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Efficacy of Modified Atmosphere and Thermal Treatments for Disinfestation of Storage Pests Infesting Walnut Kernel

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Abstract—*Walnuts are a potential export commodity from India. About 2,665.87 MT of walnuts worth of Rs.136.45 crores were exported during 2014-15. However, the major concern in their production, storage and marketing is pest infestation. Many fumigants have so far been used for the post-harvest management of stored pests infesting walnuts. But due to restrictions in their use and environmental concerns there has been a need to develop ecofriendly and non-chemical approaches. These comprise high temperature and modified atmosphere (MA) treatments. Disinfestation using MA involves the alteration of the atmospheric gases such as CO_2, O_2 and N_2 to render the atmosphere in the stores lethal to pests. A bioassay was conducted to investigate the efficacy of all MA treatments on the adults of important pests of walnut viz, Cadra cautella, Tribolium castaneum, Trogoderma granarium and Oryzaephilus surinamensis. Exposure of walnut kernel infested with adults of these storage pests were subjected to MA treatments of different gas compositions viz., MA_1 ($40\%CO_2+60\%N_2$); MA_2 ($50\%CO_2+50\%N_2$) and MA_3 ($60\%CO_2+40\%N_2$) at two temperatures 28 ± 1^0C & 35 ± 1^0C. The treatment mortality data was then subjected to probit analysis to achieve time of 99% kill (LT_{99}). The comparisons were made on the basis of LT_{99} values, the susceptibility of all the test insects to the MA treatments tested showed the following trend: C. cautella< O. mercator< T. castaneum< T. granarium. The times needed to achieve 99% kill (LT_{99}) of the least tolerant insect species i.e. C. cautella treated with three MA treatments at 28^oC were 21.07,12.57 and 15.47 h, respectively, whereas at 35^oC LT_{99} values were 19.09, 10.77 and 9.34 h, respectively.*

Keywords*: Disinfestation, Walnut kernel, Modified Atmosphere, Storage pests, Thermal treatments.*

1. INTRODUCTION

Among all nuts, Walnut (*Juglans regia)* is an excellent example of non-utilization of existing resources, is highly nutritious and has a very good export potential and long shelf life. It accounts for 4.7% share among the major horticultural commodities exported from India. Approximately 8,000 MT dried in-shell walnuts worth US$ 270,000 is annually exported from India with major destinations being Spain, UK, Egypt Germany, USA, the Netherlands and France **[8].** Generally, 45-50 percent of the production is consumed domestically and the remaining is exported. More than 95 percent of the walnuts are exported as kernels viz., 40 percent light halves; 40 percent amber halves/ light broken and the balance as amber halves **[2].** However, insect-pests are important constraints encountered during their storage and marketing.

Seventeen pests are reported to affect walnut during storage worldwide, of which nine are reported from India **[5].** The economically most significant of these pests include almond moth, *Cadra* c*autella* (Walker) (Lepidoptera: Pyralidae); red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae); grain beetle *Oryzaephilus mercator* (Fauvel) (Coleoptera: Silvanidae), and khapra beetle, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae). These storage pests cause direct damage to walnut kernel and thereby reduce the product quality through larval feeding and webbing, contaminate with faecal matter, mold growth and causes degradation of product. Thus, the post-harvest management of walnuts is essential for it

s procurement for longer periods and is also essential to meet the phytosanitary requirements of importing countries viz., Spain, Germany, UK, Egypt and the Netherlands. In view of the emerging trend of non-chemical approach to integrated pest management, which excludes all kind of chemical pesticides, Modified Atmospheric (MA) present a viable and more desirable option to solve the problem of storage pest infestation. Numerous workers **[3, 7, and 8]** extensively investigated the effect of various combinations of atmospheric gases on stored product insects. Different scientists tried different gases like high N_2 concentration, increased CO_2 concentration (hypercarbia) and (reduced O_2 concentration (anoxia). Carbon dioxide was found to be more effective for MA than N_2 because CO_2 stimulates insect respiration while displacing O_2 **[4]** with N_2 kills insects by asphyxiation. In view of this efficacy of different compositions of modified

atmosphere (nitrogen and carbon dioxide) were studied against the adults of *C. cautella*, *O. mercator*, *T. castaneum* and *T. granarium* infesting walnut kernels.

2. MATERIALS AND METHODS

The MA facility at Division of Post-Harvest Technology, IARI, New Delhi was used and different gas compositions tested were MA_1 (40%CO_2+60%N_2); MA_2 (50%CO_2+50%N_2) and MA_3 (60%CO_2+40%N_2) respectively. Specially designed exposure containers were prepared for conducting experiments. Glass containers of 1 litre capacity were used and arrangements were made for inlet and outlet of gasses through silicon tubing of o.d. 6 mm and i.d. 4 mm.and to make them gas tight. To make them gas tight, suba seal stopper of o.d. 18 mm was fitted in the middle of the cap of the containers with the help of driller for taking gas samples. The micropipettes were used of 1 ml capacity and are burnt at the tips to close their ends in order to insert at the ends of the inlet and outlet at the time of exposure.

