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Research Note

Repellency effects of an ozone-producing air purifier against medically important insect vectors

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Abstract. An indoor ozone-producing air purifier was evaluated for its repellency effects on *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, *Musca domestica* and *Periplaneta americana* adults using a modified tunnel cage. The first set of testing consisted of both bait and the air purifier, whereas only bait was offered in the second testing set. The highest percent repellency was recorded among *Cx. quinquefasciatus* (83.23%) while *Ae. aegypti* (68.56%) was the least repelled by the air purifier tested. No mortality was observed in all testings. These results illustrated the potential use of the ozone-producing air purifier as a personal protection insect repellent device in premises. Nevertheless, in order to prevent dengue infection which is transmitted by both *Ae. aegypti* and *Ae. albopictus*, ozone-producing air purifier should be utilized concurrently with other vector control methods.

Indoor air purifiers are popular among consumers nowadays. Besides their effectiveness in eliminating odors and microbial agents, those indoor air purifiers have been claimed as safe and beneficial especially for those suffering from asthma and allergic reactions (Hubbard *et al.*, 2005; Britigan *et al.*, 2006). A number of air purifiers are available in the market worldwide. Air purifiers apply various technologies including ozone production, mechanical filtration and electrostatic precipitation (Hart *et al.*, 2011). Among these, air purifiers that produce ozone during operation become the main concern as ozone is an indicator of air pollution and is regulated by many health-related standards (Britigan *et al.*, 2006).

Ozone-producing air purifiers usually need high voltage to ionize air molecules. In addition, air purifier systems are usually incorporated with a catalyst system in order to convert any entrained or internally produced CO to less dangerous CO_2 (Millar & Mouldey, 2008).

Previous studies had reported on the possible efficient sterilization of air which could be accomplished by the use of ozone. Ozone had been found to inactivate pathogens like bacteria and viruses rapidly and efficiently both in water and airstreams (Kowalski *et al.*, 1998).

Furthermore, ozone-producing air purifiers are often believed to possess a repellency effect against certain insects such as mosquitoes and flies. However, little studies had been done worldwide to support this idea. Hence, this study was performed to evaluate the repellency effects of an ozone-producing air purifier against *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, *Musca domestica* (house flies) and *Periplaneta americana* (cockroach).

An anion air sterilizer and purifier machine with a digital remote control (model JQ-208) was tested in this study. Its weight is 1.4 kg with a size of 298 mm x 210 mm x 100 mm which can be used effectively within an area of 20 to 60 m^2. JQ-208 requires an input voltage of AC220 Volts / 50 Hertz and input power of 18 Watts. Its ozone output is 500 mg/h while its anion output is 10 million/cm^3. It was laid on a stable work bench throughout this study but it could actually be hung up when in use.

According to the manufacturer, JQ-208 is to be used in purifying air inside a house within 10 to 30 minutes. This machine is believed to be able to kill germs, bacteria and viruses. It is also claimed to be effective in clearing bad indoor smells such as smoke, dust and stink as well as poisonous elements from furniture or decoration such as formaldehyde, toluene, painting and ammonia.

Other than that, JQ-208 is also claimed to possess repellency effects against medically important insects such as mosquitoes, flies and cockroaches.

Laboratory strain of sugar-fed 3 – 5 days old *Ae. aegypti*, *Ae. albopictus* and *Culex quinquefasciatus* adult females starved overnight were used to assess the repellency effects of JQ-208. Both male and female sugar-fed *Musca domestica* and water-fed *Periplaneta americana* starved overnight were employed in this study.

A modified tunnel cage with two compartments separated by a layer of plastic closing was used in this study (Figure 1). The first compartment with shorter flying space was where the insects to be tested were released into the cage. The second compartment which comprised a longer flying space was where the bait was placed to attract the insects to fly or move towards it.

