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PREPARATION OF GRAIN FORMULATIONS OF NOMURAEA RILEYI (FARLOW) SAMSON AND EVALUATION OF VIABILITY OF SPORES

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ABSTRACT

Grain formulations of *N. rileyi* were prepared by mass producing the fungus on six different grain media *viz.,* rice, wheat, jowar, bajra, maize and ragi for evaluating the viability of *N. rileyi* conidia during 2013-14. After observing full sporulation on grain media (20 days after inoculation), the spore mass along with media were dried, pulverized, sieved and packed in polythene bags. These formulations were stored at two different conditions *i.e.,* refrigeration (at 4ºC) and incubation (at 25ºC). The viability of *N. rileyi* conidia were recorded at monthly intervals upto 90 days. The results indicated that viability of spores decreased gradually with increase in storage period. Among the grain formulations tested, maize grain formulation has shown higher viability upto 90 days of storage (86.67 to 83.33 per cent germination). The lowest of 63.33-60 per cent germination of conidia was recorded with ragi grain formulation due to its low nutrient status. In rice, jowar, wheat grain formulations 66.67 to 76.67 per cent germination was noticed. Under the storage condition of refrigeration, slightly higher germination percentage was noticed in all the formulations.

KEY WORDS: Conidial germination, Days after storage, Grain formulations, Viability

INTRODUCTION

Among the various methods recommended for controlling of insect pests, biological control methods are inevitable and they are effective, ecofriendly and economical components of IPM. *Nomuraea rileyi* is an ideal entomopathogenic fungus and also an important mortality factor of many lepidopteran insects throughout the world (Lingappa and Patil, 2002). It occurs mainly in cooler months *i.e.,* in *rabi* season**.**

N. rileyi is unable to form epizootics under low relative humidity conditions with higher temperature. Development of suitable formulations of *N. rileyi* would be of significant importance to use against problematic lepidopteran insect pests in different crops grown in *rabi* season.

MATERIAL AND METHODS

Grain formulations of *N. rileyi* were prepared by using broken grains of sorghum, bajra, jowar, rice, wheat and ragi during 2013-14. Each grain of 30 g was soaked with one per cent yeast extract solution (30 ml 30 g^{-1}) for overnight, in plastic troughs (each grain separately in each trough). After soaking, the grains were filled in conical flasks of 250 ml capacity, then the flasks were plugged with cotton and autoclaved at 15 psi and 121°C for 30 minutes. After cooling, circular agar disc of 10 mm diameter was taken from the actively growing *N. rileyi* culture on SMAY plates and inoculated one into each bottle. The flasks were incubated in BOD chamber at 22°C.

After observing full sporulation on grain media (20 days after inoculation), the solid grains along with spore mass of *N. rileyi* was dried and grinded with a mixer grinder. It was sieved for removing coarse material. The material was separated into two halves after adding of 0.02 per cent of tween-20. One half was stored in refrigerator (at 4°C) and another half in incubator (at 25°C).

For testing the viability of conidia in each formulation, 0.5 gm of material was weighed separately and mixed with 100 ml of sterile distilled water (after adding 2-3 drops of Tween- 20) in 250 ml beakers. Later, this suspension was serially diluted for three times to get 1×10^5 spores ml^{-1} concentration. Two to three drops of spore suspension was placed in cavity slide. The cavity

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slide, was placed in the humidity chamber which was prepared by arranging moistened cotton in petriplates and it was incubated at 22°C. At 12 hours intervals, the spore suspension was observed under microscope for counting the germinated spores. The germination percentage of *N. rileyi* was calculated based on the number of spores germinated in spore suspension of each formulation. The germination tests were carried out at the time of storage and later at monthly intervals upto 90 days after storage (DAS).

RESULTS AND DISCUSSION

1. Viability of *N. rileyi* conidia in grain formulations stored at refrigerated conditions (at 4°C)

Thirty days after storage (30 DAS): Mean conidial germination was 86.67 per cent at 30 days after storage. Maize grain formulation recorded more than 90 (93.33) per cent of germination, where as rice and jowar grain formulations recorded 90 per cent of conidial germination with no significant difference. Wheat, bajra and ragi recorded 86.67, 83.33 and 76.67 per cent of conidial germination respectively.

Sixty days after storage (60 DAS): Only maize grain formulation recorded 90 per cent of conidial germination even after 60 days of storage, followed by 86.67, 83.33, 80, 76.67 and 70 in rice, jowar, wheat, bajra and ragi grain formulations respectively.

Ninety days after storage (90 DAS): Maize grain formulation was found superior among all the grain formulations by recording higher conidial germination of 86.67 per cent followed by rice grain formulation (80 per cent). Jowar, wheat and bajra recorded 76.67, 73.33 and 66.67 per cent of conidial germination respectively. The least conidial germination of 63.33 per cent was recorded with ragi grain formulation (Table 1).

2. Viability of *N. rileyi* conidia in grain formulations incubated at 25°C:

Thirty days after storage (30 DAS): The mean conidial germination recorded was 86.11 per cent at 30 days of storage. Maize grain formulation recorded 93.33 per cent of conidial germination followed by rice grain (90 per cent). Jowar and wheat grain formulations were statistically on par with each other by recording 86.67 per cent of conidial germination of *N. rileyi*, where as bajra and ragi grain formulations recorded 83.33 and 76.67 conidial germinations respectively (Table 2).

