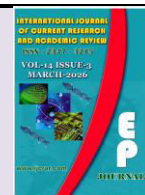




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Assessment of the Toxicity of Lead Nitrate on Germination and Vegetative Growth of *Pisum sativum* L.

Sunil Kumar* and Astik kumar Buts

Department of Botany, D.S. College Aligarh, U.P., India (Affiliated to Dr. Bhimrao Ambedkar University, Agra)

*Corresponding author

Abstract

The present study was conducted to assess the toxicological effects of lead nitrate on morphological traits of *Pisum sativum* L. using varieties IPF-99-25 (Adarsh) and IPFD-1-10 (Prakash) in M₁ and M₂ generations. The healthy seeds of both varieties of *Pisum sativum* were treated with 0.1%, 0.2%, 0.3%, 0.4% and 0.5% concentration of Lead nitrate before sowing in the experimental field. The obtained plants were considered as M₁ generation. The seeds obtained from M₁ generation were further treated with corresponding concentration of Lead nitrate and sown in experimental plot to obtain M₂ generation. Seeds were treated with 0.1 to 0.5% concentrations of lead nitrate prior to sowing. Observations revealed that seed germination, seedling survival, plant height and number of branches declined progressively with increasing concentrations. Delay in flowering and slight extension in harvesting period are also recorded at higher doses. Effects were mild at lower concentrations (0.1–0.2%) but became markedly adverse above 0.2% indicating a threshold for toxicity. At 0.5% treatment concentration, the reduction in seedling survival, height of the plants declined upto 36%. while no. of branches declined upto 51%. These findings clearly reflect the toxic impact of lead nitrate on germination and vegetative growth features of *Pisum sativum*.

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Pisum sativum, Lead nitrate, Germination, Vegetative growth and Toxicity

Introduction

Field pea (*Pisum sativum* L.) is one of the most significant legume crops cultivated in India. Pulses are known as “wonder crops” because they provide numerous advantages to humans and the environment. They play an important role in natural resource management, environmental safety and crop diversification which helps in achieving sustainable agriculture. Pulses are also an affordable and rich source of protein for millions of people. It is a member of the family Leguminosae and is mainly cultivated in cooler temperate regions, especially between the Tropic of Cancer to Mediterranean region. Pea grains are incredibly nutrient-dense. They contain about 27.8%

protein, 42.65% complex carbohydrates along with essential vitamins, minerals, dietary fiber and antioxidant compounds (Urbano *et al.*, 2003).

Plants absorb heavy metals from the soil through the root and from the atmosphere through over ground vegetative organs (Kabala *Et Al.*, 2009). The quantity or level of heavy metals absorption in plants depends not only on the concentration of the metals in the physical and chemical composition of the soil (Jankiewicz, B. and Adamczyk, D., 2010) but also varies in different plant species (Yargholi *et al.*, 2008). Some authors have reported the existence of differences in accumulation of heavy metals in plant cultivars, organs and tissues (Filipović-Trajković, R., Ilić, S. Z. and Šunić, L., 2012).

The heavy metals are a serious problem for plants as they greatly affect the metabolic activities of plants. Lead pollution mainly occurs due to mining and smelting activities the use of lead-based paints, gasoline and explosives as well as the disposal of Pb-contaminated sewage and waste materials.

Buts *et al.*, (2013) also reported the toxic effect of CdCl_2 on *Lens esculentus*. They studied upto two generations treating seeds of it with different concentration of Cadmium chloride. They reported that toxic effect increases with increase in the treatment concentration in both generations on all vegetative growth parameters and productivity but the losses incurred were recovered slightly in 2nd generation in lower treatment concentration. The productivity declined more than 50% among 0.5% treatment concentration treated plants.

Chandra Shekar *et al.*, (2011) reported that lower concentration of Cd treatment showed enhanced percentage of germination, survival percentage, plant height, root length, early flowering, more pollen viability increase in total chlorophyll content but the higher concentration of cadmium treatments showed inhibitory effect in *Lycopersicon esculentus* Mill.

