U. of K.J. Agric. Sci. 18(2), 268-275, 2010

A Note on the Incidence of *Bacillus thuringiensis* in Fruit Fly Larvae in the Sudan

Omar A.A. Sidahmed[1], Mohamed M. Khider[2] and Awad K. Taha[3]

[1]Department of Plant Protection, Faculty of Agriculture, Omdurman Islamic University, P.O. Box 382 Omdurman - Sudan,
***E-mail*: a.omerahmed@yahoo.com**
[2]Department of Immunology, Faculty of Medical Laboratory Sciences, National Ribatt University, Khartoum-Sudan
[3]Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology, Shambat - Sudan

Abstract: This study was conducted to investigate and identify the reason for the death of wild fruit fly larvae in the laboratory. The toxic activity of the presumptive bacterial isolate from fruit fly larvae was examined on the invasive fruit fly *Bactrocera invadens* larvae. The isolate was identified as *Bacillus thuringiensis* (*B.t.)* on the bases of morphological characteristics and biochemical tests. Additionally, the *B t.* isolate was sent to Poland for more investigations. Over 80% of the treated larvae were dead under room conditions compared with untreated larvae where no mortality was recorded after eight days. Furthermore, symptoms of the infection appeared on the body of treated larvae, while no symptoms appeared on untreated larvae.

Key words: *Bacillus thuringiensis;* fruit fly; identification; biochemical tests; Sudan

---

Over 4000 species of fruit flies (Diptera: Tephritidae) are known in the world. Approximately 250 of them are of economic importance and are associated with fruits and vegetables. Fruit flies cause severe losses (more than 30%) to horticultural production worldwide (Mohammed and Taha 2008). In Sudan, nearly 40 fruit fly species were recorded; the most serious ones are those attacking mango (*Mangifera indica*), guava (*Psidium guajava*) and *Citrus* spp. (Ali *et al*. 2008). In recent years, fruit

production has been seriously hampered, mainly, because of the sudden and persistent outbreaks of some fruit fly species. Infestation exceeded 80% in some of the seriously infested areas. In the River Nile State, the damage was estimated to range from 85 % to 98 % (Gubara and Abu Elgasim 2004). In 2007, the damage due to fruit flies became so severe to the extent that they were added to the list of the notorious national pests of the Sudan (Ali *et al*. 2008). Moreover, there is no standing recommendation for the control of fruit flies in Sudan, and insecticide spraying may not be encouraged because of the risk of fruit contamination (Mohamed and Ali 2008).

Bacillus thuringiensis (*B.t.*), anaerobic Gram-positive and spore-forming rod, is well known for synthesis of parasporal crystalline proteins with unique activity against a variety of insect pests affecting crops and forest trees. It was shown that representatives of the bacilli are widely distributed and colonize many habitats like soil, plant materials, air or water (Swiecicka 2003). Elyass *et al*. (2009) reported that *B.t.* strains are ubiquitous in Sudan's environment. They obtained samples of *B.t.* from different habitats (e.g. soil, water, plants and dead insects); the dead insect larvae and pupae represented 24.2 % of *B.t.* isolates. The colonies of *B.t.* isolates were white to cream coloured, flat and some of them were mucoid. All isolates were Gram-positive, produced central spores with no swelling of the cell. Also, most of them were motile (Elyass *et al*. 2009). The present study aimed to investigate and identify the reasons for the death of wild fruit fly larvae in the laboratory.

In the laboratory, experiments were conducted for rearing fruit fly larvae from infected guava fruits (*Psidium guajava*) brought from a local market in Khartoum State during August 2009. It is notable that some of the emerging fruit fly larvae showed changes in colour and died within a few days. Infected larvae were transferred to Petri dishes for observation. The symptoms of dead larvae appeared as change in colour from white to brown or black, became flaccid and died. Dead larvae were inoculated in Sabouraud Dextrose Agar (HIMEDIA, India) at 37˚C for 24 hours. After

the incubation period, growth of the bacterial colonies and a fungal colony were seen on the surface of the plate. The fungus was identified as *Aspergillus flavus* depending on morphological characters. The bacteria were subcultured in different culture media, such as Nutrient Agar (HIMEDIA, India), MacConkey Agar (HIMEDIA, India) and Blood Agar (Pronadisa Microbiological Culture Media & Diagnostic Reagent) to investigate the morphological characteristics of colonies. Identification of presumptive isolates was conducted in the laboratories of the Faculty of Medical laboratory Sciences, National Ribatt University, Sudan.

