# CHAPTER 8
# ELEMENTAL AND PHYTOCHEMICAL ANALYSIS

## 8.1 Elemental analysis

There was generally a decrease in the contents of elements *viz* carbon, nitrogen, sodium, magnesium, sulfur, phosphorous, potassium and calcium in the seedlings/ leaves with the increasing concentrations of IMI in the substratum. Seed soaking with EBR resulted in increasing the elemental contents in plants grown in IMI amended substratum.

### Carbon content (C)

As compared to the control (38.03%), C content in 10 days old *B.* juncea seedlings decreased to 26.67% in the seedlings grown in Petri-plates containing 250 mg $L^{-1}$ IMI. However, seed soaking with 100 nM EBR resulted in increase in C content, which was enhanced to 37.22% in seedlings grown under IMI toxicity (Table 8.1.1, Fig. 8.1.1).

**Table 8.1.1 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on carbon content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| **Treatments** | | **Carbon content (% dr. wt.)** | | |
|---|---|---|---|---|
| **IMI (mg $L^{-1}$)** | **EBR (nM $L^{-1}$)** | | | |
| 0 | 0 | 38.03±1.18 | | |
| 0 | 100 | 40.58±2.03 | | |
| 150 | 0 | 33.25±2.58 | | |
| 150 | 100 | 45.22±2.89 | | |
| 200 | 0 | 31.77±2.22 | | |
| 200 | 100 | 41.26±4.49 | | |
| 250 | 0 | 26.67±1.93 | | |
| 250 | 100 | 37.22±3.80 | | |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 8.97** | | |
| $F_{EBR}$ | | 55.95*** | | |
| $F_{IMI \times EBR}$ | | 3.28* | | |
| HSD | | 7.99 | | |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | **$\beta_{IMI}$** | **$\beta_{EBR}$** | |
| C (%) = 36.18 - 0.0250 IMI + 0.0863 EBR | | - 0.3887 | 0.7173 | 0.8160*** |
| *, ** and *** indicate significant at p<0.05, p<0.01 and p<0.001 respectively. r = multiple correlation coefficient. | | | | |

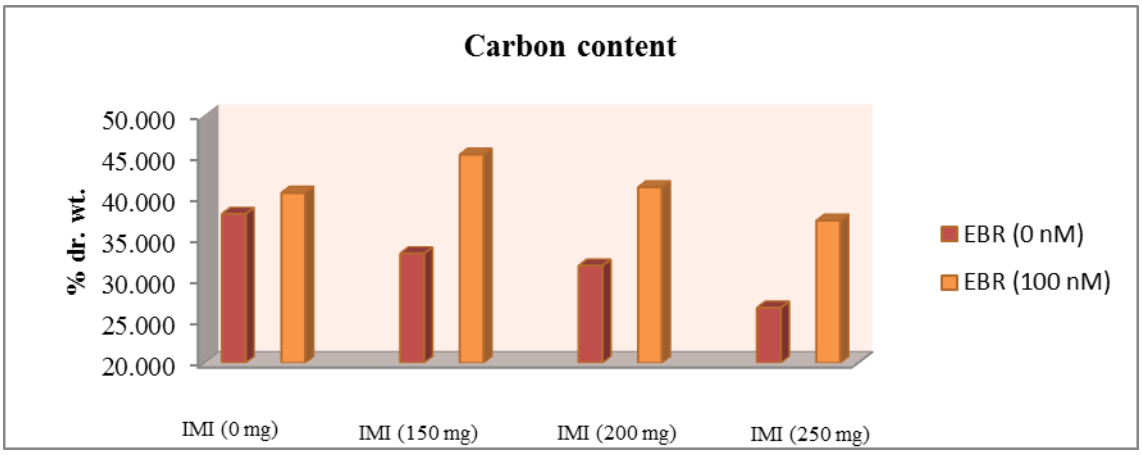


**Fig. 8.1.1 Effect of seed soaking with EBR on carbon content in *B. juncea* seedlings grown under IMI toxicity.**

In 30 days old *B. juncea* plants, C content was maximum reduced to 26.14% in plants grown in soils amended with 350 mg IMI $Kg^{-1}$ soil, when compared to the control plants (35.12%). But increase in the C content (28.96%) was observed in plants raised from 100 nM EBR treated seeds and grown in pots containing 350 mg IMI $Kg^{-1}$ soil (Table 8.1.2, Fig. 8.1.2).

**Table 8.1.2 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on carbon content in the leaves of *B. juncea* L. plants grown in imidacloprid (IMI) amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Carbon content (% dr. wt.) | | |
|---|---|---|---|---|
| IMI (mg $Kg^{-1}$) | EBR (nM $L^{-1}$) | 30 DAS | 60 DAS | 90 DAS |
| 0 | 0 | 35.12±1.47 | 39.30±0.20 | 35.49±0.32 |
| 0 | 100 | 35.98±3.30 | 35.35±0.65 | 38.70±0.68 |
| 250 | 0 | 31.92±0.30 | 34.78±0.49 | 33.10±0.60 |
| 250 | 100 | 35.93±1.12 | 38.85±0.85 | 42.85±0.71 |
| 300 | 0 | 29.42±1.61 | 32.71±0.62 | 27.74±0.86 |
| 300 | 100 | 32.79±0.36 | 37.93±1.94 | 40.30±0.63 |
| 350 | 0 | 26.14±0.54 | 29.02±0.57 | 23.55±0.79 |
| 350 | 100 | 28.96±2.37 | 35.38±0.55 | 28.17±1.96 |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 25.46*** | 40.74*** | 206.9*** |
| $F_{EBR}$ | | 15.85** | 65.50*** | 386.0*** |
| $F_{IMI \times EBR}$ | | 0.95 | 41.89*** | 32.40*** |
| HSD | | 4.81 | 2.50 | 2.65 |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| C (%) = 34.91 - 0.0189 IMI + 0.0277 EBR | | - 0.6967 | 0.3777 | 0.7925*** |
| C (%) = 36.44 - 0.0111 IMI + 0.0292 EBR | | - 0.4551 | 0.4461 | 0.6373** |
| C (%) = 34.97 - 0.0022 IMI + 0.0754 EBR | | - 0.4664 | 0.5870 | 0.7498*** |

** and *** indicate significant at p<0.01 and p<0.001. r = multiple correlation coefficient. DAS = days after sowing.

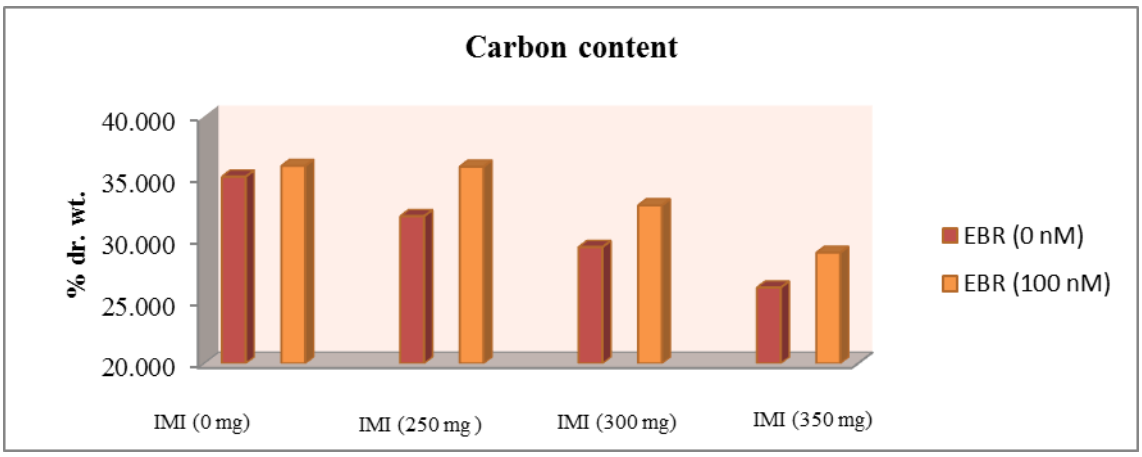


**Fig. 8.1.2 Effect of seed soaking with EBR on carbon content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.**

In plants (60 DAS), grown in soils mixed with 350 mg IMI $Kg^{-1}$ soil, the C content decreased to 29.02% as compared to 39.30% in control plants. Further increase in the C content (35.38%) was observed in plants germinated from EBR (100 nM) soaked seeds and grown under IMI toxicity (Table 8.1.2, Fig. 8.1.3).

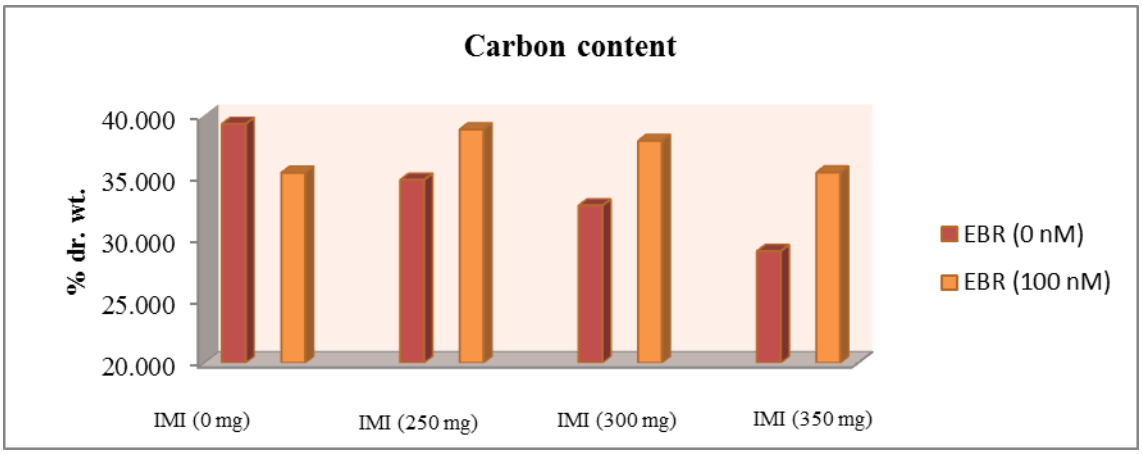


**Fig. 8.1.3 Effect of seed soaking with EBR on carbon content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.**

In the leaves of 90 days old plants of *B. juncea* plants grown under IMI toxicity (350 mg IMI $Kg^{-1}$ soil), the C content was reduced to 23.55% when compared to 35.49% in control plants. Seed soaking with 100 nM EBR before sowing resulted in increase in C content to 28.17% in plants grown in soils amended with 350 mg IMI $Kg^{-1}$ soil (Table 8.1.2, Fig. 8.1.4).

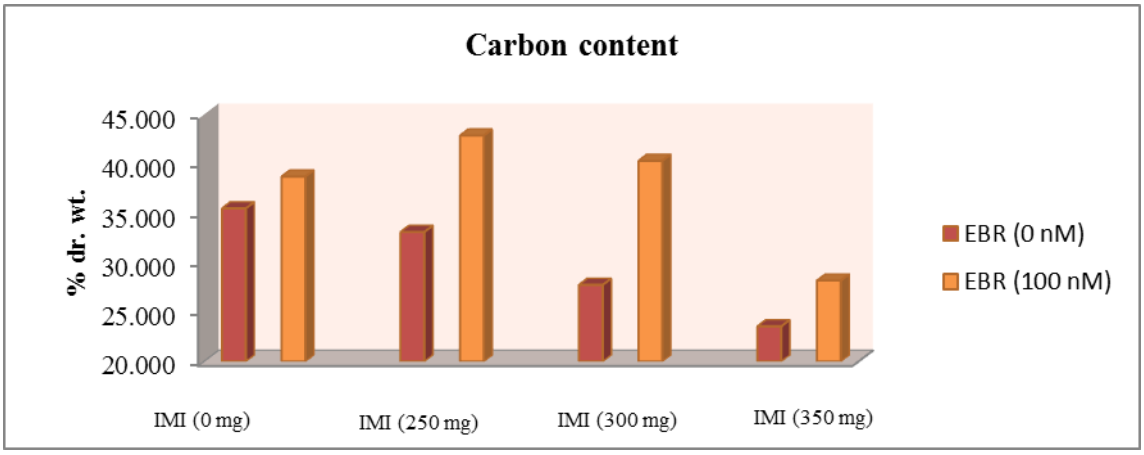


**Fig. 8.1.4 Effect of seed soaking with EBR on carbon content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.**

Two-way ANOVA and Tukey's HSD showed significant difference for C contents in the seedlings and leaves of *B. juncea* plants raised from EBR treated seeds and grown under IMI toxicity. MLR analysis revealed that IMI application reduced the C content (indicated by negative $\beta_{IMI}$), whereas EBR seed soaking enhanced the C content (indicated by positive $\beta_{EBR}$) as shown in table 8.1.1 and 8.1.2. High correlation was obtained in between simulated and experimental values using ANN model (Fig. 8.1.5)

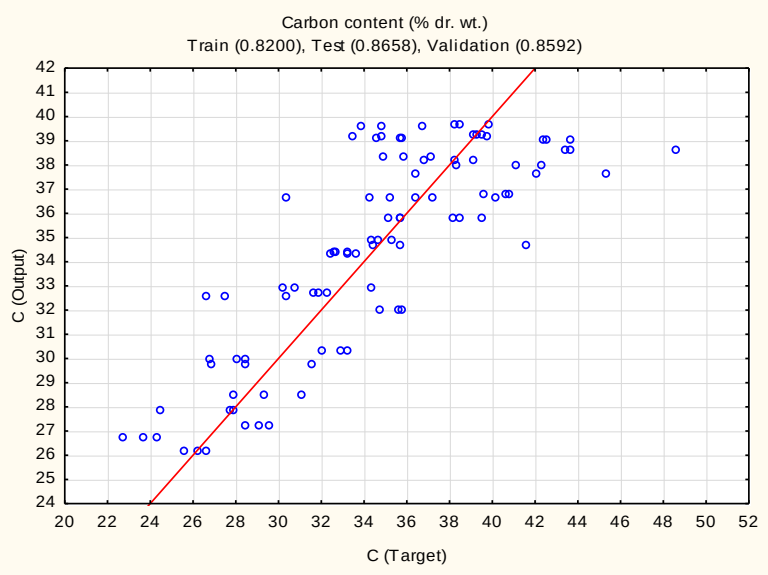


**Fig. 8.1.5 Correlation between target (experimental) and output (simulated) carbon contents using ANN model ($p<0.001$).**

**Nitrogen content (N)**

It was observed that maximum reduction in N content (3.59%) occurred in 10 days old seedlings of *B. juncea* grown in Petri-plates containing 250 mg IMI $L^{-1}$, as compared to the control (6.26%). Seed soaking with 100 nM EBR before germination in IMI containing Petri-plates resulted in increase of N content to 5.27 % in the seedlings (Table 8.1.3, Fig. 8.1.6).

