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EFFECT OF SOME DISINFECTANTS ON LARVAL HAEMOLYMPH PROTEIN PATTERN OF THE SILKWORM, *BOMBYX MORI* L. LARVAE

M. F. EL-SAYED[1], EL-SAYED MEGAHED [2], A. A. EL - SHEAKH[1] AND A. A. ZANON[1]

1 . Plant Protection Research Institute , Agricultural Research Centre, Dokki, Egypt.
2 . Faculty of Agriculture, Zagazig University , Egypt.

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Abstract

The biochemical effect of five bed disinfectants namely Papsol, New-Ceresan, Sodium hypochlorite, Formalin and Calcium hypochlorite on the electrophoretic protein pattern in the haemolymph of *Bombyx mori* L. 4th and 5th instar larvae was investigated. The haemolymph protein pattern of the 4th and 5th larval instars revealed quantitative and qualitative differences between treated and untreated larvae.

The haemolypmph protein pattern of untreated larvae showed seven bands which varied significantly in density and migratory behaviour. The haemolymph protein pattern of treated larvae showed significant variation within the five disinfectants.

INTRODUCTION

Larvae of the silkworm, *Bombyx mori* L. are susceptible to indigenous diseases. Losses due to diseases occur in all silkworm rearing areas , although the severity may vary from place to another . Total failure of the cocoon silk yield may also occur. It is therefore necessary to implement a strict policy that would avoid disease transmission, and to increase resistance of the silkworms by using external disinfectants.

Disinfectants used on *B. Mori* reduce larval mortality rate, improve cocoon indices and filament characteristics (Israngkul *et al.*, 1972; Jayaramaiah *et al.*,

1986; Hosny *et al.*, 1986).

The present work aims at investigating changes in the electrophoretic protein pattern of the haemolymph of 4th and 5th instar larvae of *B. mori* due to the use of five disinfectants , namely Papsol, New - Ceresan, Sodium hypochlorite, Commercial formalin and Calcium hypochlorite.

MATERIALS AND METHODS

Experiments were conducted on the Korean hybrid 155x156 of silkworm eggs. Rearing was carried out under the laboratory conditions of 26±1% R.H. The usual procedure of rearing took place at the same site where the silkworm has been previously reared during previous seasons. No precautions were taken to disinfect the rearing room or equipment and tools prior to rearing.

Disinfectants used

1. Papsol : active ingredient para formaldehyde 3%
2. New-Ceresan : active ingredient formaldehyde.
3. Sodium hypochlorite : specific gravity 1.15 at 20°C., contains 8+2% Cl as active alkalinity NaOH max. 8%.
4. Commercial Formalin : 34 - 38% with max. 0.04% activity.
5. Calcium hypochlorite : chlorinated lime of bleaching powder contains min. 33% available chlorine.

Concentrations used

As the bed- disinfectants Papsol and New-Ceresan are powders, 2g/30cm were prepared for dusting on the body surface of the larvae according to Israngkul *et al* ., (1972) .

Concerning the bed disinfectant solutions, Sodium hypochlorite, Formalin and Calcium hypochlorite, 0.2% were prepared according to Hosny *et al.*, (1986) and Jawale and Tayade (1987).

The powders were dusted through mesh cloth holes, while solutions were applied directly on the larval body using a sprayer.

Six hundred newly hatched larvae representing six replicates and their control were sorted. Each replicate or control was put on a wooden tray (2x20x3cm) to be reared separately. Fresh mulberry leaves were offered four times daily during larval period.

Electrophoretic identification

Poly acrylamide gel electrophoresis was employed for the separation of haemolymph protein components according to the method adopted by Davis (1964).

Identification of the zones

The protein bands were classified according to mobility resolution (Mr) value in relation to the tracking dye (bromophenol blue) which was assigned as Mr of 100. The starting point of the gel was given the value of zero. Thus, the migration of each protein band in a gel was determined according to its position or to its mobility resolution value that fell in between zero and hundred.

