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Posted Date: 15 June 2023

doi: 10.20944/preprints202306.1096.v1

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Article

A Novel Approach to Management of *Spodoptera frugiperda* J.E. Smith for Small-Holder Farmers Using Virus Extract from Larvae Treated with Baculovirus under Field Conditions

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Simple Summary: Maize is the staple food in Sub-Saharan Africa and source of livelihoods to millions of smallholder farmers. Constraints in production that include pests lead to loss of production and hunger. Since its arrival in Kenya in 2016, Fall armyworm (FAW) has caused huge destruction. Chemical control is the preferred choice by farmers despite its negative effects. Baculoviruses offer a sustainable alternative to pesticides. However, their cost and special storage requirements make them unattractive to smallholder farmers especially where repeat applications are required. The potential of use of virus extracts from FAW larvae treated with a commercial baculovirus product has not been documented. This study, therefore, evaluated the efficacy of virus extracts from larvae treated with Littovir, a commercial product under laboratory and filed conditions. Under laboratory conditions, the virus extract caused varying mortality in different FAW instars with the highest mortality recorded in 1st–3rd instars. Under field conditions the virus extract produced maize yield comparable to commercial insecticides. This study has highlighted potential of virus extracts from larvae treated with a commercial product. This approach offers affordable means of controlling FAW since farmers need to purchase the commercial product once and use extracts from treated larvae for repeat applications.

Abstract: A Fall armyworm (FAW) is a major pest of maize and causes huge losses. Chemical control is the commonly used strategy FAW among farmers. Efficacy of baculovirus against FAW has been proven, however, farmers may not afford the products. The use of farmer produced baculovirus mixtures could provide an opportunity for a nature-based solution for FAW at low cost. This study evaluated the potential of virus extracted from FAW larvae treated with a commercial baculovirus (Littovir) for the management of FAW under laboratory and field conditions. In Laboratory, the virus extracted from 25, 50, 75 and 100 FAW larvae caused varied mortality on FAW instars. The highest mortality (45%) on 1st-3rd instars was caused by Littovir followed by virus extract from 100 FAW larvae (36%). Under field conditions, even though virus extracts did not offer adequate protection against the FAW damage, the maize yield was comparable to commercial insecticides treated plots. This study has shown the potential of use of virus extracts for management of FAW. This would offer the farmers a sustainable and affordable option for management of FAW as it would require the farmers to purchase the commercial baculovirus once and collect larvae from treated plots for repeat applications.

Keywords: keyword Fall armyworm; damage; Littovir; insecticides; maize; yield; virus extracts; baculoviruses; NPV

1. Introduction

Maize (*Zea mays* L.) is the main staple food crop in sub-Saharan Africa (SSA), and is mainly grown by small-holder farmers [1,2]. The smallholder, resource poor, farmers produce mainly for subsistence and all they require is affordable and sustainable production system to feed themselves. Maize pests pose the greatest challenge to productivity among smallholder farmers and fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is one of the most destructive pests [3,4]. The pest is polyphagous and since its arrival in Africa, has left destruction of hundreds of acres of maize plantations with economic losses estimated at US$ 9.6bn [5,6,7,8,9]. A number of management and adaptation strategies, including hand picking, crop rotation, early planting, application of soil or wood ash in the whorls and planting early maturing crops, have been applied to reduce damage and crop loss [10,11]. However, chemical control remains popular among farmers and produces better gain yields compared to other control strategies [10,11,12]. In Kenya a number of insecticides are have been registered for use against FAW since its invasion. These include: Diazinon, Alpha Cypermethrin, Chlorpyrfos, Diflubenzuron Triclorfon (Dipterex), Chlorantraniliprole, Spinetoram, Emamectin benzoate, Indoxacarba and Lambda Cyhalothrin (https://www.pcpb.go.ke/crops/). Despite their popularity, pesticides have negative and undesirable effects including, human health, food safety concerns emanating from pesticide residues, development of resistance by the pest and they also affect beneficial non-target organisms [11,13]. The potential of the use of baculoviruses, particularly the *S. frugiperda* multiple nucleopolyhedrovirus (SfMNPV) has been demonstrated and some commercial products are available in various countries including in Kenya [14,15,16]. Integrated pest management and use of microbial based biopesticides like baculoviruses is a good alternative to synthetic insecticides. The baculoviruses have a number of advantages that include specificity, have no health risks, are simple to apply, can be formulated in a number of ways and do not harm beneficial non-target organisms [17,18]. Efficacy of baculoviruses against FAW and other lepidopterans has been reported in several studies [19,20].

