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Research Article

Influence of Ozone Treatment on Wheat (*Triticum aestivum*) Germination During Bulk Storage

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Abstract

The current experiment was conducted to understand gaseous ozone exposure time and cycle effect on wheat seed germination during storage. The bulk storage of wheat grain has a major issue of insects and pests which is controlled by gaseous ozone treatment in closed storage system. Ozone is the acceptable and economically viable technique for treating grains during storage for its residue-free and environment-friendly nature. In this research article, the ozone gas treatment given to the wheat grain during bulk storage and evaluated its influence on seed germination. A pilot-scale ozone disinfestation system for wheat grains was developed. The two-factorial experimental design on the influence of the parameters of the technological process of ozone treatment on the germination qualities of wheat seeds was carried out. Wheat grains were treated by gaseous ozone with various time durations (0 min, 30 min, 60 min, 90 min and 120 min) and at various frequency cycles (7 days, 14 days, 21 days). The experiment has recorded observation on the effect of ozone treatment time and the ozone frequency cycle on the germination percentage of wheat grain. The data demonstrated that ozone used in bulk storage enhances seed germination percentage. On the other hand, excess ozone can also cause some negative effects on germination. This study provided new insights into how stored wheat grain responds to ozone treatment and highlighted the impact of treatment time durations and frequency cycle for seed germination.

Keywords: Ozone, wheat, germination, bulk storage, ozone exposure time, ozone cycle.

1. Introduction

The storage of grains plays an important role in Indian economy. The storage of grains is practiced from the age of the start of civilization.Wheat is the second most staple food crop of India after Rice. Wheat contributes nearly one-third of the total food grain production(Ref?). India holds the second rank among the wheat growing countries of the world. As per GAIN Report USDA, India is heading for third consecutive record wheat harvest with marketing year (MY) 2019/20 (April/March) production forecast at 100 million metric tons (MMT). The largestwheat growing states in India are Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan, Bihar and Gujarat. Maintaining threshold temperature, proper humidity within the storage environment are the important problems faced in the bulk storage structure. The primeobjective of bulk storage is to prevent stored grain from deterioration of quality. Insects consume large amounts of stored grain and cause other deteriorating factors that will affect the economic value of the stored grain. Due to the infestation of insects and pests, areduction in germination percentage of wheat grain occurs. The risingworld population necessarily increases the food requirement. At the identical time, agricultural land has been decreased drastically because of rapid urbanization and industrialization which severely affects the land availability for the growers. However, the enhancement of seed germination has the potential to secure the food safety of masses by improving crop production. The traditionally followed chemical methods to boost seed germination have major limitations including being environmentally unhealthy, time-consuming, and are labour-intensive.

Ozone is a strong oxidant that has been widely utilized in gas form and mixed with water as a decontaminant in the food and beverages, medical and water supply industries. Ozone is used as an alternative for pest control and reduction of microorganisms including mold and mycotoxin in stored grain (Mendez *et al.*, 2003). Artificially produced ozone can decompose rapidly therefore it cannot be accumulated or transported. Thus it should be continuous generated (Miller *et al.,* 1978). Ozone may easily generated onsite where it's required, by either corona discharge (CD), ultraviolet (UV) or electrolysis of water (Kim *et al.*, 1999). Chemical insecticides offer more production and germination compare to control treatment (Kumar *et al.*, 2021) but have many side effects after consuming. Ozone offers several safety advantages over chemical pesticides. There are neither any repositories of toxic chemicals, nor risks of residual pesticides from chemical mixing or disposal, additionally no packaging waste being produced. Ozone has ashort half-life so that it reverts to naturally occurring oxygen and not leaving any residue on the product. The main objective of this study was to determine the effect of ozone on the germination qualities of ozone treated bulk stored wheat. The seed coating of insecticide offers better result during storage and give 92% germination in wheat after 14 month storage(Chaturvedi *et al.*, 2021). But the chemical insecticides have their own disadvantages tothe human body. So the ozone is preferred to prevent losses during the storage of wheat grains.

Ozone is used in agricultural practices as a germination enhancer and strong antimicrobial agent. Ozone, in limited quantity, enhances seed germination rate. Sudhakar *et al.* (2011) reported that the injection of low doses of O_3 in tomato seeds (*Lycopersicon esculentum*) increases the germination rate and produced seedlings with longer roots. The reason behind good root growth in ozone treated seedlings could be attributed to the increased production of jasmonic acid (Violleau*et al.* 2008).Mason (2006) has worked on controlling stored grain insects with ozone fumigation and they measured insect mortality and seed germination of corn. They concluded that seed germination isnot affected by continuous and repeated treatment with ozone.

