

Laboratory Studies on the Effect of Nitrogen gas (N₂) on Mortalities of the Cowpea Weevil, *Callosobruchus maculatus* (F.) (Coleoptera : Bruchidae) in Iraq *

Hussam Aldin A. Mohammed¹, Maha T. AL-Ani²

Dept. of plant protection, College of Agriculture, University of Baghdad, Iraq

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Abstract— A laboratory studies were conducted at the college of Agriculture, University of Baghdad to investigate the effect of nitrogen gas (N₂) at low pressure of 160 and 180 mm mercury and a nitrogen gas (98.5%) on mortality rates of different developmental stages of the cowpea weevil, *Callosobruchus maculatus* (F.) from egg to adult, with different exposure periods of 1, 5, 6 days. Results indicated that egg, larval, pupal and adult mortalities increased with increasing exposure periods, the complete mortality rates for all stages were achieved after 5-6 days. However, these treatments have no significant effect on seed germination, The use of these information in control programs for this pest, also discussed.

Keywords— cowpea weevil, *callosobruchus maculatus* (F.), Physical control, Nitrogen gas, Low pressure, Iraq.

I. INTRODUCTION

Cowpea, *Vigna unguiculatis* important grain legume grown in many parts of the world. It has a high nutritive value of protein ranged from 17 – 34 %. As well as fat, carbohydrates and minerals such as iron, calcium and phosphorus (Al-qaisi, 2000; Shaw, 2007). Cowpea may be infested by different insect pests causing considerable damage and considered to be one of the limiting factors in cowpea production. The cowpea weevil, *C. maculatus* is a key pest of grain legumes in food stores, it has a wide host range including cowpea, chickpeas, lentils, peas and beans (Aslam et al. 2002). Infestation began in the field when infested seeds were harvested and stored, the insect continues to feed as a hidden infestation and emerges as an adult causes secondary infestation and a total destruction to seeds and decrease their germination (Bhall et al., 2008).

Pesticides and fumigants have been extensively used all over the world to control legume grain infestation. However, chemical methods are being restricted globally

because of toxic residue and their adverse effect on environment and human health (Bhalla et al. 2008).

Different biological control methods such as, cultural, physical and biological were used by various workers (Van, 2011; Ismail, 2006; Trioesle et al., 2015 and Yamane, 2013). The use of controlled atmosphere vacuum, heating, colling and sterilizing, requires relatively advanced techniques (van, 2014).

This paper was aimed to find an alternative, ecofriendly method to insecticides and fumigants used in the present time by using nitrogen gas for the control of this pest in legume grain stores.

II. MATERIALS AND METHODS

This study was conducted in the insect research laboratory at Dept., of Plant Protection, College of Agriculture / University of Baghdad. Different stages of Cowpea weevil, *Callosobruchus maculatus* (F.) were obtained from a laboratory colony reared on cowpea seeds for this purpose and maintained under control conditions of 26 ± 2°C and humidity of 70 ± 5% in the incubator. Infested cowpea seeds were prepared by releasing 1-day old adults (10 pairs for 100 seeds) for a period of 24 h for oviposition / egg laying in culture jars. Experiment was carried out using cowpea seeds infested with different developmental stages of *C. maculatus*. As the development occurs inside seeds, these comprised of egg stage 1-day after oviposition, larval -19 days after oviposition, pupal stage -22 days after oviposition and 1-day old adults (Bhalla et al., 2008). And 10 seeds of cowpea infested with each of the development of stage of *C. maculatus* per replicate were spread in the base of petri dishes were exposed to N₂ Gas at low pressure levels (150 and 180 mm Hg) in the anaerobic bacteria breeding container capacity of (1-2) Kg. All development stages were exposed to N₂ gas (98.5%) for different periods of 1, 5, 6 days then the insect were transferred to petri dishes (9 cm) covered with muslin cloth and closed

