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NEW RECORD OF ENTOMOPATHOGENIC BACTERIA, *Bacillus aryabhattai* STRAIN B8W 22, ISOLATED FROM *Cassida vittata* Vill. AND ITS PATHOGENICITY AGAINST THIS INSECT IN EGYPTIAN SUGAR BEET FIELDS

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ABSTRACT: *Cassida vittata* Vill. (Coleoptera : Chrysomelidae) is a very important insect pest to sugar beet crop. Its larvae and adults feed upon sugar beet leaves causing significant defoliation, consequently reduce root weight and sugar percent (%) to this crop. Entomopathogenic microorganisms, mainly entomopathogenic bacteria, is a vital factor of the natural mortality against insects. Research on microbial pathogens of insects is increasing to find out environmental friendly alternatives to hazardous chemical insecticides. This investigation was done at the Experimental Farm of Sakha, Agricultural Research Station, Kafr Elsheikh Governorate, Egypt during 2018 and 2019 seasons. Obtained results showed that 11(29.72%) *C. vittata* larvae out of 37 individuals died naturally with *Bacillus aryabhattai.* Six (29.72%) *C. vittata* adults out of 25 individuals died naturally due to exposure to 10^8 cfu/ml suspension, which used for laboratory and field tests. In a laboratory test, the mortalities of larvae were 20.00, 30.00 and 45.00% after 3, 7 and 10 days post-treatment, respectively. Whereas, the mortalities of adults were 10.00, 25.00 and 40.00% after 3, 7 and 10 days post-treatment, respectively. Also, in a field test, the reduction of larvae were 12.86, 29.79 and 43.99% for 3, 7 and 10 days after treatment, respectively. Reduction of adults were 14.06, 27.05 and 39.84% for 3, 7 and 10 days after treatment, respectively. This strain was identified by GATC (biotech sequence company, Germany), for the first time in Egypt. These results conclude that this strain is an effective biocontrol agent against *C. vittata* larvae and adults in sugar beet fields.

Key words: *Bacillus aryabhattai* strain B8W 22, *Cassida vittata*, Egyptian sugar beet.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) (Family: Chenopodiaceae) is a main source of sugar in Egypt and all over the world. In Egypt, the total grown area of sugar beet about 620000 faddans in 2018 season (**Anonymous, 2018**). Sugar beet plants are subjected to attack with numerous insect pests beginning from seed germination up to maturity and harvest (**Shalaby, 2001; El-Dessouki *et al*., 2014; Bazazo *et al*., 2015; Rashed, 2017; Abbas, 2018**). *Cassida vittata* Vill. is a very important insect pest to sugar beet crop which negatively affect the crop foliage and consequently, reduce the crop productivity (**El-Mahalawy, 2011; Rashed, 2017**). The severe infestation of sugar beet with *C. vittata* caused significant reduction of 40.10 and 56.20% in root weight and sugar content, respectively (**Abo-Saied Ahmed, 1987**). *C. vittata* larvae and adults feed upon sugar beet leaves causing significant defoliation to this crop (**Bazazo, 2010; Bazazo *et al.,* 2012; Sherief *et al*., 2013; El-Dessouki *et al.,* 2014; Abbas, 2018**). **Amin *et al.* (2008)**

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indicated that the initial appearance of *C. vittata* populations was in the last week of March and extended until the second week of May.

Fortunately, the sugar beet fields have several natural predators, parasitoids and entomopathogenic factors that should be conserved to keep the natural balance in the field. **Whipps and Lumsden (2001)** emphasized the importance of insect pathogenic organisms in insect pest control. **El-Husseini (2006)** reported that insects have been known since long ages to be infected by entomopathogenic microorganisms that form an important factor of the natural mortality. **Tikar *et al.* (2008)** proved that *Bacillus* spp. produce crystalline proteins during its stationary phase of growth which are lethal to coleopterous insect. Finally, **Ramanujam *et al.* (2014)** indicated the microbial pathogens of insects is increasing considerably in recent times to find out environmental friendly alternatives to hazardous chemical insecticides. The most successful insect pathogen used for insect control is the bacterial agents.

Thus, the current investigation was carried out to investigate the role of entomopathogenic bacteria, *Bacillus aryabhattai* strain B8W22 in natural mortality of *C. vittata* larvae and adults in fields. Also, the pathogenicity of this strain against the insect pest was assessed under laboratory and field conditions.

