



Effect of Ash, Crude Protein and Nitrogen in Traditional Varieties of Rice Against *Sitophilus oryzae* (L.) Infestation in Storage

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The management of rice weevil infestations in traditional rice varieties presents unique challenges. Traditional storage practices and methods may not always align with contemporary insect pest management strategies furthermore, the genetic diversity of traditional rice varieties means that insect pest management solutions need to be tailored to specific varieties and local conditions.

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Twelve rice varieties were collected from different Farmers of Telangana State. The selected varieties, after collection, were cleaned by removing physical impurities if any and thereafter they were kept in an incubator at a temperature of 55°C for four hours to kill the existing insects without damaging the viability of the seeds. In this study, the reduced adult emergence in moderately resistant varieties could be linked to their elevated ash content. This high ash content in the seeds may deter insects due to potential toxicity, rendering these varieties less preferred for feeding, growth, and emergence by the grub, and high crude protein and nitrogen content enhances nutritional content and attractiveness, promoting growth and reproduction and increases egg laying resulting in more extensive damage to the stored rice. The present results of ash, crude protein and nitrogen content can guide the selection and breeding of traditional rice varieties with lower weevil susceptibility and perform storage practices that minimize rice weevil infestations and also improves traditional storage practices by identifying natural pest resistance and proper hermetic storage practices.

Keywords: Storage practices; physical impurities; rice weevil infestations; pest management.

1. INTRODUCTION

Rice (*Oryza sativa* Linnaeus.) is one of the most important food globally, providing sustainability for millions of world's population, considering that the most crucial crop globally. In Telangana it is cultivated nearly area 3186.40 lakh hectares, production 10217.13 lakh tons and productivity 3206 kg per hectare during 2020-2021 [1]. Among districts of Telangana, in area Nalgonda ranks first whereas in production Nizamabad ranks first [2]. Rice ranks, second most essential cereal crop in the world. It has influenced the cultures, diets, and economies of billions globally. Central to national well-being and food security, rice holds a critical position in India. Following China, India stands as the top producer and a leading exporter of rice worldwide [3].

Despite technological advancements boosting food grain production, significant losses occur due to poor storage practices. It is evaluated that 60-70% of the country's food grain kept in traditional household storage methods, including bamboo baskets, mud structures, gunny sacks, and modern bins [4]. India losses 12 to 16 million metric tons of food grains annually in post-harvest management and the World Bank assesses that it could feed most of the nation's poor. These losses are valued at over fifty thousand crores per year [5]. In India, insect pests are estimated to damage 6.5% of the total stored grains [6,7]. In storage warehouses and godowns, fumigants like methyl bromide and aluminium phosphide are commonly used to control storage pests including rice weevil. One widely utilized fumigant is phosphine (hydrogen phosphide) which is extensively employed worldwide to disinfest stored commodities, ensuring food integrity and facilitating

international trade by eradicating pests and residues [8]. However, the use of chemical pesticides like methyl bromide raises concerns due to its ability to deplete the ozone layer. Host resistance can play an important role in minimizing damage by containing several biochemicals that include phenol total sugars, reducing sugars, ash and nitrogen of stored rice by *S. oryzae* [9]. Chemical composition of the rice grains plays an important role in finding the relative resistance to insect pest attack [10]. Storage grains might contain high resistance to insect pests because of the absence of essential growth nutrients or the presence of constituents that severely affect insect growth and development [11].

Traditional rice varieties, cultivated for centuries are integral to the cultural and agricultural heritage of many regions. These varieties often exhibit unique traits such as resilience to local environment conditions and resistance to certain diseases and pests. Despite their benefits, the susceptibility of these varieties to storage insect pests like the rice weevil has not been thoroughly investigated.

The management of rice weevil infestations in traditional rice varieties presents unique challenges. Traditional storage practices and methods may not always align with contemporary insect pest management strategies furthermore, the genetic diversity of traditional rice varieties means that insect pest management solutions need to be tailored to specific varieties and local conditions. Identifying and utilizing resistant traditional varieties can provide an environmentally friendly alternative to chemical pest control preserving both agricultural biodiversity and traditional farming practices [12].

