

CONTROL OF *SITOPHILUS ORYZAE* (L.) BY MALATHION AND TOW OF THE INERT GASES IN COMPARATIVE STUDY

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ABSTRACT: This study was conducted in the laboratory pests of stored product to evaluate and compare the efficiency of one of the most known pesticides (malathion) as a chemical protectant for stored grains and the controlled atmosphere method using CO₂ or N₂ gases against the rice weevil, *Sitophilus oryzae* (L.). The obtained results could be summarized as follows :

- 1.The tested concentrations of malathion (2, 3, 4, 5 and 6 ppm) caused different levels of mortality of *S. oryzae* adults exposed to treated wheat grains. The percentages of mortality after 4 days were 84, 88, 92, 97 and 98% for 2, 3, 4, 5 and 6 ppm., respectively.
- 2.CO₂ pure had induced 100% mortality for adults after 1 day of exposure and after 2 days for eggs and larvae and after 4 days for pupae. The adults were the most susceptible stage to pure CO₂, while pupae were the most tolerant to the gas under laboratory conditions.
- 3.Carbon dioxide was more effective at the highest temperature (30°C) than at the lowest one (20°C).
4. The residual effect of malathion treated grains after one month were 40, 66.47, 51.23 and 100% mortalities for eggs, larvae, pupae and adults, respectively. After two months the mortalities were 62.57, 89.35, 80.21 and 100% for the different stages of the same order. The mortality percentages due to both gases separately were 100% for all tested stages after one or after two months of tightly storage.

Key words: Inert gases, malathion, *Sitophilus oryzae*, modified atmosphere, control.

INTRODUCITON

Attention has been focused on the problems arising from the wide use of chemical pesticides for pest control (specially of stored products) by the authorities concerning the public health and environment. World Health Organization (WHO) and Environmental Protection Agency (EPA) who emphasized upon using of alternative methods for pest control. Malathion was one of the most chemical protectants which widely recommended in many parts of the world against insect pests of stored products. Now a days consumers expect pesticides residue free food products or with much reduced residue levels. Modified atmospheres have an important role in integrated pest management systems that are pesticide free (Banks *et al.*, 1991). Many studies have been conducted to control the stored product pests by using this gas singly or in mixtures with oxygen and nitrogen (Hashem, 2000). The action of CO₂, as inert gas, lies in its availability, relative convenience, and safety of application beside the fact that it does not leave toxic residues (Jay, 1980 and Aamir, 2006).

The present work deals with the efficiency of CO₂ against the different stages of the rice weevil,

S. oryzae in comparison with malathion as the most known chemical protectant against stored product insect pests.

MATERIALS AND METHODS

Rearing Technique of Test Insect

The original cultures of the rice weevil, *S. oryzae* were started through batches of adults collected from infested stored grains.

Wheat grains variety Sakha 93 were used as a rearing host. Fresh wheat grains were firstly examined to insure and free of toxic residues then it were tightly packaged in plastic bags and kept at -13 °C. in a deep freezer for 48 hr. at least to kill insects and mites that might be present. The kernels were conditioned with respect to the water contents to be about 12% before starting the rearing. Dry yeast was added to the wheat grains at a rate for 12 gm/kg and mixed thoroughly. The stock cultures were set up by introducing 500 adults of the insect into each 1 kg. glass jar half filled with the prepared medium and tightly covered with muslin secured by rubber bands. The jars were kept in an incubator at 27 ± 1 °C. and 60 ± 5% r.h. (Miller *et al.*, 1969). The adults were permitted to oviposit in these cultures for 3 days and

then removed by sifting through a No. 10 or 12 sieve. The medium with eggs was returned to jars and the jars were put again at the same conditions. When the newly adults were emerged (F1), large batches were sieved, mixed thoroughly and introduced into new prepared rearing jars as mentioned above to initiate new cultures. The following procedures were conducted to obtain the different stages experiments.

Egg stage

Batches of 500 adults each of 1 – 2 week. old were sieved out from cultures and introduced to 1 kg glass jars half filled with wheat grains mixed with yeast (conditioned wheat grains + dry yeast). After two days of egg laying the adults were removed and transferred to other new prepared jars and the medium were returned again to jars. Samples of 100 wheat grains (containing 0-2 day old eggs) were taken randomly. These samples were put separately inside wire gauze cages to be ready for gas exposure or malathion treatment. Four replicates were prepared for each treatment as well as for the control.

