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# NOVEL FUMIGATION METHOD FOR THE MANAGEMENT OF STORAGE PESTS OF RICE

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**Abstract**–Owing to the advantage of on-site production and utilization of acetylene gas ($C_2H_2$), it would be more advantageous to use acetylene in place of $CO_2$ and $N_2$ in controlled atmosphere storage of food grains to inhibit respiration of insects. In this study, the effect of acetylene on the control of *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum* and *Callusobruchus maculatus* in the storage of paddy grains (CV.TRY 3) was assessed. Paddy grains with known number of insects in a small container [conical flask (500 ml)] with airtight closure were filled with 100 percent acetylene gas. The storage pests were assessed for mortality at an interval of 24 h for 120 h. The containers opened after bioassay was left for progeny production up to 4 months. The pests and the duration of exposure and their interactions have shown significance with acetylene gas filling. For achieving 100% mortality of *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum* and *Callusobruchus maculatus*, in the storage of paddy grains, the exposure time required were 111, 139, 153 and 139 h, respectively. The progeny of these storage pests were found in-significant with respect to long term storage period up to 4 months. The mean number of progeny production of adult and grub of these storage pests ranged from 1.7 to 7.7 and 0 to 1.7, respectively. In the scale-up (long term storage) studies conducted using the [bigger container plastic bins (25 kg)] up to 4 months, showed no survival of the adult / grubs and progenies production were seen. The storage for 30 days with acetylene gas ensured 100 per cent mortality of storage grain pests.

## INTRODUCTION

The total preventable post harvest losses of food grains in India, as estimated by the Ministry of Food and Civil Supplies, Government of India is 10 per cent of the total production. This would be enough to feed about 70 million people *ie.*, 1/3$^{rd}$ of the India's poor (Basavaraja *et al.,* 2007). Paddy or rice, occupies the maximum space under warehouse in southern states of India. The major menace of paddy under storage condition is lesser grain borer, *Rhyzopertha dominica*, rice weevil, *Sitophilus oryzae*, rice moth, *Corcyra cephalonica* and Angoumois grain moth, *Sitotroga cerealella*, particularly, *R. dominica* feeds on raw rice predominantly. The consumers look for a food products free from pesticides or / with much reduced residue levels through pest management. Airtight storage of damp grain preserved germination for a short period and prevented the growth of spoilage moulds during longterm storage (White *et al.,* 1993). Modified atmospheres have an important role in the pesticide free insect pest management (Banks *et al.,* 1991). Modified atmosphere is generated by decreasing the oxygen level or increasing the carbon dioxide or nitrogen concentration in the atmosphere thereby interfering with normal respiration of insects (Jayas *et al.,* 1991). Controlled atmospheric storage in containers is practiced mainly during shipment in order to prevent ripening, microbial deterioration and protect from stored grain insect pests. Controlled atmosphere is accurately maintaining the composition of selected gases such as carbon dioxide, oxygen and nitrogen at specified concentration under normal pressures or under partial vacuum. The effectiveness of modified or controlled atmospheres in controlling insect pests depends on abiotic factors such as air tightness, gaseous composition, relative humidity, temperature, length of exposure and gas pressure and biotic factors such as insect species, life stage size and distribution of infestation. Both the factors are required to be optimized for creating an environmental condition that is lethal to the insect pests found in the stored grain. Earlier researchers conducted several empirical studies to define atmospheric conditions for controlling various stored-grain insects (Banks *et al.,* 1991, Jayas *et al.,* 1991, Donahaye *et al.,* 1996). It is highly essential to have safe storage of fruits, vegetables and food grains in the food processing industry without harmful chemicals, while minimizing the quantitative and qualitative losses. Grain handlers are in a situation to find an alternative due to toxic and environmental effects produced by conventional chemicals and raising consumer demand for organic products. Hence, FSSAI have enforced strict food laws and legislation against food processors. Ozone is a potential alternative for traditional sanitizer in fruits or vegetable preservation where phosphine resistance is being prevailing among storage pests at an alarming level. Ozone is active against a range of microorganisms, mycotoxins and insect pests at relatively low concentrations (Pandiselvam *et al*., 2016). Adaptation of ozone fumigation in large-scale grain processing industry has few practical limitations such as temperature, relative humidity, grain moisture, bed thickness, generation of high concentration ozone, diffusion of ozone gas into the grain column and half-life of ozone. Ozone offers unique advantages for the food processing industry with desirable effects on the biochemical properties and leaving no residues. Hence, ozone is a potential alternative to conventional insecticides and fumigants in food processing industries (Pandiselvam *et al.* 2015). However, the storage pests' management using controlled / modified atmospheres remains unpopular among the farmers and processors, due to the leakage of storage structures, filling and transportation of gases to the site and monitoring mechanism of gas composition through instruments. In this context, acetylene ($C_2H_2$), an industrial gas used along with oxygen for producing flame in welding and cutting application, is considered to replace the availability of oxygen to the insects during storage when used to fill the void volume. Production of acetylene gas from calcium carbide, on site is easier compared to production of other gases, which does not involve filling and transportation. Thus, the present study was undertaken at laboratory level to study the feasibility of using acetylene as an alternate gas for $N_2$ and $CO_2$ in the controlled atmosphere storage of paddy grains.

