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Hermetic storage of de-hulled little millet at sub-atmospheric pressure: Effect on mortality rate of *Tribolium castaneum*

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Abstract
Hermetic storage system is an airtight non chemical method of storage used to store grain and to prevent losses due to infestation by insects and pests. Presence of insects causes both qualitative and quantitative losses to de-hulled millets. *Tribolium castaneum* is the major storage pest found during the storage of de-hulled millets. A study has been undertaken to evaluate the mortality rate of *Tribolium castaneum* at different pressure levels inside the hermetic storage bin. Two hermetic storage bins made of stainless steel (SS 304) each with 10 kg capacity were used for the study. One factor design is used to select the low pressure level (760 mm of Hg, 620 mm of Hg, 480 mm of Hg, 320 mm of Hg and 200 mm of Hg) for optimising the pressure to achieve highest insect mortality. Among the various pressure levels, 200 mm of Hg pressure achieved 100% mortality of *Tribolium castaneum* adult in 3 days and also reported the lowest average temperature of 27.8 ± 1.08 °C. It was also observed that the increase in pressure increases the period to achieve 100% mortality. The result showed that the pressure, temperature, relative humidity influences significantly ($p < 0.01$) the mortality of *Tribolium castaneum* adult in hermetic storage of de-hulled little millet.

Keywords: Hermetic storage, low pressure, mortality, temperature and relative humidity

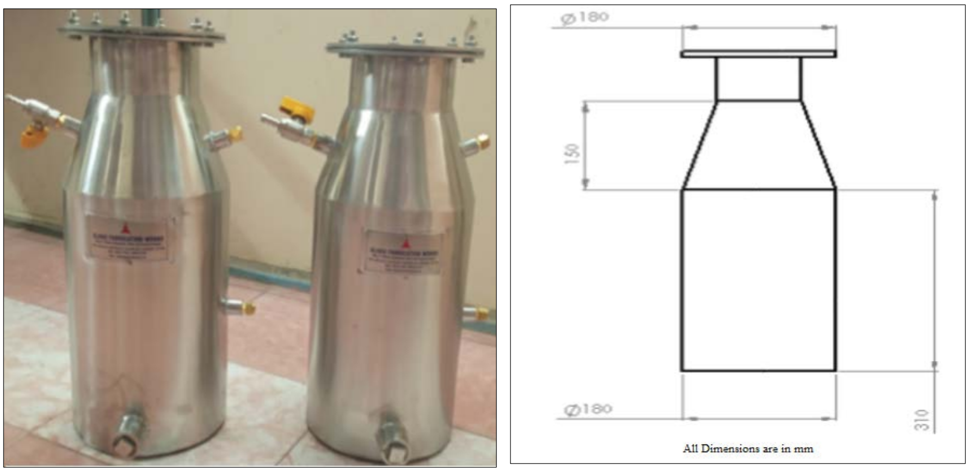
Introduction
Hermetic storage is a non-chemical method of storage and an airtight sealed system used to store the grain. The hermetic storage retains grain quality, minimize the loss of grain during storage and protect from the pest and insect, which cause damage to grain (Kuraloviyan *et al*., 2020) [9]. Hermetic metal silo and hermetic bag are cost effective methods for small farmers to store grain and also an alternative for the usage of chemical (Manandhar *et al*., 2018) [10]. Hermetic storage of grain could result in reduction of storage losses (Baribusta *et al*., 2020) [4]. The pesticide treatment contains chemical compound such as deltamethrin and fenithoin as well as it leads to the development of insect *Sitotroga cerealella* (Mubayiwa *et al*., 2021) [13]. In post-harvest losses, the storage losses contributes highest percentage of grain loss of 15-26% whereas 13-20% loss occurred in processing and 15% loss in field (Manandhar *et al*., 2018) [10].
Tribolium castaneum is the common storage pest that has been known to cause damage to a wide variety of stored grain (Hagstrum, 2017) [7]. *Tribolium castaneum* is the major pest found in stored grain that causes significant losses by secreting quinones that render the grain unfit for consumption (Deb *et al*., 2020) [5]. *Tribolium castaneum* larvae and adult both damage grain with most damage occurring during the hot and humid season, which favour rapid insect population growth (Singh and Yadav, 1995) [6]. In developing countries, the grains are stored widely in woven bag which was not efficient in controlling of insect and molds.
Low pressure in the system reduces the oxygen in storage atmosphere, which control the insects in stored grains. The low pressure is an alternative to chemical fumigants such as phosphine and methyl bromide. The low pressure exposure increases the mortality of all life stages of *Tribolium castaneum* (Mbata *et al*., 2001) [11]. The lethal time for *Tribolium castaneum* adults was 15.1 h at 25 °C and pressure of 1 kPa. When the temperature was decreased to 15 °C °C, the lethal time for *Tribolium castaneum* was 30.8 h at pressure of 1 kPa (Kucerova *et al*., 2013) [8]. The chemical method cause adverse impact to health, whereas physical method is used storage purpose. When the pressure is decreased from 20 kPa to 10 kPa at 37 °C and 75% relative humidity, percentage of insect mortality was increased (Prasantha *et al*., 2020) [16].

