POTENTIAL OF OZONE TO TREAT INSECTS IN STORED PADDY

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Abstract - *The most common insect in stored paddy that is known to cause serious quantitative and qualitative damage to paddy is Rhyzopertha dominica. The larva and adult feeding in and on grain kernels leaves only dust and thin brown shells. The currently available chemical fumigants are not eco-friendly and also recent studies are reporting that insects are developing resistance to these chemicals. Consumers are increasingly demanding good quality grain that is free from live insects and chemical residues caused by controlling pests. This study discusses the use of ozone to kill insects in paddy.*

Key Words: paddy, ozone, insects, storage, mortality, fumigation

1.INTRODUCTION

Paddy is the most important crop in India for domestic consumption and continues to be an important source of food. However, in the recent years insect infestation within the stored product has become a major concern to the grain industry which creates serious quality issues in stored paddy. Around 25% of the post-harvest loss of paddy in India is due to insect infestation. With limited control options and increased potential for resistance to current insecticides, alternate methods are needed for management of insects in stored grains.

1.1. Paddy

Rice botanically belongs to *Oryza sativa* L. of Gramineae family (Varnamkhasti *et al.*, 2007) [1]. Bernas (2011) [2] reported that rice is a source of vitamins and minerals, and is also good in nutritional values, low in fat and has no additives. It also provides energy and is rich in nutrients and has low glycemic index.

Manivannan (2016) [3] stated that CO 51 is a fine grain rice variety that is expected to cater the current market demands. It is a short duration paddy variety that offers the advantage of being a non-lodging variety. According to The Hindu news reports, CO 51 variety with white, medium slender rice is good for cooking and has a milling percentage of 69 per cent and head rice recovery of 63 per cent.

1.2. Stored grain pests

Studies have reported that three stored product insects that were found to commonly occur together, and for which pheromones are available, were the warehouse beetle (*Trogoderma variabile*) which uses a sex pheromone (Cross *et al*., 1976) [4], lesser grain borer, (*Rhyzopertha dominica*) (Williams *et al*., 1981) [5] and the red flour beetle (*Tribolium castaneum*) (Suzuki & Mori, 1983) [6], which use an aggregation pheromone.

According to Mason and John (2010) [7] the principal pests that cause damage are the adult and larval stages of beetles, and the larval stage of moths.

Naik and Geethanjali (2011) [8] reported that the major pests of stored grains are beetles (*Rhzopertha dominica*, *Callosobrunchus* spp, *Trogoderma granarium, Tribolium confusum*), weevils (*Acanthoscel idesobtectus*), moths (*Corcyra cephalonica*) and rodents.

1.2.1. Insect pests in paddy grain

Yunus and Ho (1980) [9] reported that about 187 species of insects have been found on paddy, among which only few have become serious pests. Insects in stored rice are classified as either primary or secondary insects. Primary insects are those insects whose larvae feed entirely within the kernels of the grain. These include the rice weevil, angoumois grain moth and lesser grain borer. Secondary insects feed from the outside of the grain even though they may chew through the outer coat and devour the inside. Two of the most prevalent secondary insects are the saw-toothed grain beetle and the rust-red flour beetle (Muir, 2000) [10]. Prakash *et al*. (1987) [11] said that that during storage, paddy rice is attacked by insect pests including angoumois grain moth; *Sitotroga cerealella*, lesser grain borer; *Rhyzopertha dominica*, rust-red flour beetle; *Tribolium castaneum*, rice weevil; *Sitophilus oryzae* and grain beetles; *Cryptolestes* spp.

Kiritani (2000) [12] reported that the insect fauna in paddy fields is composed of resident, migratory and aquatic species each corresponding to the continuous cropping of rice in the same field.

According to Trematera *et al*. (2004) [13] *Sitophilus oryzae* was the most abundant species in the paddy storage facility, other beetles collected in remarkable numbers were primary pests, such as *Rhyzopertha dominica* and secondary pests, such as *Cryptolestes ferrugineus* and *Oryzaephilus surinamesis*. It was concluded that various species showed very variable distribution and depending on pest and year, all parts of the facility appeared infested.

