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The efficacy of biogas to protect stored grains from insect pests

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Abstract: Stored grains such as rice and wheat (and other grains/pulses) are prone to pest infestation mainly by *Sitophilus oryzae*, *Tribolium castaneum* and *Rhyzopertha dominica* in India and more than 30 % of harvested grain is lost to stored grain pests. Protection of the grains by creating an oxygen deficit atmosphere by using carbon-dioxide or biogas is an alternative. In this study, biogas was used as a 'fumigant' against *S .oryzae*, *T. castaneum* and *R. dominica* reared on *rice (Oryza sativa) and wheat (Triticum aestivum;* Semolina and whole wheat flour) grain types, respectively. The optimum biogas flow rate required to remove the oxygen from an empty container and partially grain filled container was found to be 40 ml per minute sustained up to a time leading to an equivalent of three times the volume of the grain container. Using these fumigation conditions 100 % adult mortality was observed in *T. castaneum* and *R. dominica* within 24 hours and *S. oryzae* within 48 hours. Farmers in rural India have been using biogas plants to meet their kitchen energy needs. Some surplus gas could be used for fumigation of stored agro-products making this process inexpensive, environment friendly as well as acceptable to a growing 'organic food market'.

Key words: Stored grain pest; biogas fumigation; oxygen-deficit environment; insect mortality

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1. Introduction: Stored grains and cereals require proper storage in order to avoid insect infestation. Improper and ineffective storage is a prelude to grain damage - both in quantity and quality - which brings huge losses to the farmers. Insect infestation also promotes microbial and fungal activity that can lead to decreased nutrition, malodor and unacceptable color of the grains that renders it undesirable for human consumption **[1]**. The Indian subcontinent, with its humid and sub-humid climate, creates a favorable environment for insect adaptation, multiplication and propagation in stored grains. Nearly 10 % of the grains produced every year are infested by pests and this loss amounts to 20 million tons every year. Granary weevil (*S. granarius)*, red flour beetles *(T. castaneum*), rice weevil (*S. oryzae*) are the common pests that infest various forms of rice and wheat used in India **[2]**. Methods used to eradicate pests in grains include the use of grain protectants, contact pesticides and fumigants. Chemicals containing pyrethriod and organophosphorous compounds that protect against frost, rust and insects are called grain protectants. Contact pesticides are quite popular among small farmers for chemical control of stored-product pests. They can be used as dust formulations that are mixed with liquid surface treatments that include Malathion, Lindane, Pirimiphos methyl, Fenitrothion etc. **[3]**. However, grain protectants and contact pesticides are toxic to humans due to the chemical residues left over on the food grains and the insects are known to develop resistance to them thereby restricting their widespread usage **[4, 5]**. Over a period of time, farmers have moved away from biomass, bamboo and adobe based storage structures to HDPE containers to store grains. Thus, fumigation is a common method of stored product pest management with the use of certain chemicals in gaseous forms to disinfest grains. Fumigants can be applied in-situ and flows throughout the stored product. It is relatively inexpensive and convenient;

hence, preferred over other methods of control **[6]**. For effective fumigation, the fumigant needs to be maintained for an adequate period of time at a concentration which is toxic to the insect pest **[7]**. Prolonged exposure to the chemical fumigants like methyl bromide and phosphine increases resistance of pests leading to an ineffective control in the long run **[8, 9]**. Moreover, phosphine is a potent environment hazard as it depletes the ozone layer **[10]**. Agricultural and food industries use modified atmospheres (MA) or controlled atmospheres (CA) for preservation of raw materials **[11]**. MA technology, such as lower oxygen (O_2) concentration and higher carbon dioxide (CO_2) in a closed environment, reduces the rate of respiration and metabolism of the insects. Carbon dioxide (CO_2) is used to flush out oxygen from the grain storage vessel, occupying the bottom part of the storage vessel due to its greater density than O_2, thus pushing the oxygen to the top and out of the vessel. Lower oxygen environment or anoxic atmosphere with ≤ 1 % of oxygen can be created by flushing out the oxygen from an air tight room/silo **[12]**. Long exposure periods to low levels of oxygen leads to stresses of hypoxia and hypercarbia that slows insect development and it finally leads to their death **[13, 2]**. The fumigant diffusion rate varies linearly with cross-sectional volume in accordance to Fick's law of diffusion. Considering an ideal situation of circular trachea and Fick's gas diffusion, it creates a hypoxic atmosphere that restricts the oxygen reaching the tissues **[14]**.