**Table 8.1.3 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on nitrogen content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Nitrogen content (% dr. wt.) | | |
|---|---|---|---|---|
| IMI (mg $L^{-1}$) | EBR (nM $L^{-1}$) | | | |
| 0 | 0 | 6.26±0.53 | | |
| 0 | 100 | 8.38±1.16 | | |
| 150 | 0 | 5.99±0.67 | | |
| 150 | 100 | 8.10±0.61 | | |
| 200 | 0 | 4.96±0.41 | | |
| 200 | 100 | 7.67±0.68 | | |
| 250 | 0 | 3.59±0.66 | | |
| 250 | 100 | 5.27±1.39 | | |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 14.75*** | | |
| $F_{EBR}$ | | 40.23*** | | |
| $F_{IMI \times EBR}$ | | 0.39 | | |
| HSD | | 2.34 | | |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| N (%) = 6.65 - 0.0097 IMI + 0.0215 EBR | | - 0.5301 | 0.6291 | 0.8226*** |
| *** indicates significant at p<0.001. r = multiple correlation coefficient. | | | | |

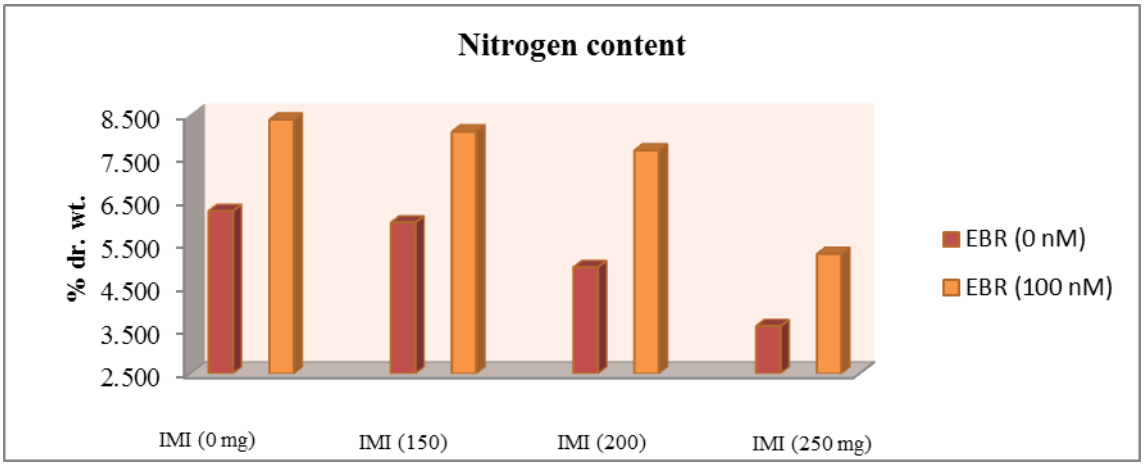


**Fig. 8.1.6 Effect of seed soaking with EBR on nitrogen content in *B. juncea* seedlings grown under IMI toxicity.**

In the leaves of 30 days old *B. juncea* plants, the maximum decrease in N content was 1.96% observed at 350 mg IMI $Kg^{-1}$ soil as compared to the control plants (4.83%). Moreover, seed soaking with 100 nM EBR enhanced the N content to 2.15% in the leaves of plants grown in soils amended with 350 mg IMI $Kg^{-1}$ soil (Table 8.1.4, Fig. 8.1.7).

**Table 8.1.4 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on nitrogen content in the leaves of *B. juncea* L. plants grown in imidacloprid (IMI) amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Nitrogen content (% dr. wt.) | | |
|---|---|---|---|---|
| IMI (mg $Kg^{-1}$) | EBR (nM $L^{-1}$) | 30 DAS | 60 DAS | 90 DAS |
| 0 | 0 | 4.83±0.14 | 5.32±0.39 | 1.72±0.04 |
| 0 | 100 | 4.97±0.17 | 5.53±0.37 | 2.08±0.20 |
| 250 | 0 | 3.84±0.15 | 3.54±0.19 | 1.55±0.03 |
| 250 | 100 | 4.03±0.18 | 4.17±0.17 | 2.42±0.17 |
| 300 | 0 | 2.88±0.09 | 2.53±0.08 | 1.16±0.08 |
| 300 | 100 | 3.58±0.15 | 3.95±0.24 | 2.24±0.06 |
| 350 | 0 | 1.96±0.27 | 1.84±0.17 | 0.73±0.10 |
| 350 | 100 | 2.15±0.06 | 2.39±0.25 | 1.61±0.10 |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 315.9*** | 175.4*** | 61.35*** |
| $F_{EBR}$ | | 20.37*** | 46.01*** | 291.7*** |
| $F_{IMI \times EBR}$ | | 3.80* | 6.05** | 10.96*** |
| HSD | | 0.46 | 0.72 | 0.32 |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| N (%) = 4.94 - 0.0069 IMI + 0.0030 EBR | | - 0.8814 | 0.1430 | 0.8930*** |
| N (%) = 5.22 - 0.0085 IMI + 0.0071 EBR | | - 0.8927 | 0.2754 | 0.9342*** |
| N (%) = 1.61 - 0.0014 IMI + 0.0080 EBR | | - 0.3584 | 0.7456 | 0.8273*** |

*, ** and *** indicate significant at p<0.05, p<0.01 and p<0.001 respectively. r = multiple correlation coefficient. DAS = days after sowing.

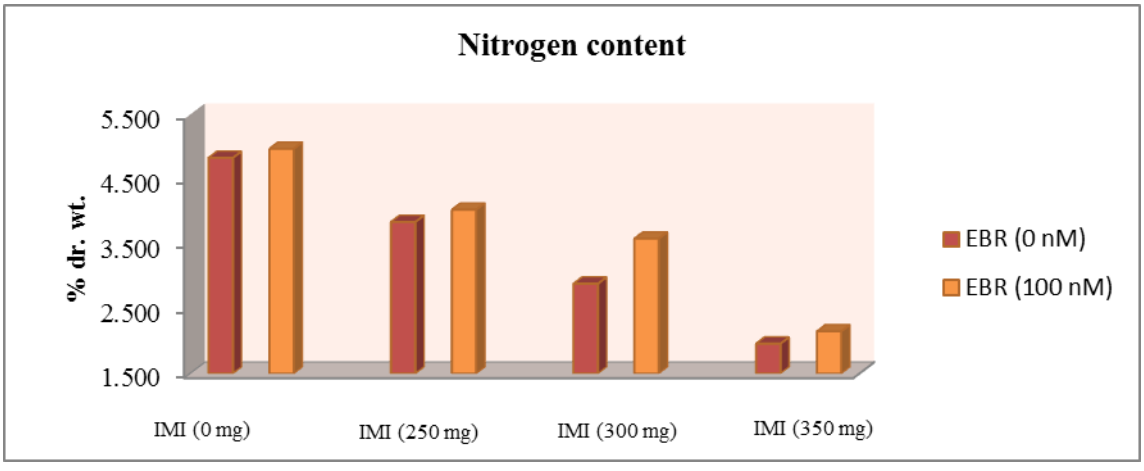


**Fig. 8.1.7 Effect of seed soaking with EBR on nitrogen content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.**

In 60 days old plants grown in soils amended with 350 mg IMI $Kg^{-1}$ soil, N content was maximum decreased to 1.84% when compared to nitrogen content in the leaves of control plants (5.32%). Additionally, seed soaking with 100 nM EBR resulted in increase in N content to 2.39% under IMI toxicity (Table 8.1.4, Fig. 8.1.8).

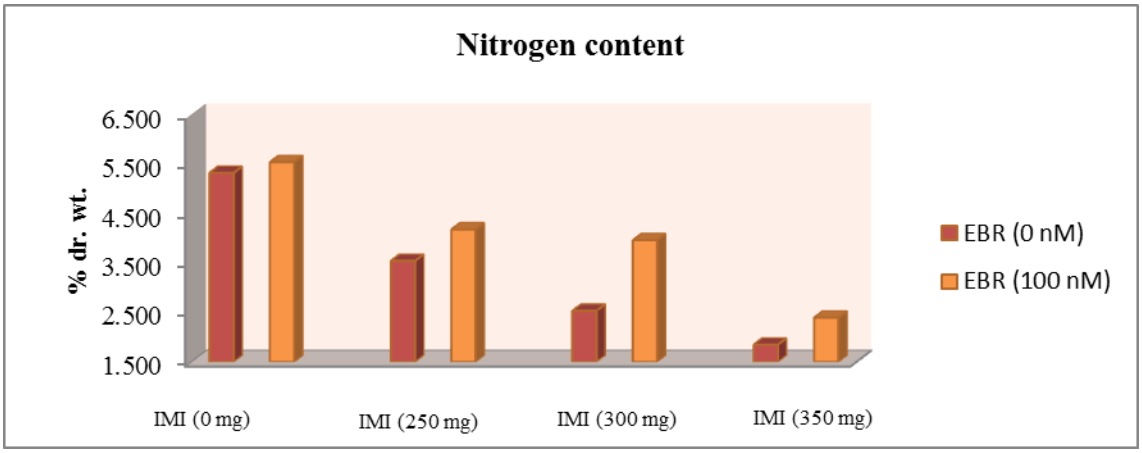


**Fig. 8.1.8 Effect of seed soaking with EBR on nitrogen content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.**

Content of N in the leaves of 90 days old plants grown under IMI toxicity (350 mg IMI $Kg^{-1}$ soil) was maximum reduced to 0.73% in comparison to N content of control plants (1.72%). But, in plants raised from 100 nM EBR soaked seeds, N content was enhanced to 1.61% under IMI toxicity (Table 8.1.4, Fig. 8.1.9).

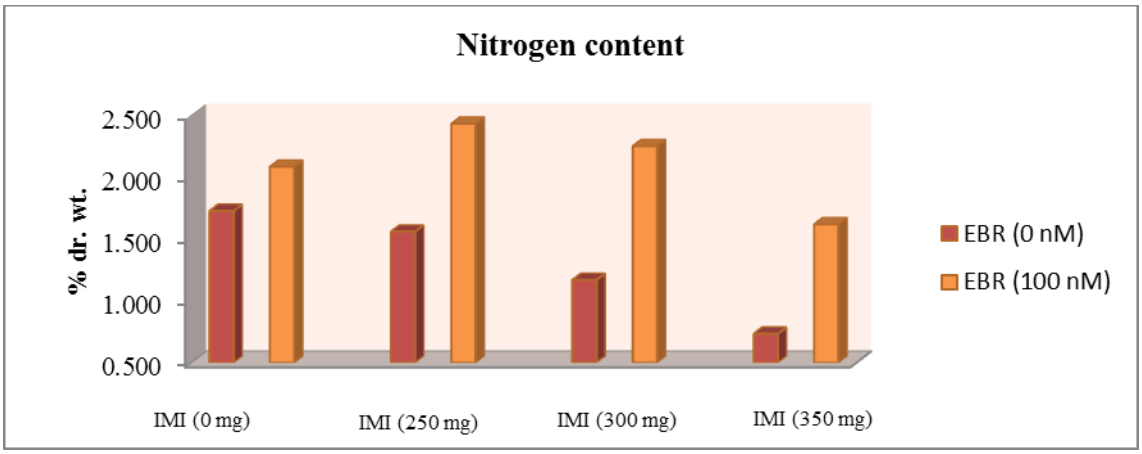


**Fig. 8.1.9 Effect of seed soaking with EBR on nitrogen content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.**

Data analysis using two-way ANOVA and Tukey's HSD showed significant differences for contents of N in plants raised from EBR treated seeds and grown in IMI amended soils, as compared to control plants (Table 5.2.1). MLR analysis also revealed that IMI concentration decreased N content (indicated by negative $\beta_{IMI}$ values) whereas seed soaking with EBR increased the N content in plants (indicated by positive $\beta_{EBR}$ values) (Tables 8.1.3 and 8.1.4). Analysis of data using ANN showed that model can simulate the data at very high level of significance ($p<0.001$) as shown in Fig. 8.1.10.

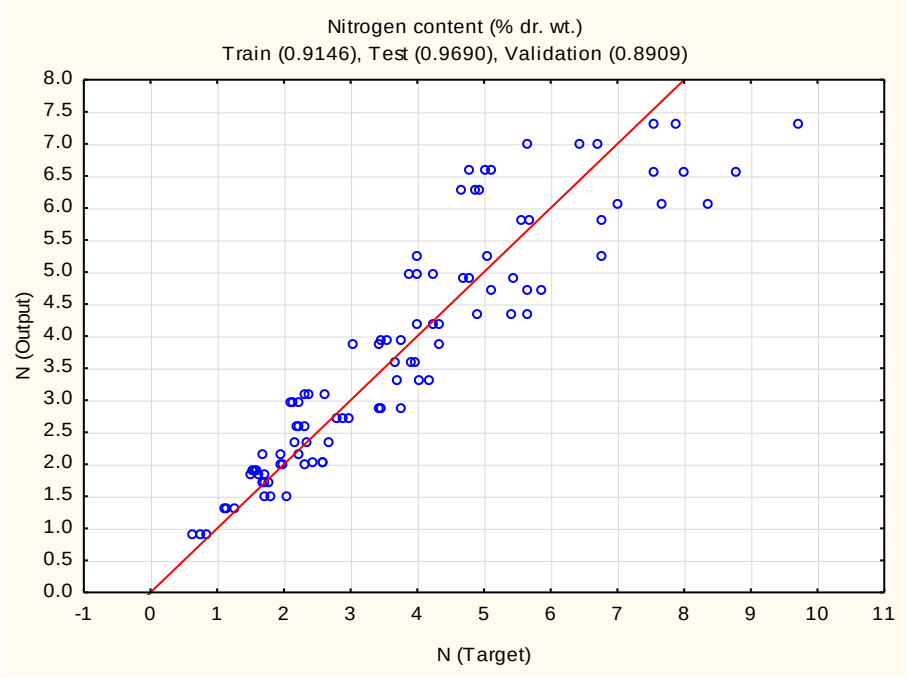


**Fig. 8.1.10 Correlation between target (experimental) and output (simulated) nitrogen contents using ANN model ($p<0.001$).**

## Sodium content (Na)

As compared to the 10 days old control seedlings of *B. juncea* (0.14%), Na content was maximum decreased to 0.04% in the seedlings grown in 250 mg IMI $L^{-1}$ solutions. However, seed soaking with 100 nM EBR resulted in increase in Na content to 0.13% in seedlings grown under IMI toxicity (Table 8.1.5, Fig. 8.1.11).

**Table 8.1.5 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on sodium content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Sodium content (% dr. wt.) |
|---|---|---|
| IMI (mg $L^{-1}$) | EBR (nM $L^{-1}$) | |
| 0 | 0 | 0.14±0.031 |
| 0 | 100 | 0.15±0.015 |
| 150 | 0 | 0.07±0.006 |
| 150 | 100 | 0.15±0.025 |
| 200 | 0 | 0.05±0.012 |
| 200 | 100 | 0.13±0.015 |
| 250 | 0 | 0.04±0.006 |
| 250 | 100 | 0.13±0.035 |
| **Two-way ANOVA** | | |
| $F_{IMI}$ | | 10.54*** |
| $F_{EBR}$ | | 57.01*** |
| $F_{IMI \times EBR}$ | | 5.11* |
| HSD | | 0.06 |
| **Multiple linear regression** | | |

| MLR equation | β-regression coefficients | | r |
|---|---|---|---|
| | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| Na (%) = 0.12 - 0.0003 IMI + 0.0006 EBR | - 0.5072 | 0.6893 | 0.8557*** |

* and *** indicate significant at p<0.05 and p<0.001. r = multiple correlation coefficient.

