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Aphicidal activity of five plant extracts applied singly or in combination with entomopathogenic bacteria, *Xenorhabdus budapestensis* against rose aphid, *Macrosiphum rosae* (Hemiptera: Aphididae)

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ABSTRACT

The rose aphid *Macrosiphum rosae* (L.) is now a globally spread insect species damages rose plants affecting quality and productivity. Botanical insecticides are excellent alternative to synthetic pesticides, as they have minimal environmental persistence and toxicity, and are more compatible with the biocontrol agents than synthetic pesticides. This study aimed to evaluate extracts of five plant species i.e. *Citrullus colocynthis*, *Tagetes erecta*, *Rosmarinus officinalis*, *Thymus vulgaris*, and *Withania somnifera*; and entomopathogenic bacteria (EPB), *Xenorhabdus budapestensis* against *M. rosae*, as individual and concomitant treatments to determine their compatibility under laboratory conditions. Results indicated that the five plants extracts and EPB applied individually had immense contact or residual toxicity against *M. rosae*. Methanol extract of *T. erecta* significantly proved to be more effective as aphicide than ethanol and acetone extracts of five tested plants. Similarly, the results also show a direct, significant relationship between the mortality rates and both EPB cell suspension concentration and exposure time when applied individually. Moreover, three days after treatment, the combination of EPB and each plant extract resulted in a significantly higher *M. rosae* mortality than the EPB or plant extract alone. We conclude that five plants extracts especially *T. erecta* had compatible capacity with EPB, thus it could be used in integrated aphid management programs.

1. Introduction

Rose is known as the "Queen of Flowers" over the world (Datta, 1997). In the floriculture industry, the rose is the most significant crop. Roses are used as cut flowers, potted plants, and garden plants, with an annual value of $10 billion. Their petals are also used as a source of natural scents and flavorings, which contributes to their economic value. Furthermore, cut rose flower is regarded as one of the best cash crop ornamental flowers. The damask rose (*Rosa damascene*) is the most important species used to produce rose water, attar of rose, concrete, and essential oils; all of which are valuable and important base material for perfume and cosmetic industries (Zuker et al., 1998; Ayci et al., 2005). *R. damascena trigintipetala* Mill. is grown at Taif, Saudi Arabia, where a unique local variety known as the Taify rose is valued for its oil content

