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# Paclobutrazol-induced augmented productivity of an endangered medicinal plant safed musli (*Chlorophytum borivilianum* Sant. *et* Fernand.)

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**Abstract**
In the present investigation an attempt was made to improve productivity of safed musli by using paclobutrazol (PBZ), a potent growth regulating chemical having promising influence on tuber yield. Foliar application with 100 and 200 µg $ml^{-1}$ of PBZ was found to improve plant potential of safed musli and this was measured in terms of some reliable physio-biochemical parameters along with some selective yield attributes. Our results showed that the foliar application of PBZ reduced plant height, increased number of branches and leaves per plant. PBZ-induced enhanced growth parameters were associated with augmented metabolic status of the plant species as evidenced from higher chlorophyll and protein contents, significantly enhanced catalase along with reduced protease activities in leaves. The positive influence of PBZ was also recorded from the yield attributes as evidenced from the increased number and fresh weight of tubers per plant as well as length and circumference of the tubers, in comparison to control ones. Thus, it can be concluded that the PBZ may be used as a potent chemical for augmented productivity of this high-value endangered plant.

**Keywords:** *Chlorophylum borivilianum*, safed musli, paclobutrazol, physiochemical changes, productivity

## Introduction
The genus *Chlorophytum* Ker Gawl., under the family Asparagaceae (formerly in family Liliaceae), includes more than 215 species, 6 subspecies and 8 varieties which are distributed throughout the tropical and subtropical parts of the world [1]. In India the genus is represented by 21 species among which 2 are ornamental plants, 18 are strictly wild and only *Chlorophytum borivilianum* is wild as well as cultivated as medicinal plant.
Fleshy tubers of *C. borivilianum* are used in Ayurvedic medicines and widely used as a natural sex tonic and is an integral part of more than 100 herbal drug formulation [2]. Their aphrodisiac properties have proved very much useful for the people suffering from erectile dysfunction. Drugs prepared from safed musli are potential enough to increase general body immunity, curative of natal and post-natal problems, increase working capacity, delay aging process, possess anti-inflammatory and anti-oxidant activity. It is also useful in rheumatoid arthritis, diabetes mellitus and post-menopausal syndrome [3-4]. At present, the estimated global demand of safed musli is approximately 40,000 tons per year but current production is 5500 tons per year only [5]. Its huge demand and inadequate supply, makes it a very costly herb. The Department of Indian System of Medicines and Homoeopathy, Ministry of Health and Family welfare, National Medicinal Plants Board (NMPB) identified safed musli as a high value and high demand medicinal crop, and thus it is rightly called the 'root of gold'. NMPB, New Delhi also identified safed musli as the sixth important herb among 28 medicinal plants to be protected, promoted and preserved. Keeping these in mind an attempt was made to undertake a comprehensive research work to address the problems of safed musli in our laboratory and agricultural field, and the present communication deals with the efficacy of a potent PGR paclobutrazol (PBZ) on augmentation of its tuber productivity.
Paclobutrazol (2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1H-1, 2, 4-trizol-1-yl)-pentan-3-ol] is a triazolic group of plant growth regulator. The growth regulating properties of paclobutrazol are mediated by reduced stem elongation [6], increasing root length and diameter as well as dry matter accumulation [7]. Reduced stem elongation may be due to inhibitory effect of paclobutrazol on oxidative reactions of gibberellins biosynthesis [8]. Paclobutrazol also can influence the chlorophyll biosynthesis, delay the senescence and prolong the metabolic activity of many plants [9].
As the global demand of safed musli is increasing day by day and it is depleting rapidly from the Indian forests conservation, mainstream cultivation in different agroclimatic zones of India

as well as its productivity improvement are the prime mandate of research with this plant for availability of raw materials. The major constraint in the cultivation of safed musli is long tuber dormancy (6-7 months), shortage of planting material, very poor germination (only 5-13%) rate [10]. Although micropropagation is becoming increasingly popular as an alternative means in the propagation of safed musli, the conventional methods of vegetative propagation have reached general acceptability. So, mainstream cultivation and its productivity improvement are vitally needed for availability of raw materials.

