have complex processing. Fast and economical assays that can be performed with standard PCR instruments are highly desirable for diagnostic analyses and for scientific studies of large numbers of pests. Individual *R. dominica* from distinct geographic populations can be discriminated into resistant homozygote, resistant heterozygote and susceptible homozygote from electrophoretogram after ARMS-PCR assay. Our TaqMan@ MGB probe assay could discriminate P49S mutation in DLD gene according to fluorescence labeling intensity variation and confirmed ARMS-PCR result as well. Our results show that the rapid detection of phosphine resistance in *R. dominica* populations in China provides important information to grain industries for decision-making in pest management strategies. In addition, our results suggest that this method could be applied for the detection of phosphine resistance in other grain pests, such as *T. castaneum* and *Sitophilus oryzae*, whose DLD genes have been sequenced. Our methods could be conducted on dead insects or insect fragments. Indeed, we evaluated consumable, running and capital cost for each method. The ability to quickly diagnose the resistance of these strains would be of great benefit. Furthermore, ARMS-PCR method for identifying the resistance locus mutation provides an opportunity to valuate level of phosphine resistance in other key pest species such as *Cryptolestes ferrugineus*, *S. oryzae* and *Sitophilus zeamais*. In addition, this technology could be extended to solve other pesticides resistance. The development of ARMS-PCR does not require generation of phosphine gas in the laboratory; also does not need collection and culture of field populations. Furthermore, the results are easier to assess with naked eye.

Keywords: Phosphine resistance, Lesser grain borer, Taqman@ probe, ARMS-PCR, Dihydrolipoamide dehydrogenase

Determınatıon of toxıcıty of gaseous ozone agaınst adult stages of German Cockroach (*Blatella Germanıca* L.)

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In this study, the effects of two different concentrations of ozone gas (16.7 and 33.3 mg / L) against *Blatella germanica* adults at different exposure times (10, 20, 30, 40 and 50 minutes) were investigated under laboratory conditions. It was determined that the ozone gas had a noticeable effect on mortality of *B. germanica* adults. In general, ozone gas caused higher paralyisis-mortality rates of *B. germanica* adults than mortality rates of *B.germanica* adults at both concentrations and all exposure times. A concentration of 33.3 mg / L of ozone gas with 40 and 50 minute exposure times killed all cockroach adults after 24 hours. On the other hand, 16.7 mg / L concentration of ozone gas with 50 minute exposure time killed 90% of the *B. germanica* adults after 24 hours. When ozone gas is evaluated in terms of exposure time to *B. germanica* adults, the concentration of 33.3 mg / L of ozone gas with 10-20 minute exposure times caused 65 % adult mortality, with 30 minute exposure time caused 90% adult mortality and with 50 minute exposure times caused 100 % adult mortality after 24 hours. At a concentration of 16.7 mg / L of ozone gas, as the exposure times increased, the adult mortalities gradually increased after 24 hours and the adult mortality reached 90% with 50 minute exposure times. All these results show that ozone gas (33.3 mg / L) with 40-50 minute exposure times can successfully control *B.germanica* adults.

Keywords: Ozone gas, *Blatella germanica*, mortality, biological efficacy.

1. Introduction

Cockroaches are insect species that have remained unchanged since ancient times (Appel, 1995). There are approximately 3,500 species of cockroach in the world (Atkinson et al, 1991). Most types of cockroaches are insect species that live in outdoor environments. However, a few cockroach species are found in the living areas of insects. One of the cockraoch species found in people's

habitats is the German cockroach, *Blatella germanica* (L.), which is one of the most common cockroach species all over the world. It is easily distributed when indoor and outdoor temperature and humidity are suitable for this species.

In addition to the psychological effects to humans, this species has a considerable medical importance, as it harbors bacteria, fungi, helminths, protozoa, viruses that can cause diseases in humans (Mullen et al., 2002). Cockroaches also cause asthma in many people with allergies (Roberts, 1996). The control of German cockroaches is traditionally made with inorganic and synthetic organic insecticides (Rust et al., 1993). Due to adverse effects of the use of intensive chemical insectides in control of the German cockroach, an alternative control method which is not harmful to the environment, humans and animals is needed.

