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Effect of soybean plant phenols and flavonoid on the mean leaf area consumed by *Spodopteralitura* and *Spilosoma obliqua* larvae

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Abstract: The aim of the present investigation was to study the effect of soybean plant phenols and flavonoid content on the mean leaf area consumed by *Spodopteralitura* and *Spilosoma obliqua* larva. Phenols and flavonoid content in methanolic leaf extract of thirty three genotypes of soybean were determined by spectrophotometrically. The highest and lowest phenolic content were found in genotypes JS-20-41(2.2±0.073 mg/g) and CSB 904 (0.45 ±0.11 mg/g), respectively. While the highest and lowest flavonoid content was found in genotypes SL 979 4.686± 0.062 mg QE/ g, respectively. In correlation study a highly significant negative correlation was observed between mean leaf area consumed (cm^2) by *S. litura*, phenol content (-0.741) and flavonoid content (-0.737) similarly a highly significant negative correlation was observed between mean leaf area consumed by *S. obliqua*, phenol content (-0.728) and flavonoid content (-0.736) in leaves. Hence it can be concluded that, the genotypes which were having higher phenol and flavonoid content in their leaves offered resistance against *S. litura* and *S. boliqua* in soybean.

Keywords: Flavonoids, Phenols, Soybean, *Spodopteralitura, Spilosoma obliqua*

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is known as "Golden bean", it is a versatile and enthralling crop with countless possibilities of not only getting better agriculture but also supporting industries (Ali, 2008). It contains primary organic and inorganic metabolites such as protein, oil, carbohydrates, minerals and secondary metabolites such as alkaloids and phenolics, including lignin and isoflavones. Primary metabolites are essential for growth, development, and reproduction, but secondary metabolites such as phenolics are associated with plant defense and survival mechanisms under biotic and abiotic stress environment factors such as drought, heat, herbivory and diseases.Phenolics, including phenolic acid, lignin, flavonoids and isoflavones, have been reported to have significant role in seed mechanical damage resistance and disease resistance. Flavonoidsare one of the largest classes of plant phenolic and it develop a defence mechanism in plants for biotic and abiotic stress, like production of flavonoids in plants increases as a result of the exposer to UV-B radiation, it may offer a measure of protection by screening out harmful UV-B radiation (Saviranta *et al*., 2010). Most plants contain an array of flavonoids, whose fingerprints often differ among families, genera and species. Tannins are phenolic compounds found in the leaves of numerous plant species that are known to defend plants against attack from herbivores (Barbehenn *et al.,* 2006). It is found that the phenolic compounds play an important role in plant resistance through antioxidant activity and free radical scavenging activity against pathogens which are intimately connected with reactive oxygen species (Mittapalli*et al*., 2006). The plants produce a high diversity of natural products or secondary metabolites with a prominent function in the protection against predators and microbial pathogens on the basis of their toxic nature and repellence to herbivores and microbes (Schafer *et al*., 2009).

Thus, the aim of this study was to examine leaf extracts of thirty three soybean genotypes for their phenol and flavonoid content as antifeedent compounds and to find out their correlation with mean leaf area consumed by *Spodopteralitura* and *Spilosoma obliqua,*so as to conclude that whether phenol and flavonoid contents in soybean plant act as resistant factor or not.

MATERIALS AND METHODS

The experiment was conducted in Department of Entomology and Department of Plant breeding and genetics GovindBallabh Pant University of Agriculture and Technology, Pantnagar.

Plant materials: Plant material (leaves)of thirty three genotypes viz., CSB 904, DS 2705, DS 2706, DS 2708, DSb 19, DSb 21, JS 20-41, JS 20-69, JS 20-71, KBS 22-2009, KDS 378, KDS 695, KDS 699, KDS 705, KDS 708, MACS 1340, MACS 1394, MACS

1407, MACS 1416, MAUS 612, MAUS 614, NRC 92, NRC 93, NRC 94, PS 1518, RKS 113, RVS-2001-18, SL 958, SL 979, SL 982, SL 688, PS 1092, PS 1347 of soybean were obtained from Crop Research Centre Pantnagar. The leaves were shade dried and vacuum packed until used.

