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Quantification of plant biochemicals from certain genotypes of maize and their effect on different degree of infestation of maize spotted stem borer, *Chilo partellus* (Swinhoe)

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Abstract
A total number of twenty-five maize genotypes namely, Shaktiman-1, Shaktiman-2, Shaktiman-3, Shaktiman-4, Shaktiman-5, Devaki, Laxmi, Suwan, RHM-1, RHM-2, RHM-3, Deep Jwala, P-3535, P-3533, P-3550, P-3555, Dekalb-9188, Dekalb-9170, New Cross 76×11, New Cross 72×70, New Cross 73×11, New Cross 73×74, New Cross 52×65, New Cross 53×52 and New Cross 50×58 were assessed for their biochemical contents including Chlorophyll-a, Chlorophyll-b, total Chlorophyll and protein and their effects on *Chilo partellus* infestation. Results have presented a negative correlation between plant age and total content of Chlorophyll-a, b and total Chlorophyll while a positive correlation found between the injury and content of all the assessed biochemicals.

Keywords: Biochemicals, chlorophyll, protein, maize, infestation

Introduction
Maize is a major crop of many regions of tropics and subtropics, also known as queen of cereals, accounts for 4.98% of the total cropped agricultural area and it's ranked the third most yielding major food grain crop among all the cultivated cereals, after wheat and rice in India, especially in the regions of Andhra Pradesh and Bihar. Maize crop has very much importance due to its ability to survive and great contains genetic diversity and it can be grown in various ecological zones [12], moreover it is gaining importance owing to being a commercial/industrial crop as it is being used in many agro-based industries, where a number of products are being manufactured out of its grains namely popcorns, Kurkure and many more [9]. Queen of cereals, the maize crop has high yield potential and responds to various agro-management practices. Low yield of maize is owing to many constraints including ecological and biological factors like pests, but imbalanced use of fertilizers, traditional sowing methods and insect pests attack are more important. Significant contribution in low yield has been due to the insect pests attack [20]. Maize (*Zea mays* L.) is a future crop and staple food for millions of people in different parts of the world specially in north America. Maize grains as well as plants can be used for many purposes such as human consumption (39%), animal feed and fodder (25%), poultry feed (25%) and industrial products (starch, alcohol and popcorn) (15%) [3]. The countries with large maize growing areas are India, Indonesia, Italy, Argentina, Brazil, China, Hungary, Mexico, Philippines, South Africa, Rumania, United States and Yugoslavia. It was introduced in India from Central America in the beginning of seventeenth century. Important maize growing states in India are Andhra Pradesh, Madhya Pradesh, Maharashtra, Karnataka, Punjab, Bihar, Rajasthan and West Bengal. In Bihar, maize occupies an area of around 0.65 million hectares with annual production about 22.26 million tonnes [4]. Potentially, maize cultivation is gaining importance in Bihar due to increasing demand as animal feed and fodder and raw material for industries.

Numerous cultural and physical and biochemical factors or compound enhance the insect-pests problems in field as well as in polyhouse conditions either by the way of manipulating the environment, favorable for growth, reproduction and development of insects including traditional cultural methods, unrestricted use of chemicals (insecticides) and non-judicial use

of fertilizers [14].

Chemical fertilizers like NP and K, sulphur and zinc are fundamental plant macro nutrients for their growth and development which play basic roles in metabolism of plants and energy production, and significantly enhance the grain yield as well as biological yield. On the other hand, crop growth rate reduces under nitrogen stress that leads to decrease in kernel number and grain yield [24]. Some minor grains like millet crop grown on high rates of nitrogen survives and following crop damages due to increased borer population amplified as compared to low rates of nitrogen fertilizer.

The average maize per hectare productivity in India is 2.5 t/ha which is lesser in compared to U.S. (9.6 t/ha.). In spite of the increasing the cropping area for this crop, the production and productivity is still very low which may be due to several known and unknown reasons, *viz*. environmental factors, poor mechanization, insect-pests and diseases etc. Among these insect-pests are one of the major limiting factors for low yielding of maize.

