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Effect of different types of abiotic stress on the growth and productivity of linseed (*Linum usitatissimum* L.)

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Abstract
The objective of this study is to evaluate the effect of UV radiation, different concenterations of Copper salts and auxin on the growth and productivity of linseed (*Linum usitatissimum* L.) using some morphological and phytochemical markers. Different treatment affects the growth and productivity of linseed differently in concentration dependent manner. UV radiation treatment affects the growth and productivity of the plant adversely by inhibiting the seed viability and plant productivity. The copper salt affects the growth and productivity of linseeds in concentration dependent manner. The low and medium concentrations of copper ions enhances the seed viability and conversion of seed to seedling without any promising effect on the growth and productivity of the plant but the higher copper concentrations adversely affect the seed germination plant growth and productivity. The auxin treatment show promising effect on the growth and productivity of the plant resulting in about 15 to 20% increment in productivity. The UV-B is adversely affecting the growth and productivity of plant by destabilizing DNA. The plant needs elements like copper for its growth and development as it is integral cofactor of large number of enzyme and coenzymes, electron carrier protein involved in essential metabolic activities of photosynthesis, respiration, DNA synthesis, gene regulation etc. But the presence of this essential element in high concentration may impose inhibitory influence on plant growth and productivity. Similarly the lower concentration of phytohormone auxin stimulate plant growth development and productivity but the concentration above optimal level impose adverse effect on linseed. Thus the knowledge of optimal dose of exogenously supplied micronutrient and growth regulator is essential to favorably induce the plant growth and yield

Keywords: linum, anthrone, metallozyme, morphological, CTAB

Introduction

In present scenario healthy food and good health is a topic of concern due to drastic change in life style. Now-a day's people show high interest in food source which are rich in good quality of fat, proteins, dietary fibers, vitamins and minerals. Linseed is among one of the important traditional plant with immense neutraceutical and medicinal value due to its diverse pharmacological effects (Umer *et al*, 2017). Linseed is gaining popularity as functional neutraceutical due presence of abundance of high quality of fatty acids, alpha linolenic acid an important omega fatty acid along with dietary fibres, proteins, lignans, phytoestrogens, vitamins and minerals having promising effect on cancer, cardiovascular diseases, obesity and other human ailments (Katare *et al*, 201). 'Linseed' (*Linum usitatissimum* L.) belongs to dicotyledonous family Linaceae. The genus *Linum* includes approximately 250 species (Fatma *et al*, 2016). It is an annual herbaceous plant growing up to the height of 15-30 inches, morphologically characterized by an erect stem bearing many lateral branches arising from the base of the stem. *L. usitatissimum* completes its life cycle in about 100 days. Depending upon the variety of the plant flowers may be white, blue, pink or violet and bear diploid chromosome number of 2n =30 (Nag *et al*, 2015) [20]. The mature fruits of linseed are ball like capsule with segments. Seeds are reddish to deep brown in color. The colour of seed is determined by amount of tannin present in pigmented cells of seed coat (Katare *et al*. 2012) [13].

The medicinal importance of plant is due to presence of many pharmacologically active phytochemical substances like alkaloids, glycosides, Resins, flavanoids, volatile oil, gum, Tannin, fatty acids, lignans etc. (Bekal *et al*, 2015) [4]. Linseed contains nearly 38-40% fat, 23-25% protein, 15-20% carbohydrate of which one third parts consisted of mucilage and 20-80 % fiber. Omega-3 fatty acid is the chief constituent of the fat content of the seed. Oil contains minerals, amino acid and vitamins like A, B, D and E (Hanna *et al*., 2017). Several research studies carried out to evaluate the effects of ultraviolet-B radiation on field grown linseed crop exhibited that ultraviolet-B radiation mainly affects the growth, productivity and chlorophyll content of the linseed crop plant (Goyal *et al*, 1991) [9]. The exposure of plant to UV radiation may results in chlorosis and necrosis of leaves by inhibiting the synthesis of chlorophyll content (Zhao *et al*, 2003) [36]. The metallic elements like copper, zinc, manganese are essentially required in photosynthetic organism in trace amount but their higher concentrations may induce metal toxicity (Fernandes & Henriguez, 1991; Baron *et al*, 1995) [3]. Copper, an essential trace element is required for the proper activities of large number of proteins, enzymes and metallozymes (Burkhead *et al*, 2009) [5] in plants but its high concentration may adversely affect cellular biomolecules like chlorophyll by replacing the magnesium ion from it (Kupper *et al*, 2002) [15] or by replacing metallic cofactor like zinc from essential enzymes (Valasta *et al*, 2018; Laporta *et al*, 2020) [17]. Excess of copper may adversely affect photosynthesis by substituting Mg^{++} under low radiance with dark phase by phenomenon called shade reaction (Kupper *et al*, 2002, 2003) [15]. Experimental studies using copper on linseed

