

Fecundity Dynamics of the Khapra Beetle, *Trogoderma granarium* Everts (Col.: Dermestidae) Under Cold Atmospheric Plasma in Laboratory

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Abstract

The khapra beetle, *Trogoderma granarium* Everts (Col.: Dermestidae), an important warehouse insect pest, responded positively to cold atmospheric plasma under constant environmental conditions. We supposed that 120s plasma treatment acted as a hormesis factor on the beetle's one-day-old larvae that could trigger higher fecundity in subsequent adults than their untreated counterparts. We applied time series analysis looking for expected short-term fluctuations in time series of fecundity due to triggering effect of plasma application against the pest. Moreover, the best combination of autoregressive and moving average processes was chosen to construct the fecundity prediction model. As expected, moving average process could smooth out fecundity data series under plasma effect and among different parameter combinations, first order coupled Autoregressive-Moving average ARMA (1,1) and pure Autoregressive AR(1) models were the best fitted ones to the fecundity data in plasma and control series, respectively.

Keywords: Plasma Jet, Oviposition, ARMA, Moving Average, Hormesis

1. Introduction

The khapra or cabinet beetle with a worldwide distribution is an economic pest of stored products mainly in tropical and semi-tropical regions which originated from ancient stores in southern Asia and then reached to malt industry in Italy and soon after to the US after World War II [1,2]. However, nowadays, this pest is considered as a quarantine species by many countries in particular wheat producer ones [3]. This insect pest feeds under hot and dry environmental conditions on various stored crops such as wheat mostly at larval stage [4].

Plasma is formed after ionizing gas through which active species such as electrons, ions and neutral molecules with disinfection and detoxification properties are produced in both cold and hot types [5-7]. Effects of cold atmospheric plasma on cigarette

beetle, *Lasioderma serricorne* Fabricius (Coleoptera: Ptinidae) by revealed more than 75% sterility after application of 45s cold atmospheric plasma against newly emerged adult beetles [8]. Application of plasma against different life stages of various insect pests' species as a killing technique is tested widely as referenced already in [9]. However, hormesis effects like those observed after chemicals trace or low-dose irradiation in some arthropods has not yet reported in plasma studies [10].

Despite reducing effect of up to 60s cold atmospheric plasma on reproductive potential of the carob moth, *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) which led to considerable collapsed moth' fertility, we noticed that khapra beetle, *Trogoderma granarium* Everts (Col.: Dermestidae), responded positively to 120s cold atmospheric plasma under the same environmental

conditions [9]. Due to larval diapause and cryptic activity of the beetles inside hidden microhabitat in stores, applicability of either chemical or non-chemical strategies against this pest toward its eradication is not highly promising; however, modified atmospheres through variations in chemicals like CO₂ showed good results [3].

Therefore, application of cold atmospheric plasma technology specially using room scale electrodes with periodic pulses in warehouses toward eradication of local small population of the pest could be an outstanding density-independent technique in future through either direct killing or manipulating the atmosphere of the stores and production of more destructive elements to the beetles in the environment. Accordingly, as usual, escaped individual or less treated insects could respond differently to trace amount of every treatment that can reverse the results of any management strategy. We supposed that 120s plasma treatment acted as a hormesis factor on the beetle's first instar larvae that could trigger higher fecundity at their adulthood than their untreated counterparts. Therefore, we applied time series analysis looking for expected short-term fluctuations in time series of the fecundity due to triggering effect of plasma application against the pest. Complementarily, the best combination of autoregressive and moving average terms was chosen to construct the fecundity prediction model that could be

taken into account in plasma-based management programs against this pest in future.

2. Materials and Methods

2.1. Insect Rearing

A small colony of the *T. granarium* was obtained from University of Tehran and the beetles were reared on broken walnuts (with shells) under constant environmental conditions of 25 ± 1 °C, $35 \pm 5\%$ RH and photoperiod 16/8 (L/D). Adults emerged in the rearing cages were collected by an oral aspirator and then transferred to the egg laying transparent glasses on a piece of paper (4 cm diameter \times 10 cm height). Newly deposited eggs were collected daily for either continuing the rearing procedure or conducting the experiments.

