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Effectiveness of *Solanum nigrum* Extract and Chlorfenapyr against *Spodoptera frugiperda* and *Tetranychus urticae* under Laboratory Conditions

Sharshar, A. H.[1*]; A. A. Farag[2] and E.A. El Shamy[3]

[1]Weed Survey, Ecology and Physiology Department at Sakha station- Weed Research Central Laboratory, ARC. Giza. Egypt.
[2]Department of Cotton Pesticides Evolution, Plant Protection Research Institute, Agriculture Research Centre, 12619 Giza, Egypt.,
[3]Department of Agric., Zoology and Nematology, Fac. of Agric., Al-Azhar Univ., Elsadat, Egypt

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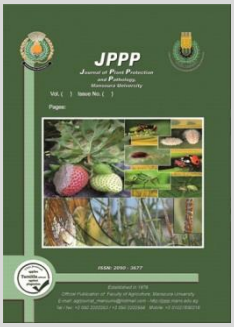

ABSTRACT

This study evaluated the efficacy of *Solanum nigrum* aqueous extract and Chlorfenapyr, and their combinations against *Spodoptera frugiperda*, Smith. (Lepidoptera: Noctuidae) (second and fourth instars) and *Tetranychus urticae*, Koch. (Acarina: Tetranychidae) (adult) across various developmental stages. Treatments were applied under laboratory conditions. This study was conducted during the summer season of 2024 at the Weed Research Laboratory in collaboration with the Plant Protection Research Institute, Sakha Agricultural Research Station,, Agricultural Research Center (ARC), Egypt. Mortality percentages were recorded post-treatment. The results demonstrated that Chlorfenapyr, whether used alone or in combination with the aqueous extract, significantly increased mortality rates in both pests compared to the extract alone. The highest mortality (up to 100% after 10 days post-treatment) was achieved using the aqueous extract of *Solanum nigrum* + Chlorfenapyr alone or at the higher combination dose (120 mg/L), particularly during the later assessment intervals. In contrast, the aqueous extract alone exhibited moderate insecticidal activity. Temporal mortality patterns indicated that all treatments achieved their highest efficacy 10 days post-treatment, with effects gradually improving over time, suggesting its potential use as a natural component in pest management strategies. Statistical analyses confirmed significant differences among the treatments. These findings highlight the value of combining botanical extracts with Chlorfenapyr in integrated pest management (IPM) programs, enhancing both efficacy and environmental sustainability.

Keywords: Aqeous extract*, solanum nigrum, Spodoptera frugiperda,Tetranychusurticae,* Chlorfenapyr.

INTRODUCTION

Solanum nigrum is considered a harmful weed that negatively affects crop productivity by competing for vital resources. It also contains toxic compounds such as solanine, which can cause health issues in humans and animals if consumed, particularly from unripe plant parts (Holm *et al.,* 1991; Edmunds and Marshall, 2002). However, recent studies have shown that aqueous extracts from parts of *Solanum nigrum*, especially the leaves, possess bioactive compounds like alkaloids and phenolics with insecticidal and acaricidal properties. These extracts have demonstrated significant efficacy in suppressing the growth and feeding activity of the fall armyworm **(***Spodoptera frugiperda***)**, and in reducing the reproductive rate of the two spotted spider mite, *Tetranychus urticae* (Senthi *et al.,* 2006; Ahmed and El-Kady 2020). This highlights the potential of *Solanum nigrum* aqueous extract as a natural biopesticide**,** offering an eco-friendly alternative within integrated pest management (IPM) strategies.

