Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(1): 224-228

Received: 06-11-2016
Accepted: 07-12-2016

Shiva Shankar Bhattarai
Department of Horticulture and Plant Protection, Tribhuvan University, Institute of Agriculture and Animal Sciences, Nepal

Correspondence
Shiva Shankar Bhattarai
Department of Horticulture and Plant Protection, Tribhuvan University, Institute of Agriculture and Animal Sciences, Nepal

The lignin effect to the inactivation curve of *Phthorimaea operculella* Granulovirus (*Phop*GV) from different bioassays in laboratory condition

Shiva Shankar Bhattarai

Abstract
Laboratory experiment was conducted in completely randomised design to determine the inactivation curve that consisted of five different virus concentrations (20, 3.33, 0.55, 0.0925 and 0.015432 LE/l) of purified and raw type of viruses which were added with lignin (1%) to play UV (ultraviolet) protectant role and without mixing lignin were also prepared and again the solutions were applied to potato and egg bioassay. These solutions were subjected to artificial UV radiation through UV lamp for different time i.e., 0, 2, 4, 8, 16, 32 and 64 minutes. *Phop*GV larvas were harvested from different bioassays: purified *Phop*GV in egg, raw *Phop*GV in egg, purified *Phop*GV in potato and raw *Phop*GV in potato. Hiatt modelling gave the common intercept between the lignin treated and non-treated i.e., -1.53288 which indicated that there was no difference in the effect of lignin on the activity of different type of virus, i.e. raw and purified virus. The parameters of egg and potato bioassay i.e., intercepts, inactivation coefficients (*k1*, *k2*) and proportion of virus particles against increased resistance (log γ) had the same value i.e., -1.53288, *k1*= 0.538571, *k2*= 0, 0.065673 and log γ = -1.67081 respectively. This pointed out that these bioassays had no differences in the inactivation curves. Half inactivation speed with lignin was 4.58 days compared to 0.559 days without lignin in lab condition. The decrease in inactivation speed in case of lignin applied in lab condition proved the reduction of potato tuber moth with virus combined with lignin through the use of low dosage.

Keywords: Raw, purified, *Phop*GV, lignin, egg, potato

1. Introduction
Among more than 40 insect species that have been found to be associated with potato crops in Nepal, the potato tuber moth (PTM), *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) is one of the most important insect pests of potato [18]. Its infestation starts right from the field during the plant growth [11]. During control of the pest, misuse of pesticides causes harmful effects on human beings and the environment [26]. Naturally occurring granulovirus *Phthorimaea operculella* infecting Granulovirus (*Phop*GV, Baculoviridae) has been standing out as one prime candidate of microbial bio-control, which is effective in controlling PTM and can play a significant role in integrated management of this pest in stored tubers and in field crops as well [14, 15]. Granulosis viruses are effective for the control of Lepidopteran order and the host range in the order includes closely related species [20]. *Phop*GV was first isolated in Sri Lanka [24] and later in other countries [27]. In Australia, the isolation of virus was confirmed in 1964 [21], in South Africa, it was isolated in 1974 [5] and in Peru in 1988 [20]. However, it has been proved from numerous findings that granulosis virus if deposited on foliage are inactivated by direct sunlight and the main reason for this is ultraviolet (UV) radiation as the main factor which reduces the activity of virus [10]. Griego *et al*. (1985) and Davidson *et al*. (1969) have found that pathogen use in the field is limited by its rapid inactivation due to Ultraviolet (UV) radiation. The identification of effective adjuvant for field application of *Phop*GV is of interest for commercial viability and utility of this technology. Lignin based formulation mixed with *Cp*GV provided significant UV protectant compared to *Cp*GV alone due to its ability to absorb UV wavelength of light thus reducing virus degradation [2]. The lignin effect towards purified SeMNPV (*Spodoptera exigua* multiple embedded nucleopolyhedrovirus) was found as a good absorber for both UVB and UVA radiation [7]. The capacity of lignin as UV protectants would vary upon different factors such as diet, media upon larva is reared and type of virus [8]. This research of lignin towards different media (egg and potato) and type of virus (raw and purified) is the prospects for finding out the most appropriate conditions.

