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Ozone Impacts in Suppressing Stored Grain Insect Pests in Maize: A Clean and Safe Control Strategy Compared with Phosphine Toxin-Gases

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ABSTRACT

The study was conducted under greenhouse (Temp. between 27 - 32 ºC and relative humidity 65%) and laboratory conditions of the Industrial Complex of the Equaquimic Company, located in the city of Quevedo province Los Ríos, at km. 4.5 via Quevedo – Valencia, coordinates 0°58'57.1" S and 79°26'28.9"W. to evaluate the mortality percentages of 20 ppm ozone dose under different exposure times (30, 45 and 60 minutes) on three of the most dangerous stored grain insect pests: *Sitophilus zeamais* (Motschulsky), *Rhyzopertha dominica* (Fabricius) and *Tribolium castaneum* (Herbst); separately for each insect with corn grains and without grains by direct exposure to each pest. Three experiments were performed, one for each insect species in a Complete Randomized Design (RCBD) in factorial arrangement (Time and condition). The results indicated that there were no significant differences in the interaction for insect mortality. The mortality was significantly affected by exposure time when corn was used or not. The mortality of ozone under 60 minutes of the exposure was recorded (< 90%), while 30 minutes, the shortest exposure time, showed average mortality of 81%; respectively for each insect species. In general, the average mortality in all exposure periods, with corn was 81%, while without corn was 98%for each insect species. These results demonstrate the efficacy of ozone as an alternative viable tool to control - stored grain insects.

INTRODUCTION

Maize (*Zea mays* L.) is an important crop all over the world with total production ca.1133.8 million tons (FAO, 2019) and is directly and indirectly consumed as a daily routine diet for both humans and animals. In Ecuador, maize plays an effective role in food security, e. g. yellow corn is mostly produced in the coastal region as a transitory crop in relation to the sown area (300,000 ha) (Zambrano *et al.*, 2019). However, 80% of yellow corn production is destined for the production of balanced feed. Maize cultivars are vulnerable to an invasion of various primary and secondary insect pests, which negatively influence the

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production and quality of corn kernel (Hernández, *et al.,* 2009) and the loss caused by storage insect pests could reach more than 10% during production and 10 to 20% in post-harvest (García-Lara *et al.,* 2003). The primary pest like the corn weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) and the secondary pests such as the Chestnut beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the lesser grain borer, *Rhyzopertha dominica* (F) (Coleoptera: Bostrychidae) directly cause a decrease in the commercial value, quality losses, and nutritional value in the grains during the storage period (García-Lara *et al.,* 2007).

The alternative method to chemical control and the intensive use of phosphine should be applied to prevent and minimize the negative effects of insecticide residue, and hazardous effects on the environment (Pérez *et al.,* 2010). In this regard, ozone (O_3) is a gas molecule consisting of three oxygen atoms with a half-life of 12 hours, in a gaseous state and at atmospheric pressure, which can transform or decay into two oxygen molecules quickly without leaving residues (Hardin *et al.,* 2009). This gas has been used as an ecological option to efficiently manage the populations of insect pests and to keep the environment healthy (Kells *et al.,* 2001). The effectiveness of ozone to cause mortality to insect pests is based directly on the concentration or the used dose and the time of exposure (Isikber and Athanassiou, 2015) as its use in low doses with the appropriate protective equipment does not affect human health (Rozado *et al.,* 2008).

Therefore, the study aimed to evaluate the mortality percentages of aluminium phosphide and 20 ppm ozone under different exposure times (30, 45 and 60 minutes) on three of the most dangerous stored grain insect pests, *S. zeamais, R. dominica and T. castaneum*, separately for each insect alone or mixed with corn grains.

MATERIALS AND METHODS

1.Experimental Design:

Three experiments were conducted on *S. zeamais, R. dominica and T. castaneum*, one for each insect species in a randomized complete design (RCD) in factorial arrangement (time and condition) according to the method of Cevallos (2020). The number of treatments for each pest was 8 and implemented as follows: A number of 20 adults of each species (replicate) were exposed to 20 ppm o ozone dose in two conditions under three different times (30, 45, and 60 min.). One condition was insect pests with corn, and the other condition was the insect pest without corn (A total of 6 treatments). Two other treatments were done with phosphine as in ozone treatment. Each treatment was repeated 4 times and the total number of insect species was 1920 individuals.