Larval stage

The wheat grains containing 0-2 day old eggs were left to obtain larvae explained below two days

as mentioned above in case of egg stage were used for rearing the larval stage as follows: Wheat grains with 0 – 2 days old egg were maintained at the same conditions (27 ± 1 °C. and $60 \pm 5\%$ r.h.) for a period of 3 weeks to get up approximately the mid period of the larval stage. (sample of grains were soaked in water for one day and dissected thoroughly to ensure existence and age of larvae). Batches of 100 grains each were taken randomly from these grains and put inside wire gauze cages (4 x 8 cm) to be ready for treatments.

Pupal stage

Another batches of wheat grains containing 0-2 day old eggs were left to reach up the pupal stage. These grains were kept in the same glass jars at the same conditions for one month, then, the pupae were developed (some grains were dissected to ensure the presence of pupal stage). Batches of 100 grains each (5 gm approximately) were taken randomly from these grains and put in cages as mentioned above in larval stage to be ready for different gases or to malathion treatments.

Adult stage

A quantity of medium with 0-2 days old eggs was thus maintained in the same jars of culture at the

same condition of rearing until emergence of adults.

Batches of 30 adults (1-2 week old) each were collected from cultures using a fine brush and glass tubes these batches were transferred to wire gauze cages and used for testing. Large batches of adults were sieved from the available cultures, mixed thoroughly and used for initiation new cultures, 500 adults approximately for each jar as described before.

Gases Experiments

Vessels of insect exposure to gases

Wire gauze cages (exposure unit)

Copper wire gauze of 60 mesh/inch were used for making the cages of insect exposure to gases. These cages were

cylindrical in shape (4 cm diameter x 7 cm height) supported at the bottom with a plastic base and at the other end with a plastic ring, covered with a punctured covers or muslin cloth (Fig. 1).

Plastic bags (exposure chamber)

The known urin collection bags (call also drainage bags in field of medicine) were used as gas exposure chambers (Fig. 2). These bags are rectangular in shape (18 x 23 cm) and 2000 ml volume. Each bag has two tubes (hoses) at the both ends. One of the two tubes is long (inlet tube) at the front of the bag and the other tube (outlet tube) is short and located at the other end of the bag. Each tube has at its free end a built in valve and a cover which could be tightly closed.

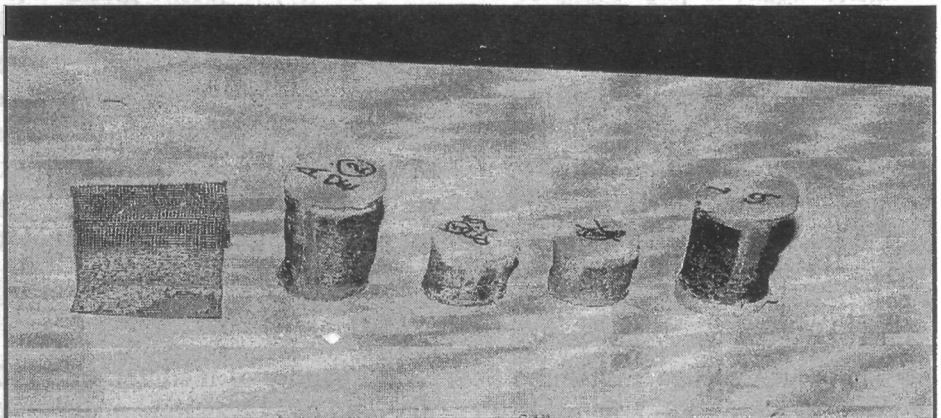


Fig. 1. Gas exposure units

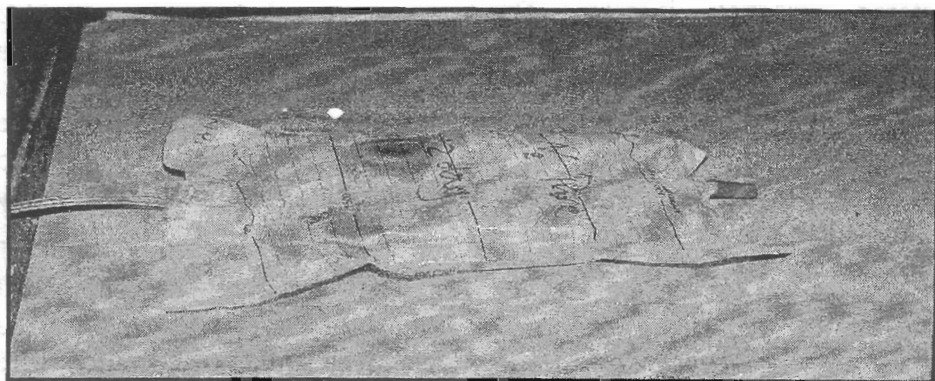


Fig. 2. Gas exposure chamber

Gas exposure procedures

The cages of the different stages of *S. oryzae* prepared as mentioned above were put (four-five replicates for each stage) in the plastic bags through a lateral cut and the bags were tightly sealed using a hand pressing sealer.