Hence, the study was undertaken to evaluate the efficacy of hermetic storage system for de-hulled little millet on the mortality of *Tribolium castaneum* at different pressure levels.

Materials and Methods
Test Insect
Tribolium castaneum (adult), the test insect was collected from the Department of Entomology, TNAU, Coimbatore. The adults were cultured in plastic jar containing 1 kg of de-hulled little millet as feed. The jar was closed by using a muslin cloth in order to avoid the entry of other insects. The insects were cultured in little millet to produce new adult emergence where the newly emerged adults after a period of 30 days were taken up for the further study. The microscopic view of *Tribolium castaneum* was visualized using sterozoom microscope for the confirmation of the particular species, which was shown in Fig. 1.

Fig 1: Microscopic view of *Tribolium castaneum*

Hermetic storage bin
Two hermetic storage bins were made by using stainless steel (SS 304) of thickness 2 mm. The hermetic bins with height and diameter of 500 mm ×180 mm were fabricated for the study. The bins were used for the study of insect mortality at five different pressure levels *viz*., 760 mm of Hg, 640 mm of Hg, 480 mm of Hg, 340 mm of Hg and 200 mm of Hg. The holding capacity of the bin was 10 kg, which was shown in Fig.2 (a). The bin consists of four ports fitted with gas septum, gas valve, vacuum pressure gauge and dummy for sampling. The gas septum was used to measure the gas composition inside the bins, the gas valve was fitted with vacuum pump to generate the required pressure levels. The vacuum pressure gauge was used to measure the pressure level inside the bin. The sample port was placed at bottom of the bin for sample collection. The schematic diagram of the hermetic storage used for the study, is shown in Fig. 2(b).



(a) (b)

Fig 2: (a) Hermetic storage bin; (b) Schematic diagram of Hermetic storage

Pressure decay test
The most common method used in leak testing of storage structure is the pressure decay test (Kuraloviyan *et al*., 2020) [9]. The test was conducted to confirm the airtightness inside the hermetic bin. Any pressure loss in the storage system is an indication of leakage. Hence, to determine the leakage or

pressure drop, the hermetic storage bin was pressurised with compressed air using compressor and negative pressure was exerted by using 0.5 hp vacuum pump. The positive pressure of about 1400 Pa was created in the bin and decrease in pressure was noted for every 5 minutes interval for a period of 45 minutes using digital manometer. The bin was evacuated to create a negative pressure of 840 Pa and increase in the pressure level was noted for every 5 minutes interval for a period of 30 minutes using digital manometer.

Raw material
The fresh de-hulled little millet procured from a reputed processor was used for the study. About 7 kg of de-hulled little millet was used for the study. The samples collected at regular intervals were studied for the insect mortality. The engineering properties of de-hulled little millet namely moisture content, bulk density, true density, porosity, angle of repose and thousand grain mass were determined.

Engineering properties of de-hulled little millet
Moisture content
Moisture content of the de-hulled little millet was analysed using direct method (AACC, 2000) [1]. 5 g of de-hulled little millet was placed in petridish and kept in hot air oven at 130 ±1 °C for 1 hour. The change in weight of the sample was noted. The moisture content was calculated by using formula (Jeevarathinam *et al*., 2021)

$$\text{Moisture content, dry basis } (\%) = \frac{Initial\ weight\ of\ sample\ (g) - Final\ weight\ of\ sample (g)}{Final\ weight\ of\ sample (g)} \quad \text{------ (1)}$$

Bulk density
Bulk density of the de-hulled little millet sample was found using the mass-volume relationship formula (Balakrishnan *et al*., 2020, Mohsenin, 1986) [21, 12].

$$\text{Bulk density} = \frac{Mass\ of\ grain\ (kg)}{Volume\ of\ grain\ (m^3)} \quad \text{------ (2)}$$

True Density
True density of grain is the ratio of mass to true volume of the grain. The water displacement method (Mohsenin, 1986) [12] was used to find the true density of de-hulled little millet.

$$\text{True Density} = \frac{Mass\ of\ displaced\ water\ (kg)}{Density\ of\ water\ (m^3)} \quad \text{------ (3)}$$

