Vulnerability of different life stages of *Sitophilus oryzae* insects in stored rice grain to ozone treatment and its effect on physico-chemical properties in rice grain

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Abstract
Ozone has emerged as a reactive gas with insecticidal activity with the capability to manage insect pests in stored grains. The study focused on the effect of moisture content with exposure time and ozone concentration in achieving mortality of different life stages of *Sitophilus oryzae*. The ozonation process was done with a moisture content (MC) of 12%–16%, ozone concentration of 1.0–2.0 g/m^3^ and exposure time of 2–6 h. The responses studied were percentage mortality of eggs, larvae, pupae, and adults. The ozone effect on rice physico-chemical, spectral FTNIR (Fourier transform near infrared spectrophotometer), and morphological attributes (X-ray micro CT) of optimized samples were compared with control sample. The adult stage of *S. oryzae* was the least vulnerable stage to the ozonation process among eggs, larvae, and pupae, whose mortality ranged from 44.13% to 99.02%. The optimized condition with the desirability of 0.99, MC of 12.31%, ozone of 1.93 g/m^3^, and time of exposure 5.86 h for mortality of different life stages of *S. oryzae* was obtained. The standard error mean and SD were in the range of 2.81–1.91 and 3.67–3.21, respectively. Protein, amylose, and MC of ozone-treated rice had lower values in comparison to fresh rice. Microstructural changes in ozonated samples were distinct with X-ray micro CT images and FTNIR peaks. The loss in the micro structure was attributed to the insect infestation and degradation of protein molecules due to oxidation of the disulphide bonds during ozone treatment.

Keywords
exposure, infestation, mortality, ozone, spectral

1 | INTRODUCTION

Rice is regarded as a staple food in many parts of the world. However, losses due to insect infestation are quite alarming to the rice industry (Biancolillo et al., 2019). The increased resistance of pests to insecticides (Benhalima et al., 2004) demands auxiliary methods which would help in the effective management of pests in rice grains. Thus, to forgo the use of chemicals, one of the prime contenders that are used in...
recent times is ozone, which has turned out to be convincing in terms of efficacy against insect pests in grains (Blancolillo et al., 2019; Gupta, Meghwal, Prabhakar & Garg, 2021; Gupta, Meghwal & Prabhaka, 2021; Srivastava et al., 2021). The mode of action of ozone on insects is still unknown, but some pieces of evidence suggest that the insect’s respiratory system is eventually a target (Li et al., 2015; Tiwari et al., 2010). Highly reactive ozone is a strong oxidizing agent and is classified as "GRAS" (Generally Recognized As Safe) by the United States Environmental Protection Agency (US-EPA). Ozone readily breaks down into oxygen, hence the disposal need is eliminated as compared to hazardous chemicals (Guzel-Seydim et al., 2004; Meghwal et al., 2013). Hence, from the safety point of view, ozone-treated products can be readily consumed (Gau et al., 2005). Ozone has been used to treat a range of food products such as fruits and vegetables, dairy products, cheese, orange and apple juices, and food grains (Tiwari et al., 2010; Tzortzakis et al., 2017; Varga et al., 2016; Zhu, 2018). Nowadays, ozone application has been put forth for grain products as they are produced in large quantities and are considered a staple food (Tiwari et al., 2010; Zhu, 2018).

A few studies have reported the effect of ozone treatment on grain insect mortality (Isikber et al., 2009, 2015; Kells et al., 2001; Leesch, 2003). The effect of ozone on the respiration pattern of Sitophilus zeamais was studied by Sousa et al. (2017) who found that 50 ppm of ozone leads to 1.09-fold in mortality. Subramanyam et al. (2017) studied R. dominica mortality in wheat with ozone treatment (0.42 and 0.84 g/m³) and found 99% mortality of adults life stage can be achieved. Moreover, Trombete et al. (2017) established the effect of ozone on the chemical properties, minerals, and rheological property of wheat at ozone concentration (60 mg/l) and found that no significant structural alteration was there with ozone.

