Methyl isothiocyanate used as a grain fumigant

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Abstract

Farmers of villages in Niger, traditionally incorporate dried leaves of *Boscia senegalensis* to their silos of 'niébé' (cowpeas) to protect them against insect pests. After separation analysis and antennography the natural product responsible for this insecticide property was identified as methyl isothiocyanate (MITC). MITC is presented as crystals which sublime at ambient temperature. Its molecular weight is 73 g/mol and so lies between CH_3Br and PH_3. The biological efficacy assays of MITC on *Sitophilus granarius* (all stages) were done at 20°C (60% r.h.) in 4 hours exposure time and different concentrations. All gas concentrations were measured by gas chromatography using a TSD detector. Statistical analysis was done with Statistical Analysis System.

Studies on the biological efficacy of MITC on all stages showed that 99% of mortality is obtained between 3 and 10 g.hours/m^3 and that the pupa is the most tolerant stage. Other biological experiments showed that we obtained the same efficacy at 10°C. Sorption experiments were also done with various filling ratios (F.R.), different concentrations and four hours exposure time. Even at a very low filling ratio, the sorption is high (e.g. 26% F.R., [C] = 2 g/m^3 gives 77.8% sorption) and with filling ratios above 50%, the sorption is better than 90%. Mass spectrometric analysis has shown that MITC sorbed on grain doesn't degrade to other compounds. MITC is an interesting molecule. A very high rate of sorption allows the application of MITC either by admixture with the grain during loading or by a recirculation system. That sorption is persistent due to the very low desorption rate if the grain remains in the bin. This also allows the use of MITC in leaky silos. The results indicate that dosages between 20 and 40 g/m^3 during 24 hours following application methods at a temperature higher than 10°C, should be effective.

Introduction

Farmers of villages in Niger traditionally incorporate dried leaves of *Boscia senegalensis* to their silos of 'niébé' (cowpea, a native legume) to protect them against insect pests. After separation, analysis and antennography the natural product responsible for this insecticide property was identified (Oger et al. 1989) as methyl isothiocyanate (MITC).

Owing to its broad spectrum of activity, i.e. against soil fungi, insects, weed seeds and nematodes (Pieroh et al. 1959), MITC was commercialised as a soil fumigant by Schering in the late fifties. Today, it is no longer applied alone in its active form, but as compounds such as the soil fumigants Dazomet or Metham sodium which liberate MITC in the soil.

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The fumigant properties of isothiocyanates against insects have been known for many years. After the First World War, a lot of assays were done in the laboratory to determine the biological efficacy of 2-propenyl-isothiocyanate on *Sitophilus oryzae, Tribolium confusum,* and *Plodia interpunctella* tested at a minimum concentration (Niefer et al. 1925). Both 2-propenyl and ethyl isothiocyanate killed all *Sitophilus oryzae* exposed for 24 hours at 20 g/m^3, and exposure of wheat to even higher concentrations did not prevent germination (Roarl and Cotton 1929).

Considering the insecticide properties of isothiocyanates at low concentrations and the actual application of MITC through *Boscia senegalensis* in Africa to protect stored crops, it was decided to investigate more closely this molecule in the laboratory, in order to estimate its possible use as a grain fumigant. Two series of assays were done. First, the conditions of application of MITC were studied to develop some standardised methods for measuring the gas concentrations inside the test chambers and for quantifying the sorption on grain, and thus to target the optimal range of conditions for MITC use. Then, the biological efficacy of MITC was tested at 20°C on all life stages of one major pest of stored wheat: *Sitophilus granarius.*

Materials and Methods

Materials

Methyl isothiocyanate

At room temperature (20°C), MITC (CH_3-N=C=S) is found as colourless crystals with a pungent horse radish-like odour, a molecular weight of 73 g/mol and a density of 3060 g/m^3. It melts at 35°C (solubility in water of 7.6 g/L at 20°C) and boils at 117–119°C (vapour pressure of 2.7 kPa and saturated concentration of 75.6 g/m^3 at 20°C).

