Formulation of a Granulovirus-based Biopesticide for Managing the Potato Tuber Moth in Stored Potatoes in Nepal

Sunil Aryal[1], Marc Sporleder[2], Yagya Prasad Giri[1], Jürgen Kroschel[3]

[1]Nepal Agricultural Research Council (NARC), Entomology Division, Nepal

[2]International Potato Center (CIP), Agroecology/IPM Sub-Program, Global Program of Integrated Crop and Systems Research Program, Kathmandu, Nepal

[3]International Potato Center (CIP), Agroecology/IPM Sub-Program, Global Program of Integrated Crop and Systems Research Program, Lima, Peru

Abstract

This study evaluated the efficacy of a talcum-formulated biopesticide based on granulovirus (*Phop*GV) for protecting stored potatoes against potato tuber moth. Different virus concentrations, product application rates and two formulation methods were tested. *Phop*GV showed 10-times higher efficacy in the wet-mixed than in the dry-mixed formulation. Dry-mixing requires less production time and space but the virus concentration needs to be increased in the formulation to adjust for reduced efficacy.

Introduction

The potato tuber moth, *Phthorimaea operculella* (Zeller), is one of the major pests causing significant economic losses during potato storage. The granulovirus infecting *P. operculella* (*Phop*GV) has been used as a dust-formulation for protecting stored potatoes in several South American and North African countries. Commonly, 20 infected larvae are mixed in 1 liter water with 1 kg talcum and the dried product is applied at a rate of 5 kg per ton of stored potatoes[1]. In Nepal, a *Phop*GV was isolated in 2008 and in vivo multiplied for further propagation as a biopesticide. Due to variation in biological activity of *Phop*GV isolates, the objective of our study was to determine the optimal virus concentration in a talcum product formulation. In addition, we tested a dry-mixing formulation method.

Materials and Methods

Two series of bioassays were conducted: (i) 3 application rates of talcum (5, 7, and 10 g kg^{-1} potato) with 6 virus concentrations from 0.7 to 2.24×10^{-4} larval equivalents (LE) kg^{-1} potato and talcum used alone with 5 application rates between 1.5 and 15 g/kg potato. (ii) 4 application rates (3, 5, 8 and 12 g/kg) with virus concentrations from 7×10^{-3} to 6.8×10^{-6} LE kg^{-1}.

Wet formulation: for each concentration the virus suspension was mixed with talcum (1:1 w/w), plated out and crushed after drying (3 days) using a spatula.

Dry formulation: virus suspensions with a 20-fold (series 1) and 60-fold increased (series 2) virus concentration were dry-mixed with talcum alone to obtain similar virus levels in the powder as in wet formulation (in series 1 dry-formulation was only tested at an application of 5 g kg^{-1} potato).

Bioassays: 50 neonate *P. operculella* larvae were placed on 100 g treated potato (4 replications per treatment, completely randomized). Virus concentration-mortality lines and relative potencies were determined through Probit regression for each product application rate and formulation type in a parallel line assay. Mortalities observed were adjusted for natural and talcum-caused mortality.

Figure 1. Wet mixing of the *Phop*GV-talcum preparations in Petri dishes (A).; blender used for dry mixing (B); treating potatoes with product in plastic bag (C); Treated potatoes used in bioassay (D).

Results and Discussion

*Phop*GV revealed regression lines with a common slope of 1.3 (0.24) (Fig. 2). Talcum-caused mortality could also be described by a probit regression line (Fig. 2D, Table1). The wet-formulated product of *Phop*GV revealed a LC_{50}-value of 0.30 ($CL_{95\%}$: 0.25-0.35) LE ton^{-1} potato, independent of the talcum application rate. For the dry-formulated product, LC_{50}-values were variable ranging from 1.34 to 6.55 LE ton^{-1} potato, corresponding to relative potencies of 0.22 and 0.04-0.14 for the 20 and 60-fold increased virus concentration preparation, respectively, compared to the wet formulation (Table 1).