Looking forward, each of the studies conducted so far has not elaborated on the mortality rate of Sitophilus oryzae in different life stages in rice concerning ozone treatment and the time of exposure. So, the study was undertaken to demonstrate and outstrip the relationship between the mortality of S. oryzae different life stages with ozone concentration, exposure time, and moisture content of the rice grain. Thus, an objective was set to achieve maximum mortality of eggs, larvae, pupae, and adult life stages of S. oryzae.

2  |  MATERIAL AND METHODS

2.1 | Sample procurement and composition

Freshly harvested rice grain of variety ‘Swarna’ was procured from the farm of Agricultural & Food Engineering Department, Indian Institute of Technology, Kharagpur and cleaned by a pneumatic separator to remove any presence of foreign particles. The rice grains were exposed to a temperature of − 20°C for at least one week so as to obliterate any chances of insects before the start of the experiment. Initial moisture content (MC) was determined through the oven drying method as per the AOAC standards on a wet basis (wb). The rice grain was conditioned to 12%, 14%, and 16% (wb) MC by adding the required amount of water. MC of the conditioned rice grain samples was verified after 24 h of addition of the required amount of water (Srivastava et al., 2018a, 2018b, 2019a, 2019b).

2.2 | S. Oryzae insect rearing

Homogeneous insect population of variety S. oryzae was collected from the Entomology Department of GB Pant University, Pantnagar, India. S. oryzae insects were reared in an 8 kg rice container at the rate of 10 S. oryzae adults per kg and stored in an incubator maintained at 26 ± 1°C and 70 ± 5% relative humidity (RH) for 45 days. S. oryzae adults were placed on the rearing media and allowed to lay eggs. The eggs were separated from adults by using 100 number sieve (0.149 mm ASTM-11). Further, larvae and pupae were obtained by sieving the bulk rice culture with five number sieves (4.00 mm ASTM E-11). After collection of adults, larvae, and pupae, 100 each were reared on 1 kg rice placed in the glass jar in which ozone treatment was to be carried out (Mishra et al., 2019; Srivastava et al., 2018a, 2018b, 2019a, 2019b).

2.3 | Ozone treatment

Gaseous ozone with a purity of about 99.5% was triggered by means of a corona discharge ozone generator (QZ-AIR(I), ISM 5 Series, Creative QZ-AIR Pvt. Ltd. Noida, Uttar Pradesh, India) with a maximum ozone production capacity of 5 g/h (Mishra et al., 2019) by using compressed oxygen gas (O2) as the input in the ozone generator (dimensions: 600 × 700 × 250 mm). The ozone generating module had cooling fans that bring fresh air from one side and throw the heated air from the other side, which helped in keeping the modules under normal temperature and ensured efficient operation of the ozone generator. The ozone flow was regulated using a flow meter. The ozone dose was computed by multiplication of treatment time of ozone with concentration and divided by the volume of the chamber. Generated ozone through an ozone generator was introduced into an acrylic cylindrical chamber (12 cm × 28 cm) containing 1 kg of infested rice (Figure 1). Care was taken to introduce ozone from the bottom of a chamber so as to have a uniform distribution of ozone inside the chamber. The outlet pipe from the cylindrical chamber was positioned on top and was kept airtight during the entire ozone treatment process. When one set of ozone exposure duration was finished, then the valve present in the outlet pipe was opened and bubbled in the water tank so as to neutralize the ozone traces. The rice grains with S. oryzae insects were positioned over the perforated floor inside the chamber and were treated with different ozone concentration and exposure time as per the experimental design.