Porosity
The porosity of grain is defined as the bulk volume of void or air fraction to given sample volume. It was calculated using formula given by Balakrishnan *et al*. (2020) [21] and Sahay and Singh (1996) [17].

$$\text{Porosity, \%} = \left(1 - \frac{Bulk\ density}{True\ density}\right) \times 100 \quad \text{----- (4)}$$

Angle of repose
The angle of repose is termed as the angle between the base and slope of the cone formed on a free vertical fall of de-hulled little millet over a horizontal plane. It was estimated by using method described by Balakrishnan *et al*. 2020 [21] and Mohsenin (1986) [12].

$$\text{Angle of repose, } \theta = \tan^{-1}\left(\frac{2 \times \text{Height of the heap formed (mm)}}{\text{Diameter of the disc (mm)}}\right) \quad \text{------(5)}$$

Thousand grain mass
Thousand grains were counted using digital seed counter with ten replication. The counted de-hulled little millets were weighed on digital electronic scale. The mass of thousand grain was noted. The average value with standard deviation was calculated.

Experimental protocol
Fresh de-hulled little millet was filled in the hermetic storage bin and 300 adult *Tribolium castaneum* were incorporated into the hermetic storage bin. The data logger was placed inside the hermetic bin and closed tightly. The vacuum pump was used to reduce the pressure inside the bin. The air pressure inside the hermetic storage bin was monitored using vacuum pressure gauge (0-760 mm of Hg). After attaining the desired pressure, vacuum pump was turned off and valve was closed to arrest vacuum inside the bin. The mortality rate of *Tribolium castaneum* was evaluated at five different pressure levels (760 mm of Hg, 620 mm of Hg, 480 mm of Hg, 340 mm of Hg and 200 mm of Hg). One factor analysis is used to select different levels of responses for optimizing the parameters. The data logger measured the temperature and relative humidity inside the bin at regular interval. The percentage of insect mortality was calculated using the formula

$$\text{Percent adult mortality} = \frac{No\ of\ adults\ dead}{No\ of\ adult\ released} \times 100 \quad \text{------(6)}$$

Statistical analysis
The level of response for the insect mortality was designed with one factor response design using Design Expert 12.0. The statistical analysis was done to test the significant difference, regression, ANOVA, and best fit predictive model. ANOVA and significance difference were evaluated using RStudio-2021.09.0-351 (Jeevarathinam *et al*., 2021) [18]. The regression plot and predictive model were done using Microsoft excel 2016.

Results and Discussion
Pressure decay test for hermetic storage bin
The pressure was reduced from 1450 Pa was to 1340 Pa and 1350 Pa in Bin 1 and Bin 2 respectively. After 30 minutes, pressure inside both the bins were constant shown in Fig 3 (a), (b). For negative pressure, the pressure was reduced to 680 Pa (bin 1) and 690 Pa (bin 2) from 840 Pa and observed that constant pressure was maintained after 20 minutes as shown in Fig.3 (c), (d). For smaller storage structure, a half pressure test lasting for 1.5 minutes was found to be satisfactory for hermetic storage (Bank and Annis, 1980). In this study, the pressure holding time was attained at for 45 and 30 minutes for Positive pressure and negative pressure respectively. The results of the decay test were judged to be satisfactory by previous study (Navarro *et al*., 1990, Navarro, 1998, Kuraloviyan *et al*., 2020) [15, 14, 9]. The significant difference ($p < 0.01$) between pressure and time was observed.