The baculovirus infection starts when the FAW ingest occlusion bodies (OBs) on the leaf surface that have been treated with the baculovirus. The midgut of the larvae is alkaline in nature and dissolves the OBs releasing the virions that bind to and infect the epithelial cells of the midgut after crossing the peritrophic membrane (PM) [21]. After some time, the affected cells in the midgut produce a second virus phenotype, named the budded virus (BV), which causes systemic infection [22]. Research has shown that the efficiency of baculovirus is influenced by the type of baculovirus formulation [23].

Baculoviruses have a limitation because they do not cause acute death of the pest, but mortality is observed days after applications, however, this is not a major challenge in maize because the crop can relatively withstand moderate defoliation without compromising yield [18]. Additionally, some biopesticides are expensive and small-holder farmers may not afford repeated applications that enhances efficacy. The solution to this problem can offer an exciting and sustainable control option that is affordable to the resource poor smallholder farmers.

Therefore, this study evaluated the efficacy of virus extracts from FAW larvae sprayed with a commercial baculovirus product, to test an approach that would reduce the cost of FAW control by small-holder farmers [18]. In this approach, farmers would only need to buy the commercial product once and use virus extracts form treated larvae for subsequent application. The virus product used in this study (Littovir) has been tested in a number of African countries against FAW and is registered in Cameroon, Morocco and Tunisia [20]. Littovir is a *Spodoptera littoralis Nucleopolyhedrovirus* (NPV).

 doi:10.20944/preprints202306.1096.v1

2. Materials and Methods

Study site and experimental plots

The experiment was carried out at Kenya Agricultural and Livestock Research Organization (KALRO) Muguga, Kiambu county between August 2020-October, 2021. The station lies at an altitude of 1675 m above sea level, longitude 36.6579649E and latitude -1.2551409S. During the experimental period the mean daily temperature was 21°C while mean annual precipitation was 136.63mm.

Maize seedlings

Eight maize seeds, variety H514, per pot were planted in pots measuring 17cm (diameter) and 17cm (depth) and placed in the open to allow germination after which thinning was done to leave 4-seedlings per pot. After 7 days post germination, the seedlings were transferred to a greenhouse to avoid infestation by FAW and other pests. They were regularly watered and used to feed FAW larvae.

Fall armyworm colony

The initial FAW colony was established by collecting FAW larvae from infested maize plants, in the open field at KALRO Muguga, and then transferred to the laboratory for rearing. FAW were transferred, using a soft camel brush to pick 1^{st} to 3^{rd} instars while larger instars (4^{th}-6^{th}) were picked with soft forceps, into lunch boxes (22cm (length) and 15cm (width)) lined with paper towel to absorb moisture from the maize leaves. The lunch boxes had tops with fine net to allow for air circulation. The net apertures were small enough not to allow larvae to escape. The FAW larvae were fed with cultivated maize leaves of variety H514. The maize leaves were harvested and cleaned with water, then air dried for 10 minutes to remove excess moisture before being fed to the larvae. The larvae were fed every 2 days. The mean temperature in the FAW rearing room was 28°C and RH 80%. A thermostatic heater was used to maintain favourable temperatures for FAW growth and development since the night temperatures could drop to as low as 8°C.

Laboratory evaluation of efficacy of virus extracts from FAW larvae sprayed with Littovir

Inoculum preparation

a) Initial inoculum-treatment with baculovirus (Littovir)

Initial inoculum was prepared by suspending 6µl of Littovir in 200ml of water and left for 10 minutes. Maize seedlings were prepared by cutting them from the base and cleaning them. They were then immersed in a basin containing 200ml Littovir suspension for 5 minutes. Subsequently, they were air dried for 10 minutes to remove excess moisture and transferred into the aerated lunch boxes. Twenty (20) FAW larvae (1^{st}-6^{th} instar) were then transferred into the containers and kept at room temperature. Maize seedlings were replaced regularly to avoid cannibalism among the larvae. Mortality was recorded daily for 7 days and dead larvae were collected and stored in a fridge at 4°C for later use. This treatment was replicated 4 times to get enough larvae to extract the virus for bioassays. In the laboratory experiment, FAW instars were grouped into 2 i.e. 1^{st}-3^{rd} instars and 4^{th}-6^{th} instars before being subjected to treatment. Each group contained 30-40 instars. This was done to avoid cannibalism of the younger instars by the older ones.

b) Virus extract from FAW larvae treated with baculovirus (Littovir)

When preparing virus extract from FAW larvae, the larvae were first placed in the freezer at 4°C for 20 minutes to immobilize them. The larvae were then transferred into a glass vial and crushed using a pestle into a paste. Virus was extracted from dead larvae by suspending the crashed larvae in 10 ml of tap water to form the suspension.

