

Physico-chemical basis of insect-resistance of traditional rice varieties against *Sitophilus oryzae* (L.)

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Based on popularity and specialty, seven traditional rice varieties were tested for resistance reactions in terms of physico-chemical properties against rice weevil, *Sitophilus oryzae* and compared with the improved high yielding rice varieties during 2019-2021. The result revealed that the aromatic rice variety *Kon joha* was the least suffered (11.67%) under the husked condition, whereas the aromatic traditional rice variety *Bokul joha* was the least suffered (53.00%) under the de-husked conditions at 18 days after infestation. Amongst the traditional varieties, the *Black rice* variety suffered maximum weight loss of 60.16% under de-husked conditions. All tested physical and biochemical parameters revealed a significant positive correlation with weight loss ($r=0.87$), total soluble protein ($r=0.86$), 1000 grain weight ($r=0.74$), moisture content ($r=0.54$), germination loss ($r=0.89$) and seed vigor loss ($r=0.54$) at 180 days of infestation under husked condition, while significant positive correlation with weight loss ($r=0.91$), starch content ($r=0.98$), crude protein content ($r=0.94$), L/B ratio ($r=0.88$), 1000 grain weight ($r=0.50$) and moisture content ($r=0.87$) at 180 days of infestation under de-husked condition. The rice grains possessing a high percentage of starch and protein, but a low per cent of crude fat was found more prone to infestation by *S. oryzae*.

Keywords: Chemical, Correlation, Infestation, Physical, Resistance, Rice, *Sitophilus oryzae*

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Rice, *Oryza sativa* L. (Family: Poaceae) is an important cereal crop serving as the staple food of over two billion people. Asia shares about 90% of the world's total rice production amounting over 700 million tons¹, which experiences 2-34% post-harvest losses². To safeguard food and nutritional security, especially in rice across the world, emphasis has been raised to address the popping-up constraints with efficient resource utilization. Increased production and reduced post-harvest loss could play a major role in this context, meeting not only the demand-supply deficits in rice but also to attain food security in developing countries like India. The demand for rice in India will continue to increase because of burgeoning population pressure and an increase in urbanization which gets complicated with the change in socio-economic and physical environment related to rice production along with over-exploitation of natural resources to meet the demand. Simultaneously, efforts are being made to counteract the hardship by evolving

new technological capsules to address the constraints. Cereal grains are exposed to maximum post-harvest losses among all agricultural commodities, ranging from 50%-60% in India due to technical inefficiency³. Post-harvest losses of cereals could broadly be categorized as qualitative (including nutritional, seed viability, and other commercials) and quantitative that vary with the crop concern. The quantitative losses include direct feeding of insects that cause a reduction in weight of grains during storage and the qualitative loss includes changes in chemical properties of grains, loss in nutritional properties, consumer preference, and commercial value⁴.

Rice grains were more prone to the infestation of insect pests and mites after harvest, as they get stored for a varied time with minimal protection at both farmers' level and commercial godowns⁵. Insect pest infestation on rice grains during storage not only reduces the quality of rice but also affects its germination. Over 1200 species of pests are found causing damage to the stored products⁶, of which rice weevil, *Sitophilus oryzae* (L.) (Coleoptera:

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Curculionidae) is a major one causing severe seed damage (18.30%) to stored cereals including rice, wheat, sorghum, maize, oats, barley, cotton, linseed, etc.^{7,8}. In India, *S. oryzae* causes about 6.5% loss to stored and milled rice seed⁹. Attack of *S. oryzae* could also lead to secondary infestation of mites and fungi, causing loss of nutritive value and commercial value of the product¹⁰.

A wide variety of heritable traits are found associated with the rice plant against herbivory leading towards avoidance, resistance, or tolerance with a known effect on host preference and performance. Scientific research on the resistance reaction of different rice varieties including traditional as well as newly developed rice varieties is very much scanty. Information on resistance reaction could help in reducing colonization of insect pest as well as post-harvest yield loss on many occasions. Hence, our present investigation aimed at screening of some traditional, elite, and popular rice varieties of Assam, India for physical and biochemical properties influencing resistance against *S. oryzae* during storage.

