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Studies on the effect of ozone on mortality of insects in stored paddy: Changes in milling qualities upon storage

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Abstract
This study aims to demonstrate the effectiveness of ozone as a fumigant in paddy. The efficacy of ozone against *Rhyzopertha dominica* adults in paddy grain was studied at different concentrations and moisture contents. The results indicated that exposure time of ozone required to achieve 100 per cent mortality of *Rhyzopertha dominica* adults in paddy grains at a moisture of 12.40% (w.b.) were 540, 360 and 270 min at 500, 1000 and 1500 ppm of ozone. Grains at moisture content of 14.20% treated under the same conditions required 610, 390 and 300 min to achieve complete mortality.
It is seen from the milling studies that maximum total milling recovery for grains at 12.40% and 14.20% moisture content on the 60th day of storage was 64.90% and 62.68% respectively when treated at a doze of 500 ppm. Head rice recovery was also highest (85.44% and 88.03%) under the same conditions. Highest broken amount was found to be 12.50% and 13.62% in grains at moisture content of 12.40% and 14.20% respectively, when treated at 1500ppm.

Keywords: Paddy, fumigation, ozone, storage, milling, head rice, brokens

1. Introduction
Rice (*Oryza sativa* L.) is one among the oldest cultivated crop and ranks as the most widely grown food grain, serving as the staple food for about half of the world's population. According to the USDA Foreign Agricultural Service, India produced about 105 million tonnes of paddy rice from 43.5 million hectares in 2016-17. Out of the total production the post-harvest loss of rice is estimated to be as high as 16% [1].
Grain storage plays a very important role in preventing losses which are caused mainly due to weevils, beetles, moths and rodents. Insect pests are a greater problem in regions like India where the high relative humidity coupled with warm temperature makes a conducive environment for insect multiplication. The estimated loss due to insects in India is around 3% of the country's production. Insect infestations in grain not only cause loss in quantity, but also quality losses and lower crop value. The most effective way to reach pests in their most remote hiding places is through fumigation, the use of poisonous gases to kill pests in an enclosed area**.** Ozone is a potential fumigant of stored grain. Ozone is also a powerful antimicrobial substance due to its potential oxidizing capacity. Notably, when ozone is applied to food, it leaves no residues since it decomposes quickly. These multifunctional attributes make ozone an attractive candidate for controlling insects and fungi in stored grain. It is known that ozone treatments can kill stored grain insects, including maize weevil (*S. zeamais*), rice weevil (*S. oryzae*), red flour beetle (*T. castaneum*), confused flour beetle (*T. confusum*), Indian meal moth (*P. interpunctella*) and Mediterranean flour moth (*E. kuehniella*). Gaseous ozone has reported to inactivate fungal spores on barley [2] and on stored wheat by [3].
With these points in view, an investigation was with the following objectives:

- To study the effect of ozone treatment on mortality of insects in paddy stored in different household bins
- To optimize the process variables *viz*. ozone concentration and exposure time with respect to insect mortality
- To study the milling qualities of stored paddy

2. Materials and Methods
In this chapter, the details of the materials used and methodology adopted to study the effect of

ozone treatment on insect mortality and the procedures followed for the determination and analysis of milling and cooking qualities of the ozone treated paddy grain are explained in brief.

2.1. Paddy
Paddy (CO 51) was purchased from the Central farm, Tamil Nadu Agricultural University, Coimbatore. The grains were manually cleaned to remove all foreign materials such as dust, dirt, small broken and immature kernels. Moisture content of the grain was also determined. The cleaned grains were stored in gunny bags.

2.2. Culturing of insects
250 g of wheat grain at 13-14% moisture content was taken in clean sterilised plastic jars. The wheat sample was stored at -20°C for 5 days to kill any pest stage that might have been present in the sample. 2 g of dry yeast (Bakers) was added to the medium and the contents mixed thoroughly. Fifty pairs of active adult *Rhyzopertha dominica* adults collected from the Paddy Breeding Station, Tamil Nadu Agricultural University was released into the jars for the preparation of insect culture. Plastic boxes containing insect loaded grain samples were covered using Khada cloth and tightened with rubber fasteners. The boxes were subsequently placed in an insect growth chamber maintained at room temperature of 27–30°C and 75–76% RH for 5 days to allow the insects to lay eggs. Nearly 500-600 adults were obtained in 50-60 days.
In order to get uniformly aged adults, 100 g of wheat samples were taken and 25 adult insects were introduced for egg laying. The insects were removed within 1 to 2 days and the culture was left undisturbed. After a period of 50 days, same aged adults were obtained. Insects of the same age were used throughout the study to determine mortality rates.

