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Annals of Biological Research
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CODEN (USA): ABRNBW

Study of the effect of *Beauveria bassiana* (Vuil,) on the biochemistry and structure of the cuticle of *Schistocerca gregaria* (Forskal)

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ABSTRACT

This study was undertaken in order to help identify the biological activity of the entomopathogenic Beauveria bassiana against the cuticle of adult individuals and 5th stage larvae of Schistocerca gregaria viewpoint biochemistry and structure. The application of entomopathogenic B.bassiana with the dose 1.69 × 105 spores / ml on the cuticle of L5 and adults of S.gregaria showed a significant reduction in the dry weight of the cuticle from 36, 50mg to 32.93 mg in L5 and 46.49 mg to 42.31 mg in adults. The same is true for the chitin content is increased from 7.38 mg to 6.44 mg in L5 and 7.41 mg to 4.20 mg in adults. For proteins, the amount dropped to 29.12 mg 26.48 mg in L5 and 39.08 mg to 37.88 mg in adults. The larval cuticle of the fifth stage of S.gregaria highlighted by differential staining with Heidenhain azan , shows the presence of the epicuticle , the exocuticle and endocuticle. The epicuticle colored red is as a thin layer on the surface of the cuticle and L5 S.gregaria exocuticulaire least orange colored thinner than the first part of a colorless and a greater thickness corresponds to the endocuticle. The application of the integument B.bassiana 5th larval stage S.gregaria was dependent of an apparent disturbance on the structure of the cuticle.Indeed the third day of treatment it has been observed a complete disappearance of the epicuticle and a sharp decline in the thickness of the exo and endocuticle and that compared to the control larvae cuticle or three layers are distinct and exposed with a visibly thicker. The breakdown of cuticular layers larvae treated is probably due to degradation by proteolytic enzymes and chitinolytic the infectious unit of our fungal strain, spore which was spotted a few cells in parts of the cuticle digested.

Key words: *Schistocerca gregaria*, *Beauveria bassiana*, cuticle treatment, biological control.

INTRODUCTION

Since the advent of agriculture there are more than 10 000 years, humanity is facing a formidable enemy and full of resources, the grasshopper gregariapte or locust. Normally solitary, these insects originate from the deserts of West Africa to India transform when some conditions are met, voracious in huge swarms that leave a trail of devastation behind them.

All time, farmers and plant protection services have worked to push bands and swarms making noise or smoke, by picking, burying and burning insects. Swarms have always imposed their law: that of number. In fact, they can extend for hundreds of kilometers and after billions of winged [1].

Man has always wondered where these animals come from and how they survived. It was not until the mid-twentieth century it was discovered that the insect light brown lonely living in the desert was the same species as the desert of red and yellow color invasions.

Algeria of all time is recorded among the countries where the locusts has a great economic importance, The most harmful locust species in the country remain Locust *Schistocerca gregaria* (Forskal, 1775) ; the migratory locust *Locusta migratoria* (Linné, 1758) and the Moroccan locust *Dociostaurus maroccanus* (Thunberg, 1815).

In Algeria, as in the world, since the biology of these locusts was understood, only chemical pesticides and aerial spraying have been used to combat the apocalyptic plague and reduce infestations. However, use of pesticides on a large scale has also raised real concerns for health and environment, Locust chemical pesticides are not only specific to locusts, they often have a detrimental effect on their natural enemies, on birds and insects of agronomic utility [2]. An awareness of these problems has led research institutes to provide the biocontrol agents to reduce the use of chemical pesticides. Mention the last locust infestation in El Oued (south of Algeria) region where a large quantity of biopesticide *Metarhizium anisopliae* spores variety acridum (Green –Muscle) was spread under the auspices of the FAO and the National Institute of Plant Protection of Algeria (El harrach). In fact, the biological control, specifically by use of entomopathogenic microorganisms is a very promising alternative to ensure effective pest control by natural ubiquity of microbial in ecosystems their wide variety, their easy dissemination, specificity of action and also their persistence in the environment. The micro-organisms used in microbial control belong to several taxa namely viruses, bacteria, micro-fungi, nematodes and protozoa. The use of microbial insecticides increases rapidly from 10 to 25% per year [3]. By the same author; among the microorganisms used, more than 700 species of micro-fungi are entomopathogenic and play an important role in the natural control of insect populations. The largest number of pathogens is in the class Zygomycetes, but most employees come from Deuteromycetes (imperfect Fungi), such as *Beauveria, Metharizium, Verticillium, Hirsutella, Entomophtora* and *Entomophaga*. They have considerable agronomic interest in the fight against crop pests and are therefore studied more extensive [4]. In this sense several studies have been made in the Department of Agricultural and Forest Zoology of the National Agronomic Institute of El Harrach (Algeria). Among them we quote those of, treating the biological activity of *Metharhizium anisopliae* against *Schistocerca gregaria* [5], [6]. The study of the the influence of *Beauveria bassiana* on the digestive tract, the cuticle and eggs of *Schistocerca gregaria* and *Locusta migratoria* [7],[8], [9], [10] ,[11]. The study the effect of two entomopathogenic on CBC and the cuticle of *Locusta migratoria* [12].