Maize is subjected to be attacked or infested by many sucking and chewing insect-pests. It was observed and estimated that about 250 insect species are found to be associated with maize in field conditions as well as in the storage [17]. Among all the insect-pests attacking the maize crop, lepidopteron group of pests, particularly the stem borers are major constraint to the productivity of maize crop. In Asia and Africa, *C. partellus* is economically the most notorious pest of maize and sorghum [7]. These insects are of great economic importance in most maize growing areas throughout the world. Of these, spotted stem/stalk borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is a major cause of damage in *kharif* season [15, 26, 18, 8, 10]. In severe case, it can cause losses upto 75 per cent [22].

Biochemical factors are largely responsible for imparting resistance into the maize plants against many insects including *Chilo partellus* (Swinhoe). The major plant Biochemicals like Chlorophyll-A, Chlorophyll-B, total Chlorophyll and protein are present in maize plant tissues have a significant role in insect-pest resistance and tolerance. So, there is need to explore the utilization of different level of resistance/tolerance through assessing the comprehensive relationship of injury/damage caused by *Chilo partellus* in promising genotypes of maize in view of major nutrition factors. There is need to explore the possibilities of increasing the productivity and production through better understanding of some key constraints of its production and the present study, Quantification of Plant Biochemicals from Certain Genotypes of Maize and their effect on Different Degree of Infestation of Maize Spotted Stem Borer, *Chilo Partellus* (Swinhoe) was made to find out relationship of the biochemicals against different degrees of infestation of maize spotted stem borer, *Chilo partellus* (Swinhoe) in popular genotypes of maize in Bihar.

Materials and Methods

All the selected common and promising genotypes were sown in the two rows of each during *Kharif* in the month of June, 2017 and 2018 at research farm, Dr. Rajendra Prasad Central Agricultural University Pusa, Samastipur Bihar. The test insect *i. e. Chilo partellus* was reared in laboratory and neonate larva were used for artificial infestation in the field. The artificial infestation was done at 25 DAS.

Laboratory culture of *Chilo partellus* was developed by collecting larvae and pupae during the first fortnight of July, 2017 and 2018. These collected immature larvae were reared in two feet healthy maize stalk and pupae were kept in iron net made moths emergence cages with median hanging flag [16]. The emerged moths from moths emerging cages were transferred to egg laying cages or egg laying box of iron net made and coated with wax paper inside. Mated female moths laid eggs throughout lined white papers inside the cages after 2-3 days of mating. These laid egg-masses were pale brown to yellow and turned into a blackish and dark black headed stage prior to hatching after 2-3 days of oviposition. The black headed stage of egg masses with paper bits were transferred into semi-synthetic diet vials for their further growth and development. The rearing process repeated to develop stock culture of neonate larvae. All the plants in both rows were infested with five numbers of neonate larvae at evening hours. The grown maize germplasms under test were closely examined at a regular interval of seven days.

Genotypes

SN	Genotypes	SN	Genotypes
1.	Shaktiman-1	14.	P-3533
2.	Shaktiman-2	15.	P-3550
3.	Shaktiman-3	16.	P-3555
4.	Shaktiman-4	17.	DeKalb-9188
5.	Shaktiman-5	18.	DeKalb-9170
6.	Devaki	19.	New Cross (76X11) Pool 17 QPM-B7XCML-165
7.	Lakshmi (Check Resistant)	20.	New Cross (72X70) CML-163-7-2XCML-196
8.	Suwan	21.	New Cross (73X11) Dholi pop -65XCML-165
9.	RHM-1	22.	New Cross (73X74) Dholi pop -65XCML-373
10.	RHM-2	23.	New Cross (52X65) HKI-1105x2006-6-CML-471
11.	RHM-3	24.	New Cross (53X52) Dholi pop -65XHKI-1105
12.	Deep Jwala	25.	New Cross (50X58) HKI-586XPop Dholi
13.	P-3535		

List of genotypes (Common and Promising)

The observations pertaining to the different types of symptoms were done according to methodology adopted and also their categorization into different degree of susceptibility and degree of resistance to maize spotted stem borer was ascertain by counting or measuring the number of damaged/infested leaves (1- 9 scale) by adopting the standard method suggested by Guthrie *et al*., 1960 [13]. The plant leaves injuries were recorded at 30 and 60 days after emergence of crop. The total mean of score of overall leaves injury of the

insect-pest to a particular germplasm was rated as follows.

