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Screening of indigenous Chak-hao (*Oryza sativa* L.) genotypes of Manipur for resistance reactions against rice leaf folder, *Cnaphalocrocis medinalis* (Guenee)

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Abstract
Rice genotypes indigenous to Manipur were screened for resistance reactions against rice leaf folder at the research field of College of Agriculture, Central Agricultural University, Imphal, Manipur. The experimental design followed was Randomized Bock Design, with three replications and 19 genotypes (treatments). Insect infestation was recorded at 30, 45, 60, 75 and 90 DAT. Considering the average of five observations *i.e.* at 30, 45, 60, 75 and 90 DAT, the lowest percent leaf infestation was observed in Chak-hao Manam Nungshibi with 3.58 percent. Wairi Chak-hao showed highest percent leaf damage with 5.27 percent. For finding the resistance reactions, biochemical parameters such as total sugars, reducing sugars, total phenols and orthodihydroxy phenol were analysed. The result showed that sugar content and insect infestation were positively co-related and phenol content and insect infestation were negatively co-related.

Keywords: Chak hao, rice leaf folder, Manipur, indigenous, screening

Introduction
Chak hao rice, also known as black rice, is a traditional rice variety mainly cultivated in the North-eastern region of India, especially in Manipur state. The dark purple to black color of the rice grain is due to the presence of high levels of anthocyanin, which is known to be potent antioxidant (Nam *et al.*, 2005) [11]. Chak hao rice has higher levels of protein, fat, fibre, and minerals such as calcium, iron, and zinc compared to other commonly cultivated rice varieties in Manipur (Singh *et al.*, 2016) [14]. These rice genotypes have a long history of cultivation in Southeast Asian countries such as China, India and Thailand (Kong *et al.*, 2003) [9]. In Manipur, a diverse array of this rice genotypes are available but mostly with purplish colour grain. The unique purple colour of Chak-hao is due to high deposition of anthocyanin in the outer pericarp, seed coat and aleurone layer (Chaudhury, 2003) [6]. The literal meaning of Chak-hao is delicious rice, Chak-rice; hao-delicious (Roy *et al.*, 2014) [12]. The area under cultivation of Chak-hao is comparatively very low, approximately it ranges 60-70 ha only in Manipur. They are poor yielding as compared to hybrid varieties and traditional varieties. (Borah *et al.*, 2018) [3]. However, they are more profitable in terms of economic returns.
In tropical Asia only, about 20 species of rice insect pest are of major importance and of regular occurrence (Grist and Lever, 1969) [8]. But several species that were earlier considered as minor pest have recently become major pests (Dale, 1994) [7]. In India, approximately 100 insect species feed on rice and 20 of these are considered to be major pests, causing 30% yield loss. Among these, rice leaf folder, *Cnaphalocrocis medinalis* Guenee is one of the most destructive insect-pest occurring throughout the country causing yield loss of about 10-60 percent (Chatterjee and Mondal, 2016) [4].
Usually, local varieties are well adapted in the local conditions and also mostly resistant/ tolerant to the pests and diseases. Hence, it necessitates evaluation of the reaction of the indigenous Chak-hao rice genotypes against the changing pest status. Further, looking into the increasing market demand of the Chak-hao rice, it becomes necessary to identify the genotype which is resistant/ tolerant against the major insect pests and give higher yield than the other genotypes for popularising among the farmers and also for use in the future genetic improvement programmes.

Materials and Methods
A field experiment was carried out at the Entomological Research Farm, College of

Agriculture, CAU, Imphal located at 24° 45´ N latitude and 93° 56 E´ longitude to evaluate 19 Chak-hao rice genotypes (including the susceptible check) and find their resistance reaction against rice leaf folder in present rice ecosystem of Manipur. Experimental design followed was Randomized Block Design, each Chak-hao rice genotypes were transplanted in 3 rows and each row consisted of 20 hills. After every 10 genotypes, three rows of susceptible check, Leimaphou was also transplanted. At the beginning and end of the plot, three rows of susceptible check were also transplanted to increase the pest pressure. The similar pattern was replicated three times. However, sequence of genotypes were randomised.

