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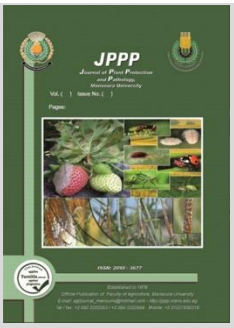
Pathogenicity of New Isolates of Entomopathogenic Fungi *Metarhizium anisopliae*, Metsch. from Sinai-Peninsula against Wheat Aphid *Schizaphis graminum* (Rondani), (Hemiptera: Aphididae) under Lab. Condition.

Altahawi, A.G.[1*]; A. A. M. Ibrahim[1]; F. M. H. Eid[1]; S. A. Ahmed[2] and H. M. Mahfouz[2]

[1]Plant Prot. Res. Inst., Agric. Res. Center-Dokki-Giza-Egypt.
[2]Fac. of Environ. Agric. Sci., Arish Univ.



ABSTRACT

Four native isolates of entomopathogenic fungi *Metarhizium anisopliae* (M 01, M 02, M 06 and M 09) were isolated from Sinai Peninsula and evaluated against wheat aphid, *Schizaphis graminum* (Rondani). Four-concentrations of spores suspensions for every fungal-isolate 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia/ml were used against adult aphid (one day old). The results showed that (M02) was the most effective according to LC_{50}.

Keywords: entomopathogenic fungi, *Metarhizium anisopliae*, wheat aphid, *Schizaphis graminum*, Sinai Peninsula

INTRODUCTION

Wheat-aphid *Schizaphis graminum* (Rondani) which caused directly-losses of yields by sap sucking from invested plants and indirectly-losses as a transporter of fungal and viral diseases, (Abro, *et al*., 2004). In Upper Egypt, the wheat damage caused by aphid-insects was recorded mostly infestations reached up to "23%" (El-Heneidy and Adly, 2012). The bird-cherry or oat-aphid *Rhopalosiphum padi* L.; green-bug *S. graminum; Rhopalosiphum maidis* Fitch. and *Sitobion avenae* F. one of the most important types of insect-aphids in Arab Republic of Egypt (El-Fatih, 2000). Aphids are commonly controlled by pesticides. Though, pesticide-resistance has researched to finding a new method to insect-aphids control. In addition to, randomly using for the several synthetic-insecticides to mammalians protects from dangerous health-hazards. The integrated pest management concluded entomopathogenic-fungi as a biological control agent, can be used in controlling against aphids attack, which in turn works often led to the natural-mortality of population density. Such as, the entomopathogenic fungi (EPF), *Metarhizium anisopliae*, Metsch., which is used as a pathogen for many pests and is used within the Integrated-Pest-Management as biological-control in all parts of the world. (Sandhu, et al., 2012), therefore the wheat-aphid *M. anisopliae*, considered one of a favorable method for biological-control against insect-pests (Zibaee *et al*., 2011).,

The current investigation aim to studding and suggestion some alternatives methods within the Integrated-Pest-Management "IPM" to controlling of wheat-aphid, *S. graminum* by determining the toxicity of four isolates of entomopathogenic fungi, *Metarhizium anisopliae* (Metsch.) against *S. graminum* adult females under laboratory conditions to evaluate their effects and efficiency as natural insecticides.

MATERIALS AND METHODS

Rearing of Wheat Aphid:

Aphid colonies were maintained according to (El-Gendy, 2009). The Laboratory strain of wheat aphid, *Schizaphis graminum* (Rondani) was obtained from a colony cultured at Biological control Department, Plant Prot. Res. Inst., Agric. Res. Centre, Dokki-Giza-Egypt. These strains were reared for several generations under laboratory conditions on wheat plants in plastic pots. Aphid colonies were prevented from external contamination by placing infested plants in cages covered with a muslin cloth.

Bioassay:

Four conc. of spores suspension 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia/ml, were prepared in (0.1%) TritonX-100 (added as surfactant) for each of entomopathogenic fungi isolates, *Metarhizium anisopliae* (M 1, M 2, M 6 and M 9) were isolated in "Bio-insecticides Production Unit" Plant Prot. Res. Inst., Agric. Rese. Center - Giza-Egypt in previous research (Altahawi *et al*. 2020). Spray method was used to test the virulence of fungi isolates. "Abbott's formula", Abbott 1925, used in correct of mortalities%. Ldp line software "Bakr, 2000" used to calculating the values of "LC_{50}, LC_{90} and slope" according to "Finney, 1971".

