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Biophysical and biochemical mechanism of resistance to *Spodoptera litura* in groundnut (*Arachis hypogaea* L.)

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Abstract
Spodoptera litura (*S. litura*) is an important insect pest affecting the yields of groundnut worldwide and host plant resistance is a key pest management strategy. Understanding the mechanism of resistance to *S. litura* would help in developing resistant cultivars. In the present study, biophysical (trichome density, relative water content, specific leaf weight, epicuticular wax) and biochemical (reducing, non-reducing and total sugars, protein and phenol content) parameters were analysed in the eight selected *S litura* resistant groundnut genotypes and four checks along with a wild species *Arachis monticola* L. Significant negative correlation was observed between *S. litura* damage and specific leaf weight, midrib, leaf lamina trichome density, wax content, protein and phenol content while, significant positive correlation with reducing and total sugar. Among the *S. litura* resistant genotypes, ICG 928 had higher leaf lamina (140 mm^{-2}) and midrib (180 mm^{-2}) trichome density, specific leaf weight (2.5 g/dm^2), wax content (0.36 $\mu g/cm^2$), protein (4.91 mg/g), and phenol content (3.77 mg/g) compared to susceptible check JL 24 which had higher relative water content (73.3 %), reducing sugar (11.32 mg/g), total sugar (12.75 mg/g) and lower leaf lamina (60 mm^{-2}) and midrib trichome(30 mm^{-2}) density, specific leaf weight (2.2 g/dm^2), wax content (0.2 $\mu g/cm^2$), protein (2.39 mg/g), and phenol content (2.03 mg/g). These biophysical and biochemical components can be effectively utilized in breeding for resistance against *S. litura* in groundnut.

Keywords: Groundnut, host plant resistance, *S. litura*, biophysical, biochemical

Introduction
Biotic and abiotic stresses such as insect attack, pathogen infection, higher or lower temperature, drought and water logging affect the plant growth. Insect pests drastically reduce the crop yield and cause loss of over US$ 14 billion worldwide annually [1]. Furthermore, enormous and indiscriminate use of insecticides causes adverse effect on non-target organisms (predators and parasitoids), pesticide residue in food, pest resurgence, development of insect resistance, toxic effects on human beings and environmental pollution [2]. In this context, host plant resistance is one of the important and eco-friendly approaches of keeping the pest populations below economic injury levels. Improving host plant defence to insects will result in reduced losses due to herbivores, less insecticides use, better crop yields and safer environment [3].

Groundnut is an important oilseed crop in the World and is an important source of digestible proteins, cooking oil and also vitamins [4]. Insect pests are important constraints in achieving higher groundnut yields. In India, the annual yield losses by insect pests in groundnut are about 15 per cent which accounts for about 1.6 million tonnes and 25.27 billion rupees [5]. Among the insects, polyphagous pest *S. litura* (Lepidoptera: Noctuidae) is an important pest of groundnut besides affecting tobacco, cotton, pulses and several vegetables crops [6]. It has been reported that an infestation level of one larva per plant during the seedling or flowering stage resulted in 20% yield loss [7]. Severe outbreak of the pest results in 30-40% loss in pod formation [8]. In India, transitional tract of Karnataka (Dharwad) has been identified as hot spot for *S. litura* during *kharif* season, where yield loss to an extent of 66.6 per cent was reported in groundnut [9]. Larvae feed gregariously on leaves and fresh growth causing extensive damage [10].

Though many effective insecticide molecules are suggested to combat *Spodoptera*, they are not eco-friendly and add to the cost of cultivation especially in semi-arid tropics where farmers

Grow groundnut as subsistence crop. In this regard, breeding for inbuilt resistance occupies importance and is an amenable approach. Many genotypes viz., M 45, M 28-2, NC Ac 343 and ICGV 91180 (11)(12), ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 (13) have been identified as resistant sources to S. litura in groundnut. Many mechanisms may be involved in making a genotype as resistant. Higher enzymatic (POD, PPO, PAL, SOD, APX, LOX and CAT) activity, more amounts of condensed tannins, hydrogen peroxide, protein was found in resistant genotypes compared to susceptible genotypes [13]. Significant correlation noted between damage due to S. litura and plant morphological characteristics such as main stem thickness, hypanthium length, leaf let shape and length, leaf hairiness, standard petal length and petal markings, basal leaf let width, main stem thickness and hairiness, stipule adnation length and width, and peg length [14]. Prasad [13] established the role of protein content of groundnut seed in S. litura resistance and reported that resistant varieties viz., NC Ac 343 (252 µl/ml of extract), R 9227 (270 µl/ml), ICGV 86031 (194 µl/ml) had higher protein content and susceptible checks like TMV 2 (150 µl/ml) and JL 24 (133 µl/ml) had lower protein content. But there is no much work on the understanding of the biophysical and biochemical factors associated with resistance to S. litura in groundnut leaves which will help in deterring the attack by S. litura. In this context, the present study is aimed at analysing the biophysical and biochemical mechanisms of resistance to S. litura in groundnut.

Material and methods

A set of eight groundnut genotypes with less than 10 % leaf damage compared to JL 24 (45.7 %) due to S. litura were selected from the 184 groundnut mini core genotypes and 44 elite groundnut genotypes evaluated for their reaction against S. litura during 2017 rainy season at hot spot location, Dharwad. The details of these genotypes along with checks are provided in Table 1. These eight genotypes along with resistant (ICG 2271) and susceptible (JL 24) checks and popular released groundnut foliar disease resistant cultivars GPBD 4 and G 2-52, and one wild species Arachis monticola maintained at All India Coordinated Research Project on Groundnut, Dharwad, were assessed for biophysical and biochemical components of resistance to S. litura during post rainy season 2018 at 50 days after sowing (DAS) that coincides with grand growth stage with flower initiation at which infestation by S. litura normally results in huge yield loss. Each genotype was sown in a row of 3 m length in 2 replications with 30 × 10 cm spacing in randomized complete block design (RCBD) at Main Agriculture Research Station, University of Agricultural Sciences, Dharwad, India (15o 13' N, 75o 07' E, 678 m above MSL, and 800 mm average annual rainfall) following standard package of practices excluding insecticide sprays.