Table 1: Details of Chak-hao genotypes

Treatments	Name of Chak-hao rice genotypes
T_1	Pong Chak-hao
T_2	Kom Chak-hao
T_3	Kotha Chak-hao
T_4	Chettamo Chak-hao
T_5	Chak-hao Poireiton
T_6	Wahong Chak-hao
T_7	Ching Chak-hao Angangba
T_8	Chak-hao Sempak
T_9	Chak-hao Amubi
T_{10}	Chak-hao Tatha
T_{11}	Chak-hao Angangbi
T_{12}	Chak-hao Heimang
T_{13}	Kom Chak-hao Macha
T_{14}	Chak-hao Manam Nungshibi
T_{15}	Chak-hao Napduina
T_{16}	Wairi Chak-hao
T_{17}	Chak-hao Mongkhangi
T_{18}	Chak-hao Taniangban
T_{19}	Leimaphou (KD 2-6-3)

Leaf folder infestation was recorded as percent damage leaves. Observations were recorded from 10 randomly selected hills in each replication. In each selected hill, the total number of leaves and total number of damage leaves by Leaf folder were counted and converted into percent damage leaves with the following formulae:

$$\text{Percent damage leaves} = \frac{\text{Number of damage leaves per hill}}{\text{Total number of leaves per hill}} \times 100$$

Observations were recorded at 30, 45, 60, 75, and 90 DAT.

Biochemical basis of Resistance

Based on leaf samples collected from 60 days old plants of resistant and susceptible gene pools, the following biochemical constituents were determined: total sugars, reducing sugars, total phenols, and orthodihydroxy phenols. The procedures followed are discussed as under.

Alcohol extraction of Plant tissues

A sample of leaves was collected from a 60-day-old plant of the Chak-hao rice genotype. Each sample was washed and dried in the shade. One gram each of plant samples of all genotypes were taken in separate conical flasks and 15 ml of 80 percent ethanol was added. These were refluxed on a hot water bath for 30 minutes before being analyzed. The extracts were cooled after boiling. After decanting the supernatants into new flasks, the residues were again re-extracted with hot ethanol and decanted. Extracts were filtered through Whatman No.1 filter paper and then made up to a known volume with 80 percent ethanol. Samples were prepared from the extracts after they were stored in refrigerator at 4 °C.

Total soluble Sugar estimation

According to the Anthrone method for determining total soluble sugars (Sadasivan and Manickam, 1996), working standard solutions were prepared by dissolving 10 ml glucose stock solution in 100 ml water. Working standard solutions of 0.2, 0.4, 0.6, 0.8 and 1 ml, along with 0.1 ml aliquot samples (alcoholic free extracts), were pipetted out into test tubes and the volume was made up to 1 ml with distilled water. In each sample, anthrone reagent was added in a volume of 4 ml, followed by adding water to make a total volume of 25 ml. After 1 minute in a boiling bath, they were cooled and colour developments were measured at 630 nm using spectrophotometer. Total soluble sugars were calculated by drawing standard graph with glucose as standard.

Estimation of reducing Sugars by DNS method

The working standard glucose solutions were prepared by dissolving 10 ml glucose stock solution in 100 ml water. Standard solutions of 0.2, 0.4, 0.6, 0.8 and 1 ml, as well as 0.1 ml aliquot samples (ethanol free extract) of selected genotypes were pipetted into test tubes, which were subsequently filled with distilled water to a volume of 1 ml. In addition to 3 ml of DNS, 25 ml of distilled water was added to each sample. After boiling for 1 minute, they were cooled and the colour developed was measured using a spectrophotometer at 510 nm. Reducing sugars content was calculated by drawing a standard graph with glucose as standard.

Estimation of total Phenols

Total phenol was estimated using the method as given by Malik and Singh, 1980. The working standard catechol solution was prepared by dissolving 10 ml catechol stock solution in 100 ml water. Working standard solutions of 0.1, 0.2, 0.3, 0.4 and 1 ml were pipetted out in a series of test tubes, along with 0.1 ml aliquot samples (alcoholic free extract) of selected genotypes, and the volume was made up to 1 ml with distilled water. To 1 ml of sample extract, 0.2 ml FCR reagent and 2 ml 20% Na_2CO_3 nd distlled water were added making the volume upto 25 ml. It was kept in a boiling water bath for 1 minute, cooled and the colour developed was measured at 650 nm using spectrophotometer. Total phenol content was calculated by drawing a standard graph with catechol as standard.