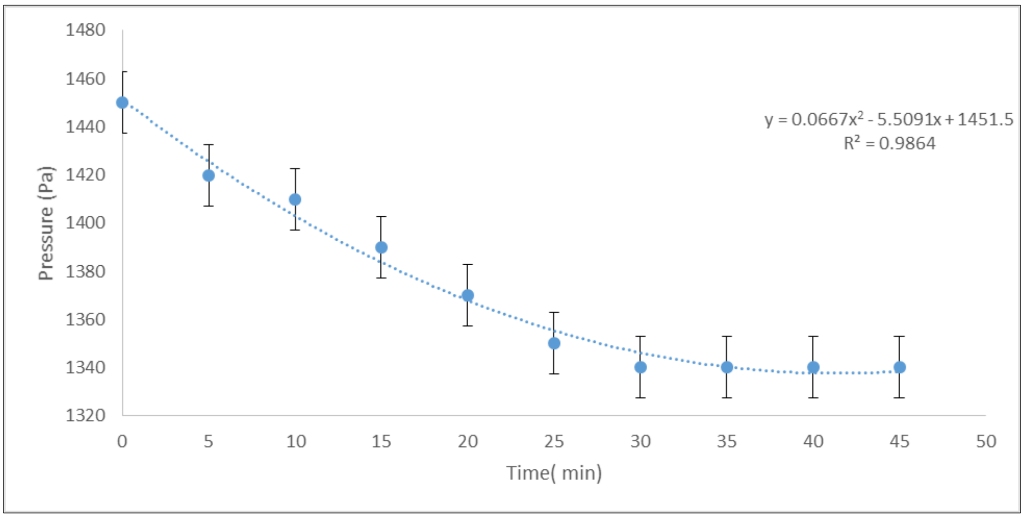


Fig 3: (a) Decay test- Positive pressure-bin 1

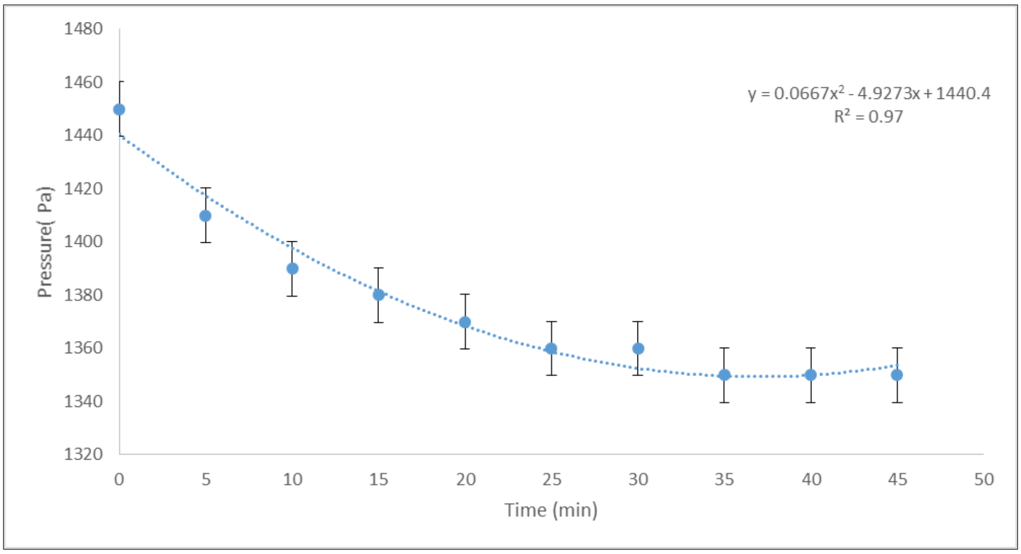


Fig 3: (b) Decay test- Positive pressure-bin 2

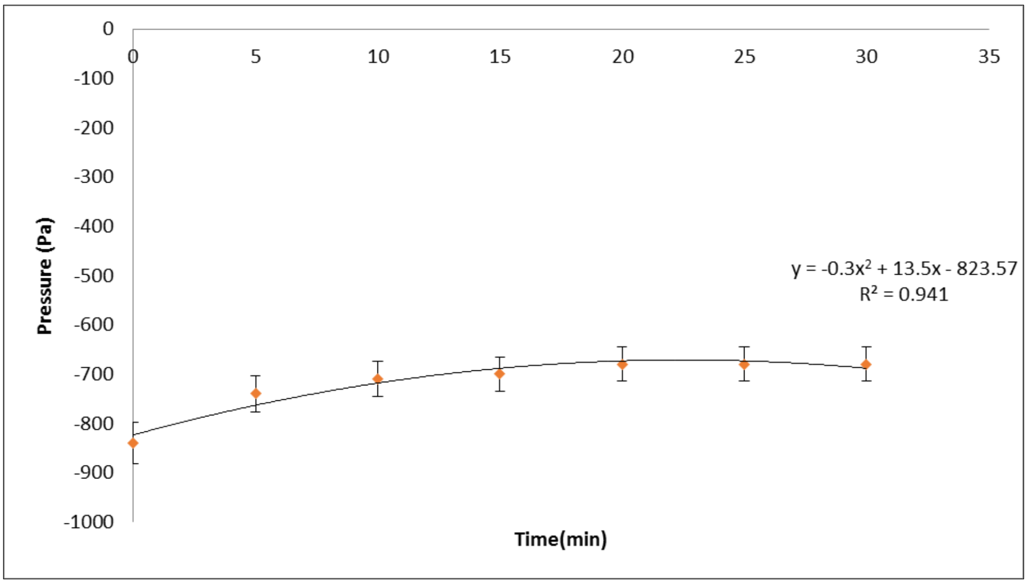


Fig 3: (c) Decay test- Negative pressure-bin 1

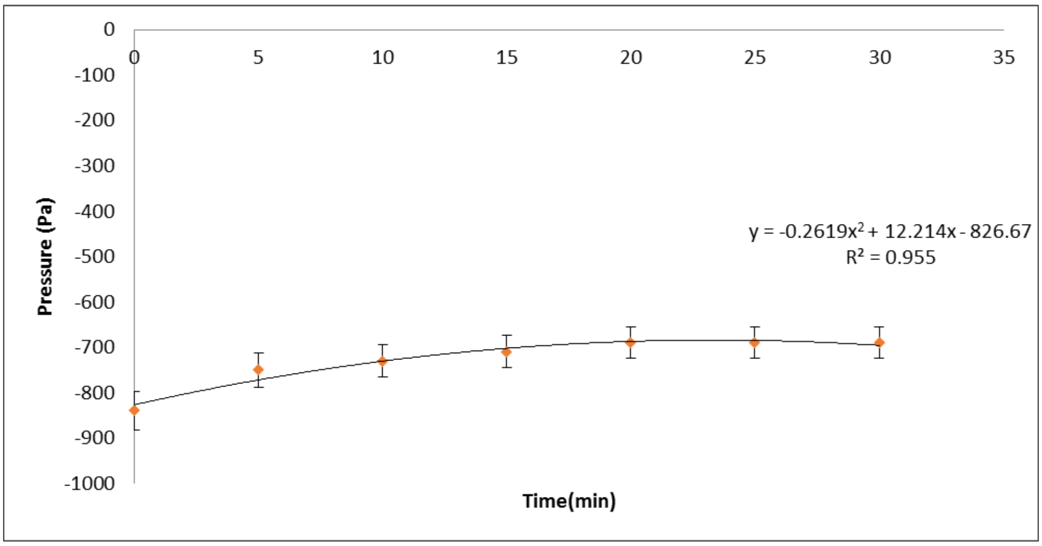


Fig 3: (d) Decay test- Negative pressure-bin 2

Mortality of *Tribolium castaneum* at different sub-atmospheric pressure levels

Resultsof sub-atmospheric pressure treatments showed that pressure directly influence the survival of *Tribolium castaneum* adult. At low pressure the survival ratio of *Tribolium castaneum* decreased. Among the various pressure level, 200 mm of Hg pressure achieved 100% mortality at 3rd day. At the pressure 320 and 480 mm of Hg, the mortality of insect shown similar response, where 100% insect mortality was achieved at 6th day. But these pressure shown different mortality rate (88 ± 10.61 and 49 ± 10.23) at 3rd day. For the pressure levels 760 and 620 mm of Hg, 100% mortality were achieved at 12th and 18th day respectively.

