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Effect of Seed Priming and Foliar Treatment of Melatonin on Stress Alleviation in *Brassica juncea* L. Grown in Waste Water

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Seed germination and seedling growth are vital plant stages that can be affected by stresses such as drought and aging, which cause deterioration and reduce seed viability. The rapid increase in the industries at or along the banks of rivers is leading to serious health hazards for the population consuming the contaminated water and the crops irrigated by the contaminated water. The present study was conducted to determine the effect of melatonin (100 µm) treatment *viz*., seed priming, foliar spray and seed priming + foliar spray on the plants grown in the waste water. In the experiment, significant reductions in the morphological and yield traits were recorded in the plants

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grown in waste water. A reduction in shoot length, root length, dry weight, number of leaves, number of internodes, pod length, number of pods, number of seeds and 100 seed weight was observed in the plants grown in waste water as compared to the plants irrigated with tap water.. Melatonin (MT)-treated plants also showed significant improvements in morphological and yield parameters as compared to the control. The highest increase was observed in fresh weight of the plants by the co-application of MT(seed priming + foliar spray) in comparison to the control plants. Further, MT-treated plants exhibited less reduction in morphological and yield components under waste water irrigation as compared to plants grown in waste water without MT treatment. The plants grown in waste water under foliar MT(100 µm) treatment showed increase in shoot length, fresh weight, dry weight, number of leaves, number of internodes, number of pods, pod length and number of seeds per pod in comparison to plants grown in waste water only. The study revealed that the foliar MT (100 µm) treatment was most effective in increasing the morphological and yield parameters of mustard grown under waste water compared to seed priming and seed priming + foliar spray treatment.Application of MT at concentration of 100 µmol L^{-1} by foliar spraying can be helpful in alleviation of stress in mustard growing in waste water. This can be further used in harnessing the photoremediation properties of *Brassica juncea* L. and reclamation of the soil.

Keywords: Mustard; melatonin; seed priming; foliar treatment; waste water.

1. INTRODUCTION

Seeds are the units of sexual reproduction of plants, and their function is to propagate, perpetuate, and disperse the species to which they belong. Seed germination is a vital stage in plant development and can be considered as a determinant for plant productivity. Many seeds are capable of surviving dehydration at maturity, and in this state, they can survive for long periods (up to hundreds of years in some cases) and resume growth when rehydrated. However, deteriorative chemical processes continue in dry seeds, resulting in their gradual loss of vigor and eventual death (García-Cánovas et al., 2024; Sun et al., 2025). *Brassica juncea* is an oilseed crop and contributes the second highest in domestic edible oil production. In India, *B. juncea* is grown in Rabi season from September-October to February-March. It grows in sandy loam to clay loam soils but thrives best on light loam soils. It does not tolerate water logging conditions or heavy soils. Soil having a neutral pH is ideal for its proper growth and development. *B. juncea*, also known as Indian mustard, is a species of Brassicaceae family (Mustard family). Among the plants of the *Brassica* species, the *Brassica juncea* deserves special attention because of its relevance to the process of phytoextraction of heavy metals from soil (Rathore et al., 2019). Remediation of heavy metal-contaminated soil using plants has attracted much attention in the recent past due to its environment-friendly approach and low cost (McGrath et al., 2002).

The rapid increase in the industries at or along the banks of rivers is leading to serious health hazards for the population consuming the contaminated water and the crops irrigated by the contaminated water (Sankhla et al., 2016). Industrial wastewater contains highly variable compounds and volume depending on the type of industry producing those (Mokarram et al., 2020). Some industries like mining and quarrying, pulp and paper etc. are water-intensive units and release a large amount of waste water. Some industries produce biodegradable waste whereas some industries like petrochemical, chemical manufacturing and mining industries produce non-biodegradable and toxic waste (Sharma et al., 2024a; 2025). These compounds are very difficult to treat. Further, a number of plants are grown in polluted sites (Alsherif et al., 2022). Subsequently, their growth and yield is negatively affected by the hazardous chemicals present in polluted sites or by the irrigation of waste water due to overproduction of reactive oxygen species and oxidative stress (Sharma et al., 2024a). To overcome this negative impact and thrive under adverse situations, plants have developed innate defence mechanisms (Muthamilarasan and Prasad, 2013). Besides the innate mechanisms, several exogenous plant growth regulators/promoters and biomolecules have also been tried to enhance the crop productivity under stress conditions (Maurya et al., 2024; Sharma et al., 2024b).

