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Entomopathogenic Nematodes against Insect Pests of Rice

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Abstract*— *Rice is one of the important staple crop in the world. Rice pests cause yield reduction as well as value of the crop. A number of insect pests that attack rice plants account for yield losses. In the rice agro ecosystems, many types of entomopathogens such as nematodes, fungi, bacteria, and viruses can reduce pest population. More emphasis should be placed on using an IPM approach where biological control with entomopathogens is also one of the main components. Entomopathogenic nematodes (EPN) as a safe alternative to the use of insecticides against insect pests in IPM of rice.

***Keywords*— Rice, Insect pests, Biological control, Entomopathogenic Nematodes.**

I. INTRODUCTION

Rice being a tropical plant is also adaptable to a broad range of climatic, edaphic and cultural conditions. It provides 20% of the per capita energy and 15% of the per capita protein for humans worldwide (Mikkelsen and Datta, 1991). Rice is grown in more than a hundred countries, with a total harvested area of approximately 158 million hectares, producing more than 700 million tons annually. Nearly 640 million tons of rice is grown in Asia representing 90% of global production (Way and Bowling, 1991). Rice production should be increased to supply a rapidly expanding population; however, it has been hindered by a number of diseases and insect pests. Moreover, rapid changes in rice production technologies have created greater frequencies of pest epidemics (Reissig *et al*., 1986).

II. INSECT PESTS STATUS

A number of insect pests that attack rice plants account for yield losses of 24% worldwide. Insect pest cause at least 20 per cent field losses in rice in India (Pathak *et al.*, 1982). More than 70 species of insect pests are known to feed on rice, and at least 20 of them can seriously affect rice production. A variety of factors can contribute to pest outbreaks, including climatic factors, improper irrigation, high rates of nitrogen fertilizer application, overuse of insecticide. Insect pests attack all parts of the rice plant at all growth stages and some serve as vectors of viruses that adversely affect the plant.

Brown planthopper (*Nilaparvata lugens*) and white-backed planthopper (*Sogatella furcifera*) are serious pests of rice. They occur in tropical to temperate areas with high reproductive potential and can cause extensive damage through their feeding activity and transmission of viral diseases like rice grassy stunt and ragged stunt. Nymphs and adults suck sap from the base of plants, just above the waterline. In heavy infestation, these planthoppers can cause hopperburn resulting in browning and wilting of some or all tillers in a hill (Kuno, 1968). Most of the rice varieties are susceptible to this pest.

The rice stem borer, *Chilo suppressalis* has been one of the most important pests. It can cause significant damage by reducing tiller number. Females are capable of laying eggs in masses near the base of rice leaves or leaf sheaths. The larvae penetrate tillers and feed on the inner surface of the stem walls, interrupting the movement of water and nutrients. The central leaves of damaged tillers of young plants turn brown (called dead hearts).If the damage occurs after formation of spikelets, panicles turn white (called whiteheads) and no grain filling occurs. The plant often dies and the larvae move to another stem.

Rice water weevil (*Lissorhoptrus oryzophilus*) the larvae cause much more damage, as they feed on roots and prune them, than the adult weevils feeding on leaves. The yield loss could reach up to70-80% with heavy infestation.

III. MANAGEMENT OF INSECT PESTS

Control of insect pests has primarily depended on the application of chemical insecticides. Chemical insecticides are expensive, besides other disadvantages, including secondary pest resurgence, insecticide resistance, environmental pollution, and impact on nontarget organisms. The utilization of pathogenic microorganisms holds a high possibility for the suppression of rice insect pests (Otake, 1979; Chatterjee *et al.* 1983; Pathak *et al*., 1982; Heong, 1983). In recent years more emphasis has been placed on using an IPM approach where biological control is also one of the main components. In the rice agro ecosystems, many types of entomopathogens such as nematodes, fungi, bacteria, and viruses can reduce pest population.

Nematodes have been found associated with most of the insect orders. There are more than 3100 natural associations between insects and nematodes involving 11 orders of nematodes and 23 orders of insects. The association may range from a phoretic relationship to obligate entomoparasitism leading to host death, sterility, reduced fecundity or delayed development. Some that are associated with one host and its special ecology are highly specialized and difficult to propagate on an artificial medium while other less specialized forms have wide host range, can be mass produced on artificial media, and are currently used for control of agricultural pests. Entomopathogenic nematodes (EPN) as a safe alternative to the use of insecticides in IPM of different crops, including rice, have gained worldwide attention (Table 1).