In order to use safer and 'human health friendly' gases as a method to control insect pests few studies on the use of biogas as a fumigant have been reported. Biogas fumigation does not affect seed germination, seedling vigor or grain quality and grain cooking quality. Palaniswany & Dakshinamurthy **[15]** used biogas produced from cow dung having 60 % methane and 30 – 35 % carbon dioxide as a fumigant and achieved 100 % mortality within 4 - 6 hours of incubation. Mohan & Gopalan **[16]** used biogas to control *Callosobrunchus chinensis* storage insect of pulses, in seeds and grains of pigeon pea and achieved 100 % mortality of eggs, grubs and adults within a period of 10 days of fumigation. Subramanya et al. **[17]** used both biogas and carbon dioxide at a rate of 2 liters per minute in a 500 ml saline bottle (flow equivalent to 240 volume equivalent of the container changed per hour) for control of rice weevils (*S. oryzae*) infestations and achieved 100 % mortality after 21 hours of continuous exposure of the pests to carbon dioxide.

Most of the previous studies on the use of biogas as a fumigant were restricted to a specific type of grain and its respective insect pests; therefore, it was not clear if its application can be extended to other pests and grains. More importantly, the flow rate and volumes of biogas used for fumigation were extremely high and the rationale not very clear. In order to enable this technique to be standardized, there is a need to determine the minimum volume of biogas to be used as well as an experimentally verify the underlying process so as to be applicable under typical field conditions. The present study was carried out to experimentally determine the application of biogas as a fumigant for three different types of grain commonly grown in India which differ in properties and their respective grain insects. Secondly, to estimate the optimum flow rate and volume of biogas required to flush out the oxygen/air from an empty or partially filled grain storage container to create an oxygen-deficit environment. Finally, the mortality of the insects located throughout the grain volume and not restricted to specific areas (radial movement of gas) with the variations in grain volume in the container.

2. Materials and methods:

2.1 Experimental setup: Insect cultures of *S. oryzae* (rice weevil), *T. castaneum* (red flour beetle) and *R. dominica* (lesser grain borer) were procured from Bio-Control Research Laboratories (BCRL), a division of Pest Control (India) Pvt. Ltd. Cultures were reared for 6 - 8 months at ambient temperature

23 ºC – 30 ºC with humidity 60 - 80%. Grains of *O. sativa* (rice), milled *T. aestivum* (semolina and whole wheat flour) were used for the rearing of *S. oryzae*, *T._castaneum* and *R. dominica*, respectively. The cultures were allowed to undergo nearly three generation cycles to yield the required numbers of adult insects and occasional sub-culturing was done to yield a flourishing culture of adults. The fumigation experiments were carried out in tapered cylindrical glass bottles of inner diameter (6.9 cm), height (17 cm) and total volume of (650 ml) respectively (saline bottles). The glass bottles were fitted with a rubber cork and sealed with wax to ensure an air tight environment. Two stainless steel tubes (17 cm and 10 cm) long of (6 mm and 4 mm) diameters were used as the inlet and outlet for the biogas flow (Figure 1). A compressed biogas cylinder (standard biogas) was procured from CHEMIX-India, Bengaluru that had a biogas composition of methane (57.43 %) and carbon dioxide (42.57 %). The flow of biogas from the cylinder into the glass bottles was controlled through a 3-stage manual flow controller that was connected to a gas flow meter to measure the flow rate of biogas. The composition of biogas (CH_4 and CO_2) was determined in a gas chromatograph procured from Mayura Analytical, Bengaluru, India using Haysep A column with nitrogen as a carrier, oven temperature of 80 ºC, flame ionization detector (FID) set to 300 ºC. Oxygen and nitrogen were measured in the same gas chromatograph using a molecular sieve column, hydrogen as carrier with an oven temperature of 80 ºC using a thermal conductivity detector.

The physical properties of the grains like diameter, height, volume and bulk density were determined. In case of rice, the length and breadth of ten grains were measured and a mean value was chosen. For semolina and milled wheat the dimensions were measured under a precalibrated light microscope using a stage micrometer and the mean value was chosen. The bulk density was measured using a specific gravity bottle at ambient conditions.

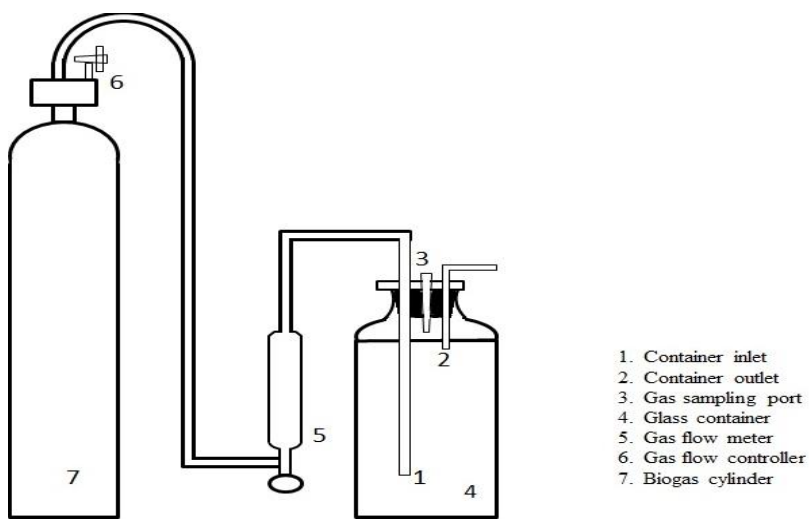

Figure 1: Schematic diagram of the experimental set-up.

