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Influence of different media on growth, biomass production, sporulation and concentrations of liquid form of *Nomuraea rileyi* inoculum's on its growth, development and bioefficacy against *Spodoptera litura*

■ S.D. PATIL

Department of Entomology, Agricultural Research Station, Niphad, NASIK (M.S.) INDIA

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ABSTRACT

The entomopathogenic fungus, *Nomuraea rileyi* (Farlow) Samson was mass produced in different liquid media. The nine media of various nutrient sources were evaluated to find out most suitable medium for growth, biomass and viability of *N.rileyi.* Sabouraud's dextrose broth with yeast extract proved to be superior which gave significantly highest cfu ($8.33x10^8$/ml) and biomass (6.10g). The next best medium was Sabouraud's maltose broth with yeast extract and potato dextrose broth with yeast extract which registering cfu count of ($7.33x10^8$ and $5.67x10^8$cfu/ml) and biomass (5.63 and 4.20g), respectively. The lowest (21.67%) medium surface coverage and least biomass (1.04g) and cfu ($2.33x10^8$/ml) were registered in medium with malt extract. Thus, considering growth, development and viability of *N.rileyi* Sabouraud's dextrose broth with yeast extract (SDY) emerged as the most potential medium for biomass production and sporulation. The growth of *N.rileyi* increased with increase in concentration of inoculums in Sabouraud's dextrose broth with yeast extract. *N.rileyi* ($2x10^9$) 90.0 per cent produced highest biomass (11.17g). However, it was at par with 30.0 to 80.0 per cent *N.rileyi* producing the fungal biomass of 10.57 to 11.07g, respectively. The biomass at 10 DAI was lowest 7.63g in concentration of 10.0 per cent. Maximum ($21.67x10^8$ cfu/ml) cfu count was registered in 50 per cent concentration of *N.rileyi* aqua suspension. However, it was at par with that in 40 per cent ($20.67x10^8$cfu/ml) and 30 per cent ($19.67x10^8$cfu/ml) inoculums of aqua suspension. The increase in concentration of *N.rileyi* culture from 10 per cent (pH 8.04) to 90 per cent (pH 8.84) there was gradual increase in pH as compared the SDY medium pH (6.46) measured before adding the inoculum. Studies carried out under laboratory condition to know dose mortality response between different instars of *S.litura* and *N.rileyi* indicated that fungus performed better at its higher. Concentration ($1.8x10^7$cfu/ml) compared to lower concentrations *viz.*, $2.0x10^6$ to $1.6x10^7$cfu/ml. the *N.rileyi* was found to be highly effective to early instars of *S.litura*.

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Author for Correspondence :
Email: saurushrutu@gmail.com

INTRODUCTION

The use of entomopathogenic fungi, due to their amenability to mass production has potential in future strategies in insect pest management. Most of the orders of the insects are more or less susceptible to fungal diseases. *Nomuraea rileyi* (Farlow) Samson Moniliales, Moniliaceae is an entomofungus of cosmopolitan nature.It is being regularly observed in epizootic form on *Spodoptera litura* (Fab.) in crops like cotton,soybean and groundnut in cooler months (Manjula *et al*., 2003). *N.rileyi* infects mainly Lepidoptera, particularly economical important and polyphagous noctuid insect pests. *N.rileyi* is an entomopathogen causing natural mortality in as many as 51 Lepidopteran insects throughout the world (Lingappa and Patil, 2002). *N.rileyi* frequently cause epizootics in nature, is one promising because of its wide spread occurrence and relative abundance due to its wide host range which included many catterpiller pests. The pathogenicity of fungi towards insects has been mainly attributed to various hydrolytic enzymes such as chitinase, proteases and lipases. Progress of research on *N.rileyi* in India is slow though the results of the few studies have revealed that *N.rileyi* as a potential mycoinsecticide (Vimla Devi *et al.*, 2002). Several technics for the mass production of entomogenous fungi where design to yield infective conidia in large quantities. Wadyalkar *et al*. (2003) reported that potato dextrose broth was found to be the best in spore production for *Metarhizium anisopliae.*

Studies on the suitability of artificial media for growth and sporulation of *N.rileyi* were limited. Hence the present study was taken up to determine the suitability of media for mass culturing, standardization of concentration and pathogenicity of *N. rileyi* against different larval instars of *S. litura* under laboratory condition.

