

# Enhancing the Insecticidal Potential of Baculovirus by Overexpressing the Mammalian B-Galactosyl Binding Protein Galectin-1

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## Research Article

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# Abstract

Developing bio-pesticides is an important area of research in agriculture for which viruses are an essential tool. Infection by entomological pathogenic viruses kills agricultural pests, and viral progenies are disseminated to infect more pests, eventually achieving long-term pest control in the field. Of the current virus-based pest control models, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is the most studied. AcMNPV belongs to the family *Baculoviridae* and can infect many lepidopterans. Although AcMNPV has been previously demonstrated as a potential pest-control tool, its long virus infection cycle has made field application challenging. To overcome this hurdle, we generated a recombinant baculovirus that can express mammalian galectin-1, which is a galactoside-binding protein that binds to the peritrophic matrix in the midgut of pest lepidopterans and induces perforation of the membrane. Galectin-1 has been shown to affect immune responses in mammals, including humans, but to our knowledge, the effect of galectin-1 on insect immune systems has not been previously reported. Hosts infected with this recombinant virus exhibited reduced appetite and died sooner in both laboratory and small-scale field studies, suggesting that the overexpression of galectin-1 can more efficiently eliminate pest hosts. In addition to disrupting the integrity of the peritrophic matrix, the immune system of hosts infected with recombinant baculovirus carrying the *galectin-1* gene was suppressed, making hosts more vulnerable to secondary infection. These results demonstrated that the potential of baculovirus for pest control can be improved by using a recombinant baculovirus that overexpresses mammalian galectin-1 in hosts.

## Key Message

- Baculoviruses facilitate recombinant protein production and play important roles in pest management.
- Mammalian galectin-1 exhibited activity toward insect larvae via midgut peritrophic membranes.
- Mammalian galectin-1 immune gene expression and cell-mediated immunity in *Spodoptera litura*.
- Baculovirus insecticidal activity was synergistically enhanced by co-expression of galectin-1.

## Introduction

Pest control has been important to conventional agriculture, and chemical-based pesticides are widely used for this purpose. Even though chemical pesticides are highly efficient at eliminating pests, the widespread use of pesticides causes environmental pollution and, due to a lack of target specificity, may cause adverse reactions in animals that ingest it while eating the crops (Bonmatin et al., 2015, Goulson, 2013, Henry et al., 2012, vanderWerf, 1996). Bio-pesticides, such as bacteria, fungi, and viruses, have become alternative tools for pest control because they are less likely to be biohazards in the environment and have a more specific host range (Copping and Menn, 2000). However, bio-pesticides are unable to completely replace chemical pesticides due to their lack of efficiency and underdevelopment of industrialized production. One method for improving bio-pesticide efficiency is to genetically modify

pathogens to overexpress toxins, hormones, and specific enzymes to expedite the infection process (Copping and Menn, 2000).

*Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) belongs to the family *Baculoviridae*. Baculoviruses are double-stranded DNA viruses with a genome size ranging from 88 to 153 kb (Burgess, 1977). These viruses mainly infect invertebrates and have been reported to infect more than 600 insect species (Martignoni, 1986). There are two phenotypes in the life cycle of baculoviruses: budded virus (BV) and occluded virus (ODV). After being ingested by larvae, occlusion bodies are passed down to the midgut, where they are dissolved by the alkaline condition of the gut, releasing ODVs. These ODVs are taken up by microvilli and subsequently enter host cells to commence infection to produce BV progenies (Blissard, 1996). BVs then infect cells in blood and other tissues, such as fat, muscle, and epithelium, resulting in systemic infection (Granados and Lawler, 1981, Blissard and Rohrmann, 1990). Owing to their specific host range, baculovirus used as bio-pesticides would not pose a threat to the environment or non-target organisms. Nevertheless, it takes 7 days for baculoviruses to complete their infection cycle and kill the host, which is an issue limiting their applicability as bio-pesticides.

Galectin-1 is an animal lectin that can bind to  $\beta$ -galactoside, which is present in muscle and fat tissues in humans; these cells have receptors for  $\beta$ -galactoside on their surfaces (Barondes et al., 1994, Cho and Cummings, 1995). Galectin-1 mediates several physiological functions of these cells, including growth, differentiation, and adhesion (Camby et al., 2006, Adams et al., 1996, Ahmad et al., 2004). The toxicity of galectin-1 in lepidopteran larvae has also been demonstrated (Chen et al., 2009). When diamondback moth (*Plutella xylostella*) larvae were fed with galectin-1-coated leaves, they became anorexic and exhibited 60% mortality in 4 days. Galectin-1 was able to bind to chitins on the peritrophic matrix in the midgut, disrupting the integrity of the midgut. This disruption not only resulted in anorexia in *P. xylostella* larvae but also allowed pathogens to enter and invade the hemocoel. Galectin-1 suppresses immune responses in mammals (Allione et al., 1998, Blaser et al., 1998) and can induce apoptosis in the human T cell line HPB-ALL by binding to a cluster of differentiation proteins (CDs) located on the T cell surface with the highest affinity toward CD45 (Perillo et al., 1995). Binding of CD45 by galectin-1 has been shown to induce apoptosis in immune cells (Ong et al., 1994, Stillman et al., 2006). Nevertheless, to the best of our knowledge, the effect of galectin-1 on the immune system of insects has not been reported.

To enhance the efficiency of baculoviruses as bio-pesticides in the field, a recombinant baculovirus carrying the *galectin-1* gene under the control of the *polyhedrin* promoter was constructed (Maeda, 1989, Miller, 1988, Weyer et al., 1990, Bonning and Hammock, 1996). We assessed the efficiency of this recombinant virus as a bio-pesticide against *Spodoptera litura* (tobacco cutworm) larvae. Single feeding of the recombinant virus to the larvae could decrease larval appetite, and continuous feeding was sufficient to cause 70% mortality in 5 days. Gene expression analysis suggested that the expression of genes involved in the Toll pathway was suppressed, increasing the infection efficiency of the recombinant virus. Tests in simulated field trials and other common pests also confirmed that recombinant viruses combined with Gal-1 have high insecticidal abilities.

# Materials And Methods

## Cells and viruses

The *S. frugiperda* IPLB-Sf-21 (Sf-21) cell line was cultured in TC-100 insect medium containing 10% heat-inactivated fetal bovine serum. Sf-21 monolayers were used for propagation of wild type AcMNPV or recombinants baculovirus. The titers of the virus clones were estimated by both conventional TCID<sub>50</sub> and quantitative PCR methods (Lin et al., 2020).

## Generation of recombinant virus

*Gal-1* gene fragment was amplified from Chinese hamster ovary cells (CHO cells) and subsequently cloned into T&A™ Cloning Vector using T&A™ Cloning Vector Kit (Yeastern Biotech). T&A™ cloning vector containing *Gal-1* gene was digested with XhoI and XbaI and the insert was cloned into pBacPAK8 (Takara), generating pABpRpGal1. Plasmid pABpRpGal1 was purified using High Pure Plasmid Isolation Kit (Roche) and co-transfected with BacPAK viral DNA (Takara) into *S. frugiperda* cell line IPLB-Sf21 using lipofectamine (Invitrogen) as transfection reagent, generating vABpRpGal1. Recombinant baculovirus vABpRpGal1 was amplified in IPLB-Sf21 cells and the titre was determined using quantitative real-time polymerase chain reaction, qPCR).

## Western blot analysis of Galectin-1 expression

*S. litura* cell line SL1A cells ( $1 \times 10^5$  cells/well) were seeded in a 24-well plate and infected with recombinant virus at MOI (multiplicity of infection) = 1. Forty-eight hours after infection, supernatant was removed and cells were lysed with 100 mL RIPA buffer. Cell lysate was mixed with equal volume of Laemmli sample buffer (Bio-rad) and sample was resolved in 10% SDS-polyacrylamide gel using Wet/Tank Blotting Systems (Bio-rad). Resolved sample was transferred onto a PVDF membrane (Millipore) (Wu et al., 2018). Expression of Galectin-1 was detected using either rabbit anti-6xHis antibody (LTK) or anti-Galectin-1 antibody as primary antibody, followed by secondary goat anti-rabbit IgG antibody (Merck) or anti-mouse IgG antibody. Signal was detected using Western HRP Substrate (Millipore) and quantified using UVP ChemStudio (analytikjena).

