Indian Journal of Entomology 85(4): 997-1000 (2023) DoI. No.: 10.55446/IJE.2021.358

EVALUATION OF ADJUVANTS ON GROWTH AND VIRULENCE OF *METARHIZIUM RILEYI* AGAINST *SPODOPTERA LITURA* (F.)

Gurmehar Kaur Grewal* and Neelam Joshi*

*Department of Microbiology; [1]Department of Entomology
Punjab Agricultural University, Ludhiana 141004, Punjab, India
*Email: neelamjoshi_01@pau.edu (corresponding author)

ABSTRACT

This study evaluated the effect of adjuvants viz. glycerol, boric acid, carboxy methyl cellulose (CMC) and Tween-80 on the growth of *Metarhizium rileyi* (Farlow) Kepler, Rehner and Humber isolates. Their virulence against *Spodoptera litura* (F.) larvae has also been assessed. The isolates were observed to exhibit maximum surface area coverage and biomass with glycerol @ 5% and CMC @ 0.5%. Further, NIPHM isolate of *M. rileyi* supplemented with mixture of adjuvants- glycerol @ 5% and CMC @ 0.5% when evaluated against *S. litura* larvae gave maximum mortality (63.33%) and increase over control (without adjuvants- 2.69%). This study demonstrates the possible role of adjuvants in enhancing the growth of *M. rileyi* and its virulence against *S. litura.*

Key words: *Spodoptera litura*, *Metarhizium rileyi*, adjuvants, carboxy methyl cellulose, glycerol, boric acid, Tween-80, mixtures, virulence, surface coverage, biomass, mortality

Entomopathogenic biocontrol agents are ecofriendly alternatives to pesticides for managing crop pests. With the increasing emphasis on environmental protection and sustainable agriculture, biopesticides have become essential alternatives (Dhakal and Singh 2019). Entomopathogenic fungi for biological plant protection has an important role in sustainable IPM (Sinha et al., 2016). The fungus *Metarhizium rileyi* (Farlow) Kepler, Rehner and Humber, formerly known as *Nomuraea rileyi* (Kepler et al., 2014) is an entomopathogenic fungus that infects several important pests of crops including many noctuids. However, microbes lose their viability under unfavourable conditions like temperature, humidity and ultraviolet radiations (Raypuria et al., 2019). The shelf-life and performance of formulations can be improved by adding suitable adjuvants that may act as UV protectants, wetting agents, adhesives or nutrients which can be beneficial for the growth and viability of the fungus (Patil and Jadhav, 2016). The application of adjuvants can provide protection and stimulate the establishment of antagonist on the host surface (Guijarro et al., 2018). There is a need for basic research on the standardization of suitable adjuvants for the development of myco-formulations (Ritika et al., 2019). *Metarhizium rileyi* has a lot of potential as a promising entomopathogen. There is a need for detailed studies to understand and develop economic production and formulation techniques to elevate the status of this fungus in pest control (Fronza et al., 2017). Hence, the present study to evaluate the effect of few adjuvants on the growth of *M. rileyi* and its efficacy against *S. litura*.

MATERIALS AND METHODS

Two *M. rileyi* isolates viz. MTCC4254 and MTCC10395 procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India and one isolate procured from National Institute of Plant Health Management, Hyderabad, India (NIPHM isolate) were used. These isolates were grown and maintained on Sabouraud maltose agar with yeast extract (SMAY) (mycological peptone 1%, maltose 4%, agar 2%, yeast extract 1% and chloramphenicol 0.5%), and refrigerated till further use. The conidial suspensions were prepared by scraping the fungal mat and suspending it in 100 ml distilled water. The conidial suspension was vortexed for 5 min to produce a uniform suspension and filtered through a muslin cloth. The fungal suspension was quantified to 10^8 conidia/ml using a haemocytometer. Adjuvants at concentrations viz. glycerol (1%, 2%, 5%), carboxymethyl cellulose (CMC) (0.1%, 0.2%, 0.5%), tween-80 (0.1%, 0.2%, 0.5%) and boric acid (1%, 2%, 5%) were sterilized in hot air oven at 160°C for 1 hr and added individually to optimum concentration (1x 10^8 cfu/ ml) of *M. rileyi* and incubated at 25± 2°C. One ml of this formulated suspension was then added to 100 ml Sabouraud maltose broth (SMB) and incubated

at 25± 2°C for 10 days following the methodology of Patil and Jadhav (2016). The surface area coverage was recorded according to Ritika et al. (2019) on 7th day after inoculation (DAI) and the fungal biomass was recorded 10 days after inoculation. There were ten treatments with three replicates each.

