JBiopest 7(Supp.):106-115(2014)

Evaluation of entomopathogenic fungus, *Nomuraea rileyi* (Farlow) samson for the control of groundnut *Spodoptera litura* (F.) and its compatibility with synthetic and botanical pesticides

R.K. Patil[1*], Y.S. Bhagat[2], B. Halappa[1] and R.S. Bhat[2]

ABSTRACT

The entomopathogenic fungus, *Nomuraea rileyi* (Farlow) Samson was isolated from the cadavers of *Spodoptera litura* (F.) collected from groundnut field of University of Agricultural Sciences, Dharwad. rDNA-ITS sequence of *N. rileyi* isolate IOF1 showed considerable homology with those of other isolates. Pathogenicity of *N. rileyi* isolate IOF1 against *S. litura* was studied by topical application of spores at a concentration of 10^8 conidia per litre to first to fifth instars. Early instars were highly susceptible with a mortality of 70.17 percent, which decreased significantly as the age of the larvae advanced. Lethal time for 1st to 5th instars of *S. litura* was 130.71, 137.77, 148.04, 235.65 and 263.10 hrs, respectively. *In vitro* compatibility of *N. rileyi* with fungicides, insecticides and botanicals at their recommended dosages was studied. Among the fungicides, carbendazim and mancozeb resulted in complete growth inhibition (100%), whereas tridemefon and cyperconazole inhibited the growth by 49.65% and 55.25%, respectively. Growth inhibition with insecticides endosulfan and dichlorvos was high (52.77% and 50.68%, respectively). However, Methomyl and λ-Cyhalothrin resulted in low (11.81%) growth inhibition. Among the botanical extracts, both *Annona squamosa* and *Polyalthia longifolia* showed higher growth inhibition (55.76%) when compared to those of *Vinca rosea* (2.40%) and *Adhatoda vasica* (4.85%). Hence, the new isolate IOF1 of *N. rileyi* is an effective entomopathogenic fungus that can be used against *S. litura*.

MS History: 15.12.2013 (Received)-3.3.2014 (Revised)-15.5.2014 (Accepted)

Key words: *Nomuraea rileyi*, *Spodoptera litura*, rDNA-ITS sequence, bioassay, compatibility.

INTRODUCTION

Tobacco caterpillar, *Spodoptera litura* (F.), is one of the most destructive pests of various crops and is more or less of universal occurrence except in regions where extremes of climate prevail. It has been reported to feed on 112 cultivated food plants all over the world (Mousa *et al*., 1980) of which 40 are grown in India (Basu, 1981; Muthukrishnan *et al.*, 2005) including tobacco, tomato, cotton, chilly, okra, cauliflower, castor, groundnut, soybean, maize and black gram. Control of *S. litura* using insecticides has become difficult because of the development of resistance. Biological control of insect pests is one of the most important components of Integrated Pest Management (IPM), wherein entomopathogens such as bacteria, viruses and fungi are exploited against insect pests. These biological agents including entomopathogenic fungi can provide an alternative and more environmentally friendly approach for the control of pests. Fungal diseases are known to cause insect mortality naturally (Gupta, 2003; Roberts and Humber, 1981; Vimaladevi and Prasad, 2001). Entomopathogenic fungi are potential agents for pest control due to their specificity, mode of action and ease of application. This has promoted the evaluation of the entomopathogenic fungi as biocontrol agents in many countries.

Unlike bacteria or viruses, fungi infect insects by breaching the host cuticle. The insecticidal activity *Nomuraea rileyi* (Farlow) Samson to lepidopteran pests was reviewed by Ignoffo during 1981 and he

concluded that the fungus was a candidate for further development as a microbial insecticide. A recent study on the pathogenesis of *N. rileyi* against *S. litura* showed that the infection process starts with adhesion of conidia on the insect cuticle (Srisukchayakul *et al*., 2005). The germ tube penetrates through the cuticle, causes lysis of endocutilce followed by development of hyphal bodies in the hemocoel which convert to invasive mycelia and causes death of the host. At the end of the infection cycle, mycelia emerge from the cuticle and produces conidiophores (Srisukchayakul *et al.,* 2005). Also *N. rileyi* secretes a proteinaceous substance inhibiting larval moult and metamorphosis (Kiuchi, 2003). Entomopathogenic fungi also possess added advantage over other microbial control agents as they are capable of attacking all developmental stages of insects including pupal stages (Anand *et al.,* 2009; Ferron, 1978). Most importantly, these entomopathogenic fungi are the principal pathogens on sucking pests since these hosts cannot ingest other pathogens like bacteria or viruses that infect through gut wall. Apart from this virtually all insect orders are susceptible to fungal diseases. However, of the 700 species of entomopathogenic fungi currently known only ten species have been are presently being developed for biocontrol (Roberts and Hajek, 1992).