1.3. Fumigation

Fumigation is the only way to eliminate pests completely from a food grain without leaving pesticide residues. Kells *et* *al*. (2001) [14] found that there are only two registered fumigants for stored food; phosphine and methyl bromide. Phosphine reacts with atmospheric water to produce hydrogen phosphide gas or phosphine. Phosphine has inhibitory effect on insect respiration and is unique in that it is toxic to insects only in the presence of oxygen. A major disadvantage with phosphine is time required to eliminate the target pest, which may range from 3 to 7 days (Bond *et al.*, 1969) [15].

The popularity of methyl bromide as a fumigant is largely attributable to its high toxicity to many pests, the variety of settings in which it can be applied, its ability to penetrate the fumigated substances, and its rapid dissipation following application (Yang *et al.*, 1995) [16].

Rodriguez (2001) [17] reported that if the phosphine gas concentration is 50-100 ppm, at the end of 96 hour fumigation, there will be 100% insect mortality rate.

Studies also conclude that *R. dominica* has developed resistance to all approved organophosphorus insecticides; chlorpyrifos methyl, fenitrothion, pirimiphos methyl, and malathion (Navarro *et al.*, 1986 [18] ; Collins, 2006) [19].
Nayak *et al*. (2007) [20] reported that phosphine fumigation is the major pest management option in Australia and it is used to disinfest up to 80% of the stored grain. Phosphine possesses several advantages over other fumigants; including its low cost, versatility in application and most importantly, its global acceptance as a residue-free treatment.

1.5. Ozone

Ozone was first discovered by the European researcher C. F. Schonbein in 1839. It was first used commercially in 1907 for municipal water supply treatment in Nice and in 1910 in St. Petersburg (Kogelschatz, 1988) [21]. It is the triatomic state of oxygen, having chemical formula O_3.

Studies conducted by various researchers report that, at room temperature, ozone decomposes rapidly and thus, do not accumulate substantially without continual ozone generation (Peleg, 1976; [22] Miller *et al*., 1978) [23].

Ozone is readily detectable at 0.01 to 0.05 ppm level (Miller *et al*., 1978) [24]. In pure water, ozone rather quickly degrades to oxygen, and even more rapidly in impure solutions (Hill and Rice, 1982) [25]. Ozone solubility in water is 13 times that of oxygen at 0–30℃ and it is progressively more soluble in colder water. It is found in low concentration in nature and has a longer half-life in the gaseous state than in aqueous solution (Rice, 1986) [26].

Gaseous ozone has a pungent, characteristic odour described as similar to fresh air after a thunderstorm. At ordinary temperatures ozone is a blue gas, but at concentrations at which it is normally produced the colour is not noticeable. At -112℃, ozone condenses to a dark blue liquid. Liquid ozone is easily exploded, if greater than 20% ozone to oxygen mixture occurs (Coke, 1993) [27].

1.5.1 Generation of ozone

Ozone is formed in the stratosphere, in photochemical smog and by UV sterilization lamps, high voltage electric arcs, and gamma radiation plants (Mustafa, 1990) [28]. In addition to electrical discharge method and photochemical method (UV radiation), ozone can be produced by chemical, radiochemical, and electrolytic methods (Kim *et al*., 1999) [29].

1.5.1.1. Corona discharge

The procedure of electrical gas discharge, also named Corona discharge (Fig. 1), was developed by Siemens in 1857. It is nowadays widely applied for ozone production.

There are two electrodes in corona discharge, one of which is the high tension electrode and the other is the low tension electrode (ground electrode). Electrons are accelerated across air gap so as to give them sufficient energy to split the oxygen-oxygen double bond producing atomic oxygen (Barlow, 1994) [30].