plant has shown its impact on various aspect of growth, biomass and enzymatic activities, as high concentration of copper (Cu) have shown significant decrease in biomass and growth in many plants (Saleem *et al*, 2020) [27]. The growth regulators like auxin and gibberellins produce profound effect on yield, fiber quality and quantity in *L. usitatissimum*. Gibberellic acid reported to induce the stem elongation but adversely affects the stem diameter while the Indole acetic acid (auxin) have shown the opposite effect by promoting the increment in stem girth by inhibiting the stem elongation and thus enhancing the fiber yield and adversely affecting its quality (Silva *et al*, 2005) [8].

Material and Methods

Seeds of Garima variety of Linseed (*Linum usitatissimum* L.), selected for present study were sown in the field at Panchanan Maheshwari garden, Ewing Christian College, Prayagraj. The experimental field was divided into seven plots and each plot was sown with equal number of seeds (50 each). The seed samples were divided into seven groups *viz*. A, B, C, D, E, F and G, each group of seed samples were given different sets of treatments in order to analyse the effect of each of these treatments on growth, chemistry and DNA content of plant. Each of the seed samples were sown in separate plot under identical edapho-climatic conditions.

2.1. The seeds were sown during the month of November 2019 and allowed to grow and flower during February 2020.

- The seed sample of group A was sown under normal condition and it was treated as controlled.
- The seed sample of group B was sown in field after treated it with UV radiation (254nm wave length) for three hour under laminar air flow cabinet.
- The seed sample of group C was sown in the field after treated it with 50 mg/l concentration of $CuSO_4$ solution.
- The seed sample of group D was sown in the field after treated it with 100 mg/l concentration of $CuSO_4$ solution.
- The seed sample of group E was sown in the field after treated it with 500 mg/l concentration of $CuSO_4$ solution.
- The seed sample of group F were sown in the field and treated with 20 mg/l concentration of Indole acetic acid (auxin) on growing apical part of the plant after 20 days of germination.
- The seed sample of group G were sown in the field and treated with 1 mg/l concentration of indole acetic acid (auxin) on growing apical part of the plant after 20 days of germination.

Cultivated seeds were left to grow till the end of the season and experiments were performed to investigate the effect of different treatments.

Estimation of chlorophyll content

The chlorophyll content of plants growing in each of the seven experimental plots was estimated. The chlorophyll of plant samples of each of the plots was extracted in acetone and chlorophyll content was estimated at absorption wavelength of 663 nm and 645 nm respectively using uv-spectrophotometer as suggested by Arnon (1949) [2] and Witham *et al* (1971) [33]. Amount of chlorophyll a and b determined by using formulae as suggested by Arnon.

mg chlorophyll a/gm tissue $=12.7(A_{663}) - 2.69(A_{645}) \times V/1000 \times W$

mg chlorophyll b/gm tissue $=22.9\ (A_{645}) - 4.68(A_{663}) \times V/1000 \times W$

mg total chlorophyll /gm tissue $=20.2(A_{645}) +8.02(A_{663}) \times V/1000 \times W$

A= absorbance at specific wavelength
V= final volume of chlorophyll extract in 80% acetone.
W= fresh weight of tissue extract

Estimation of carbohydrate content

Carbohydrate content was estimated by Anthrone method as per methodology suggested by Hedge and Hofreiter (1962) using Uv-vis-spectrophotometer. With the help of graph concentration of standard is obtained. By putting the value of concentration and absorbance in the formulae final concentration of carbohydrate in each plot was obtained.