Materials and Methods

The present investigation to evaluate resistance reactions of traditional rice varieties of Assam against *Sitophilus oryzae* was conducted in the Department of Entomology, Assam Agricultural University (AAU), Jorhat, Assam, India during 2019-21.

Sampling of rice varieties

Altogether seven traditional rice varieties of state Assam were selected for comparative assessment toward *S. oryzae* resistance during storage in comparison to the high yielding improved rice varieties based on popularity, availability, amylose content and resilient characteristics (Table 1). About 2 kg of freshly harvested rice seeds were collected

Table 1 — List of rice varieties selected for the experimentation along with their categorization

| S/No. | Category | Group | Rice variety |
|-------|-----------------------|-----------------------|------------------------------------------|
| 1 | Traditional varieties | Glutinous rice | <i>Aghoni Bora, Chakua Bora</i> |
| | | Specialty rice | <i>Chakua, Black Rice</i> |
| | | Scented rice | <i>Kon joha, Bokul joha, Keteki joha</i> |
| 2 | Improved varieties | High yielding | <i>Ranjit, Bahadur, Shraboni</i> |
| | | Submergence tolerance | <i>Ranjit sub-1, Bahadur Sub-1</i> |
| | | Short duration | <i>Luit, Dishang</i> |
| | | Staggered planted | <i>Gitesh</i> |
| | | Semi dwarf | <i>Jaya</i> |

from Regional Agricultural Research Station (RARS), Titabar (Latitude: 26.5721° N, Longitude: 94.1808°E), Instruction cum Research Farm (ICR Farm), AAU, Jorhat (Latitude: 26.7509° N, Longitude: 94.2037°E) and other localities of Jorhat district of Assam (Latitude: 26.7509°N, Longitude: 94.2037°E). Collected rice grains were dried under direct sunlight to attain uniform moisture content, cooled under shade, and stored in air-tight plastic containers. Half of the collected rice grains were de-husked and kept separately in air-tight plastic containers for subsequent studies.

Mass culture of test insect

The mother culture of *S. oryzae* was obtained from the Post-Harvest Laboratory, AAU, Jorhat during 2019-20 and released (Male: Female sex ratio - 1:1) on freshly harvested insect-free rice grains (var. *Ranjit*) kept in plastic containers (Capacity: 1 liter) for 24 h to lay egg on rice grains and removed thereafter to obtain uniformly aged test insect. The lids of the containers were replaced with a muslin cloth to provide proper aeration to the insects.

Grain damage (%)

A total of 50 g of husked and de-husked rice grains were collected randomly from each of the selected rice varieties and placed separately in a plastic container (5 cm diameter). Altogether, 10 pairs of neonate adults of *S. oryzae* separated based on the rostrum characteristics as suggested by Richards¹¹ and Halstead¹² were released on to each of the containers having rice grains and covered with muslin cloth for proper aeration. The containers were kept at room temperature and humidity; and data on seed damage were recorded at 15, 30, 60, 90 and 180 days after release. Grain damage (%) was calculated based on insect exit holes and partially or completely eaten germ point or endosperm, as per the formula proposed by Padmasri *et al.*¹³ mentioned below.

Grain infestation (%) = (Total number of seeds infested / Total number of seeds observed) x 100

Grain weight loss (%)

Initially, 50 g of husked and de-husked rice grains were placed on plastic containers and 10 pairs of neonate adults were released as per the method described above. The grains were subjected to the test insect for about 6 months (180 days), after which the grains were weighed again and the weight loss (%)

was estimated using the formula proposed by Ngatia and Kimondo¹⁴.

$$\text{Weight loss (\%)} = [(W_1 - W_2) / W_1] \times 100$$

Where, W_1 = Initial weight taken, W_2 = Final weight taken

Physical parameters of rice seed

Length-Breadth (L/B) ratio

The length and breadth of 10 numbers of randomly selected rice grains of each variety were recorded using graph paper and the length/breadth (L/B) ratio was calculated.