The air inside the bag was allowed to get out by gently pressing on the bag and then the bag was connected to gas cylinder (CO_2 or N_2) through a rubber hose and the long tube of the bag. The gas was allowed to purge very slowly inside the bag for about 20 – 30 seconds to ensure completely exchanging the air inside the bag with the gas without any pressure inside the bag. Valves of the treated bags were tightly closed and the bags were kept under the tested conditions for the tested periods of gas exposure. At the end of exposure, the bags were aerated

and the wire gauze cages were taken out from the bags and examined immediately for recording the death of adult stage. The cages of the developmental stages of *S. oryzae* (egg, larvae and pupae) were maintained in the incubator at the tested temperature until adult emergence and the percentages of mortality of these developmental stages were calculated, corrected using abbot, formula (Abbot, 1925) and recorded. The data obtain were statistically analyzed according to (Finney, 1971), using the computer (probit analysis program).

Comparative Study Between (malathion) and the Inert Gases for Controlling *S. oryzae*

An experiment was conducted in the laboratory to compare the efficacy of inert gases (CO_2 and N_2) and malathion and one of the most known protectant against the

stored product, insects. This experiment was conducted as follows :

Toxicity of malathion against adult stage of *S. oryzae*

Treated grains technique was used for evaluation the toxicity of malathion to *S. oryzae* adults. Serial concentrations of malathion were prepared (using talc powder as a diluent) on the base of weight of insecticide to weight of grains. Quantities of wheat grains (Sakha 93) 50 gm. each of about 13% water content were put in vials of 3.5 x 6.5 cm. and mixed thoroughly with the suitable concentration of malathion powder. Five concentrations of malathion were tested to give graduated mortality (%) to establish the concentration mortality regression line. Four replicates were used for each concentration as well as for the untreated. Grains of untreated replicates were treated with talc powder only.

Twenty five adults of 1-2 week-old were introduced to each vial, the vials were covered with muslin secured with rubber bands and kept at $30 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ r.h. Mortality counts were recorded daily for a period of 4 days and the cumulative mortality percentages

were recorded and corrected according to Abbott's formula (Abbott, 1925). The concentration mortality regression lines were statistically analyzed according to the method described by Finney (1971).

The different developmental stages of *S. oryzae* which were reared as mentioned before were exposed separately to inert gases and malathion as follows :

Twelve samples of 300 wheat grains (15 gm) each infested with the different developmental stages of *S. oryzae* (three for each stage). These replicates were treated with malathion powder at the concentration that kill 98% of adults (6 ppm according to the previous tests) and put separately in wire gauze cages. Another 12 samples were treated with talc powder only to be used as control and tightly sealed. These replicates were introduced into urin bags (three replicates of each insect developmental stage for one bag). Other three groups of 12 similar samples each one of which was treated with CO_2 , the second group was treated with N_2 and the third group was treated with the normal atmospheric air to be used as untreated. Method of gas exposure was described previously. All

these bags were kept at 30 ± 1 °C in an incubator.

The treated bags by malathion and the two tested gases and their untreated controls were opened after one month to record the mortality of adult stage and the mortality of the other developmental stages. Similar experiment was conducted exactly but the plastic bags (exposure chambers) were opened after two months.

RESUTLS AND DISCUSSION

The efficiency of malathion, CO₂ and N₂ treatments against the different stages of the rice weevil, *S. oryzae* under laboratory conditions could be discussed as follows :

Toxicity of Malathion

Data presented in Table 1 showed that the tested concentrations of malathion caused different levels of mortality (%) of *S. oryzae* adults. The of mortality (%) after one day were 23, 31, 40, 76 and 80% for the corresponding concentrations of 2, 3, 4, 5 and 6 ppm, respectively.