The fall Armyworm, *Spodoptera frugiperda* (Lepidoptera:Noctuidae) belonging to the family Noctuidae, is a highly destructive, migratory pest with a wide host range exceeding 80 plant species. Maize is considered its preferred host, making this pest a significant threat to food production and security, especially in tropical and subtropical regions (Goergen *et al.,* 2016; Montezano *et al.,* 2018). Since its first detection in Africa in 2016, *S. frugiperda* has rapidly invaded Asia and the Middle East, including Egypt, where it now poses a new and serious challenge to local agriculture.Current management strategies heavily rely on synthetic insecticides. However, the overuse and misuse of these chemical compounds have resulted in several negative outcomes, such as the development of insecticide resistance, bioaccumulation in the ecosystem, adverse effects on beneficial and non-target organisms, and long-term soil and water contamination (Popp *et al.,* 2013; Sparks and Nauen, 2015). Moreover, the economic burden on farmers due to the frequent application of insecticides highlights the urgent need for safer, more sustainable pest control alternatives.In recent years, botanicals have emerged as viable options within Integrated Pest Management (IPM) programs. Plant-derived compounds offer several advantages: they are biodegradable, exhibit lower toxicity to humans and the environment, and often possess multiple modes of action, reducing the likelihood of resistance development (Isman, 2006; Pavela and Benelli, 2016). Extracts from medicinal and wild plants have shown promising insecticidal, antifeedant, and growth-inhibitory effects against a wide range of insect pests.*Solanum nigrum* L. (Black Nightshade), a common weed distributed throughout Egypt and many parts of the world, belongs to the Solanaceae family and is known for its rich content of secondary metabolites such as alkaloids, flavonoids, glycosides, and saponins. Several studies have highlighted the potential insecticidal and larvicidal activity of *Solanum* species against agricultural pests (Dhileepan *et al.,* 2012 and

* **Corresponding author.**
E-mail address: Alysharshar1@gmail.com,
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Singh *et al.,* 2016). However, limited data are available on the use of *S. nigrum* extracts against *Spodoptera frugiperda* under Egyptian conditions.

Therefore, the present study was undertaken to evaluate the efficacy of the aqueous extract of *Solanum nigrum* against different larval instars of the fall armyworm under laboratory conditions. The study aims to contribute to the development of environmentally friendly pest control methods and to reduce the dependency on chemical insecticides, thereby minimizing the environmental footprint of pest management practices.

MATERIAL AND METHOD

This work was carried out in the Weed Research Laboratory in collaboration with the Plant Protection Research Institute, Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Agricultural Research Center (ARC), Egypt, and was carried out in sterile plastic dishes (covered with gauze), in four replicates. In an effort to explore eco-friendly alternatives to conventional pesticides or to reduce their application rates, thereby mitigating the environmental pollution associated with pesticide use.The life cycle of the fall armyworm (*Spodoptera frugiperda*) consists of four main stages: egg, larva, pupa, and adult moth. The cycle begins when the female lays her eggs on plant leaves, which hatch after 2-4 days, releasing larvae that begin feeding on the leaves. The larvae go through six instars before transforming into the pupal stage. The larval stage is the most destructive to crops due to the significant consumption of leaves, which weakens the plants and affects their growth, as Food and Agriculture Organization (FAO). (2020), Nagoshi *et al.,* (2017) and University of Florida, IFAS Extension.(2021).

Insecticides

Chlorfenapyr(erylpyrrol derivative), 4-bromo-2(4-chlorophenyl -1-(ethoxymethyl)-5-(trifluoromythyl)-1H-pyrrole-3-carbonitrile, fantyextera application rateof rate 240 cm^3 /fed.was supplied by the Plant Protection Research Institute, Dokki – Agricultural Research Center – Egypt**.**

Source of materials:

The aqueous extract of *Solanum nigrum* was utilized to evaluate its effect on the developmental stages of the fall armyworm and the two-spotted spider mite. This work was carried out at Sakha Agricultural Research Station. To establish a culture of *Spodoptera frugiperda* field strain, egg patches were collected from untreated maize plants at Sakha Agricultural Research Farm, and transported to the laboratory at 27°C and 70% RH. On maize plants, neonate larvae of *S. frugiperda* were reared in the laboratory for 2 generations until used, using plastic pots (500 ml) covered with a clean piece of muslin cloth. While *T. urticae* was collected from cotton plants and transported to the laboratory at 27°C and 70% RH. On caster bean leaves (*Ricinus communis*), they were reared in the laboratory for 2 generations until application. *Solanum nigrum* specimens were collected from the Sakha Research Farm during the summer season of 2024 to prepare the extract. Maize plants, the primary host of the fall armyworm, were also collected to serve as a food source during the study.