2.2 Gas flow and displacement: In the field conditions, biogas is stored in typical biogas plants at low ambient pressures of 500 - 1000 Pa (in floating drum models) and up to 4000 Pa (in fixed dome models). In order to use the information in this study it would be very difficult for biogas plant owners to measure gas flow rates using flow meters and standardize fumigation by biogas. We therefore adopt a simple reporting method for flow rate and total gas allowed to flow – a method similar to that adopted in airconditioning or mushroom houses etc, where in the flow rate is measured as the number of air changes in one hour and total volume of gas used could be the total number of air changes carried out. As there is no air changes carried out, the units of flow rate and total gas used are expressed as number of gas displacements achieved per hour and total number of gas displacements achieved. As the flow rates used here are similar to that achievable in the field, the information used here could easily be translated to action in the field.

2.3 Biogas fumigation: The biogas fumigation experiment was carried out in three steps that are as follows: a) The air was displaced from the empty fumigation bottles using biogas flowing at various flow rates of 10, 20, 30, 40, 50 ml/min representing 1, 2, 3, 4 and 5 times the volumes of the container displaced per hour. Two replicates were maintained to account for the variations in gas fill. At each flow rate, the biogas content in the empty fumigation bottles was analyzed using gas chromatography. b) The air displacement from the containers having three different types of grains namely *Oryza sativa* (rice), semolina and whole wheat flour was studied. These grains were filled in three different grain volumes of one fourth, one-half and three fourth the volume of the fumigation bottles in order to represent various states of grain filled in a container. After the fumigation process the gas composition inside the container was analyzed without physically disturbing the grain and also after shaking the container 5-6 times in order to estimate the trapped air in the void spaces of the grains. c) The mortality of three different insect pests was studied by adding ten adult insects from each of the mixed-age cultures. The study of the mortality of each type of insect pest was conducted at two different grain volumes filled up to one-fourth and one-half of the container and mixed to get random placement of the insects. After this, biogas was bled into the containers in order to displace the air at three times the volume of container at a flow rate of 40 ml/min [specify volume displacement rate]. Insect mortality with one and two day gas exposure was examined and reported. Insect mortality after fumigation was carried out by the methods followed by Subramanya et al. **[18]** and Wang et al. **[2]**. The insects were removed from the fumigation bottle and kept separately in a container at ambient conditions under constant supervision for 24 hours. The insects which did not show any movement for 24 hours were then prodded with a soft brush to observe any movement and thus identified as dead. Experiments were conducted in triplicates to account for variation.

2.4 Statistical analysis: A two way ANOVA was carried out for the mortality of insect pests. Two different factors that affect the pest's mortality such as the grain fill volume, exposure time in days and the combined effect of grain fill volume and time (days) were analysed using the two way ANOVA. The F value was compared at the significance level of $P < 0.05$.

3. Results and discussion: The results of the experiments conducted for determining the extent of air replacements achievable in empty containers, in containers with different levels of filling and at various levels of flow rates and total biogas used is reported here. The displacement of oxygen (air) by biogas from a simulated empty grain container was studied by bleeding biogas equal to once (1V), twice (2V) and thrice (3V) the volume of the empty container at 10, 20, 30, 40, 50 ml/min flow rates respectively. This was equivalent to 1x, 2x, 3x, 4x and 5x volume displacements or gas changes per hour (Figure 2); a level that is easily achievable in the field. Two replicates were maintained to account for variations in gas fill. At 1V the increase in biogas flow rate from 10 to 50 ml/min does not increase the air displacement and attained a maximum of 54 % at 50 ml/min (5x). This shows that at a one volume equivalent of gas displacement (1V), an increase in flow rate does not improve the level of air displacement. However at 2V, the air displacement from the container increased for all the flow rates with the 40 ml/min (4 volumes displaced per hour, VDPH) achieving 100 % air removal. At 3V the volume of air displacement from the containers did not show any considerable difference from 2V with the 40 ml/min achieving 100 % air removal. However, at 50 ml/ min flow rate with 2V and 3V displacement, the oxygen removal decreased by 7 % and 6 % respectively. This may be attributed to the accumulation of air molecules in the certain pockets of the head space. Therefore, in order to achieve complete air removal, the total biogas volume used for displacement of air needs to be greater than two volumes (2V) of the container at a flow rate of 40 ml/ min (4 VDPH).