## MATERIALS AND METHODS

### Raw materials

Paddy grains (CV TRY 3), harvested, dried and cleaned during the crop season 2019- 2020was made available from the university research farm. It was ensured that the grains were free from field carried over pest and in the moisture content range of 10-14 per cent. The test insects required for the study, *Sitophilus oryzae, Rhizopertha dominica, Tribolium castaneum* and *Callusobruchus maculates* were obtained from susceptible population maintained for about 25 generations at the Department of Plant Protection, Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural university, Tiruchirappalli, Tamil Nadu.

### Storage studies in glass jars

The initial experiments to study the feasibility of using acetylene as an alternate gas in controlled atmosphere storage were conducted using 500 ml airtight smaller conical flasks and larger bins. Rubber corks of size suitable for the flasks were provided with two holes of 6 mm diameter using drill / corkborer to fit a glass tube of 6 mm external diameter. Two "L" shaped glass tubes with the ends having 50 mm length were fit to provide inlet and outlet for gas. The other ends of the tubes, 50 mm and 150 mm were placed such that they fit to the conical flask to facilitate uniform distribution of gas into the flask. Rubber tubes attached to the glass tubes facilitate to provide valve to regulate and retain the gas inside the flask. The flasks were well-cleaned and dried moisture free, and filled with the cleaned paddy grain. Ensured that the grain was filled-up to the bottom of the rubber cork and no space is left unfilled. Before closing with the cork fitted with inlet and outlet for gas, ten numbers of the adult insects as mentioned above were placed in the flask and closed airtight, with labels. Acetylene gas from the cylinder was delivered into each conical flask filled with paddy and insect through the inlet and allowed the gas to exit through the outlet to ensure that the pore volume is completely filled with acetylene gas and both inlet and outlet were well closed using the pinch cork. Control experimental samples were prepared by filling with grains and insects and closed airtight without gas filling. These conical flasks were stored at 30-35°C temperature and 65-70% relative humidity for assessing the mortality of insects (Fig.1).

**Experimental plan**

The following experimental plan was adopted for our study.

| Variables | Particulars | Levels | Particulars |
|---|---|---|---|
| Independent variables | Insects | 4 | i. *Sitophilus oryzae* |
| | | | ii. *Rhyzopertha dominica* |
| | | | iii. *Tribolium castaneum* |
| | | | iv. *Callusobruchus maculatus* |
| | Gas filling | 2 | i. 100% gas (Treatment) |
| | | | ii. 0% gas (control) |
| | Exposure duration | 5 | i. 24 h |
| | | | ii. 48 h |
| | | | iii. 72 h |
| | | | iv. 96 h |
| | | | v. 120 h |
| | Replications | 10 | 10 insects/replication |
| Dependent variables | Mortality (%) | | |
| | Progeny production | - | |

**Scale-up storage in bins**

Grain storage in plastic bins of 25 kg capacity was conducted following the experiments in glass jars. The plastic containers were airtight with lid, packing gasket and lock arrangement. Inlet and outlet values were fitted at bottom and top, respectively along the height of the bin for filling acetylene gas. These containers were filled with paddy grains upto 90% of its volume (18 to 23 kg) with 25 insects and acetylene gas from cylinder was delivered into the bin. The complete filling of gas in the pore volume was ensured by the acetylene gas exit through the outlet valve and both valves were closed well (Fig.2). The storage bins, with three replications and control were placed in ambient condition (30-35°C and 65-70% relative humidity) for assessing the mortality of insects after a long-term storage of four months. All these experiments were performed at Modified Atmosphere Packing Laboratory, Agricultural Engineering College and Research Institute, Kumulur and Storage Entomology Laboratory, Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Tiruchirappalli, Tamil Nadu.