RESULTS AND DISCUSSION

Virulence of entomopathogenic isolates against aphid, *S. graminum*:

Four isolates of the entomopathogenic fungi, *Metarhizium anisopliae* were chosen according to the highest mortality of the previous experiment (M 01, M 02,

* **Corresponding author.**
E-mail address: amado.goda@gmail.com
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M 06 and M 09) to study susceptibility of adult *S. graminum* to entomopathogenic fungi *M. anisopliae* isolates. Percentage mortality values after exposing to concentrations-series (1×10^5, 1×10^6, 1×10^7 and 1×10^8 spores/ml) illustrated in (Table 1 and Fig. 1) after 10-days from treatments. Mortality% showed gradual-increasing over with increasing of spores-conc. and exposure-time. Low-conc. of (1×10^5 spores/ml) resulted (48, 53, 32 and 38%) for (M1, M2, M6 and M9) respectively, after "10-days" from treatments, but high-conc. of (1×10^8 spores/ml) resulted (88, 91, 76 and 79%) for (M1, M2, M6 and M9) respectively. When mortality was assessed after the same consecutive days, respectively. Fig (2), illustrated that, sporulation of *M. anisopliae* on cadavers of adult stage of *S. graminum* which prove the pathogenicity of the entomopathogenic fungi against aphid.

Table 1. Mortality% of *S. graminum* treated with series concentrations of (M 01, M 02, M 06 and M 09) isolates after ten days of treatment.

	Line Name	Concentrations			
		1×10^5	1×10^6	1×10^7	1×10^8
Mortality %	M 01	48	60	74	88
	M 02	53	69	82	91
	M 06	32	42	60	76
	M 09	38	45	67	79

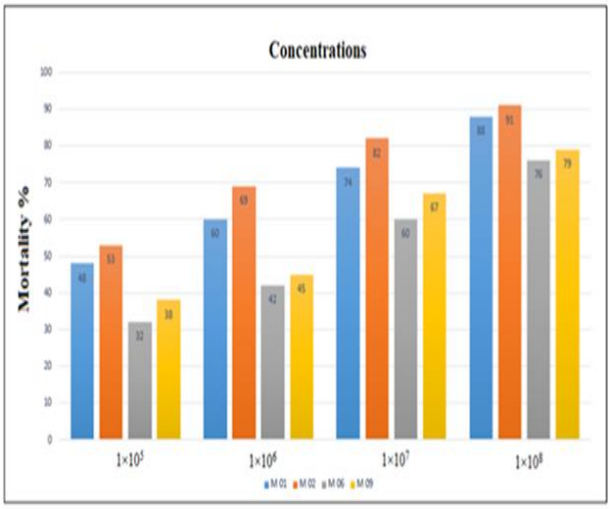

Fig .1. Mortality% of *S. graminum* treated with series concentrations of (M 01, M 02, M 06 and M 09) isolates after ten days of treatment.

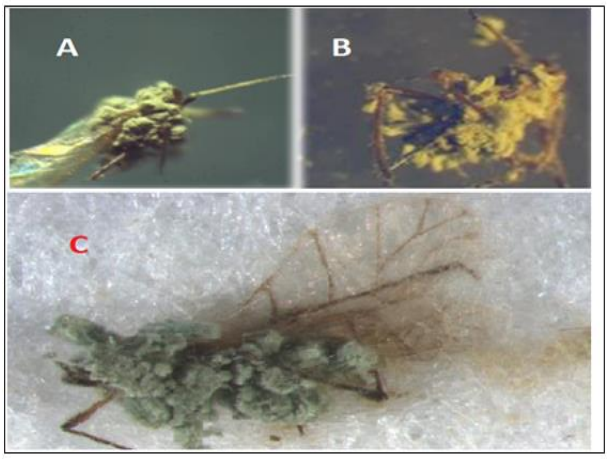

Fig .2. Cadavers of aphid covered by spores of *M.anisopliae*

Results presented in Table (2), and illustrated in Fig. (3) revealed that, M02 isolate give the most effect against *S. graminum*, the LC_{50} value of M2 was $1x10^7$ spores/ml "slope 0.278",while it give the greater LC_{50} value for M01, M06 and M09 which were ($2.7x10^7$, $2.3x10^8$,and $7.8x10^7$) spores/ml respectively. Mortality means were 7.45, 10.3, 7.35 and 8.47 for M1, M2, M6 and M9 respectively. Results obtained indicated that, the M2 isolate give highly-mortality in short time, the LT_{50} value was 3.94 days, while the isolates others M1, M6 and M9, the LT_{50} values were 4.32, 5.1and 5.04 days, respectively as shown in Table (3) & Fig(4).