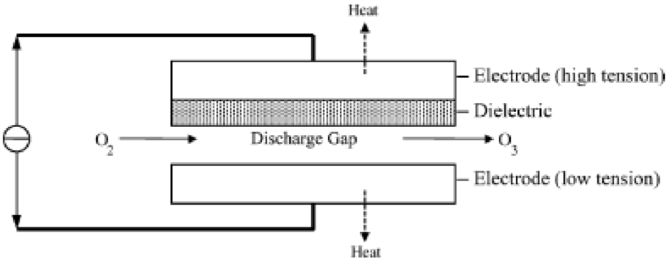

Fig. 1 Schematic diagram of corona discharge method of ozone generation

(Source: Rice *et al*., 1981) [31]
The two atoms produced by collision react with other diatomic oxygen molecules to form ozone according to following equation.

$3O_2 + \text{Energy} \rightarrow 2O_3$
Mahapatra *et al*. (2005) [32] reported that corona discharge is the only practical and safe method for large scale ozone generation.

1.5.1.2. Photochemical method

Dohan and Masschelein (1987) [33] reported that photochemical generation of ozone by UV irradiation of an oxygen-containing gas is a balance between ozone formation and the photolytic decomposition of ozone. Air is passed over a UV lamp from which a small portion of oxygen is converted into ozone by high energy radiation. Generally, a wavelength of 185 nm is normally used, although under ideal conditions maximum ozone concentration of 0.1% is obtainable.

Muthukumarappan *et al*. (2000) [34] reported that in the ultraviolet method of ozone generation, ozone is formed when oxygen is exposed to UV light of 140–190 nm wavelengths.

1.5.1.3. Electrolytic method

Electrodes used for ozone generation should consist of electro-chemically stable material and should possess high electrical conductivity. Hence, precious metals or metallic oxides in their highest oxidation state are applicable for electrochemical ozone production. The desired quantities of ozone and hydroxyl ions for the oxidation of unwanted materials can be varied through variation of current (Fryda *et al*., 2003) [35].

Chen *et al*. (2008) [36] reported that, with this technology up to 750 µg ozone can be produced per litre. The quantity of ozone produced by electrolysis depends on the charge density in the electrolysis cell, temperature (at high temperatures fast decomposition of ozone takes place) and the electrolyte used.

1.5.1.4. Radiochemical method

In radiochemical ozone production the dissociation step is initiated by high-energy products of radiochemical decay reactions. Usually isotopes such as ^{137}Cs, ^{60}Co or ^{90}Sr are used for excitation of circulating air in a water-cooled closed system. High energy irradiation of oxygen by radioactive rays can promote the formation of ozone. Whilst high yields have been achieved under specific conditions using oxygen, the best results from an air flow, through the system at atmospheric pressure has been approximately 3 to 4 mg/m^3. The process has complications in filtering harmful isotopes and it is not viewed with potential use in commercial applications (Heim and Glas, 2011) [37].

1.6. Safety aspects of ozone

Hoof (1982) [38] reported that in humans, ozone primarily affects the respiratory tract. Symptoms of ozone toxicity includes headache, dizziness, burning sensation in the eyes and throat, sharp taste and smell, and cough. Chronic symptoms were said to be weakness, decreased memory, increased prevalence of bronchitis and increased muscular excitability.

However, the USDA granted approval for the use of ozone in recycling poultry chill-water, after more than a decade of research to support such use (Sheldon and Brown, 1986) [39].

Studies show that the strong oxidizing activity of ozone is expected to cause some deleterious effects on the treated produce such as physiological injury and oxidative stress in plant tissues (Forney, 2002) [40]. Some of the symptoms of these injuries are browning, pitting, increased decay and weight loss and fruit discoloration among others (Forney, 2003) [41].

Certain studies also report that exposure to high concentrations of ozone can cause some detrimental health effects. In the United States, the Federal Occupational Safety and Health Administration (OSHA), and in the United Kingdom, the Health and Safety Executive specified a 0.1 ppm threshold for continuous exposure in the workplace environment during an eight-hour day/40-hour work week period and 0.3 ppm for a 15 min period (Karaca and Velioglu, 2007) [42]. To protect workers from ozone, an effective and reliable ozone monitoring and alarm system and proper personnel protective equipment was suggested.