The full potential of entomopathogenic fungi has not been approached. However, previous studies indicate that the insect mycopathogen *N. rileyi* a dimorphic hypomycete causes fungal epizootics in population of several noctuid pests (Ignoffo *et al*., 1989; Tang and Hou, 1998; Vargas *et al*., 2003). Moreover, previous bioassays have shown that *N. rileyi* isolates from different geographical locations and different hosts vary in their virulence and specificity (Boucias *et al.,* 1982; Ignoffo and Boucias, 1992; Tigano, 1995; Vimaladevi *et al.,* 2003). However, the virulence of entomopathogenic fungi is affected by environmental factors such as temperature, humidity, light and solar radiation. The optimum temperature for conidia to germinate and penetrate into noctuid insect body for *N. rileyi* is 20 to 30°C (Gardner, 1985; Tang and Hou, 2001). Also the optimum humidity for conidial germination, infection and sporulation is 95 to 100 per cent (Tang and Hou, 2001). Therefore it is important to identify potential entomopathogenic fungal isolates which further can be exploited as myco-insecticide for specific geographic locations. In this investigation our main objective was to study the insecticidal activity of *N. rileyi* isolate against *S. litura*. Thus, it can be exploited as myco-insecticide for the control of *S. litura* which is a major pest in groundnut in the specific geographic locations which lies in the transition belt where conditions are favourable for the entomopathogenic fungi to cause spectacular epizootics during *kharif* season.

MATERIAL AND METHODS

Isolation and Identification of *N. rileyi* Isolate

N. rileyi was isolated from *S. litura* cadavers collected from Groundnut field at Main Agriculture Research Station (MARS), University of Agricultural Sciences, Dharwad. The dead larvae collected from the fields were surface sterilized by immersing in 0.5 % $HgCl_2$ for 2 min followed by rinsing in three changes of sterile water. The surface sterilized diseased specimens were cut in a sterile watch glass and a small portion of the infected tissue was transferred to a sterile culture plate containing Saborauds Maltose Agar Media (SMAY) method followed by Bell (1975). The plates were incubated at room temperature 26 ± 1°C for a week and the colonies that came up were further purified by repeated subculture on SMAY media. The isolates that came up on the SMAY medium were identified as *N. rileyi* by microscopic examination according to the outlines given by Samson *et al.* (1988) and maintained as pure culture. The microscopic examination revealed that the vegetative hyphae of *N. rileyi* appeared smooth septate, hyaline and conidiophores were erect and septate. Branches formed near a septum were developed in whorls each giving rise to 2 to 4 phialides. Conidia were single smooth ellipsoidal.

Preparation of Fungal Culture of *N. rileyi* Isolates

SMAY media was used for the multiplication of the fungus as well as spores. The cultured plates were incubated at 28°C for 7 to 10 days. Spores obtained on the SMAY media were screened for their insecticidal activity against third instar larvae of *S. litura*.

The fungal spores from SMAY plates were mixed with distilled water and 0.02% Tween-80 to produce the spore suspension. The number of conidia was determined with a Neubauer haemocytometer. Finally, through serial dilution method spore suspensions containing 1×10^8 conidia/ ml were obtained. The *S. litura* culture that was used for the bioassays was raised from field collected larvae and maintained under laboratory conditions in the Institute of Organic Farming at University of Agricultural Sciences, Dharwad.

Bioassays of *N. riley*

Insecticidal activity of *N. rileyi* isolates to *S. litura* was studied through larval bioassays. Spore suspension of 1.2×10^6 conidia/ml of *N. rileyi* was topically applied against all five instars. They were fed with fresh castor leaves. Five replicates of 20 larvae were tested. Controls consisted of larvae applied with 500 µl of distilled water and 0.02% Tween-80 solution. The number of dead larvae was recorded on the fifth to eight day of spore inoculation and finally the percent mortality for each of the isolates was computed. The data were subjected to statistical analysis of variance using SAS Online Doc 9.1.3. (SAS Institute Inc., Cary, NC) and mean separation was carried out using DMRT (Duncan's Multiple Range Tests) to find significant difference among the isolates. Median lethal time (LT_{50}) was estimated using Proc Life Reg procedure and data were fitted to a Weibull distribution (SAS OnlineDoc 9.1.3). The differences in pathogenicity and LT_{50} for *N. rileyi* against *S. litura* were compared using the Fisher's least significant difference test ($\alpha \leq 0.05$) (Table 1).