The total mean of score of overall leaves injury of the insect-pest to a particular germplasm was rated as follows.

Sl. No.	Injury symptoms	Score
1	Plant leaves showing no any of infestation symptoms.	1
2	1 to 2 Plant leaves with pinholes.	2
3	3-4 plant leaves with holes	3
4	1/3 of the plant leaves showing insects infestation symptoms.	4
5	1/2 of the plant leaves with insect infestation symptoms.	5
6	2/3 plant leaves with damage symptoms and the holes becoming window.	6
7	Plant leaves with long window and plant growth are stunted.	7
8	Whole leaves displaying heavy infestation and plant growth is stunted.	8
9	Plants Showing Dead-heart	9

The observations pertaining to the different groups were assessed for percent infestation. Extent of pest infestation for each genotype was calculated by using the formula,

$$\text{Per-cent plant infestation} = \frac{\text{Number of infested plant/two rows}}{\text{Total number of plant/two rows}} \times 100$$

For calculating infestation over the resistant check (Lakshmi), the modified formula of Abbott, 1925 has been used.

$$\text{Corrected Infestation per cent} = \frac{\text{Per cent Infestation in Treatment} - \text{Per cent Infestation in Check}}{100 - \text{Per cent Infestation in Check}} \times 100$$

Estimation of plant chlorophyll content [6]. The chlorophyll content has been estimated by adopting the method suggested by Arnon (1949). About 1 gr of leaf samples were taken and cut into tiny pieces and homogenized in a pre-cooled mortar and pestle using 80% of Acetone. A little amount of calcium carbonate was added at the time of grinding the samples. Later on, the plant extracts were centrifuged at 3000 rpm for a time of 15 min and the volume made up to 25 ml with 80% of Acetone. The clear solutions were transferred to the colorimeter tubes and the optical density was recorded at 645 nm and 663 nm, against an 80% acetone blank in Shimadzu 35 Double Beam spectrophotometer (UV 240). The levels of chlorophyll 'a' and chlorophyll 'b' were assessed by using the formula given below:

Amount of Chlorophyll a [milligrams/milliliter] = 12.7 A_{663} - 2.69 A_{645}

Chlorophyll b [mg/ml] = 22.9 A_{645} - 4.68 A_{663}

Where:

A_{645} = absorbance at a wavelength of 645 nm

A_{663} = absorbance at a wavelength of 663 nm.

Total Chlorophyll [mg/mL] = Chlorophyll a + Chlorophyll b.

Protein estimation

Sample preparation: Three plants were randomly selected and cut from the base at root before. These plants were brought to the laboratory and then chopped into very small pieces. Later chopped material was mixed thoroughly and dried in the oven at 60 ^{0}C for a total period of 8 hours. These dried samples were again grinded upto finer dust. From these grinded samples, 5 gm of grinded material was taken for estimation of Nitrogen for the analysis of protein. The protein was calculated by the formula followed by Winkleman *et al*. 1954 [25]

Protein per cent = Nitrogen per cent×6.25

Results

The results presented in Table 1, contains chlorophyll a, b and total chlorophyll, protein, insect leaf injury score, total infestation and corrected per cent of infestation.

