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# Preparation of Certain Dry Formulations of *Nomuraea rileyi* and Their Evaluation in Terms of Viability of Conidia

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**ABSTRACT**

Dry formulations of *Nomuraea rileyi* were prepared by using different carriers viz., Talc powder, Wheat flour, Corn flour, Broken rice flour and Vermiculite. On the Day of Storage (ODS), the highest conidial germination percent of 94.50 was recorded with the inert material, corn flour. In talc formulation, 93.00 per cent germination was recorded. The mean conidial germination at the time of storage was 91.59 per cent. The viability of *N.rileyi* conidia was reduced with the storage period. Vermiculite formulation and wheat flour formulation failed to record more than 50 per cent of conidial germination with in 4 and 5 months after storage. The talc and corn flour formulations were able to hold 42.75 and 44.00 per cent of conidia in viable conditions. If the storage was for only two months, the conidial viability was highest with corn flour and rice flour.

**Key words :** Corn flour, Nomuraea rileyi, Rice flour, Talc, Wheat flour, Vermiculite, Viability.

The inappropriate use of chemical pesticides continues to pose threats to environment and human health. There is an obvious need to find effective alternative approaches with minimal deleterious effects. The bioagents used as pesticide are environmentally safe, selective, specific in their action and easily biodegradable. Among the bioagents the best green alternative is microbial agents like viruses, bacteria, fungi etc. The entomopathogenic fungi have great potential for integrated pest management programmes due to their specifity, mode of action, ease of application. Of these, *Nomuraea rileyi (*Farlow) Samson is an important entomofungal pathogen causing regular epizootics in lepidopteran caterpillars in different parts of India including Andra Pradesh (Manjula *et al.* 2003). It shows a high degree of pathogenicity to several lepidopteran caterpillars under laboratory conditions. Environmental conditions greately affects these fungi decreasing the field viability and persistence. These fungi failed to form the epizootics under low relative humidity with higher temperature. In combating this problem, development of suitable formulation plays an important role. In formulation, priority is the retention of viability and virulence of the infective units during storage and application. Formulation is mandatory in order to enhance spore application and efficacy (Angus and Luthy, 1971; Soper and Ward, 1981). Formulation is nothing but a technical concentrate of an organism that has been formulated. The present study was conducted to see the duration of retention of viability of *Nomuraea rileyi* spores in the formulations which is an important criteria to use as bioagent against insect pests.

## MATERIAL AND METHODS

The studies were carried out during 2010-2011 at the Department of Entomology, S.V. Agricultural college and Regional Agricultural Research Station, Tirupati. The *N.rileyi* culture available in the Department was retrieved and mass multiplied on Saboraud's maltose agar medium fortified with one percent yeast extract (SMAY). After attainment of sufficient quantity of *N.rileyi* culture, spores on agar media were harvested under asceptic conditions. The spore mass of 2 g weight was taken for mixing with inert material in each treatment.

### Preparation of inert material and formulation making:

For preparation of dry formulations of *N.rileyi* the inert materials viz., Talc powder, Wheat flour, Corn flour, Broken rice flour and Vermiculite, were selected. Talc powder, wheat flour and corn flour were taken as such they are available. Broken rice flour was obtained by grinding the broken rice to get powder. Vermiculite was collected from a tank in

Table 1. Viability of *N.rileyi* in terms of germination of conidia in dry formulations.