Concentration of carbohydrate content present in sample is obtained by using the formulae

Concentration of unknown = absorbance of unknown × concentration of standard/absorbance of standard

Value of carbohydrate = (concentration of carbohydrate in mg / volume of sample) ×100

Test for the presence of copper

The copper absorption by plants of plot C, D and E was estimated by using colour intensity of thin root section.

Statistical analysis

- Average height of plant was calculated by arithmetic mean- $X= \sum fx/\sum f$
- Germination percentage(%)= number of seedling/total number of seeds×100

DNA isolation

DNA was isolated from leaves without using liquid nitrogen by C-TAB method as per methodology suggested by Sharma *et al*. (2003) [28]

Results

Effect of different treatment on morphological growth and development of *L. usitatissimum* (Table 1)

- **Germination time:** The seed sown in each of the plots A to G except B and E exhibit relatively similar seed germination time and pattern. The seeds of plot B treated with UV radiation show delayed germination.
- **Germination % of seeds:** Highest seed germination percentage was recorded in plot C (100%) followed by plot D (96%), A (92%), G (91%), F (89%), E (68%) and B (64%). The lowest seed germination percentage and seed viability is exhibited by seed sown in plot B treated with UV radiation.
- **Height of plants:** Plants of plot F treated with 20 mg/l concentration of auxin exhibited maximum decline in plant height and Plants of plot G treated with 1 mg/l concentration of auxin showed maximum plant height as compare to the controlled plants sown in plot A (Figure 2).
- **Flowering time:** The plants that emerged from seeds treated with 20mg/l concentration of auxin grown in plot F and the plants of plot E in which seeds were

treated with 500mg/l concentration of $CuSO_4$ exhibit characteristic decline in flowering time as compare to control and the plants given other treatments.

- **Flower size:** Largest flower size exhibited by the plants of plot B treated with UV radiations. Plants of plot F treated with0.20mg/l concentration of auxin exhibit considerable decline in flower size.
- **Leaf morphology:** The leaves of plot A, B and G have wider leaves and were bright green in colour. Plot C, D and E plants have narrower and longer leaves. Plot F plants have small, slightly broad and dark green coloured leaves. The leaf blade show characteristic variation in plants of plots C to F.
- **Capsule number:** Maximum capsule number is exhibited by plants of plot G and minimum number of capsule number is exhibited by plants grown in plot B and plot E.

Fig 1: Normal healthy leaves of controlled plant

Fig 2: longer and narrower leaves of $CuSO_4$ treated plants

Fig 3: small and broad leaved auxin treated plants)

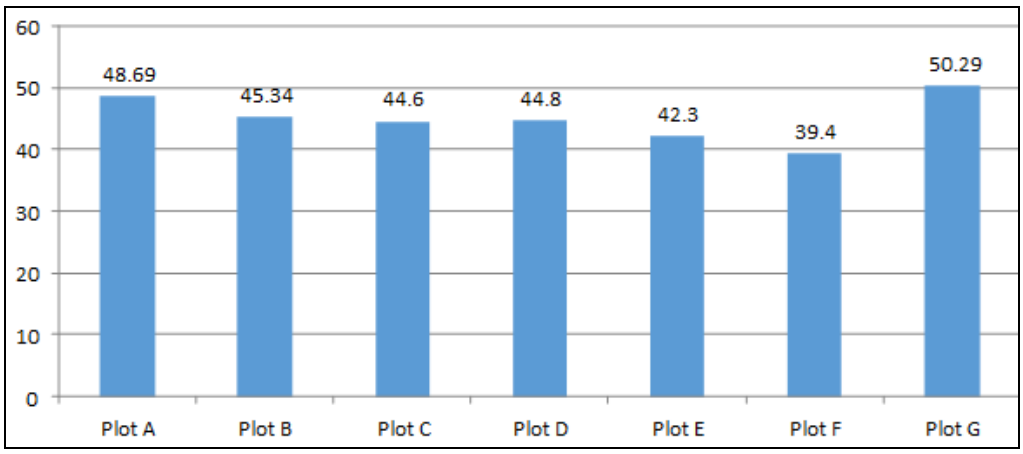

Fig 4: Effect of different treatments on average height of plants of *L. usitatissimum*

