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Vegetable waste extracts as enhancers of baculovirus infections

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ABSTRACT

Vegetable waste extracts (VWE) contain a great variety of antioxidants such as polyphenols, which have shown to potentiate baculovirus infections, making them ingredients for pest control ingredients. In the present study, the mortality enhancement of different vegetable extracts obtained from food residues when combined with baculoviruses was evaluated. Extracts from spent coffee (E2), rosehip (E17), asparagus (E28), artichoke (E29), beet stalks (E32) and banana peel (E37) were selected as they increased mortality of Spodoptera littoralis nucleopolyhedrovirus (SpliNPV) in second instar *S. littoralis* larvae, when comparing with the virus inoculation alone. Extracts were assayed at 1 % w/v. In *S. littoralis*-SpliNPV system, the selected extracts reduced the median lethal concentration (LC_{50}) of SpliNPV against second instar larvae. The E37 extract presented the highest potentiation, as it reduced the LC50 13.61 times, while the rest of the extracts presented LC50 reductions from 3.71 to 7.72-fold. In *Spodoptera exigua*-SeMNPV (Spodoptera exigua multiple nucleopolyhedrovirus) system, none of the extracts decreased the LC_{50} of SeMNPV. In contrast, in *Spodoptera frugiperda*-SfMNPV (Spodoptera frugiperda multiple nucleopolyhedrovirus) system, E2 showed the greatest potentiating effect. In the heterologous systems, none of the extracts tested increased the effective host range of SfMNPV, AcMNPV (Autographa californica multiple nucleopolyhedrovirus), and MbMNPV (Mamestra brassicae multiple nucleopolyhedrovirus) in second instar *S. littoralis* larvae. Thus, the viral enhancing effect of VWE was host-pathogen and instar dependent. However, the potentiation effect of the extracts could not be directly related with the antioxidants content of the extracts.

1. Introduction

Chemical insecticides have participated in the increase of agricultural production by protecting crops from pests at a relatively low cost. Despite that, their indiscriminate use has led to several troubles, including environmental pollution, ozone depletion, development of resistance in target pests, minimization of non-target populations, and impact on human health (Ansari et al., 2014; Braak et al., 2018; Haddi et al., 2020; Midingoyi et al., 2019; Mulé et al., 2017; Serrão et al., 2022; Ziółkowska et al., 2021). Insect pathogenic viruses, mainly those included in the *Baculoviridae* family, have proven to be useful bioinsecticides against insect pests under different agronomical conditions (Deshayes et al., 2017; Grzywacz, 2017; Lacey, 2017; Moscardi, 2003). They are naturally occurring pathogens, highly specific to insects, some of them being infectious to only one or two insect species (Grzywacz, 2017). Hence, they are safe for vertebrates, humans and non-target organisms, and contribute to the maintenance of the biodiversity in agroecosystems (Landwehr, 2021).

However, baculovirus-based bioinsecticides have some limitations compared to chemical insecticides. The cost associated with the mass production is one of the major drawbacks to the use of baculoviruses as biocontrol agents. Baculoviruses require active host cells for their replication since they are obligate intracellular pathogens. Consequently, viral production must be accomplished in susceptible insect cells (Reid et al., 2023). Until now, the only feasible methods for the development of baculovirus-based insecticides have been *in vivo* (Grzywacz et al., 2023; Reid et al., 2023). *In vivo* production involves inoculation of huge numbers of larvae, their rearing during virus infection, and the

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collection and purification of Occlusion Bodies (OBs) from larval carcasses. This process is laborious and expensive, making it difficult for baculovirus-based products to compete with chemical insecticides in the market (Reid et al., 2023). The use of synergistic compounds in baculovirus formulations have proved to decrease lethal doses (Behle and Birthisel, 2023; Hay et al., 2020). For these reasons, the search for synergistic products that reduce the amount of baculovirus active matter needed is key to making this type of bioinsecticide feasible. All of this has led in recent years to exhaustive research in pursuit of the most appropriate formulations (Behle and Birthisel, 2023). Several synthetic and natural compounds have been evaluated for decades as enhancers of insecticidal activity (host spectrum, pathogenicity, and virulence) and as sunscreens against baculoviruses (Hayakawa et al., 2000). Among the different products tested, optical brighteners produced, by far, the greatest enhancement of baculovirus infection under laboratory conditions (Hamm and Shapiro, 1992; Shapiro and Argauer, 2001; Shapiro and Robertson, 1992). Moreover, the viral-enhancing effect was more pronounced in fourth instar (Okuno et al., 2003; Shapiro and Argauer, 2001), so they could solve the problem associated with the overlapping larval generations in agroecosystems, since a very low viral concentration could handle all pest instars. However, optical brighteners are chemically synthesized substances that, when used at concentrations of 1 %, have shown to have negative effects on useful fauna and agroecosystems (Goulson et al., 2000, 2003). Their inclusion in "bio" products would therefore be unacceptable. For this reason, the most recent trend is oriented towards the search for natural substances such as plant extracts.

Fruit and vegetable wastes contains a great variety of bioactive compounds like flavonoids, tannins, flavanols, vitamins, fatty acids, minerals, anthocyanins, volatiles and pigments (Ben-Othman et al., 2020; Jiménez-Moreno et al., 2019; Kumar et al., 2017), with several biological activities such as antioxidant, anti-inflammatory, cardio protective, immune-modulatory and anti-cancerous properties (Gascón et al., 2018; Jiménez-López et al., 2020). These compounds are important for plant physiology and defense against attack by insects and herbivores (Friedman, 2002; Korpan et al., 2004), and have also been used as plant protection products against different pathogens (Shaalan et al., 2005; Stankovic et al., 2020; Waliwitiya et al., 2009).

Plant compounds that are known to have insecticidal effect are alkaloids, flavonoids, glycosides, esters and fatty acids (Hikal et al., 2017). These compounds could be grouped in three categories: alkaloids, polyphenols and terpenoids (Mann and Kaufman, 2012). Polyphenols, secondary metabolites of plants, are toxic to insects, fungi, bacteria, weeds and nematodes. They consist of different types of compounds, which include flavonoids, anthocyanins, phytoalexins, tannins, lignans and coumarins (Stankovic et al., 2020). The insecticidal effects of plant-derived compounds could be classified as repellents, feeding deterrents/antifeedants, toxics, growth retardants, chemosterilants or attractants (Rattan, 2010). In addition, some plant bioactive compounds have a potentiating effect in combination with synthetic or natural insecticides (Shaalan et al., 2005; Stankovic et al., 2020; Waliwitiya et al., 2009). For instance, soybean flavonoids have been tested as enhancers of baculovirus pathogenicity, significantly improving Anagrapha falcifera multiple nucleopolyhedrovirus (AnfaMNPV) activity against *Trichoplusia ni* (Hay et al., 2020).

In the present study, the potentiation activity of several plant extracts obtained from different food residues when combined with baculovirus was evaluated in order to include them in the formulation of bioinsecticides.

2. Materials and methods

2.1. Insect and viruses

In the present study, second and fourth instar larvae of *S. littoralis*, *S. exigua* and *S. frugiperda* were used. Second instar larvae were assayed as in field insecticide treatments are directed to young larvae to prevent them from reaching later instars, which are the ones that produce the damage (*Simón* et al., 2015). Fourth instar larvae were also used in some assays to evaluate the potentiating effect of the extracts in older instar, and determine if a single viral dose could manage both instars. All of them were obtained from a healthy laboratory colony maintained on semi-synthetic diet based on wheat germ (*Greene* et al., 1976). Colonies were maintained in the insectary facilities at the Public University of Navarre that included several rearing chambers under specific rearing conditions: 25 ± 1 °C, 60 % RH and 16/8 light/dark regime. The viruses used were Spodoptera littoralis nucleopolyhedrovirus (SpliNPV), Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV), Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV), Autographa californica multiple nucleopolyhedrovirus (AcMNPV) and Mamestra brassicae multiple nucleopolyhedrovirus (MbMNPV). All of them were obtained from the virus collection of the Microbial Bioinsecticides group at the Public University of Navarre.