However, the specific objectives of this study were to test the effect of PBZ on growth, metabolism and productivity of this high value endangered medicinal plant. In fact, the present study aims to improve tuber productivity of safed musli by using PBZ as a potential chemical having promising influence on tuber productivity of some other plants [11, 12].

## Material and Methods

Experimental plant material (healthy wet tubers with crown) of safed musli (*Chlorophytum borivilianum* Sant. *et* Fernand.) were procured from Jeevan Herbs & Agro Farms, Sagar, M.P. Paclobutrazol (PBZ), used as chemical manipulating agent, was purchased from Sigma Chemical Co., USA. Field experiment was done in the departmental research field at Burdwan University. Planting materials were used with a portion of the crown. Tubers with disc were planted on raised beds at spacing of 30 cm between the rows and 20 cm between the plants in the first week of May, 2016. Normal irrigation was given at 8-10 days interval. At post flowering stage (after 20 days of plantation) a single foliar spray was given with 100 and 200 µg $ml^{-1}$ aqueous solution of PBZ along with distilled water as control. Data on the plant height, number of branches and leaves per plant were taken after 60, 80 and 100 days at plantation. The mean value of 10 uniformly grown randomly selected plants was considered for recording the data.

For extraction and estimation of chlorophyll leaf tissues (100 mg) were immersed in 5 ml methanol and kept in a refrigerator for 24 h. The supernatant was decanted off and leaf samples were rinsed repeatedly with a little volume of methanol until they were completely free from green colour. Thus, the final volume of methanol was made up to 10 ml and the intensity of the green colour was measured at 650 nm using a spectrophotometer. Total chlorophyll contents in leaves of 60, 80 and 100 days old plants were measured following the method of Arnon (1949) [13].

Protein extraction and estimation was done from the same leaf samples. Hundred mg leaf samples were homogenized using a mortar and pestle with 80% ethanol and centrifuged at 6000 *g* for 10 min. Phenol free pellet was made by thorough successive washing with 10% cold trichloroacetic acid (TCA, w/v, twice), ethanol (once), ethyl alcohol: chloroform (3:1, v/v once) and finally with solvent ether following the method of Kar and Mishra (1976) [14].

Then the pellet was evaporated to dryness to remove the ether. The protein was then solubilized by treating with 0.5 N NaOH at 80 °C for 1 h. A volume of 4 ml was made with the extraction medium i.e. 80% ethanol. Protein content was then estimated by reacting protein solution with Folin phenol reagent and measuring the OD value at 650 nm according to the method of Lowry *et al*., (1951)[15]. Quantitative determination was made by comparing the OD values with a standard curve previously prepared using bovine serum albumin (BSA Fraction-v, Sigma Chemical Co., USA).

To analyse catalase activity 500 mg leaf of each treatment along with control was homogenized with 8 ml of chilled 0.1 M phosphate ($Na_2HPO_4$ / $NaH_2PO_4$) buffer (pH 6.5). The homogenate was centrifuged at 3000 *g* for 15 min followed by 10000 *g* for 20 min in cold condition, then the supernatant taken and the volume was made up to 10 ml with the same buffer, and this was used as crude enzyme source. The enzyme activity was determined following the method of Snell and Snell (1971) [16].

modified by Biswas and Choudhuri (1978)[17]. To estimate catalase activity 2 ml 0.05 M $H_2O_2$ was mixed with 1 ml of the above extract and incubated the reaction mixture at 37 °C for 2 min. The reaction was stopped by adding 2 ml 0.1% titanium sulphate in 25% $H_2SO_4$ (v/v). The mixture was centrifuged at 4000 *g* for 20 min. Supernatant became yellow coloured and the intensity was measured at 420 nm. The blank was prepared by inactivating (heat killed) enzyme with the addition of titanium sulphate prior to $H_2O_2$ addition.