Biophysical components of resistance

Biophysical traits viz., trichome density on leaf blade, midrib and leaf lamina, specific leaf weight, relative water content and wax content were studied from the top fully opened leaflets of these selected genotypes at 50 DAS.

Trichome density on midrib, leaf lamina and leaf blade

Trichomes on midrib were analyzed by following the procedure Maite *et al*. [15] wherein five fully developed leaves from each genotype were heated with 20 ml of distilled water in a test tube for five minutes in an oven at 85 °C. After five minutes, water of test tube was decanted and added with 20 ml of 96 per cent ethyl alcohol and the samples were heated again for 10 minutes at 80 °C. The alcohol was decanted and the same procedure was repeated 3 times so that chlorophyll content was removed completely. Finally, 20 ml of 90 per cent concentrated lactic acid was added and heated again at 85 °C for 45 minutes upon which, the leaf segments were cleared. Later, the test tubes were cooled and stored for further observation. To observe the midrib hair density, leaf segments were mounted on glass slide and a drop of lactic acid was put on it and observed under stereoscopic binocular microscope (Scope imager, version 9.0) at 10X magnification and number of trichomes on one mm length of midrib was counted. Similarly, the number of laminar trichomes (abaxial surface) and leaf blade trichomes were counted and expressed as number of trichomes on one mm square of leaf lamina and leaf blade.

Specific leaf weight

The specific leaf weight (SLW) indicates the leaf thickness and was determined by method as suggested by Radford [16] and is expressed as g dm-2.

$$SLW = \frac{\text{Leaf dry weight (g)}}{\text{Leaf area (dm2)}}$$

Relative water content

Relative water content was estimated by the method of Kramer [17]. This parameter was estimated in the healthy leaf and 10 discs were made by using cylinder of 0.75 cm2 area. Weight of the discs was taken and expressed as fresh leaf weight (g).Then discs were immersed in water for 4 hours to absorb water and later, discs were taken out and turgid leaf discs were weighed and expressed as turgid leaf weight (g). After that, discs were taken and kept in oven for 48 hours at 70° C for drying. Later, disc weight was taken and expressed as dry weight (g). Relative water content of the leaf in percentage (%) was calculated by using the following formula.

$$\text{Relative water content (\%)} = \frac{\text{(Fresh disc weight - Dry disc weight)}}{\text{(Turgid disc weight - Dry disc weight)}} \times 100$$

Wax content of leaf

The leaf wax was determined by using colorimetric method Ebercon *et al*. [18]. Ten groundnut leaf discs of 0.75 cm2 area was in each genotype and immersed in 15 ml of chloroform for 15 seconds. The extract was filtered and evaporated to dryness on a boiling water bath until choroform smell vanishes. Five ml of acidic potassium dichromate was added to the samples and placed on boiling water bath for 30 minutes. After cooling, 12 ml of deionized water was added. The solution was allowed for 15-20 minutes for the development of colour and optical density of the sample was read at 590 nm in spectrophotometer.

Wax was quantified by using the standard curve prepared by using carbowax 3000 (polyethylene glycol 3000) and expressed as microgram per centimetre square (µg/cm2) area (both surfaces-since wax is present on both the surfaces).

Biochemical components of resistance

The third fully opened healthy leaf from the top in each genotype was taken at 50 DAS in each replication. From this leaf, two grams of leaf sample was collected from each genotype and leaf extract (aliquot) was prepared and biochemical constituent's viz., sugar, phenols and proteins were estimated from each of the selected genotypes.

Preparation of leaf extract in alcohol (aliquot)

The aliquot was prepared by taking two grams of fresh leaf sample and cutting into small pieces and immersing in alcohol (distilled ethanol 80%). The pieces of leaf tissues were ground thoroughly in a pestle and mortar with a little ethanol and passed through the muslin cloth and the extraction procedure was repeated one more time. The filtrates were pooled and filtered through Whatman No. 41 and volume was made to 20 ml with 80 per cent ethanol. The filtrate was clarified by adding 2 ml of saturated lead acetate and 3 ml of di-sodium hydrogen phosphate and allowed over night for settling down of the tissues and then filtered through Whatman No. 42 filter paper. The final volume of clear filtrate was made to 25 ml with 80 per cent ethanol. This constituted the stock solution from which aliquot was drawn for the estimation of sugar, phenols and protein content. The absorbance of each chemical constituent in a sample was measured using spectrophotometer.

For sugar and protein estimation, 1 ml of aliquot was taken from the filtrate and put in the hot water bath until alcohol smell of sample was completely evaporated so as to avoid error in the estimation of sugar and protein. Then sample was utilized for the estimation of sugar and protein. But in phenol estimation removal of alcohol was not mandatory because alcohol content was not having influence on the phenol content of sample.

Estimation of sugar

Standard stock solution was freshly prepared by dissolving 100 mg of D-glucose in a small quantity of distilled water and making volume to 100 ml with distilled water which contained 1 mg of glucose per ml. A 10 ml was taken from this and diluted to 100 ml with distilled water which contained 100 micro g of D-glucose per ml to make the working solution. Reducing sugar in filtrate was estimated by following the procedure as given Nelson [19] (Nelson-Somogyi's method).

The working standard solution was put in different concentration (ml) like 0, 0.2, 0.4, 0.6, 0.8 and 1 and test samples were put in 0.1 and 0.3 ml. All the test tube volume was made to 1 ml by adding the distilled water. Then, 1 ml of alkali copper reagent was added and mixed well. These test tubes were kept in boiling water bath for 20 min. After heating, the test tubes were cooled under tap water without shaking. 1 ml of arseno-molybdate was added in all the test tubes and mixed immediately and volume was made to 20 ml by distilled water. Blue colour was developed. Samples were read at 510 nm against the blank reagent at 100 per cent transmittance (% T).