Estimation of Orthodihydroxy phenol

According to Arnow method (Arnow, 1937) [2] for determining orthodihydroxy phenolworking standard catechol solution were prepared by dissolving 10 ml catechol stock solution in 100 ml water. Working solutions of 0.1, 0.2, 0.3, 0.4 and 1 ml each along with 0.1 ml aliquot sample (alcoholic free extract) of the selected genotypes were pipetted out in a series of test tubes and the volume was made up to 1 ml with distilled water. To 1 ml of sample extract, 1 ml of arnow reagent, 2 ml NaOH and 1 ml of 0.05 N HCl and volume was made upto 25 ml by adding distilled water. It was kept in a boiling water bath for 1 minute, cooled and colour developed

was measured at 515 nm using spectrophotometer. Ortho dihydroxy phenol content was calculated by drawing standard graph with glucose as standard.

Statistical Analysis

Statistical testing of significance through analysis of variance was performed after appropriate transformation of mean values obtained from the various experiments.

Result and Discussion

At 30 DAT, the infestation of leaf folder was low and the damage leaves ranged from 2.67 percent in Kotha Chak-hao to 5.56 percent in susceptible check (Table 2). However, at 45 DAT the infestations was increased and recorded highest damage leaves of 5.73 percent in Chak-hao Tatha and it was followed by Wairi chak-hao (5.6 percent), Ching chak-hao Angangba (5.56 percent), Chak-hao Amubi (5.5 percent) and Chak-hao Poireiton (5.34 percent) in descending order. The lowest incidence of Leaf folder at 45 DAT was observed in Chak-hao Manam Nungshibi (3.69 percent) and it was followed by Kotha chak-hao (3.7 percent), Chak-hao Taniang ban (3.86 percent) Kom Chak-hao (3.93 percent) and Chak-hao Sempak (4.07 percent) in ascending order. The infestation of leaf folder was still high at 60 DAT also and the percent damage leaves ranged from 4.43 in Kom chak-hao to 6.61 in Wairi chaka-hao. The infestation level slightly increased from 75 DAT and damage leaves ranged from 4.52 percent in Chak-hao Manam Nungshibi to 6.57 percent in Chak-hao Tatha. At 90 DAT, the infestation of Leaf folder damage leaves ranged from 2.49 percent in Chak-hao Manam Nungshibi to 3.79 percent in Wairi Chak-hao.

None of the genotypes tested were free from leaf folder infestation. Lowest incidence of mean infestation of leaf folder was also observed in Chak-hao Manam Nungshibi with 3.61 percent damage leaves and highest in wairi chak-hao with 5.27 percent other than the standard check, Leimaphou with 7.59 percent. The incidence of leaf folder in Kom Chak-hao and Kotha Chak-hao were comparable with Chak-hao Manam Nungshibi. Although Chak-hao Angangbi, Chak-hao Heimang and Chettamo Chak-hao recorded lower incidence of leaf follder, they were significantly higher than Chak-hao Manam Nungshibi.

The present finding is almost in agreement to Chatterjee *et al.* (2011) [5], where they conducted a field trial on screening of 51 genotypes against rice leaf folder *Cnaphalocrocis medinalis.* The genotypes CSR 23, TNAU 831311, ARC 6626, ADT 46, SB 319, AGANNI and ASD 16 had minimum infestation of leaf folder. While TN 1, Kavya, Choorapundy, RP 4621-1842 and LF 293 received maximum damage at 50 DAT. Observation on 80 DAT revealed that the leaf folder infestation was minimum in IC 115737, AGANNI, IC 155876, ARC 5982, IF 88, CR-MR-1523, SB 436, SB 55 and TN1.

Table 2: Percent damage leaves by Leaf folder in Chak-hao rice gentoypes during *Kharif*, 2017.