2.3. Ozone fumigation system
Ozone experimental setup consists of oxygen concentrator, ozone generator, ozone analyser and ozone destructor. General characteristics of each component are given below:

2.3.1. Oxygen concentrator
The Oxygen concentrator (Invacare perfectO_2 - IRC5PO2V) used for the experiment makes use of molecular sieve and pressure swing adsorption methodology to produce oxygen gas as output. Ambient air entering the device is first filtered to remove traces of nitrogen and other gases from air, and is then compressed. The air after compression is directed towards one of the two nitrogen absorbing sieve beds. The device operates at 230 V AC, 50 Hz supply with power consumption of 300 W and has a maximum flow rate of 5 lpm. Oxygen concentration level of output gas ranged from 87 to 95.6%.

2.3.2. Ozone generator
Ozone generator (Faraday Ozone - L30G) having dimension of 420 x 210 x 370 mm operates using an air cooled ozone cell. The generator has a maximum ozone production of 30 g/h and power consumption of 230 W. The generator works on the principle of corona discharge method of generating ozone. The flow of oxygen from concentrator into the generator is controlled by a flow meter which is attached to the generator. The pure oxygen supplied is split and converted into ozone with the help of an electrode provided in the generator. The generated ozone can be supplied to the product containing system for treatment.

2.3.3. Ozone analyzer
The ozone analyzer (BMT Messtechnik Gmbh - BMT 964) used is a microprocessor based dual beam photometer for measuring the content of ozone. The device has a maximum power consumption of 15 W with a voltage range of 12 to 36 V DC. The analyzer measures UV radiation in the measurement channel, UV radiation in the reference channel, temperature and pressure in the cuvette. Ozone concentration is displayed in percentage weight of ozone (% wt/wt), grams of ozone per normal cubic meter of sample gas (g/Nm^3) or ppm_v on a 16 character alphanumeric display.

2.3.4. Ozone destructor
The Faraday ozone destructor unit (Faraday Ozone - DES OZ-01) is a durable 304 stainless steel chamber connected with the ozone analyser using 6 mm fittings that easily converts used ozone gas into oxygen before venting into the atmosphere. Technical specifications are: Power input-230 V AC, 50 Hz, Power consumption- 25 W, Operating range- 10 g/h. The unit utilizes thermal-catalytic method to remove excess ozone. The catalyst used is a transition MnO_2 material, because it is not consumed by ozone gas and acts as a true catalyst. Ozone destructor unit turns ozone back into oxygen and eliminates the need for complicated and costly external ducting.

2.4. Experimental design
The experimental design was prepared to study the effect of ozone dosage on mortality of *Rhyzopertha dominica* adults in stored paddy and to further evaluate the quality characteristics of the treated grain. Three levels of ozone doses and two levels of moisture contents of grains were used in the experimental design.
The levels of ozone concentration was selected and evaluated based on the study conducted by Pandiselvam [4]. Ozone concentration ranging from 500 ppm to 1500 ppm was found to give efficient results in terms of mortality rates and biochemical quality characteristics. The recommended moisture content for safe and long storage of grains is 14 per cent (w.b.) or lesser. Hence the levels of moisture content selected for this study were 12.40 and 14.20 percentage (w.b.).
Based on the above conditions storage studies were conducted for a period of 60 days in order to study the effect of treatment on milling and cooking quality of grain. The details of the experimental design followed in the study are presented below:

2.4.1. Independent variables
1. Ozone concentration (500, 1000 and 1500 ppm)
2. Moisture content (12.40% and 14.20% w.b.)
3. Storage period (0, 30, 60 days)

2.4.2. Dependent variables
1. Insects mortality, %
2. Milling qualities
    - Total milling recovery, %
    - Head rice recovery, %
    - Percentage broken
3. Cooking qualities
    - Cooking time, min
    - Swelling index
    - Water uptake, g/g

