**Pathogenicity of subculture-I, II and insect cultures of Nomuraea rileyi (Farlow) Samson against castor semilooper, Paralellia algira Linnaeus**

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**Abstract**

The larvae of II, III and IV instars of *P. algira* treated with subculture-I, II and insect cultures of *N. rileyi* resulted larval mortalities showing positively correlated with all the concentrations of the three cultures. First sub-culture and insect culture were almost equally efficacious in causing the disease. Slightly lowered mortalities were recorded with subculture II. Reduction in larval mortalities was noticed with advancement of the age in *P. algira* larvae. Almost 100 percent larvae were dead with 1x10^8 spores ml^-1 concentration when treated in II instar and it was reduced by 5-10 percent when treated in III and IV instars significant reduced virulence were resulted in two conidial transfers of *N. rileyi* against to *P. algira*.

**Keywords:** Nomuraea rileyi, subculture, insect cultures, Paralellia algira, pathogenicity

**Materials and Methods**

**Culturing of test insect and pathogen**

Cultures of *P. algira* were maintained in the laboratory by using sterilized rearing containers. The eggs of *P. algira* were collected from castor plants from dry land farm, wet land farm at S.V. Agricultural College, Tirupati. After hatching, larvae were reared on castor leaves in the laboratory. Fresh food material was provided every time. At pre-pupal stage, the larvae were transferred to troughs containing fine sterile soil for the pupation and kept in the wire cages. The emerged adults were provided...
with suspended cotton swabs dipped in the solution of water and honey in the ratio of 3:1 for feeding the adults. The four sides of wire cages were covered with butter paper for oviposition. Eggs were collected and freshly hatched neonates were separated into the troughs containing fresh leaves of castor for experimental use. The N. rileyi available in the department was passed through the insect larvae (Spodoptera litura) and then mass produced on Saboraud’s maltose agar fortified with yeast. First, second subcultures and insect culture of N. rileyi were used for the bioassay studies.

**Application of N. rileyi**

Stock suspensions of 1x10^8 spores ml⁻¹ were prepared in distilled water, measuring the density of spores with Neubaur haemocytometer and a compound microscope. Then, serial dilutions were prepared. Castor leaves were cleaned with cotton swab and placed into plastic troughs lined with filter paper inside. Seven concentrations of N. rileyi viz., 1x10^8, 1x10^7, 1x10^6, 1x10^5, 1x10^4, 1x10^3 and 1x10^2 spores ml⁻¹ were used for infecting the larvae under each culture. For each concentration, 10 uniform sized 2, 3 and 4 instars of just moulted larvae were selected with the help of hand automizer. N. rileyi spore suspensions were sprayed on the larvae in petriplates separately for the treatments. Cap after 5 mins, the treated larvae were transferred into troughs. The experiment was replicated thrice and carried out under room temperature of 25±2°C and 80 percent relative humidity. Untreated control was also maintained with water spray on larvae. Daily observations on symptoms of infection, larval mortalities were recorded.

**Results and Discussion**

The maximum mean larval mortality of 96.55 and 95 percent were obtained with 1x10^8 spores ml⁻¹ concentrations of subculture-I and insect culture (Table 1), while 92.22 percent mean mortality was obtained with subculture-II (Table 2). In all the three cultures, death of larvae was gradually reduced with the lowering of concentration of spores. More than 50 percent larval mortality was recorded with the concentrations 1x10⁴ and above. A significant difference in mortality was observed in all the instars tested with various concentrations. From the observations, the subculture-II of N. rileyi was recorded little fewer efficacies towards larval mortality when compared to the insect and subculture-I. Hence, significant reduced virulence was resulted in two conidial transfers of N. rileyi against P. algira. This findings confirms the previous reports by Morrow et al. (1989) indicated that serial sub-culturing of N. rileyi alters both growth and development on in vitro and in vivo substrates. According to him, six conidial transfers on SMAY plates, resulted in reduced virulence against Anticarsia gemmatalis larvae and after 16th conidial transfer, progeny conidia became avirulent. Similarly reduced virulence on repeated conidial subculturing of Metahizium anisopliae against H. armigera was reported by Pallavi et al. (2008).