For bioassay studies, *Spodoptera litura* larvae and eggs were collected from cabbage and reared in the laboratory on cabbage leaves placed in glass jars covered with muslin cloth at ambient environmental conditions (25± 2°C). The food was changed daily. Conidial suspension was prepared by individually supplementing three *M. rileyi* formulations @ 1x 10^8 cfu/ ml with a mixture of adjuvants (glycerol @5% + CMC @0.5%). Laboratory bioefficacy study was carried out against 2nd instar larvae of *S. litura* by leaf-dip method according to Devi et al. (2003). Leaves were dipped in this supplemented conidial suspension, and larvae were released on to the treated leaves kept in plastic containers. Each replication consisted of 20 larvae, and there were six replications. Fresh untreated leaves were provided daily. The control larvae were kept on leaves treated with *M. rileyi* alone (without adjuvants). The cumulative % mortality and increase in mortality over control (*M. rileyi* without adjuvants) was recorded 5, 7 and 10 days after treatment (DAT). The dead larvae were transferred to petri plates containing moist paper to confirm the cause of death. The data were subjected to one-way ANOVA in SPSS 16.0. Means were compared by Tukey's post-hoc test (p=0.05).

RESULTS AND DISCUSSION

Growth of *M. rileyi* isolates on media supplemented individually with adjuvants was recorded as surface area covered and biomass produced; surface area covered was recorded at 7th day after inoculation (Table 1). In *M. rileyi* (NIPHM isolate), growth in media supplemented with adjuvants ranged from 35.0 to 98.33%, and surface area covered were statistically significant over control (without adjuvants) except for treatment supplemented with boric acid; in this isolate, glycerol @ 5% (98.33%) gave maximum surface growth and at par with glycerol @ 2% (95.0%); glycerol and CMC supported the maximum growth on all days of observation. Biomass observed after 10 days of treatment revealed that *M. rileyi* NIPHM isolate produced maximum biomass (1.50 g/ 100ml) in media supplemented with glycerol @5%; this was followed by CMC @ 0.5% (0.95 g/ 100ml); and the least (0.48 g/100ml) was in media supplemented with boric acid @ 5%. All isolates of *M. rileyi* recorded maximum surface area and biomass in media supplemented with a higher concentration of glycerol (5%) and CMC (0.5%). These results are similar to those obtained by Patil and Jadhav (2016) who evaluated various chemicals and oils, and combinations thereof, as adjuvants with *N. rileyi*; maximum biomass was on CMC @ 0.5% followed by glycerol @2%. The maximum surface area and biomass of *M. anisopliae* were observed with glycerol @ 10% by Boruah et al. (2015); Raypuria et al., (2019) studied its compatibility with various adjuvants and observed minimum growth

Table 1. Effect of adjuvants on growth of *M. rileyi* isolates

Treatments	Concentration (%)	NIPHM		MTCC 4254		MTCC 10395	
		Surface area at 7 DAI	Biomass at 10 DAI	Surface area at 7 DAI	Biomass at 10 DAI	Surface area at 7 DAI	Biomass at 10 DAI
M. rileyi+ Glycerol	1	93.33^{ba}	0.78^{dcb}	75.00^{ba}	0.79^{cb}	86.67^{a}	0.70^{ba}
	2	95.00^{a}	0.90^{cb}	88.33^{a}	0.81^{cb}	91.67^{a}	0.77^{a}
	5	98.33^{a}	1.50^{a}	93.33^{a}	0.99^{a}	95.00^{a}	0.82^{a}
M. rileyi+ CMC	0.1	80.00^{cba}	0.68^{ed}	86.67^{a}	0.70^{dc}	88.33^{a}	0.67^{ba}
	0.2	90.00^{ba}	0.77^{dcb}	91.67^{a}	0.72^{dcb}	90.00^{a}	0.71^{ba}
	0.5	93.33^{ba}	0.95^{b}	93.33^{a}	0.88^{ba}	93.33^{a}	0.76^{a}
M. rileyi+ Tween 80	0.1	51.67^{ed}	0.57^{fe}	50.00^{dcb}	0.56^{ed}	31.67^{b}	0.37^{d}
	0.2	66.67^{dc}	0.64^{fed}	60.00^{cb}	0.60^{ed}	33.33^{b}	0.55^{cb}
	0.5	75.00^{cb}	0.71^{ed}	75.00^{ba}	0.65^{dc}	45.00^{b}	0.70^{ba}
M. rileyi+ Boric Acid	1	50.00^{ed}	0.74^{edc}	50.00^{dcb}	0.69^{dc}	41.67^{b}	0.67^{ba}
	2	40.00^{e}	0.69^{ed}	43.33^{dc}	0.64^{dc}	31.67^{b}	0.66^{ba}
	5	35.00^{e}	0.48^{f}	38.33^{dc}	0.55^{ed}	30.00^{b}	0.46^{dc}
M. rileyi (Control*)	-	46.67^{e}	0.47^{f}	30.00^{d}	0.45^{e}	40.00^{b}	0.44^{dc}