RESULTS AND DISCUSSION

1. Electrophoretic protein pattern of the haemolymph of the 4th larval instar of *B. mori* L.

The haemolymph protein pattern of the 4th larval instar of *B. mori* L. revealed quantitative and qualitative differences between treated and untreated larvae (Fig.1).

The protein pattern of untreated larvae showed seven bands which varied signifcantly in their densities and migratory behaviour. B6 appeared to be the major band having the largest density.

Refering to the number of bands, Papsol appeared to be the most effective bed-disinfectant. It showed five bands, and the major band B6 existed at the same density and Mr as that of the untreated. The slowest moving band B1 was present in

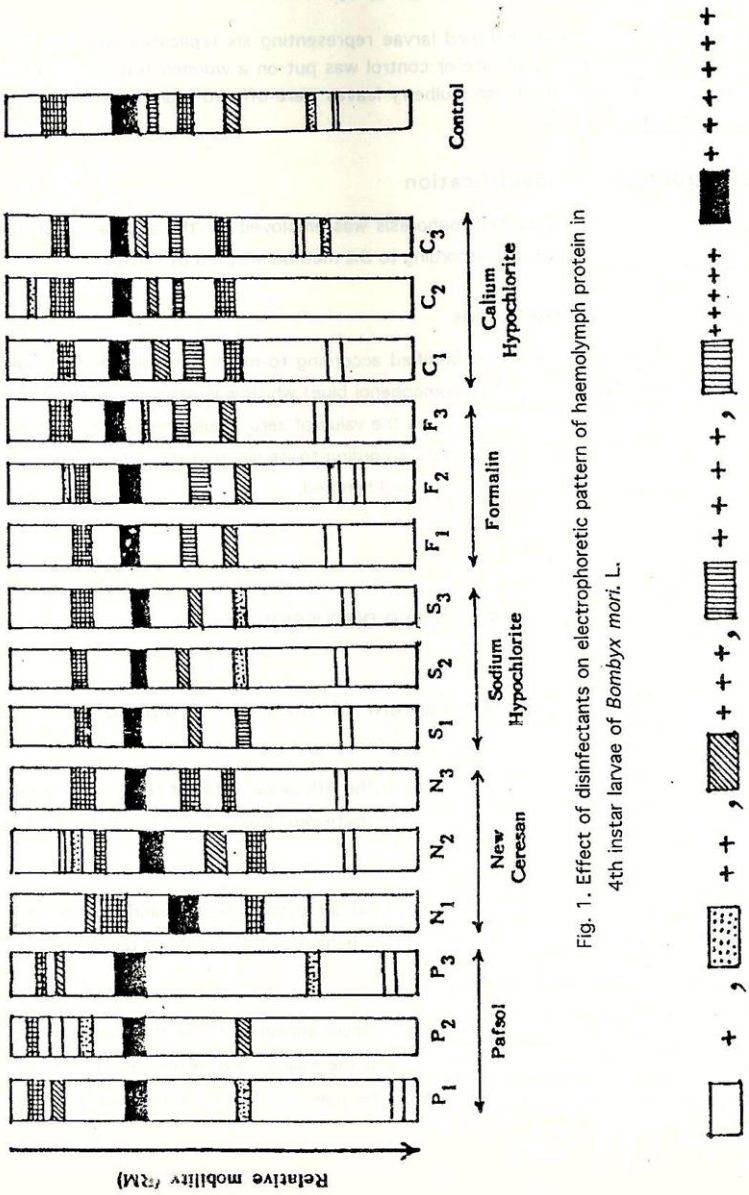

Fig. 1. Effect of disinfectants on electrophoretic pattern of haemolymph protein in 4th instar larvae of *Bombyx mori*. L.

larvae treated with Papsol and absent in the Control . The slow moving band B2 existed in low density when compared with the control . The fast moving band B22 was absent in the control but was found in Papsol treatment(lower and higher concentration, B7 and B3). In the region between the fast and the slow moving bands, few bands varying in number and density were observed. It could be concluded that B1 was a characterestic band for Papsol. Its existence could be attributed to the fact that Papsol induced the synthesis of that specific protein band.