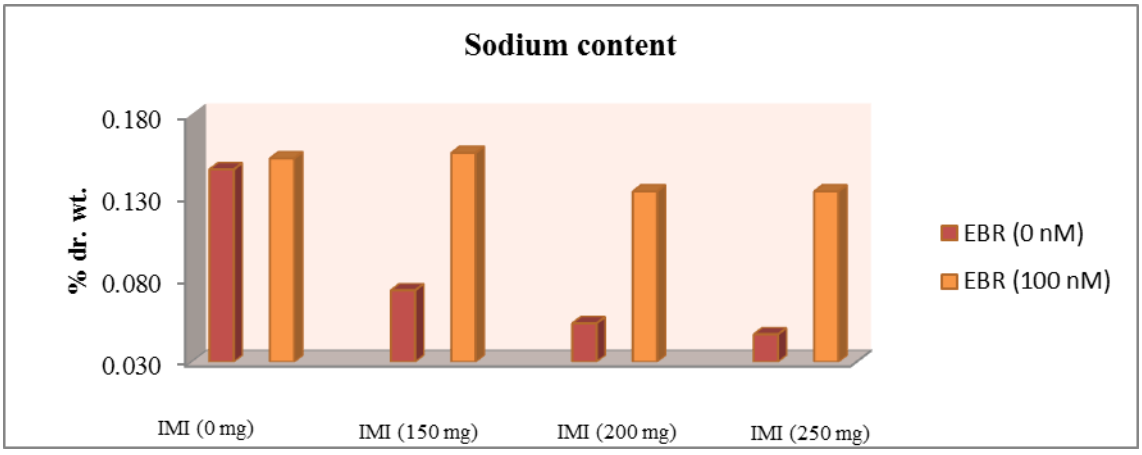


**Fig. 8.1.11 Effect of seed soaking with EBR on sodium content in *B. juncea* seedlings grown under IMI toxicity.**

In the leaves of 30 days old control plants, Na content was 0.33% which was reduced to 0.20% in the plants grown in soils mixed with 350 mg IMI $Kg^{-1}$ soil. In plants raised from EBR (100 nM) treated seeds and grown under IMI toxicity (350 mg IMI $Kg^{-1}$ soil), Na content was further enhanced to 0.23% (Table 8.1.6, Fig. 8.1.12).

**Table 8.1.6 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on sodium content in the leaves of *B. juncea* L. plants grown in imidacloprid (IMI) amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Sodium content (% dr. wt.) | | |
|---|---|---|---|---|
| IMI (mg $Kg^{-1}$) | EBR (nM $L^{-1}$) | 30 DAS | 60 DAS | 90 DAS |
| 0 | 0 | 0.33±0.03 | 0.45±0.04 | 0.13±0.006 |
| 0 | 100 | 0.35±0.02 | 0.49±0.02 | 0.14±0.015 |
| 250 | 0 | 0.25±0.01 | 0.26±0.03 | 0.09±0.015 |
| 250 | 100 | 0.46±0.02 | 0.33±0.03 | 0.13±0.015 |
| 300 | 0 | 0.23±0.02 | 0.21±0.03 | 0.06±0.015 |
| 300 | 100 | 0.33±0.02 | 0.25±0.04 | 0.11±0.021 |
| 350 | 0 | 0.20±0.03 | 0.12±0.02 | 0.03±0.006 |
| 350 | 100 | 0.23±0.04 | 0.16±0.02 | 0.08±0.025 |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 30.37*** | 95.29*** | 27.93*** |
| $F_{EBR}$ | | 59.94*** | 10.43** | 29.82*** |
| $F_{IMI \times EBR}$ | | 13.88*** | 0.19 | 1.77 |
| HSD | | 0.07 | 0.08 | 0.04 |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| Na (%) = 0.31 - 0.0003 IMI + 0.0009 EBR | | - 0.4324 | 0.5359 | 0.6886*** |
| Na (%) = 0.46 - 0.0009 IMI + 0.0005 EBR | | - 0.9339 | 0.1825 | 0.9515*** |
| Na (%) = 0.13 - 0.0002 IMI + 0.0004 EBR | | - 0.7206 | 0.4701 | 0.8606*** |

** and *** indicate significant at p<0.01 and p<0.001. r = multiple correlation coefficient. DAS = days after sowing.

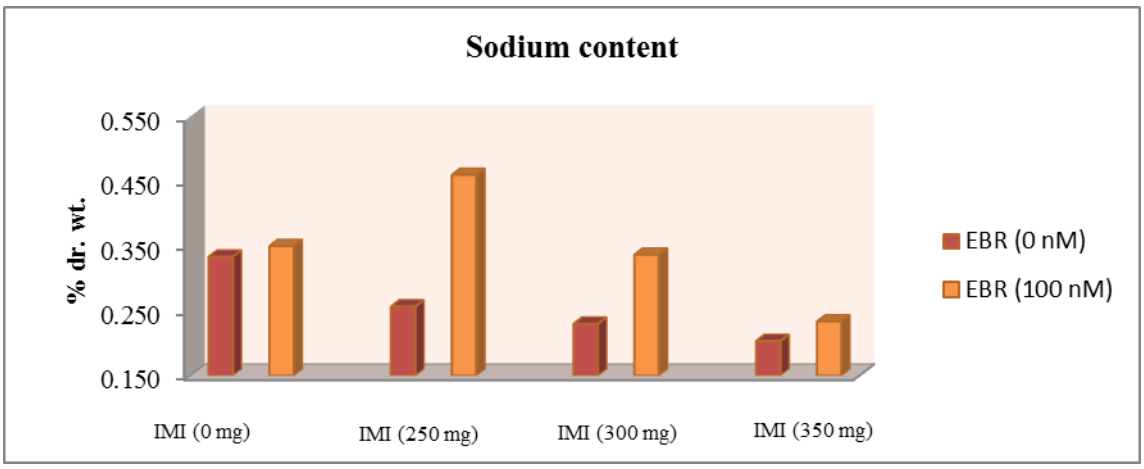


**Fig. 8.1.12 Effect of seed soaking with EBR on sodium content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.**

Content of Na in the leaves of 60 days old plants grown under IMI toxicity (350 mg IMI $Kg^{-1}$ soil) was reduced to 0.12% as compared to 0.45% in the leaves of control plants. EBR (100 nM) seed soaking resulted in increase of Na content under IMI toxicity, which was enhanced to 0.16% (Table 8.1.6, Fig. 8.1.13).

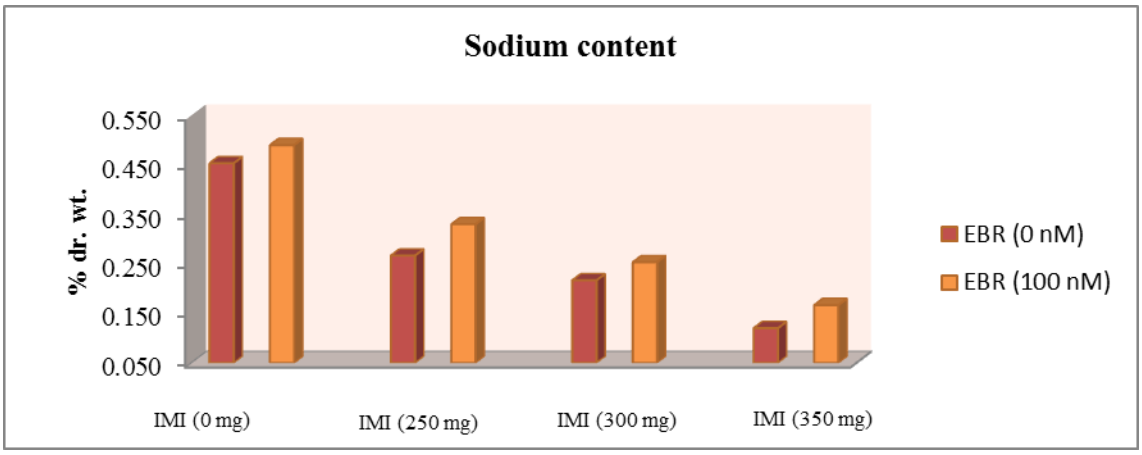


**Fig. 8.1.13 Effect of seed soaking with EBR on sodium content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.**

Minimum Na content in the leaves of 90 days old plants grown in IMI amended soils ((350 mg IMI $Kg^{-1}$ soil) was 0.03% as compared to Na content in the leaves of 90 days old control plants (0.13%). Seed soaking with 100 nM EBR further enhanced Na content to 0.08% under IMI toxicity (Table 8.1.6, Fig. 8.1.14).

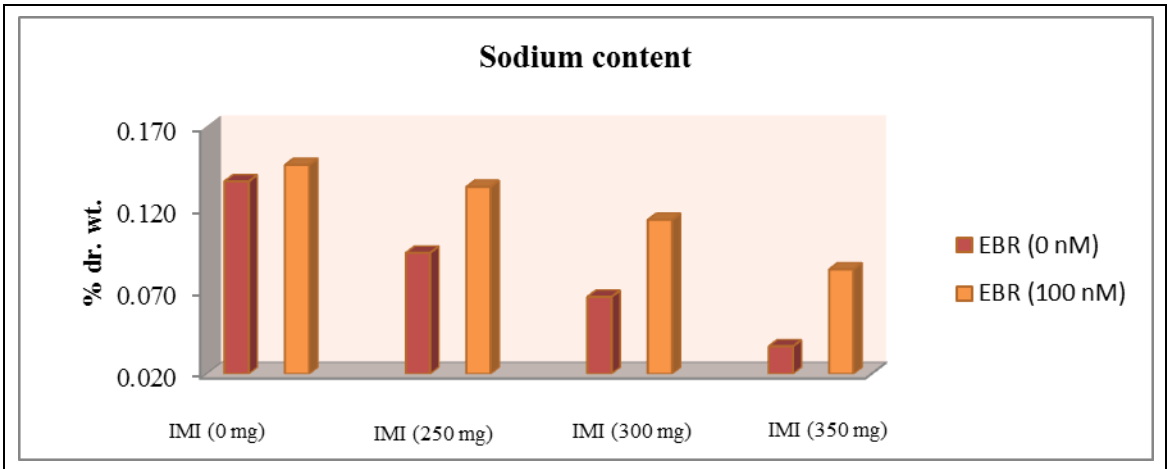


**Fig. 8.1.14 Effect of seed soaking with EBR on sodium content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.**

Significant differences in Na contents in the seedlings/leaves of *B.* juncea plants were observed using two-way ANOVA and Tukey's HSD. MLR revealed the role of IMI and EBR in Na content of leaves. IMI application reduced the Na content as shown by negative $\beta_{IMI}$ values, whereas seed treatment with EBR enhanced the Na content in the leaves of *B. juncea* leaves as indicated by positive $\beta_{EBR}$ values (Table 8.1.5 and 8.1.6). High correlation was observed between target and output values after analyzing the data using ANN (Fig. 8.1.15)

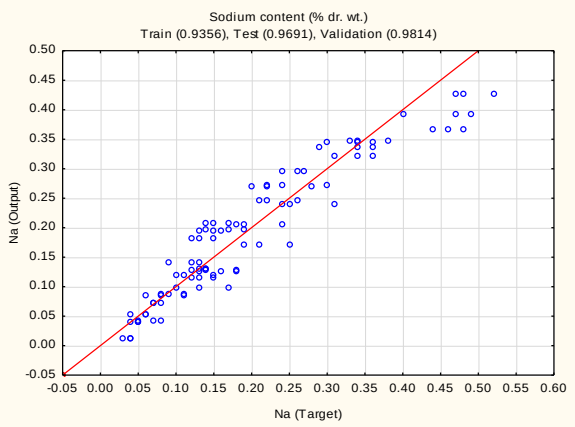


**Fig. 8.1.15 Correlation between target (experimental) and output (simulated) sodium contents using ANN model (p<0.001).**

## Magnesium content (Mg)

In 10 days old control seedlings, Mg content was 0.29% which was decreased to 0.12% in seedlings grown in Petri-plates supplemented with IMI solution (250 mg IMI $L^{-1}$). Seed soaking with 100 nM EBR before germination in Petri-plates containing 250 mg IMI $L^{-1}$ resulted in increase of Mg content to 0.18% in the seedlings (Table 8.1.7, Fig. 8.1.16).

**Table 8.1.7 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on magnesium content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Magnesium content (% dr. wt.) |
|---|---|---|
| IMI (mg $L^{-1}$) | EBR (nM $L^{-1}$) | |
| 0 | 0 | 0.29±0.04 |
| 0 | 100 | 0.31±0.04 |
| 150 | 0 | 0.23±0.03 |
| 150 | 100 | 0.34±0.02 |
| 200 | 0 | 0.20±0.04 |
| 200 | 100 | 0.30±0.03 |
| 250 | 0 | 0.12±0.03 |
| 250 | 100 | 0.18±0.04 |
| **Two-way ANOVA** | | |
| $F_{IMI}$ | | 17.84*** |
| $F_{EBR}$ | | 19.02*** |
| $F_{IMI \times EBR}$ | | 1.53 |
| HSD | | 0.11 |
| **Multiple linear regression** | | |

| MLR equation | β-regression coefficients | | r |
|---|---|---|---|
| | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| Mg (%) = 0.28 - 0.0005 IMI + 0.0007 EBR | - 0.5895 | 0.4519 | 0.7427*** |

*** indicates significant at p<0.001. r = multiple correlation coefficient.

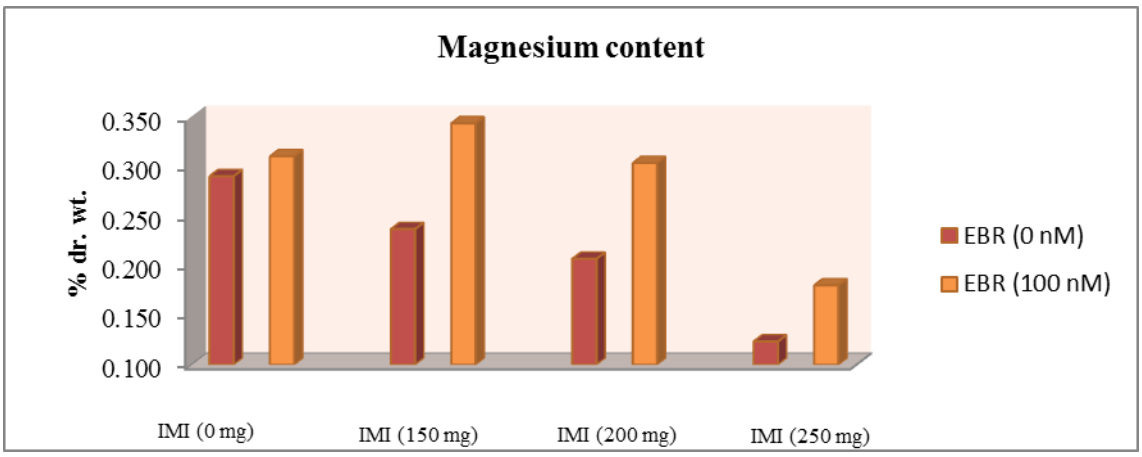


**Fig. 8.1.16 Effect of seed soaking with EBR on magnesium content in *B. juncea* seedlings grown under IMI toxicity.**

In the leaves of 30 days old plants grown in IMI amended soils (350 mg IMI $Kg^{-1}$ soil), Mg content was reduced to 0.20% as compared to Mg content of control plants (0.50%). However, in plants raised from EBR (100 nM) soaked seeds, Mg content was increased to 0.37% under IMI stress (Table 8.1.8, Fig. 8.1.17).