**Influence of larval age on N. rileyi infection**

The maximum (100 percent) infection was recorded in second and third instars of P. algira with higher concentration (1x10^8 spores ml⁻¹) of sub-culture-I of N. rileyi. Fourth instar P. algira was also susceptible upto to 93.66 percent with the above concentration. More than 50 percent second instar larvae were dead even with 1x10⁵ spores ml⁻¹. Whereas, it was 34.00 and 19.00 percent with respect to third and fourth instars respectively. Similarly second and third instar larvae were observed to be more susceptible than fourth instar to second subculture also.

The observed mortalities with 1x10⁴ to 1x10⁵ spores ml⁻¹...
Table 1. Mortality of *P.algira* larvae due to subculture-I of *N.rileyi*

<table>
<thead>
<tr>
<th>Concentration of <em>N.rileyi</em> (Spores ml⁻¹)</th>
<th>Percent larval mortality</th>
<th>Mean (% larval mortality)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Instar II</em></td>
<td><em>Instar III</em></td>
</tr>
<tr>
<td>1 x 10⁴</td>
<td>100.00(90.00)</td>
<td>97.33(84.52)</td>
</tr>
<tr>
<td>1 x 10³</td>
<td>92.66(74.39)</td>
<td>79.66(53.20)</td>
</tr>
<tr>
<td>1 x 10²</td>
<td>79.33(53.20)</td>
<td>56.66(44.04)</td>
</tr>
<tr>
<td>Untreated control</td>
<td>0.00(0.00)</td>
<td>0.00(0.00)</td>
</tr>
</tbody>
</table>

SEm ± CD (P=5%)

- Instars 0.72 2.05
- Concentrations 1.18 3.35
- Instar x concentrations 2.04 5.81

Figures in the parentheses are angular transformed values. *Mean of three replications. Figures indicated with same alphabet(s) are statistically insignificant.

The larval mortalities obtained in the present study were in the range of 34-90 percent for II instar, 27 to 82 percent for III instar and 16-79 percent for IV instar of *P.algira*.

When insect culture was applied to second and third instar larvae of *P. algira*, 100 and 95 percent mortalities were obtained at higher concentration i.e. 1x10⁸ spores ml⁻¹. Larval mortality of 90.00 percent recorded in case of IV instar larvae. The concentrations 1x10⁴ to 1x10⁷ spores ml⁻¹ recorded 60 to 91 percent, 40-82 percent and 51 to 84 percent in second, third and fourth instars respectively. The lowest concentration i.e., 1x10⁵ spores ml⁻¹ also recorded nearly 27 percent larval mortality in all the three instars. In all the three types of cultures tested, as the age of the larvae advanced there was decrease in mortality rate. The fungal spores need to germinate and penetrate through the integument for infection to occur, the increased toughness of the cuticle in grown up larvae prohibits fungal development further. The above observations are in accordance with the findings of Habib and Patel (1990) who reported that third instar larvae of *S.fragiperda* was susceptible than fourth instar when infected with *N.rileyi* with concentrations of 1.03x10⁷ and 1.2x10⁷ conidial spores ml⁻¹ applied topically to maize leaves on which larvae were fed. Susceptibility decreased with increasing the age of *S.littoralis* to *N.rileyi* (Fargues and Rodriguez, 1980). Boman (1981) reported chemical constituents vary with increasing the larval age results in hardening of the cuticle and increased hormonal defense mechanisms to the microbial infections, leads to lesser susceptibility of later instars. Khan and Rajak (1986) reported that, first two instars of *H.armigera* were highly susceptible to *B.bassiana*. The present results showed significant pathogenicity of *N. rileyi* to the semi-looper, *P. algira* and corroborate with the findings of several workers on effects of entomopathogenic fungi on lepidopteran larvae.