One ml of alcohol free extract was taken and added with 1 ml of 1N H2SO4 to hydrolyze non-reducing sugar and boiled well which upon cooling under running water, 1-2 drops of phenophthalein indicator was added. Then, 1 ml of 1N NaOH was added drop wise till solution turned pink. Then, 1N H2SO4 was added till pink colour disappears. The volume was made to 5 ml by distilled water. From this, 0.3 and 0.5 ml of extract was taken and the Nelson Somogyi's method was followed as was done for estimation of reducing sugar and absorbance was read at 510 nm.

Estimation of protein

Protein content in the extract was estimated by following Lowry's method [20]. The readings at 660 nm were measured and standard graph was drawn and amount of protein in the sample was calculated and expressed as milligram per gram of leaf sample (mg/g).

Estimation of total phenol

Total phenol was estimated by using Folin-Ciocalteau reagent method of Bray and Thorpe [21]. Stock catechol solution was prepared by dissolving 50 mg of catechol in distilled water and making the volume to 50 ml with distilled water. This solution contained 1 mg of catechol per ml. Working standard solution was prepared by taking five ml of stock standard solution and diluting to 100 ml with distilled water. This working solution contained 1 mg of catechol per ml.

In a series of test tubes, 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard solution was taken and 0.3 and 0.5 ml of aliquot was taken in two different test tubes and volume was made to 1 ml by adding distilled water. Later, 1 ml of 1 N FCR was added to all test tubes. The content was mixed well by shaking. After shaking, 2 ml of 2 per cent sodium carbonate was added. The mixture was shaken well and placed on a hot water bath for one minute. The test tubes were cooled immediately under running water and volume was made to 15 ml with distilled water. Colour absorption was measured at 650 nm in spectrophotometer.

Statistical significance of genotypes for each biophysical and biochemical parameter was calculated over susceptible check JL 24 at critical difference of 5 per cent and 1 per cent level of probability.

Results

Eight genotypes (ICG 928, ICG 76, ICG 2777, ICG 12276, ICG 4412, ICG 9905, Dh 216 and ICGV 93468) were selected from the 184 groundnut mini core and 44 elite groundnut genotypes showing less than 10 % leaf damage compared to susceptible check JL 24 (45.7%) due to S. litura under field condition at hot spot location, Dharwad during 2017 rainy season (Table 1).

Table 1: Selected groundnut genotypes and their reaction to *S litura* during *kharif* 2017

Sl. No.	Genotype	Biological status	Leaf damage by *Spodoptera litura* (%)	Yield per plant (g)
1	ICG 928	Germplasm accession	4.7	26.4
2	ICG 76	Germplasm / Landrace	7.7	26.5
3	ICG 2777	Germplasm / Landrace	8.0	31.6
4	ICG 12276	Germplasm / Landrace	7.5	27.6
5	ICG 4412	Germplasm accession	10.0	27.2
6	ICG 9905	Germplasm accession	8.7	28.3
7	Dh 216	Advanced Breeding line	7.0	19.8

8	ICGV 93468	Advanced Breeding line	9.5	16.7
9	JL 24	Susceptible check	45.47	15.6
10	ICG 2271	Resistant check	7.85	16.2
11	GPBD 4	Popular cultivar	25.65	16.4
12	G 2-52	Popular cultivar	14.92	12.1

Biophysical profile of the selected resistant groundnut genotypes

Among the resistant genotypes, ICG 928 (54.2%) and Dh 216 (55.4%) had significantly less relative water content as compared to susceptible check JL 24 (73.3%) and GPBD 4 (71%). The relative water content was very less (45.7 %) in case of wild species *A. monticola* (Table 2).There was significant positive association of *S. litura* damage with leaf relative water content (Table 3).

Some of the resistant genotypes viz., ICG 928, ICG 76, ICG 4412, Dh 216 and ICGV 93468 had higher specific leaf weight (2.5 to 2.8 g/dm2) similar to that found in resistant check ICG 2271(2.8g/dm2) and wild species, Arachis monticola (2.9g/dm2), while other resistant genotypes viz., ICG 2777, ICG 12276 and ICG 9905 had less specific leaf weight (2.1g/dm2) similar to that of susceptible cultivar JL 24 (2.2g/dm2; Table 2). There was negative correlation between S. litura damage and specific leaf weight (Table 3).

Both the midrib trichome and laminar trichome density varied greatly among the genotypes (Table 2). Resistant genotypes had higher midrib trichome density (130-260 per mm2) (Plate 2) and laminar trichome density (120-260 per mm2) (Plate 1) as compared to susceptible check JL 24 (60 mm-2 & 30 mm-2, respectively). There was significant negative correlation of midrib and laminar trichome density with *S. litura* damage. There was no much difference in number of leaf blade trichomes between resistant and susceptible genotypes (Table 2). There was also non-significant correlation between leaf blade trichome density and S. litura damage (Table 3).

Wax content in leaf was significantly higher in resistant genotypes (0.28 to 0.36 ug/cm2) compared to susceptible check JL 24 (0.20 ug/cm2; Table 2). The wax content was highest in case of wild groundnut species *Arachis monticola* (0.40ug/cm2). There was significant negative correlation (-0.765) between S. litura damage and leaf wax content (Table 3).