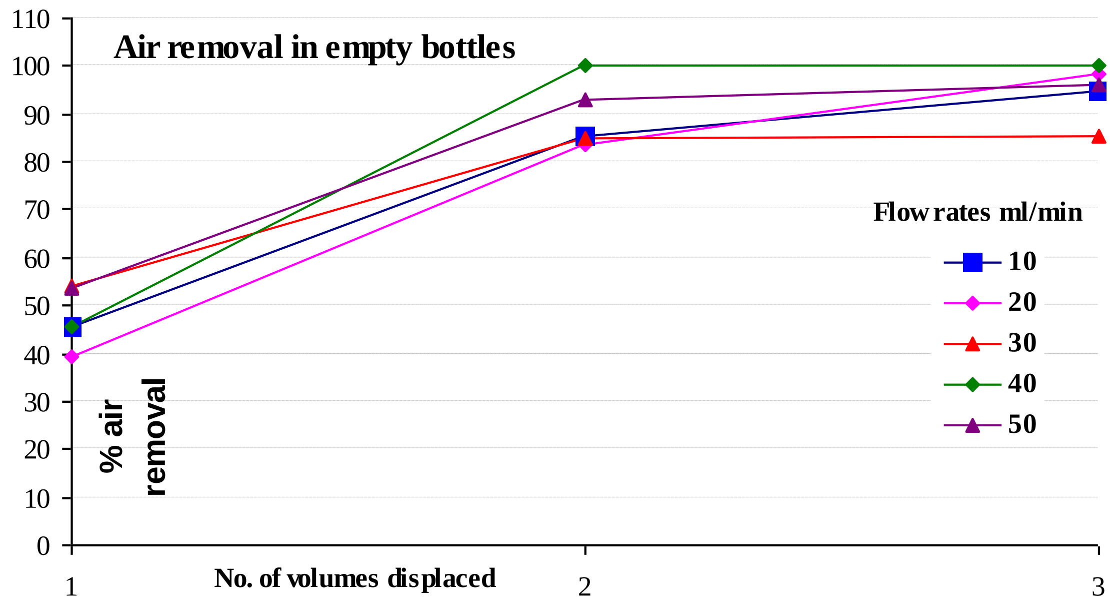

Figure 2: The extent of air displacement achieved at different flow rates and gas displacement volumes achieved.

Three different types of grain products were used in the study namely, rice, semolina and milled wheat flour – these also represent the different particle size and concomitant fumigation needs that are likely to be encountered under conventional grain and grain product storage. Their measured physical properties are presented in Table 1. The grain bulk can be considered analogous to a packed bed that is filled with beads having a known porosity and interstitial voids in between the grains. Rice is ellipsoid to capsular in shape with an average (smaller) diameter of 2 mm. In the case of semolina, the particles were granular and spheroid in shape with an average diameter of 1 mm. In the third case, namely, milled wheat, the particles are much smaller measuring much less than 1 mm (Table 1). With the decrease in particle size from rice grains to milled wheat flour, the inter-grain voids/spaces increases and the bulk density decreases. The increase in interstitial voids would therefore trap comparably more air that would require higher amount of biogas to displace all the air from the container. In addition to the above, the air removal from the container would be strongly influenced by the volume of grain occupying the container – or the grain filling volume. At 25 % grain filling volume, the least oxygen replacement was found in the case of milled wheat flour, followed by rice with 88.02 %, 91.18 % respectively (Figure 3a and 3c). In case of rice grains (Figure 3a) the change in composition of head space gas soon after flushing and upon mixing the contents indicated 1 – 2 % change indicating the absence of any need to mix the contents of the grain bin to obtain uniform kill throughout the bin. Whereas semolina achieved 100 % of air displacement at 25 % grain filling volume (Figure 3b) indicating the importance of physical structure of grain in fumigation process. In order to account for voids where biogas could not have reached, the stored grain products were thoroughly mixed by shaking the bottle. This was expected to bring out the air trapped in the voids into the 'head space' and the composition of the gas taken before and after mixing would indicate the completeness of the displacement achieved in the first stage, i.e. before mixing the contents. After mixing the contents of the bottle, the level of air displaced measured in percentage air displaced decreased to 85.33 %, 90.46 % in milled wheat flour and rice, respectively (Figure 3a and 3c). However Semolina continued to show 100 % air removal indicating complete air removal (Figure 3b). The completeness of biogas filling the voids for various levels of grain filling was flushing the required quantity of biogas and estimating the biogas content of the headspace gas soon after. The extent to which all voids in the grain was not filled with biogas was estimated by mixing the contents of the container and estimating the drop in biogas content of head space. In cases only 2 - 4% improvement was obtained on mixing which indicated that a reasonably uniform spread of biogas occurred in the test containers.