The present finding revealed that when pressure inside the hermetic system was lowered, the mortality rate of *Tribolium castaneum* get increased. Table 1 summarizes the insect population at three-day intervals at various pressure levels. *Tribolium castaneum*is more susceptible to low vacuum pressure (Mbata *et al.*, 2004) [11]. The higher pressure is less effective in mortality of insect. It has been demonstrated that the low partial pressure of oxygen, which results in hypoxia, is the primary cause of insect death when exposed to low pressure (Alder *et al.*, 2000). Prasantha *et al.*, 2020 [16] reported the vacuum pressure 10 kPa has higher mortality than 20 kPa vacuum pressure. The significant difference ($p< 0.01$) between pressure and insect mortality was observed.

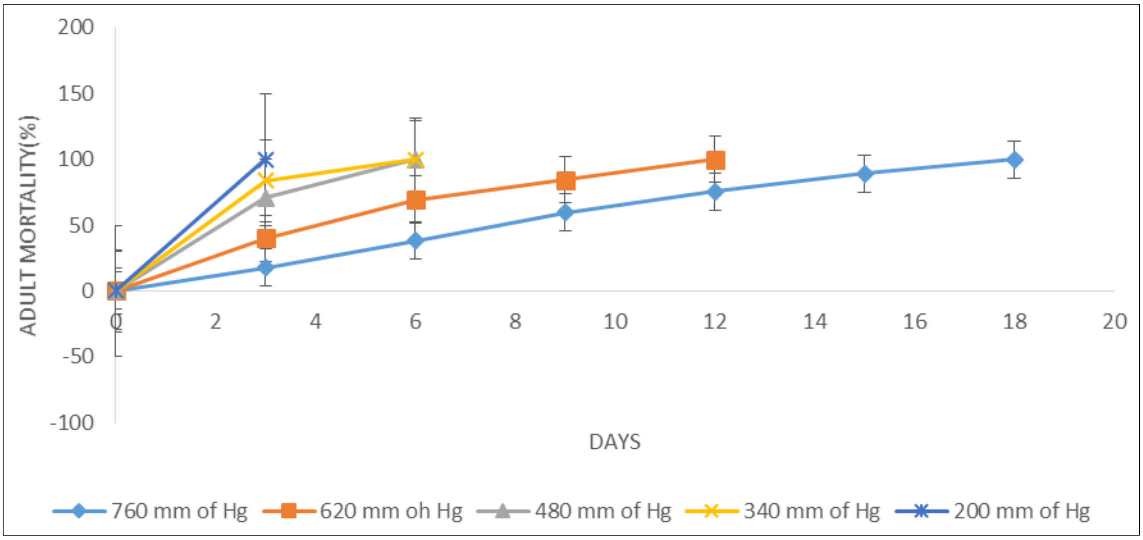


Fig 4: Effect of mortality rate of *Tribolium castaneum* at various pressure levels