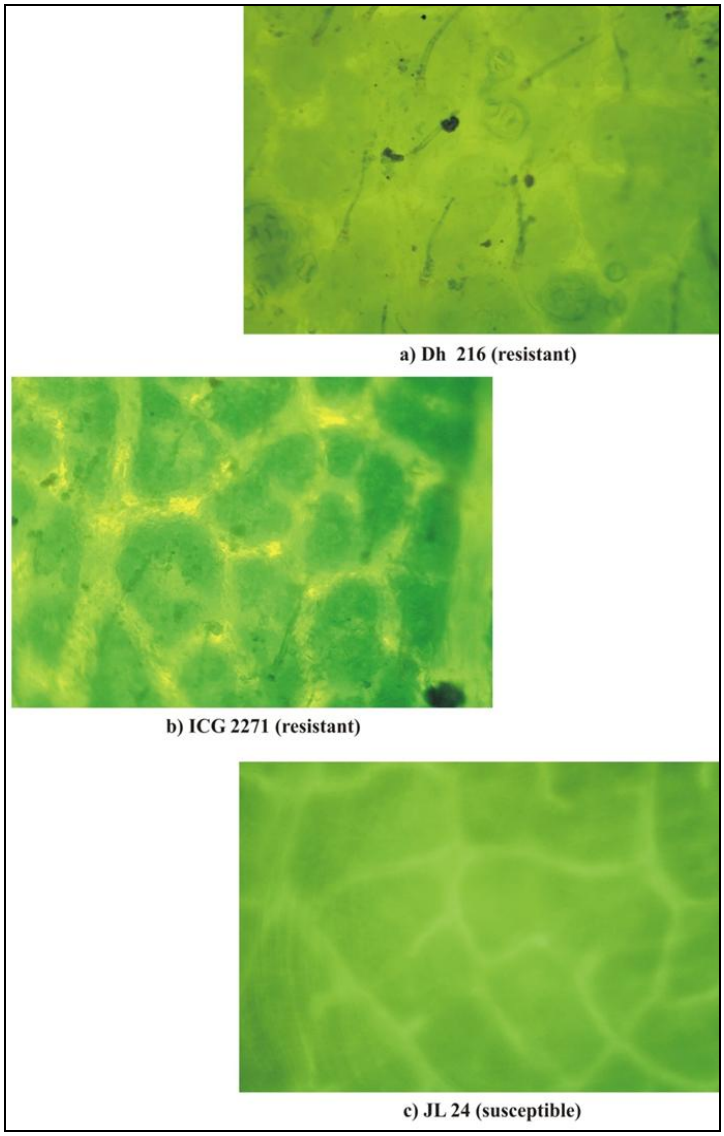

Plate 1: Laminar trichome density in resistant and susceptible groundnut genotypes

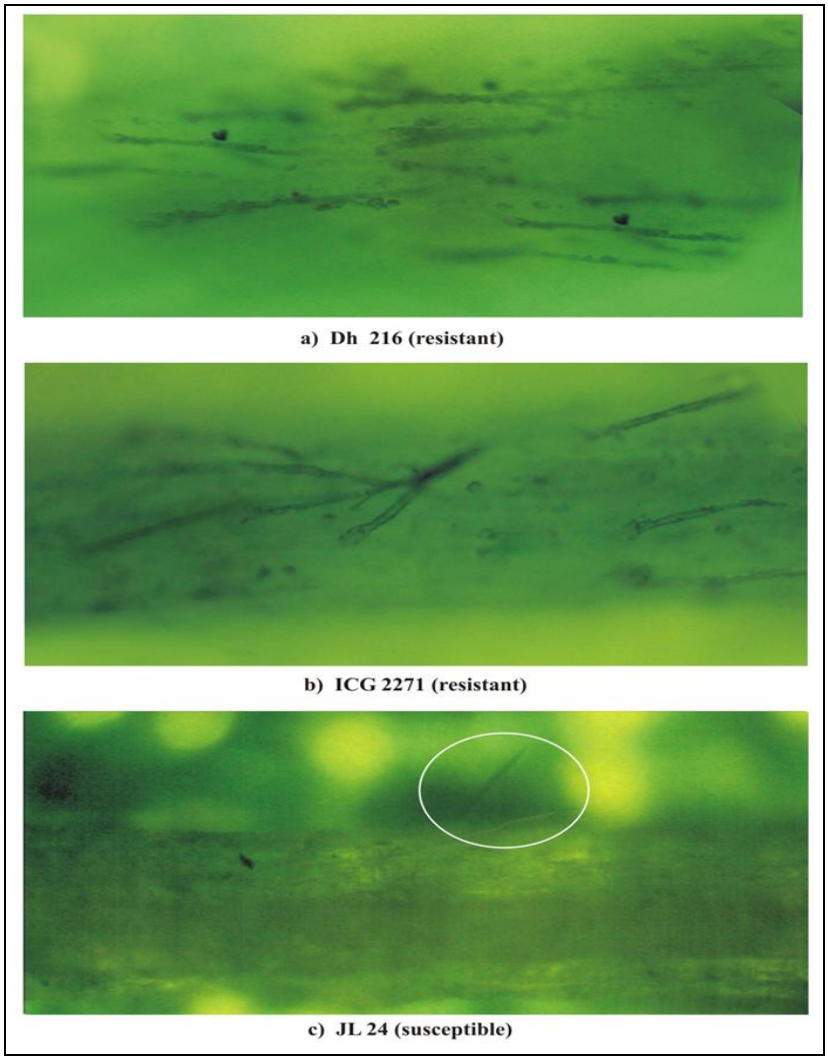

Plate 2: Midrib trichome density in selected resistant and susceptible groundnut genotypes

Table 2: Biophysical and biochemical components of resistance to *Spodoptera litura* in selected genotypes at 50 days after sowing