MATERIALS AND METHODS

This study was carried out at the Experimental Farm of Sakha Agricultural Research station, Kafr Elsheikh Governorate, Egypt during 2018 and 2019 seasons .The cultivated area about one faddan was sown with Raspoly cultivar on 15th November during both seasons.

Monitoring Dead Larvae and Adults

Dead larvae and adults were monitored from 10 March to 25 April, 2018. Every five days intervals, examination were done for 20 plants per inspection .They taken out and preserved in small sterilized vials by a fine brush to be dissected for mortality factor.

Isolation of the Entomopathogenic Bacteria

According to **Quesnel (1971)**, pathogen infected larvae and adults were surface sterilized with 0.5% sodium hypochlorite for two minutes to avoid any pollution, then washed in distilled water three times and dried between two layers of filter papers. The sterilized insect were inoculated onto PDA (Potato Dextrose Agar medium) and incubated at 28°C for overnight. The pathogen isolates were picked up, purified and stored in PDA at 4°C for further studies and identification. The Petri dishes with growing bacteria (Fig. 1) were washed with distilled water. Then, a suspension of the growing bacteria was adjusted to 10^8 cfm/ml of water using the hemocytometer to be used in the laboratory and field studies.

Identification of the Entomopathogenic Bacteria

The entomopathogenic bacteria was identified by GATC company (Germany) by the aid of Sigma Scientific Services Company (Egypt), as *Bacillus ayabhattai* strain B8 W22 16S ribosomal RNA gene, partial sequence with a high similarity of 99% for the first time in Egypt.

Laboratory test

Infested leaves with *C. vittata* larvae and adults were collected from field for using in laboratory test. Twenty individuals each of stage, (5 individuals x 4 Petri dishes) for each date, having a piece of sugar beet leaf treated with *B. aryahbattai* spores suspension by hand sprayer (1 liter). The mortalities (%) were recorded 3, 7 and 10 days after treatment. Date of spraying was 1st April, 2019. The bacteria was reisolated from the dead insects.

Field test

An area of sugar beet about (100 m^2) was divided into four replicates, sprayed with *B. aryabhattai* spores suspension by hand sprayer (2 liter). Another area was left as a check. The reductions of larvae and adults due to bacterial infection were recorded 3, 7 and 10 days after treatment. Date of spaying was 15th April, 2019. The reductions were calculated by **Henderson and Tilton formula (1955)**:

Reduction (%) = 1 – (No. of larvae in check plots before spray / No. of larvae in check plots after spray × No. of larvae in treated plots after spray / No. of larvae in treated plots before spray) × 100

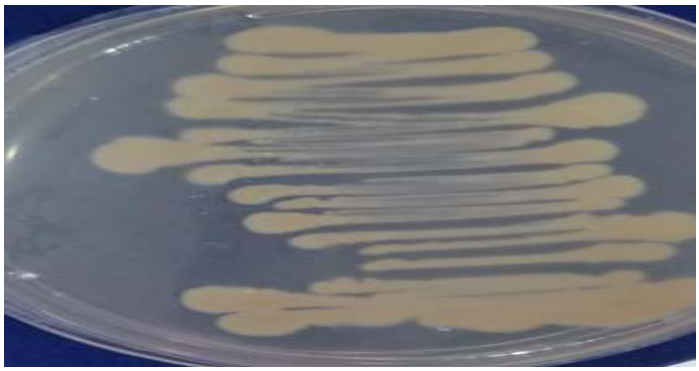

Fig. 1. *Bacillus aryabhattai* spores

RESULTUS AND DISCUSSION

Monitoring the Natural Mortality of *Cassida vittata* Larvae with *Bacillus aryabhattai*

Data in Table 1 indicate that the total number of dead larvae throughout the season was 37 individuals. 11 out of 37 individuals died with *B. aryabhattai* with percentage (29.72%).

Monitoring the Natural Mortality of *Cassida vittata* Adults with *Bacillus aryabhattai*

As shown from obtained data in Table 2, the total number of dead adults throughout 2019 season was 25 individuals. 6 out of 25 individuals died with *B. aryabhattai* with percentage (24.00%).

