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Studies on the Applicability of Tosylchloramide Sodium (Halamid®) to Silkworm Rearing Industry as a useful Disinfectant*

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Tosylchloramide Sodium (Halamid®)의 蠶室 蠶具 및 蠶體消毒藥으로서의 應用可能性에 關한 硏究

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Summary

During the autumn-rearing season of 1971, at the silkworm rearing house at the college of agriculture, Seoul national university in Korea, the authors carried out a series of experiments (1) on the susceptibility of four important silkworm pathogens (*Aspergillus flavus*, *Bacillus thuringiensis*, *Aspergillus oryzae*, and *Isaria farinosa*) to Halamid (Tosylchloramide Sodium), (2) on the acute toxicity of Halamid to silkworm larvae at every instar, (3) on the inhibiting effect of Halamid to the attack of two silkworm infections, fungal muscardine and viral jundice, and the chronic toxicity of Halamid to silkworm larvae, and (4) on the yield and quality of the raw silk harvested from the Halamid treated silkworms.

As the results of the experiments the authors found that Halamid could be applied usefully to silk worm rearing industry as an effective disinfectant for both silkworms themselves and their envir onmental artıcles, rearing houses and tools, also that Halamid exerts its disinfectant effect most efficiently as a disinfectant for silkworms when sprayed in 2% or 3% aqueous solution every second day throughout all the instars of larval stage.

Introduction

Silkworms (*Bombyx mori L.*) are continuously invaded by various pathogens, such as fungi, bacteria and viruses, during their larval stage. For successful silkworm rearing, silkworms should be kept from the infection of the pathogens and silkworm diseases should be treated, if infection occurs. In case of silkworms, external prophylaxis is much more reasonable and economical than internal treatment because their body sizes are very small and silkworms grow very rapidly.

In the Far Eastern countries, silkworm rearing industry have long been one of the very popular and

* Tosylchloramide Sodium = Tosylchloramidum Natricum = Sodium Paratoluenesulfonchloramide, $CH_3C_6H_4SO_2N(Na)Cl \cdot 3H_2O$. Halamid (The registered trade name of Tosylchloramide Sodium, of Koninklijke Zwavelzuurfabrieken v/h Ketjen N.V. Amsterdam, Holland, CHEMICAL DIVISION OF AKZO.) was kindly supplied by Taipyong Chemical Co. Ltd., Seoul, Korea.

important industries, and in the other Asian countries and African countries, this industry is gaining in public favour as one of the profitable industries. By the authors' opinion, however, there is still not a very useful disinfectant of great safety to both man and silkworms in the silkworm rearing field, although there are some disinfectants for silkworm only, of low effectiveness or of high toxicity[1] to silkworms, and some other disinfectants for the silkworm rearing houses and tools only, of poor usefulness or of strong toxicity to man. Hereby, the authors paid special attention to the application of the excellent and universal disinfectant effect of Halamid to the silkworm rearing field.

The universal disinfectant effect of Halamidis well known all over the world and has been usefully applied to various fields over a wide range such as medical field, veterinary practice, dairy industry, fish processing industry, fish trade field, mink farming, meat processing industry, table-poultry industry, and so forth.

To investigate the applicability of Halamid to silkworm rearing industry, the authors carried out a series of experiments at the silkworm rearing house of the college of agriculture, Seoul national university during the autumn-silkworm rearing season of 1971.

As the results of the present experiments ((1) examining the susceptibility of certain silkworm pathogens to Halamid (2) investigating the acute toxicity of Halamid to silkworm larvae, (3) observing the inhibiting effect of Halamid to the attack of certain silkworm infections and the chronic toxicity of Halamid to silkworm larvae, and (4) inspecting the yield and quality of the raw silk obtained from the Halamid treated silkworms), the authors discovered the fact that Halamid could be applied usefully to silkworm rearing industry as an excellent and universal disinfectant for both silkworms themselves and their environment, namely, silkworm rearing houses and tools.

Materials and methods

1. Examining the susceptibility of certain silkworm pathogens to Halamid

Of four purified pathogens examined in this examination; Bacillus thuringiensis and Isaria farinosa were obtained from the sericultural experiment station, office of rural development in Korea, and Aspergillus flavus and Aspergillus oryzae, from the department of food technology, college of agriculture, Seoul national university.

