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FIELD APPLICATION OF *BACILLUS THURINGIENSIS VAR KURSTAKI* (DIPEL-2X) AGAINST SOFT AND HARD TICKS.

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SUMMARY

Field application of *Bacillus thruringiensis (B.t.)* var.. *kurstaki* (Dipel-2X) against hard tick, *Boophilus annulatus* and soft tick, *Argas (persicargas) persicus* was probed. Hard tick died in infected calves after six days post spraying with Dipel-2X while soft tick succumbed in its nymphs after six weeks post treatment. The indirect effect of Dipel-2X on *Argas (p.) persicus* was also investigated. Egg laying of tick fed on hens injected with 20 and 40 mg Dipel-2X was clearly affected. Molecular structures of hemolymphs collected from ticks got Dipel-2X infected blood meals did not change as demonstrated by SDS-PAGE.

Key words: *Bacillus thuringiensis* var. *kurstaki* (Dipel-2X), *Argas (p.) persicus, Boophilus annulatus,* soft tick, hard tick, field application, hemolymph, egg laying, SDS-PAGE.

INTRODUCTION

Ixodoidea, including soft and hard ticks are recognized world-wide as major vectors parasitizing birds and mammals respectively. They are considered one of the most important biological vectors, transmitting diseases to man and animals.

Due to the increased environmental chemical pollution, the scope of biological control of external parasites is greatly increased and attracted the attention of scientists. The use of *Bacillus thuringiensis* in insect control as an alternative compound to chemical insecticides is presently being given considerable attention due to its feasibility of application and high specificity of its toxin which is non toxic to man and most other non target insects. Many successful field trials have been carried out using *B.t.* as spray against Citrus red mite, black fly and mosquitoes (Hall et al., 1971; Oswald, 1973; Lawrence and Albert, 1984; Ree et al., 1983 and Liu, 1986). The field persistence of formulation of *B.t.* found to be significantly greater in areas with hot and dry climate than in the other areas where conditions were cool and humid (Dudley et al., 1974). Whereas Dulmage and Aizawa (1982) and Phyllis and Russell (1989) indicated that strains of *B.t* are ubiquitous soil microgranism.

No literatures are available dealing with the effect of *B.t.* against soft and hard ticks except Hassanian et al., 1997 and Abdel Gaffar et al., 1997 who initiated a research to determine the effect of serial dilutions of three varieties of *B.t. (kurstaki, israelensis* and *thuringiensis)* against different stages of soft and hard ticks. Also the protein alterations of the treated-tick hemolymph were mentioned.

The present study is considered as a continuation of these previous trials and concerns with the field application of *B.t. kurstaki* on soft and hard ticks.

MATERIAL AND METHODS

The spray solution was prepared by dissolving 10 and 20 gm Dipel-2X per liter of water according to Hussanain et al. (1997) for spraying soft and hard ticks respectively. Soft ticks were sprayed in their cracks while hard ticks were sproyed on animals. The solution was thoroughly agitated with magnetic stirrer for 15 min just before use and electric sprinkler was used.

Field application of Dipel-2X against hard ticks.

Total number of 33 infected calves with hard ticks, *Boophillus annulatus* with tick index 89.2, were subjected to field experiment. Five of these animals were kept as non sprayed control group while twenty eight infected animals were sprayed for two successive days in-door with Dipel-2X (20 gm/L water) and animals were observed daily for tick mortalities for ten days.

Field applicatio[illegible] of Dipel-2X against soft tic

Infected fowl house with soft ticks, *Argas* (*persicus* was chosen for field application t during summer. The cracks and walls of t chambers of chicken house were sprayed with prepared solution of dipel-2X (10 gm/L) twi with one week interval. The sprayed chick chamber (12 crack/chamber) were observ weekly for 3 months. Another two chambers the chicken house were kept as control no sprayed ones (12 crack/chamber). The effect Dipel 2x on *Argas p. persicus* was evaluated b holing three cracks weekly to determine th mortality percentages. Ticks in representativ control cracks were counted (weekly) similarly t determine the number of tick population in thes cracks and the observation lasted for 3 months.

Experimental assay of Dipel-2X as an acaricide,

Fifteen layer's hens weighing about one kg were divided into five equal groups. Hens in first four groups were separately injected subcutaneously with 1 ml dist. water containing 5, 10, 20 and 40 mg Dipel-2X respectively. The fifth group was kept as non-treated control. After one week post injection each hen was tied thoroughly in separate cage and five adult unfed females were put under each hen's wing to feed. Complete feeding lasts for 30-45 min and then engorged females dropped off. Each fed female was kept in a separate plastic tube, incubatd at 29°C ± 2 and 75% relative humidity and observed daily for egg

laying. This assay was conducted to evaluate the effect of dipel-2X on tick fertility indirectly through blood meals from *B.t.* injected hens.