Compatibility of *N. rileyi* with synthetic and botanical pesticides

In vitro studies were conducted by using poison food technique. Potato Dextrose Agar (PDA) medium was used with requisite quantity of fungicides, insecticides and botanicals to get desired concentration. Culture of *N. rileyi* grown on SMAY media for seven days at the optimum temperature for growth inhibition studies. Small disc (0.8 cm) of the fungus culture was cut with sterile cork borer and transferred aseptically in the centre of petridish containing poison medium with different chemicals with suitable check. Each treatment was replicated five times. Colony diameter of the fungus was measured at three, five and seven days of inoculation and compared with check to establish the toxicity (Nene and Thapliyal, 1997).

Genotyping of *N. rileyi* isolate:

DNA Amplification and Sequencing

Genomic DNA was purified from mycelium using the cetyltrimethylammonium bromide (CTAB) extraction method of Rogers and Bendich (1988). A single pair of primers was used to PCR amplify the internal transcribed spacer (ITS) regions ITS_1 and ITS_2 along with the central 5.8S rDNA. The primers used were the universal eukaryotic ITS primers ITS1 (5´-TCC GTA GGT GAA CCT GCG G-3´) and ITS4 (5´-TCC TCC GCT TAT TGA TAT GC-3´) (White *et al.*, 1990). The PCR products were checked using agarose gel electrophoresis and as expected produced a product of about 600 bp. Bands were excised and then purified using QIAGEN Gel Extraction kit according to the manufacturer's instructions (Qiagen, Hilden, Germany) prior to sequencing or were sequenced directly using consensus primers. ITS purified fragment was sequenced in both directions with the primers ITS_1 and ITS4 by commercial sequencing center, Bangalore Genei (India) Pvt. Ltd., Bangalore using ABI 3130 XL sequencer. Sequence assembly was done by using Vector NTI Advance 10 software. Selected sequences available from Gen Bank were also included in the analysis. Amplified ITS sequences were compared with the Gen Bank Nucleotide Database (http://www.ncbi.nlm.nih.gov) using the algorithm BlastN (Altschul *et al.*, 1997). A consensus tree was constructed after 1,000 bootstraps resampling steps by UPGMA method with p-distance using the software Mega 5 (Tamura *et al.*, 2011).

RESULTS

Pathogenicity levels of *N. rileyi* against *S. litura*

N. rileyi was found to be pathogenic against *S. litura*. The mortality of *S. litura* topically applied with the

spore suspension is and median lethal time (LT_{50} in days) values of *N. rileyi* isolates against first to fifth instars of *S. litura* are shown in table 1. Bioassays of *N. rileyi* isolate of *S. litura* origin against *S. litura* showed that as the larval stage advanced the mortality also decreased significantly. Results showed that early instars are highly susceptible with a mortality of 96.25 per cent. Lowest mortality of 20 per cent was recorded in 5th instar. The median lethal time (LT_{50}) values for 1st to 5th instars were 130.71, 137.77, 148.04, 235.55 and 263.10 h, respectively.

Table 1. Pathogenicity of *Nomuraea rileyi* **(**10^8 Conidia/l) to *Spodoptera litura* mortality (%)

Instar	Different hours after application					
	120	**144**	**168**	**192**	**Mean**	**LT_{50}**
First	41.80a	67.24a	77.56a	96.25a	70.71a	130.71
Second	33.68b	61.50b	75.26a	87.50b	64.48b	137.77
Third	28.50c	59.48b	64.63b	71.25c	55.96c	148.04
Fourth	11.20d	17.58c	25.32c	37.50d	22.90d	235.55
Fifth	3.35e	7.90d	11.25d	20.00e	10.62e	263.10

In a column, means followed by the same alphabet do not differ significantly (P=0.05) by DMRT