IV. MODE OF ACTION

Mermithids are a large group of obligate entomopathogenic nematodes that are considered important regulators for some insect populations (Kaiser,1991). Mermithid parasitism results in nutritional depletion, retarded growth, organ disruption, reduced fecundity or sterility and death. The newly hatched second stage mermithid is the infective stage (pre-parasite).Once the mermithid contacts the plant hopper nymph, it uses its stylet to penetrate through the cuticle into the host hemocoel to initiate the parasitic phase. The 3rd and 4th stage juveniles occur in the hemocoel.Two to three weeks after parasitization, the 4th stage juvenile (post parasite) exits its adult host by boring through the thin intersegmental area of the abdominal segment, causing death of the host. After emergence, the postparasite burrows into the soil, molts, and overwinters as an adult (Sutanov *et al*., 1990; Vandergast and Roderick,2003; Kamminga *et al*.2012).

Steinernematidae and Heterorhabditidae have attracted most attention as they contain the EPNs *Steinernema* and *Heterorhabditis*. The nematodes musualistically associated with insect-pathogenic bacteria. The bacteria *Xenorhabdus* and *Photorhabdus* (Family: Enterobacteriaceae) are symbiotically associated with *Steinernema* spp. and *Heterorhabditis* spp. respectively. These bacteria are ecologically obligate to EPNs, with specific mechanisms of pathogenicity and their existence in free form in nature is believed to last a very short while due to photo and thermo sensitivity. These nematode bacterium associations meet many criteria for augmentative control of insect pests through inundative releases including: broad host range; ability to kill hosts rapidly : a durable infective stage capable of storage; distribution; and persistence; available mass inexpensive mss production technologies; no evidence of insect immunity; safety to plants and vertebrates; and application with existing spray equipment. The third stage dauer juvenile (DJ) occurs free in the soil and its role is to seek out and infect an insect larva. These free-living, non-feeding juveniles and developmentally arrested third stage juvenile ranging in length from 0.4mm to 1.1mm. *Steinernema* gains entry to the insect larva through natural openings (mouth, anus and spiracles). In addition to these modes of entry, *Heterorhabditis* also gains entry by abrading the intersegmental membranes of the insect. Once in the haemocoel of the insect the DJ releases cells of a symbiont bacterium that it carries in its intestine. The insect haemolymph provides rich medium for the bacterial cells and these begin to grow, release toxins and exoenzymes and kill the insect. The insect dies rapidly, usually within 24-48 h. Generally life-cycle of entomopathogenic nematodes is completed within 12-15 days at room temperature. Depending on the availability of food resource, both heterorhabditis and steinernematids generally complete 2-3 generations within insect cadaver and emerge as infective juveniles to seek new hosts.

PARASITISM / BIOEFFICACY

Imamura (1932) reported that Mermithidae were parasitic on *Chilo simplex.* Grewal *et al*., (2006) recorded it in Asia. Pena & Shepared (1985) recorded 50% parasitisation of BHP by *Hexamermis* sp. in Phillippines. Heong (1983) reported that an entomopathogenic nematode *Amphimermis unka* caused high mortality of hoppers pest in China. Natural incidence of parasitism by *Hexamermis* sp. on BPH was first reported in south India by Manjunath (1978) and in eastern india by Satpathi (1999). Ramani, (2003) also reported that *Hexamermi*s sp. was most important nymphal /adult parasitoid from India. Satpathi *et al*., (2008) studied in detail about the factor affecting abundance of parasitic nematode *Hexamermis* sp. in eastern India. Jayanthi *et al*., (1987) recorded parasitism (12%) by mermithids in rice planthopper. *Rhabditis* sp. (*Oscheius* sp.) was found to be effective against egg mass and neonate larvae of *Scirpophaga incertulas* (Padmakumari *et al*.,2007;2008).