After 2 days exposure, the cumulative mortality percentages took the same trend of the first day

recording 52% for 2 ppm and increased to 85% for 6 ppm. Mortality increased gradually as the exposure period was increased reaching to 98% after 4 days of exposure to 6 ppm. LC₅₀ values clearly decreased as the exposure period of adults to the treated grains increased recording 3.745, 1.792, 0.4299 and 0.725 ppm for 1, 2, 3 and 4 days of exposure periods, respectively. LC₉₀ values took the same trend also recording 8.564, 7.0815, 3.969 and 2.997 ppm after 1, 2, 3 and 4 days of exposure, respectively. It could be concluded that treatment of wheat grains with malathion at the concentration of 6 ppm was effective to induce nearly complete kill of all existed adults.

Efficiency of Carbon Dioxide

Under laboratory conditions

Data in Table 2 indicated that the developmental stages and adults of *S. oryzae* exhibited different levels of susceptibility to CO₂ at different exposure period. The mortality percentages of insects after 6 hrs. of gas exposure were 33.27, 39.42, 20.23 and 45.8% for eggs, larvae, pupae and adults, respectively. Cumulative mortality (%) increased in the subsequent periods of exposure by different rates reaching 100% after

24 hrs. exposure for adults and 48 hrs. for eggs, larvae and 89.4% for pupae. Adults were the most susceptible stage while pupae were the most tolerant stage, larvae and eggs were in the middle position in their susceptibility. LT_{50} values were 9.064, 7.865, 13.956 and 6.854 hrs. for eggs, larvae, pupae and adults, respectively. LT_{90}

values took exactly the same trend of LT_{50} recording 24.586, 19.678, 49.031 and 15.475 hrs. for eggs, larvae, pupae and adults, respectively. Slope values of the regression lines revealed that larvae and adults were more homogeneous in their susceptibility to CO_2 than eggs and pupae.

Table 1. Cumulative mortality percentages of *S. oryzae* adults exposed to wheat grains treated with malathion dust at $30 \pm 1^\circ C$ and $60 \pm 5\%$ R.H. for different exposure period

Concentrations (in ppm)	Exposure periods (in days)			
	1	2	3	4
Untreated	0	0	0	0
2	23	52	83	84
3	31	70	85	88
4	40	80	89	92
5	76	83	92	97
6	80	85	95	98
LC_{50}	3.745	1.792	0.4299	0.725
LC_{90}	8.564	7.0815	3.959	2.997
Slope	3.567	2.148	1.329	2.0799

Table 2. Mortality (%) of the different stages of *Sitophilus oryzae* exposed to pure carbon dioxide at $30^\circ C$.

Treated stages	Exposure periods (in hrs)					LC_{50}	LC_{90}	Slope
	6	12	24	48	72			
Eggs	33.27	59.53	88.173	100	100	9.064	24.586	2.957
Larvae	39.42	65.13	95.7	100	100	7.865	19.678	3.218
Pupae	20.23	41.93	72.299	89.4	100	13.956	49.0312	2.348
Adults	45.8	73.84	100	100	100	6.854	15.475	3.623

Under different degrees of temperature

Table 3 show the effect of temperature on the efficiency of CO₂ gas against the different stages of *S. oryzae* at different exposure periods. It was observed that efficiency of CO₂ gas clearly increased as the tested degrees of temperature increased. At 20°C and after 1 day exposure, adults were the most sensitive stage recording the highest mortality percentage (89.32%) followed by larval stage (81.19%) and egg stage (70.83%) while the pupal stage was the most tolerant stage (55.73%). In the second day and the subsequent days, the mortality took the same trend of the first day reaching 100% mortality after the 4th day for adults and after the 6th day for eggs and larvae. Mortality of pupae reached to 99.9% after 6 days of gas exposure. LT₅₀ values were 0.315, 0.427, 0.520 and 0.935 days for adults, larvae, eggs and pupal stages, respectively. LT₉₀ values took exactly the same trend of LT₅₀. Slope values of the regression lines revealed that eggs and pupae were the most heterogeneous stages from their susceptibility point of view. The other two stages (adult and larvae) were more homogeneous for their susceptibility.

At 25 °C the relative sensitivity of the different stages to CO₂ gas remained as that of the previous temperature, but the percentages of mortality were increased than that of 20 °C.

At 30 °C, mortality of (all stages) increased comparing with the temperatures less than 30°C. A complete mortality of adults was occurred after 1 day exposure only while the other three stages were completely died after 4 days. Slope values of the regression lines were 2.715 for pupae, 4.026 and 4.321 for eggs and larval stages indicating a higher degree of homogeneity for the susceptibility of egg and larvae to CO₂ gas.

Comparison Between Malathion, Carbon Dioxide and Nitrogen for Controlling *S. oryzae*

Data presented in Table 4 show the efficiency of malathion , CO₂ and N₂ for controlling the rice weevil.

