



Asian Journal of Plant Sciences

ISSN 1682-3974

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

Effect of *Pseudomonas fluorescens* for the Management of Insecticide Resistant *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae)

P. Duraimurugan and A. Regupathy
Department of Agricultural Entomology,
Tamil Nadu Agricultural University, Coimbatore-641 003, India

Abstract: In the present study, the talc-based formulation of *Pseudomonas fluorescens* strain (Pfl) was tested against *H. armigera* in cotton, okra and pigeonpea. The susceptibility of *H. armigera* fed on *P. fluorescens* treated and untreated plants was bioassayed against cypermethrin on cotton bolls, okra fruits and pigeonpea pods. The susceptibility of third instar larvae of *H. armigera* to cypermethrin, fed on *P. fluorescens* treated plants did not differ significantly with the untreated plants. However, there was variation in the protein banding pattern among the *P. fluorescens* treated and untreated plants with or without infestation of *H. armigera*. Protein bands of molecular weight 83 and 40 kDa in cotton, 130 and 52 kDa in okra were observed in the *Pseudomonas* treated plants with infestation of *H. armigera*.

Key words: *Pseudomonas fluorescens*, *Helicoverpa armigera*, cotton, okra, pigeonpea

INTRODUCTION

Helicoverpa armigera Hubner has become India's number one agricultural pest^[1]. In India, it causes severe damage to a variety of crops like cotton, pigeonpea, okra, chickpea, tomato and sunflower. The damage by *H. armigera* is estimated at more than Rs 2000 crores (\$ 450 m) nationally with 15% decline in the cotton yield^[2]. More than 75% of the insecticides used in cotton are being targeted towards *H. armigera*^[3]. Of which, synthetic pyrethroids constitute 50-70%^[4]. This high selection pressure led to the development of resistance in *H. armigera*. The utilization of plant's own defense mechanism is the subject of current interest in the management of pests and diseases. Induced protection of plants against various pests and pathogen by biotic and abiotic inducers has been reported in many crops^[5]. Of these, the induced protection by selected strains of non-pathogenic, root-colonizing Plant Growth Promoting Rhizobacteria (PGPR) has been shown to be capable of inducing pest and disease resistance in addition to promoting plant growth. This phenomenon is commonly referred to as rhizobacteria mediated Induced Systemic Resistance (ISR). However, the reports on the use of PGPR for induced resistance against arthropod pests are limited. In the present study, a talc-based powder formulation was tested to assess the susceptibility of *H. armigera* to synthetic pyrethroids after feeding on inducing agent *P. fluorescens* treated plants.

MATERIALS AND METHODS

Susceptibility of *H. armigera* to synthetic pyrethroids after feeding on *P. fluorescens* treated plant sources: To assess the PGPR mediated induced systemic resistance against the susceptibility of *H. armigera* to synthetic pyrethroids after feeding on inducing agent treated plants, a pot culture study was undertaken by using completely randomized design with four replications. The talc-based formulation of PGPR, *Pseudomonas fluorescens* (Strain Pfl) containing 2.5 to 3.0×10^8 cfu g⁻¹ obtained from the Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, India was applied as seed treatment, soil application and foliar spray. The dry talc powder was mixed with the seeds of cotton, okra and pigeonpea at the rate of 20 g kg⁻¹ of seeds along with rice gruel at the rate of 50 mL kg⁻¹ of seeds^[6]. Five gram of talc-based formulation per pot was added 30 days after planting^[7]. The talc based product was dissolved in water (20 g L⁻¹) and allowed to settle for 1 h, filtered through muslin cloth and the filtrate was sprayed 30 days after planting^[8]. Untreated checks without bacterial treatment were also maintained. Foliar spray with water 30 days after planting was carried out in the untreated checks.