List of Chak-hao rice genotypes	Mean percent damage leaves recorded at					*Mean percent damage leaves
	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	
Pong Chak-hao	3.14 (1.91)	4.52 (2.24)	5.58 (2.47)	5.33 (2.41)	3.05 (1.88)	4.32 (2.18)
Kom Chak-hao	2.76 (1.81)	3.93 (2.10)	4.43 (2.22)	5.2 (2.39)	2.64 (1.77)	3.79 (2.06)
Kotha Chak-hao	2.67 (1.78)	3.7 (2.05)	4.67 (2.27)	4.79 (2.30)	2.69 (1.79)	3.70 (2.04)
Chettamo Chak-hao	3.17 (1.92)	4.63 (2.26)	5.56 (2.46)	6.19 (2.59)	3.3 (1.95)	4.57 (2.23)
Chak-hao Poireiton	3.76 (2.06)	5.34 (2.42)	6.56 (2.66)	6.44 (2.63)	3.42 (1.98)	5.10 (2.35)
Wahong Chak-hao	3.48 (1.99)	4.48 (2.23)	5.24 (2.40)	6.3 (2.61)	3.07 (1.89)	4.51 (2.22)
Ching Chak-hao Angangba	3.5 (2.00)	5.56 (2.46)	6.42 (2.63)	6.37 (2.62)	3.59 (2.02)	5.09 (2.35)
Chak-hao Sempak	3.07 (1.89)	4.07 (2.14)	4.56 (2.25)	4.82 (2.31)	2.55 (1.75)	3.81 (2.07)
Chak-hao Amubi	3.72 (2.05)	5.5 (2.45)	6.64 (2.67)	6.18 (2.58)	3.51 (2.00)	5.11 (2.35)
Chak-hao Tatha	3.82 (2.08)	5.73 (2.50)	6.59 (2.66)	6.57 (2.66)	3.42 (1.98)	5.23 (2.37)
Cak-hao Angangbi	3.64 (2.03)	4.63 (2.26)	5.78 (2.51)	5.62 (2.47)	3.06 (1.89)	4.55 (2.23)
Chak-hao Heimang	3.65 (2.04)	4.52 (2.24)	5.69 (2.49)	5.65 (2.48)	3.26 (1.94)	4.55 (2.24)
Kom Chak-hao Macha	3.54 (2.01)	5.16 (2.38)	5.36 (2.42)	5.71 (2.49)	3.33 (1.96)	4.62 (2.25)
Chak-hao Manam Nungshibi	2.69 (1.79)	3.69 (2.05)	4.68 (2.27)	4.52 (2.24)	2.49 (1.73)	3.61 (2.01)
Chak-hao Napduina	3.65 (2.04)	4.45 (2.22)	5.34 (2.42)	5.67 (2.48)	3.43 (1.98)	4.51 (2.23)
Wairi Chak-hao	3.88 (2.09)	5.6 (2.47)	6.61 (2.67)	6.48 (2.64)	3.79 (2.07)	5.27 (2.39)
Chak-hao Mongkhangi	3.71 (2.05)	4.63 (2.26)	5.58 (2.46)	6.19 (2.59)	3.29 (1.95)	4.68 (2.26)
Chak-hao Taniang Ban	2.67 (1.78)	3.86	4.68	4.92	2.51	3.73

		(2.09)	(2.27)	(2.33)	(1.73)	(2.04)
Leimaphou	5.56 (2.46)	7.97 (2.91)	9.39 (3.14)	9.80 (3.21)	5.24 (2.41)	7.59 (6.54)
S.Ed(±)	0.04	0.07	0.06	0.06	0.31	1.20
CD	0.09	0.14	0.13	0.12	0.62	2.38

Figures in parentheses are square root transformed values.
*Mean of five replications.
Basis of resistance against Leaf Folder of Chak-hao rice genotypes

Total soluble sugars content in leaf folder resistant and susceptible genotypes

The total soluble sugars present in the six selected susceptible genotypes (including susceptible check) and five resistant genotypes are presented in table 3. Among the susceptible genotypes the total soluble sugars content ranged from 5.23 to 6.61 percent. Whereas, in the resistant genotypes ranged from 3.19 to 3.88 percent. The highest total soluble sugars content was recorded in Chak-hao Poireiton (6.61 percent) and lowest in Chak-hao Sempak (3.19 percent).