MATERIAL AND METHODS

Fungus culture :

The pure fungus culture of *N. rileyi* was made, available from isolates in Biocontrol Lab of Entomological centre, College of Agriculture, Pune.

Laboratory studies with nine culture media having three replications in Completely Randomized Design were carried out in the biological control laboratory, Department of Entomology, MPKV, Rahuri during 2009 to 2012.

Medium for mass production :

The present study was conducted for evaluation of nine media of various nutrient sources on the basis of per cent surface coverage and biomass. Nine different culture media with their respective composition per 1000 ml sterilized distilled water were used, *viz.*, Sabouraud's dextrose (SD) broth + 1 per cent Yeast extract, Sabouraud's maltose (SM) broth + 1 per cent Yeast extract, Potato peptone (PP) broth, Yeast extract glucose (YEG) broth, Potato dextrose (PD) broth, Potato maltose (PM) broth, Malt extract (ME) broth, Potato glucose (PG) broth and Potato dextrose (PD) broth + 1 per cent Yeast extract were prepared. The each empty saline bottles were filled with 40 ml medium. As such different media bottles were sterilized under 15 lbs pressure at 121^0C for 15 minutes. Each bottle was inoculated with fungal culture ($2x10^8$cfu/ml) and incubated for 10 days at room temperature 25 + 2^oC. The individual fungal mat was separated using pre-weight Whatman No.1 filter paper. The observation on per cent surface coverage on 3,7 and 10 days and biomass on 10^{th} day were recorded (Hall and Bell, 1961).

Standardization of concentration of inoculums :

The fresh fungus cultured on Sabouraud's dextrose (SD) broth + Yeast extract medium, incubated for 10 days at 25 ± 2^0 C ,was harvested in a plastic container and grinded with sterilized blender for 3 minutes. Series of dilutions of the duly grinded fresh culture of *N.rileyi* from 10 to 90 per cent concentration of bioactive ingradient (BAI) were made using distilled water as diluent. The stock samples were stored in 100 ml autoclaved sterilized saline bottle. Each preparation was evaluated for its potential for growth, development and viability of *N.rileyi* up to 10 days and bioefficacy of standardized aqua suspension (AS) 30 per cent v/v of *N.rileyi*. The experiments were replicated thrice in Complete Randomized Design.

Testing of cfu count :

The method suggested by Feng *et al.* (1990) was used. The autoclaved Sabouraud's dextrose agar with yeast extract (SDA) medium in petridishes, (100 mm diameter) was inoculated with the help of micropipette by releasing 1 ml *N.rileyi* suspension prepared in the distilled water in laminar flow cabinet. Other petridishes with the medium were prepared in similar manner and

inoculated with various dilutions in the series (10^1 to 10^{10} cfu/ ml) at 27 ± 1^0C. After 48 hrs from the 10 samples in each petridishes the numbers of colonies/petridishes were counted and cfu/ml was calculated.

Bioefficacy of standardized concentration against *S.litura* :

Laboratory experiment was carried out in Complete Randomized Design and three replications. Ten uniform sized second and third instar larvae were selected and taken in a glass container along with castor leaves as food which were directly sprayed with 10 ml desired concentration of conidial suspension *viz.*, $2x10^6$ to $1.8x10^7$ cfu/ml using hand atomizer and allowed to dry for about 15 minutes. Each larvae was transferred to a separate plastic vial (6 x 4cm) treated with antibiotics to avoid growth of other micro-organisms. Each vial containing moist filter paper at bottom with treated food. Fresh untreated castor leaves were provided to the larvae at every 24 hrs. Each treatment consisted of 10 larvae and replicated thrice. The treated larvae were incubated at room temperature at 25 ± 10°C and RH of 70 ± 10 per cent. The larval mortality was recorded at an interval of 24 hours up to 10 days. Per cent mortality was calculated and corrected by formula given by Abbott (1925) and then by arc sin square root transformation (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Medium for mass production :