Table 1: Chlorophyll a, b and total chlorophyll contents, protein and their relationship with *Chilo partellus* infestation

Sl. N.	Name of the Genotypes	Chlorophyll [mg/ml]						Protein %	Mean leaves injury score	Total Infestation %	Corrected Infestation %
		After 25 days of Emergence			At Tasseling Stage						
		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Chlorophyll a	Chlorophyll b	Total Chlorophyll				
1.	Shaktiman-1	4.30	1.15	5.45	4.15	1.05	5.20	14.06	2.35	25.50	8.11
2.	Shaktiman-2	3.90	1.05	4.55	3.50	0.80	4.30	14.37	2.45	21.50	3.18
3.	Shaktiman-3	4.25	1.30	5.55	4.10	1.15	5.25	18.74	3.20	26.00	8.73
4.	Shaktiman-4	4.05	1.15	5.20	3.90	1.05	4.95	14.53	2.65	22.00	3.79
5.	Shaktiman-5	4.50	1.10	5.60	4.15	0.90	5.05	21.87	4.00	40.75	26.92
6.	Devaki	4.90	1.40	6.00	4.50	1.15	5.65	14.68	2.90	29.10	12.55
7.	Lakshmi (C) Resistant	4.25	0.85	5.40	3.50	0.90	4.40	12.50	2.95	18.92	0.00
8.	Suwan	4.50	1.15	5.65	3.95	0.95	4.90	13.12	3.05	25.25	7.80
9.	RHM-1	4.10	1.20	5.30	3.55	0.95	4.50	22.81	3.55	23.25	5.34
10.	RHM-2	5.20	1.45	6.65	4.80	1.20	6.00	25.34	6.65	50.00	38.33
11.	RHM-3	3.45	0.90	4.35	3.20	0.80	4.00	12.18	1.65	15.01	-4.82
12.	Deep Jwala	3.80	1.05	4.85	3.15	0.90	4.05	9.78	1.80	15.72	-3.94
13.	P-3535	4.50	0.95	5.45	4.25	0.90	5.15	11.25	1.85	26.02	7.52
14.	P-3533	4.10	1.15	5.25	3.30	1.00	4.30	12.81	2.15	19.25	0.40
15.	P-3550	5.05	1.40	6.45	4.55	1.15	5.70	25.00	6.70	44.05	30.99

16.	P-3555	4.55	1.50	6.05	4.25	1.35	5.60	25.62	6.60	34.02	18.62
17.	DeKalb 9188	4.55	1.15	5.70	4.40	1.00	5.40	12.81	2.95	39.50	25.38
18.	DeKalb 9170	4.30	1.10	5.40	4.10	1.00	5.10	13.43	2.35	25.00	7.49
19.	New Cross (76X11)	4.20	1.05	5.25	3.80	0.85	4.65	14.06	6.65	23.35	5.46
20.	New Cross (72X70)	3.45	1.10	4.55	3.30	0.90	4.20	24.71	2.25	13.90	-6.19
21.	New Cross (73X11)	4.70	0.95	5.65	4.30	1.00	5.30	9.78	1.85	18.90	-0.02
22.	New Cross (73X74)	3.95	0.95	4.90	3.65	0.75	4.40	13.12	2.40	17.70	-1.50
23.	New Cross (52X65)	3.70	0.95	4.65	3.15	0.90	4.05	7.90	1.60	15.75	-3.90
24.	New Cross (53X52)	4.10	1.05	5.15	3.60	1.00	4.60	12.81	2.90	24.52	6.90
25.	New Cross (50X58)	4.20	1.15	5.35	3.85	1.15	5.00	11.56	2.40	19.90	1.20
F Test		Sig.	NS	Sig.	Sig.	Sig	Sig.	Sig.	Sig.	Sig	-
CD (at 5%)		0.79	NS	0.90	0.45	0.28	0.48	2.75	1.27	8.85	-
Sem (±)		0.29	0.06	0.38	0.19	0.08	0.18	0.90	0.43	2.98	-