In order to make our contribution, we have conducted research on a promising biological control agent against crop pests, the *Beauveria bassiana* (Bals.). The present study examines the activity of entomopathogenic acridicide towards the locust and migratory locust. On individuals of *S. gregaria* we monitored its effect on the cuticle of perspective structure and biochemistry.

MATERIALS AND METHODS

l.1 biological material:

l.1.1 fungal strain:

The bioassay was conducted mainly on the fungus *Beauveria bassiana* isolated from dead individuals locust recovered in December 1996 in South Algerian [13].

l.1.2 Locust:

The study of the biological activity of *B. Bassiana* is conducted on the locust *Schistocerca gregaria*. People used to come from the town of Ain Zegumir, Zaouit Kunta located at the province of Adrar (Algeria).

➢ **Breeding of locusts:**

Breeding is reproduced in the laboratory of the Department of Agricultural and Forest Zoology of the National Institute of Agronomic of El Harrach (ENSA) in rectangular cages made of wood and covered with a metal mesh fine mesh. Volume of 56 cm × 43 cm × 24 cm, cages are fitted with sliding glass doors for easy cleaning and replacement of food, The bottom of the cage is equipped with nest boxes containing moistened sand, The breeding is conducted under an average temperature of 30 ± 2 ° C and a relative humidity of 75%, the food is a mixture of cabbage, wheat and wheat bran.

METHODS

1.2.1 Treatment:

To administrate the fungal solution containing *B.bassiana* the locusts we selected 50 lethal dose defined by HALOUANE (2008) in imagos *S. gregaria* treated with entomopathogenic *B.bassiana* that evaluates to 1.69×10^5 spores/ml.

Individuals used for the biochemical study of the cuticle are the fifth larvae instar (L5) *S.gregaria* aged 8 days and adults aged 10 days from the operation described above. Histological study has been conducted only on the 5th larval stage just after molting.

The application of the fungal solution (fungal spores contained in sterilized distilled water is performed by topical application using a micro syringe. A quantity of 2µl is deposited above the pronotum of all individuals experienced locusts. Control individuals were treated with sterilized distilled water.

1.2.2 Techniques:

- Determination of cuticular chitin-protein:

Individuals distributed in two batches, control and treatment were sacrificed on the third day after treatment. The abdomen is disconnected from the rest of the body, and its rear end. Using fine forceps, removing everything viscera, muscles and adipose tissue. The abdominal sterna are then placed between two sheets of filter paper to remove the remaining tissue can adhere to the cuticle.

The technique that is based on determining the chitin-protein Bordereau and Anderson (1978) cited by Abbasi and Hamza (1995) [14].

To confirm statistically the effect of *B. Bassiana* amounts of protein and chitin in the abdominal sternal cuticle L5 and adults *S.gregaria* the 3rd day of treatment we used analysis of variance to sole criterion for classification.

Histology of the cuticle:

The histological technique is performed according to the method of Gabe (1968) [15].

RESULTS AND DISCUSSION

II.1- Effect of *B. bassiana* on the biochemistry of cuticle 5th instar larvae and adults of *S. gregaria:*

The results for the quantities of chitin and protein content in the abdominal sternal cuticle 5th stage larvae and adults *S.gregaria* control and treated by the entomopathogenic *B.bassiana* are included in the graphs of Figures n°1 and 2. Through the results shown in Figures 1 and 2, it appears that the treatment *B.bassiana* significantly decreased ($P < 0.01$) the quantities of chitin and protein contained in the abdominal sternal cuticle L5 (7.38 ± 1.14 to 6.44 ± 1.28 for chitin and 29.12 ± 0.98 to 26.48 ± 0.48 for protein) and adults (7.41 ± 1,37 to 4.20 ± 0.77 for chitin and 39.08 ± 0.68 to 37.88 ± 0.46 for protein) of *S.gregaria* in the treated group compared to the control series. Also it is noted that the quantities of chitin and proteins of the cuticle adults are significantly higher than those of larvae before and after treatment ($P < 0.1$).

