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REVIEW ARTICLE

CONTROL OF THE COCONUT PEST *ORYCTES RHINOCEROS* L. USING THE *ORYCTES* VIRUS

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Abstract—The coconut palm is an important plantation crop in India, where it is cultivated on 1.796 million hectares. The rhinoceros beetle, *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae) is a serious pest of coconut throughout India and southeast Asia, causing an estimated 10% yield loss in the crop. Successful biological control of this pest could be achieved using the non-occluded *Oryctes* virus (syn. *Baculovirus oryctes* or *Oryctes baculovirus*). This review provides an account of this microbial agent, its biology, effects and impact, production and maintenance and alternative hosts, particularly in the context of the Indian situation. It also proposes future areas for investigation on the virus, in order to achieve its commercial viability and more widespread use.

Key words: coconut palm, *Oryctes rhinoceros*, *Oryctes* virus, microbial control, India

Resumé—Le palmier cocotier est une importante culture de plantation en Inde, où il est cultivé sur 1,796 millions d'hectares. Le scarabée rhinocéros, *Oryctes rhinoceros* L. (Coleoptera : Scarabaeidae) est un sérieux ravageur du cocotier en Inde et en Asie du Sud Est, responsable de pertes de récolte estimées à 10%. Un contrôle biologique efficace de ce ravageur pourrait être obtenu en utilisant le virion non inclus du virus d'*Oryctes* (syn. *Baculovirus oryctes* ou baculovirus de l'*Oryctes*). Cette revue bibliographique fait le point sur cet agent microbien, sa biologie, son efficacité et son impact, sa production et sa maintenance ainsi que sur ses hôtes alternatifs, en particulier dans le contexte de l'Inde. Elle propose également de futurs domaines de recherches sur ce virus, afin d'aboutir à sa viabilité commerciale et à une plus grande utilisation.

Mots Clés: palmier cocotier, *Oryctes rhinoceros*, le virus de l'*Oryctes*, Control microbien, Inde

INTRODUCTION

Of the total of 547 insect and mite species recorded on the coconut palm (Kurian et al., 1979), the rhinoceros beetle, *Oryctes rhinoceros* L., is among the major pests that cause serious damage to palms of all ages. Its adults bore

into the growing spear leaf cluster, spathes and petioles and chew off the soft internal tissue, which is pushed out as fibres through the holes. When open, the fronds show geometric V-shaped cuts caused by the feeding of the beetle. A yield reduction of about 10% occurs due to the drying of the inflorescence of the injured spathes (Nair, 1986) accompanied by indirect loss caused by reduction in the photosynthetic activity and weakening of petioles. The holes made by the

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beetle also serve as entry points for another important pest, the red palm weevil (*Rhynchophorus ferrugineus*), and host fungal pathogens. A method to assess the beetle's damage has been developed by Ramachandran et al. (1963) and Zelazny (1979).

Oryctes rhinoceros is stout and black in colour, 35–50 mm in length, 14–21 mm in breadth and has a cephalic horn, which is longer in males. Female beetles have dense reddish brown hairs on their pygidium. Eggs are laid in decaying organic matter, such as farmyard manure, cowdung heaps, dry and dead lodged palm trunks, decaying palm stumps and compost pits so that on hatching, grubs get plenty of food (Abraham, 1994). Recently, it has been observed that coir pith, accumulating as waste from local coir industries in Kerala, India, is a favoured breeding site of the pest (Gopal and Sathiamma, 2000). In addition to coir pith, vermicompost also harbours this pest (unpublished data). The life cycle of *O. rhinoceros* is completed in an average of 171 days (Nirula, 1955).

In 1963, an investigation of the diseases of *O. rhinoceros* was conducted in Malaysia (prev. Malaya), Fiji and Western Samoa. This survey resulted in the discovery of a viral pathogen of the pest, namely *Baculovirus oryctes* (syn. *Rhabdionvirus oryctes*) (Huger, 1966), whose infection was locally called The Malaya Disease. The disease was later reported in the Philippines, Indonesia, Sumatra Island, Mauritius and West Kalimantan. Zelazny (1981) reported the presence of this virus in India, and Mohan et al. (1983) reported on its incidence (54 %) in a natural population of rhinoceros beetles sampled from different locations in Kerala, India. The pathogen was later used successfully in many South Pacific and Indian Ocean Islands (Caltagirone, 1981).