Treatment

There were 6 treatments as follows:

(1) Littovir, (2) untreated control, (3) virus extracted from 25 larvae, (3) virus extracted from 50 larvae, (4) Virus extracted from 75 larvae and, (6) virus extracted from 100 larvae. Twenty (20) FAW larvae (1st-6th instar) were used in bioassays and each treatment was replicated 3 times.

Field evaluation of efficacy of virus extracts from FAW larvae sprayed with a commercial baculovirus product.

Initial inoculum for field evaluation and seed bed preparation

Land was ploughed and harrowed 1 month before start of the rainy season. Levelling was done to ensure the land was even. A plot measuring 30 x 50 meters was planted earlier than the experimental plot. Maize was planted at 75 cm inter row by 25cm interplant. All agronomic practices for maize production were carried out according to recommendations. The plants were naturally infested by FAW in the field. After infestation, the plants were sprayed with Littovir at the recommended rate of 3ml in 20 litres of water. This was the source of initial inoculum for extraction of virus from FAW larvae for field experiments.

Engeo 247SC (Sygenta) and Escort 1.9EC (Green life crop protection)

The insecticides (pesticides) used as positive control in this study are commonly used for control and management of FAW in Kenya. Engeo (141 g/litre Thiamethoxam and 106 g/litre Lambda-cyhalothrin) was applied at the recommended dose rate of 8 ml /20lt of water or 150ml / Ha in 500 litres of water while Escort (Emamectin benzoate 19 g/l) was applied at the recommended dose rate of 25ml/20lt of water or 500 ml/ha in 400 litres of water. The insecticides were acquired from agrodealers in Nairobi, Kenya. Prior to application of the pesticides, they were thoroughly mixed with tap water and applied using separate knapsack sprayers to avoid contamination.

Soil and weather conditions in Muguga

Muguga area has an average temperature of 16° C with daily temperatures rarely exceeding 28°C or falling below 8°C (Muguga Meteorological Station). Muguga is near the equator and therefore there are minimal day length variations. Muguga area has gently sloping hills and well drained clay-loam soils. The fertile soils in the area are originally from lava and are generally very deep [24].

Crop

Maize variety H614 acquired from local agrodealers was planted in plots measuring 10 x 10m. Two seeds per hole were planted and allowed to germinate. The intra plant and inter row spacing was 30cm by 75cm respectively. After germination, thinning was done to leave 1 seedling per hole. Total plant population was 333 plants per plot. All the agronomic practices were carried out safe for application of pesticides. The experiment was carried out between April and October 2021.

Treatments, Layout and Design

Following the laboratory experimental results where virus extracts from 25, 50 and 75 larvae caused low mortalities, they were dropped in the field experiment and instead virus extracts from 100, 150, 200 and 250 FAW larvae were used in the field experiment. There were 8 treatments as follows: (1) Littovir; (2) untreated control; (3) Engeo-synthetic insecticide; (4) Escort -synthetic insecticide; (5) virus extracted from 100 FAW larvae; (6) virus extracted from 150 FAW larvae; (7) virus extracted from 200 FAW larvae and (8) virus extracted from 250 FAW larvae. The experimental design was a completely randomized block design. Each treatment was replicated 5 times, hence there were 40 experimental plots. Treatment applications were applied weekly for 5 weeks.

Evaluation of Treatments

FAW Infestation Assessment

Immediately after maize germination, FAW pheromone traps (FAW lure from Farmtrack Consulting Ltd) were installed at the rate of 4 traps per hectare to monitor FAW infestation. Twenty (20) maize plants per treatment were randomly selected from each experimental plot. The plants were thoroughly examined on the leaves and whorls for presence of FAW larvae and eggs. The larvae found on the plants were counted and recorded. Sampling was carried out weekly and begun 4 weeks after germination.