2.2. Selection and obtaining VWE

The VWE were obtained from a great variety of food by-products. Twenty-five plant matrices were selected in order to obtain 38 different extracts to be tested for their possible enhancement effect. All plant matrices were obtained from household-generated food waste, except for seeds (flax, pumpkin, chia and sesame), rosehips and *Moringa oleifera*, which were not waste. These extracts were used in different bioassays to determine if they contribute to the improvement of baculoviruses-induced mortality. Table 1 summarizes the sources of the different extracts and the composition of the extraction solvents.

Prior to extraction, vegetable residues were dried in a stove at 30 °C until constant weight and, once dried, all raw materials were ground and sieved (<300 μm). The different powders obtained from each vegetable waste were kept in a plastic bottle at room temperature until their extraction. The extraction was carried out at 40 °C in Erlenmeyer flasks with orbital shaking (250 rpm) for 24 h in a stove (Ing Climas, Barcelona, Spain). The solvent used for the extraction consisted of different percentages of ethanol/water (Table 1). Most of the extracts were obtained with 96 % ethanol in order to obtain extracts with a powdery, non-sticky texture. In the cases where the percentage of ethanol was modified, it was done with the aim of improving the texture of the extract obtained so that it would be easier to handle. In all cases, the extract:solvent ratio was 1:100 (w/v). All the extracts were centrifuged with a Sorvall ST8 centrifuge (Thermo Scientific, Waltham, MA, USA) during 15 min at 8000 rpm. Immediately, the extracts were filtered through a filter paper and the ethanol solvent was removed on a rotary evaporator. Finally, the extracts were dissolved in approximately 5–10 mL of water and lyophilized (Telstar Cryodos freeze drier, Madrid, Spain). The resulting extracts were kept at 5 °C until the bioassay performance. Finally, based on the extraction yield and solubility of the extracts, a preliminary screening was made. Fig. S1 in Supplementary material shows the final appearance of the selected extracts.

2.3. Screening of VWE as sensitizers of baculovirus infection

In the first screening, the enhancing effect of VWE was evaluated using SpliMNPV on the homologous host *S. littoralis* (Table 2). The selected extracts were tested in different days during several months in seven different assays (Table 2). Second or fourth instar larvae were used depending on the assay. Larvae were deposited in artificial diet contaminated superficially with different concentrations of SpliNPV (depending on the bioassay) and with extracts at a final concentration of 1 % (w/v). Surface contamination was selected as bioassay method since in a previous assay with the droplet feeding method did not produce synergism (Fig. S2 in Supplementary material). Viral concentrations were determined using a Neubauer improved counting chamber

Table 1
Characteristics of the 38 extracts assayed.

Number	Vegetable source	Part/format	Extraction solvents (EtOH:H_2O)	Extraction yield (%)
E1	Coffee[a]	Spent capsules	50:50	8.6
E2	Coffee[a]	Spent capsules	75:25	8.2
E3	Rose hips	Entire	50:50	43.8
E4	Rose hips	Entire	75:25	47.7
E5	Peanut	Hulls	50:50	8.4
E6	Peanut	Hulls	75:25	8.7
E7	Garlic	Husks	50:50	8.4
E8	Garlic	Husks	75:25	7.0
E9	Flax	Seeds	96:4	39.5
E10	Pumpkin	Seeds	96:4	38.7
E11	Cabbage	Peel and stems	96:4	38.0
E12	Eggplant	Peel and stems	96:4	26.1
E13	Orange	Peel	96:4	37.6
E14	Potato	Peel	96:4	11.7
E15	Kiwi	Peel	96:4	60.4
E16	Coffee	Spent capsule	96:4	11.1
E17	Rosehip	Entire	96:4	35.0
E18	Peanut	Hulls	96:4	5.2
E19	Garlic	Husks	96:4	4.5
E20	Tangerine	Peel	96:4	33.1
E21	Pear	Peel	96:4	51.4
E22	Lemon ginger tea[b]	Spent commercial tea	96:4	6.5
E23	Black chai tea[c]	Spent commercial tea	96:4	9.8
E24	Avocado	Peel and pits	96:4	11.8
E25	Chia	Seeds	96:4	30.9
E26	Sesame	Seeds	96:4	53.6
E27	*Moringa oleifera*	Commercial leaves powder	96:4	19.1
E28	Asparagus	Non-edible parts	96:4	25.7
E29	Artichoke	Non-edible parts	96:4	12.2
E30	Beet	Leaves	96:4	16.2
E31	Beet	Skin	96:4	37.5
E32	Beet	Stalks	96:4	22.0
E33	Beet	Entire	96:4	25.6
E34	Coffee	Spent capsules	85:15	9.4
E35	Lemon ginger tea[b]	Spent commercial tea	85:15	7.8
E36	Strawberry	Leaves	96:4	30.9
E37	Banana	Peels	96:4	37.7
E38	Tomato	By-products	96:4	29.8

[a] Commercial coffee capsules elaborated with a mixture of robusta (*Coffea canephora*) and Arabic (*Coffea arabica*) coffee.
[b] Ingredients: ginger, lemongrass, lemon peels, lemon myrtle, liquorice.
[c] Ingredients: tea, cinnamon, cardamom, ginger, aromas, cloves, chicory aniseed, pepper.

(Paul Marienfeld GmbH and Co. KG, Lauda-Königshofen, Germany), and serial dilutions to the desired viral concentration.

The first assay showed in Table 2 (date 2021.04.16) included the extracts E1, E2, E3, E4, E9, E10, E11, E12, E13, E14, E17 and doses of 1×10^4 in second instar larvae. Fourth instar larvae were inoculated at 2×10^6 OBs/mL but the increase of mortality was not as clear as in second instar with selected extracts (Fig. S3 in Supplementary material). For this reason, of the two types of larvae, it was decided to continue the studies with second instar larvae and therefore no longer use the fourth instar ones. Due to the low mortality observed in the first assay, in the rest of assays two other viral concentrations (either 2×10^4 or 5×10^4 OBs/mL) were tested (see Table 2).

Second instar larvae were starved overnight and then allowed to ingest the diet contaminated with OBs at different concentrations (Table 2) and extracts at 1 % (w/v). Positive control larvae were fed a diet containing only the virus at the same concentrations, and negative control larvae were fed a diet containing water and extracts at 1 % (w/v) without virus. No mortality was recorded of negative control larvae and no differences in weight were observed, indicating that extracts did not exert any adverse effect on the larvae. Viral stocks were diluted to the appropriate viral concentration and aliquots of 1 mL of solution were prepared: one including the virus and another including the virus and the extract. Thirty-five microliters of these suspensions were overlaid on the diet in 28-well bioassay plates, and stirred for its homogenization in a balancer. In this way, three 28-well plates of insects were used in each test for each extract. After 10 min, one larva was individually deposited in each well. Larvae were incubated at 25 °C during 10 days until death or pupation. Three repetitions for each treatment were performed.

2.4. Potentiation effect of selected VWE on homologous systems

Based on the results obtained in the previous section, some extracts were selected by their ability to enhance SpliNPV infection. First, to evaluate and confirm the potentiation, a concentration mortality response assay was performed applying just two viral concentrations of each virus, SpliNPV, SeMNPV and SeMNPV, in combination with the extracts. A low and a high viral concentration were applied based on previous assays. For SpliNPV the doses were 1×10^4 and 2×10^4 OBs/mL, for SeMNPV were 5×10^2 and 1×10^3 OBs/mL and, finally, for SfMNPV, viral concentrations were 5×10^3 and 2×10^4 OBs/mL.