As for New-Ceresan treated larvae, the haemolymph protein patterns showed 5,7 and 5 bands at the three different concentrations (N1, N2 and N3), respectively. The lower and the higher concentrations (N1 and N3) were characterized by the absence of B7 and B2 bands in both treatments , while the middle concentration (N2) was characterized by the absence of B7 and the existence of B2 but in low density.

In the three diagrams representing the haemolymph protein pattern of Sodium hypochlorite treated larvae, B1 and B2 bands were absent at the three concentrations showing qualitative and quantitative differences among treatments compared with the control . Sdium hypochlorite treated larvae were also characterized by the absence of B7 band.

The haemolymph protein pattern of Formalin treatment at the three different concentrations showed 5,7 and 6 bands, respectively. B7, B8 and B20 bands were absent , while B2 band was absent only at the lower concentration.

As illustrated in Fig1, larval haemolymph protein pattern of Calcium hypochlorite showed appreciable differences when compared with the control . B3, B4 and B5 bands were absent in the haemolymph protein pattern of Calcium hypochlorite treated larvae.

2 . Electrophoretic protein pattern of the haemolymph of the 5th larval instar

The haemolymph protein bands of the 5th larval stage differed in case of *B. mori* L . than in other insects. It was recorded by McCormick and Scott (1966) to be 19 fractions in case of *Locusta migratoria.* Chen and Levenbook (1966) working on *Phormia ricina* detected 19 protein bands. Nielson and Mills (1968) reported 15 protein bands in the blood of the American cockroach. Abdel Nabi (1983) recorded

26 protein zones in blood of silkworm *Philosamia recini* fifth instar larvae prior to cocoon formation . In the present study only seven bands were separated at the end of the 5th instar untreated larvae.

As shown in Fig 2, there were quantitative and qualitative differences in the haemolymph protein pattern of the 5th larval instar of both treated and untreated larvae. The control showed seven bands, while Sridhara and Anathasamy (1963) reported six protein zones in the haemolymph of the natural larvae of *B. mori.* The control zones varied significantly in their densities and migratory behaviour, as B6 band was major with the highest density.

Papsol-treated larvae had only 5 bands. The major band B6 appeared at the same density and relative mobility as in the control. The slowest moving band B1 existed in Papsol-treated larvae while it was absent in the control. The slow moving band B2 appeared in Papsol protein patterns in high density. Different bands which varied in number and density were observed between fast and low migrating bands. So, as in the case of fourth larval instar, it could be concluded that B1 band might be characteristic of Papsol treatment because it was absent in the control.

In case of New Ceresan, the bands of the concentrations N1 and N3 were characterized by the absence of B1 band . While at the moderate concentration N2, the existence of B1band was observed. At the same time, B3 band was detected with both the lower and higher concentrations N1and N3 but with different densities.

Sodium hypochlorite disinfectant affected haemolymph protein resulting in the existence of 8 and 9 bands in treated larvae and seven bands in the control. Haemolymph of treated larvae was characterized by the absence of B10 band as compared with the control.

Following the three diagrams representing Formalin treatments , Formalin did not affect the number of bands at the concentrations 0.2 and 0.4% B6, which is the major band in control treatment was absent at the three concentrations. Other bands with different densities and relative mobilities appeared in Formalin treatment.

As illustrated in Fig.2, B2 , B14 and B18 bands were detected in the control treatment , but were absent in Calcium hypoclorite treated larvae at the three concentrations. This absence might be due to the inhibitory effect of Calcium hypochlor-