2.5. Determination of insect mortality
Mortality rate of insects was determined by counting the number of dead insects out of the total number of insects. Mortality was ensured by complete lack of movement after prodding. Control samples were also examined for mortality. Throughout the study mortality rate was calculated using formula [5];

$$\text{Mortality (\%)} = \frac{\text{No. of dead insects}}{\text{Total no of insects}} \text{x} 100 \quad \text{------3.2}$$

2.6. Experimental procedures
2.6.1. Trials in household bins
In order to determine the exposure time required for complete mortality of insects in household bins, trials were conducted in a 15 kg stainless steel bin with provision for ozone inlet at the bottom and outlet at the top. To distribute ozone gas throughout the bulk of the grain an L-shaped perforated PVC pipe was placed vertically at the centre, such that it covered the entire bin depth. Paddy was fed into the bin and insect cages positioned at the bottom, middle and top locations.
Insect cages were made in cylindrical shape using acrylic material with dimensions 40 x 50 x 3 mm (height x diameter x thickness). Bottom portion of the cage was covered using filter cloth in order to allow ozone gas to enter through beneath. 30 g of paddy was filled into the cage along with and 20 insects. The top end of the cage was then covered using Khada cloth and tightened with cotton threads before positioning into the bin.
Flow rate was set to 4 lpm in the oxygen concentrator and ozone gas was allowed to pass from the generator to the bin. Trials were conducted at three different concentrations (500, 1000, 1500 ppm) by adjusting the ozone variable knob positions. The used up ozone gas from the outlet of the bin was sent to the ozone destructor for safe disposal. Exposure time was kept equal to the time corresponding to complete insect mortality.

2.7. Quality analysis
Treated paddy samples along with control were collected before storage and at monthly intervals during the storage period, to analyse its moisture content, milling qualities and cooking qualities. All quality analysis experiments were replicated thrice.

2.7.1. Milling qualities
Milling quality of rice grains is important to both, producers and consumers as the market price of rice is largely dependent on milling performance. The quality of rice depends upon total milling recovery and the proportion of head and broken rice on milling.
Milling experiments were carried out in the Rice Quality Laboratory, Paddy Breeding Station, Tamil Nadu Agricultural University. The moisture content of grains was determined prior to milling. Milling studies were conducted using 100 g of paddy grains. The samples were cleaned manually to remove all foreign matters.
Rough rice was first dehulled using McGill laboratory sheller to obtain brown rice. The dehulled rice was then polished using McGill No. 2 miller for a 60 sec milling cycle. The polished grains were then sorted to head rice and broken using an oscillating grading sieve. The weights of sample obtained after each operation was recorded. The weights were used to determine the milling characteristics such as Total milling recovery (TMR), Head Rice Recovery (HRR), Broken Rice Percentage (BRP).

2.7.2. Total milling recovery (TMR).
The Total Milling Recovery (TMR) is defined as the ratio of the weight of milled rice to the total weight of rough rice multiplied by hundred. It was calculated by the following formula suggested by [6]:

$$\text{TMR (\%)} = \frac{W_{mr}}{W_p} \text{x} 100 \qquad \text{-----3.3}$$

Where
W_{mr}: Weight of milled rice, g
W_p: Total weight of paddy, g

2.7.3. Head rice recovery (HRR)
Head rice recovery is defined as the ratio of the weight of polished head rice to the weight of total milled rice expressed in percentage. The percentage of head rice was calculated using the formula given by Trigo-Stockli and Pederson (1994) [6]:

$$\text{HRR (\%)} = \frac{W_{hr}}{W_{mr}} \text{x} 100 \qquad \text{------3.4}$$

Where
W_{hr}: Weight of head rice, g
W_{mr}: Weight of milled rice, g

2.7.4. Broken rice percentage (BRP)
Broken Rice Percentage is defined as the ratio of the weight of polished broken rice to that of the total milled rice multiplied by hundred. Broken Rice Percentage was calculated as given by [7]:

$$\text{BRP (\%)} = \frac{W_{br}}{W_{mr}} \text{x} 100 \qquad \text{-----3.5}$$

Where
W_{br}: Weight of broken rice, g
W_{mr}: Weight of milled rice, g

3. Results and Discussion
The results of experiments conducted for studying the mortality of insects in stored paddy using ozone gas. The effect of ozone treatment on milling and cooking qualities during the storage of grains were also tabulated, statistically analyzed and discussed in this chapter.