tightly with rubber bullets and then placed in the incubator at a temperature of $26 \pm 2^\circ$ and a relative humidity of $70 \pm 5\%$. The experiment were laid out according to Complete Randomized Design (CRD) and 3 replicates for each treatment. Healthy cowpea seeds were also subjected to these doses treatments as above and to germination test. The effect of cumulative impact of N₂ gas on different developmental stages on the percentage of mortality rates of eggs, larva, pupa, and adults were calculated. Data were analyzed using SAS program (2012). All the comparisons was considered significant when $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Results in Tables 1 revealed that their were a significant differences in the mortalities of egg, larva, pupa and adults in the tretments of N₂ gas under low pressure of 150 mm Hg and control treatment. Mortality rates of 90, 66.66, 73.33 and 93.33 % respectively were achived after 24 hr from exposure to N₂ gas. Its obvious that eggs and adults were more sensitive to this treatment more than larvae and

pupal stage during the first 24 hr. However, complete mortalities of developmental stages from the egg – adult were reached after 5 – 6 days from exposure. These results agree with that of Ismail (2006) who found that the adult, stage of the cowpea weevil, *C. maculatus* was the most sensitive followed by the egg stage, larva, then pupa, and complete mortalities of all these stage were obtained after 6 days from exposure to N₂ gas and carbon dioxide at low pressure (160 mm Mercury). This results also conside with Al-Hadidi (2002) that the adults stage of the flower beetles, *Tribolium castenum* evertsand *Trogoderma granariumis* the more sensitive to N₂ gas under low pressure followed by eggs, larva and pupa respectively.

Table.1: Effects of exposure to N₂ gas on the different development stages of cowpwa weevil, *C.maculatus* under low pressure of (150 mm Mercury)

Exposure period (days)	%egg mortality	%larval mortality	% pupal mortality	%Adult mortality
Control	0.00	0.00	0.00	0.00
1	90.00	66.66	73.33	93.33
5	100.00	96.66	100.00	100.00
6	100.00	100.00	100.00	100.00
L.S.D	9.41	7.68	5.43	5.43

Results in Table (2) show a significant differences in the mortalities of all developmental stages of the cowpea weevil, *C. maculatus* from egg to adult in the treatments of N₂ gas and low pressure of 180 mm mercury and the control treatments. Mortalities of 93.33, 76.0, 93.0 and 96.0 were obtained after 24 hr from the exposure to N₂ gas for the eggs, larva, pupa and adults respectively. Mortality rates were increased for all developmental stages by the increasing pressure from 150 mm mercury to 180 mm mercury during the first 24 hr from the treatment. However, Their were no significant effect was

observed from the previous experiment above when exposure poroid increased to 5 and 6 days, as a complete mortalities of all developmental stages were obtained. It seems that amount of pressure is the effecting factor which may speed the rate of mortality than the exposure time which need a further investigation in the future. Mbata (1994) observed the same results that when *C.subiunotatus* and *C.maculatus* were exposed to an atomosphere saturated with carbon dioxide and a temperature of 32°C and relative humidity of 70 %

Table.2: Effects of exposure to N₂ gas on different developmental stages of cowpea weevil *C. maculatus* under low pressure of (180mm mercury).

Exposure period (days)	%egg mortality	%larval mortality	% pupal mortality	%adult mortality
Control	0.00	0.00	0.00	0.00
1	93.33	76.66	93.33	96.66
5	100.00	100.00	100.00	100.00
6	100.00	100.00	100.00	100.00
L.S.D	5.43	5.43	5.43	5.43

Results in Table (3) indicated that there were no significant differences in the percentage of cowpea seeds germination between the cowpea seed exposed to N₂ gas from (1 – 6) days and the control treatment under low pressure of 150 and 180 mm mercury

Oyebanji et al., (2015) found that using the atmospheric pressure, a high seed germination rate up to (88.2%) during the storage and noticed that the longer the seed storage period, the lower the germination rate.

Table.3: Effects of N₂ gas on the germination of cowpea seeds

Treatment (Bar)	Period (Day)	% of cowpea seed germination
Control	1-6	91
5	1	93
	5	95
	6	98
L.S.D		7.19
10	1	95
	5	98
	6	100
L.S.D		7.42

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