This review discusses the work done on this pathogen in various coconut-growing countries, with particular emphasis on India.

THE VIRUS

In 1966, Huger (1966) placed the *Oryctes* virus in a new genus and named it *Rhabdionvirus oryctes*. Further studies on the virus structure strengthened its inclusion under *Baculoviridae* and it was named *Baculovirus oryctes* (Payne, 1974; David, 1975). Franckii et al. (1991) put the occluded NPVs and GVs of *Baculoviridae* under the sub-group *Eubaculovirinae* and non-occluded

ones like *Baculovirus oryctes* under *Nudibaculovirinae*. However, this pathogen has recently been removed from the *Baculoviridae* and placed under its own virus category—*Oryctes* (Evans and Shapiro, 1997).

Oryctes viruses consist of a rod-shaped nucleocapsid (220–240 × 80–110 nm) surrounded by an envelope (Mohan and Gopinathan, 1989b). The genome is a double-stranded super coiled DNA molecule of Mr 60–92 × 10⁶ Daltons (Monsarrat et al., 1973a; Revet and Monsarrat, 1974; Payne et al., 1977). Detailed research on purification of the virus (Monsarrat et al., 1973 b; Payne, 1974; Zelazny et al., 1985) and the comparison of genomic DNA of various virus isolates using restriction endonucleases (Crawford et al., 1985) has been reported. The genomic DNA of the Indian isolate of the *Oryctes* virus was found to have a melting temperature of 71.8 °C (in 0.1 × SSC) corresponding to 49.92 % G + C content. This isolate also revealed an antigenic property different from the Philippines isolate PV 505 (Mohan and Gopinathan, 1989a) and comprised of a total of 48 viral proteins as against the 39 of the PV 505 (Mohan and Gopinathan, 1989b). Based on physical mapping of its genome (Mohan and Gopinathan, 1991) and the restriction patterns after endonuclease digestion, the taxonomic status of the Indian isolate (OBV-KI) was proposed as a variant of the *Oryctes* virus (Mohan and Gopinathan, 1992).

Site of infection

The *Oryctes* virus, like other viruses, is able to gain entry into the host through ingestion of contaminated food. After gaining entry, it reaches the nuclei of midgut epithelial cells of larvae and adults (Payne, 1974), cells of larval fat bodies, the haemocytes, and the testicular and ovarian cells (Monsarrat et al., 1973a; Majumder and Jacob, 1993), where it replicates. In the 1st to 4th hour post-infection period, virus adsorption into the plasma membrane and uptake in cytoplasmic vesicles occur. Seven to 12 hours post-infection, viral replication in the clear area of the hypertrophied nucleus occurs, and finally, at 16 hours or more, virus release from the plasma membrane takes place (Crawford and Sheehan, 1985).

Symptoms of the *Oryctes* virus disease

Both the grub and the adult stages of *O. rhinoceros* are infected by the *Oryctes* virus.

Effect on grubs

Infected grubs become lethargic and consume less food from the 2nd day of infection, in all three instars. Their consumption index, growth rate and efficiency of conversion of digested and ingested food is reduced (Paulose et al., 1997). They finally stop feeding and come to the surface of the feed; the abdomen becomes turgid and glassy with chalky white spots. As the virus multiplies, the fat body disintegrates and the haemolymph content increases. The midgut also becomes devoid of food and gets filled with white mucoid fluid which causes translucency of the dorsal midgut line when seen against a bright light (Huger, 1966). The increase in turgor pressure in the abdomen sometimes leads to extrusion of the rectum. Infected grubs die within 15–20 days, and do not pupate. The period of lethal infection depends on the larval stage and surrounding temperature, occurring faster at high temperatures of about 32 °C (Zelazny, 1972).

The median periods of lethal infection for the 1st-, 2nd- and 3rd-instar grubs were observed to be 8.5 ± 0.2 , 12.6 ± 1.0 and 22.5 ± 2.8 days respectively (Zelazny, 1972). However, the order of susceptibility among the three larval stages was reported to be I > III > II (Mohan et al., 1985 a). On dissection of the infected grub, a swollen midgut filled with a white fluid is seen. In addition, the infection causes changes in the haemocyte count, particularly that of granular cells and plasmocytes (Vincent et al., 1988), protein, amino acid and sugar content of the host (Biju et al., 1993).

