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Effect of Brassinosteriods on the Seed Germination and Seedling Parameters of *Plumbago Zeylanica* L.

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Abstract

Plumbago zeylanica L. is a very useful medicinal herb of angiosperms belonging to the family plumbaginaceae. Brassinosteroids are potential plant growth regulators (PGRs) capable of positively modulating different physiological responses. The ability of BRs in enhancing the medicinal value in plants is an on-going process and various studies are being carried out across the globe in finding the capability of these potential PGRs in increasing the growth and yield of medicinal plants. The present study was undertaken to understand the potentiality of homobrassinolide, one of the potential BRs in improving the seed germination and seedling growth *Plumbago zeylanica* L. Supplementation of homobrassinolide to *Plumbago zeylanica* L improved the seed germination percentage with respect to the parameters studied like Germinability (% G), Germination Rate Index (GRI), Coefficient Velocity of Germination (CVG) and Relative Seed Germination (RSG). Further, it was observed that application of homobrassinolide also improved the seedling growth in *Plumbago zeylanica* in terms of Relative Root Elongation (RRE), Growth Index (GI) and Vigour Index (VI).

Key words Co –efficient of velocity of germination; germinability; germination rate index; growth index *Plumbago zeylanica* L.; relative root elongation; relative seed germination; vigour index

1.0. Introduction

Plumbago zeylanica L. is an important medicinal plant of angiosperms from the family plumbaginaceae. This species is native to South Asia and is widely distributed in tropical and subtropical belts of the globe [1-3]. It is widely distributed in India and grows mostly as wild plant from Central India to West Bengal, Maharastra and in all districts of Tamilnadu, Andhra Pradesh, Telangana, Karnataka and Kerala. The plant has several vernacular names in the country viz. *chiktraka/chitramol* (Hindi), *agni/vahini* (Gujrati), *chitramoolam/kodiveli* (Telugu), *chita* (Tamil), *chitramula/bilichitramala* (Kannada), Vellakeduveli (Malayalam).

Different parts of *Plumbago zeylanica* L. are being used since time unknown. This herb is referred to as *rasayan* in ayurveda. The plant is utilized for its preventive role against enlarged liver and spleen. It is a suggested rejuvenator and used in chronic colds and cough [4]. It has also been used to treat digestive disorders like loss of appetite and indigestion. The flowers are used to improve digestion [5]. Leaves have aphrodisiac property and are used in curing the infections as well as dysentery, in treatment of scabies etc. Seeds are used in preparation of decoction which is given to reduce muscular pain [6- 7]. According to Chopra et al. [8], roots are used to treat skin diseases, diarrhoea, dyspepsia, piles, and even in leprosy. Roots are also used as an appetizer, antiperiodic, and in urinary tract infections. Further, roots are also beneficial in treating disease like rheumatism, laryngitis etc. [8, 9].

The research study on brassinosteroids (BRs) enhancing the medicinal value in plants is an on-going process [10, 11]. The capability of these potential PGRs in increasing the growth and yield of medicinal plants is the need of the hour. Application of brassinolide resulted in mitigation of drought in *Xanthoceras sorbifolia* seedlings [12]. Swamy et al. [13] observed that supplementation of 28-homobrassinolide alleviated the toxic levels of lead and increased plant growth in *Trigonella feonu-graecum*. Recently, Pramod et al. [14] observed that exogenously applied 24-epibrassinolide and brassinazole improved the xylogenesis and micro distribution of cell wall polymers in *Leucaena leucocephala* (Lam) De Wit. Further, Kong et al. [15] reported that brassinosteroid homeostasis is critical for the functionality of the *Medicago truncatula* pulvinus. The present study was undertaken to understand the potentiality of homobrassinolide in improving the seed germination and seedling growth *Plumbago zeylanica* L.

2.0. Material and Methods

Healthy seeds of uniform size of *Plumbago zeylanica* L. were selected by a primary method of floating them in water and viable seeds, which settled down in water, were taken for study. The seeds were dried and scarified with sand. Surface sterilization of the seed was done using 0.5%(v/v) sodium hypochlorite and later washed thoroughly with sterile distilled water. The seeds were soaked for 24 hours in either of the following mentioned below:

Distilled water (Control) 4µM, 8µM or 16µM of Homobrassinolide (HBL). From each treatment, twenty seeds were taken and placed in sterile petridishes layered with germination paper. The petridishes were supplied with 5 ml of distilled water/ 4µM/ 8µM or 16µM of Homobrassinolide (HBL) test solutions. The seeds were allowed to germinate in dark at room temperature. On the 4th day of the experiment, 5 ml more of test solutions were added. The number of seeds germinated was counted at the end of 12, 24 and 36 hours. Appearance of radicle was taken as the criteria for germination. The seed germination parameters are mentioned below

2.1. *Germinability (% G)*

The numbers of *Plumbago zeylanica* L. seeds germinated were calculated using the formulae.