In finer tests, the LC_{50} and the potency ratios were determined using homologous host-pathogen systems SpliNPV-*S. littoralis*, SeMNPV-*S. exigua* and SfMNPV-*S. frugiperda*. The pathogenicity of SpliNPV, SeMNPV and SfMNPV in combination with the extracts was compared with that of the viruses alone in concentration-mortality bioassays using second and fourth instars larvae of *S. littoralis*, *S. exigua* and *S. frugiperda*, respectively. For this, bioassays using the surface contamination method with one of five concentrations of OBs were performed to inoculate larvae. Considering the mortality obtained in the concentration mortality response assay, in second instar larvae for SpliNPV-*S. littoralis* and SfMNPV-*S. frugiperda* systems, the concentrations were 1×10^6, 3.33×10^5, 1.11×10^5, 3.70×10^4 and 1.23×10^4 OBs/mL (3-fold dilution). For SeMNPV-*S. exigua* system, the concentrations were 1×10^5, 3.33×10^4, 1.11×10^4, 3.7×10^3 and 1.23×10^3 OBs/mL (3-fold dilution). In fourth instar larvae, the viral concentrations used for SpliNPV and SfMNPV were 1×10^7, 2×10^6, 4×10^5, 8×10^4 and 1.6×10^4 OBs/mL and for SeMNPV, 1×10^6, 2×10^5, 4×10^4, 8×10^3 and 1.6×10^3 OBs/mL were chosen. These concentration ranges were previously determined and killed between 95 % and 5 % of the experimental insects. Virus-induced mortality was compared to that produced by viruses in combination with 1 % of the selected extracts. Negative control larvae were treated with 1 % extract but without OBs. One millilitre of suspension was prepared for each treatment and viral concentration, and 35 μL were over dispensed on the diet surface in 28-well bioassay plates. Larvae were individually deposited and reared at 26 ± 1 °C. Mortality data were recorded daily until insects had either died from nucleopolyhedrovirus infection or pupated. The bioassay was performed three times using different batches of larvae.

2.5. Potentiation effect of selected VWE on heterologous systems

For heterologous system, a concentration mortality response assay was performed. Three heterologous viruses infective to *S. littoralis* larvae were tested: the broader viruses Autographa californica multiple nucleopolyhedrovirus (AcMNPV), Mamestra brassicae multiple nucleopolyhedrovirus (MbMNPV) and the SfMNPV that showed low infectivity. Three viral concentrations were applied: high (1×10^7 OBs/mL), medium (1×10^5 OBs/mL) and low (1×10^3 OBs/mL). Bioassays were performed by surface contamination. Two microcentrifuge tubes were prepared for each virus and concentrations, one including 1 mL suspension of the virus at the concentration indicated and the other 1 mL suspension including the virus at the same concentration and 1 % of extracts E2, E17 and E37. In this case, these three extracts were selected as in SpliNPV-*S. littoralis* system produced the highest potentiation. Thirty-five microliters of suspension were overlaid in the diet in 28-well bioassay dish and after 10 min stirring to homogenize the

Table 2
Percentages of mortality induced by vegetable waste extracts when added to SpliNPV in second instar larvae of *S. littoralis*.

	Bioassays											
Date	2021.04.16	2021.05.04		2021.05.11		2021.06.04		2021.06.16		2021.06.25		2021.07.26
OBs/mL	1×10^4	2×10^4	5×10^4	1×10^4	2×10^4	1×10^4	2×10^4	1×10^4	2×10^4	1×10^4	2×10^4	2×10^4
Positive control	4	45	63	26	56	36	60	30	39	6	40	45
E1	9	–	–	–	–	–	–	–	–	–	–	–
E2	20	86**	92**	–	–	–	–	–	–	–	–	86**
E3	7	–	–	–	–	–	–	–	–	–	–	–
E4	13	–	–	36*	60	–	–	–	–	–	–	–
E9	16	–	–	20	43	–	–	–	–	–	–	–
E10	9	–	–	–	–	–	–	–	–	–	–	–
E11	4	–	–	–	–	–	–	–	–	–	–	–
E12	16	–	–	39**	73**	–	–	–	–	–	–	–
E13	9	–	–	–	–	–	–	–	–	–	–	–
E14	4	–	–	–	–	–	–	–	–	–	–	–
E15	2	–	–	–	–	–	–	–	–	–	–	–
E16	–	–	–	–	–	–	–	27	55	–	–	–
E17	18*	80**	89**	–	–	–	–	–	–	–	–	80**
E18	–	–	–	32	60	–	–	–	–	–	–	–
E19	–	–	–	36*	81**	–	–	–	–	–	–	–
E20	–	–	–	31	56	–	–	–	–	–	–	–
E21	–	–	–	21	63	–	–	–	–	–	–	–
E22	–	–	–	–	–	100[a]	100[a]	–	–	–	–	–
E23	–	–	–	30*	57	–	–	–	–	–	–	–
E24	–	–	–	–	–	30	54	–	–	–	–	–
E25	–	–	–	–	–	30	55	–	–	–	–	–
E26	–	–	–	–	–	37	50	–	–	–	–	–
E27	–	–	–	52**	87**	–	–	–	–	–	–	–
E28	–	–	–	–	–	57	76	–	–	–	–	76*
E29	–	–	–	–	–	57	76	–	–	–	–	76*
E30	–	–	–	–	–	42	55	–	–	–	–	–
E31	–	–	–	–	–	43	65	–	–	–	–	–
E32	–	–	–	–	–	63*	81**	–	–	–	–	–
E33	–	–	–	–	–	52*	70	–	–	–	–	–
E34	–	–	–	–	–	–	–	44	35	–	–	–
E35	–	–	–	–	–	–	–	33	36	–	–	–
E36	–	–	–	–	–	–	–	–	–	13**	56**	–
E37	–	–	–	–	–	–	–	–	–	18**	69**	64**
E38	–	–	–	–	–	–	–	–	–	5	43	–

* p-Values <0.10 (extract *vs* corresponding control).
** p-Values <0.05 (extract *vs* corresponding control).
[a] Not considered for being dissolved in ethyl lactate (toxic by itself).

sample in the surface, one individual second instar *S. littoralis* larva was deposited individually and reared at 26 ± 1 °C. The bioassays were repeated thrice and the mortality was registered daily until insects died or pupated.

2.6. Determination of total polyphenol and flavonoid content

The selected extracts were chemically characterized. Total polyphenol and flavonoid content were determined by spectrophotometric methods. The initial concentration of the extracts to be analyzed was approximately 5 mg/mL but, depending on the extract, it was necessary to apply different dilutions. The samples were analyzed three times. The total polyphenol content of the VWE was determined by Folin Ciocalteu method according to the protocol explained in Singleton et al. (1999). The absorbance was measured at a wavelength of 765 nm. The standard used for the preparation of the calibration curve was gallic acid at concentrations ranging from 0.21 to 4.15 mM. The determination coefficient (R^2) was 0.998. The results were expressed as mg gallic acid equivalents/g extract. The flavonoid content was measured following the aluminum chloride assay previously described by Chandra et al. (2014). For the analysis, a calibration curve was prepared with quercetin at concentrations ranging from 3.03 to 30.3 μg/mL. The R^2 of the calibration curve was 0.998. The absorbance was determined at 420 nm. The final results were displayed as mg quercetin equivalents/g extract.

2.7. Identification and quantification of the phenolic composition by HPLC-DAD-FLD

High-performance liquid chromatography (HPLC) was used to identify and determine the concentration of the phenolic compounds present in the selected extracts. The HPLC instrument consists of two 510 pumps, a 717 Plus autosampler, a 996-photodiode array detector and a 474-fluorescence detector (Waters Div., Milford, MA, USA). The software Empower 2.0 was used to control the instrument and for data processing. A Zorbax Eclipse Plus reversed-phase column (250 × 4.6 mm; particle size 5 μm, Agilent, Santa Clara, CA, USA) was used at 30 °C. Before analysis, 23.9–34.2 mg of every single extract were dissolved in 350 μL of methanol by using an ultrasound bath (Ultrasons-HD, Selecta, Barcelona, Spain). All the samples were filtered by using 0.45 μm PTFE syringe filters and each extract was analyzed in triplicate. To analyze the phenolic composition of the VWE, the method of Barros et al. (2014) was taken as a reference. Two mobile phases were used: A (water:formic acid 85 %, 99:1 v/v) and B (acetonitrile:formic acid 85 %, 99:1 v/v). The formic acid (98–100 % purity) was from Scharlab (Barcelona, Spain) and the acetonitrile (HPLC quality, 99.9 % purity) from PanReac AppliChem (Barcelona, Spain). The gradient had a duration of 60 min and was as follows (time in minutes; % A): (0; 95 %), (15; 85 %), (22; 80 %), (25, 80 %) (35; 70 %), (45; 50 %), (50; 5 %), (55; 95 %) and (60; 95 %). The injection volume was 10 μL and the flow rate was 1 mL/min. The identification of each phenolic compound was