2. Preparation of The Grain:

The yellow corn kernels were obtained from the San Camilo Grain Plants with free traces of storage insecticides. Firstly, the corn grains were stored in cold rooms (10-12 °C and 55–60% R.H.) to prevent grain infestation. Before placing the grains in the containers; the moisture content was 12.3% as recorded by Steinlite SL-95moister meter.

3. Insect Pests Breeding and Multiplication:

Adults of insect pest cultures were obtained from the San Camilo grain plants and separated into species. A new hundred adults of *S. zeamais*, *R. dominica* or *T. castaneum* were placed separately in 2 L containers supported with 500 g of corn and 50 g of flour which served as an insect food source. The containers were incubated under 27 ± 3°C temperature, 84% R.H., and 12 L:12D photoperiod. The corn grains were weighted using Ker 1000-2-gram scale. (Morales-Velasco, 2019).

4. Distribution of Insects in Each Treatment:

Once the offspring of the insects were obtained, groups of 20 non-sexed adult individuals aged between 30 and 35 days for each treatment were chosen and placed in plastic containers with a capacity of 1 L. The transfer of the 20 insects was manually handled by a plastic spoon to avoid any damage to the insects. Depending on the treatment, 400 g of corn was placed in each container, and grain-free insects were also placed inside the containers.

5. Bioassays of Ozone (concentration and application time):

The ozone was obtained through an ozone generator (1000 mg $h^{-1)}$ Enaly brand, model 1000BT-12. This equipment met the characteristics required to comply with the experiment. The concentration and verification of the dose was carried out with an ozone concentration meter brand Forensics in The Seed Laboratory of Equamic Company to determine the appropriate time for 50% mortality. The dose of 20 ppm of ozone showed more than 50% of mortality after 30 minutes and the exposure times were determined to be 30, 45 and 60 minutes.

5. Application of Ozone:

The ozone generating machine, model 1000BT-12, was used with a 1/4-inch silicone hose and the ozone was allowed to circulate inside the plastic jars with a capacity of 1000 ml. In the bottles corresponding to treatments a dose of 20 ppm was applied in three exposure times 30, 45 and 60 minutes and a wire mesh tape was placed between the lid of the bottle and container to avoid ozone leakage. Each bottle has two holes in the upper part, in one the silicone hose (1/4 of an inch) was placed where the ozone circulated and in the other hole (covered with wire mesh tape) was the ozone detector to verify the dose in each treatment. Through the hole that was made by the ozone dosing, some gas was allowed to escape, in order to release pressure (created by gas) inside the bottle.

The hole where the dosing was carried out gets covered with a gray cloth adhesive tape when it meets the ozone application time in correspondence to application time to prevent the gas from escaping, after 24 hours of having completed the exposure time, the bottles were opened.

6. Application of Aluminium Phosphide (gastoxin) in Bottles:

In order to calculate the dose of aluminum phosphide which can be used, a weight of 400 g of corn and 20 insects of each species were placed in each jar, separately with a total of 240 insects. For one ton of corn six gastoxin tablets are placed and in an empty space, two pills per m^3 are used. The weight of each gastoxin tablet is 3 g. For the calculation of gastoxin in grain mass, a rule of three was made, if in 1´000,000 g of corn 18 g of gastoxin was used, in 400 g of corn 0.07 g was used. In the case of empty space, the following formula was applied to calculate the volume of empty space inside the container.

Data: Radius: 5 cm; Height: 7 cm

Formula: $V = \pi . r^2 . h$ ($V = 3.1416 \times 5^2 \times 7cm$) = 549.78 cm.