Reducing sugar content in leaf folder resistant and susceptible genotypes

The reducing sugars content in the susceptible genotypes ranged from 2.32 to 2.73 percent. The lowest contents were recorded in Wairi Chak-hao (2.32 percent) and Ching Chak-hao Angangba (2.31 percent). Whereas the highest content was recorded in Chak-hao Poireiton (3.86 percent). Out of the five resistant genotypes, Chak-hao Taniang Ban (2.29 percent) recorded highest content and it was followed by Kom Chak-hao (2.11 percent), Chak-hao Sempak (2.10 percent), Kotha Chak-hao (2.06 percent), and Chak-hao Manam Nungshibi (1.99 percent) in descending order.

Total phenols content in leaf folder resistant and susceptible genotypes

The total phenols content was relatively high in resistant genotypes. The highest total phenols content was recorded in Kotha Chak-hao (4.63 percent) and it was followed by Kom Chak-hao (4.60 percent), Chak-hao Taniang Ban (4.20 percent), Chak-hao Manam Nungshibi (4.15 percent) and Chak-hao Sempak (3.80 percent) in descending order. The total phenols content was lowest in Chak-hao Amubi (1.90 percent).

Ortho dihydroxy phenols content in leaf folder resistant and susceptible genotypes

In the susceptible genotypes, the ortho dihydroxy phenols content was relatively lower compared to resistant genotypes. The susceptible genotypes *viz.* Leimaphou (1.11 percent), Chak-hao Tatha (1.27 percent), Wairi Chak-hao (1.32 percent), Ching Chak-hao Angangba (1.37 percent), Chak-hao Amubi (1.1.43 percent) and Chak-hao Poireiton (1.48 percent) recorded lower ortho dihydroxy phenol content in ascending order. Whereas the resistant genotypes *viz.*, Kom Chak-hao (2.73 percent), Kotha Chak-hao (2.71 percent), Chak-hao Taniang Ban (2.11 percent), Chak-hao Manam Nungshibi (1.99 percent) and Chak-hao Sempak (1.95 percent) recorded higher ortho dihydroxy phenol content in descending order.

Among the selected rice genotypes, total soluble sugars content in selected resistant genotypes were significantly lower than the selected susceptible genotypes. Similarly the reducing sugar content in susceptible genotypes were higher in comparison to the resistant genotype. Similar findings were also observed by Ahmad *et al.* (2006) [1]. They reported that high amount of total and bound phenols contain were found to be imparting resistance against Rice leaf folder. However the total soluble sugars and reducing sugars content in present investigation is supported by Ram Singh *et al.* (2004) [15], they reported that the total soluble sugars and reducing sugars contain was higher in susceptible genotypes in comparison to resistant genotypes. Similar findings were also reported by Borah *et al.* (2018) [3] where the absence of severe insect infestation was not observed in chak-hao due to antocyanins and phenolic contents.

Table 3: Biochemical constituents in leaf folder resistant and susceptible Chak-hao rice genotypes of Manipur

Chak-hao rice Genotypes	*Percent infestation of leaf folder	Biochemical constituents (percent)			
		**Total soluble sugars	**Reducing sugars	**Total phenols	**Ortho dihydroxy phenols
Kotha chak-hao	3.70 (2.04)	3.88 (2.09)	2.06 (1.60)	4.63 (2.26)	2.71 (1.79)
Chak-hao Manam Nungshibi	3.61 (2.01)	3.62 (2.03)	1.99 (1.58)	4.15 (2.16)	1.99 (1.58)
Chak-hao Taniang Ban	3.73 (2.04)	3.28 (1.94)	2.29 (1.67)	4.20 (2.17)	2.11 (1.61)
Kom chak-hao	3.79 (2.06)	3.30 (1.95)	2.11 (1.61)	4.60 (2.26)	2.73 (1.80)
Chak-hao Sempak	3.81 (2.07)	3.19 (1.92)	2.10 (1.61)	3.80 (2.07)	1.95 (1.57)
Wairi chak-hao	5.27 (2.39)	6.10 (2.49)	2.32 (1.68)	2.29 (1.67)	1.32 (1.35)
Chak-hao Poireiton	5.10 (2.35)	6.61 (2.68)	2.42 (1.70)	2.27 (1.66)	1.48 (1.41)
Chak-hao Amubi	5.11 (2.35)	5.31 (2.40)	2.46 (1.72)	1.90 (1.54)	1.43 (1.39)
Chak-hao Tatha	5.23 (2.37)	5.43 (2.44)	2.73 (1.80)	2.96 (1.86)	1.27 (1.33)
Ching Chak-hao Angangba	5.09 (2.35)	5.23 (2.39)	2.32 (1.68)	2.41 (1.71)	1.37 (1.36)
Leimaphou	7.59 (3.25)	5.93 (2.47)	3.86 (2.09)	1.93 (1.56)	1.11 (1.27)
S.Ed(±)	0.18	0.04	0.06	0.06	0.05
CD	0.36	0.08	0.12	0.12	0.11