Aspergillus flavus, Aspergillus oryzae and Isaria farinosa were cultured at pH 5.6, at 28°C., for 96 to 120 hours, and with Sabouraud dextrose agar and Sabouraud dextrose broth (without agar from Sabouraud dextrose agar). Bacillus thuringiensis was cultured at pH 5.4, at 30°C., for 72 to 96 hours, and with nutrient agar and nutrient broth and a medium for phenol coefficient (beef extract 5 Gms., sodium chloride 5 Gms., peptone 10 Gms., and distilled water q.s. ad. 1,000 ml.)

2. Investigating the acute toxicity of Halamid to silkworm larvae

The strain of the silkworms which were reared for this investigation was Moran-Daedong for the autumn-rearing. The silkworms had been raised at the standard rearing conditions, i.e., at younger instars at 25°~27°C., and at 80~90% r.h. (relative humidity), and at advanced instars at 22°~23°C., and at 65~70% r.h.

Halamid was sprayed thoroughly on the body surface of silkworm larvae in aqueous solution of various concentrations with a hand sprayer, and Halamid solutions were always prepared freshly just before spraying.

In this investigation, the acute toxicity was estimated by the numbers of the larvae which died within 24 hours after a single spraying of a Halamid solution to the healthy silkworm larvae which had not been treated with any Halamid solution before.

3. Observing the inhibiting effect of Halamid to the attack of certain silkworm infections and the chronic toxicity of Halamid to silkworm larvae

The strain of the silkworms used in this observation, the conditions of silkworm rearing and the method of spraying Halamid solutions are just the same as the above mentioned.

In this observation, the effect of Halamid and the toxicity of the chemical was compared with those of

Table I. The susceptibility of certain silkworm pathogens to Halamid

Pathogen	Duration of contact	Conc. of Halamid 0.5%	1.0	2.0	3.0	4.0	5.0
Aspergillus flavus	5 min.	+	+	+	+	+	+
	10	+	+	+	+	+	−
	15	+	+	+	+	−	−
	20		+				
	25		+				
	30		−				
Bacillus thuringiensis	5	+	+	+	−		
	10	+	+	−	−		
	15	+	+	−	−		
	20		+				
	30		−				
Aspergillus oryzae	5	+	+	−			
	10	+	−	−			
	15	−	−	−			
Isaria farinosa	5	+	+	−			
	10	±	±	−			
	15	−	−	−			

The symbol (−) means that growth of the pathogen was inhibited.

formalin, because the disinfectant effect and chronic toxicity of formalin to silkworms have been well established in silkworm industry in spite of its low usefulness. Formalin is normally sprayed on silkworm larvae in 1% aqueous solution on younger instars (1st, 2nd and 3rd) and in 2% on advanced instars (4th and 5th).

A special observation on small scale was carried out by inoculating certain silkworm pathogens directly to silkworm larvae for observing the inhibiting effect of Halamid to the attack of the silkworm infections. In this observation, purified nuclear polyhedrosis virus obtained from the sericultural experiment station was fed on silkworm larvae by way of spraying the virus suspension of 10^7/ml. concentration of inclusion body to mulberry leaves to be fed. And the two pathogens for muscardine, Isaria farinosa and Spicaria pracina, obtained from the same station as above were inoculated in a suspension of 10^7/ml. concentration immediately after ecdysis at 2nd instar with an atomizer.

Every experimental procedure was repeated thrice with a group of 100 silkworms of each for reliable results.

4. Inspecting the yield and quality of the raw silk obtained from the Halamid treated silkworms

The work of inspecting the quality of the raw silk was completed by the specialists at the sericultural experiment station.

Results

1. The suspectibility of certain silkworm pathogens to Halamid

Table I shows the susceptibility of four silkworm pathogens to Halamid. The inhibited growth of the pathogens by Halamid is shown in the eleven pathotographs of Fig. 2.

Taking into consideration the fact that Aspergillus flavus is known as the most resistant silkworm pathogen[2] to the disinfectants known, a 2% or 3% solution of Halamid is enough to inhibit the growth of all fungal and bacterial silkworm pathogens from a practical view of point.