If in 100,000 cm^3 of empty space 6 g of gastoxin is applied, in 550 cm^3 of empty space inside the containers 0.04 g was applied. The dose of gastoxin to be applied is 0.07 g in grain mass plus 0.04 g in empty space, a total of 0.11 g of gastoxin in each relative control. In the control bottles, no holes were made for normal conditions, and also gastoxin bottles were to be remained closed for high toxicity evaluation.

***2.7.* The Mortality percentages of insect pests:**

The mortality of insect pests was calculated according to the formula of Duso *et al.* (2008): $\%M = \frac{MFN}{NIV}$

Where:

%M = The percentage of mortality from treatment.

NMF= The number of individuals killed at the end of treatment.
NVI= The number of living individuals at the start of treatment.

8. Data Analysis:

A Generalized Binomial Linear Model (Demétrio *et al.*, 2014) was used for mortality data. A normal mean graph with a simulated envelope with the hnp package (Moral *et al.*, 2017) was used to assess the goodness of fit of the model. When the Deviation Analysis indicated significant differences ($p < 0.05$), the means were compared using contrast models with the glht function of the multi-comp package (Hothorn *et al.*, 2008). All analyses were performed using R software (R Core Team, 2021). Observations of the combination of the Time factor (absolute control and chemical control) with maize and without maize were excluded from the analysis because they did not present variability.

RESULTS AND DISCUSSION

1. Ozone and Mortality of *S. Zeamais, R. dominica,* and *T. castaneum:*

For the three insect pests, there were no significant differences found in the interaction between the exposure time of ozone and insect pests in both conditions with corn and without corn (Table 1& Fig. 1) (p = 0.8931, 0.6436, and 0.741; respectively for *S. Zeamais*, *R. dominica*, and *T. castaneum*). However, mortality percentages were affected by the exposure time ($p < 0.0001$) and condition ($p < 0.0001$).

The highest mortality percentages for the three insect species were obtained with 60 minutes of exposure to ozone, and the least with 30 minutes for both conditions of insect pests with corn and without corn (Table 1&Fig. 1). Among all treatments, at 60 minutes with corn, it has been observed that ozone showed high efficiency with *S. Zeamais* (97.50 ± 1.44), and without corn was (100.00 ± 0.00) with all tested three insect pests. The lowest mortality occurred at 30 minutes in conditions corresponding to corn and without corn with *T. castaneum* (65.00 ± 2.04 and 96.25 ± 1.25; respectively).

The results showed that the effect of ozone on the mortality of the pests can be attributed to the ozone acting in damaging the respiratory system of the target pests. This occurs more when there are increases in temperature because insects increase their respiratory rate which facilitates the entry of gas into their body (Pereira *et al.*, 2008).

The highest percentages of mortality were obtained from experiments when corn was not used. These results could be obtained due to the effectiveness of ozone in producing mortality in insects which is related to stored product characteristics and chemical components of storage structures (Hardin *et al.*, 2009). In addition, since there is no physical barrier to ozone such as corn that absorbs part of the gas, it can penetrate much faster with high quantity through the cuticle of the insect.

Table (1): Effect of ozone concentration [20 ppm] and exposure time on the mortality of S. zeamais, R. dominica, and T. castaneum (Mean ± SE) treated with corn and without corn.

Insect pest& Treatment	Mortality (%) corresponding to Exposure time [Min.] – with corn			Mortality (%) corresponding to Exposure time [Min.] – without corn		
	30	45	60	30	45	60
S. zeamais	68.75 ± 1.25	82.50 ± 1.44	97.50 ± 1.44	97.50 ± 1.44	98.75 ± 1.25	100.00 ± 0.00
***Chemical control**	100 ± 0.00			100 ± 0.00		
***Control**	0.00 ± 0.00			0.00 ± 0.00		
R. dominica	67.50 ± 1.44	81.25 ± 1.25	96.25 ± 1.25	97.50 ± 1.44	97.50 ± 1.44	100.00 ± 0.00
***Chemical control**	100 ± 0.00			100 ± 0.00		
***Control**	0.00 ± 0.00			0.00 ± 0.00		
T. castaneum	65.00 ± 2.04	80.00 ± 2.89	96.25 ± 1.25	96.25 ± 1.25	97.50 ± 1.44	100.00 ± 0.00
***Chemical control**	100 ± 0.00			100 ± 0.00		
***Control**	0.00 ± 0.00			0.00 ± 0.00		