3.1. Mortality of *Rhyzopertha Dominica* adults in paddy grains stored in household bins
The results of mortality studies of *Rhyzopertha Dominica* adults for various ozone concentrations in household bin is shown in Fig. 1. From the figure it was observed that mortality rates increased with increase in the concentration of ozone.
Similar results were reported by [8] while determining the susceptibility of stored product insects to high concentrations of ozone at different exposure intervals. It is also reported that much higher concentrations and longer exposure time was needed to obtain full control of insects [9].

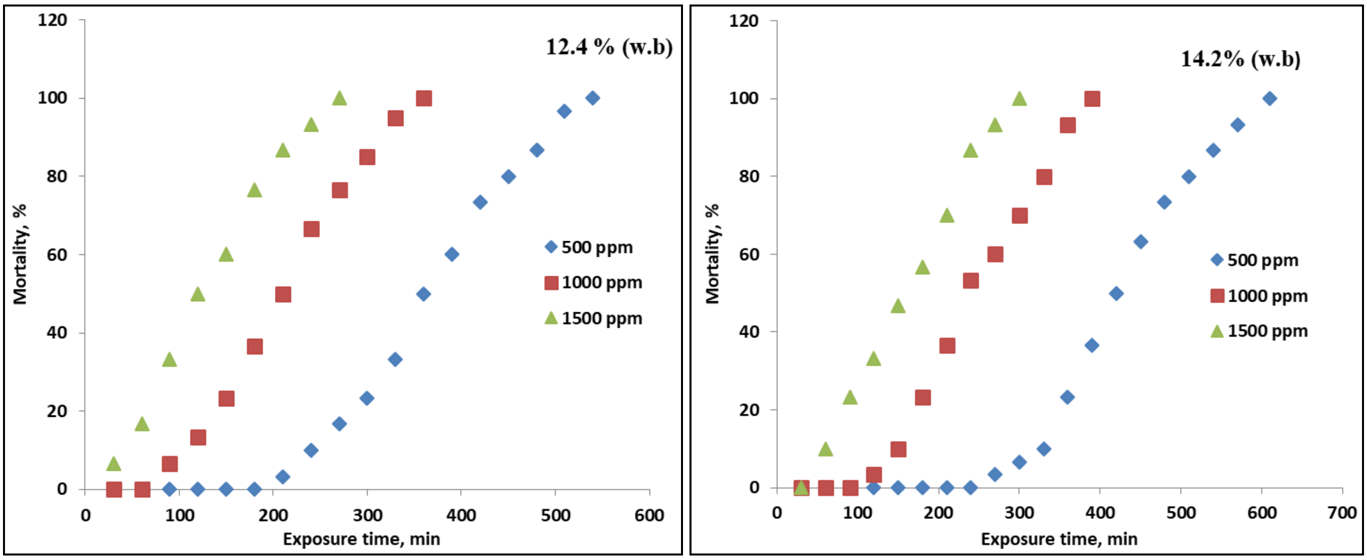

Fig 1: Mortality of *Rhyzopertha dominica* adults in paddy for various ozone concentration and exposure time

The exposure time required for achieving complete mortality of insects at different ozone concentration and moisture content is presented in Table 1. From the table it was found that highest exposure period of 610 min was required when concentration of ozone was 500 ppm and moisture content (w.b.) of paddy was 14.2%. Among the two moisture contents (12.4% and 14.2% w.b.), time taken for 100 per cent mortality of *Rhyzopertha dominica* adults was less at lower moisture content. It was deduced that at higher moistures the movement of ozone within grain layers is slowed down which requires more exposure time for insect mortality.
It is studied the efficacy of ozone for preservation of food grains and reported that exposure time becomes higher during moist conditions [10].