Hemolymph was also collected in separate capillary tubes from the above mentioned fed ticks by amputating its legs (Hefnawy, 1972). Hemolymph were centrifuged at 10,000 rpm for 10 min and stored at -20°C until use. Protein content of the collected hemolymph was determined according to Lowery et al. (1951).

Electrophoretic studies on the hemolymph proteins of *Argas (p) persicus* fed on *B.t.* infected hens using sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS- PAGE) was conducted according to Laemmli (1970).Molecular weight standards were phosphorylase B (97 KD), bovine serum albumin (66 KD), ovalbuumin (45 KD), carbonic anhydrase (29 KD) and ج-lactalbumin (14 KD). All were electrophoresed in sample buffer.

RESULTS

The effect of Dipel-2X with the concentration of 20 gm/L on *Boophilus annulatus* mortality was displayed in table 1. within the first three days of Dipel-2X spray, the tick index was still 89.2 as no tick mortalities were produced. After four days of exposure the lethal effect of Dipel-2X started to appear as the tick index reached to 27.1 and 10.1 after four and five days of spraying respectively (Table 1). The maximum lethality of Dipel-2X was observed after six days post spraying where all treated animals became free from ticks. the moribund ticks are still found in situ on animals and need gentle movement to drop off.

The moribund ticks were paralyzed first and still alive after two days psot spraying and succumbed after 4 days where the integument of the sprayed ticks became balckened and flaccid.

Representative cracks full of *Argas. p. persicus* in fowl houses were sprayed with 10g/L Dipel-2X water suspension and observed weekly for tick mortality percentages. Sprayed ticks were clearly affected with Dipel-2X showing mortality percentages reached to 76.5 after 2 weeks post spraying (Table 1). The effect was gradually increased (87.3-96.0) to reach its optimal value after six weeks by complete death of treated ticks (Table 1).

Table 1 : Mortalities of *Boophilus annulatus* & *Argas (p.) persicus* sprayed with Dipel-2X.

Boophilus annulatus		*Argas (p.) persicus*	
Time (Day)	Tick index	Time (Week)	mortality (%)
2	89.2	2	76.5
3	89.2	3	87.3
4	27.1	4	96.0
5	10.1	5	96.0
6	-	6	100.0

The indirect effect of *B.t.* on *Argas p. persicus* was evaluated by injecting hens subcutaneously with different concentrations of Dipel-2 X and then allowing ticks to get *B.t.* infected blood meals. The comparison which was held between egg masses of *Argas persicus* female ticks fed on hens injected with 5, 10, 20 and 40 mg Dipel-2X was interesting. the results cleared that egg mass deposition among tick females fed on hens injected either with 5 or 10 mg Dipel-2X was similar to control ticks fed on non injected hens which ranged between (65-80 eggs). However, tick females fed on hens injected with 20 mg Dipel-2X showed reduction in egg masses reached to 15-20 eggs. Failure of egg laying was observed by tick females fed on hens injected with 40 mg Dipel-2X.

Polyacrylamide gel electrophoresis was employ to identify constituent hemolymph proteins *Argas P. persicus* fed on hens previously injec with 10, 20 and 40 mg Dipel-2X. In additi control hemolymph collected from Argas fed non-injected birds was also separated. Aliquots hemolymph (≃ 30µg) were suspended in t appropriate sample buffer and resolved by 10 one-dimensional SDS-PAGE slab gel.

Control hemolymph was resolved into a numb of molecular entities of which the most salie were 91.2, 66, 53.7, 30,29, 14 and 12 K components as shown in Fig. 1, 2, 3 lane A Unexpectedly, similar electrophoretic profiles t control were observed with hemolymphs collecte from ticks fed on hens injected with 10 mg Dipe 2X (Fig. B), 20 mg (Fig. 2B) and 40 mg (Fig. 3B

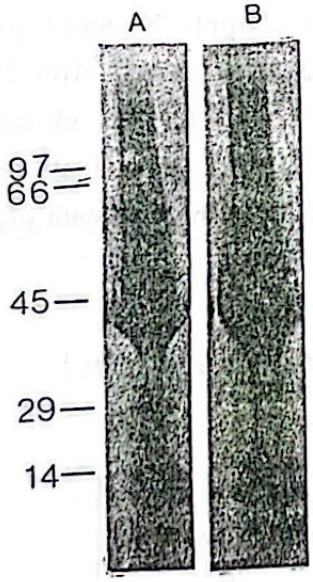

Fig. 1: SDS-PAGE of control hemolymph (A) and 10 mg treated hemolymph. (B).

