The benefits of antagonists in the suppression of disease symptoms has been widely reported by several authors (Wells *et al.*, 1972; Baker and Cook, 1974; Papavizas and Lumsden, 1980). The genus *Trichoderma* appears to include many species capable of parasitizing plant pathogenic fungi. Chet and Baker (1981) reported that *T. hamatum* conidial treatment reduced the incidence of damping off due to *R. solani* and *Pythium* in peas and radish, respectively.

Richard (1981) succeeded in his attempts in commercialising a *Trichoderma*-based mycofungicide for the control of seed borne disease. Elad *et al* (1982) observed that *T. harzianum* as seed treatment reduced *R. solani* infection in cotton and the method was widely used and found promising in Israel. Richard (1983) evolved the use of *T. viride* pellets for the control of dutch elm disease. The present studies also showed that the *Trichoderma* seed pelleting of cotton seeds reduced the seedling disease of cotton besides enhancing the germination rate of seeds.

Key words : *Trichoderma harzianum*, *T. viride*, cotton seed pelleting, *Rhizoctonia solani*

REFERENCES

Baker, K. F. and Cook, R. J. 1974. *Biological control of plant pathogens*. San Francisco, Freeman, 533 pp.

Chet, I. and Baker, R. 1981 Isolation and Biocontrol potential of *Trichoderma hamatum* from soil naturally suppressive to *Rhizoctonia solani*. *Phytopathol*, 71 286-290.

Elad, Y., Kalfon, A. and Chet, I. 1982. Control of *Rhizoctonia solani* in cotton by seed-coating with *Trichoderma* spores. *Plant soil*, 66, 279-282.

Papavizas, G. C. and Lumsden, R D. 1980. Biological control of soil borne fungal propagules. *Ann. Rev. Phytopathol.*, 18, 389-413.

Ramakrishnan, G. 1981. *Studies on seedling disease of cotton caused by Rhizoctonia solani* Kuhn. Ph. D. Thesis, Tamil Nadu Agric. Univ., Coimbatore, 129 pp.

Richard, J. L. 1981. Commercialization of a *Trichoderma*-based mycofungicide ; some problems and solutions. *Biocontrol News Inf.*, 2, 95-98.

Richard, J. L. 1983. Field observations on the biocontrol of Dutch elm disease with *Trichoderma viride* pellets. *Eur. J. For Pathol*, 13, 60-62.

Sivan, A., Elad, Y. and Chet, I. 1984. Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. *Phytopathol.*, 74, 498 501.

Wells, H. D., Bell, D. K. and Jaworski, C. A. 1972. Efficacy of *Trichoderma harzianum* as a biocontrol for *Sclerotium rolfsii*. *Phytopathol.*, 62, 442 447.

J Biol. Control, 1 ( ), 67-70, 1987

Serological Characterisation of Nuclear Polyhedrosis Virus of *Spodoptera litura*

K. NARAYANAN

Indian Institute of Horticultural Research, Bangalore, Karnataka 560 089

With increasing attention being given to the possible use of NPV of *Spodoptera litura* in India for biological control (Jayaraj *et al.*, 1979; Ramakrishnan *et al.*, 1981), more sensitive, specific and quantitative serological methods are required to detect and monitor viruses *in vivo* and in the physical environment Hence, this study was undertaken to determine the

serological properties of NPV of *S. litura* and their relationship to other baculoviruses employing some of the serological tests like agglutination (A) haemagglutination (HA), haemagglutination inhibition (HI), and agar gel diffusion