Tanda and Kaya (1993) indicated that bacteria are prokaryotic, unicellular organism varying from less than 1um to several lengths. Most of the insect pathogenic bacteria occur in different families such as Bacillaceae. Member of Bacillaceae, particularly *Bacillus* spp. have received maximum attention as microbial control agents. In insect, bacteria disrupt the digestive system by producing endotoxins that are often specific to the particular insect pest. Also, **El-Husseini (2006)** showed that the entomopathogenic bacteria an important factor of the natural mortality to insect pests. **Ramanujam *et al*. (2014)** reported that the main advantages of these biocontrol agents are their specificity to target pests, safety to the non-target organism. They do not cause ill effects on environment and human health and can be used against pests which develop resistance to the conventional insecticides. They fit as ideal components in integrated pest management (IPM) and also in organic farming system.

Pathogenicity of *Bacillus aryabhattai* Against *Cassida vittata* Larvae and Adults in Laboratory Tests

Data in Table 3 show that the mortalities of larvae were 20.00, 30.00 and 45.00% after 3, 7 and 10 days post treatment, respectively. The overall mortalities of larvae were 31.66%. As shown from obtained results in Table 4 the mortalities of adults were 10.00, 25.00 and 40.0% after 3,7 and 10 days post - treatment, respectively. The overall mortalities of adults were 25.00%.

Bravo *et al.* (2011) demonstrated that the most successful insect pathogen used for insect control is the bacteria, *Bacillus* spp. Each one of the strains produces different mix of toxins and specifically kills one or a few related species of insects.

Effect of *Bacillus aryabhattai* on *Cassida vittata* Larvae and Adults in the Field

Data in Table 5 indicate that the reduction of *C. vittata* larvae were 12.86, 29.79 and 43.99% for 3,7and 10 days after treatment, respectively. Overall mean of reductions was 28.88%.

As shown from obtained data in Table 6 the reduction of *C.vittata* adults were 14.06, 27.05 and 39.84 for 3,7and 10 days after treatment, respectively. Overall mean of reductions were 26.98%. **Shalaby (2001)** reported that the application of biocide, *Bacillus thuriengiensis* var. *Kurstaki* (Dipel 2x) eliminated 19.01 and 27.78 of *Cassida vittata* Vill in 1998/1999and 1999/2000 seasons, respectively.

Table 1. Natural mortality of *Cassida vittata* larvae collected from 20 sugar beet plants/each date, due to *Bacillus aryabhattai* effect during 2018 season

Date	No. of dead larvae	No. of dead larvae with bacteria	Dead larvae with bacteria (%)
10/ 3	3	1	33.33
15/ 3	4	1	25.00
20/ 3	3	1	33.33
25/ 3	5	2	40.00
30/3	5	0	0.00
5/ 4	0	0	0.00
10/ 4	6	3	50.00
15/ 4	6	2	33.33
20/ 4	5	1	20.00
25/ 4	0	0	0.00
Total	37	11	-
(%)	-	29.72	-

Table 2. Natural mortality of *Cassida vittata* adults collected from 20 sugar beet plants/each date, due to *Bacillus aryabhattai* effect during 2018 season

Date	No. of dead adults	No. of dead adults with bacteria	Dead adults with bacteria (%)
10/ 3	0	0	0.00
15/ 3	0	0	0.00
20/ 3	3	1	33.33
25/ 3	4	1	25.00
30/3	4	0	0.00
5/ 4	3	0	0.00
10/ 4	6	2	33.33
15/ 4	2	1	50.00
20/ 4	3	1	33.33
25/ 4	0	0	0.00
Total	25	6	-
(%)	-	24.00	-

Table 3. Mortality of *Cassida vittata* larvae due to the effect of *Bacillus aryabhattai* suspension (10^8 cfu/ml water), 2019 season

Duration	No. of treated larvae	No. of dead larvae	Mortality of larvae (%)
3	20	4	20.00
7	20	6	30.00
10	20	9	45.00
Total	60	19	31.66

Table 4. Mortality of *Cassida vittata* adults due to the effect of *Bacillus aryabhattai* suspension (10^8 cfu/ml water), 2019 season

Duration	No. of treated adults	No. of dead adults	Mortality of adults (%)
3	20	2	10.00
7	20	5	25.00
10	20	8	40.00
Total	60	15	25.00

