P-ISSN: 3009 – 7487
E-ISSN: 3009 – 7886

JOURNAL OF PLANT AND FOOD SCIENCES June 2025

# Precipitin test to determining the relationship between some insect predators and fall armyworm and red spider mites

**El Shamy, E. A.[1], Abdel Hady, M. A. H.[2], Abdelaziz, Yasmen A.[3], Amer, Nahed S.[3], Elsadawy, Amany A.[3], El-Shazly, Safaa A.[3], Farag, A. A.[4]**

1- Department of Agriculture, Zoology and Nematology, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt.
2- Dept of Agric. Zoology and Nematology. Fac. of Agric. Al-Azhar Univ. Egypt.
3- Department of Agriculture, Zoology and Nematology, Faculty of Agriculture (Girls branch), Al-Azhar University, Cairo, Egypt.
4- Department of cotton pesticides evaluation, plant protection research institute, ARC, Giza, Egypt.

*** Corresponding author:** Abdel Hady, Mohamed Abdel hady Hassan
**E-mail**: mohamed_abdelhady.5@azhar.eud.eg https://orcid.org/0009-0009-7018-964X
Received: Received: 3rd February 2025; in revised form: 3rd February 2025/ Accepted: 10th May 2025/ Published: 1st June 2025
DOI: 10.21608/jpfs.2025.356784.1024

## ABSTRACT

This study was conducted to evaluate the relationship between the antiserum of the insect predator against the fall armyworm and some cotton pests. The double diffusion test in agar was used to determine which larvae predator, the green lacewing, *Chrysoperla carnea*, was feeding on the fall armyworm, *Spodoptera frugiperda*, the cotton mealy bug, *Phenacoccus solenopsis*, and the two-spotted spider mite, *Tetranychus urticea*, in the field. The precipitin technique was observed in agar gel for the insect pests. The reaction between the antiserum of *Chrysoperla carnea* against antigens *S. frugiperda*, *P. solenopsis*, and *T. urticea* showed positive reactions represented by 5, 6, and 3 precipitin lines, respectively. It devoured 48.65 spotted spider mite nymphs and 56.33 armyworm 1st instar larvae during 7.99 and 8.99 days, respectively. The negative reaction was recorded when antiserum of *Chrysoperla carnea* was reacted against the antigen of the other predator, *Scymnus interruptus*. Sharp reactions between the antiserum of the fall armyworm pest *S. frugiperda* and the predators' antigens, *Coccinella undecimpunctata*, *Metasyrphus corolla*, *Cydonia veciniisis*, and *Paedrus alfierii, were* represented by 5, 4, 2, and 1 precipitin lines, respectively. This method indicated variations between the insect pests and their predators, and all antisera gave positive serological reactions with homologous antigens and negative reactions with heterologous antigens.

**Keywords:** Precipitin, antiserum, antigens, insect predator.

## INTRODUCTION

Serological tests are considered one of the most important and accurate methods used recently in discovered relationship between the fall armyworm and some cotton pests and their predators. Feeding capacity on woodlice was examined serologically to demonstrate the difference in quantitative and predation efficiency between predators and insect pests (Sutton, 1970). Serological methods employed in pest-predator determining what proportion of antigen and antisera of predators has consumed on pest (Lund, 1977). The precipitin test which is relied on the reaction between pest antigen and predator antisera (Ohiagu, 1978).

Precipitin test may be to qualify the antigens as relationship by their activity with antisera produced against them (Cassaro *et al*., 2001). The ladybird and the lacewing are serious predators of the cotton insect pests causing severe damage for immature stage and adults, the predators used as a biocontrol agent against different insect pests infesting different plants (Boraei *et al*., 2005). The double diffusion test has proved useful in insect predators and this test has sensitivity to determine consumption of the insect pests investigation and strong reaction between pests and their predators (Redoan *et al*., 2016). The fall armyworm, *S. frugiperda* is one of the most important newly discovered prospector insect pests and it has many plant hosts and feeding on all parts of the plants randomly in Egypt (Salem *et al*., 2021). It caused a lot of damage to the local product, along with other pests that affect the crop.