Table 1: (Effect of different treatment on morphological growth and productivity of L. *usitatissimum*)

PLOT	Germination time	Germination %	Height (cm)	Flowering time	Flower size	Leaf morphology	Capsule no/plant
A	On time	92	48.7	Normal	Maximum	Wider	20
B	Delayed	64	45.34	Late	Maximum	Wider	12
C	On time	100	44.6	Late	Medium	Narrower	18
D	On time	96	44.8	Late	Medium	Narrower	17
E	Delayed	68	42.3	Too late	Medium	Narrower	10
F	On time	84	39.4	Too late	Minimum	Small slightly broader	17
G	On time	87	50.29	Normal	Normal	Wider	23

Effect of different treatment on Biochemical attributes of *L. usitatissimum*

- **Effect of copper treatment:** The root section of the plants of plot C, D and E which were treated with 50 mg/l, 100 mg/l and 500 mg/l conc. of $CuSO_4$, respectively, were taken and were treated with NaOH solution. After treating with NaOH solution blue colour appeared on the root section which indicates that the copper was absorbed by the plants from the soil (Fig. 4).

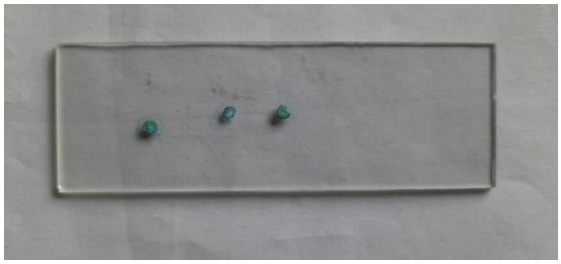

Fig 4

- **Chlorophyll estimation:** it was observed that the plants of plot A had maximum chlorophyll value followed by plot G, plot F, plot B, plot C and D then plot E plants. Plot E plants had minimum chlorophyll value (Fig.5a, b)

Fig 5: a (Chlorophyll extracted from plants of each plot)

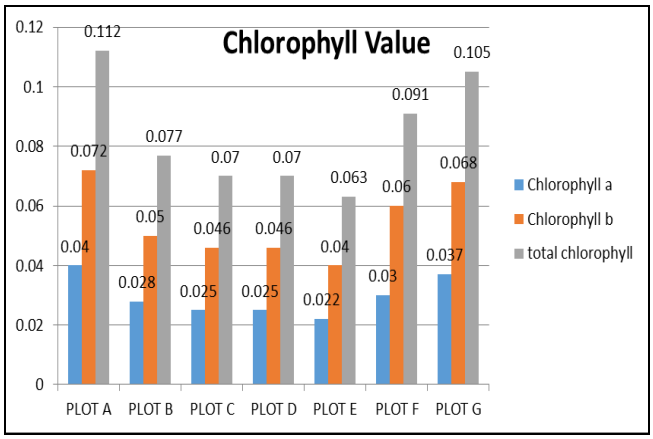

Fig 5: b (chlorophyll content in different extract)

- **Carbohydrate estimation:** After performing carbohydrate test by Anthrone method on seeds of different plots following value of carbohydrate were obtained (Table 3 and 4, Fig. 6)

Table 3: Standard value of glucose solution and optical density as measured through Uv-vis-spectrophotometer

Volume of standard solution in ml	Optical Density at 630 nm	Concentration in mg/ ml
0 ml	0	0
0.2 ml	0.21	0.2
0.4 ml	0.41	0.4
0.6 ml	0.60	0.6
0.8 ml	0.78	0.8
1.0 ml	0.99	1.0

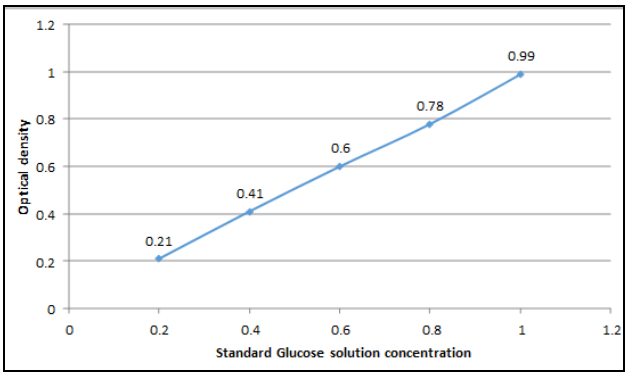

Fig 6: (Graph between optical density and concentration for standard glucose solution)