Considering the understanding of the biochemical parameters of traditional varieties of rice is crucial for management of storage pest because these parameters determine a variety susceptible or resistance to rice weevil infestation and this helps in selecting and developing traditional rice varieties that are naturally less prone to weevil attacks, reducing post-harvest losses.

2. MATERIALS AND METHODS

Twelve traditional rice varieties were collected from various Farmers of Telangana State. The selected varieties, after collection, were cleaned by eliminating physical impurities and those varieties were kept in an incubator at a temperature of 55°C for four hours to kill the early stages of insects if any without damaging the viability of the seeds [13]. Then the moisture content of the test traditional varieties (under three replications) were standardized to equal by keeping the test varieties in the desiccator having saturated solution of potassium hydroxide for 21 days [14]. These pre-conditioned test varieties were used in the experiment.

In this research *MTU 1010 (resistant variety) and *BPT 5204 (susceptible variety) were used as control for comparison, ensuring that the results of an experiment variables are tested rather than external factors there by enhancing accuracy of the findings.

2.1 Mass Multiplication

The test insect (rice weevil) were massively multiplied on paddy BPT 5204 and for screening studies newly emerged seven days old adult weevils were used. The female and male sexes of the adult weevils were identified according to the traits given by Halstead [15] which states that rice weevils containing long rostrum having narrow holes along rostrum arranged in particular rows and not contacting each other were considered as females. Whereas, short rostrum with wide holes which are arranged irregularly and not in a row and often contacting each other along the rostrum are considered as males [16].

2.2 Screening

For each replication, ten grams of seed were placed in small plastic tubes (7.5 cm x 5 cm) with minute perforations on the lid. Freshly emerged, seven-day-old adult weevils (eight females and four males) with 34 were introduced into each tube to infest the ten grams of seeds for each

test varieties [17]. They were placed in an incubator set at a temperature of 26±2°C and a relative humidity of 70±5 percent. The weevils were permitted to lay eggs in the seeds for a duration of two weeks before being removed. The plastic tubes were returned to a desiccator and kept there until the emergence of the F1 adults.

2.3 Mean Adult Emergence

The count of adults emerging from each treatments from each replication was recorded daily and subsequently removed from their respective tubes. Counting persisted until weevils ceased emerging from the seeds. The mean adult emergence was evaluated by pooling the data.

2.4 Mean Development Period

The mean development period of test insect (rice weevil) against traditional varieties was evaluated as per Howe [18]. Each treatment undergone three replications.

$$D = \frac{\sum (A \times B)}{C}$$

A = Number of new adults (rice weevil) emerged on nth day. B = number of days required for their adult emergence (rice weevil). C = Total number of adults (rice weevil) emerged during experimental period. D = Mean developmental period in days.

2.5 Per Cent Seed Damage

At the conclusion of the experiment, the quantity of seeds damaged by the weevil in each treatment in each replication was tallied and then converted into a percentage of damaged seeds.

2.6 Ash Content (Per Cent)

The ash content of the test varieties was determined according to the AOAC [19] guidelines and reported as a percentage. Each treatment undergone three replications. For this analysis, 5grams finely ground sample of the test varieties were utilized and initially charred with in silica crucibles. Once charring was completed, the crucibles were subjected to a muffle furnace at 600°C for a duration of 2 hours. Subsequently, the final weight of the sample was determined by subtracting the initial weight, and the result was converted into a percentage.

$$\text{Ash (\%)} = \frac{\text{mass of ash}}{\text{Initial mass of the sample}} \times 100$$

2.7 Nitrogen

The nitrogen content of the grain samples was analyzed following the micro-Kjeldahl method recommended by AOAC [20], employing a Kjelplus auto analyzer (Plate 3.14). Initially, 0.2g of sample was digested in presence of 2g of catalyst mixture (copper sulphate and potassium sulphate in 1:5 ratio) and 10 ml of conc. Sulphuric acid at 420°C for 2 hours. After cooling, the distillation was carried out in auto distillation system (loaded with 4% boric acid and 40% sodium hydroxide). The distillate obtained was titrated against 0.1N HCL till appearance the of pink colour. The per cent nitrogen was calculated as follows.

$$\text{N}_2 (\%) = \frac{(\text{Titre value of test varieties sample} - \text{Titre value of blank}) \times 14 \times \text{Normality of HCl (0.1)} \times 100}{(\text{Weight of sample} \times 1000)}$$

2.8 Crude Protein Content

The crude protein content was calculated by multiplying the nitrogen per cent obtained with the factor 6.25 [21] and expressed in percentage.