Buts *et al.*, (2014) evaluated the morphological and productivity response on *Abelmoschus esculentus* under the stress of lead nitrate. They reported that lead nitrate treatment showed decrease in percentage of germination, survival percentage, plant's height and pod formation. The deleterious effects increased with increase in the treatment concentrations. The maximum losses in productivity was noted in 0.5% treatment concentration in both generation taken for study.

Seed germination and early seedling growth of *Albizia lebbeck* and *Leucaena leucocephala* were markedly suppressed under the application of 500 and 700 ppm concentrations of Pb and Cd. The greatest decrease was recorded under 700 ppm of Pb indicating that Pb has a strong toxic potential at this level of concentration. The minimal inhibition was detected with 500 ppm of Cd. Both species showed the least level of tolerance at 700 ppm Pb. However *Leucaena leucocephala* showed comparatively higher tolerance to both Pb and Cd than *Albizia lebbeck* (M. Z. Iqbal and Y. Shazia, 2003).

Materials and Methods

In the present investigation two cultivars of *Pisum sativum* viz. IPF 99-25 (Adarsh) and IPFD 1-10

(Prakash) have been taken for study. *Pisum sativum* contains $2n=14$ chromosomes and it belong to family Leguminosae. The seeds of the plant have been procured from ICAR-Indian Institute of Pulse Research, Kanpur. Lead nitrate were used to examine its effect on *Pisum sativum*. Various concentrations of lead nitrate are taken for seed treatment. Lead nitrate is the white or colourless translucent crystals. It is poisonous and its density is 4.53. Lead nitrate is soluble in water, slightly soluble in alcohol and methanol, insoluble in concentrated nitric acid and its aqueous solution is slightly acidic.

Healthy seeds of uniform size and shape were selected for lead nitrate treatment. The dry dormant seeds were soaked in water for six hours. Then these soaked seeds were placed in separate-petridishes containing lead nitrate solution of 0.1%, 0.2%, 0.3%, 0.4% and 0.5% for three hours. After treatment, the seeds were sown in well maintained experimental plots with a distance of 10 cm between plants and 30 cm between rows. A control group of 100 seeds, soaked in water for six hours, were also sown in the experimental plot. The rows were in the east-west direction which will be most effective in absorbing the sunlight. The gap between the rows will provide space for each row to move during side dressing and during harvest time.

Contrary to this in the field, the hypocotyls and cotyledons coming above the soil surface indicated germination. Facility for regular operation and irrigation was also provided. Chemical as well as other fertilizers were not used. This is to avoid confusion. In the field, seeds were placed in the first week of November and final harvesting was done during the period March last to April first week. In between the above periods, morphological characteristics were analyzed in respect of germination percentage, seedling survival, number of branches and plant height. The seeds were collected after harvesting and stored in glass containers separately with specific symbols & regarded as M_1 generation.

The stored seeds of M_1 generation were again treated with the corresponding concentration i.e. 0.1%, 0.2%, 0.3%, 0.4% and 0.5% of lead nitrate by the same methods as mentioned above for M_1 generation and sown in the prepared field. This second generation was termed as the M_2 generation. Germination, survival of seedlings and morphological traits were measured in the same way as in the M_1 generation. Phenotypic variations and their corresponding frequencies were observed and finally the rate of induced variability in quantitative traits for both M_1 and M_2 generations were calculated.