For each species, 200gm of walnut kernel was infested with 30 adults per replicate were used. Four replicates for each level of MA and exposure period with an equal number of untreated controls.

The required percentage of gas mixtures (CO_2 and N_2) was blended in the blender and flushed into the exposure containers and simultaneously tested for gas concentration by using CO_2 and O_2 analyzer (PBI Dansensor: Model-Checkmate II). After the desired concentration was reached, gas in and gases out tubes were held tightly with the help of pinch cocks. After the completion of the treatment, CO_2 and O_2 concentrations were once again checked for gas retention. Gas retention was checked after every 2 h till 24 h.

After treatment the exposure containers were placed in a BOD incubator at temperatures of 28± 1^{o}C & 35± 1^{o}C and R.H. 60-65% for the respective exposure periods from 2-24 h. Mortality was assessed just after the treatment and till 24 h to ensure no survival. Percent mortalities were determined within a range of 2h to 24h exposure time.

2.1 Insect Mortality Assessment

The samples infested with adults for different durations from 2- 24 h were observed for their survival, moribund state and dead, immediately after the exposure period and at regular intervals till 24h to ensure complete mortality with no survival. Mortality percentage of each stage was calculated as the percentage of dead insects relative to total treated insects for each exposure period.

2.2. Statistical Analysis

Treatment mortality was corrected for control mortality using the Abott (1925) formula. After calculating the corrected mortality for time–mortality responses, treatment means were subject to probit analysis of SPSS version 14.0. Data was analyzed as log-transformed dosage mortality regression lines in order to calculate the regression estimates and time to achieve 99 per cent mortality (LT_{99}).

3. RESULTS AND DISCUSSION

The adults of four test insects viz., *C. cautella*, *O. mercator*, *T. castaneum* and *T. granarium* tested were subjected to four MA treatments namely: MA_1: 40% CO_2 and 60% N_2, MA_2: 50% CO_2 and 50% N_2 and MA_3: 60% CO_2 and 40% N_2 at 28^{0}C and 35^{0}C. The results of the observed mortalities were subjected to probit analysis in order to calculate LT_{99} values. The comparisons were made on the basis of LT_{99} values at both temperatures.

The LT_{99} and 95% fiducial limits at 28± 1^{o}C are presented in Table 1 and at 35 ± 1^{o}C are presented in Table 2.

Table 1: The effect of different MA treatments on mortality as expressed in LT_{99} (hours to obtain 99 % mortality) (Fiducial limits)a at 28± 1oC

Species of the stored pest	MA1 (40% CO2 +60% N2)	MA2 (50% CO2 +50% N2)	MA3 (60% CO2 +40% N2)
C. Cautella	21.07 (18.38-25.30)	12.57 (10.58-16.55)	15.47 (12.74-20.21)
O. mercator	23.50 (20.92-27.14)	19.24 (15.85-26.32	16.53 (13.75-22.51)
T. castaneum	302.45 (145.99-1735.64)*	721.78*	43.84 (33.78-68.96)
T. granarium	89.18 (72.48-119.07)	45.67 (35.82-65.84)	48.60 (33.67-69.84)

[a]Fiducial limits (LFL-UFL) were calculated at P ≤ 0:05 level.
*Wide confidence limits

Table 2: The effect of different MA treatments on mortality as expressed in LT_{99} (hours to obtain 99 % mortality) (Fiducial limits)[a] at 35 ± 1^{o}C

Species of the stored pest	MA1 (40% CO2 +60% N2)	MA2 (50% CO2 +50% N2)	MA3 (60% CO2 +40% N2)
C. Cautella	19.94 (16.54-25.40)	10.77 (7.84-19342)	9.34 (8.94-11.54)
O. mercator	20.49 (15.82-33.43)	12.49 (11.16-15.08)	11.98 (10.09-16.42)
T. castaneum	371.71*	416.28*	32.34 (28.43-39.05)
T. granarium	4.14 (3.68-8.87)	15.82 (5.98-25.33)	38.09 (24.08-238.83)

[a]Fiducial limits (LFL-UFL) were calculated at P ≤ 0:05 level.

The close scrutiny of the result shows that the times required to achieve 99% kill (LT_{99}) of the least tolerant insect species i.e. *C. cautella* treated with three MA treatments at 28^{o}C were 21.07,12.57 and 15.47 h, respectively, whereas at 35^{o}C LT_{99} values were 19.09, 10.77 and 9.34 h, respectively. These comparisons based on LT_{99} values show the following trend of susceptibility of test insects: *C. cautella*< *O. mercator* < *T. castaneum*< *T. granarium.*

It was also observed that more the per cent of CO_2 and less was time taken for mortality of the stored pests **[5]**. In general, it was observed from the present study that as the temperature increased the mortalities increased and vice-versa. Published information **[1, 10]** on the effect of MA on different storage pests also supported the results that at a given atmospheric gas composition, the higher the temperature, the shorter will be the exposure period needed to achieve same level of insect mortality.

4. CONCLUSION

The effect of modified atmosphere treatments was more pronounced at high temperatures and the percent of CO_2 in MA treatment is very crucial for the quick knockdown of the walnut stored pests under study.

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