Agamermis species infecting insects have been reported in North America (Cobb *et al*.,1923; Christie,1936), Asia (Kaburaki and Imamura 1932; Choo *et al*.1995), Australia (Baker and Poinar,1995), Africa (Igbinosa,1988), China (Bao et al.,1992) and Europe (Rubtsov,1969;1977). *Agamermis unka* is the most important and common natural enemy in temperate regions. *Agamermis* species live in the soil and infect hosts from the soil directly or after short migration up to plant stem (Nickle, 1981; Choo *et al*., 1995). *Agamermis unka* was first collected by Esaki and Hasimoto,1931 from BPH and WBPH at Oita, Japan and described as a new species (Kaburaki and Imamura,1932; Fuse and Sato,1968). Esaki and Hasimoto (1931) found that >40% BPH and >70% of WBPH populations were parasitized. Mermithid nematodes have received attention as possible biocontrol agents of brown planthopper (BPH). A number of studies were initiated to determine its role as a mortality factor in plant hopper population. *Agamermis* parasitism castrates the reproductive organs of BPH and WBPH. In Philippines, 50% parasitism of BPH by an unidentified mermithid was recorded during the wet season (Otake, 1979), but parasitism was low throughout the year and its impact as a natural control agent of BPH was negligible. Parasitism of the host usually occur at the lower part of the rice stem where most planthoppers are found (Cho *et al*.,2002; Choo and Kaya, 1990;1991;1993). In Korea, *Agamermis unka* is a major natural enemy of the brown planthopper, *Nilaparvata lugens*(Stal) (Hemiptera: Delphacidae), and the whitebacked leafhopper, *Sogatella furcifera* (Horvath) (Hemiptera: Delphacidae), and has been widely studied regarding future inoculative releases and conservation approaches to mange populations in rice (Choo and Kaya,1994).When the pre parasites of *A. unka* were released at a mermithid to BPH of 10:1, parasitism of BPHs ranged from 33 to 63% (Choo *et al*.,1995). *Agamermis* can be redistributed by artificial releases. *Agamermis unka* is an important mortality factor in planthopper populations in Korea. It kills the adult and reduces the fecundity of the females. Males of BPH and WBPH are susceptible to parasitism by *A.unka* (Kuno,1968). As egg production and hatchability of *Agamermis* are high, inoculative releases into areas where the mermithid population is low or nonexistent appear feasible. To affect plant hopper populations; the mermithid must parasitize a high number of progeny of the migrating population. Both the short-winged (brachypterous form) and the long-winged (macropterous form) adults are susceptible to mermithid parasitism, but the brachypterous form (57%) had higher parasitism than the macropterous form(8%)(Choo *et al*.,1989).The brachypterous form is usually found lower on the rice plant where the mermithid is more likely to encounter it. *A. unka* would be most effective when the migrating adult insects produce few progeny and the parasitic stage of the mermithid occurs in high numbers. About 30% of the natural controls of brown plant hopper in eastern India are due to parasitic nematode (Satpathi *et al*., 2008). A control strategy would be to reduce the number of progeny produced by migrating adults. This can be accomplished through an integrated manner with chemical or biological insecticides, resistant cultivars, cultural methods, or a combination of these control tactics. Before an integrated pest management system can be incorporated in the field, further studies on the biology of the nematode and its compatibility with current control tactics are needed. Rice water weevil adults can be parasitized by mermithid, as has been reported in native regions (Bunyarat *et al*., 1977).These parasites may reduce fecundity and cause high mortality in infected adults. Rice blue beetle (*Leptispa pygmaea*) was reported to be parasitized by *Hexamermis* (Patel and Shah,1988). This mermithid is already established in the rice fields in Korea, appears to be compatible with some chemical pesticides, and reduces the fecundity of its host (Choo *et al*., 1998). Cultural practices such as tilling and irrigation can increase the performance of *A.unka*. We have to enhance the effectiveness of the naturally occurring mermithid into an IPM program to reduce BPH population. Genus identification through molecular technique can help predict and infer mermithid biology, which can ultimately assist in rearing protocols, if mermithids are to be used for future research and incorporation into current management protocols.