MITC was obtained in a crystal block, which could be divided into separate parts to get the required quantities of compound.

Gas analysers

Gas chromatograph Varian 3300
Detector: Thermionic Specific detector
Column: chromosorb 101, 80/100 mesh, 2 m × 1/8 inch i.d.
GC conditions:
Gas flow rates: Air: 175 mL/minute; H_2: 4 mL/minute; N_2: 30 mL/minute
Temperatures: Column : 200°C; Injector: 200°C; Detector: 230°C
Attenuation 2, sensitivity 10^{-10}
Retention time: 3 minutes
Integration card: STAR CS + STAR chromatograph Workstation System (V.3)
Mass spectrometer — NERMAG R10-10C
Electronic impact (E.I.) coupled with GC Delsi Di 700
Column DB5 60 m × 0.33 mm i.d.
Conditions: E.I.: 70; evmass range: 33 to 350 a.m.u.
Temperatures: column: 200°C; Inj: 200°C; Interface: 200°C; Source: 150°C

Insects

A laboratory population of *Sitophilus granarius* was used, bred in the Laboratoire des Denrées Stockées (Cenon, France). The tested insects were grown in the dark, at a temperature of 28°C and in a relative humidity of 75%. Under these conditions, the life cycle was completed in 35 days.

At day 0, 400 adults of *Sitophilus granarius* were put inside 1 kg untreated wheat for reproduction and egg-laying. Seven days later, the grain was sieved in order to remove the adults. At day 35, the first emergencies of adults were observed. At day 52, the population was ready for the experimentation and all life stages of the insect were present.

Methods

Sublimation rate

The sublimation rate of MITC at 20°C was determined in order to estimate the loss occurring between the weighing of the crystals and their introduction in the fumigation chambers, and thus a better estimate of the real dose injected.

The evolution of MITC with time was monitored for three different weights of crystal (100, 200 and 1000 mg) with three replicates/quantity. The crystals were weighed on an electronic balance (sensitivity in the order of 0.1 mg) and the decrease in weight was continuously monitored for 250 minutes using a RS 232 linkage to a computer.

Qualitative and quantitative analysis of MITC

Since this compound is known for its extreme sorption on many materials including steel and glass, MITC has to be measured in a solvent.

A linear relationship was found with methanol (CH_3OH) as solvent for the range of MITC concentrations 0.005 to 8 g/L, when an attenuation of 2 and a sensitivity of 10^{-10} were applied. Therefore these conditions were chosen as standard. A 1 g/L solution of MITC in methanol was used as the reference for the calculation of gaseous concentrations in the samples of atmosphere taken from the fumigation chambers. Before each series of measurements, the chromatograph was calibrated using five aliquots of 5 µL of methanol, each containing 5 µg of MITC.

Gas sampling from the test chambers was done directly by removing 0.5 mL with a syringe for chambers whose volumes ranged below 2 L. For chambers of 125 and 300 L, samples were taken in 500 mL blisters, which have been previously subjected to vacuum. 5 µL were then removed with a syringe and very quickly ($\leq$ 3 seconds) transferred onto the chromatograph, to avoid any sorption on the glass. Data were either expressed in terms of concentrations (g/m^3) or converted in terms of CT product (CTP) ($g.hours/m^3$).

Sorption: relationship between concentration-filling ratio-CTP-fumigation technique

Assays with homogenisation. The assays were done inside 2 L glass jars containing grain at various filling ratios (e.g. 0.05; 0.013 ; 0.26 ; 0.52 ; 0.75 and 0.95) under the following conditions: temperature of 20°C (±1°C); relative humidity (r.h.) of 70% (±5%) ; moisture content (m.c.) of the grain of 13.5% (±0.5%) and specific weight (s.w.) of 775 g/L (±10 g/L).

Six different concentrations of gas were tested: 1, 2, 4, 8 and 16 g/m^3 for 4 hours and 10 g/m^3 for 24 hours exposure time, with four replicates/treatment. After filling up the jars, the crystals were deposited on the surface and immediately and thoroughly mixed with the wheat.

