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PREPARATION OF GRAIN FORMULATIONS OF *NOMURAEA RILEYI* (FARLOW) SAMSON AND TESTING VIRULENCE AGAINST 3RD INSTAR OF *SPODOPTERA LITURA*

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ABSTRACT
Lab experiment was conducted at department of Entomology, S.V Agricultural College, Tiruati during 2013-14 to evaluate the efficacy of grain formulations of *Nomuraea rileyi* against 3rd instar larvae of *Spodoptera litura*. Among the different treatments tested, Maize grain formulation recorded highest virulence (ranging from 57 to 67 percent at 4ºC and 47 to 57 percent at 25ºC). Lowest virulence was recorded in Ragi grain formulation (ranging from 30-40 at 4ºC and 20 to 27 at 25ºC). The grain formulations stored at 4ºC were shown 10 percent higher response with regard to virulence when compared to grain formulations stored at 25ºC. The mortalities gradually reduced with concentrations (showing least at 1×10^5spores ml^{-1} and highest at 1×10^8spores ml^{-1}).The larval mortalities are positively associated with concentrations and negatively associated with storage period.

INTRODUCTION

The cutworm *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), is a major destructive polyphagous pest of subtropical and tropical crops, causing serious economical losses (Rao *et al.,* 1993). Concerns about the negative effects of chemical insecticides have led to emphasis on alternative strategies for pest control. Pest management involving biocontrol agents is assuming prominence and have been considered as an important strategy in insect population reduction. Among the various fungal biological control agents, *Nomuraea rileyi* is an ideal dimorphic hypomycete, causes fungal epizootics in several noctuid pests (Ignoffo *et.al.,*1989). It is a important mortality factor of many lepidopteran insects throughout the world (Lingappa and Patil, 2002). It occurs in epizootic form in the humid regions and easily multiplied at a low cost and hence it has a great potential for developing as a myco-insecticide. Inoculum production at competitive cost is the primary step in popularizing any microbial agent. Therefore, in this study six different grain media were tried for mass multiplication to make it very cost efficient and to prepare the grain formulations of the fungus *N. rileyi* by adding additives like Tween-20. In this connection, the present investigations were, therefore undertaken to evaluate the virulence of grain formulations of *N. rileyi* against 3rd instar larvae of *S. litura*.

MATERIALS AND METHODS

In order to prepare grain formulations of *N. rileyi* (initial culture was maintained on SMAY medium), the fungus was mass multiplied on six different broken grain media *i.e.,* sorghum, bajra, jowar, rice, wheat and ragi. For mass production of *N. rileyi* on grain media the protocol of Babineeraja (2007) was followed. Each grain of 30 g was soaked with one per cent yeast extract solution (30mL $30g^{-1}$) 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, 121ºC 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 22ºC.

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 4ºC) and another half in incubator (at 25ºC).

Testing the virulence of *N. rileyi* in grain formulations against *S. litura*

To conduct virulence studies, the test insect *S. litura* was maintained on natural feed *i.e.,* on castor leaves throughout the lab experiment. Virulence studies were conducted at 30 days interval upto 90 days. For this, Spore suspensions of 1×10^8 to 1×10^5spores ml^{-1} concentrations of all the six grain formulations were prepared. Initially stock suspensions (*i.e.,* 1×10^8 spores ml^{-1} was prepared after dissolving the 0.5g of

each grain formulation in 100 ml distilled water and filtered through muslin clothe, by adjusting the spore count of *N. rileyi* with the help of Neubauer haemocytometer. From these stock suspensions, serial dilution of 1×10^7 to 1×10^5 spores ml^{-1} were prepared. All the four concentrations of each six (sorghum, bajra, jowar, rice, wheat and ragi.) grain based suspensions or formulations of *N. rileyi* were used for infecting laboratory reared 3rd instar larvae by leaf application method with the help of hand automizer (Swetha, 2011). For each treatment, ten *S. litura* larvae were allowed to crawl on the treated castor leaves and feed on the first day. From next day onwards, fresh castor leaves were supplied to the larvae. Daily observations were recorded on larval mortalities. Each treatment was replicated thrice. An untreated control was maintained for comparison. The larval mortality due to formulations of *N. rileyi* was expressed as per cent mortality using the formula.

$$\text{Per cent mortality} = \frac{\text{Number of larvae dead}}{\text{Total number treate}} \times 100$$

RESULTS AND DISCUSSION

The data regarding various grain formulations (at various concentrations, at different time periods) and their efficacy against 3rd instar larvae of *S. litura* were represented in Table 1.