Limited research has been performed in the field of ozone application in seeds for detecting germination change. Savi*et al.* (2014) worked with 40, 60, 90, and 120 min to O_3 gas exposure time in wheat storage. They also recorded that up to 120 min exposure did not affect the quality and seed germination. They also included that the O_3 treatment affected wheat germination after 180 min of exposure and reduced up to 12.5%. The increased temperatures during storage caused lower or no germination. (Freeman, 1980). The ozone gas exposure time increased the seed germinationin alpine plants (Abeli*et al.*, 2016). The O_3 may stimulate DNA-repair mechanisms and antioxidant activity or dormancy breaking effects in hydrated seeds. The applicationof ozone in enhancing the germination of winter wheat seeds revealed that besides improving the germination, the germinating energy of the seeds are also enhanced (Avdeeva *et al.*2018).

The higher degree of inactivation of microorganism is done with ozone at higher temperatures but reduction in germination percentage in room storage due to high temperature and humidity.(Farooq *et al.*, 1977). High relative humidity and temperature negatively correlate with germination percentage.The sorghum seed germination percentage is 100 % after 6 months at -5°C temperature when 25-30°C storage temperature decrease the germination percentage up to 37%.The higher grain moisture and temperature negatively correlate with grain germination percentage as observed by many researchers (Owolade*et al.*, 2011). O_3 gas causes degradation of chemical constituents present in the grains. Seed germination loss may occur from excessive use of O_3 gas. (Tiwari *et al.,* 2010).

Gaseous ozone treatment in barley had not significant effect on germination energy up to 30-min ozone exposure time.The largest decreases of 61% to 53% in germination with increased exposure time after 30 min. (Kottapalli*et* *al.*, 2004). The reduction in germination in barley grain which were heavily infected with *F. graminearum* and 96% germination percentage detected in barley (Beattie *et al.*, 1998).From their result it could be proven that the response of ozone and fungal infection was responsible for causing reduced germination energy. Violleau*et al.* (2007) worked on ozone treatment (20 g/m^3) on corn seed during 6.8 or 20.5 minutes. Germination tests were started immediately or 48h after treatment. They resulted that ozone treated seeds have higher germination percentage than untreated one. They also observed faster start of germination for treated samples than untreated one. This early germination start led to have more germinated seeds with large root in treated samples than untreated at 4 and 5 days. The ozone is a strong antimicrobial agent as well as a germination enhancer.Ozone can enhances seed germination rate if used in limited quantity (Pandiselvam*et al.*, 2020).Chattha *et al.* (2015) worked on wheat grain storage and its quality changed during storage in a ferrocement bin. They resulted that storage duration is directly influenced to the germination percentage of wheat. They got germination percentage of wheat grain decreased up to 65% with respect to the storage duration.

2. Materials and Methods

The freshly harvested cleaned wheat grains (GW 496) were collected from Junagadh, Gujarat, India. The unwanted materials present in wheat grains, if any, were removed. The samples hadan initial moisture content of 10.40% (wb). The moisture content was determined using the AOAC-935.29 method (AOAC-935.29).

In the bulk storage, the wheat grain samples (20 kg) were filled in bins and subjected to different ozone exposure time (30, 60, 90, 120 min) and ozone frequency cycle (7, 14, 21 days). The conditions, at which maximum mortality was achieved, were considered for the final treatment.

The wheat seed germination was measured using method given by the International Seed Testing Association (1985). Ozone treated and control sampleswere collected monthly to measure germination percentage under laboratory conditions for the period of 30 days (August-2021) to 120 days (November-21) of storage. The standard method stipulates that in seed control laboratories, the germination ability of wheat is observed at a constant temperature of +20 °C. For detecting germination, germination sheet roll method was used in the laboratory.Dry seeds (100 pieces in each batch) were laid out on a film covered with a layer of germination paper. The paper was moistened with water. Seeds were placed at a distance of 5 cm from the highest edge and 1 cm from one another. It was covered with the same roll of germination paper and formed into a roll. The seed roll was placed vertically in a chamber during germination which was then moistened completely with water. The seeds germinated in a ventilated chamber at a constant temperature of 20-25 °C. The seeds were inspected on the sixth day. Rotten seeds and seeds that gave abnormal shoots were removed. Well germinated seedlings were calculated out of 100 seed.

$$\text{Germination (\%)} = \frac{\text{seeds germinated}}{\text{total seeds}} \times 100$$

Fig. 1 Germination of ozone treated and untreated wheat grains after 7 days

2.1 Statistical analysis

For determination of accurate effect of ozone on seeds, two factorial experiment was established. All observation of germination percentage were analyzed using analysis of variance (ANOVA). The main results were expressed in terms of mean standard deviation and values of $p < 0.05$ were considered statistically significant.