**Table 8.1.8 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on magnesium content in the leaves of *B. juncea* L. plants grown in imidacloprid (IMI) amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Magnesium content (% dr. wt.) | | |
|---|---|---|---|---|
| IMI (mg $Kg^{-1}$) | EBR (nM $L^{-1}$) | 30 DAS | 60 DAS | 90 DAS |
| 0 | 0 | 0.50±0.02 | 0.65±0.02 | 0.42±0.03 |
| 0 | 100 | 0.51±0.03 | 0.64±0.03 | 0.43±0.03 |
| 250 | 0 | 0.32±0.04 | 0.38±0.01 | 0.33±0.01 |
| 250 | 100 | 0.54±0.05 | 0.44±0.02 | 0.37±0.01 |
| 300 | 0 | 0.26±0.02 | 0.27±0.01 | 0.27±0.02 |
| 300 | 100 | 0.44±0.05 | 0.43±0.03 | 0.30±0.02 |
| 350 | 0 | 0.20±0.02 | 0.22±0.03 | 0.18±0.03 |
| 350 | 100 | 0.37±0.03 | 0.38±0.02 | 0.24±0.03 |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 36.10*** | 202.9*** | 74.01*** |
| $F_{EBR}$ | | 87.75*** | 71.59*** | 11.98** |
| $F_{IMI \times EBR}$ | | 8.45** | 0.92 | 0.92 |
| HSD | | 0.11 | 0.07 | 0.07 |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| Mg (%) = 0.44 - 0.0006 IMI + 0.0015 EBR | | - 0.6228 | 0.6079 | 0.8703*** |
| Mg (%) = 0.60 - 0.0009 IMI + 0.0009 EBR | | - 0.9074 | 0.3114 | 0.9594*** |
| Mg (%) = 0.42 - 0.0006 IMI + 0.0004 EBR | | - 0.8616 | 0.2177 | 0.8886*** |
| ** and *** indicate significant at p<0.01 and p<0.001. r = multiple correlation coefficient. DAS = days after sowing. | | | | |

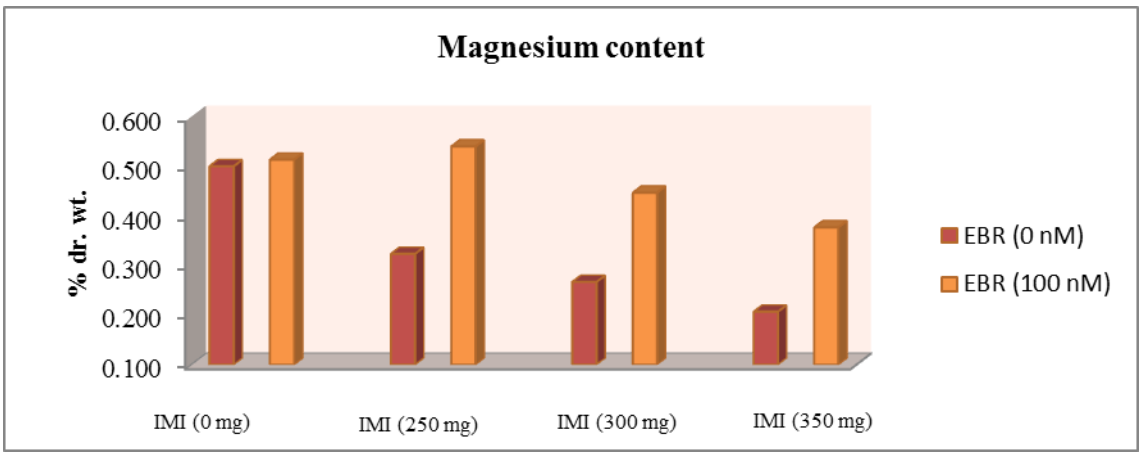


**Fig. 8.1.17 Effect of seed soaking with EBR on magnesium content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.**

In 60 days old plants, in comparison to the control (0.65%), Mg content was reduced to 0.22% in plants grown in soils supplemented with 350 mg IMI $Kg^{-1}$ soil. Seed soaking with EBR (100 nM) enhanced the Mg content to 0.38% in the leaves of plants grown in soils amended with 350 mg IMI $Kg^{-1}$ soil (Table 8.1.8, Fig. 8.1.18).

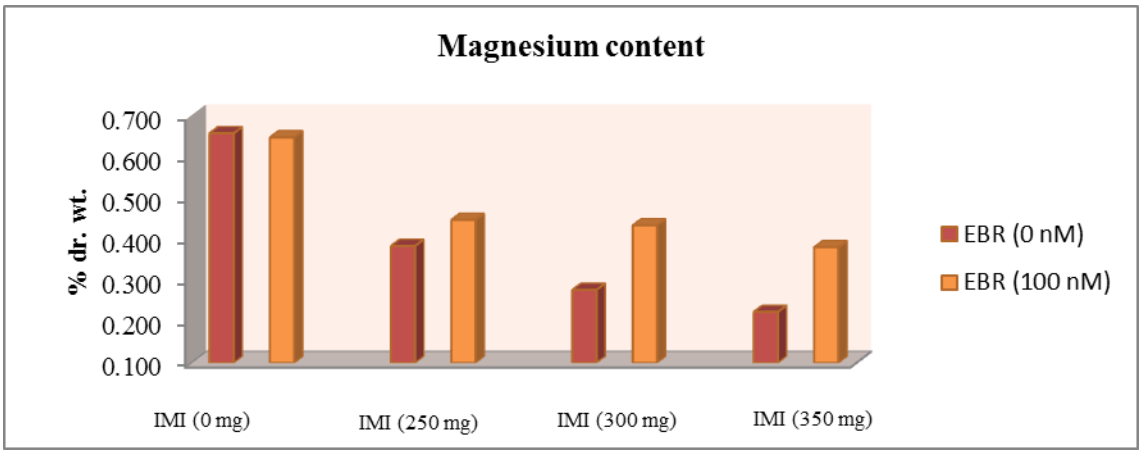


**Fig. 8.1.18 Effect of seed soaking with EBR on magnesium content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.**

Content of Mg in the leaves of 90 days old plants grown in IMI containing soils (350 mg IMI $Kg^{-1}$ soil) was decreased to 0.18% in comparison to Mg content of control plants (0.42%). Increment in Mg content (0.24%) was observed in plants raised from EBR (100 nM) treated seeds and grown in soils amended with 350 mg IMI $Kg^{-1}$ soil (Table 8.1.8, Fig. 8.1.19).

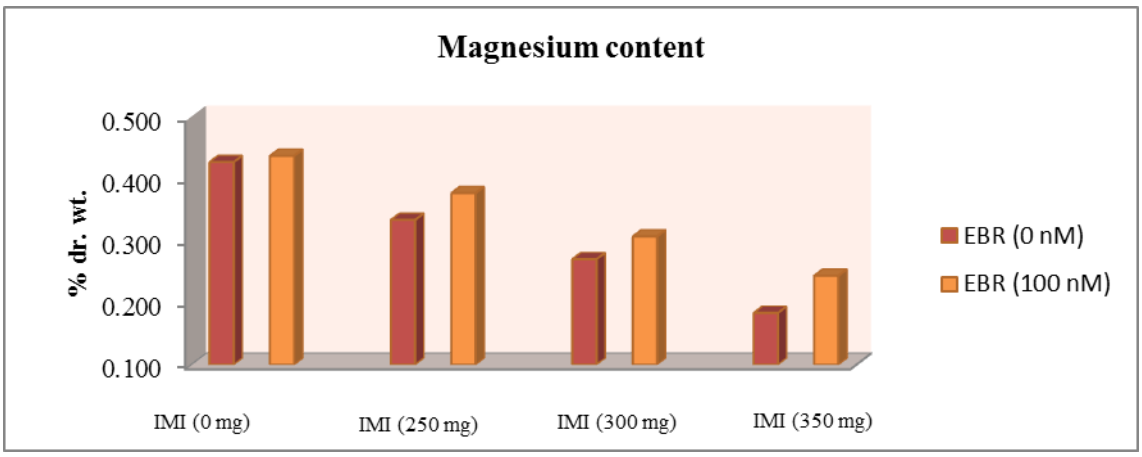


**Fig. 8.1.19 Effect of seed soaking with EBR on magnesium content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.**

Analysis of data using two-way ANOVA and Tukey's HSD showed significant differences in Mg content indicating the effect of IMI and EBR on Mg content in *B. juncea* plants. Furthermore, MLR analysis of data explained the effects of IMI and EBR on Mg content. Negative $\beta_{IMI}$ values indicated reduction in the content of Mg with IMI application, whereas positive $\beta_{EBR}$ values suggested increase in Mg content in *B. juncea* plants raised from EBR treated seeds (Table 8.1.7 and 8.1.8). Data analysis using ANN model showed that simulated (output) and experimental (target) values were highly correlated (Fig. 8.1.20).

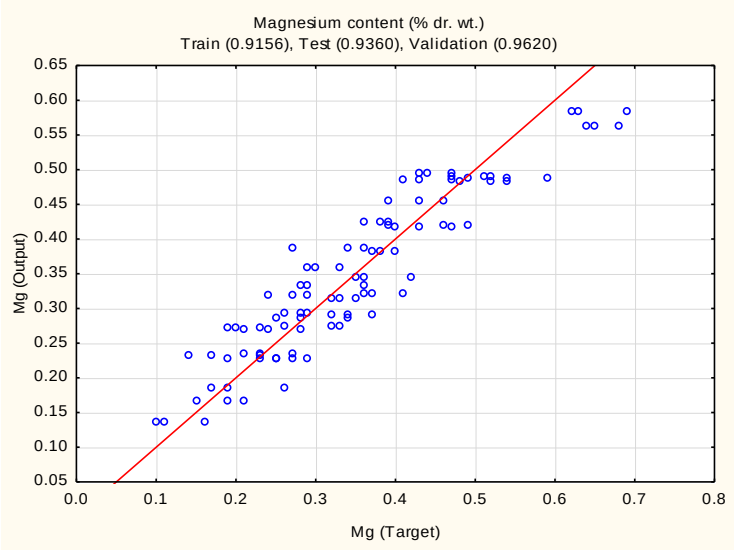


**Fig. 8.1.20 Correlation between target (experimental) and output (simulated) magnesium contents using ANN model ($p<0.001$).**

**Phosphorous content (P)**

It was observed that as compared to control (0.74%), P content in 10 days old *B. juncea* seedlings was maximum decreased to 0.47% as a result of IMI toxicity (250 mg IMI $L^{-1}$). Seed soaking with 100 nM EBR resulted in increase in P content which was enhanced to 0.62% in seedlings grown under IMI toxicity (Table 8.1.9, Fig. 8.1.21).

**Table 8.1.9 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on phosphorous content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Phosphorous content (% dr. wt.) | | |
|---|---|---|---|---|
| IMI (mg $L^{-1}$) | EBR (nM $L^{-1}$) | | | |
| 0 | 0 | 0.74±0.08 | | |
| 0 | 100 | 0.77±0.12 | | |
| 150 | 0 | 0.61±0.10 | | |
| 150 | 100 | 0.70±0.12 | | |
| 200 | 0 | 0.55±0.10 | | |
| 200 | 100 | 0.80±0.05 | | |
| 250 | 0 | 0.47±0.06 | | |
| 250 | 100 | 0.62±0.07 | | |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 5.26* | | |
| $F_{EBR}$ | | 11.47** | | |
| $F_{IMI \times EBR}$ | | 1.49 | | |
| HSD | | 0.27 | | |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| P (%) = 0.70 - 0.0007 IMI + 0.0013 EBR | | - 0.5106 | 0.4902 | 0.7078*** |

*, ** and *** indicate significant at p<0.05, p<0.01 and p<0.001 respectively. r = multiple correlation coefficient.

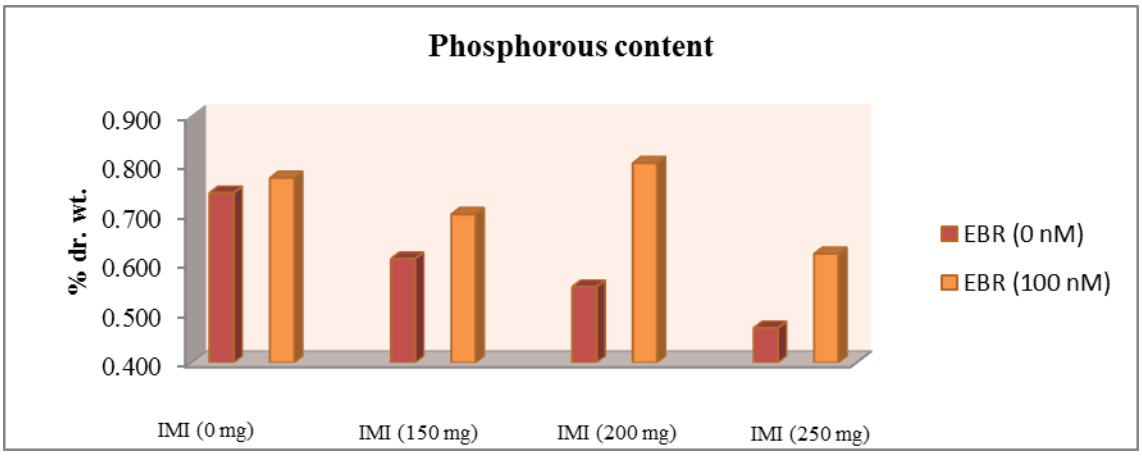


**Fig. 8.1.21 Effect of seed soaking with EBR on phosphorous content in *B. juncea* seedlings grown under IMI toxicity.**

As compared to the P content in control plants (0.25%), minimum P content (30 DAS) was observed (0.16%) in plants grown in soils mixed with 300 mg IMI $Kg^{-1}$ soil. Seed soaking with 100 nM EBR resulted in increase in P content to 0.26% in plants grown in soils amended with 300 mg IMI $Kg^{-1}$ soil (Table 8.1.10, Fig. 8.1.22).

**Table 8.1.10 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on phosphorous content in the leaves of *B. juncea* L. plants grown in imidacloprid (IMI) amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Phosphorous content (% dr. wt.) | | |
|---|---|---|---|---|
| IMI (mg $Kg^{-1}$) | EBR (nM $L^{-1}$) | 30 DAS | 60 DAS | 90 DAS |
| 0 | 0 | 0.25±0.02 | 0.89±0.05 | 0.24±0.02 |
| 0 | 100 | 0.27±0.01 | 0.87±0.03 | 0.27±0.00 |
| 250 | 0 | 0.27±0.02 | 0.52±0.05 | 0.21±0.01 |
| 250 | 100 | 0.35±0.03 | 0.60±0.08 | 0.41±0.06 |
| 300 | 0 | 0.23±0.02 | 0.37±0.04 | 0.21±0.00 |
| 300 | 100 | 0.34±0.02 | 0.43±0.05 | 0.32±0.03 |
| 350 | 0 | 0.16±0.02 | 0.33±0.03 | 0.21±0.03 |
| 350 | 100 | 0.26±0.03 | 0.41±0.04 | 0.26±0.02 |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 16.24*** | 121.1*** | 6.46** |
| $F_{EBR}$ | | 56.16*** | 5.03* | 61.44*** |
| $F_{IMI \times EBR}$ | | 3.57* | 1.40 | 9.14** |
| HSD | | 0.07 | 0.13 | 0.08 |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| P (%) = 0.24 - $5.06\times10^{-5}$ IMI + 0.0008 EBR | | - 0.1089 | 0.6531 | 0.6621*** |
| P (%) = 0.86 - 0.0014 IMI + 0.0005 EBR | | - 0.9570 | 0.1138 | 0.9637*** |
| P (%) = 0.22 - $1.03\times10^{-5}$ IMI + 0.0010 EBR | | - 0.0203 | 0.7031 | 0.7034*** |

*, ** and *** indicate significant at p<0.05, p<0.01 and p<0.001 respectively. r = multiple correlation coefficient. DAS = days after sowing.