In India, efforts were made during 1970s to study the effectiveness of exotic EPNs, *S.carpocapsae* (DD-136) against insect pests of rice (Rao *et al*., 1968). However, the nematode was not able to become established in field trials. *S. carpocapsae* (DD-136) causing up to 98% mortality and fast multiplication on 5th instar larvae of *Cnaphalocrocis medinalis* (Srinivas and Prasad,1991). Choo *et al.* (1989) reported that *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* are very effective against the rice yellow stem borer, *Scirpophaga incertulas* causing mortality and proved EPN as a potential biocontrol agent in rice eco system. *S. carpocapsae* or *H. bacteriophora* were sprayed at the rates of 250, 500, 1000 or 2000 IJs for *S. carpocapsae* and 100, 200, 400 or 800IJs for *H. bacteriophora*. Seven days after treatment, showed that both nematodes were effective causing more than 91% mortality. On the other hand, mortality was 42.6% and 63.1% when stems were dipped into a nematode suspension at the rate of 100 or 200 IJs of *H. bacteriophora.* Because the moist habitat of rice stems were favorable to nematode survival and searching abilities, entomopathogenic nematode, were confirmed to be a potential biological control agents against rice stem borers (Choo *et al*., 1991). Prasad *et al*., (2006) reported that *H.indica* caused 100% mortality of rice leaf folder, *Cnaphalocrocos medinalis* within 18 to 20 hours of exposure. Padmakumari *et al.* (2008) reported that lethal time of 19.8 h was recorded by *H. indica* and 37.8 h by *S. asiaticum* on *C. medinalis* in a bioassay study. Combined inoculation of *H. indica* and *S. asiaticum* each at 75 IJs/larva resulted in faster larval mortality on *C. medinalis* (24.6 h). The percent larval mortality caused by *H. indica* alone was resulted significantly more (60%) than *S. asiaticum* (40%) on *C. medinalis*. Progeny produced by *H indica* and *S. asiaticum* 4843 and 4330 IJs/larva respectively on *C. medinalis* larva (Sankar *et al*., 2009). *Heterorhabditis indica* caused 66.67 to 91.67 per cent mortality at concentrations of 5IJs to 9IJs in the grubs of *L. pygmaea* (Karthikeyan, and Jacob, 2009). Spraying of *H.indica* @ 3000 IJs /ml was effective in reducing the white ear incidence,@ 2500 IJs /ml was also equally effective in reducing the gall midge incidence at 25 DAT, *H. indica* applied @ 3000 IJs /ml was the superior treatment at 25 days after transplanting while at the later stage (55 days after transplanting), a lower dose @ 2500 IJs /ml was found to be sufficient to bring about significant control of leaffolder (Karthikeyan and Jacob,2010). Among the three entomopathogenic nematodes evaluated for their biological control, maximum reduction of BPH was observed with *Steinernema glaseri* followed by *Metarhabditis amsactae* isolate Drr-Ma2 (Annon.2015).Rice stem borer (*Chilo suppressalis*) was reported to managed by *S.carpocapsae* Pocheon strain, *S.carpocapsae* Iksan strain, *S.monticolum* Hwasun strain, *H.megidis* Hwasun strain in Korea (Jung *et al*.,2018). Efficacy of EPNs was evaluated was evaluated against the African white rice stem borer, *Maliarpha separatella*. Significant virulence was obtained with all the nematodes species at 200 IJs after 48 hours of exposure in the following order *H.indica* > Ex Nakuru (local isolate) > *S.carpocapsae* > Ex Mombosa (local isolate) > *S.karii* (Kega *et al*.,2013;2020). *Steinernema carpocapsae* was found to cause mortality against rice water weevil, *Lissorhoptrus oryzophilus* under laboratory setting but failed to work in the field in Japan (Nagata,1987).In Cuba, there was success using *Steinernema* spp. against the rice water weevil with up to 80% control in field trials(Carbonell, 1983; Meneses, 1983).In California, research with both *S.carpocapsae* and *Heterorhabditis* spp. found that nematodes provided control of rice water weevil larvae when applied to drained soil that was reflooded 8 d later (Grigarick and Oraze,1990).In China, the Otio strain of *S. feltiae* was found to cause high mortality for larvae (Sun *et al*.,2006) and mortality rate was affected by time and dose. Efficacy of *S.feltiae*, *S. carpocapsae* A24 strain, *S.glaseri* NC 34 strain, *H.bacteriophora* and *H.zealandica* have also been detected in adult weevils (Kisimoto *et al*.,1987). Mortality of 82.5% and 97.5% was observed in adults of *L. oryzophilus* treated with *S.feltiae* and *H.bacteriophora*, respectively, at 10d after incubation with nematodes (Li *et al*.,2007). However, the widespread application or adoption of nematodes against rice water weevil in Asia or North America has not been possible for economic reasons (Choo and Rice, 2007).

V. CONCLUSION

Understanding the ecological and behavioral relationships between the nematode and insects could result in proper use of compatible insecticides or other biological control agents in providing an integrated approach to insect management. *In vivo* production of mermithid has been accomplished with the mermithids from the banded cucumber beetle (Creighton and Fassuliotis, 1982) and from mosquitoes (Peterson, 1984). Similarly, if BPH can be mass produced easily, *in vivo* production of the mermithid may be used to augment natural population. In addition to production, methods to store the eggs and adults and timing of introduction into BPH populations need to developed. Using the conservation of naturally-occurring population of mermithid, there is a need to implement an effective IPM programme. By understanding the biology and ecology of these entomopathogens, we may be able to use them effectively in the integrated pest management of rice through augmentation or inundative release. The microclimate of rice culture with high humidity and moderate temperature is also conductive for the survival, movement, tracking and invasion of the host by EPN and their establishment as a bio-control agent. However, there is a need for development of suitable delivery mechanisms including formulation technology for field application of this EPN. Studies are required to evaluate their bioefficacy against other rice pests as well.

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