Phytochemicals in *Brassica juncea* L. seedlings under imidacloprid-epibrassinolide treatment using GC-MS

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**ABSTRACT**

*Brassica juncea* L. seedlings were screened for the presence of compounds of medicinal importance under imidacloprid (IMI) stress in binary combination with 24-epibrassinolide (24-EBL). The compounds were identified using gas chromatography-mass spectroscopy (GC-MS). The compounds identified were 4,5-epithiovaleronitrile, 2-naphthoic acid-methyl ester, L-(+)-ascorbic acid 2, 6-dihexadecanoate, phytol, 9,12-octadecadienoic acid (Z,Z), 9-octadecenoic acid, alpha-tocopherol, ergosta-5,22-dien-3-beta-ol, campesterol and gamma-sitosterol. Multiple linear regression (MLR) analysis of data revealed that increase in IMI concentrations results into decline in the concentrations of these compounds, whereas seed pre-soaking with 24-EBL significantly decreased the IMI toxicity and enhanced the phytochemical concentrations.

**Key words:** 24-epibrassinolide, imidacloprid, brassinosteroids, *Brassica juncea*, Indian mustard.

**INTRODUCTION**

*Brassica juncea* L. is a source of micronutrients, antioxidants, vitamins etc. and is generally consumed as vegetable and seed oil. It is also known for its medicinal value and is used to cure various diseases [1,2]. Green leaves of *B. juncea* are known to reduce diabetic disorders like comorbid anxiety [3]. Mental disorders during diabetes and anti-hyperglycemic properties of methanolic extracts have also been reported [1-3, 4,5]. Compounds like alkanes, ketones, glucosinolates, iso-thiocyanates, polyphenols, brassinosteroids have been reported in *B. juncea* [6-11]. Glucosinolates, iso-thiocyanates and brassinosteroids (BRs) are known for their anti-cancerous properties [9,10,12]. Several authors [13-18] reported that application of pesticides cause toxicity to the plants, which results in reduced growth, photosynthesis, phytochemical levels and yield of plants. These authors further reported that BRs increased these parameters in *B. juncea* under pesticide toxicity. Keeping in mind the protective role of BRs in pesticide toxicity, present study aimed to observe the effect of 24-epibrassinolide (24-EBL) on the phytochemical concentrations under imidacloprid (IMI) pesticide stress in *Brassica juncea* L.

**EXPERIMENTAL SECTION**

**Plant material**

Seedlings of *Brassica juncea* L. were raised in Petri-plates with filter paper as substratum and supplemented with different concentrations of IMI (0.00, 150, 200 and 250 mg/L water IMI) and grown in seed germinator (25 ± 0.5°C, 16 h photoperiod). To observe the effect of BRs on phytochemicals in the presence of IMI, seeds were treated with 100 nM 24-EBL for 8 h before sowing. Phytochemical analysis was done after 10 days of seed sowing.

**Sample preparation**

0.5 g of fresh seedlings were homogenised with 0.5 ml of extractant (1 % acetic acid in acetonitrile) and 0.2 g of MgSO₄and 0.1 g of NaOAc was added (To remove excessive water from sample). The mixture was then centrifuged.
at 13000 RPM for 15 min. To the upper centrifuged layer (0.25 ml), 0.035 g of MgSO₄ and 0.015 g of primary secondary amine (to remove excessive fatty acids and sugars) were added. The mixture was again centrifuged at 13000 RPM for 15 min. Upper phase was used for GC-MS analysis.

Analysis using GC-MS
8 µl of sample was injected into Shimadzu GC-MS-QP2010 Plus for phytochemical analysis.

Conditions: Carrier gas: helium, initial column oven temperature 50°C, then raised to 125°C at 25°C/min and finally increased to 300°C at 10°C/min and held for 15 min. Injection temperature: 250°C, injection mode: splitless and column flow: 1.70 ml/min. DB-5ms analytical column was used. Ion source temperature: 200°C and interface temperature: 280°C. Compounds were identified using NIST08s and Wiley7 library.

Statistical Analysis
Data was statistically analysed using multiple linear regression (MLR) and unitless β-regression coefficients using self-coded software.

RESULTS AND DISCUSSION
GC-MS analysis of B. juncea seedlings resulted in the identification of 10 common compounds in all the treatments (Table 1). In the present experiment, it was observed that with increasing IMI concentration, the phytochemical concentration was decreased, however application of 100 nM 24-EBL resulted in recovery of these phytochemicals. MLR analysis also revealed that 100 nM 24-EBL significantly enhanced the phytochemical concentration, which was earlier decreased with the application of IMI. Concentration of IMI regressed negatively on the area all phytochemicals, whereas application of 100 nM EBL regressed positively on the area these phytochemicals (Table 2). 24-EBL was reported to enhance the phytochemical levels of B. juncea leaves under IMI stress [17]. All the compounds detected were reported to have medicinal importance. 4,5-Epithiovaleronitrile was found to be effective against Aedes aegypti [19]. 2-Naphthoic acid derivatives were reported to be effective against cancer cells [20]. L- (+)-Ascorbic acid 2,6-dihexadecanoate was found to have antimicrobial and anti-tumour applications [21-23]. Antibacterial activity of phytol was reported from the extracts of Ipomoea pes-caprae [24]. 9,12 octadecadienoic acid (ZZ) was also observed to possess antimicrobial, antiarthritic and anti-inflammatory properties [23,25] and was found to be present in medicinal plant Cissus vitiginea [26]. Among the identified compounds, 9-octadecenoic acid has antibacterial and antimicrobial properties, gamma-sitosterol has anti-inflammatory and antiasthma activities and alpha-tocopherol has antiplasmodic, antimicrobial and anti-inflammatory properties [21,27]. Ergosta-5,22-dien-3-beta-ol from mushroom was observed to possess antimicrobial activity [28]. Campesterol from Cenchrus setigerus extracts was observed to possess antimicrobial activity [29].
CONCLUSION

Present study establishes the stimulatory effect of 24-epibrassinolide on various phytochemical levels under imidacloprid toxicity, which supports the protective role of BRs under pesticide toxicity.

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