The polyhedral antigen was prepared as follows. NPV infected *S. litura* larvae stored at 5 C were triturated using a solution containing 0.14 M sodium chloride, 0.0115 M sodium citrate, and 0.001 M phenyl-2-thiourea. The triturate was filtered through several layers of muslin cloth. Polyhedral inclusion bodies thus obtained were purified by repeated differential centrifugation and saline water washes, freeze dried and stored at 5° C and used as needed in the study. Virions and/or soluble polyhedral protein antigens were obtained from purified polyhedral occlusion bodies (POB) (50 mg) subjected to 30 minutes of solubilization with 6 ml of 0.05 M sodium carbonate and 4 ml of 0.05 M sodium chloride and by differential centrifugation (Shapiro and Ignoffo, 1970). Antiserum to intact polyhedra was prepared in rabbits by subcutaneous injections at 1 ml and 2 ml at an interval of 14 days. The total quantity of virion and soluble polyhedral protein antigen injected into each rabbit was 3.11 mg and 16.66 mg respectively. The animals were bled at weekly intervals

Two-fold serial dilutions of antiserum were made in saline and the agglutination test was performed following the method of Tanada (1954) using perspex plate. For performing haemagglutination (HA) test, pooled fresh blood from several chicks were drawn into Alsever's solution, centrifuged, and the pellet of red blood cells (RBCs) were washed thrice with phosphate buffer and finally 0.5% suspensions of RBCs were prepared in normal saline. Two-fold serial dilutions of polyhedral suspensions were made in saline in perspex plate and the test was performed following the method of Clarke and Covals (1968). The highest dilution of polyhedral suspension producing 100% (HA) was considered one HA unit. In the case of doubtful results, the perspex plates were incubated at 5°C overnight and observed for HA the next day. The normal saline and erythrocytes suspensions were kept as controls.

For conducting haemagglutination inhibition, the polyhedral occlusion bodies were diluted in saline solution to contain 4 HA units and the test was performed following the method of Clarke and Covals (1968). For gel diffusion test, 1% agar containing 8% saline and preservative, i.e merthiolate (1:1000), was poured on a clear petri dish to a thickness of 8 to 10 mm and allowed to air dry. Wells were made after removing the agar. The centre well was filled with antiserum and the side wells were filled with appropriate antigen (Table 1) and the test was performed as per Shapiro and Ignoffo (1970).

It is evident from the results that the antibody titre in the case of insoluble antigen like POB of *S. litura* by means of agglutination was found to be 1:160 (Table 1). Similar agglutination property has been reported by Tanada (1954) in the case of *Pieris rapae* L. polyhedral virus.

Table 1. Results of some of the serological responses of nuclear polyhedrosis virus of *Spodoptera litura*

Test performed	Response	Titre
1) Agglutination	+	1: 160
2) Haemagglutination	+	1: 80*
3) Haemagglutination inhibition	+	1: 320
4) Gel diffusion		
Antigen		
i) Polyhedra of NPV of *S. litura*	+	
ii) Capsule of GV of *S. litura*	—	
iii) NPV-diseased larval extract of *S. litura*	+	
iv) Healthy larval extract of *S. litura*	—	
v) Polyhedra of NPV of *C. cephalonica*	—	
vi) Polyhedra of NPV of *S. litura* containing a drop of alkali	+	

(* Data extracted from Narayanan, 1985)

Though Narayanan (1985) had earlier reported the haemagglutinating property of POB of NPV of *S. litura*, it is evident from the present results that specific antibodies produced against intact polyhedra of NPV of *S. litura* inhibited the haemagglutinating property at a titre of 1:320. Similar haemagglutination inhibition properties have been reported in the case of NPVs of *H. zea* (Shapiro and Ignoffo, 1970)

The specificity of the serological responses by means of gel diffusion test is also summarised in Table 1. It is evident from the absence of cross reaction between polyhedra of NPV of *S. litura* and capsules of GV of *S. litura* as well as NPV of *Corcyra cephalonica* Stainton another heterologous virus, that the antigenic character of polyhedral protein is determined by its virus. In this connection it is worth to mention that Longworth *et al.* (1972) have reported the existence of two proteins in the occlusion bodies of GV of *Pieris brassicae* L. Protein A was found in occlusion body protein and protein B at the surface of the occlusion body and the enveloped virus particle. The positive cross reaction noted in the well containing alkali solubilized POB, may be due to antigen arising from the partial degradation of polyhedra during sodium carbonate treatment. NPV of *S litura* recorded in India was found to be serologically similar to that of *Spodoptera* sp. found in New Zealand in tests in immuno-osmophoresis test with antisera prepared against polyhedral protein (Scotti, P.D., personal communication). It has been found by Krywienczyk and Bergold (1961) that the virus and its polyhedral protein are only weakly if at all serologically related, and that the relationship could be due to mutual contamination. They also demonstrated that the polyhedral protein antigen system was a very complex one.