The cuticle of locusts mainly contains chitin (polymer of N. Acetyl glucosamine) and proteins are strongly related there, together form the microfiber that is also wrapped in a protein coats more or less compact [16]. The cuticle is also composed of glycoprotein, of poly hydric phenol, enzymes, lipids, pigments, and inorganic components [17]. Fibrous elements polysaccharide (chitosan) included in a protein matrix in this two-phase form compound the outer support structure of an insect.

The mechanical properties of the cuticle depend on levels, distribution and composition of each phase [18]. Proteins contribute significantly to the strength and rigidity of the cuticle [19].

Glycoprotein complex as more or less stable proteins represent 25% to 37% of the dry weight of the cuticle Orthoptera [20].

In other insect orders the percentage of protein in the cuticle is much higher, it is close to that of grasshoppers. It is: 63.3% larvae 'pharates' *Tenebrio molitor* [21], 72.0% In *Rhodnius prolixus*, 71.4% in *Triatoman phyllosoma pallidipenis*, and 35.28% in *Calliphora vomitoria* [19], [22].

From the results obtained, proteins are the major constituents of the abdominal sternal cuticle locust during the fifth instar larvae and adult stage (Figures n°1 and 2). The protein secretion follows a decreasing pace during the developmental stages of the locust [23]. This author has mentioned a protein in the cuticle of L5 (84%) than observed in adults (78%) values are close to those obtained in our experiment.

The 3rd day of treatment with *B.bassiana*, there was a significant reduction ($p < 0.05$) in the amount of proteins in the cuticle control individuals compared to individuals treated 29.12 ± 0.98 mg to 26 48 ± 0.48 mg in L5 larvae and 39.08 ± 0.68 mg to 37.88 ± 0.46 mg in adults.

This decrease is dependent on protein degradation by the action of extracellular proteinase (trypsin) secreted by appressoria during penetration hyphae as shown on the infection of *Manduca sexta* by *M. anisopliae* [24].

Similarly, a significant reduction in rate of cuticular proteins in larvae and adults *S.gregaria* treated *M.anisopliae* [5]. Some IGRs insects such chlorfluazuron, cyromazine have an inhibitory effect on synthesis of all cuticular proteins [25], [26]. While others like flucycloxuron and Triflumuron have no effect as was underlined Morsli (1994) cited by [27] Abbasi and Hamza (1995) respectively on *Panaeus kerathurus* and *Tenebrio molitor*.

Furthermore, chitin is an important component in the insect cuticle. The excess or deficiency of chitin in any morphogenetic cycle can produce harmful and deadly effects to insects [27]. Since chitin is a characteristic of arthropods, particularly a potential and promising target for a specific insecticide seed coat appears to be related to some aspects of the metabolism of chitin. This could include the biosynthesis or degradation of the latter [28].

Cuticular chitin content differs from one species to another and from one stage to another, the larvae of *Tenebrio molitor* contain 21.7% of chitin by dry weight of the cuticle, 22.0% are from the cuticle femurs of imagos of *Locusta migratoria* [29] 11.5% and 11.6% of the dry weight of the cuticle c in *Rhodnius prolixus* and *Triatoma phyllosoma pallidipenis* [30] and 48.4% of the dry weight of the larval cuticle of *Calliphora vomitoria* [19].

Our study has also shown that the quantities of chitin contained in the abdominal cuticle of adults are significantly higher than those in the larval cuticle L5.

Our results are consistent with those found by [23], which recorded a cuticular chitin content of 13.08% in L5 and 16.80% in adult locusts.

Chitin can be dissolved and hydrolyzed by several body chemicals such as dilute acids, concentrated mineral acids, formic acid, sodium hypochlorite, alcohol, ether solvents and fats etc ... We reported that many compounds disrupt chitin synthesis. It was also reported that several compounds reported as inhibitors of chitin synthesis are rather ineffective and act only at high concentrations as is the case of acyl ureas and buprofezin compound that is a specific product Homoptera [29].