**Fig. 1** Bioassay in airtight smaller container

**Fig. 2.** Bioassay in airtight bigger containers

**Bioassay of storage and progeny production**

The gas filled conical flasks with paddy, insects were opened at the specified time intervals of 24, 48, 72, 96, and 120 h as per the experimental plan and the number of insect alive and dead were counted. With all the insects removed from the storage, the conical flasks as per the experimental design were left with cork closed, keeping the inlet and outlet valves opened to the atmosphere for progeny study upto four months. Three flasks were opened to assess the progeny in every month upto four months and the presence of the storage pests was recorded. The storage in the large sized bins were opened after a long-term storage of four months. In both experiments in the progeny assay, the type of pest and their counts were recorded. This method of exposing adults on a commodity for a given time, then removing them and assessing mortality and progeny production, or by leaving the adults on the commodity continuously, was the methodology followed (Arthur and Morrison, 2020).

**Data analysis**

All the mortality data were statistically analyzed for significance (ANOVA) and students 't' test. The mortality data with gas exposure time were fitted to linear regression models and the significance of the models were tested following standard techniques. Using this models, the lethal time for 50% and 99% mortality were studied.

## RESULTS AND DISCUSSION

After 24 of exposure, mortality among the storage pests ranged from 3 per cent (*R.dominica* to 25 per cent (*T. castaneum*). More than 50 per cent mortality was recorded after four days of exposure in all the exposed insects except *T.castaneum.*

**Mortality of stored grain pests in air tight small glass jars**

Mean mortality of 96 to 98 per cent was recorded among various storage pests over exposed duration of 120 h. (Table 2). As seen from the plot of the

**Table 2**. Mean mortality of the storage pests at indicated exposure duration for paddy grains filled with acetylene gas

| Sl. No. | | Insect Exposure duration, h | Percent mortality* |
|---|---|---|---|
| 1. | *Tribolium castaneum* | 0 | 0 (0) |
| 2. | | 24 | 25 (5.27) |
| 3. | | 48 | 41 (7.38) |
| 4. | | 72 | 68 (7.89) |
| 5. | | 96 | 86 (5.16) |
| 6. | | 120 | 98 (4.21) |
| 7. | *Sitophilus oryzae* | 0 | 0 (0) |
| 8. | | 24 | 11 (7.38) |
| 9. | | 48 | 18 (6.32) |
| 10. | | 72 | 46 (6.99) |
| 11. | | 96 | 69 (7.38) |
| 12. | | 120 | 98 (4.22) |
| 13. | *Rhyzopertha dominica* | 0 | 0 (0) |
| 14. | | 24 | 3 (4.83) |
| 15. | | 48 | 13 (4.83) |
| 16. | | 72 | 26 (5.16) |
| 17. | | 96 | 67 (4.83) |
| 18. | | 120 | 96 (5.16) |
| 19. | *Callusobruchus maculatus* | 0 | 0 (0) |
| 20. | | 24 | 6 ( 5.16) |
| 21. | | 48 | 28 (7.89) |
| 22. | | 72 | 38 (7.89) |
| 23. | | 96 | 70 (4.74) |
| 24. | | 120 | 98 (4.21) |

* mean of 10 replications and standard deviation given in parenthesis.

**Table 1.** Analysis of variance of percent mortality with other experimental variables.

| Source | Sum of Squares | df | Mean Square | F |
|---|---|---|---|---|
| Corrected Model | 468519.0 | 39 | 12013.3 | 495.96** |
| Intercept | 269361.0 | 1 | 269361.0 | 1.11 ** |
| Insect | 8165.0 | 3 | 2721.6 | 112.36** |
| Time | 105311.5 | 4 | 26327.8 | 1087** |
| Gas | 242064.0 | 1 | 242064.0 | 9993** |
| Insect * gas | 8590.0 | 3 | 2863.3 | 118.21** |
| Time * gas | 97788.5 | 4 | 24447.1 | 100.9** |
| Insect * time | 3522.5 | 12 | 293.5 | 12.12** |
| Insect * time * gas | 3077.5 | 12 | 256.4 | 10.59** |
| Error | 8720.0 | 360 | 24.2 | |
| Total | 477239.0 | 399 | | |

R Squared =0.982 (Adjusted R Squared = 0.980) exposure duration to acetylene gas and the percent mortality of storage pests in Fig.3, the percent mortality was showing a linear relationship with exposure duration for all the pests studied with regression coefficient of above 0.90. The plots were extrapolatedfor 100% mortality and liner models werefitted for all the storage pests studied. From the plot shown (Fig.3) It is seen that *Rhyzopertha dominica* offered more resistance for the exposure to acetylene gas with minimum mortality followed by other pests *Callusobruchus maculatus*, *Sitophilus oryzae* and *Triboliumcastaneum* pests.