G = Total No. of Seeds Germinated X 100 /Total No. of Seeds Sown

2.2. *Germination Rate Index (GRI)*

The Germination Rate Index (GRI) of the *Plumbago zeylanica* L. was calculated by employing the formulae

GRI = No. of normal seedlings of days x/ Days X X= No. of days from seed soaking

2.3. *Coefficient Velocity of Germination (CVG)*

The coefficient velocity of germination (CVG) in *Plumbago zeylanica* L. was calculated employing the formulae given by Kotowski [16].

CVG=Sum of n x 100/ Sum of (nt);

Where, n = No. of seeds emerged on the day and t= time or days of soaking

2.4. *Relative Seed Germination (RSG)*

The RSG of the seed germination of *Plumbago zeylanica* L. was calculated as = No. of seeds germinated in the extract x100 No. of seeds germinated in the control

2.5. *Seedling Growth*

The seedling growth studies of *Plumbago zeylanica* L. were observed for 15 days (on every alternate day i.e., 2^{nd} day, 4^{th} day, 6^{th} day, 8^{th} day, 10^{th} day, 12^{th} day and 15^{th} day). The following parameters were

calculated in the seedlings of *Plumbago zeylanica* L.

2.6. *Relative Root Elongation (RRE)*

The relative root elongation (RRE) of *Plumbago zeylanica* L. seedlings was calculated as = Mean root elongation in the extract x 100 Mean root elongation in the control

2.7. *Growth Index (GI)*

The Growth Index (GI) of *Plumbago zeylanica* L. seedlings was calculated as = % seed germination x % root elongation/100

2.8. *Vigour Index (VI)*

The Vigour Index (VI) of *Plumbago zeylanica* L. seedlings was calculated based on the formulae given below.

VI = Germination % x Seedling length

3.0. Results

3.1. *Seed Germination Percentage of Plumbago zeylanica* L.

The effect of Homobrassinolide (HBL) supplied at 4µM/ 8µM or 16µM on the seed germination percentage of *Plumbago zeylanica* L. is shown in Figure 3.1 and Image 3.1. All concentrations of homobrassinolide increased the germination percentage (G%) against the control in *Plumbago zeylanica* L. The germination percentage increased around 65%, 40%, 35% on supplementation of 16µM, 8µM and 4µM HBL compared to the control on the second DAS. The germination percentage was 75%, 50%, 40% with 16µM, 8µM and 4µM concentrations respectively compared to the control on the fourth DAS.

Image 3.1. Seed Germination of *Plumbago zeylanica* L.

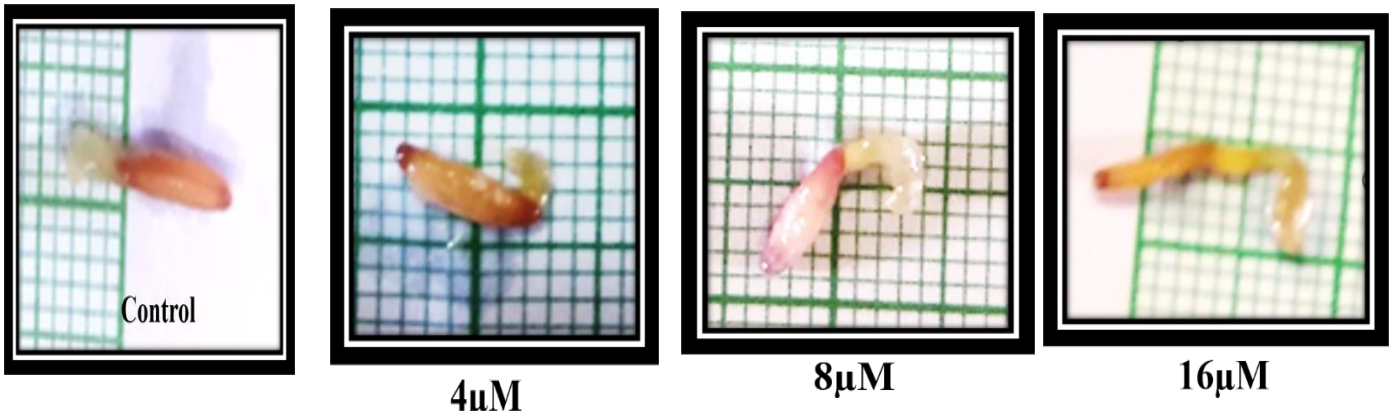
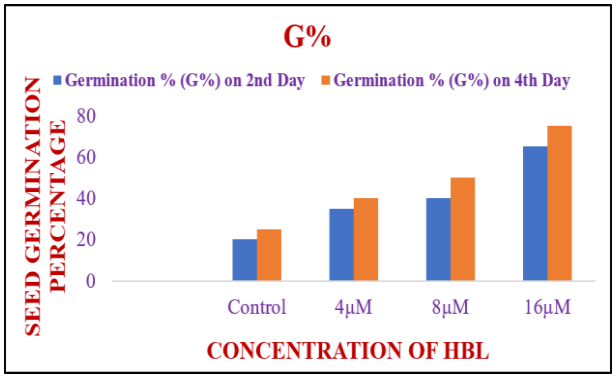