2. The acute toxicity of Halamid to silkworm

Table II. Acute toxicity of Halamid to silkworm larvae at every instar

(The numbers in the table indicate the numbers of the silkworm larvae which died within 24 hours after a single spraying.)

Conc. of Halamid	Group of 15 silkworm larvae	1st instar		2nd		3rd		4th				5th					
		2nd day	3rd day	2 nd	3* rd	1 st	3 rd	1** st	2 nd	3 rd	4 th	1 st	2 nd	3 rd	6 th	7 th	8 th
Control	I	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1%	I	0	0	0	0	0											
	II	0	0	0	0	0											
	III	0	0	0	1	1											
2	I	0	0	0	0	0											
	II	0	0	0	0	0											
	III	0	0	1	0	0											
5	I	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
	II	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	III	3	3	0	1	0	0	3	0	0	0	0	0	0	0	0	0
10	I	4	0	0	4	0	0	4	1	1	0	0	0	0	0	0	0
	II	7	7	0	2	1	0	6	3	0	0	0	0	0	0	0	0
	III	2	5	4	5	2	0	4	2	0	0	0	0	0	0	0	0
15	I	5	7	1	4	1	0	8	7	0	0	1	0	0	0	0	0
	II	9	7	0	5	1	0	3	0	0	0	1	0	0	0	0	0
	III	5	0	3	4	0	0	4	4	0	3	1	0	0	0	0	0

* Sprayed just before sleeping.

** Sprayed before first feeding after ecdysis.

larvae

In table II the results of the experiment on the acute toxicity of Halamid at every instar are shown. Silkworm larvae are able to tolerate well to a single spraying of Halamid solutions although some of the larvae at first and second instars died from 10% and 15% Halamid solutions. In general, just before sleeping and before first feeding after ecdysis the silkworm larvae seemed to have a liability to be poisoned by Halamid because of their weakened condition.

The maximum solubility of Halamid in water is 15.5% at 20°C. according to the authors' measurement. Silkworm larvae did not eat the mulberry leaves sprayed or soaked by any of the Halamid solutions of varions concentrations. The Halamid solutions discolor the mulberry leaves soon and the starting time and degree of the discolaration varied widely with the concentration of Halamid (Fig. 1). These characters of Halamid on the solubility in water and the tendency of discoloring mulberry leaves seemed to serve as a prevention against a excess spray of Halamid solutions which may lead to an acute intoxication of silkworms by Halamid. The refusal of silkworm larvae to eat the Halamid sprayed mulberry leaves requires to spray Halamid solutions after the larvae ate up or stopped to eat the fresh and intact leaves fed beforehand. And this refusal of silkworm larvae, unfortunately, means that Halamid could not be used to disinfect the mulberry leaves to be fed like the cases of all other disinfectants for silkworms.

3. The inhibiting effect of Halamid to the attack of certain silkworm infections and the chronic toxicity of Halamid to silkworm larvae

Table III indicates the inhibiting effect of Halamid

Table III. The inhibiting effect of a single spraying of a Halamid solution to the attack of muscardine and jundice in the silkwormswhich were inoculated with the pathogens

Pathogen inoculated	Conc. of pathogen-suspension	Name of infection induced	Conc. of Halamid	Rate of incidence of infection
Spicaria pracina	10^7/ml.	Muscardine	1 %	83.7%
			2	34.0
			(Formalin 2%)	37.5
			control	92.7
Isaria farinosa	10^7/ml.	Muscardine	1	59.3
			2	15.9
			(Formain 2%)	24.0
			control	88.3
Nuclear polyhedrosis virus	10^7/ml. (of inclusion body)	Jundice	1	30.5
			2	23.3
			(Formalin 2%)	17.0
			control	50.0

to the attack of fungal muscardine and viral jundice in the silkworms which were artificially inoculated with the respective pathogens.

The results shown in table III indicate that a single spraying of 2% Halamid solution has a definite inhibitory effect to the attack of muscardine and jundice. Taking into account the fact that the concentration of muscardine pathogens in natural state in 10^4/ml., the inhibiting effect of Halamid to the infection should be very reliable.