However, in 1997, ozone was granted Generally Recognized as Safe (GRAS) status and in 2001, it received full USFDA approval as a direct contact food sanitizing agent (Tzortzakis *et al*., 2007) [43]. It does not possess many of the troublesome properties of other chemical treatments that have been tested for food protection and preservation.

1.7. Ozone in food processing

Studies were conducted to explore the different applications of ozone in food industry. It was found that treating fruits and vegetables with ozone is suitable for increasing its shelf-life (Rice *et al*., 1982) [44].

Restaino *et al*. (1995) [45] investigated the antimicrobial effects of ozonated water against food related microorganisms and concluded that ozone effectively killed gram positive bacteria such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, and Gram negative bacterias including *Pseudomonas aeruginosa* and *Yersinia enterocolitica*. It was also determined that ozone destroyed the yeasts *Candida albicans* and *Zygosaccharomyces bacilli* and spores of *Aspergillus niger*.

Guzel-Seydim *et al*. (2004) [46] worked on the efficacy of ozone to reduce bacterial populations in food components and significant log cycle reductions in the *E. coli* populations at 10 min were observed in buffer, starch, locust bean gum, caseinate, and whipping cream.

Onions were treated with ozone during storage. It was found that mould and bacterial counts were greatly decreased without any change in chemical composition and sensory quality (Song *et al*., 2000) [47].

Hampson and Steven (2004) [48] reported that for washing of broccoli with water containing up to 1 ppm dissolved ozone, the contact time necessary for one log-fold reduction in aerobic plate count microorganisms was 6 min. It was concluded that the potential utility of ozone in food processing lies in the fact that ozone is a 52% stronger oxidant than chlorine.

For minimally processed fruit and vegetables, ozone was applied in aqueous phase during the washing step. Positive results were reported, especially when ozone was used in combined treatments. Beltrán *et al*. (2005) [48] studied the effects of washing potato strips with ozonated water (20 mg/l-min) combined with storage for up to 14 days in MAP or under vacuum at 4°C. The authors stated that respiration rate was not affected by the ozone treatment, and browning was controlled by the combination of dipping in ozonated water along with vacuum storage.

Zhang *et al*. (2005) [49] concluded that in the food industry, ozone can be applied for several purposes like food preservation, extension of shelf-life, equipment sterilization, elimination of undesirable flavours produced by bacteria during both storage and shipping etc.

Habibi Najafi and Khodaparast (2008) [50] concluded that promising results indicated the efficacy of ozone to reduce the microbial populations in date fruits. *E. coli* and *S. aureus* were not found on cultured plates inoculated with the treated samples after treatment with 5 ppm concentration for 60 min.

Ozone has also found use as a food preservative and could also be used as a chemical treatment for storing high-moisture grains. White *et al*. (2010) [51] concluded that ozone treatments at 2.4 and 4.8 mg/min for 24 hours were effective at reducing dry matter loss in 26% moisture maize stored for 30 days at 15.5°C

Akata *et al*. (2015) [52] reported that the level of yeast and mould population naturally present on mushrooms was reduced to more than 1.43 log after ozonation at 5.3 mg/l for 45 min.

1.7.1. Ozone as a fumigant

Several studies have established 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*) (Kells *et al*., 2001; [53] Isikber and Öztekin, 2009 [54]). Gaseous ozone was reported to inactivate fungal spores on barley by Allen *et al*. (2003) [55] and on stored wheat by Wu *et al*. (2006) [56]. After a 5 min fumigation of wheat and barley with ozone at concentrations of 6000 and 3000 ppm respectively, 96% of fungal spores were found to be inactivated.