Figure 3.1. Effect of Homobrassinolide on Germination percentage on seeds of *Plumbago zeylanica* on 2 DAS and 4 DAS

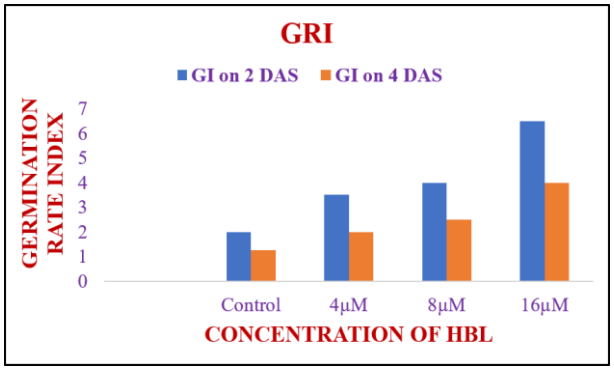


***** Mean ± S.E (n=9); Homorassinolide = HBL

3.2. *Germination Rate Index (GRI) of Plumbago zeylanica L.*

The effect of homobrassinolide supplied at 4µM/ 8µM or 16µM on the germinationrate index (GRI) of *Plumbago zeylanica* L. is shown in Figure 3.2. The germination rate index increased in all the treatments of homobrassinolide in *Plumbago zeylanica* L. GRIwas 6.5%, 4%, 3.5% with 16µM, 8µM and 4µM concentrations respectively whencompared to the control on the second DAS. GRI was 3.75%, 2.5%, 2% with 16µM, 8µMand 4µM concentrations respectively when compared to the control on the fourth DAS.

Figure 3.2. Effect of Homobrassinolide on Germination Rate Index on 2 DAS and 4 DAS



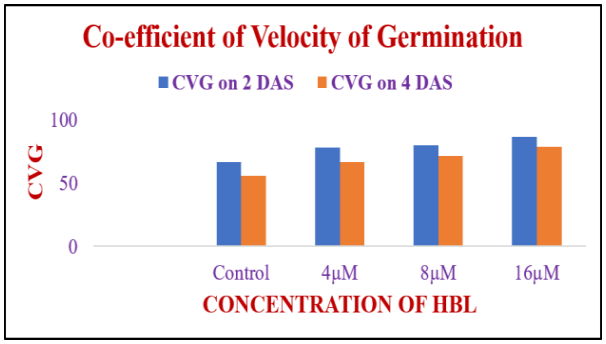
***** Mean ± S.E (n=9); Homobrassinolide = HBL

3.3 Co-efficient of Velocity of Germination (CVG) of Plumbago zeylanica L.

The effect of Homobrassinolide supplied at 4µM/ 8µM or 16µM on the co-efficient of velocity of germination (CVG) of *Plumbago zeylanica* L. is shown in Figure 3.3. Co – efficient of velocity of germination (CVG) also significantly increased in all the treatments of homobrassinolide in *Plumbago zeylanica* L. The CVG was 86%, 80% and 77% with 16µM, 8µM and 4µM concentrations respectively over

control on the second DAS. CVG observed was 78%, 71% and 66% with 16µM, 8µM and 4µM concentrations respectively over control on the fourth DAS.