3. Results and Discussion

In laboratory of CAET, Junagadh Agricultural University, Junagadh, an experiment was set to identify the effect of various time of exposures of ozone and frequency cycle of ozone on bulk storage of wheat. Wheat seeds were planted and germinated in accordance with requirements for determining the germination qualities of the seed. In addition to treated seeds, control seeds were germinated.

Table 1: Influence of ozone on seed germination of wheat (%)

	Storage period (Germination %)			
	30 days	**60 days**	**90 days**	**120 days**
Control	99.5	96.5	94.5	93.5
Exposure time (T)				
30 min	98.167	98.667	97.5	96.667
60 min	99.167	99.5	98.5	97.33
90 min	97.667	98.333	97.167	96.5
120 min	97.5	98.167	96.667	95.667
CD	0.867	0.636	0.861	0.861
SEM	0.276	0.204	0.276	0.276
Ozone Cycle (C)				
7 days	97.375	98	97	96.375
14 days	98.125	98.75	97.125	95.875
21 days	98.875	99.25	98.25	97.375
CD	0.746	0.551	0.746	0.746
SEM	0.239	0.177	0.239	0.239
T*C				
CD	N/A	N/A	N/A	N/A
SEM	0.479	0.354	0.479	0.479
C.V. %	0.68	0.506	0.694	0.701

The result of the experiment on the effect of ozone gas on germination percentage of wheat seeds of *GW 496*are presented in Table 1.There was a significant difference ($p < 0.05$) in germination percentage of untreated wheat grains and ozone treated samples (Table 1). A decrease from99.5% to 93.5% in germination percentage was observed for control sample for the storage period of 30 days to 120 days whereas decrease from 99.17 to 95.67 % in germination percentage was observed in ozone treated sample for the storage period of 30 days to 120 days. Avdeeva*et al.*, (2018) found similar trend in storage of wheat and they recommended ozone treatment.A higher rate of loss in germination content of control sample may be attributed to the hidden infestation in addition to grains own metabolic activity. There might be some eggs, which hatched over the period of storage and damaged grain over the period of storage and at room temperature. The study also revealed that ozone treatment helped to holding germination rate in grains for a longer duration during storage without allowing further deterioration as compared to control samples. Similar results were reported by Wu J. *et al.* (2006).

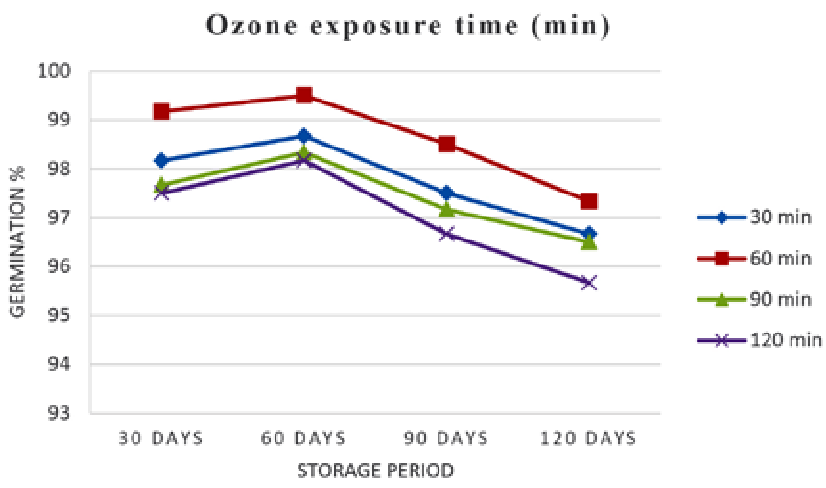

Fig. 2 Effect of Ozone exposure time (min) on germination percentage of wheat up to 120 days of storage

From the result, exposure time of ozone significantly affectsthe germination percentage of wheat grains. From 30 min to 60 min of exposure of ozone to wheat grains in bin affect positively and increase the germination percentage. If ozone exposure time increase after 60 min then germination percentage start to decrease and 90 min and 120 min of exposure time of ozone decrease the germination rate. The reduction rate of germination after 60 min is lower than control because of lethal effect on insect and infestation is under control. So, exposure time has significant effect on germination. Similar result reported by (Normov *et al.,* 2019).