Results and Discussion

The results Obtained in the present study have been shown in table 1 to 4 and figure (A-D) and expressed together with the discussion in separate heading as under:-

Effect of Lead Nitrate on seed germination

In M₁ generation, the average seed germination of *Pisum sativum* (Pea) Variety IPF-99-25 are 90%, 88%, 85%, 81% & 68% in 0.1%, 0.2%, 0.3%, 0.4% & 0.5% concentration of lead nitrate respectively in comparison to 94% under control. In M₂ generation, the average seed germination of *Pisum sativum* are 88%, 84%, 81%, 79% & 72% in 0.1%, 0.2%, 0.3%, 0.4% & 0.5% treatment concentration respectively in comparison to 92% under control (Table 1 & 2). In variety IPFD-1-10 the germination were 88, 85, 84, 76, and 68 in M₁ generation and 90, 88, 84, 81, & 72 in M₂ generation in 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% treatment concentration of Lead nitrate respectively (Table 3 & 4). In control the germination percentage were 94% & 92% in M₁ & M₂ generation respectively.

Lead nitrate shows a concentration-dependent effect which create hindrance in seed germination in both of *Pisum sativum* varieties in both generations taken for study. The IPF-99-25 & IPFD-1-10 (M₁ generation) variety showed decreasing germination percentage with increasing treatment concentration. The M₂ generation also showed a similar pattern. The reduction is less in treatment concentrations of 0.1% and 0.2%. But the decline became more apparent after 0.3% concentration. The 0.5% concentration showed maximum inhibition which proved that higher doses produced greater toxic effects.

In variety IPFD-1-10, a similar pattern was observed in both M₁ and M₂ generations. Germination percentage decreased progressively with increasing concentrations of lead nitrate. The inhibitory effect was moderate up to 0.2% concentration, but reduction in germination was more apparent at 0.4% and 0.5% treatment concentrations. This indicates that higher concentrations of lead nitrate adversely affect seed viability and germination capability (Fig.1).

Therefore, the data showed that Lead nitrate adversely affect the seed germination in both generations of *Pisum sativum*. The reduction in seed germination is gradual as per increase in concentration of lead nitrate. Seed

germination was inversely related to the treatment concentrations of lead nitrate. This finding is similar to the finding of Buts *et al.*, 2011 in (Lentil).

Effect of lead nitrate on seedling survival

The seedling survival of variety IPF-99-25 are 85%, 83%, 80%, 77% & 62% in M₁ generation and 86%, 84%, 82%, 75%, 65% in M₂ generation among 0.1%, 0.2%, 0.3%, 0.4% and 0.5% treatment concentration of Lead nitrate treated seed respectively (Table 1 & 2).

Similarly in variety IPFD-1-10, the seedling survival percentage are 84, 82, 79, 74 & 66 in M₁ generation and 88, 84, 80, 76 & 69 in M₂ generation among 0.1%, 0.2%, 0.3%, 0.4% & 0.5% treatment concentration of Lead nitrate treated seeds respectively (Table 3 & 4).

The seedling survival percentage in control are 92 & 90 in M₁ generation and 91 & 90 in M₂ generation in varieties IPF-99-25 & IPFD-1-10 respectively (Fig.2).

The results clearly show that seedling survival in *Pisum sativum* declined progressively with increasing treatment concentrations of lead nitrate in both varieties and in both generations. In both M₁ and M₂ generations, a gradual decrease in the survival percentage was encountered from lowest to highest level of treatment in both varieties i.e. IPF-99-25 with more prominent drop at 0.4% and 0.5% concentrations. The reduction remained relatively moderate up to 0.3% whereas higher concentrations caused a marked decrease in seedling survival. This trend suggests that increasing levels of lead nitrate exert toxic effects on early seedling establishment thereby reducing the survival capability of the plants.