The ultimate suitability of the medium for mass production of the entomopathogenic fungi was determined on the basis of growth, development and viability at 10 DAI, considering surface coverage, biomass development and cfu count, respectively. The surface coverage, biomass development and cfu count in the test media ranged from 21.67 to 100.0 per cent, 1.04 to 6.10g and $2.33x10^8$ to $8.33x10^8$cfu/ml, respectively. The nine media of various nutrient sources (Table 1) were evaluated to find out most suitable medium for growth, biomass and viability of *N.rileyi*.

Sabouraud's dextrose broth with yeast extract was significantly superior medium for the growth among all

Table 1 : Influence of the test media on growth, development and viability of *N.rileyi*

Tr. No.	Treatments	Surface coverage (%) on 3 DAI	7 DAI	10 DAI	Biomass (g/40 ml)	Sporulation (cfu) (x 10^8/ml)
T_1	Sabouraud's dextrose broth +Yeast extract	61.67 (51.77)*	100.00 (90.00)	100.00 (90.00)	6.10	8.33 (2.97)**
T_2	Sabouraud's maltose broth +Yeast extract	58.33 (49.78)	100.00 (90.00)	100.00 (90.00)	5.63	7.33 (2.80)
T_3	Potato peptone broth	11.67 (20.00)	23.33 (28.86)	21.67 (27.76)	1.47	3.67 (2.04)
T_4	Yeast extract glucose broth	31.67 (34.27)	90.00 (71.56)	98.33 (82.51)	4.47	5.33 (2.42)
T_5	Potato dextrose broth	28.33 (32.14)	66.67 (54.76)	96.67 (79.53)	2.97	3.67 (2.04)
T_6	Potato maltose broth	20.00 (26.56)	43.33 (41.15)	63.33 (52.71)	1.60	3.33 (2.03)
T_7	Malt extract	11.67 (20.00)	16.67 (24.12)	21.67 (27.76)	1.04	2.33 (1.68)
T_8	Potato glucose broth	21.67 (27.76)	63.33 (52.71)	71.67 (57.86)	1.97	3.67 (2.04)
T_9	Potato dextrose broth + Yeast extract	58.33 (49.78)	90.00 (71.56)	100.00 (90.00)	4.20	5.67 (2.48)
	S.E ±	1.58	2.33	2.37	0.11	0.10
	C.D.(P=0.05)	4.69	6.92	7.05	0.34	0.29

* Figures in parentheses indicate arc sin values. DAI = Days after inoculation

** Figures for cfu in parentheses are √n+0.5

the nine test media. It showed maximum (61.67%) surface coverage at 3 DAI. It was at par with Sabouraud's maltose broth with yeast extract (58.33%) and potato dextrose broth with yeast extract (58.33%). At 7 DAI, the both media recorded cent per cent surface coverage. However, at 10 DAI Sabouraud's dextrose broth with yeast extract was most adequate as judged from significantly highest cfu count ($8.33x10^8$/ml) and biomass (6.10g) (Fig. 1). The next best media were Sabouraud's maltose broth with yeast extract and potato dextrose broth with yeast extract registering cfu count of $7.33x10^8$/ml and $5.67x10^8$/ml with the biomass of 5.63 and 4.20g, respectively.

The lowest per cent surface coverage at 3 (11.67%), 7 (16.67%) and 10 (21.67%) DAI with least biomass (1.04g) and viability ($2.33x10^8$cfu/ml) at 10 DAI was recorded in malt extract. Thus, during the present investigation considering growth, development and viability of *N.rileyi* Sabouraud's dextrose broth with yeast extract (SDY) emerged as the most potential medium for the biomass production.