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and adaptability to the local climate. Aphids, thrips, whiteflies, and various lepidopteran larvae are some of the insects that damage roses. The buds, leaves, and flowers of rose plants are extremely vulnerable to these insect infestations. As a result, infestations with these insect pests limit rose yields (Karlik and Tjosvold, 2003). Rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae); potato aphid, *M. euphorbiae* (Thomas); cotton aphid, *Aphis gossypii* Glov.; and green peach aphid, *Myzus persicae*; are all major aphid pests that infest and harm rose plants (Karlik and Tjosvold, 2003). *M. rosae* is a key pest of rose, causing direct damage by sap sucking which results in deformed leaves and new bloom stems, stunted growth, gall formation and changing the composition of plant biochemicals (Jalalizand et al., 2012; Singh et al., 2014). Indirect loss is incurred, however, when honeydew is secreted, which promotes mold growth on blossoms and leaf surfaces, lowering plant photosynthetic activity and thus yield (Jalalizand et al., 2012). Therefore, aphid infestation lowers the market value of rose flowers and has a negative impact on plant flowering capacity, resulting in losses of 20–40 % (Jayma and Ronald, 1992). Aphids are also responsible for the transmission of various plant viruses, as well as providing entry points for fungal spores and bacteria inside rose plants through holes punched during feeding (Chau et al., 2004). Taif's rose has been known to have severe *M. rosae* infestations (Sayed and Montaser, 2012; Sayed and Alghamdi, 2017). Growers frequently use synthetic chemical insecticides to protect flowers from aphid infestation. Synthetic pesticides are usually expensive and leave long-lasting residues on exposed surfaces, even though they provide immediate and effective control for the time being; besides this sucking insect pest associated with rose plant has been long known to develop resistance (Metcalf, 1980). Furthermore, the scenario demands sustainable solutions due to other issues such as health risks, negative side effects, insect comeback and disturbance, and environmental pollution caused by the continued use of synthetic chemical pesticides (Parmar, 1993; Freeha et al., 2017). One such option that has the potential to change modern day insect pest control is the employment of natural and biodegradable compounds, predators, parasitoids, and entomopathogenic microorganisms (Ghodke et al., 2013; Dixit et al., 2015). Secondary metabolites such as phenols, flavonoids, quinones, terpenoids, alkaloids, and tannins are produced by plants to protect themselves from herbivorous and microbial attacks. Due to their efficiency against various life stages of many insect pests, extracts or essential oils of medicinal or aromatic plants are frequently utilized for pest management (Ahmed et al., 2020). There is an increasing requirement for new active compounds and ingredients for pest control that have less negative environmental effects (Rodríguez-González et al., 2019). Botanical pesticides and plant extracts have recently demonstrated a significant role in pest control due to their low cost, lack of residual effects, environmentally friendly nature, wide availability, and high toxicity against numerous insect pests such as aphids. Furthermore, due to their molecule complexity, they are unlikely to cause pesticide resistance in diseases and pests (Bedini et al., 2020). Medicinal plant extracts have been successfully utilized against a range of hemipteran pests, particularly various aphid species (Vishwanath, 2002; Sood et al., 2005). Many plant-derived essential oils, such as neem, rosemary, lavandula, thyme, and ziziphora, have insecticidal properties that cover a broad range of soft-bodied arthropod pests (Murray, 2006; Alexenizer and Dorn, 2007). Bacteria have long been regarded as potential pest control alternatives to conventional chemical insecticides. Secondary metabolites (SMs) or small-molecule natural products are synthesized by these microorganisms. Agrochemical, food, and pharmaceutical sectors are all interested in many of these SMs since they have a diversity of biological functions. Entomopathogenic bacteria (ENB) have attracted a lot of attention as biological control agents over the last two decades. The Gram-negative bacteria *Xenorhabdus* spp., which are members of the Enterobacteriaceae family and are symbiotically coupled with entomopathogenic nematodes of the Steinernematidae family (Boemare et al., 1993; Forst et al., 1997), have demonstrated a composite relationship between these bacteria and their nematodes. The extreme toxicity against several insect species has been justified as a result of their association (Akhurst, 1983; Herbert and Goodrich-Blair, 2007; Herbert et al., 2009). As nematodes infest their insect hosts, they release symbiotic bacteria from their intestinal tracts into the heomocoel, where the bacteria grow and kill the insects within 48 h. The bacteria subsequently turn the insect into suitable food for nematode and release toxins (Akhurst and Bedding, 1986) that keep the dead insect from decaying while the nematodes feed and multiply on it (Sicard et al., 2004; Yang et al., 2012). The bacterium *Xenorhabdus* has been proven to grow successfully in the laboratory, and both cell suspensions and cell-free supernatants of this bacterium have been reported to have a detrimental effect on several insect pests, causing mortality. *Xenorhabdus szentirmaii* is a unique source of antimicrobial peptides that are effective against practically all known plant pathogens (Fuchs et al., 2014). Many biocontrol agents are affected by the majority of synthetic pesticides. These control agents interact in a synergistic, additive, or antagonistic approach. Synergistic interactions would improve EPB efficacy while lowering insecticide adverse effects. Plant-derived insecticides are more effective when used in combination with microbial or synthetic insecticides than when used alone (Isman, 2006). As a result, integrated pest management (IPM), which combines biocontrol agents and biopesticides, is gaining popularity and has been shown to be an environmentally friendly management strategy in which biocontrol agents can be mixed with plant-derived extracts (Kalita and Hazarika, 2018). Although there have been numerous studies on the toxicity effects of plant extracts and EPBs applied singly on insect pests, little work has been carried out to find the lethal combined effects of those two agents on aphid control. Therefore, this study was aimed to evaluate, for the first time, the compatibility of extracts from five medicinal plant species located in the Taif region of Saudi Arabia and EPB, *Xenorhabdus budapestensis*, against the rose aphid, *M. rosae*, under laboratory conditions.