made by the double coincidence of the ultraviolet-visible spectrum at the characteristic wavelength of the standard and its retention time. The standards used in the calibration curves were from Sigma-Aldrich (San Luis, MO, USA), Extrasynthese (Genay, France), Merck (Hohenbrunn, Germany) and PhytoLab (Vestenbergsgreuth, Germany). The phenolic compounds identified in the extracts were: caffeic acid (98.8 %, Sigma-Aldrich), ferulic acid (99.1 %, Merck), protocatechuic acid (98.9 %, Sigma-Aldrich), chlorogenic acid (99.8 %, Phytolab), neochlorogenic acid (99.5 %, Sigma-Aldrich), ellagic acid (98.9 %, Sigma-Aldrich), *p*-coumaric acid (99.9 %, Sigma-Aldrich), vanillic acid (99 %, Sigma-Aldrich), apigenin (95.4 %, Sigma-Aldrich), syringic acid (98.5 %, Sigma-Aldrich), cinnamic acid (100 %, Merck), catechin (100 %, Extrasynthese), epicatechin (96.4 %, Sigma-Aldrich), taxifolin (87.1 %, Sigma-Aldrich), kaempferol (98.8 %, Sigma-Aldrich), quercetin derivate (100 %, Sigma-Aldrich), procyanidin B1 (96.1 %, Extrasynthese) and B2 (95.7 %, Extrasynthese). For the quantification, different calibration curves with the corresponding standard of the identified compound were used. The determination coefficients were $R^2 > 0.99$ in all cases.

2.8. Statistical treatments

The screening procedure was completed by means of an inferential procedure. The analysis was done independently for each day of assay. Each day, two different viral concentrations were considered and mortality produced by the virus combined with the extracts was calculated with respect to the mortality induced by the control (virus alone). The percentage of mortalities obtained after applying two viral concentration in combination with extracts in homologous systems were analyzed with Dunnett test. Mortality data produced by heterologous viruses in *S. littoralis* larvae was also analyzed by Dunnett test. Both Dunnett tests were carried out using the R 4.2.2 (R Core Team, 2018) and the desctools package v3.0. The potentiation effect of selected VWE on homologous systems, by means of virus-induced mortality, were subjected to probit analysis using the Polo-Plus program (LeOra Software, 1987) to calculate the LC_{50} values and the potency ratios. Kruskal-Wallis test was applied to determine the significant differences ($p < 0.05$) in polyphenol and flavonoid content and in individual phenolic content of the different VWE. The Kruskal-Wallis tests were performed using SPSS statistical software (IBM SPSS Statistics version 25.0, IBM Corp, Armonk, NY, USA).

3. Results

3.1. Obtaining VWE

In Table 1 shows the extraction yields of the VWE analyzed. The extraction yields varied widely among the different extracts, ranging from 4.5 % (E19, garlic husks extracted with 96 % ethanol) to 60.4 % (E15, kiwi peel extracted with 96 % ethanol). The extracts obtained from coffee, peanut, garlic, ginger infusion and black chai tea resulted in yields of <10 %, while extracts from kiwi, sesame and pear gave yields of >50 %. Most of the extracts showed sticky texture, except for extracts obtained from coffee, peanut, garlic, tangerine, black chai tea and beet skin, which had powder-like consistency. Of the extracts prepared, those obtained from coffee and lemon ginger tea with 96 % ethanol as solvent (E16 and E22), along with those from beet leaves and stalks (E30 and E32), peanut hulls (E5, E6 and E18), tomato (E38), and different seeds (E9, E10, E25 and E26) were difficult to resuspend in water. All of these properties were considered in a first screening of extracts, before performing the efficacy studies. Therefore, garlic and peanut extracts (E5, E6, E7 and E8) were not considered for further studies given the low yield of garlic extract, and the low yield and difficult handling of peanut extracts.

3.2. Screening of VWE as enhancers of baculovirus infection

The mortality increase obtained for each extract can be found in Table 2. As it can be seen, different extracts demonstrate to improve the mortality of the virus at one of several viral concentrations: E2, E9, E12, E17, E19, E27, E28, E29, E32, E36 and E37. Among them, the efficiency of the rosehip extract (E17), which was tested four times and at three different concentrations, was noteworthy: when applied along with 1×10^4 OBs/mL in second instar larvae, increased the mortality of SpliNPV from 4 % to 18 %. In the second bioassay, when 2×10^4 and 5×10^4 OBs/mL were used together with the rosehip extract in second instar larvae, mortality reached 80 % and 89 %, respectively, almost twice the mortality observed in positive control larvae (from 45 to 80 % and from 63 to 89 %). In the last assay, the mortality observed in second instar larvae was almost twice that of control larvae (from 45 to 80 %), when applying a dose of 2×10^4 OBs/mL together with the extract. Coffee and banana extracts (E2 and E37 respectively) also stand out for their high significance in different assays. On the other hand, E22 was not considered for statistical analysis despite producing 100 % of mortality because larvae that ingest this extract died just after ingestion, indicating that E22 was toxic. This toxicity was attributed to the ethyl lactate used as solvent for this extract due to its low water solubility. In view of these results, and based on different aspects (significance of the mortality enhancement, extraction yield, ease of handling, solubility and/or availability of the vegetable by-product throughout all the year), 6 extracts were selected for further tests among those that increased the mortality: E2, E17, E28, E29, E32 and E37.

3.3. Potentiation effect of selected VWEs on homologous systems

Previously, lower concentrations of the extracts (0.5 % and 0.1 %) were assayed in second *S. littoralis* larvae using two SpliNPV concentrations (2×10^4 and 2×10^5 OBs/mL). These concentrations did not produce a sensitization effect (Fig. S4 in Supplementary material). Thereafter, the selected extracts were used at 1 % to evaluate their capacity to increase the mortality produced by SpliNPV, SeMNPV and SfMNPV OBs on their respective homologous hosts.

The increase in larval mortality induced by the extracts varied in the different homologous systems studied SpliNPV, SeMNPV and SfMNPV (Fig. 1). In SpliNPV-*S. littoralis* system, at low concentration only E28 and E29 were able to increase viral mortality, while at higher concentrations all the extracts except E17 induce higher mortality (Fig. 1A). In contrast, in the SeMNPV-*S. exigua* system, a slightly higher mortality was only observed in the presence of extracts E29 and E37 at low concentration. A higher viral concentration did not induce an increased mortality (Fig. 1B). Finally, in SfMNPV-*S. frugiperda* system, at low concentration none of the extracts improve the mortality of control virus, while at high dose E17 and E37 were the extracts that induced a mortality enhancement (Fig. 1C).

Subsequently, a finer bioassay was performed to calculate the LC_{50} and the potency of the different treatments applied in order to determine the extract that showed the greatest potentiation of the virus (Table 3). First, LC_{50} values and potency ratios were calculated on the SpliNPV-*S. littoralis* system. All the extracts reduced the LC_{50}, as the fiducial limits of the potency ratios did not include the 1. The banana peel extract (E37), produced the largest increase in pathogenicity, reducing the LC_{50} 13.61 times. Thereafter, based on the results obtained in SpliNPV-*S. littoralis* system, extracts E2, E17 and E37 were assayed in SeMNPV-*S. exigua* and SfMNPV-*S. frugiperda* systems. In SeMNPV-*S. exigua* system, none of the tested extracts enhanced the pathogenicity, that is, the addition of E2, E17 and E37 at 1 % did not decrease the LC_{50} of the virus. In fact, the E17, extracted from rosehips, decreased the pathogenicity of SeMNPV by half. Finally, in SfMNPV-*S. frugiperda* system, E2 and E37 extracts induced a greater pathogenicity. E2, extracted from coffee waste, decreased 7 times the LC_{50}, while the banana peel extract (E37) reduced it 4 times, without showing significant differences between them.