The present investigation a double diffusion test in agar was conducted to compare the predation efficiency and preference of *Chrysoperla carnea* on some cotton pests, thus the relationship between the antiserum of *S. frugiperda* and the antigen of the predators accompanying it in nature.

## MATERIALS AND METHODS

In the present study, a simultaneous relationship was found between the cotton pests and some important predators such as the lacewing and the ladybird. Accordingly, this relationship was clarified by conducting some serological techniques that illustrate this relation through precipitin reactions that appear in the form of precipitin lines between the pests antigens and the predators antiserum or vice versa, that feed on them in the field or laboratory.

### Cultures of *S. frugiperda, T. urticae* and *P. solenopsis:*

The fresh eggs patched of *S. frugiperda* were collected from maize fields at the farm of Sakha Agricultural research station, 2024 season. Also, *T. urticae* and *P. solenopsis* were collected from cotton fields, in to same farm of Sakha. The fields did not treat with any insecticides and then transported to the laboratory of Sakha, Plant Protection Research Institute, separately. The egg patches were placed in glass jars until hatching. The newly hatched larvae is placed in clean glass jars, fed on castor been every. While adult of the spotted spider mite, *T. urticae* and mealybug, *P. solenopsis* were brought to the laboratory on cotton plants for sucking plant juices. The newly hatched 1$^{st}$ instar larvae of S. *frugiperda* and 2$^{nd}$ instar nymphs of *T. urticae* and *P. solenopsis* were used in the laboratory experiments.

### Predator rearing:

Adults of the green lacewing, *Chrysoperla carnea* were collected from non-treatment cotton fields and transferred to the laboratory, they were placed in clean glass jars containing black twisted paper for laid eggs and a piece of cotton saturated with a sugar solution to feed adults (Khoder, 2005). The increased of breeding process, the eggs of *Chrysoperla carnea* were obtained from Predators Mass Rearing Laboratory, Faculty of Agriculture, Cairo University, Egypt. The newly hatched 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ larvae were put individually in a petri dish with 1$^{st}$ instar larvae of *S. frugiperda* and 2$^{nd}$ instar nymphs of *T. urticae* for feeding. Four replicates were made for each stage. The devoured individuals were daily recorded.

### Antigen preparation:

Twenty mg of immature stage both of *Chrysoperla carnea* and *S. frugiperda* to prepare antiserum and 5 mg of the other insects for antigen production. The larval stage and adults for all the insect species added to send were crushed by mortar pestle. The powders were mixed with sodium chloride solution 1%, 0, 2 m sodium azide to prevent the possible pollution and the solution was put in refrigerated overnight at 4$^{o}$ c to dissolve soluble protein,

subsequently was centrifuged at 3000 r.p.m. for 15 minute and filtered through a Millipore sterilizing. The suspension was used for injected or can a stored in a freezer at -20 $^{0}$c until used. The adjuvant was mixed with suspension in the ratio of 1:1 was emulsion neatly (Petterson, 1975).

**Antiserum preparation:**

The production of antiserums used two individual New Zealand white rabbits. They were bred under laboratory conditions until the individual reached 2.5 to 3 kg. Each rabbit was injected with one species of insect. The first injection starts with 0.5 ml of antigen, and the second injection is 1 ml, and the quantity is gradually increased until the last injection of 5 ml. The total number of injections was 10 injections; accordingly, each rabbit was injected with 27 ml of the prepared antigen, and then the rabbit was left for 10 days after the last injection. The blood samples were taken from the rabbit's ear. The plasma was separated from the blood samples. Petri dishes were prepared in which pure agar was cooked on fire, and then 5 holes were made to be used for the insect antigens (3 ml) and antisera (3 ml). The antigen and antisera were placed at 40°C for a period of 24 h until the interaction between the antigen and antisera was discovered (Boraei, 1984).