Table 1: Population of *Tribolium castaneum* adult at various pressure levels

Days	760 mm of Hg	620 mm of Hg	480 mm of Hg	340 mm of Hg	200mm of Hg
0	300	300	300	300	300
3	247 ± 7.78	181 ± 12.24	88 ± 10.61	49 ± 10.23	0
6	185 ± 10.61	92 ± 11.51	0	0	N/A
9	121 ± 16.75	47± 9.79	N/A	N/A	N/A
12	73 ± 11.22	0	N/A	N/A	N/A
15	32 ± 4.96	N/A	N/A	N/A	N/A
18	0	N/A	N/A	N/A	N/A

*N/A – not applicable

Effect of temperature and relative humidity on insect mortality

Fig.5. depicts the change in temperature inside the hermetic storage bin. Lowest average temperature (27.81 ± 1.08 ℃) was recorded at 200 mm of Hg pressure. The average temperature of ambient environment was about 27.4 ± 1.35 ℃. The temperature fluctuation was higher in ambient condition than in hermetic bins. The average temperature inside the hermetic bin at various pressure level was found to be 27.89 ± 1℃ (320 mm of Hg), 28.19 ± 1.07 ℃ (480 mm of Hg), 28.5 ± 0.73 ℃ (620 mm of Hg) and 28.74 ± 0.95 ℃ (760 mm of Hg). The results clearly shows that the temperature is influenced by pressure. By decreasing the pressure, the temperature also decreases gradually. The results obtained from the hermetic storage system was in accordance to the ideal gas equation (PV=mRT) that a direct relationship exists between temperature and pressure. The significant difference ($p < 0.01$) was observed between pressure and temperature.

The change in relative humidity (RH) inside the hermetic storage bin at different pressure levels were shown in Fig 6. As like temperature, the deviation of RH at ambient environment was higher about 70.93 ± 7.11%. The average RH was high at 200 mm of Hg pressure and found to be 73.05 ± 0.91%. The average RH inside hermetic storage at various pressure levels were 64.53 ± 2.11% (760 mm of Hg), 67.6 ± 0.47% (620 mm of Hg), 71.6 ± 0.17% (480 mm of Hg) and 72.70 ± 0.40% (320 mm of Hg) respectively. The result obtained from the study showed an inverse relationship between pressure and relative humidity. The significant difference ($p < 0.01$) was observed between pressure and relative humidity.