The trend of the chronic toxicity of Halamid to silkworms is shown in the 5th column of table IV. The intoxication by continuous spraying of Halamid was negligible with exception of case of 5% Halamid solution. The chronic toxicity of formalin to silkworm larvae could not be disregarded.

4. The yield and quality of the raw silk harvested from Halamid treated silkworms

Table IV shows the yield of cocoons by the Halamid treated silkworms and the quality of the cocoons.

The results of table IV generally indicate that 3% Halamid solution secures the largest yield of cocoons, that every day spraying brings more yield of cocoons than the case of every second day spraying, that in standpoint of the quality of cocoon and raw silk, every second day spray is better than the case of every day spraying, that the lower the concentration of Halamid is, the better the quality of cocoon and raw silk is, and that the effects by 2% Halamid solution are much the same as those by 3% Halamid solution.

Discussion

Silkworms are reared under the constant threat of various silkworm pathogens during their growing period of about one month, and they require a great deal of manual labor. So, a drug, to be qualified for a good disinfectant for silkworms, must have foundamentally the following characters; (1) It must exert a certain and universal killing effect on the various pathogens. (2) It must not be harmful to silkworms. (3) It must have no influence upon the yield and quality of the cocoons to be harvested afterwards. (4) It must not be poisonous to man.

Considering the results of the present work, it is clear that 2% or 3% aqueous solution of Halamid sprayed every second day throughout all the instars of larval stage satisfies the above characters. 2% or 3% solution of Halamid sprayed every day and 5% solution of the chemical are not recommendable because of their tendency to induce toxic effect to silkworm larvae and low quality of raw silk.

And to become a useful disinfectant, a drug must possess the following adventages; viz. low price, good chemical stability, simplicity on handling, easiness of purchase and inertness to metals, clothes, etc.

Table IV. The yield and quality of the cocoons harvested from Halamid treated silkworms

scale of rearing	Duration of spraying	Intervals of spraying	Conc. of Halamid (%)	Mortality due to apparent intoxication	Indices of cocoon yield (control 100)	Percentage of cocoon layer	Percentage of reelability	Percentage of raw silk yield
a laboratory scale*	1stto 5 th instar	every day	0.5	0	101.4	18.0	73	17.19
			1.0	0	103.1	17.7	73	17.46
			2.0	0	108.2	17.5	68	17.62
			3.0	0.7	108.5	16.9	76	17.71
			5.0	10.0	106.6	16.6	77	17.10
			(Formalin) 1.0-2.0	2.0	98.6	16.5	76	17.10
			control	0	100.	18.1	78	18.46
		every second day	0.5	0	101.3	17.5	86	17.40
			2.0	0	103.0	18.1	81	17.89
			5.0	0.3	106.5	18.0	81	17.35
			5.0	2.3	106.7	18.0	81	17.88
			control		100.	18.1	78	18.46
a practical rearing scale**	1st to 5th instar	every day	2.0		132.3	19.7	66	18.13
			control		100.	20.1	68	17.99
		every second day	2.0		111.8	19.8	72	17.30
			control		100.	20.1	68	17.79

* 300 silkworm larvae of each treatment.
** About 10,000 silkworm larvae of each treatment.

Fortunately, the fact that Halamid fulfils the above advantages is already well known. Nevertheless, it should be remembered that like all other cases of general or ordinary disinfectants Halamid exerts its excellent killing effect to various silkworm pathogens on the outside of silkworm bodies only, never to the inside of the bodies. Spraying the Halamid solutions must be planned to prevent the infection by the pathogens.

The authors still have not carried out the experiment on a large scale in practical silkworm rearing field, and have not examined the susceptibillity to Halamid of all of the silkworm pathogens already found, also have not investigated the residual disinfectant effect of Halamid as a disinfectant for silkworms and their environmental articles.

Although more studies are intended to be carried out on the usefulness of Halamid in silkworm rearing in dustry, the authors still believe that Halamid will become the most useful disinfectant for both silkworms themselves and silkworm rearing houses and tools.