**Table (1): Antiserum and antigens tested for determine prey-predator relationship.**

| Antiserum and antigens | Species |
|---|---|
| Antiserum 1 | *S. frugiperda* |
| Antiserum 3 | *Chrysoperla carnea* |
| Antigen 2 | *C. undecimpunctata* |
| Antigen 4 | *T. urticae* |
| Antigen 5 | *M. corolae* |
| Antigen 6 | *P. alferii* |
| Antigen 7 | *C. vecini isis* |
| Antigen 8 | *Sc. Interruptus* |
| Antigen 9 | *P. solenopsis* |
| Antigen 10 | *S. frugiperda* |

## RESULTS AND DISCUSSION

**Feeding efficiency of *Chrysoperla carnea* larvae on *T. urticae* and *S. frugiperda* under laboratory conditions:**

The results showed in table (2) the potential status of *Chrysoperla carnea* larvae fed on *T. urticae*. The three larval instars of *Chrysoperla carnea* were devoured 8.66 individuals (34.3%), 16.3 individuals (54.43%) and 23.66 individuals (59.2%) respectively, with daily mean of 5.21, 7.00 and 5.91 individuals each day. As shown in table (2), the time needed by *Chrysoperla carnea* larvae to feed of *S. frugiperda* larvae were 11.33 individuals (56.65), 21.00 individuals (70.00) and 27.00 individuals (67.50) larvae in its 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ instar larvae, with average daily devoured of 5.66, 7.89 and 6.23 larvae, respectively through 8.99 days.

**Table (2): Feeding capacity of *Chrysoperla carnea* larvae on *S. frugiperda* larvae and *T. urticae* first nymph under laboratory conditions.**

| Prey species | Larvae stages predator | No. of consumed | Consumed (day) | % consumed | Stages duration |
|---|---|---|---|---|---|
| *T. urticae* | 1st instar | 8.66 ± 1.09 | 5.21 | 34.3 | 1.66 ± 0.91 |
| | 2nd instar | 16.33 ± 1.21 | 7.00 | 54.43 | 2.33 ± 1.20 |
| | 3rd instar | 23.66 ± 0.91 | 5.91 | 59.2 | 4.00 ± 0.87 |
| | Total | 48.65 ± 2.13 | 18.12 | | 7.99 ± 2.02 |
| *S. frugiperda* (larvae) | 1st instar | 11.33 ± 2.02 | 5.66 | 56.65 | 2.00 ± 1.34 |
| | 2nd instar | 21.00 ± 1.81 | 7.89 | 70.00 | 2.66 ± 1.52 |
| | 3rd instar | 27.00 ± 2.01 | 6.23 | 67.50 | 4.33 ± 1.61 |
| | Total | 59.33 ± 1.99 | 19.78 | | 8.99 ± 1.68 |

**The relationship between of *Chrysoperla carnea* and some cotton pests and armyworm by precipitin test:**

Results obtained in Table (3) and illustrated in Fig. (1), indicated that more numerous reactions were fridge antigens, particularly for antiserum *Chrysoperla carnea* larval instar when tested with antigen *P.solenopsis* nymphs instars he gave 6 precipitin lines. When the antigens the fall armyworm, *S. frugiperda* and the two spotted spider mite, *T. urticae* reacted with anti -3- serum they gave 5 and 3 precipitin lines, respectively. While negative activity was observed when antiserum of *Chrysoperla carnea* was reaction against the other predators antigens, *Sc. interruptus* gave a negative reactions (zero precipitin lines).