For each treatment, concentrations were measured on the surface. For experiments lasting 4 hours, eight measures were spread out on the exposure time; for the exposure time of 24 hours, the measures were taken each hour up to 5 hours then at 8, 19 and 24 hours fumigation.

Comparison between fumigation without homogenisation and fumigation in layers. The assays were done inside food containers (125 L bins) 75% filled with wheat and MITC was applied at a rate of 50 g/m^3, either for 4 hours or for 24 hours exposure time and assays were repeated twice. The following external conditions were recorded: temperature 21°C, 60% r.h., 14% m.c. and 765 g/L s.w.

Two techniques of application were applied and compared: application of the crystals on the surface of the grain after filling up; and application during the filling up, i.e. 1 g of MITC on each layer of 10 kg wheat.

Measurements were taken from three locations (top, middle and bottom) at the same times as for the assays with homogenisation.

Influence of the initial dosage (fumigation in layers). The same procedure as described in the second technique of application (above) was followed with the same two exposure times (4 and 24 hours) but using three different initial MITC concentrations (6.5, 20 and 50 g/m^3).

Influence of a recirculation system. This experiment was done at 20°C inside a small silo of 300 L equipped with a ventilator (nominal flow rate of 150 m^3/hour; water column of 60 mm) and 95% filled with grain.

After introduction of a MITC dose of 10 g/m^3, the air–gas mixture was recirculated for 1 hour and the fumigation lasted either 4 or 24 hours. Measurements were taken at four points (top; upper middle; lower middle; bottom) at the same times as for assays with homogenisation (above).

Desorption from wheat after fumigation

Some trials were carried out to determine the best method to observe the desorption rate of MITC from the grain after the end of the fumigation exposure. Comparison was made between methanol and hexane as solvent with 50 g of wheat exposed during 4 hours to a MITC concentration of 1,2,4,8 and 16 g/m^3. The grain was spread onto the laboratory bench, then immersed in 50 mL of solvent for 0, 4, 24 and 48 hours. Methanol was found to be the best compound.

The stability of the molecule after desorption was then checked by mass-spectrometry under the conditions in the section above on gas analysers.

Biological efficacy of MITC on Sitophilus granarius *at 20°C*

Samples of 100 g wheat containing on average 200 insects were used as replicates. They were prepared 24 hours before the beginning of the experiment. The procedure described in the section on assays with homogenisation was observed, using an exposure time of 4 hours, four replicates of the treatment, controls and MITC concentrations of 1, 2, 4, 8 and 16 g/m^3.

At the end of the exposure time, the glass jars were opened up to allow desorption of fumigant from the grain and each sample was subsequently sieved to remove the adults. All adults present in a sample were transferred into a single Petri dish containing 5 g of untreated grain and kept in the dark breeding room (first series of lots). The remaining grain, which contained all hidden life stages (egg, larvae and pupae) was collected into a separate container and also kept in the breeding room (second series of lots).

After 7 days, the percentage of adult mortality was calculated for each lot of the first series. At 7, 14, 21 and 28 days, each lot of the second series was sieved again; the adults were removed and counted; the grain was collected and put back again in the breeding room. A last adult counting was done at 35 days. Each count of adults corresponded to a different initial life stage subpopulation submitted to MITC. Thus

adults counted at 7 days originated from pupae; at 14 days from old larvae; at 21 and 28 days from young larvae and at 35 days from eggs.

These numbers were compared to those found in the control lots and percentages of mortality could therefore be estimated for each life-stage subpopulation. The probit procedure available from the statistics package of Statistical Analysis System (SAS Institute Inc., Cary, NC) was applied to determine the CTP which killed 50, 99 and 99.997% of each subpopulation.

Results and Discussion

Sublimation rate

A quantity of 1 g crystals needed more time to achieve complete sublimation than a quantity of 100 mg but the sublimation rate was greater. A delay of 5 seconds was necessary between the weighing and the introduction of the crystals inside the fumigation chambers. Regarding the sublimation rate, the loss of MITC could equal 0.2% of the initial quantity for 1 g and 0.15% for 100 mg. These losses were then neglected in the subsequent studies.

