EXPERIMENTAL RESULTS
## EXPERIMENTAL RESULTS

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EXPERIMENTAL RESULTS

Experimental results are presented herein mainly as changes in growth and photosynthetic characteristics, sulfur and nitrogen assimilation, components of antioxidant system and yield characteristics of mustard cultivars as influenced by different concentrations of cadmium, sulfur and nitrogen.

4.1 Experiment 1: Screening of Mustard Cultivars Treated with Different Levels of Cadmium

The Experiment was conducted to study the effect of five concentrations of Cd viz., 0, 25, 50, 100 and 150mg kg\(^{-1}\) soil on Cd accumulation, growth, photosynthetic and yield characteristics of five mustard cultivars, namely Alankar, Varuna, Pusa Bold, Sakha and RH30. Tolerance index of the five cultivars of mustard was calculated and cultivars were designated as Cd tolerant and Cd non-tolerant on the basis of their performance under Cd stress. Results are described below in detail.

4.1.1 Growth characteristics

The effect of Cd on growth characteristics was found significant at the three sampling stages. The interaction of Cd treatments and cultivars was also found significant at 60 and 90DAS but was non-significant at 30DAS for all the growth characteristics studied (Figures 2-5). The increase in Cd concentration decreased the growth characteristics of all the five cultivars at all sampling stages. The observations recorded at all the sampling stages showed similar pattern of cultivar response to Cd treatments. The pattern of decrease in growth characteristics for the cultivars was RH30 > Sakha > Pusa Bold > Varuna > Alankar. Maximum reduction in growth characteristics was noted with 150mg Cd kg\(^{-1}\) soil followed by 100, 50 and 25mg Cd kg\(^{-1}\) soil at all growth stages.

Among cultivars, Alankar showed lesser decrease in growth characteristics followed by Varuna and Pusa Bold, whereas, RH30 and Sakha exhibited greater reduction in growth characteristics under Cd stress.

In Alankar, root length was decreased by 9.1, 15.1, 24.2, 37.1% at 30DAS; 7.1, 11.9, 20.2, 32.1% at 60DAS and 5.2, 10.4, 18.0, 27.7% at 90DAS due to 25, 50, 100, and 150mg Cd kg\(^{-1}\) soil, respectively, over their respective control. Contrarily, a higher decrease of 17.0, 27.0, 40.0, 58.0% at 30DAS; 16.4, 30.3, 45.0, 60.6% at 60DAS and 13.0, 23.7, 33.6, 42.6% at 90DAS in root length of RH30 was noted with
Fig. 2. Effect of Cd treatments (T) on root length (cm plant$^{-1}$) of five cultivars (C) of mustard (*Brassica juncea* L.) at 30, 60 and 90 days after sowing (DAS).
Fig. 3. Effect of Cd treatments (T) on shoot length (cm plant\(^{-1}\)) of five cultivars (C) of mustard (Brassica juncea L.) at 30, 60 and 90 days after sowing (DAS).
Fig. 4. Effect of Cd treatments (T) on leaf area (cm² plant⁻¹) of five cultivars (C) of mustard (*Brassica juncea* L.) at 30, 60 and 90 days after sowing (DAS).
Fig. 5. Effect of Cd treatments (T) on plant dry mass (g plant⁻¹) of five cultivars (C) of mustard (*Brassica juncea* L.) at 30, 60 and 90 days after sowing (DAS).

- 30DAS
  - LSD₀.₀５ (C×T) = NS
- 60DAS
  - LSD₀.₀５ (C×T) = 0.28
- 90DAS
  - LSD₀.₀５ (C×T) = 0.57
25, 50, 100 and 150mg Cd kg\(^{-1}\) soil, respectively, over their respective control (Figures 2,6).

The decrease in shoot length followed the same pattern as observed in root length. In comparison to control, shoot length of Alankar decreased by 5.4, 10.2, 17.4, 26.2% at 30DAS; 4.6, 8.9, 14.7, 24.8% at 60DAS and 3.4, 6.5, 12.5, 20.4% at 90DAS with 25, 50, 100 and 150mg Cd kg\(^{-1}\) soil, respectively. In RH30, reductions of 10.3, 22.7, 29.6, 47.0% at 30DAS; 14.3, 26.8, 30.6, 50.2% at 60DAS and 8.4, 20.3, 25.6, 42.2% at 90DAS were noted with 25, 50, 100 and 150mg Cd kg\(^{-1}\) soil, respectively, over their respective control (Figures 3,6).

When compared to control, leaf area of Alankar decreased by 8.6, 15.5, 29.3, 37.9% at 30DAS; 9.5, 15.5, 30.5, 40.0% at 60DAS and 5.0, 7.7, 16.9, 26.5% at 90DAS with 25, 50, 100 and 150mg Cd kg\(^{-1}\) soil, respectively. Whereas, RH30 showed reductions of 16.7, 26.2, 38.1, 54.7% at 30DAS; 21.9, 30.9, 40.8, 58.1% at 60DAS and 15.9, 21.6, 32.7, 42.5% at 90DAS with 25, 50, 100 and 150mg Cd kg\(^{-1}\) soil, respectively, over their respective control (Figures 4,7).

The trend of plant response to Cd treatments in terms of plant dry mass was almost similar to those observed for the individual growth characteristics. Plant dry mass of Alankar decreased by 6.0, 11.3, 25.3, 33.3% at 30DAS; 5.4, 9.5, 21.2, 29.8% at 60DAS and 3.4, 7.1, 20.3, 26.9% at 90DAS due to 25, 50, 100 and 150mg Cd kg\(^{-1}\) soil, respectively, in comparison to their respective control. While, in RH30, plant dry mass was decreased by 13.2, 21.5, 39.6, 54.5% at 30DAS; 15.5, 25.3, 41.4, 60.3% at 60DAS and 9.6, 18.1, 31.6, 43.7% at 90DAS with 25, 50, 100 and 150mg Cd kg\(^{-1}\) soil, respectively, over their respective control (Figures 5, 7). The trend of sensitivity of mustard cultivars to Cd toxicity in terms of tolerance index (calculated on the basis of plant dry mass) was: RH30 >Sakha >Pusa Bold >Varuna >Alankar (Figure 8).

4.1.2 Photosynthetic characteristics
Photosynthetic characteristics (total chlorophyll content, carotenoids content, carbonic anhydrase activity, net photosynthetic rate and stomatal conductance) of the five mustard cultivars decreased significantly with the increasing Cd concentration at all growth stages (Figures 9-13). The interaction of Cd treatments and cultivars was significant at all the sampling stages except for carotenoids content which remained non-significant at 30DAS. The greatest significant reduction in photosynthetic characteristics was recorded with highest Cd level (150mg Cd kg\(^{-1}\) soil) in all the
Fig. 6. Per cent change in root and shoot length of five cultivars of mustard (*Brassica juncea* L.) due to 150mg Cd kg⁻¹ soil over control at 30, 60 and 90 days after sowing (DAS).
Fig. 7. Per cent change in leaf area and plant dry mass of five cultivars of mustard (Brassica juncea L.) due to 150mg Cd kg⁻¹ soil over control at 30, 60 and 90 days after sowing (DAS).
Fig. 8. Tolerance index of five cultivars of mustard (*Brassica juncea* L.) treated with 150mg Cd kg⁻¹ soil. Tolerance index was calculated as percentage of the plant dry mass obtained in 150mg Cd kg⁻¹ soil and control at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates ± S.E.
cultivars. The pattern of decrease in photosynthetic characteristics was: RH30 > Sakha > Pusa Bold > Varuna > Alankar.

Maximum reduction in total chlorophyll and carotenoids content was observed in RH30 and least in Alankar under Cd stress at all sampling stages. In Alankar, total chlorophyll content was decreased by 13.7, 21.6, 32.7, 44.4% at 30DAS; 10.9, 17.4, 22.3, 35.3% at 60DAS; 13.0, 20.0, 28.0, 41.0% at 90DAS and in carotenoids content by 5.3, 17.4, 21.1, 32.6% at 30DAS; 4.3, 17.1, 25.2, 34.2% at 60DAS; 4.8, 18.6, 25.7, 38.3% at 90DAS with 25, 50, 100 and 150mg Cd kg\(^{-1}\) soil, respectively, over their respective control. RH30 showed greater reduction in total chlorophyll content, which was 20.3, 31.6, 43.6, 60.1% at 30DAS; 26.3, 41.3, 52.6, 67.7% at 60DAS; 26.9, 46.1, 53.8, 69.2% at 90DAS and carotenoids content decreased by 16.0, 33.3, 40.7, 49.3% at 30DAS; 19.7, 34.3, 44.5, 54.0% at 60DAS; 18.7, 34.9, 45.5, 55.3% at 90DAS with 25, 50, 100 and 150mg Cd kg\(^{-1}\) soil, respectively, over their respective control (Figures 9, 10, 14).