Table 1: Exposure time required at different moisture contents for achieving complete mortality of *Rhyzopertha Dominica* adults

Ozone concentration (ppm)	Moisture content (%) (w.b.)	Exposure time (min)
500	12.4	540
	14.2	610
1000	12.4	360
	14.2	390
1500	12.4	270
	14.2	300

Probit analysis of adults was also studied, the results of which are presented in Appendix B.1. It was observed that the lethal time progressively decreased with increasing ozone concentration. LT_{50} and LT_{99} values recorded for 12.4% moisture content (w.b.) at 500, 1000 and 1500 ppm were 365.513, 207.813 and 126.752 min; 566.587, 376.234 and 289.271 min respectively. At 14.2% moisture content LT_{50} and LT_{99} values were 428.322, 246.951 and 158.673 min; 626.265, 422.494, and 328.213 min at 500, 1000 and 1500 ppm, respectively.
The effect of ozone treatment and moisture content on exposure time required to achieve complete mortality was analyzed statistically and ANOVA is presented in Appendix C.1. The ANOVA results indicated that ozone concentration and moisture content had significant effect on exposure time at 1% level. The interaction between ozone concentration and moisture content was also found to be significant at a significance level of 5%.

3.2. Quality parameters of ozone treated grains

Storage studies were conducted for a period of 60 days and quality studies were conducted after regular intervals of 30 days. The effects of ozone treatment on the milling and cooking qualities of grains are reported in following sections.

3.2.1. Effect of ozone gas on milling qualities of grains during storage

The total milling recovery, head rice recovery and broken rice percentage of ozone fumigated paddy grains, with different ozone concentration at different moisture contents is presented in Table 2 and Table 3.
From the table it was observed that the milling recovery of grains decreased with increase in storage period. The highest milling recovery on the 60th day of storage was found to be 64.90% and 62.68% for grains at a moisture content of 12.40% and 14.20% respectively, when ozone gas at 500 ppm concentration was used. Total milling recovery of control sample (without ozone treatment) was recorded as 63.80% and 61.60% for grains at initial moisture content (w.b.) of 12.40% and 14.20% respectively.
Head rice recovery of the ozone treated grains was found to be greater than the control sample even after 60 days of storage. It was also observed that percentage of head rice decreased with increase in storage period. Paddy treated using ozone gas at a concentration of 500 ppm showed highest values of head rice, 88.44% and 88.03% after 60 days of storage at moisture contents of 12.40% and 14.20%, respectively.
The broken percentage of samples at 12.40% moisture content (w.b.) treated using 500, 1000 and 1500 ppm doze of ozone were found to be 10.83, 11.75 and 12.50 per cent, respectively after 60 days of storage. In case of samples at 14.20% moisture content (w.b.) treated under the same conditions broken percentage was 11.80, 12.23 and 13.62 per cent, respectively. In case of control samples at 12.40% and 14.20% (w.b) percentage broken values were found to be 13.00 and 14.12% respectively on the 60th day.
It was observed from the table that at lower moisture content the milling yield and head rice yield of paddy was higher. Broken percentage was found to decrease with decrease in moisture content. These could be attributed to that at lower moisture content, the hardness of grains increased, leading to more resistance against compressive loading [11].

Decrease in percentage of head rice and increase in percentage of brokens with storage period could be attributed to the biological ageing phenomenon (Trigo-Stockli and Pederson, 1994) [6]. The increase in broken percentage with higher ozone concentrations may be due to the reduced hardness of grains at higher concentrations.

Higher insect infestations can directly cause comparative lower total milling yield and head rice yield of paddy during storage. The higher values of total milling yield and head rice yield in ozone treated samples may be due to reduced infestation. It is reported that grains stored in insecticide incorporated bags had higher milling yields and head rice yields when compared to grains stored in untreated bags [12].

It is also reported that ozone does not alter the milling yield of soft and hard wheat or change its kernel pigments [13]. Ozone treatment in maize also was found to have no effect on the dry and wet milling performance and it gave similar yields as that of control treatment.

From the results it was observed that the grains treated at an ozone concentration of 500 ppm gave maximum milling recovery and head rice recovery. Broken rice percentage was also found to be least when compared to other treatments. Hence, grains treated using ozone at a concentration of 500 ppm irrespective of the moisture content and storage days was found to be most acceptable with regard to its milling qualities.