Thousand grain weight (g)

A thousand numbers of grains of each of the selected rice variety was weighed by using micro balance (Make: Sartorius, Model: BS 224 S) and replicated thrice.

Moisture content (%)

The pre- and post-infestation moisture content (%) of each rice variety was recorded by using a digital grain electronic moisture meter (Make: Satake, Model: SS- 5).

Seed germination (%)

A total of 25 numbers of rice grains of each of the variety was collected randomly at pre- and post-infestation and kept on the germinator (Make: Tempstar, Model: KSG-001) for 14 days to record the percentage germination. Each of the treatments was replicated four times and seed germination (%) was calculated by using the formula proposed by Padmasri *et al.*¹³.

$$\text{Seed germination (\%)} = (\text{Number of seeds germinated} / \text{Total number of seeds observed}) \times 100$$

Seed vigor index

The seed vigor index was measured as per the method proposed by Abdul-Baki and Anderson¹⁵, in which, 10 numbers of randomly selected rice grains of each variety were placed on a germination plate, allowed to grow for 14 days in the germinator (Make: Tempstar Model: KSG-001) and replicated thrice. The seedling length (mm) of the selected rice varieties was recorded after 14 days of plating by using a graph paper. Data on per cent seed germination and seedling length (mm) were utilized to compute seed vigor index as per the formula mentioned below.

$$\text{Seed Vigor Index} = \text{Seed germination (\%)} \times \text{Seedling length (mm)}$$

Biochemical parameters of rice seed

Starch content

The starch content was estimated by the method proposed by Chopra and Konwar¹⁶, wherein each of the de-husked rice seed samples was grounded to a fine powder and treated with 80% ethyl alcohol (Make: Changshu Yangyuan Chemical) to remove sugars. The products were treated with 52% perchloric acid (Make: Finar) to hydrolyze the starch into glucose and finally dehydrated to hydroxy-methyl furfural, which turns into a green-colored product on addition of anthrone reagent. The solution was subjected to spectro-photometry at 630 nm (Make: Chemito, Model: UV 2600) and the value obtained from the standard curve was multiplied by the factor 0.9 (Morris factor) to get the exact starch content in the sample. The estimation was carried out in triplicate and all the mean was expressed in g per 100 g of moisture-free sample.

Crude protein

The total nitrogen content of the selected rice samples was carried out with the Kjeldahl method proposed by Scales & Harrison¹⁷, which was later converted to protein by multiplying the nitrogen percentage by a factor of 5.95. The experiment was replicated thrice, and the protein content was expressed as a percentage on a moisture-free basis. Total nitrogen was calculated using the following formula:

$$\text{Total Nitrogen (g/100 g sample)} = [(a-b) \times \text{Normality} \times 14 \times 100] / [\text{weight of sample (g)} \times 100]$$

Where, a = mL of standard acid for sample. b = mL of standard acid for blank

$$\text{If total nitrogen value is X, protein content in 100 g} = [X / 5.95]$$

Total soluble protein

The total soluble protein was estimated by Folin-Ciocalteu/ Lowry Method proposed by Lowry *et al.*¹⁸, where the protein reacts with the Folin-Ciocalteu reagent to give a colored complex whose intensity was measured spectrophotometrically under 660 nm (Make: Chemito, Model: UV 2600) against the blank.

Crude fat

To estimate the crude fat content, 5 g of moisture-free powdered rice sample was placed on a Soxhlet apparatus and extracted using petroleum ether (BP-60-80°C) for 6 h until a clear solvent was obtained¹⁹. After removal of solvent through evaporation, the residue was oven-dried at 80°C, cooled in desiccators and weighed to calculate the crude fat content (%) on moisture-free basis using the following formula-

$$\text{Crude Fat Content (g/100 g sample)} = \left[\frac{\text{Weight of the either extract (g)}}{\text{Weight of sample (g)}} \right] \times 100$$

Ash

To estimate the ash content, 5 g of moisture-free powdered rice seed sample was taken in a silica crucible, charred in a low Bunsen flame, and ignited at 600°C for 6 h in a muffle furnace. Each of the rice seed sample was replicated thrice and the data on ash content (%) in moisture-free ignited rice sample was recorded using the following formula-

$$\text{Ash content (g / 100 g sample)} = \left[\frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \right] \times 100$$