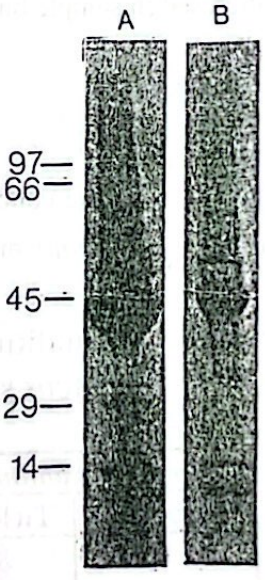

Fig. 2: SDS-PAGE of control hemolymph (A) and 20 mg treated hemolymph. (B).

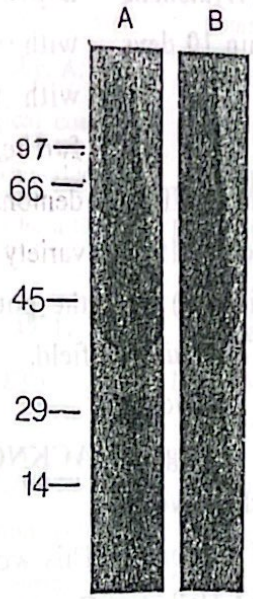

Fig. 3: SDS-PAGE of control hemolymph (A) and 40 mg treated hemolymph. (B).

The hemolymph of *Argas* Argas fed fed on birds injected with 5 mg *B.t. showed* similar electrophoretic profile to that of 10 mg *B.t.* fed ticks.

DISCUSSION

The present study was carried out to investigate he possibilities of using Dipel-2X (*B. t. kurstaki)* gainst soft and hard ticks in field trials and onsiderd the first field trial in biological control f ticks. The selection of adult stage of both tick pecies is attributed to the severe damages caused y these serious ectoparasites due to their blood eeding and the long period they parasitize on nimals. Also engorged females are the stages responsible for egg deposition and consequently the continuation or cycling of different stages. The effect of Dipel-2X on soft ticks., *Argas (p.) persicus* was interesting, the representive sprayed cracks in the hen house were free from ticks after 6 weeks post spray. The data showed that 76.5%, 87.3 and 96% mortality reached after 2,3 and 4 weeks respectively. This study is an extension to previous laboratory work done by Hassanain et al., 1997 who revealed that soft ticks, *Argas (p.)* persicus succumbed after 5 days post tratemnt with Dipel-2X.

Concerning the effect of Dipel-2X against hard tick *Boophilus annulatus,* the present trial revealed that sprayed calves became free from tick after 6 days. Also Hassanain et al. 1997 stated

during laboratory experiment on engorged and unfed males and females of hard ticks, *Hyalomma dromedarii* that ticks succumbed within 10 days post treatment with Dipel-2X.

No literatures are available dealing with the effect of *B.t.* on soft and hard ticks, but Burn et al., 1991 used another microbe (Enterobacteriaceae) as microbial pathogen against hard ticks, *Boophilus microplus,* they found that the microbe produced a genital infection and/or death of engorged females. Meanwhile previous studies dealt with the lethal effect of *B.t.* were focused on some agriculture mites, acarina (Heimple, 1967, Hall et al., 1971, Neal et al., 1987 and Hoy and Ouyang, 1987). They found that *B.t.* (B-exotoxin) is highly effective against citrus red mites *Panonychus citri,* the two spotted spiders mites, *Tetranchus urticae* and the pacific spider mite, *T. pacificus* and *T. cinnabarinus.*

The notable effect of Dipel-2X on egg laying of *Argas (p.) persicus* deserved further investigation. Searching for reasons behind this effect. Examination of protein structures of the hemolymphs, the extracellular fluid responsible for the vital activities of ticks is adopted. collected hemolymphs from *Argas* fed on hens injected with, 10, 20 and 40 mg Dipel-2X were analyzed by 10% SDS-PAGE. Actually protein banding of these hemolymphs did not change proving that the effect of Dipel-2X on egg laying did not concern with hemolymph structures. Although Dipel-2X was shown to induce drastic changes in the hemolymph components of the same *Argas* species when it was sprayed directly with this bacterial variety (Abdel Ghaffar et al., 1997).

These results indicated that the indirect effe Dipel-2X on *Argas p. persicus* may be associ with other organs than hemolymph and prob with tick reproductiv organs. Consequen further investigations are necessary t demonstrate the mode of action of this bacte variety on soft and hard ticks as well and to all the potential acaricide use of Dipel-2X in t field.

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