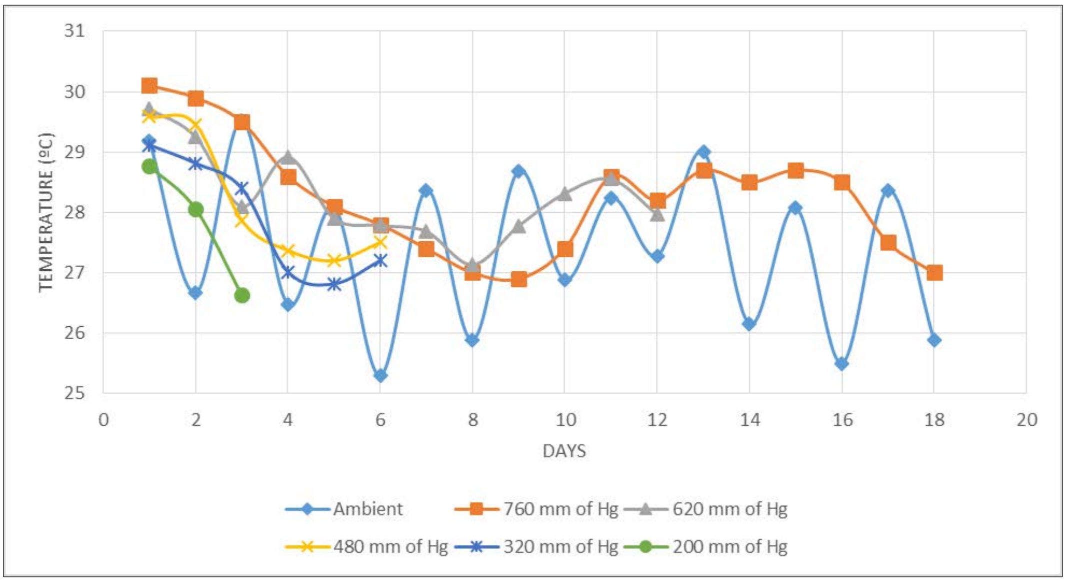


Fig 5: Changes in temperature during insect mortality at various pressure levels

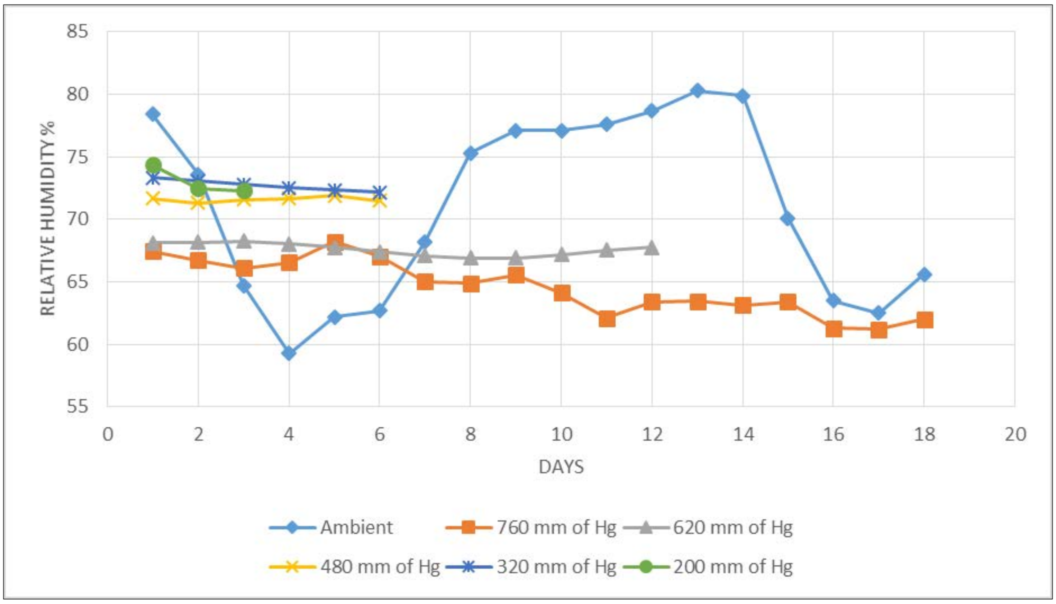


Fig 6: Changes in relative humidity during insect mortality at different levels

Engineering properties of Little Millet

To develop the hermetic storage system, the engineering properties of de-hulled little millet namely bulk density, true density, porosity, angle of repose and thousand grain mass were studied at moisture content of 12% d.b.The values were found to be 832.83 kg/m^3, 1362.4 kg/m^3, 38.9%, 27º and 2.21 ± 0.02g. Similar findings were reported by Balasubramanian and Viswanathan (2010) [2] for bulk density, true density, porosity and angle of repose for minor millet ranges from 868.1 to 477.1 kg/m^3, 1988.7 to 884.4 kg/m^3, 63.7 to 32.5% and 25.0 to 38.2° at moisture content ranging between 11.1 to 25% d.b. and also by Balakrishnan *et al*. (2020) [21] for turmeric and Krishnan Kumar *et al*. (2021) [20] for cassava tubers confirms the findings of this study.

Table 2: ANOVA for pressure decay test, temperature, and relative humidity

	SS	df	MS	F value	P-value
Positive pressure decay test bin1	9146281	1	6106551	6312*	2e-16
Positive pressure decay test bin2	9173351	1	9173351	14393*	2e-16
Negative pressure decay test bin1	1876848	1	1876848	1075*	4.1e-13
Negative pressure decay test bin2	1928458	1	1928458	1248*	1.69e-13
Insect mortality	278150.619	6	46358.44	13.38*	2.5e-07
Temperature	3605.54	6	600.9233	94.83598*	9.91e-29
Relative humidity	49857.33	6	8309.554	414.3743*	7.95e-55

* p is significant at 1% level ($p < 0.01$)

Conclusion

The engineering properties identified was mainly used for the design and development of hermetical storage bin to evaluate the mortality rate of *Tribolium castaneum* at different pressure levels. The mortality of *Tribolium castaneum* adult was mainly dependent on the pressure inside the hermetic storage system. At sub-atmospheric pressure level of 200 mm of Hg, 100% mortality was achieved in three days whereas at the pressure 760 mm of Hg, 100% mortality was achieved at 18th day. The results also proved that mortality of *Tribolium castaneum* adult was higher at lower pressure when compared to higher pressure. The pressure was directly proportional to temperature and inversely proportional to relative humidity inside the hermetic storage system. The hermetic storage system showed minimum deviation of temperature and relative humidity with respect to ambient temperature and relative humidity. The stainless steel has low thermal conductivity and anticorrosive property which last for many years. Thus the low pressure hermetic storage system was effective for storage of little millet and also protects them from insect.