2. Materials and Methods

2.1 Sources of neonates and potato

Potato tuber moth neonates were obtained from the colony maintained at NARC Entomology Division laboratory using the rearing system previously described [3]. Potatoes were brought directly from the farmers' field ensuring free of any chemicals. The potato variety used was Kufri Sindhuri which was of late variety that matched the research duration.

2.2 Laboratory uses

Laboratory experiment was done by using various chemicals and equipments during the entire research duration. Tween-80 was used as surfactant for the uniform spreading of virus particles in the solution. Rectangular boxes of 0.5 litre capacity (5 cm depth, 15 cm in length and 10 cm width) were used as the rearing materials. Sodium hypochlorite was used as a disinfectant against pathogen while rearing PTM. Micropipette of 100 microliter was used for pouring virus suspension over the PTM eggs. Lignin sulphonic acid sodium salt (Himedia Laboratories, Pvt. Ltd, India) was used as an UV protectant. UV lamp (Model number- Ultra-vitalux 230V-E27/ES, 300W, Osram company, Germany) of wavelength 280-320nm was used as the source of artificial radiation.

2.3 Preparation of solution

Purified virus (ultra-centrifugation on a sucrose gradient, as described by Sporleder (2003) [23] was obtained from CIP (International Centre of Potato), Lima, Peru. PTM larvae infected with *Phop*GV were sent to CIP and submitted to ultra-centrifugation. One eppendorf tube contained the virus obtained from 100 larvae. The viral suspension was mixed to 1 litre water to obtain 100 LE/litre purified solution and further diluted to obtain the required concentrated purified solution. Twenty fully developed *Phop*GV infected larvae were collected and crushed in a mortar with the pestle and diluted in 1 liter of water, thus preparing a solution of 20LE/liter as a raw *Phop*GV suspension. In the first set of bioassays, the highest concentration was 20 LE/liter for all larval ages tested. Further concentrations were diluted in a repeated logarithm scale using a dilution factor 6 and thus four lower concentrations i.e., 3.33, 0.55, 0.0925 and 0.015432 LE/L were obtained. Few drops of Tween 80 was added as surfactant in the solution. Lignin solution was prepared by mixing lignin sulphonic acid sodium salt for 1% with every concentrated solution. Lignin 1% was mixed with water only to prepare control lignin. Only water was counted as control only without mixing anything.

2.4 Viral solution application

The prepared viral solution of different concentration were exposed to UV lamp at different time were taken out from the exposure. For egg bioassay, the viral suspensions of different concentration were poured over PTM eggs using the micropipette that holds 100 micro liter of solution. When the eggs hatched the neonates consumed the viral particles attached to the egg shell and later were transferred to potato tubers and their survival was assessed after 14 days and again after 20 days subsequently. Similarly these UV treated solutions were directly treated to potato and a day larva was released to those treated potatoes that fed upon different concentrated viral particles.

2.5 Bioassay process

Rectangular boxes of 0.5 litre capacity (5 cm depth, 15 cm in length and 10 cm width) were used as the rearing apparatus. Rectangular hole (3 x 2 cm^2) was cut in the centre of the lid of each boxes for ventilation and sealed with polyester mesh that could facilitate air exchange. Different concentrated viral solutions treated eggs were kept over the filter paper that absorbs extra moisture in the setup. Those treated and dried eggs were kept inside the plastic petri dishes which were covered with a lid and tied with a parafilm wax over it so that no larvae escaped out of it. The larvae came out after hatching of the eggs and fed upon the egg chorion that took up the virus particles attached to the egg shell and caused viral infection in the larvae. Similarly, the treated potatoes were fed to a day PTM larvae. On feeding those treated potatoes the larva took up the virus particles attached to the potato. The inactivation of *Phop*GV was found out with respect to time among all the solutions.