2.4 | Design of experiment and its optimization

The mortality of different life stages of S. oryzae by ozone treatment was done using a three-level factorial design and three factor with
five center points. Response surface methodology was used for the optimization process using Design Expert 9.0 (StatEase) software taking into account three process parameters, namely moisture content (12%-16%) on a wet basis, the concentration of ozone (1.0-2.0 g/m³; 1 g/m³ ozone in the air by volume = 467 ppm), and exposure time (2–6 h). The response variables were percentage mortality of eggs, larvae, pupae, and adults. The regression models were developed for the optimization process from the factorial design for the response variables that had a lack of fit non-significant (p > 0.05). The quadratic model was thus selected for the optimization process as it best fitted (Table S1). The optimized regression model was validated by point prediction values at optimized conditions. The effect of ozone treatment (ozonation) on rice physico-chemical, spectral, and morphological attributes of the optimized ozone-treated rice grain was compared with the control sample.

2.5 | Physico-chemical attributes

The control and optimized ozone-treated rice samples were evaluated for proximate analysis by following standard AOAC protocols. Minerals such as iron (Fe), sodium (Na), copper (Cu), magnesium (Mg), manganese (Mn), potassium (K), calcium (Ca), zinc (Zn), and selenium (Se) were done by a transversely heated graphite atomic absorption spectrometer (AAAnalyst™ 700, Perkin-Elmer, Inc., Shelton, USA). Prior to analysis, the samples of rice were digested with triacid (sulphuric, nitric, and perchloric acid) in the ratio of 1:3:2 in an automated Kjeldahl digester (Gerhardt Vapodest 20 GmbH & Co. KG, Germany) until the solution was colorless. After digestion, the solution volume was made up to 100 ml and filtered through a Whatman filter paper no. 2 to remove the residues. The filtrates were taken for mineral composition analysis. Thousand kernel weight of rice grain were counted using an automated counter machine. The amylose content was measured by following colorimetric protocol at a wavelength of 590 nm with iodine as a reagent, which produces a blue color complex with amylose. The hardness of ozone-treated rice and control samples was done through a texture analyzer (Model TA-XT2i, Stable Microsystems, UK). The change in color in terms of L*, a*, and b* was measured by bench top spectrophotometer (Model CM-5, Konica Minolta Sensing, Tokyo).

2.6 | Spectral attributes by FTNIR

The ozone treatment effect on functional bonds present in the rice grain during disinfection was done by Fourier transform near infrared spectrophotometer (FTNIR; Bruker Optics™, Germany) and Opus 5.5 software. The spectra (absorbance) were recorded in wavenumber range 4000 to 1200 cm⁻¹ by the PbS detector by taking an average of 130 scans for each sample (Srivastava et al., 2019a, 2019b). A total of 45 spectral samples of ozone-treated rice were taken, which were further averaged using the spectral signature bands in Opus software. Thus, averaged spectra were taken for the ozone-treated sample with respect to the control samples of rice grains.

2.7 | Microstructural attributes

The effect of ozone on the microstructure was studied by X-ray micro computed tomography (CT) machine ‘Phoneix V| tome | xm’ (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) machine at a magnification of 24x (Srivastava et al., 2020). The voltage and current was kept at 80 kV, 100 μA, respectively. A total of 500 slices of each image in 100 ms were acquired via X-ray micro CT and analyzed by VG Studio Max 2.2 software (Schoeman et al., 2016).

3 | RESULTS AND DISCUSSION

In the current study, the vulnerability of different life stages of S. oryzae due to ozone treatment mostly had an effect on the moisture (% wb),