FAW Leaf Damage Assessment

Leaf damage assessment was carried out once before treatment application and thereafter once every week. Twenty (20) maize plants were randomly selected from each experimental plot. The plants were thoroughly examined on the leaves and whorls for FAW damage. The damage was scored using a damage scale: where 1= no damage to any ears; 2= Tip (<3cm) damage to 1-3 ears; 3= Tip damage to 4-7 ears; 4= Tip damage to 7 and more ears and damage to 1-3 kernels below ear tips on 1 to 3 ears; 5= Tip damage to 7 and more ears and damage to 1-3 kernels of 4 to 6 ears; 6= Ear tip damage 7-10 ears and damage to 1-4 kernels below tips of 7 to 10 ears; 7= Ear tip damage to 7-10 ears and damage to 4-6 kernels destroyed on 7-8 ears; 8= Ear tip damage to all ears and 4-6 kernels destroyed on 7-8 ears; 9= Ear tip damage to all ears and 5 or more kernels destroyed below tips of 9-10 ears [25]. Sampling was carried out weekly, 4 weeks after germination.

Maize Grain Yield Assessment

Maize grain yield was assessed at the end of the experiment. Maize was harvested from all the experimental plots and dried to remove excess moisture. The maize cobs were then threshed using a thresher, cleaned, and weighed. The yield data were then recorded. The gain yield was computed in kilograms per hectare and extrapolated to ton ha^{-1}.

3. Results

Statistical Analysis

The FAW infestation, damage assessment scores, and maize grain yield were first log transformed before subjecting the data to analysis of variance (ANOVA), and means separated using Tukey HSD. Leaf and expressed in kilograms per hectare, which was extrapolated to ton ha^{-1}. Data were analyzed using R software version 4.1.2.

Results

Laboratory experiment

Effect of baculovirus (Littovir) on different FAW developmental stages

Mortality of the 1st-3rd instars varied significantly among the treatments ($F=12.3$, $df=4$, $P=0.02$). Littovir caused the highest mortality at 44.79% while the virus extract from 25 FAW larvae caused the lowest mortality at 8.3% (Table 1). The mortality induced by all the treatments was however less than 50% and therefore LT50 was not calculated. The mortality also varied significantly across the days (7 days of data collection), $F=11.7$, $df=4$, $p<0.001$.

Table 1. Mean FAW 1st-3rd instar mortality induced by different virus treatments.

Treatment	FAW instar	Mean mortality +SE
Virus extract from 25 larvae	1st-3rd	8.3± 1.3d
Virus extract from 50 larvae	1st-3rd	16.46 ± 2.3dc
Virus extract from 75 larvae	1st-3rd	22.5 ± 4.2c
Virus extract from 100 larvae	1st-3rd	36.22 ± 4.8b
Littovir	1st-3rd	44.79 ± 5.3a

Means followed by the same letter within a column are not significantly different by Tukey HSD at $P < 0.05$.

The mortality induced in the 4-6th instars was also significantly different across the different treatments $F=9.3$, $df=4$, $p<0.001$. A similar trend where Littovir caused highest mortality while virus extract caused the lowest mortality was observed (Table 2). However, the treatments induced lower mortalities in 4-6th FAW instars compared to 1st-3rd instars.

Table 2. Mean FAW 4th-6th instar mortality induced by different virus treatments.

Treatment	FAW instar	Mean mortality +SE
Virus extract from 25 larvae	4-6th	5.21 ± 0.21c
Virus extract from 50 larvae	4-6th	9.58 ± 0.80bc
Virus extract from 75 larvae	4-6th	12.08 ± 1.39bc
Virus extract from 100 larvae	4-6th	15.42 ± 1.60ab
Littovir	4-6th	21.70 ± 2.11a

Means followed by the same letter within a column are not significantly different by Tukey HSD at P < 0.05.

Field experiments

Effects of treatments on FAW damage on maize crop

The FAW damage on maize showed significant variation across the different treatments; F = 11.01, df = 7, p<0.001 (Table 3). The damage increased with time and was less in early weeks of infestation, and continued even after treatment applications. Fall armyworm larvae damage varied significantly across the weeks; F = 5.28, df = 4, p<0.001. Among the treatments, the synthetic pesticides Escort and Engeo were more effective in protecting maize against damage by FAW compared to the baculovirus (Littovir) and the virus extracts from 100, 150, 200 and 250 FAW larvae (Table 3). There was no significant difference between damage on maize crops treated with Littovir, virus extracts from 100, 150, 200, 250 FAW larvae and untreated control (Table 3).