This study was conducted in two different sets. The first set consisted of the test insects, bait and JQ-208 while the second set consisted of only the insects and bait. For the first set, JQ-208 was located next to the second compartment of the cage where the bait was placed. White mice were used as baits for all species of mosquitoes tested while sugar and water were used as baits for house flies, *Musca domestica* and *Periplaneta americana*.

Fifty (50) sugar fed 3 – 5 days old female mosquitoes were employed for each replicate, whereas 25 males and 25 females of *Musca domestica* and *Periplaneta americana* were used per replicate. The number of mosquitoes or house flies landed on the bait within 15 minutes as recommended by the manufacturer was counted. At least 5 replicates were tested for each set in this study.

Results obtained were subjected to statistical analysis. Percent repellency was calculated as [(C – T) / C] x 100, where: C = Mean of unrepelled insects in the untreated group; T = Mean of unrepelled insects in the treated group.

Table 1 shows the mean number of mosquitoes, house flies and cockroaches landed on the bait within 15 minutes of

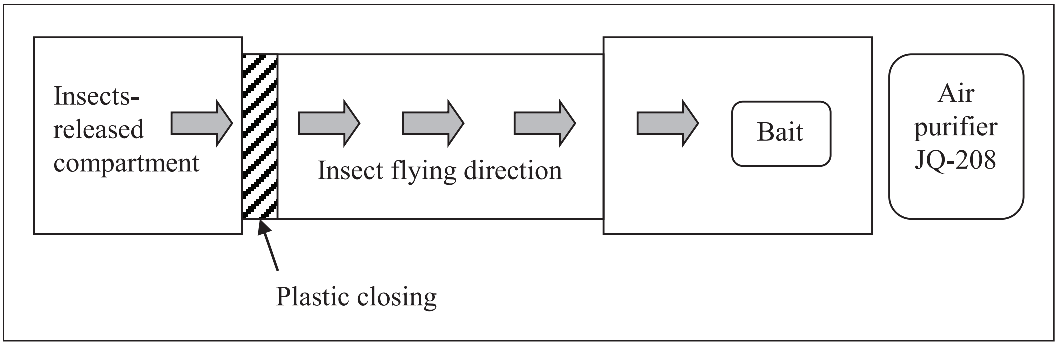

Figure 1. A schematic diagram of a modified tunnel cage used to evaluate the repellency effects of JQ-208.

Table 1. Mean number of mosquitoes, house flies and cockroaches landed on the bait within 15 minutes of exposure time

Type of test / Species of insects	Set 1 (insects + bait + JQ-208) Mean ± S.E.	Set 2 (insects + bait) Mean ± S.E.	Wilcoxon Test	% Repellency
Ae. aegypti	53.17 ± 15.81	169.08 ± 40.69	$p < 0.05$	68.56
Ae. albopictus	20.25 ± 4.55	88.58 ± 9.02	$p < 0.05$	77.14
Cx. quinquefasciatus	2.67 ± 1.50	15.92 ± 2.86	$p < 0.05$	83.23
Musca domestica	28.30 ± 5.36	100.30 ± 15.60	$p < 0.05$	71.78
Periplaneta americana	6.92 ± 1.66	35.00 ± 1.77	$p < 0.05$	80.23

S.E. = Standard Error

exposure time. Results obtained demonstrated repellency effects of ozone from JQ-208 against all species of insects tested and ranged between 68.56% and 83.23%. The highest percent repellency of JQ-208 was observed among *Cx. quinquefasciatus*, followed by *Periplaneta americana* (80.23%). The least repelled insect was *Ae. aegypti*. No mortality was observed in all testings. These findings indicated the potential use of JQ-208 as an insect repellent device. However, other control methods should be implemented simultaneously with the use of JQ-208 indoors as its repellency effect among *Ae. aegypti*, an indoor breeder of dengue vector was not as high as against other insects tested.