Amylose

The Amylose content was determined by the method described by Sowbhagya & Bhattacharya²⁰. In this method, 100 mg moisture free powdered samples were placed in a volumetric flask and 1 mL of alcohol and 10 mL of NaOH were added to it and left overnight at room temperature. Five milliliters of sample solution were dispersed in 10 mL NaOH (1N) and diluted to 100 mL. Then, 5 mL of aliquot were taken in a 100 mL volumetric flask, 3 drops of phenolphthalein indicator was added to it and made the volume up to 50 mL. Dilute hydrochloric acid was added drop wise with shaking till the colour was just discharged. Later, 2 mL of iodine solution was added, and the volume was made to 100 mL. The intensity of color developed was measured in a spectrophotometer at 620 nm wavelength (Make: Chemito, Model: UV 2600) against the reagent blank after 30 min of rest and repeated thrice. The amylose content was estimated by-

$$\text{Amylose content (per 100 g dry weight)} = \left[\frac{R}{A} \right] \times \left(\frac{a}{r} \right) \times \left(\frac{1}{5} \right) \times 100$$

Where, R = 620 nm reading for sample dispersion, A = 620 nm reading for standard amylose solution, a = Amount of the standard amylose taken, r = Amount of sample taken.

Amylopectin

The amylopectin content was determined by subtracting the percentage of the amylose from 100 on moisture-free sample basis²¹.

Results

On subjecting selected rice varieties including the traditional rice varieties of Assam for a period of 180 days of storage with artificial inoculation of *S. oryzae*, the seed infestation percentage was found to get varied between 24.67-11.67% and 93.67-53% in husked and de-husked grains across the rice varieties, respectively (Fig. 1 and Fig. 2). The seed damage caused by *S. oryzae* was found less in traditional varieties as compared to the improved varieties. Both *Kon joha* (husked) and *Bokul joha* (de-husked) varieties showed the least damage by *S. oryzae* recording only 11.67% and 53.00% seed damage, respectively at 180 DAI. The seed weight loss found varied between 8.81-2.90% and 60.16%-12.41% in husked and de-husked grains, respectively (Fig. 3). The lowest weight loss percentage was recorded in the case of *Kon joha* (2.90%) and *Bokul joha* (12.41%) as they suffered from elast insect infestation.

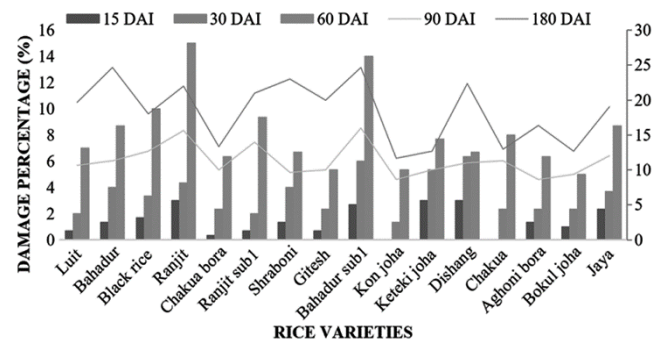


Fig. 1 — Grain damage reaction of rice varieties against *Sitophilus oryzae* (in husked rice) at different time interval

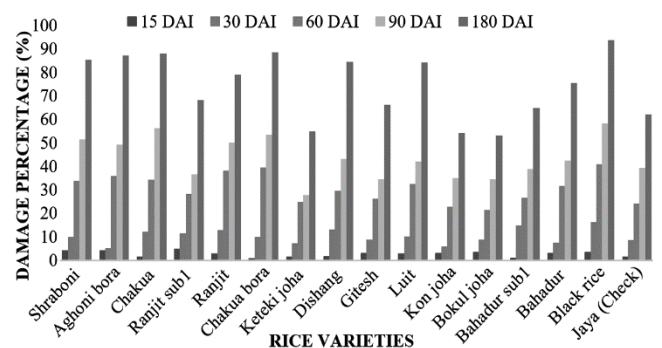


Fig. 2 — Grain damage reaction of rice varieties against *Sitophilus oryzae* (in de-husked rice) at different time interval