When the grain filling volume was 50 %, the trend of higher air displacement was observed with milled wheat flour, rice and semolina showing an air removal of 88.44 %, 94.14 % and 100 % respectively, in the head space prior to mixing the contents. After mixing the grains products maintained in the bottles, the percentage of air displaced fell marginally to 85.97 % and 93.71 % in the case of milled wheat flour and rice. There was no change in the head space gas composition in the case of semolina and remained at 100 %. This suggested that a grain filling volume of 50 % improved the level of air displacement by biogas compared to a lower level of grain filling volume of 25 %. When the grain filling volume was increased to 75 %, the air removal was found to be 70.14 % and 91.15 % in case of milled wheat flour and rice which was significantly lower than 50 % grain filling volume. After mixing the contents, the air displacement was reduced to 66.75 % and 90.14 % for milled wheat flour and rice, respectively. In both the cases, about 1 – 3 % of the air in the voids is not displaced in a one time operation of displacing air from the container by biogas by the method of gas bleeding practiced in this study. Therefore, the concentrations of air in the head space increases after mixing the grains in the container. However in the case of semolina, the air displacement remained unchanged at 100 % before and after the mixing operation. The air displacement by biogas penetration into the voids was found to be the highest in case of containers filled up to 50 % of the grain filling volume compared to the 25 % and 75 % of levels of fill volumes. This suggests that the ideal practice of fumigation of stored grains with biogas could be achieved at a 50 % grain filling volume. The decrease in air displacement at 75 % fill volume in the case of milled wheat flour and rice is not well understood from available data and can be attributed to the higher inter granular space or voids. Higher, non-uniform and randomly distributed void spaces can trap air making it difficult for biogas to flush out the air completely from the voids. In all three cases, it was observed that at a particle diameter close to one mm, there was 100 % air removal from the grain bulk. The above set of observations can also be visualized as an ideal situation with uniformly shaped particles of 1 mm diameter in a packed bed reactor. A particle diameter of 0.3 mm gave nearly the same oxygen scavenging/removal percentage in both 25 % and 50 % grain volumes. With particle diameter of 0.3 mm and 2 mm it was observed that the air removal was marginally lower in comparison with particle diameter of 1 mm.

Table 1: Physical properties of rice, semolina and milled wheat.

Type grain	Diameter ($1x10^{-3}$ m)	Height ($1x10^{-3}$m)	Assumed shape	Volume ($1x10^{-9}m^3$)	Bulk density ($1x10^3$ kgm^{-3})
Rice	2	5.07	Ellipsoidic spheroid	$\pi r^2 h = 63.71$	0.915
Semolina	0.9	-	Sphere	$4\pi r^3 = 9.16$	0.805
Milled wheat	0.3	-	Sphere	$4\pi r^3 = 0.339$	0.71

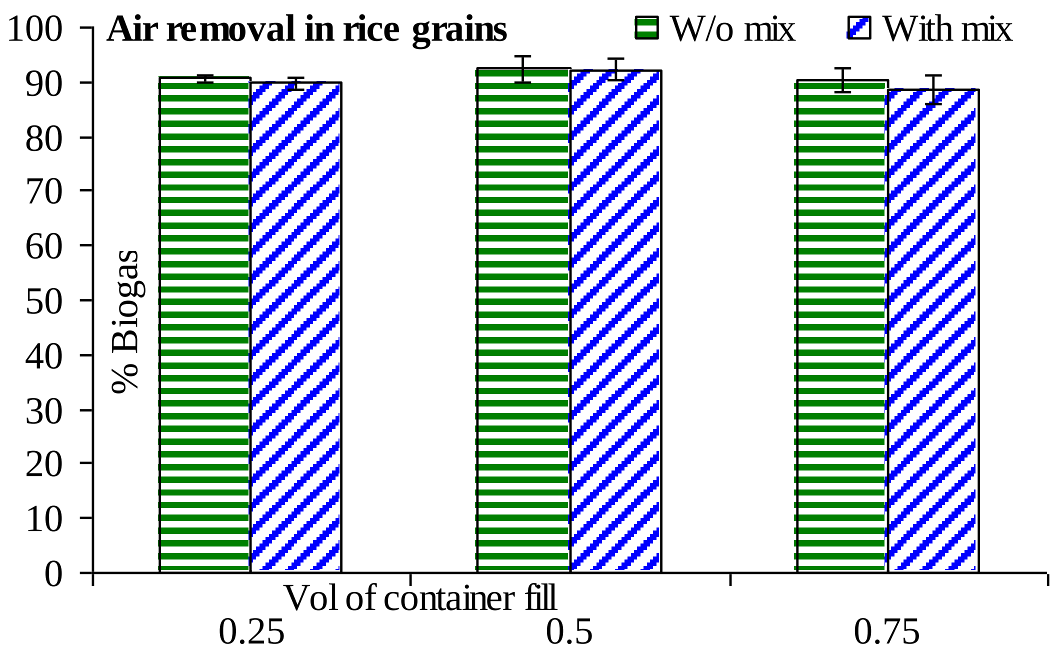

Figure 3a: Impact of the rice grain fill volume 25 % (0.25), 50 % (0.5) and 75 % (0.75) on the extent of air flushed out of the test containers for rice grains.