Sixty days after storage (60 DAS): All the formulations were statistically different to each other with respect to conidial germination. The mean conidial germination after 60 days of storage was 76.67 per cent. The highest conidial germination was recorded in maize (86.67 per cent)

Ninety days after storage (90 DAS): Maize grain formulation was found superior by recording 83.33 per cent of conidial germination followed by rice grain formulation (80 per cent). Least conidial germination (60 per cent) was recorded with ragi grain formulation. The viability of conidia in wheat and bajra grain formulation were 70 and 66.67 per cent. The mean conidial germination was 72.78 per cent, at 90 days of storage.

In the present study, the refrigerator stored grain formulations shown 4 per cent higher response with regard to viability at 90 days of storage, when compared to incubator stored formulations.

In both storage conditions *i.e.,* at incubator and refrigerator stored conditions, maize grain formulation showed higher viability when compared to other formulations. The superiority of maize grain to act as carrier material for *N. rileyi*, may be due to the higher (80 per cent 100 g^{-1}) carbohydrate and protein content (11 per cent 100 g^{-1}). Reddy (2008) recorded 13.7 per cent of moisture content 100 g^{-1}, carbohydrate content of 79 per cent 100 g^{-1} and protein content of 6.8 g 100 g^{-1} present in rice grains. In sorghum grains, 72.4 per cent of carbohydrate and 10.4 g 100 g^{-1} of protein were recorded.

Next best treatments after maize grain formulation were rice grain formulation and sorghum grain formulation which recorded 80 and 80, 76.67 and 76.67 per cent viability of *N. rileyi* conidia even after 3 months of storage in refrigerator and incubator stored conditions respectively.

Swetha (2010) reported that after 30 days of storage, corn flour mixed *N. rileyi* conidia (67.50%) showed relatively higher mortality of *Spodoptera litura* Fabricius larvae followed by broken rice flour (62.50%). After 3 months, they recorded 32.50 per cent and 25.00 per cent mortalities respectively.

Ragi grain formulation recorded least germination percentage of spores of *N. rileyi* in both storage conditions. This may be due to the fiber content (3.6 per cent 100 g^{-1}) present in ragi grains and also clumping of grains at the time of autoclaving of grain media. Kulkarni (1999) reported that less amylase content (6-18 per cent) and formation of clumping in ragi grain were responsible for

Table 1. Viability of *N. rileyi* in terms of germination of conidia in refrigerator stored grain formulations

Formulations	Per cent germination of *N. rileyi* spores		
	30 DAS	60 DAS	90 DAS
Maize	93.33[a] (75.10)	90.00[a] (71.62)	86.67[a] (68.64)
Rice	90.00[ab] (71.58)	86.67[ab] (68.62)	80.00[b] (63.45)
Jowar	90.00[ab] (71.58)	83.33[bc] (65.93)	76.67[c] (61.12)
Wheat	86.67[c] (68.64)	80.00[d] (63.44)	73.33[d] (61.13)
Bajra	83.33[d] (65.92)	76.67[e] (61.15)	66.67[e] (54.75)
Ragi	76.67[e] (61.15)	70.00[f] (56.79)	63.33[f] (52.73)
General mean	**86.67**	**81.11**	**74.44**
SE(m)	**0.83**	**0.73**	**0.74**
C.D.(0.05)	**2.31**	**2.03**	**2.06**

DAS: Days After Storage
The values are means of three replications.
Figures in the parentheses are angular transformed values.
Mean followed by same letter in the column do not differ significantly by DMRT (p = 0. 0 1)

Table 2. Viability of *N. rileyi* in terms of germination of conidia in incubator stored grain formulations

Formulations	Per cent germination of *N. rileyi* spores		
	30 DAS	60 DAS	90 DAS
Maize	93.33[a] (75.10)	86.67[a] (68.60)	83.33[a] (65.91)
Rice	90.00[ab] (71.67)	83.33[b] (65.91)	80.00[ab] (63.45)
Jowar	86.67[bc] (68.67)	80.00[c] (63.55)	76.67[b] (61.12)
Wheat	86.67[bc] (68.64)	73.33[d] (58.93)	70.00[c] (56.80)
Bajra	83.33[c] (65.91)	70.00[e] (56.80)	66.67[c] (54.75)
Ragi	76.67[d] (61.13)	66.67[f] (54.74)	60.00[d] (50.78)
General mean	**86.11**	**76.67**	**72.78**
SE(m)	**1.04**	**0.66**	**0.79**
C.D.(0.05)	**2.78**	**1.83**	**2.22**

DAS: Days After Storage
The values are means of three replications.
Figures in the parentheses are angular transformed values.
Mean followed by same letter in the column do not differ significantly by DMRT (p = 0. 0 1)

lower (interfered with efficient harvest of spores and thus led to low productivity) conidial yield production of *N. rileyi* in ragi grain formulation.

Ramegowda (2005) confirmed corn flour as more efficient inert material for preparation of *N. rileyi* formulation. He also reported that the broken rice flour also showed considerably higher larval mortalities of *Helicoverpa armigera* Hubner (47.00 to 62.50 per cent) upto two months, then reduced mortalities up to four months thereafter no mortalities were found.

Nankinga and Moore (2000) reported that application of *Beauveria bassiana* maize flour formulated wettable powder at the rate of 2×10^6 conidia per ha proved most effective in reducing the banana weevil (*Cosmopolites sordidus*) population by 65.72 per cent within 8 weeks after a single application.

Babi Neeraja (2008) reported that the crushed sorghum and rice grains with one per cent yeast extract comparatively as favorable food media for the faster as well as higher spore production of *N. rileyi* in 15 days period (2.4×10^9 and 2.1×10^9 spores ml^{-1} respectively). Maize and Bajra also produced considerably good sporulation. Ragi and wheat grains have proved inferior for spore production.

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