Preparation of plant materials:

The aqueous extract of the Black Nightshade, *S.nigrum* was prepared following standard extraction procedures. The plant material was first air-dried in the shade for 10 days, then oven-dried at 70°C until a constant weight was achieved. The dried material was finely ground using an electric grinder to obtain a uniform powder. A quantity of 20 grams of this powder was added to 100 milliliters of distilled water to prepare a 20% (w/v) aqueous extract. The mixture was thoroughly stirred, filtered through cheesecloth, and the resulting filtrate was used for subsequent bioassays adapted from (Singh *et al.,* 2016).

Ten larvae from each of the second and fourth instars were placed in separate sterile plastic pots. Corn leaves were immersed in the aqueous extract for half a minute, and then air-dried on blotting paper for 1 minute before being placed in the pots as a food source for the larvae. The pots were covered with a clean piece of muslin cloth. This procedure was carried out in four replicates (10 larvae/replicate) for each treatment. The larvae were starved for six hours, and observations were made to record the number of live larvae after 1, 3, 7, and 10 days. Also, for the two-spotted spider mite, there were four replicates (10 adults/replicate) for each treatment. The experiment in plastic pots included four treatments besides the untreated (control), arranged in a completely randomized design with four replicates, as follows:

1- Water extract of *Solanum. nigrum* at 20% conc. (w/v).
2- Water extract of *S. nigrum* at 20% conc. (w/v) + Chlofenopyr SC 24 % at rate 37.5 mg/L.
3- Water extract of *S. nigrum* at 20% conc. (w/v). + Chlofenopyr at rate 75mg /L..
4- Chlofenopyr SC 24 % at rate 150mg/L.
5- Untreated (control)

Statistical analysis:

The experiments were arranged in a Randomized Complete Design with four replicates. The data were statistically analyzed using the analysis of variance method as described by Gomez and Gomez (1984**)**. Mortality percentages were recorded 48hrs post-treatment. Mortality percentages were corrected according to Abbott's equation (Abbott,1925). Statistically significant differences were determined by one-way analysis of variance (ANOVA) (SPSS.

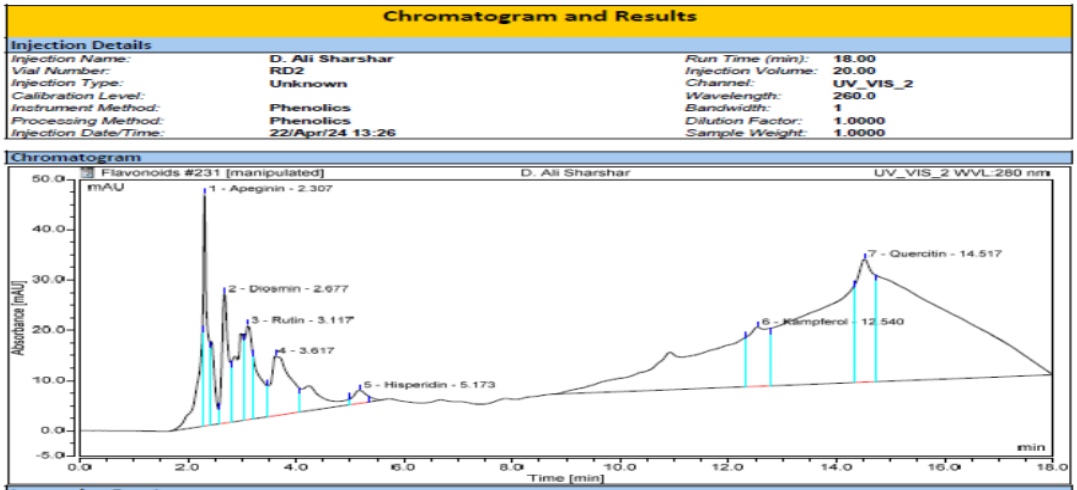
RESULTS AND DISCUSSION

The impact of the aqueous extract of *Solanum nigrum* (w/v), either alone or combined with Chlorfenopyr at full, half, or quarter doses, was evaluated on the live count of fall armyworm larvae under laboratory conditions. The experiment, conducted in four replicates, extended over a period of 10 days, with the first observation recorded 24 hours after the initiation of the treatment.