## Maintenance of *S. litura*

*S. litura* (Tobacco cutworms) were purchased from a farm which breed these insects in the laboratory for generations (New Taipei City, Taiwan). The artificial feed for these insects was prepared as follows: a) mix 90 g of kidney bean powder, 36 g of yeast extract, 33 g of wheat germ powder and 480 mL of sterile water in a blender for 5 minutes; b) dissolve 036 g L-cysteine (PanReac AppliChem), 3.6 g L-ascorbic acid (PanReac AppliChem), and 0.225 g streptomycin sulfate (Bioshop) in 60 mL of sterile water; c) pour b) into blender containing a) and mix well; d) dissolve 9.9 g agar completely in 300 mL of water by boiling; and e) pour d) into c) and homogenize for 5 minutes. The content was poured into container and allowed to hardened and kept at 4°C until use. Tobacco cutworms were kept in round and transparent plastic

boxes (8cm(L) x 5.5 cm(H)) at 26±1°C with alternating light : dark cycle at 12 hrs : 12 hrs. Feed was refreshed daily.(Tang et al., 2021)

### **Measurement of survival rate, appetite and body length**

Second-instar *S. litura* larvae were divided into 4 groups, control, wild-type baculovirus (wt AcMNPV), recombinant vABpRpGal1 (vABpRpGal1) group and commercially available biological insecticides (*Bacillus thuringiensis* (0.3 kilograms per hectare)). Larvae in wt AcMNPV and vABpRpGal1 groups were fed with 1 x 10<sup>6</sup> pfu virus/larva. For single feeding experiment, larvae were fed with virus on day one of the experiment. For continuous feeding experiment, fixed sum of viruses was given daily. Larvae were observed for 7 days and mortality and body length of experiment larvae in each group were recorded daily. For appetite measurement, every larva which survived in the single feeding experiment was placed on one leaf and the area ingested by the larva after 24 hours was recorded.

### **Relative gene expression analysis**

SL1A cells were seeded in a 24-well plate and infected with wild-type AcMNPV or recombinant vABpRpGal1 at MOI = 1. Infected cells were harvested 48 hours post infection and total RNA was extracted using Total RNA Mini Kit (Geneaid) following manufacturer's protocol. RNA concentration was determined using NanoDrop 2000 (Thermo Fisher Scientific). First-strand cDNA was obtained using PrimeScript<sup>®</sup> RT reagent kit (Takara). Sample was prepared to include: 2 mL of 5x PrimeScript buffer, 0.5 mL of PrimeScript RT enzyme mix, 0.5 mL of oligo dT primer, 2 mL of random hexamer, 100 ng of RNA and RNase-free water. Sample was incubated at 37°C for 15 minutes, followed by 5 seconds at 85°C and then cooled down to 4°C. Relative expression level of target gene in samples was measured by quantitative real-time polymerase chain reaction. Each sample was set up to include 10 mL of SYBR green (Bioline), 0.8 mL of forward primer, 0.8 mL of reverse primer, 1 mL of cDNA as template, and 7.4 mL of sterile double-distilled water. Reaction was carried out with the following program: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 sec and extension at 60°C for 30 sec (Lu et al., 2020). Sequences of the primer pairs used are listed in table 1.

### **Phagocytosis activity assay**

Fourth instar larvae were injected with phosphate buffered saline (PBS, control), 1 x 10<sup>6</sup> pfu wild-type AcMNPV (wt), or 1 x 10<sup>6</sup> pfu recombinant vABpRpGal1 virus (vABpRpGal1). Forty-eight hours after injection, hemocytes were collected from hemolymph and seeded in a 96-well plate (4 x 10<sup>6</sup> cells/well). FITC-conjugated *E. coli* were added per well and the plate was incubated in the dark for 1 hr. Cells were washed 2 – 3 times with phosphate buffered saline. Staining solution containing 50 mL of 0.4% trypan blue and 50 mL of TC100 culture medium was added per well and cells were stained for 10 min. After washing with PBS, cells were stained with 300 mM DAPI (Thermo Fisher) for 30 minutes. Stained cells were washed 2 – 3 times with PBS and re-suspended in 100 mL of TC-100 culture medium. They were observed under fluorescence microscope to determine the ratio of *E. coli* to hemocytes (Wan et al., 1993, Chang et al., 2020).

## Semi-Field trial

Eight to ten lettuce seedlings were planted with equal spacing in a containing (57cm x 37cm x 19cm). Wild-type AcMNPV and vABpRpGal1 solution containing 1% Tween20 was sprayed onto seedlings in wt and vABpRpGal1 group ( $1 \times 10^9$  pfu virus/larva), respectively, and seedlings in control group were sprayed with sterile water. One hour after spraying, 40 or 80 second-instar *S. litura* larvae were placed on each seedling and observed for 2 or 3 days. The number of surviving larvae was recorded daily and unfound larva was counted as dead. Leaves of each seedling were photographed after experiment. (Cory et al., 1994)

## Statistical analysis

Statistical difference among groups in survival rate was analyzed using Log-rank test. Quantitative PCR (qPCR) data in gene expression analysis was converted to relative expression level ( $2^{-DDCt}$ ) and statistical difference in relative expression level was analyzed using student's t-test. A *p* value less than 0.05 ( $p < 0.05$ ) was considered statistically different and marked with an asterisk (\*) (Hu et al., 2018).

# Results

## Generation of recombinant baculovirus carrying Gal-1 gene

To investigate whether the overexpression of galectin-1 could improve the biopesticide potential of baculovirus, a recombinant baculovirus, vABpRpGal1, carrying the *Gal-1* gene under the control of the very-late *polyhedrin* promoter and *dsRed* gene as a reporter gene was generated. A control virus carrying only the *dsRed* gene was also generated (Fig. 1A). IPLB-Sf-21 cells infected with vABpRpGal1 exhibited red fluorescence 48 h post infection (Fig. 1B), and the expression of Gal-1 in infected cells was confirmed by western blot analysis (Fig. 1C).

## Recombinant baculovirus carrying gal-1 gene significantly induced higher mortality in *S. litura* larvae

To evaluate the toxicity of recombinant vABpRpGal1 in *S. litura* larvae, the virus was added once to the artificial feed on day one of the experiment, larvae were observed for 7 days, and the mortality was recorded daily. There was no difference between the vABpRpGal1 and control virus groups in the survival rate (over 95% for both) (Fig. 2A). Larvae fed with vABpRpGal1 were smaller (Fig. 2B), and therefore, the surviving larvae were placed on sweet potato leaves (one leaf per larva) to assess their appetite. The larvae from the control virus group ingested more than 50% of the leaves, whereas the larvae from the vABpRpGal1 group ingested less than 50% of the leaves (Fig. 2C and 2D), indicating that vABpRpGal1 reduced larval appetite.

To evaluate the effect of continuous vABpRpGal1 exposure in larvae, viruses were added to the artificial feed daily for 7 days. The larvae fed with the control virus had a survival rate of 80% on day 7, whereas larvae fed with vABpRpGal1 had a survival rate of less than 40% on day 5 and less than 20% on day 7

(Fig. 3A). The analysis of body length showed that, starting on day 3 of the experiment, larvae in virus groups exhibited slower growth rates compared to those in the control group, and larvae in the vABpRpGal1 group started to show signs of growth arrest on day 5 and had the shortest body length on day 6 (Fig. 3B and 3C). These results suggested that galectin-1 could negatively influence the appetite and growth of *S. litura* larvae.