* The data were not considered in the analysis, because they did not demonstrate variability.
* Chemical control: Phosphine toxin-gases

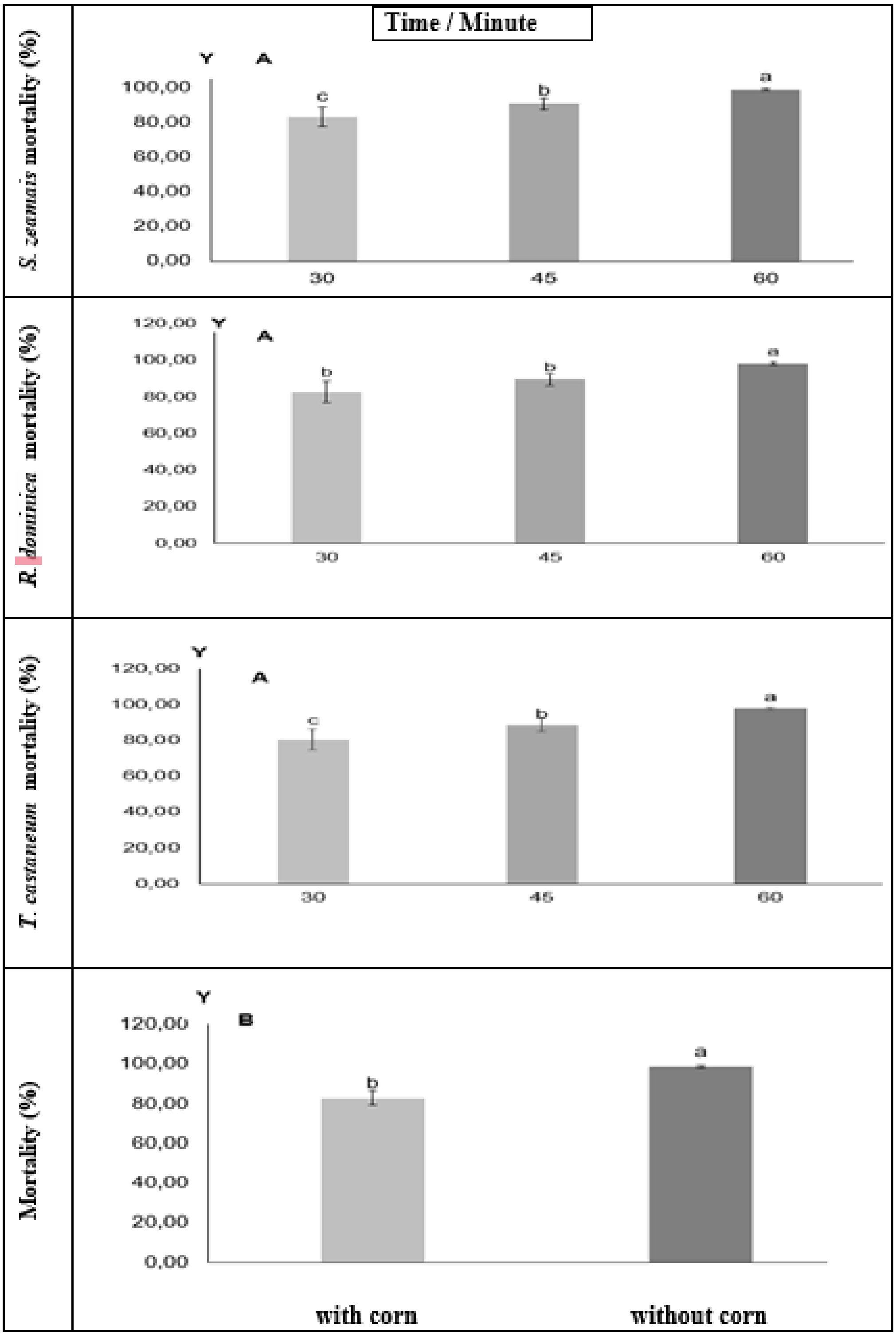

Fig. 1: The mortality percentages of *S. zeamais*, *R. dominica*, and *T. castaneum* caused by ozone at different exposure times (A) and condition (B), columns with the same letters do not present different significance ($p < 0.05$).