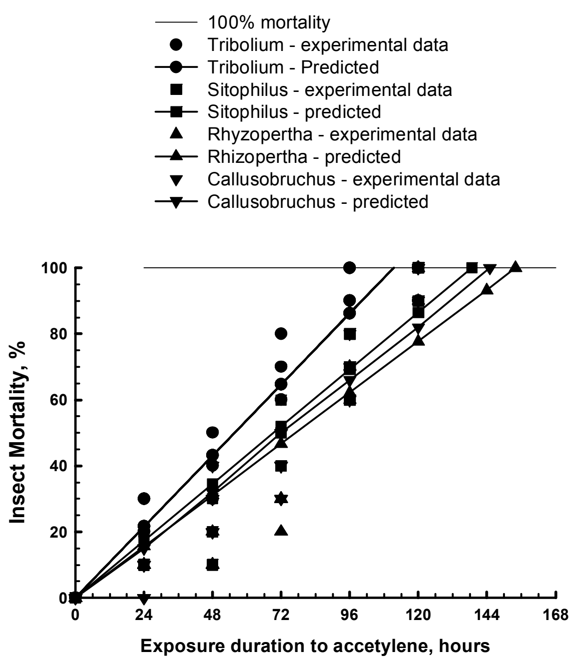


**Fig.3: Mortality of indicated insects with exposure duration.**

For reaching 100% mortality of *Tribolium castaneum, Sitophilus oryzae, Rhyzopertha dominica* and *Callusobruchus maculatus*, in the storage of paddy grains, the exposure time required are 111, 139, 153 and 139 hours, respectively (Table 3). The duration required to achieve 50% mortality in these storage pests under acetylene filled storage condition are 56, 69, 77 and 70 h. The linear models relating the mortality and the exposure duration proposed for the storage pests of this study are given in Table 4. The proposed models had the highest fit of above 0.9 with least error components. Using these models, the exposure time required for any desired level of motility can be estimated.

**Table 3**. Lethal time for the indicated storage pests for paddy grains filled with acetylene gas.

| Storage pest | Lethal time for 50%, h | Lethal time for 100%, h |
|---|---|---|
| *Tribolium castaneum* | 56 | 111 |
| *Sitophilus oryzae* | 69 | 139 |
| *Rhyzopertha dominica* | 77 | 153 |
| *Callusobruchus maculatus* | 70 | 139 |

**Progeny production and scale-up studies in larger storage bins**

The progeny studies in the glassware containers were conducted for a period up to four months and recorded the progeny at monthly intervals. The effect of storage pests and the exposure duration were related to the progeny production through analysis of variance (Table 5). The duration of exposure and its interaction with insects were found insignificant. The non-significance of the exposure duration with progeny production showed that the progeny production does not continued beyond the initial period of 30 days. The storage for 30 days with acetylene filled ensures the 100 % mortality of storage grain pests.

The mean number of progeny production of adult and larvae of these storage pests is given in Table 6. From the table it is seen that the progeny production of adults and grub ranged from 1.7 to 7.7 and 0 to 1.7, respectively. Progeny development and kernel damage were seen affected in brown rice than rough rice with when treated with infra red rays. Howver, an infrared ray intensity of 10.84 kW/m$^2$ increased the number of overall progeny, the amount of adults, and frass produced on CL152 brown rice (Rachel *et al.*, 2019). Shelled corn treated with 1 or 3 ppm of Entrust negatively affected progeny emergence of all maize weevil, *Sitophilus zeamais* Motsculsky, red flour beetle, *Tribolium castaneum* (Herbst) and sawtoothed grain beetle, *Oryzaephilus surinamensis* Linnaeus (Jonathan *et al.*, 2007). In the scale-up (long term storage) studies conducted in the plastic bins as explained in the above sections, the bins were emptied and sieved the grains to separate the insect pests introduced at the beginning of the trial. Upto the period of four months, no survival of the pests / grub and progeny production were seen. However, minimum level of progeny production were observed.

