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Defensive Role of Fibre Fractions in Rice Genotypes against Rice Leaf Folder *Cnaphalocrocis medinalis* (Guenee)

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***Abstract*:** Rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) is a major insect pest in rice and can cause serious damage under favourable conditions. Host plant resistance is an economical, feasible and eco-friendly option for management of this pest. Fibre fractions viz. cellulose, hemicellulose and lignin were quantified in seven genotypes and their effect on larval and pupal parameters was studied. The total larval duration ranged from 15.1-19.6 days in these genotypes. Prolonged duration was in TKM6 and IET22155 (19.5 and 19.6 days, respectively), while percentage pupation (54.0% and 64.0%, respectively) was significantly less on these genotypes. Final instar larval weight was less in W1263, TKM6 and IET22155 (22.7-24.9 mg) than in other genotypes (>25.5 mg). Pupal weight varied from 17.1-22.9 mg and TKM6 and IET22155 recorded lesser pupal weight (17.1 and 18.8 mg, respectively). The cellulose and hemicellulose content was higher in TKM6, W1263 and IET22155, while lignin content was higher in TKM6, W1263 and JGL21066. Positive correlations were observed between larval duration and cellulose and hemicellulose. While negative correlations of larval weight with cellulose and hemicellulose was recorded. The per cent pupation was negatively correlated to lignin. Higher amount of the fibre fractions has negatively impacted the pupal weight.

***Keywords*:** *Cnaphalocrocis medinalis*, Rice leaffolder, Fibre fraction, Resistance, Cellulose, Hemicellulose, Lignin

Rice is the staple food of more than 60 per cent of the world population. India has the largest area among rice growing countries and stands second in production following China. More than 100 species of insects attack this crop in Asia and 20 of these are of economic importance and yield loss due to these insect pests has been estimated at about 25 per cent (Sharma et al 2017). In India, losses incurred by different insect pests of rice are reported to the tune of 15,120 million rupees which is 18.60 per cent of total losses (Chandramani et al 2010). Rice leaffolder (RLF), once considered as minor pest but due to application of high doses of nitrogen fertilizers and non-judicious use of insecticides causes RLF outbreaks (Punithavalli et al 2013). Due this pest, the losses in seed yield have been reported from 20-50% at tillering and flowering stages, respectively (Padmavathi et al 2013). Various chemicals recommended for the control of RLF does not achieved the desired control and their indiscriminate use causes resistance, resurgence and residue problems (Wang et al 2009). Therefore, the use of host plant resistance is the most effective and safest measure for RLF management as it is less expensive and ecologically safe method and can be easily adopted by farmers. By understanding the mechanism of biochemical activities responsible for resistance; these can be incorporated into the cultivated plants and this will help to breed crop varieties that support lower RLF population or that can better tolerate insect infestation. Plant genotypes, either due to environmental stress or genetic makeup, possess physiological and biochemical differences which alter the nutritional value for plant feeding insects that in result make host plant unfavorable to phytophagous insects (Mitchel et al 2016). The rice genotypes have also shown such diversity in antibiosis and biochemical factors influencing the biology of the insect pests (Muduli et al 2021). At present, the information about the host plant resistance mechanism due to the crude fibres i.e. cellulose, hemicellulose and lignin in rice genotypes is very limited and there has been a large scope to work on this area. Therefore, the present studies are undertaken to estimate the effect of the fibre fractions on larval weight, pupal weight and per cent pupation of RLF.

MATERIAL AND METHODS

All the studies related to biological parameters of *C. medinalis* on seven rice genotypes was carried out at Rice Research Farm, Punjab Agricultural University (PAU), Ludhiana (30°54'N and 75°48'E, 247 m above mean sea level) during 2016-17 wet crop season. The analysis of fibre fractions at constitutive and induced levels was carried out in Animal Nutrition Laboratory, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana during 2017-18 wet crop season.

Raising of test plants and *C. medinalis* population: The

seeds of the test genotypes (TKM6, IET22155, RP4918-142, JGL21066 and W1263) were obtained from Indian Institute of Rice Research, Hyderabad and TN1 and PR121 from Rice section, PAU. These test genotypes were grown separately in earthen pots (20 cm diameter). The insect culture was multiplied in Rice Entomology Laboratory, Dept. of Plant breeding & Genetics on TN1 plants. The seeds of TN1 were sown in pots (20 cm diameter) at periodic intervals to ensure continuous supply of fresh green plants. These pots were placed in the insect rearing cages and insect larvae collected from field were introduced in the cages on 30 days old plants (DOPs). The food plants were changed regularly to maintain the insect culture. Adults emerging in the rearing cages were transferred to new cages provided with TN1 plants and 10 per cent honey solution for oviposition to maintain the insect culture.