2.9 Statistical Analysis

The data underwent square root and angular transformation and analyzed utilizing a completely randomized design (CRD). The data which is obtained were analyzed by Analysis of variance (ANOVA) and INDOSTAT statistical software used for analysis. Percentage data collected were transformed to angular transformation.

3. RESULTS AND DISCUSSION

3.1 Mean Adult emergence

The number of rice weevil adults emerged from various test traditional varieties ranged from 3.3 to 26.0.

- Lowest mean adult emergence: MTU 1010 (3.3)
- Moderate mean adult emergence: Kabirajsal (8.3), BPT 5204 (8.7), Rajamudi (9.7), Karupukavuni (14.3), Rajbhog (16.0), Bahurupi (17.3), and Ambemohar (20.0).
- Highest mean adult emergence: Navara (26.0), Manipuriblack (22.0) and Chittimuthyalu (21.7).

Similar findings were recorded by the earlier workers. Kiran [7] where least number of rice weevils had emerged from rice variety JGL 3844 (3.00) Whereas, significantly maximum number of rice weevil adults had emerged from JGL 11118 (17.33) in rice varieties.

Majid Marani et al, [21] identified in rice cultivar that the average number of *S. oryzae* adults emerging from distinctive rice varieties ranged from 14.8 to 40.3 caused by *S. oryzae*.

3.2 Mean Development Period (Days)

The mean development period of rice weevil observed in various treatments ranged from 32.3 to 45.6 days.

- Lowest mean development period: Navara (32.3 days)
- Moderate mean development period: BPT 5204 (34.0 days), Kalabati(34.3days), Manipuriblack (35.3 days) Ambemohar (36.6), Chittimuthyalu (37.3) Bahurupi (38.6) and MTU 1010 (39.6 days) and Rajbhog (41.0).
- Highest mean development period: Kabirajsal (45.6 days) Rajamudi (43.3 days), and Karupukavuni (42.0 days)

The answers are compatible with those of earlier researchers. kiran et al., (2020) the variety having highest mean development period MTU 1001 (48.83 days) and lowest found in the variety JGL 11118 (35.84 days).

Yevoor [22] identified a minimum developmental period (39.00 days) of rice weevil on the maize hybrid SAT, which had a high F1 offspring. In contrast, a maximum developmental period (45.00 days) were observed on DHM-11, which had the minimum F1 progeny.

3.3 Seed Damage (Per Cent)

Seed damage in traditional rice varieties ranged from 1.3 to 11 per cent (Table 1).

- Lowest seed damage: Kabirajsal (1.3%)
- Moderate seed damage: MTU 1010 (3.3), Rajamudi (3.7%), Kavuni (4.3%), Rajbhog (4.7) Bahurupi (6.0), BPT 5204 (7.0), Ambemohar (7.7) and Manipuriblack (7.7).
- Highest seed damage: Navara (11.0%) Kalabati (9.3%) and Chittimuthyalu (8.0).

- The answers are compatible with those of earlier researchers. Kiran et al. [7] the variety JGL 11118 showed highest seed damage of (10.82%) and lowest found in variety JGL 3844 (1.38%).

Majid Marani et al. [21] identified in rice cultivars that minimal seed damage (2.04%) occurred in the least preferred paddy variety, TH-1, whereas the most preferred variety, Keshwari, exhibited more seed damage (11.08%) caused by *S. oryzae*.

3.4 Ash (Per Cent)

The ash content of various traditional rice test varieties ranged from 0.04 to 1.44 per cent.

