig.5: Effects of phosphorus on sturdiness of Cucumis sativus in arsenic solution culture. T0= Control, T1=2ppm As, T2= 2ppm As+10ppm P, T3= 5ppm As, T4= 5ppm As+10ppm, P, T5= 10ppm As, T6= 10ppm As+10ppm P.