Table 2. LC_{50} and mortality means between (M01, M02, M06 and M09) isolates on *S. graminum*.

Lines-name	Lc_{50} "spres/ml" Lower limit – Upper limit	The index	The slope	LC_{90} "spores/ml"	Mean±SE
M 01	$2.7x10^7$ $5.7x10^9$ - $1.68x10^{11}$	35.51	0.278	$1.13x10^{12}$	7.45±0.85 bc
M 02	$1 x10^7$ $3x10^6$– $1.65x10^{10}$	100	0.253	$1.13x10^{12}$	10.3±2.35 a
M 06	$2.3x10^8$ $3.8x10^9$ - $6.9x10^{10}$	4.23	0.29	$6.05x10^{12}$	7.35±0.86b
M 09	$7.8x10^7$ $1.3 x10^9$ – $1.02 x10^{10}$	12.46	0.319	$8.8x10^{11}$	8.475±0.99bc

a-b-c "Means within same row having significantly superscripts different at level "$P \leq .05$"
The index compared with (M2)

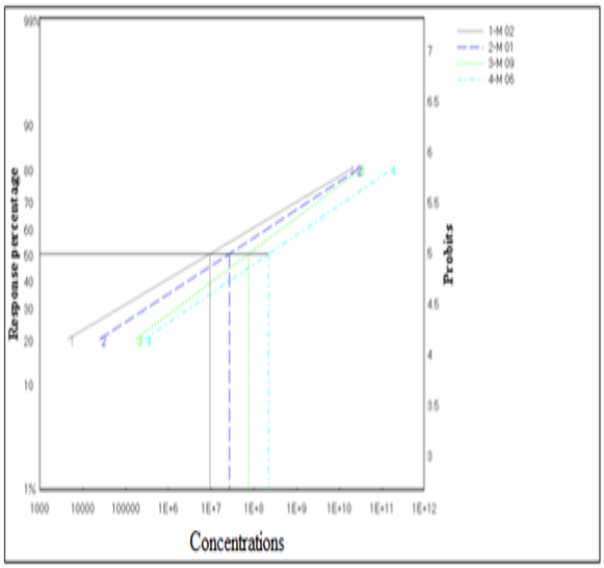

Fig .3. LC_{50} regression lines of (M01, M02, M06 and M09) isolates on *S. graminum*

Table 3. LT_{50} of entomopathogenic fungus isolates (M01, M02, M06 and M09) isolates on *S. graminum*.

Lines-name	Lc_{50} "spres/ml" Lower limit - Upper limit	The index	The slope	LT_{90} "Days"
M 01	4.32	91.36	3.98	9.06
M 02	3.94	100	3.91	8.34
M 06	5.1	77.33	3.6	11.79
M 09	5.04	78.19	3.48	11.58

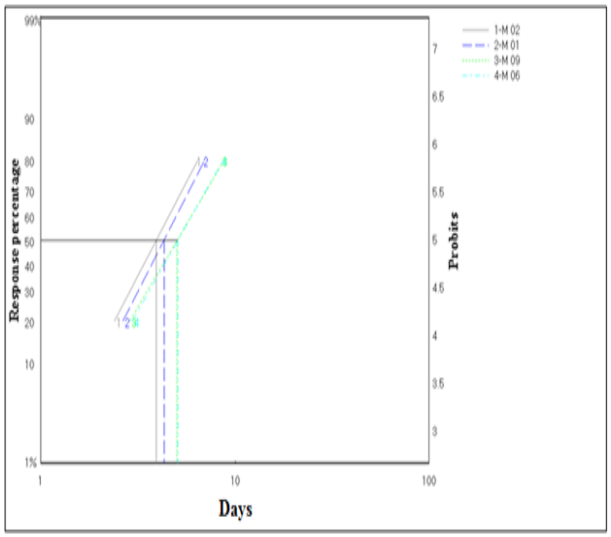

Fig .4. LT_{50} regression lines of (M01, M02, M06 and M09) isolates at concentration 1× 10^8 spores/ml against *S. graminum*

One way ANOVA statistical analysis indicated that significant levels of effect ($F_{3,156}$=2.96; $P \leq .05$) between isolates M01, M02, M06 and M09 on adult aphid.