It was in conformity with Manjula and Krishnamurthy (2005) who obtained excellent growth of the *N.rileyi* in medium with similar ingredients. Im *et al*. (1988) found that yeast extract was necessary for mycelial growth while dextrose was required for sporulation. In the present study dextrose peptone and yeast extract used in SDY medium might have contributed for the significantly superior growth biomass production and sporulation of *N.rileyi*. This is in concordance with earlier work of Sharma *et al*. (2002) who reported the good growth and sporulation of *Beauveria* spp. with Sabourad's liquid medium and also recorded maximum production of biomass of *M.anisopliae.*

Standardization of inoculum concentration as bioactive ingredient :

The Sabouraud's dextrose broth with yeast extract grown *N.rileyi* cultures with $2x10^9$cfu/ml was used as stock bioactive ingredient to standardize its concentration in aqua suspension (AS) formulation. The growth was judged from per unit surface coverage while development was evaluated from biomass produced and viability from cfu/ml. The results revealed that the growth of *N.rileyi* increased with increase in concentration of inoculums in Sabouraud's dextrose broth with yeast extract. The

Table 2 : Effect of concentrations of bioactive ingredient of *N.rileyi* on growth and development in SDY medium

Tr. No.	*N. rileyi* ($2x10^9$ cfu/ml) conc.(%)	Surface coverage (%) on 3 DAI	7 DAI	10 DAI	Biomass g/40 ml medium	Sporulation (cfu) ($x10^8$/ml)	pH at 10 DAI
T_1	10.0	21.67 (27.76)*	100.00 (90.00)	100.00 (90.00)	7.63	5.67 (2.48)**	8.04
T_2	20.0	25.00 (30.00)	100.00 (90.00)	100.00 (90.00)	9.50	11.33 (3.44)	8.07
T_3	30.0	28.33 (32.14)	100.00 (90.00)	100.00 (90.00)	10.57	19.67 (4.49)	8.13
T_4	40.0	28.33 (32.14)	100.00 (90.00)	100.00 (90.00)	10.73	20.67 (4.60)	8.21
T_5	50.0	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	10.83	21.67 (4.71)	8.45
T_6	60.0	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	11.03	17.33 (4.22)	8.64
T_7	70.0	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	11.07	16.33 (4.10)	8.75
T_8	80.0	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	11.07	15.33 (3.98)	8.79
T_9	90.0	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	11.17	14.67 (3.89)	8.84
	S.E ±	1.42	-	-	0.26	0.10	-
	C.D. (P=0.05)	4.21	-	-	0.78	0.29	-

*Figures in parentheses indicate arc sin values. DAI = Days after inoculation

** Figures for cfu in parentheses are √n+0.5

highest (33.33%) growth in the form of per cent surface coverage was observed in 90.0 per cent concentration of *N.rileyi* ($2x10^9$) at 3 DAI (Table 2). However, it was at par with concentration 80.0, 70.0 and 60.0 per cent (31.67% each), 40.0 per cent (30.0%), 30.0 per cent (28.33%) and 20.0 per cent (28.33%). All the treatments (10.0 to 90.0 % inoculums) recorded cent per cent surface coverage at 7 and 10 DAI. *N.rileyi* ($2x10^9$) 90.0