2.6 UV exposure

The UV radiation was emitted by UV lamp to different solutions at distance of 1 meter and radiating for about 5 minutes. Inspection of the setup was done to check that the lamp didn't get off automatically. In the first set of bioassays, for the highest concentration of 20 LE/liter, the lowest number of mortality occurred for 64 minutes and further time is halved in a logarithmic manner. All the five concentrations of solution (both raw and purified), control only and control lignin were exposed to different time i.e., 0, 2, 4, 8, 16, 32 and 64 minutes and later treated to eggs and potato tuber subsequently for their respective bioassays.

2.7 Data collection

The condition **of** failure to pupate after an overall incubation period of 14 days is considered as mortality. The number of absent larvae under control mortality and control lignin mortality was counted as natural mortality.The infected larvae were counted after 14 days of larvae inoculation. The larva showing typical whitish, full bodied and sluggish movement were identified as viral infected larvae.

2.8 Statistical analysis

After collection of data, they were managed in excel spreadsheet. The concentration of the solutions was log transformed at the base of 10. The correction for natural mortality was done using Abott's (1925) [1] formula.

$$Adjusted\ \ mortality\ \ (\%) = \frac{observed\ \ mortality\ \ (\%) - control\ \ mortality\ \ (\%)}{100 - control\ \ mortality\ \ (\%)} \times 100 \quad (1)$$

Exponential half-life, or half-inactivation time of the virus, $t_{1/2}$ (Rutherford, 1907) [22] calculated using equation:

- $t1/2$ = log 0.5 / b (single exponential decay) where b is the slope of the regression obtained after plotting the log activity (log R_x) against the time of exposure
- $t1/2$ = ln(2) / $k1$ or $k2$, where k1, k2 are the inactivation.

The data followed a bisegmented inactivation curve which is as follows,

Activity = (1-γ)*EXP(-($k1$+$k2$)* t)+ γ*EXP(-$k2$* t)

Where't' is the radiation time in minutes, $k1$, $k2$ are the inactivation coefficients and γ is the proportion of virus particles with increased resistance against UV.

2.9 Comparing models

Hiatt modeling (1964) [9] was used for analyzing the collected data where the change in Akaike Information Criterion (AIC) compared models giving the estimation for the goodness of fit. Further statistical evidence was given by F-test and probability test. Probability is calculated and denoted by P_{calc} which is compared with traditional P value, i.e. 0.05 where model is resulted out as significantly better if P_{calc} >0.05 [17]. Computer software used during analysis was MS-Excel.

3. Results and Discussion

3.1 Inactivation effect on the bioassays

Using Hiatt modelling (1964) [9], models with AIC= -13.5 value was significantly better with F-test ratio 0.20 and probability test 0.6561 to the second most fitted model. Table i showed that the best fitted model had the inactivation coefficient denoted by *k1* was same for all the bioassay methods in the experiment, i.e. $k1_{lignin} = k1_{without\ lignin} = 0.538$ that corresponded to a half inactivation speed of 0.559 min. The second inactivation coefficient *k2* was different for lignin treated and untreated virus, where $k2_{lignin} = 0$, no further inactivation, $k2_{without\ lignin} = 0.0657$ corresponding to the half inactivation speed of 4.58 min. These differences in inactivation speed between lignin treated and untreated resulted the differences in inactivation curve which is shown in both the figure i and figure ii. The common intercept between the lignin treated and non-treated as shown in the table i indicated that there was no difference in the effect of lignin on the activity of different type of virus, i.e. raw and purified virus. So, both raw and purified virus gave similar inactivation curve for both egg and potato bioassay as given in the figure i and figure ii. According to Kroschel, 1995 [13], neither raw nor purified virus preparation displayed any effect 17 days after application. The parameters from the best fitted model as shown in the table i showed that both egg and potato bioassay have significantly similar effect on the inactivation curve which is shown in figure i and figure ii. It was observed that *Phop*GV with lignin suspension usually had a greater effect than *Phop*GV without lignin [12], which we observed in our study that inactivation speed was reduced in case of lignin treated compared to non-lignin treated under lab condition. Lignin in the field tests extended the residual activity of *Anagrapha falcifera* Kirby, nucleopolyhedrovirus (AfMNPV) [16, 4] and in the laboratory tests with simulated sunlight [25].