- Lowest ash content: Navara (0.04%), Bahurupi (0.07%) and Manipuriblack (0.09%)
- Moderate ash content: karupukavuni (0.12%) Chittimuthyalu (0.17%) Rajbhog (0.27%), Rajamudi (0.28%) Ambemohar (0.33%) and Kalabati (0.45%).
- Highest ash content: Kabirajsal (0.57).

From results it was considered that varieties with minimum adult emergence, lower seed damage and maximum mean development period identified good amount of ash viz., Kabirajsal (0.57%), Kalabati (0.45%), Ambemohar (0.33%), Rajamudi (0.28%) and Rajbhog (0.27%). Similarly, Ramakrishna [23] It has been identified that ash percentage in maize exhibits a significant negative correlation with the adult emergence of *S. oryzae*. Conversely, the highly infested variety BPT 5204, despite having a substantial ash content (1.27%), likely experiences greater infestation due to other factors, such as elevated levels of crude protein, total and reducing sugars.

3.5 Nitrogen (Per Cent)

- Lowest Nitrogen content: BPT 5204 (1.3%), Rajamudi (1.04%),
- Moderate Nitrogen content: Manipuriblack (1.06%) Kabirajsal (1.09%), MTU 1010 (1.09%), Bahurupi (1.11%), Chittimuthyalu (1.15%), Navara (1.15%), Karupukavuni (1.16%), Rajbhog (1.17%).
- Highest Nitrogen content: Kalabati (1.20%) and Ambemohar (1.18%).

These elevated levels likely rendered these varieties particularly favourable for the growth of

rice weevils, consequently resulting to increased adult emergence, seed damage, and weight loss, while varieties containing elevated nitrogen content have less preference by weevil due to other factors such as high phenol and ash content.

3.6 Crude Protein (Per Cent)

- Lowest Crude protein content: Rajamudi (6.52%), Manipuriblack (6.65%).
- Moderate Crude protein content: Kabirajsal (6.83%), Bahurupi (6.91%), MTU 1010 (6.93%), Karupukavuni (7.0%), BPT (7.4%), Navara (7.18%), Chittimuthyalu (7.20%), Rajbhog (7.31%).
- Highest Crude protein content: Kalabati (7.53%) and Ambemohar (7.35%).

Similar findings were reported Mebarkia et al. [24] discovered that wheat variety Arz, which has a lower protein content (9.27%), experienced a smaller percentage of weight loss due to *Sitophilus* infestation. Conversely, the susceptible variety Siete Cerros, with a higher protein content (16.63%), recorded a comparatively greater percentage of weight loss.

Kiran et al. [7] the variety JGL 17004 showed highest crude protein of (8.62%) and lowest found crude protein in variety JGL 11470 (6.93%).

Majid Marani et al. [21] identified positive correlations between protein content in rice and both adult emergence and weight loss caused by *S. oryzae*.

Rizwana et al. [25] identified positive correlations between protein content in rice grain weight loss and both and progeny emergence caused by *Sitotroga cerealella*. Similarly, Murad and Batool [26] observed that wheat genotypes containing elevated protein levels were highly susceptible to *Sitotroga cerealella*, as these genotypes exhibited a significantly maximum number of emerged adults, along with increased grain damage and percentage grain weight loss.

The present findings was also found with Kiran et al. [7] the variety JGL 17004 showed highest crude protein of (8.62%) and lowest found crude protein in variety JGL 11470 (6.93%). Majid Marani et al., [21] identified positive correlations between protein content in rice and both adult emergence and weight loss caused by *S. oryzae*.

Table 1. Adult emergence, Mean development period and Seed damage percentage in various treatments

Treatments	Name of the treatment	Mean adult emergence (days)	Mean developmentalperiod	Seed damage (%)
T1	Kalabati	24.7	34.3	9.3 (17.7)
T2	Manipuriblack	22.0	35.3	7.7 (16.06)
T3	Karuppukavuni	14.3	42.0	4.3 (11.5)
T4	Navara	26.0	32.3	11.0 (18.4)
T5	Rajamudi	9.7	43.3	3.7 (11.5)
T6	Ambemohar	20.0	36.6	7.7 (16.0)
T7	Chittimuthyalu	21.7	37.3	8.0 (17.1)
T8	Rajbhog	16.0	41.0	4.7 (11.5)
T9	Kabirajsal	8.3	45.6	1.3 (6.5)
T10	Bahurupi	17.3	38.6	6.0 (14.1)
T11	*MTU 1010	3.3	39.6	3.3 (10.4)
T12	**BPT 5204	8.7	34.0	7.0 (16.4)
	Standard error mean ±	0.36	0.40	0.34
	CD (P= 0.05)	1.05	1.19	0.99
	CV (%)	3.87	1.84	4.22
	*Resistant check			
	**Susceptible check			