Larval and pupal parameters of *C. medinalis*: All the experiments related to above studies were evaluated under screen house conditions in completely randomized design (CRD). The experiments were replicated five times and five potted plants of each genotype having pot size of 20 cm constituted one replication. Two neonate larvae (1st instar) from the insect culture were released on 50 DOPs of test genotypes. Plants were then covered with mylar cages and kept in screen house at 28±2°C and 75±5% RH conditions. These plants were observed daily during larval development and total time taken by the larva to pupate was recorded to calculate larval duration and last instar larvae were collected to measure the weight. Then, number of pupae formed was counted to calculate percentage pupation and newly formed pupae were collected and pupal weight was recorded.

Fibre fractions: A separate set of experiments were laid out to study various fibre fractions in leaf blades of 50 and 70 DOPs of different genotypes under constitutive and induced conditions and such studies were replicated five times. Five fourth instar larvae were used per replication to get infested plants for biochemical analysis in induced resistance conditions. Standard methods of AOAC (2000) were followed for the determination of cellulose, hemicellulose and lignin.

Determination of hemicellulose: Hemicellulose was calculated after determining neutral detergent fibre (NDF) and acid detergent fibre (ADF). To determine NDF, half gram of dried and ground rice leaves sample was taken in a spoutless beaker and 50 ml of neutral detergent solution (NDS) was added to the sample. The mixture was heated on a hot plate and the liquid content was filtered through sintered glass crucible mounted on suction flask. Vacuum was created using suction pump and residue was washed with hot distilled water and acetone. The crucible was then kept at 100^{0}C in hot air oven for overnight. The crucible was weighed after cooling in a desiccator**.** The NDF of the sample was calculated by the given formula:

NDF (%) =W_b-W_a/ W_o x 100

Where, W_a = Weight of oven dried crucible; W_o = Initial weight of sample (dried & ground leaves); W_b = Weight of oven dried sample and crucible

To determine ADF, half gram of dried and ground paddy straw sample was taken in a spoutless beaker and 50 ml of acid detergent solution (ADS) was added to the sample. The mixture was heated on a hot plate and the liquid content was filtered through sintered glass crucible mounted on suction flask. Then vacuum was allowed from a suction pump and washing with hot distilled water and acetone was done. The crucible was kept at 100^{0}C in hot air oven for overnight. The crucible was weighed after cooling in a desiccator**.** The ADF of the sample was calculated by the given formula:

ADF (%) =W_b-W_a/ W_o x 100

Where, W_a = Weight of oven dried crucible; W_o = Initial weight of sample (dried & ground leaves); W_b = Weight of oven dried sample and crucible

Then hemi-cellulose was obtained by subtracting ADF from NDF

Hemicellulose (%) = NDF(%) – ADF(%)

Determination of cellulose and lignin: Cellulose and lignin were calculated after determining acid detergent lignin (ADL).To determine ADL, cold solution of 72% H_2SO_4 (w/w) was added to the residue of ADF. The lumps were broken with a glass rod. The crucible was refilled with the solution as the acid drains. Then suction was applied to wash the contents of the crucible with hot distilled water until the washings were acid free to pH paper. The crucible was heated at 100^{0}C in hot air oven. Dried sample was cooled and weighed. Then cellulose content of the sample was calculated.

Cellulose (%) = W_b-W_c/ W_o x 100

Where, W_o = Initial weight of sample (dried & ground leaves); W_b = Weight of oven dried fibre (ADF) and crucible; W_c = Weight of 72% H_2SO_4 treated sample and silica crucible

To determine lignin content, the crucible containing 72% H_2SO_4 treated sample was ignited at 600^{0}C in muffle furnace for 3 hour and the crucible was placed in oven at 100^{0}C for 1 hour after removing it from furnace. The crucible was cooled in a desiccator and weighed. Lignin was determined as given below:

Lignin (%) = W_c-W_d/ W_o x 100

Where, W_o = Initial weight of sample (dried & ground paddy straw); W_c = Weight of 72% H_2SO_4 treated sample and crucible; W_d = Weight of furnace burnt sample and crucible

Statistical analysis: The data from different larval and pupal experiments were subjected to analysis using statistical software SPSS (IBM 2011). The data of fibre fractions was

subjected to factorial CRD. Pearson's correlation coefficients were determined to find relationship between fibre fractions and antibiosis factors.