Table 4: Carbohydrate content of *L. usitatissimum* as determined through Anthrone method using UV-vis-spectrophotometer

Sample source	Optical Density at 630 nm.	Concentration in mg	Value of carbohydrate in 100 mg sample
Plot A	0.31	0.313	31.3
Plot B	0.25	0.252	25.2
Plot C	0.23	0.232	23.2
Plot D	0.22	0.202	20.2
Plot E	0.19	0.191	19.1
Plot F	0.26	0.262	26.2
Plot G	0.29	0.292	29.2

From table-4 it can be observed that the highest carbohydrate conc. was present in plot A plants followed by plot F, plot B, plot C, plot D and lowest carbohydrate concentration was present in plot E plants.

- **Electrophoretic analysis of DNA:** After performing electrophoresis it was observed that the DNA obtained from plot A (controlled) plant was run on agarose gel and perfect band pattern was observed under UV trans illuminator. DNA obtained from plot B (UV treated) plant was also run on electrophoretic gel but the band pattern obtained was ahead of the loading dye. On the other hand DNA obtained from plot C ,plot D and plot E (0.05g/l, 0.10g/l and 0.50g/l conc. of $CuSO_4$, respectively) did not run on the electrophoretic gel and no band pattern were obtained. DNA obtained from Plot F and plot G (0.20g/l and 0.01g/l concentration of Auxin) plants was run on the electrophoretic gel and suitable band pattern was observed under uv-transilluminator.

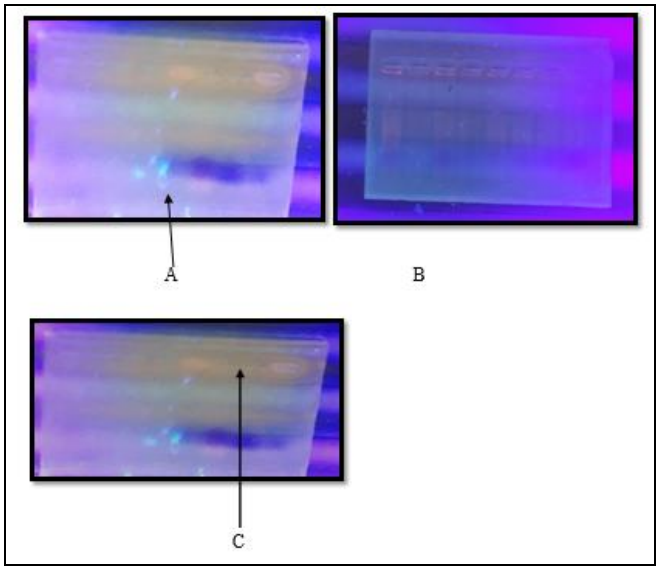

Fig 7: (A) DNA band ahead of loading dye in UV treated plants, (B) Normal DNA band pattern of untreated plant, (C) $CuSO_4$ treated plants lacking DNA bands