The obtained results in this study showed that all tested isolates infected aphid treated with conc. (1×10^5, 1×10^6, 1×10^7 and 1×10^8 spores/ml) respectively. Pathogenicity of fungus increased with the increase of concentration and time. The results were compatible with Vu *et al.*(2007), where, the all isolates pathogenicity was reconfirmed and accumulated-mortality rates ranged among "33.3 and 100%" within (7 days), in addition, Aranya *et al.* (2010), who found highest concentration of 10^8 spores/ ml, at the LT_{50} = 5.54 days.

Also, results were compatible with, (Lee *et al.* 2015, and Aker and Abaci 2016) who proved that the use of *M. anisopliae* can provide protection against green peach aphid *Myzus persicae* and hazelnuts aphids *Myzocallis coryli*, respectively and it can be effective as biocontrol agent.

The present results were consistent with laboratory bioassay studies carried out by Entesar *et al* (2020), evaluated four native of entomopathogenic fungi isolates *B. bassiana* and *M. anisopliae* on wheat-aphid *S. graminum* R. at three concentrations (1×10^6 , 1×10^7 and 1×10^8 conidia).Isolate (B1) was more effective against adults, The LC_{50} values of B1 were 3.11×10^6 spores/ml while M1, M2, and B2 revealed greater LC_{50} value, 6.09×10^6 , 2.32×10^7 and 1.15×10^8 spores/ml, respectively.

Results were in same trend with Sahar *et al*. (2016), when evaluated four-conc. 1×10^6, 1×10^7, 1×10^8 and 1×10^9 spores/ml., for each entomopathogenic fungi, *B. bassiana*, *M. anisopliae*, *P. lilacinus* and *L. antillanum* against the adults of cowpea aphid, *A. craccivora*. Also the results were in harmony with Inas (2020) where *M. anisopliae*, have high toxicity and mortality rates against Cabbage Aphids, *Brevicoryne brassica* occurred after 3rd days from treatments. The maximum percent of mortality (100%) occurred after the 10th day from treatment with the 3rd concentration (1 x 10^7 spores/ m). Furthermore Tang *et al.* (2019) suggested that, the entomopathogenic fungi *M. anisopliae* is a good as a bio-control agent against *S. furcifera* and *N. lugens* with (LT_{50}) of approximately four-days were observed with high conidial-conc. of (1×10^8 conidia/ml) of *M. anisopliae*, with alternative strategy for pest control. On the same line, these results were compatible with (Ogarkov and Ogarkova, 1997;, and Ismail, *et al.*, 2016) they found that, the most common fungi used for insect control belong to the genera *Beauveria*, *Metarhizium*, *Paecilomyces*, *Verticillium*, *Aschersonia*, and *Conidiobolus.*

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قدرة الإمراض لعزلات جديدة من الفطر الممرض للحشرات *"Metarhizium anisopliae"* المعزول من شبه جزيرة سيناء ضد من القمح *"Schizaphis graminum"* تحت الظروف المعملية

أحمد جوده الطحاوي[١]، أحمد عدلي محمد ابراهيم[١]، فوزي محمد حسن عيد[١]، سيد على أحمد[٢] و حاتم محمد محفوظ [٢]

[1]معهد بحوث وقاية النباتات ، مركز البحوث الزراعية ، الدقي ، الجيزة

[2]كلية العلوم الزراعية البيئية ، جامعة العريش ، شمال سيناء

تم اختيار أربع عزلات محلية من فطر *Metarhizium anisopliae* الممرض للحشرات وهي (M 01, M 02, M 06 and M 09) من مجموعة من العزلات معزولة مسبقاً من شبه جزيرة سيناء وتم تقييمها معمليا ضد من القمح *Schizaphis graminum* وقد تم استخدام أربعة تراكيزات من معلق الجراثيم لكل من العزلات الفطرية الأربعة 1×10^{5} و 1×10^{6} و 1×10^{7} و 1×10^{8} جرثومة/مل ضد الحشرة الكاملة عمر يوم ، وقد أظهرت النتائج أن العزلة M 02 كانت الأكثر فاعلية كما أظهرت خطوط السمية LC_{50}