Fig. (1) Illustrated that flavonoids are natural plant compounds that have shown insecticidal properties in recent studies. They can act as feeding deterrents, disrupt insect growth, or interfere with enzymes essential for insect survival. Research suggests that compounds like Quercetin and Kaempferol are effective against various pests. Therefore, flavonoids may serve as eco-friendly alternatives to synthetic pesticides as (Pereira *et al*., 2024) and Goławska *et al*., 2023)

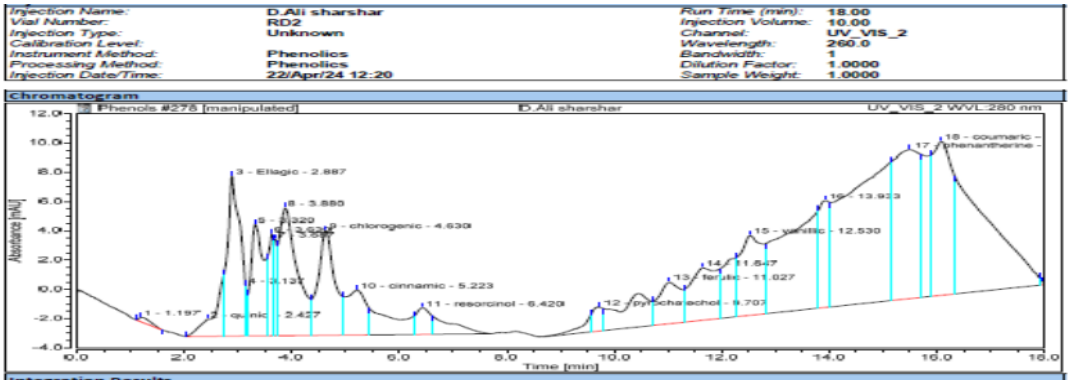
Fig. (2) Illustrated that Phenolic compounds are secondary metabolites found in plants and are known for

their antioxidant, antimicrobial, and insecticidal activities. The chromatographic analysis revealed the presence of various phenolic acids such as Chlorogenic, Ellagic, Vanillic, and P-coumaric acid. These compounds have been reported to affect insect physiology by disrupting digestion, enzyme activity, and growth. Therefore, Phenolics could be promising natural alternatives to synthetic insecticides as Tsao (2010) and War *et al.,* (2012).



No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount mg
1	Apeginin	2.307	3.538	46.153	11.98	32.66	1.5982
2	Diosmin	2.677	3.648	25.693	12.36	18.18	0.7627
3	Rutin	3.117	2.775	18.741	9.40	13.26	0.8555
4	3.617	4.954	11.872	16.78	8.40	n.a.
5	Hisperidin	5.173	0.667	2.620	2.26	1.85	0.8349
6	Kampferol	12.540	5.132	11.786	17.38	8.34	0.4011
7	Quercitin	14.517	8.813	24.469	29.85	17.31	0.5195
Total:			**29.528**	**141.333**	**100.00**	**100.00**	

Fig.1. Flavonoids are natural plant compounds on the aqueous extract of *Solanum nigrum*



No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount mg
1		1.197	0.089	0.302	0.27	0.30	n.a.
2	quinic	2.427	0.847	1.176	2.55	1.18	0.0007
3	Ellagic	2.887	2.974	10.919	8.97	10.98	0.0010
4		3.137	0.111	3.473	0.33	3.49	n.a.
5		3.320	2.017	7.617	6.08	7.66	n.a.
6		3.630	0.890	6.949	2.68	6.99	n.a.
7		3.887	0.322	6.580	0.97	6.62	n.a.
8		3.880	3.483	8.730	10.50	8.78	n.a.
9	chlorogenic	4.630	2.602	7.141	7.85	7.18	0.0007
10	cinnamic	5.223	1.259	3.076	3.80	3.09	0.0026
11	resorcinol	6.420	0.535	1.809	1.61	1.82	0.0002
12	pyrochatechol	9.707	0.363	1.643	1.10	1.65	0.0000
13	ferulic	11.027	1.336	2.844	4.03	2.86	0.0001
14		11.647	2.110	3.577	6.36	3.60	n.a.
15	vanillic	12.530	2.659	5.446	8.02	5.48	0.0002
16		13.933	1.572	7.292	4.74	7.33	n.a.
17	phenantherine	15.487	5.534	10.165	16.69	10.22	0.0015
18	coumaric	16.073	4.428	10.508	13.36	10.57	0.0002
19		17.973	0.026	0.186	0.08	0.19	n.a.
Total:			33.156	99.433	100.00	100.00	