Protease activity was estimated following the method described by Snell and Snell (1971) [16] after necessary modification. Five hundred milligram (500 mg) of seed of each sample was taken and homogenized with 5 ml chilled phosphate buffer (pH 6.5) in cold condition. The homogenate was then centrifuged at 5000 *g* for 10 min. The supernatant was taken and the volume was made up to 10 ml with phosphate buffer, and this was used as crude enzyme source. One ml of enzyme solution was mixed with 0.1 ml of 0.1 M $MgSO_4$ and 1 ml of 50 µg $ml^{-1}$ Bovin Serum Albumin, and then kept the set up in an incubation at 37 °C for 1 h. Then the reaction was stopped with 50% TCA. After that reaction mixture was centrifuged at 10000 *g*. Supernatant was rejected and pellet was taken. The pellet was dissolved in 1 ml of 1 M NaOH solution and 1 ml of distilled water was added to it. The mixture was then incubated at 80 °C for 15 min. After incubation the mixture was diluted to 10 times. One ml Cu-tartrate and NaOH - $Na_2CO_3$ (1:10) mixture was added with 1 ml of diluted solution and waited for 10 min. Thereafter 10 ml of Folin reagent was added to it. The intensity of blue colour was measured at 650 nm.

Data on the numbers of tuber per plant, tuber fresh and dry weight per plant, average tuber length and circumference were taken after harvest in the month of January 2017.

Each experiment was done in three replicates and the experimental results were expressed as mean ± standard deviation (SD).

## Results

### Effect of PBZ on plant height (Fig. 1)

Result showed that with the advancement of plant age, plant height was increased in all treatments including control one at least up to 80 days of plant age. Data also revealed that plant height was maximum in control sample in all the selected observation days of plant age. A significant decrease in the plant height was noted both in the PBZ treated (100 and 200 µg $ml^{-1}$) plants over control in all the selected observation days, and the magnitude of the decrease in plant height was found to be maximum in 200 µg $ml^{-1}$.

It was also clear from the data that the effectiveness of PBZ was remarkable at the early stage of the plant development and it tend to become weaker with the advancement of plant age.

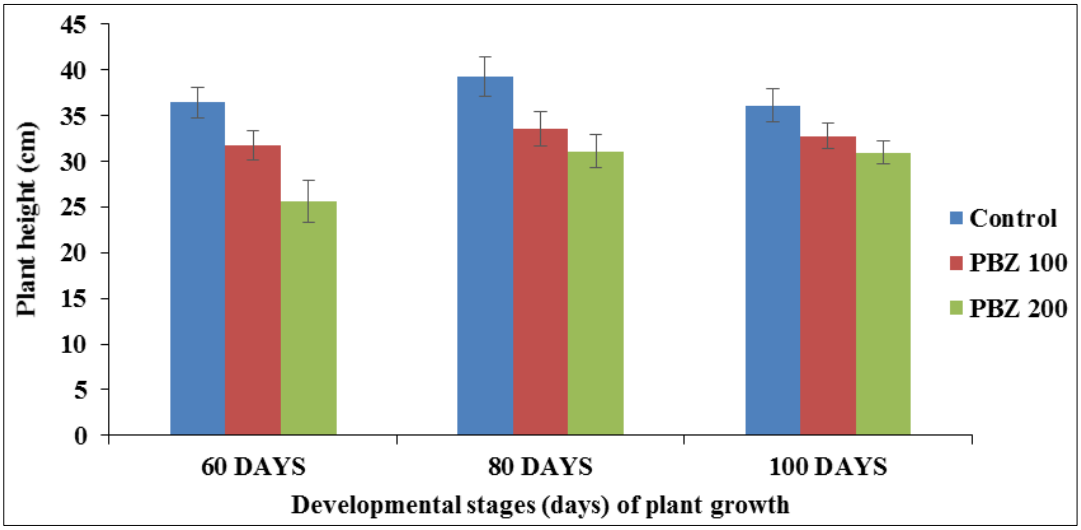


**Fig 1:** Effect of PBZ on changes of plant height of *C. borivilianum* at different developmental stages

**Effect of PBZ on number of branches per plant (Fig 2)**

Fig. 2 shows that the number of branches per plant was increased with the age of the plants up to 100 days of plant age in all treatments excluding control one. A significant increase in the number of branches per plant was recorded both in the concentrations of PBZ treatment over control. Highest number of branches per plant was seen in 200 µg $ml^{-1}$ PBZ treated plant in all the selected observation days.