Chemicals: Methanol, Standards of phenolic acids (gallic acid) and of flavonoids (quercetin), Folin- Ciocalteu's phenol reagent, aluminium chloride (AlCl3), was procured from Sigma Chemical Co. All other chemicals used were of analytical grade and purchased locally.

Preparation of leaf extracts: Leaf extracts were prepared according to a standard protocol. Prepared dry leaf powder (5 g) was transferred to dark-coloured flasks and mixed with 100 ml of methanol and stored at room temperature. After 24 h, infusions were filtered through Whatman No. 1 filter paper and residue was re-extracted with equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40 ^{o}C using Rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4 ^{o}C.

Determination of total phenolic contents in the leaf extracts: The concentration of phenolics in leaves extracts was determined using spectrophotometric method (Singleton and Rossi, 1999)with modifications. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The samples were thereafter incubated in a thermostat at 45 ^{o}C for 45 min. The absorbance was determined using spectrophotometer at λ max = 765 nm. Same procedure was repeated for the standard solution of gallic acid and the calibration line was construed

Determination of flavonoid concentrationsin theleaf extracts: The content of flavonoids in leaves extracts was determined using spectrophotometric method (Quettier *et al.,* 2000) with slight modifications. The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The absorbance was determined using spectrophotometer at λ max = 415 nm.

No choice experiment: The antifeedant activity of 33 genotypes of soybean was evaluated against 4th instar larvae of *S. litura*and *S. obliqua* under laboratory conditions (29±5ºC, RH 83±5%) using 'no-choice' feeding technique (Belles *et al.,* 1985 and Kumar, 1993).

Statistical analysis: The experiments were conducted in completely randomized design (CRD) (Gomez and Gomez, 1984) and the means were separated by using STPR 3 software. The correlation between different parameter was analyzed by using STPR-5 software.

RESULTS AND DISCUSSION

The total phenol content in the leaves extract of soy-

Table 1. Total phenolic and flavonoids content in soybean genotypes.

S.N.	Genotypes	Phenolics(mg/g)	Flavonoids (mg/g)
1	CSB 904	0.45 ± 0 .011	0.91 ± 0.004
2	DS 2705	0.90 ±0.008	1.96 ± 0.027
3	DS 2706	1.22 ± 0.011	2.31 ± 0.028
4	DS 2708	1.45 ±0.044	2.78 ± 0.021
5	DSb 19	1.27 ±0.043	2.55 ± 0.022
6	DSb 21	1.52 ± 0.015	3.56 ± 0.039
7	JS-20-41	2.20 ±0.073	4.22 ± 0.030
8	JS-20-69	1.56 ± 0.050	3.31 ± 0.060
9	JS-20-71	0.50 ±0.045	1.02 ± 0.032
10	KBS-22-2009	1.09 ± 0.035	2.09 ± 0.009
11	KDS-378	0.63 ± 0.037	1.32 ± 0.056
12	KDS-693	1.24 ± 0.021	2.90 ± 0.053
13	KDS-699	0.81 ± 0.002	1.59 ± 0.014
14	KDS 705	1.46 ± 0.051	2.58 ± 0.047
15	KDS 708	1.60 ± 0.002	3.18 ± 0.022
16	MACS 1340	0.62 ± 0.020	1.18 ± 0.021
17	MACS 1394	0.58 ± 0.016	1.16 ± 0.009
18	MACS 1407	0.87 ± 0.027	1.69 ± 0.028
19	MACS 1416	0.94 ± 0.029	1.89 ± 0.036
20	MAUS 612	1.68 ± 0.008	3.76 ± 0.017
21	MAUS 614	0.93 ± 0.026	1.80 ± 0.024
22	NRC 92	1.17 ± 0.028	2.79 ±0.029
23	NRC 93	1.22 ± 0.033	2.56 ± 0.043
24	NRC 94	0.61 ± 0.030	1.17 ± 0.061
25	PS 1518	0.88 ± 0.024	1.73 ±0.031
26	RKS 113	1.22 ± 0.027	2.78 ± 0.029
27	RVS 2001-18	1.75 ± 0.057	3.98 ± 0 .031
28	SL 958	1.23 ± 0.008	3.53 ± 0.014
29	SL 979	1.99 ± 0.009	4.69 ± 0.062
30	SL 982	1.26 ± 0.006	3.51 ± 0.067
31	SL 688	1.39 ± 0.041	2.88 ± 0.045
32	PS 1092	0.90 ± 0.031	1.83 ± 0.006
33	PS 1347	1.27 ± 0.041	3.57 ± 0.056