2.2. Plasma Treatment

The cold atmospheric plasma based on gliding arc discharge (GAD) was applied following the procedure described in [9]. The atmospheric cold plasma based on GAD after application of high electric potential between two metal electrodes produces strong sparks. The flow of air expands on the electric discharge zone that prevents the electric discharge zone from overheating. In this experiment, a power supply with 0-15kV voltage and 50 kHz sine frequency was used to produce the discharge (Figure 1).

GAD plasma

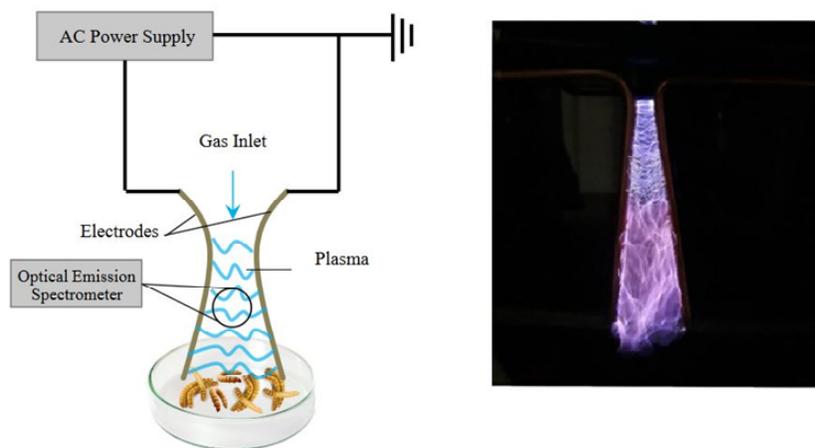


Figure 1: Atmospheric Cold Plasma Set Up for Direct Exposure of the Larvae of the *T. granarium*. Left and Right Show Schematic and Real Plasma Environments Used in this Research, Respectively

Forty 1-day-old larvae were treated inside plasma environments simultaneously exposed directly to sparks and the time of exposure was controlled by a stopwatch manually (Figure 1). Different plasma treatments of 20, 60, 120 and 180 seconds were applied and soon after the treated larvae were transferred into a 15 cm diameter Petri dish containing artificial diet.

2.3. Study of Biological Parameters of the Pest Under Different Plasma Treatments

Along emergence of adults in plasma and control cohorts, males

and females were gradually transferred to small cylindrical cages (4 cm diameter \times 10 cm height) for mating and laying eggs till the death of the last female was recorded daily. Pre-tests on 20 first instar larvae in 20, 60, and 180s plasma treatment showed that 20 and 60s had no effect on the immatures' biology and all became adults easily. However, in 180s treatment, larval development time prolonged to more than 3 months and more than 7 molting events were observed before pupation. To study population characteristics and avoid long lasting larval period, times between 60 and 180s were applied. Hatching rate of plasma treated eggs was almost zero

and so first instar larvae were taken as the experimental insects. Also, due to hidden activity of the experimental larvae inside diet, immature stage durations were not recorded. Accordingly, population rate of increase r , was calculated by equation (3).

3. Statistical analysis

The repeated-measures ANOVA procedure using AnovaRM () function was performed to detect statistical difference of fecundity and r between plasma and control treatments. 40 first instar larvae were studied in each treatment until the death of the last female. As oviposition behavior has periodicity of one generation, without loss of generality, we assumed that the pattern recognized for the current generation would be nearly the same for subsequent generations just to visualize the current generation pattern more robustly. Therefore, we used the current data as pseudo-values for the next three generations, yet no conclusion was made about those later three. Seasonality and potential moving average were detected using the autocorrelation (ACF) and partial autocorrelation (PACF) functions plot focusing on the first quarter of the plots related to real data series. Augmented Dickey-Fuller (ADF) test was used to examine stationarity in both plasma and control time series data. After assessing ACF and PACF, autoregressive (AR) and moving average (MA) processes were estimated and various formulae of ARMA were fitted to the data looking for the best predictive one. Time series models were developed to explore the long-term consequences of plasma effect on fecundity dynamics and have the form of equation (2) where r is as equation (1),

$$r = \ln \left(\frac{N_{t+1}}{N_t} \right) \quad (1)$$

$$r(t) = f(X)(\pm \epsilon t) + g(Z) \quad (2)$$

$f(X)$ is a function for density dependence, $g(Z)$ describes density independent process, and exogenous component ϵt was modeled as a random process [11]. Here, AR(P) was written using backward operator B as equation (3)

$$\phi(B) \times X_t = Z_t \quad (3)$$

where B acts on time index as equation (4).