Fig. 2. Phenolic are natural plant compounds on the aqueous extract of *Solanum nigrum*

The results presented in Table (1) and Figs. (3 and 4) show that all treatments significantly reduced the numbers of various developmental stages of both *S.frugiperda* and *T.urticae* compared to the control group over the four evaluation periods (First day, 3, 7, and 10 days after treatment); Where Showed that aqueousextract alone (0.1/L) a moderate reduction in pest populations, with a gradual decline in larval and adult numbers, especially after 7 and 10 days; However, the effect was relatively limited compared to other treatments;While giving aqueousextract(0.1/L) + Chlorfenapyr (37.5mg\L.)treatment improved effectiveness over the extract alone, with more notable reductions in pest numbers by 7 and 10days, This suggests a synergistic interaction between the two components, the table also shows that using the aqueous extract + Chlorfenapyr (75mg/L) treatments showing the lowest pest counts across all stages and evaluation periods; In some cases, pest numbers reached zero by the 10th day, indicating a strong joint effect at this concentration, also Chlorfenapyr alone (150mg/L.) showed high efficacy but was slightly less effective than the combination of aqueous extract and Chlorfenapyr at 75mg/L., supporting the idea that combination treatments enhance pest control, this is compared to untreated treatments which gave the highest pest counts, reinforcing the effectiveness of all tested treatments.

The results presented in Table (2) and Figs. (3 and 4) show that the results of the study clearly demonstrate that Chlorfenapyr, whether applied alone or in combination with

the aqueous extract of *Solanum nigrum*, was significantly more effective against *Spodoptera frugiperda* and *Tetranychus urticae* across all developmental stages and time intervals compared to the extract alone. The highest efficacy was observed in the treatment with Chlorfenapyr alone, which achieved up to 100% mortality in adult stages by the 7th and 10th days, confirming its potent insecticidal action through the disruption of mitochondrial electron transport, as documented by Ishaaya *et al.,* (2002). When Chlorfenapyr was combined with the aqueous extract, especially at higher concentrations, a noticeable synergistic effect was evident, resulting in nearly equal efficacy to Chlorfenapyr alone. This synergy aligns with Isman (2006), who noted that botanical compounds can enhance the effectiveness of synthetic insecticides when used together. On the other hand, the aqueous extract of *Solanum nigrum* alone showed moderate insecticidal activity that increased over time, Edmunds and Marshall (2002), indicating its potential role as a natural pesticide within integrated pest management (IPM) programs. Such botanical-based solutions have been recognized for their eco-friendly and sustainable approach to pest control, as supported by Tripathi *et al.*, (2009). Finally, the untreated control group confirmed the validity of the findings, showing no mortality in any pest stage.

Table 1. Effect of Aqueous Extract of *Solanum nigrum*, Chlorfenapyr, and Their Combination on *Spodoptera frugiperda* and *Tetranychus urticae* at Different Developmental Stages Over Time under laboratory condations