Ozone is one of important factors that affect the survival of humans, plants, animals and insects. Ozone possesses a radiomimetic property (Erdman & Hernandez, 1982). Ozone has been used not only for potable-water production, water treatment and wastewater treatment, but also as virucide for poliomyelitis (Venosa, 1972; Diaper, 1977; Culp *et al.*, 1978; Blogoslawski & Rice, 1975; Murphy & Orr, 1975). Ozone acts as germicidal (Beard, 1965), a highly powerful oxidizing agent as well as a general mutagenic agent (Zelac *et al.*, 1971).

In addition, it is believed that ozone plays an important role in repelling insects. Although ozone effects on insects in nature are hard to be evaluated, some observations under simulated system are possible (Beard, 1965). Even though there are no studies on the effects of ozone on mosquitoes that could be captured so far, few studies on the ozone effects against other insects had been reported worldwide.

In 1965, Beard reported that house flies exposed to ozone-enriched environment varied extensively in numbers and was generally at a higher level than house flies reared in ambient air. Nevertheless, complete mortalities were observed in the last three generations of ozone-treated house flies once the ozone treatment was stopped and diffusive ventilation took place. In contrast, the control population of house flies remained unaffected. These findings illustrated that house flies could not adapt to a changed environment.

Moreover, in the same study, Beard also demonstrated that ozone-treated female house flies laid lesser number of eggs compared to the control population due to their short life span. However, high concentration of ozone totally inhibited egg laying. Moreover, house flies exposed to both high ozone concentration and abnormally high temperature for two to three days died before egg laying. These findings showed that long exposure in highly concentrated ozone environment could reduce house flies fecundity which could be applied as one of the vector control strategies.

Similar observations were obtained by Levy *et al.* (1972) where there was an abnormal egg laying behaviour which caused an increased number of eggs laid by ozoned *Musca domestica* adult females. Egg hatch was also slightly decreased in *Musca domestica* and stable flies; *Stomoxys calcitrans* (L.), but not in *Drosophila melanogaster* Meigen. However, egg hatching, larval molting, pupation, adult emergence and physical characteristics of all three insect species tested were not affected by the ozone treatment at all.

After about a decade, Akey (1982) conducted a study on the effects of ozone on the biting gnats. A complete mortality of the biting gnats; *Culicoides variipennis* adult females was observed upon an hour of ozone exposure at 600 ppm.

In the same year, Erdman & Hernandez (1982) also observed mortality in *Drosophila virilis* exposed to 30 ± 2 ppm ozone for four hours and more on or before day 25 post-exposure. In fact, their studies had illustrated that ozone treatment caused an increase in the percentage of dominant lethal in *Drosophila virilis* during spermatogenesis.

More than twenty years later, researchers started to regain interests in the effects of ozone in insects. Studies by Hollingsworth & Armstrong in 2005 demonstrated that ozone fumigation at ≅200 ppm in 100% carbon dioxide environment at 37.8°C for 30 minutes caused 47.9% and 98.0% mortalities in ornamental crops: longtailed mealybug, *Pseudococcus longispinus* Targioni Tozzetti and western flower thrips, *Frankliniella occidentalis* (Pergande), respectively.

In addition, Lu *et al.* (2009) reported on the reduction in the respiration rate of lesser grain borer, *Rhyzopertha dominica* (F.) and rust-red flour beetle, *Tribolium castaneum* (Herbst) adults which are common stored-product insects due to the increase of ozone concentration. Moreover, studies by Niakousari *et al.* (2010) demonstrated complete mortalities of larvae and adults of both Indian meal moth, *Plodia interpunctella* and sawtooth grain beetle, *Oryzaephilus surinamensis* that infested Kabkab dates, *Phoenix dactylifera* L. upon ozone treatment at a concentration of more than 2,000 ppm for 2 hours.

Not only that, studies by James (2011) showed that neonates and adults of greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) which is an insect pest in honey bee hives were dead within only a few hours of ozone exposure. At the same time, Bonjour *et al.* (2011) found that ozone treatment applied at 70 ppmv for more than 4 days was effective against adults of *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Liposcelis bostrychophila* Badonnel and *Liposcelis paeta* Pearman which are major grain pests in stored wheat. Additionally, later studies by Sousa *et al.* (2012) demonstrated that ozone treatment reduced walking activity of the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae).