Since UV radiation is the major hindrance to use granuloviruses in the plant protection, there is an urgent need to discover more of the UV protectants. This research focuses on the identification of effective protectants' combination with correct media and type of larva in order to improve the commercial viability and utility of the technology. Previously, lignin products were shown as their role to protect granulovirus [25] whereas the present study confirms the efficacy of lignin under suitable conditions.

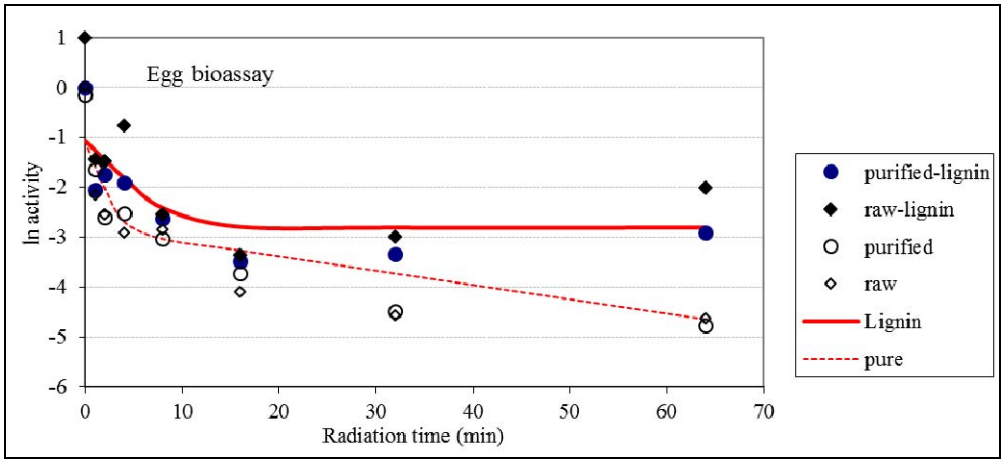

Fig I: Inactivation curve comparison with and without lignin in egg bioassay under lab experiment

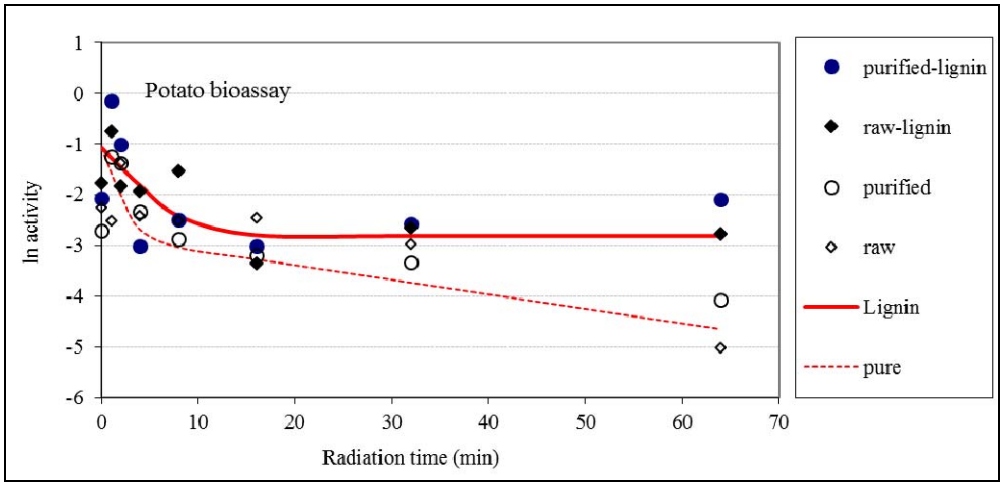

Fig II: Inactivation curve comparison with and without lignin in potato bioassay under lab experiment