Table 5. Reduction in *Cassida vittata* larvae due to the effect of *Bacillus aryabhattai* suspension (10^8 cfu/ml water) in the field during 2019 season

Treatment		*B. aryabhattai* suspension	Control
Before spray	Total	51	52
	Mean	12.75	13.00
After 3 days	Total	47	55
	Mean	11.75	13.75
	Red.(%)	12.86	-
After 7 days	Total	42	61
	Mean	10.50	15.25
	Red.(%)	29.79	-
After 10 days	Total	39	71
	Mean	9.75	17.75
	Red.(%)	43.99	-
Overall mean of reductions		28.88	-

Table 6. Reduction in *Cassida vittata* adults due to the effect of *Bacillus aryabhattai* suspension (108 cfu/ml water) in the field during 2019 season

Treatment		*B. aryabhattai* suspension	Control
Before spray	Total	32	33
	Mean	8	8.25
After 3 days	Total	30	36
	Mean	7.50	9
	Red.(%)	14.06	-
After 7 days	Total	29.00	41
	Mean	7.25	10.25
	Red.(%)	27.05	-
After 10 days	Total	28	48
	Mean	7	12
	Red.(%)	39.84	-
Overall mean of reductions		26.98	-

Molecular identification of *B. aryahattai* B8W22

Sigma Scientific Services Company Egypt made sequencing to the Polymerase chain Reaction (PCR) product on GATC Company (Germany), by use ABI3730XL DNA sequencer by using forward and reverse primers. Based on DNA sequences using r DNA, results showed that the Bacillus isolate belonged to *Bacillus aryabhattai* strain B8W22 16S ribosomal RNA gene, partial sequence with a high similarity of 99% phylogenetic tree analysis was performed to obtain strain and the result is presented in Fig. 2.