### **Galectin-1 increases the vulnerability of *S. litura* larvae to baculovirus by suppressing the immune system**

Galectin-1 was shown to bind to receptors on the T cell surface in mammals and subsequently induce apoptosis, resulting in a suppressed immune system. This is also a tactic used by cancer cells to avoid immune system attacks. To evaluate the effect of vABpRpGal1 infection on the immune response of *S. litura*, the expression of genes involved in the Toll pathway was measured as pathogen-induced humoral immunity in insects mediated mainly via the Toll pathway. Upstream toll-like receptors and downstream anti-bacterial peptide cecropin expression levels increased in larvae infected with the control virus, suggesting that pathogen infection elicited an immune response. In contrast, the expression levels of upstream toll-like receptors (Toll), Myd88, Pelle, and downstream anti-bacterial peptide cecropin were lower in the vABpRpGal-1 group (Fig. 4A), suggesting that Gal-1 had an inhibitory effect on the Toll pathway. Similar results were obtained when the expression of genes involved in the IMD pathway, including *pgrp*, *fadd*, and *relish*, was analyzed (Fig. 4B), indicating that Gal-1 suppressed the expression of genes involved in the immune response of host larvae.

Phagocytosis is the primary cell-mediated immune response in insects. To evaluate the effect of Gal-1 on cell-mediated immunity, the phagocytic activity of hemocytes collected from *S. litura* larvae 48 h after infection with baculovirus was measured using FITC-labeled *E. coli*. Phagocytosed *E. coli* emitted green fluorescence when observed under a fluorescence microscope, while the fluorescence from un-phagocytosed *E. coli* was masked by trypan blue dye. Fluorescence microscopy images showed green fluorescence in the control and control virus groups, whereas significantly less green fluorescence signals were detected in the vABpRpGal1 group (Fig. 4C, left). Phagocytic activity (phagocytosis) was determined based on the ratio of the number of fluorescent cells to the total number of cells. The phagocytic activity was inhibited following viral infection, and the activity was further inhibited by the expression of Gal-1 (Fig. 4C, right). Combining these results, we concluded that Gal-1 exerted an inhibitory effect on both humoral and cell-mediated immune responses.

### **Recombinant baculovirus expressing Gal-1 has potential as a bio-pesticide in the field**

To evaluate the potential of recombinant baculoviruses expressing Gal-1 as bio-pesticides, a field trial was conducted. Eight to ten lettuce plants were planted in a box (40 cm × 60 cm, Fig. 5A), and the leaves were sprayed with a solution containing control virus, vABpRpGal1, or sterile water for the control group. In total, 40 or 80 *S. litura* larvae were evenly distributed on the leaves. To evaluate the toxicity of recombinant vABpRpGal1 in *S. litura* larvae, leaves were sprayed with virus once on day 1 of the experiment. Larvae were observed for 1 to 7 days, and the mortality was recorded daily. The survival rate was measured and photographs were taken of the leaves ingested by the larvae after 2 days (cabbage)

and 3 days (lettuce). Ingested lettuce photographs showed that larvae without viral infection ingested almost all the leaves, whereas larvae infected with the control virus ingested approximately half of the leaves, and larvae infected with vABpRpGal1 ingested the fewest leaves (Fig. 5B). The survival rate for the control and control virus groups was 95% and 85%, respectively, whereas it was only 52.5% for the vABpRpGal1 group (Fig. 5C). The other two lettuce replicates showed the same trend (Fig. 5D and 5E).

Similar results were obtained when cabbage was used as the food source, with a survival rate of 97.5% for the control, 82.5% for the control virus, and 70% for the vABpRpGal1 groups (data not shown). These results indicated that overexpression of Gal-1 increased the lethality of baculovirus in *S. litura* larvae. To evaluate the bio-pesticide potential of recombinant vABpRpGal1 in other pests, a field experiment was conducted using fall armyworm (*Spodoptera frugiperda*) and diamondback moth larvae, and similar results were obtained as those with *S. litura* larvae (Fig. 6A and 6B). When the experiment was conducted using insects with economic value, such as honeybees, recombinant vABpRpGal1 did not exert any toxicity (Fig. 6C), suggesting its safety in the field for non-host insects. Generally, using viruses to control pests results in worse outcomes compared to using bacteria. The insecticidal efficiency of recombinant baculovirus expressing Gal-1 was compared with another bio-insecticide, *Bacillus thuringiensis*, and the results showed that the insecticidal effects (growth and survival rate) of the Gal-1 virus did not differ significantly from those of *B. thuringiensis* (Fig. 6D). Thus, the constructed recombinant virus expressing Gal-1 exhibits potential for practical application as a bio-pesticide.

## Discussion

The use of baculoviruses as bio-pesticides has been proposed and tested for over two decades (Moscardi, 1999, Szewczyk et al., 2006). The occluded form of baculovirus is stable in the environment, and virus particles are released after it is ingested by the host to initiate infection (Braunagel et al., 2003). However, a major shortcoming of baculoviruses as bio-pesticides is that they cannot eliminate pests as efficiently as chemical pesticides. Through genetic engineering, recombinant baculoviruses can be generated to carry gene(s) that can help increase the efficiency of the baculovirus as a bio-pesticide (Kitts and Possee, 1993, Summers, 2006, Inceoglu et al., 2001, Stewart et al., 1991). In the present study, a recombinant baculovirus carrying the mammalian *galectin-1* gene and expressing the Gal-1 protein vABpRpGal1 was generated, and its potential as an efficient bio-pesticide was evaluated. Single infection with vABpRpGal1 did not increase the mortality of *S. litura* larvae, but did affect the appetite of the larvae (Fig. 2D). This result may be due to the disruption of the peritrophic matrix in the midgut mediated by galectin-1, which resulted in the larvae being smaller (Fig. 2C) and possibly dying before metamorphosis.

In addition to disrupting the structure of the peritrophic matrix in the midgut, galectin-1 has been shown to suppress the activation of T cells in mammals, a strategy utilized by cancer cells to escape attack from the immune system (Rubinstein et al., 2004, He and Baum, 2006, Kovacs-Solyom et al., 2010). However, the effect of galectin-1 on the immune system in insects has not yet been investigated. The expression profile of immune response genes in *S. litura* larvae infected with vABpRpGal1 showed that the expression of genes involved in the Toll and Imd pathways, both of which are involved in humoral

immune response, was suppressed (Fig. 4A and 4B). Furthermore, a phagocytic activity assay showed that the cell-mediated immune response was also significantly suppressed in larvae infected with vABpRpGal1. The mechanism underlying phagocytosis inhibition mediated by galectin-1 in insects is yet to be elucidated, but it may be similar to the inhibition of T cell activation. Suppression of the host immune system not only expedites the baculovirus infection cycle in *S. litura* larvae but may also increase their vulnerability to other pathogens in the field. Galectin-1 has been shown to bind to various glycoproteins on the cell surface (Ahmad et al., 2004), and it is likely that physiological functions other than pest immune response are also affected by galectin-1. Further research is needed to fully understand the overall effect of overexpressing galectin-1 on insects.

To increase their efficiency as bio-pesticides, recombinant baculoviruses were engineered to express proteins that would help achieve this goal, and several toxin proteins were tested. A recombinant baculovirus carrying AaIT, a scorpion toxin that acts on the nervous system, was able to paralyze larvae and stop them from eating (Mccutchen et al., 1992). Scorpion toxin is a good candidate for enhancing baculovirus efficiency (Maeda et al., 1991, Popham et al., 1998). However, it poses a biohazard risk because the dead larvae may be ingested along with crops, which could induce allergic reactions in humans. In addition to toxins, proteins that are toxic only to insects have also been tested. A recombinant baculovirus expressing the kernel mitochondrial protein URF13 not only suppressed larval growth but also induced death sooner (Korth KL, 1993). Proteins that could disrupt physiological barriers were also tested. Recombinant baculoviruses that express proteases ScathL, from *Sarcophaga peregrina*, and keratinase, from *Aspergillus fumigatus*, can degrade tissue structure causing the infected larvae to become anorexic (Gramkow et al., 2010). However, humans that ingest infected larvae may also be affected by these proteases. The midgut is the target for insecticidal crystal proteins of *B. thuringiensis*, and a recombinant baculovirus expressing insecticidal crystal proteins was shown to enhance the lethality of the baculovirus in host larvae (Ribeiro and Crook, 1993, Chang et al., 2003, Pang et al., 1992, Bai et al., 1993). In most of these previous studies, recombinant baculoviruses were directly injected into the host larvae to ensure infection, and virus infection was initiated in the hemolymph. In the present study, the recombinant baculovirus was administered orally via the ingestion of leaves to simulate field conditions. When administered orally, the virus must pass through the esophagus to the gut and finally reach the hemolymph. Nevertheless, a mortality rate of over 50% was recorded on day 5 post-infection (Fig. 3A), suggesting that recombinant baculoviruses expressing galectin-1 have potential as bio-pesticides. Further research is required to determine the effect of galectin-1 on humans and other non-target organisms once ingested.