**Table (3): The number of precipitin lines detected during the serological reactions between some antigens and antiserum.**

| Types of antigens | Anti- *S. frugiperda* -serum | | Anti- *Chrysoperla carnea*- serum | |
|---|---|---|---|---|
| | Reactions | No. of precipitin lines | Reactions | No. of precipitin lines |
| *T. urticae* (4) | - | - | ++ | 3 |
| *C. undecimpunctata* (2) | +++ | 5 | - | - |
| *P. solenopsis* (9) | - | - | +++ | 6 |
| *P. alferii* (6) | + | 1 | - | - |
| *C. vecini isis* (7) | ++ | 4 | - | - |
| *Sc. Interruptus* (8) | - | - | - | 0 |
| *M. corolae* (5) | + | 2 | - | - |
| *S. frugiperda* (10) | - | - | +++ | 5 |

+ Low relation- ++ mod. relation- +++ high relation

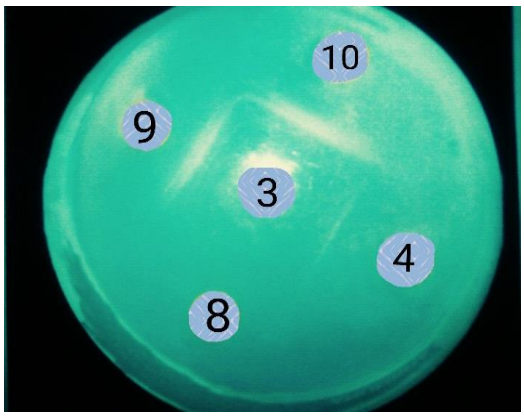


**Fig.(1). Determination of precipitin lines in agar double diffusion test when anti- *Chrysoperla carnea*-serum interacts against the antigens of *S. frugiperda P., T. urticea* and *Scymnus. Interruptus.***

**The relationship between of *S. frugiperda* and associated predators by precipitin test:**

Data presented in table (3) and illustrated in Fig. (2) Obviously revealed that strong reactions were discovery them when antiserum of *S. frugiperda* (1) was assayed with the antigens of the predators, *C. undecimpunctata* (2) and *C. vecini isis* (7) and grouped from cotton plants, hence sharp reaction (5 precipitin lines with *C. undecimpunctata* and 4 precipitin lines with *C. vecini isis*) were discovered. However, only 2 and 1 bands were detected when it was tested (*S. frugiperda*) antisera against the predator's antigens, *M. corolae* and *P. alferii*. The antiserum of the predator to insect pests makes the success of the predation efficiency under field and laboratory conditions, the antiserum would produce a detectable activity only if the predators were copping while eating on a prey.

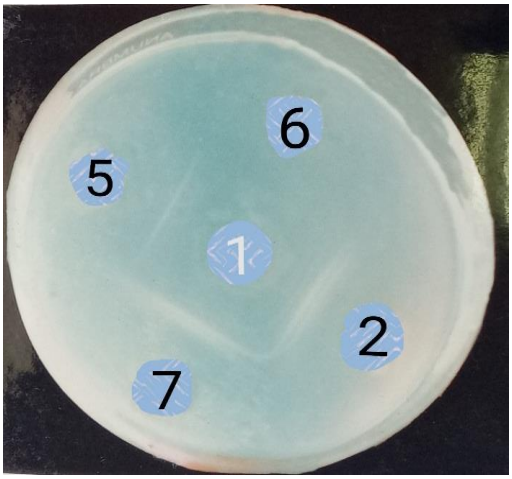


**Fig.(2): Determination of precipitin lines in agar double diffusion test when anti- *S. frugiperda* (1)- serum interacts against the antigens of *C. undecimpunctata* (2), *P. alferii* (6), *C. vecini isis* (7) and *M. corolae* (5).**