Healthy grubs on the other hand are active, feed vigorously, remain beneath the feed and show a clear dark midgut line on their dorsal surface. The midguts of healthy grubs are usually filled with black feed.

Effect on adults

Adults infected with the *Oryctes* virus also become lethargic and stop feeding. Their midgut is filled with a white mucoid fluid (healthy beetles have a very thin, brown midgut containing very little clear brownish fluid). The longevity of infected adults is reduced to 25 days instead of the normal 75 and fecundity of the females to one or two eggs instead of 15 in healthy ones (Zelazny, 1973a). Infected beetles also excrete up to 0.3 mg of virus-contaminated faeces a day, and thus spread the pathogen in nature (Monsarrat and Veyrunes, 1976).

Diagnosis of the *Oryctes* virus disease

The presence of the virus in an infected host can be detected by the following methods.

Smears

Air-dried smears of midgut fluid and midgut epithelial cells fixed in methanol for 2–5 min, stained in 3% Giemsa stain for 45–60 min and finally rinsed in distilled water (Zelazny, 1973b) show large clumps of cells with purple-stained hypertrophied nuclei (18–28 μ m) and sparse blue cytoplasm. Also in many cases, a homogeneously stained deep pink circular band along the periphery of the nucleus is seen. In contrast, the number of free cells in the smears of healthy midguts is significantly lower, with small (7.5 – 12.5 μ m), pink nuclei (Mohan et al., 1983). This diagnostic method is reliable, rapid and economical.

Immuno-osmophoresis (IOP)

This technique is used as a rapid method for the detection of the virus. A slight modification of the procedure of John (1965) is followed. A volume of 4.5 ml of 0.8 % warm 'oxoid' agar in 0.045 M phosphate buffer (pH 7.4), containing 0.1 % sodium azide is pipetted onto a Formvar 15/95 E (0.2 % w/v in chloroform) coated microscope slide. To set the agar film, the slide is kept overnight in a moisture chamber under refrigeration. Using a gel cutter, pairs of small wells (2 mm diam.) are cut 1 cm apart in 2 or 3 vertical rows. The vertical rows of the wells on the cathodic end constitute the antigen wells and the ones near the anodic end form the antiserum wells. The antigen wells are filled with the virus-infected midgut aspirates, and antiserum obtained from rabbit inoculated with *Oryctes* virus is placed in the opposite wells. Both the midgut aspirates of the healthy grubs/ beetles and the normal rabbit serum serve as controls. The IOP is carried out for 70 minutes with a current of 12–15 mA and voltage of 10 V/cm. Phosphate buffer (0.045 M, pH 7.4) used for agar gel is also used as an electrolyte in the buffer tank. A precipitin line of growing intensity with time is noticed after the run. However, maximum intensity is obtained after 30 minutes incubation at room temperature (Mohan and Pillai, 1983).

An enzyme linked immunosorbent assay (ELISA) for detecting the *Oryctes* virus has also been reported (Longworth and Carey, 1980; Mohan and Gopinathan, 1989a; Rajamannar and Indiravathi, 2000).

Electron microscopy (EM)

One millimetre-thick midgut slices are fixed in 2 % glutaraldehyde in 0.2 M, pH 7.2 phosphate buffer and stained with 1% osmium tetroxide and finally embedded in Epon-araldite. When examined under an electron microscope, bacilliform virus particles of 220–240 × 80–110 nm, with each nucleocapsid enveloped in distinct membrane, are observed (Mohan et al., 1983; Mohan and Gopinathan, 1992).

Examination of beetle excreta

Examination of beetle excreta is used to detect virus infection in beetles (Monsarrat and Veyrunes, 1976). Virus-infected beetles are kept in plastic containers with 5 ml of phosphate buffer saline (PBS) (0.01 M, pH 7.0, NaCl 0.85 %), just enough to be level with the distal end of the abdomen of the beetles. The buffer contains streptomycin (250 mg/l), penicillin (200 mg/l) and oxytetracycline (100 mg/l) to prevent bacterial contamination. The faecal matter collected is centrifuged at 500 rpm for 10 minutes. The sediment is resuspended in 0.2 ml PBS and examined with 3 % Giemsa staining (Mohan et al., 1985 b).