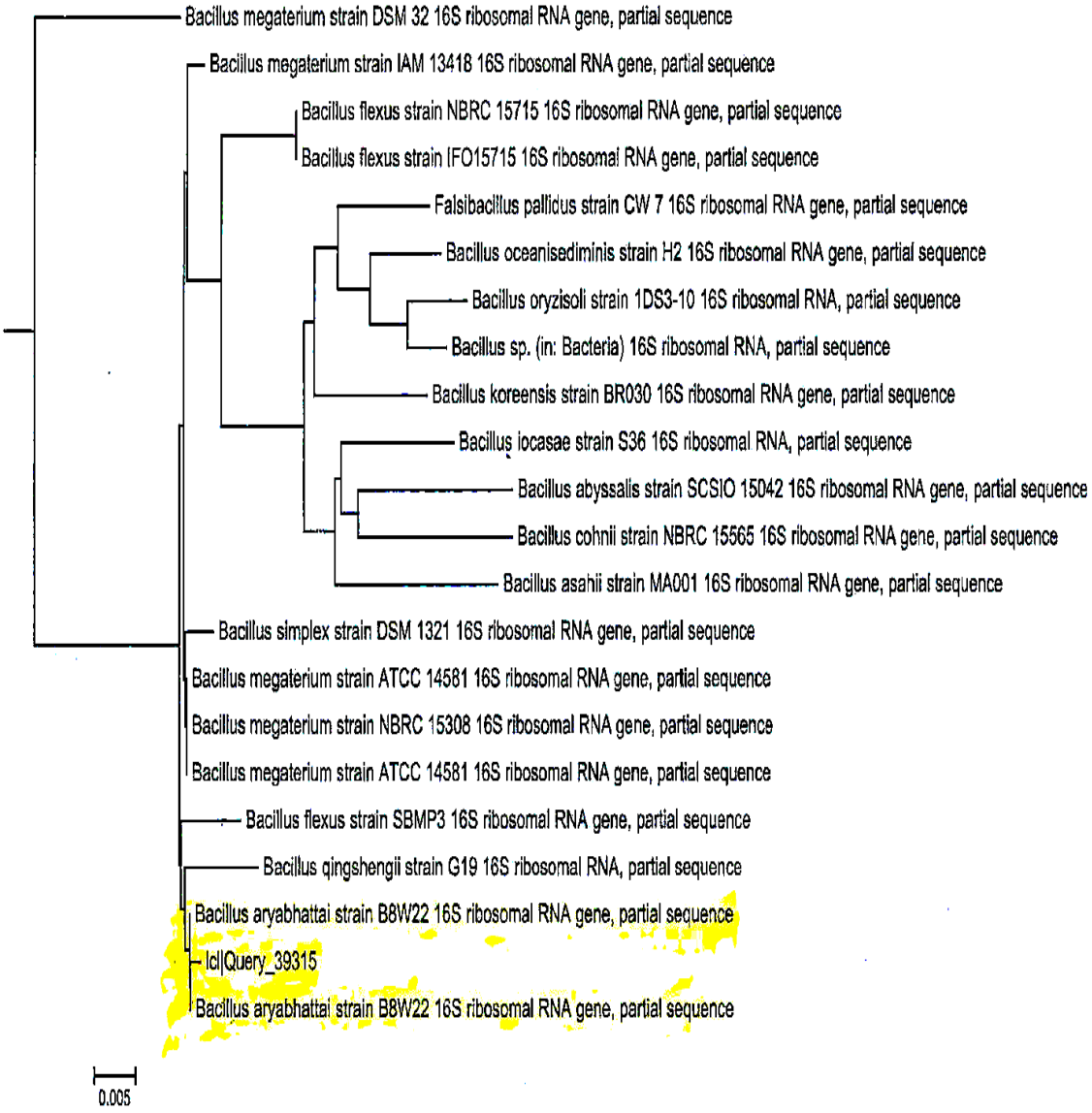

Fig. 2. Phylogenetic tree of the nucleotide sequences of the PCR product of *Bacillus aryabhattai* B8W22 16S

REFERENCES

Abbas, N.M. (2018). Integrated pest control of sugar beet. M.Sc. Thesis, Fac. Agric., Kafr Elsheikh Univ., 93.

Abo-Saied Ahmed, A.M. (1987). Studies on the insects of sugar beet in Kafr El-Sheikh Governorate, Egypt. Ph.D. Thesis, Fac. Agric., Tanta Univ., 160.

Amin, A., A. Helmi and S. El-Serwy (2008). Ecological studies on sugar beet insects at Kafr El-Sheikh Governorate, Egypt. Egypt. J. Agric. Res., 86 (6): 2129-2139.

Anonymous (2018). Annual report of 2018 season. Council for Sugar Crops, Ministry of Agriculture and Land Reclamation, Egypt, 30.

Bazazo, K.G. (2010). Studies on some insect pests and natural enemies in sugar beet fields at Kafr El-Sheikh region. Ph.D. Thesis, Fac. Agric., Tanta Univ., 139.

Bazazo, K., A. Ibrahim and R. El-Shafey (2012). First isolation of the entomopathogenic fungi, *Stachybotrysy* sp. from naturally infected tortoise beetle, *Cassida vittata* Vill. (Coleoptera: Chrysomelidae) in sugar beet fields in Egypt. J. Plant Prot. and Path., Mansoura Univ., 3 (6): 501-509.

Bazazo, K., A. Ibrahim and R. El-Shafey (2015). A new strain of entomopathogenic bacteria, *Bacillus thuringiensis* Bt407 isolated from diseased *Pegomyia mixta* Vill. larvae and pathogen virulence against the insect pest. Egypt. J. Plant Pro. Res., 3 (1): 20-28.

Bravo, A., S. Likitvivatanavong, S. Gill and M. Soberon (2011). *Bacillus thuriengiensis*:A story of a successful bioinsecticide. Insect Biochem. and Molec. Biol., 41: 423-431.

El-Dessouki, S., S. El-Awady, K. El-Khawass, M. Mesbah and W. El-Dessouki (2014). Population fluctuation of some insect pests infesting sugar beet and associated predatory insects at Kafr El-Sheikh Governorate. Ann. Agric. Sci., 59 (1): 119-123.

El-Husseini, M.M. (2006). Microbial control of insect pests: Is it an effective and environmentally safe alternative. Arab J. Pl. Prot., 24 (2): 162-169.

El-Mahalawy, N.A. (2011). Ecological and biological studies on some sugar beet insects. M.Sc. Thesis, Fac. Agric., Tanta Univ., 135.

Henderson, G. and E. Tilton (1955). Test with acaricide against the brown wheat mite. J. Econ. Entomol., 84: 157-161.