Figures in parentheses are angular transformed values

Table 2. Ash content, nitrogen and crude protein (%) in different treatments

Treatments	Name of the variety	Ash content (%)	Nitrogen (%)	Crude protein (%)
T1	Kalabati	0.45	1.20	7.53
T2	Manipuriblack	0.09	1.06	6.65
T3	Karuppukavuni	0.12	1.16	7.0
T4	Navara	0.04	1.15	7.18
T5	Rajamudi	0.28	1.04	6.52
T6	Ambemohar	0.33	1.18	7.35
T7	Chittimuthyalu	0.17	1.15	7.20
T8	Rajbhog	0.27	1.17	7.31
T9	Kabirajsal	0.57	1.09	6.83
T10	Bahurupi	0.07	1.11	6.91
T11	*MTU 1010	1.44	1.09	6.93
T12	**BPT 5204	1.27	1.3	7.4
	Standard error mean \pm	0.0088	0.0043	0.0265
	CD (P= 0.05)	0.0124	0.0125	0.0775
	CV (%)	3.5	0.655	0.6513
	*Resistant check			
	**Susceptible check			

4. CORRELATION STUDIES

4.1 Ash Content (%)

Ash content possessed significant positive relationship with mean development period (0.03). But, it had significant negative relationship with adult emergence (-0.69), weight loss (-0.47), susceptibility index (-0.17) and grain damage (-0.28)

In this study, the reduced adult emergence in moderately resistant varieties could be linked to their elevated ash content. This high ash content in the seeds may deter insects due to potential toxicity, rendering these varieties less preferred for feeding, growth, and emergence by the grub [27].

Similarly, Vishwamitra [28] documented a notable negative correlation between adult emergence and ash percentage, seed damage

(%), and per cent weight loss due to *C. chinensis* in red gram varieties.

4.2 Crude Protein and Nitrogen Content (%)

Protein and nitrogen had non-significant and negative correlation with mean development period (-0.55) and (-0.54). Whereas, they had non significant positive relationship with adult emergence (0.39) and (0.12), seed damage (0.54) and (0.42).

The results align with the findings of Murthy [29], indicating a positive correlation between the protein content of sorghum varieties and the emergence of adult *S. oryzae*. Yadav [30] also noted that protein content exhibited a positive relationship with grain damage and percentage weight loss in wheat. Kiran [12] also noted that protein and nitrogen had non-significant and negative correlation with mean development period (-0.28).

Table 3. Biological parameters for Adult emergence and Seed damage

Biological Parameters	Adult emergence (number)	Mean development period (days)	Seed damage (%)
Physico - chemical charecters			
Ash (%)	-0.69*	0.03	-0.28
Nitrogen (%)	0.12	-0.54	0.42
Crude protein (%)	0.39	-0.55	0.54