S. No	Genotype	*Spodoptera* damage (%)	Wax (µg/ cm^2)	Specific leaf weight (g/dm^2)	Relative water content (%)	Trichome density (Numbers per mm^2)			Reducing sugar (mg/g)	Non-reducing sugar (mg/g)	Total sugar (mg/g)	Protein (mg/g)	Phenol (mg/g)
						Leaf blade	Mid rib	Leaf lamina					
1	ICG 928	5.00	0.36	2.5	54.2	140	180**	140**	4.75**	3.11	7.87**	4.91**	3.77**
2	ICG 76	6.85	0.38	2.8**	59.0	150	240**	220**	5.37**	1.06	6.44**	5.22**	4.10**
3	ICG 2777	7.65	0.37	2.1	66.4	140	180**	170**	7.05**	1.79	8.85**	4.12**	2.93**
4	ICG 12276	8.40	0.37	2.1	66.8	170	130**	160**	6.55**	2.44	9.00**	3.80**	2.76**
5	ICG 4412	9.00	0.37	2.5*	52.1	130	180**	180**	4.75**	2.44	7.20**	3.64**	3.49**
6	ICG 9905	9.35	0.28	2.1	55.7	200	130**	260**	4.99**	4.71	9.71**	2.85	3.19**
7	Dh 216	7.00	0.31	2.8**	55.4	180	220**	120**	5.52**	3.99	9.51**	5.73**	3.96**
8	ICGV 93468	9.50	0.28	2.8**	56.1	160	200**	120**	4.60**	4.47	9.07**	5.20**	3.53**
	Checks												
9	ICG 2271	7.85	0.27	2.8**	59.1	180	210**	200**	5.33**	2.33	7.67**	6.81**	3.13**
10	JL 24	43.45	0.20	2.2	73.3	140	60	30	11.32	1.43	12.75	2.39	2.03
11	GPBD 4	25.65	0.26	2.2	71.0	160	70	90	8.80**	3.99	12.77	2.81	2.51*
12	G 2-52	14.92	0.36	2.7	65.9	270	140**	140**	6.47**	2.18	8.65**	3.49**	2.82**
13	*A. monticola*	0.00	0.395	2.9	45.7	280	260**	170**	3.13**	1.48	4.62**	9.34**	5.34**
Mean		11.89	0.32	2.49	59.4	176	169	154	6.05	2.72	8.77	4.63	3.35
Minimum		0.00	0.20	2.05	49.4	130	60	30	3.14	1.07	4.62	2.38	2.03
Maximum		43.45	0.39	2.87	76.1	280	260	260	11.32	4.71	12.77	9.34	5.34
CD (5%)		4.24	0.03	0.29	10.2	57.1	46.8	49.6	0.90	0.48	0.96	0.54	0.46
CD (1%)		5.95	0.04	0.41	14.4	80.0	65.6	69.5	1.26	0.68	1.35	0.77	0.65
CV (%)		16.37	4.48	5.39	7.9	14.8	12.6	14.7	6.84	8.20	5.05	5.43	6.38

*&** - indicates the superiority of the genotype over the susceptible check genotype (JL 24) at 5 per cent and 1 per cent level of probability, respectively

Table 3: Phenotypic and genotypic correlation of biophysical and biochemical parameters and *Spodoptera litura* damage

Traits	Reducing sugar	Non-reducing sugar	Total sugar	Protein	Phenol	Wax	Specific leaf weight	Relative water content	Leaf blade trichome	Mid rib trichome	Leaf lamina trichome	Colour of leaf	*Spodoptera* damage
Reducing sugar	1	-0.165	0.845***	-0.663**	-0.812**	-0.604**	-0.517**	0.859**	-0.336	-0.803**	-0.626**	-0.281	0.900**
Non-reducing sugar	-0.167	1	0.38	-0.228	-0.091	-0.373	-0.163	-0.187	-0.054	0.181	0.028	0.157	-0.077
Total sugar	0.844**	0.387	1	-0.744**	-0.809**	-0.765**	0.573**	0.701**	-0.344	-0.849**	-0.569**	-0.177	0.800**
Protein	-0.688**	-0.239	-0.774**	1	0.829**	0.364	0.705**	-0.598**	0.420*	0.810**	0.248	0.226	-0.6305**
Phenol	-0.842**	-0.112	-0.849**	0.856**	1	0.563**	0.626**	-0.768**	0.401	0.828**	0.376	0.301	-0.721**
Wax	-0.637**	-0.385	-0.805**	0.395*	0.598**	1	0.175	-0.464*	0.210	0.583**	0.486*	0.296	-0.737**
Specific leaf weight	-0.576**	-0.180	-0.637**	0.770**	0.697**	0.228	1	-0.499**	0.333	0.684**	0.054	0.357	-0.404*
Relative water content	0.978**	-0.218	0.879**	-0.759**	-0.935**	-0.507**	-0.702**	1	-0.2248	-0.745**	0.512*	0.226	0.776**
Leaf blade trichome	-0.360	-0.104	-0.395*	0.498**	0.393*	0.241	0.452*	-0.204	1	0.250	0.154	0.106	-0.230
Mid rib trichome	-0.850**	-0.205	-0.907**	0.886**	0.923**	0.592**	0.841**	-0.962**	0.220	1	0.487*	0.370	-0.790**
Leaf lamina trichome	-0.733**	0.016	-0.676**	0.256	0.441*	0.513**	0.115	0.704**	0.205	0.517**	1	-0.085	-0.699**
Colour of leaf	-0.305	0.137	-0.210	0.230	0.305	0.360	0.361	-0.310	0.07	0.428*	-0.103	1	-0.334
Relative chlorophyll content (SPAD)	0.427*	0.025	0.414*	-0.503**	-0.511**	-0.03	-0.119	0.532**	-0.382	-0.332	-0.130	0.1432	0.197
Spodoptera damage	0.955**	-0.080	0.850**	-0.646**	-0.770**	-0.765**	-0.417*	0.922**	-0.299	-0.862**	-0.738**	-0.372	1

*& **- Significant at 5 and 1 per cent level of probability, respectively Values above the diagonal represent phenotypic correlation while, below the diagonal represent genotypic correlation

Biochemical profile of the selected resistant groundnut genotypes

Resistant genotypes had significantly less amount of reducing (3.13-7.05 mg/g) and total sugar (4.62-9.71 mg/g) as compared to susceptible check JL 24 (11.32 and 12.75 mg/g, respectively; Table 2). There was significant positive correlation between reducing and total sugar with S. litura damage (Table 3). There was no significant difference in content of non-reducing sugar between resistant and
Susceptible genotypes (Table 2).

Higher protein content of 2.85-9.34 mg/g was recorded in resistant genotypes as compared to lower 2.03 and 2.51 mg/g in susceptible genotypes including check JL 24 (2.39 mg/g; Table 2). Significant negative correlation was observed between S. litura damage and protein content (Table 3).

Total phenol content was significantly higher in resistant genotypes viz., ICG 76 (4.1 mg/g) and in wild species of groundnut A. monticola (5.34 mg/g) compared to susceptible genotype JL 24 (2.03 mg/g) and GPBD 4 (2.51 mg/g) (Table 2). Total phenol had significant negative association with S. litura damage (Table 3).

Discussion

Plant resistance to insect pest is a complex phenomenon, which is the result of interaction between many factors in the plant. These may be biophysical or biochemical factors. Biophysical factors like leaf succulence, leaf thickness, leaf trichome density, wax content and biochemical factors like reducing sugar, non-reducing sugar, total sugar, protein and phenol content were estimated in the resistant genotypes for their possible role in imparting resistance to S. litura in groundnut.