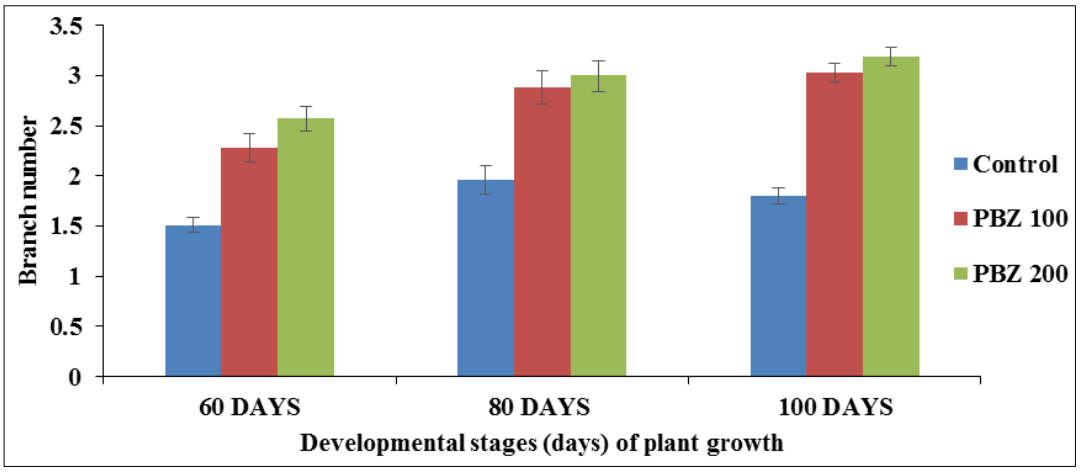


**Fig 2:** Effect of PBZ on changes of branch number per plant of *C. borivilianum* at different developmental stages

**Effect of PBZ on leaf number per plant (Fig 3)**

Results also showed that the leaf number per plant was increased with the age of the plants at least up to 80 days regardless of the treatments including control one, and thereafter it was decreased. PBZ treatment more or less significantly decreased the leaf number per plant and the reduction was found concentration dependent.

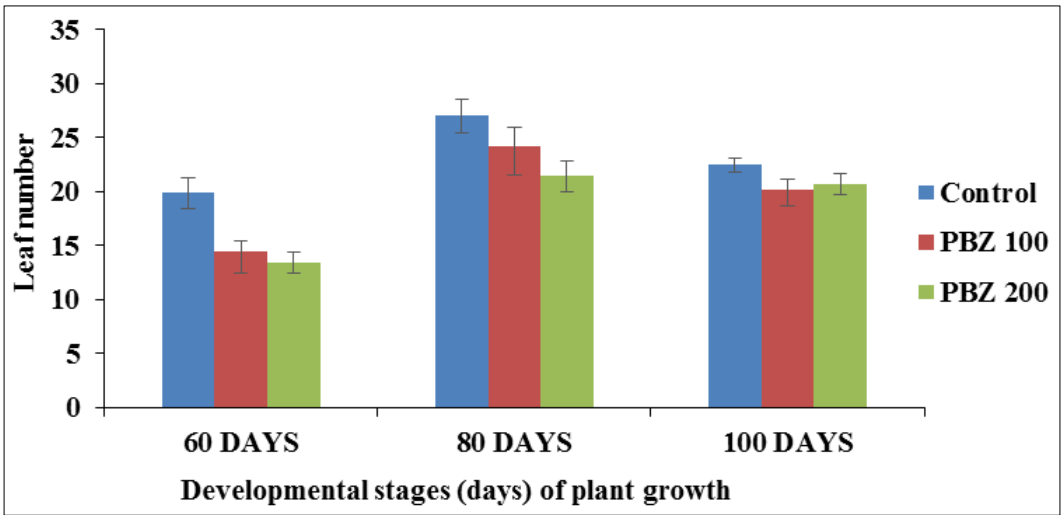


**Fig 3:** Effect of PBZ on changes of leaf number per plant of *C. borivilianum* at different developmental stages

**Effect of PBZ on total leaf area per plant (Fig 4)**

Fig 3 shows that the total leaf area per plant was increased with the age of the plants at least up to 80 days of plant age irrespective of the treatments including control one and thereafter, it was decreased. Interestingly the magnitude of decrease after 80 days was found to be much higher in control sample in comparison to PBZ-treated ones. From our data it was quite apparent that PBZ significantly deceased total leaf area per plant at all the developmental stages and the magnitude of decrease was concentration dependent.