2. Materials and methods

2.1. Insects

The adults of rose aphid, *M. rosae* were originally collected from common rose farms of Taif region, Saudi Arabia. These aphids were reared on potted Taify rose plants grown in a growth chamber (25 ± 2 °C, 65 ± 3 % R.H. and a photoperiod of 16:8h L:D) for two months (several generations) before conducting the experiments. All experiments were performed under the above mentioned conditions in laboratory.

2.2. Plant extracts

Five medicinal plant species from four different families were collected in January 2021 from their natural habitat in the Al-Shafa region, Taif Province, as illustrated in Table 1. These tested plants were morphologically identified by the Herbarium Unit at the Biology Department, Faculty of Science, Taif University, Saudi Arabia. The collected fresh parts (1 kg from each plant species) were washed thoroughly under running tap water, cut into small pieces, fully shade dried for 10 days, and used for extraction. Dried plant materials were homogenized to fine powder by a mortar and pestle. Following Ahmed et al. (Ahmed et al., 2020) with some

Table 1
The five medicinal plants used in the present study.

No.	Scientific Name	Common Name	Family Name	Extracted Part
1	*Citrullus colocynthis* (*Cc*)	Colocynth	Cucurbitaceae	Leaves
2	*Tagetes erecta* (Te)	African marigold	Asteraceae	Flowers
3	*Rosmarinus officinalis* (Ro)	Rosemary	Lamiaceae	Leaves
4	*Thymus vulgaris* (Tv)	Thyme	Lamiaceae	Leaves
5	*Withania somnifera* (Ws)	Ashwagandha	Solanaceae	Leaves

modifications, extractions were performed thrice at room temperature for three days by using the cold extraction/solvent extraction method. In brief, five grams of fine powder from each plant was extracted with 100 ml 95 % of different organic solvents i.e. ethanol, methanol and acetone. Afterward, each extract was centrifuged at 5000 rpm for 15 min and filtered 3 times with Whatman filter paper No. 1. Then, the volume was reduced by concentrating the filtered material through the rotary evaporator at 30 °C. Next, the filtrate was allowed to dry for 12 h in a fume hood at 28 °C. The dry extracts were dissolved in 1 % aqueous solution of dimethylsulfoxide (DMSO) and adjusted to a final concentration of 1000 μg/mL and then, the extracts were stored in glass bottles at 4 °C for further bioassays.

2.3. Gas chromatography–mass spectrometry (GC–MS) analysis of T. erecta flowers

The methanol extract of *T. erecta* flowers was analyzed with an Agilent GC–MS instrument using an Agilent HP-5MS column (30 m length) with helium as the carrier gas at a flow rate of 7 ml min^{-1}. Agilent HP-5 ms is a (5 %-phenyl)-methylpolysiloxane phase with low bleed characteristics that is ideal for GC/MS. The column is bonded, cross-linked, and the solvent rinsable, with excellent inertness for active compounds with improved signal-to-noise ratio for better sensitivity and mass spectral integrity. The oven was set at the following temperatures: initial, 70 °C; ramp with 5 °C min^{-1} to 310 °C; hold for 1 min at 310 °C; ramp to 70 °C with 5 °C min^{-1}. For the determination of the compounds, the analytical method was used: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