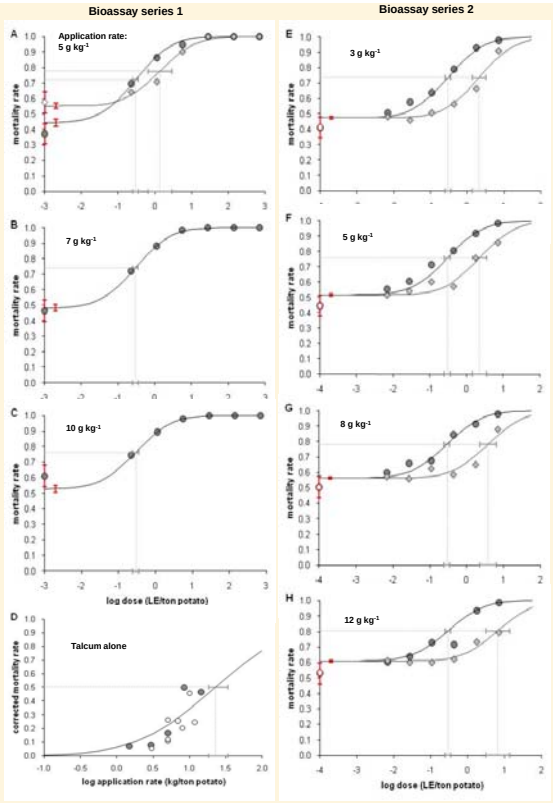

Figure 2. Mortality curves obtained for the different virus talcum preparations. Points: observed data (dots: wet formulation; diamonds: dry formulation). In D: open dots are data observed in controls. LC_{50}-values are indicated (scattered lines; bar represent $CL_{95\%}$). Open dots on x-axis: observed control mortalities (red bars: $CL_{95\%}$ for natural mortality observed and the model estimate.

Table 1. Estimated parameters, LC_{50}-values with their relative potencies (toxicity ratios) and goodness of fit for *Phop*GV in different virus-talcum preparations tested using a probit regression model.

Preparation	Product appl. rate (kg/ton potato)	N[a]	Estimated talcum-caused mortality[b] (%) (±SE).	Slope (±SE)	Intercept (±SE)	df	Chi^2	P	LC_{50} ($CL_{95\%}$) (LE/ton potato)	Toxicity ratio ($CL_{95\%}$)
Wet	all	8400	variable[c]	1.298 (±0.245)	0.687 (±0.043)	41	44.5	0.33	0.30 (0.25-.035)	1
dry (60-fold)	3	1200	15.6 (±6.8)	"	-0.413 (±0.118)	5	5.8	0.32	2.08 (1.36-3.18)	0.142 (0.097-0.209)
dry (60-fold)	5	1200	22.5 (±4.1)	"	-0.452 (±0.126)	5	5.5	0.36	2.23 (1.41-3.50)	0.133 (0.089-0.198)
dry (60-fold)	8	1200	30.1 (±3.2)	"	-0.758 (±0.149)	5	8.5	0.13	3.84 (2.26-6.54)	0.077 (0.047-0.126)
dry (60-fold)	12	1200	37.4 (±4.6)	"	-1.060 (±0.182)	5	2.0	0.85	6.55 (3.13-13.8)	0.045 (0.025-0.083)
dry (20-fold)	5	1200	22.5 (±4.1)	"	-0.167 (±0.122)	5	7.2	0.20	1.34 (0.80-2.22)	0.221 (0.159-0.305)
									(kg/ton)	
Talcum alone	all	2600		1.146 (±0.393)	-1.557 (±0.032)	11	12.7	0.312	22.8 (18.3-34)	

[a] N = number of test insects; [b] expected mortality due to physical protection of tubers by talcum coverage; mortalities and their SE's are the estimates resulting from the Probit model for talcum alone; independent interaction between talcum and *Phop*GV is assumed; [c] not determined in the table because different talcum rates were applied.

Conclusions

The product should be applied at a rate of 5 g kg^{-1} potato. The wet-formulated product should contain 3.6 LE *Phop*GV kg^{-1} of talcum (corresponding to LC_{99}). Dry formulation might be simpler to produce; however, the virus concentration should be increased approximately 10 times to adjust for reduced homogenous virus distribution in the formulation and thus reduced potency.

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