The *Oryctes* virus disease can be confirmed by conducting a laboratory bioassay wherein the midgut of a diseased beetle or grub is homogenised in a minimum volume of phosphate buffer (0.001 M, pH 8.5) containing antibiotics (streptomycin, 0.5 g/l; aureomycin, 0.3 g/l; chloramphenicol, 0.3 g/l) and clarified by centrifugation. This homogenate is fed orally to healthy grubs (5 per container) using a syringe and these are maintained in moist autoclaved cow dung or a cow dung-sawdust mixture (2:1 w/w). They are then observed for the appearance of characteristic translucency of the thoracic region and death due to virus infection, which usually occurs within five weeks. The control samples remain alive during this period. If even one inoculated grub in a test group exhibits typical disease symptoms, the bioassay is considered to be positive (Zelazny, 1978; Mohan et al., 1983).

PRODUCTION, MAINTENANCE AND STORAGE OF THE VIRAL INOCULUM

Production and maintenance of the *Oryctes* virus can be done on an *O. rhinoceros* cell culture (Quiot et al., 1973), in *Spodoptera frugiperda*, and in *Aedes albopictus* cell lines (Kelly, 1976). However, the latter two have been disputed (Crawford, 1981). Cell lines derived from *Heteronychus arator* F. support the replication of this virus (Crawford, 1982; Crawford and Sheehan, 1985).

The virus is also produced and maintained by propagation in live grubs/beetles of *O. rhinoceros*. Eight virus-killed grubs are homogenised in a blender and mixed with sterilised sawdust, cow dung or cow dung-sawdust mixture in a plastic box (61 × 27 × 41 cm). One hundred to 150 healthy grubs are released into the box and allowed to feed for 5–7 days, then transferred to a similar box with sterilised feed until death (Bedford, 1976). An alternative feed in the form of coir pith/ waste has been used and it was established that sterilised coir waste serves as a better feed than cow dung because of its lower bacterial contamination (Gopal and Sathiamma, 2000). Alternatively, the virus-infected midgut of the beetle/ grub can be excised, homogenised in phosphate buffer saline (pH 7.0) and fed to healthy grubs or beetles which are then maintained in sterilised feed as described earlier.

A production method currently in use by the Philippines Coconut Authority is described. The midguts of 5 beetles inoculated with the virus are homogenised on ice, suspended in 2 ml buffer (50 mM Tris-HCl, pH 8.5, 100 mM NaCl, 1 mM EDTA and 10 mM Na₂SO₃), centrifuged for 10 min at 5000 × g and loaded on a 15-mm-wide chromatography column packed with controlled-pore glass (Sigma, 700 A pore size, 120–200 mesh size) to a height of 80 cm. The virus suspension is eluted out from the column with 50 mM Tris-HCl buffer. The first clear 60 ml are discarded and the next 20 ml of turbid eluate containing the virus are collected. Sucrose is added to achieve a concentration of 10% (w/v). This is filtered through 0.22 mm Millipore membrane filter and dispensed in 2 ml lots of sterile serum vials. This virus suspension can be stored at room temperature for several weeks (Zelazny et al., 1987).

Virus-packed cadavers can be stored indefinitely at -40 °C (Bedford, 1976). The virus as ground cadavers has reduced activity and is

inactivated within 2 weeks under ambient conditions. The virus-containing cell culture fluid and the sterile virus filtrate retain infectivity for up to 20 weeks when refrigerated or for up to two weeks when stored at room temperature under tropical conditions (Zelazny et al., 1987). The purified virus suspension is inactivated by heating at 70 °C for 10 min, or by adding a 1% solution of formaldehyde or Dettol™ (Zelazny, 1972).

TRANSMISSION OF THE VIRUS

The *Oryctes* virus is perpetuated in nature by infected hosts. It is transmitted mostly during mating, when healthy individuals come into oral contact with the virus defecated by infected beetles, or when healthy and infected beetles feed together. The beetles also pass infection to healthy grubs when they visit breeding sites (Zelazny, 1976). This mode of transmission is supported by a mathematical model where rates of 6 different transmission pathways of the *Oryctes* virus were estimated and transmission from infected to susceptible feeding adults was proved to be the dominant route (Hochberg and Waage, 1991). Infected beetles excrete virus-contaminated faecal matter from the 3rd and the 9th day after infection into the surroundings (Mohan et al., 1985b) and the rate of spread of the virus in the field is estimated to be about 1 km /month (Jacob, 1996).