According to the methods of Martin and Travers (1989), Health Protection Agency (2007) and Elyass *et al*. (2009), various available procedures were conducted to identify the isolated bacteria, e.g. (i) smears of isolate were examined for their Gram reaction under a light microscope, (ii) smears were prepared from an isolate and stained with modified Ziehl-Neelsen (M-ZN) which is a special stain to show the spore and (iii) ten biochemical tests were conducted on the isolate; namely, catalase production, esculin hydrolysis, citrate utilization test, anaerobic growth, production of indole, kliger iron agar (KIA test), lecithinase production, urease test, starch hydrolysis test, oxidase test and growth at 5^{o}C, 37^{o}C and 40^{o}C. For bioassay test, 20 wild larvae of the invasive fruit fly *Bactrocera invadens* of the same size were put in a sterilized Petri dish (9 cm in diameter) and used as a replicate. The experiment was arranged in a complete randomize design with three treatments and four replicates. Each replicate contained about 10 grammes of crushed carrot treated with water only and two concentrations of isolate (10^{0} and 10^{-1}) by dipping in treatments for five minutes. The preparation of the isolate concentrations (spores) from the agar were floated on 20 ml of sterile distilled water and shaken for one minute. The suspension was stored in sterile scintillation vial under room condition until it was tested (Martin and Travers 1989). The toxic activity of the isolate against larvae was tested by observation of the symptoms and number of dead larvae in the replicates.

The bacterial isolates from fruit fly larvae were identified as *Bacillus thuringiensis* depending on morphological characteristics, Gram and spore stains and biochemical tests. The morphological characteristics of a single colony were described in different cultures as (i) on Nutrient Agar medium which produced white to cream-coloured, flat and mucoid colonies, with a ground-glass appearance (Fig.1-A), (ii) on Blood Agar medium which produced large, irregular, dry colonies, opaque in colour and β haemolytic after 48 hours (Fig 1-B), (iii) on MacConkey Agar medium which produced large, dry, irregular, translucent and non- lactose fermenting colonies (NLF). A single colony on MacConky Agar culture appeared in different forms from first to third day. In the first day, the colony was convex, wet and translucent; but in the third day it was flat, dry and grayish to green colour. The isolate was Gram-positive with sub-terminal spore in which the spore appeared colourless, but in the spore stain (M-ZN) it appeared to have red colour. The isolate was capable of growth at 37^{o}C and 40^{o}C, but there was no growth at 5^{o}C until 72 hours after culturing.

Six biochemical tests (catalase production, esculin hydrolysis, starch hydrolysis test, anaerobic growth, lecithinase production (Fig. 2-A) and urease test) gave positive reactions, while the other tests (citrate utilization, indol production, oxidase test and Kliger iron agar (KIA test)) gave negative results. Dr. Izabela Swiecicka (Personal Communication 2010) affirmed that the isolate is *B. thuringiensis,* because it is from dead larvae and the tests and colony appearance seemed to be typical for *B.t*.

The toxic activity of the isolate concentrations on fruit fly larvae was more effective with mortality of about 80 % recorded after eight days. The symptoms appeared at the 3rd day after treatment. Treated larvae became inactive, flaccid, stopped feeding and died after a few days. The body turned brown to black as it decomposed (Fig.2-B). The untreated larvae were alive after eight days; also, no changes in colour and body were recorded.

In conclusion, the results of these experiments were recorded only as observations on the effects of the isolate on fruit fly larvae. Therefore, the isolate was sent to the Department of Microbiology, Institute of Biology, University of Bialystok, Poland, for more investigations. These will include biochemical tests and PCR primers. Also, more investigations are needed on the toxic effects of the isolate against fruit flies and other insects.

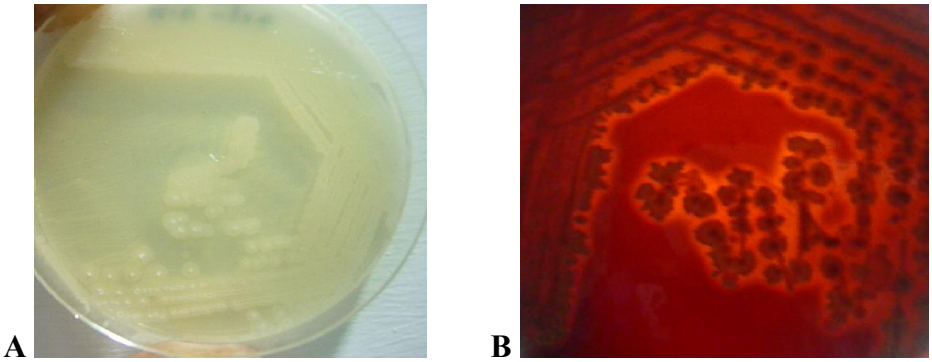

Fig.1. The *B. thuringiensis* isolate, **A** = Growth on a nutrient agar culture and **B** = Hemolysis in blood agar