In terms of physical parameters, the traditional varieties like Black rice, *Chakua* and *Chakua bora* showed a higher L/B ratio of 3.22, 3.11, and 3.11, respectively; as against a lower L/B ratio of 2.42, 2.55, and 2.66 in *Bokul joha*, *Kon joha*, and *Keteki joha*, respectively (Fig. 4). The highest thousand grain weight was recorded in the case of *Bahadur*, while the lowest in *Bokul joha* (Fig. 4). The final moisture content varied from 12.80% to 14.51% in the case of husked rice grains (Fig. 4), while 13.15% to 15.35 % in the case of de-husked grains across the varieties. The rice seeds were subjected to a germination test both before and after infestation at 180 DAI (Fig. 5) and the seeds of improved rice variety *Bahadur* suffered a higher degree of infestation by *S. oryzae* recording the lowest germination (71.04%) was recorded at 180 DAI. The highest per cent germination (41.40) was recorded in Black rice as it suffered less due to *S. oryzae* infestation. The seed vigor loss of the germinated seeds was recorded at 14 days after germination and was found varied from 48.21% to 79.06% (Fig. 5).

The starch content of the selected rice varieties on dry weight basis were found varied between 68.36% to 82.14% (Fig. 6). Traditional rice variety black rice

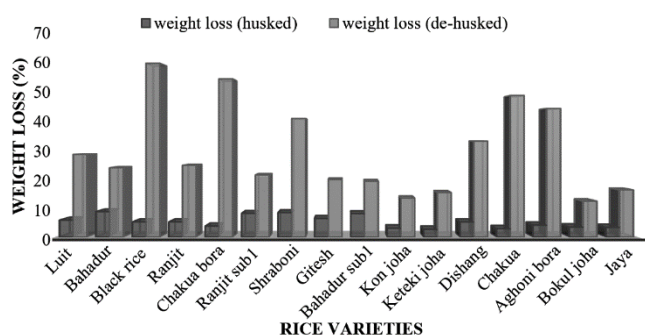


Fig. 3 — Seed weight loss (%) after 180 days of infestation by *S. oryzae*

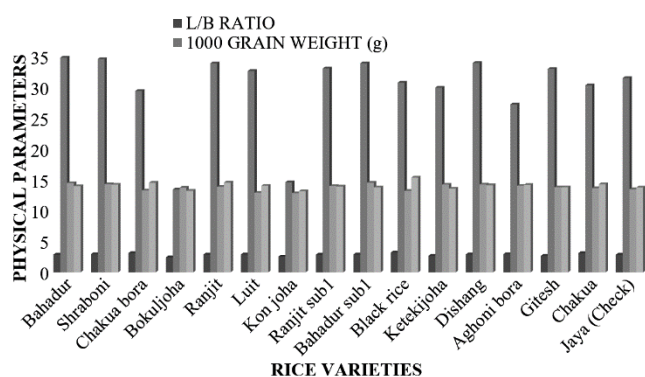


Fig. 4 — Physical properties of selected rice varieties of Assam

recorded the highest starch content of 82.14%, followed by other traditional rice variety *Chakua bora* (80.08%), *Chakua* (79.38%), *Aghoni bora* (79.01%). The lowest level of starch content was recorded in the case of traditional rice variety *Bokul joha* (68.36%), followed by *Kon joha* (69.23%) and *Aghoni bora* (79.01%). The total soluble protein content of rice varieties tested was found to be the highest in the improved rice variety *Bahadur* (6.42%) and the lowest in *Bokul joha* (4.65%). The crude protein content amongst the rice varieties was found to be varied within a range of 7.35% to 10.49%. The traditional and popular rice varieties such as *Chakua bora*, *Bahadur*, and *Aghoni bora* were found to have 9.83, 9.27%, and 9.17% of crude protein, respectively. A higher level of crude fat was recorded in traditional rice variety *Keteki joha* (3.53%). The amylose content amongst the rice varieties was found to be varied within the range of 1.75% to 25.27%. In our present investigation, categorization of the rice varieties based on amylose content was done as per the classification proposed by Bhattacharya *et al.*²² and the data revealed that none of the varieties could be categorized as high (>25%) amylose content, while two varieties each could be categorized under low (*Chakua* and Black rice) and