Compatibility of *N. rileyi* with synthetic and botanical pesticides

The compatibility studies indicated that fungicides were more toxic (Table, 2) to the entomopathogenic fungus followed by insecticides (Table 3). While botanical pesticides were least antagonistic (Table 4). All the seven fungicides inhibited the growth of the fungus significantly on all the days of observation as compared to control. Among them, carbendazim and mancozeb were found highly detrimental to the fungus at both the concentrations tested (Table, 2). They did not allow any growth of fungus whereas others inhibited the growth from 55.25 to 76.92 per cent. Tridemefon and cyperconazole were comparatively safe to the fungus with less inhibition at the recommended as well as reduced dosages. These were followed by hexaconazole, propiconazole and chlorothalonil. Similarly, among the seven insecticides evaluated for their inhibitory effect on growth of *N. rileyi*, endosulfan and dichlorvos were found to be highly detrimental compared to others. The former insecticide at both the recommended and reduced dosages and later at the recommended dosage inhibited the fungal growth to the tune of 50.58 to 52.77 per cent. Inhibition by other insecticides ranged from 15.98 to 35.42 per cent at recommended concentration. Out of thirteen plant products evaluated, *A. squamosa* seed extract (5%), *P. longifolia* (5%) and *P. hysterophorus* (10%) inhibited growth to the extent of 50 to 55 per cent after seven days of inoculation. Inhibitory effect of other botanicals varied between 2.4 to 17.58 per cent. The least inhibitory effect (2.4%) was noticed in *V. rosea* followed by *S. indica* (5%), NSKE (5%), *V. negundo* (5%), neem oil (1%), *A. Mexicana* (5%), pongamia oil (1%), *C. inerme* (5%) and *A. calamus* (5%).

Genotyping of *N. rileyi* isolate

ITS sequence variation was not found between 4 isolates of *N. rileyi* Ma1 (JQ991621.1), MAFF 83007(AB268359.1), CBS 806.71(AY624205.1) and IOF1. The ITS sequences of various isolates of *N. rileyi* are clustered together with *N. rileyi* IOF1 isolate. *N. rileyi* IOF1 showed 99 per cent similarity with CG129 (EU553337.1), 98 per cent similarity with ASRA3 (JQ686668.1). Phylogenetic tree constructed using ITS sequences clearly distinguished these isolates from *N. rileyi* IOF1 isolate.

Phylogenetic results revealed that there are two major clusters, cluster A comprising *N. rileyi* isolates Ma1 (JQ991621.1), MAFF 83007(AB268359.1), CBS 806.71(AY624205.1) , CG 129(EU553337.1) and IOF1, whereas cluster B comprises ASRA3 (JQ686668.1). Biological control, particularly by entomopathogenic fungi is important for reducing the population density of pests in IPM programs. Therefore, preservation of entomopathogens that occur naturally or introduced for insect control should be observed (Oliveira *et al.,* 2003). In addition, we must understand the compatibility of entomopathogenic fungi with other crop production techniques such as the use of insecticides which may inhibit to a smaller or larger extent the development and reproduction of pathogen (Malo, 1993; Rogerio *et al.,* 2005). Co- application of entomopathogenic fungi with low doses of insecticides is gaining importance in insecticide resistance management in insect pest of crops as a component of IPM programme. In this context, this study provides useful information on the compatibility between the fungal biological control agents and plant-based insecticides and plant extracts which are normally used in pest management. This study also reveals that the mixture of either commercial botanicals or plant extracts and fungicides can be used for field application along with these fungi. Moreover, tested botanicals and fungi have been used in pest management and these components can be integratively used in Bio Intensive Pest Management (BIPM). Only limited reports are available on the use of *N. rileyi* on *S. litura*. In the present study, *N. rileyi* was found to be highly infective to early instars of *S. litura* than later instars. The present finding is in conformity with Manjula and Krishna Murthy (2005) who reported that the highest larval

Table 2. Effect of fungicides on growth and development of *N. rileyi*

Treatments	Colony size of *N. rileyi* (mm)					
	3 DAI	%inhibition	5 DAI	% inhibition	7 DAI	% inhibition
Carbendazim 0.05%	0.00 j	100.00	0.00 g	100.00	0.00 g	100.00
Carbendazim 0.025%	0.00 j	100.00	0.00 g	100.00	0.00 g	100.00
Mancozeb 0.2%	0.00 j	100.00	0.00 g	100.00	0.00 g	100.00
Mancozeb 0.1%	0.00 j	100.00	0.00 g	100.00	0.00 g	100.00
Cyperconazole 0.1%	11.67 e	53.32	14.00 c	57.14	18.67 d	60.83
Cyperconazole 0.05%	12.67 d	49.32	15.67 b	52.03	21.33 c	55.25
Propiconazole 0.1%	9.33 g	62.68	11.00 de	66.33	11.00 f	76.92
Propiconazole 0.05%	10.67 f	57.32	12.00 d	63.26	14.00 e	70.63
Chlorothalonil 0.2%	6.67 i	73.32	8.67 f	73.46	12.00 f	74.48
Chlorothalonil 0.1%	8.00 h	68.00	10.33 e	68.38	14.33 e	69.94
Tridemefon 0.1%	14.33 c	42.68	14.33 c	56.14	21.33 c	55.25
Tridemefon 0.05%	15.33 b	38.68	15.67 b	52.00	24.00 b	49.65
Hexaconazole 0.1 %	10.00 fg	60.00	12.00 d	63.26	14.00 e	70.63
Hexaconazole 0.05%	12.00 de	52.00	13.67 c	58.15	15.33 e	67.84
Untreated Check	25.00 a	-----	32.67 a	-----	47.67 a	-----