| Formulations | Per cent germination of *N.rileyi* spores (DAS-Days after Storage, ODS- On the Day of Storage) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ODS | 30 DAS | 60 DAS | 90 DAS | 120DAS | 150DAS | 180 DAS | 210 DAS | 240 DAS |
| Talc | 93.00$^{ab}$ | 90.00$^{a}$ | 84.38$^{ab}$ | 80.00$^{a}$ | 75.00$^{a}$ | 59.75$^{b}$ | 55.35$^{a}$ | 50.50$^{a}$ | 44.00$^{a}$ |
| | (74.74) | (71.62) | (66.82) | (63.44) | (60.00) | (50.62) | (48.07) | (45.29) | (41.99) |
| Wheat flour | 90.26$^{c}$ | 85.00$^{b}$ | 81.75$^{c}$ | 73.65$^{c}$ | 68.50$^{c}$ | 52.50$^{c}$ | 42.25$^{d}$ | 34.00$^{d}$ | 30.00$^{d}$ |
| | (71.84) | (67.21) | (64.71) | (59.11) | (55.86) | (46.43) | (40.54) | (35.67) | (33.21) |
| Corn flour | 94.50$^{a}$ | 90.00$^{a}$ | 83.90$^{b}$ | 78.50$^{b}$ | 72.50$^{b}$ | 65.50$^{a}$ | 51.00$^{b}$ | 48.75$^{b}$ | 42.75$^{b}$ |
| | (76.58) | (71.64) | (66.34) | (62.38) | (58.37) | (54.03) | (45.57) | (44.28) | (40.40) |
| Broken rice flour | 90.69$^{bc}$ | 85.36$^{b}$ | 86.00$^{a}$ | 79.00$^{b}$ | 64.50$^{d}$ | 51.50$^{d}$ | 45.73$^{c}$ | 41.75$^{c}$ | 33.00$^{c}$ |
| | (72.23) | (67.51) | (68.03) | (62.73) | (53.43) | (45.86) | (42.55) | (40.25) | (35.06) |
| Vermiculite | 89.50$^{c}$ | 82.00$^{c}$ | 75.00$^{d}$ | 66.45$^{d}$ | 58.38$^{e}$ | 46.25$^{e}$ | 40.50$^{e}$ | 32.90$^{e}$ | 24.63$^{e}$ |
| | (71.14) | (64.90) | (60.00) | (54.61) | (49.82) | (42.85) | (39.52) | (35.00) | (29.75) |
| General Mean | 91.59 | 86.47 | 82.21 | 75.52 | 67.78 | 55.10 | 46.97 | 41.58 | 34.88 |
| | (73.31) | (68.58) | (65.18) | (60.45) | (55.50) | (47.96) | (43.25) | (40.10) | (36.08) |
| S.Ed. | 1.16 | 0.89 | 0.96 | 0.44 | 0.41 | 0.38 | 0.50 | 0.24 | 0.43 |
| C.D.(0.05) | 2.46 | 1.89 | 2.04 | 0.95 | 0.88 | 0.81 | 1.07 | 0.52 | 0.92 |

- The values are means of four replications.
- Figures in the paranthesis are angular transformed values.
- The values indicated by the same alphabet are not significantly different.

Rompicherla mandal. The clumps of vermiculite were broken with stone and made into powder. Hundred ml of distilled water was added to 100 gm of each carrier, and the mixtures were autoclaved for 20 minutes under 15 psi pressures at 121$^{0}$c. Then the mixtures were placed in an oven at 120$^{0}$c for three hours on each day for seven days until completely dry. After the carriers were completely dried, they were powdered, cooled and mixed each with 2 g of harvested *N.rileyi* spores under asceptic conditions in laminar air flow chamber along with 2-3 drops of Tween 20 as a wetting agent. The prepared formulations of *N.rileyi* were transferred to sterilized polythene bags separately, tightly packed and incubated at 20$^{0}$c.

**Testing the viability of prepared dry formulations:**

Equal weights of dry formulations were taken and definite quantity of stock suspensions of spores were prepared by adding wetting agent and filtering through double layered muslin cloth. Then diluted spore suspension was taken in to cavity slide. The humidity chamber was prepared by arranging moistened cotton in petriplates. The cavity slide along with spore suspension was placed in the prepared humidity chamber and it was incubated at 20 ± 4$^{0}$c. At six hours and twelve hours intervals, the spore suspension was observed under microscope for counting the germinated spores for testing the viability of spores with different carriers.

Under the microscopic observation the lemon shaped spores of *N.rileyi* and the conidia showing germ tube among them were counted in one microscopic field. By these observations the per cent germination of spores were calculated using the formulae

$$\text{Per cent germination conidia} = \frac{\text{No.of conidia germinated}}{\text{Total no fo conidia observed}} \times 100$$

The data on percent germination was recorded in monthly intervals for each formulation and were subjected to suitable statistical analysis to draw the inference.