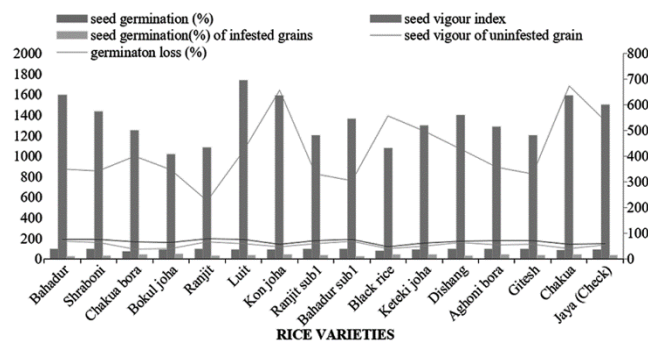


Fig. 5 — Loss of Germination (%) and seed vigor loss (%) of rice varieties before and after infestation by *S. oryzae*

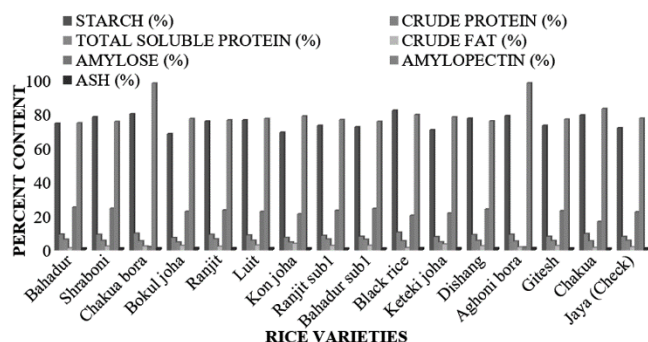


Fig. 6 — Biochemical parameters of rice varieties

waxy (*Aghoni bora* and *Chakua bora*) (Table 2). The highest ash content (Fig. 6) was recorded in the case of *Bokul joha* (1.37%), followed by *Aghoni bora* (1.31%) and *Keteki joha* (1.29%). A lower level of ash content was recorded in the case of traditional variety *Chakua* (1.02%).

In our experiment, grain characteristics were correlated to their vulnerability to weevil infestation to find out the special feature that could be included in various rice breeding programmes (Table 3) and a very significant positive correlation observed between the L/B ratio, 1000 grain weight and moisture

percentage with seed damage percentage was recorded at 180 DAI in de-husked rice with a coefficient of correlation was found to be 0.88, 0.50 and 0.87, respectively. A significant positive correlation was observed between the seed damage and germination loss (%) and seed vigor loss (%) with correlation coefficient of 0.89 and 0.54, respectively at 180 DAI (Table 3).

A significant positive correlation between starch, soluble protein, crude protein content and damage percentage was also recorded with a correlation coefficient of 0.98, 0.86 and 0.94, respectively at 180 DAI (Table 4), whereas the ash and crude fat content shown a non-significant negative correlation with that of seed damage percentage after 180 days of infestation. The amylose content of rice grains under observation was positively correlated to the damage percentage in husked grains, whereas negatively correlated in de-husked grains at 180 DAI with a correlation coefficient of 0.48 and -0.41, respectively. In contrast, the amylopectin content showed a negative correlation ($r=-0.48$) with the damage

Table 2 —Categorization of selected rice varieties based on amylose content (Srinang *et al.*⁴¹)

| Category (% amylose) | Name of the rice variety |
|----------------------|-------------------------------------------------------------------------------------------------------------------------------|
| Waxy (0-2.9) | <i>Aghoni bora, Chakua bora</i> |
| Low (10-19.9) | <i>Chakua, Black rice</i> |
| Medium (20-24.9) | <i>Jaya, Bokul joha, Gitesh, Keteki joha, Shraboni, Ranjit sub-1, Kon joha, Bahadur sub-1, Dishang, Ranjit, Bahadur, Luit</i> |
| High (>25) | Nil |