Discussion

Linum usitatissimum exhibit considerable alteration in growth, development and productivity pattern in response to diverse treatment. The treatments of linseed plant with Copper sulphate solution, auxin and ultraviolet radiation produces significant effect on the morphology, phytochemistry of the plant but the extent of alteration in these parameters depends upon the dosage of the treatment. Treatment with ultraviolet radiation and $CuSO_4$ solution at concentrations of 50mg/l and 100 mg/l respectively resulted in almost similar effects except the seed germination rate. UV treatment adversely inhibited the rate of seed germination while the treatment of seeds with low concentration $CuSO_4$ solution favorably promoted the germination and viability of the seeds of the plant indicating that copper ion induces the seed germination by either activating the enzymes or metallozymes and coenzymes involved in seed germination of *L. usitatissimum*. Treatment with UV radiation resulted in delayed germination time, decreased germination percentage, reduction in plant height, capsule number, and chlorophyll and carbohydrate content of the plant (Table 1, Fig. 5a, b). The present result is in consonance with the findings of previous authors (Goyal *et al* 1989). The treatment of linseed with UV-B radiation inhibits the plant growth and photosynthesis by adversely affecting the metabolic process involved in synthesis of photosynthetic pigments and reduction in carbon assimilation. Similar results are obtained in other plant species by several authors (Mark and Tevini, 1997; Rai *et al* 2017) [18, 22]. There may be more than one mechanism by which ultraviolet radiation induces adverse affects on the plant growth and development UV radiation may inhibit stem elongation by adversely affecting the biosynthesis of Indole acetic acid (IAA a natural auxin by destabilizing and destroying its precursor tryptophan or by breaking down and depredating IAA (Ros and Tevini,1995) [26]. Auxin indirectly promotes the biosynthesis of Gibberellic Acid at internodes by inducing biosynthesis of GA_3 oxidase and by inhibiting GA_2 oxidase biosynthesis. The GA_3 oxidase catalyse the conversion of GA_{20} to $GA_1$by hydroxylation of carbon 3 which induces tallness in plant. Thus UV radiation treatment inhibits the elongation of plant by destabilizing the synthesis of both auxin and gibberellins (O'Neill and Ross, 2002). UV radiation may affect the growth and productivity of plants by inhibiting the synthesis of photosynthetic pigments like chlorophyll and carotenoids or by their their degradation through lipid per-oxidation or by inhibiting the expression of gene coding for chlorophyll binding proteins on photosynthetic lamellae of chloroplast (Casti and Walbot 2003) [6]. The UV treatment also results in decline accumulation of carbohydrate content in seeds. The UV radiation inhibit the accumulation of carbohydrate in seeds destabilizing the Sucrose–H^+ transporters adversely affecting the process of apoplastic phloem loading in herbaceous plants like *Linum usitatissimum* (Rai *et al*.2017) [22]. The low concentration of copper sulphate (50mg/l) enhances the seed viability and resulting 100 % seed germination but the percentage of conversion of seed to seedling decline with increasing concentrations and at high concentration (500mg/l) it reduces to 68%. The present experimental result is in consonance with the finding of other authors (Yruela, 2009 and Zafar *et al*, 2018) [35].

Copper is an important micronutrient of plants. It is an important cofactor of large number of enzymes and metallozymes involved in important biological and physiological activities of plants like photosynthesis, chlorophyll, and carbohydrate synthesis, oxidative phosphorylation and nucleic acid synthesis (Fernandes and Henriques, 1991; Burkhead *et al*, 2009; Adrees *et al*, 2015) [5, 1]. Thus the optimal concentration of copper induces seed viability and germination by enhancing the efficiency of copper containing metallozymes involved in seed germination and plant growth. The optimal concenteration of copper increases photosynthetic ability by enhancing the efficiency of mobile electron transporter plastocyanin (Raven *et al*, 1999) [24]. Though Cu is needed essentially in trace amount but its high concenteration may induce oxidative stress adversely affecting cellular biomolecules destroying and destabilizing thyalokoid membrane protein and adversely affecting photosynthetic ability of plants (Rehman *et al*, 2019) [25]. The high concentration of copper in plants may lead to substitution and replacement of magnesium in chlorophyll molecules under low radiance by the phenomenon called shade reaction (Kupper, 2002, 2003) [16]. It results in chlorophyll damage adversely affecting photosynthetic reactions particularly associated with photosystem II (Mohanty *et al*, 1989; Kupper, 2003) [19. 16] Thus high concentration of Copper adversely affects the plant growth, carbon assimilation and reproductive ability by adversely affecting the photosynthetic ability of the plant under oxidative stress induced by copper toxicity in plants.