DAI-Days After Inoculation. In a column, means followed by same alphabet do not differ significantly (P=0.05) by DMRT

mortality of 91.2 per cent was obtained in the first instar of *S. litura* and 95 per cent in the second instar of *H. armigera* with the highest concentration of 1 x 10^9 of *N. rileyi* spores ml/l.

Vimala Devi and Prasad (1994) conducted field studies and found that *N. rileyi* was effective against *S. litura* as foliar spray and soil application. Likewise, Sridhar and Prasad (1996) recorded up to 36.9 per cent infection of *N. rileyi* on *S. litura* in groundnut fields in Andhra Pradesh. *N. rileyi* was a key natural mortality factor of *S. litura* populations in coastal Andhra Pradesh (Sridhar and Prasad, 1996). Vimala Devi *et al.* (2002) reported larval mortality when *S. litura* was reared on castor leaves inoculated with 2 x 10^8 spores ml/ l. Navi *et al.* (2006) indicated that *N. rileyi* caused higher mortality of *S. litura* under field conditions. In India, it is frequently observed in tomato, cabbage, field bean, banana and pigeon pea ecosystems as a natural epizootic on *H. armigera*, *S. litura* and *T. ni* (Gopalakrishnan and Mohan, 1997). The data on mortality response of different instars of *S. litura* to *N. rileyi* indicate that the infection is dependent on age of the larvae. The larval mortality was highest in the first instar and least in the ultimate instar after 8 days of infection. The LT_{50} also increased with the age the larvae to the infection.

Table 3. Effect of insecticides on growth and development of *N. rileyi*

Treatment	Colony size of *N. rileyi* (mm)					
	3 DAI	%inhibition	5 DAI	% inhibition	7 DAI	% inhibition
Monocrotophos 0.05%	18.67 cd	25.32	24.67 c	24.48	36.33 e	24.31
Carbendazim 0.025%	21.00 b	16.00	26.33 b	19.40	37.67 d	21.52
Chrolopyriphos 0.04%	16.00 e	36.00	21.67 d	33.67	30.00 h	35.42
Chloropyriphos 0.02%	17.67 d	29.32	22.33 d	31.65	31.33 g	34.73
Endosulfan 0.07%	13.67 fg	45.32	16.33 e	50.00	22.67 i	52.77
Endosulfan 0.035%	14.33 f	42.68	16.67 e	48.97	22.67 i	52.77
Dichlorvos 0.04%	13.00 g	48.00	16.33 e	50.00	23.67 i	50.58
Dichlorvos 0.02%	18.00 d	28.00	21.67 d	33.67	35.00 e	27.08
Methomyl 0.025%	19.33 c	22.68	24.33 c	25.52	40.33 c	15.98
Methomyl 0.0125%	20.67 b	17.32	25.33 bc	22.46	42.33 b	11.81
λ- Cyhalothrin 0.01%	13.33 f	46.68	24.33 c	25.52	40.33 b	11.81
λ- Cyhalothrin 0.005%	12.67 g	49.32	22.67 d	30.60	33.00 fg	33.33
Phosphamidon 0.04 %	13.33 f	46.68	24.33 c	25.52	36.33 e	24.31
Phosphamidon 0.02%	20.67 b	17.32	25.67 bc	21.43	39.33 c	18.06
Untreated Check	25.00 a	-----	32.67 a	-----	48.00 a	-----

DAI-Days After Inoculation. In a column, means followed by same alphabet do not differ significantly (P=0.05) by DMRT

The highest LT_{50} value of 263.10 h was obtained for the 5th instar which was reduced nearly half for first instar larvae.

Studies carried out understand the compatibility of entomopathogen with fungicides, insecticides and botanicals, revealed that botanicals were least inhibiting to the fungus followed by insecticides in contrast to all test fungicides were highly inhibitory to fungus. Present findings are in conformity with findings of many workers (Ignoffo *et al.,* 1975; Garcia and Ignoffo, 1979; Gardner *et al.,* 1979; Ross and Brady, 1985; Silva *et al.,* 1993; Gopalkrishnan and Mohan, 1991; Tang and Hou, 1998 and Kulkarni, 1999). Variation in inhibition by different pesticides is due to inhibitory compounds present in the pesticides or toxicity level of pesticide.

