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Research Article

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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 increasedthese 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 $MgSO_4$and 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 $MgSO_4$ 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^{o}C, then raised to 125^{o}C at 25^{o}C/min and finally increased to 300^{o}c at 10^{o}C/ min and held for 15 min. Injection temperature: 250^{o}C, injection mode: splitless and column flow: 1.70 ml/min. DB-5ms analytical column was used. Ion source temperature: 200^{o}C and interface temperature: 280^{o}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 IMIregressed 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 (Z,Z) 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 activites and alpha-tocopherol has antiplasmodic, antimicrobial and anti-inflammatory properties [21,27]. Ergosta-5,22-dien-3-beta-ol from mushroom was reported to be antimicrobial compound [28]. Campesterol from *Cenchrus setigerus* extracts was observed to possess antimicrobial activity [29].

Table 1. Compounds detected in 10 days old *Brassica juncea* L. seedlings using GC-MS

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	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	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

CN = control, T1 = 100 nM 24-EBL, T2 = 150 mg/L water IMI, T3 = 200 mg/L water IMI, T4 = 250 mg/L water IMI, RT = retention time.

Table 2. Multiple linear regression (MLR) analysis showing effect of seed pre-soaking with 24-EBL on phytochemicals in 10 days old *Brassica juncea* L. seedlings grown in IMI supplemented solution

Multiple regression equation	β-regression coefficient		Multiple correlation coefficient	
	β_1	β_2	r	p
$Y_1 = 4\times10^6 - 7184.984\ X_1 + 25487\ X_2$	– 0.4151	0.7870	0.8898	0.01
$Y_2 = 3\times10^6 - 8383.838\ X_1 + 14052\ X_2$	– 0.6133	0.5495	0.8235	0.05
$Y_3 = 2\times10^7 - 52944.65\ X_1 + 42141\ X_2$	– 0.8478	0.3607	0.9213	0.01
$Y_4 = 2\times10^7 - 63946.16\ X_1 + 99781\ X_2$	– 0.6925	0.5776	0.9018	0.01
$Y_5 = 1\times10^8 - 513540.6\ X_1 + 337524\ X_2$	– 0.9114	0.3201	0.9660	0.001
$Y_6 = 1\times10^8 - 445012.6\ X_1 + 452266\ X_2$	– 0.8248	0.4480	0.9386	0.01
$Y_7 = 5\times10^6 - 15259.52\ X_1 + 17811\ X_2$	– 0.7897	0.4927	0.9308	0.01
$Y_8 = 4\times10^6 - 14621.87\ X_1 + 22136\ X_2$	– 0.7153	0.5788	0.9201	0.01
$Y_9 = 2\times10^7 - 47283.28\ X_1 + 41805\ X_2$	– 0.6350	0.3001	0.7023	0.10
$Y_{10} = 3\times10^7 - 80764.52\ X_1 + 143524\ X_2$	– 0.6353	0.6035	0.8763	0.01

X_1 = IMI treatment, X_2 = 24-EBL treatment, β_1 = beta regression coefficient for IMI,β_2 = beta regression coefficient for 24-EBL, 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, Y_8 = Ergosta-5,22-dien-3-beta-ol, Y_9 = Campesterol, Y_{10} = gamma-Sitosterol.

CONCLUSION

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

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