FIGURE 1  Schematic diagram for ozone treatment of S. oryzae infested rice grains (Mishra et al., 2019)
The mechanism of ozonation in the rice grain involves the reaction of the ozone (O3) molecule with the rice grain mass where it decomposes rapidly and moves through the entire rice grain mass making it saturated. Later, if any trace amount is left after it reaches a saturation point in the entire grain mass, it readily decomposes in the water tank attached for the O3 decomposition (Figure 1). The optimization of the ozone treatment was done via the quadratic model as it was chosen on the basis of the highest order polynomial where the additional terms were significant and the model was not aliased. The quadratic model ANOVA regression analysis in terms of the sum of squares, F-value, and p-value for different life stages of S. oryzae insect disinestation by ozone treatment is shown in Table 1. The regression models developed for the efficacy of mortality of different life stages of S. oryzae were found to be best fit with \( R^2 \) values 0.979, 0.978, 0.977, and 0.973 for eggs, larvae, pupae, and adults, respectively. The model F-value of 241.82 implicates that the quadratic model is significant and only 0.01% chances are there that this large F-value might be due to noise. P-values less than 0.05 indicate model terms were also significant. In this case, A, B, C, AB, and AC are significant model terms. The validation of the developed regression model by point prediction values for mortality of different life stages of S. oryzae in response to optimized ozone treatment is shown in Table 3. The developed equation in terms of actual factors (eggs, larvae, pupae, and adults) can be used to make predictions about the response (mortality) for given levels of each factor (ozone conc., time of exposure, and moisture content). The effect on mortality (%) of S. oryzae eggs, larvae, pupae, and adults with ozone concentration (g/m³) and moisture content (%) in stored rice grains is shown in Figures 2–5, respectively.
### TABLE 2
Regression equations developed for the efficacy of mortality of different life stages of *S. oryzae* in response to optimized ozone treatment

| Mortality (%) | Regression equations | R² | Adjusted R² | CV (%) | S.D. |
|---------------|----------------------|----|-------------|--------|------|
| Eggs          | +89.83 −22.67A + 6.098 + 2.87C + 3.37AB + 1.49AC − 0.8687BC − 13.37 A² − 1.14 B² + 0.4078 C² | 0.979 | 0.975 | 4.09 | 3.21 |
| Larvae        | +89.51 −22.97A + 6.18B + 3.10C + 3.17AB + 1.35AC − 0.7166 BC − 12.85 A² − 1.53 B² − 0.0816 C² | 0.978 | 0.976 | 4.11 | 3.20 |
| Pupae         | +89.22 −23.04 A + 6.27 B + 3.31 C + 3.04 AB + 1.26 AC − 0.5305 BC − 12.43 A² − 1.64 B² − 0.5779 C² | 0.977 | 0.974 | 4.25 | 3.31 |
| Adults        | +88.35 −23.74 A + 6.44 B + 3.28 C + 2.76 AB + 1.27 AC − 0.6686 BC − 12.20 A² − 1.74 B² − 0.6548 C² | 0.973 | 0.969 | 4.78 | 3.67 |

*A* is Moisture content (%), *B* is Ozone conc. (g/m³), and *C* is Time of Exposure; *CV* is Coefficient of variation; *SD* is Standard deviation.

### TABLE 3
Validation of the developed regression model by point prediction values for mortality of different life stages of *S. oryzae* in response to optimized ozone treatment

| Mortality (%) | Predicted mean | 95% CI low for mean | 95% CI high for mean | SE mean | S.D. |
|---------------|----------------|---------------------|----------------------|---------|------|
| Eggs          | 101.63         | 97.80               | 105.46               | 1.91    | 3.21 |
| Larvae        | 102.36         | 98.41               | 106.30               | 1.97    | 3.30 |
| Pupae         | 102.05         | 98.22               | 105.88               | 1.91    | 3.21 |
| Adults        | 102.49         | 98.12               | 106.87               | 2.18    | 3.67 |

*SE* is Standard error mean; *CI* is Confidence interval; *SD* is Standard deviation.