RESULTS AND DISCUSSION

Larval duration: The total larval duration indicated significant variation in different genotypes (Table 1). The total larval duration was maximum in genotype IET22155 (19.6 days) followed by TKM6 (19.5) and W1263 (18.4). The other genotypes were in descending order for larval duration as JGL21066, RP4918-142, PR121 and TN1 (Table 1). As fibres affect the nutritional value of insect diet, so the longer total larval duration in some genotypes could be due to higher amount of cellulose and hemicellulose in these genotypes. High level of fibres increase bulk density of the diet and make it difficult to ingest adequate levels of nutrients and water to complete life cycle (Santiago et al 2013). The results are supported by study of Wang et al (2020) that suggested longer larval duration of potato tuber moth, *Phthorimaea operculella* on *Solanum tuberosum* having highest cellulose content (20%) while, shorter larval duration was observed on *S. lycopersicum* and *Physalis alkekengi* having lower cellulose amount of 11 and 8 per cent, respectively.

Larval weight and per cent pupation: The significant variation was observed in the weight of final instar of larvae feeding on different genotypes. The least larval weight was on resistant check TKM6 (22.7 mg) followed by IET22155 (23.4 mg) and W1263 (24.9 mg) and these genotypes were at par. The other genotypes were significantly at par to each other and JGL21066 had the highest larval weight among these genotypes (Table 1).

The per cent pupation varied significantly and was maximum in susceptible check TN1 (82%) followed PR121 (78%), RP4918-142, JGL21066 and W1263 and was minimum among IET22155 (64%) and TKM6 (54%) (Table 1). The variation in larval survival among genotypes could be due to the concentration of lignin in leaves of various genotypes. Lignin is the end product of the phenyl propanoid pathway that accumulates in the plant tissues after injury that triggers the pathway. Its deposition also makes plant tissues tougher and less palatable that make it difficult for the insect to feed on the plant (Armani et al 2020). Wang et al (2020) also observed higher larval survival on *S. tuberosum* (83%) having lower lignin content (4%) but larval survival was much lower on resistant *Lycium barbarum* (42%) that had higher lignin content (18%).

Pupal weight: The pupal weight in different genotypes varied significantly from 17.1 mg to 22.9 mg and minimum pupal weight was recorded in TKM6 (17.1 mg) followed by IET22155 (18.8 mg) and JGL21066 (20.6 mg). The pupal weight in other genotype viz., PR121, RP4918-142, W1263 and TN was in ascending order (Table 1). The variation in the pupal weight could also be due to the factors that were involved in affecting the larval weight. Wang et al (2020) observed that pupal weight was higher on plants having lower lignin content and vice-versa. However, present study revealed strong negative correlation of pupal weight with that of hemicellulose and cellulose and a weak negative correlation with that of lignin content Table 5).

Fibre fractions in leaves of different rice genotypes at constitutive and induced levels: Fibre fractions viz. cellulose, hemicellulose and lignin were analyzed in un-infested (constitutive) and infested (induced) leaves of 50 and 70 DOPs of selected genotypes.

Cellulose at constitutive and induced levels: The amount of cellulose in 50 days old uninfested plants varied significantly from 24.1 per cent in PR121 to 27.5 per cent in TKM6 (Table 2). In the infested plants, the cellulose content decreases after feeding and highest per cent decrease was in RP4918-142 (19.1%) followed by IET22155 (17.4%) but minimum decrease was in W1263 (10.9%). The decrease in cellulose content after infestation could be due to feeding by the larvae. In 70 DOPs, similar trend was observed and W1263 (33.4%) had highest cellulose content and PR121 (28.2%) had minimum cellulose content in uninfested plants (Table 2). After infestation, the cellulose content decreased in

Table 1. Larval duration, larval weight, per cent pupation and pupal weight of *C. medinalis* on rice genotypes