The decrease in carbonic anhydrase activity in Alankar was 8.6, 14.5, 32.7, 40.7% at 30DAS; 9.1, 16.4, 36.8, 44.7% at 60DAS and 9.3, 20.3, 39.4, 45.2% at 90DAS with 25, 50, 100 and 150mg Cd mg\(^{-1}\) soil, respectively, over their respective control. Carbonic anhydrase activity in RH30 was decreased by 22.1, 35.0, 55.4, 67.5% at 30DAS; 26.6, 46.9, 58.3, 70.6% at 60DAS and 29.4, 48.8, 63.5, 78.9% at 90DAS with 25, 50, 100 and 150mg Cd mg\(^{-1}\) soil, respectively, over their respective control (Figures 11, 15).

In comparison to control, net photosynthetic rate of Alankar was decreased by 7.9, 14.3, 32.5, 37.7% at 30DAS; 7.1, 13.2, 30.8, 33.8% at 60DAS and 5.9, 12.5, 27.6, 29.9% at 90DAS with 25, 50, 100 and 150mg Cd kg\(^{-1}\) soil, respectively, over their respective control. Whereas, in RH30, it was decreased by 12.4, 27.9, 46.1, 53.5% at 30DAS; 14.3, 32.0, 47.4, 54.7% at 60DAS and 14.1, 33.3, 47.8, 60.7% at 90DAS with 25, 50, 100 and 150mg Cd kg\(^{-1}\) soil, respectively, over their respective control (Figures 12, 16).

The decrease in stomatal conductance followed the same pattern as observed in net photosynthetic rate. When compared with the respective control, reduction in stomatal conductance in Alankar was 9.7, 16.1, 25.2, 40.3% at 30DAS; 11.3, 17.7, 30.0, 42.3% at 60DAS and 9.8, 20.2, 30.4, 45.5% at 90DAS with 25, 50, 100 and 150mg Cd kg\(^{-1}\) soil, respectively. RH30 showed decrease in stomatal conductance by 16.4, 26.5, 49.8, 57.8% at 30DAS; 18.9, 30.4, 52.6, 60.3% at 60DAS and 21.0, 34.9,
Fig. 9. Effect of Cd treatments (T) on total chlorophyll content (mg g$^{-1}$ FW) of five cultivars (C) of mustard (Brassica juncea L.) at 30, 60 and 90 days after sowing (DAS).
Fig. 10. Effect of Cd treatments (T) on carotenoids content (mg g⁻¹ FW) of five cultivars (C) of mustard (Brassica juncea L.) at 30, 60 and 90 days after sowing (DAS).
Fig. 11. Effect of Cd treatments (T) on carbonic anhydrase activity (mmol CO$_2$ g$^{-1}$ FW) of five cultivars (C) of mustard (Brassica juncea L.) at 30, 60 and 90 days after sowing (DAS).
Fig. 12. Effect of Cd treatments (T) on net photosynthetic rate (μmol CO₂ m⁻² s⁻¹) of five cultivars (C) of mustard (Brassica juncea L.) at 30, 60 and 90 days after sowing (DAS).
Fig. 13. Effect of Cd treatments (T) on stomatal conductance (mmol m$^{-2}$ s$^{-1}$) of five cultivars (C) of mustard (*Brassica juncea* L.) at 30, 60 and 90 days after sowing (DAS).
Fig. 14. Per cent change in total chlorophyll content and carotenoids content of five cultivars of mustard (*Brassica juncea* L.) due to 150mg Cd kg$^{-1}$ soil over control at 30, 60 and 90 days after sowing (DAS).
Fig. 15. Per cent change in carbonic anhydrase activity of five cultivars of mustard (*Brassica juncea* L.) due to 150mg Cd kg⁻¹ soil over control at 30, 60 and 90 days after sowing (DAS).
Fig. 16. Per cent change in net photosynthetic rate and stomatal conductance of five cultivars of mustard (*Brassica juncea* L.) due to 150 mg Cd kg⁻¹ soil over control at 30, 60 and 90 days after sowing (DAS).
54.2, 68.1% at 90DAS with 25, 50, 100 and 150mg Cd mg⁻¹ soil, respectively, in comparison to their respective control (Figures 13,16).

4.1.3 Cadmium accumulation

Root and leaf Cd accumulation increased with the increase in soil Cd concentration and was significant at 30, 60 and 90DAS (Figures 17-18). The effect of Cd treatment and cultivar interaction was also found significant at all the growth stages. For every concentration used in the study, Cd concentration was more pronounced in the roots than the leaves in all the five cultivars. The trend of Cd accumulation in root and leaf of five cultivars was: RH30 >Sakha >Pusa Bold >Varuna >Alankar at all growth stages.

Among cultivars, RH30 accumulated highest amount of Cd in root and leaf, whereas, Alankar accumulated least. Maximum accumulation of Cd in the root of RH30 was 354.2, 403.7, 452.8μg Cd g⁻¹ DW and in the leaf the accumulation was 137.1, 205.6, 130.2μg Cd g⁻¹ DW at 30, 60 and 90DAS, respectively of plants treated with 150mg Cd kg⁻¹ soil. Root and leaf of Alankar accumulated 273.1, 330.8, 381.2μg Cd g⁻¹ DW and 70.7, 95.3, 51.2μg Cd g⁻¹ DW at 30, 60 and 90DAS, respectively, when treated with 150mg Cd kg⁻¹ soil (Figures 17-19).

4.1.4 Yield characteristics

The increasing concentration of Cd significantly decreased the yield characteristics of all the five cultivars (Figure 20). The interaction of Cd treatments and cultivars was also significant. Maximum decrease in yield characteristics was noted with 150mg Cd kg⁻¹ soil. The cultivars RH30 and Sakha exhibited similar and greatest decrease in yield characteristics, whereas, Alankar showed lowest decrease followed by Varuna and Pusa Bold. The order of performance for yield characteristics of cultivars was Alankar >Varuna >Pusa Bold >Sakha >RH30.

In Alankar, the decrease in number of siliqua per plant was 11.5, 22.7, 30.4, 35.7%; number of seeds per siliqua was 10.6, 22.7, 32.2, 33.8%; 1000 seed weight was 13.7, 25.0, 35.8, 41.4% and seed yield was 13.7, 21.6, 28.1, 32.5% due to 25, 50, 100 and 150mg Cd kg⁻¹ soil, respectively, over their respective control. In contrast, RH30 showed greater reduction in number of siliqua per plant which was 24.5, 35.5, 43.5, 53.8%, number of seeds per siliqua was decreased by 25.2, 39.3, 51.1, 55.9%, 1000 seed weight by 25.6, 37.4, 53.2, 60.0% and seed yield by 22.4, 35.3, 45.4, 52.7% with to 25, 50, 100 and 150mg Cd kg⁻¹ soil, respectively (Figures 20-21).
Fig. 17. Effect of Cd treatments (T) on root Cd content (µg g⁻¹ DW) of five cultivars (C) of mustard (Brassica juncea L.) at 30, 60 and 90 days after sowing (DAS).
Fig. 18. Effect of Cd treatments (T) on leaf Cd content (μg g⁻¹ DW) of five cultivars (C) of mustard (*Brassica juncea* L.) at 30, 60 and 90 days after sowing (DAS).
Fig. 19. Per cent change in root Cd content and leaf Cd content of five cultivars of mustard (*Brassica juncea* L.) due to 150mg Cd kg⁻¹ soil over control at 30, 60 and 90 days after sowing (DAS).
Fig. 20. Effect of Cd treatments (T) on number of siliqua per plant, number of seeds per siliqua, 1000 seed weight (g) and seed yield (g plant$^{-1}$) of five cultivars (C) of mustard (*Brassica juncea* L.) at harvest i.e., 120 days after sowing (DAS).
Fig. 21. Per cent change in number of siliqua per plant, number of seeds per siliqua, 1000 seed weight and seed yield of five cultivars of mustard (*Brassica juncea* L.) due to 150mg Cd kg⁻¹ soil over control at harvest i.e., 120days after sowing (DAS).
4.2 Summary of Experiment 1

- The accumulation of Cd in root and leaf was found Cd dose dependent. The accumulation was maximum in RH30 followed by Sakha, Pusa Bold, Varuna and Alankar.
- Cadmium treatments resulted in an overall reduction in growth, photosynthetic and yield characteristics in mustard cultivars with more pronounced decrease with the 150mg Cd kg⁻¹ soil treatment.
- Among cultivars, RH30 showed greater reduction in growth, photosynthetic and yield characteristics, whereas, least decrease in these characteristics was found in Alankar.
- The trend of sensitivity of mustard cultivars to Cd toxicity was: RH30 >Sakha >Pusa Bold >Varuna >Alankar.
- On the basis of overall performance of the cultivars under Cd stress, Alankar emerged as Cd tolerant and RH30 as Cd non-tolerant.