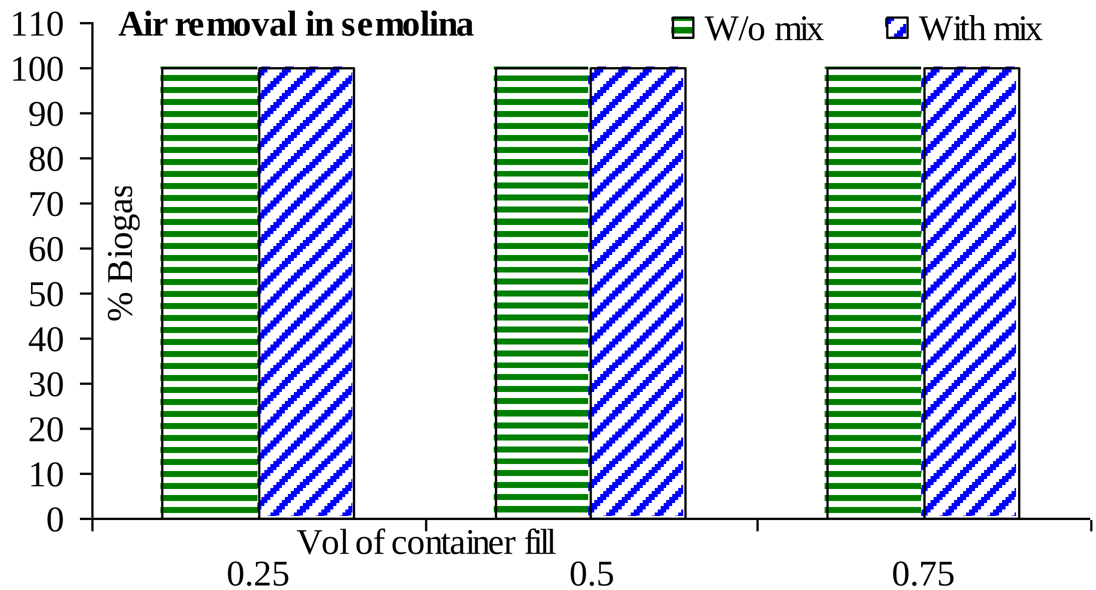

Figure 3b: Impact of the Semolina fill volume of 25 % (0.25), 50 % (0.5) and 75 % (0.75) on the extent of air flushed out of the test containers for Semolina.

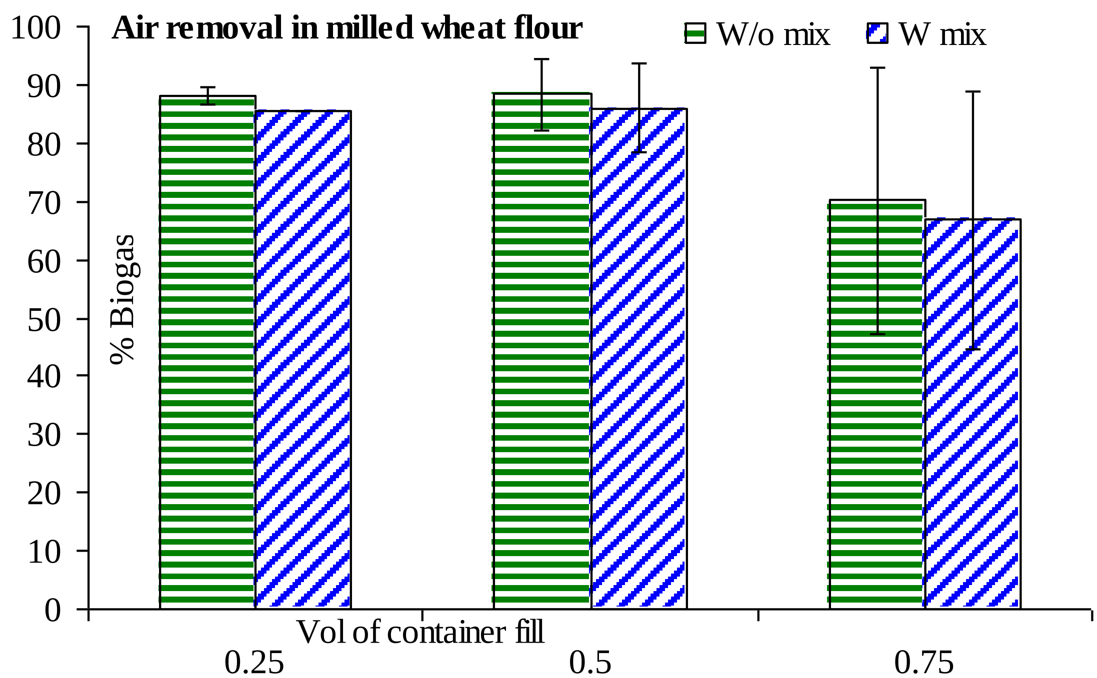

Figure 3c: Impact of the milled wheat flour fill volume of 25 % (0.25), 50 % (0.5) and 75 % (0.75) on the extent of air flushed out of the test containers for milled wheat flour.