Genotype	Total larval duration (Days) (Mean±SE)	Larval weight (mg) (Mean±SE)	Per cent pupation (Mean±SE)	Pupal weight (mg) (Mean±SE)
PR121	15.5±0.5^{a}	25.9±0.4^{c}	78.0±3.7^{c}	22.0±0.7^{cd}
RP4918-142	16.5±0.5^{ab}	25.5±0.9^{bc}	76.0±5.1^{c}	22.1±0.5^{cd}
W1263	18.4±0.2^{bc}	24.9±1.0^{abc}	74.0±2.4^{c}	22.9±0.6^{d}
JGL21066	16.7±1.0^{ab}	26.2±0.8^{c}	74.0±5.0^{c}	20.6±0.4^{bc}
IET22155	19.6±0.4^{c}	23.4±0.8^{ab}	64.0±5.1^{b}	18.8±0.8^{ab}
TKM6	19.5±0.9^{c}	22.7±0.6^{a}	54.0±6.8^{a}	17.1±0.8^{a}
TN1	15.1±0.5^{a}	26.1±0.5^{c}	82.0±3.7^{c}	22.9±0.7^{d}

Means within a column followed by same letter are not significantly different at $p \leq 0.05$ according to LSD

70 DOPs. Cellulose is the main component of cell wall, insoluble in water and highly stable polymer (Taylor 2008).

Hemicellulose at constitutive and induced levels: The similar trend was followed for hemicellulose content in all the genotypes and there was reduction in the hemicellulose amount after the insect infestation and was lowest in PR121 (30.0%) followed by TN1 and highest in TKM6 (34.2%) in 50 DOPs (Table 3). The decrease in hemicellulose amount in leaves among different genotypes could be due to the consumption of the primary cell wall by the larvae during the feeding process. After infestation, there was 11.7 to 22.8 per cent decrease in hemicellulose and highest hemicellulose content after infestation was found in TKM6 (28.4%) and lowest in JGL21066 (24.5%) (Table 3). In 70 days old uninfested plants, hemicellulose content in TN1 and PR121 was significantly less (35.1 and 35.4%, respectively) than other genotypes viz., TKM6, IET22155, W1263, RP4918-142 and JGL21066 having hemicellulose amount from 40.9 to 43.3 per cent (Table 3).

In the infested plants, TN1, PR121 and JGL21066 had significantly less hemicellulose content (29.1, 30.6 and 33.9%, respectively) than W1263 (38.5%), TKM6 (38.3%), IET22155 (37.4%) and RP4918-142 (36.7%) (Table 3). Hemicellulose refers to a diverse class of polysaccharides which differ from the cellulose because this fraction is amorphous and easily soluble or hydrolysable in alkaline or acid solutions (Somerville et al 2004).

Lignin at constitutive and induced levels: The lignin content in 50 DOPs uninfested plants varied significantly and was maximum in JGL21066 (3.7%) followed by TKM6 (Table 4). Whereas, W1263, IET22155, TN1, PR121 and RP4918-142 has lower lignin content. Slight numerical increase was recorded in lignin content after insect feeding in various genotypes in both 50 and 70 DOPs. The per cent increase in lignin content in 50 DOPs of different genotypes after insect infestation varied from 5.0 to 9.4 per cent. However, in 70 DOPs there was increase in the amount of lignin in all the genotypes. The highest lignin content in uninfested plants was again in JGL21066 (4.3%) and TKM6 (4.2%) and lowest in TN1 (3.5%) and RP4918-142 (3.2%) (Table 4). Likewise, under infested conditions lignin content was highest in JGL21066 (4.6%) and TKM6 (4.5%). The genotypes

Table 2. Cellulose content (Per cent of dry weight) before and after infestation by *C. medinalis* in the leaves of 50 and 70 days old plants of selected genotypes