Quesnel, B. (1971). Microscopy and Microbiol. 5A:1-103, London Academic Press.

Ramanujam, B., G. Ramanujam, G. Sivakumar, M. Mohan and M. Yandigeri (2014). Management of insect pests by microorganisms. Pro.Indian Natn., Sci., Acad., 80 (2): 455-471.

Rashed, M.(2017). Toxicological studies on some insect pests of sugar beet in in Kafr EL. Sheikh Governorate, M.Sc. Thesis, Fac. Agric., Kafr EL. Sheikh Univ., Egypt, 107.

Shalaby, G.A. (2001). Ecological studies on some important sugar beet pests and natural enemies and their control. Ph.D. Thesis, Fac. Agric., Tanta Univ., 141.

Sherief, E., A. Said, F. Shaheen and H. Fouad (2013). Population fluctuation of certain pests and their associated predator insects on sugar beet in Sharkia Governorate, Egypt. Egypt J. Agric., Res., 91 (1): 139-150.

Tanda, Y. and H. Kaya (1993). Insect Pathology. Academic press Inc., Harcourt Brace Jovanovich Publishers, San Diego.

Tikar, S., M. Mendki, K. Chandel, B. Parashar and P. Shri (2008). Susceptibility of immature stages of Aedes aegypti vector of Denugue and Chikingunya to insecticide from India Parasitol. Res., 102: 907-913.

Whipps, J. and R. Lumsden (2001). Commercial use of fungi as plant disease biological control: status and prospects. In: Butt. T.; C. Jackson and N. Magan (eds.). Fungi as biocontrol agents. Biddless Ltd., Guildford and King's Lynn, UK.

تسجيل لسلالة جديدة من البكتريا الممرضة للحشرات *Bacillus aryabhattai* strain B8W 22 معزولة من خنفساء البنجر وفاعليتها ضد هذه الحشرة فى حقول بنجر السكر المصرية

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تعتبر حشرة خنفساء البنجر من أهم آفات محصول بنجر السكر حيث تتغذى اليرقات والحشرات الكاملة على الأوراق وبالتالى تتسبب فى انخفاض وزن الجذور ونسبة السكر، تشكل الممرضات الحشرية وبصفة خاصة البكتريا عاملاً حيوياً للموت الطبيعى للحشرات فى الحقول حيث تتزايد الأبحاث الخاصة بهذه الممرضات لتكون بديلاً صديقاً للبيئة عن المبيدات الكيماوية الضارة، لذلك أجرى هدا البحث فى المزرعة البحثية بمحطة البحوث الزراعية بسخا، محافظة كفر الشيخ – مصر خلال موسمي الزراعة ٢٠١٨ و ٢٠١٩ وأظهرت النتائج ما يلى: ١١ يرقة من ٣٧ و٦ حشرات كاملة من ٢٥ ماتوا طبيعياً بسبب هذه السلالة وذلك بنسبة ٢٩,٧٢ و٢٤,٠٠% على التوالي، تم عمل معلق من جراثيم هذه السلالة بتركيز 10^{8} جرثومة/مل ماء للاختبارات المعملية والحقلية، كانت نسب موت اليرقات فى المعمل ٢٠ ، ٣٠ و٤٥% بعد ٣ و٧ و١٠ يوماً من المعاملة على التوالي، وكانت نسب الموت هي ١٠ ، ٢٥ و٤٠% بعد ٣ و ٧ و ١٠ أيام من المعاملة على التوالي للحشرات الكاملة، وفى التجربة الحقلية كانت نسب انخفاض اليرقات ١٢,٨٦، ٢٩,٧٩ و٤٣,٩٩% بعد ٣، ٧ و١٠ أيام من المعاملة على التوالى، وكانت نسب اخفاض الحشرات الكاملة هي ١٤,٠٦ ، ٢٧,٠٥ و٣٩,٨٤% بعد ٣، ٧ و١٠ أيام من المعاملة على التوالى للحشرات الكاملة، تم تعريف هذه السلالة لأول مرة فى مصر بواسطة شركة (جى ايه تى سى) بألمانيا، فى النهاية تلخص النتائج السابقة أن هذه السلالة أحد العوامل الحيوية الفعالة ضد اليرقات والحشرات الكاملة لخنفساء البنجر.

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