Effect of Lead nitrate on Plant height

Average height of plants in variety IPF-99-25 (Adarsh) in M₁ generation are 199.38 cm, 194.75 cm, 183.71 cm, 172.36 cm and 138.5 cm in 0.1%, 0.2%, 0.3%, 0.4% and 0.5% treatment concentration of lead nitrate respectively in comparison to 206.39 cm in control. In M₂ generation, the value for the same criteria are 195.40 cm, 193.77 cm, 184.07 cm, 174.98 cm and 146.93 cm respectively in comparison to 207.70 cm in control (Table: 1 & 2). While in variety IPFD-1-10 (Prakash) in M₁ generation, average height of plants are 102.77 cm, 96.47 cm, 89.87 cm, 81.87 cm and 68.54 cm in 0.1%, 0.2%, 0.3%, 0.4% and 0.5% concentration treatment of lead nitrate respectively in comparison to 108.55 cm in control.

Table.1 Effects of Lead nitrate on *Pisum sativum* Variety IPF-99-25 in M₁ Generation

Treatments (Lead Nitrate)	Germination Of Seeds in the field	Seedling Survival	Height of the plant (cm) $\pm$ SD	Number of Branches per plant $\pm$ SD
0.1%	90%	85%	199.38 $\pm$ 25.17	4.89 $\pm$ 2.71
0.2%	88%	83 %	194.75 $\pm$ 28.78	4.39 $\pm$ 2.42
0.3%	85%	80 %	183.71 $\pm$ 23.58	3.28 $\pm$ 0.45
0.4%	81%	77 %	172.36 $\pm$ 17.05	3.19 $\pm$ 0.28
0.5%	68%	62 %	138.5 $\pm$ 22.5	3.05 $\pm$ 7.5
Control	94%	92 %	206.39 $\pm$ 48.60	6.30 $\pm$ 2.70

Table.2 Effects of Lead nitrate on *Pisum sativum* Variety IPF-99-25 in M₂ Generation

Treatments (Lead Nitrate)	Germination Of Seeds in the field	Seedling Survival	Height of the plant (cm) $\pm$ SD	Number of Branches per plant $\pm$ SD
0.1%	88%	86 %	195.40 $\pm$ 44.04	5.06 $\pm$ 2.43
0.2%	84%	84 %	193.77 $\pm$ 35.11	4.34 $\pm$ 1.69
0.3%	81%	82 %	184.07 $\pm$ 39.10	4.14 $\pm$ 1.55
0.4%	79%	75 %	174.98 $\pm$ 39.58	3.90 $\pm$ 1.74
0.5%	72%	65 %	146.93 $\pm$ 26.71	3.88 $\pm$ 1.52
Control	92%	90 %	207.70 $\pm$ 30.10	6.16 $\pm$ 1.10

Table.3 Effects of Lead nitrate on *Pisum sativum* Variety IPFD-1-10 in M₁ Generation

Treatments (Lead Nitrate)	Germination Of Seeds in the field	Seedling Survival	Height of the plant (cm) $\pm$ SD	Number of Branches per plant $\pm$ SD
0.1%	88	84%	102.77 $\pm$ 21.93	3.00 $\pm$ 1.52
0.2%	85	82 %	96.47 $\pm$ 1.33	2.98 $\pm$ 0.97
0.3%	84	79 %	89.71 $\pm$ 17.60	2.86 $\pm$ 0.77
0.4%	76	74 %	81.87 $\pm$ 20.18	2.54 $\pm$ 1.60
0.5%	68	66 %	68.54 $\pm$ 15.97	2.47 $\pm$ 1.52
Control	94	91 %	108.55 $\pm$ 24.19	3.09 $\pm$ 0.83

Table.4 Effects of Lead nitrate on *Pisum sativum* Variety IPFD-1-10 in M₂ Generation

Treatments (Lead Nitrate)	Germination Of Seeds in the field	Seedling Survival	Height of the plant (cm) $\pm$ SD	Number of Branches per plant $\pm$ SD
0.1%	90	88 %	102.11 $\pm$ 21.57	3.08 $\pm$ 1.49
0.2%	88	84 %	95.19 $\pm$ 6.60	3.03 $\pm$ 1.33
0.3%	84	80 %	89.47 $\pm$ 3.71	2.83 $\pm$ 1.22
0.4%	81	76 %	82.5 $\pm$ 13.71	2.63 $\pm$ 0.92
0.5%	72	69 %	70.60 $\pm$ 19.84	2.45 $\pm$ 1.23
Control	92	90 %	107.06 $\pm$ 11.42	3.16 $\pm$ 0.53

Figure.1 Percentage variation in germination of *Pisum sativum* treated with Lead Nitrate in respect to control in M₁ & M₂ generation.