The different treatments on cotton, pigeonpea and okra are T₁-cotton treated with *P. fluorescens*, T₂-cotton untreated, T₃-okra treated with *P. fluorescens*, T₄-okra

untreated, T₃-pigeonpea treated with *P. fluorescens*, T₆-pigeonpea untreated. *H. armigera*, first instar larvae of *H. armigera* were released on to the bolls of cotton, pods of pigeonpea and fruits of okra and the introduced part of the plants were covered with polyethylene cover and allowed to feed. The larvae grown upto early third instar were bioassayed for susceptibility to cypermethrin by following the bouquet bioassay/foiar residue/terminal bud bioassay method^[9]. The LC₂₅ and LC₅₀ values of cypermethrin on cotton bolls, okra fruits and pigeonpea pods were determined through probit analysis^[10].

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis of crude protein of *Pseudomonas* treated plants against *H. armigera*:

The total protein was assessed at molecular level for the systemic leaves of *P. fluorescens* induced cotton, okra and pigeonpea plants with and without infestation of *H. armigera* and it was compared with the untreated plants. The leaf (with and without feeding of *H. armigera*) tissues were collected and immediately homogenized. One gram of powdered leaf samples was homogenized with 1 mL of 0.1 M sodium phosphate buffer (pH 7.0) under 4°C. The homogenate was centrifuged for 20 min at 10000 rpm. The supernatant was used for the SDS-PAGE^[11].

RESULTS AND DISCUSSION

Susceptibility of *H. armigera* to synthetic pyrethroid after feeding on *P. fluorescens* treated plant sources:

There was no significant difference in the susceptibility of *H. armigera* fed on *P. fluorescens* treated and untreated host plants as the LC₅₀ values were comparable (Table 1). The LC₅₀ value of cypermethrin for the *P. fluorescens* induced plants was 4.50, 4.07 and 5.28 µg/larva, while the normal plants registered a LC₅₀ of 4.75, 3.90 and 4.95 µg/larva on cotton bolls, okra fruits and pigeonpea pods, respectively.

In addition to direct antagonism and plant growth promotion, certain isolates of fluorescent pseudomonads, interestingly bring about induction of systemic resistance against infection by herbivores^[5,12]. Delivery of *P. fluorescens* through seed, soil, root or foliage leads to the reduction in the incidence of pests^[6,7]. But in the present investigation there was no significant difference in the susceptibility of *H. armigera* which fed on *P. fluorescens* treated and untreated plants as the LC₅₀ values for cypermethrin were comparable (Table 1). This is contrary to the reports of Murugan^[6], who observed that the lower level LC₅₀ value of the quinalphos, chlorpyrifos and profenophos to *H. armigera* on the jasmonic acid and *P. fluorescens* induced tomato plants compared to normal plants. Unlike the pathogens, *P. fluorescens* did not kill the insects through the host plant. Its application brings some physiological changes in host plants that prevent the insects from feeding^[13]. This may be the reason, why *Pseudomonas* induced plant infested with *H. armigera* did not vary with the concentration mortality response with the normal plants.

