Original Research Article

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

**ABSTRACT**

Mustard is a very important oil seed crop of India. Due to rapid urbanization and industrialization, a lot of untreated sewage and industrial effluents are discharged into the water bodies. Thus leading to water and soil pollution .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, a significant reduction in the morphological and yield traits were recorded in the plants grown in waste water. The plants under treatment of MT showed improvement in morphological and yield parameters as compared to the control.MT treated plants exhibited less reduction in morphological and yield components under waste water irrigation compared to plants without MT treatment. 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.

Key words: Mustard ,Melatonin, Seed priming, Foliar treatment, Waste water

**1. INTRODUCTION**

*B. juncea* is an oilseed crop and contributes 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 thrive best on light loam soils. It does not tolerate water logging conditions or heavy soils. Soil having 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* deserve special attention because of its relevance to the process of phytoextraction of heavy metals from soil [1]. 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 [2].

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[3].Industrial waste water contains highly variable compounds and volume depending on the type of industry producing those [4]. 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. These compounds are very difficult to treat.

Melatonin is one of the earliest molecules whose existence can be traced back to the origin of life. Melatonin’s initial function was likely as a free radical scavenger [5]. Melatonin serves as a first line of defence against oxidative stresses. 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, melatonin is involved in multiple functions in the growth and development of the plants ranging from seed germination to senescence [5].Endogenous as well as exogenous melatonin can increase the adaptability of plants to various stresses [7]. Further, supplementation of melatonin to plants may offer stress protection against various pollutants. However, there are no reports related to the role of melatonin in stress amelioration in Indian mustard grown in the polluted sites.

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

**2. MATERIAL 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 the plastic pots(diameter: 20 cm, height: 15 cm) filled with 4 kg of soil. The soil in the pot will be irrigated by the treated(wastewater collected from Maggar khud, Kathua stored in a refrigerator (4 ◦C) until the moment of testing)and untreated(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 melatonin solution (MT) at 20 °C under dark for 18 h. Subsequently, the seeds were removed from the solutions and washed with distilled water for 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 MEL 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 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 of root and shoot was measured immediately using digital balance. Plant samples were oven dried at 70 °C for 48 h to measure the dry mass of root and shoot.

Number of pods and Number of Seeds per 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 were 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.

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

2.1 The following treatments (T0 – T8) are:

T0: Control (Irrigation with Tap water).

T1: Irrigation with wastewater.

T2: Melatonin (Seed priming Treatment) and irrigation with Tap water.

T3: Melatonin (Seed priming Treatment) and irrigation with Waste water.

T4: Melatonin (Foliar spray) and irrigation with Tap water.

T5: Melatonin (Foliar spray) and irrigation with Waste water.

T6: Melatonin (Seed priming Treatment + Foliar spray) and irrigation with Tap water.

T7: Melatonin (Seed priming Treatment + Foliar spray) and irrigation with waste water.

Statistical analysis: All data was analyzed using one-way ANOVA while Tukey’s posthoc 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 treated with co-application of Melatonin 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 melatonin(foliar spray) showed increase in shoot length as compared to plants without the treatment. Melatonin treatment as seed priming and 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 at 100 DAS. Therefore, melatonin treatment in the form of foliar spray was found to be most effective to improve the shoot length. The results are in conformity with the results of Park et al.,2021[8]that plant heightwas enhanced in 38 day old salinity stressed plants under melatonin treatment.

Figure 1: Effect of seed priming and foliar treatment of melatonin on (a) shoot length and (b) root length 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 test.

The data in Figure 1(b) shows the root length of mustard under the impact of contaminated water and melatonin (seed priming +foliar spray) treatments . The maximum root length was observed in the plants subjected to co-application of Melatonin(seed priming+ foliar spray) followed by plants under treatment of melatonin(foliar spray). A decrease in the root length was observed in plants grown in waste water as compared to plants under control conditions. Melatonin treatment in 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 at el.,2017[9] that root length increased by 14% in cucumber seedlings under nitrate stress by melatonin application and Dai et al.,(2020)[10] showed that melatonin promoted taproot and lateral root growth under drought stress in two different varieties of mustard

3.2 Number of Leaves and number of internodes

Lowest number of leaves were recorded in the plants irrigated by waste water. Plants under treatment of melatonin 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 melatonin. Maximum increase was recorded with melatonin as seed priming treatment followed combined Treatment(seed priming and foliar spray) and foliar treatment .Arena et al., 2025[11] also reported increase in number of green leaves in two varieties of Broccoli under drought stress by melatonin application.

Figure 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 test.

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 melatonin treatment is effective in increasing the number of internodes in plant under waste water stress. The highest increase was recorded with seed priming treatment of melatonin. Foliar treatment and combined treatment(seed priming and foliar ) also showed good results in this parameter. However the highest number of internodes were recorded in plants under combined treatment(seed priming and foliar) of melatonin.