### TABLE 4
Comparison of physico-chemical properties of control and optimized ozone-treated rice grains

| Parameters                  | Control rice grain | Optimized ozone treated rice grain |
|-----------------------------|--------------------|-----------------------------------|
| Protein (%)                 | 4.89 ± 0.03        | 3.87 ± 0.09                       |
| Moisture (%)                | 12.05 ± 0.09       | 10.75 ± 0.31                      |
| Fat (%)                     | 0.44 ± 0.14        | 0.49 ± 0.23                       |
| Ash (%)                     | 0.67 ± 0.09        | 0.72 ± 0.09                       |
| Fiber (%)                   | 1.33 ± 0.11        | 1.11 ± 0.11                       |
| Carbohydrate (%)            | 79.84 ± 0.08       | 79.99 ± 0.05                      |
| Amylose content (%)         | 15.27 ± 0.01       | 12.64 ± 0.11                      |
| Color                       |                    |                                   |
| L*                          | 76.33              | 79.11                             |
| a*                          | 1.48               | 1.47                              |
| b*                          | 23.28              | 23.31                             |
| Hardness (kg)               | 13.89 ± 0.01       | 14.25 ± 0.09                      |
| 1000 kernel weight          | 10.45 ± 0.21       | 9.21 ± 0.08                       |
| Minerals (mg/kg)            |                    |                                   |
| Iron (Fe)                   | 15.33 ± 0.06       | 15.11 ± 0.09                      |
| Sodium (Na)                 | 66.21 ± 0.13       | 65.35 ± 0.17                      |
| Copper (Cu)                 | 5.06 ± 0.17        | 3.79 ± 0.08                       |
| Magnesium (Mg)              | 153.78 ± 0.08      | 153.34 ± 0.11                     |
| Manganese (Mn)              | 76.56 ± 0.15       | 75.46 ± 0.22                      |
| Potassium (K)               | 268.21 ± 0.09      | 268.33 ± 0.11                     |
| Calcium (Ca)                | 84.79 ± 0.12       | 82.56 ± 0.21                      |
| Zinc (Zn)                   | 13.21 ± 0.10       | 12.45 ± 0.09                      |
| Selenium (Se)               | 0.29 ± 0.21        | 0.23 ± 0.14                       |

Values are mean ± standard deviation of three replicates on a dry weight basis.
**FIGURE 2**  Effect on mortality (%) of *S. oryzae* eggs with (a) ozone concentration (g/m³) and moisture content (%) (b) time of exposure (h) and moisture content (%) in stored rice grains
**FIGURE 3** Effect on mortality (%) of *S. oryzae* larvae with (a) ozone concentration (g/m³) and moisture content (%) (b) time of exposure (h) and moisture content (%) in stored rice grain
FIGURE 4 Effect on mortality (%) of S. oryzae pupae with (a) ozone concentration (g/m³) and moisture content (%) (b) time of exposure (h) and moisture content (%) in stored rice grains
FIGURE 5  Effect on mortality (%) of \textit{S. oryzae} adults with (a) ozone concentration (g/m$^3$) and moisture content (%) (b) time of exposure (h) and moisture content (%) in stored rice grains
3.1 Vulnerability of *S. oryzae* eggs with the process parameters

Eggs of *S. oryzae* were the most vulnerable stage to the ozonation process, whose mortality ranged from 46.55% to 99.89%. Figure 2a shows the effect on mortality (%) of *S. oryzae* eggs with ozone concentration (g/m³) and moisture content (%). When the moisture content was increased from 12% to 16%, the efficacy in terms of egg mortality decreased even if the concentration of ozone was increased (Mishra et al., 2019; Shah et al., 2015). One of the prime reasons for the significant negative effect of moisture on the *S. oryzae* eggs mortality can be explained by the fact that the grain with higher moisture content takes a longer time to get saturated with O₃, which obviously decreases the half-life period in comparison to rice grains treated with lower moisture content. Moreover, once the diffusion of O₃ in the rice grain occurs and it gets saturated, it would require more time for the fumigation leading to higher saturation time. Hence, more the saturation time less would be the mortality rate of eggs for a particular time interval. Similar findings were reported by other researchers as well that with an increase in moisture, the effectiveness of the ozone process ceases (Shah et al., 2015). Figure 2b shows the effect on mortality (%) of *S. oryzae* eggs with the time of exposure (h), and moisture content (%) in stored rice grains. The exposure time had a significant effect on *S. oryzae* eggs mortality in comparison to other life stages (larvae, pupae, and adults) of *S. oryzae*, that is, higher mortality rate was achieved in less time and ozone concentration in comparison to other life stages. This may be attributed to the fact that eggs of *S. oryzae* form clusters on the rice grain surface and thus are more exposed to the O₃ molecule. The regression equation developed for the efficacy of mortality of eggs in response to optimized ozone treatment has an $R^2$ value of 0.979, coefficient of variation (CV) of 4.09, and standard deviation (SD) of 3.21, respectively, indicating that the developed model can easily predict the responses in terms of ozonation process quite effectively (Table 2).