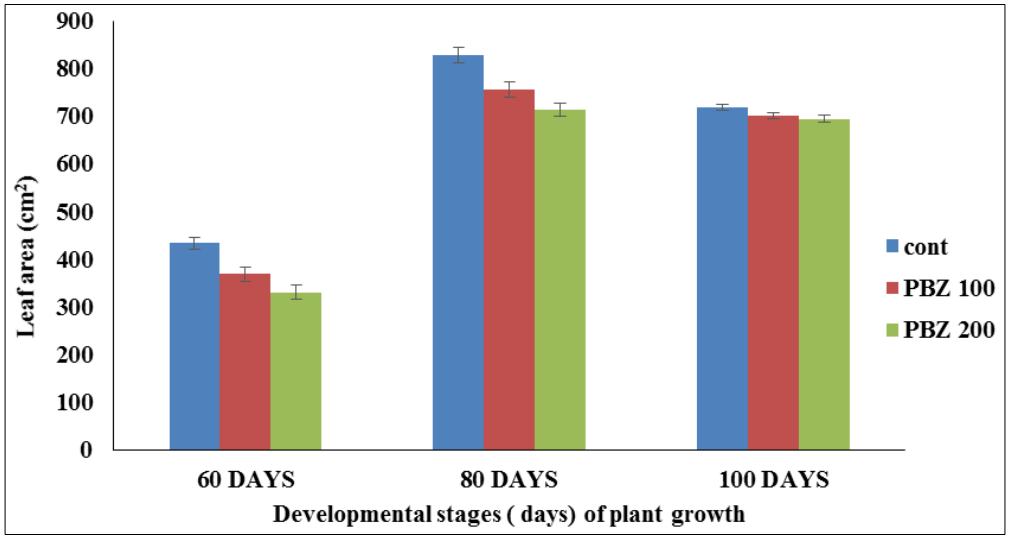


**Fig 4:** Effect of PBZ on changes of total leaf area per plant of *C. borivilianum* at different developmental stages

**Effect of PBZ on chlorophyll and protein contents of leaves (Table 1)**

It is evident from the data in table 1 that chlorophyll contents of the leaves progressively increased up to 80 days of plant age and after that a significant reduction of chlorophyll was found in control set. However, in PBZ treated plants the chlorophyll content was progressively increased up to 100 days, and PBZ at 200 µg $ml^{-1}$ show the better result over PBZ 100 µg $ml^{-1}$ and control. Data also revealed that the protein content of the leaves showed identical trends as in chlorophyll content.

**Table 1:** Effect of PBZ on changes of chlorophyll and protein contents of *C. borivilianum* leaves at different developmental stages.

| Treatments | Chlorophyll (mg $g^{-1}$fr. wt.) | | | Soluble protein (mg $g^{-1}$fr. wt.) | | |
|---|---|---|---|---|---|---|
| | Developmental stages(days) of plant growth | | | | | |
| | 60 | 80 | 100 | 60 | 80 | 100 |
| Control(0 µg $ml^{-1}$) | 1.21±0.07 | 1.64±0.11 | 1.38±0.15 | 11.89±0.58 | 16.28±0.88 | 14.26±1.09 |
| PBZ (100 µg $ml^{-1}$) | 1.43±0.08 | 2.06±0.16 | 2.18±0.07 | 13.44±0.62 | 18.48±0.85 | 18.54±1.75 |
| PBZ (200 µg $ml^{-1}$) | 1.95±0.16 | 2.31±0.19 | 2.42±0.20 | 15.52±0.69 | 19.40±0.73 | 20.33±2.42 |

**Effect of PBZ on catalase and protease activity of leaves (Table 2)**

Results showed that catalase activity was decreased with the age of the plants developed from PBZ treatments including control. But in each selected developmental stages catalase activity was higher in PBZ treated plants over control, and increased catalase activity was found concentration dependent.