After one month exposure the mortalities (%) of tested stages with malathion were 40, 66.47, 51.23 and 100% for egg, larvae, pupae and adults, respectively. Malathion was less effective against eggs, larvae and pupae of *S. oryzae* but was very effective

Table 3. Mortality (%) of the different stages of *S. oryzae* exposed to pure carbon dioxide at different temperatures for different exposure periods

Tested stages	Exposure periods (in days)				LC ₅₀	LC ₉₀	Slope	Temperature degrees
	1	2	4	6				
Eggs	70.83	84.67	90.32	100	0.520	2.724	1.782	20 C
Larvae	81.19	90.34	98.71	100	0.427	1.623	2.210	
Pupae	55.73	71.84	82.92	99.9	0.935	4.137	1.985	
Adults	89.32	95.63	100	100	0.315	1.092	2.374	25 C
Eggs	82.09	93.54	98.8	100	0.415	1.468	2.337	
Larvae	93.21	98.56	100	100	0.282	0.846	2.684	
Pupae	63.46	79.73	95.32	100	0.781	2.616	2.441	30 C
Adults	94.6	99.9	100	100	0.472	0.859	4.933	
Eggs	87.24	99	100	100	0.522	1.086	4.026	
Larvae	96.36	99.9	100	100	0.384	0.761	4.321	30 C
Pupae	70.93	85.73	99.2	100	0.671	1.990	2.715	
Adults	100	100	100	100	-	-	-	

Table 4. Mortality (%) of the different stages of *S. oryzae* as exposed to malathion, pure carbone dioxide and pure nitrogen at constant temperature of 30 ± 1 °C

Treatment	Exposure periods	Tested stages			
		Eggs	Larvae	Pupae	Adults
Malathion (6ppm)	One month	40	66.47	51.23	100
		CO ₂	100	100	100
		N ₂	100	100	100
Malathion (6ppm)	Two month	62.57	89.35	80.21	100
		CO ₂	100	100	100
		N ₂	100	100	100

against adults at the tested concentrations. The mortality (%) due to the treatment (carbon dioxide and nitrogen treatment) were 100% for all tested stages.

After two months of infested grains storage treated with the tested chemicals the percentages of mortality induced by malathion were 62.57, 89.35, 80.21 and 100% for eggs, larvae, pupae and adults, respectively, while the tested gases completely killed all stages recording 100% mortality at the end of the tested period.

The protective and curative control of wheat as any other cereal from the rice weevil or any other insect pests of stored products were mainly based on preventive measure and the use of chemical productants and fumigant. Inert gases represents specially during the last three decades the most suitable and safe method for controlling pests of stored products. The findings of this study of both malathion and inert gases are in agreement with those recorded by many investigators. Alganatay (1974), Suchita *et al.* (1989) and Biswas *et al.* (2001) found that malathion was one of the most effective compounds against the adults of *Sitophilus oryzae*. Also, Kljajic

and Peric (2006) applied the insecticide malathion against *Sitophilus granaries* adults, and reported that malathion had the least toxic effect on the weevils originating from Belgrade Port and Kikinda.

However, in the present study malathion was effective against adults (which exposed directly to grains treated by the insecticide dust) but not effective against the other stages (eggs, larvae and pupae) because these stages live inside grains.

The tested gases (CO₂ and N₂) were more effective than malathion when the infested grains were treated with these chemicals and tightly stored for limited periods.

The obtained results concerning the efficacy of CO₂ against the tested stages are in agreement with those obtained by Zakladnoi (1976) who reported that pure CO₂ was more rapidly toxic than pure nitrogen to the adults of *Sitophilus oryzae* at all three experimental temperatures (20, 25 and 35°C). Jay (1984) noticed that increase in temperature results in a higher mortality in case of insect treatment with the atmospheric gases and could be useful in reducing the treatment period. Locatelli and Daolio (1991)

investigated the effectiveness of CO₂ at different temperature (20, 25, 30 and 40°C) against *Sitophilus oryzae* and *Rhyzopertha dominica* and found that few eggs of *R. dominica* less than 1% could survive at 20 °C for 48 h., while 100% mortality was recorded within 24 h. at 30°C. and within. At 40 °C, the total larval mortality was recorded within 30, 24, 24, 12 and. At 20, 25, 30, 35 and 40 °C, respectively. Pupae were less sensitive to CO₂ than the other stages. Total adult mortality occurred within 24, 12, 12 and 6h. at 20, 25, 30 and 35°C, respectively. Suss and Locatelli (1991) mentioned that carbon dioxide under high temperature was toxic than at low temperature. If the temperature is below the optimum for insect development, its metabolism is slower and thus less oxygen is required, the authors added that increase in temperature resulted in higher mortality and could be useful in reducing the treatment period. Some food stuffs could be subjected to changes in organoleptic quality and the use of increased temperature for reducing treatment time will be restricted to some products only.