Values are expressed as mean values of three replications ± standard deviation

Table 2. Effect of 33 genotypes of soybean on feeding behaviour of 4th instar larvae of *S. obliqua,* Bihar hairy caterpillar.

S. N.	Genotype name	MLAC (cm^2)	Feeding percentage (%)	Antifeedant activity (%)	Feeding inhibition %	Preference index (C)	Antifeedant category
1	CSB 904	18.03 (4.30)	72.1	0.79	2.01	0.98	Slightly antifeedant
2	DS 2705	13.00 (3.67)	51.98	6.155	18.165	0.82	Slightly antifeedant
3	DS 2706	9.13 (3.10)	36.5	10.28	34.57	0.65	Moderately antifeedant
4	DS 2708	2.17 (1.63)	8.66	17.705	79.315	0.21	Extremely antifeedant
5	DSb 19	4.22 (2.17)	16.86	15.515	63.315	0.36	Strongly antifeedant
6	DSb 21	4.07 (2.13)	16.28	15.67	64.355	0.35	Strongly antifeedant
7	JS 20-41	1.94 (1.56)	7.76	17.94	81.26	0.18	Extremely antifeedant
8	JS 20-69	2.73 (1.79)	10.92	17.1	74.62	0.25	Extremely antifeedant
9	JS 20-71	12.71 (3.63)	50.84	6.46	19.24	0.81	Slightly antifeedant
10	KBS 22-1009	9.94 (3.23)	39.76	9.41	30.745	0.69	Moderately antifeedant
11	KDS 378	15.92 (4.05)	63.66	3.04	8.215	0.92	Slightly antifeedant
12	KDS 693	2.21 (1.64)	8.84	17.655	78.925	0.21	Extremely antifeedant
13	KDS 705	2.27 (1.66)	9.08	17.59	78.42	0.21	Extremely antifeedant
14	KDS 708	10.15 (3.26)	40.58	9.195	29.825	0.70	Moderately antifeedant
15	KDS 99	15.95 (4.05)	63.8	3	8.11	0.92	Slightly antifeedant
16	MACS 1340	16.27 (4.09)	65.06	2.665	7.14	0.93	Slightly antifeedant
17	MACS 1394	17.00 (4.18)	67.98	1.89	4.95	0.95	Slightly antifeedant
18	MACS 1407	3.55 (2.01)	14.18	16.235	68.275	0.32	Strongly antifeedant
19	MACS 1416	5.60 (2.46)	22.4	14.04	54.035	0.46	Strongly antifeedant
20	MAUS 612	6.77 (2.69)	27.08	12.795	46.98	0.53	Moderately antifeedant
21	MAUS 614	5.78 (2.50)	23.1	13.855	52.935	0.47	Strongly antifeedant
22	NRC 93	2.32 (1.68)	9.28	17.54	77.995	0.22	Extremely antifeedant
23	NRC 92	5.47 (2.44)	21.86	14.185	54.89	0.45	Strongly antifeedant
24	NRC 94	14.86 (3.91)	59.42	4.17	11.63	0.88	Slightly antifeedant
25	PS 1518	5.89 (2.52)	23.56	13.73	52.225	0.47	Strongly antifeedant
26	RKS 113	2.25 (1.65)	8.98	17.615	78.63	0.21	Extremely antifeedant
27	RVS 2001-18	1.84 (1.52)	7.34	18.055	82.185	0.17	Extremely antifeedant
28	SL 979	1.71 (1.48)	6.82	18.195	83.34	0.16	Extremely antifeedant
29	SL 982	2.11 (1.61)	8.42	17.765	79.83	0.20	Extremely antifeedant
30	SL 958	6.08 (2.56)	24.3	13.535	51.095	0.49	Strongly antifeedant
31	SL688	2.00 (1.58)	7.98	17.885	80.78	0.19	Extremely antifeedant
32	PS1092	4.17 (2.16)	16.66	15.57	63.635	0.36	Strongly antifeedant
33	PS1347	2.05 (1.59)	8.18	17.835	80.345	0.19	Extremely antifeedant
34	BRAGG	18.77 (4.38)	75.06	0.00	0.00	1.00	Preferred plant
	CD at 5%	0.496					
	F value	**					