$$B \times X_t = X_{t-1} \quad (4)$$

Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used to compare models' fitting capability. All statistical procedures were carried out using Python 3.11.7 packaged by Anaconda (Jupyter notebook 7.0.8) and some plots are prepared in Microsoft Office Excel.

4. Results and Discussion

Application of 120s cold atmospheric plasma against 24h old eggs of the beetle led to high mortality of the insects and just one adult beetle emerged out of 40 eggs as the initial cohort (unpublished data). Therefore, as mentioned above, first instar larvae were picked as the experimental insect stage to be treated under such plasma conditions. Results showed increased total fecundity of plasma treated insects in comparison to the control one ($F_{10,10} = 2.6227$, $p < 0.0721$) (see Figure 2A, and B for trends of fecundity).

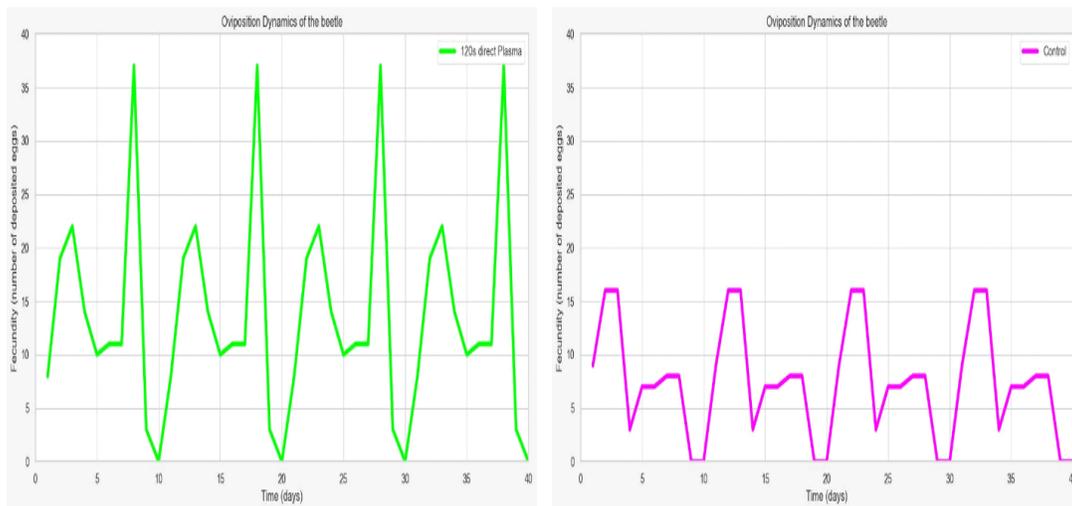


Figure 2: Time Series Plots of the *T. granarium* Fecundity Under Plasma (a) and Control (b) Treatments.

This observation could be dedicated to the triggering effect of cold atmospheric plasma as a hormesis mechanism against this pest; however, complementary experiments are needed to be done to prove this hypothesis. Moreover, population growth rate of the

beetles r in plasma treatment was not significantly different from the control value ($F_{7,7} = 1.3564$, $p < 0.3489$) (Figure 3A, and B for trends of r).