Biophysical characters in plants interfere with host selection, feeding, ingestion, digestion, mating, oviposition and behaviour of insect pests. The biophysical traits like trichome density on leaf, leaf succulence, leaf thickness, and wax content play a vital role in imparting resistance to insect pests with either positive or negative impact on its feeding habit or life cycle. In order to determine the role of biophysical traits towards resistance against S. litura, leaf samples were collected at 50 DAS which was coinciding with higher incidence of S. litura at Dharwad. They were analysed for different biophysical parameters. Earlier, Patil [13] has reported higher incidence of *S. litura* at Dharwad at peak flowering to peg initiation stage of the crop during rainy season.

Relative water content

Leaf relative water content indicates the leaf succulence in a particular genotype. In the present study, relative water content was significantly less in resistant genotypes which make these genotypes less-palatable for feeding by S. litura. Increased relative water content in leaf facilitates the increased level of feeding by *S. litura* due to thinner cell walls or higher turgidity of the tissues [22]. Waterman *et al*. [23] observed that most of the herbivores feed preferentially on leaves with high relative water content. The present results are in agreement with the findings of Patil *et al*. [24] who revealed the significant positive association between low relative water

content of leaf and *S. litura* resistance in *Arachis monticola* L. Sesane *et al*. [25] also revealed significant positive association between relative water content and *S. litura* damage in soybean genotypes.

Specific leaf weight

Specific leaf weight is another biophysical parameter which indicates the relative thickness of the leaf. No specific relationship between resistant genotypes and specific leaf weight indicates that this parameter may not be important in imparting resistance in the genotypes studied against *S. litura* in groundnut. Earlier Deshnmukh *et al*. [26] Vishalakshi [27] and Reddy [28] reported comparatively thicker leaves in leaf miner resistant genotypes than in susceptible groundnut genotypes. Dhaliwal and Singh [29] and Lakshminarayanan *et al* [30] reported that toughness and thickness of various plant parts adversely affected the penetration and feeding by insects. Praveen [31] reported that leaf miner damage had significant negative correlation with specific leaf weight in groundnut. Wightman and Ranga Rao [32] observed that the newly hatched larvae rejected leaves of ICGV 86031 and suggested that leaf toughness acts as a major factor in rejecting the feeding through scraping by neonate larvae. Prasad and Gowda [11] reported that reduction in larval weight reared on ICGV 86031 could be due to toughness of the leaves. Chandramani *et al*. [33] noted that maximum leaf thickness in rice leaf lead to resistance to major insect pests in rice. Sesane *et al*. [25] also reported existence of a negative correlation between defoliators damage and specific leaf weight in soybean. Contrasting results about specific leaf weight on imparting resistance could be due to different feeding habit of S. litura and leaf miner in groundnut or different mechanism operating in different crops or genotypes.

Trichome density

Among the biophysical parameters, trichome density at both midrib and lamina were reported to have a major role in imparting resistance to defoliators and sucking pests. Negative and significant correlation between damage due to *S. litura* and trichomes on midrib and leaf lamina indicate the role of trichomes in imparting resistance to *S. litura*. The present findings are in conformity with the results of RangaRao [34] and Vishalakshi [35] who recorded significantly more number of trichomes on midrib as well as on leaf lamina in resistant genotypes to leaf miner. Near to midrib of the leaf, S. litura prefer to lay eggs. So, midrib trichome density hinders in the oviposition of the insect, thus imparting resistance to *S. litura*. Leaf laminar trichome density hinders insect movement and feeding activity. Thus, leaf laminar trichome density acts as a physical barrier for insect damage. But, leaf blade trichome density did not have any adverse effect on S. litura damage. This could be associated with feeding habit of *S. litura* which will not prefer feeding from the leaf blade.

Epicuticular wax

The epicuticular wax content is associated with both biotic and moisture deficit stress resistance in several crop species and also in groundnut. Wax interferes with the feeding activity of insect that make insect to reject host because of gummy substance of leaf. Though no reports on the impact of wax content on Spodoptera resistance in groundnut is reported in literature, the interaction between *B. oleracea* (cabbage, broccoli, cauliflower) and *Plutella xylostella* (L.) (Lepidoptera) highlight the interface between plant waxes and herbivore resistance [36, 37, 38]. This non-preference behaviour causes a lack of establishment, reduced feeding and increased larval mortality [39, 40]. Thus, epicuticular wax imparts resistance to insect pests in groundnut and especially against *S. litura*.

Among the various biophysical traits, wax content, specific leaf weight, trichome density at midrib and leaf lamina can be utilized in the indirect selection and breeding of *S. litura* resistant groundnut genotypes.

Biochemical parameters

Biochemical parameters such as sugar, protein and phenol in the plant parts play a key role in imparting insect resistance. The differential amount of these biochemical constituents makes a genotype as susceptible or resistant. Hence, analysis of the biochemical parameters in the resistant genotypes will be useful in understanding the mechanism of resistance to a particular insect pest. This information would help a breeder to effectively incorporate this trait of important mechanism against a particular insect pest.

Sugar

Among different biochemical constituents, sugar (reducing and total sugar) contributes greatly for resistance or susceptibility of the host. Lower amount of reducing (Fig. 1) and total sugar in the resistant genotypes indicates the role of sugar content in making a genotype resistant or susceptible. Earlier, Praveen [31] reported significant and positive association between reducing sugar, total sugar and leaf miner damage in groundnut variety KRG 1. Similarly, Vishalakshi [35] and Senguttuvan and Sujatha [41] reported increased leaf miner damage with higher soluble sugar. Nazeem [22] also reported reducing, non-reducing and total sugar contributing greatly to susceptibility of the host to insect pests. Susceptibility was associated with higher sugar content in leaf and also in other plant parts because sugar act as feeding stimulant for insects which was documented [42, 43]. There was no correlation between non-reducing sugar and damage due to S. litura indicating no role of non-reducing sugar in imparting resistance against *S. litura* in groundnut.