Melatonin (MT) is one of the earliest biomolecules whose existence can be traced back to the origin of life. Its initial function was likely as a free radical scavenger (Zhao et al., 2019). MT serves as a first line of defence against oxidative stresses and thus, regulates plant stress tolerance responses (Kour et al., 2024; Maurya et al., 2024). Plants, being sessile organisms are more exposed to environmental stresses; it is presumed that to compensate for this, they have greater levels of melatonin than animals (Tan et al., 2012). In addition to providing protection against oxidative stress, MT is involved in multiple functions in the growth and development of plants ranging from seed germination to senescence (Zhao et al., 2019). Endogenous as well as exogenous MT can increase the adaptability of plants to various stresses (Hattori et al., 1995). Further, supplementation of MT to plants may offer stress protection against various pollutants. However, there are no reports related to the role of MT in stress amelioration in Indian mustard grown in the polluted sites.

In this study, we determined the effects of MT treatments *viz*. seed priming, foliar spray and combination of both in stress amelioration in *B. juncea* due to waste water. Different morphological and reproductive parameters were measured. Understanding the role of MT in improving the performance of *B. juncea* under stress due to waste water can offer valuable insights for developing sustainable strategies to enhance crop productivity.

2. MATERIALS AND METHODS

The seeds of certified variety (Giri Raj variety) of *Brassica juncea* were procured from Sher-e-Kashmir University of Agriculture Sciences and Technology, Jammu. Seeds were surface sterilized by dipping in 0.4% sodium hypochlorite solution for 15 min followed by repeated rinsing with distilled water. Seeds of *B. juncea* were grown in the month of October in plastic pots (diameter: 20 cm, height: 15 cm) filled with 4 kg of soil. The soil in the pot was irrigated by the waste water collected from Maggar khud, Kathua stored in a refrigerator (4°C) and normal irrigation. The trial consisted of eight treatments, three replicants and a randomized block design.

Seed priming: The seeds were surface sterilized by soaking in 0.4% sodium hypochlorite solution for 15 min followed by repeated rinsing with distilled water. The seeds were then soaked in 100 μ M MT solution (MT) at 20°C under dark for 18 h. Subsequently, the seeds were removed from the solutions and washed with distilled water several times.

Foliar spray of melatonin (100 μ M) was applied on 30, 35, 60, 65, 90 and 95 DAS. Three replicates were maintained in each treatment. Sampling was performed at 40, 70 and 100 DAS to access the various morphological and reproductive parameters. The yield characteristics were observed at harvest (140 DAS).

Hormone preparation: A stock solution of MT was prepared by dissolving its required amount in ethanol. The stock was diluted using double distilled water (DDW) to get the desired concentration (100 μ M) used in the experiment.

Growth characteristics: The plants were carefully removed from each pot, submerged in water and shaken vigorously to remove the adherent soil. After washing for 2–3 times, plants were kept on a blotting sheet to remove the adherent water molecules. Growth parameters such as root and shoot length were assessed, using a metric scale. The number of leaves and number of internodes were counted for each plant. Fresh biomass (fresh weight) of root and shoot was measured immediately using a digital balance. Plant samples were oven-dried at 70 °C for 48 h to measure the dry mass (dry weight) of root and shoot.

Number of pods and Number of Seeds per pod (Seed_pod): Random samples of three plants were collected from each pot for number of pods per plant and number of seeds per pod.

Length of pod: Random sample of three plants was collected from each pot, four pods were selected in each, out of which two were terminal and two matured were selected and average length was calculated.

100 seed weight: With the help of electronic seed counter 100 seeds were counted and weight was recorded.

2.1 The Following Treatments (T_0 – T_7) are

- T_0: Control (Irrigation with tap water).
- T_1: Irrigation with waste water.
- T_2: Melatonin (Seed priming Treatment) and irrigation with tap water.
- T_3: Melatonin (Seed priming Treatment) and irrigation with waste water.