Sorption

Relationship between initial dosage – filling ration – CTP – 4 hour fumigation

For the experiments lasting 4 hours, an inverted exponential relationship was found between the CTP and the filling ratio at constant dosage values (with values of the coefficient of determination, $r^2 > 0.9$) (Table 1; Fig. 1).

An exponential relationship was found between the sorption percentage of MITC on the grain and the filling ratio (Table 2). Even at a very low filling ratio, the sorption is high and close to 100% with filling ratios greater than 50%.

At constant filling ratio values (i.e. same surfaces of contact), a positive linear relationship was found between the CTP and the introduced dose ($r^2 > 0.99$) and the slope coefficients of these straight lines were negatively correlated to the filling ratio values (Table 3).

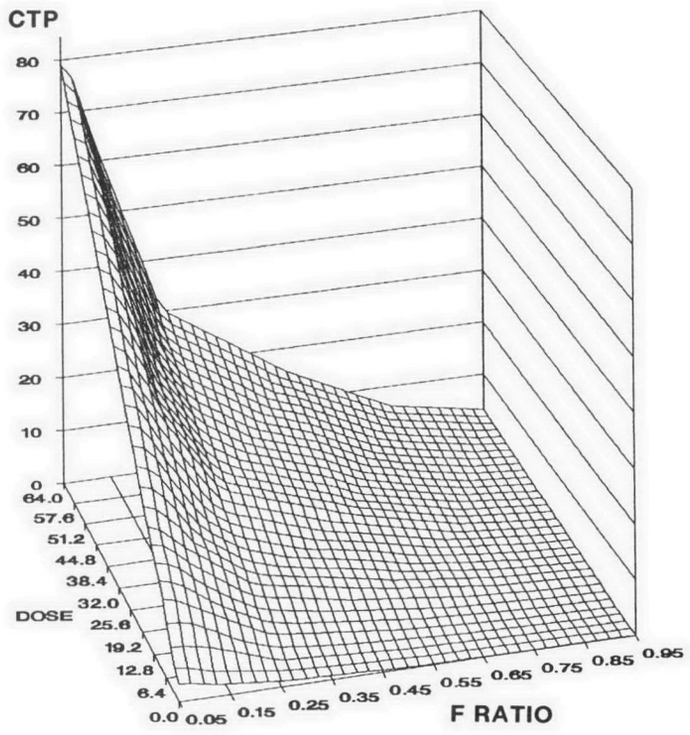

Fig. 1. CTP of methyl isothiocyanate resulting from various combinations of dosage and filling ratio. CTP = f([C], F Ratio). 4 hours fumigation; temperature = 20°C; CT g.hours/m^3; dose: dosage g/m^3; F ratio:filling ratio.

For the experiments lasting 24 hours, an exponential relationship was also observed between the sorption percentage and the filling ratio. The sorption percentages were of the same

Table 1. CTP (expressed in g.hours/m^3) obtained in a 4-hour fumigation with homogenisation, for various filling ratios and various concentrations of methyl isothiocyanate.

Filling ratio	Initial dosages in g/m^3						
	1	2	4	8	16	32	64
0	3.16	6.32	12.64	25.28	50.56	101.1	202.2
0.065	3.1	4.6	7	13	21	40.71	79.11
0.13	2	3	6	10	16	31.38	61.14
0.26	1	1.4	2	4	8	15.4	30.44
0.52	0.2	0.4	0.7	1.5	4	7.79	15.79
0.75	0.05	0.11	0.19	0.46	1.60	3.05	6.25
0.95	0.02	0.04	0.07	0.17	0.77	1.5	3.1

Table 2. Percentage of sorption obtained in a 4-hour fumigation (expressed in percentage of losses). Fumigation with homogenisation, various filling ratios and various concentrations.