**M. rileyi* without adjuvant; DAI= Days after inoculation; Mean value followed by same letter (a, b, c, d, e) in vertical column not significantly different (Tukey's post hoc test, p=0.05)

inhibition with CMC, also a higher concentration of CMC (0.5%) supported maximum radial growth and highest spore load. The growth of the fungus on higher concentration of CMC was attributed to the possible release of cellulolytic enzymes by *M. anisopliae* on CMC, which are necessary in the natural biodegradation to carbon and are therefore, essential for growth of microorganisms (Betty et al., 2013). The properties of CMC as an adjuvant include sticker, binder and stabilizer, while glycerol acts as humectant, carrier and osmotic protectant (Raypuria et al., 2019).

Babu et al. (2014) conducted compatibility studies of *M. anisopliae* with pesticides, insecticides and botanicals and reported that boric acid had a detrimental effect on growth of all fungal isolates. Boric acid inhibited fungal growth in *P. variotii* and *Trichoderma* strains (Ang et al., 2011). Surfactants reduce the surface tension of aqueous solutions by wetting of surfaces. However, the antifungal activity of surfactants depends on the length of the alkyl chain (Jackson et al., 2009). Various types of adjuvants that can improve spore adhesion or provide protection from adverse environmental conditions are often necessary to improve the performance of a biocontrol agent. However, some adjuvants can also be toxic to certain fungi (Wyss et al., 2004).

Against 2nd instar larvae of *S. litura,* after 5 days of treatment (DAT), the cumulative % mortality was not significantly different among isolates (Table 2); after 7 DAT, maximum mortality was observed with *M. rileyi* NIPHM (46.67%) followed by MTCC 4254 (40.00%); and maximum mortality (63.33%) was in *M. rileyi* NIPHM followed by MTCC 4254 (58.33%) at 10 DAT; though not statistically significant, an increase in mortality over control (*M. rileyi* without adjuvants) was observed in all isolates. The increase in mortality over control in *M. rileyi* NIPHM, MTCC 4254 and MTCC 10395 isolates was observed to be 3.71%, 4.35% and 5.01% at 7 DAT and 2.69%, 2.94% and 3.85% at 10 DAT respectively. Boruah et al. (2015), evaluated the efficacy of bioformulation of *M. anisopliae* against cowpea aphid under pot and field conditions. They observed mortality of 30.16% when supplemented with glycerol @ 10%; 50.00% when supplemented with sunflower oil @ 0.5 %; and up to 80.00% when supplemented with both glycerol @ 10% + sunflower oil @ 0.5% after thirty days of spraying. It was suggested that the addition of adjuvants and oils reduced conidial desiccation and increased the efficacy. Williams et al. (2000) studied the potential of different adjuvants for increasing the virulence of *Verticillium lecanii* and observed an additive improvement in infection with glycerol. Glycerol is a polyhydric alcohol that plays an important role in fungal growth when humidity fluctuates (Jennings 1995).

The production of hydrolytic enzymes by the microorganism is one of the mechanisms involved in biocontrol. The type of nutrients and their amount in the formulation must allow sufficient production of these hydrolytic enzymes (Stack et al., 1988). The increase in mortality observed in present study when supplemented with adjuvants, though not statistically significant, might be due to an increased level of hydrolytic enzymes secreted by the fungus in presence of these adjuvants that act as nutrients, adhesives, etc. Kim et al. (2010) described the role of certain adjuvants in promoting the aphicidal activity of *Beauveria bassiana* enzyme precipitate. It is concluded from the present study that media supplemented with glycerol (@ 5%) and CMC (@ 0.5%) improved growth of *M.*

Table 2. Mortality of *S. litura* larvae by *M. rileyi* supplemented with adjuvants

Treatments	% mortality								
	5 DAT			7 DAT			10 DAT		
	Control*	With Adjuvants**	% increase over control	Control*	With Adjuvants**	% increase over control	Control*	With Adjuvants**	% increase over control
M. rileyi NIPHM	30.00 (33.02)	31.66	5.53	45.00 (42.10)	46.67	3.71	61.67 (51.78)	63.33 (52.77)	2.69
M. rileyi MTCC 4254	26.67 (31.05)	28.33	6.22	38.33 (38.17)	40.00	4.35	56.67 (48.84)	58.33 (49.81)	2.94
M. rileyi MTCC 10395	26.67 (30.98)	26.67	0	33.33 (35.20)	35.00	5.01	43.33 (41.14)	45.00 (42.10)	3.85
CD (5%)	8.96	NS		9.69	NS		6.90	7.26	

Critical difference (CD), p= 0.05; DAT= days after treatment; **M. rileyi* formulation without adjuvants; ***M. rileyi* supplemented with CMC @ 0.5% + glycerol @ 5%; Values in parenthesis arc sine transformed values.

rileyi. Further field studies in understanding the role of adjuvants in virulence can lead to development of newer methods of production and application of mycoformulations.