- T_4: Melatonin (Foliar spray) and irrigation with tap water.
- T_5: Melatonin (Foliar spray) and irrigation with waste water.
- T_6: Melatonin (Seed priming Treatment + Foliar spray) and irrigation with tap water.
- T_7: Melatonin (Seed priming Treatment + Foliar spray) and irrigation with waste water.

Statistical analysis: All data was analyzed using one-way ANOVA while *Tukey's HSD (Honest Significant Difference) post hoc* test was applied for mean separation at probability level p = 0.05. Results are reflected as the mean±SE.

3. RESULTS AND DISCUSSION

3.1 Shoot Length and Root Length

The maximum shoot length was observed in plants grown in irrigation water (tap water) and treated with co-application of MT in form of seed priming and foliar spray. Plants irrigated with waste water showed minimum shoot length at 40 and 70 days after sowing. The plants irrigated with waste water under the treatment of MT (foliar spray) showed an increase in shoot length as compared to plants without the treatment of MT.MT treatment as seed priming and a combination of seed priming and foliar spray in the plants irrigated by waste water also showed increased shoot length at 40 and 70 DAS but a sharp decline in the shoot length was observed in these plants at 100 DAS. Therefore, MT treatment in the form of foliar spray was found to be most effective in improving the shoot length. The results are in conformity with the results of Park et al., (2021) that plant height was enhanced in 38 day old salinity-stressed plants under MT treatment.

The data in Fig. 1(b) shows the root length of mustard under the impact of waste water and MT (seed priming +foliar spray) treatments. The maximum root length was observed in the plants grown in irrigation water subjected to co-application of MT (seed priming+ foliar spray) followed by plants under treatment of MT (foliar spray). A decrease in the root length was observed in plants grown in waste water as compared to plants under control conditions. MT treatment in the form of seed priming as well as foliar spray were found to be effective in increasing the root length in mustard. The result is in conformity with result reported by Zhang et al., (2017) that root length increased by 14% in cucumber seedlings under nitrate stress by MT application and Dai et al., (2020) showed that MT promoted taproot and lateral root growth under drought stress in two different varieties of mustard.

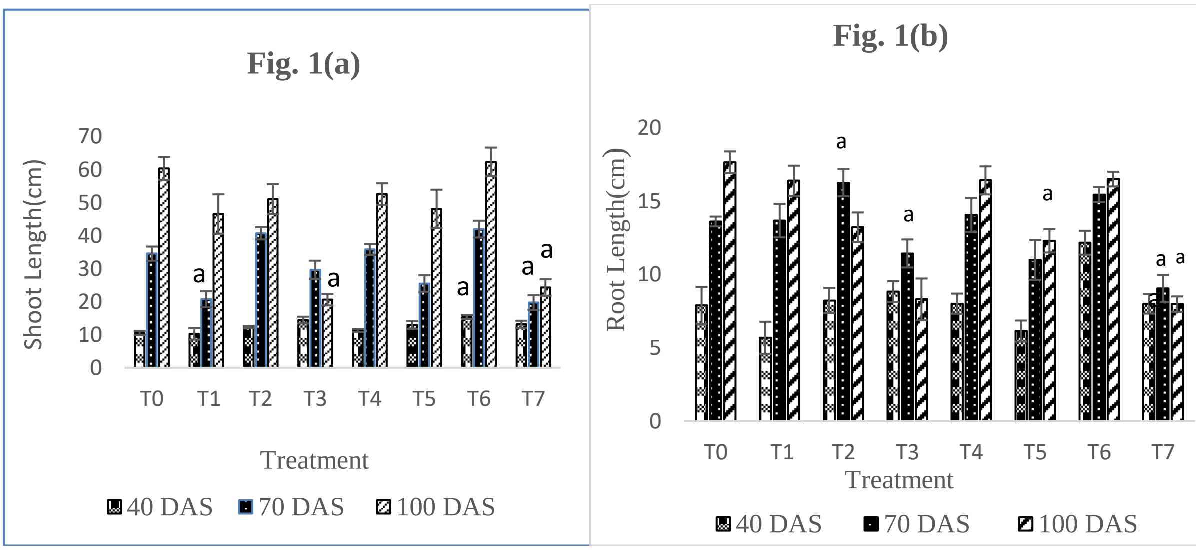

Fig. 1. Effect of seed priming and foliar treatment of melatonin on (a) shoot length (cm) and (b) root length (cm) of 40, 70 and 100 days mustard grown in waste water. Values show the mean± SE and significant difference at p=0.05. Albhabet 'a' indicate statistical difference from control in accordance with *Tukey's HSD (post hoc)* test