Bioassays were also performed in fourth instar larvae. In this case, only the extracts that produced the greatest potentiation effect with SpliNPV (E2 and E37) in second instar *S. littoralis* larvae were assayed

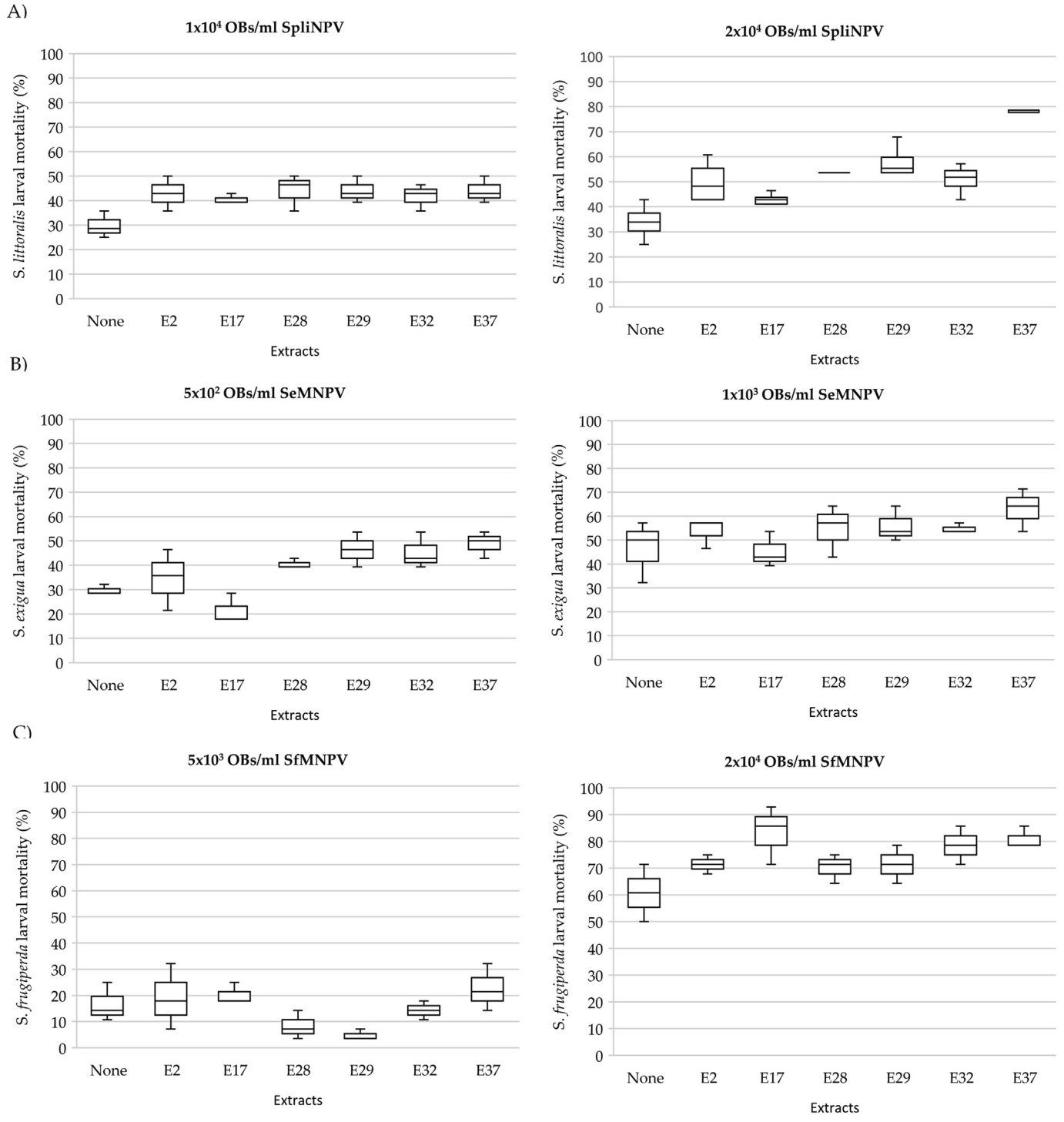

Fig. 1. Percentage of larval mortality induced by SpliNPV, SeMNPV and SfMNPV in homologous hosts in second instars in presence of absence of 1 % extracts E2, E17, E28, E29, E32 and E37. Two viral concentrations were applied for each virus. The mortality obtained is indicated above the bars. The error bars indicated standard error.

in the three systems. None of the selected extracts increased the pathogenicity of homologous viruses in this larval instar (Table 4). Therefore, the mortality increase was only observed in second instar larvae. It appears that potentiation is host and instar dependent.

3.4. Potentiation effect of selected VWEs on heterologous systems

E2, E17 and E37 were tested in combination with three heterologous viruses AcMNPV, SfMNPV and MbMNPV in second larval instar of *S. littoralis* (Fig. 2). Three viral concentrations (1×10^7, 1×10^5 and 1×10^3 OBs/mL) were applied. The lowest viral concentration, 1×10^3 OBs/mL, did not induce larval mortality. AcMNPV at 1×10^5 OBs/mL produced 2 % of mortality, similar to the mortality observed in presence of E2, E17 and E37 (Fig. 2A). SfMNPV at 1×10^5 OBs/mL induced 13 % of mortality in second instar larvae of *S. littoralis*, similar to the mortality observed with the addition of E2, E17 and E37 (Fig. 2B). In the case of MbMNPV, although it may appear that both E17 and E37, in combination with 1×10^5 OBs/mL, produced higher mortality than the control,

Table 3
LC_{50} values (OBs/mL) and potency ratios of SpliNPV, SeMNPV and SfMNPV on homologous second instar species in presence or absence of the selected extracts.

Specie	Virus	Extract	Intercept ± SE	Slope ± SE	Chi-square (χ^2)	LC_{50} (OBs/mL)	Fiducial limits (95 %)		Potency ratio	Fiducial limits (95 %)	
							Upper	Lower		Upper	Lower
S. littoralis	SpliNPV	–	1.35 ± 0.54	1.01 ± 0.11	2.25	4.18×10^4	2.85×10^4	5.73×10^4	1	–	–
		E2	2.70 ± 0.64	0.88 ± 0.13	3.08	5.42×10^3	4.98×10^2	1.39×10^4	7.72	3.20	18.61
		E17	2.50 ± 0.66	0.93 ± 0.14	2.64	5.67×10^3	2.05×10^3	1.04×10^4	7.33	3.16	17.01
		E28	1.53 ± 0.66	1.10 ± 0.14	1.76	1.13×10^4	6.12×10^3	1.71×10^4	3.71	2.03	6.79
		E29	2.72 ± 0.57	0.83 ± 0.12	0.67	9.35×10^3	3.88×10^3	1.63×10^4	4.47	2.08	9.61
		E32	2.13 ± 0.69	1.02 ± 0.15	0.53	6.23×10^3	2.53×10^3	1.08×10^4	6.71	3.10	14.50
		E37	3.18 ± 0.6	0.81 ± 0.14	1.08	3.07×10^3	6.47×10^2	6.98×10^3	13.61	4.36	42.60
S. exigua	SeMNPV	–	1.50 ± 0.16	2.07 ± 0.31	2.04	4.18×10^3	2.95×10^3	5.76×10^3	–	–	–
		E2	2.50 ± 0.36	1.99 ± 0.11	2.14	5.98×10^3	4.26×10^3	8.28×10^3	0.70	0.44	1.11
		E17	2.11 ± 0.74	1.81 ± 0.25	2.06	8.40×10^3	5.85×10^3	1.19×10^4	0.50	0.31	0.80
		E37	1.89 ± 0.82	1.88 ± 0.21	1.72	3.00×10^3	1.48×10^3	4.87×10^3	1.39	0.73	2.66
S. frugiperda	SfMNPV	–	0.17 ± 0.25	1.16 ± 0.21	2.16	1.00×10^5	5.87×10^4	1.68×10^5	–	–	–
		E2	1.43 ± 0.29	1.10 ± 0.25	2.77	1.46×10^4	5.31×10^4	1.92×10^5	6.88	2.59	18.26
		E17	1.26 ± 0.48	0.94 ± 0.20	0.94	1.02×10^5	2.12×10^5	4.02×10^5	0.98	0.45	2.15
		E37	1.18 ± 0.26	1.40 ± 0.26	1.50	2.64×10^4	1.33×10^4	4.21×10^4	3.80	1.83	7.92

Goodness-of-fit tests were non-significant for *S. littoralis*-SpliNPV ($\chi^2 = 4.02$; df = 6; p = 0.67) for *S. exigua*-SeMNPV ($\chi^2 = 6.18$; df = 3; p = 0.14) and for *S. frugiperda*-SfMNPV ($\chi^2 = 2.02$; df = 3; p = 0.57).

no significant differences were found (Fig. 2C). At 1×10^7 OBs/mL, the mortality obtained in the presence of the extracts was similar to that of the control treatment (without extracts) in the three cases.