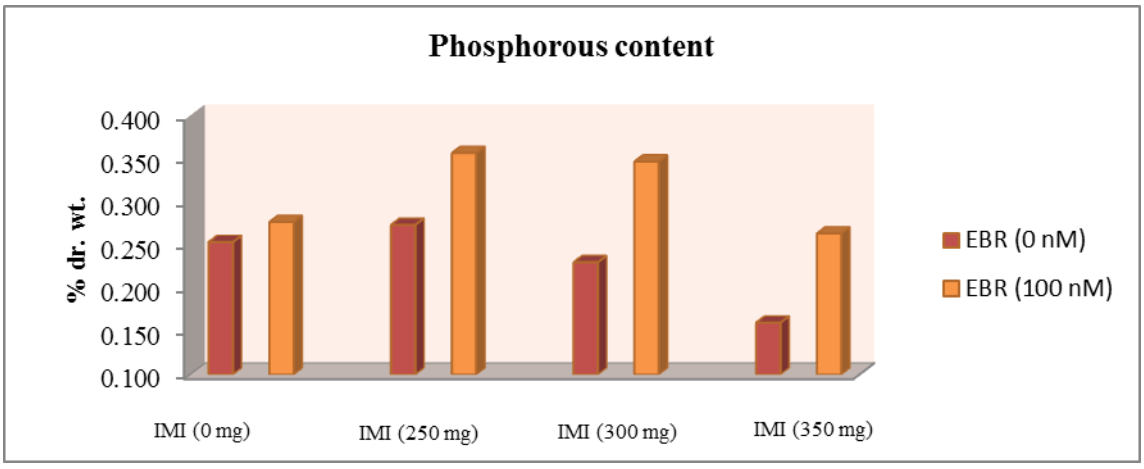


**Fig. 8.1.22 Effect of seed soaking with EBR on phosphorous content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.**

When compared to the leaves of 60 days old control plants (0.89%), P content was maximum reduced to 0.33% in plants grown in soils mixed with 300 mg IMI $Kg^{-1}$ soil. An enhancement in P content to 0.41% was noticed in plants germinated from EBR (100 nM) soaked seeds and grown in pots containing 300 mg IMI $Kg^{-1}$ soil (Table 8.1.10, Fig. 8.1.23).

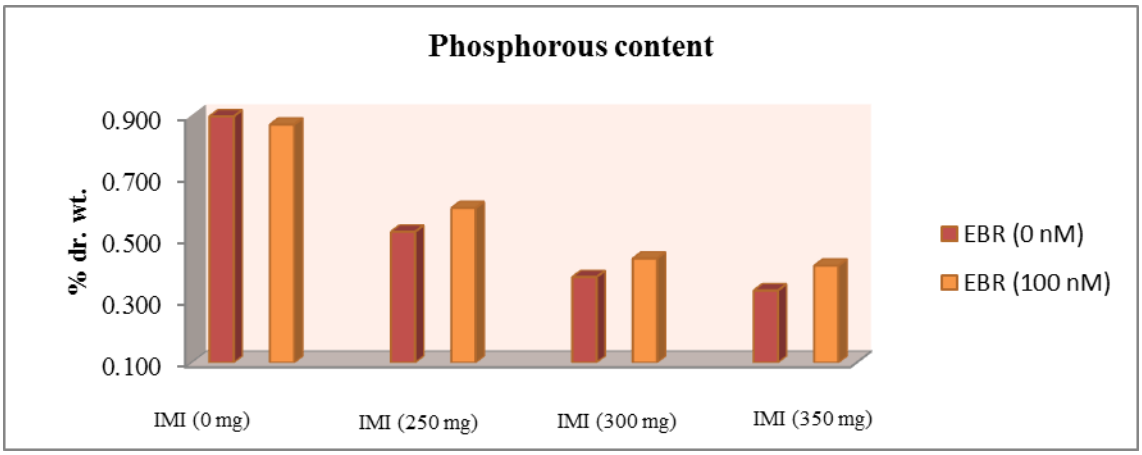


**Fig. 8.1.23 Effect of seed soaking with EBR on phosphorous content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.**

In the leaves of 90 day old control plants, P content was 0.24%, which was reduced to 0.21% in plants grown under IMI toxicity (350 mg IMI $Kg^{-1}$ soil). However, P content was observed to increase (0.265) in plants raised from EBR (100 nM) treated seeds and grown under IMI toxicity (Table 8.1.10, Fig. 8.1.24).

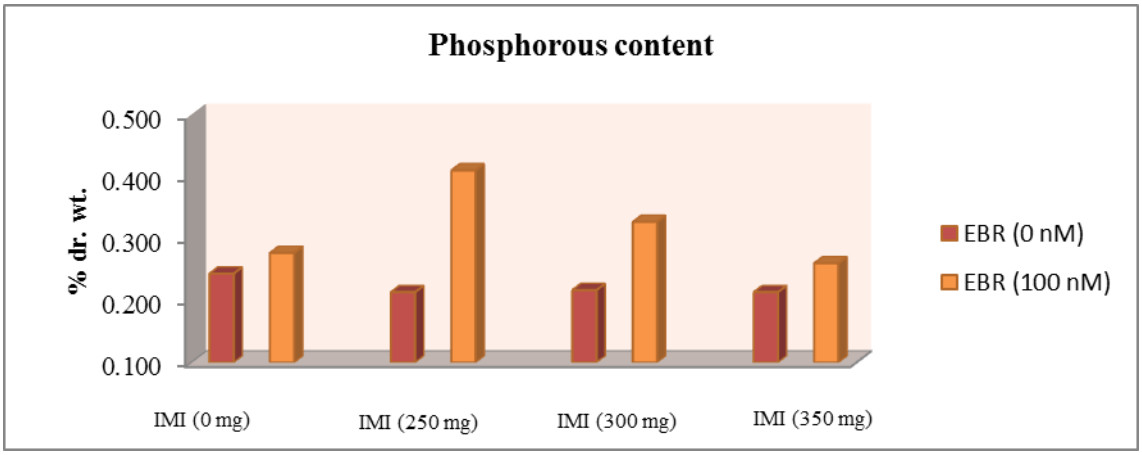


**Fig. 8.1.24 Effect of seed soaking with EBR on phosphorous content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.**

Two-way ANOVA and Tukey's HSD showed significant differences in P contents of *B. juncea* plants under normal and IMI stress conditions. Furthermore, MLR analysis revealed that IMI application via substratum reduced the P content (indicated by negative $\beta_{IMI}$ values) whereas seed treatment with EBR before sowing enhanced the P content in *B. juncea* plants under IMI toxicity which is indicated by positive $\beta_{EBR}$ values (Table 8.1.9 and 8.1.10). ANN model showed high correlation between target and output phosphorous contents (Fig. 8.1.25)

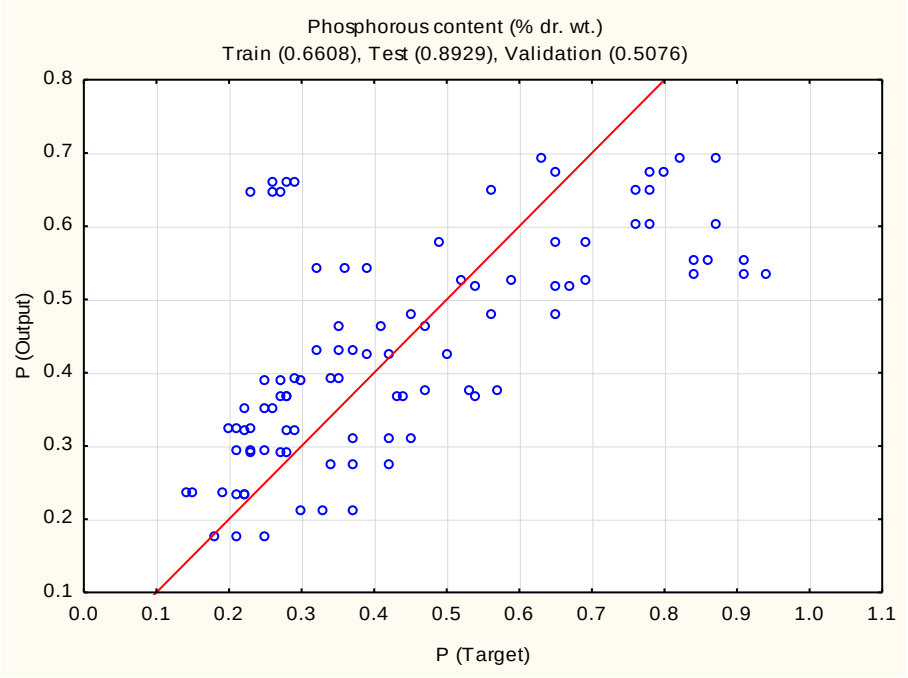


**Fig. 8.1.25 Correlation between target (experimental) and output (simulated) phosphorous contents using ANN model ($p<0.001$).**

## Sulphur content (S)

As compared to control seedlings (0.97%), S content was maximum decreased to 0.36% in the seedlings grown in Petri-plates added with 250 mg $L^{-1}$ IMI. Seed soaking with 100 nM EBR resulted in increase in S content which was increased to 0.56% in seedlings grown under IMI toxicity (Table 8.1.11, Fig. 8.1.26).

**Table 8.1.11 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on sulphur content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Sulphur content (% dr. wt.) |
|---|---|---|
| IMI (mg $L^{-1}$) | EBR (nM $L^{-1}$) | |
| 0 | 0 | 0.97±0.19 |
| 0 | 100 | 1.09±0.16 |
| 150 | 0 | 0.78±0.11 |
| 150 | 100 | 0.80±0.12 |
| 200 | 0 | 0.72±0.13 |
| 200 | 100 | 0.81±0.11 |
| 250 | 0 | 0.36±0.07 |
| 250 | 100 | 0.56±0.09 |
| **Two-way ANOVA** | | |
| $F_{IMI}$ | | 19.13*** |
| $F_{EBR}$ | | 4.25 |
| $F_{IMI \times EBR}$ | | 0.49 |
| HSD | | 0.37 |
| **Multiple linear regression** | | |

| MLR equation | β-regression coefficients | | r |
|---|---|---|---|
| | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| S (%) = 1.01 - 0.0020 IMI + 0.0011 EBR | - 0.7921 | 0.2317 | 0.8252*** |

*** indicates significant at p<0.001. r = multiple correlation coefficient.

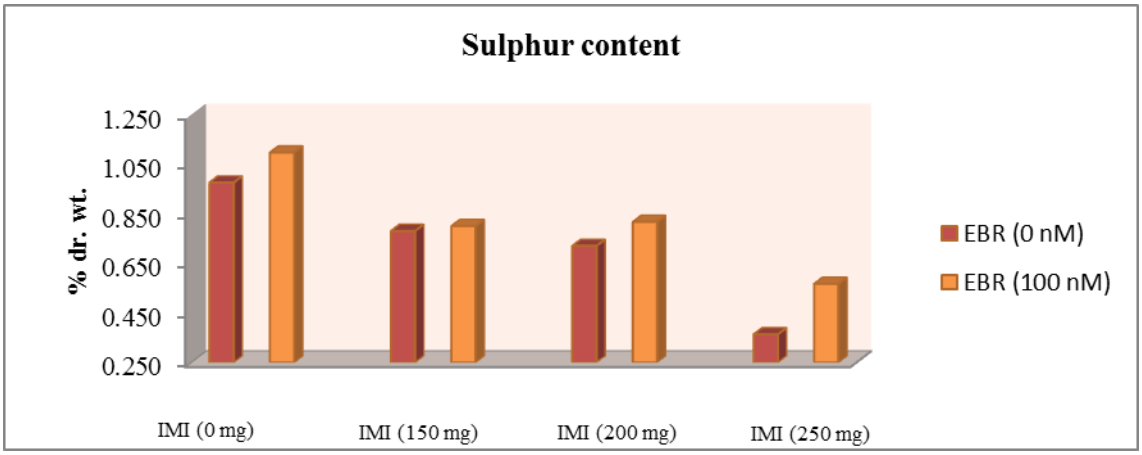


**Fig. 8.1.26 Effect of seed soaking with EBR on sulphur content in *B. juncea* seedlings grown under IMI toxicity.**

In the leaves of 30 days old *B. juncea* plants, S content was reduced to 0.33% in plants grown in pots amended with 350 mg IMI $Kg^{-1}$ soil, as compared to S content in control plants (0.63%). Increase in S content to 0.63% was observed in plants raised from 100 nM EBR treated seeds and grown in pots amended with 350 mg IMI $Kg^{-1}$ soil (Table 8.1.12, Fig. 8.1.27).

**Table 8.1.12 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on sulphur content in the leaves of *B. juncea* L. plants grown in imidacloprid (IMI) amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Sulphur content (% dr. wt.) | | |
|---|---|---|---|---|
| IMI (mg $Kg^{-1}$) | EBR (nM $L^{-1}$) | 30 DAS | 60 DAS | 90 DAS |
| 0 | 0 | 0.63±0.06 | 0.64±0.03 | 0.53±0.02 |
| 0 | 100 | 0.69±0.05 | 0.69±0.07 | 0.57±0.05 |
| 250 | 0 | 0.50±0.03 | 0.55±0.05 | 0.37±0.03 |
| 250 | 100 | 0.94±0.07 | 0.68±0.02 | 0.56±0.05 |
| 300 | 0 | 0.42±0.03 | 0.44±0.03 | 0.25±0.04 |
| 300 | 100 | 0.82±0.05 | 0.64±0.07 | 0.37±0.05 |
| 350 | 0 | 0.33±0.03 | 0.31±0.05 | 0.18±0.03 |
| 350 | 100 | 0.63±0.06 | 0.38±0.04 | 0.23±0.04 |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 21.39*** | 41.90*** | 76.24*** |
| $F_{EBR}$ | | 181.2*** | 28.04*** | 31.14*** |
| $F_{IMI \times EBR}$ | | 15.16*** | 2.25 | 3.71* |
| HSD | | 0.15 | 0.14 | 0.12 |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| S (%) = 0.54 - 0.0003 IMI + 0.0030 EBR | | - 0.2338 | 0.7684 | 0.8032*** |
| S (%) = 0.64 - 0.0007 IMI + 0.0012 EBR | | - 0.6533 | 0.3986 | 0.7653*** |
| S (%) = 0.53 - 0.0008 IMI + 0.0010 EBR | | - 0.7807 | 0.3294 | 0.8474*** |

* and *** indicate significant at p<0.05 and p<0.001. r = multiple correlation coefficient. DAS = days after sowing.