摘 要

1971 년 가을누에 飼育期間동안 서울大學校 農科大學 蠶室에서 (1) 4 가지 重要한 누에 病原體(*Aspergillus flavus, Bacillus thuringiensis, Aspergillus Oryzae* 및 *Isaria farinosa*)의 Halamid (Tosylchloramide Sodium) (할라미드; 한국내 登錄商品名 하라밀®)에 대한 感受性에 관하여 (2) 각 齡期에서의 누에 幼虫에 대한 Halamid의 急性毒性에 관하여 (3) 곰팡이로 인한 硬化病과 Virus로 인한 膿病의 發病에 대한 Halamid의 抑制效果와 Halamid의 누에 幼虫에 대한 慢性毒性에 관하여 (4) Halamid로서 處理된 누에에서 얻은 生絲의 量과 質에 관하여 일련의 實驗을 실시하였다.

이번 實驗의 結果로서 筆者들은 Halamid를 蠶室, 蠶具 및 蠶體 消毒藥으로서 有效하게 쓸수 있다는것, 그리고 Halamid는 2%나 3%의 水溶液狀態로서 누에 幼虫에 대하여 全齡期를 통해서 하루걸러 뿌릴때에 蠶體消毒藥으로서의 效果를 實驗한 다른 條件의 경우보다는 더욱 잘 발휘한다는 것을 알아 내었다.

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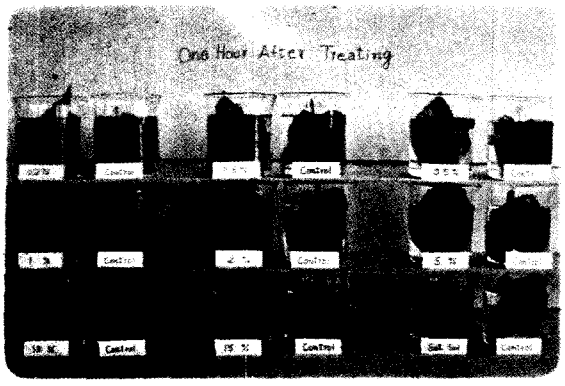
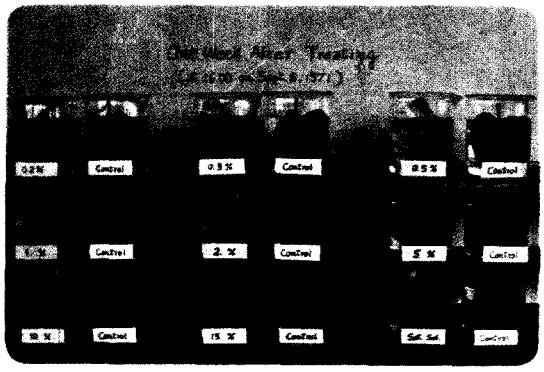
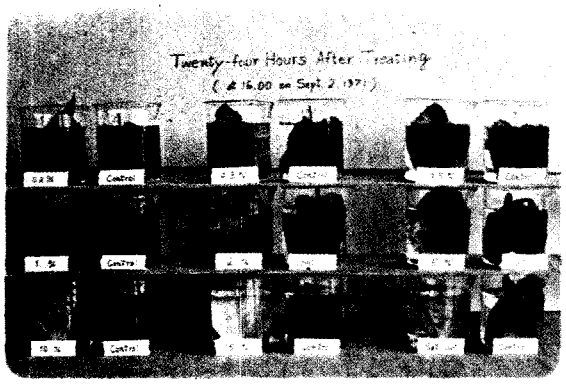