3.5. Total polyphenol and flavonoid content of the selected VWE

Total polyphenol and flavonoid content were determined in selected VWE (Fig. 3), with the aim of determining if these compounds were responsible for the sensitization of insect larvae to baculovirus infection produced by those extracts. The polyphenol content clearly varied among the different VWE (Fig. 3A). The highest content was found in E2, obtained from coffee spent capsules and extracted with ethanol 75 %. On the other hand, the extract E37 obtained from banana peel had the lowest total polyphenol content of the six selected extracts.

The flavonoids content obtained also varied markedly among the different extracts (Fig. 3B). The extracts E2 (spent coffee extract) and E29 (artichoke extract) presented the highest content of flavonoids and polyphenols. The extracts E28 (asparagus extract) and E37 (banana peel extract), which presented similar values, were the extracts with the lowest flavonoid content. Finally, and in contrast to the results obtained for total polyphenols, E17 (rosehip extract) showed lower values of total flavonoid concentration than E32 (beet stalks extract).

3.6. Phenolic composition

The phenolic composition of the selected extracts is shown in Table 5. Some of the compounds identified were present in more than one of the selected extracts, as is the case of caffeic, chlorogenic and neochlorogenic acids (in E2 and E29), protocatechuic acid (in E2, E17 and E29), catechin and procyanidin B2 (in E17 and E32) or ferulic acid (in E2 and E28). The main compounds found in the extract from spent coffee (E2) were neochlorogenic and chlorogenic acids. In the rosehip extract (E17) a wide variety of phenolic compounds were identified, being catechin, procyanidin B1 and quercetin derivate the main ones. In the asparagus extract (E28) ferulic, *p*-coumaric and vanillic acids were identified, but the latter could not be quantified because it was found in very small amounts. The major compound present in artichoke extract (E29) was, undoubtedly, chlorogenic acid. The amount of this compound in the extract accounted for almost the 95 % of the phenolic compounds identified by chromatography. In the banana peel extract (E37), only a small amount of cinnamic acid was identified.

4. Discussion

The aim of the present study was to evaluate the potentiating effects of VWE on baculovirus infection in different species of lepidoptera, with the aim of including them in bioinsecticide formulations. Certain plant flavonoids stand out because they improve the biological and physical properties of baculoviruses (Hay et al., 2020). In addition, the utilization of agri-food waste to produce value-added products could be a sustainable solution to reduce the environmental impact and could be considered as a management strategy within the circular economy concept (Paini et al., 2022).

First, several bioassays were carried out with 34 extracts in second instar *S. littoralis* due to the availability of larvae (see Table 2). In this screening E2, E17, E28, E29, E32 and E37 were selected as potential

Table 4
LC_{50} values (OBs/mL) and potency ratios of SpliNPV, SeMNPV and SfMNPV on homologous fourth instar species in presence or absence of selected extracts.

Specie	Virus	Extract	Intercept ± SE	Slope ± SE	Chi-square (χ^2)	LC_{50} OBs/mL	Fiducial limits (95 %)		Potency ratio	Fiducial limits (95 %)	
							Upper	Lower		Upper	Lower
S. littoralis	SpliNPV	–	1.61 ± 0.80	0.90 ± 0.15	1.61	7.22×10^4	3.05×10^4	1.37×10^5	–	–	–
		E2	1.56 ± 0.79	0.90 ± 0.15	1.03	8.28×10^4	3.61×10^4	1.56×10^5	0.87	0.32	2.38
		E37	1.01 ± 0.83	1.00 ± 0.16	2.49	9.57×10^4	4.65×10^4	8.66×10^5	0.76	0.29	1.96
S. exigua	SeMNPV	–	1.81 ± 0.73	1.08 ± 0.17	2.10	7.69×10^3	3.87×10^3	1.34×10^4	–	–	–
		E2	2.92 ± 0.65	0.93 ± 0.12	1.54	9.42×10^3	4.40×10^3	1.73×10^4	0.82	0.34	1.99
		E37	1.22 ± 0.76	1.18 ± 0.18	2.24	1.09×10^4	6.11×10^3	1.83×10^4	0.70	0.31	1.57
S. frugiperda	SfMNPV	–	1.83 ± 0.69	1.09 ± 0.19	1.61	1.75×10^6	9.95×10^5	3.36×10^6	–	–	–
		E2	1.03 ± 0.76	0.93 ± 0.18	0.60	2.83×10^6	1.48×10^6	6.99×10^6	0.62	0.24	1.56
		E37	1.47 ± 0.99	0.88 ± 0.16	1.01	2.02×10^6	1.04×10^6	4.82×10^6	0.86	0.34	2.18

Goodness-of-fit tests were non-significant for *S. littoralis*-SpliNPV ($\chi^2 = 0.27$; df = 2; p = 0.87) for *S. exigua*-SeMNPV ($\chi^2 = 1.21$; df = 2; p = 0.55) and for *S. frugiperda*-SfMNPV ($\chi^2 = 0.79$; df = 2; p = 0.67).

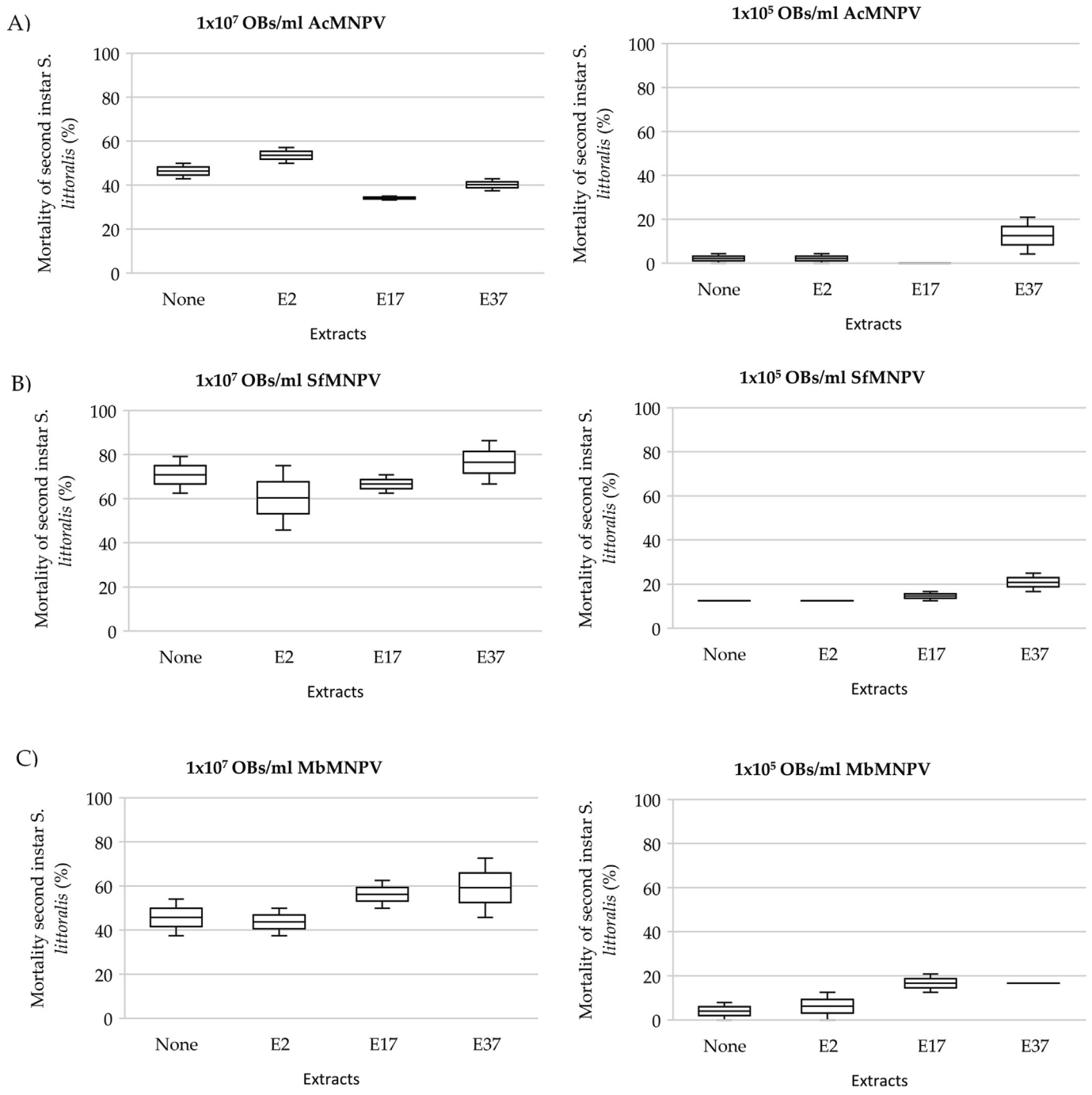

Fig. 2. Percentage of larval mortality induced by AcMNPV, SfMNPV and MbMNPV in heterologous second instar *Spodoptera littoralis*. Three viral concentrations were tested 1×10^7, 1×10^5 and 1×10^3 OBs/mL in presence of E2, E17 and E37 at 1 %. The mortality obtained is indicated above the bars. The error bars indicated standard error.