El-Lakwah *et al.* (1992) mentioned that CO₂ was more effective at the higher temperature than the lower one. and the mortality of insects were increased

generally as the concentration of CO₂ was increased. Ofuya and Reichmuth (1993) studied the sensitivity of adults, eggs, larvae and pupae of *C. maculatus* and *Acanthoscelides obtectus* in cowpea to pure CO₂ at 25 and 32 °C. and the 27 ± 5% R.H. and found that irrespective of temperature all adults of both bruchids were killed within 1 day of exposure to pure CO₂ atmosphere. All eggs were killed in 5 days at 25 °C and in 3 days at 32 °C. All larvae and pupae were also killed after 4 and 6 days of exposure at 32 and 25 °C. respectively.

Hashem (2000) stated that high CO₂ contents were relatively more toxic for all developmental stages of *C. maculatus* than N₂. Rajendran *et al.* (2002) studied the storage of basmati rice under CO₂ rich atmosphere for 4 months. The treatment resulted in 100% mortality of all stages of *Tribolium castaneum*.

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مكافحة سوسة الأرز باستخدام الملاثيون وأثنان من الغازات الخاملة فى دراسة مقارنة

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أجريت دراسات معملية بمعمل بحوث آفات المخازن بالقسم لتقييم ومقارنة كفاءة أحد المبيدات الحشرية المعروفة (الملاثيون) كمبيد واقي للحبوب المخزونة وطريقة التخزين فى الأجواء المعدلة باستخدام غاز ثانى أكسيد الكربون أو النيتروجين وذلك لمكافحة سوسة الأرز ويمكن تلخيص النتائج المتحصل عليها كالاتى :

١- أوضحت النتائج أن التركيزات المختبرة من الملاثيون (٢ ، ٣ ، ٤ ، ٥ ، ٦ جزء / مليون مبيد/ حبوب)) أدت إلى حدوث مستويات مختلفة من السمية بالنسبة للطور الكامل لسوسة الأرز المعرضة لحبوب قمح معاملة بالمبيد. كانت نسب الموت بعد ٤ أيام ٨٤ ، ٨٨ ، ٩٢ ، ٩٧ و ٩٨% للتركيزات المختبرة على التوالي.

٢- تسبب غاز ثانى أكسيد الكربون فى حدوث موت بنسبة ١٠٠% للأطوار الكاملة واليرقات وبعد ٣ أيام للعدارى. كانت الحشرات الكاملة أكثر الأطوار حساسية للغاز بينما كان طور العذراء أقل الأطوار حساسية للغاز وذلك تحت الظروف المعملية.

٣- كان غاز ثانى أكسيد الكربون أكثر فعالية على درجات الحرارة العالية عنه على درجات الحرارة المنخفضة. وكانت فترة التعريض عامل مهم جداً للوصول إلى نسبة موت مرتفعة حيث أنه كلما زادت فترة التعريض زادت نسبة الموت.

٤- وفى تجارب المقارنة بين المبيد والغازات لتقدير الفعالية فى مكافحة وجد أنه بعد شهر من تعرض الحشرات لحبوب معاملة بالملاثيون فى أوعية مغلقة كانت نسبة الموت (٤٠ ، ٤٧ ، ٦٦ ، ٢٣ ، ٥١ ، ١٠٠%) للبيض ، اليرقات ، العذارى والحشرات الكاملة على التوالي. وكانت نسب الموت بعد شهرين (٦٢ ، ٧٥ ، ٨٩ ، ٣٥ ، ٢١ ، ٨٠ ، ١٠٠%) وذلك لجميع الأطوار المختبرة وبنفس الترتيب. كانت نسب الموت نتيجة التعرض لكلا الغازين منفردين (ثانى أكسيد الكربون ، النيتروجين) هى ١٠٠% لكل الأطوار المختبرة سواء بعد شهر أو شهرين من التعريض.

مما سبق يتضح أن استعمال كلا الغازين فى مكافحة سوسة الأرز كان أفضل من استعمال المبيد تحت نفس ظروف التخزين.