3.3 Fresh weight and dry weight

The maximum fresh weight was observed in plants withco-application of melatonin(seed priming+ foliar spray).The plants under irrigation with waste water recorded a significant increase in the fresh weight by melatonin treatment. The highest increase was observed by foliar treatment of melatonin. A parallel observation was made in *Brassica napa* under cobalt stress by Ali et al.,2023[12] 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.

Figure 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 statistical difference from control in accordance with Tukey’s HSD test.

The highest dry weight was recorded in plants with co-application of melatonin in form of seed priming and foliar spray at 40, 70 and 100 DAS followed by plants under foliar treatment of melatonin. Plants irrigated by contaminated water showed least dry weight but it increased significantly with foliar treatment of melatonin. Whereas co-application of melatonin in form of seed priming and foliar spray was found to be ineffective for dry weight viz a viz plants irrigated by contaminated water. A similar result was drawn by Wang et al., (2022)[13] that foliar treatment is more effective any other treatment for increasing biomass under stress. Furthermore,Zhang at el.,2017[9]confirmed that exogenous melatonin showed increases of 10.49% in shoot DW , 57.14% in root DW , 14.67% in DW 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 under melatonin treatment in form of foliar application and co-application of seed priming and foliar application. Plants irrigated by contaminated water showed significant decrease in the number of pods but the treatment with melatonin as foliar application and co-application of seed priming and foliar spray showed good results. Whereas melatonin treatment in the form of seed priming was found to be ineffective for plants under contaminated water stress. The result is in parlance with result obtained by Khan et al.(2020)[14] in two varieties of rapeseeds under drought stress with respect to seed priming with melatonin and giberillic acid.

Figure 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 test.

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 under co-application of melatonin as seed priming and foliar spray. Melatonin in form of foliar spray also showed good results followed by seed priming treatment of melatonin. Plants irrigated with contaminated water recorded decreased pod length at 100 and 140 DAS. Melatonin 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 increase in pod length viz a viz plants irrigated by contaminated water.

3.5 Number of seeds\_pod and seed weight\_100

The highest number of seeds per pod were observed in mustard plants under the treatment of melatonin in form of foliar spray. The plants under control conditions showed good results whereas the plants grown in waste water produced lesser number of seeds per pod. The melatonin treatment was found to be effective to increase the yield in waste water in form of foliar spray followed by co-treatment of foliar and seed priming treatment. A similar result was found in soyabean plants w.r.t. seed number by Wei at el.(2015)[15]

Figure 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 test.

Under control conditions, the plants recorded good seed weight per hundred. The highest seed weight \_100 was recorded in the plants under foliar treatment of melatonin. Irrigation with waste water decreased the seed weight .In a parallel investigation on two different varieties of *Brassica* ,Khan et al.,2020[14] found drastic decrease in the seed weight -1000 due to drought stress which was alleviated by Seed priming with MT. In our study, the seed weight -100 was increased significantly in plants irrigated by waste water by melatonin treatment in form of seed priming and foliar spray. Similarly foliar application of melatonin(100uM) helped increase the yield by 34.23% in Broccoli under drought stress by Sardar et al.(2024)[16]

**4. CONCLUSION AND FUTURE PROSPECTS**

It was observed that all the parameters under study showed significant decrease when the mustard plants were irrigated with contaminated water as compared to the control as reported by Rampal,R.K.(2019) in case of contaminated water. Previous studies showed that exogenous application of melatonin increased the growth as well as yield parameters of mustard in control as well as stress condition. The treatment of melatonin 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[21]**.** As reported by Wang et al., (2022)[13]foliar application of melatonin was found to be most effective method in alleviating the stress due to contaminated water in mustard. Co-application of melatonin as seed priming and foliar spray showed good result in shoot length, root length, number of leaves, number of internodes and biomass viz a viz plants irrigated by contaminated water. It was observed that plants grown after seed priming with 100 µM Melatonin, showed a significant increase in all growth parameters at 40 DAS in case of plants irrigated with tap water as well as contaminated water. However, seed primed plants exhibit drastic decrease in the parameters at 70,100 and 140 DAS. It was observed that exposed 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[19] in rapeseed under drought stress.

Application of Melatonin 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. In line with our observation, Zargara et al., 2022 recommended the use of melatonin for increasing stress resilience and growth of plants in cd contaminated soils.

References;

1. Rathore, S. S., Shekhawat, K., Dass, A., Kandpal, B. K., & Singh, V. K. (2019). Phytoremediation mechanism in Indian mustard (Brassica juncea) and its enhancement through agronomic interventions. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *89*, 419-427.

2. McGrath, S. P., Zhao, J., & Lombi, E. (2002). Phytoremediation of metals, metalloids, and radionuclides.

3. Sankhla, M. S., Kumari, M., Nandan, M., Kumar, R., & Agrawal, P. (2016). Heavy metals contamination in water and their hazardous effect on human health-a review. *Int. J. Curr. Microbiol. App. Sci*, *5*(10), 759-766.