candidates in baculovirus-based formulations not only because they enhanced the mortality induced by SpliNPV but also because they showed advantages for massive production (high extraction yield, ease of handling, solubility and availability of the by-product). Later, those extracts were used in three homologous systems SpliNPV-*S. littoralis*, SeMNPV-*S. exigua* and SfMNPV-*S. frugiperda* (see Fig. 1). In SpliNPV-*S. littoralis* system (Fig. 1A), the potentiating effect of the extracts selected in the previous study was confirmed. However, higher mortalities were obtained compared with the previous assays, probably due to differences in larva susceptibility. Gut microbial community modulates armyworms susceptibility to baculovirus infection (Donkersley et al., 2023). Moreover, differences in susceptibility might also reflect natural variation in response (Wang et al., 2023). As bioassays were not performed at the same time, different generations of larvae were used and those larvae might show differences in their response to baculovirus infection. Given those differences, a finer test was performed by calculating the LC_{50} (see Table 3). The selected six extracts enhanced the biological activity of SpliNPV and SfMNPV baculoviruses on homologous hosts, decreasing up to 14-fold the LC_{50} values. The extract obtained from banana peel (E37) increased the SpliNPV pathogenicity 14 times in second instar larvae, while the spent coffee extract (E2) improved the pathogenicity of SfMNPV 7 times. This means that by including these extracts in insecticidal formulations, the active matter required would be reduced, decreasing the production

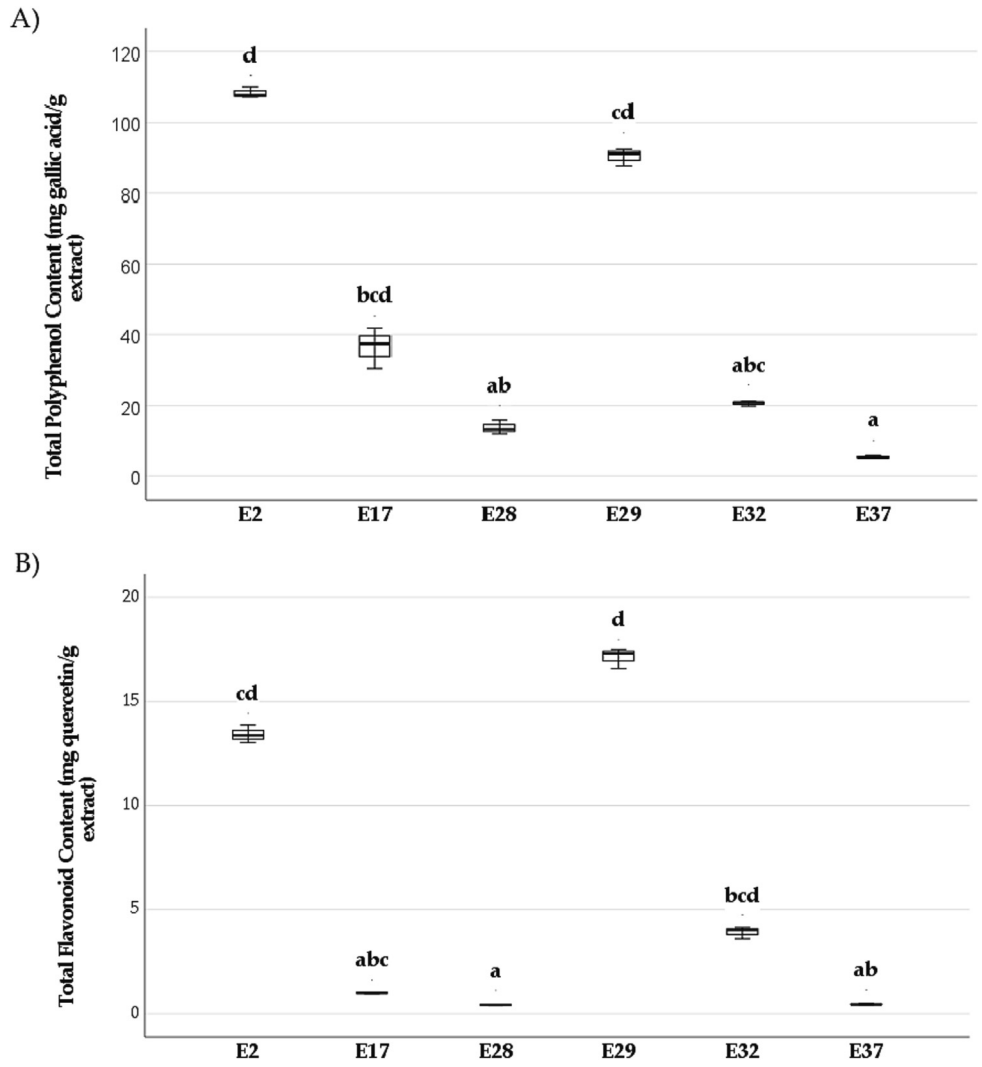

Fig. 3. Total polyphenol (mg gallic acid equivalents/g extract) and flavonoid content (mg quercetin equivalents/g extract) of the selected extracts: E2 (coffee spent capsule 75 % ethanol), E17 (rose hips 96 % ethanol), E28 (asparagus 96 % ethanol), E29 (artichoke 96 % ethanol), E32 (beet stalks 96 % ethanol), E37 (banana peel 96 % ethanol). Different letters in the bars indicate significantly different results among the extracts (Kruskal-Wallis, significance level: 0.05).

costs related to the massive production of SpliNPV and SfMNPV. Similarly, different extracts from neem seed kernel were effective in increasing the pathogenicity of *Pieris brassicae* granulovirus against homologous host (Bhandari et al., 2009), although the effect on heterologous hosts was not tested. Among the different extracts tested by these researchers, the petroleum-ether extract resulted in the

Table 5
Phenolic composition (μg/mg extract) of the selected extracts (mean ± SD).

	Coffee extract (E2)	Rosehip extract (E17)	Asparagus extract (E28)	Artichoke extract (E29)	Beet stalks extract (E32)	Banana peel extract (E37)
Caffeic acid	0.180 ± 0.051b	nd	nd	0.080 ± 0.020a	nd	nd
Ellagic acid	nd	0.094 ± 0.003	nd	nd	nd	nd
Ferulic acid	0.059 ± 0.010b	nd	0.041 ± 0.002a	nd	nd	nd
Coumaric acid	nd	nd	0.110 ± 0.003	nd	nd	nd
Protocatechuic acid	0.215 ± 0.044ab	0.129 ± 0.015a	nd	0.322 ± 0.013b	nd	nd
Apigenin	nd	nd	nd	0.141 ± 0.006	nd	nd
Epicatechin	nd	0.102 ± 0.009	nd	nd	nd	nd
Catechin	nd	0.413 ± 0.075b	nd	nd	0.067 ± 0.006a	nd
Quercetin-derivate	nd	0.286 ± 0.039	nd	nd	nd	nd
Syringic acid	nd	nd	nd	0.241 ± 0.041	nd	nd
Neochlorogenic acid	1.968 ± 0.218b	nd	nd	0.420 ± 0.022a	nd	nd
Chlorogenic acid	1.689 ± 0.096a	nd	nd	22.149 ± 6.601b	nd	nd
Cinnamic acid	nd	nd	nd	nd	nd	0.007 ± 0.001
Procyanidin B1	nd	0.357 ± 0.060	nd	nd	<0.030*	nd
Procyanidin B2	nd	<0.018*	nd	nd	nd	nd

Different letters in the same row indicate significantly different results among the extracts (Kruskal-Wallis, $p < 0.05$); nd, not detected.
* The quantification was not possible because values were below the quantification limit.

highest LC_{50} reduction, while aqueous and methanol extracts showed a lower enhancing effect. Petroleum ether is a very polluting solvent, so it is better to avoid it when you want to obtain products that are going to be released into the environment. In the present work, only Generally Recognized as Safe (GRAS) solvents have been used to obtain all the extracts with the aim of reducing the contamination when releasing them along with baculoviruses in the field, and ensuring that they will not have any toxic effect on ecosystems or human health.