The values in parentheses are square root transformed values
*Mean of five replications
**Mean of three replications

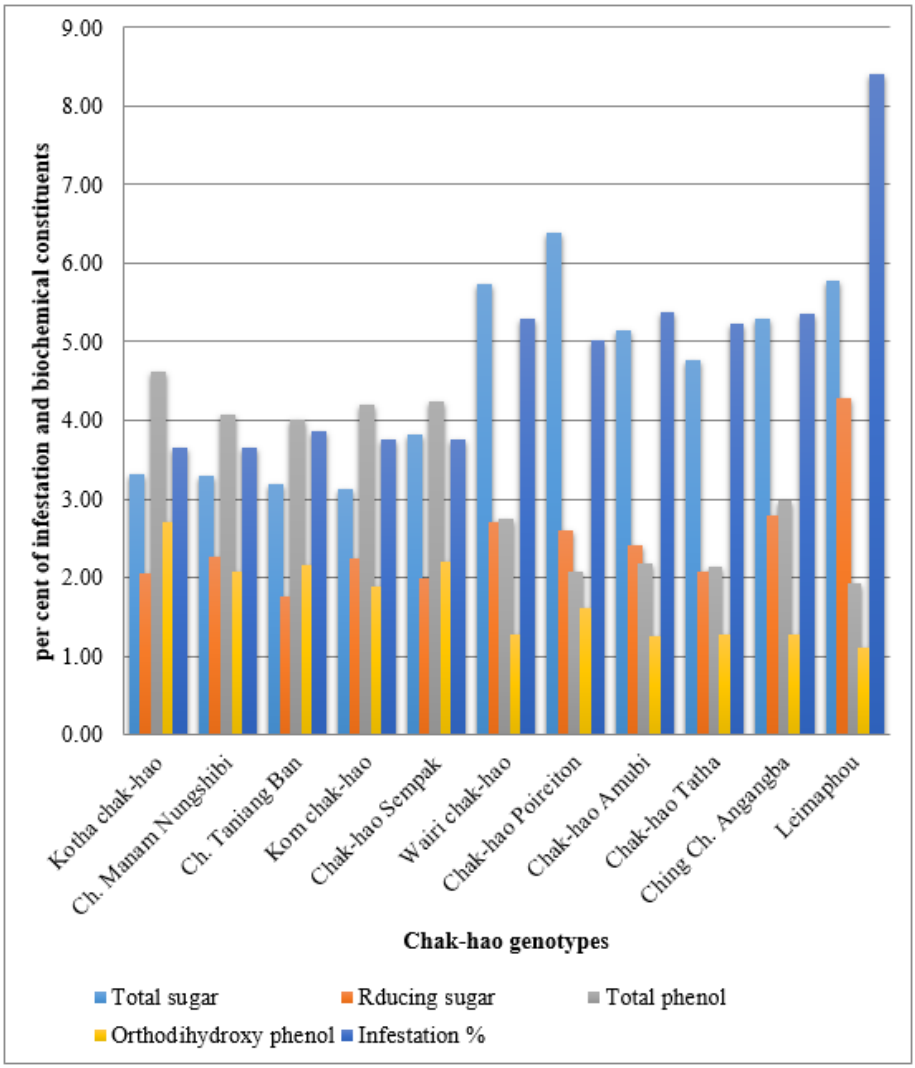

Fig 1: Graphical representation between percent leaf folder infestation and biochemical constituents.

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