3.2 Vulnerability of *S. oryzae* larvae with the process parameters

Larvae of *S. oryzae* were the second most vulnerable stage to the ozonation process, whose mortality ranged from 45.82% to 99.77%. Figure 3a shows the effect on mortality (%) of *S. oryzae* larvae with ozone concentration (g/m³) and moisture content (%). Figure 3b shows the effect on mortality (%) of *S. oryzae* larvae with the time of exposure (h), and moisture content (%) in stored rice grains. The mortality rate with respect to the egg stage of *S. oryzae* was a bit lower and with an increase in moisture content, higher O₃ concentration and time were required. One of the probable reasons for the aforesaid effect is based on the fact that when the moisture content is more than O₃, it is readily converted to O₂. Thus, the amount of O₃ available for larvae is lowered. Moreover, as larvae spend most of their time inside the rice grain and carry out their metabolic activity by chewing the grain by their mandibulate mouthparts, they require a higher time of exposure and O₃ concentration for reaching out the same mortality rate as required by the eggs. The regression equation developed for the efficacy of mortality of larvae in response to optimized ozone treatment had an $R^2$ value of 0.978, CV of 4.11, and SD of 3.20, respectively, indicating that the developed model can easily predict the responses in terms of the ozonation process quite effectively (Table 2).

3.3 Vulnerability of *S. oryzae* pupae with the process parameters

Pupae of *S. oryzae* were the third most vulnerable stage to the ozonation process, whose mortality ranged from 45.78% to 99.57%. Figure 4a shows the effect on mortality (%) of *S. oryzae* pupae with ozone concentration (g/m³) and moisture content (%). Figure 4b shows the effect on mortality (%) of *S. oryzae* pupae with the time of exposure...
(h) and moisture content (%) in stored rice grains. As per the observations discussed above, a similar trend was followed also by the pupae stage of *S. oryzae*, that is, with an increase in moisture content, the efficiency of the ozonation process for achieving the mortality rate was decreased. Thus, moisture content plays an effective role in regulating the mortality rate of the pupae stage of *S. oryzae*. When the moisture content was increased from 12% to 16%, the mortality rate decreased due to the depletion of O$_3$. In an aqueous solution, O$_3$ reacts with dissolved substances, leading to the formation of secondary oxidants (–OH radicals), which further react with solutes to form a chain reaction resulting in depletion of O$_3$. The regression equation developed for the efficacy of mortality of pupae in response to optimized ozone treatment has an $R^2$ value of 0.974, CV of 4.25, and SD of 3.31, respectively, indicating that the developed model can easily predict the responses in terms of the ozonation process quite effectively (Table 2).
**3.4 | Vulnerability of *S. oryzae* adults with the process parameters**

The adult stage of *S. oryzae* was the least vulnerable stage to the ozonation process among eggs, larvae, and pupae, whose mortality ranged from 44.13% to 99.02%. Figure 5a shows the effect on mortality (%) of *S. oryzae* adults with ozone concentration (g/m$^3$) and moisture content (%). Figure 5b shows the effect on mortality (%) of *S. oryzae* adults with the time of exposure (h) and moisture content (%) in stored rice grains. Moisture content had a significant negative effect on the mortality of adults. Thus, the effectiveness of ozone decreased, when the stored rice that was to be treated was of higher moisture content. Adult *S. oryzae* have the abdominal spiracles, also covered by the coadunate elytra (King, 2014; Plarre, 2013) which help them escape from the O$_3$ treatment for several hours as they can close their spiracles and remain alive inside the rice grain. Thus, a higher amount of O$_3$ and exposure time was required to achieve a 99.02% of mortality rate. The regression equation developed for the efficacy of mortality of adults in response to optimized ozone treatment has an $R^2$ value of 0.969, CV of 4.78, and SD of 3.67, respectively, indicating that the developed model can easily predict the responses in terms of the ozonation process quite effectively (Table 2).