** Highly significant at 5 % level, Figures in parentheses are √x+1 value.

Table 3. Effect of 33 genotypes of soybean on feeding behaviour of 4th instar larvae of tobacco caterpillar, *S. litura*(Fab.).

S. N.	Genotype name	MLAC (cm^2)	Feeding Inhibition %	Feeding percentage (%)	Antifeedant activity (%)	Preference index (C)	Antifeedant category
1	CSB 904	17.49 (4.24)	3.57	69.94	1.38	0.97	Slightly antifeedant
2	DS 2705	12.48 (3.60)	20.17	49.92	6.72	0.80	Slightly antifeedant
3	DS 2706	7.42 (2.81)	43.40	29.68	12.11	0.57	Moderatlyantifeedant
4	DS 2708	1.89 (1.54)	81.76	7.54	18.01	0.18	Extremely antifeedant
5	DSb 19	3.78 (2.06)	66.55	15.10	16.00	0.34	Strongly antifeedant
6	DSb 21	3.48 (1.99)	68.81	13.92	16.31	0.31	Strongly antifeedant
7	JS 20-41	1.64 (1.46)	83.98	6.54	18.28	0.16	Extremely antifeedant
8	JS 20-69	2.15 (1.62)	79.45	8.60	17.73	0.21	Extremely antifeedant
9	JS 20-71	11.82 (3.50)	22.74	47.28	7.42	0.77	Slightly antifeedant
10	KBS 22-1009	7.82 (2.88)	41.20	31.28	11.69	0.59	Moderately antifeedant
11	KDS 378	13.81 (3.78)	15.27	55.24	5.29	0.85	Moderately antifeedant
12	KDS 693	1.97 (1.56)	81.05	7.86	17.93	0.19	Extremely antifeedant
13	KDS 705	2.08 (1.60)	80.10	8.30	17.81	0.20	Extremely antifeedant
14	KDS 708	8.47 (2.99)	37.85	33.88	10.99	0.62	Moderately antifeedant
15	KDS 99	15.07 (3.94)	11.00	60.28	3.95	0.89	Slightly antifeedant
16	MACS 1340	15.19 (3.96)	10.56	60.76	3.83	0.90	Slightly antifeedant
17	MACS 1394	15.55 (4.00)	9.42	62.20	3.44	0.91	Slightly antifeedant
18	MACS 1407	2.51 (1.73)	76.46	10.02	17.35	0.24	Extremely antifeedant
19	MACS 1416	4.65 (2.26)	60.36	18.58	15.07	0.40	Strongly antifeedant
20	MAUS 612	5.95 (2.53)	51.87	23.80	13.68	0.48	Strongly antifeedant
21	MAUS 614	5.03 (2.35)	57.74	20.12	14.66	0.43	Strongly antifeedant
22	NRC 93	2.13 (1.62)	79.67	8.50	17.76	0.21	Extremely antifeedant
23	NRC 92	4.32 (2.19)	62.69	17.26	15.42	0.37	Strongly antifeedant
24	NRC 94	13.05 (3.68)	18.03	52.20	6.11	0.82	Slightly antifeedant
25	PS 1518	5.18 (2.38)	56.76	20.72	14.50	0.31	Strongly antifeedant
26	RKS 113	2.04 (1.59)	80.45	8.14	17.85	0.20	Extremely antifeedant
27	RVS 2001-18	1.54 (1.42)	84.84	6.16	18.38	0.15	Extremely antifeedant
28	SL 979	1.42 (1.38)	85.98	5.66	18.52	0.14	Extremely antifeedant
29	SL 982	1.84 (1.52)	82.20	7.34	18.07	0.18	Extremely antifeedant
30	SL 958	5.72 (2.49)	53.30	22.88	13.92	0.47	Strongly antifeedant
31	SL688	1.75 (1.49)	83.00	6.98	18.16	0.17	Extremely antifeedant
32	PS1092	3.51 (2.00)	68.58	14.04	16.28	0.39	Strongly antifeedant
33	PS1347	1.78 (1.50)	82.73	7.10	18.13	0.23	Extremely antifeedant
34	BRAGG	18.78 (4.38)	0.00	75.10	0.00	1.00	Preferred plant
	CD at 5%	0.554					
	F value	**					