Table I: Showing the parameters of the best fitted model of lab experiment

Assay	UV screen	Intercept	k1	k2	Log γ	Deviance	N	P	ΔAIC
Egg	Lignin	-1.53288	0.538571	0	-1.67081	12.98	32	5	-13.5
Potato	Lignin	-1.53288	0.538571	0	-1.67081				
Egg	without Lignin	-1.53288	0.538571	0.065673	-1.67081				
Potato	without Lignin	-1.53288	0.538571	0.065673	-1.67081				

k1, k2 are the inactivation coefficients, log γ is the proportion of virus particles with increased resistance against UV, Deviance is the sum of squares of data, N is the number of data points, P is the number of parameters, ΔAIC= N x ln(SS2/SS1)+ 2 ΔDF, where N is the number of parameters points, SS2 and SS1 are the sum of squares of simple and complicated models respectively, ΔDF is the change in degree of freedom.

4. Conclusion

It was concluded that there was no any difference in the performance of raw and purified virus and thus gave the similar inactivation curve. The lignin could reduce the inactivation of pathogen due to solar radiation equally in both raw and purified virus. Similarly, both egg and potato bioassay gave significantly similar effect on the inactivation curve. Thus, lignin had no differences on the inactivation curve from different bioassays i.e., purified *Phop*GV in egg, raw *Phop*GV in egg, purified *Phop*GV in potato and raw *Phop*GV in potato.

5. Acknowledgement

The author warmly acknowledges International Potato Center (CIP), Peru for financial supports and Nepal Agriculture Research Council (NARC) for technical supports during whole experiment.