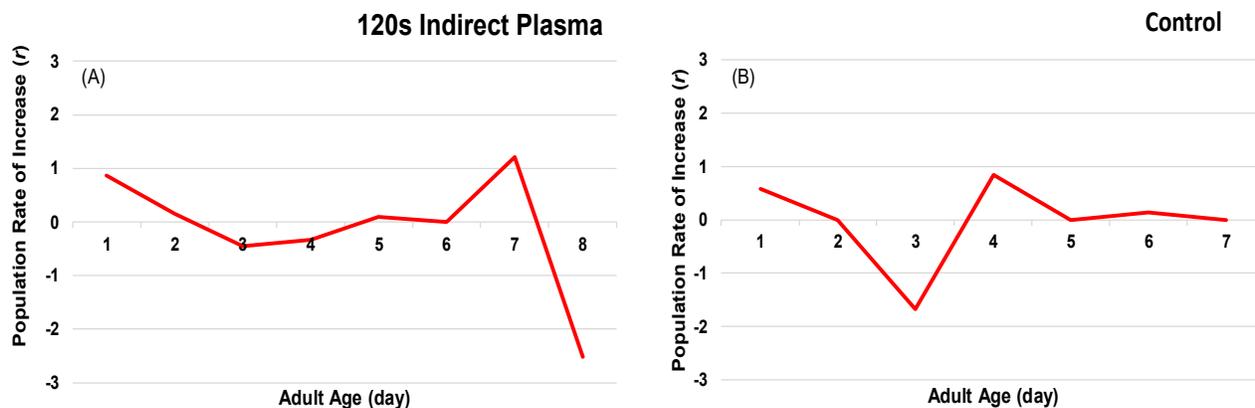


Figure 3: Daily Dynamics of Population Growth Rate r of the *T. granarium* in Plasma (a) and Control (b) Treatments

Through the experiments, deformed eggs (Figure 4A) were observed repeatedly especially in treatments longer than 120s and

all were discarded throughout data series and normal eggs (Figure 4B) were examined throughout.

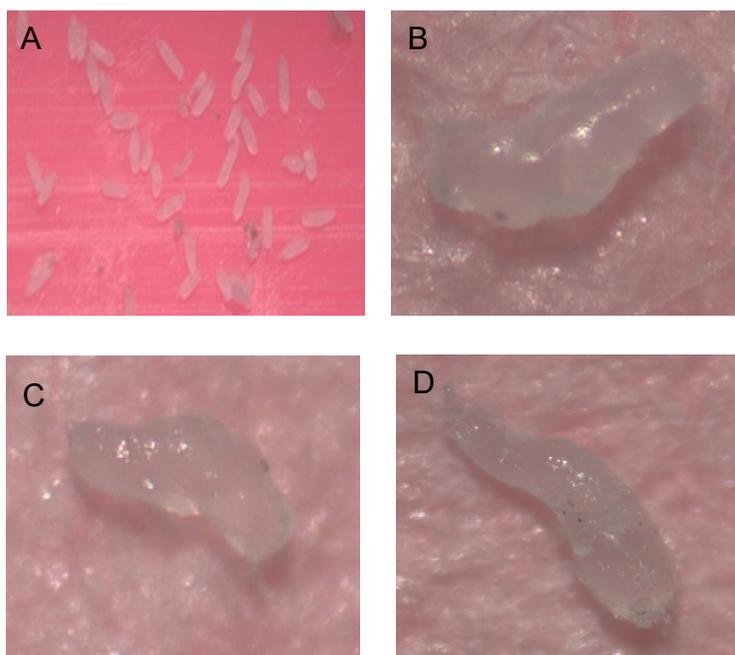


Figure 4: Normal (A) and Three Deformed Eggs of *T. granarium* (C, D, and E) Observed in 120s Plasma Treatment

However, observed deformed eggs in higher exposure times could promise probable control of the pest by more powerful plasma and should be examined in future studies. Augmented Dickey-Fuller (ADF) test showed that both time series of fecundity in plasma and control treatments were stationary and did not need differencing or any kind of transformation (ADF Statistic $\sim -767e+10$; p-value: 0.00; ADF Statistic $\sim -4864e+10$; p-value: 0.00, respectively). Autocorrelation functions (ACFs) of both

plasma and control series showed stationarity and seasonality (Figure 5A, and C). Significant early lags in ACFs imply possible first order autoregressive AR process for both series. In order to recognize the best experimental models, partial autocorrelation functions (PACFs) were also depicted. As can be seen, early lags of 2 to 4 are significant and so PACFs imply possible first order moving average MA (1) process in plasma series (Figure 5B).