The goal of genetically modifying baculoviruses is to replace chemical-based pesticides/insecticides in the field. In the present study, the recombinant baculovirus expressing galectin-1 reduced the appetite of *S. litura*, *S. frugiperda*, and *P. xylostella* larvae, increased their mortality rate in the field trial (Figs. 5 and 6), and showed no effect on honeybees. These results strongly suggested that incorporating the *galectin-1* gene into the baculovirus genome can significantly improve the bio-pesticide potential of baculoviruses in the field.

# Declarations

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## Author Contributions Statement

Guarantors of the integrity of the entire study, study concepts, and manuscript preparation: C.Y.L., Y.C.L., Y.L.W., and R.N.H. Study design, data acquisition/analysis, literature research, and manuscript preparation: C.Y.L., Y.C.L., Y.L.W., and R.N.H. Data acquisition/analysis, manuscript editing, and revision: C.Y.L., Y.L.W., and R.N.H. All authors reviewed the manuscript.

## Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## Code availability

Software applications are mentioned in the methods, and programs are publicly available.

## Competing interests

The author(s) declare no competing interests.

# References

1. ADAMS, L., SCOTT, G. K. & WEINBERG, C. S. 1996. Biphasic modulation of cell growth by recombinant human galectin-1. *Biochimica Et Biophysica Acta-Molecular Cell Research*, 1312, 137-144.
2. AHMAD, N., GABIUS, H. J., SABESAN, S., OSCARSON, S. & BREWER, C. F. 2004. Thermodynamic binding studies of bivalent oligosaccharides to galectin-1, galectin-3, and the carbohydrate recognition domain of galectin-3. *Glycobiology*, 14, 817-825.
3. ALLIONE, A., WELLS, V., FORNI, G., MALLUCCI, L. & NOVELLI, F. 1998. beta-galactoside-binding protein (beta GBP) alters the cell cycle, up-regulates expression of the alpha- and beta-chains of the

- IFN-gamma receptor, and triggers IFN-gamma-mediated apoptosis of activated human T lymphocytes. *Journal of Immunology*, 161, 2114-2119.
4. BAI, C., DEGHEELE, D., JANSENS, S. & LAMBERT, B. 1993. Activity of Insecticidal Crystal Proteins and Strains of *Bacillus-Thuringiensis* against *Spodoptera-Exempta* (Walker). *Journal of Invertebrate Pathology*, 62, 211-215.
  5. BARONDES, S. H., CASTRONOVO, V., COOPER, D. N. W., CUMMINGS, R. D., DRICKAMER, K., FEIZI, T., GITT, M. A., HIRABAYASHI, J., HUGHES, C., KASAI, K., LEFFLER, H., LIU, F. T., LOTAN, R., MERCURIO, A. M., MONSIGNY, M., PILLAI, S., POIRER, F., RAZ, A., RIGBY, P. W. J., RINI, J. M. & WANG, J. L. 1994. Galectins - a Family of Animal Beta-Galactoside-Binding Lectins. *Cell*, 76, 597-598.
  6. BLASER, C., KAUFMANN, M., MULLER, C., ZIMMERMANN, C., WELLS, V., MALLUCCI, L. & PIRCHER, H. 1998. beta-galactoside binding protein secreted by activated T cells inhibits antigen-induced proliferation of T cells. *European Journal of Immunology*, 28, 2311-2319.
  7. BLISSARD, G. W. 1996. Baculovirus-insect cell interactions. *Cytotechnology*, 20, 73-93.
  8. BLISSARD, G. W. & ROHRMANN, G. F. 1990. Baculovirus Diversity and Molecular-Biology. *Annual Review of Entomology*, 35, 127-155.
  9. BONMATIN, J. M., GIORIO, C., GIROLAMI, V., GOULSON, D., KREUTZWEISER, D. P., KRUPKE, C., LIESS, M., LONG, E., MARZARO, M., MITCHELL, E. A. D., NOOME, D. A., SIMON-DELISO, N. & TAPPARO, A. 2015. Environmental fate and exposure; neonicotinoids and fipronil. *Environmental Science and Pollution Research*, 22, 35-67.
  10. BONNING, B. C. & HAMMOCK, B. D. 1996. Development of recombinant baculoviruses for insect control. *Annual Review of Entomology*, 41, 191-210.
  11. BRAUNAGEL, S. C., RUSSELL, W. K., ROSAS-ACOSTA, G., RUSSELL, D. H. & SUMMERS, M. D. 2003. Determination of the protein composition of the occlusion-derived virus of *Autographa californica* nucleopolyhedrovirus. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 9797-9802.
  12. BURGESS, S. 1977. Molecular weights of Lepidopteran baculovirus DNAs: derivation by electron microscopy. *Journal of General Virology*, 37, 501-510.
  13. CAMBY, I., LE MERCIER, M., LEFRANC, F. & KISS, R. 2006. Galectin-1: a small protein with major functions. *Glycobiology*, 16, 137r-157r.
  14. CHANG, J. H., CHOI, J. Y., JIN, B. R., ROH, J. Y., OLSZEWSKI, J. A., SEO, S. J., O'REILLY, D. R. & JE, Y. H. 2003. An improved baculovirus insecticide producing occlusion bodies that contain *Bacillus thuringiensis* insect toxin. *Journal of Invertebrate Pathology*, 84, 30-37.
  15. CHANG, Y., TANG, C.-K., LIN, Y.-H., TSAI, C.-H., LU, Y.-H. & WU, Y.-L. 2020. Snellenius manilae bracovirus suppresses the host immune system by regulating extracellular adenosine levels in *Spodoptera litura*. *Scientific reports*, 10, 1-11.
  16. CHEN, S. J., CHEN, N. T., WANG, S. H., HSU, J. C., DING, W. H., KUO-HUANG, L. L. & HUANG, R. N. 2009. Insecticidal action of mammalian galectin-1 against diamondback moth (*Plutella xylostella*). *Pest Management Science*, 65, 923-930.