Fumigation was carried out with three different pest species with grain filling volume of 25 % and 50 % (Figure 4a and Figure 4b). The extent of biogas volume was kept constant as per the previous gas study experiments attaining 100 % biogas fill. The survival of ten adult insects from each of the mixed-age cultures were monitored for the two levels of grain filling (one-fourth and one-half). Insect mortality with one day and two day gas exposure was monitored. The results indicate that *S. oryzae* needed a biogas exposure of two days, whereas *T. castaneum* and *R. dominica* needed only one day exposure to achieve 100 % mortality. This was observed in both cases of 25 % (Figure 4a) and 50 % (Figure 4b) of grain filling volumes. The experimental results were also substantiated by the two-way ANOVA test (Table 2) that showed a non significant value (ANOVA: F1, 8 = 15.99; p < 0.05) for *S. oryzae whereas T. castaneum and R. dominica had a significant value of* (ANOVA: F1, 8 = 0.99; p < 0.05) and ($F_{1,8} = 0$; $p < 0.05$) respectively. This suggests that the *T. castaneum* and the *R. dominica* are more susceptible than the *S. oryzae* and are killed within the first 24 hour of gas exposure (disinfestation attempts). After one day biogas exposure, in the case of *S .oryzae* the one-fourth volume of grain fill achieved 93.33 % of insect mortality. However, kill was even lower in the case of 50 % grain filling, achieving about 80 %. The two-way ANOVA results for *S.oryzae* (ANOVA: F1, 8 = 3.99; p < 0.05), *T. castaneum* (ANOVA: F1, 8 = 1; p < 0.05) and *R. dominica* (ANOVA: F1,8 = 0; p < 0.05) had a significant value indicated that grain filling volume had no effect on *R. dominica* with a little increase in its effect in *T. castaneum* and *S. oryzae*. Overall the fill volume had minimum effect on the insect mortality indicating that the air trapped in the grain voids are not sufficient enough to sustain the insect's survival. However, in all the three grain types and two levels of grain filling and the three types of pests, 100 % kill was achieved for all insect types after 2d of exposure to biogas. It was observed that *S.oryzae* was the most robust insect type followed by *T. castaneum* and R. *dominica* was the most susceptible insect to the biogas fumigation.

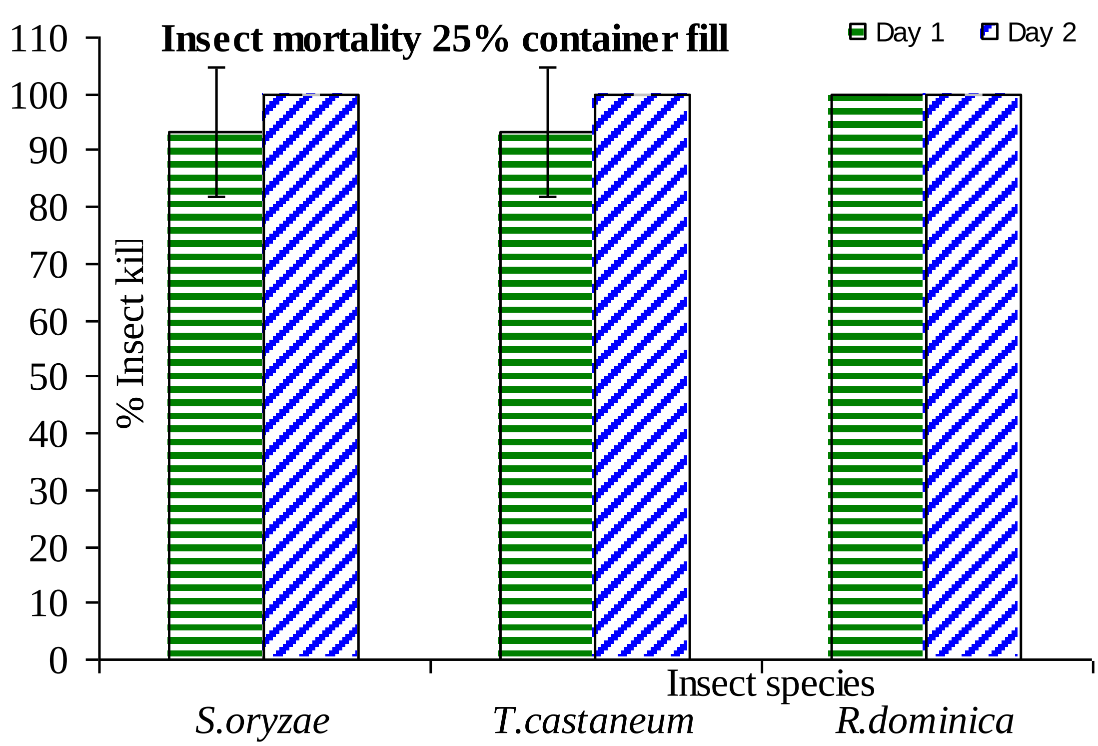

Figure 4a: Impact of 25 % grain fill volume on the insect kill obtained for a gas exposure period of one and two days