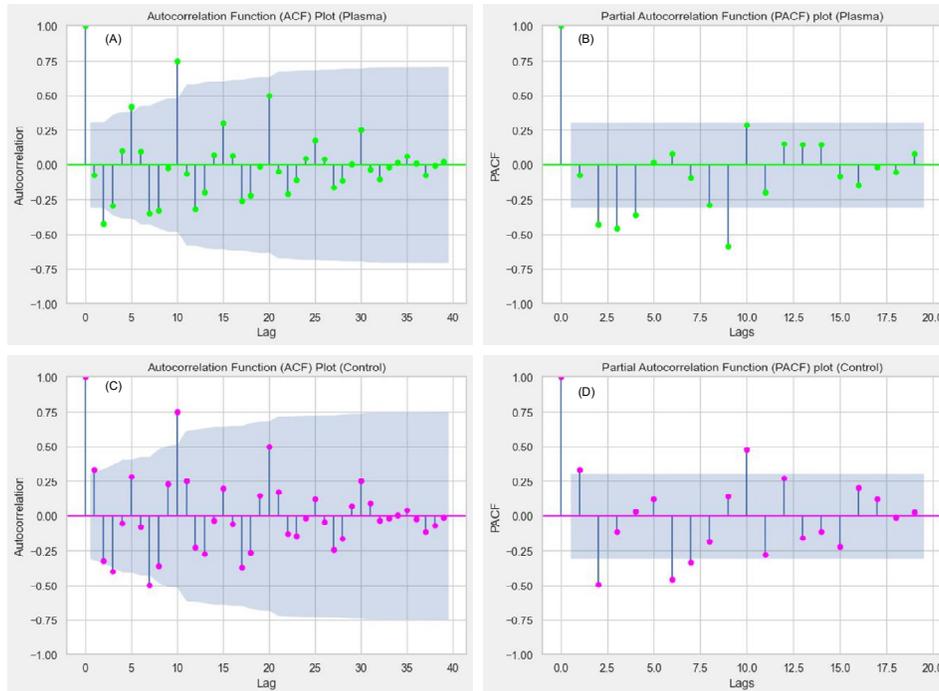


Figure 5: Autocorrelation Functions (ACF) (A, and C) and Partial Autocorrelation Functions (PACF) (B, and D) of the *T. granarium* Under Plasma and Control, Respectively.

Among different parameter combinations for ARMA, (p,q)=(1,1) was the best fitted to the plasma data series (Table 1).

ARMA results for plasma model (1,1)						
	Coeff	SE	z	P > z	[0.025	0.975]
AR(1)	1.000	0.000	4456.281	0.000	1.000	1.000
MA(1)	-0.9983	0.229	-4.355	0.000	-1.448	-0.549
Sigma2	102.9995	0.002	4.62e+04	0.000	102.995	103.004
AIC	309.096					
BIC	314.163					

Table 1: Arma Model Results Fitted to the *T. granarium* Fecundity Time Series in Cold Atmospheric Plasma Treatment

Accordingly, the best model fitted to the fecundity time series under plasma effect was evaluated as equation (5).

$$(1 - B) \times \ln(x_t) = 102.995 + (1 + 0.9983 \times B) \times z_t \quad (5)$$

Among different parameter combinations for ARMA, (p,q)=(1,0) was the best fitted to the control data series. However, moving average term was not significant in the control series as expected and so was removed from the model (Figure 5D) (Table 2).

ARMA results for control model (1,0)						
	Coeff	SE	z	P > z	[0.025	0.975]
AR(1)	0.7708	0.091	8.431	0.000	0.592	0.950
Sigma2	32.4343	10.599	3.060	0.002	11.661	53.208
AIC	257.589					
BIC	260.967					

Table 2: Arma Model Results Fitted to the *T. granarium* Fecundity Time Series in Control Treatment

Accordingly, the best model fitted to the fecundity time series under control treatment was evaluated as equation (6).

$$(1 - 0.7708 \times B) \times \ln(x_t) = 32.4343 + z_t \quad (6)$$

Figure 6A, and B show the ARMA model predictions based on the data series of the beetle fecundity in plasma and control treatments, respectively. Clearly, both models are predictive satisfactorily in comparison to real data series. Figure 6A shows that moving average process successfully described instantaneous effect of plasma species produced in 120s on the fecundity dynamics of the pest.