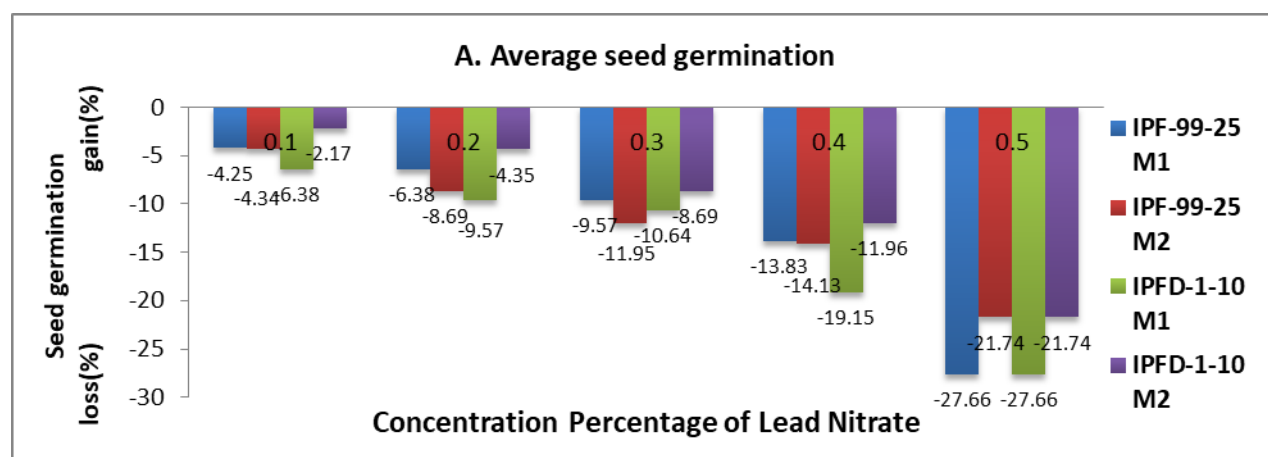


Figure.2 Percentage variation in Seedling survival of *Pisum sativum* treated with Lead Nitrate in respect to control in M₁ & M₂ generation.