** Highly significant at 5 % level, Figures in parentheses are √x+1 value.

Table 4. Simple correlation coefficient between biochemical constituents of soybean genotypes leaves and mean leaf area consumed (cm^2).

Chemical compounds	Mean leaf area consumed by *S. litura*	Mean leaf area consumed by *S. obliqua*
Phenols	-0.741 **	-0.728**
Flavonoids	-0.737**	-0.736**

** Highly significant at 1%

bean genotypes was expressed as µ moles gallic acid equivalent (GAE) per gram extract. The phenol content in the leaves varied from 2.2 ± 0.073 to 0.45 ± 0.011 mg/g of leaves extract Table 1. The highest phenolic content recorded in genotypes JS-20-41(2.2 ± 0.073 mg/g) followed by SL 979, RVS 2001-18, MAUS 612, with 1.998±0.009, 1.751 ± 0.057 and 1.69 ± 0.008 mg/ g respectively, whereas, the lowest total phenolic content recorded in genotypes namely CSB 904 (0.45 ±0.11 mg/g) followed by 0.500 ± 0.045, 0.583 ± 0.016, 0.612 ± 0.030, 0.622 ± 0.020 mg/g of leaf extract respectively for JS 20-71, MACS 1394, NRC 94, MACS 1340 genotypes.

Flavonoids are also one of important biochemical component for protection crop against herbivores. The flavonoid content was expressed as µ mole Quercetin Equivalents (QE) g^{-1} dry wt. Total flavonoid content in methanolic soybean leaf extract ranged from 0.913 ± 0.004 to 4.686 ± 0.062 mg QE/ g Table 1. The genotypes differed with respect to flavonoid content in their leaf extract. Maximum flavonoids recorded in genotypes SL 979 4.686± 0.062 mg QE/ g followed by 4.225±0.030, 3.976±0.031 and 3.560± 0.039 mg QE/ g for JS -20-41, RVS 2001-18 and DSb 21 and PS 1347 respectively for leaf extract of soybean genotypes. Minimum total flavonoids were observed in CSB 904 (0.913± 0.004 mg QE/ g) followed by JS 20-71 (1.022±0.032), MACS 1394 (1.160± 0.009), MACS 1340 (1.181±0.021) and NRC 94 (1.18± 0.061) mg QE/ g of leaf extract respectively.