	Initial dosages in g/m^3						
Filling ratio	1	2	4	8	16	32	64
0.065	1.9	27.2	44.6	48.6	58.5	59.7	60.9
0.13	36.7	52.5	52.5	60.4	68.4	69.0	69.8
0.26	68.4	77.8	84.2	84.2	84.2	84.8	84.9
0.52	93.7	93.7	94.5	94.1	92.1	92.3	92.2
0.75	98.4	98.2	98.5	98.2	96.8	97.0	96.9

order as those recorded during the fumigations of 4 hours for a dose of 8 g/m^3 (Table 4).

In these laboratory trials, with a 4-hour fumigation, it seems impossible, due to sorption, to obtain the CTP necessary to kill insects when the filling ratio is above 50%.

Comparison between fumigation without homogenisation and fumigation in layers

Regarding the first technique of fumigation without homogenisation, the maximum concentrations obtained at all measuring points were reached very quickly (15–20 minutes). A considerable heterogeneity was observed in the bin characterised by very low CTPs in the middle and the bottom (Table 5; Fig. 2). The maximum concentration obtained on the bottom was only 1/100th of the initial dose.

In contrast, with the second technique of fumigation in layers, maximum concentrations were obtained much more slowly, with a peak occurring only after 5 hours. Since CTPs obtained were lower on the top compared to the first technique, they were much more even. The differences between CTPs observed in the lower layer and those in the top layer could be the result of the difference of filling ratio between the top and the bottom, the empty volume being on the top.

The CTPs obtained with the first method are never enough in the grain mass to kill insects even at 24 hours. With the second method, the CTPs obtained at 24 hours are high enough, and nearly enough at 4 hours exposure time.

The quick natural diffusion of MITC associated with its rapid sorption on grain shows that an admixture layer by layer improves the even distribution of the fumigant. This reflects the traditional practice in Africa.

Influence of the initial dosages in layer fumigation

Even with a fumigation in layers, the CTPs obtained on the bottom are lower than those obtained on the top. According to sorption assays and mathematical calculations, for a filling ratio of 0.75, we would expect CTPs of 0.5, 1.85 and 4.85 g.hours/m^3, respectively, for initial dosages of 6.5, 20 and 50 g/m^3, 4 hours exposure time). These calculated CTPs are lower than those obtained on the top, and equal to or greater than those obtained on the middle and bottom (Table 6). The difference observed between calculated and actual values could be the result of a better availability of free gas in the top layers.

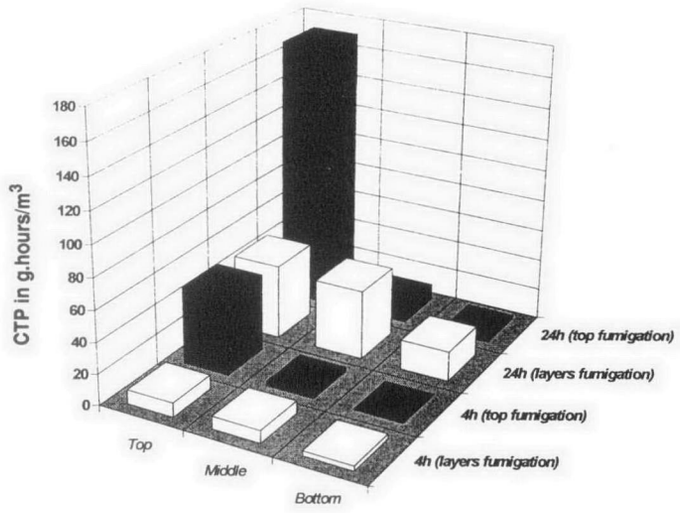

Fig. 2. Influence of fumigation in layers on CTP.

Recirculation system in MITC fumigation

The influence of a 1 hour recirculation system on the MITC sorption is important and allows the distribution of equal concentration at all levels. A CTP of 5 g.hours/m^3 (Table 7; Fig. 3) is not enough to kill insects at all stages. Nevertheless, if the fumigation was done at 10 g/m^3 in layers, the CTP obtained should have been 0.9 g.hours/m^3 (CTP = 102 exp$^{(-0.04(F\ Ratio))}$, $r^2 = 0.98$)

Table 3. Relationship between CTP and introduced dose. y = CTP (g.hours/m^3); x = initial dosage (g/m^3), 4 hours fumigation with homogenisation.