It can be inferred from the previous results that clear precipitin lines appear when the predators that feed on them in large quantities and precipitin lines does not appear with the heterologous antigens. In the present study, it could be concluded that *Chrysoperla carnea* is very voracious feeders on *S. frugiperda* and *T. urticae* and compare the preference of the green lacewing larvae on the two pests under laboratory conditions. However, the predation efficiency of *Euborellia annulipes* on eggs *S. frugiperda* increasing and consumed 11.06, 49.02, 122.7, 148.09 and 374.9 eggs from the $1^{st}$ to $5^{th}$ instar, respectively. The predatory potential on the first instar of this pest increased during to their development, the highest devoured of $2^{nd}$ instar by *E. annulipes* occurred in the $4^{th}$ and $5^{th}$ in stars, which revealed daily consumption of 12.8 and 15.1 larvae, respectively. These finding are in line same with a previous study which revealed *Chrysoperla carnea* larvae feeding an average of 12.00, 25.00 and 46.50 larvae during 7.75 days of *Spodoptera littoralis* (Boraei *et al*., 2005). A previous study indicated that the feeding capacity for four stages larval of *Coccinella undecimpunctata* with 45.83 individual of *T. urticae* during 14 days (Farag, *et al*., 2022).

These results agreed with a previous finding which clarified that the clearest strong reaction was observed with homologous antigens and the reaction were negative with the other heterologous antigens (Boraei *et al*., 2005). Guedes *et al.* (2007) observed the tests with the gut concluded weak precipitation, probably because the consumption had direct suffered enzyme reaction at in this region. The fewness of reaction may be due to a dipped attraction of the antiserum to antigen. These results are in accordance with the findings of Khoder, (2005). In this study, it was observed that the predators were eating on aphids on the plant gave positive reaction when activity with antigen aphid.

Food selection and food preferences related to the availability of pest in the environment (Cruz, 2015). Asserting the favoring of this predator by aphid, it has been reported that the negative findings may not average that the predator did not feed the pest (Redoan *et al.*, 2016). It was also observed that *S. frugiperda* antigen when reacted with antiserum of *Lagria villos* gave a strong reaction and the digestive tract of the lygaeidae or antiserum used to assess the macerate of the whole insect. Accordingly, the obtained results indicated analogy the serological technique and predation adequacy of *C. undecumpanctata* and *Chrysoperla carnea* on some insect vermin of cotton plants. Conclusions, it is possible to prepare antiserum specific for *S. frugiperda and Chrysoperla carnea* to determine the relationship between prey and their predators. Serological technique in laboratory discovery of the feeding preference of *Chrysoperla carnea* by *S. frugiperda*, *P. solenopsis* and *T. urticae*. Regarding the type of prey, the precipitin test has the sensitivity to react positively to the antigen of the prey. Through the precipitin test, positive reaction observed after consuming predator of prey. Serological techniques, besides being a simple and inexpensive tool, are highly efficient in determining the relationship between pests and their predators. These predators as candidates for use in biological control programs for *S. frugiperda.*

**Conclusion:**

In the present study, it could be concluded that *Chrysoperla carnea* is a very voracious feeder on *S. frugiperda* and *T. urticae,* and the preference of the green lacewing larvae on the two pests under laboratory conditions is compared. However, the predation efficiency of *Euborellia annulipes* on eggs of *S. frugiperda* is increasing. In addition, it was observed that the predators that were eating aphids on the plant gave a positive reaction when interacting with the antigen aphid. So, it is possible to prepare antiserum specific for *S. frugiperda* and *Chrysoperla carnea* to determine the relationship between prey and their predators. Regarding the type of prey, the precipitin test has the sensitivity to react positively to the antigen of the prey. Through the precipitin test, a positive reaction was observed after consuming predator or prey. Serological techniques, besides being a simple and inexpensive tool, are highly efficient in determining the relationship between pests and their predators. These predators are candidates for use in biological control programs for *S. frugiperda*. This research can recommend the following:

- Use *Chrysoperla carnea* as a biological control agent for managing *Spodoptera frugiperda* and *Tetranychus urticae*.
- Integrate serological techniques (e.g., precipitin test) to monitor predator-prey relationships.
- Conduct field studies to validate lab findings and improve practical application.
- Promote sustainable pest management by reducing pesticide use and encouraging biological control.
- Develop mass-rearing programs for natural predators like *Chrysoperla carnea*.