Table 3 — Correlation of seed damage with physical properties of rice varieties at different time intervals

| Type | Time interval (DAI) | Physical properties of rice varieties | | | | |
|-----------|---------------------|---------------------------------------|-----------------------|----------------------|----------------------|----------------------|
| | | L/B Ratio | 1000 grain weight (g) | Moisture content (%) | Germination loss (%) | Seed vigour loss (%) |
| Husked | 15 | -0.01 ^{NS} | 0.39 | 0.47 | 0.17 | 0.56* |
| | 30 | 0.10 | 0.50 | 0.51* | 0.22 | 0.70** |
| | 60 | 0.33 | 0.49 | 0.48 | 0.25 | 0.31 |
| | 90 | 0.31 | 0.52* | 0.47 | 0.25 | 0.28 |
| | 180 | 0.23 | 0.74** | 0.54* | 0.89** | 0.54* |
| De-husked | 15 | -0.18 | -0.14 | 0.02 ^{NS} | - | - |
| | 60 | 0.84** | 0.44 | 0.92** | - | - |
| | 90 | 0.85** | 0.29 | 0.86** | - | - |
| | 180 | 0.88** | 0.50* | 0.87** | - | - |

NS= non-significant, * - Correlation is significant at the 0.05 level, ** - Correlation is significant at the 0.01 level, DAI- Days after infestation, L/B Ratio- Length/Breadth Ratio

Table 4 — Correlation of seed damage caused by *S. oryzae* with weight loss and biochemical properties of rice varieties at different time intervals

| Type of grain | Time intervals (DAI) | Weight loss | Biochemical properties of rice varieties (%) | | | | | | |
|---------------|----------------------|---------------------|----------------------------------------------|-----------------------|-----------------------|---------------------|-----------------|---------------------|----------------------|
| | | | Starch content | Crude protein content | Total soluble protein | Crude fat content | Amylose content | Amylopectin content | Ash content |
| Husked | 15 | 0.07 ^{NS} | -0.09 ^{NS} | -0.07 ^{NS} | 0.31 | -0.02 ^{NS} | 0.32 | -0.32 | 0.32 ^{NS} |
| | 30 | 0.23 | 0.01 ^{NS} | 0.02 ^{NS} | 0.32 | -0.04 ^{NS} | 0.37 | -0.37 | 0.08 ^{NS} |
| | 60 | 0.33 | 0.09 ^{NS} | 0.20 | 0.71** | -0.23 | 0.28 | -0.28 | -0.19 |
| | 90 | 0.42 | 0.04 ^{NS} | 0.15 | 0.78** | -0.20 | 0.38 | -0.38 | -0.31 |
| | 180 | 0.87** | 0.15 | 0.18 | 0.86** | -0.32 | 0.48 | -0.48 | -0.30 |
| De-husked | 15 | -0.03 ^{NS} | -0.01 ^{NS} | 0.01 ^{NS} | -0.27 | -0.07 ^{NS} | 0.13 | 0.20 | -0.01 ^{NS} |
| | 30 | 0.36 | 0.42 | 0.45 | 0.18 | -0.28 | 0.30 | 0.01 ^{NS} | -0.002 ^{NS} |
| | 60 | 0.87** | 0.92** | 0.95** | 0.16 | -0.59 | -0.47 | 0.43 | 0.10 |
| | 90 | 0.91** | 0.91** | 0.90** | 0.39 | -0.70 | -0.42 | 0.27 | -0.04 ^{NS} |
| | 180 | 0.91** | 0.98** | 0.94** | 0.27 | -0.64** | -0.41 | 0.36 | 0.028 ^{NS} |

NS= non-significant, * Significant at the 0.05 level, ** Significant at the 0.01 level, DAI- Days after infestation

percentage in husked grains and positive correlation ($r=0.36$) in de-husked grains.