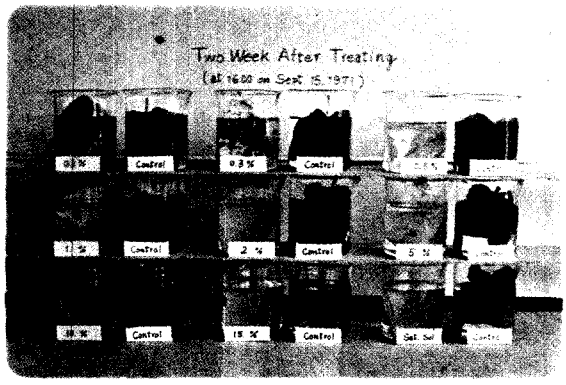
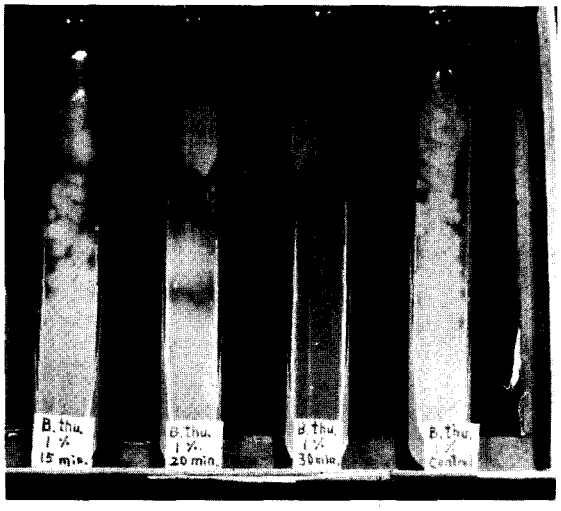
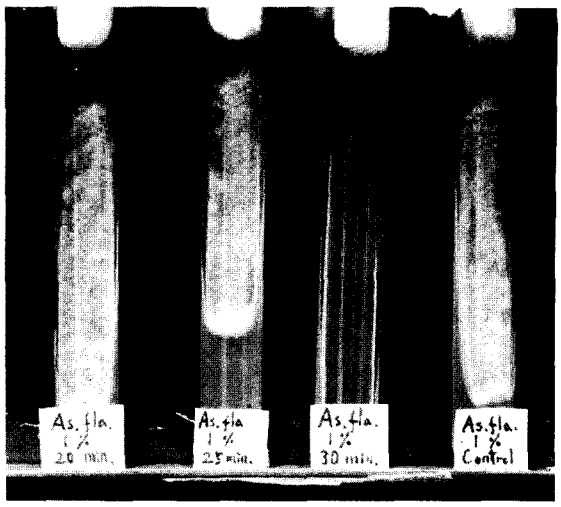
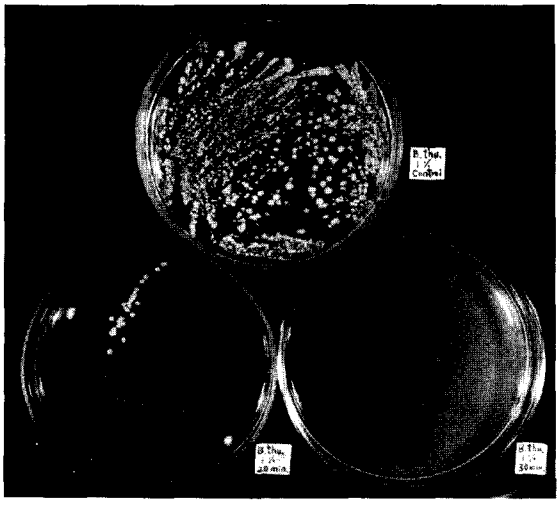


Fig. 1. The discoloration of mulberry leaves by Halamid solutions.







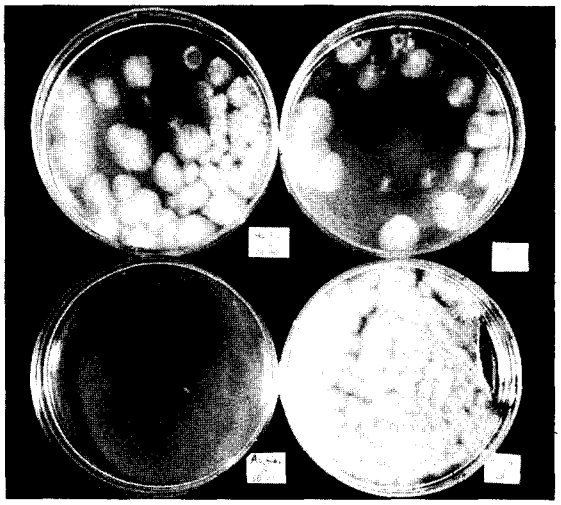


Fig. 2. Eleven photographs showing growth of four silkworm pathogens under the influence of Halamid