4. Mokarram, M., Saber, A., & Sheykhi, V. (2020). Effects of heavy metal contamination on river water quality due to release of industrial effluents. *Journal of Cleaner Production*, *277*, 123380.

5. Zhao, D., Yu, Y., Zhao, Z., Sharma, R., & Reiter, R. J. (2019). Melatonin synthesis and function: evolutionary history in animals and plants. *Frontiers in endocrinology*, *10*, 441357.

6. Tan, D. X., Hardeland, R., Manchester, L. C., Korkmaz, A., Ma, S., Rosales-Corral, S., & Reiter, R. J. (2012). Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. *Journal of experimental botany*, *63*(2), 577-597.

7. Hattori, A., Migitaka, H., Iigo, M., Itoh, M., Yamamoto, K., Ohtani-Kaneko, R.,et al(1995). Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. *Biochemistry and molecular biology international*, *35*(3), 627-634.

8. Park, H. S., Kazerooni, E. A., Kang, S. M., Al-Sadi, A. M., & Lee, I. J. (2021). Melatonin enhances the tolerance and recovery mechanisms in Brassica juncea (L.) Czern. under saline conditions. *Frontiers in plant science*, *12*, 593717.

9. Zhang, R., Sun, Y., Liu, Z., Jin, W., & Sun, Y. (2017). Effects of melatonin on seedling growth, mineral nutrition, and nitrogen metabolism in cucumber under nitrate stress. *Journal of Pineal Research*, *62*(4), e12403.

10. Dai, L., Li, J., Harmens, H., Zheng, X., & Zhang, C. (2020). Melatonin enhances drought resistance by regulating leaf stomatal behaviour, root growth and catalase activity in two contrasting rapeseed (Brassica napus L.) genotypes. *Plant Physiology and Biochemistry*, *149*, 86-95.

11. Arena D, Ben Ammar H, Rodriguez VM, Velasco P, Calì R, Ciccarello L, Branca F. The Role of Melatonin in Modulating Morphometric Parameters and Bioactive Compounds of *Brassica**oleracea* L.var. *italica* Plenck Under Drought Stress. Agronomy. 2025; 15(2):279. https://doi.org/10.3390/agronomy15020279)

12. Ali, S., Gill, R. A., Ulhassan, Z., Zhang, N., Hussain, S., Zhang, K., et al (2023). Exogenously applied melatonin enhanced the tolerance of Brassica napus against cobalt toxicity by modulating antioxidant defense, osmotic adjustment, and expression of stress response genes. *Ecotoxicology and Environmental Safety*, *252*, 114624.

13. Wang, Y., Gun, S., Li, Y., & Qu, L. (2022). Meta-analysis of effects of melatonin treatment on plant drought stress alleviation. *Agriculture*, *12*(9), 1335

14. Khan, M. N., Khan, Z., Luo, T., Liu, J., Rizwan, M., Zhang, J., et al (2020). Seed priming with gibberellic acid and melatonin in rapeseed: Consequences for improving yield and seed quality under drought and non-stress conditions. *Industrial Crops and Products*, *156*, 112850.

15. Wei, W., Li, Q. T., Chu, Y. N., Reiter, R. J., Yu, X. M., Zhu, D. H., ... & Chen, S. Y. (2015). Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. *Journal of experimental botany*, *66*(3), 695-707.

16. Sardar, H., Shafiq, M., Naz, S., Ali, S., Ahmad, R., & Ejaz, S. (2024). Enhancing drought tolerance in broccoli (Brassica oleracea L.) through melatonin application: Physiological and biochemical insights into growth, photosynthesis, and antioxidant defense mechanisms. *Biocatalysis and Agricultural Biotechnology*, *59*, 103256.

17. Rampal, R. K. (2019, April 7). *Impact of Combined Industrial Effluents  from Kathua on Some Ecophysiological  Parameters of Phaseolus vulgaris L*. <http://hdl.handle.net/10603/237495>

18. Sadak, M. S., Abdalla, A. M., Abd Elhamid, E. M., & Ezzo, M. I. (2020). Role of melatonin in improving growth, yield quantity and quality of Moringa oleifera L. plant under drought stress. *Bulletin of the National Research Centre*, *44*, 1-13.

19. Pillai, B. V., Kagale, S., & Chellamma, S. (2011). Enhancing productivity and performance of oil seed crops under environmental stresses. In *Crop Stress and its Management: Perspectives and Strategies* (pp. 139-161). Dordrecht: Springer Netherlands.

20. Zargar, T. B., Mir, A. R., Alam, P., & Hayat, S. (2022). Melatonin alleviates cadmium-induced toxicity by modulating antioxidant defence mechanisms, growth and photosynthesis in Brassica juncea. *Russian Journal of Plant Physiology*, *69*(6), 121.