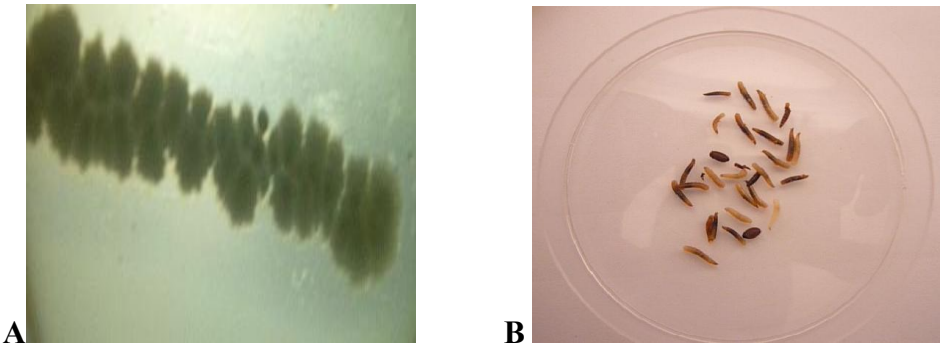

Fig.2. The *B. thuringiensis* isolate, **A** = a zone of egg yolk precipitation and B = toxicity symptoms on larvae

REFERENCES

Ali, E.E.; Abbas, A.M.; Ahmed, H.M.; Abdurrahman, A.A. and Abdelmajid, F.M. (2008). Fruit fly review in Sudan from 1960 to 2008. *Workshop of National Project for Fruit Flies in Sudan*. Khartoum, Sudan, August 2008.

Elyass, M.E.; Mahdi, A.A. and Hamza, A.A. (2009). Isolation and characterization of *Bacillus thuringiensis* from various habitats in five locations in the Sudan. *University of Khartoum Journal of Agricultural Sciences* 17(2), 283- 296.

Gubara, S. and Abu Elgasim, M. (2004). Fruit flies. Plant Protection Administration, Ministry of Agriculture and Forestry Bulletin, Sudan. November 2004.

Health Protection Agency (2007). Identification of *Bacillus thuringiensis*. National Standard Method; BSOP. ID 9 Issue 2.1. http://www.hpa-standardmethods.org.uk/pdf.

Martin, P.A.W. and Travers, R.S.; (1989). Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. *Applied and Environmental Microbiology* 55(10), 2437-2442.

Mohamed, A.H. and Ali, E.A. (2008). Evaluation of para- pheromones and three components food baits for mass trapping of fruit flies in fruit trees. The 78th Meeting of the National Pest and Diseases Committee, Agricultural Research Corporation, Wad Medani, Sudan.

Mohamed, A.H. and Taha, A.K. (2008). Phermones, para pheromones and food lures in fruit fly management. *Workshop of National Project for Fruit Flies in Sudan*. Khartoum, Sudan, August 2008.

Swiecicka, I. (2003). Molecular typing by pulsed-field gel electrophoresis of Bacillus *thuringiensis* from root Voles. *Current Microbiology* 46, 256–260.

وجود *Bacillus thurengiensis* في يرقات ذبابة الفاكهه في السودان

عمر احمد عبدالله سيداحمد[1] ومحمد محمود خضر[2] وعوض خلف الله طه[3]

[1] قسم وقاية المحاصيل، كلية الزراعة، جامعة امدرمان الإسلامية، امدرمان- السودان.

[2] قسم المناعة ، كلية علوم المختبرات الطبية، جامعة الرباط الوطني، الخرطوم- السودان.

[3] قسم وقاية النباتات، كلية الدراسات الزراعية، جامعة السودان للعلوم والتكنولوجيا، شمبات- السودان.

مؤجز البحث: أجريت هذه الدراسة لبحث وتعريف سبب موت يرقات ذبابة الفاكهه البريه في المعمل. فحص النشاط السام للعزلة الإفتراضية ضد يرقات ذبابة الفاكهه الغازيه *Bactrocera invadens* . أكدت نتائج الدراسة أن العزلة هي *Bacillus thuringiensis* اعتمادا علي الوصف المورفولوجي والإختبارات الكيموحيوية، وارسلت عينات من هذه البكتيريا الي بولندا لمزيد من التحقق. مات أكثر من 80% من اليرقات المعاملة في درجة حرارة الغرفة مقارنة مع اليرقات غير المعاملة التي لم تسجل موتا ً بعد ثمانية ايام من المعاملة. ظهرت أعراض الإصابة علي أجسام اليرقات المعاملة بينما لم تظهر أي أعراض علي اليرقات غير المعاملة.