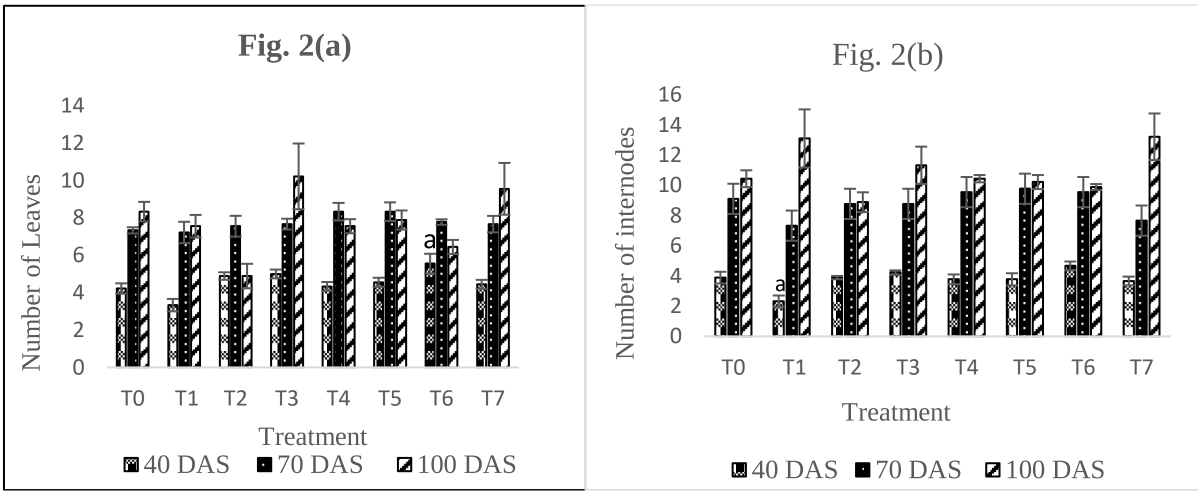

Fig. 2. Effect of seed priming and foliar treatment of melatonin on (a) number of leaves and (b) number of internodes of 40, 70 and 100 days mustard grown in waste water. Values show the mean+ SE and significant difference at p=0.05. Albhabet 'a' indicate statistical difference from control in accordance with *Tukey's HSD (post hoc)* test