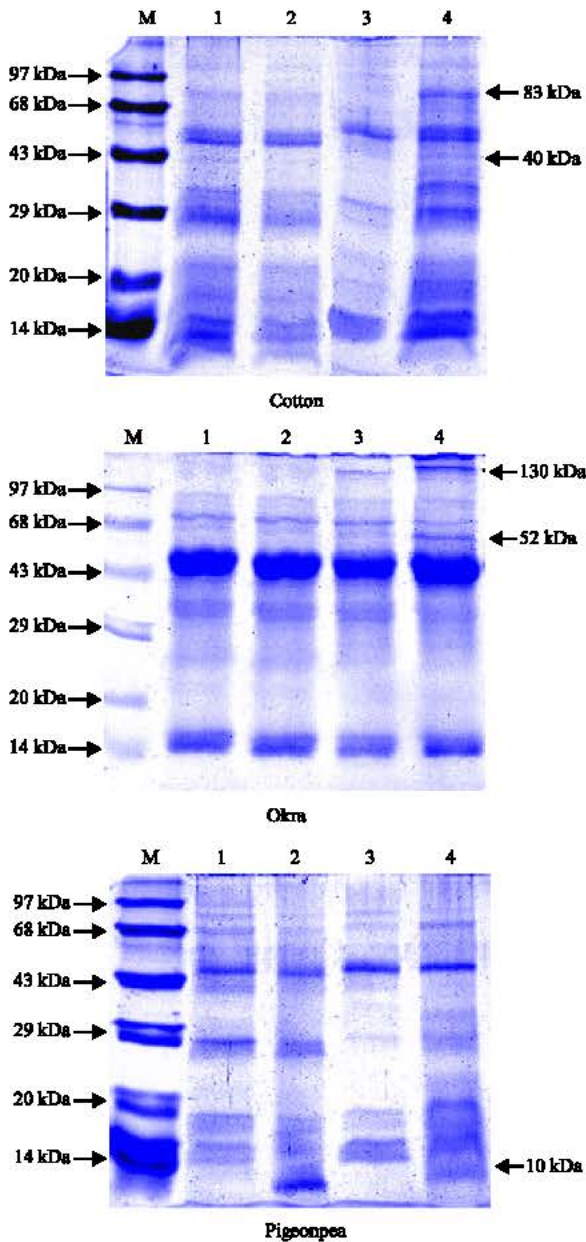
SDS-PAGE analysis of crude protein of *Pseudomonas* treated plants against *H. armigera*:

It was found that there was variation in the protein banding pattern among the *Pseudomonas* treated and untreated plants with or without infestation (Fig. 1). The intensity of bands were very high in *H. armigera* infested *Pseudomonas* treated plants, where as it was less in case of *Pseudomonas* untreated plants without infestation. *Pseudomonas* induced proteins with molecular weight of 83 and 40 kDa against *H. armigera* in cotton. In okra, it was noticed that protein with low relative mobility of 130 and 52 kDa with high intensity was induced. In pigeonpea, there was no induction of protein. However, 10 kDa protein was observed in *Pseudomonas* treated and untreated plants only after *H. armigera* infestation (Fig. 1).

Table 1: Susceptibility of *H. armigera* larvae to cypermethrin after feeding on *P. fluorescens* treated and untreated plants

Treatments	LC ₅₀ (µg/larva)	Friducial limits		LC ₉₅ (µg/larva)	Friducial limits		λ ²	Regression equation
		LL	UL		LL	UL		
<i>P. fluorescens</i> untreated plant								
Cotton bolls	4.75	4.02	5.66	19.85	13.50	41.17	1.025	-1.79 + 2.62 x
Okra fruits	3.90	3.11	4.71	21.04	13.40	53.84	0.903	-1.32 + 2.24 x
Pigeonpea pods	4.95	4.17	5.98	22.20	14.53	50.98	2.010	-1.75 + 2.52 x
<i>P. fluorescens</i> treated plant								
Cotton bolls	4.50	3.70	5.48	23.29	14.65	60.59	1.526	-1.50 + 2.30 x
Okra fruits	4.07	3.28	4.92	21.72	13.77	55.92	1.315	-1.38 + 2.26 x
Pigeonpea pods	5.28	4.38	6.65	28.12	16.80	84.14	1.535	-1.63 + 2.26 x

LL-Lower Limit; UL -Upper Limit



M: Medium range protein marker
 1: Untreated plant without infestation of *H. armigera*
 2: Untreated plant with infestation of *H. armigera*
 3: *P. fluorescens* pretreated plant without infestation of *H. armigera*
 4: *P. fluorescens* pretreated plant with infestation of *H. armigera*

Fig. 1: SDS-PAGE analysis of proteins induced by *H. armigera* in *P. fluorescens* pretreated cotton, okra and pigeonpea

The crude protein profile of *P. fluorescens* treated plants after infestation with *H. armigera* showed variation in the protein banding pattern compared to untreated plants with or without infestation. Radjacomare *et al.*^[7] reported that the *Cnaphalocrocis medinalis* infested *Pseudomonas* treated rice plants induced proteins with molecular weight of 41 kDa compared to the untreated plants. This is in agreement with the present investigation. However the induced proteins observed in the present study were not sufficient to make susceptibility in *H. armigera* against pyrethroids.