4.3 Experiment 2: Study of Alleviation of Cd-induced Effects with Sulfur Application in Cd-tolerant and non-tolerant Mustard Cultivars

Experiment 2 was conducted on the basis of the findings of Experiments 1. As observed in Experiment 1, Alankar emerged as Cd-tolerant and RH30 as Cd non-tolerant cultivar. The treatment 150mg Cd kg⁻¹ soil caused maximum decrease in the observed characteristics and 50mg Cd kg⁻¹ soil was moderate toxic in effects. Therefore, the present Experiment was aimed to study the alleviation potential of elemental S (0, 50 and 100mg S kg⁻¹ soil) under the moderate (50mg Cd kg⁻¹ soil) and extreme (150mg Cd kg⁻¹ soil) Cd levels by studying the changes in Cd accumulation, growth and photosynthetic characteristics, sulfur and nitrogen assimilation, components of antioxidant system and yield characteristics of tolerant (Alankar) and non-tolerant (RH30) mustard cultivars.

Detailed results of this Experiment are presented in the following section.

4.3.1 Growth characteristics

Growth characteristics (root length, shoot length, leaf area and plant dry mass) decreased in both the cultivars treated with Cd and the decrease was proportional to the concentration of Cd (50 and 150mg Cd kg⁻¹ soil). Sulfur application nullified the effects of 50mg Cd kg⁻¹, but could not ameliorate the effects of 150mg Cd kg⁻¹ soil. Ironically, the application of 50mg S kg⁻¹ soil proved most effective in improving growth characteristics of both the cultivars treated with 50mg Cd kg⁻¹ soil. Whereas,
no such effect was observed for the supplementation of 100 mg S kg\(^{-1}\) soil (Figures 22-23).

Both the levels of Cd (50 and 150 mg Cd kg\(^{-1}\) soil) significantly reduced the growth characteristics of both the cultivars. The extent of decrease was greater in non-tolerant cultivar RH30 than the tolerant cultivar Alankar. In Alankar, the variation in the reduction of plant dry mass was 10.5 and 35.7% at 30DAS; 7.9 and 27.9% at 60DAS and 7.1 and 27.9% at 90DAS due to 50 and 150 mg Cd kg\(^{-1}\) soil, respectively, in comparison to their respective control. In RH30, the decrease in plant dry mass due to 50 mg Cd kg\(^{-1}\) soil was about 25% and 50% due to 150 mg Cd kg\(^{-1}\) soil at all sampling times (Figures 24-25).

The application of 50 mg S kg\(^{-1}\) soil completely overcome the effects of 50 mg Cd kg\(^{-1}\) soil and also increased the growth characteristics. This increasing effect of S was seen in Alankar. Root length, shoot length, leaf area and plant dry mass of Alankar treated with 50 mg Cd kg\(^{-1}\) soil were increased by 1.5, 3.9, 1.6, 1.4% at 30DAS; 3.3, 4.7, 4.3, 2.6% at 60DAS and 2.6, 3.6, 3.9, 1.9% at 90DAS, respectively, over their respective control due to 50 mg S kg\(^{-1}\) soil (Figures 22-25).

In RH30, supplementation of 50 mg S kg\(^{-1}\) soil reduced the decrease in growth characteristics caused by 50 mg Cd kg\(^{-1}\) soil (Figures 22-25). The effect of supplementation of 100 mg S kg\(^{-1}\) soil in the alleviation of the effects of 50 mg Cd kg\(^{-1}\) soil was lesser than 50 mg S kg\(^{-1}\) soil. Furthermore, no ameliorative effect of 100 mg S kg\(^{-1}\) soil was observed in plants treated with 150 mg Cd kg\(^{-1}\) soil (Figures 22-25).

4.3.2 Photosynthetic characteristics

Significant reductions in photosynthetic characteristics (total chlorophyll content, carotenoids content, carbonic anhydrase activity, net photosynthetic rate and stomatal conductance) were noted with 50 and 150 mg Cd kg\(^{-1}\) soil in both the cultivars. However, the extent of decrease was higher in non-tolerant cultivar RH30 than Alankar (tolerant). The application of 50 mg S kg\(^{-1}\) soil alleviated and improved the photosynthetic characteristics of both the cultivars treated with 50 mg Cd kg\(^{-1}\) soil. The application of 100 mg S kg\(^{-1}\) soil proved ineffective in the alleviation of 150 mg Cd kg\(^{-1}\) soil-induced toxicity (Figures 26-28, 29-31).

Lesser decrease in photosynthetic characteristics was noted when plants were treated with 50 mg S kg\(^{-1}\) soil plus 50 mg Cd kg\(^{-1}\) soil in comparison to 50 mg Cd kg\(^{-1}\) soil alone. In Alankar, supplementation of 50 mg Cd kg\(^{-1}\) soil treated plants with 50 mg S kg\(^{-1}\) soil limited the decrease in total chlorophyll and carotenoids content and
Fig. 22. Effect of sulfur (S) application on root and shoot length (cm plant\(^{-1}\)) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 23. Effect of sulfur (S) application on leaf area (cm$^2$ plant$^{-1}$) and plant dry mass (g plant$^{-1}$) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 24. Per cent change in root and shoot length of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress with sulfur (S) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 25. Per cent change in leaf area and plant dry mass of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress with sulfur (S) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 26. Effect of sulfur (S) application on total chlorophyll content (mg g⁻¹ FW) and carotenoids content (mg g⁻¹ FW) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 27. Effect of sulfur (S) application on carbonic anhydrase activity (nmol CO₂ g⁻¹ FW) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 28. Effect of sulfur (S) application on net photosynthetic rate ($\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) and stomatal conductance (mmol m$^{-2}$ s$^{-1}$) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (*Brassica juncea* L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 29. Per cent change in total chlorophyll content and carotenoids content of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress with sulfur (S) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 30. Per cent change in carbonic anhydrase activity of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (Brassica juncea L.) under Cd stress with sulfur (S) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 31. Per cent change in net photosynthetic rate and stomatal conductance of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress with sulfur (S) doses at 30, 60 and 90 days after sowing (DAS).
stomatal conductance caused by 50mg Cd kg\(^{-1}\) soil alone. Finally, the decrease noted in total chlorophyll and carotenoids content and stomatal conductance was 7.9, 2.7 and 8.0% at 30DAS; 4.6, 2.7 and 7.1% at 60DAS and 3.3, 0.7 and 5.3% at 90DAS, respectively. Similar effect was noted for carbonic anhydrase activity and net photosynthetic rate, which was decreased by 1.7 and 0.6% at 30DAS and 1.5 and 0.4% at 90DAS, respectively. At 60DAS, the application of 50mg S kg\(^{-1}\) soil not only reduced the adverse effects of Cd (50mg Cd kg\(^{-1}\) soil) but overcome the effect and increased the carbonic anhydrase activity and net photosynthetic rate. The increase was found to be 0.3 and 1.5% at 60DAS (Figures 29-31).

In RH30, the application of 50mg S kg\(^{-1}\) soil only reduced the adverse effects of 50mg Cd kg\(^{-1}\) soil at all the growth stages. The decrease in total chlorophyll content, carotenoids content, carbonic anhydrase activity, net photosynthetic rate and stomatal conductance was 19.4, 21.8, 20.0, 14.6, 15.0% at 30DAS; 22.6, 18.8, 21.5, 13.6, 16.2% at 60DAS and 25.0, 25.4, 24.6, 15.9, 20.7% at 90DAS, respectively, over their respective control (Figures 26-31). Whereas, no ameliorative effect of 100mg S kg\(^{-1}\) soil was observed in plants treated with 150mg Cd kg\(^{-1}\) soil in both the cultivars (Figures 29-31).

4.3.3 Sulfur and nitrogen assimilation
Sulfur and nitrogen assimilation varied differentially under Cd stress. Cadmium stress increased the ATP-sulfurylase activity and S content, whereas, nitrate reductase activity and N content were decreased in both cultivars at all growth stages (Figures 32-33). ATP-sulfurylase activity and S content increased in Cd treated plants and the increases were higher in Alankar than RH30. In Alankar, the increase in ATP-sulfurylase activity and S content due to 50 and 150mg Cd kg\(^{-1}\) soil was 39.2, 44.5% and 21.9, 25.5% at 30DAS; 35.4, 45.5% and 21.3, 24.7% at 60DAS and 27.3, 36.1% and 15.0, 20.0% at 90DAS, respectively, in comparison to their respective control. This increase in RH30 was 22.6, 30.3% and 7.4, 12.0% at 30DAS; 23.4, 33.8% and 10.4, 13.3% at 60DAS and 20.0, 25.5% and 5.0, 10.1% at 90DAS, respectively, in comparison to their respective control (Figure 34).

The application of 50mg S kg\(^{-1}\) soil further increased the ATP-sulfurylase activity and S content of Cd treated plants of both the cultivars. Maximum significant increase was noted with 50mg S kg\(^{-1}\) soil and 50mg Cd kg\(^{-1}\) soil treated plants. The effect of 100mg S kg\(^{-1}\) soil plus 50mg Cd kg\(^{-1}\) soil was almost similar to 50mg S kg\(^{-1}\)
Fig. 32. Effect of sulfur (S) application on ATP-sulffurylase activity (μmol Pi mg⁻¹ protein min⁻¹) and sulfur content (%) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 33. Effect of sulfur (S) application on nitrate reductase activity (nmol NO₂ g⁻¹ FW h⁻¹) and nitrogen content (%) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are the mean of three replicates.
Fig. 34. Per cent change in ATP-sulfurylase activity and sulfur content of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress with sulfur (S) doses at 30, 60 and 90 days after sowing (DAS).
soil plus 50mg Cd kg$^{-1}$ soil. Further, no ameliorative effect of 100mg S kg$^{-1}$ soil was observed in plants treated with 150mg Cd kg$^{-1}$ soil.