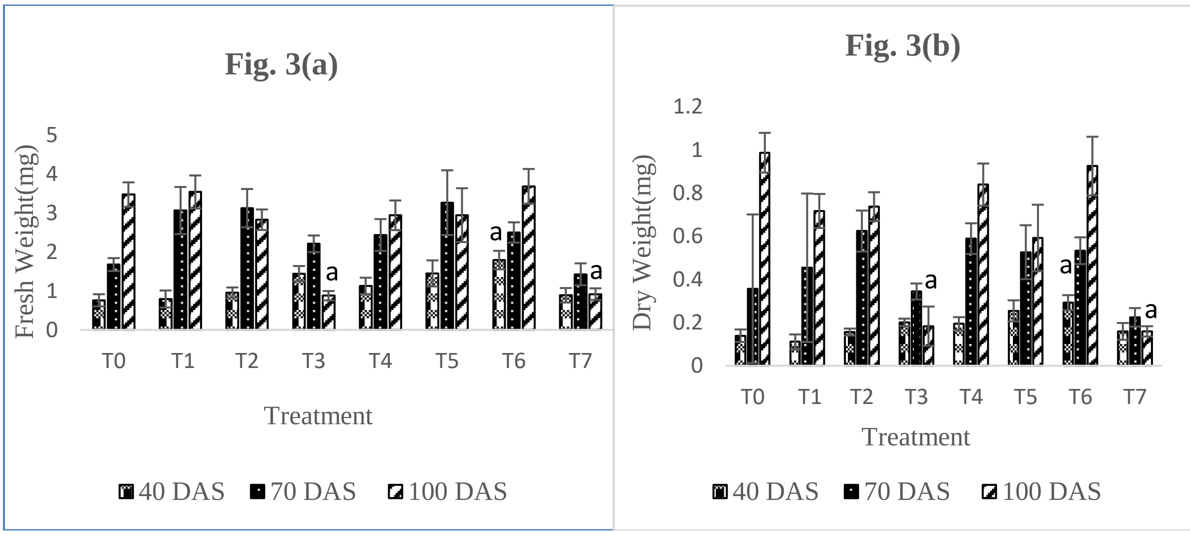

Fig. 3. Effect of seed priming and foliar treatment of melatonin on (a) fresh weight and (b) dry weight of 40, 70 and 100 days mustard grown in waste water. Values show the mean+ SE and significant difference at p=0.05. Albhabet 'a' indicate the statistical difference from control in accordance with *Tukey's HSD (post hoc)* test

3.2 Number of Leaves and Number of Internodes

Lowest number of leaves was recorded in the plants irrigated by waste water. Plants grown in irrigation water under treatment of MT as seed priming and foliar spray showed higher number of leaves at 40 and 70 DAS. A significant increase was observed in plants irrigated by waste water by the treatment of MT. Maximum increase was recorded with MT as seed priming treatment followed by combined treatment (seed priming and foliar spray) and then by foliar treatment. Arena et al., (2025) also reported an increase in number of green leaves in two varieties of Broccoli under drought stress by MT application.

A decrease in the number of internodes was observed in the plants irrigated by waste water in comparison to plants under control conditions. The results show that MT treatment is effective in increasing the number of internodes in plant under waste water. The highest increase was recorded with seed priming treatment of MT.

Foliar treatment and combined treatment (seed priming and foliar) also showed significant results in this parameter. However, the highest number of internodes was recorded in plants grown in irrigation water under combined treatment (seed priming and foliar) of MT.

3.3 Fresh Weight and Dry Weight

The maximum fresh weight was observed in plants grown in irrigation water along with co-application of MT (seed priming+ foliar spray). The plants under irrigation with waste water recorded a significant increase in the fresh weight by MT treatment. The highest increase was observed by foliar treatment of MT. A parallel observation was made in *Brassica napa* under cobalt stress by Ali et al., (2023) that exogenous applied MT notably at 100 µM MT dramatically increased leaf fresh/dry weight and root fresh/dry weight by (18/14%) and (25/23%) respectively.

The highest dry weight was recorded in plants grown in irrigation water with co-application of MT in form of seed priming and foliar spray at 40, 70 and 100 DAS followed by plants under foliar treatment of MT. Plants irrigated by waste water showed least dry weight but it increased significantly with foliar treatment of MT. Whereas co-application of MT in form of seed priming and foliar spray was found to be ineffective for dry weight *viz-a-viz* plants irrigated by waste water. A similar result was drawn by Wang et al., (2022) that foliar treatment is more effective than any other treatment for increasing biomass under stress. Furthermore, Zhang et al., (2017) confirmed that exogenous MT showed increases of 10.49% in shoot dry weight, 57.14% in root dry weight, 14.67% in dry weight in cucumber seedlings with nitrate stress.

3.4 Number of Pods and Pod Length

The maximum number of pods was observed in control treatment. This was followed by plants grown in irrigation water under MT treatment in form of foliar application and co-application of seed priming and foliar spray. Plants irrigated by waste water showed a significant decrease in the number of pods but the treatment with MT as foliar application and co-application of seed priming and foliar spray showed good results. Whereas MT treatment in the form of seed priming was found to be ineffective for plants grown in waste water. The result is in parlance with the result obtained by Khan et al. (2020) in two varieties of rapeseeds under drought stress with respect to seed priming with MT and gibberellic acid.