17. CHO, M. J. & CUMMINGS, R. D. 1995. Galectin-1, a Beta-Galactoside-Binding Lectin in Chinese-Hamster Ovary Cells .1. Physical and Chemical Characterization. *Journal of Biological Chemistry*, 270, 5198-5206.
18. COPPING, L. G. & MENN, J. J. 2000. Biopesticides: a review of their action, applications and efficacy. *Pest Management Science*, 56, 651-676.
19. CORY, J. S., HIRST, M. L., WILLIAMS, T., HAILS, R. S., GOULSON, D., GREEN, B. M., CARTY, T. M., POSSEE, R. D., CAYLEY, P. J. & BISHOP, D. H. L. 1994. Field Trial of a Genetically Improved Baculovirus Insecticide. *Nature*, 370, 138-140.
20. GOULSON, D. 2013. REVIEW: An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology*, 50, 977-987.
21. GRAMKOW, A. W., PERECMANIS, S., SOUSA, R. L. B., NORONHA, E. F., FELIX, C. R., NAGATA, T. & RIBEIRO, B. M. 2010. Insecticidal activity of two proteases against Spodoptera frugiperda larvae infected with recombinant baculoviruses. *Virology Journal*, 7.
22. GRANADOS, R. R. & LAWLER, K. A. 1981. In vivo pathway of Autographa californica baculovirus invasion and infection. *Virology*, 108, 297-308.
23. HE, J. & BAUM, L. G. 2006. Endothelial cell expression of galectin-1 induced by prostate cancer cells inhibits T-cell transendothelial migration. *Laboratory Investigation*, 86, 578-590.
24. HENRY, M., BEGUIN, M., REQUIER, F., ROLLIN, O., ODOUX, J. F., AUPINEL, P., APTEL, J., TCHAMITCHIAN, S. & DECOURTYE, A. 2012. A Common Pesticide Decreases Foraging Success and Survival in Honey Bees. *Science*, 336, 348-350.
25. HU, Y. T., TANG, C. K., WU, C. P., WU, P. C., YANG, E. C., TAI, C. C. & WU, Y. L. 2018. Histone deacetylase inhibitor treatment restores memory-related gene expression and learning ability in neonicotinoid-treated Apis mellifera. *Insect Mol Biol*, 27, 512-521.
26. INCEOGLU, A. B., KAMITA, S. G., HINTON, A. C., HUANG, Q. H., SEVERSON, T. F., KANG, K. D. & HAMMOCK, B. D. 2001. Recombinant baculoviruses for insect control. *Pest Management Science*, 57, 981-987.
27. KITTS, P. A. & POSSEE, R. D. 1993. A Method for Producing Recombinant Baculovirus Expression Vectors at High-Frequency. *Biotechniques*, 14, 810-&.
28. KORTH KL, L. C. 1993. Baculovirus expression of the maize mitochondrial protein URF13 confers insecticidal activity in cell cultures and larvae. *Proc Natl Acad Sci U S A*. 1993;90(8):3388-92.
29. KOVACS-SOLYOM, F., BLASKO, A., FAJKA-BOJA, R., KATONA, R. L., VEGH, L., NOVAK, J., SZEBENI, G. J., KRENACS, L., UHER, F., TUBAK, V., KISS, R. & MONOSTORI, E. 2010. Mechanism of tumor cell-induced T-cell apoptosis mediated by galectin-1. *Immunology Letters*, 127, 108-118.
30. LIN, Y.-H., TAI, C.-C., BROŽ, V., TANG, C.-K., CHEN, P., WU, C. P., LI, C.-H. & WU, Y.-L. 2020. Adenosine Receptor Modulates Permissiveness of Baculovirus (Budded Virus) Infection via Regulation of Energy Metabolism in Bombyx mori. *Frontiers in immunology*, 11, 763.
31. LU, Y. H., WU, C. P., TANG, C. K., LIN, Y. H., MAAROUFI, H. O., CHUANG, Y. C. & WU, Y. L. 2020. Identification of Immune Regulatory Genes in Apis mellifera through Caffeine Treatment. *Insects*, 11.

32. MAEDA, S. 1989. Expression of Foreign Genes in Insects Using Baculovirus Vectors. *Annual Review of Entomology*, 34, 351-372.
33. MAEDA, S., VOLRATH, S. L., HANZLIK, T. N., HARPER, S. A., KEI, M. M., MADDOX, D. W., HAMMOCK, B. D. & FOWLER, E. 1991. Insecticidal Effects of an Insect-Specific Neurotoxin Expressed by a Recombinant Baculovirus. *Virology*, 184, 777-780.
34. MARTIGNONI, M. E. 1986. *A catalog of viral diseases of insects, mites, and ticks*, US Department of Agriculture, Forest Service, Pacific Northwest Research Station.
35. MCCUTCHEN, B. F., CHOUDARY, P., FOWLER, E., HAMMOCK, B. D. & MAEDA, S. 1992. Development of a Recombinant Baculovirus Expressing an Insect Selective Neurotoxin - Potential for Pest-Control. *Abstracts of Papers of the American Chemical Society*, 203, 46-Agro.
36. MILLER, L. K. 1988. Baculoviruses as Gene-Expression Vectors. *Annual Review of Microbiology*, 42, 177-199.
37. MOSCARDI, F. 1999. Assessment of the application of baculoviruses for control of Lepidoptera. *Annual Review of Entomology*, 44, 257-289.
38. ONG, C. J., CHUI, D., TEH, H. S. & MARTH, J. D. 1994. Thymic Cd45 Tyrosine Phosphatase Regulates Apoptosis and Mhc-Restricted Negative Selection. *Journal of Immunology*, 152, 3793-3805.
39. PANG, Y., FRUTOS, R. & FEDERICI, B. A. 1992. Synthesis and Toxicity of Full-Length and Truncated Bacterial CryIvδ Mosquitocidal Proteins Expressed in Lepidopteran Cells Using a Baculovirus Vector. *Journal of General Virology*, 73, 89-101.
40. PERILLO, N. L., PACE, K. E., SEILHAMER, J. J. & BAUM, L. G. 1995. Apoptosis of T-Cells Mediated by Galectin-1. *Nature*, 378, 736-739.
41. POPHAM, H. J. R., PRIKHOD'KO, G. G., FELCETTO, T. J., OSTLIND, D. A., WARMKE, J. W., COHEN, C. J. & MILLER, L. K. 1998. Effect of deltamethrin treatment on lepidopteran larvae infected with baculoviruses expressing insect-selective toxins mu-Aga-IV, As II, or Sh 1. *Biological Control*, 12, 79-87.
42. RIBEIRO, B. M. & CROOK, N. E. 1993. Expression of Full-Length and Truncated Forms of Crystal Protein Genes from *Bacillus-Thuringiensis* Subsp *Kurstaki* in a Baculovirus and Pathogenicity of the Recombinant Viruses. *Journal of Invertebrate Pathology*, 62, 121-130.
43. RUBINSTEIN, N., ALVAREZ, M., ZWIRNER, N. W., TOSCANO, M. A., ILARREGUI, J. M., BRAVO, A., MORDOH, J., FAINBOIM, L., PODHAJECER, O. L. & RABINOVICH, G. A. 2004. Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection: A potential mechanism of tumor-immune privilege. *Cancer Cell*, 5, 241-251.
44. STEWART, L. M. D., HIRST, M., FERBER, M. L., MERRYWEATHER, A. T., CAYLEY, P. J. & POSSEE, R. D. 1991. Construction of an Improved Baculovirus Insecticide Containing an Insect-Specific Toxin Gene. *Nature*, 352, 85-88.
45. STILLMAN, B. N., HSU, D. K., PANG, M., BREWER, C. F., JOHNSON, P., LIU, F. T. & BAUM, L. G. 2006. Galectin-3 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death. *Journal of Immunology*, 176, 778-789.

46. SUMMERS, M. D. 2006. Milestones leading to the genetic engineering of baculoviruses as expression vector systems and viral pesticides. *Insect Viruses: Biotechnological Applications*, 68, 3-73.
47. SZEWCZYK, B., HOYOS-CARVAJAL, L., PALUSZEK, M., SKRZECZ, W. & DE SOUZA, M. L. 2006. Baculoviruses - re-emerging biopesticides. *Biotechnology Advances*, 24, 143-160.
48. TANG, C. K., TSAI, C. H., WU, C. P., LIN, Y. H., WEI, S. C., LU, Y. H., LI, C. H. & WU, Y. L. 2021. MicroRNAs from *Snellenius manilae* bracovirus regulate innate and cellular immune responses of its host *Spodoptera litura*. *Communications Biology*, 4.
49. VANDERWERF, H. M. G. 1996. Assessing the impact of pesticides on the environment. *Agriculture Ecosystems & Environment*, 60, 81-96.
50. WAN, C. P., PARK, C. S. & LAU, B. H. S. 1993. A Rapid and Simple Microfluorometric Phagocytosis Assay. *Journal of Immunological Methods*, 162, 1-7.
51. WEYER, U., KNIGHT, S. & POSSEE, R. D. 1990. Analysis of Very Late Gene-Expression by *Autographa Californica* Nuclear Polyhedrosis-Virus and the Further Development of Multiple Expression Vectors. *Journal of General Virology*, 71, 1525-1534.
52. WU, P. C., LIN, Y. H., WU, T. C., LEE, S. T., WU, C. P., CHANG, Y. & WU, Y. L. 2018. MicroRNAs derived from the insect virus HzNV-1 promote lytic infection by suppressing histone methylation. *Sci Rep*, 8, 17817.

## Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

## Figures

Fig. 1

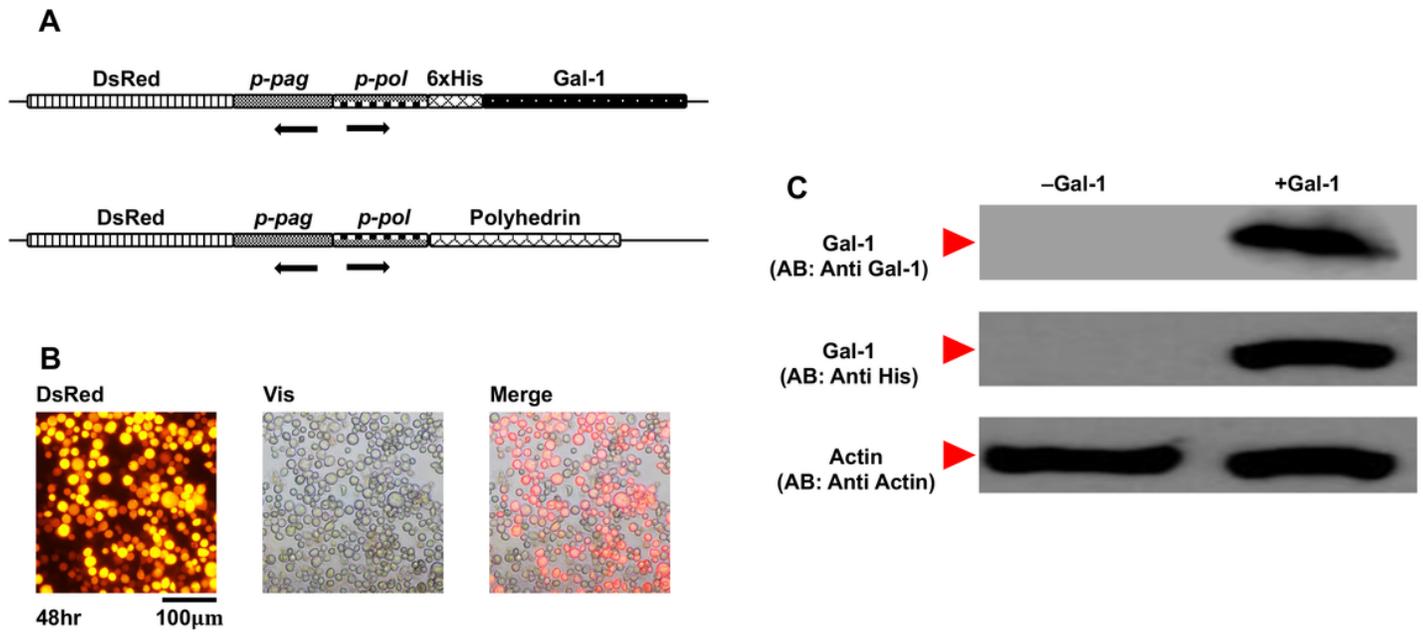
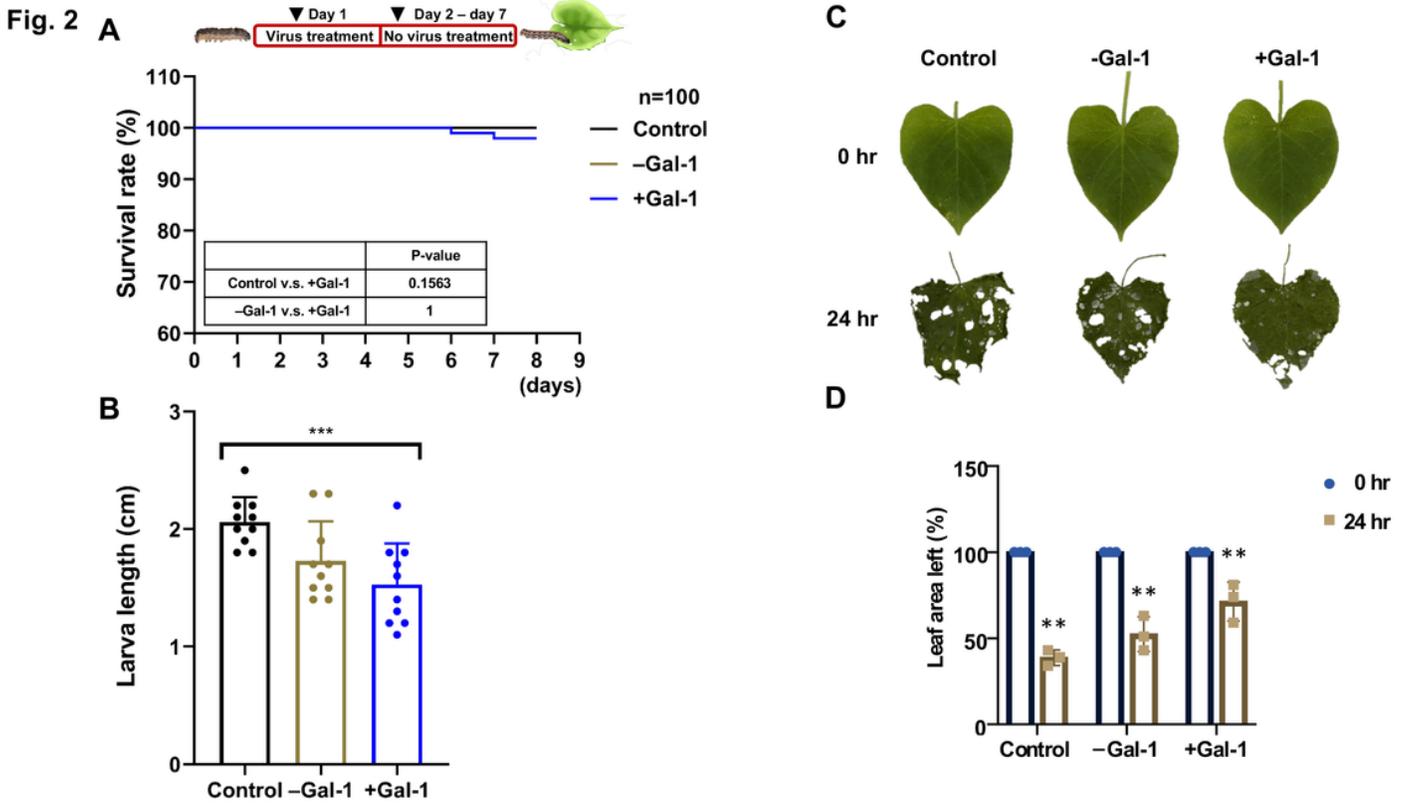


Figure 1

Construction of recombinant viruses vABpR and vABpRpGal1. (A) Expression cassette for vABpRpGal1 (top) and vABpR (bottom) at the polyhedrin locus. DsRed: red fluorescence protein; *p-pag*: *pag* promoter; *p-pol*: polyhedrin promoter; 6xHis: 6 repeats of histidine; Gal-1: galectin-1 protein. (B) IPLB-Sf21-AE cells infected with recombinant vABpRpGal1 (MOI = 1) 48 h post infection. (C) Galectin-1 expression in Sf21 cells infected with vABpR or vABpRpGal1. Sf21 cells were infected with vABpR or vABpRpGal1, and galectin-1 expression was confirmed by western blot analysis (antibodies Anti-Gal1 and Anti-His).



**Figure 2**

Effect of single feeding of recombinant viruses vABpR and vABpRpGal1 on the appetite, growth, and survival rate of *S. litura* larvae. (A) Survival rate of *S. litura* larvae after single feeding of vABpR and vABpRpGal1. Second instar larvae were fed with approximately  $1 \times 10^6$  pfu/larva of vABpR, vABpRpGal1, or sterile water and observed for 7 days. Survival rate among experimental groups was analyzed using the log rank test. There were 100 larvae on day 1 of the experiment. Control: larvae were fed with sterile water; -Gal-1: larvae were infected with vABpR on day 1; +Gal-1: larvae were infected with vABpRpGal1 on day 1. (B) Body length of larvae 7 days after virus infection. The body length of 10 larvae from each group was measured. The data are presented as mean  $\pm$  SD, and difference in body length among groups was analyzed using student's t-test. \* =  $p < 0.05$  was considered significantly different. (C) Leaves not ingested by larvae on day 7 after virus infection. (D) Measurement of leaf area not ingested by larvae in each group on day 7 after virus infection. Three leaves from each group were measured, and the data are presented as mean  $\pm$  SD. \*\* =  $p < 0.005$  was considered significantly different.