Bonjour *et al*. (2008) [57] tested the effect of ozone on six species of stored grain insect pests at three different concentrations (25, 50 and 70 ppm) for 1 to 4 days exposure durations. It was observed that rice weevil, *Sitophilus oryzae* adults suffered 100% mortality after 2 days exposure at 50 ppm or after 4 days exposure at 25 ppm. *Tribolium castaneum* adults suffered 100% mortality after 4 days exposure at 50 ppm concentration. No *T. castaneum* progeny were produced after 2 to 4 days exposure at 70 ppm. For lesser grain borer, *R. dominica* , rusty grain beetle, *Cryptolestes ferrugineus*, and sawtoothed grain beetle, *Oryzaephilus surinamensis*, 100% mortality was not achieved and progeny were produced at all ozone levels.

McDonough *et al*. (2011) [58] reported that the most ozone-tolerant stages of *T. castaneum* were pupae and eggs, which required a treatment of 180 min at 1800 ppm ozone to reach 100% mortality. Eggs of *P. interpunctella* also required 180 min at 1800 ppm ozone to reach 100% mortality. Ozone treatments of 1800 ppm for 120 min and 1800 ppm for 60 min were required to kill all adult *S. zeamais* and adult *S. oryzae*, respectively. The results indicated that high ozone concentrations reduce the treatment times significantly.
Studies conducted by Tiwari *et al*. (2010) [59] reported that ozone offers unique advantages for food grain processing with minimal or desired effects on the physicochemical properties. Hence ozone treatment is a potential greener alternative to conventional fumigants.

According to Hansen *et al*. (2012) [60] full control of the majority of tested insects was obtained with 35 ppm for 6 days. Full mortality of the internal stages of *Sitophilus spp.* and *R. dominica* required approximately 135 ppm for 8 days. Pandiselvam (2015) [61] reported that paddy grains at a moisture content of 12.35%, when treated with ozone at 1500 ppm for 120, 150, and 210 min showed 100 per cent lethality of *S. oryzae, T. castaneum,* and *R. dominica* respectively. *R. dominica* was found to be the most resistant of the insects tested

Pawar *et al*., (2015) [62] found that, concentration of 50 ppm ozone for three days resulted in 92–100% mortality of adult red flour beetle, *Tribolium castaneum*, adult maize weevil, *Sitophilus zeamais*, and larval moth, *Plodia interpunctella*.
Anandakumar *et al*. (2016) [63] reported that the 99% lethal time (LT99) of the larva, pupa and adult of *Laisoderma serricorne* at 150, 250, 350 and 450 ppm treatments were 609.37, 544.90, 496.64 and 187.20 min; 628.80, 581.64, 522.78 and 429.33 min; 745.33, 610.18, 544.98 and 422.98 min respectively. The egg of *L. serricorne* did not attain any mortality in 150, 250, 350 ppm, whereas at 450 ppm it attained 70% mortality.

Xinyi *et al*. (2017) [64] reported that 3 to 4 hours exposure to 200 ppm ozone resulted in 100% final mortality of *Sitophilus spp*., whereas *O. surinamensis* required 6 to 10 hours of exposure to ozone.

1.8. Inactivation mechanism

Liu et al. (2007) [65] reported that ozone is found to cause oxidative tissue damage even at low concentrations, resulting in DNA strand breaks, alteration of pulmonary function, bronchial responsiveness, membrane oxidation or mutations in vivo.

Microbial inactivation by ozone was reported to be a complex process which acts upon various cell membrane and cell wall constituents along with cell content constituents (*e.g.* enzymes and nucleic acids). Microorganisms were found to be inactivated by disruption or disintegration of the cell envelope leading to cell lysis. Both molecular ozone and the free radicals produced by its breakdown play a part in this inactivation mechanism (Cullen *et al.,* 2009) [66].

Xinyi *et al*. (2017) [67] reported that ozone can cause oxidative damage to insects, and to deal with this oxidative stress, one of the strategies insects use is to lower the respiration rate by breathing discontinuously.

3. CONCLUSION

As ozone gas is internationally generally recognized as safe (GRAS) and does not leave residues in food, could be a promising method of fumigation in storage units to avoid insect infestation and to ensure food security to the consumer. There are no evidences for the presence of any major genes resistant to ozone identified so far.

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