However, in SeMNPV-*S. exigua* system, the addition of extracts did not improve the baculovirus biological activity. When comparing the pathogenicity of SeMNPV, SpliNPV and SfMNPV on homologous hosts, SeMNPV is, by far, the most pathogenic virus (Pudjianto et al., 2016). Highly specific baculoviruses, such as SeMNPV or Helicoverpa armigera single NPV (HearSNPV) are extremely pathogenic for homologous hosts (Arrizubieta et al., 2022), masking a possible potentiation effect. In fourth instar larvae, there was not observed an increase in the mortality percentage when using the selected extracts. Therefore, the effectiveness of VWEs as mortality enhancers appears to be host-pathogen and concentration dependent.

Several studies demonstrate that the insect sensitivity to a specific molecule depends on the species and, in some cases, sensitivity is mainly related with the action of a specific chemical structure such as a functional group (Regnault-Roger, 1999). The insecticidal, repellent and fumigant activities of several VWE varied against two different grain pests (Saroukolai et al., 2010; Teke and Mutlu, 2021). Furthermore, the potentiation effect of VWE seems to be species-specific. Similarly, a mixture of α-chaconine + α-solanine was the most toxic against *Sitophilus oryzae*, while in controlling *S. oryzae* and *Tribolium castaneum*, the most effective mixture was α-chaconine + α-tomatine (Spochacz et al., 2018).

In addition to evaluating the effect of the extracts in homologous systems, it was also evaluated in heterologous systems with the aim of increasing the effective host range of broad-spectrum viruses. Unlike to other microbial control agents (entomopathogenic bacteria, fungi and nematodes), baculoviruses are only infectious for a small number of insect species (theoretical host range). Some of these species, on the other hand, cannot be effectively controlled (effective host range) even when high concentrations of active ingredient (OBs) are used, making them economically or commercially unfeasible. Because of this, all baculoviruses that have been developed as microbial insecticides characteristically have a high degree of specificity. The high specificity has been one of the factors that hindered a greater commercialization of baculovirus-based insecticides (Reid et al., 2023). Our results have shown that the activity of heterologous viruses was not enhanced in presence of VWEs, and only the pathogenicity of non-highly infectious homologous viruses was improved in the presence of some extracts.

The polyphenol and flavonoid contents were evaluated in the selected extracts (E2, E17, E28, E29, E32 and E37) in order to correlate them with the sensitization effect over infected larvae. In the SpliNPV-*S. littoralis* system, the banana peel extract (E37) showed the highest potentiation of the insecticidal activity (14-fold), although its total polyphenol and flavonoid contents were clearly lower (5.48 mg gallic acid/g extract and 0.46 mg quercetin/g extract, respectively) than that of other extracts such as coffee extract (E2) (108.33 mg gallic acid/g extract and 13.42 mg quercetin/g extract) or artichoke extract (E29) (90.44 mg gallic acid/g extract and 17.13 mg quercetin/g extract), which enhanced insecticidal activity 8 and 4-times, respectively. In contrast, in SfMNPV-*S. frugiperda* system, the spent coffee extract (E2) produced the highest potentiation of the insecticidal activity (7-times), greater than the activity enhancement observed with rosehip extract (E17) (no potentiation) or banana peel extract (E37) (4-times). It is very likely that not all the compounds present in each extract are responsible for the enhancing effect observed. However, the isolation of active ingredients could be detrimental since some compounds have less effectiveness when they are separated due to the existence of many synergistic relationships in botanical preparations (Shaalan et al., 2005), which clearly affect the potentiation level in the different systems.

In the extracts from rosehips (E17), artichoke waste (E29) and beet stalks (E32), some flavonoids were found, which are known to protect plants against herbivores and pathogens (Falcone Ferreyra et al., 2012). The flavonoids identified in these extracts were catechin (E17 and E32), epicatechin (E17), procyanidin B1 (E17 and E32) and B2 (E17), apigenin (E29) kaempferol (E17), taxifolin (E17) and quercetin derivate (E17). Therefore, it is possible that the action of these compounds is related to the increase in mortality. On the other hand, other antioxidants such as phenolic acids could also interact with baculoviruses, increasing their larvicidal action. Cinnamic acid was the only phenolic compound identified in the banana peel extract (E37), which was the extract that produced the greatest enhancement of SpliNPV pathogenicity on homologous hosts. Therefore, despite being found in low concentration, its presence could be related to its insecticidal effect. The larvicidal activity of banana peel has been evaluated in different works. For instance, Joy and Madhavan (2022) studied the larvicidal activity of banana peel extracts against the larvae of the malaria vector, *Anopheles stephensi*. After testing these extracts on fourth instar larvae, these authors concluded that banana peel extracts have a potent mortality effect against this type of mosquito. Ann and Arizo (2018) found that ethanolic extracts from the peel of *Musa acuminata* (a species of banana native to Southern Asia) caused 100 % mortality in *Aedes aegypti* larvae, another mosquito responsible for the transmission of some diseases such as zika or dengue. These authors observed damage in the midgut of *A. aegypti* larvae and suggested that the larvicidal activity could be related to the presence of bioactive compounds in the extracts. Since in the present work only cinnamic acid has been identified in the banana peel extract, it would very interesting to identify other types of phenolic compounds such as coumarins or lignans, which may also have insecticidal activity. Likewise, it would be important to determine the mechanism of action of these antioxidants on the midgut of insects. On the other hand, the spent coffee extract (E2) showed a greater enhancement of the larvicidal activity in SfMNPV-*S. frugiperda* system. This extract was rich in chlorogenic and caffeic acids, which are known to be involved in the defense mechanism of plants against nematode infections (Giebel, 1982). Both phenolic acids were also present in the artichoke extract (E29). In fact, the content of chlorogenic acid in artichoke waste extract was clearly higher, but the enhancing capacity of the coffee extract (E2) was greater than that of E29. Thus, the contribution of chlorogenic acid to the increase in larvae mortality is not clear or there is some other component in the artichoke extract that interacts with this acid, preventing its action or exerting an antagonistic effect. Therefore, it would be interesting to perform more exhaustive analyses of the spent coffee extract in order to determine if these phenolic acids are actually responsible for the potentiation of the observed effects of baculovirus infection. A viable strategy to determine the enhancing effect of each of the phenolic compounds with baculoviruses could be to conduct bioassays using commercial standards of the antioxidants that appear to be more effective.

5. Conclusions

Agri-food waste is an increasingly worrying environmental and economic problem and, therefore, value-added alternatives for these residues must be sought. In this work, the enhancer effects of different plant extract from agri-food waste on the larvicidal action of different baculoviruses have been determined. The extracts of spent coffee (E2), rosehip (E17), asparagus (E28), artichoke (E29), beet stalks (E32) and banana peel (E37) were selected since they increased the mortality of SpliNPV in second instar *S. littoralis* larvae. The potentiation of the baculovirus infection observed for these extracts in the present work was host-pathogen and instar dependent.

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CRediT authorship contribution statement

Blanca Martínez-Inda: Formal analysis, writing original draft and editing.

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Irene Esparza: Methodology, data revision, writing, reviewing and editing.

Jose Antonio Moler: statistical analysis and writing original draft.

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All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

All authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aoas.2023.11.001.

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