Filling ratio	Relationship between CTP and [C]	r^2
0.065	$y = 1.2x + 2.31$	0.99
0.13	$y = 0.93x + 1.62$	0.98
0.26	$y = 0.47x + 0.36$	0.99
0.52	$y = 0.25x - 0.21$	0.98
0.75	$y = 0.1x - 0.15$	0.99
0.95	$y = 0.05x - 0.1$	0.99

Desorption from wheat after fumigation

Only preliminary experiments have been done. The rate of desorption varied considerably according to the exhaust gas method used.

- With ventilation, desorption is very quick, two-thirds MITC concentration on grain is lost in 4 hours and only traces remain after 24 hours.
- In contrast, when the grain is left in the bins, after 24 hours only 50% is lost and the actual concentrations on grain remain at that level for some days.

MITC sorbed on grain does not degrade to other compounds as mass spectrometer analysis showed.

Table 4. Relationship between sorption percentage and filling ratio for a 24-hour fumigation, dosage 10 g/m^3.

Filling ratio	Control	0.065	0.13	0.26	0.52	0.75	0.95
CTP	190	91.5	56.3	33.6	14.6	2.4	0.9
% of sorption		51.9	70	82	92	98.7	99.5

Table 5. Influence of fumigation in layers on CTP (4 and 24 hours fumigation, filling ratio 0.75, dosage of 50 g/m^3)

	CTP obtained in 4 hours			CTP obtained in 24 hours		
	Top	Middle	Bottom	Top	Middle	Bottom
Top fumigation	50.1	3.2	0.7	171	10.5	2.3
Fumigation in layers	9.1	8	3.1	47.8	44.6	19

Table 6. Influence of the dosage on CTP in fumigations by layer (CTPs expressed in g.hours/m^3).

	Bin A 6.5 g/m^3			Bin B 20 g/m^3			Bin C 50 g/m^3		
	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
CTP 4 hours	3.6	1	0.6	9.6	1.2	0.6	9.1	8	3.1
CTP 24 hours	8	2.6	3.1	25.5	7.5	6.2	47.8	44.6	19

Table 7. Recirculation influence on methyl isothiocyanate fumigation (CTP in g.hours/m^3)

	Top	Middle top	Middle bottom	Bottom
CTP 24 hours	5.7	4.7	4.1	5.7

Biological efficacy of MITC on *Sitophilus granarius* at 21°C

In general, the slope of the curve is very high (Fig. 4). For eggs, the mortality lies between 10 and 90% with a CTP of 1.2–4; for young larvae, the mortality lies between 10 and 90% with a CTP reaching 2–4.9; for old larvae, the mortality lies between 10 and 90% with a CTP reaching 1.8–4.5; for pupae, the mortality lies between 10 and 90% with a CTP reaching 0.8–4.7.

The biological efficacy of MITC on all stages shows that 99% of mortality is obtained between 3–10 g.hours/m^3 and that the pupal stage is the most tolerant (Table 8; Fig. 4).

Other biological experiments show that the same efficacy is obtained at 10°C (Table 9).

Conclusion

MITC is an interesting molecule from several points of view. Its diffusion is very rapid but the sorption on grain takes place immediately after its introduction. As a result, if the fumigant is applied as usual on the surface of the grain after loading, the resulting concentrations are very low. With an initial dosage of 50 g/m^3, a 24 hour exposure time and a filling ratio of 0.75, the resulting CTP obtained at the top of the bin is 171 g.hours/m^3 and only 2.3 g.hours/m^3 at the bottom, which is not enough to control all stages *of S. granarius* since a CTP of 10 g.hours/m^3 is needed.

For fumigations in layers, like applications of contact insecticides, better homogeneity in terms of CTP is obtained. Nevertheless, fumigations of 24 hours with an initial dosage of 20 g/m^3 (filling ratio 0.75) give a CTP superior to those desired in the top of the bins (25.5 g.hours/m^3) while in the bottom the CTP are too low to kill insects (6.2 g.hours/m^3). As a result, it would be necessary to apply more MITC in the bottom layers and gradually less upwards.