Table 3. Mean crop damage as influenced by treatments.

Treatment	Mean crop damage +SE
Escort	1.72 ± 0.20a
Engeo	2.00 ± 0.07ab
Littovir	2.73 ± 0.37bc
100 FAW larvae	2.80 ± 0.23c
200 FAW larvae	2.84 ± 0.27c
250 FAW larvae	2.87 ± 0.36c
Untreated control	3.30 ± 0.43c

Means followed by the same letter within a column are not significantly different by Tukey HSD at P < 0.05.

Maize grain yield

The only maize grain yield obtained at the end of the experiment that differed significantly was between Escort and the non-treated control; F=2.7, df=7, p=0.023 (Table 4). Escort produced the highest yield at 4.38 tons ha-1. Escort produced one and half times more yield than the untreated control while Littovir produced 1.3 times more yield compared to untreated control despite there being no significant difference in the maize grain yield. Among the baculovirus treatments, Littovir produced about 3.1 tons ha-1 even though there was no significant difference in the yield among the baculovirus treatments. The maize yield did not seem to be influenced by the damage because even though Escort (insecticide) was able to offer better protection against the maize crop compared to the other treatments, this was not reflected in the yield.

Table 4. Mean gain yield as influenced by treatments.

Treatment	Mean grain yield +SE
Escort	4.38 ± 0.45a
Engeo	4.05 ± 0.27ab
Littovir	3.08 ± 0.45ab
100 FAW larvae	2.98 ± 0.36ab

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 15 June 2023 doi:10.20944/preprints202306.1096.v1

200 FAW larvae	2.88 ± 0.40ab
250 FAW larvae	3.04 ±0.45ab
Untreated control	2.43 ± 0.45b

Means followed by the same letter within a column are not significantly different by Tukey HSD at P < 0.05.

4. Discussion

Since its arrival in Kenya in 2016, the fall armyworm has spread to the entire country causing huge damage and associated yield losses [26,27], and smallholder farmers are the most affected because they lack adequate resources to manage the pest. Management of the pest relies mostly on the use of chemical (synthetic) pesticides which are associated with health and environmental risks and high cost especially to the resource poor small-holder farmers. It is therefore important to develop affordable and sustainable strategies for management of the pest. This study evaluated the potential of using virus extract from larvae treated with a commercial baculovirus (Littovir) under laboratory and field conditions. In the laboratory, both the Littovir and virus extracted from FAW larvae induced mortality with the highest mortality induced by Littovir followed by virus extracted form 100 FAW larvae. The induced mortality was higher in 1st-3rd instars compared to 4-6th instars even though the mortality was below 50% in all the treatments. This variation may have resulted from young instars being more aggressive feeders as compared to older instars, thus more likely to pick up more baculovirus in the process of feeding. Baculoviruses need to be ingested in order to kill an insect. Additionally, the young instars have not developed defense mechanisms and hence they are more susceptible to virus infection. Under field conditions, inducing infections of FAW resulting in dead caterpillars is an important source of inoculum for the occurrence and maintenance of epizootics [15,16]. The epizootics are desirable in biological control because the dead cadavers can enhance the spread of a virus to healthy non-infected populations [20].

In the field, FAW damage and yield was evaluated against the eight treatments. Among the treatments Escort (insecticide) produced better protection against FAW damage. Littovir performed the same as Engeo (insecticide) though there was no significant difference between the baculovirus and the untreated control. Increasing the number of treated larvae from which virus was extracted and used as a treatment did not offer any additional advantage. In fact, there was no significant gain in terms of crop protection from damage on maize crop between 100 and 250 larvae treatments. Even though the baculovirus (Littovir) treatment did not offer sufficient protection against FAW damage, it performed equally well and was comparable to Engeo, a synthetic pesticide. Fall armyworm damage on maize crop has been shown to be influenced by cropping systems and agricultural practices and varies between monocrops and intercrops with monocrops having more damage [28,11]. The maize in this study was planted as a monocrop and there were neigbouring monocrop maize fields near the trial sites. This may have increased the FAW population pressure resulting in the damage. Fall armyworm pheromone traps were installed immediately after germination to monitor FAW infestation. Treatment commenced four weeks later after germination, and this was to allow for the crop to attain uniform height and infestation levels. This implies that considerable damage had happened by the time treatment application commenced, hence making it difficult to detect significant differences in damage after the treatments were applied. The similar performance between the insecticides and the baculovirus in protecting the crop against FAW damage could be explained by the FAW larvae feeding deep in the whorl of young maize plants, and hence a high volume of liquid insecticide may be required to obtain adequate penetration resulting in better protection against further damage. Additionally, baculovirus is slow acting and some damage occurred between treatment application and action of the baculovirus. Additionally, maize is characterized by many functional leaves, and can compensate the photosynthesis to ensure better crop yield, foliar damage not withstanding and especially when infestation occurs at early stages the crop growth under good agronomic practices [6,29]. In addition to this, FAW larvae are known to shift feeding preference from leaf tissues to tassel, silk and ears and this has an influence on the damage caused by FAW as crop advances in age.