Treatments	Rate / fed	No. of after treatment											
		First day			3 days			7 days			10 days		
		S. frugiperda		*T. urtica*	*S. frugiperda*		*T. urtica*	*S. frugiperda*		*T. urtica*	*S. giperda*		*T. urtica*
		2nd	4th	adult	2nd	4th	adult	2nd	4th	adult	2nd	4th	adult
1- Water extract of *Solanum. nigrum* at 20% conc. (w/v)	20L	8.00	4.50	3.00	4.75b	3.75	2.25	2.75b	1.75	1.00 b	1.50	0.75	0.50
2- Water extract of *Solanum. nigrum* at 20% conc. (w/v) + Chlofenopyr SC 24 % at rate 37.5 mg/L.	20L +37.5 mg/L	8.00	4.75	3.00	3.50 b	2.50	1.50	1.75b	1.50	0.75c	0.50	0.50	0.25
3- Water extract of *Solanum. nigrum* at 20% conc. (w/v). + Chlofenopyr SC 24 % at rate 75mg /L.	20L +75 mg/L	7.75	4.50	2.00	2.50 b	1.75	1.00	1.25c	0.75	0.00	0.50	0.00	0.00
4- Chlofenopyr SC 24 % at rate 150mg/L.	150 mg/L	2.50	3.00	1.25	1.50 c	1.75	0.25	0.50c	0.50	0.00	0.50	0.00	0.00
Untreated (control)	-	10.00	5.00	5.00	10.00a	5.00	5.00	10.00	5.00	4.50	10.00	5.00	4.50

Table 2. Mortality Percentage of *Spodoptera frugiperda* and *Tetranychus urticae*a after Treatment with the aqueous extract *Solanum nigrum* and Chlorfenapyr under laboratory condations

Treatments	Rate / fed	No. of after treatment											
		First day			3 days			7 days			10 days		
		S. frugiperda		*T. urtica*	*S. frugiperda*		*T. urtica*	*S. frugiperda*		*T. urtica*	*S. frugiperda*		*T. urtica*
		2nd	4th	Adult	2nd	4th	adult	2nd	4th	adult	2nd	4th	adult
1- Water extract of *Solanum. nigrum* at 20% conc. (w/v)	20 L	20.0 b	10.0 b	40.0 c	52.5 d	35.0 c	45.00 d	72.5 d	65.0 d	77.7 c	85.0 b	85.0 c	88.8 c
2- Water extract of *Solanum. nigrum* at 20% conc. (w/v) + Chlofenopyr SC 24 % at rate 37.5 mg/L.	20 L + 37.5 mg/L	20.0 b	5.0 c	40.0 c	65.0 c	50.0 b	70.0 c	82.5 c	70.0 c	83.3 b	95.0 a	90.0 b	94.4 b
3- Water extract of *Solanum. nigrum* at 20% conc. (w/v). + Chlofenopyr SC 24 % at rate 75mg /L.	20 L +75 mg/L	22.5 b	10.0 b	60.0 b	75.0 b	65.0 a	80.0 b	87.5 b	85.00 b	100.0 a	95.5 a	100.0 a	100.0 a
4- Chlofenopyr SC 24 % at rate 150mg/L.	150 mg	75.0 a	40.0 a	75.0 a	85.0 a	65.0 a	95.0 a	95.0 a	90.00 a	100.0 a	95.5 a	100.0 a	100.0 a
Untreated (control)	-	0	0	0	0	0	0	0	0	0	0	0	0