Table 8. Biological efficacy of methyl isothiocyanate against *S. granarius* at 21°C (CTP necessary to obtain a given mortality)

	Eggs	Young larvae	Old larvae	Pupae
CT 50	2.21	3.16	2.91	1.98
CT 99	6.64	7.14	6.44	9.8
CT Probit 9	14.5	13	11.5	16

Table 9. Biological efficacy of methyl isothiocyanate against *S. granarius* at 10°C (CTP necessary to obtain a given mortality)

	Eggs	Larvae	Pupae
CT 50	0.5	1.46	0.52
CT 99	8	5.45	6.3

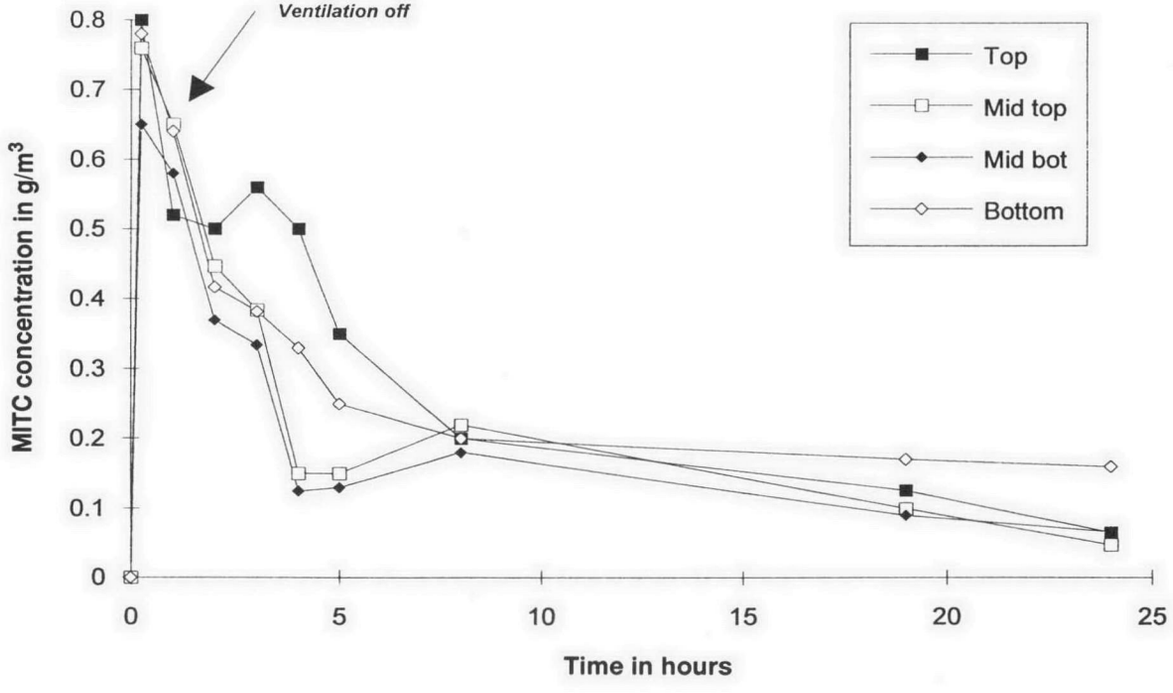

Fig. 3. Influence of recirculation system on methyl isothiocyanate concentration.