Cadmium treatments significantly decreased NR activity and N content of both the cultivars but the decrease was greater in RH30 than Alankar. The activity of NR and N content of Alankar was decreased by 14.6, 11.0% and 27.2, 25.4% at 30DAS; 15.5, 10.6% and 29.9, 25.6% at 60DAS and 21.0, 17.2% and 34.4, 30.4% at 90DAS with 50 and 150mg Cd kg$^{-1}$ soil, respectively, in comparison to their respective control. Whereas, in RH30 the decrease in NR activity and N content was 26.4, 19.4% and 48.1, 38.6% at 30DAS; 34.7, 22.5% and 50.0, 39.4% at 60DAS and 37.7, 27.7% and 58.6, 45.7% at 90DAS with 50 and 150mg Cd kg$^{-1}$ soil, respectively, in comparison to their respective control (Figure 35).

The reductions in NR activity and N content due to Cd were lowered by S supplementation. Lesser decrease in NR activity and N content was observed when 50mg Cd kg$^{-1}$ soil treated plants were given 50mg S kg$^{-1}$ soil in comparison to 50mg Cd kg$^{-1}$ soil alone. In Alankar, the decrease in NR activity and N content due to 50mg Cd kg$^{-1}$ soil was limited to 1.8 and 1.7% at 30DAS; 1.1 and 1.4% at 60DAS and 8.8 and 10.1% at 90DAS with 50mg S kg$^{-1}$ soil. In RH30, these decreases were restricted to 15.1 and 7.9% at 30DAS, 18.6 and 13.2% at 60DAS and 20.9 and 16.5% at 90DAS with 50mg S kg$^{-1}$ soil (Figure 35).

4.3.4 Cadmium accumulation

Cadmium content in the root and leaf of both the cultivars increased with the increasing Cd concentration in soil. Non-tolerant cultivar (RH30) accumulated more Cd in root and leaf than tolerant cultivar Alankar (Figure 36). In comparison to root, the content of Cd was less in the leaf of both the cultivars due to S supplementation. The application of 50mg S kg$^{-1}$ soil maximally lowered the Cd content in the root and leaf of both the cultivars treated with 50mg Cd kg$^{-1}$ soil. The application of 100mg S kg$^{-1}$ soil also lowered the Cd content in root and leaf of 50mg Cd kg$^{-1}$ soil treated plants but the values obtained were almost similar to that of 50mg S kg$^{-1}$ soil plus 50mg Cd kg$^{-1}$ soil. Application of 100mg S kg$^{-1}$ soil did not decrease the Cd content in root and leaf of 150mg Cd kg$^{-1}$ soil treated plants.

Cadmium content in the root and leaf of Alankar was 110.8, 27.7 and 274.3, 70.0µg g$^{-1}$ DW at 30DAS; 141.3, 40.7 and 331.5, 95.8µg g$^{-1}$ DW at 60DAS and 184.4, 24.5 and 380.5, 50.1µg g$^{-1}$ DW at 90DAS due to 50 and 150mg Cd kg$^{-1}$ soil, respectively. The addition of 50mg S kg$^{-1}$ soil maximally lowered the Cd content in
Fig. 35. Per cent change in nitrate reductase activity and nitrogen content of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress with sulfur (S) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 36. Effect of sulfur (S) application on root Cd content and leaf Cd content (µg g⁻¹ DW) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
root and leaf of plants treated with 50mg Cd kg\(^{-1}\) soil and the values were 85.5 and 18.8\(\mu\)g g\(^{-1}\) DW at 30DAS; 106.7 and 25.4\(\mu\)g g\(^{-1}\) DW at 60DAS and 154.6 and 18.3\(\mu\)g g\(^{-1}\) DW at 90DAS, respectively (Figure 37).

In RH30, Cd content in the root and leaf was 135.8, 63.3 and 357.5, 138.1\(\mu\)g g\(^{-1}\) DW at 30DAS; 190.7, 92.6 and 488.3, 206.2\(\mu\)g g\(^{-1}\) DW at 60DAS and 286.1, 62.4 and 556.4, 132.3\(\mu\)g g\(^{-1}\) DW at 90DAS due to 50 and 150mg Cd kg\(^{-1}\) soil, respectively. The combined application of 50mg S kg\(^{-1}\) soil and 50mg Cd kg\(^{-1}\) soil lowered the Cd content in root and leaf and the values were 115.3 and 49.8\(\mu\)g g\(^{-1}\) DW at 30DAS; 161.6 and 72.1\(\mu\)g g\(^{-1}\) DW at 60DAS and 260.2 and 53.3\(\mu\)g g\(^{-1}\) DW at 90DAS, respectively (Figure 37).

4.3.5 Thiobarbituric acid reactive substances and hydrogen peroxide content
Significant Cd-dose dependent increase in TBARS and H\(_2\)O\(_2\) content was observed in both cultivars and the extent of increase was found greater in RH30 than Alankar. Maximum increase in TBARS and H\(_2\)O\(_2\) content was noted with 150mg Cd kg\(^{-1}\) soil in both the cultivars at all growth stages. Application of 50mg S kg\(^{-1}\) soil maximally lowered the TBARS and H\(_2\)O\(_2\) content of 50mg Cd kg\(^{-1}\) soil treated plants at all growth stages. The values obtained for TBARS and H\(_2\)O\(_2\) content with 100mg S kg\(^{-1}\) soil plus 50mg Cd kg\(^{-1}\) soil were almost similar to the values obtained in plants treated with 50mg S kg\(^{-1}\) soil plus 50mg Cd kg\(^{-1}\) soil. Application of 100mg S kg\(^{-1}\) soil did not lower the TBARS and H\(_2\)O\(_2\) content of 150mg Cd kg\(^{-1}\) soil treated plants (Figure 38).

In Alankar, the increase in TBARS and H\(_2\)O\(_2\) content was 146.3, 317.6% and 74.8, 142.6% at 30DAS; 168.1, 324.4% and 85.1, 145.2% at 60DAS and 173.3, 363.7% and 86.4, 150.5% at 90DAS due to 50 and 150mg Cd kg\(^{-1}\) soil, respectively, over their respective control. Supplementation of 50mg S kg\(^{-1}\) soil to plants treated with 50mg Cd kg\(^{-1}\) soil significantly lowered the TBARS and H\(_2\)O\(_2\) content by 41.4 and 28.4% at 30DAS; 34.9 and 25.9% at 60DAS and 62.4 and 33.1% at 90DAS, respectively, in Alankar (Figure 39).

The content of TBARS and H\(_2\)O\(_2\) in RH30 was increased by 224.8, 412.4% and 144.2, 197.7% at 30DAS; 339.4, 427.8% and 156.0, 206.5% at 60DAS and 403.5, 445.1% and 161.5, 228.0% at 90DAS due to 50 and 150mg Cd kg\(^{-1}\) soil, respectively, over their respective control. Whereas, supplementation with 50mg S kg\(^{-1}\) soil lowered the TBARS and H\(_2\)O\(_2\) content by 162.9 and 99.7% at 30DAS; 180.2 and
Fig. 37. Per cent change in root Cd content and leaf Cd content of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress with sulfur (S) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 38. Effect of sulfur (S) application on thiobarbituric acid reactive substances (nmol g\(^{-1}\) FW) and hydrogen peroxide content (\(\mu\)mol g\(^{-1}\) FW) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (\textit{Brassica juncea} L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 39. Per cent change in thiobarbituric acid reactive substances and hydrogen peroxide content of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress with sulfur (S) doses at 30, 60 and 90 days after sowing (DAS).
100.9% at 60DAS and 250.3 and 112.5% at 90DAS, respectively, in plants treated with 50mg Cd kg\(^{-1}\) soil (Figure 39).

**4.3.6 Components of antioxidant system**

**4.3.6.1 Enzymatic antioxidants**

The response of antioxidant enzymes was differential to Cd stress in both the cultivars. In general, S application (50mg S kg\(^{-1}\) soil) maximally increased the activities of catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) under Cd stress (50mg Cd kg\(^{-1}\) soil). Whereas, the application of 50mg S kg\(^{-1}\) soil lowered the increase in SOD activity caused by 50mg Cd kg\(^{-1}\) soil alone (Figures 40-41).