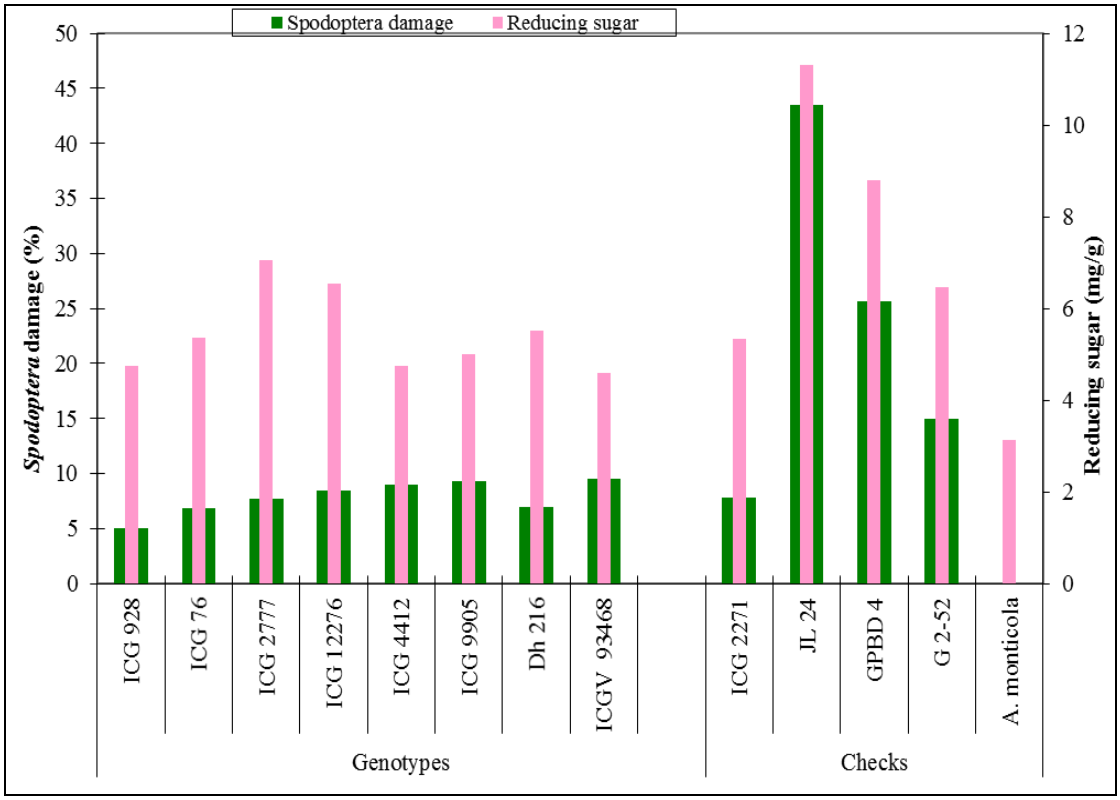

Fig 1: Reducing sugar content in *S. litura* resistant and susceptible groundnut genotypes

Protein

Protein content in leaf is one of the important biochemical components in imparting insect resistance in plants. Higher protein content in the resistant genotypes (Fig. 2) indicates the role of protein in making a genotype as resistant or susceptible. The results of the present study are in agreement with Prasad [13] who reported that protein content was more in resistant genotypes than in susceptible genotypes. They have also reported that there was significant negative correlation between protein content in the leaf and S. litura damage. Higher amount of proteins could be attributed to the greater activity of plant defensive enzymes and production of other plant defensive proteins [13].

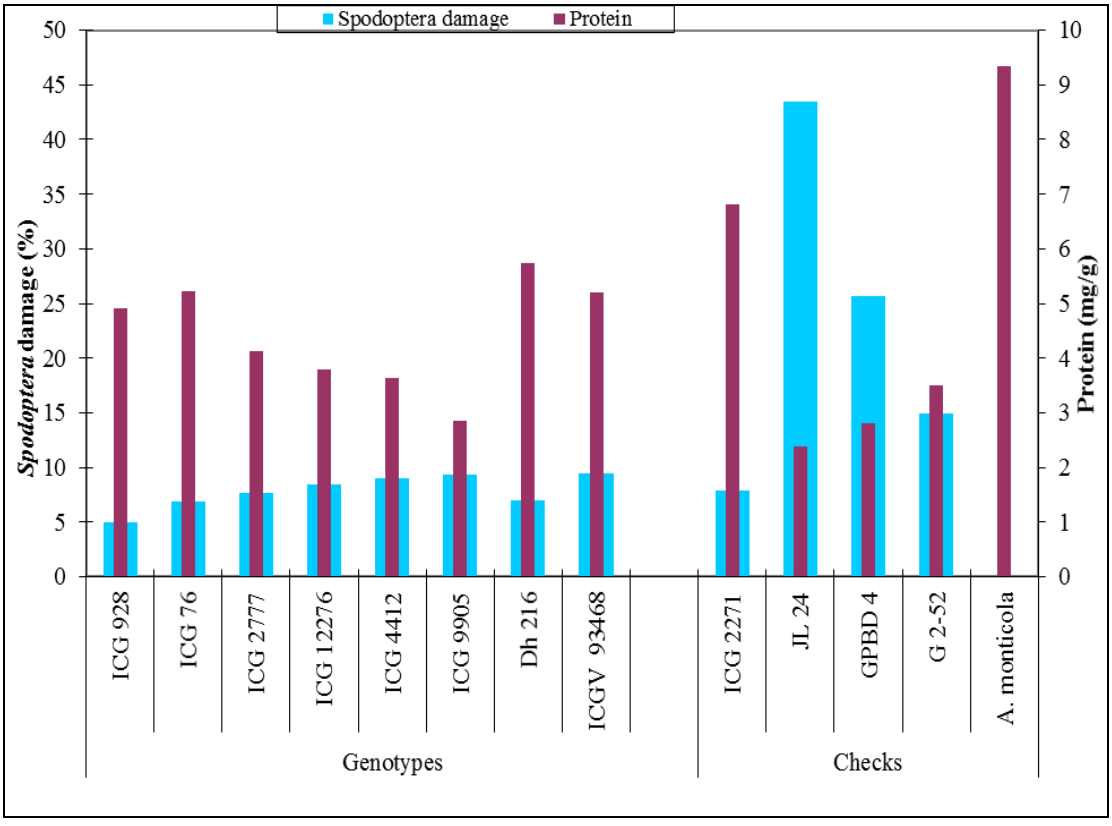

Fig 2: Protein content in *S litura* resistnat and susceptible groundnut genotypes