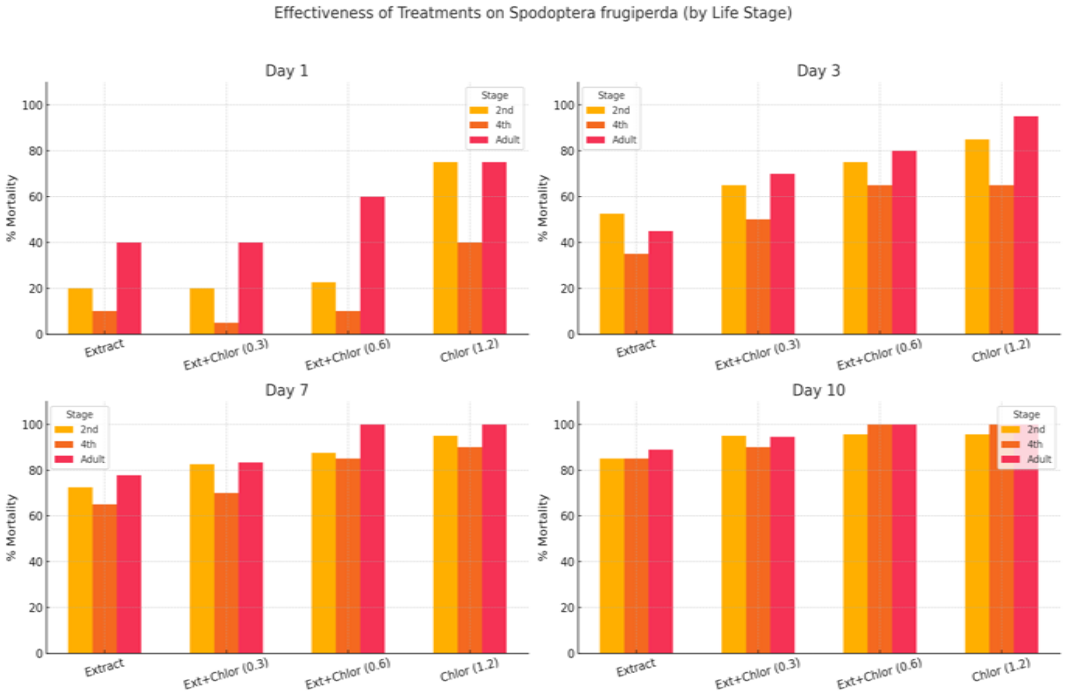


Fig. 3. illustrates the change in the number of *S. frugiperda*2nd instar larvae over time under different treatments.

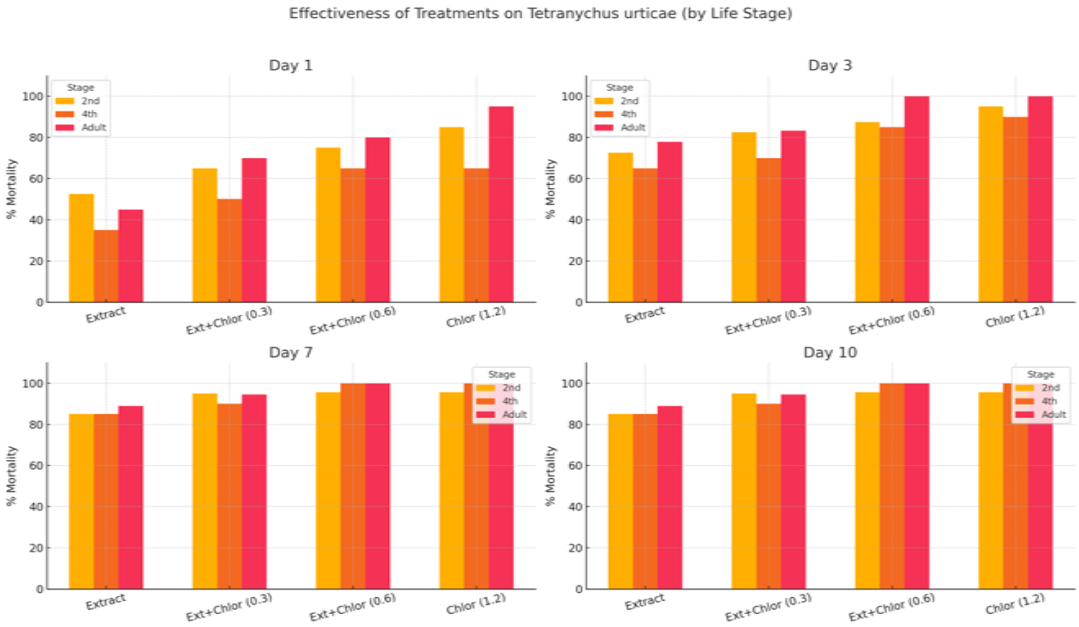


Fig. 4. Illustrates the change in the number of *Tetranychusurticae*over time under different treatments.