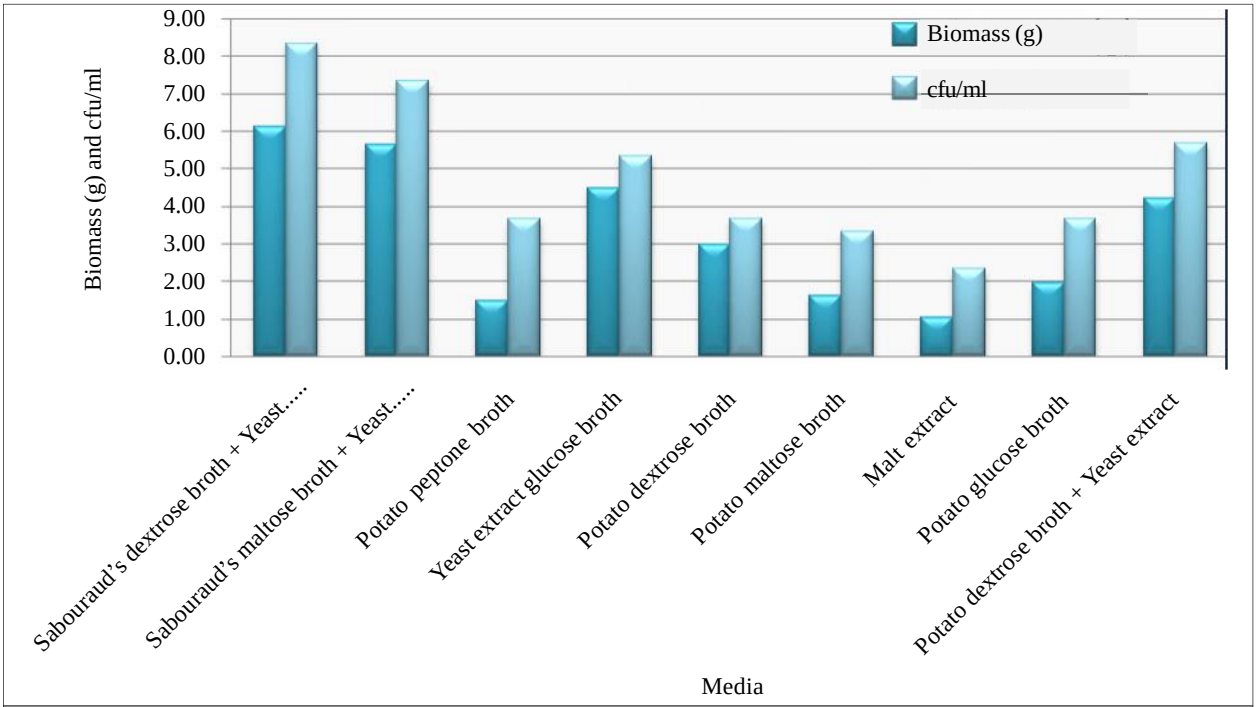

Fig. 1 : Influence of the test media on biomass and viability of *N. rileyi*

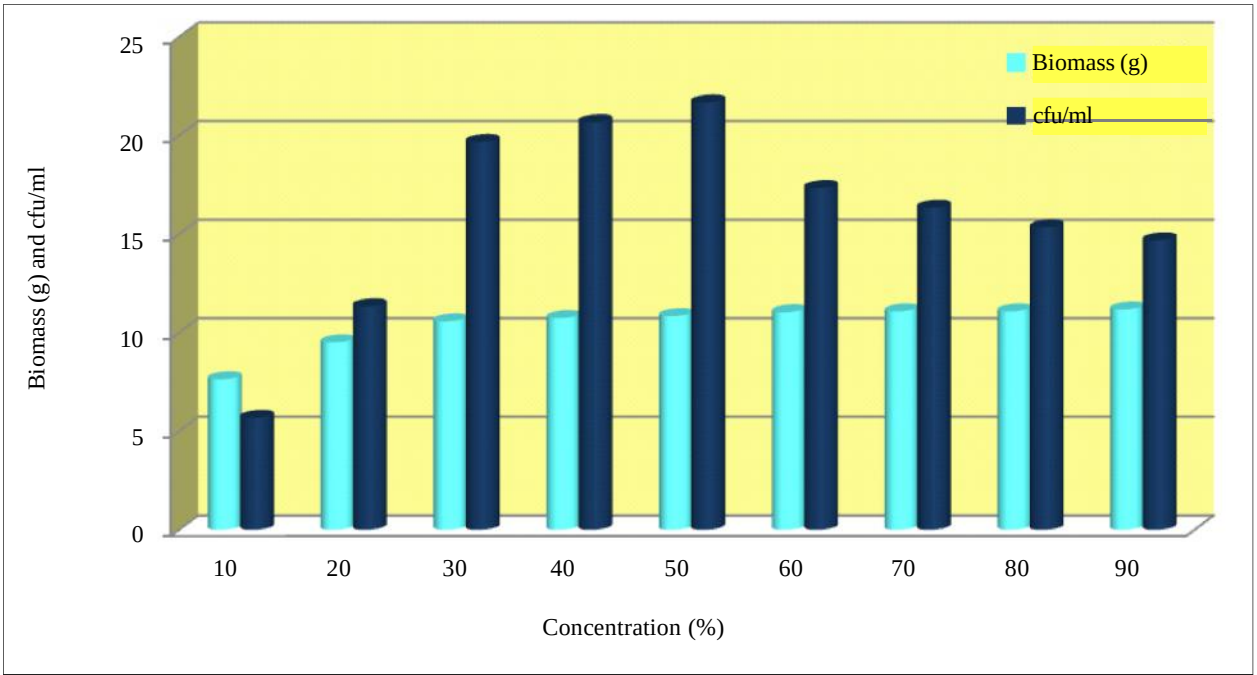

Fig. 2 : Effect of concentrations of bioactive ingredient on biomass and validity of the inoculums

per cent produced highest biomass (11.17g). The biomass at 10 DAI was lowest 7.63g in concentration of 10.0 per cent. However, it was at par with 30.0 to 80.0 per cent *N.rileyi* producing the fungal biomass of 10.57 to 11.07g, respectively. Maximum ($21.67x10^8$ cfu/ml) cfu count was registered in 50 per cent concentration of *N.rileyi* aqua suspension. However, it was at par with that in 40 per cent ($20.67x10^8$cfu/ml) and 30% ($19.67x10^8$cfu/ml) inoculums of aqua suspension (Fig.2).

The data in Table 2 clearly showed that at 10 DAI with increase in concentration of *N.rileyi* culture from 10 per cent (pH 8.04) to 90 per cent (pH 8.84) there was gradual increase in pH as compared the SDY medium pH (6.46) measured before adding the inoculum.

Thus, it showed narrow difference in biomass development in the at par treatments. It indicated that the increase in concentration from 1 to 9 times, there was no proportionate increase in biomass produced. It established that the constant quantity of medium with increased pressure of inoculum determined the quantity of biomass produced. So, increase in concentration of the inoculum in same quantity (40 ml) of medium could not produced proportionately higher biomass of the inoculum on the