Genotype	50 days old plants		70 days old plants	
	Uninfested (Mean±SE)	Infested (Mean±SE)	Uninfested (Mean±SE)	Infested (Mean±SE)
PR121	24.17±0.74^{a}	20.37±0.49^{a}	28.23±0.41^{a}	24.13±0.18^{bc}
RP4918-142	27.20±1.14^{bc}	22.00±0.97^{ab}	28.30±0.87^{a}	24.13±0.52^{bc}
W1263	27.07±0.67^{bc}	24.10±1.42^{c}	33.40±0.74^{b}	28.60±0.74^{d}
JGL21066	25.60±0.35^{ab}	21.73±0.68^{ab}	29.60±0.92^{a}	23.03±1.05^{ab}
IET22155	26.77±0.82^{c}	22.10±0.56^{b}	29.63±0.97^{a}	25.20±1.19^{c}
TKM6	27.53±0.93^{c}	23.83±0.32^{c}	32.20±0.78^{b}	29.60±0.99^{d}
TN1	24.83±0.43^{a}	20.80±0.75^{ab}	28.63±0.47^{a}	22.40±0.29^{a}

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

Table 3. Hemicellulose content (per cent of dry weight) before and after infestation by *C. medinalis* in the leaves of 50 and 70 days old plants of selected genotypes

Genotype	50 days old plants		70 days old plants	
	Uninfested (Mean±SE)	Infested (Mean±SE)	Uninfested (Mean±SE)	Infested (Mean±SE)
PR121	30.00±0.76^{a}	26.47±0.12^{bcd}	35.47±0.38^{a}	30.63±0.78^{a}
RP4918-142	31.30±0.44^{ab}	25.03±0.94^{ab}	43.37±1.36^{c}	36.77±1.01^{c}
W1263	33.20±0.71^{bc}	27.03±0.56^{c}	41.83±0.87^{bc}	38.50±0.78^{c}
JGL21066	31.80±1.36^{ab}	24.53±0.99^{ab}	40.93±0.39^{b}	33.97±0.59^{b}
IET22155	31.50±1.17^{ab}	27.80±1.00^{d}	41.67±1.11^{bc}	37.47±1.13^{c}
TKM6	34.20±1.01^{c}	28.47±1.58^{d}	42.47±0.59^{bc}	38.33±0.80^{c}
TN1	30.93±0.58^{a}	25.43±0.93^{abc}	35.17±0.48^{a}	29.16±0.84^{a}

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

RP4918-142 (3.5%) and TN1 (3.6%) recorded the minimum lignin content after infestation (Table 4). Per cent increase in lignin content among genotypes varied from 3.7 to 9.5 per cent after insect infestation. Lignin is the most resistant polymer in nature and plays an important role in plant growth and development by providing structural support for land plants and as a resistance mechanism to biotic and abiotic stresses (Wang et al 2020). The increase in the lignin amount after insect infestation was due to the triggering of the defense mechanism via phenyl propanoid pathway that results in the formation of lignin, final product of phenyl propanoid pathway (Boerjan et al 2003). Yanni et al (2011) observed that lignin content in maize leaves increased from 34.4 to 39.3 g/kg in non-Bt variety and 31.6 to 33.6 g/kg in Bt variety after the infestation of European corn borer (ECB). Similarly, Barros-Rios et al (2011) investigated that maize line (EP39) resistant to ECB was less damaged by the pest having lignin content of 69 g/kg than susceptible line (EP47) with lignin content of 50 g/kg.

Correlation between antibiosis parameters and fibre fractions: The Pearson's correlation analysis showed a significant positive correlation of larval duration with that of cellulose (r = 0.63) (Fig. 1) and hemicellulose and lignin means with increase in the dietary fibre in the plant leaves it take more time for the insect to complete its larval phase (Table 5). However, the final larval weight has negative correlation with cellulose and hemicellulose (Fig. 2) (Table 5). Larval survival was negatively correlated to lignin content in leaves (Fig. 3) i.e. higher lignin content has fatal effect on developing larvae but cellulose and hemicellulose has no significant effect on larval survival. Pupal weight was found to have negative correlation with cellulose and hemicellulose (Table 5).

Table 5. Pearsons correlation between antibiosis parameters and biochemical factors

Characters	Cellulose	Hemi-cellulose	Lignin
Larval duration	0.63**	0.62**	0.42*
Larval weight	-0.43**	-0.41*	-0.12
Per cent pupation	-0.30	-0.27	-0.36*
Pupal weight	-0.49**	-0.50**	-0.34