When plants are grown in soil containing toxic level of Cu it was reported that the plants accumulated reactive oxygen species like hydrogen peroxide and free oxide radicals The copper induced oxidative stress increases DNA instability by promoting its destruction as observed in DNA isolated from plant samples grown in plot C to E is in consonance with the findings of Mostafa and Fujiya, 2013. The DNA instability caused by copper induced oxidative stress may be due to increased level of proline content in proteins. Proline is an amino acid that destabilise secondary (α helix and β plated sheet) and tertiary structure of protein. The high concentration of proline adversely affects the stability of protein and enzyme responsible DNA stability (Saleem *et al.*, 2020) [27]. The linseed plants treated with higher

concenteration of copper sulphate solution (500mg/l) have shown considerable inhibition in plant growth, seed germination, biomass, stunned root and leaf development and change in DNA structure as observed by several autors on different plant species (Yruela,2009; Zafar *et al.* 2018) [35]. Copper is an important micronutrient of plants. In higher plants Cu is essential for many biological and physiological activities like photosynthesis, chlorophyll, and carbohydrate synthesis and nucleic acid formation. It is also an important co-factor for many enzymes of Electron Transport Chain (Adrees *et al.* 2015) [1]. High concenteration of Cu responsible for destruction of thylakoid membrane of chloroplast adversely affecting photosynthesis and carbon assimilation (Rehman *et al.* 2019) [25]. This may inhibit the accumulation and subsequent translocation of carbohydrate from leaves to reproductive parts of plants adversely affecting the development of capsule in *L. usitatissimum.* The auxin treatment show opposite effect on morphological as well as biochemical parameters considered in the study. The higher concentration of IAA (0.20g/l) resulted inhibited growth, delay in flowering time, Reduction in flower size, leaf size, chlorophyll and carbohydrate content of the plant. The low concentration (0.01g/l) of IAA show considerable induction of growth and height of the plant, there was no much delay in flowering time, flower size was also normal, chlorophyll and carbohydrate content were higher in comparison to other treatments. Normal band pattern of DNA was obtained on agarose gel at both the concentration of Auxin. The effect of auxin treatment on *L. usitatissimum* is in consonance with that reported in previous studies (Rastogi *et al*, 2018). Phytohormone is the principal factor that regulates plant growth and development. Indole acetic acid (a natural auxin) regulate plant growth and development by stimulating cell expansion and cell division (Kende and Zeevaart, 1997) [14]. However the higher concentration of IAA produce reversal effect by inhibiting by inhibiting shoot and root growth and expansion of leaf (Sterling and Hall, 1997) [29]. At higher concentration on the growth sites auxin induces the synthesis of ethylene by stimulating enzyme amino cyclopropane carboxylic acid synthase (ACC synthetase) by activating gene for ACC synthesis (Taiz and Zeiger, 1998; Wei etal, 2000) [30, 32]. The de-novo synthesis of auxin induced ethylene exhibit growth inhibition in plants (Wei *et al*, 2000) [32]. Sometime cyanides are also produced as co-product during oxidation of ACC by ACC oxidase and they act as phytoinhibitors (Hansen and Grossmann, 2000). The auxin induced stimulation of ethylene synthesis may sometime accompanied by accumulation of absicisic acid (ABA) , a growth inhibitor in shoots of the plant that adversely affects CO_2 assimilation and photosynthetic efficiency and ability of plants stimulating plant growth inhibition (Hansen and Grossmann, 2000)

Conclusions

Plants growing under diverse environmental stresses show characteristic variation in their morphology, growth and differentiation pattern. The appropriate dosage of some of these a-biotic stresses may favorably affect the growth and productivity of plants but the presence of high dose may impose adverse effect by inducing toxicity. Copper is an essential micronutrient needed for the growth of plant as it act as cofactor of large number of enzyme involved in metabolic reactions of photosynthesis, respiration, DNA synthesis, activator of gene expression etc. The optimal concentration stimulates the plant development and productivity but the high concentration of copper may induce toxic effect adversely affecting photosynthesis and carbon assimilation in *L. usitatissimum*. Phytohormone auxin is essentially needed for growth and differentiation of plants but exogenous supply above optimum requirement may inhibit plant growth by stimulating the synthesis of growth inhibitor ethylene. The physical dosage of ultraviolet radiation always inhibits the growth and development by destabilizing the DNA in *L. usitatissimum*. Thus the quantitative analysis of the requirement of optimum dosage of even essential micronutrient and growth factor is essential for exogenous supplementation to favorably promote the plant growth and productivity and any indiscriminate approach may prove to be futile.

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