Kulkarni and Lingappa (2002), applied *N.rileyi* at different concentrations viz., 1.2x10⁸ to 1.2x10⁴ conidia L⁻¹.
Fig. 1. Mortality of *P. algira* larvae due to subculture-II of *N. rileyi*

Table 2. Mortality of *P. algira* larvae due to *N. rileyi*

<table>
<thead>
<tr>
<th>Concentration of <em>N. rileyi</em> (Spores ml⁻¹)</th>
<th>Percent larval mortality</th>
<th>Mean (% larval mortality)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Instar II</em></td>
<td><em>Instar III</em></td>
</tr>
<tr>
<td>1 x 10⁵</td>
<td>100.00(90.00)</td>
<td>95.00(79.45)</td>
</tr>
<tr>
<td>1 x 10⁷</td>
<td>91.33(72.88)</td>
<td>78.66(62.54)</td>
</tr>
<tr>
<td>1 x 10⁸</td>
<td>84.66(67.01)</td>
<td>79.00(62.83)</td>
</tr>
<tr>
<td>1 x 10⁷</td>
<td>75.66(60.74)</td>
<td>54.66(47.68)</td>
</tr>
<tr>
<td>1 x 10⁴</td>
<td>58.33(49.82)</td>
<td>42.00(40.39)</td>
</tr>
<tr>
<td>1 x 10⁵</td>
<td>45.33(42.31)</td>
<td>38.33(38.25)</td>
</tr>
<tr>
<td>1 x 10²</td>
<td>34.33(35.84)</td>
<td>26.33(30.85)</td>
</tr>
<tr>
<td>Untreated control</td>
<td>0.00(0.00)</td>
<td>0.00(0.00)</td>
</tr>
</tbody>
</table>

Mean: 61.20(52.33) 51.75(45.25) 55.25(46.83) 56.06(48.13)

SE of CD (P=5%)

Instars: 0.71 2.03
Concentrations: 1.17 3.32
Instars x concentrations: 2.02 5.76

Figures in the parentheses are angular transformed values. * Mean of three replications. Figures indicated with same alphabet(s) are statistically insignificant on the larvae of different noctuids viz. *Spodoptera litura*, *Achoea janata*, *Cydia pychora* Meyrick, *Mythimna separata* Walker, *Earias vitella* F. and *Galleria mellonella* L. highest concentration of 1.2x10⁸ spores ml⁻¹ resulted in maximum mortalities of all the noctuids. They also stated that *S. litura* and *A. janata* were relatively susceptible species than others tested. Rao and Phadke (1977) found that a dense aqueous spore suspension of the fungus, *N. rileyi* caused 100.00 percent mortality of *S. litura* larvae. Lezama et al. (1993) reported 100 percent mortality of II, III, IV and V instars of *Spodoptera frugiperda*, at 1x10⁸ spores ml⁻¹ concentration. Vimaladevi (1994), recorded
cumulative mortality of 88-97 percent in S. litura with N. rileyi at 2x10\(^{11}\) spores ml\(^{-1}\). Gopalakrishnan and Narayanan (1989) sprayed N. rileyi spores on III instar larvae of H. armigera and observed 100 percent mortality.

Goh et al. (1992) stated that application of N. rileyi at 1x10\(^{7}\) spores ml\(^{-1}\) concentration caused 50-76% mortality in first to fourth instar larvae of S. litura, whereas fifth instar was susceptible upto 36 percent only. Faria et al. (1993), reported the hundred percent infection of N. rileyi in a population of Anticarsia gemmatalis (Hubner) in soybean crop in Federal district, Brazil in 1990-91. The findings of the present study had concluded that N. rileyi is pathogenic to castor semilooper, Paralellia algira. Repeated conidial transfers lead to reduced virulence of the fungus and virulence is regained when it is passed through any host insect.

References