Ozone is a form of three atomic oxygen (O3) molecule. Ozone is produced as a bluish or colorless gas characterized by fresh clean odor in the air following the thunder storm. Ozone is an unbalanced gas and quickly converts oxygen to temperatures above 35 ° C. Therefore, it must be produced during use and can not be stored after it is produced. There is a striking characteristic odor that many people in ozone can notice even at very low concentrations (0.02 ppm by volume) (Kim et al., 2003). Commercially, mostly ozone is produced with pure oxygen or airborne corona current generators (Kim et al., 2003). The control of insects with ozone gas has started with stored product pests. Isikber and Öztekin (2009) tested ozone gas on some stored product pests. These are the application of ozone gas to the insects with products and without product environment. The toxicity data obtained from studies with ozone gas in unfilled environment (empty volume) revealed significant differences between ozone gas sensitivities of developmental periods of *Ephestia cautella* and *Plodia interpunctella*. Ozone application in unfilled environment (empty volume) caused 100% death of *E. cautella* and *P. interpunctella* in all life stages, with the exception of the egg. The toxicity results obtained in study showed that *Tribolium confusum* is generally more resistant to ozone gas than *Ephestia kuehniella.*

To our knowledge, there are no studies on the effect of ozone against *B. germanica*. For this reason, in this study, we determine the optimum ozone concentration and ozonation duration by using the osmotic gas in the adult control of German cockroach.

2. Materials and Methods

Insect

Colonies of *B. germanica* were reared in plastic containers (60 liter) and maintained at room temperature. The cockroaches were provided with water in glass tubes with cotton stoppers and dry dog food. Each container was provided with paper egg cartons as shelter. The adult cockroaches (5-10 days old) were tested for each bioassays at 25 (± 2) ºC and 50 (±5) % relatıve humidity.

Ozone gas fumigation chamber

In empty volume applications, 3 liters of glass jars with a metal cover of 9 cm in diameter were used in all of the biological tests. These hatches have 2 entry pipes, 0.5 cm in diameter and 3 cm in length. A 5 cm long silicone hose is connected to the end of each record (one of the hoses is connected to the vacuum pump and the other to the ozone generator). One of the hoses was provided with ozone gas from the other hole while the air in the glass jar where the biological tests were conducted was discharged. Thus, the ozone gas was periodically circulated in the glass jar. The adjustment of the ozone gas concentration is adjusted according to the flow rate of the pure oxygen gas. The flow rate of the oxygen gas is controlled by the flowmeter placed between the oxygen tube and the ozone generator.

Biological tests and Empty volume applications

For empty volume applications, biological tests were carried out in 3 l metal-lined glass jars (fumigation chamber) at a temperature of 26 ± 1 and 65 ± 5% relative humidity. In all tests, *B. germanica* adults were used. The adults used in the biological tests were placed in 3 l jars where ozone fumigation was carried out and a small amount of food was added to the bottles. The solution was prepared by adding 10 ml of purified water to 100 g of MgNO2 (Magnesium Nitrate) to keep the media noodle in the jars constant at 65 ± 5%. This solution was soaked in a jar until wetted to a drying paper size of 5 x 2 cm. As the individuals exposed to ozone gas were placed in glass jars, the air in the jars used in the tests was evacuated to 760 mm Hg by low pressure pump (KNF, Germany). As the air in the jars is evacuated, the hoses on the covers of the jars are closed with the help of plastic clips to prevent gas in and out of the jars. After taking the air in the jars used in the biological tests, ozone gas is delivered to the ozone generator of the oxygen gas with the help of the flowmeter and ozone gas is produced. In order to produce ozone gas at different concentrations, the oxygen gas flow was set at 5 and 10 l / h and the flow rate was monitored from the flowmeter screen. When the desired flow rate is reached, the clips in the lids of the jars are opened and the produced ozone gas is directed to the fumigation chamber and the pressure inside the jar is filled with ozone gas until reaching normal pressure conditions. Since ozone gas is not a stable gas, it quickly transforms into oxygen form due to the effect of temperature and relative huminity. Therefore, ozone gas application has been completely applied to biological tests. In empty volume applications, application times were determined as 10, 20, 30, 40 and 50 minutes and the air in the jars where the tests were carried out once every 30 minutes in 40 and 50 minute applications was evacuated by vacuum pump and ozone gas was applied again. Experiments were carried out in 3 replicates of 10 individuals each time, leaving 3 controls for each trial. Upon completion of the application period, the ozone gas applied jars were ventilated and the insects were removed from the jars. The adults exposed to ozone gas were placed in 1 l glass jars and a small amount of food that was not exposed to ozone gas were added to the bottles. Dead and alive individuals were counted 1 hour, 6 hours and 24 hours after the termination of the experiments.