ACKNOWLEDGMENTS

The financial support from the Common Fund for Commodities (CFC) Europe, International Cotton Advisory Committee (ICAC) USA and Natural Resource Institute (NRI), UK is acknowledged.

REFERENCES

1. Regupathy, A., K. Kranthi, J. Singh, A. Iqbal, Y. Wu and D.A. Russell, 2004. Patterns and Magnitude of Insectivore Resistance Levels in India, Pakistan and China. In: J.V. Biljon and D. Joubert (Eds.) Proc. World Cotton Conference III, 9-13, March, Cape Town, South Africa, pp: 1215-29.
2. Regupathy, A., D.S. Rajavel, S. Rajkumar and D. Russel, 1999. Present status of insecticides resistance in *Helicoverpa armigera* and its management in Tamil Nadu, India. In: Proc. ICAC-CCRI Regional Consultation Insecticide Resistance Management in Cotton, June 28-July 1, Multan, Pakistan, pp: 48-55.
3. Banerjee, S.K., K.S. Turkar and R.R. Wanjari, 2000. Evaluation of newer insecticides for the control of bollworms in cotton. *Pestology*, 24: 14-16.
4. Jayaswal, A.P., 1989. Management of American bollworm on cotton in Andhra Pradesh. *Indian Farming*, 17: 6-7.
5. Zehnder, G.W., J.R. Murphy, E.J. Sikora, and J.W. Kloepper, 2001. Application of rhizobacteria for induced resistance. *European J. Plant Path.*, 107: 39-50.
6. Murugan, M., 2003. Role of induced resistance in the management of major insect pests of tomato (*Lycopersicon esculentum* Mill.) and okra (*Abelmoschus esculentus* (L.) Moonch). Ph.D. Thesis. Tamil Nadu Agricultural University, Coimbatore, India, pp: 230.

7. Radjacommar, R., R. Nandakumar, A. Kandan, S. Suresh, M. Bharathi, T. Raguchander and R. Samiyappan, 2002. *Pseudomonas fluorescens* based bio-formulation for the management of sheath blight and leaf folder insect in rice. Crop Protection, 21: 671-677.
8. Vidhyasekaran, P., P. Sethuraman, K. Rajappan and K. Vasumathi, 1997. Development of powder formulation of *Pseudomonas fluorescens* to control pigeonpea wilt. Biological Control, 8: 166-171.
9. Ramasubramanian, T. and A. Regupathy, 2003. Laboratory measured resistance and field control of *Helicoverpa armigera* (Hub.) by thiodicarb. Asian J. Plant Sci., 2: 1175-1178.
10. Finney, D.J., 1962. Probit Analysis. Cambridge University Press, Cambridge, pp: 318.
11. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
12. Pieterse, C.M.J., J.A. Van pelt, S.C.M. Vanwees, J.Ton, K.M. Leonkloosterziel, J.J.B. Keurentjes, B.W.M. Verhagen, M. Knoester, S. Vander, P.A.H.M. Bakker and L.C. Van Loon, 2001. Rhizobacteria-mediated induced systemic resistance, triggering, signaling and expression. European J. Plant Pathol., 107: 51-61.
13. Lewis, P.A, R.L. Lampaman and R.L. Metcalf, 1990. Kairomonal attractants for *Acalymma vittatum* (Coleoptera: Chrysomelidae). Environ. Entomol., 19: 8-14.