Total phenol

Among all the biochemical components, phenols play a vital role in imparting resistance to insect pests in plant [44]. Higher total phenol content in resistant genotypes shows vital role of phenol content in imparting resistance to S. litura in groundnut genotypes (Fig. 3). Nazeem [22] reported that correlation between phenol content and defoliator damage and population had significant negative association. Sahoo and Patnaik [45] also observed a negative association between total phenol content and pod borer damage in pigeon pea. Rao [46] showed that more phenol content in the leaf reduced the incidence of S. litura in groundnut. Strong negative association between per cent foliage damage by leaf miner and larval population with total phenol content in the leaf was reported in groundnut [33]. RangaRao [34] Vishalakshi [35] and Senguttuvan and Sujatha [41] reported higher phenolic content in leaf which contributed for resistance against leaf miner. Resistance in wild species of groundnut against S. litura was associated with presence of several quercetindi-glycosides and caffeoylquine acids [47]. Total phenol acts as a defensive source against insect attack because oxidation of phenols produces toxic quinones, which covalently bind to leaf proteins thereby inhibiting protein digestion in herbivores [48]. Thus, the present investigation showed that total phenol concentration as a leading and reliable indicator of resistance against *S. litura*.

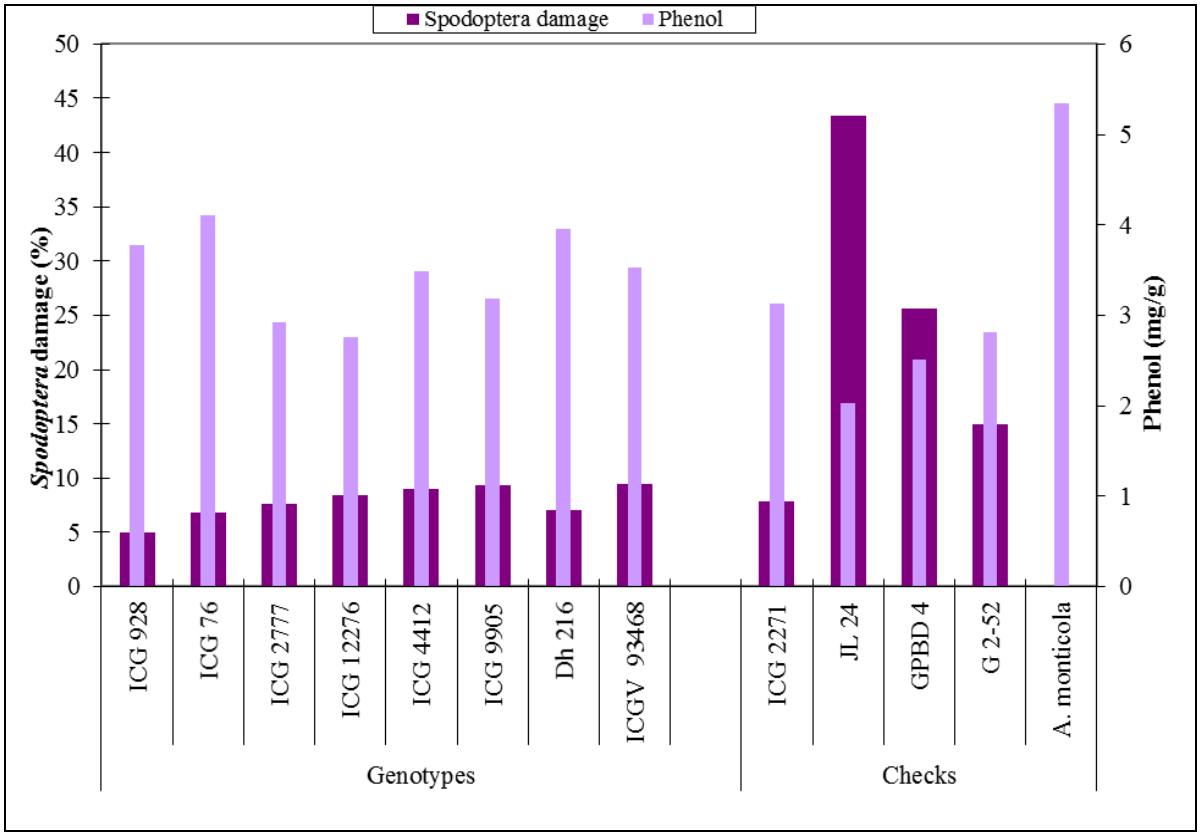

Fig 3: Phenol content in *S litura* resistant and susceptible groundnut genotypes

Conclusion

Biophysical (Avoidance/Non preference) and biochemical parameters (antibiosis) play a vital role in imparting resistance in crop plants. There was significant phenotypic and genotypic negative correlation between *S. litura* damage and specific leaf weight, midrib trichome density, leaf lamina trichome density and wax content. On the contrary, there was significant positive correlation with relative water content. Among the various biophysical traits, wax content, specific leaf weight, trichome density at midrib and leaf lamina can be utilized in the indirect selection of *S. litura* resistant genotype. In case of biochemical factors, there was significant phenotypic and genotypic positive correlation between *S. litura* damage and reducing sugar, total sugar while, significant negative association with protein content and phenol content. Among the biochemical traits, phenol content can be utilized as indirect selection criteria for identifying resistant genotypes in groundnut against *S. litura*. The present study has shown the importance of various biochemical and biophysical components in imparting resistance against Spodoptera litura in groundnut. Further, these components of resistance can be employed in resistance breeding programme against *S. litura*. The genotypes with different mechanisms of resistance could be hybridized to pool the genes to enhance the level and effectiveness of resistance.

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