Table 4. Effect of botanicals on growth and development of *N. rileyi.*

Treatment	Colony size of *N. rileyi* (mm)					
	3 DAI	%inhibition	5 DAI	% inhibition	7 DAI	% inhibition
***Annona squamosa* 5%**	12.00 e	50.68	14.00 e	57.14	24.33 g	55.76
***Parthenium hysterophorus* 10%**	19.67 c	19.15	27.67 bc	15.30	27.67 f	49.69
***Parthenium hysterophorus* 5%**	20.67 bc	15.04	27.67 bc	15.30	27.67 f	49.69
***Adhatoda vasica* 5%**	23.67 a	2.70	31.67 a	3.06	52.33 bc	4.85
***Vinca rosea* 5%**	24.33 a	0.00	32.67 a	0.00	53.67 ab	2.40
***Polyalthia longifolia* 5%**	15.33 d	36.99	21.33 d	34.71	24.33 g	55.76
***Vitex negundo* 5%**	21.00 b	13.68	36.67 bc	18.36	51.33 c	6.67
***Acorus calamus* 5%**	19.33 c	20.55	26.33 c	19.40	45.33 e	17.58
***Clerodendron inerme* 5%**	19.67 c	19.15	27.67 bc	15.30	45.67 e	16.96
***Argemone Mexicana* 5%**	20.67 bc	15.04	28.67 b	12.24	51.30 c	6.72
***Stachytarphita indica* 5%**	20.33 bc	16.44	27.33 bc	16.34	52.33 bc	4.85
Neem oil 1%	21.33 b	12.32	30.67 a	6.72	51.33 c	6.67
Pongamia oil 1%	19.67 c	19.15	27.33 bc	16.33	47.33 d	13.94
NSKE 5%	27.67 a	2.71	32.00 a	2.00	51.67 c	6.05
Untreated Check	24.33 a	-----	32.67 a	-----	55.00 a	-----

DAI-Days after Inoculation; In a column, means followed by same alphabet do not differ significantly (P=0.05) by DMRT

Comparative analysis of ribosomal RNA (rDNA) gene sequence information can be used to clarify natural evolutionary relationships over a wide taxonomic range (Pace *et al.,* 1986). Ribosomal RNA genes (rDNA) typically exist as a tandem repeat that includes coding regions which are conserved to varying degrees as well as highly divergent spacer regions. These spacer regions or internal transcribed spacer sequences (ITS), have been widely used in fungal systematics (Bowman *et al.*, 1992; Hibbett, 1992; Driver *et al.*, 2000). We therefore decided to use rDNA-ITS sequencing to identity *N. rileyi* entomopathogenic fungal isolate and to its clarify the taxonomic relationships. Multiple alignment of the ITS sequences of various isolates of *N. rileyi* along with *N. rileyi* IOF1 clearly indicated the differences in nucleotide sequences of all the three species which differentiated these species from each other. In the multiple alignment both ends of the ITS region showed variation in nucleotide sequences. These ends are actually ITS-1 and ITS-2 regions. Perfect homology was observed in nucleotide sequences in central region which is actually the 5.8S rDNA region.

REFERENCES

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J., 1997. Gapped BLAST and PSI-BLAST:A new generation of protein database search programs. *Nucleic Acids Research.* **25**(17):389-402.

Anand, R., Prasad, B. and Tiwary, B. N. 2009. Relative susceptibility of *Spodoptera litura*

pupae to selected Entomopathogenic fungi. *Bio Control*, **54**:85-92.

Basu, A. C. 1981. Effect of different foods on the larval and post larval development of moth of *Prodenia litura* (Fab.). *Journal of Bombay Natural History Society*, **44**: 275–288.

Bell, J. V. 1975, Production and pathogenicity of the fungus *Spicaria rileyi* from solid and liquid media. *Journal of Invertebrate Pathology,* **26**:129-130.

Boucias, D. G., Schoborg, E. A. and Allen, G. E. 1982. The relative susceptibility of six noctuid species to infection by *Nomuraea rileyi* isolated from *Anticarsia gemmatalis*. *Journal of Invertebrate Pathology,* **39**:238-240.

Bowman, B. H., Taylor, J. W., Brownlee, A. G., Lee, J., Lu, S. D. and White, T. J. 1992. Molecular evolution of the fungi: relationships of the *Basidiomycetes*, *Ascomycete*s and *Chytridiomycetes*. *Moolecular Biology and Evolution,* **9**:285-296.