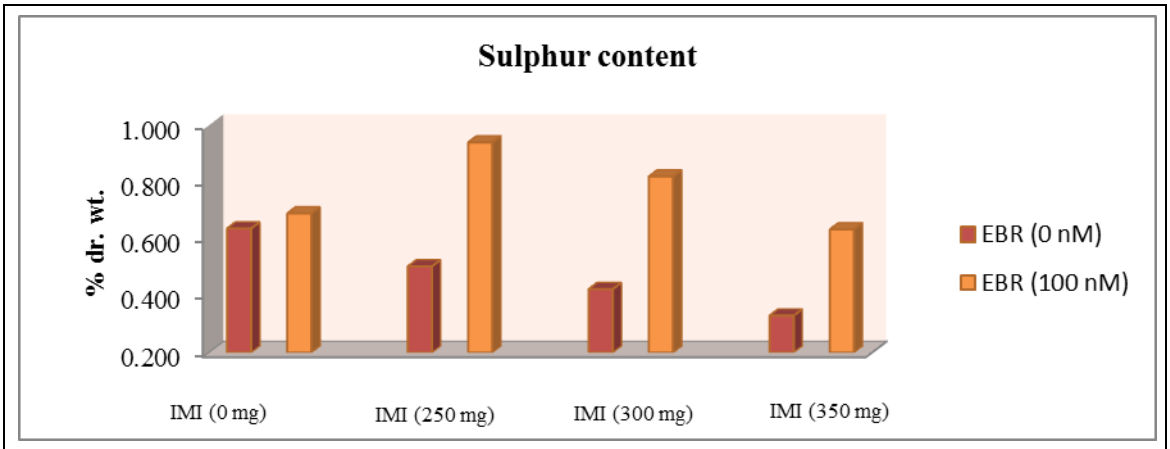


**Fig. 8.1.27 Effect of seed soaking with EBR on sulphur content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.**

In the leaves of 60 days old plants grown in soils mixed with 350 mg IMI $Kg^{-1}$ soil, S content was reduced to 0.31% as compared to 0.645% in the control plants. An enhancement in the S content to 0.38% was observed in the leaves of plants germinated from EBR (100 nM) soaked seeds and grown under IMI toxicity (Table 8.1.12, Fig. 8.1.28).

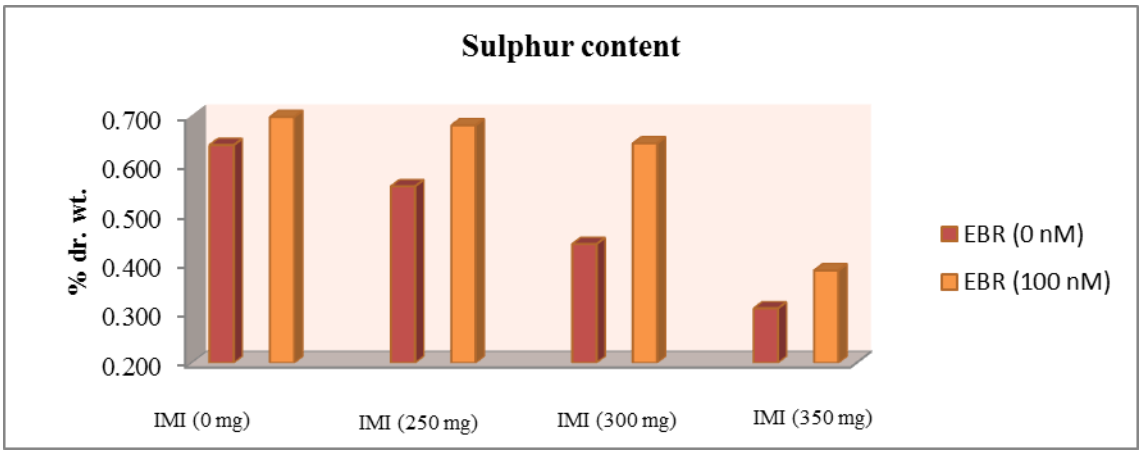


**Fig. 8.1.28 Effect of seed soaking with EBR on sulphur content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.**

Similarly, in the leaves of 90 days old *B. juncea* plants grown in pots containing 350 mg IMI $Kg^{-1}$ soil, S content was decreased to 0.18% when compared to 0.53% in the control plants. Seed soaking with 100 nM EBR and growing in pots amended with 350 mg IMI $Kg^{-1}$ soil resulted in increase in S content to 0.23% (Table 8.1.12, Fig. 8.1.29).

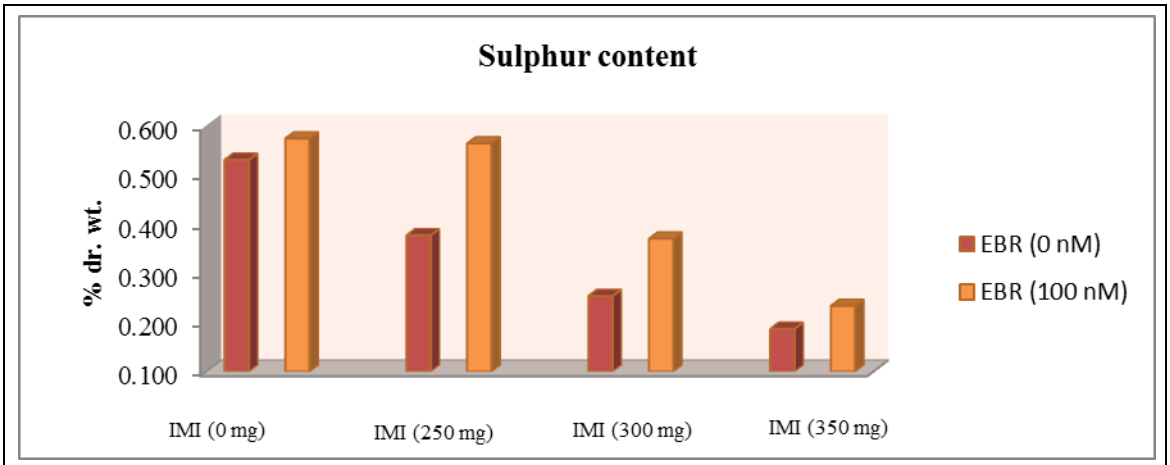


**Fig. 8.1.29 Effect of seed soaking with EBR on sulphur content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.**

Analysis of data using two-way ANOVA and Tukey's HSD showed significant differences in S contents in the seedlings as well as leaves of *B. juncea* plants raised from EBR treated seeds and grown under IMI toxicity. MLR analysis revealed that IMI reduced the S contents (indicated by negative $\beta_{IMI}$), whereas EBR seed soaking before sowing enhanced the S contents (indicated by positive $\beta_{EBR}$) as shown in table 8.1.11 and 8.1.12. Data analysis using ANN revealed that ANN model simulated the experimental data at high level of significance ($p<0.001$) as shown in Fig. 8.1.30.

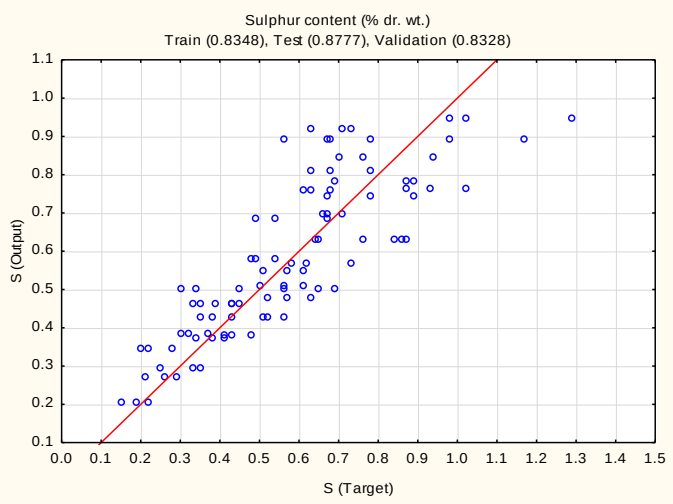


**Fig. 8.1.30 Correlation between target (experimental) and output (simulated) sulphur contents using ANN model (p<0.001).**

## Potassium content (K)

It has been observed that K content in control 10 days old seedlings was 0.66% which was reduced to 0.35% in the seedlings grown in 250 mg IMI $L^{-1}$ solutions. Seed soaking with 100 nM EBR before germination in IMI containing Petri-plates resulted in increase in K content to 0.47% (Table 8.1.13, Fig. 8.1.31).

**Table 8.1.13 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on potassium content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Potassium content (% dr. wt.) | | |
|---|---|---|---|---|
| IMI (mg $L^{-1}$) | EBR (nM $L^{-1}$) | | | |
| 0 | 0 | 0.66±0.05 | | |
| 0 | 100 | 0.72±0.05 | | |
| 150 | 0 | 0.56±0.09 | | |
| 150 | 100 | 0.69±0.17 | | |
| 200 | 0 | 0.46±0.08 | | |
| 200 | 100 | 0.63±0.06 | | |
| 250 | 0 | 0.35±0.04 | | |
| 250 | 100 | 0.47±0.06 | | |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 10.65*** | | |
| $F_{EBR}$ | | 10.86** | | |
| $F_{IMI \times EBR}$ | | 0.36 | | |
| HSD | | 0.25 | | |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| K (%) = 0.66 - 0.0010 IMI + 0.0012 EBR | | - 0.6575 | 0.4258 | 0.7833*** |
| ** and *** indicate significant at p<0.01 and p<0.001. r = multiple correlation coefficient. | | | | |

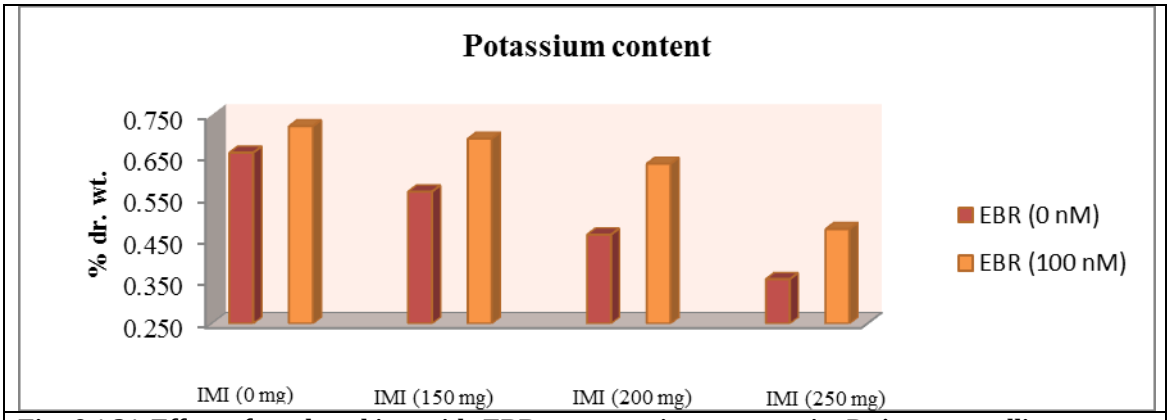


**Fig. 8.1.31 Effect of seed soaking with EBR on potassium content in *B. juncea* seedlings grown under IMI toxicity.**

In the leaves of 30 days old *B. juncea* plants, the maximum decrease in K content was 0.78% observed at 350 mg IMI $Kg^{-1}$ soil as compared to the control plants (2.76%). However, seed soaking before sowing with 100 nM EBR enhanced the K content to 1.33% in the leaves of plants grown in pots containing 350 mg IMI $Kg^{-1}$ soil (Table 8.1.14, Fig. 8.1.32).

**Table 8.1.14 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on potassium content in the leaves of *B. juncea* L. plants grown in imidacloprid (IMI) amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Potassium content (% dr. wt.) | | |
|---|---|---|---|---|
| IMI (mg $Kg^{-1}$) | EBR (nM $L^{-1}$) | 30 DAS | 60 DAS | 90 DAS |
| 0 | 0 | 2.76±0.10 | 3.50±0.08 | 2.41±0.19 |
| 0 | 100 | 2.49±0.07 | 3.87±0.30 | 2.60±0.17 |
| 250 | 0 | 2.18±0.09 | 2.67±0.19 | 2.28±0.35 |
| 250 | 100 | 3.33±0.03 | 4.01±0.28 | 2.91±0.14 |
| 300 | 0 | 1.76±0.09 | 2.10±0.08 | 1.99±0.25 |
| 300 | 100 | 2.24±0.26 | 2.41±0.03 | 2.57±0.38 |
| 350 | 0 | 0.78±0.22 | 1.69±0.22 | 1.43±0.23 |
| 350 | 100 | 1.33±0.10 | 2.45±0.36 | 1.93±0.07 |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 170.4*** | 73.69*** | 16.65*** |
| $F_{EBR}$ | | 64.39*** | 56.14*** | 22.58*** |
| $F_{IMI \times EBR}$ | | 23.87*** | 6.53** | 0.96 |
| HSD | | 0.41 | 0.63 | 0.69 |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| K (%) = 2.61 - 0.0033 IMI + 0.0048 EBR | | - 0.5824 | 0.3115 | 0.6605*** |
| K (%) = 3.48 - 0.0044 IMI + 0.0069 EBR | | - 0.7202 | 0.4236 | 0.8356*** |
| K (%) = 2.39 - 0.0016 IMI + 0.0048 EBR | | - 0.4521 | 0.4969 | 0.6718*** |

** and *** indicate significant at $p<0.01$ and $p<0.001$. r = multiple correlation coefficient. DAS = days after sowing.

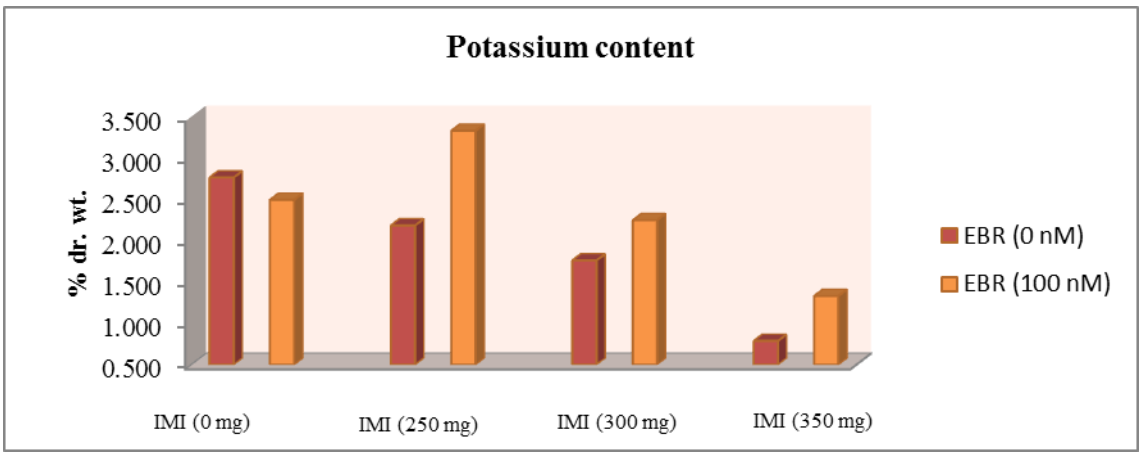


**Fig. 8.1.32 Effect of seed soaking with EBR on potassium content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.**

In 60 days old plants grown in pots amended with 350 mg IMI $Kg^{-1}$ soil, K content was decreased to 1.69% when compared to K content in the leaves of control plants (3.50%). Seed soaking with 100 nM EBR before sowing resulted in increase in K content to 2.45% under IMI toxicity (Table 8.1.14, Fig. 8.1.33).