No virus infection occurs in grubs hatching from eggs that are surface-contaminated with the virus or laid by virus-infected females (Zelazny, 1976), but the observations of Monsarrat et al. (1974) indicate transovarial transmission of the *Oryctes* virus.

APPLICATION OF THE VIRUS

A practical method of virus dissemination is through the release of virus-infected adults (Bedford, 1981). An inoculum is prepared by homogenising 1 g infected larval tissue in 1 litre of phosphate buffer (0.05 M, pH 8.0) and 3% sucrose, which amounts to 31.6 LD₅₀ dose (Mohan et al., 1989). Healthy beetles collected from the field are allowed to wade through virus inoculum contained in a basin for 30 min. The beetles can also be inoculated with the sterile viral filtrate prepared as described by Zelazny et al. (1987). After this swim treatment beetles are confined together for 12 to 24 hrs, and released after dusk

at the rate of 15 inoculated beetles per hectare (Mohan et al., 1989). This method eliminates the disadvantages of other methods of application, where a compost heap or a split coconut log heap is prepared artificially and inoculated with virus-killed larval tissues (Bedford, 1981).

The infected beetles disperse widely before death, spreading the disease directly into the wild population, and contaminating breeding sites which may contain larval broods and other beetles, including the palm crowns (Zelazny, 1976).

OTHER HOSTS OF THE VIRUS

Several of related agricultural pests have been found to be variably susceptible to the *Oryctes* virus. These include *Oryctes nasicornis*, *O. monoceros* and *O. boas* (Huger, 1966; Julia and Mariau, 1976 and Purrini, 1989), *Scapanes australis grossipunctatus* (Bedford, 1973), *Papuana uninodis* (Zelazny et al., 1988) and *Xylotrupes gideon* (Dangar et al., 1994). *Sternonchetus mangiferae*, a curculionid pest of mango nut, has been described to be infected with a virus and showed symptoms similar to these of *Oryctes* virus infection (Shukla et al., 1984). On the other hand, contradictory observations have been reported in the case of pathogenicity towards the cashew stem and root borer by the *Oryctes* virus (Bakthavatsalam and Sundararaju, 1990).

SAFETY TEST

In a safety test conducted in France, no pathogenicity of the *Oryctes* virus was observed on eight tissues: two human and two pig cell cultures, and one each from mouse, hamster, fish and calf. Similar results were obtained in living inoculated mice and in organs of mice up to 60 days after inoculation (Anonymous, 1973; Gourreau et al., 1979; 1982). There are no reports of the *Oryctes* virus being infective to the natural enemies of its homologous pest.

IMPACT OF THE VIRUS

The impact of the virus can be assessed directly by observing the fresh leaf, spathe or inflorescence damage on coconut palms, trapping of the beetles and diagnosing for virus disease and also by

recording the population of the host at breeding sites (site occupancy test). The results can be meaningful only when the above parameters are recorded before the release of the virus. Detailed methods of survey have been described by Young (1974), Bedford (1976), Mohan et al., (1983, 1989) and Mohan and Pillai (1993).

A drastic reduction in beetle population and crop damage has been reported in the South Pacific Islands, Fiji, Mauritius, Seychelles and Papua New Guinea where the *Oryctes* virus was released specifically to suppress the pest population (Marschall, 1970; Hammes, 1971; Young, 1974; Bedford, 1976; Monty, 1978; Gorick, 1980; Lomer, 1986). The results of successful control in several localities in India, namely Minicoy Island, Lakshadweep (Mohan et al., 1989); Androth Island, Lakshadweep (G. B. Pillai, pers. commun.); Chittilappily, Thrissur (Biju Babjan et al., 1995); and in Andaman Islands, (Jacob, 1996) are summarised in the Tables 1, 2, 3 and 4 respectively. In oil palm plantations of Kerala the virus has shown good microbial control of the rhinoceros beetle (Dhileepan, 1994).