The higher percentage of mortality observed in all experiments with the exposure time of 60 minutes may be due to the effectiveness of ozone in producing mortality to insects is closely related to its concentration and the time of exposure (Isikber and Athanassiou, 2015). Additionally, the presence of corn inside the containers caused a decrease in the area of ozone which resulted in a low effect in mortality compared with containers containing insects without corn where the gas could move freely without decomposing managing to produce higher mortality percentages. In this regard, Kells *et al.* (2001) indicates that the ozonation process begins with a first phase, in which ozone degrades by reacting with the inert sites that have the grains on their surface, moving through the grains with a slow movement. Then, the second stage takes place, where the gas flows freely between the grains, decreasing its rate of degradation and having direct contact with pests.

In addition to the reason for the presence of corn in getting low mortality of *S. zeamais, R. dominica and T. castaneum* in containers with corn, also it could be associated with ozone exposure times that were used in the present research, which may be insufficient, for the gas to meet the saturation phase described by Kells *et al.* (2001). This phase is necessary for the gas to move freely and have contact with insects. The dynamics of ozone through grains may be related to the characteristics of grain types, such as their intergranary space, grain temperature and humidity, as well as ozone concentration and flow rate (Isikber and Athanassiou, 2015). According to Kells *et al.* (2001), the reduction of the first stage of ozonation is the key to ensuring the effectiveness of ozone as an insecticide.

Our results are similar to the findings of Solano *et al.* (2017), who found significant differences between the percentage of mortality in empty silos (65.44%) and silos with corn (26%); as well as, between the different exposure times, where mortality increased from 24.2 to 66.6% as the time increased during their research on evaluating the effect of ozone on the adults of cigarette weevil, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), using prototypes of silos with corn and without corn, with a concentration of 20 ppm of ozone during 10, 15, 30 and 45 min. of exposure.

CONCLUSION

Ozone at doses of 20 ppm causes mortality of over 80% for the three insect pests: *Sitophilus zeamais, Rhyzopertha dominica* and *Tribolium castaneum*. The mortality (%) of each insect pest increases as long as the exposure time to ozone increases. The industries of stored grains should be motivated to use ozone as an ecologically safe alternative to chemicals or insecticides for controlling insect pests attacking stored grains to avoid toxic residuals of insecticides and their hazards to human health.

REFERENCES

Cevallos, S. F. (2020). Evaluación del efecto de ozono (O3) en el control de gorgojo Pagiocerus frontalis (F.), en granos almacenados de maíz suave (*Zea mays* L.) en la provincia Cotopaxi, Campus Experimental Salache, Proyecto Granos Andinos Pp. 83.

Demétrio, C. G. B., Hinde, J., & Moral, R. A. (2014). Models for Overdispersed Data in Entomology BT - Ecological Modelling Applied to Entomology (C. P. Ferreira & W. A. C. Godoy (Eds.); pp. 219–259). Springer International Publishing. https://doi.org/10.1007/978-3-319-06877-0_9

Duso, C., Malagnini, V., Pozzebon, A., Castagnoli, M., Liguori, M., & Simoni, S. (2008). Comparative toxicity of botanical and reduced-risk insecticides to Mediterranean populations of Tetranychus urticae and Phytoseiulus persimilis (Acari Tetranychidae, Phytoseiidae). *Biological Control*, 47(1), 16–21.

FAO. (2019). SMIA - Perspectivas de Cosechas y Situación Alimentaria 4, Diciembre 2018.

García-Lara, S, Burt, A. J., Serratos, J. A., Díaz Pontones, D. M., Arnason, J. T., & Bergvinson, D. J. (2003). Defensas naturales en el grano de maíz al ataque de *Sitophilus zeamais* (Motsch, Coleoptera: Curculionidae): Mecanismos y bases de la resistencia.