Other than that, sensory modalities play important roles in insects to locate their food sources. Food-seeking behaviour in insects involves a complex and integrated systems of olfactory, visual and thermal (Kang *et al.*, 2011; Lima *et al.*, 2014; Mysore *et al.*, 2014; Vinauger *et al.*, 2014). By consideration of the role of JQ-208 in clearing odour and other environmental pollutants, it is also possible that odour of baits had also been eliminated by JQ-208 which eventually enhanced the efficacy of its repellency effects against tested insects.

Besides insects, the effects of ozone exposure on other living organisms such as humans and plants remain uncertain. As such, studies by McKenzie *et al.* (1977) on 26 normal male volunteers demonstrated that exposure of 0.4 ppm ozone for 4 hours did not show any clear effect on human cytogenetic. Moreover, studies by Paradisi *et al.* (2009) found that there were no improvements in semen quality of infertile men.

Nevertheless, due to its highly reactive triatomic molecule of oxygen, ozone has been claimed to be among the most potent oxidizer that can cause damage in cell membranes of organisms (Stecher, 1968; Akey, 1982; Hollingsworth & Armstrong, 2005). For instance, studies by Diaz-Llera *et al.* (2002) showed that ozone stimulates DNA damage in human peripheral leukocytes in vitro. Furthermore, studies by Pacini *et al.* (2003) and Palli *et al.* (2009) in Florence, Italy found a significant positive correlation between ozone concentrations and oxidative DNA damage in the epithelial cells of nasal mucosa and circulating lymphocytes, respectively. However, any correlation between ozone poisoning and oxygen poisoning is still unclear (Clark, 1959).

On the other hand, ozone treatment at high concentration and high temperature is toxic to plants as it triggers peroxidation and denaturation of membrane lipids (Loreto *et al.*, 2001).

The use of air purifiers to produce ozone indoors has been reported to cause increased concentrations of fine particulate matters (Hubbard *et al.*, 2005; Huttunen *et al.*, 2010). However, the release and persistence of by-products due to reactions between ozone and indoor materials are varied (Poppendieck *et al.*, 2007a, b; Huttunen *et al.*, 2010). For example, furniture cleaning using either ozone alone or combination of steam wash and ozone was effective against microbes but does not disintegrate the fungal DNA detected in the dust (Huttunen *et al.*, 2010). Meanwhile, studies by Hubbard *et al.* (2005) showed that reactions between ozone and terpenes facilitated the growth of indoor secondary pollutants in residential indoor atmospheres which is potentially harmful to humans.

The ozone-producing air purifier had been proven to be effective against main contaminant and air-borne disease agents such as *Aspergillus conidia* (Desoubeaux *et al.*, 2014). However, the use of ozone treatment still has its own limitation. Materials to be used in the machine or apparatus in the air purifier for ozone production should not deteriorate upon ozone exposure (Akey, 1982). Hence, exposure of lower ozone concentrations for longer duration could be effective.

In conclusion, an air sterilizer and purifier, JQ-208 possesses repellency effects of more than 50% but less than 90% against *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, *Musca domestica* and *Periplaneta americana*. These findings illustrated its effectiveness as an insect repelling device which could be used as an alternative control method of medically important insects. Nevertheless, further studies covering other aspects are still needed to be carried out before it can be used in residential indoor settings as evidences on the potentially harmful effects of ozone generation in residential environments especially against humans have been reported as well (Hubbard *et al.*, 2005). Future studies should cover wider range of ozone concentration and exposure duration in an actual standard size room. An ozone sensor should also be utilized to detect and measure the level of ozone released from the device tested. It is crucial to confirm the safety of ozone production by JQ-208 against humans, targeted insects and plants at all stages.

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