In no choice feeding experiment for *S. litura* the minimum feeding was observed with SL 979 (1.42 cm^2) and maximum in CSB 904 (17.48 cm^2) over check (Bragg=18.77 cm^2), while the minimum and maximum feeding was found withSL 979 (1.71 cm^2), and CSB 904 (18.03 cm^2) respectively against larvae of *S. obliqua* over control (MLAC=18.76 cm^2). On the basis of preference index DS 2708, JS 20-41, JS 20-69, KDS 693, KDS 705, NRC 93, RKS 113, RVS 113, RVS 2001-18, SL 979, SL 982, SL 688 and PS 1347 genotypes were found to be extremely antifeedant while DSb 19, DSb 21, MACS 1407,MACS 1416, MAUS 614, NRC 92, PS 1518, SL 958 and PS 1092 were found strongly antifeedant and DS 2706, KBS 22-2009, KDS 708 and MAUS 612 were found to be moderately antifeedant, while the remaining genotypes where found slightly antifeedant Tables 2 and 3.

In the present study a fairly high degree of association was found between mean leaf area consumed and with some of important biochemical constituents in soybean genotypes Table 4. A highly significant and negative correlation was observed between MLAC (cm^2) by *S.litura* and *S.obliqua* and Phenol content in leaves (r= -0.741**) and (-0.728**) respectively, flavonoid content in leaves (r = -0.737**) and (-0.736**), respectively.

Thus it can be concluded that the genotypes which were having higher biochemical parameters namely Phenol and flavonoids in their leaves offered resistance against *S.litura* and *S.boliqua* in soybean. Other authors also found that phenols and falvonoids plays aimportant role in plant defence system. (War *et al*., 2011) reported that the plant phenol constitute one of the most common and widespread group of defensive compound which play a major role in host plant resistance against herbivores, including insects. Phenol limits the entry of herbivore by blocking physically or increasing the leaf toughness that reduces the feeding by herbivors, and also decreases the nutritional content of the leaf (Johnson *et al*., 2009). The concentration of the toxic phenolic compounds in the plant is a key factor in deterrence and it is the accumulation of phenols in particular parts of the plant which represent a feeding barrier (Castellanos and Espinosa, 1997 and Zagrobelny*et al.*, 2004). (Simmonds and Stevensoni, 2001) reported that flavonoids shows antifeedant and antibiotic activity towards the larvae of *H. armigera.* (Kondo *et al.,* 1992) also concluded that flavonoids areone of the largest classes of plant phenolic and perform very different functions in plant system including pigmentation and defense from insect herbivory. Girija*et al.*(2008) reported that total phenols exhibited highly significant negative association (-0.763) with per cent pod damage followed by cellulose (-0.706), malic acid (-0.684), pod husk thickness (-0.668), lignin (-0.627) and number of trichomes (-0.596), while hemicellulose showed negative correlation (-0.266) with per cent pod damage although non-significant.Handley *et al.* (2005) reported that trichome density negatively affects the ovipositionalbehavior, feeding and larval nutrition of insect pests. In addition, dense trichomes affect the herbivory mechanically, and interfere with the movement of insects and other arthropods on the plant surface, thereby, reducing their access to leaf epidermis (Howe and Jander 2008). The Glandular trichomes secrete secondary metabolites including flavonoids, terpenoids, and alkaloids that can be poisonous, repellent, or trap insects and other organisms, thus forming a combination of structural and chemical defense (He *et al*., 2011).

Conclusion

A highly significant negative correlationswas observed

between mean leaf area consumed (cm^2) by *S.litura* (-0.741) and *S.obliqua* (-0.728)and Phenol (-0.737) & flavonoid (-0.736) content in leaf extract of soybean genotypes respectively. Thus it can be concluded that, the genotypes which were having higher Phenol and flavonoid content in their leaves offered resistance against *S.litura* and *S.boliqua* in soybean.

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