Larval mortalities of 3rd instar of *S. litura* with grain formulations of *N. rileyi* stored under refrigeration (at 4°C)

After 30 days of storage, maize grain formulation resulted in higher larval mortality of 67 to 77 per cent at different concentrations used against 3rd instar larvae of *S. litura*, when compared to all the formulations. After sixty days of storage, the highest larval mortality of 70 per cent was obtained with maize grain formulation of *N. rileyi* at 1×10^8spores ml^{-1}. The mean mortalities in rice grain formulation ranges from 53-63 per cent, followed by sorghum 50-60, wheat 53-43, Bajra 37-47 and ragi 40-50 per cent respectively. Where as at 90 days of storage, the mean per cent larval mortality obtained was 44.40. The larval mortality recorded with maize grain formulation was 57-67 per cent, with rice grain formulation, 50-57 per cent, with sorghum grain formulation 47-53, with wheat 37 to 47, with bajra and ragi grain formulations 30-40 per cent (Table1.).

Larval mortalities of 3rd instar of *S. litura* with grain formulations of *N. rileyi* stored under incubator (25°C)

More than 40 per cent larval mortality was recorded with all the concentrations of all the grain formulations at the end of 30 days of storage. At the end of 60 days of storage, highest (63 per cent) and lowest (30 per cent) larval mortality was recorded with maize and ragi formulations at 1×10^8 and 1×10^5spores ml^{-1} concentrations respectively. Similarly, at 90 days of storage, the larval mortality recorded with maize grain formulation was 47-57 per cent, with rice grain formulation, 40-47 per cent, with sorghum grain formulation 37-43, with wheat 27 to 33, with bajra 23-30 and with ragi 20-27 per cent.

In both storage conditions *i.e.,* at incubator and refrigerator stored conditions, maize grain formulation shown higher virulence 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 content and an amount of protein content of 11.1 per cent

Table 1: Virulence of grain formulations of *N. rileyi* against 3rd instar larvae of *S. litura*

Refrigerator stored grain formulations (At 4°C)			Incubator stored grain formulations(At 25 °C)					
SL.No.		Treatments	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
1	Maize	$1x10^8$spores ml^{-1}	76.67^{a}(61.22)	70.00^{a}(56.79)	66.67^{a}(54.78)	73.33^{a}(59.00)	63.33^{a}(52.78)	56.67^{a}(48.85)
		$1x10^7$ spores ml^{-1}	73.33^{ab}(59.00)	66.67^{ab}(54.78)	63.33^{ab}(52.78)	70.00^{ab}(56.79)	60.00^{ab}50.77)	53.33^{ab}(46.92)
		$1x10^6$spores ml^{-1}	70.00^{abc}(56.79)	63.33^{abc}(52.78)	60.00^{abc}(50.77)	66.67^{abc}(54.78)	56.67^{abc}(48.85)	50.00^{abc}(45.00)
		$1x10^5$spores ml^{-1}	66.67^{bcd}(54.78)	60.00^{bcd}(50.77)	56.67^{bcd}(48.85)	63.33^{bcd}(52.78)	53.33^{bcd}(46.92)	46.67^{bcd}(43.08)
2	Rice	$1x10^8$ spores ml^{-1}	70.00^{abc}(56.79)	63.33^{abc}(52.78)	56.67^{bcd}(48.85)	66.67^{abc}(54.78)	56.67^{abc}(48.85)	46.67^{bcd}(43.08)
		$1x10^7$ spores ml^{-1}	66.67^{bcd}(54.78)	60.00^{bcd}(50.77)	53.33^{cde}(46.92)	63.33^{bcd}(52.78)	53.33^{bcd}(46.92)	43.33^{cde}(41.15)
		$1x10^6$spores ml^{-1}	63.33^{cde}(52.78)	56.67^{cde}(48.85)	50.00^{def}(45.00)	60.00^{cde}(50.77)	50.00^{cde}(45.00)	40.00^{def}(39.23)
		$1x10^5$spores ml^{-1}	60.00^{def}(50.77)	53.33^{def}(46.92)	50.00^{def}(45.00)	56.67^{def}(48.85)	46.67^{def}(43.08)	40.00^{def}(39.23)
3	Sorghum	$1x10^8$ spores ml^{-1}	66.67^{bcd}(54.78)	60.00^{bcd}(50.77)	53.33^{cde}(46.92)	66.67^{abc}(54.78)	53.33^{bcd}(46.92)	43.33^{cde}(41.15)
		$1x10^7$ spores ml^{-1}	63.33^{cde}(52.78)	63.33^{abc}(52.78)	53.33^{cde}(46.92)	63.33^{bcd}(52.78)	50.00^{cde}(45.00)	40.00^{def}(39.23)
		$1x10^6$spores ml^{-1}	60.00^{def}(50.77)	56.67^{cde}(48.85)	50.00^{def}(45.000	60.00^{cde}(50.77)	46.67^{def}(43.08)	36.67^{efg}(37.22)
		$1x10^5$spores ml^{-1}	56.67^{efg}(48.85)	50.00^{efg}(45.00)	46.67^{efg}(43.08)	60.00^{cde}(50.77)	43.33^{efg}(41.15)	36.67^{efg}(37.22)
4	wheat	$1x10^8$ spores ml^{-1}	60.00^{def}(50.77)	53.33^{def}(46.92)	46.67^{efg}(43.08)	56.67^{def}(48.85)	43.33^{efg}(41.15)	33.33^{fgh}(35.22)
		$1x10^7$ spores ml^{-1}	56.67^{efg}(48.85)	50.00^{efg}(45.00)	43.33^{fgh}(41.15)	53.33^{efg}(46.92)	40.00^{fgh}(39.23)	30.00^{ghi}(33.21)
		$1x10^6$spores ml^{-1}	53.33^{fgh}(46.92)	46.67^{fgh}(43.08)	40.00^{ghi}(39.23)	50.00^{fgh}(45.00)	40.00^{fgh}(39.23)	26.67^{hij}(31.00)
		$1x10^5$spores ml^{-1}	50.00^{gh}(45.00)	43.33^{ghi}(41.15)	36.67^{hij}(37.22)	46.67^{gh}(43.08)	36.67^{ghi}(37.22)	26.67^{hij}(31.00)
5	Bajra	$1x10^8$ spores ml^{-1}	56.67^{efg}(48.85)	46.67^{fgh}(43.08)	40.00^{ghi}(39.23)	53.33^{efg}(46.920	40.00^{fgh}(39.23)	30.00^{ghi}(33.21)
		$1x10^7$ spores ml^{-1}	53.33^{fgh}(46.92)	43.33^{ghi}(41.15)	36.67^{hij}(37.22)	50.00^{fgh}(45.00)	36.67^{ghi}(37.22)	26.67^{hij}(31.00)
		$1x10^6$spores ml^{-1}	50.00^{gh}(45.00)	40.00^{hi}(39.23)	36.67^{hij}(37.22)	46.67^{gh}(43.08)	33.33^{hi}(35.22)	23.33^{ij}(28.78)
		$1x10^5$spores ml^{-1}	46.67^{h}(43.08)	36.67^{i}(37.22)	30.00^{j}(33.21)	43.33^{h}(41.15)	33.33^{hi}(35.22)	23.33^{ij}(28.78)
6	Ragi	$1x10^8$ spores ml^{-1}	53.33^{fgh}(46.92)	50.00^{efg}(45.00)	40.00^{ghi}(39.23)	53.33^{efg}(46.92)	40.00^{fgh}(39.23)	26.67^{hij}(31.00)
		$1x10^7$ spores ml^{-1}	50.00^{gh}(45.00)	46.67^{fgh}(43.08)	36.67^{hij}(37.22)	50.00^{fgh}(45.00)	36.67^{ghi}(37.22)	23.33^{ij}(28.78)
		$1x10^6$spores ml^{-1}	46.67^{h}(43.08)	43.33^{ghi}(41.15)	33.33^{ij}(35.22)	46.67^{gh}(43.08)	33.33^{hi}(35.22)	20.00^{ij}(26.57)
		$1x10^5$spores ml^{-1}	46.67^{h}(43.08)	40.00^{hi}(39.23)	30.00^{j}(33.21)	40.00^{i}(41.15)	30.00^{i}(33.21)	20.00^{ij}(26.57)
		Untreated check	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.000.00)
		General mean	56.67(48.30)	50.53(44.68)	44.40(41.09)	54.53(47.03)	43.06(40.31)	33.73(34.65)
		SE(m)	1.59	1.51	1.52	1.62	1.56	1.65
		C.D.(0.05)	4.51	4.29	4.31	4.61	4.45	4.70