*Significant at 5 and** 1per cent level of significance

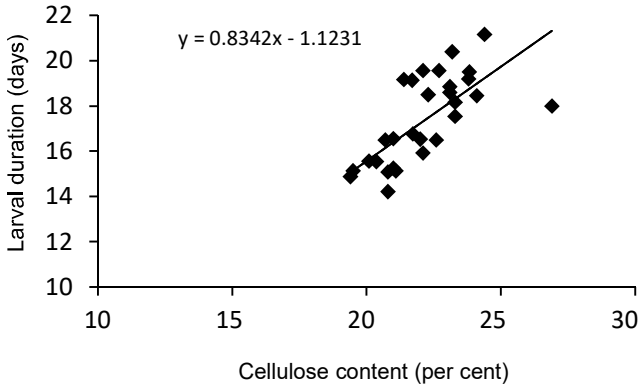

Fig. 1. Correlation between cellulose content and larval duration

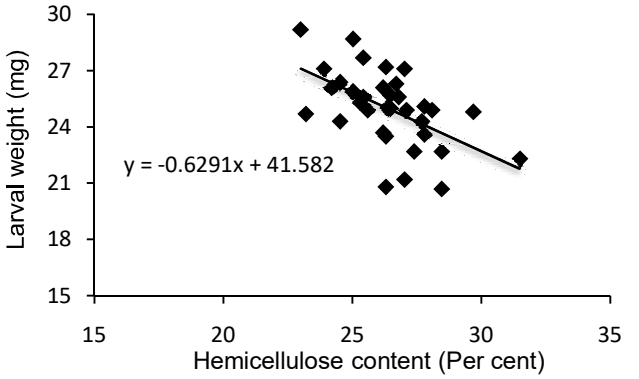

Fig. 2. Correlation between hemicellulose content and larval weight

Table 4. Lignin content (per cent of dry weight) before and after infestation by *C. medinalis* in the leaves of 50 and 70 days old plants of selected genotypes

Genotype	50 days old plants		70 days old plants	
	Uninfested (Mean±SE)	Infested (Mean±SE)	Uninfested (Mean±SE)	Infested (Mean±SE)
PR121	3.13±0.09^{a}	3.33±0.07^{a}	3.63±0.03^{bc}	3.83±0.09^{b}
RP4918-142	3.07±0.12^{a}	3.36±0.12^{a}	3.23±0.09^{a}	3.50±0.15^{a}
W1263	3.43±0.13^{b}	3.70±0.10^{b}	3.80±0.12^{c}	4.07±0.03^{c}
JGL21066	3.73±0.09^{c}	4.07±0.17^{c}	4.30±0.17^{d}	4.60±0.20^{d}
IET22155	3.17±0.12^{a}	3.43±0.12^{a}	3.44±0.09^{ab}	3.77±0.09^{b}
TKM6	3.67±0.13^{bc}	3.93±0.18^{bb}	4.26±0.09^{d}	4.53±0.09^{d}
TN1	3.17±0.12^{a}	3.33±0.09^{a}	3.50±0.11^{b}	3.63±0.07^{ab}

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

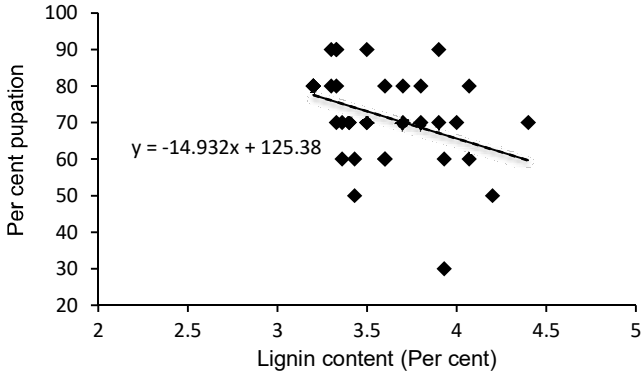

Fig. 3. Correlation between lignin content and per cent pupation

CONCLUSIONS

The genotypes that had higher amount of fibre fractions in their leaves provide some kind of resistance to the plants against RLF as the insect took more time to complete its larval stage on such genotypes. The last instar larval weight was also less on the genotypes having higher cellulose and hemicelluloses content. On the contrary, cellulose and hemicelluloses did not significantly affect larval survival but higher lignin content resulted in significantly lesser number of larvae turning into pupae. However, pupal weight was significantly reduced by greater amount of cellulose and hemicelluloses but lignin content had no significant effect on pupal weight of the RLF. These promising factors could be analyzed to screen the promising genotypes against RLF and against other rice lepidopteran insect pests.

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