Table 1. Effect of *Oryctes rhinoceros* on crop damage following the introduction of *Oryctes* virus in Minicoy, India (Mohan et al., 1989)

Period of survey	% Crop damage		
	Leaf	Spathe	Spindle
<i>Pre-release</i>			
Apr. 1983	55.83	25.90	29.56
<i>Post-release</i>			
Jan. 1984	45.43	8.86	8.27
Nov. 1984	25.57	1.95	1.84
Sept. 1985	12.89	1.61	2.90
CD 5 %	5.40	6.14	9.72
CV (%)	9.36	16.73	31.32
SEM	1.75	1.88	3.16

Table 2. Performance of the *Oryctes* virus in Androth Island of Lakshadweep, India (G.B. Pillai, pers. commun.)

Period of survey	% Crop damage			<i>Oryctes</i> virus incidence (%)
	Leaf	Spathe	Fresh Spindle	
<i>Pre-release</i>				
Apr. 1988	55.0	7.3	3.5	00.0
<i>Post-release</i>				
Dec. 1988	43.0	3.0	15.7	35.6
Jan. 1990	13.5	3.2	5.9	60.6

Table 3. Effect of re-release of the *Oryctes* virus in an already infected contiguous area at Chittilappily, Thrissur, India (Biju Babjan et al., 1995)

Period of survey	% Damage			
	Palms	Leaf	Fresh Spathe	Spindle
<i>Pre-release</i>				
Jul. 1989	100	34.4	12.5	68.18
<i>Post-release</i>				
Feb. 1990	64.29	23.76	0.00	50.00
Jul. 1990	64.71	27.07	0.00	17.65
Mar. 1991	89.47	33.96	6.52	0.00
Aug. 1992	22.73	6.66	0.00	0.00

Table 4. Percentage of the *Oryctes* virus infection in beetle population at Sipighat, Andamans, India (Jacob, 1996)

Observations	% Virus infection
<i>Pre-release</i>	
Mar. 1987	0 % (n = 81)
<i>Post-release</i>	
Dec. 1987	53 % (n = 53)
Dec. 1988	77 % (n = 17)
1989–1991	61 % (n = 18)

n = number of beetles

FURTHER AREAS FOR INVESTIGATION

Although detailed investigations have been carried out on some aspects of the *Oryctes* virus, several areas remain to be researched in depth. The development of recombinant *Oryctes* viruses having quicker speed of kill, as has been done with the baculoviruses of lepidopteran pests (Bonning and Hammock, 1996), would improve its efficiency and adoption by the farmers. Traps that act as attractants for the pest and which contain microbial control agents (Klein and Lacey, 1999) might be explored for *Oryctes* virus delivery. Studies on storage of the virus using, for instance, preservatives that enable the virus to retain its viability/ infectivity for longer periods at ambient conditions, and the formulation of commercial products, are also needed.

Ecological studies of the pathogens of *O. rhinoceros* in relation to biotic and abiotic factors need to be strengthened, in order to regulate their application for better control of the pest. Habitat management by retaining dead standing coconut palms (up to 5 per ha.) could improve virus transmission (Zelazny and Alfiler, 1986; Zelazny et al., 1992).

Work in the Philippines has indicated a significant positive correlation between virus incidence and rainfall, and a marginal negative correlation with temperature (Villacarlos and Betonio, 1990). Investigations currently being carried out in Kerala, India indicate a low incidence of this viral agent in nature, at least during certain seasons, and the presence of an opportunistic bacterial pathogen in *Oryctes* grubs, which act as a limiting factor to the virus's perpetuation (Gopal and Gupta, 2000). Hence, a detailed survey is required on the host-pathogen interaction in nature so that augmentative release of the biocontrol agent can be taken up at the most appropriate time.

Studies on the evolution rate and genomic changes in the *Oryctes* virus (Crawford and Zelazny, 1990), field comparison of different virus strains (Zelazny et al., 1990), and virulence of virus isolates (Alfiler, 1990) are desirable wherever *O. rhinoceros* is major pest.

Cataloguing of the *Oryctes* virus using primers developed for PCR studies (Richards et al., 1999) and maintaining them as a repository for further use, would be highly desirable. From such repositories, strains could be selected and tested for efficiency and suitability for the management of the pest occurring in different ecological zones, and would also help tide over incidences of the host insect developing resistance to the pathogen, as has been suspected in some places (Zelazny et al., 1989).

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