3. Conclusion

In this study, the toxicity of ozone gas against adults of *B. germanica* was demonstrated at two different application concentrations and different application times. In this study, as a result of the biological tests, ozone gas was generally observed in both the ozone gas concentration and the paralysis-mortality rate in all the application periods in the adults of *B. germanica*, was higher than the death rate. This has shown that ozone gas is a knockdown feature on this insect which leads to death. When the concentration of 33.3 mg / L of ozone gas was applied to adults of *B. germanica* with 40 and 50 minutes, respectively, it killed all cockroaches after 24 hours of application. On the other hand, when a concentration of 16.7 mg / L of ozone gas was applied to *B. germanica* adults for 50 minutes, it killed 90% of the adults of *B. germanica* after 24 hours of application. When the ozone gas is evaluated in terms of exposure time to *B. germanica* adults, the concentration of 33.3 mg / L of ozone gas with 10-20 minute exposure times caused 65 % adult mortality, with 30 minute exposure time caused 90% adult mortality and with 50 minute exposure times caused 100 % adult mortality after 24 hours. At a concentration of 16.7 mg / L of ozone gas, as the exposure times increased, the adult mortalities gradually increased after 24 hours and the adult mortality reached 90% with 50 minute exposure times. At a concentration of 16.7 mg / L of ozone gas, as the exposure times increased, the adult mortalities gradually increased after 24 hours and the adult mortality reached 90% with 50 minute exposure times. All these results have shown that ozone gas has potential for controlling *B. germanica* and may be an alternative to the synthetic chemicals used in the control of this insect. However, a comprehensive study of the ozone gas applicability in the natural habitat of the German cockroach and the determination of its effect on different factors when applied in natural conditions is necessary.

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Does the lower concentration of anticoagulants affect the efficacy of rodenticide baits?

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Extended abstract

Rodents belong to dominant synanthropic pests in agriculture environment, where cause wide range of damages by feeding on crops, gnawing of materials and faecal/urine contamination (Frankova et al. 2016, Stejskal et al. 2016). Rodents are predominatly controlled by anticoagulant-based rodenticides (AR) with the chronic mode of action (e.g. Frankova et al. 2017). Their delayed efficacy prevents rodents to connect the consumption of the bait with subsequent toxic effects and thus, favours them over other chemical rodenticides. On the other hand, application of ARs is permitted under strict regulation (Regulation (EU) No 528/2012) as ARs are considered as PBT (i.e. persistency, bioaccumulativity and toxicity) substances which pose environmental risks.

In addition, EU Commission recently adopted reclassification of ARs products (Commission Regulation (EU) 2016/1179; shall apply from 1 March 2018) - rodenticides with anticoagulant of 30 ppm or more must be labelled as "toxic to reproduction" and will be available to professional use only. Currently, it concerns seven of the eight approved anticoagulants, which contain 50 ppm of active substance. This Regulation leads manufacturer to produce rodenticide baits with a decreased concentration of anticoagulants to avoid a reclassification of products.

We focused on the testing efficacy of standard (50 ppm) and lower (25 ppm) concentration of anticoagulant in two brodifacoum-based baits in wild house mouse (Mus musculus). The laboratory no-choice feeding tests showed 100% mortality (mean survival time was 5.3 ± 2.1 days) for both concentrations. The consequent field experiments confirmed the previous laboratory results for the new baits with the lowered concentration (i.e. 25 ppm): during the three-week application period we found a significant decrease of both the tested bait and monitoring non-toxic bait consumption. Our study shows promising efficacy of products with the lowered concentration of brodifacoum. Nevertheless, there is a work ahead rodent scientists to illuminate the new baits efficacy in rodent populations with the decreased physiological sensitivity or increased resistance to anticoagulants.

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COMMISSION REGULATION (EU) 2016/1179 of 19 July 2016 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures

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