Driver, F., Milner, R. J. and Trueman, J. W. H. 2000. A taxonomic revision of *Metarhizium* based on a phylogenetic analysis of rDNA sequence data. *Mycological Research,* **104**:143-150.

Ferron, P., 1978. Biological control of insect pests by entomopathogenic fungi. *Annual Review Entomology,* **23**:409-442.

Garcia, C. and Ignoffo, C.M. 1979. Sensitivity of *Nomuraea rileyi* from cadavers of cabbage semilooper, *Trichoplusia ni. Journal of Invertebrate Pathology*, **30**:114-116.

Gardner, W. A. 1985. Effect of temperature on the susceptibility of *Heliothis zea* larvae to *Nomuraea rileyi*. *Journal of Invertebrate Pathology,* **3**:2-10.

Gopalkrishnan, C. and Mohan, K.S. 1991. Studies on indigenous entomogenous fungi affecting insect pests of horticultural crops, Annual report, 1990-91. Indian institute of Horticultural Research, Hesaragatta, Bangalore, pp 45.

Gupta, V. P. 2003. Natural occurrence of the entomopathogenic fungus *Nomuraea rileyi* in the soybean green semilooper, *Chrysodeixis acuta*, in India. Online. *Plant Health Progress doi*, 10.1094/PHP-2003-0113-01-HN.

Hibbett, D. S. 1992. Ribosomal RNA and fungal systematics. *Transaction of Mycological Society of Japan*, **33**:533-556.

Ignoffo, C. M. 1981. The fungus, *Nomuraea rileyi* as a microbial insecticide. Pages 513-538 in: Microbial Control of Pests and Plant Diseases, 1970-80 H. D. Burges, ed. Academic Press, London, UK.

Ignoffo, C. M. and Boucias, D. B. 1992. Relative activity of geographical isolates of *Nomuraea* bioassayed against the cabbage looper and velvetbean caterpillar. *Journal of Invertebrate Pathology,* **59**:215-217.

Ignoffo, C. M., Garcia, C. and Samson, R. A. 1989. Relative virulence of *Nomuraea* spp. (*N. rileyi*, *N. atypicola* and *N. anemonoides*) originally isolated from an insect, a spider and soil. *Journal of Invertebrate Pathology,* **54**:373-378.

Kiuchi, M., Yasui, H., Hayasaka, S. and Kamimura, M. 2003. Entomogenous fungus *Nomuraea rileyi* inhibits host insect moulting by C22-oxidizing inactivation of hemolymph ecdysteroids. *Archives of Insect Biochemistry* and *Physiology,* **52**:35-44.

Kulkarni, N.S. 1999. Utilization of fungal pathogen *Nomuraea rileyi* (Furlow) Samson on the management of Lepidopteran pests. *Ph.D thesis, University of agricultural Sciences, Dharwad.* **PP**.154.

Manjula, K. and Krishna Murthy, K. V. M. 2005. Efficacy of *Nomuraea rileyi* against different instars of *Spodoptera litura* and *Helicoverpa armigera*. *Annals of Plant Protection Sciences*, **13**: 347–350.

Malo, A. R. 1993. Estudio sobre la compatibilidad del hongo *Beauveria bassiana* (Bals.) Vuill. con formulaciones comerciales de funguicidas e insecticidas. *Review of Colombian Entomology.* **19**: 151-158.

Mousa, M. A., Zaker, M. A. and Koppy, F. 1980. Abundance of cotton leaf worm, *Prodenia litura* (Fab.) in relation to host plants I. Host plants and their effect on biology. *Bulletin of Entomological Society, Egypte,* **44**: 241–251.

Muthukrishnan, N., Ganapathy, N., Nalini, R. and Rajendran, R. 2005. Pest Management in

Horticultural Crops. *New Madura Publishers, Madurai*. **PP**. 325.

Navi, S. S., Patil, R. K., Kulkarni, K. A. and Rachappa, V. 2006. Efficacy of *Nomuraea rileyi* (Farlow) Samson, neem seed kernel extract (NSKE), quinalphos and varietal resistance for the management of *Spodoptera litura* (Fab.). *Journal of Entomological Research*, **30**: 207–212.

Nene, Y.L. and Thapliyal, P.N., 1997. Fungicides in plant diseases control, Oxford and IBH publishing Co. Pvt. Ltd. New Delhi, pp531.

Rogerio, A., Depieri, S., Martinez, S., Menezes, A. O., 2005. Compatibility of the Fungus *Beauveria Bassiana* (Bals.) Vuill. (Deuteromycetes) with extracts of Neem Seeds and Leaves and the emulsible Oil. *Neotrophis* of *Entomology,* **34**(4): 601-606.