The activity of SOD increased with increasing Cd concentration in soil and extent of increase was greater in RH30 than Alankar. Sulfur supplementation lowered the Cd induced increase in SOD activity of both the cultivars. In Alankar, significant increase in SOD activity was 51.3, 71.6% at 30DAS; 43.4, 57.2% at 60DAS and 42.7, 33.9% at 90DAS due to 50 and 150mg Cd kg\(^{-1}\) soil, respectively, over the control. However, the activity of SOD was reduced to 24.5, 27.5 and 26.8% at 30, 60 and 90DAS, respectively, when the plants were given 50mg S kg\(^{-1}\) soil in the presence of 50mg Cd kg\(^{-1}\) soil (Figure 42).

The activity of SOD was increased by 71.4, 116.1% at 30DAS; 76.4, 100.0% at 60DAS and 50.3, 69.2% at 90DAS in RH30 due to 50 and 150mg Cd kg\(^{-1}\) soil, respectively, over the control. Its activity was restricted to 40.3, 33.8 and 28.5% at 30, 60 and 90DAS, respectively, with the addition of 50mg S kg\(^{-1}\) soil to 50mg Cd kg\(^{-1}\) soil-treated RH30 plants (Figure 42).

The activity of CAT was increased with the increasing Cd concentration in the soil at 30, 60 and 90DAS in Alankar. Whereas, in RH30, the increase in CAT activity was noted only at 30DAS. At later stages (60 and 90DAS), Cd significantly decreased the CAT activity. Sulfur supplementation further increased the Cd induced increase in CAT activity at all growth stages in tolerant cultivar Alankar. In RH30, addition of S lowered the decrease in CAT activity only at 60 and 90DAS, whereas, slight increase in its activity was noted at 30DAS.

Significant increase in CAT activity of Alankar due to 50 and 150mg Cd kg\(^{-1}\) soil was 33.3, 56.4% at 30DAS; 24.1, 42.1% at 60DAS and 23.6, 28.3% at 90DAS, respectively, in comparison to their respective control. Addition of 50mg S kg\(^{-1}\) soil
significantly increased the CAT activity by 40.2, 48.3 and 30.2% at 30, 60 and 90DAS, respectively; in Alankar treated with 50mg Cd kg⁻¹ soil (Figure 42).

In RH30, the increase in CAT activity was 10.7 and 13.9% at 30DAS, whereas, its activity was decreased by 20.4, 36.7% at 60DAS and 18.1, 35.3% at 90DAS due to 50 and 150mg Cd kg⁻¹ soil, respectively. S supplementation (50mg S kg⁻¹ soil) increased the CAT activity by 16.1% at 30DAS, while, at 60 and 90DAS its application only lowered the decrease in CAT activity by 9.2 and 10.6%, respectively, in plants treated with 50mg Cd kg⁻¹ soil (Figure 42).

Supplementation of 50mg S kg⁻¹ soil significantly and maximally increased the APX activity of both the cultivars treated with 50mg Cd kg⁻¹ soil at all growth stages.

The increase in APX activity of Alankar due to 50 and 150mg Cd kg⁻¹ soil was 70.4, 128.4% at 30DAS; 158.4, 193.1% at 60DAS and 109.7, 144.7% at 90DAS, respectively, over control. Further, the addition of 50mg S kg⁻¹ soil caused maximal significant increase of 200.7, 231.8 and 175.0% in APX activity at 30, 60 and 90DAS, respectively, of plants treated with 50mg Cd kg⁻¹ soil (Figure 43). However, in RH30 lesser increase was observed in plants treated with 50mg Cd kg⁻¹ soil or these plants supplemented with 50mg S kg⁻¹ soil (Figure 43).

Glutathione reductase activity of both the cultivars was increased under Cd stress. Maximal significant increase in GR activity was noted in both the cultivars treated with 50mg Cd kg⁻¹ soil. Treatment of 150mg Cd kg⁻¹ soil significantly decreased the GR activity in non-tolerant cultivar RH30. Addition of S (50mg S kg⁻¹ soil) further increased the GR activity of both the cultivars at all growth stages and extent of increase was higher in Alankar than RH30.

The increase in GR activity of Alankar due to 50 and 150mg Cd kg⁻¹ soil was 61.4, 40.6% at 30DAS; 55.3, 38.3% at 60DAS and 39.5, 26.3% at 90DAS, respectively, over the control. Treating these plants with 50mg S kg⁻¹ soil further increased the activity, which was maximum compared to other S treatments. Similar response of RH30 was noted with respect to Cd and S treatments (Figure 43).

4.3.6.2 Non-enzymatic antioxidants
Cadmium treatments significantly decreased the ascorbic acid content at all growth stages and greater decrease was found in RH30 than Alankar. Supplementation of Cd-treated plants with S lowered the Cd-induced reduction in ascorbic acid content of both the cultivars at all growth stages and 50mg S kg⁻¹ soil maximally lowered the
Fig. 40. Effect of sulfur (S) application on superoxide dismutase activity (Units mg\(^{-1}\) protein) and catalase activity (Units mg\(^{-1}\) protein) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90days after sowing (DAS). Data are mean of three replicates.
Fig. 41. Effect of sulfur (S) application on ascorbate peroxidase activity (Units mg\(^{-1}\) protein) and glutathione reductase activity (Units mg\(^{-1}\) protein) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (*Brassica juncea* L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 42. Per cent change in superoxide dismutases activity and catalase activity of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (Brassica juncea L.) under Cd stress with sulfur (S) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 43. Per cent change in ascorbate peroxidase activity and glutathione reductase activity of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress with sulfur (S) doses at 30, 60 and 90 days after sowing (DAS).
Ascorbic acid content. Treatment of 100mg S kg\(^{-1}\) soil to 50mg Cd kg\(^{-1}\) soil-treated plants also lowered the reductions but was less effective compared to 50mg S kg\(^{-1}\) soil. Application of 100mg S kg\(^{-1}\) soil did not improve the ascorbic acid content of 150mg Cd kg\(^{-1}\) soil treated plants (Figure 44).

Ascorbic acid content of Alankar was decreased by 27.9, 44.2\% at 30DAS; 24.2, 49.1\% at 60DAS and 27.2, 46.0\% at 90DAS with 50 and 150mg Cd kg\(^{-1}\) soil, respectively, over the control. Addition of S (50mg S kg\(^{-1}\) soil) maximally lowered the reduction in ascorbic acid content of 50mg Cd kg\(^{-1}\) soil treated plants by 11.9, 5.3 and 10.6\% at 30, 60 and 90DAS, respectively, over the control (Figure 45).

In RH30, the decrease in ascorbic acid content was 44.6, 61.2\% at 30DAS; 50.0, 64.0\% at 60DAS and 57.5, 68.7\% at 90DAS with 50 and 150mg Cd kg\(^{-1}\) soil, respectively, over the control. Supplementation of 50mg S kg\(^{-1}\) soil maximally lowered the reduction in ascorbic acid content of 50mg Cd kg\(^{-1}\) soil treated plants by 27.3, 25.8 and 30.3\% at 30, 60 and 90DAS, respectively, over the control (Figure 45).

Glutathione content increased in Cd-treated both the cultivars. The increase was higher in Alankar than RH30. Sulfur supplementation further increased the glutathione content of both the cultivars and maximum significant increase in its content was noted in plants treated with 50mg S kg\(^{-1}\) soil plus 50mg Cd kg\(^{-1}\) soil at all growth stages.

The increase in glutathione content of Alankar due to 50 and 150mg Cd kg\(^{-1}\) soil was 19.7, 24.4\% at 30DAS; 25.3, 36.0\% at 60DAS and 25.3, 31.9\% at 90DAS, respectively, over the control. Further maximal significant increase in its content occurred with the addition of 50mg S kg\(^{-1}\) soil to 50mg Cd kg\(^{-1}\) soil treated plants. RH30 also responded similarly to Cd and S treatments (Figure 45).

### 4.3.7 Yield characteristics

Cadmium treatments significantly decreased the yield characteristics (expressed as number of siliqua per plant, number of seeds per siliqua, 1000 seed weight and seed yield per plant) at harvest and the extent of decrease was greater in RH30 than Alankar. Sulfur supplementation (50mg S kg\(^{-1}\) soil) lowered the Cd (50mg Cd kg\(^{-1}\) soil)-induced reduction in yield characteristics of both the cultivars (Figure 46). The application of 100mg S kg\(^{-1}\) soil also lowered the reductions in yield characteristics caused by 50mg Cd kg\(^{-1}\) soil but was less effective than 50mg S kg\(^{-1}\) soil. Further, the application of 100mg S kg\(^{-1}\) soil did not alleviate the reduction in yield characteristics caused by 150mg Cd kg\(^{-1}\) soil.
Fig. 44. Effect of sulfur (S) application on ascorbic acid content (nmol g⁻¹ FW) and glutathione content (nmol g⁻¹ FW) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 45. Per cent change in ascorbic acid content and glutathione content of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress with sulfur (S) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 46. Effect of sulfur (S) application on number of siliqua per plant, number of seeds per siliqua, 1000 seed weight (g) and seed yield (g plant$^{-1}$) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (*Brassica juncea* L.) under Cd stress (T) at harvest i.e., 120days after sowing (DAS). Data are mean of three replicates.
Fig. 47. Per cent change in number of siliqua per plant, number of seeds per siliqua, 1000 seed weight and seed yield of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress with sulfur (S) doses at harvest i.e., 120 days after sowing (DAS).
Number of siliqua per plant, number of seeds per siliqua, 1000 seed weight and seed yield per plant of Alankar due to 50 and 150mg Cd kg\(^{-1}\) soil was decreased by 22.7, 22.8, 26.2, 21.6% and 35.7, 33.8, 42.4, 32.5%, respectively, over the control. Application of 50mg S kg\(^{-1}\) soil maximally lowered the 50mg Cd kg\(^{-1}\) soil-induced reductions in these characteristics, which were 8.3, 13.3, 12.7 and 10.8%, respectively, over the control. RH30 also responded similarly to Alankar for Cd and S treatments but the cultivar was less responsive (Figure 47).