6. References

1 Abbott WS. A method for computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925; 18:265-267.
2 Arthurs SP, Lacey LA, Behier RW. Evaluation of lignins and particle films as solar protectant for the granulovirus of the codling moth, *Cydia pomonella*. Biocontrol Science and Technology. 2008; 18:829 -839
3 Aryal S. Management of the potato tuber moth, *Phthorimaea operculella* Zeller, (lepidoptera: gelechiidae) using indigenous granulovirus isolate. Thesis, M. Sc. IAAS. Tribhuvan University. 2011, 72.
4 Behle RW, Tamcz-Guerra P, McGuire MR. Field Activity and Storage Stability of *Anagrapha falcifera* Nucleopolyhedrovirus (AfMNPV) in Spray-Dried Lignin-Based Formulations. Journal of Economic Entomology. 2003; 96:1066-1075.
5 Broodryk SW, Pretorius LM. Occurrence in South Africa of a granulosis virus attacking potato tuber moth, *Phthorimaea operculella* Zeller, (lepidoptera: gelechiidae). Journal of Entomological Society of Southern Africa. 1974; 37:125-128.
6 Davidson MM, Takla MFG, Reader JK, Butler RC, Wratten SD, Conner AJ. Evaluation of field grown potato lines transgenic for a cry1ac9 gene conferring resistance to potato tuber moth. Journal of New Zealand Plant Protection. 1969; 55:405-410.
7 El Salamouny S, Shapiro M, Ling KS, Shephard BM. Black tea and lignin as ultraviolet protectants for the Beet Armyworm Nucleopolyhedrovirus. Journal of Entomological Science. 2009; 44(1):50-58.
8 Griego VM, Martignoni ME, Claycomb AE. Inactivation of nuclear polyhedrosis virus (Baculovirus subgroup A) by monochromatic UV radiation. Journal of Applied Environmental Microbiology. 1985; 49(3):709-710.
9 Hiatt CW. Kinetics of the inactivation of viruses. Bacteriological Review. 1964, 28.
10 Jaques RP. Stability of entomopathogenic viruses. Miscellaneous Publications of the Entomological Society of America. 1977; 10:99-116.
11 Joshi SL. Comparative life cycle of the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) on potato tubers and foliage and its economic loss in yield. Journal of Entomological Society of Nepal. 1989; 1:59-69.
12 Krieg A. Interactions between pathogens. *In*: H. D. Burges and N. W. Hussey (eds.) Microbial Control of Insects and Mites. Academic Press, London, 1971, 459-466
13 Kroschel J. Integrated pest management in potato production in Yemen with special reference to the integrated biological control of the potato tuber moth (*Phthorimaea operculella* Zeller). Tropical Agriculture, 8. Margraf Verlag, Weikersheim, Germany. 1995; 227.
14 Lacey LA, Arthurs SP. An Overview of Microbial Control of the Potato Tuber Moth. *In*: Integrated Pest Management for the Potato Tuber Moth, *Phthorimaea operculella* (Zeller) - a Potato Pest of Global Importance (Kroschel, J and Lacey, L. A. eds.). Tropical Agriculture 20, Advances in Crop Research 10. Margraf Publishers, Weikersheim, Germany. 2009, 33-48.
15 Lacey LA, Kroschel J, Wraight SP, Goettel MS. An introduction to microbial control of insect pests of potato. Fruit, Vegetable, and Cereal Science and Biotechnology. 2009; 3:20-24.
16 McGuire MR, Tamez-Guerra PT, Behie RW, Street DA. Comparative field stability of selected entomopathogenic virus formulations. Journal of Economic Entomology. 2001; 94:1037-1044.
17 Motulsky HJ. Prism 5 Statistics Guide. GraphPad Software. San Diego CA, 2007.
18 NPRP (Nepal Potato Research Program). Annual report. National Potato Research Program. NARC, Khumaltar, Nepal. 2004, 05, 70.
19 Payne CC. Insect pathogenic viruses as pest control agents. *In*: Biological Plant and Health Protection (Franz JM, Fischer G. Eds.) Stuttgart. 1986, 183-200.
20 Raman KV, Alcazar J. Biological control of potato tuber moth, *Phthorimaea operculella* (Zeller) using a granulosis virus in Peru. *In*: Proceedings of the Second Triennal Conference of the Asian Potato Association, June 12-26, Kunming, China, 1988.
21 Reed EM. A granulosis virus of potato moth. The Australian Journal of Science. 1969; 31:300.
22 Rutherford FRS. The production and origin of radium. Philosophical Magazine Series 1907; 14(84):6.
23 Sporleder M. The granulovirus of the potato tuber moth *Phthorimaea operculella* (Zeller) characterisation and prospects for effective mass production and pest control. Advances in crop research, Margraf Verlag, Weikersheim, Germany. 2003, 3.
24 Steinhaus EA. Bacterial infections of potato tuber moth

larvae in an insectary. Journal of Economic Entomology. 1945; 38:718-719.
25 Tamez-Guerra P, Rodriguez-Padilla C, Galán-Wong LJ. Solar Radiation (UV) Protectant for Microbial Insecticides. *In*: Petroski RJ (ed.) Semiochemicals in Pest and Weed Control American Chemical Society, Washington. 2005, 127-147.
26 Thapa RB. Pesticide pollution and integrated pest management. *In*: Neupane FP (ed.) Proceeding of National Seminar on Integrated Pest Management in Nepal, 25-26 September 2002. Himalayan Resources Institute, New-Baneshwor, Kathmandu, Nepal. 2003, 175 -197.
27 Zeddam JL, Pollet A, Mangoendiharjo S, Ramadhan TH, Lopez Ferber M. Occurrence and virulence of a granulosis virus in *Phthorimaea operculella* (Lep., Gelechiidae) populations in Indonesia. Journal of Invertebrate Pathology. 1999; 74:48-54.