As per shown in fig.1, ozone exposure time at 60 min have higher germination percentage all over the storage period of 120 days compare to other treatments. Germination percentage in starting of storage increase with ozone application up to 60 days and then germination start to decrease with increase of storage duration. Thus, 60 min exposure time gives the best result of germination percentage during storage of 120 days.

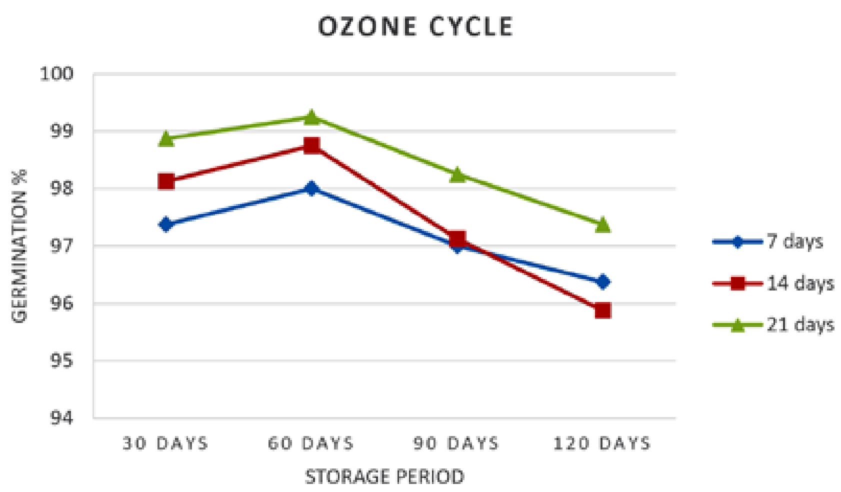

Fig. 3 Effect of Ozone cycle (days) on germination percentage of wheat up to 120 days of storage

Effect of ozone cycle at frequent time on germination percentage is significant. From 7 days frequent cycle to 14 days frequent cycle of ozone, germination percentage start to increase and at 21 days cycle of ozone it gives more germination then above both cycle. So, 21 days cycle gives best result as compare to 7 and 14 days cycle. The reason behind this is frequently high exposure of ozone decrease the germination percentage due to oxidation.

As per shown in fig 2, ozone cycle of 21 days gives higher germination percentage compare to 7 days and 14 days ozone cycle treatment over the storage period of 120 days. Germination percentage is increased with storage period up to 60 days storage period at regular ozone cycle. After reaching the pick point of germination, its start to decrease with storage time increment because of higher ozone frequency adversely affect the germination percentage of wheat.

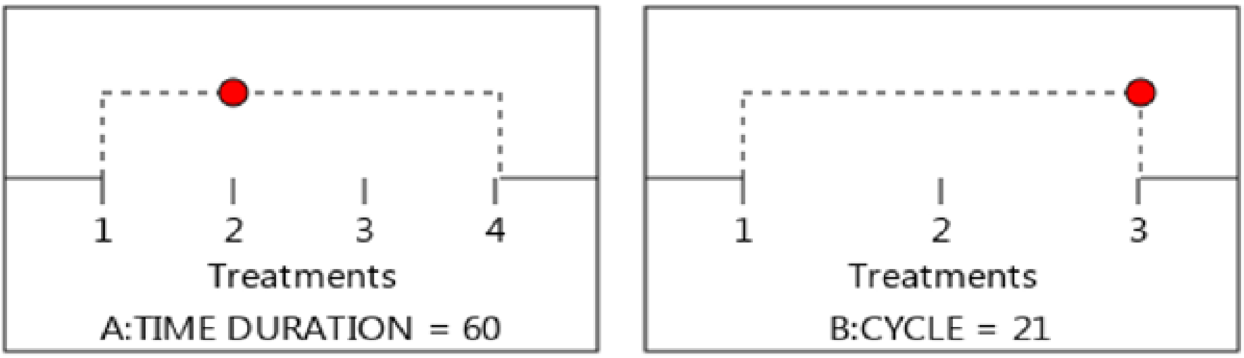

Fig. 4 Optimization parameter of ozone treatment for maximum germination percentage

Thus, the result of experiments performed treating wheat seed with ozone makes it clear that ozone improvesthe germination percentage of wheat seed up to its peak point after that if we increase ozone exposure then germination starts to decrease. As shown in Fig.4, optimal parameters for treating wheat seed with ozone to stimulate germination should be exposure time 60 min and ozone cycle at 21 days recommended.