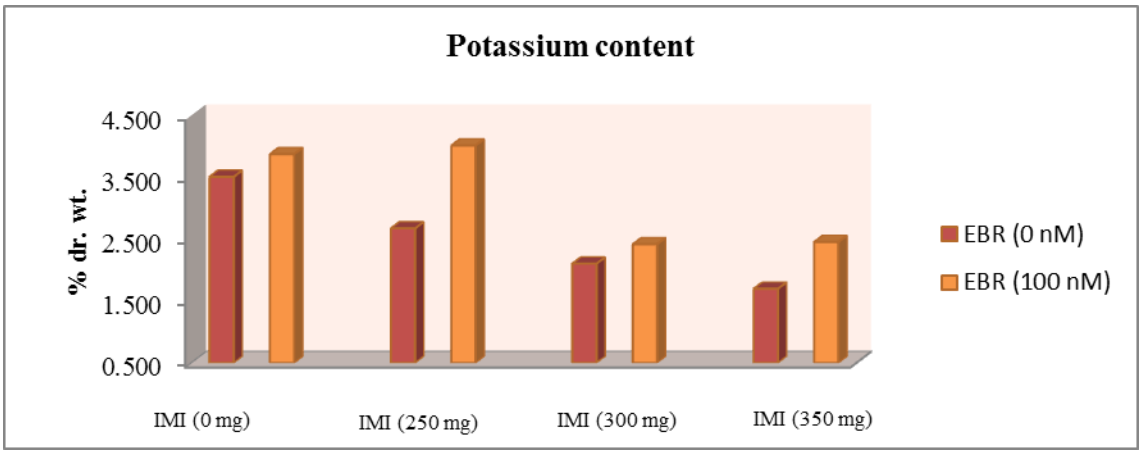


**Fig. 8.1.33 Effect of seed soaking with EBR on potassium content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.**

Content of K in the leaves of 90 days old plants grown under IMI toxicity (350 mg IMI $Kg^{-1}$ soil) decreased to 1.43% when compared to K content in control plants (2.41%). In plants raised from 100 nM EBR soaked seeds, K content was increased to 1.93% under IMI toxicity (Table 8.1.14, Fig. 8.1.34).

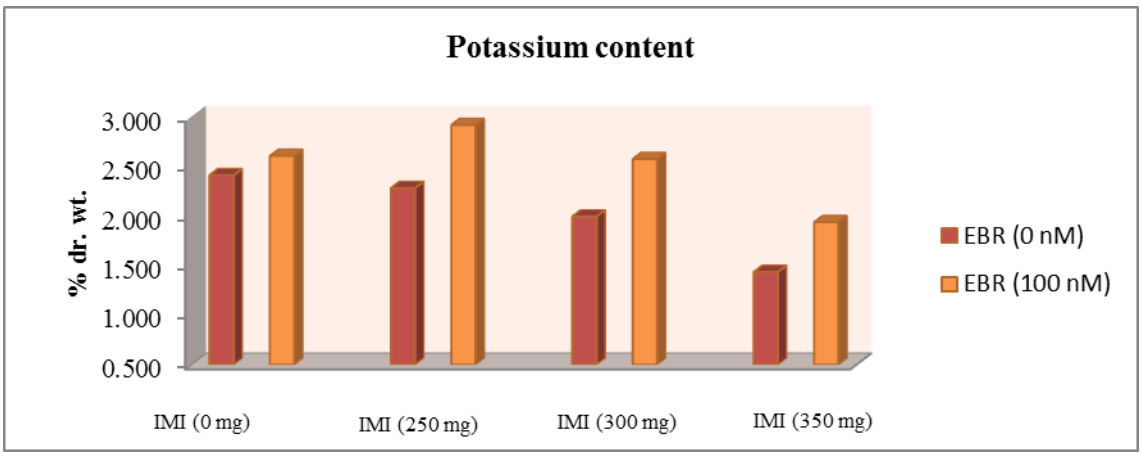


**Fig. 8.1.34 Effect of seed soaking with EBR on potassium content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.**

Data analysis using two-way ANOVA and Tukey's HSD showed that K contents were significantly different in plants raised from EBR treated seeds and grown in IMI amended soils, as compared to the control plants (Table 5.2.1). MLR analysis revealed that IMI concentrations in Petri-plates and soils decreased K contents (indicated by negative $\beta_{IMI}$ values) whereas seed soaking with EBR increased the K contents in plants (indicated by positive $\beta_{EBR}$ values) (Tables 8.1.13 and 8.1.14). The target and output values from ANN for K contents were highly correlated as shown in Fig. 8.1.35.

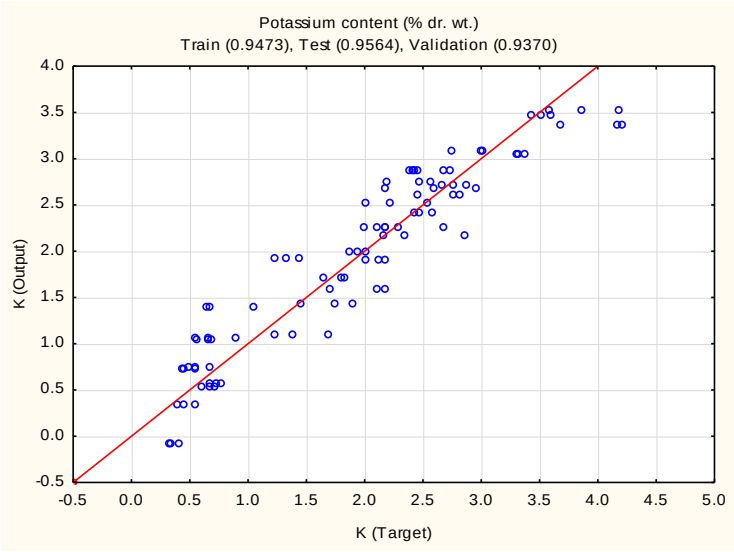


**Fig. 8.1.35 Correlation between target (experimental) and output (simulated) potassium contents using ANN model ($p<0.001$).**

## Calcium content (Ca)

As compared to the Ca content in 10 days old control seedlings of *B. juncea* (0.52%), Ca content was maximum decreased to 0.25% in the seedlings grown in Petri-plates containing 250 mg IMI $L^{-1}$ solutions. Seed soaking with 100 nM EBR resulted in increase in Ca content to 0.48% in seedlings grown under IMI toxicity (Table 8.1.15, Fig. 8.1.36).

**Table 8.1.15 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on calcium content in 10 days old *B. juncea* L. seedlings grown in imidacloprid (IMI) containing Petri-plates. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Calcium content (% dr. wt.) | | |
|---|---|---|---|---|
| IMI (mg $L^{-1}$) | EBR (nM $L^{-1}$) | | | |
| 0 | 0 | 0.52±0.11 | | |
| 0 | 100 | 0.56±0.16 | | |
| 150 | 0 | 0.47±0.09 | | |
| 150 | 100 | 0.52±0.13 | | |
| 200 | 0 | 0.40±0.06 | | |
| 200 | 100 | 0.66±0.11 | | |
| 250 | 0 | 0.25±0.06 | | |
| 250 | 100 | 0.48±0.09 | | |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 3.13 | | |
| $F_{EBR}$ | | 10.74** | | |
| $F_{IMI \times EBR}$ | | 1.69 | | |
| HSD | | 0.31 | | |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| Ca (%) = 0.48 - 0.0005 IMI + 0.0015 EBR | | - 0.3237 | 0.5104 | 0.6044** |
| ** indicates significant p<0.01. r = multiple correlation coefficient. | | | | |

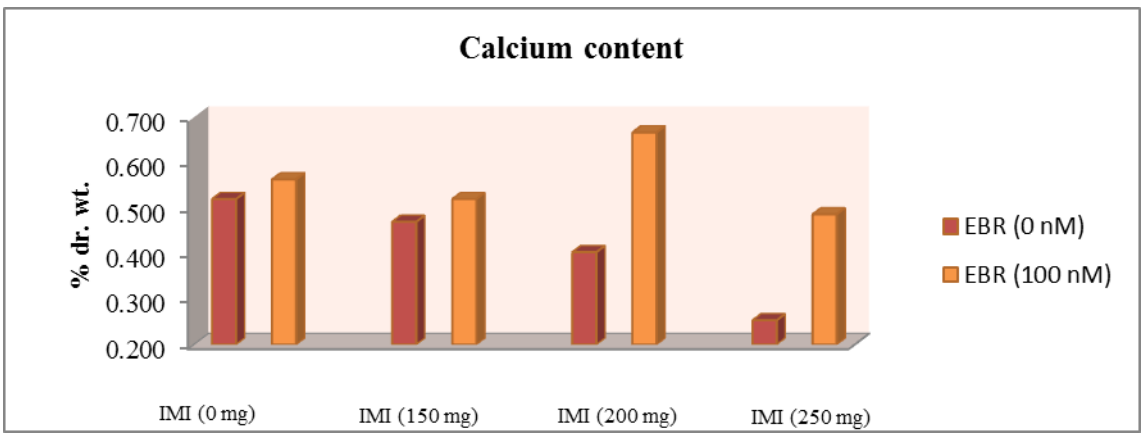


**Fig. 8.1.36 Effect of seed soaking with EBR on calcium content in *B. juncea* seedlings grown under IMI toxicity.**

In the leaves of 30 days old control plants, Ca content observed was 2.18% which was maximum decreased to 0.89% in the plants grown in soils mixed with 350 mg IMI $Kg^{-1}$ soil. Moreover, in plants raised from EBR (100 nM) treated seeds before sowing in pots containing 350 mg IMI $Kg^{-1}$ soil, Ca content was enhanced to 1.88% (Table 8.1.16, Fig. 8.1.37).

**Table 8.1.16 Effect of seed pre-soaking with 24-epibrassinolide (EBR) on calcium content in the leaves of *B. juncea* L. plants grown in imidacloprid (IMI) amended soils. Data are Mean±SD (n=3), Two-way ANOVA, Tukey's HSD and multiple linear regression analysis (MLR).**

| Treatments | | Calcium content (% dr. wt.) | | |
|---|---|---|---|---|
| IMI (mg $Kg^{-1}$) | EBR (nM $L^{-1}$) | 30 DAS | 60 DAS | 90 DAS |
| 0 | 0 | 2.18±0.04 | 1.81±0.14 | 1.44±0.05 |
| 0 | 100 | 2.62±0.12 | 2.18±0.32 | 1.31±0.02 |
| 250 | 0 | 1.81±0.12 | 1.78±0.28 | 1.22±0.11 |
| 250 | 100 | 2.70±0.25 | 2.64±0.17 | 2.60±0.20 |
| 300 | 0 | 1.05±0.06 | 1.47±0.16 | 1.17±0.16 |
| 300 | 100 | 2.34±0.43 | 2.52±0.28 | 1.89±0.30 |
| 350 | 0 | 0.89±0.17 | 0.68±0.30 | 0.89±0.22 |
| 350 | 100 | 1.88±0.07 | 1.14±0.34 | 1.41±0.20 |
| **Two-way ANOVA** | | | | |
| $F_{IMI}$ | | 33.60*** | 30.09*** | 18.12*** |
| $F_{EBR}$ | | 121.1*** | 41.08*** | 67.70*** |
| $F_{IMI \times EBR}$ | | 4.52* | 2.29 | 17.09*** |
| HSD | | 0.56 | 0.74 | 0.52 |
| **Multiple linear regression** | | | | |
| **MLR equation** | | **β-regression coefficients** | | **r** |
| | | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| Ca (%) = 2.05 - 0.0025 IMI + 0.0090 EBR | | - 0.5218 | 0.6941 | 0.8684*** |
| Ca (%) = 1.84 - 0.0018 IMI + 0.0069 EBR | | - 0.3658 | 0.5161 | 0.6325** |
| Ca (%) = 1.19 - $4.2\times10^{-5}$ IMI + 0.0062 EBR | | - 0.0108 | 0.5979 | 0.5980** |

*, ** and *** indicate significant at p<0.05, p<0.01 and p<0.001 respectively. r = multiple correlation coefficient. DAS = days after sowing.

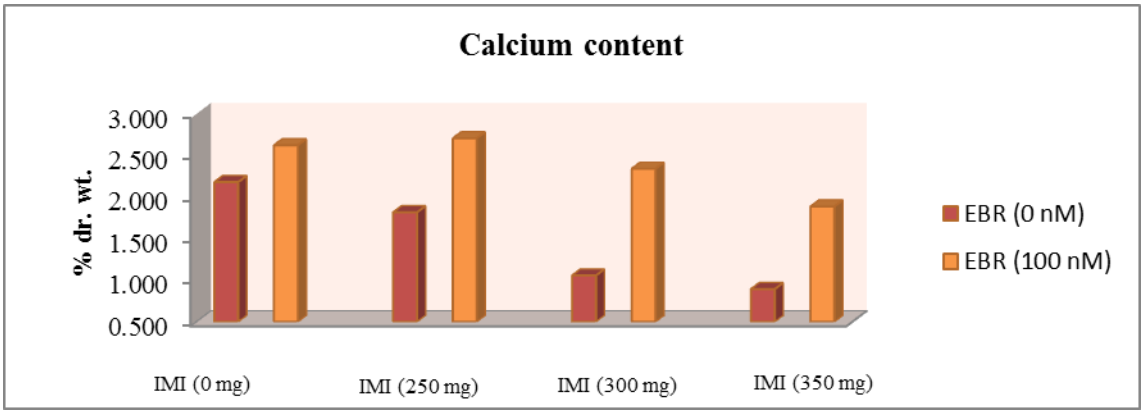


**Fig. 8.1.37 Effect of seed soaking with EBR on calcium content in the leaves of *B. juncea* plants (30 DAS) grown under IMI toxicity.**

Ca content in the leaves of 60 days old plants grown under IMI toxicity (350 mg IMI $Kg^{-1}$ soil) declined to 0.68% as compared to Ca content of 1.81% in control plants. Furthermore, EBR (100 nM) seed soaking before sowing in IMI containing soils in increase of Ca content to 1.14% (Table 8.1.16, Fig. 8.1.38).

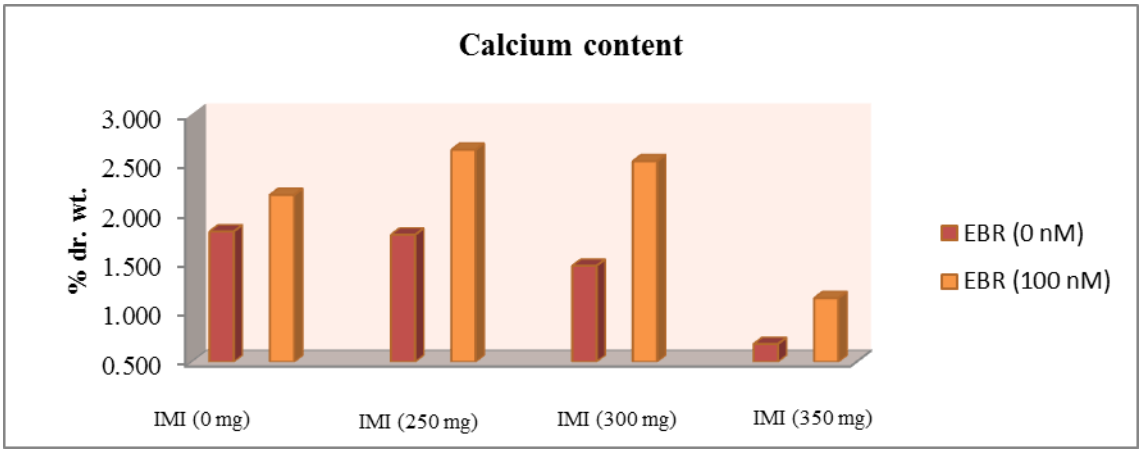


**Fig. 8.1.38 Effect of seed soaking with EBR on calcium content in the leaves of *B. juncea* plants (60 DAS) grown under IMI toxicity.**

Minimum Ca content (0.89%) was observed in the leaves of 90 days old plants grown under IMI toxicity (350 mg IMI $Kg^{-1}$ soil) when compared to Ca content in the leaves of the control plants (1.44%). Seed soaking before sowing with 100 nM EBR increased the Ca content to 1.41% under IMI toxicity (Table 8.1.16, Fig. 8.1.39).