4.4 Summary of Experiment 2

- Application of 50mg S kg\(^{-1}\) soil proved most effective in alleviating the effects of 50mg Cd kg\(^{-1}\) soil on growth, photosynthetic, biochemical and yield characteristics of Alankar and RH30 cultivars.
- Cd treatments up-regulated the S assimilation pathway and increased ATP-sulfurylase activity and S content. Whereas, N assimilation pathway was down-regulated and decrease in nitrate reductase activity and N content were observed. Sulfur supplementation (50mg S kg\(^{-1}\) soil) to plants treated with 50mg Cd kg\(^{-1}\) soil further increased the ATP-sulfurylase activity and S content and also lowered the decrease in nitrate reductase activity and N content.
- Application of 50mg S kg\(^{-1}\) soil decreased the Cd content maximally in root and leaf of both the cultivars treated with 50mg Cd kg\(^{-1}\) soil.
- Cd-induced accumulation of TBARS and H\(_2\)O\(_2\) was lowered by the application of 50mg S kg\(^{-1}\) soil.
- Responses of antioxidant enzyme activities were found differential in both the cultivars under Cd stress. S application (50mg S kg\(^{-1}\) soil) increased the activities of CAT, APX and GR of 50mg Cd kg\(^{-1}\) soil treated plants.
- Application of 50mg S kg\(^{-1}\) soil maximally lowered the 50mg Cd kg\(^{-1}\) soil-induced decrease in ascorbic acid content and increased the glutathione content in both the cultivars.
- Application of 50mg S kg\(^{-1}\) soil also lowered the Cd-induced decrease in yield characteristics of both the cultivars.
- The application of 50mg S kg\(^{-1}\) soil was found most effective in mitigating the 50mg Cd kg\(^{-1}\) soil induced reductions in growth, photosynthetic, biochemical and yield characteristics in Alankar, whereas, reductions in these characteristics were only lowered in RH30.
4.5 Experiment 3: Coordination of Sulfur and Nitrogen Application in the Alleviation of Cd-induced Effects on Cd tolerant and non-tolerant Mustard Cultivars

Experiment 3 was conducted on the basis of the findings of Experiments 2. The present Experiment was aimed to study the effectiveness of addition of N levels (40, 80 and 120mg N kg\(^{-1}\) soil) to the alleviation potential of 50mg S kg\(^{-1}\) soil on 50mg Cd kg\(^{-1}\) soil-induced reductions in Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars.

Results of this Experiment are presented in the following section.

4.5.1 Growth characteristics

Application of different N levels (40, 80 and 120mg N kg\(^{-1}\) soil) further enhanced the alleviation effect of 50mg S kg\(^{-1}\) soil in Alankar and RH30 cultivars treated with 50mg Cd kg\(^{-1}\) soil. Among the N levels used, the addition of 80mg N kg\(^{-1}\) soil to 50mg S kg\(^{-1}\) soil nullified the adverse effects of 50mg Cd kg\(^{-1}\) soil and also maximally increased the growth characteristics of Alankar. Other N levels proved less effective (Figures 48-49).

The treatment 80mg N kg\(^{-1}\) soil plus 50mg S kg\(^{-1}\) soil resulted in maximal increase in shoot length, root length, leaf area and plant dry mass by 6.5, 5.7, 5.6, 4.1% at 30DAS; 7.3, 6.9, 5.9, 5.3% at 60DAS and 5.2, 5.6, 4.8, 4.8% at 90DAS of 50mg Cd kg\(^{-1}\) soil treated Alankar plants, respectively, over their respective control (Figures 50-51).

Similar effect of the treatment (80mg N kg\(^{-1}\) soil plus 50mg S kg\(^{-1}\) soil) was observed in RH30. Cd-induced reductions in growth characteristics were lowered by all N levels applied in combination with 50mg S kg\(^{-1}\) soil. However, application of 80mg N kg\(^{-1}\) soil with 50mg S kg\(^{-1}\) soil maximally lowered the reductions in shoot length, root length, leaf area and plant dry mass and these were limited to 8.4, 8.4, 8.2, 8.5% at 30DAS; 6.6, 6.4, 8.6, 6.4% at 60DAS and 7.0, 7.7, 9.7, 6.1% at 90DAS of 50mg Cd kg\(^{-1}\) soil treated plants, respectively, over their respective control (Figures 50-51).

4.5.2 Photosynthetic characteristics

The reductions caused by 50mg Cd kg\(^{-1}\) soil in photosynthetic characteristics (total chlorophyll content, carotenoids content, carbonic anhydrase activity, net photosynthetic rate and stomatal conductance) were reversed by the application of 50mg S kg\(^{-1}\) soil at all the growth stages in both the cultivars. Ameliorative effect of
Fig. 48. Effect of sulfur (S) and nitrogen (N) application on root and shoot length (cm plant⁻¹) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 49. Effect of sulfur (S) and nitrogen (N) application on leaf area (cm² plant⁻¹) and plant dry mass (g plant⁻¹) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 50. Per cent change in root and shoot length of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress with sulfur (S) and nitrogen (N) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 51. Per cent change in leaf area and plant dry mass of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (Brassica juncea L.) under Cd stress due to sulfur (S) and nitrogen (N) doses at 30, 60 and 90 days after sowing (DAS).
different levels of N along with 50mg S kg\(^{-1}\) soil was almost similar as observed for
growth characteristics. Among the N levels used, 80mg N kg\(^{-1}\) soil along with 50mg S
kg\(^{-1}\) soil appeared as the most effective combination for reversing the Cd-induced
(50mg Cd kg\(^{-1}\) soil) reductions in photosynthetic characteristics (Figures 52-54).

Application of 50mg S kg\(^{-1}\) soil together with 80mg N kg\(^{-1}\) soil increased the
total chlorophyll and carotenoids content in Alankar by 3.2 and 3.1% at 30DAS and
5.3 and 4.8% at 60DAS but at 90DAS, this combination only lowered the reductions,
which limited to 3.9 and 2.4% in plants treated with 50mg Cd kg\(^{-1}\) soil, respectively,
over their respective control. The activity of carbonic anhydrase, net photosynthetic
rate and stomatal conductance in Alankar were increased by 4.6, 6.2, 4.0% at 30DAS;
5.7, 6.6, 5.7% at 60DAS and 3.5, 4.2, 2.3% at 90DAS, respectively, over their
respective control due to 80mg N kg\(^{-1}\) soil plus 50mg S kg\(^{-1}\) soil of 50mg Cd kg\(^{-1}\) soil-
treated plants (Figures 55-57).

Application of 50mg S kg\(^{-1}\) soil together with 80mg N kg\(^{-1}\) soil substantially increased
the ATP-sulfurylase activity and S content in both the cultivars treated with 50mg Cd
kg\(^{-1}\) soil at all growth stages. Among the combinations, 80mg N kg\(^{-1}\) soil and 50mg S
kg\(^{-1}\) soil given to 50mg Cd kg\(^{-1}\) soil treated plants resulted in maximum ATP-
sulfurylase activity and S content at all growth stages. Whereas, the same combination
maximally lowered the reductions in nitrate reductase activity and N content in both
the cultivars at all growth stages. Application of other N levels with 50mg S kg\(^{-1}\) soil
proved less effective (Figures 58-59).