Protease activity showed a reverse trend as compared to catalase activity, and it was found to increase with the age of the plants in all the samples including control one. Whereas PBZ treatments significantly decreased the protease activity in each selected developmental stages over control.

**Table 2:** Effect of PBZ on changes of catalase and protease activities of *C. borivilianum* leaves at different developmental stages.

| TREATMENTS | Catalase (unit $h^{-1}$ $g^{-1}$ fr. wt.) | | | Protease (unit $h^{-1}$ $g^{-1}$ fr. wt.) | | |
|---|---|---|---|---|---|---|
| | Developmental stages(days) of plant growth | | | | | |
| | 60 | 80 | 100 | 60 | 80 | 100 |
| Control (0 µg $ml^{-1}$) | 50.17±2.48 | 47.17±4.06 | 38.95±3.53 | 55.86±4.88 | 56.88±3.62 | 67.93±5.45 |
| PBZ (100 µg $ml^{-1}$) | 61.09±3.16 | 59.63±4.73 | 55.44±4.02 | 44.89±3.69 | 45.08±3.05 | 51.78±2.37 |
| PBZ (200 µg $ml^{-1}$) | 64.97±4.90 | 63.93±4.25 | 60.01±2.97 | 40.41±3.34 | 40.55±3.69 | 43.11±3.91 |

**Effect of PBZ on number of tuber, fresh and dry weight of tubers per plant as well as length and circumference of tuber (Table 3)**

PBZ treatment significantly increased some yield attributes like number of tuber, fresh and dry weight of tuber per plant as well as length and circumference of tuber over control. The number of tuber per plant was maximum in PBZ 200 µg $ml^{-1}$ treated plant and it was almost 55.6% higher over control and in case of PBZ 100 µg $ml^{-1}$ treated plant it was 21.77%. Fresh and dry weight of tuber per plant as well as length and circumference of tuber were also found highest in PBZ 200 µg $ml^{-1}$ followed by PBZ 100 µg $ml^{-1}$ and control. Thus, PBZ 200 µg $ml^{-1}$ treatment was found to be most effective for enhanced crop yield of safed musli.

**Table 3:** Effect of PBZ on number of tuber, fresh and dry weight of tuber per plant as well as tuber length and circumference of *C. borivilianum*.

| Treatments | Tuber number per plant | Tuber Fresh weight per plant (g) | Tuber Dry Weight per plant (g) | Average tuber length (cm) | Average tuber circumference (cm) |
|---|---|---|---|---|---|
| Control | 11.38±0.88 | 27.66±2.36 | 6.62±0.59 | 6.57±0.29 | 2.45±0.09 |
| PBZ (100µg $ml^{-1}$) | 13.66±0.86 | 41.59±3.82 | 9.88±0.71 | 7.92±0.29 | 2.65±0.27 |
| PBZ (200µg $ml^{-1}$) | 17.66±1.02 | 52.01±4.53 | 12.91±1.14 | 8.76±0.26 | 3.06±0.21 |

## Discussion

PBZ acts as a potent plant growth regulator and can alter the levels of different plant hormones by inhibiting gibberellin synthesis, decreasing ethylene production, and enhancing cytokinin and abscisic acid contents [18]. PBZ also influences the various physiological processes that ultimately modulate the productivity of crop plants. The most important physiological function of PBZ is repression of senescence with enhanced concentrations of photosynthetic pigments, alteration of hormonal balance, improvement in mineral absorption, tolerance to abiotic stresses, carbohydrate synthesis and translocation towards economic sink [6, 19]. PBZ is also reported to be related with enhancement of leaf relative water content, improvement of membrane stability index, induces antioxidant activities, increases level of proline, reduces lipid peroxidation etc. In our experiment foliar application of PBZ significantly decreased plant height and increased number of branches per plant. Thus, such observation seems to be in conformity with that of some previous workers [20-23]. In this investigation foliar application of PBZ decreased the leaf number as well as leaf area per plant. Abraham *et al.* (2008) [21] reported that application of PBZ reduced leaf number as well as total leaf area per plant of *Sesamum indicum*. Berova and Zlatev (2000) [20] reported that foliar treatment with PBZ significantly decreased number of leaves per plant along with total leaf area of tomato plants. Thus, such findings of the some workers also support the findings of our present investigation. From this study it was evident that in case of control sample total leaf number as well as total leaf area per plant was decreased after 80 days, whereas in PBZ-treated plant it was found still increasing. From such observation we can predict that PBZ might delayed the onset of plant senescence.