Discussion

Although a significant disparity in terms of physico-chemical properties observed among the traditional rice varieties, but none of the varieties were found to be totally resistant to the attack of *S. oryzae*. Both the traditional aromatic rice varieties *Kon joha* (husked) and *Bokul joha* (de-husked) showed least damage by *S. oryzae* recording only 11.67% and 53.00% seed damage, respectively after 180 DAI, which might have been regulated with a combination of characteristics, including grain hardness, nutritional value, and natural resistance²³. Many a times, the resistance against *S. oryzae* generated by husked grains was likely due to the thickness of the husk and hardness of the grain limiting the penetration of the insect into the grain, which even influence the oviposition choice of the insect²⁴. The rice grains with a compact husk suffered the least damage could be explained by the fact that the husk acted as a physical barrier to *S. oryzae*²⁴⁻²⁷, which was also found true in our present investigation exerting physical resistance to the insect infestation. Moreover, silica and lignocellulose present on the seed coat constitute a unique lignocellulose-silica network that could probably restricts insect infestation during storage. Hence, it could be recommended that rice grains should be kept un-hulled for a longer storage and only to be hulled just before consumption to escape the insect infestation during storage. The lignin in the cell wall of rice grains along with the silica creates a physical barrier might help in conferring resistance against insect feeding during storage.

Moreover, grains with high L: B ratio shows greater grain size, which alters insect infestation through space for growth, development, and infestation. *Sitophilus* spp. prefers large seeds for oviposition, as larger seeds are more likely to contain many eggs than the smaller seeds and support more space and food for weevil development²⁸⁻³⁰, which was found true in case of traditional rice varieties *Bokul joha* that suffered least due to its lower grain weight (13.41 g). A high grain moisture content indicates a conducive physiological environment for the weevil's growth and development, leading to high seed damage. Zunjare *et al.*³¹ found that grain weight loss was positively correlated with insect progeny emerging, which corroborates our present

investigation. Guenha *et al.*³² also reported that the weight loss in rice grains was positively correlated with the seed vigor loss caused by storage insects, and indeed the decrease in shoot length was more pronounced as the storage period lengthened³³.

Our observation showing a positive correlation of starch content with insect damage corroborates the key findings of Matsumoto *et al.*³⁴ and Chijindu & Boateng³⁵, who found that insects consume starch and protein from grains to develop and lay eggs. Several others also reported a positive relationship between protein content in cereals and the adult emergence of *S. oryzae*^{36,37}. From our investigation it is also evident that the insect infestation decreases with the increase crude fat content in stored grains, which was supported by Okiwelu *et al.*³⁸, who revealed a negative significant relationship ($r=-0.97$) between oil content and the degree of infestation by *S. zeamais* in stored maize. Previous research indicated that fatty acids from the groups C 18:1 and C 20:1 present on wheat kernel acts as a stimulating agent, while fatty acid groups C 15:0, C 16:1, and C 18:3 act as a deterring agent against *Sitophilus granaries*³⁹, which need further confirmation through appropriate research. Negative correlation of amylopectin with insect damage observed was in agreement with the study of Ngom *et al.*⁴⁰, who reported a negative correlation between the amylose content in maize grains and the susceptibility index ($r=-0.67$) of the bigger grain borer, *Prostephanus truncates*, as high amylose content along with high gelatinizing temperature and relative humidity (> 75%) creates a low equilibrium grain moisture content contributes toward low digestibility of raw starch.

Conclusions

From our present study it could be concluded that seed weight, grain size, grain moisture and grain weight loss are the reliable criteria for assessing resistance reaction against *S. oryzae*. Moreover, rice grains having a high percentage of starch and protein, but a low per cent of crude fat was found more prone to *S. oryzae* infestation. Traits showing resistance against *S. oryzae* could further be exploited for resistance breeding programs in the near future.

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Consent for publishing

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Conflict of Interest

The authors declare that they have no conflict of interest on the manuscript's content and study undertaken.

Author Contributions

Investigation, data recording, analysis, and writing (PD), Conceptualization of research (SK), Finalization of methodology, research supervision, review and editing of manuscript (SK), Supervision of research and logistic support (PP, BS, KP), Data analysis (HS)

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