**RESULTS AND DISCUSSION**

On the Day of Storage (ODS), the highest conidial germination percent of 94.50 was recorded with the inert material corn flour. The viability of conidia in talc formulation was on par with corn flour

formulation (93.00 %). In broken rice flour and wheat flour formulations also more than 90.00 per cent conidial germination was recorded, to which vermiculite also was on par (89.50 % germination). The mean conidial germination at the time of storage was 91.59 per cent. After 30 days of storage, conidia present in two inert materials i.e. talc and corn flour showed 90 per cent of germination (Table 1). The mean per cent conidial germination after 30 and 60days of storage was 86.47 and 82.21 respectively. After 90 Days of Storage (90 DAS), mean per cent conidial germination was reduced to 75.52 per cent. Except talc, in all the other formulations less than 80 per cent germination of *N.rileyi* conidia was observed. After 120 days of storage (120 DAS), the lowest of 58.38 per cent was recorded with vermiculite. Talc continued to be superior inert material with highest per cent of conidial germination (75.00). After 180 days of storage (180 DAS), the mean per cent conidial germination was 46.97. Conidia in talc germinated to the extent of 55.35 per cent, followed by corn flour (51.00%). The least conidial germination of 40.50 per cent was recorded with vermiculite. After 240 days of storage (240 DAS), none of the five formulations found to show 50 per cent germination. The mean per cent germination reduced to 34.88. The talc showed viability per cent of 44.00 per cent (Table 1)

The present results indicate that the viability of *N.rileyi* conidia reduced with the increase in storage period. The Broken rice flour showed less reduction of per cent of conidial germination of 12.89 from the day of storage to 90 days after storage. This was followed by talc which resulted in 13.97 per cent reduction in conidial germination from the day of storage to 90 days of storage. After 240 days of storage, in talc and corn flour comparatively lower per cent reduction of conidial germination i.e. 51.88 and 55.55 respectively was recorded (Table 1). Ramegowda (2005) evaluated the viability of *N. rileyi* conidia in the wettable powder formulation containing different carrier materials and storage environments. He mentioned that viability of spore was significantly influenced by the duration of storage.

In the present results, the talc was found to be the best carrier material for maintenance of viability of *N.rileyi* conidia. As explained by other workers, talc as good carbon source useful for the fungal conidia to be alive. On the other hand, the vermiculite showed the least germination percentage. This may be due to less nutrient status of vermiculite compared to other inert materials.

The present findings with respect to superiority of the talc are in close agreement with the findings of following two scientists. Das *et al.* (2006) formulated *Trichoderma harzianum* with different carriers viz., starch, talc and molasses in nine combinations. Among nine formulations tested, starch showed maximum sporulation upto 60 days but from 60 days onwards talc based formulation gave higher sporulation than starch. Srikanth *et al.* (2006) reported that talc formulations showed highest viability of *Beauveria brongniartii* up to 9 months.

In the present study after the talc, corn flour was observed to be the suitable media for keeping nearly 50.00 percent of *N.rileyi* conidia in viable conditions up to 210 days. Rice flour occupied next position for the favour to *N.rileyi* (41.75 %). Whereas wheat flour was able to maintain 34.00 per cent viable conidia. Richness of carbohydrates in corn and rice may be the reason. The results are similar with the observations of Mohammad *et al.* (2007) who developed formulations of *B.bassiana* with various inert materials and evaluated the efficacy. They confirmed that corn flour formulation was more efficient inert material for vectoring *B.bassiana* to flower crops.

According to (Ramegowda, 2005), Rice flour, talc and sorghum flour were best among carrier materials evaluated, while skimmed milk powder and gram flour appeared to be non-suitable for *N. rileyi*. Padmanaban *et al*. (2009) prepared wettable powder formulations of *B. bassiana* with different carriers viz., ragi flour, sorghum flour, rice chaffy grain, maize flour and wheat bran. With the results, they concluded that rice chaffy grain and maize flour formulations recorded significantly higher spore production of 6 x $10^{10}$ and 1 x $10^{9}$ cfug$^{-1}$ respectively.

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