Garcia-Lara, S., Espinosa, C., Bergvinson, D. 2007. Manual de plagas en granos almacenados y tecnologías alternas para su manejo y control. Handbook (https://repository.cimmyt.org/xmlui/handle/10883/797), CIMMYT, Mexico 1:1

Hardin, J. A., Jones, C. L., Bonjour, E. L., Noyes, R. T., Beeby, R. L., Eltiste, D. A., & Decker, S. (2009). Ozone fumigation of stored grain; closed-loop recirculation and rate of ozone consumption. *Reno, Nevada*, June 21-June 24, 2009, 1.

Hardin, J., Jones, c., Bonjour, E., Noyes, R., Beeby, R., Eltiste, D., Decker, S. (2010). Ozones fumigation of stored grain; closed-loop recirculation and the rate of ozone consumtion. *Journal of Stored Products Research*, 46: 149-154.

Hernández, C., Rodríguez, Y., & Niño, Z. (2009). Efecto del almacenamiento de granos de maiz (zea mays) sobre la calidad del aceite extraido. Información Tecnológica (https://dialnet.unirioja.es/servlet/articulo?codigo=7319620), 20 (4), 21–30.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, 50(3), 346–363. https://doi.org/10.1002/bimj.200810425

Isikber, A. A., & Athanassiou, C. G. (2015). The use of ozone gas for the control of insects and micro-organisms in stored products. *Journal of Stored Products Research*, 64, 139–145.

Kells, S. A., Mason, L. J., Maier, D. E., & Woloshuk, C. P. (2001). Efficacy and fumigation characteristics of ozone in stored maize. *Journal of Stored Products Research,* 37(4), 371–382.

Moral, R. A., Hinde, J., & Demétrio, C. G. B. (2017). Half-normal plots and overdispersed models in R: The hnp package. *Journal of Statistical Software,* 81(10). Error! Hyperlink reference not valid.

Morales Velasco, M. J. (2019). Evaluación de la actividad insecticida de aceites esenciales de menta (*Mentha pulegium*) Y eucalipto (*Eucalyptus melliodora*) para el control del gorgojo de maíz (*Sitophilus zeamais*). Thesis of pregarduation Quevedo-UTEQ (https://repositorio.uteq.edu.ec/handle/43000/3847).

Pereira, A. de M., Faroni, L. R., Sousa, A. H. de, Urruchi, W. I., & Paes, J. L. (2008). Influence of the grain temperature on the ozone toxicity to *Tribolium castaneum*. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 12 (5), 493–497.

Pérez, E. N., Hurtado, P. G., Báez, J. R. C., Torres, B. V., Ruiz, C. R. B., & Herrera-Flores, R. (2010). Utilización de extractos de plantas para el control de gorgojo pardo Acanthoscelides obtectus (Say) en frijol almacenado. *Ra Ximhai: Revista Científica de Sociedad, Cultura y Desarrollo Sostenible,* 6 (1), 37–44.

Rozado, A. F., Faroni, L. R. A., Urruchi, W. M. I., Guedes, R. N. C., & Paes, J. L. (2008). Ozone application against *Sitophilus zeamais* and *Tribolium castaneum* on stored maize. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 12 (3), 282–285.

Zambrano, M. J. L., Yanez, C., Sangoquiza, C. C. A., Limongi, Andrade, R., Alarcon, Cobeña, F., Zambrano, E., Caicedo, V. M. B., Villavicencio, L. J. P., Cartagena, A. Y. E., Parra, R., Azaquibay, I. C. R., Quimbiamba, V., Nieto, B. M. R., Lopez, G. V. A., Tapia, C., Tacan, M., Villacres, P. C. E., Garces, S., Cañarte, Bermudez, E., Pinargote, G. L. F. (2019). Situación del cultivo de maíz en Ecuador: investigación y desarrollo de tecnologías en el Iniap. *XXIII Reunión Latinoamericana Del Maíz y IV Congreso de Semillas*, 1–3.