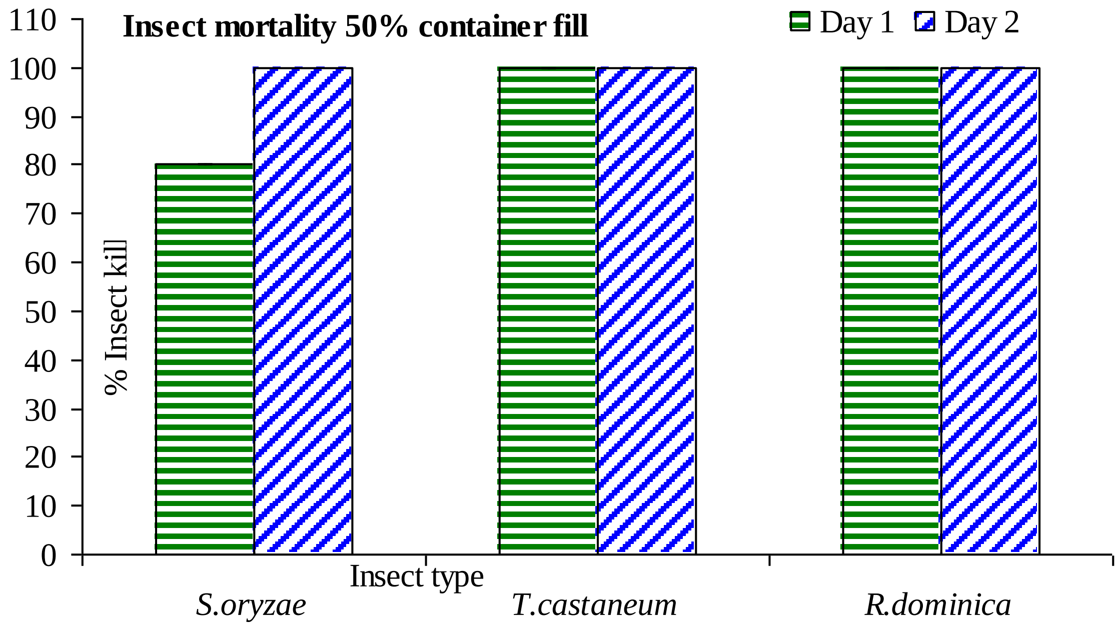

Figure 4b: Impact of 50 % grain fill volume on the insect kill obtained for a gas exposure period of one and two days.

Insect type	Parameters	Degrees of freedom	F	P
S. oryzae	Grain fill volume	1,8	3.99	<0.05
	Days	1,8	15.99 NS	<0.05
	Grain fill volume x Days	1,8	4.01	<0.05
T. castaneum	Grain fill volume	1,8	1	<0.05
	Days	1,8	0.99	<0.05
	Grain fill volume x Days	1,8	1	<0.05
R. dominica	Grain fill volume	1,8	0	<0.05
	Days	1,8	0	<0.05
	Grain fill volume x Days	1,8	0	<0.05

Table 2. The effect of grain fill volume and exposure time on the mortality of *S. oryzae*, *T. castaneum* and *R. dominica* using two-way ANOVA test (NS indicate non-significant; in all combinations of grain fill volume and exposure time the null hypothesis of no significant effect was accepted and only in the case of *S oryzae* and exposure time null hypothesis of no effect was rejected).

4. Conclusions: This research demonstrates that biogas can be used to protect harvested grains from stored grain pests by biogas assisted fumigation using simple rules for flow rate and filling. Common stored grain insect pests such as *S. oryzae*, *T. castaneum* and *R. dominica* occurring in rice, semolina and wheat flour, respectively, could be controlled completely within two days after displacing air in the storage containers with biogas. Control of these pests occurred at a wide range of grain fill levels (25 – 75 %) in the containers. Complete air displacement could be achieved even at moderate extent of fill (3 volume changes) and at a low flow rates (3 - 5 volume displacements per hour). Rural population of India has easy access to biogas either from cow dung /agricultural residues/leaf litter. Until now biogas plants have been looked upon as a source of alternative energy (CH_4) and plant nutrients (compost). However, its application as a fumigant for post harvest products can extend its application and increase the value and quality of grain stored in rural areas for self and the market. Rural areas can thus provide pesticide-free fumigation and storage of key grains in rural areas at low costs and possibly provide livelihoods to many biogas plant owners.

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