The length of pods in mustard was recorded at 100 and 140 days after sowing and tabulated. The maximum length of pods was observed in plants grown in irrigation water under the co-application of MT as seed priming and foliar spray. MT in form of foliar spray also showed significant increase followed by seed priming treatment of MT. Plants irrigated with waste water recorded decreased pod length at 100 and 140 DAS. MT treatment in form of foliar application to these plants was found to be very effective in increasing the pod length. Seed priming and foliar application in combination also showed an increase in pod length as compared to plants irrigated by waste water.

3.5 Number of Seeds per pod and 100 seed weight

The highest number of seeds per pod was observed in mustard plants grown in irrigation water under the treatment of MT in form of foliar spray.The plants under control conditions showed higher number of seeds per pod as compared to the plants grown in waste water. The MT treatment was found to be effective in increasing the yield in plants grown in wastewater under treatment of MT in form of foliar spray followed by co-treatment of foliar and seed priming treatment. A similar result was found in soybean plants with respect to seed number by Wei et al. (2015).

Under control conditions, the plants recorded good 100 seed weight. The highest 100 seed weight was recorded in the plants under foliar treatment of MT. Irrigation with waste water decreased the seed weight. In a parallel investigation on two different varieties of *Brassica*, Khan et al., (2020) found drastic decrease in the 1000 seed weight due to drought stress, which was alleviated by seed priming with MT. In our study, 100 seed weight was increased significantly in plants irrigated by waste water by MT treatment in form of seed priming and foliar spray. Similarly foliar application of MT (100 µ M) helped increase the yield by 34.23% in Broccoli under drought stress by Sardar et al. (2024).

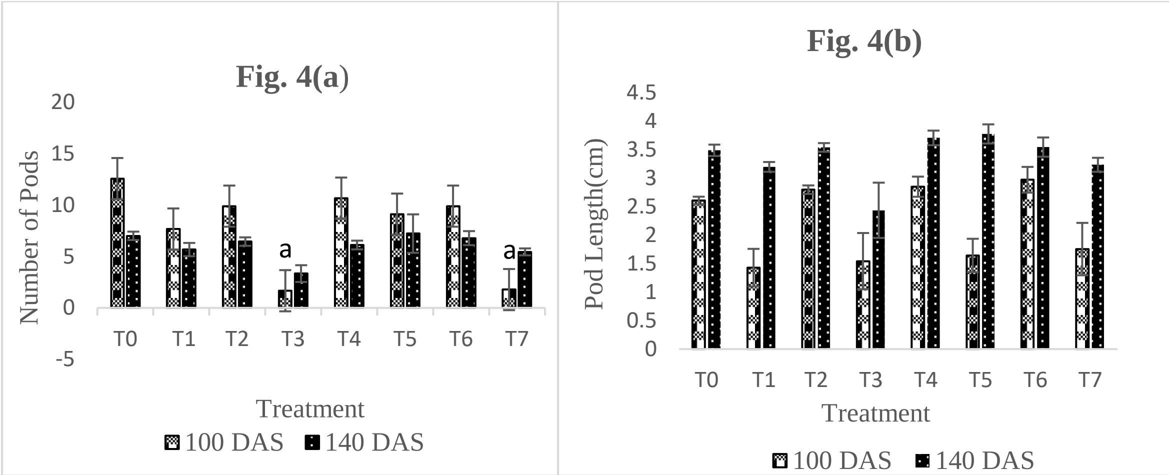

Fig. 4. Effect of seed priming and foliar treatment of melatonin on (a) number of pods and (b) pod length of 100 and 140 days mustard grown in waste water. Values show the mean+ SE and significant difference at p=0.05. Albhabet 'a' indicate statistical difference from control in accordance with *Tukey's HSD (post hoc)* test