The early stage of leaf senescence shows chlorophyll degradation and decreasing photosynthetic capacity. Nouriyani *et al.* (2012) [24] reported that application of 50, 100 and 150 mg $l^{-1}$ PBZ as a foliar spray enhanced chlorophyll, carotenoids and soluble protein content in two wheat cultivars. Abu-Muriefah (2015) [25] found that photosynthetic pigments like chlorophyll, soluble carbohydrate and soluble protein were significantly enhanced when plants were treated with PBZ. Earlier studies also revealed that PBZ treatment enhanced the chlorophyll a and chlorophyll b content of *Jatropha curcas* [26]. However, it is still not clear that, whether the PBZ-induced increase of chlorophyll is due to enhanced chlorophyll synthesis or reduced chlorophyll degradation or simply a result of 'concentrating effect' due to reduced leaf expansion [6]. In our experiment PBZ triggered the level of both chlorophyll and protein, and enhancement of these two macromolecules seemed to play a crucial role for plant potentiation which was reflected in productivity of plant, possibly by supplying higher amount of photoassimilates to the active sink of the plant i.e., tuberous roots.

Enzymatic antioxidant such as peroxidase, catalase, superoxide dismutase and ascorbate peroxidase plays a vital role in cellular defense system by maintaining the cellular redox status, protecting cell membrane integrity and inactivating ROS produced during metabolic changes [27]. Catalase helps in protecting cells from oxidative damage by catalyzing the detoxification of $H_2O_2$ to form water and oxygen [28-29]. Our present study showed that PBZ significantly enhanced the catalase activity over control. PBZ application has been reported to significantly increase enzymatic antioxidant activity in various plant species e.g. *Sesamum indicum* [30], tomato [31], rice [32] and wheat [33]. On the other hand, some degrading enzymes like protease and nuclease which degrade protein and nucleic acid respectively activated during senescence. Our result showed that PBZ significantly reduced protease activity over control. Davis *et al.* (1985) [34] reported that application of PBZ reduced the activity of protease and RNase in soybean leaves. Thus our findings corroborate the findings of some previous workers.

Our present investigation also clearly showed that foliar application of PBZ enhanced some potential yield attributes of safed musli and these cumulatively resulted in augmented tuber yield. Pervious study of Bhar *et al.*, (2008) [35] shows that NaDk, a potent plant growth regulator, has the ability to enhance the tuber yield of safed musli. Halder and Bhattacharjee (2017) [36] reported that ascorbic acid, a promising plant growth regulator as well as antioxidant, has some positive influences on growth and metabolism as well as productivity of *Chlorophytum borivilianum.* Thus, our observation corroborate the previous findings. From cumulative data it could be speculated that enhanced chlorophyll content might have promoted the photosynthetic efficacy of the plant. It is also evident from previous work that PBZ increased stomatal conductance and photosynthetic efficacy in some plants [25, 37]. Further, reports available in literature clearly show that PBZ has ability to delay the onset of senescence in soybean plant [9, 6].

## Conclusion

As a balance source-sink relationship is an important determinant for crop yield, our observation indicates that the enhanced tuber yield of safed musli might be due to the positive role of PBZ for supplying photoassimilates to the active sink (tubers) for longer duration and thus enhanced productivity. This might have been mediated by maintaining the coveted source-sink relationship induced by PBZ during the active growth phase of tuberous roots. Whatever might be the mechanism involved in PBZ-induced enhanced tuber yield, it can be concluded from our investigation that PBZ may be used for obtaining desired productivity of safed musli.

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