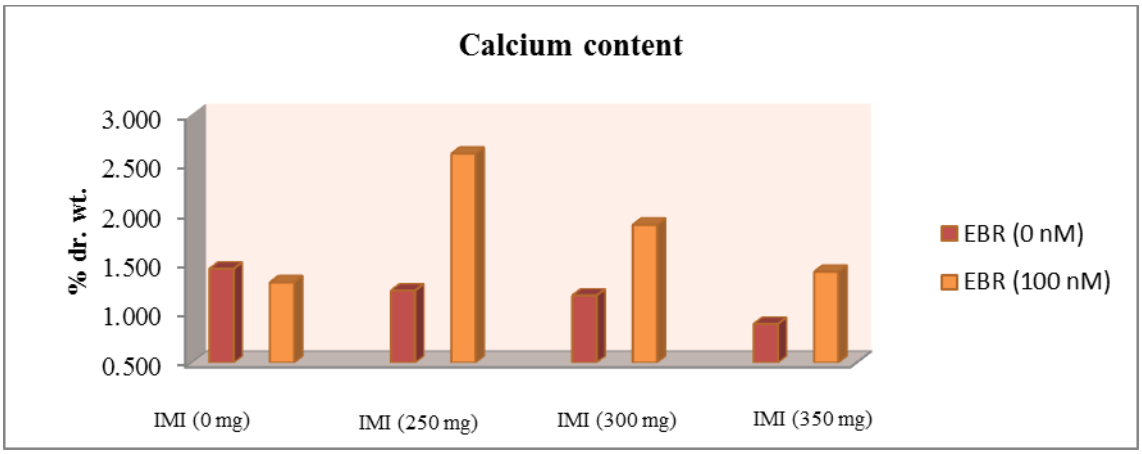


**Fig. 8.1.39 Effect of seed soaking with EBR on calcium content in the leaves of *B. juncea* plants (90 DAS) grown under IMI toxicity.**

Data analysis using two-way ANOVA and Tukey's HSD showed significant differences for Ca contents in seedling (10 DAS) and leaves of all the stages of *B. juncea* plants. MLR analysis revealed the role of IMI and EBR in calcium contents of seedlings and leaves. IMI application reduced the Ca content as shown by negative $\beta_{IMI}$ values, whereas seed treatment before sowing with EBR enhanced the Ca content in the leaves of *B. juncea* leaves as indicated by positive $\beta_{EBR}$ values (Table 8.1.15 and 8.1.16). Data analysis using ANN model showed that simulated and experimental values of Ca contents were highly correlated after taking applied IMI, EBR and DAS as inputs (Fig. 8.1.40).

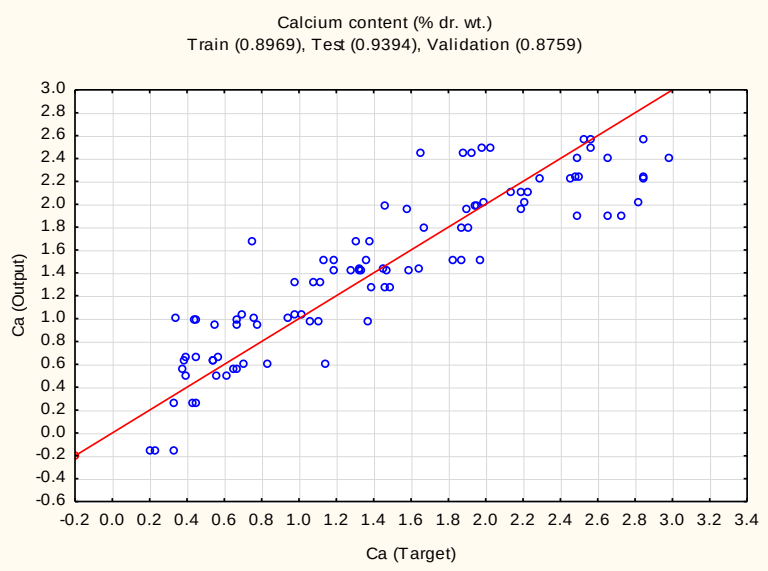


**Fig. 8.1.40 Correlation between target (experimental) and output (simulated) calcium contents using ANN model ($p<0.001$).**

## 8.2. Phytochemical analysis

It has been observed that phytochemicals in the seedlings and leaves of *B. juncea* plants were negatively affected by the application of IMI. However, seed soaking with EBR before sowing in Petri-plates and soils containing IMI resulted in the recovery of concentrations of phytochemicals which were earlier negatively affected by IMI application.

In 10 days old seedlings, increase in the concentration of phytochemicals was observed after seed treatment with EBR before germinating them in IMI containing

Petri-plates (Table 8.2.1). MLR analysis also revealed that IMI application resulted in reduction in concentration of phytochemicals as indicated by negative $\beta_{IMI}$ values, whereas EBR seed treatment resulted in recovery of reduced concentrations of phytochemicals as indicated by positive $\beta_{EBR}$ values (Table 8.2.1).

**Table 8.2.1 GC-MS analysis showing the effect of 24-epibrassinolide on various phytochemicals present in 10 days old seedlings of *Brassica juncea* L. under imidacloprid stress.**

| Name of Compound | CN | | T1 | | T2 | | T1+T2 | |
|---|---|---|---|---|---|---|---|---|
| | RT | Area | RT | Area | RT | Area | RT | Area |
| 4,5-Epithiovaleronitrile | 3.670 | 3511074 | 3.678 | 6874578 | 3.692 | 1750808 | 3.682 | 5654566 |
| 2-Naphthoic acid-methyl ester | 8.346 | 2032129 | 8.354 | 4033988 | 8.352 | 1498998 | 8.352 | 4318068 |
| l-(+)-Ascorbic acid 2,6-dihexadecanoate | 10.950 | 14989340 | 10.935 | 17903716 | 10.928 | 6649347 | 10.925 | 16938220 |
| Phytol | 12.279 | 14471356 | 12.276 | 35326403 | 12.278 | 12892907 | 12.275 | 23692944 |
| 9,12 Octadecadienoic acid (Z,Z) | 12.579 | 155507529 | 12.532 | 163857478 | 12.509 | 61096432 | 12.509 | 132755701 |
| 9-Octadecenoic acid | 16.644 | 84207315 | 16.633 | 169175923 | 16.624 | 42780777 | 16.613 | 56449312 |
| (+)-alpha-Tocopherol acetate | 19.652 | 4153670 | 19.652 | 6566489 | 19.652 | 3401194 | 19.647 | 5543080 |
| Ergosta-5,22-dien-3-beta-ol | 19.715 | 2661572 | 19.716 | 7390770 | 19.716 | 2089429 | 19.712 | 3965687 |
| Campesterol | 20.109 | 12000312 | 20.109 | 28696676 | 20.11 | 10665424 | 20.104 | 29529315 |
| gamma-Sitosterol | 20.663 | 22403575 | 20.664 | 45806051 | 20.665 | 21958924 | 20.658 | 41740037 |
| **Name of Compound** | **T3** | | **T1+T3** | | **T4** | | **T1+T4** | |
| | **RT** | **Area** | **RT** | **Area** | **RT** | **Area** | **RT** | **Area** |
| 4,5-Epithiovaleronitrile | 3.683 | 2082573 | 3.673 | 3662198 | 3.681 | 3164436 | 3.662 | 4512168 |
| 2-Naphthoic acid-methyl ester | 8.351 | 986688 | 8.353 | 1650414 | 8.356 | 887810 | 8.347 | 1024128 |
| l-(+)-Ascorbic acid 2,6-dihexadecanoate | 10.92 | 4946242 | 10.94 | 7320774 | 10.922 | 2433111 | 10.916 | 3711713 |
| Phytol | 12.27 | 10114001 | 12.285 | 12741804 | 12.283 | 6438373 | 12.274 | 12068053 |
| 9,12 Octadecadienoic acid (Z,Z) | 12.495 | 40970788 | 12.544 | 78449716 | 12.488 | 21168745 | 12.492 | 38690066 |
| 9-Octadecenoic acid | 16.605 | 13161984 | 16.648 | 80848440 | 16.611 | 3488980 | 16.609 | 18071861 |
| (+)-alpha-Tocopherol acetate | 19.641 | 1798610 | 19.656 | 2863299 | 19.654 | 781404 | 19.642 | 2286215 |
| Ergosta-5,22-dien-3-beta-ol | 19.703 | 1568114 | 19.72 | 2576327 | 19.718 | 765078 | 19.706 | 2005907 |
| Campesterol | 20.097 | 10189028 | 20.114 | 11067376 | 20.11 | 5735891 | 20.1 | 11583096 |
| gamma-Sitosterol | 20.65 | 15989505 | 20.667 | 20099408 | 20.662 | 8809360 | 20.653 | 18925456 |

**Multiple linear regression**

| MLR equation | β-regression coefficients | | r |
|---|---|---|---|
| | $\beta_{IMI}$ | $\beta_{EBR}$ | |
| $Y_1 = 4\times10^6$ - 7184.9 IMI + 25487 EBR | - 0.4151 | 0.7870 | 0.8898** |
| $Y_2 = 3\times10^6$ - 8383.8 IMI + 14052 EBR | - 0.6133 | 0.5495 | 0.8235* |
| $Y_3 = 2\times10^7$ - 52944.6 IMI + 42141 EBR | - 0.8478 | 0.3607 | 0.9213** |
| $Y_4 = 2\times10^7$ - 63946.1 IMI + 99781 EBR | - 0.6925 | 0.5776 | 0.9018** |
| $Y_5 = 1\times10^8$ - 513540.6 IMI + 337524 EBR | - 0.9114 | 0.3201 | 0.9660*** |
| $Y_6 = 1\times10^8$ - 445012.6 IMI + 452266 EBR | - 0.8248 | 0.4480 | 0.9386** |
| $Y_7 = 5\times10^6$ - 15259.5 IMI + 17811 EBR | - 0.7897 | 0.4927 | 0.9308** |
| $Y_8 = 4\times10^6$ - 14621.8 IMI + 22136 EBR | - 0.7153 | 0.5788 | 0.9201** |
| $Y_9 = 2\times10^7$ - 47283.2 IMI + 41805 EBR | - 0.6350 | 0.3001 | 0.7023 ns |
| $Y_{10} = 3\times10^7$ - 80764.52 IMI + 143524 EBR | - 0.6353 | 0.6035 | 0.8763** |

CN = control, T1 = 100 nM EBR, T2 = 150 mg IMI $L^{-1}$, T3 = 200 mg IMI $L^{-1}$, T4 = 250 mg IMI $L^{-1}$, RT = retention time. r = multiple correlation coefficient, $Y_1$ = 4,5-Epithiovaleronitrile, $Y_2$ = 2-Naphthoic acid-methyl ester, $Y_3$ = l-(+)-Ascorbic acid 2,6-dihexadecanoate, $Y_4$ = Phytol, $Y_5$ = 9,12 Octadecadienoic acid (Z,Z), $Y_6$ = 9-Octadecenoic acid, $Y_7$ = (+)-alpha-Tocopherol acetate, $Y_8$ = Ergosta-5,22-dien-3-beta-ol, $Y_9$ = Campesterol, $Y_{10}$ = gamma-Sitosterol. *, ** and *** indicate significance at $p<0.05$, $p<0.01$ and $p<0.001$ respectively, ns = not significant.

In 30 days old plants, methanolic extracts were analyzed by GC-MS to observe the effect of IMI and EBR on the concentration of various phytochemicals. It has been observed that 18 out of 21 phytochemicals were negatively affected with the application of IMI in soil. However, seed soaking with EBR resulted in increase of concentration of all the phytochemicals when EBR treated seeds were grown in soils amended with IMI (Table 8.2.2).

**Table 8.2.2 GC-MS analysis showing the effect of 24-epibrassinolide on various phytochemicals present in 30 days old plants of *Brassica juncea* L. under imidacloprid stress**

| Name of the compound | Peak area (Treatments) | | | Comparison of peak area ( %) | |
|---|---|---|---|---|---|
| | CN | T1 | T2 | CN vsT1 | T1 vs T2 |
| 3-n-Butylthiolane | 4514248 | 1786348 | 2512205 | (-) 60.43 | (+) 40.63 |
| Butanoic acid, 2-ethyl-3-hydroxy-4-(1,1-dimethylethoxy)-, methyl ester | 224975 | 196448 | 269216 | (-) 12.68 | (+) 37.04 |
| 8-Methyl-.alpha.-ionone | 2090086 | 695077 | 851778 | (-) 66.74 | (+) 22.54 |
| Phosphonofluoridic acid, (1-methylethyl)-, ethyl ester | 200270 | 188848 | 283378 | (-) 05.70 | (+) 50.05 |
| 4,7-Octadecadiynoic acid, methyl ester | 242336 | 47159 | 80058 | (-) 80.54 | (+) 69.76 |
| Pentafluoropropionic acid, hexadecyl ester | 605749 | 352547 | 407573 | (-) 41.80 | (+) 15.60 |
| Nonylphenol isomer | 1165217 | 805152 | 3622073 | (-) 30.90 | (+) 349.86 |
| n-Nonylphenol | 2456993 | 2497690 | 3181363 | (+) 01.66 | (+) 27.37 |
| Phenol, 4-nonyl- p-Nonylphenol | 3403154 | 3937508 | 4998894 | (+) 15.70 | (+) 26.95 |
| Phenol, 2-methyl-5-(1-methylethyl)- Carvacrol | 840216 | 159770 | 214994 | (-) 80.98 | (+) 34.56 |
| Tricyclo[3,1,0,0(2,4)]hexane, 3,3,6,6-tetraethyl | 920567 | 671040 | 811179 | (-) 27.11 | (+) 20.88 |
| 1,4-Methano-1H-cyclohepta[d]pyridazine, 4,4a,5,6,7,8,9,9a-octahydro-10,10-dimethyl | 1212662 | 830377 | 1108519 | (-) 31.52 | (+) 33.49 |
| p-tert-Amylphenol | 3783256 | 2653980 | 3453194 | (-) 29.85 | (+) 30.11 |
| 1,2-Epoxy-1,2,5.9,9-pentamethyl-spiro(3.5)non-5-ene | 2565575 | 2025836 | 2510582 | (-) 21.04 | (+) 23.92 |
| Linoleic acid | 1606891 | 501145 | 567894 | (-) 68.81 | (+) 13.31 |
| Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester | 16918525 | 13255456 | 16312678 | (-) 21.65 | (+) 23.06 |
| (1E)-1-Ethylidene-7a-methyloctahydro-1H-indene | 1167356 | 1751856 | 2237279 | (+) 50.07 | (+) 27.70 |
| Palmitic acid | 7012031 | 3960537 | 5114622 | (-) 43.52 | (+) 29.13 |
| Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester | 805831 | 170290 | 425167 | (-) 78.87 | (+)149.67 |
| Stearic acid | 1578269 | 962779 | 1007884 | (-) 39.00 | (+) 0 4.68 |
| Lucenin 2 | 359163 | 198236 | 912949 | (-) 44.81 | (+)360.53 |
| CN = control, T1 = 250 mg IMI $Kg^{-1}$ soil, T2 = (250 mg IMI $Kg^{-1}$ soil + 100 nM EBR), (-) = % decrease and (+) = % increase. | | | | | |