Pace, N. I., Olsen, G. I. and Woese, C. R., 1986. Ribosomal RNA phylogeny and the primary lines of evolutionary descent. *Cell*, **45**:325-326.

Roberts, D. W. and Hajek, A. E., 1992. Entomopathogenic fungi as bioinsecticides. Pages 144-159 in: Frontiers in Industrial Mycology. G. F. Letham, ed. Chapman and Hall, New York, NY.

Roberts, D. W. and Humber, R. A., 1981. Entomogenous fungi. Pages 201-236 in: The Biology of Conidial Fungi, Vol. 2. G. T. Cole and B. Kendrick, eds. Academic Press, New York, NY.

Rogers, S. O. and Bendich, A. J., 1988, Extraction of DNA from plant tissues. In: Gelvin SB, Schilperpoort RA and Verma DPS (eds) Plant Molecular Biology Manual. v. A6, Kluwer Academic Publishers, Dordrecht, **PP.** 1-10.

Ross, D.C. and Brady, U.E., 1985. Organophosphate pesticides and DMSO effect on mycelial growth and phosphatidyl choline biosynthesis in an entomopathogenic fungus. *Journal of Entomological Sciences*, **20**:287-293.

Samson, R. A., Flannigan, B., Flannigan, M. E., Verhoeff, A. P., Adan, O.C.G. and Hoekstra, E. S., 1994. Health implications of Fungi in indoor environments. Air Quality Monographs 2. Amsterdam: *Elsevier*. pp. 531-538.

Srisukchayakul, P., Wiwat, C. and Pantuwatana, S. 2005. Studies on the pathogenesis of the local isolates of *Nomuraea rileyi* against *Spodoptera litura*. *Science Asia*, **31**:273-276.

Sridhar, V. and Deva Prasad, V., 1996. Life table studies on natural population of *Spodoptera litura* (F.) on grounfnut, *Arachis hypogea* Linn. *Annuals of Plant protection Sciences*, **4**:142-147.

Silva, L.D.A., Silva, R.F.P., Heineck, M.A. and Silva, L. 1993. In vitro evaluation of the effect of different insecticides on the sporulation of the fungus *Nomuraea rileyi*. *Anais da sociedada Entomologica do Brasil*, **22:**99-103.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution***,** doi: 10.1093/molbev/msr121.

Tang, L. C. and Hou, R. F. 2001. Effects of environmental factors on virulence of entomopathogenic fungus *Nomuraea rileyi* against the corn earworm *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Applied Entomology*, **125**:243-248.

Tigano, M. S., Faria, M. R., Lecuona, R. E., Sartori, M. R., Arima, E. Y., Diaz, B. M. and De-faria, M. R. 1995. Analysis of pathogenicity and germination of the fungus *Nomuraea rileyi* isolated in Federal district. *Anais da Sociedade Entomology do Brasil*, **24**:53-60.

Vargas, L. R. B., Rossato, M., Dasilva, R. T. and De-barros, N. M. 2003. Characterization of *Nomuraea rileyi* strains using polymorphic DNA, virulence and enzyme activity. *Brazilian. Archives of Biology and Technology*, **46**:13-18.

Vimladevi, P. S. and Prasad, Y. G. 2001. *Nomuraea rileyi*: A potential mycoinsecticide. Pages 23-38 in: Biocontrol Potential and its Exploitation in Sustainable Agriculture, Vol. 2. Insect Pests. R. K. Upadyay, K. G. Mukherji, and B. P. Chamola, eds. Kluwer Academic/Plenum Publisher, New York, NY.

Vimaladevi, P. S., Prasad, Y. G., Chowdary, D. A., Mallikarjuna Rao, L. and Balakrishnan, K. 2003. Identification of virulent isolates of the entomopathogenic fungus *Nomuraea rileyi* (F.)

Samson for the management of *Helicoverpa Armigera* and *Spodoptera litura*. *Mycopathologia*, **156**:365-373.

White, T. J., Bruns, T. D., Lee, S. B. and Taylor, J. W. 1990. Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal genes. In: Innis MA, Gelfand DH, Sninsky JJ and White TJ (eds) PCR Protocols. Academic Press, New York, **PP**. 315-22.

R.K. Patil[1*], Y.S. Bhagat[2], B. Halappa[1] and R.S. Bhat[2]

[1]Department of Agricultural Entomology, [2]Department of Biotechnology. University of Agricultural Sciences, Dharwad-580 005, Karnataka, India.

*Communication author

Email: rkpatil56@yahoo.com