ACKNOWLEDGEMENTS

The author is very grateful to Mr. Nandakishore, then Technical assistant and to the staff members of Department of Veterinary Microbiology, University of Agricultural Sciences, Bangalore for their help in the conduct of the study. He is also grateful to Dr. K. L. Chadha, then Director, for the facilities provided

Key words : NPV, *Spodoptera litura*, agglutination, haemagglutination inhibition, gel diffusion.

REFERENCES

Clarke, D. H. and Covals, J. 1968. Technique for haemagglutination and haemagglutination inhibition with arthropod borne viruse . *J. Trop. Med. Hyg.*, 7, 551-573.

Jayaraj. S., Santharam, G., Narayanan, K., Soundarajan, K. and Balakurunathan, K. 1979. Effectiveness of the nuclear polyhedral virus against field populations of the tobacco caterpillar, *Spodoptera litura* on cotton. *Andhra agric. J.*, 27, 26-29.

Krywienczyk, J. and Bergold, G. H. 1961. Serological studies of inclusion body proteins by agar diffusion technique. *J. Insect Pathol.*, 3, 15-28.

Longworth, J. F., Robertson, T. S and Payne, C. C. 1972. The purification and properties of inclusion body protein of the granulosis virus of *Pieris brassicae*. *J. Invertebr. Pathol.*, 19, 42-50

Narayanan, K. 1985. Haemagglutination by certain Baculoviruses. *Curr. Sci.*, 54, 816-817.

Ramakrishnan, N., Chaudhari, K. S., Kumar, S., Rao, R. S. N. and Satyanarayanan, S. V. 1981. Field efficacy of nuclear polyhedrosis virus against the tobacco caterpillar, *Spodoptera litura*. *Tob. Res.*, 7, 129 134.

Shapiro, M. and Ignoffo, C. M. 1970. Haemagglutination by a Nucleo polyhedrosis virus of the cotton bollworm *Heliothis zea*. *Virology*, 41. 577-579.

Tanada, Y. 1954. A polyhedrosis virus of the imported cabbage worm and its relation to a polyhedrosis virus of the Alfalfa caterpillar *Ann. Entomol. Soc. Am.*, 47, 553-747.

J. Biol. Control, 1 (1), 70-71, 1987

Biological Control of Early Moth Borers of Sugarcane by *Trichogramma* in North Bihar

M. P. MISRA and A. D. PAWAR
Central Biological Control Station, Gorakhpur, Uttar Pradesh 273 001

Under the North Bihar conditions, species of moth borers, viz., *Chilo infuscatellus* Snellen *Sesamia inferens* Walker and *Raphimetopus ablutella* Zeller, occur as shoot borers of sugarcane. However, *C infuscatellus* outnumbers the other two (Misra *et al.*, 1986). In addition, *Scirpophaga excerptalis* Walker appears soon after the shoot stage of the crop and continues to cause substantial damage until July-August each year.

Use of *Trichogramma* spp. in sugarcane ecosystem has been demonstrated by Sithanantham (1980) and Tuhan and Pawar (1983) in Tamil Nadu and Punjab, respectively. At the Harinagar farm, biocontrol trials commenced in 1980 against *C. infuscatellus* (Misra *et al.*, 1984). Based on the encouraging results in a four acre field, a *Trichogramma* breeding labaratory was commissioned in June, 1984 for mass breeding of the host