**3.5 | Optimization of the ozone disinfection procedure**

The results obtained from the data analysis were used to develop an appropriate amalgam of ozone concentration (g/m$^3$), moisture content (%), and exposure time (h) for the treatment of infested rice grain. The validation of the developed regression model by point prediction values for mortality of different life stages of *S. oryzae* in response to optimized ozone treatment is shown in Table 3. The optimization of the O$_3$ treatment was done by targeting *S. oryzae* adult mortality to 99% and maximizing the mortality of eggs, larvae, and pupae. The software thus found 88 solutions of which the best solution was with desirability of 0.99, moisture content of 12.31% (wb), ozone concentration of 1.93 g/m$^3$, and time of exposure 5.86 h for mortality of 101.638% (eggs), 102.36% (pupae), 102.059% (larvae), and 102.499% (adults) different life stages of *S. oryzae*, respectively. The standard error mean and SD were in the range of 2.81–1.91 and 3.67–3.21, respectively. The regression models over predicted the responses, but the residuals values below 5% (Table 3) suggest that the developed regression models can be used to predict the responses of the ozonation process.

**3.6 | Evaluation of physico-chemical attributes of rice grain for the ozonation process**

The physico-chemical attributes of ozone treated optimized rice grain samples were recorded and contrasted with untreated rice grain (control) samples for the change in physico-chemical attributes which may alter the quality of the rice grain for its end use. Comparative evaluation of physico-chemical properties of control and optimized ozone-treated rice grains in Table 4. The moisture content (% wb) of the optimized ozone treated rice grain was lowered in comparison to untreated rice grain (control) samples which might be due to the oxidation reaction caused by O$_3$, leading to the removal of surface moisture content. The protein (%) content also decreased somewhat for the optimized ozone treated rice grain samples because of the effect of prolonged exposure to O$_2$. A decrease in protein content might be attributed to the fact that the oxidation reaction caused by O$_3$ results in loss of gluten structure which is responsible for the protein unit building (Bonjour et al., 2011; Sánchez-Maríñez et al., 1997; Sanchez, 2004). The values of ash, fat, carbohydrate, and fiber (%) were not altered for the optimized ozone treated rice grains to untreated rice grain (control) samples. The 1000 kernel weight and hardness (kg) optimized ozone treated rice grain to untreated rice grain (control) samples were increased due to surface moisture removal during the O$_3$ treatment process, thereby making the rice grain harder in nature. The amylose content was seen to decrease for the optimized ozone treated rice grain (Lee et al., 2017; Mei et al., 2016). This might be probably due to the degradation of starch molecules as a result of oxidation and radical reactions occurring because of O$_3$ treatment (Barrera et al., 2007). The ozonation process also affected the color of the rice grain, that is, it increased the lightness (L’) of the optimized ozone-treated rice grain due to the oxidation process while the a’ and b’ values were not affected. Similar findings were reported by other researchers (Marston et al., 2015; Qi et al., 2016; Wang et al., 2016) on other grains such as wheat, maize, and sorghum. Other mineral composition (mg/kg) was also not affected except selenium and copper, which might be due to the oxidation process during the ozonation process.