The statistical significance among treatments (ANOVA, Duncan's test) further supports the superior performance of Chlorfenapyr-containing treatments. Overall, the integration of botanical extracts with chemical pesticides not only enhances pest control efficacy but also contributes to safer and more sustainable agricultural practices. Regarding spider mite control, the counts conducted on the 7th and 10th days after treatment with the insecticide chlorfenapy alon at 150 mg / L of water, as well as when used at 75 mg / L combined with *solanum nigrum* extract at 20% concentration, showed a100 % mortality rate in both cases . as for the first count on day and the second count on day 3, the mortality rates were 75% and 95%, respectively, for the insecticide alon and 60 % and 80 %, respectively, for the insecticide combined with the extract. For *Spodoptera frugiperda* control, the counts conducted on the 7th and 10th days after treatment with the insecticide chlorfenapyr alone at 150 mg/l showed 95% & 90% mortality and 93% & 100% mortality in both larval ages at 7th and 10th days, respectively. Also, the mortality percentages were 75% & 90% and 85% & 95% in both larval ages, respectively. On the hand, for S. frugiperda control, the counts conducted on the 7th and 10th days after treatment with chlorfenapyr at 75 mg/l plus Solanum nigrum extract at 20% conc. showed 87.5% & 85% and 95% & 100% mortality in both larval ages, respectively. Also, the mortality percentages were 22.5% & 10% and 75% & 65% in both larval ages at 7th and 10th days, respectively. As for the rest of the treatments, they also gave significant results as previously mentioned, although they were less effective. All results are compared to the untreated control.

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I would like to express my sincere gratitude and deep appreciation for your generous scientific support and invaluable guidance, which have had a profound impact on my academic journey. You have always been a model of dedication and generosity. I pray that Allah preserves you, blesses your knowledge, and makes you a source of benefit for the country and its people. May He reward you abundantly. With utmost respect and appreciation,

Your devoted student / Dr. Aly Aly Hassan Sharshar Assistant Professor, Weed Research Central Laboratory – Agricultural Research Center

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تأثير مستخلص نبات *Solanum nigrum* ومركب Chlorfenapyr ضد دودة الحشد الخريفية والعنكبوت الأحمر ذو البقعتين تحت الظروف المعملية

علي حسن. شرشر[١]، عبد السلام اسماعيل فرج[٢] و عماد عبدالغفار الشامى[٣]

[1]قسم حصر الحشائش ، علم البيئة والفسيولوجيا، محطة سخا - المعمل المركزي لبحوث الحشائش، مركز البحوث الزراعية، الجيزة، مصر.
[2] قسم اختبار مبيدات آفات القطن، معهد بحوث وقاية النبات، مركز بحوث الزراعة، ١٢٦١٩ الجيزة، مصر،
[3]قسم علم الحيوان والنيماتودا، كلية الزراعة، جامعة الأزهر، السادات، مصر،

الملخص

تمت هذه الدراسة لتقيم فعالية المستخلص المائى لنبات *Solanum nigrum* ومركب Chlorfenapyr على الأطوار الغير كاملة لدودة الحشد الخريفية (العمر الثاني والرابع) والعنكبوت الأحمر (الطور الكامل) في معمل بحوث الحشائش ومعهد بحوث وقاية النباتات بمحطة البحوث الزراعية بسخا - مركز البحوث الزراعية. اتضح من الدراسة أن مركب Chlorfenapyr له تأثير معنوي عالى جدا على دودة الحشد الخريفية والعنكبوت الأحمر وسجل نسبة موت عالية سواء استخدم فى صورة منفردة أو فى صورة مختلطة مع المستخلص المائى Solanum nigrum مقارنتا فى حالة إستخدام المستخلص فى صورة منفردة. سجلت أعلى نسبة موت عالية جدا بعد ١٠ أيام من المعاملة (١٠٠٪) فى حالة استخدام المستخلص المائى مع مركب Chlorfenapyr فى صورة مختلطة بتركيز (١٢٠ملجرام/ لتر) وأيضا أظهر المستخلص المائى تأثير متوسط على الافاتين ومع مرور الوقت أظهرت جميع المعاملات تأثيرات عالية المعنوية بعد ١٠ أيام من المعاملة. و ذلك يمكن إستخدام المستخلص النباتى ضمن برنامج المكافحة المتكاملة لدودة الحشد الخريفية والعنكبوت الأحمر سواء فى صورة منفردة أو مختلطة مع المركب لتعزيز الفاعلية وتحقيق الإستدامة البيئية.