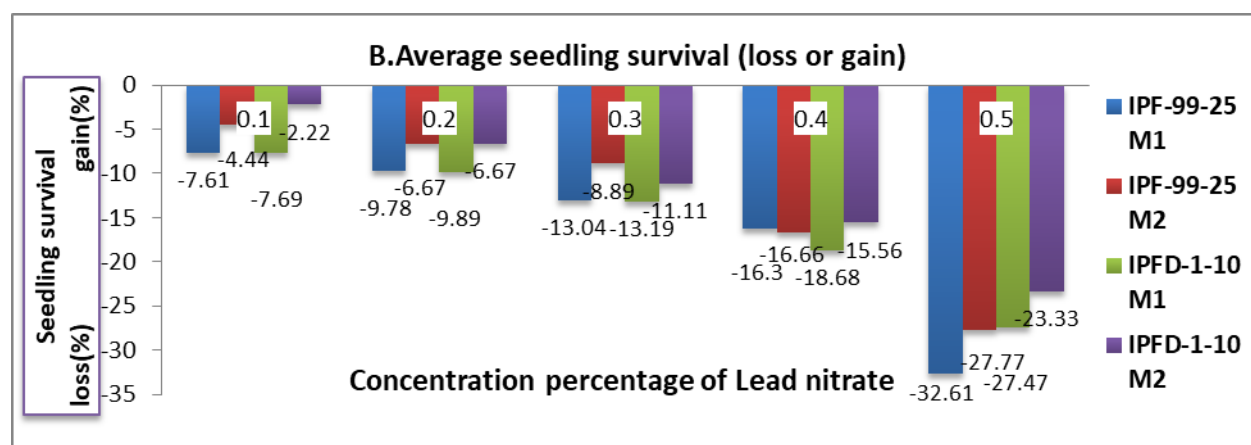


Figure.3 Percentage variation in height of the plant of *Pisum sativum* treated with Lead Nitrate in respect to control in M₁ & M₂ generation.

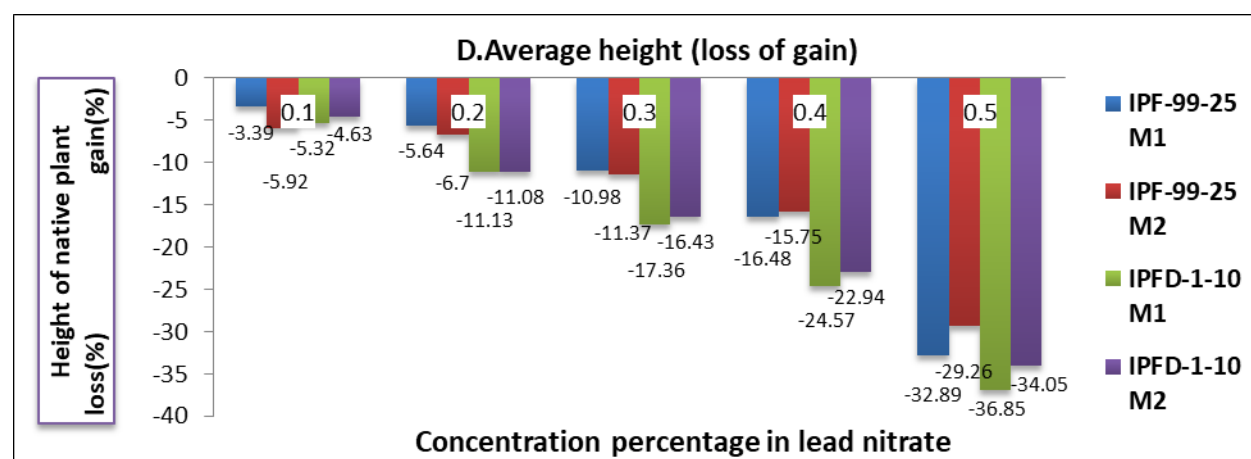
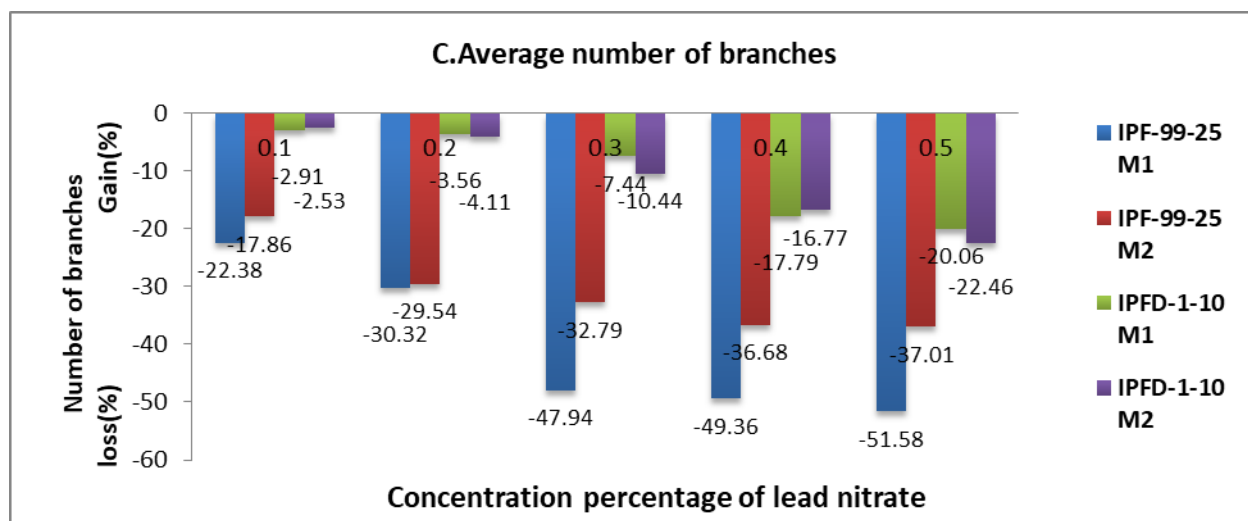