The result presented in Table 1 under the chlorophyll content after 25 days of emergence showed that total chlorophyll ranged from 6.65-4.35. Result showing that chlorophyll a found maximum in RHM-2 (5.20) followed by P-3550 (5.05) and Devaki (4.90). Minimum amount of chlorophyll a found in genotypes namely RHM-3; New Cross 72×70 (3.45) followed by New Cross 52×65 (3.70) and Deep Jwala (3.80). Chlorophyll b found to be maximum in genotypes namely, P-3555 (1.50) followed by RHM-2 (1.45) and Devaki; P-3550 (1.40). Minimum chlorophyll b was found in Lakshmi (0.85) followed by RHM-3 (0.90) and P-3535; New Cross 73×11; New Cross 73×74 and New Cross 52×65 (0.95). Total chlorophyll was found maximum in genotypes namely, RHM-2 (6.65) followed by P-3550 (6.45) and P-3555 (6.05). Minimum total chlorophyll was found in RHM-3 (4.35) followed by Shaktiman-2; New Cross–72×70 (4.55) and New Cross 52×65 (4.65).

At the stage of tasseling, maximum chlorophyll a was found in RHM-2 (4.80) followed by P-3550 (4.55) and P-3555 (4.50). Minimum chlorophyll a was found in Deep Jwala; New Cross 52×65 (3.15) followed by RHM-3 (3.20) and P-3533; New Cross 72×70 (3.30). Maximum chlorophyll b was found in P-3555 (1.35) followed by Rhm-2 (1.20) and Shaktiman-3; Devaki; P-3550 and New Cross 50×58 (1.15). Minimum chlorophyll b was found in New Cross 73×74 (0.75) Shaktiman-2; RHM-3 (0.80) and New Cross 76×11 (0.85). Total chlorophyll was fond maximum in RHM-2 (6.00) followed by P-3550 (5.70) and Devaki (5.65). Minimum total chlorophyll was found in genotypes namely RHM-3 (4.00) followed by Deep Jwala; New Cross 52×65 (4.05) and New Cross 72×70 (4.20).

total protein content per cent was varied from 7.90 per cent to 24.71 per cent in which the maximum protein was observed in genotypes namely P-3555 with 25.62 per cent protein content followed by RHM-2 with 25.34, P-3550 with 25.00 and New Cross 72×70 with 24.71 per cent protein content. Minimum protein content per cent was recorded in New Cross 52×65 with 7.90 per cent protein content followed by New Cross 73×11, Deep Jwala with 9.78, P-3535 with 11.25 and New Cross 50×58 with 11.56 per cent protein content.

The result presented in Table 1 showed that the minimum leaf injury score was occupied by New Cross 52×65 with 1.60 followed by RHM-3 with 1.65, Deep Jwala with 1.80 and P-3535, New Cross 73×11 with 1.85 mean leaf injury score which was at par with each other. The maximum mean leaf injury score was recorded in P-3550 with 6.70 mean leaf injury score followed by RHM-2 and New Cross 76×11 with mean leaf injury of 6.65 that was at par with each other, P-3555 with 6.60 and Shaktiman-5 with 4.00 mean leaf injury score.

The results presented in Table 1, showed that the mean percent of overall plant infestation and it ranged from 13.90-50.00 per cent. Among the different tested maize genotypes, the minimum percent infestation, 13.09 per cent was recorded in followed by RHM-3 with 15.01, Deep Jwala with 15.72, New Cross 72×70 and New Cross 52×65 with 15.75 per cent infestation. The maximum per cent infestation was reached upto a level of 50.00 per cent in RHM-2 followed by P3550 with infestation of 44.05, Shaktiman-5 with 40.75 and Dekalb-9188 with 39.50 per cent infestation of spotted stem borer.

The results presented in Table 1, showed that the corrected percent of plant infestation (calculated corrected infestation over the resistant check) and it was ranged from -6.19 to 30.00 over resistant check, Lakshmi. Among the different tested maize genotypes, the minimum percent corrected infestation over check, -6.19 was recorded in RHM-3 followed by New Cross 72×70 with -4.82, Deep Jwala with -3.94 and New Cross 52×65 with -3.90 per cent corrected infestation. The maximum per cent corrected infestation was recorded highest in RHM-2 with 38.33 followed by P3550 with infestation of 30.99, Shaktiman-5 with 26.92 and Dekalb-9188 with 25.38 per cent corrected infestation of spotted stem borer.