**3.7 | Evaluation of spectral attributes of rice grain for the ozonation process**

Comparison of ozonated and fresh rice grain spectral data generated by FTNIR is shown in Figure 6. The spectral attributes were evaluated to identify the chemical changes that might have occurred due to the ozonation process. The identification of the same was done via examining the intensity of the peak and position. The water (H$_2$O) stretching and bend bond (–OH) in fresh rice was observed at 6518 cm$^{-1}$ and 4549 cm$^{-1}$, respectively (Srivastava et al., 2018a, 2018b). In ozone-treated rice, the water (H$_2$O) stretching and bend bond (–OH) were a bit lower in position and observed at 5395 cm$^{-1}$ and 4512 cm$^{-1}$, respectively due to moisture removal during the ozone treatment. The intermolecular hydrogen bond due to N–H stretching of protein was found at 6892 cm$^{-1}$ (fresh rice) and was lower in intensity for ozone-treated rice (5911 cm$^{-1}$) due to oxidation reaction caused by O$_3$ (Bonjour et al., 2011; Sánchez-Maríñez et al., 1997). The starch molecule stretch pertaining to C–H stretch and C = O stretch was found higher for fresh rice (6914 cm$^{-1}$) than for the ozonated rice (5175 cm$^{-1}$). The wavenumbers of 7125, 7540, and 8359 cm$^{-1}$ were assigned to N–H stretching for amino acids and amide bonds, respectively, for fresh rice, while wavenumbers of 7033, 7291, and 8184 cm$^{-1}$ were assigned to
the ozone treated rice, respectively. A decrease in the values of protein, moisture, and starch for ozone-treated rice can possibly be due to the oxidation of protein molecules with ozone. In brief, O3 oxidizes the (OH) hydroxyl group of starch to carboxyl and carbonyl groups. The oxidation may occur because of cross-linking or degradation of starch molecules (Castanha et al., 2017, 2019; Klein et al., 2014).

3.8 | Evaluation of microstructural attributes of rice grain for the ozonation process

Micro computed tomography view of fresh rice grain and ozonated rice grain is shown in Figure 7. The defect volume (µm³) in fresh rice is almost negligible while the optimized ozone treatment with an ozone concentration of 1.93 g/m³, and exposure time 5 h gave somewhat defect volume (µm³). As ozone diffused inside the grain, it can be seen in terms of color coordinates (blue to greenish yellow) inside the grain in Figure 7b. The loss in the micro structure can be attributed to the insect infestation and degradation of protein molecules due to oxidation of the disulphide bonds by the ozone treatment (Zhu, 2018).

4 | CONCLUSIONS

Ozone treatment is an effective method that can be helpful in disinfestation of rice grains for all the life stages of S. oryzae. Eggs of S. oryzae were the most vulnerable stage to the ozonation process, whose mortality ranged from 46.55% to 99.89% followed by larvae, pupae, and adults. The adult stage was the least vulnerable stage to the ozonation process whose mortality ranged from 44.13% to 99.02%. The optimized condition for ozonation of rice included an ozone concentration of 1.93 g/m³, moisture content of 12.5% (wb), and exposure time of 5 h. The physico-chemical attributes of optimized ozone treated rice were recorded and compared with untreated rice grain (control) samples for the change in physico-chemical attributes. These physico-chemical attributes are important parameters that determine the rice quality and thereby help in determining which quality was altered more in rice and whether it is suitable for its end use or not. Effect on moisture content was prominent after ozone treatment that gave hardness to the texture of the rice grain. Some effects were also observed on protein and amylose content due to the oxidation reaction of ozone. Ozone is considered an eco-friendly, cost effective, strong oxidant that has different applications in food including food grains. Thus, in the near future ozone can be used in silos and bins to combat the infestation of rice and other cereal grains. Moreover, the findings of the study infer that ozone can be an appropriate method to disinfect grains at a larger scale and that too effectively. Although some morphological and spectral changes were reported but that too can be regulated if proper measures are taken while giving the ozone treatment. Therefore, it becomes evident that ozone can improve the functionalities of grains and their related products while maintaining food safety.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

SS and HNM conceived and designed research. SS conducted experiments. GM contributed new reagents and/or analytical tools. SS analyzed data and wrote the manuscript. All authors read and approved the manuscript.

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