Fig. 3

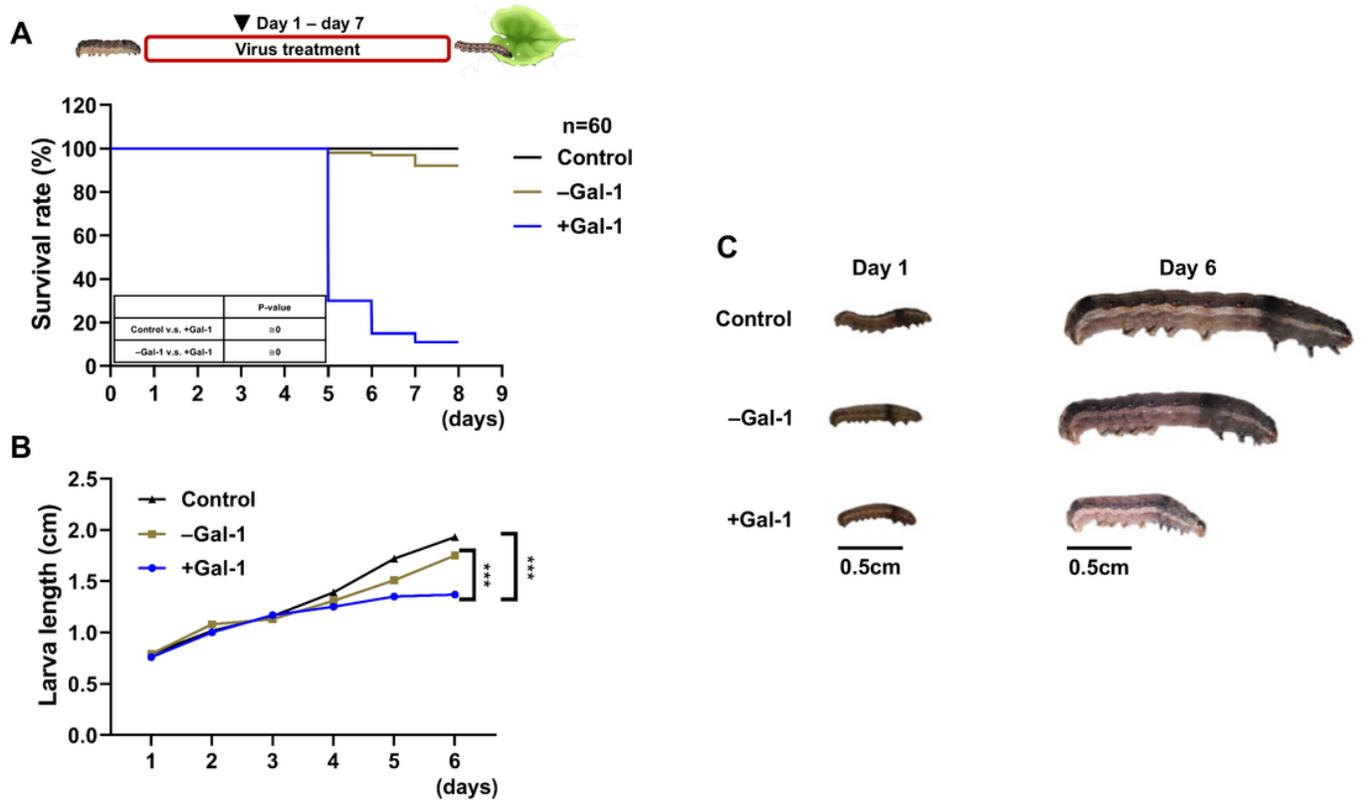


Figure 3

Effect of 7-day feeding on the appetite, growth, and survival rate of *S. litura* larvae. (A) Survival rate of *S. litura* larvae after continuous feeding of vABpR and vABpRpGal1. Second instar larvae were fed with approximately  $1 \times 10^6$  pfu/larva of vABpR, vABpRpGal1, or sterile water daily for 7 days. Survival rate among experimental groups was analyzed using the log rank test. There were 60 larvae per group on day 1 of the experiment. Control: larvae were fed with sterile water; -Gal-1: larvae were infected with vABpR; +Gal-1: larvae were infected with vABpRpGal1. (B) Body length of larvae from day 1 to day 6. The body length of 10 larvae from each group was measured. The data are presented as mean  $\pm$  SD, and body length among groups was analyzed using student's t-test. \* =  $p < 0.05$  was considered significantly different. (C) Representative larvae from each group on day 1 and day 6 of the experiment.

Fig. 4

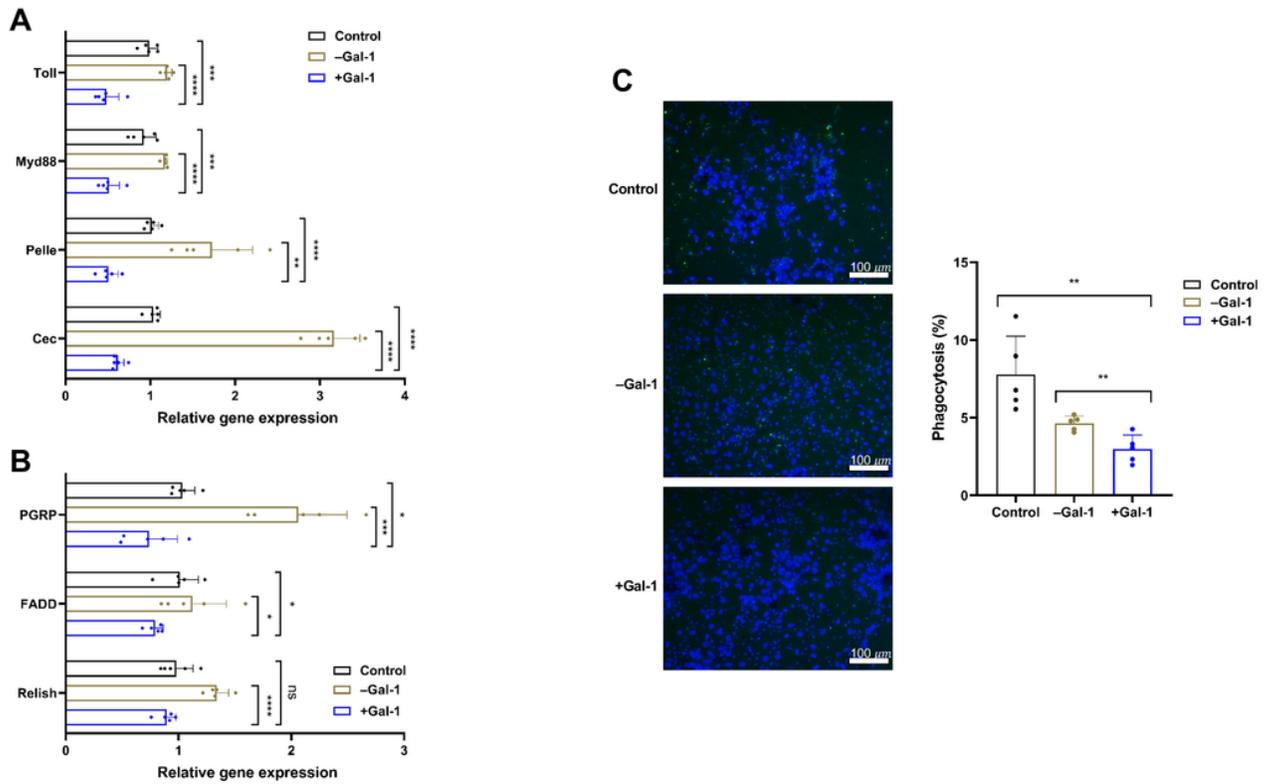


Figure 4

Recombinant baculovirus expressing galectin-1 suppresses humoral and cell-mediated immune responses in *S. litura* cell line SL1A. Expression levels of genes involved in Toll pathway (A) and IMD pathway (B) in SL1A cells not infected with baculovirus (control) and infected with vABpR (-Gal-1) or vABpRpGal1 (+Gal-1). Samples were harvested 48 h after infection for analysis. The expression level of 18S was used for normalization, and the relative expression level of genes in the control group was set to 1. The relative gene expression level in other groups was adjusted accordingly. (C) Representative images of hemocytes collected from 5th instar larvae not infected with virus (control) and infected with vABpR (-Gal-1) or vABpRpGal1 (+Gal-1) in the phagocytic activity assay (left panels). Green fluorescence: *E. coli*; blue fluorescence: hemocytes. Quantification of phagocytic activity of hemocytes is shown on the right. Hemocytes were collected from five larvae from each group for the phagocytic activity assay. Student's t-test was used to analyze the difference among experiment groups. \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ .

Fig. 5

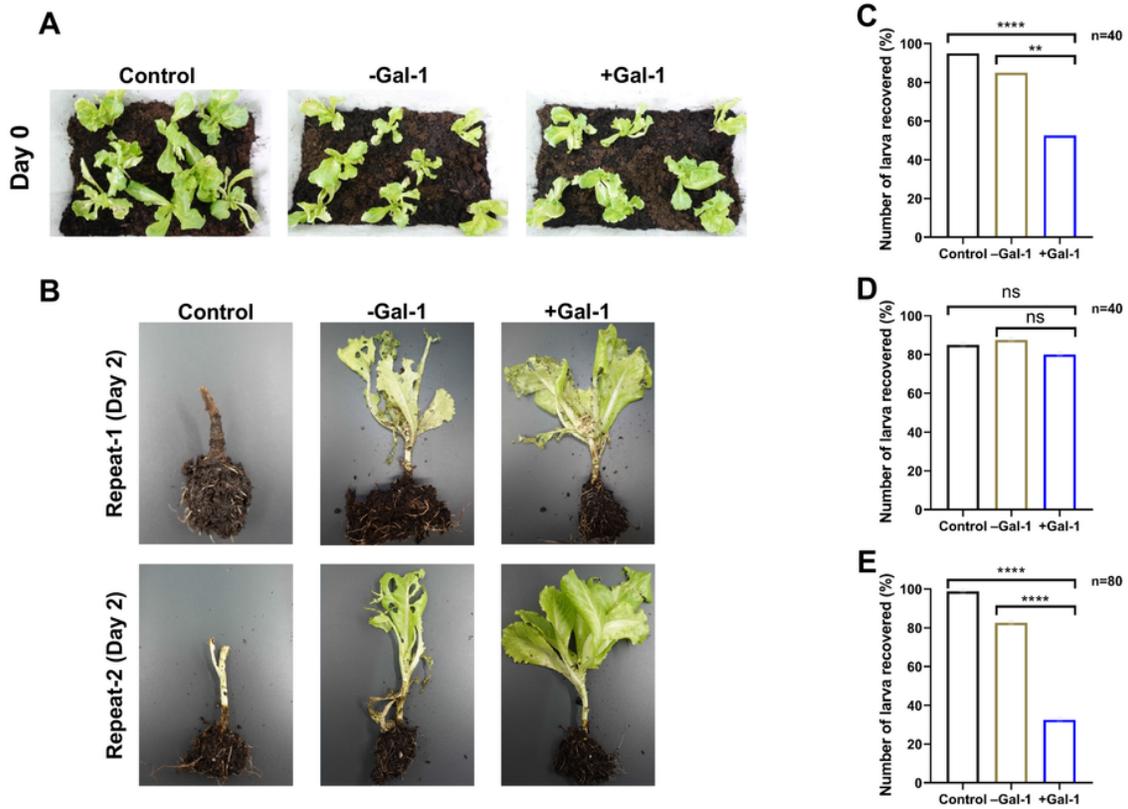
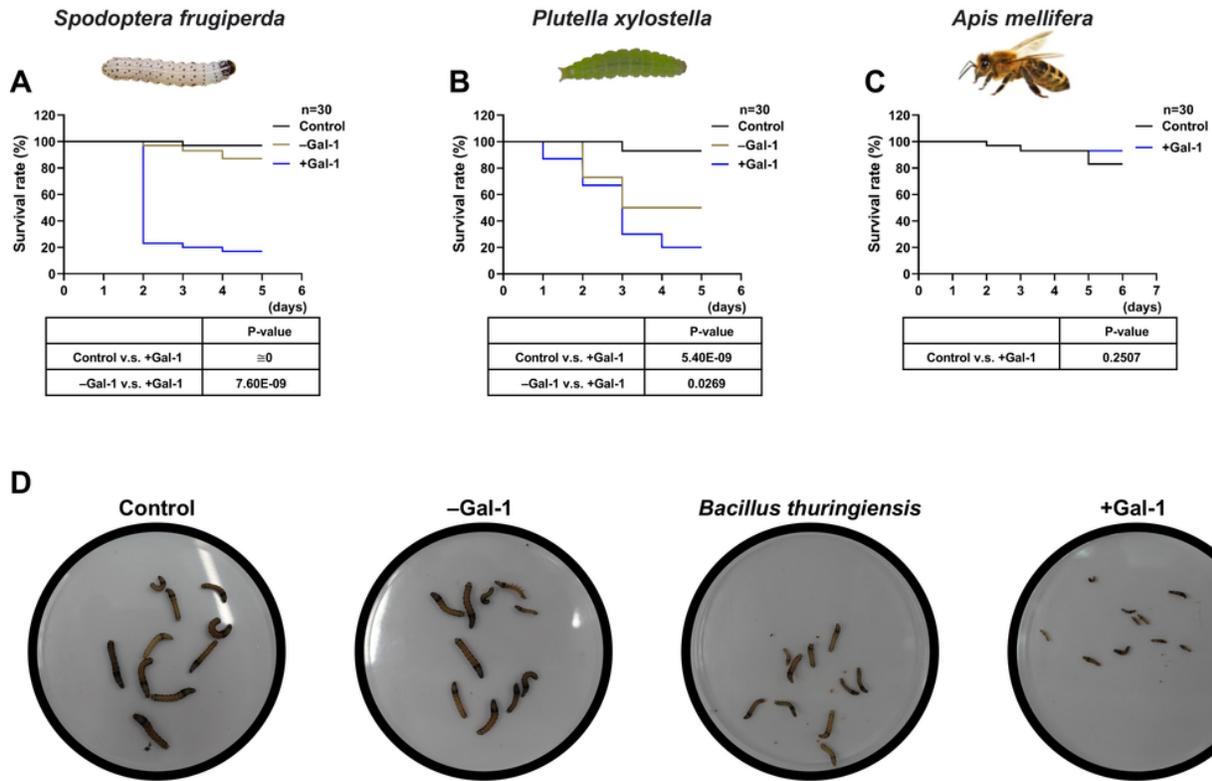


Figure 5

Recombinant baculovirus expressing galectin-1 reduces the appetite of *S. litura* larvae and increases their mortality in the field. Lettuces before (A) and after (B) ingestion by larvae not infected with virus (control) and infected with vABpR (-Gal-1) or vABpRpGal1 (+Gal-1) for 72 h. (C–E) Number of 2nd instar larvae collected from lettuces 72 h after infection.  $n = 40/\text{group}$  on day 1 of the experiment, and each larva received approximately  $1 \times 10^9$  pfu of recombinant viruses, except the control group. The data are presented as mean  $\pm$  SD, and survival rate among groups was analyzed using student's t-test. \* =  $p < 0.05$  was considered significantly different.

**Fig. 6****Figure 6**

Effect of galectin-1 on other insects. Fall armyworms (*Spodoptera frugiperda*) (A), Diamondback moth (*Plutella xylostella*) (B), and honeybees (*Apis mellifera*) (C) were fed with  $1 \times 10^6$  pfu of recombinant baculovirus vABpR (-Gal-1) or vABpRpGal1 (+Gal-1) daily for 7 days, and their survival rate was recorded daily. There were 30 larvae per group on day 1. Statistical analysis was carried out using the log rank test. (D) Body length of *S. litura* larvae from day 4 with vABpR, vABpRpGal1 and *Bacillus thuringiensis* treatment. The body length of 10 larvae from each group was measured.

## Supplementary Files

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