Maximum of seven number of *R.dominica* adult was observed after 90 days exposure and a minimum number of *T. castaneun* adults were observed after seven days of exposure. Considering grubs, no grub was seen after 120 days of exposure

**Table 4.** Linear models for the lethal time of the indicated storage pests for paddy grains filled with acetylene gas with statistical significance.

| Storage pest | Linear model | $R^2$ | SE | SEE | T | P |
|---|---|---|---|---|---|---|
| *Tribolium castaneum* | M = 0.898 (T) | 0.951 | 0.0144 | 8.116 | 62.2574 | <0.0001 |
| *Sitophilus oryzae* | M = 0.721 (T) | 0.908 | 0.0190 | 10.7068 | 37.8740 | <0.0001 |
| *Rhyzopertha dominica* | M = 0.653 (T) | 0.911 | 0.0257 | 14.4393 | 25.4553 | <0.0001 |
| *Callusobruchus maculatus* | M = 0.717 (T) | 0.908 | 0.0189 | 10.6469 | 37.8868 | <0.0001 |

M – mortality, %; T – duration of exposure, h

**Table 5.** Analysis of variance of progeny with other experimental variables.

| Source | Sum of Squares | df | Mean Square | F |
|---|---|---|---|---|
| Corrected Model | 114.583 | 15 | 7.639 | 7.801** |
| Intercept | 1752.083 | 1 | 1752.083 | 1.789E3** |
| Insect | 92.917 | 3 | 30.972 | 31.631** |
| Time | 11.417 | 3 | 3.806 | $3.887^{ns}$ |
| Insect * time | 10.250 | 9 | 1.139 | $1.163^{ns}$ |
| Error | 31.333 | 32 | 0.979 | |
| Total | 1898.000 | 48 | | |
| Corrected Total | 145.917 | 47 | | |

R Squared = 0.785 (Adjusted R Squared = 0.685)

**Table 6.** Mean progeny production of the storage pests at indicated exposure duration for paddy grains filled with acetylene gas.

| Sl. No. | Storage pest | Days of exposure for progeny | Mean Number of progeny (alive / dead) | | |
|---|---|---|---|---|---|
| | | | Adult | Larvae | Total |
| 1 | *Tribolium castaneum* | 30 | 1.7 | 0.7 | 2.3 |
| 2 | | 60 | 3.3 | 1.7 | 5.0 |
| 3 | | 90 | 4.0 | 0.0 | 4.0 |
| 4 | | 120 | 4.3 | 0.0 | 4.3 |
| 5 | *Sitophilus oryzae* | 30 | 5.7 | 1.0 | 6.7 |
| 6 | | 60 | 6.3 | 1.7 | 8.0 |
| 7 | | 90 | 6.0 | 1.0 | 7.0 |
| 8 | | 120 | 6.0 | 0.0 | 6.0 |
| 9 | *Rhyzopertha dominica* | 30 | 6.0 | 1.3 | 7.3 |
| 10 | | 60 | 6.3 | 1.3 | 7.7 |
| 11 | | 90 | 7.7 | 0.7 | 8.3 |
| 12 | | 120 | 6.7 | 0.3 | 7.0 |
| 13 | *Callusobruchus maculatus* | 30 | 3.7 | 1.7 | 5.3 |
| 14 | | 60 | 4.7 | 1.3 | 6.0 |
| 15 | | 90 | 5.3 | 0.7 | 6.0 |
| 16 | | 120 | 5.7 | 0.0 | 5.7 |

with *C. maculatus* while the maximum number of 1.7 grubs of *T. castaneum*, *S. oryzae* and *C. maculatus* were seen after 60, 60 and 30 days after exposure respectively.

## CONCLUSION

Acetylene gas is found suitable as an alternate for $CO_2$, $N_2$ and other gases for controlled atmosphere storage of food grains and control the storage pests, by reducing the availability of oxygen. For achieving 100 per cent mortality of *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum* and *Callusobruchus maculatus,* in the storage of paddy grains (CV TRY 3) the exposure duration required to acetylene gas are 111, 139, 153 and 139 h respectively. The progeny of these storage pests were found insignificant with respect to storage period, during longterm storage up to four months. Production and use of acetylene gas at storage site will ease the adoption of controlled atmosphere storage of food grains using acetylene as alternate gas.

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