*Significance at 5% level

**Significance at 1% level

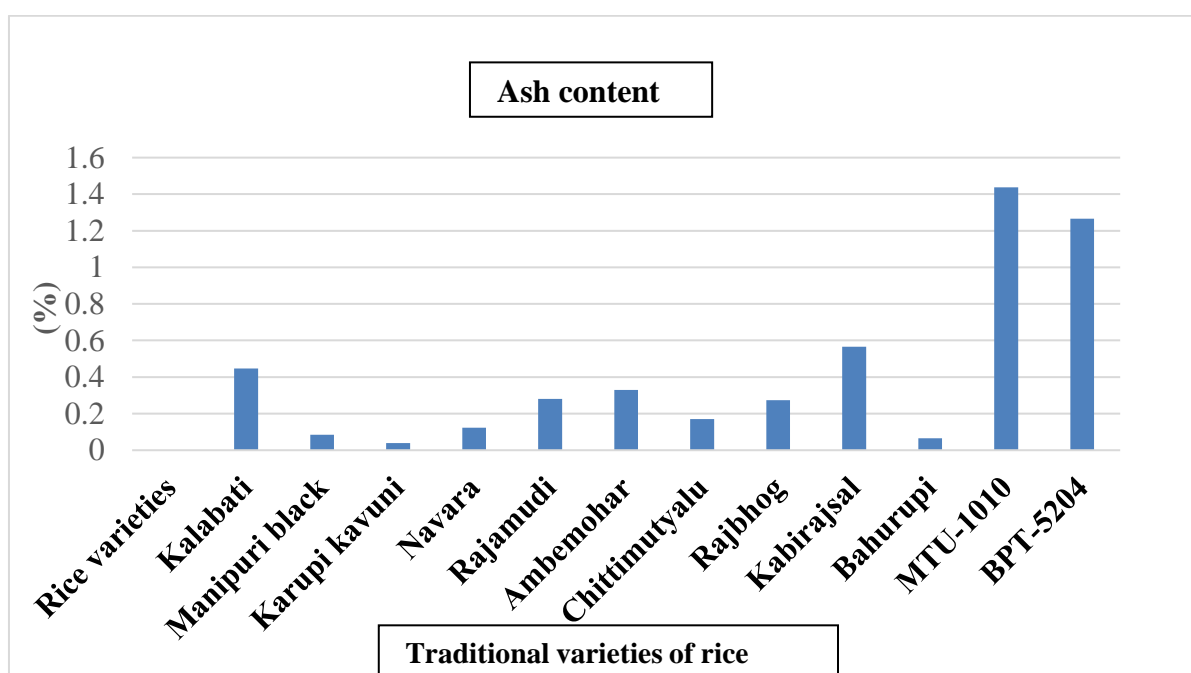


Fig. 1. Ash content in Traditional varieties of rice

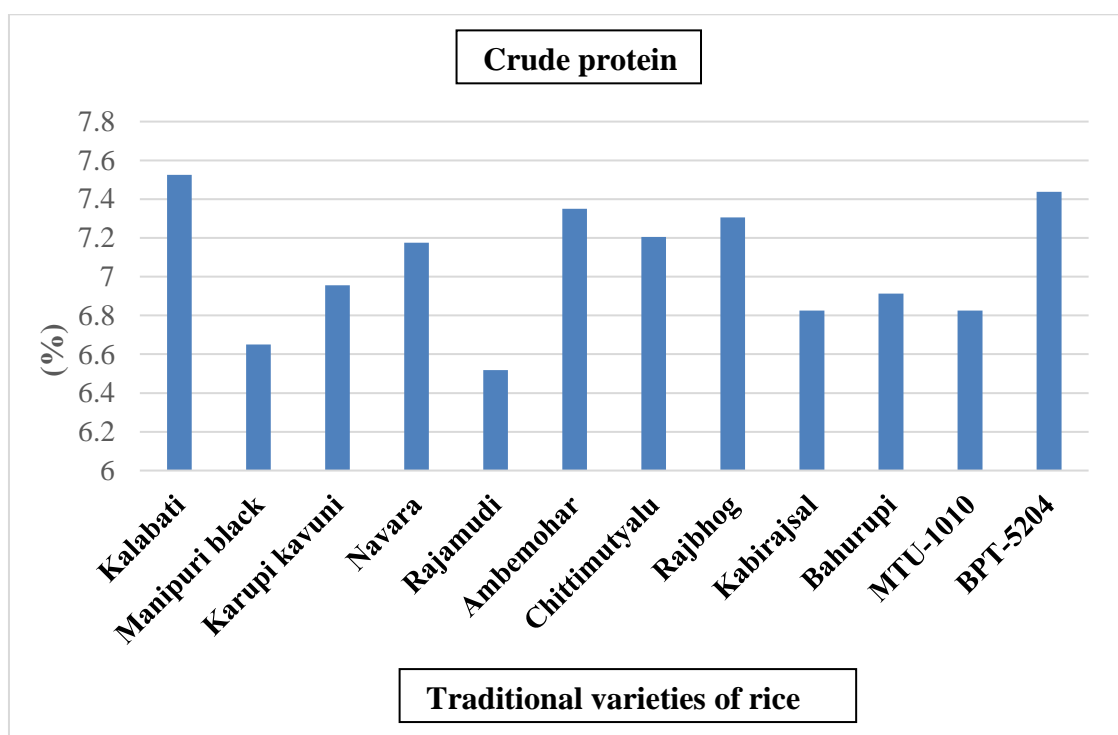


Fig. 2. Crude protein content in Traditional varieties of rice

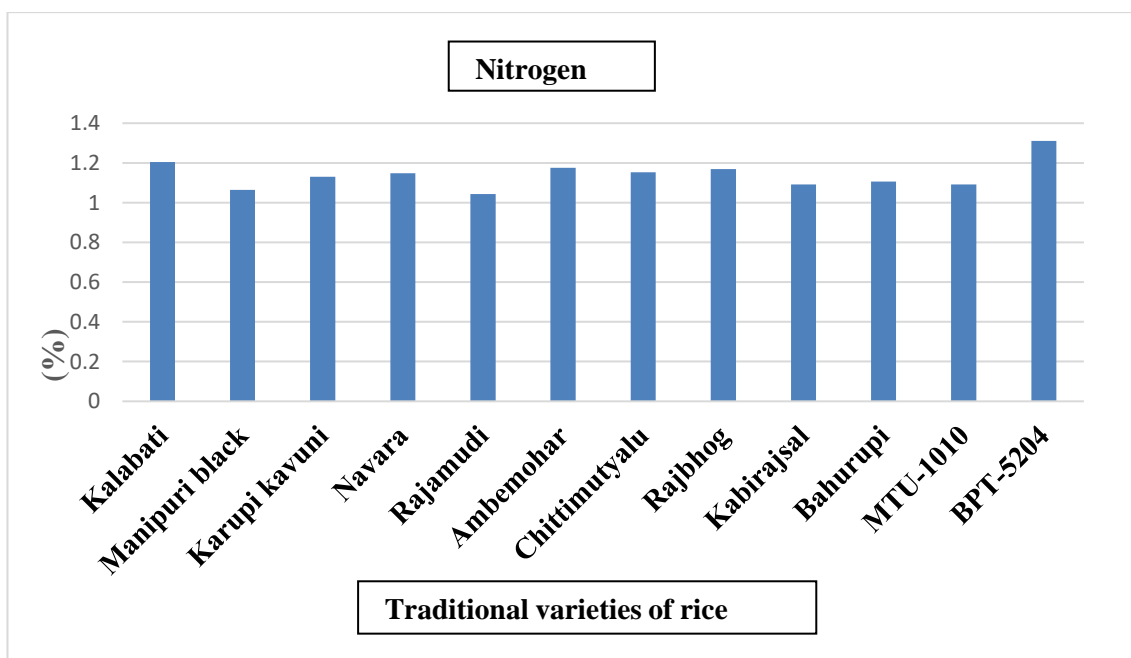


Fig. 3. Nitrogen content in Traditional varieties of rice

5. CONCLUSION

Ash content can play crucial role in deterring rice weevil through several mechanism like higher ash content in rice varieties has rougher surface on rice grain and ash content posses rich mineral content like silica and calcium which is

unpalatable for weevils making harder to digest. Elevated ash content in grains may exert toxic effects on insects, disrupting their ingestion and rendering the test variety unsuitable for feeding, development, and adult emergence [27]. Crude protein and nitrogen content enhances nutritional content and attractiveness, promoting growth

and reproduction and increases egg laying resulting in more extensive damage to the stored rice. The varieties having good content of ash or less content of nitrogen and crude protein viz., MTU 1010, Kabirajsal, Rajamudi, Rajbhog and Ambemohar can be exploited for other characters and good character can be selected in the breeding research to get resistant genotypes.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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