Maize grain yield was evaluated at the end of the season and expressed as tons ha-1. In this study, only the treatment with Escort produced yield that was significantly different from the untreated control. The yield in all the other treatments was similar to untreated control. This implies that the maize damage level influenced the grain yield. The Escort insecticide might have enhanced more crop growth and higher photosynthetic rate resulting in higher maize yield [30]. Previous studies have shown that good agricultural practices like weeding and nutrition management enables maize to compensate for the FAW damage and produce optimum yield [31,32,33].

The lack of significant differences in the maize grain yield between Engeo (insecticide) and the baculovirus (Littovir) as well as virus extracts from FAW larvae treated with Littovir demonstrates the potential of the baculovirus and the virus extracts as a sustainable FAW management strategy. This could offer resource poor smallholder farmers a sustainable and affordable FAW management option because it negates the need for repeat applications of the expensive insecticides.

5. Conclusions

This study has demonstrated the potential of virus extracted from FAW larvae treated with baculovirus in management of FAW. This provides a promising opportunity for smallholder farmers who can hardly afford repeat applications and who suffer huge losses. The fact that farmers can apply baculovirus once and collect larvae and extract the virus for repeat or subsequent applications would save farmers money. The virus extract from treated FAW larvae produced yield comparable to insecticide treated plots and this suggests that this approach would offer farmers benefits that include human safety, environmental protection and enhanced biological control of FAW since the virus is highly specific and does not affect non-target organisms and thus is compatible with integrated pest management strategy.

It is recommended that further studies be undertaken with other registered baculovirus products that have been reported to be more virulent, to determine if the virus extract from these may be more potent. There is also a need to determine how long the farmers should keep either the FAW larvae after collecting them from the field or the virus extract before being a repeat application. Further studies should also be carried to evaluate the effect of ecological parameters in the efficacy of virus extract from dead FAW larvae under different agro-ecological zones.

Author Contributions: A.M. designed and carried out the experiments, undertook data analysis and wrote the manuscript. I.R. conceptualized the research activities and developed the research ideas, A.O. reared insects and took care of plants for the experiments. D.C. provided designed and carried out field layouts, reviewed manuscript. J.G. developed the research methodology and reviewed the manuscript. B.L. provided research funds and reviewed the manuscript.

Funding: The study was conducted as part of CABIs PlantwisePlus programme, which is funded by the UK Foreign, Commonwealth and Development Office (FCDO), Netherlands Directorate-General for International Cooperation (DGIS), Swiss Agency for Development and Cooperation (SDC) and the European Commission (DG INTPA).

Data Availability Statement: Data can be provided upon request to the corresponding/lead author.

Acknowledgments: The authors acknowledge funding from CABI- an international intergovernmental organisation, and gratefully acknowledges the core financial support from our member countries (and lead organisations) including the United Kingdom (Department for International Development), China (Chinese Ministry of Agriculture), Australia (Australian Centre for International Agricultural Research), Canada (Agriculture and Agri-Food Canada), the Netherlands (Directorate-General for International Cooperation), Switzerland (Swiss Agency for Development and Cooperation), and Ireland (Irish Aid, International Fund for Agricultural Development-IFAD). See http://www.cabi.org/about-cabi/who-we-work-with/key-donors/ for full details. The authors also acknowledge Andermatt Group AG for providing the sample (Littovir) used in this study.

Conflicts of Interest: The authors declare no conflict of interest.

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