basis of the result. The concentration of 30.0 per cent was considered optimum for formulating the product having cfu of $2x10^9$/ml of *N.rileyi*. There is no published literature on optimization of concentration of the bioactive ingredient in aqua suspension formulation of entomopathogenic fungus.

Influence of concentrations on bioefficacy against *S.litura* :

The results of the bioefficacy of standardised aqua suspension (30% v/v) of *N. rileyi* against II and III instar larvae of *S.litura* are presented in Table 3.

The II instar larvae of *S.litura* :

The mortality of *S.litura* at 5, 7 and 10 DAT ranged from 6.67 to 46.67, 16.67 to 63.33 and 36.67 to 90.0 per cent; respectively against zero kill in untreated control. The trend of mortality was almost same in all observations. Results revealed that the highest (46.67%) mortality was observed in the concentration of $1.8x10^7$cfu/ml and found to be significantly superior to all the treatments at 5 DAT. However, it was at par with $1.6x10^7$cfu /ml (43.11%) and $1.4x10^7$cfu/ml (36.67%).

The mortality of larvae at 10 DAT was in the range of 36.67 to 90.0 per cent. The treatment with $1.8x10^6$cfu/ml (90.0%) was superior over rest of the treatments except that with $1.6x10^7$cfu/ml (83.33%). Thus, it was observed that there was increase in mortality of *S.litura* larvae with increase in concentration and duration of exposure. Next promising and at par treatments with optimum mortality were $1.0x10^7$, $1.6x10^7$ and $8x10^6$cfu/ml.