References

1. AACC. Approved methods, American Association of Cereal Chemists. 10th edn, Moisture determination, 44-15A, Minnasota, St Paul 2000.
2. Balasubramanian S, Viswanathan R. Influence of moisture content on physical properties of minor millets. Journal of food science and technology 2010;47(3):279-284.
3. Banks H, Annis P. Conversion of existing grain storage structures for modified atmosphere use. In Developments in Agricultural Engineering, Elsevier, 1980, 461-473.
4. Baributsa D, Ignacio MC. Developments in the use of hermetic bags for grain storage. Advances in Postharvest Management of Cereals and Grains; Maier, DE, Ed 2020.
5. Deb M, Kumar D. Bioactivity and efficacy of essential oils extracted from Artemisia annua against Tribolium *castaneum* (Herbst. 1797) (Coleoptera: Tenebrionidae): An eco-friendly approach. Ecotoxicology and environmental safety 2020;189:109988.
6. Dharam S, Yadav TD. Studies on the incidence of insect pests, practices and losses in stored wheat in Haryana. Indian Journal of Entomology 1995;*57*(2):107-115.
7. Hagstrum D. Atlas of stored-product insects and mites 2017.
8. Kucerova Z, Kyhos K, Aulicky R, Stejskal V. Low-pressure treatment to control food-infesting pests (*Tribolium castaneum*, *Sitophilus granaries*) using a vacuum packing machine. Czech Journal of Food Sciences 2013;31(1):94-98.
9. Kuraloviyan R, Balakrishnan M, Rajkumar P, Zohn Kennedy. Design and development of hermetic storage bin for de-hulled Kodo millet, Journal of Pharmacognosy and Phytochemistry 2020;29(5):512-516.
10. Manandhar A, Milindi P, Shah A. An overview of the post-harvest grain storage practices of smallholder farmers in developing countries. Agriculture 2018;8(4):57.
11. Mbata GN, Phillips TW, Payton M. Mortality of eggs of stored-product insects held under vacuum: effects of pressure, temperature, and exposure time. Journal of economic entomology 2004;97(2):695-702.
12. Mohsenin N. Physical characteristics: physical properties of plant and animal materials. Gordon and Breach Science publisher, New York 1986.
13. Mubayiwa M, Mvumi BM, Stathers T, Mlambo S, Nyabako T. Field evaluation of hermetic and synthetic pesticide-based technologies in smallholder sorghum grain storage in hot and arid climates. Scientific Reports 2021;11(1):1-13.
14. Navarro S. Pressure tests for gaseous applications in sealed storages: Theory and practice. Proceedings of 7th international working conference on stored-product protection 1998.
15. Navarro S, Donahaye E, Rindner ME, Azrieli A. Airtight storage of grain in plastic structures. Hassadeh Quarterly 1990;1(2):85-88.
16. Prasantha BR. Effects of low-pressure storage of food commodities on the mortality of adult stored product insects. Sri Lankan Journal of Biology 2020;5(1).
17. Sahay K, Singh K. Unit operations of agricultural processing, Vikas Publishing House Pvt. Ltd 1996.
18. Jeevarathinam G, Pandiselvam R, Pandiarajan T, Preetha P, Balakrishnan M, Thirupathi V, *et al*. Infrared assisted hot air dryer for turmeric slices: Effect on drying kinetics and quality parameters. LWT-Food Science and Technology 2021;144:111258.
19. Jeevarathinam G, Pandiselvam R, Pandiarajan T, Preetha P, Krishnakumar T, Balakrishnan M, *et al*. Design Development and Drying Kinetics of Infrared Assisted Hot Air Dryer for Turmeric Slices. Journal of Food Process Engineering, 2021, 13876.
20. Krishnakumar T, Sajeev MS, Pradeepika C, Namrata A, Giri Sanket J, Jeevarathinam G, *et al*. Physical and mechanical properties of short and long duration cassava (Manihot esculenta Crantz) cultivars: Implications for the

design of continuous type peeling machine. Journal of Food Process Engineering 2021, 13923.
21. Balakrishnan M, Jeevarathinam G, Aiswariya S. Physical, frictional and thermal properties of turmeric Rhizomes. International Journal of Chemical Studies, 2020;8(4):2268-2271.