Table 2: Correlation between plant biochemicals and different infestation categories

Sl. N.	Name of the Variable		Correlation with Leaf Injury Score	Correlation with Total Infestation	Student T-Test for Leaf Injury Score	Student T-Test for Total Infestation
1.	Chlorophyll- a	After 25 days of Emergence of the crop	0.588	0.799	Significant	Significant
2.	Chlorophyll- b		0.657	0.655	Significant	Significant
3.	Total Chlorophyll		0.673	0.810	Significant	Significant
4.	Chlorophyll- a	Before the Tasseling Stage	0.523	0.796	Significant	Significant
5.	Chlorophyll- b		0.499	0.499	Significant	Significant
6.	Total Chlorophyll		0.555	0.789	Significant	Significant
7.	Protein		0.701	0.607	Significant	Significant

The above Table, Table 2 presenting correlations between all the biochemicals and different categories of infestation including leaf injury score and total plant infestation. All the biochemicals found to be positively correlated with leaf injury

score as well as total plant infestation.

Discussion

The results presented in Table 1, showed a clear-cut variation in different levels of chlorophyll from the date of first observation till the tasseling stage of all the grown maize genotypes. The genotypes having maximum content of genotypes includes RHM-3; New Cross 72×70, New Cross 52×65 and Deep Jwala for chlorophyll a, Chlorophyll b found maximum in genotypes namely, P-3555, RHM-2 and Devaki; P-3550 and total chlorophyll was found maximum in genotypes namely, RHM-2, P-3550 and P-3555 at 25 days after the emergence of plants while at the time of tasseling, genotypes namely RHM-2, P-3550 and P-3555 showed maximum content of chlorophyll a, maximum chlorophyll b was found in P-3555, Rhm-2 and Shaktiman-3; Devaki; P-3550 and New Cross 50×58 and total chlorophyll found to be maximum in RHM followed by P-3550 and Devaki. However, a significant level of decrease in chlorophyll content including chlorophyll a, b and total chlorophyll has been recorded with the increasing crop age [19].

A positive correlation has been observed between infestation of *Chilo partellus* and chlorophyll a, b and total chlorophyll [19b; 23]. Genotypes having minimum amount of chlorophyll a, b and total chlorophyll showed varying degree of resistance against *Chilo partellus* infestation [11; 21; 2]. Protein shows positive correlation with all the infestation traits of maize spotted stem borer and genotypes namely P-3555 and RHM-2 shows higher level of protein content as well as infestation level [5].

Conclusion

The maize spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is an important pest of tropical maize in several countries including India. Serious crop losses have been reported, mostly in experiments conducted under artificial infestations at experimental stations. In order to develop an economical and environmentally friendly method of pest management, a large number of maize genotypes with varying levels of resistance to maize spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) have been identified. The result of correlation reveals that the genotypes with maximum amount of chlorophyll a, b, total chlorophyll and protein are more susceptible against maize spotted stem borer in compare to the genotypes having lower content of these biochemicals.

Among all the tested genotypes, the total infestation ranged from 13.90 to 50.00 per cent and many genotypes have been identified as resistant against *Chilo partellus* even after artificial infestation. Genotypes namely RHM-3 followed by New Cross 72×70 with -4.82, Deep Jwala with -3.94 and New Cross 52×65 found to be less infested with *Chilo partellus* after artificial infestation. Biochemicals play an important role in development and survival of insects. The present study shows the importance of biochemical factors including chlorophyll a, b and total chlorophyll and their impact on infestation and susceptibility levels of Chilo partellus. Results indicate that genotypes having less amount of chlorophyll including a, b and total chlorophyll and protein found to be resistant against *Chilo partellus* infestation.

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