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)

100 g^{-1} present in it, (Reddy, 2008).

On the other hand, ragi grain formulation recorded least virulence in both storage conditions. This may be due to the an amount of fibre content (*i.e.,* 3.6 per cent 100 g^{-1}) present in ragi grains and also clumping of grains at the time of autoclaving of grain media. These findings are in similar with Kulkarni (1999), who reported that less amylase content (6-18 per cent) and formation of clumping in ragi grain were responsible for lower (interfered with efficient harvest of spores and thus led to low productivity) conidial yield production of *N. rileyi* in ragi grain formulation.

With regard to different concentrations of all the formulations, 1×10^8 spores ml^{-1} concentration was found superior, then followed by 1×10^7 and 1×10^6 spores ml^{-1} concentrations. Whereas least larval mortality was recorded with 1×10^5 spores ml^{-1} concentration. These results are in agreement with that of Gundannavar (2001) who reported that cumulative mortality of *H. armigera* larvae increased with increase in concentration of spores of *N. rileyi* and exposure period. These results are also in close conformity with the findings of Swetha (2011) and Sharmila *et al*. (2015).

In the present study, with respect to mortality obtained with refrigerator and incubator stored grain formulations against 3rd instar larvae of *S. litura*, around 10 per cent deviation was observed in all the formulations at 90 days of storage. And refrigerator stored formulations shown higher virulence comparatively, this may be due to the fact that under refrigerated conditions the life activity will be at its minimum. The results are in close conformity with findings of Richard *et al*. (1982), Gupta *et al*. (2000), Rachappa (2003), Mallikarjuna (2010) and Savitha *et al*. (2012).

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