## REFERENCES

Boraei, A.H. (1984). Ecological studies on the alfalfa weevil *phytonomus variablis* Herbst (Col: Curulionidea). Ph.D These Hungarian Academy of Sciences Bodapest Keszthely Hungary. 151 pp.

Boraei, A. H., Asmhan, E.Y., El-Kady, E.M., Farag, A. A. (2005). Serological studies on the relationship between some Egyptian clover insect pests and their predators. International Conference, plant prods. Res. Inst. 34-40.

Cassaro-Silva, M., Serrao, J. E., Sousa, S. C. R., Pacheco, J. M. (2001). Identificação de predators de *Orphulella punctata* (de Geer) (Orthoptera: Acrididae) através da serologia. Revista Brasileira de Zoologia, São Paulo 18(1): 75-79.

Cruz, I. (2015). Avancos e desafios no controle bologicocom predatores e parasitoides na cultura do milho In Seminario Nacional DE Milho Safrinha Maringa. Anais Maringa Univ Estadual de Maringa 13: 647-662.

Dempster, J. P. (1960). A quantitative study of the predators on the eggs and larvae of the broom beatle *Phytodecta olivacea* Forster, using the precipitin test. J Anim Ecol Oxford 29 (1):149-167.

El-Kordy, M.W.M., Gadallah, A., Metwally, M.M., Mohamed, H. A. (1999). The use of isoelectric focusing technique for determining patterns of predation in insects. Al- Azhar J. Agric. Res. 23(2):217-232.

Farag, A.A. Abd- Rahman, H.A., Refaei, A.E., El-Shamy, E.A. (2022). Effect of cannilbamlis phenomenon on some biological aspects and predation efficiency on *Tetranychus urticae* Koch of the ladybird beetle, *Coccinella undecimpunctata* Linnaeus. J. Entomol. Zoolo. Studies. 10(3)5-10.

Guedes, B.A.M., Zanuncio, J.C., Ramalho, F.S., Serrag, J.E. (2007). Midgut morphology and enzymes of the obligate zoophytophagous stinkbug *brontocoris tabidus* (singnoret (1863) (heteroptera ; Pentatomidae). Pan Pac Entomol. 83:66-74.

Khoder, I. A. (2005). Prey -predator relationship in maize field at Kafr El-Sheikh. J Agric Res Tanta Univ. 3 (2):259- 272.

Lund, R.D., TurpIn, F.T. (1977). Serological investigation of black cutworm consumption by ground beetles. Ibid. 70:322-324.

Ohiagu, V.C.E., Boreham, P.F.I. (1978). A simple field for evaluating insect prey-predator relationships. Ent Exp Appl. 23:40-47.

Petterson, T. (1975). Technical description of a serological methods for quantitative predator efficiency studies on *Rhopalosiphum padi* L Swadisg. J Agric Res. 2: 65-69.

Redoan, A.C.M., Silva, C.R., Cruz, I. (2016). Serological use in the identification of insect predators, *Revista brasileira* de Milho Sorgo. 15(2):157-170.

Silva, A.B., Batista, J.L., Brito, C.H. (2009). Capacidade predatória de *Euborellia annulipes* (Lucas, 1847) sobre *Spodoptera frugiperda* (Smith, 1797). Acta Scientiarum Agronomy Maringá. 31(1):7-11.

Sutton, S.T. (1970). Predation on woodlice an infestation using the precipitin test. Entomol Exp Appl. 13:279-285.

Salem, Sherehan A.R., Dahi, H.F., Mahmoud, Mervat A.B. (2021). Development of the fall armywom, *Spodoptera frugiperda* (J. E. Smith) on three Host plants. J.of plant protection and pathology, Manssoura Univ., 12(4):285-289.