In nature many organizations through their enzymatic equipment can degrade chitin, such as *Bacillus* and *Chitinovorus* hyphomycete fungi *Metarhizium anisopliae* and *Beauveria bassiana* [31].

Indeed, the presence of chitinases extracellulaies type acetyl-bD-glucosminidases virulence factors in fungal entomopathogenicity developed by three entomopathogenic *M.anisopliae*, *M. flavoviride* and *B. bassiana* chitinolytic these enzymes are induced in the primary penetration hyphae into the cuticle of *Manduca sexta* [32].

Our results show a significant reduction ($p < 0.05$) of chitin content in the abdominal sternal cuticle fifth stage larvae and adults *S.gregaria* the 3rd day of treatment with *B.bassiana* 7.38 ± 1.14 to 6.44 ± 1.28 and de 7, 41 ± 1.37 to 4.20 ± 0.77 respectively . In the same way the results of the work of Raymond and *al*., (1995) showed a significant reduction of chitin content of the cuticle of beetle *Blaberus giganteus* treated by *M. anisopliae* . The same fungus has the same effect for the larvae and adults of *Locusta migratoria* and *S.gregaria* as pointed out respectively [5], [12]. For deregulators growth products the effect of diflubenzuron on the cuticle of the femur of tergite and intersegmental membrane of adult *Locusta migratoria migratorioides* causes a significant reduction in the content chitin. This reduction is complete in the intersegmental membrane [29]. The SIR8514 benzoyl phenyl urea derivative prevented chitin synthesis in the cells of *Chironomus tentans* [33] and in L3 larvae of *Lucilia cuprina* [34]. A decrease in the amount of chitin in larval of *L.migratoria* treated respectively teflubenzuron and hexaflumuron [35], [36].

Similarly, the external factors such as temperature too low or too high severely inhibit chitin synthesis in insects [37].

II.2 Effect of *B. bassiana* on the structure of the cuticle of the 5th instar larvae of *S. gregaria*:

The larval cuticle of the fifth stage *S.gregaria* highlighted by differential staining with Heidenhain azan, shows the presence of the epicuticle, the exocuticle and endocuticle.

The epicuticle colored red is as a thin layer on the surface of the cuticle and L5 *S.gregaria* exocuticulaire least orange colored thinner than the first part of a colorless and a greater thickness corresponds to the endocuticle (Fig.3a).

The application of the integument *B.bassiana* 5th instar larvae of *S.gregaria* was dependent of an apparent disturbance on the structure of the cuticle.

Indeed the third day of treatment it has been observed a complete disappearance of the epicuticle and a sharp decline in the thickness of the exocuticle and endocuticle (Fig. 3b) and that compared to the control larvae or cuticle the three layers are distinct and exposed with a thickness clearly greater (Fig. 3a).

The breakdown of cuticular layers larvae treated is probably due to degradation by proteolytic enzymes and chitinolytic the infectious unit of our fungal strain, spore which was spotted a few cells in parts of the cuticle digested (Fig.3b).

The arthropod cuticle with its underlying epidermis forms the seed coat. She plays both the role of exoskeleton and barrier between the environment and animals, it lines the whole body surface invaginates in the digestive tract and respiratory system.

The insect growth cannot be achieved without a periodic release of the cuticle, which is the phenomenon of molting. She also suffered during the development cycle when the insect in the larval stage heterometaboles going to the adults and larvae of holometabolous in pupal and then adult. From structural point of view it recognizes as the chemical composition of two main layers outer epicuticle, thin, non-chitin and internal procuticle (exocuticle and endocuticle), thicker and chitin [38], [17], [20].

The peculiarities of the cuticle to give the insect its appearance. In addition to the articulated and other sensilla, which bristle surface, a very characteristic relief can be detected: ridges, granulation, micro spines or cheating. The presence of pigments in epidermal cells or in the insect cuticle gives its color. However, many insects have structural colors, some are iridescent, changing the angle of view and they are due to interference caused by thin periodic structures on the scales or the cuticular lining of the body effects [20].

In control by differential staining L5 Heidenhain azan we come to recognize the usual structure of the cuticle with these different layers: the epicuticle, the exocuticle and endocuticle(Fig.3a). Of such structures with such aspects were observed in *Locusta migratoria* by [39] [36] using differential staining with Heidenhain azan.