In Alankar, application of 80mg N kg\(^{-1}\) soil and 50mg S kg\(^{-1}\) soil maximally
increased ATP-sulfurylase activity and S content by 82.1, 54.4% at 30DAS; 85.5,
55.5% at 60DAS and 72.6, 42.1% at 90DAS of plants treated with 50mg Cd kg\(^{-1}\) soil.
In RH30, ATP-sulfurylase activity and S content were increased by 60.0, 29.9% at
30DAS; 61.6% at 60DAS and 47.5, 21.4% at 90DAS over their respective control
(Figure 60).
Fig. 52. Effect of sulfur (S) and nitrogen (N) application on total chlorophyll content (mg g$^{-1}$ FW) and carotenoids content (mg g$^{-1}$ FW) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (*Brassica juncea* L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 53. Effect of sulfur (S) and nitrogen (N) application on carbonic anhydrase activity (mmol CO₂ g⁻¹ FW) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 54. Effect of sulfur (S) and nitrogen (N) application on net photosynthetic rate (μmol CO₂ m⁻² s⁻¹) and stomatal conductance (mmol m⁻² s⁻¹) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 55. Per cent change in total chlorophyll content and carotenoids content of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (Brassica juncea L.) under Cd stress due to sulfur (S) and nitrogen (N) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 56. Per cent change in carbonic anhydrase activity of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (Brassica juncea L.) under Cd stress due to sulfur (S) and nitrogen (N) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 57. Per cent change in net photosynthetic rate and stomatal conductance of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (Brassica juncea L.) under Cd stress due to sulfur (S) and nitrogen (N) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 58. Effect of sulfur (S) and nitrogen (N) application on ATP-sulfurylase activity (μmol Pi mg⁻¹ protein min⁻¹) and sulfur content (%) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 59. Effect of sulfur (S) and nitrogen (N) application on nitrate reductase activity (nmol NO₂ g⁻¹ FW h⁻¹) and nitrogen content (%) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 60. Per cent change in ATP-sulphurylase activity and sulfur content of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress due to sulfur (S) and nitrogen (N) doses at 30, 60 and 90 days after sowing (DAS).
The reductions in nitrate reductase activity and N content were reversed by S supplementation in Cd stressed plants. Lesser decrease in nitrate reductase activity and N content was observed when plants were given 50mg S kg\(^{-1}\) soil plus 50mg Cd kg\(^{-1}\) soil in comparison to 50mg Cd kg\(^{-1}\) soil alone. In Alankar, application of 80mg N kg\(^{-1}\) soil and 50mg S kg\(^{-1}\) soil maximally increased the nitrate reductase activity and N content by 5.2, 3.8% at 30DAS; 5.2, 4.2% at 60DAS but at 90DAS, this combination only lowered the reductions by 2.8, 5.4% in plants treated with 50mg Cd kg\(^{-1}\) soil, respectively, over their respective control. In RH30, 50mg S kg\(^{-1}\) soil alone and in combination with 80mg N kg\(^{-1}\) soil lowered the reductions in nitrate reductase activity and N content of 50mg Cd kg\(^{-1}\) soil treated plants (Figure 61).

### 4.5.4 Cadmium accumulation

In both the cultivars, increased accumulation of Cd in root and leaf due to 50mg Cd kg\(^{-1}\) soil was lowered by the application of 50mg S kg\(^{-1}\) soil alone and in combination with 40, 80 and 120mg N kg\(^{-1}\) soil at all growth stages. Application of 40mg N kg\(^{-1}\) soil plus 50mg S kg\(^{-1}\) soil also lowered the Cd accumulation in root and leaf in both the cultivars but to a lesser extent than 80mg N kg\(^{-1}\) soil. The effect of 120mg N kg\(^{-1}\) soil was equal to 80mg N kg\(^{-1}\) soil on Cd accumulation. In Alankar, combined application of 50mg S kg\(^{-1}\) soil and 80mg N kg\(^{-1}\) soil to 50mg Cd kg\(^{-1}\) soil treated plants maximally lowered the Cd content in root and leaf, which was 73.1, 14.6µg g\(^{-1}\) DW at 30DAS; 88.5, 20.6µg g\(^{-1}\) DW at 60DAS and 145.6, 15.4µg g\(^{-1}\) DW at 90DAS, respectively. Cadmium content in the root and leaf of RH30 with the same combination was 104.3, 42.9µg g\(^{-1}\) DW at 30DAS; 145.0, 61.9µg g\(^{-1}\) DW at 60DAS and 244.3, 50.3µg g\(^{-1}\) DW at 90DAS, respectively (Figures 62-63).

### 4.5.5 Thiobarbituric acid reactive substances and hydrogen peroxide content

Application of 50mg S kg\(^{-1}\) soil lowered the TBARS and H\(_2\)O\(_2\) content of 50mg Cd kg\(^{-1}\) soil treated plants at all growth stages. Supplementation of 50mg S kg\(^{-1}\) soil with different levels of N (40, 80 and 120mg N kg\(^{-1}\) soil) again lowered the contents of TBARS and H\(_2\)O\(_2\) of both the cultivars treated with 50mg Cd kg\(^{-1}\) soil at all growth stages. Combined application of 40mg N kg\(^{-1}\) soil plus 50mg S kg\(^{-1}\) soil also lowered the TBARS and H\(_2\)O\(_2\) content in both the cultivars but to a lesser extent than 80mg N kg\(^{-1}\) soil. Whereas, the effect of 120mg N kg\(^{-1}\) soil plus 50mg S kg\(^{-1}\) soil was equal to 80mg N kg\(^{-1}\) soil plus 50mg S kg\(^{-1}\) soil on TBARS and H\(_2\)O\(_2\) content. In particular, combined application of 80mg N kg\(^{-1}\) soil plus 50mg S kg\(^{-1}\) soil maximally lowered
Fig. 61. Per cent change in nitrate reductase activity and nitrogen content of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress due to sulfur (S) and nitrogen (N) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 62. Effect of sulfur (S) and nitrogen (N) application on root Cd content and leaf Cd content (µg g⁻¹ DW) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 63. Per cent change in root Cd content and leaf Cd content of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress due to sulfur (S) and nitrogen (N) doses at 30, 60 and 90 days after sowing (DAS).
the TBARS and \( \text{H}_2\text{O}_2 \) contents by 27.1, 15.2\% at 30DAS; 21.8, 11.6\% at 60DAS and 45.2, 26.5\% at 90DAS, respectively, over their respective control, in 50mg Cd kg\(^{-1}\) soil treated Alankar plants (Figures 64-65).

In RH30, combined application of 80mg N kg\(^{-1}\) soil plus 50mg S kg\(^{-1}\) soil lowered the TBARS and \( \text{H}_2\text{O}_2 \) contents by 97.1, 80.2\% at 30DAS; 90.5, 77.6\% at 60DAS and 149.2, 97.6\% at 90DAS, respectively, over their respective control, in plants treated with 50mg Cd kg\(^{-1}\) soil (Figure 65).

4.5.6 Components of antioxidant system

4.5.6.1 Enzymatic antioxidants

The increase in SOD activity in plants treated with 50mg Cd kg\(^{-1}\) soil was lowered by the application of 50mg S kg\(^{-1}\) soil alone and in combination with 40, 80 and 120mg N kg\(^{-1}\) soil in both the cultivars at all growth stages. Application of 40mg N kg\(^{-1}\) soil plus 50mg S kg\(^{-1}\) soil also lowered the SOD activity in both the cultivars but to a lesser extent than 80mg N kg\(^{-1}\) soil. Whereas, the effect of 120mg N kg\(^{-1}\) soil plus 50mg S kg\(^{-1}\) soil was equal to 80mg N kg\(^{-1}\) soil plus 50mg S kg\(^{-1}\) soil on SOD activity when treated with 50mg Cd kg\(^{-1}\) soil. In Alankar, combination of 50mg S kg\(^{-1}\) soil and 80mg N kg\(^{-1}\) soil to 50mg Cd kg\(^{-1}\) soil fed plants maximally lowered the SOD activity by 20.6, 19.5 and 25.3\% at 30, 60 and 90DAS, respectively, over their respective control. Same combination lowered the SOD activity of RH30 plants treated with 50mg Cd kg\(^{-1}\) soil by 36.7, 30.8 and 28.3\% at 30, 60 and 90DAS, respectively, over their respective control (Figure 66, 68).

In Alankar, the increase in CAT activity due to 50mg Cd kg\(^{-1}\) soil was further increased by the application of 50mg S kg\(^{-1}\) soil alone and in combination with 40, 80 and 120mg N kg\(^{-1}\) soil at all growth stages. Combined application of 50mg S kg\(^{-1}\) soil and 80mg N kg\(^{-1}\) soil to 50mg Cd kg\(^{-1}\) soil treated plants maximally increased the CAT activity by 52.2, 58.9, 34.3\% at 30, 60 and 90DAS, respectively, over their respective control. In RH30, the application of 50mg S kg\(^{-1}\) soil and 80mg N kg\(^{-1}\) soil increased the CAT activity by 23.9\% at 30DAS, while, at 60 and 90DAS its application only limited the decrease in CAT activity to 1.02 and 2.30\%, respectively, in plants treated with 50mg Cd kg\(^{-1}\) soil (Figure 66, 68).