Figure 3.3. Effect of Homobrassinolide on Co-efficient of Velocity of Germination (CVG) on 2 DAS and 4 DAS

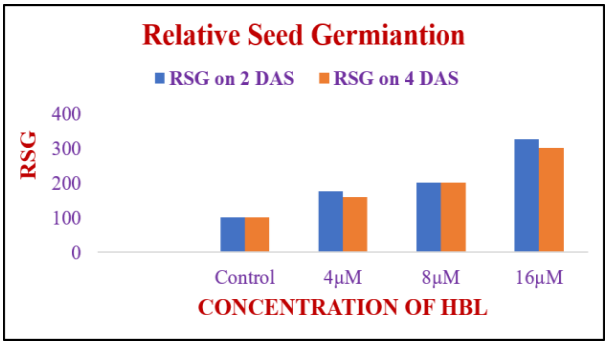


***** Mean ± S.E (n=9); Homobrassinolide = HBL

3.4. Relative Seed Germination (RSG) of Plumbago zeylanica L.

The effect of homobrassinolide supplied at 4µM/ 8µM or 16µM on the Relative Seed Germination (RSG) of *Plumbago zeylanica* L. is shown in Figure 3.4. Relative Seed Germination (RSG) in *Plumbago zeylanica* L. also increased in all the treatments with homobrassinolide. Relative Seed Germination was 325, 200 and 175 with 16µM, 8µM and 4µM concentrations respectively when compared to control on the second DAS and around300, 200 and 160 RSG with 16µM, 8µM and 4µM concentrations respectively was recorded on the fourth DAS when compared with the control.

Figure 3.4. Effect of Homobrassinolide on Relative Seed Germination (RSG) on seeds of *Plumbago zeylanica* L. on 2 DAS and 4 DAS



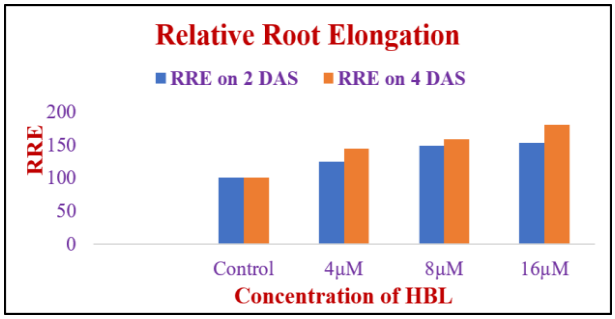
***** Mean ± S.E (n=9); Homobrassinolide = HBL

3.5. Relative Root Elongation (RRE) of Plumbago zeylanica L.

The effect of Homobrassinolide supplied at 4µM/ 8µM or 16µM on the Relative Root Elongation (RRE) of

Plumbago zeylanica L. is shown in Figure 3.5. Relative Root Elongation (RRE) also increased with all the treatments of homobrassinolide in *Plumbago zeylanica* L. RRE was 152%, 149% and 124% with 16µM, 8µM and 4µM concentration respectively on the second DAS when compared to the control. RRE was 180%, 149% and 144% with 16µM, 8µM and 4µM concentrations respectively on the fourth DAS when compared to control.

Figure 3.5. Effect of Homobrassinolide on Relative Seed Germination (RRE) on 2 DAS and 4 DAS

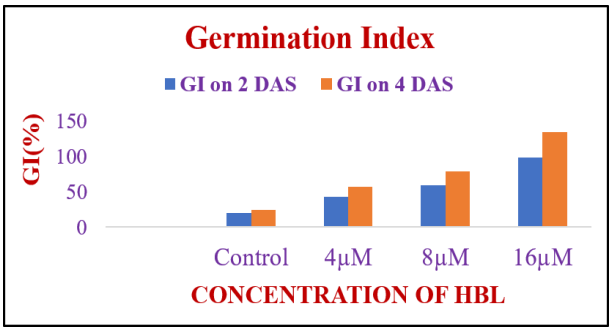


* Mean ± S.E (n=9); Homobrassinolide = HBL

3.6. Germination Index (GI) of Plumbago zeylanica L.

The effect of Homobrassinolide supplied at 4µM/ 8µM or 16µM on theGermination Index (GI) of *Plumbago zeylanica* L. is shown in Figure 3.6**.** Germination Index (GI) also significantly increased with all the treatments of homobrassinolide in *Plumbago zeylanica* L. GI was 99, 59 and 43 with 16µM, 8µM and 4µM concentrations respectively on the second DAS when compared to the control. GI was 135, 79, 57 with 16µM, 8µM and 4µM concentrations respectively on the fourth DAS when compared to control.