As. ory
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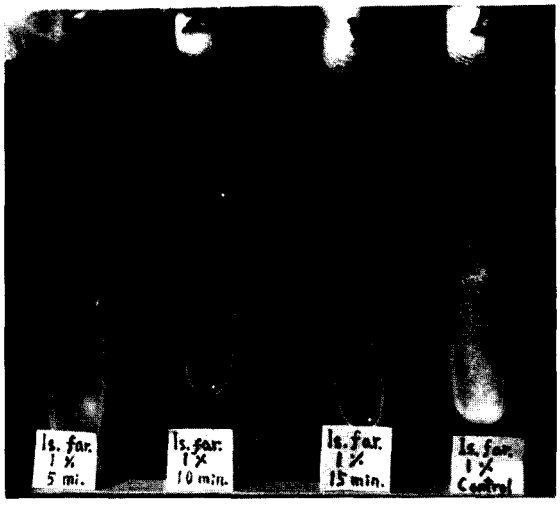
5 min.
As. ory
1%
10 min.
As. ory
1%
15 m
As. ory
1%
Control


Is. far.
1%
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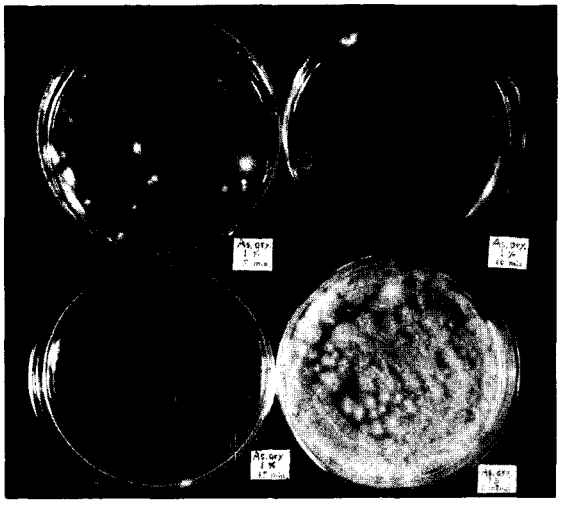

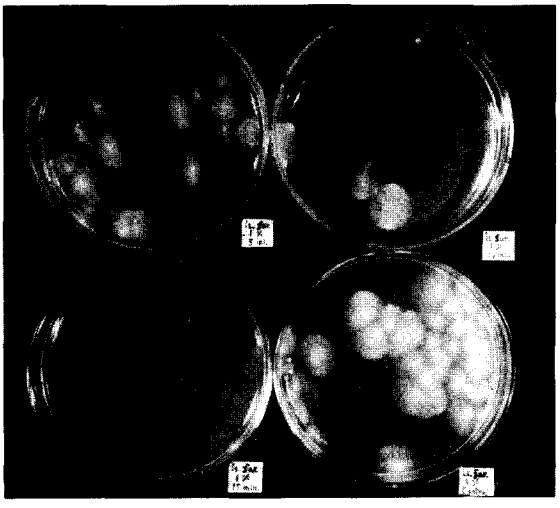

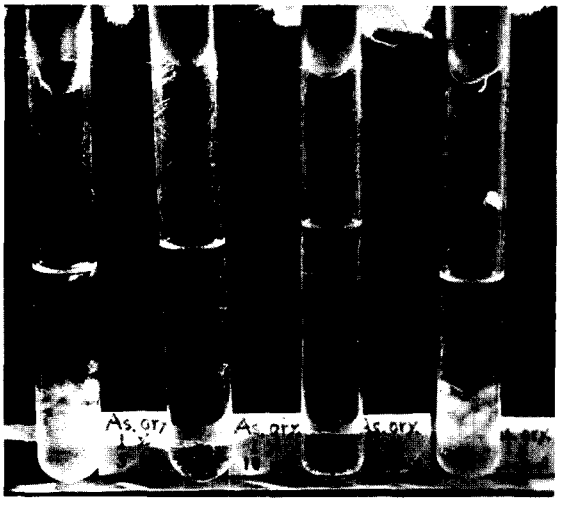
As. ory
1%