Applying *B.bassiana* severely disrupted this structure, the degeneration of these different parts (Fig.3b) is observed. This breakdown has led to a molt and external signs disrupted and blocked revealing a wrinkled appearance, wavy and dry.

These signs are similar to those observed with other inhibitors in various insects exposed to different treatments [40] evaluating the effect of chlorfluazuron, a chitin synthesis inhibitor, and also on *Choristoneura fumiferana* studying the effect of hematoporphyrin (a light-activated insecticide) on *Culex pipiens* making endocuticle and exocuticle indistinguishable.

Larvae of *Schistocerca gregaria* treated by *M.anisopliae* [5] and the analogue of juvenile hormone (JH III) [41].

However, the biochemical processes involved and disturbed structures in the mode of action differ between inhibitors. Many insect growth inhibitors impair the synthesis and deposition of chitin in the cuticle [26]. However, the lack of chitin due to inhibition of its biosynthesis is the cause of the amorphous form of the cuticle area.

Synthesis inhibitors tell the benzoyl phenyl urea inhibit the junction between the amino carbohydrate and protein that form microfibrils [38]. The mirofibrilles commonly encountered in this region consist of protein and chitin and chitin require for its development [18]. Its main architectural components are deposited chitin and probably directed at the surface of cells. In biological inhibitors tell that entomopathogenic fungi and specifically during infection *B.bassiana* in the integument of insect's process, four stages can be distinguished: adhesion, germination, differentiation and penetration.

Membership is characterized by a mechanism of recognition and compatibility with conidia of the insect integument cells [38]. This phase is divided into two distinct phases, the first passive or attachment to the cuticle is achieved through hydrophobic and electrostatic forces [39] and the second active characterized by the production of a mucilage which will generate a epicuticular modification leading to germination [31]. After the accession phase, germination will be dependent on environmental conditions and also the host physiology (biochemical composition of the cuticle) that can promote or inhibit germination [42] [31].

The penultimate stage differentiation is characterized by the production of appressorium terminal structures that will serve as the anchor point, softening point of the cuticle and promote penetration. Appressoria production is highly dependent on the nutritional value of the cuticle which will stimulate mycelial growth rather than penetration. The

last phase is the penetration of the host that is the combination of mechanical and enzymatic pressure such as lipases, proteases and chitinases [31]. To expedite the process of infection some strains produce toxins such as non-enzymatic beauvericin the beauverolides the bassianolides and isarolides. The host colonization occurs when the fungus can overcome the immune defense mechanisms of the insect and invades the hemolymph [43].

On the death of the insect, the fungus produces an antibiotic: oosporine that will allow him to overcome bacterial competition. Saprophytic stage (Fig.4) will be characterized by the mummification of the body transformed into sclerotia. Hyphae across the integument preferentially in inter segmental then covered with white cottony mycelial matting that will initiate the formation of conidia [44].

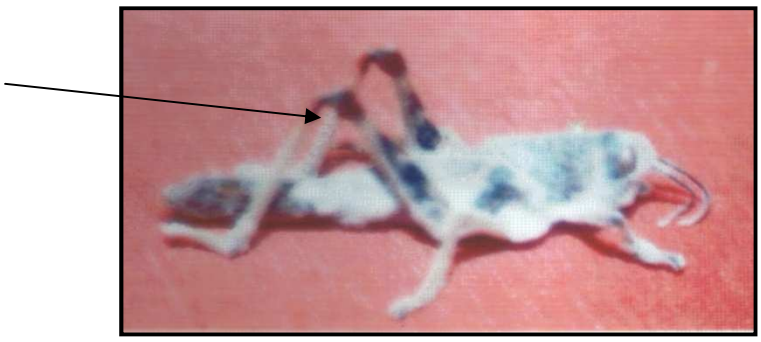

Fig. 4 - Saprophytic phase of infection by *B.bassiana*

CONCLUSION

The study of the effect on the cuticle *B.bassiana* L5 and adults *S.gregaria* showed a significant reduction in the dry weight of the cuticle from 36.50 mg to 32.93 mg in L5 and 46.49 mg to 42.31 mg in adults. The same is true for the chitin content is increased from 7.38 mg to 6.44 mg in L5 and 7.41 mg to 4.20 mg in adults. For proteins, the amount dropped to 29.12 mg 26.48 mg in L5 and 39.08 mg to 37.88 mg in adults.

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