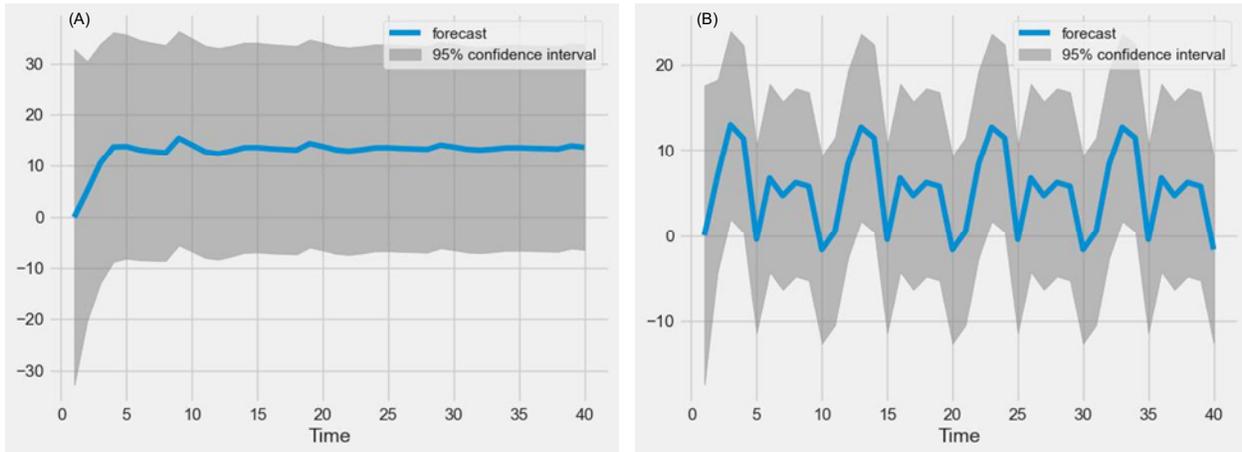


Figure 6: ARMA Simulation of (1,1) and (1,0) Models Fitted to Data Series of the *T. granarium* Fecundity Under Laboratory Conditions

Literature review showed that hormesis effect of cold atmospheric plasma has not been reported in any arthropod; however, all relevant studies suggest collapsing effect of this technique on the reproduction of the experimental insects. In particular, various coleopteran insect species that are important economically in warehouses such as *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), *T. confusum* Jacqueline du Val. (Coleoptera: Tenebrionidae), and *Lasioderma serricornis* Fabricius (Coleoptera: Ptinidae) are easily controlled by the means of plasma technique equipped with single or mixed gases such as argon, oxygen and nitrogen [8,12-14].

Trace amounts of chemicals are proved to be effective in promoting reproductive reactions in many arthropods; phenomenon known as hormesis or induced hormesis/hormoligosis [15]. Production of various chemical species inside plasma jet chamber and their stochastic contact with the beetle larvae could be considered as a process after which subsequent emerged adults are capable to reproduce more in comparison to their untreated counterparts. However, the most probable candidate from these species and the mode of their action needs to be studied in details.

At first glance, high energy electrons at either low contact frequency or low production i.e. in trace amounts could be the best nominate as a stimulant of some cells in the larval body due to their penetration ability inside the bodies; however, simultaneously, these electrons could be the only and worthy candidate as sterilizing agent against other insects' species like moths who are maybe more susceptible

than the beetles [9]. In conclusion, our study revealed that despite killing effect of the cold atmospheric plasma on the eggs of the *T. granarium*, larval treatment could enhance the reproduction of emerged adults to some extent; therefore, assessment of the aforementioned plasma technique in warehouses against this insect pest species should be taken with caution.

Fecundity dynamics were not mainly identical under both plasma and control treatments. For both treatments, dynamics were mainly governed by a delayed density dependent process AR (1) which is expected. However, when plasma acted, the fecundity showed nearly different pattern under this treatment and a moving average process MA(1) was needed to be added to the model that subsequently smoothed out fluctuations and was proved to be constructive in making a predictive ARMA model. This model predicted long-term behavior of the plasma-treated fecundity at higher general equilibrium states than the fecundity in control state.

5. Acknowledgement

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6. Disclosure Statement

All authors claim that there is no conflict of interest to declare.

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