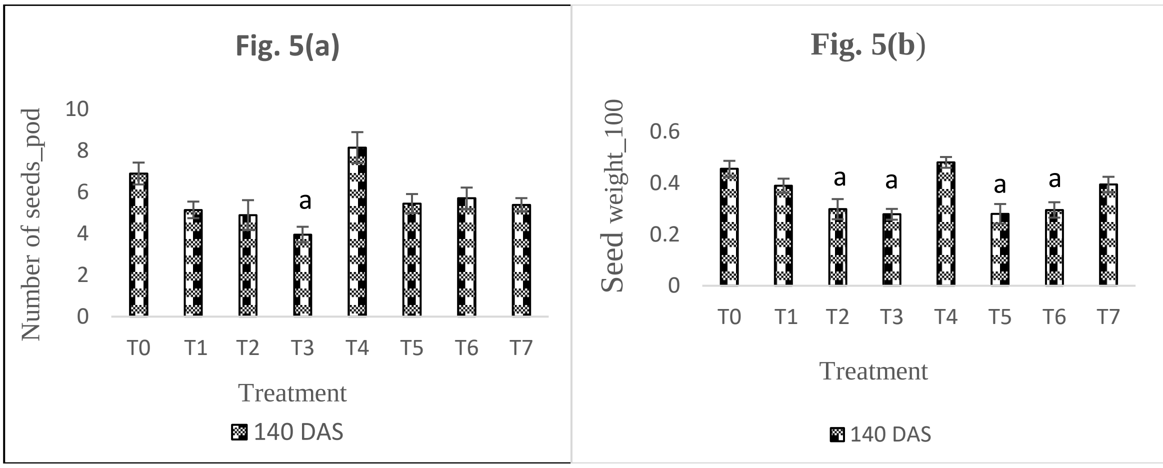

Fig. 5. Effect of seed priming and foliar treatment of melatonin on (a) number of seeds _pod and (b) seed weight_100 of mustard grown in waste water. Values show the mean+ SE and significant difference at p=0.05. Albhabet 'a' indicate statistical difference from control in accordance with *Tukey's HSD (post hoc)* test.

Therefore, it was observed in this study that all the parameters under study showed a significant decrease when the mustard plants were irrigated with waste water as compared to the control, which is also supported by Rampal, (2019) in case of contaminated water. Previous studies showed that exogenous application of MT increased the growth as well as yield parameters of mustard in control as well as stress conditions. The treatment of MT at 100 µM concentration was found effective in increasing the growth and yield parameters. This is in harmony with the results of Sadak et al., (2020). As reported by Wang et al., (2022) foliar application of MT was found to be the most effective method in alleviating the stress due to contaminated water in mustard.

Co-application of MT as seed priming and foliar spray showed siginificant results in shoot length, root length, number of leaves, number of internodes and biomass as compared to plants irrigated by waste water. It was observed that plants grown after seed priming with 100 µ M MT, showed a significant increase in all growth parameters at 40 DAS in case of plants irrigated with tap water as well as waste water. However, seed-primed plants exhibit a drastic decrease in the parameters at 70, 100 and 140 DAS. It was observed that in case of exposure to abiotic stress, the most sensitive stage in *Brassica* spp. is from flowering to pod development. This was also reported by Pillai et al., (2012) in rapeseed under drought stress.

Foliar application of MT at 100 µmol L^{-1} concentration can be helpful in alleviation of stress in mustard growing in waste water. This can be further used in harnessing the phytoremediation properties of *Brassica juncea L.* and reclamation of the soil. In line with our observation, Zargar et al. (2022) recommended the use of MT for increasing stress resilience and growth of plants in cadmium contaminated soils.

4. CONCLUSION AND FUTURE PROSPECTS

In this study, the *B. juncea* plants grown under waste water has shown significant reduction in morphological and yield parameters. The highest decrease was observed in number of pods per plant followed by shoot length in plants grown in waste water as compared to control. Further application of MT at 100 µmol L^{-1} through foliar spray was found to be most effective in ameliorating the negative impact of waste water in *B. juncea* plants. The highest increase was observed in number of pods per plant followed by pod length in plants grown in waste water by foliar application of MT with respect to plants grown in waste water only. Hence MT may be employed for enhancing the yield of mustard plants grown in areas irrigated with waste water. However, exhaustive field trials may be undertaken for different crops at varied concentrations of MT to ensure their large scale application in stress amelioration. Moreover, the detailed biochemical and molecular analysis would assist in devising the underlying mechanisms of MT-mediated stress responses in plants.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that **NO** generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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