Figure 3.6. Effect of Homobrassinolide on Germination Index (GI) on 2 DAS and 4 DAS



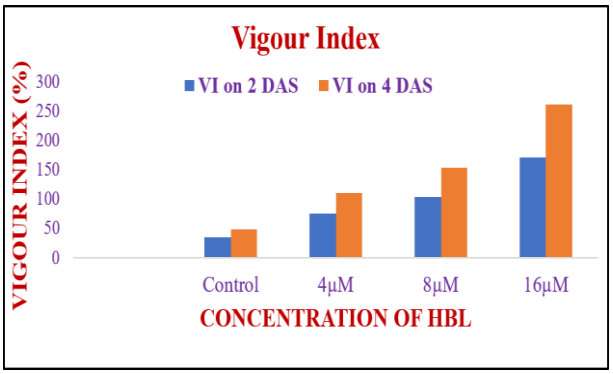
* Mean ± S.E (n=9); Homobrassinolide = HBL

3.7. Vigour Index (VI) of Plumbago zeylanica L.

The effect of Homobrassinolide supplied at 4µM/ 8µM or 16µM on the Vigour Index (CVG) of

Plumbago zeylanica L. is shown in Figure 3.7. Vigour Index (VI) increased significantly with all the treatments of homobrassinolide in *Plumbago zeylanica* L. VI was 171, 103, 75 with 16µM, 8µM and 4µM concentrations respectively on the second DAS when compared to the control. VI was 261, 153, 111 with 16µM, 8µM and 4µM concentrations respectively on the second DAS when compared to control.

Figure 3.7. Effect of Homobrassinolide on Vigour Index (GI) on 2 DAS and 4 DAS



***** Mean ± S.E (n=9); Homobrassinolide =HBL

4.0. Discussion

Germination is regarded as unprotected and risky developmental stage in the life cycle of each plant. The present study clearly demonstrates that homobrassinolide has a positive effect on the seed germination and seedling growth parameters of *Plumbago zeylanica* L. Pre-sowing or pre-treating seeds with PGRs increased the seed germination under normal and stress conditions [17]. The present study revealed that among three concentrations of homobrassinolide, 16µM concentration was most effective to increase the germination percentage (75%), germination rate index (3.75) co-efficient of velocity of germination (78%) and relative seed germination (300) in *Plumbago zeylanica* L.

Brassinosteroids were reported to enhance the elongation of hypocotyls, epicotyls in seeds of dicotyledons and coleoptiles and mesocotyls in seeds of monocotyledons at very low concentrations [18]. Brassinosteroids when employed as seed pre-sowing or priming agent in various plants under different conditions exhibited promising results on germination of seeds and growth of seedlings. In cherry plum and sloe embryos, 24- epibrassinolide increased the seed germination rate by shortening the dormancy period [19]. In *Brassica juncea*, 10^{-10} M 28-homobrassinolide and 10^{-8} M 24-epibrassinolide caused maximum increases in seed germination when compared to controls [20].

Similarly, seed pre-sowing with 24-epibrassinolide in *Brassica napus* and *Arabidopsis thaliana* enhanced the seed germination under when cultivated under salt- stress conditions [21]. Zhong et al. [22]

reported that there might exist a transcriptional hubcapable of integrating gibberellin-brassinosteroid signals to promote seed germinationin *Arabidopsis.* Recently, it was reported that pre-soaking of Cotton seeds with 1 µMepibrassinolide and grown under 200 mM sodium chloride showed a 2.5 fold increase inseed germination when compared to controls [23]. Further, soaking in brassinolide wasalso found to protect black bean sprouts from preharvest UV-B radiation influence [24]which is tune with the current study that showed that pre-sowing with homobrassinolideincreased the relative root elongation (RRE) germination index (GI) and vigour index (VI).

Seedling establishment and plant growth increases with pre-sowing seed treatment with brassinosteroids along with the increase in the seed germination was observed in earlier studies [25]. Seed pre-sowing with brassinolide in three varieties i.e., Victoria, Golden Empress, and Victor of *Medicago sativa L*. (lucerne) under sodium chloride stress increased the percentage of germination, germination index and vigour index. Further, it also increased the root length, root vigour, fresh and dry weights of root and shoot [26]. The results of the present study gave an insight on the alleviative nature of homobrassinolide in increasing the seed germination and seedling growth of *Plumbago zeylanica* L.

Conflict of Interest

The authors hereby declare that there is no conflict of interest with respect to the detailspublished in the paper.

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