The increase in APX activity due to 50mg Cd kg\(^{-1}\) soil was further increased by the application of 50mg S kg\(^{-1}\) soil alone and in combination with 40, 80 and 120mg N kg\(^{-1}\) soil in both the cultivars at all growth stages. In Alankar, combined application of 50mg S kg\(^{-1}\) soil and 80mg N kg\(^{-1}\) soil to 50mg Cd kg\(^{-1}\) soil treated
Fig. 64. Effect of sulfur (S) and nitrogen (N) application on thiobarbituric acid reactive substances (nmol g⁻¹ FW) and hydrogen peroxide content (µmol g⁻¹ FW) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (*Brassica juncea* L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 65. Per cent change in thiobarbituric acid reactive substances and hydrogen peroxide content of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress due to sulfur (S) and nitrogen (N) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 66. Effect of sulfur (S) and nitrogen (N) application on superoxide dismutases activity (Units mg⁻¹ protein) and catalase activity (Units mg⁻¹ protein) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 67. Effect of sulfur (S) and nitrogen (N) application on ascorbate peroxidase activity (Units mg\(^{-1}\) protein) and glutathione reductase activity (Units mg\(^{-1}\) protein) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 68. Per cent change in superoxide dismutases activity and catalase activity of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress due to sulfur (S) and nitrogen (N) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 69. Per cent change in ascorbate peroxidase activity and glutathione reductase activity of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (Brassica juncea L.) under Cd stress due to sulfur (S) and nitrogen (N) doses at 30, 60 and 90days after sowing (DAS).
plants maximally increased the APX activity by 297.3, 301.2, 209.1% at 30, 60 and 90DAS, respectively, over their respective control. Whereas, the increase in APX activity of RH30 by this combination was 135.0, 175.5 and 120.4% at 30, 60 and 90DAS, respectively, over their respective control (Figure 67,69).

The increase in GR activity was same as observed in the case of APX activity. In Alankar, combined application of 50mg S kg⁻¹ soil and 80mg N kg⁻¹ soil to 50mg Cd kg⁻¹ soil treated plants maximally increased the GR activity by 150.8, 157.3, 92.1% at 30, 60 and 90DAS, respectively, over their respective control. Whereas, the increase in APX activity of RH30 by this combination was 85.5, 94.0, 47.8% at 30, 60 and 90DAS, respectively, over their respective control (Figure 67,69).

4.5.6.2 Non-enzymatic antioxidants

The decrease in ascorbic acid content due to 50mg Cd kg⁻¹ soil was lowered by the application of 50mg S kg⁻¹ soil alone and in combination with 40, 80 and 120mg N kg⁻¹ soil in both the cultivars at all growth stages. Combined application of 40mg N kg⁻¹ soil plus 50mg S kg⁻¹ soil also lowered the decrease in ascorbic acid content of both the cultivars but to a lesser extent than 80mg N kg⁻¹ soil. The effect of 120mg N kg⁻¹ soil plus 50mg S kg⁻¹ soil was equal to 80mg N kg⁻¹ soil plus 50mg S kg⁻¹ soil on ascorbic acid content when treated with 50mg Cd kg⁻¹ soil. In Alankar, combined application of 50mg S kg⁻¹ soil and 80mg N kg⁻¹ soil to 50mg Cd kg⁻¹ soil treated plants lowered the decrease in ascorbic acid content by 5.4, 4.3, 7.5% at 30, 60 and 90DAS, respectively, over their respective control. Whereas, in RH30, this combination lowered the decrease in ascorbate content by 15.2, 10.1, 18.1% at 30, 60 and 90DAS, respectively, over their respective control (Figures 70-71).

The increase in glutathione content due to 50mg Cd kg⁻¹ soil was further increased by the application of 50mg S kg⁻¹ soil alone and in combination with 40, 80 and 120mg N kg⁻¹ soil in both the cultivars at all growth stages. Combination of 40mg N kg⁻¹ soil plus 50mg S kg⁻¹ soil also increased the glutathione content of both the cultivars but to a lesser extent than 80mg N kg⁻¹ soil plus 50mg S kg⁻¹ soil. Whereas, the effect of combined application of 120mg N kg⁻¹ soil plus 50mg S kg⁻¹ soil was almost equal to 80mg N kg⁻¹ soil plus 50mg S kg⁻¹ soil on glutathione content when treated with 50mg Cd kg⁻¹ soil. In Alankar, combined application of 50mg S kg⁻¹ soil and 80mg N kg⁻¹ soil to 50mg Cd kg⁻¹ soil fed plants maximally increased the glutathione content by 58.8, 60.7, 47.3% at 30, 60 and 90DAS, respectively, over their respective control. While, the same combination increased the glutathione
Fig. 7. Effect of sulfur (S) and nitrogen (N) application on ascorbic acid content (nmol g<sup>-1</sup> FW) and glutathione content (nmol g<sup>-1</sup> FW) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at 30, 60 and 90 days after sowing (DAS). Data are mean of three replicates.
Fig. 71. Per cent change in ascorbic acid content and glutathione content of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress due to sulfur (S) and nitrogen (N) doses at 30, 60 and 90 days after sowing (DAS).
Fig. 72. Effect of sulfur (S) and nitrogen (N) application on number of siliqua per plant, number of seeds per silicua, 1000 seed weight (g) and seed yield (g plant\(^{-1}\)) of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars (C) of mustard (Brassica juncea L.) under Cd stress (T) at harvest i.e., 120days after sowing (DAS). Data are mean of three replicates.
Fig. 73. Per cent change in number of siliqua per plant, number of seeds per siliqua, 1000 seed weight and seed yield of Alankar (Cd tolerant) and RH30 (Cd non-tolerant) cultivars of mustard (*Brassica juncea* L.) under Cd stress due to sulfur (S) and nitrogen (N) doses at harvest i.e., 120 days after sowing (DAS).
content in RH30 by 37.1, 40.5, 33.8% at 30, 60 and 90DAS, respectively, over their respective control (Figures 70-71).

4.5.7 Yield characteristics

The reductions in yield characteristics (number of silique per plant, number of seeds per silique, 1000 seed weight and seed yield per plant) due to 50mg Cd kg$^{-1}$ soil were lowered by the application of 50mg S kg$^{-1}$ soil alone and in combination with 40, 80 and 120mg N kg$^{-1}$ soil in both the tolerant (Alankar) and non-tolerant (RH30) cultivars. A combination of 50mg S kg$^{-1}$ soil and 80mg N kg$^{-1}$ soil maximally lowered the reductions in yield characteristics. In Alankar, lesser decrease in number of silique per plant, number of seeds per silique, 1000 seed weight and seed yield per plant due to 50mg S kg$^{-1}$ soil and 80mg N kg$^{-1}$ soil was 1.9, 5.0, 4.8 and 1.9%, respectively, over their respective control. Whereas, in RH30, the decrease remained to 19.2, 23.0, 21.0 and 20.4% due to 80mg N kg$^{-1}$ soil plus 50mg S kg$^{-1}$ soil (Figures 72-73).

4.6 Summary of Experiment 3

- Coordination of sulfur and nitrogen proved effective in mustard cultivation. A package of these nutrients not only helped in increasing crop productivity but also exhibited its potential in the alleviation of Cd effects.

- Among the N levels applied, 80mg N kg$^{-1}$ soil strengthened the effectiveness of 50mg S kg$^{-1}$ soil in the alleviation of 50mg Cd kg$^{-1}$ soil induced reductions in both the cultivars at all growth stages. The effectiveness of the combined application of 50mg S kg$^{-1}$ soil plus 80mg N kg$^{-1}$ soil was different in tolerant and non-tolerant cultivars. In Alankar (tolerant cultivar), the Cd-induced reductions in growth, photosynthetic, biochemical and yield characteristics were completely overcome, whereas, the reductions in these characteristics were only lowered by this combination in RH30 (non-tolerant cultivar).

- Application of 50mg S kg$^{-1}$ soil and 80mg N kg$^{-1}$ soil to 50mg Cd kg$^{-1}$ soil treated plants increased the ATP-sulfurylase activity and S content and also improved the NR activity and N content to a greater extent.

- Cadmium-induced increase in root and leaf Cd content was lowered by the combined application of 50mg S kg$^{-1}$ soil and 80mg N kg$^{-1}$ soil to 50mg Cd kg$^{-1}$ soil treated plants in both the cultivars at all growth stages. The extent of decrease in Cd content was more in Alankar than RH30.

- The accumulation of TBARS and H$_2$O$_2$ due to 50mg Cd kg$^{-1}$ soil was lowered by the combined application of 50mg S kg$^{-1}$ soil and 80mg N kg$^{-1}$ soil in both
the cultivars at all growth stages. The extent of decrease in TBARS and H$_2$O$_2$ content was greater in Alankar than RH30.

- The activities of antioxidant enzymes (catalase, ascorbate peroxidase and glutathione reductase) were maximally increased by the combined application of 50mg S kg$^{-1}$ soil and 80mg N kg$^{-1}$ soil in 50mg Cd kg$^{-1}$ soil treated plants. Whereas, activity of SOD was lowered by this combination of sulfur and nitrogen in both the cultivars at all growth stages.

- Combined application of 50mg S kg$^{-1}$ soil and 80mg N kg$^{-1}$ soil lowered the Cd-induced decrease in ascorbic acid content. This combination, however, increased the glutathione content in both the cultivars at all sampling stages.

- Application of 50mg S kg$^{-1}$ soil plus 80mg N kg$^{-1}$ soil reversed the reduction in yield characteristics of Alankar and values reached on par to control. Whereas, this combination only lowered the reduction in yield characteristics of RH30.