The III instar larvae of *S.litura* :

All the concentrations showed significantly higher mortality than untreated control in all the observations. The treatment with highest concentration of $1.8x10^7$ cfu

Table 3 : Influence of concentrations of 30 per cent (v/v) of *N.rileyi* culture on bioefficacy against II and III instar larvae of *S.litura*

Tr. No.	*N.rileyi* conc. (cfu/ml)	II instar Larval mortality (%)			III instar Larval mortality(%)		
		5 DAT	7 DAT	10 DAT	5 DAT	7 DAT	10 DAT
T_1	$2x10^6$	6.67 (15.00)*	16.67 (24.12)	36.67 (37.29)	3.33 (10.47)*	13.33 (21.39)	30.00 (33.21)
T_2	$4x10^6$	20.00 (26.56)	33.33 (35.24)	43.33 (41.15)	13.33 (21.39)	23.33(28.86)	36.67 (37.29)
T_3	$6x10^6$	23.33 (28.86)	33.33 (35.24)	56.67 (48.85)	16.67 (24.12)	26.67 (31.11)	50.00 (45.00)
T_4	$8x10^6$	26.67 (31.11)	36.67 (37.29)	56.67 (48.85)	20.00 (26.56)	30.00 (33.21)	50.00 (45.00)
T_5	$1.0x10^7$	30.00 (33.21)	40.00 (39.23)	60.00 (50.77)	23.33 (28.86)	33.33 (35.24)	53.33 (46.89)
T_6	$1.2x10^7$	33.33 (35.24)	43.33 (41.15)	63.33 (52.71)	26.67 (31.11)	36.67 (37.29)	56.67 (48.85)
T_7	$1.4x10^7$	36.67 (37.29)	46.67 (43.11)	66.67 (54.76)	30.00 (33.21)	40.00 (39.23)	60.00 (50.77)
T_8	$1.6x10^7$	43.33 (41.15)	53.33 (46.89)	83.33 (65.88)	36.67 (37.29)	46.67 (43.11)	76.67 (61.14)
T_9	$1.8x10^7$	46.67 (43.11)	63.33 (52.71)	90.00 (71.56)	40.00 (39.23)	53.33 (46.89)	86.67 (68.61)
T_{10}	Untreated control	0.0 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E ±	2.57	2.38	2.19	2.91	2.18	2.68
	C.D.(P=0.05)	7.69	7.14	7.29	8.73	6.55	8.03

*Figures in parentheses indicate arcsin values
DAT = Days after treatment

/ml was most promising, recording the mortality of 86.67 % at 10 DAT. However, the concentration of 1.6 x10^7cfu/ml (76.67%) was at par to it. The next promising and at par treatments were 6x10^6 to 1.4x10^7 cfu/ml resulting in 50 to 60 per cent kill of the pest. Few caterpillars in higher concentrations showed external mycosis of the entomopathogenic fungi. However, internal fungal growth was observed in body fluid of the cadaver.

In the present study, *N. rileyi* was found to be highly infective to early instars of *S.litura* than later instar. The mortality of *S.litura* increased with increase in concentrations of *N.rileyi.* The present findings is in conformity with those reported by Vimladevi (1994) and Kulkarni and Lingappa (2002). Manjula and Krishnamurthy (2005) reported similar observations for bioefficacy of *N.rileyi* against *S.litura*. Dayakar and Kanaujia (2003) observed that susceptibility of *S.litura* to *N.rileyi* decreased with increase in the age, as it was also experienced in present study. However, the concentration is required to be optimised. Considering statistically at par treatments, *N.rileyi* 8x10^6 and 1.0x10^7cfu/ml gave 56.67 and 60.0 per cent mortality of the II instar larvae against the requirement of 1.2x10^7 and 1.4x10^7cfu/ml to get equal mortality of III instar caterpillar of *S.litura*. So, concentration of 6x10^6 and 1.2x10^7cfu/ml could be considered optimum for II and III instar larvae to get more than 50 per cent mortality.

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