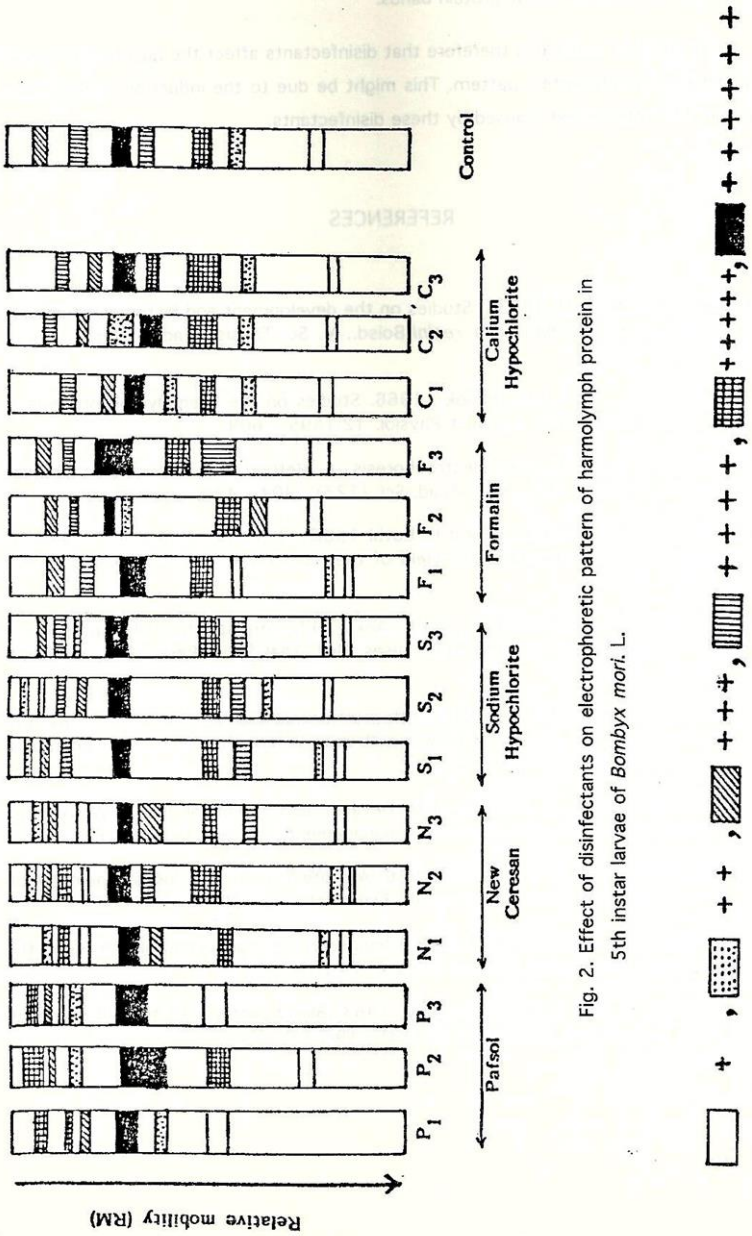

Fig. 2. Effect of disinfectants on electrophoretic pattern of harmolymph protein in 5th instar larvae of *Bombyx mori*. L.

ite on the synthesis of those protein bands.

It could be concluded therefore that disinfectants affect the quantity and quality of haemolymph protein pattern. This might be due to the induction or repression of specific protein bands caused by these disinfectants.

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تأثير بعض المطهرات علي صور بروتينات الهيموليمف في يرقات دودة الحرير

محمد فرج السيد [١] ، السيد مجاهد [٢] ، علي عبد العزيز الشيخ [١] ، عبد الحميد زنون [١]

١ – معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي
٢ – كلية الزراعة – جامعة الزقازيق.

في هذا البحث تمت دراسة التأثير الكيماوي الحيوي لخمس مطهرات وهي البابسول ، نيوسيريسان، هيدروكلوريت الصوديوم ، الفورمالين ، وهيبوكلوريت الكالسيوم علي صورة الهجرة الكهربائية لبروتينات هيموليمف العمر اليرقي الرابع والخامس لدودة الحرير.

وقد اظهرت صورة بروتينات هيموليمف العمر اليرقي الرابع والخامس اختلافات كمية وكيفية بين اليرقات المعاملة وغير المعاملة. وأظهرت بروتينات اليرقات غير المعاملة سبع مناطق فصل تختلف معنوياً في الكثافة ومعدل الهجرة أما بالنسبة لليرقات المعاملة فقد أظهرت اختلافا معنويا بين المركبات المستعملة.