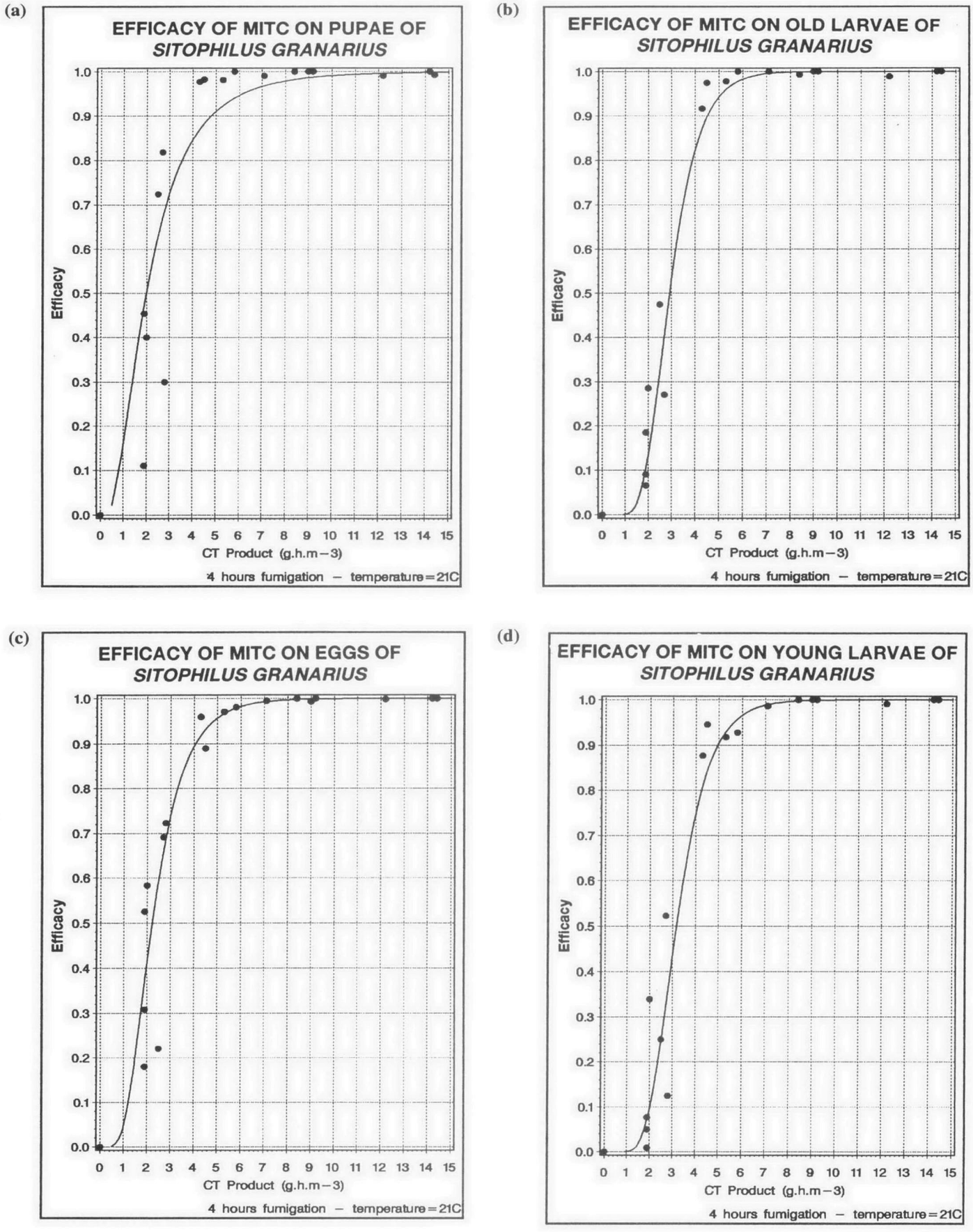

Fig. 4. Efficacy of methyl isothiocyanate on *S. granarius* at 21°C: (a) pupae; (b) larvae; (c) eggs; (d) young larvae.

The best way to obtain quickly a good homogeneity is the use of a recirculation system. In that case, fumigation at 20 g/m^3 allows control all stages of *S. granarius*.

However, its extreme sorption power gives a curative action and a protectant effect. This would allow its use in silos which are not gastight.

Knowing its anti-fungal properties, it would be interesting to carry out studies on its efficacy on the pathogen flora of grains. If it could be proved efficient, then it would be a powerful pesticide for grain, both insecticide and fungicide.

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