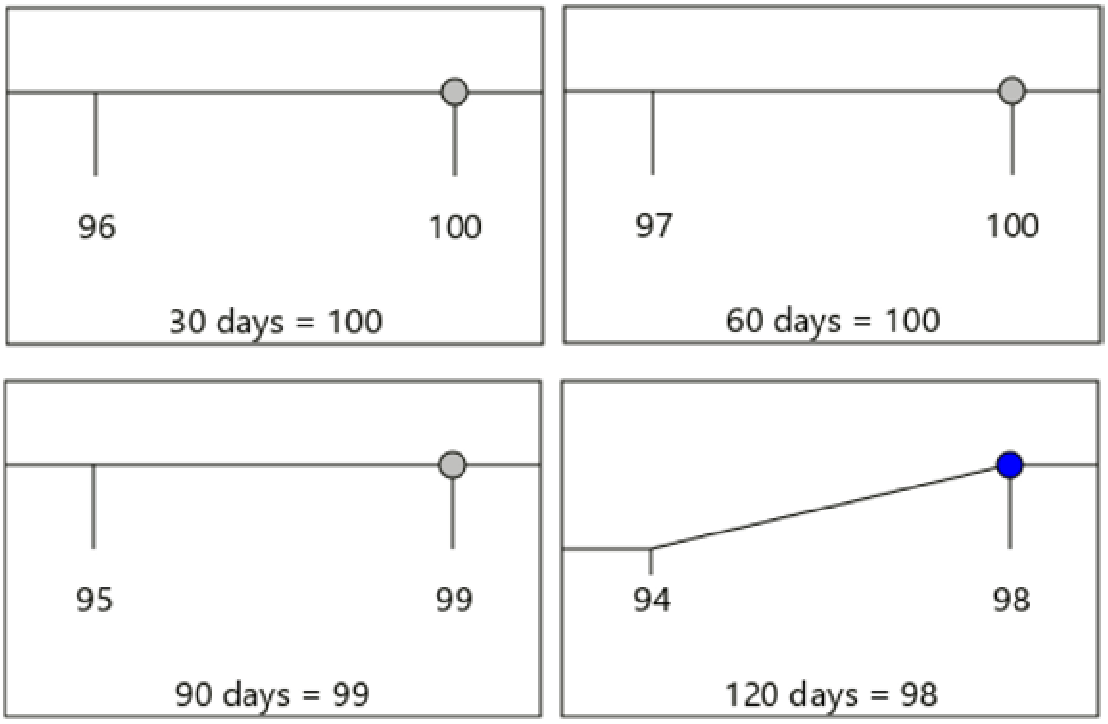

Fig. 5 Ozone germination percentage at optimum parameter of ozone treatment after 30 days, 60 days, 90 days and 120 days in wheat bulk storage

At the recommended optimal parameters for treating wheat seed with ozone, continuous observation taken after 30 days, 60 days, 90 days and 120 days. The result shows that exposure time 60 min and ozone cycle at 21 days gives best result in germination. As per given in fig.5,after 30 days of applied optimum condition of ozone, 100% seeds were germinated which is continued up to 60 days, then slightly decrease to 99% after 90 days and 98% germination after 120 days at desirability of 1, which gives best result in compare to other treatment of ozone exposure.

Conclusion

The experiments conducted in this study demonstrate the effect of ozone on wheat seed germination. Ozone has potential for use in storage insect control because it can be generated at site of application, it does not leave toxic residue after treatment and rapidly control the insect pest infestation. After all of these advantages, ozone gas treatment enhances the germination percentage of wheat seeds up to its peak point and then it start to reduce. The reduction rate is also very slow compare to control treatment. Considering the germination enhancement, ozonation at exposure time 60 min and ozone cycle at 21 days can be recommended as a non-toxic disinfectant for wheat grains.

Author contributions

Conceptualization of research (AMS & MND); Designing of the experiments (AMS & MND); Contribution of experimental materials (AMS & MND); Execution of field/lab experiments and data collection (AMS & MND); Analysis of data and interpretation (AMS & MND); Preparation of the manuscript (AMS & MND).

Conflict of interest: No

Declaration

The authors declare no conflict of interest.

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