ACKNOWLEDGEMENTS

Authors thank the Head, Department of Entomology and Head, Department of Microbiology, Punjab Agricultural University, Ludhiana, for providing facilities.

REFERENCES

Ang S S, Salleh A B, Bakar F A, Yusof N A, Zaman M Z, Heng L Y. 2011. Effect of boric acid on the growth and production of β-glucosidase in *Paecilomyces variotii*. African Journal of Microbiology Research 5(17): 2451-2454.

Babu M N, Usha J, Padmaja V. 2014. In-vitro evaluation of the entomopathogenic fungal isolates of *Metarhizium anisopliae* for compatibility with pesticides, fungicides and botanicals. International Journal of Applied Biology and Pharmaceutical Technology 5(1): 102-113.

Betty A B, Thatheyus A J, Ramya D. 2013. Biodegradation of carboxymethyl cellulose using *Aspergillus flavus*. Science International (4): 85-91.

Boruah S, Dutta P, Puzari K C, Hazarika G N. 2015. Liquid bioformulation of *Metarhizium anisopliae* is effective for the management of cowpea mosaic disease. International Journal of Applied Biology and Pharmaceutical Technology 6: 178-185.

Devi P S V, Prasad Y G, Chowdary D A, Rao L M, Balakrishnan K. 2003. Identification of virulent isolates of the entomopathogenic fungus *Nomuraea rileyi* (F) Samson for the management of *Helicoverpa armigera* and *Spodoptera litura*. Mycopathologia 156: 365-373.

Dhakal R, Singh D N. 2019. Biopesticides: A key to sustainable agriculture. International Journal of Pure and Applied Bioscience 7(3): 391-396.

Fronza E, Specht A, Heinzen H, Monteiro de Barros N. 2017. *Metarhizium (Nomuraea) rileyi* as biological control agent. Biocontrol Science and Technology 27(11): 1243-1264.

Guijarro B, Larena I, Melgarejo P, DeCal A. 2018. Surfactant effects on wettability of *Penicillium frequentans* formulations to improve brown rot biocontrol. Journal of the Science of Food and Agriculture 98: 5832-5840.

Jackson M A, Dunlap C A, Jaronski S T. 2009. Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. Bio Control 55(1): 129-145.

Jennings D H. 1995. The physiology of fungal nutrition. *Cambridge:* Cambridge University Press.

Kepler R M, Humber R A, Bischoff J F, Rehner S A. 2014. Clarification of generic and species boundaries for *Metarhizium* and related fungi through multi gene phylogenetics. Mycologia 106(4): 811-829.

Kim J S, Je Y H, Woo E O. 2010. Roles of adjuvants in aphicidal activity of enzymes from *Beauveria bassiana* (Ascomycota: Hypocreales) SFB-205 supernatant. Journal of Asia-Pacific Entomology 13(4): 345-350.

Patil S D, Jadhav R S. 2016. Effect of various adjuvants on growth and development of the entomopathogenic fungi *Nomuraea rileyi* (Farlow) Samson. International Journal of Plant Protection 9: 593-602.

Raypuriya N, Das S B, Bhowmick A K, Vibha. 2019. Compatibility of *Metarhizium anisopliae* (Metchnikoff) Sorokin, with various adjuvants. Journal of Entomology and Zoology Studies 7(1): 544-547.

Ritika, Joshi N, Sangha KS. 2019. Effect of adjuvants on *Lecanicillium lecanii* against nymphs of *Lipaphis erysimi* (Kalt). Indian Journal of Entomology 81(3): 597-602.

Sinha K K, Choudhary A K, Kumari P. 2016. Entomopathogenic fungi. Ecofriendly pest management for food security. pp. 475-505.

Stack J P, Kenerley C M, Pettit R E. 1988. Application of biocontrol agents, in biocontrol of plant diseases. CRC Press, Boca Raton. pp. 43-54.

Williams M E D C, Edmondson R N, Gill G. 2000. The potential of some adjuvants in promoting infection with *Verticillium lecanii:* laboratory bioassays with *Myzus persicae*. Annals of Applied Biology 137(3): 337-345.

Wyss G S, Charudatta R, Rosskopf E N, Littell R C. 2004. Effects of selected pesticides and adjuvants on germination and vegetative growth of *Phomopsis amaranthicola*, a biocontrol agent for *Amaranthus* spp. Weed Research 44: 469-482.

(Manuscript Received: July, 2021; Revised: January, 2022;
Accepted: January, 2022; Online Published: March, 2022)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e21159