Figure.4 Percentage variation in number of branches of *Pisum sativum* treated with Lead Nitrate in respect to control in M₁ & M₂ generation.



In M₂ generation, the value for the same criteria are 102.11 cm, 95.19 cm, 89.47 cm, 82.5 cm and 70.60 cm respectively in comparison to 107.06 cm in control (Table: 3 & 4).

The observations clearly demonstrate that plant height decreased progressively with increasing concentrations of lead nitrate in both varieties and in both generations. In variety IPF-99-25 the reduction in plant height was gradual at lower concentrations but became more pronounced at 0.4% and 0.5% indicating the inhibitory effect of higher lead nitrate levels on vegetative growth. A similar pattern was also evident in variety IPFD-1-10 where plant height declined consistently with the increase in treatment concentration. The comparatively higher reduction at elevated concentrations suggests that lead nitrate exerts a toxic influence on plant growth processes such as cell elongation and division ultimately leading to reduced plant height in *Pisum sativum* (Fig. 3).

Effect of Lead nitrate on Number of branches

In variety IPF-99-25 (Adarsh) the average number of branches is found to be 4.89, 4.39, 3.28, 3.19 and 3.05 in 0.1%, 0.2%, 0.3%, 0.4% and 0.5% concentration treatment of Lead nitrate respectively in comparison to 6.30 in control in M₁ generation. Similarly in M₂ generation, the value for the same criteria are 5.06, 4.34, 4.14, 3.90 and 3.88 respectively in comparison to 6.16 in control (Table: 1 & 2). In variety IPFD-1-10, the average number of branches are 3.00, 2.98, 2.86, 2.54 and 2.47 in 0.1%, 0.2%, 0.3%, 0.4% and 0.5% concentration

treatment of Lead Nitrate respectively in comparison to 3.09 in control in M₁ generation. In M₂ generation, the value for the same criteria are 3.08, 3.03, 2.83, 2.63, and 2.45 respectively in comparison to 3.16 in control (Table: 3 & 4).

The results indicate that the number of branches decreased gradually with increasing concentrations of lead nitrate in both varieties in both generations. In variety IPF-99-25 a clear reduction in branching was observed from 0.1% to 0.5% concentration indicating the inhibitory effect of higher lead nitrate levels on vegetative growth. A similar decreasing trend was also observed in variety IPFD-1-10 where the number of branches declined progressively with increasing treatment concentration. The comparatively lower number of branches at higher concentrations suggests that lead nitrate stress interferes with normal growth and branching processes in *Pisum sativum* (Fig.4).

On the basis of above discussion, it is clearly established that all growth parameters are adversely affected with lead nitrate. The adverse impact increases with increasing the treatment concentration of lead. Although I have used upto 0.5% concentration of lead nitrate.

Most probably higher doses above 0.5% may completely hamper the viability of seeds. This toxic effect may harmfully affect the health of human beings through bioaccumulation process in food chain & food web. Therefore awareness should be raised & care should be taken against lead nitrate pollution in nature.

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