Table 2: Effect of ozone treatment on milling qualities of paddy at 12.40% moisture content

Treatment	Total milling recovery, %		
	1st day	**30th day**	**60th day**
500 ppm	66.00	65.80	64.90
1000 ppm	65.84	65.71	64.33
1500 ppm	65.75	65.44	64.00
Control	66.10	65.20	63.80
Head Rice Recovery, %			
500 ppm	91.56	89.20	88.44
1000 ppm	91.28	88.41	88.13
1500 ppm	91.25	87.86	86.00
Control	90.77	87.42	85.10
Broken Rice Percentage			
500 ppm	8.18	10.33	10.83
1000 ppm	8.50	11.41	11.75
1500 ppm	8.56	12.02	12.50
Control	8.40	12.11	13.00

Table 3: Effect of ozone treatment on milling qualities of paddy at 14.20% moisture content

Treatment	Total milling recovery, %		
	1st day	**30th day**	**60th day**
500 ppm	65.00	64.71	62.68
1000 ppm	64.94	64.63	62.29
1500 ppm	64.89	64.36	61.64
Control	65.10	64.20	61.60
Head Rice Recovery, %			
500 ppm	91.32	89.02	88.03
1000 ppm	91.16	88.22	87.75
1500 ppm	91.11	87.66	86.21
Control	90.62	87.38	85.86
Broken Rice Percentage			
500 ppm	8.40	10.97	11.80
1000 ppm	8.80	11.71	12.23
1500 ppm	8.84	12.30	13.62
Control	8.83	12.46	14.12

3.3. Optimization of ozone concentration and duration of exposure

From the study it was found that ozone gas at all the three concentrations had the potential to kill stored grain pest. But treatments using higher concentrations (1000 ppm and 1500 ppm) resulted in some adverse effects such as acidic odour, kernel discoloration etc.

Optimal ozone concentration was selected based on maximum milling yield, minimum breakage and preferable cooking attributes. Out of the different treatments maximum milling yield and head rice recovery and minimum kernel breakage was noticed in grains treated using gaseous ozone at a concentration of 500 ppm. With respect to cooking qualities, treatment using ozone gas at 500 ppm gave results that were on par with control samples; but cooking time was slightly higher.

Thus, from the study it was concluded that grains at a moisture content of 12.40% (w.b.) treated using ozone gas at 500 ppm concentration for 540 min was most acceptable with regard to its milling and cooking qualities.

4. Summary and conclusion

Three levels of ozone concentration (500, 1000 and 1500 ppm) and two levels of grain moisture content (12.40% and 14.20% w.b.) was used throughout the study to determine mortality of insects. Trials were conducted using household bins of 15 kg capacity to estimate the mortality rates of *Rhyzopertha Dominica* adults corresponding to different exposure times.

The ozone treated grains were stored in stainless steel household bins at a temperature of 30 ± 2 °C for a period of 60 days. In household bin study, 100 per cent mortality of *Rhyzopertha dominica* adults at 500, 1000 and 1500ppm were achieved at 540, 360 and 270 min, respectively, at 12.40% moisture content (w.b.) of grains. Complete mortality of *Rhyzopertha Dominica* adults placed in paddy grains at 14.20% moisture content (w.b.) was achieved at an exposure time of 610, 390 and 300 min when treated at the same concentrations.

Milling studies of ozone treated grains indicated that the milling qualities improved upon ozone treatment. Total milling recovery and head rice recovery of the treated samples were found to be higher than the control grains and broken rice percentage was found to reduce. Highest milling recovery at the end of 60 days was observed to be 64.90% and 62.68% for grains at a moisture content of 12.40% and 14.20% (w.b.) respectively, when ozone gas at 500 ppm concentration was

used. Paddy grains treated using ozone gas at a concentration of 500 ppm showed highest values of head rice i.e. 88.44% and 88.03% after 60 days of storage at moisture contents of 12.40% and 14.20% (w.b.), respectively. Broken rice percentage of the grains at 12.40% and 14.20% moisture content (w.b.) when treated with ozone gas at the same concentration gave least amount of broken of values 10.30 and 11.80%, respectively.
Out of the different treatments, maximum milling yield and head rice recovery and minimum kernel breakage was noticed in grains treated using gaseous ozone at a concentration of 500ppm.

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