2.4. EPb

In this study, one strain of symbiotic bacterium included in the bioassay was provided from the Fodor Laboratory at Pannonia University, in Keszthely, Hungary. The symbiotic bacterium, *Xenorhabdus budapestensis* DSM 16,342 (EMA), which was isolated from the EPN *Steinernema bicornutum* was used for determining its compatibility with plant extracts against *M. rosae*. The bacterium was regularly cultivated on LBTA (Luria Bertani agar) indicator plates [10 g of peptone, 5 g of yeast extract, 5 g of sodium chloride, 15 g of agar, 25 mg of bromothymol blue, 40 mg of 2,3,5-triphenyltetrazolium chloride and 1 L of distilled water (pH 6.8)] in the dark at 25 °C. In preparing the bacterial cell suspension, as an inoculum for a 100-mL culture, a single dark blue/red colony was placed separately into test tubes containing 5 ml of LB liquid media. Exactly 100-mL aliquots of culture were shaken overnight at room temperature in 500-mL Erlenmeyer flasks before being transferred to flasks containing 400 ml of the same media and shaken at 200 rpm for five days. To obtain the bacterial pellet, the multiplied bacterial culture was centrifuged (13,000 rpm for 30 min) at 4 °C. A 0.22 m Millipore filter was used to separate the supernatant and pellet which was resuspended in sterile distilled water. Adjustment of the bacterial cell suspension at OD600 to 1.0 was performed using a spectrophotometer (SpectroStar Omega, BMC Labtech, Aylesbury, UK). A 10-fold serial dilution spread plate was used, and the bacterial suspension concentration was 10^8 (CFU/mL). Six dilutions of bacterial cell suspension were adjusted to 10^8, 10^7, 10^6, 10^5, 10^4, and 10^3 CFU/mL.

2.5. Bioassays

2.5.1. Toxicity of plant extracts

Each of the extract prepared by using different solvents (1000 μg/mL) was diluted by distilled water to obtain five concentrations of 800, 600, 400, 200 and 100 μg/mL. Contact and residual toxicity methods were adopted to evaluate the activity of each extract against *M. rosae*. For contact assay, 20 adult wingless aphids were dipped in each respective extract/concentration for 5 s and released on freshly cut leaves placed in plastic petri dishes (100 × 15 mm) with moistened cotton tissues to maintain humidity. While in residual toxicity test, fresh rose leaf discs (2 cm diameter) were cut off, dipped for 20 s in each respective extract/concentration and dried in air for half an hour. After the dipping application, 3 leaf discs were placed in a petri dish (100 × 15 mm) lined with moistened cotton tissues. Next, 20 adult wingless aphids were released on these leaf discs. For both toxicity assay tests, 1 % DMSO prepared in distilled water was used as the control. Then, the dishes were kept in a plant growth chamber at 25 ± 2 °C, 65 ± 3 % R.H. and a photoperiod of 16:8 L:D. Accordingly, each replicate (plate) contained 20 individual aphids, with a total of 5 replicates per concentration. Aphid mortality was assessed at an exposure of 24, 48 and 72 h by gentle probing with a fine brush and observing the lack of insect movement and the change in the body to a post-mortem color (Sadeghi et al., 2009). The mortality rates in the treatments were corrected with that in the control according to Abbott's formula (Abbott, 1925).

$$\textit{Corrected Mortality } \% = \left(\frac{\% MT - \% MC}{100 - \% MC}\right) \times 100 \quad (1)$$

where MT: Mortality in Treatment; MC: Mortality in Control.

2.5.2. Pathoginicity of EPB

The toxicity of EPB cell suspension was evaluated on *M. rosae* via contact and residual toxicity methods. The experiment was conducted as previously described in the plant extract bioassay with application of prepared six concentrations of bacterial cell suspension instead of plant extract and distilled water was used as control.

2.5.3. Combination activity between plant extracts and EPB

From the resulted data of bacterial bioassay, the concentration of 1×10^5 CFU/mL was used to be combined with a low concentration of each methanol extract (100 μg/mL) as contact application method against *M. rosae*. The mixture of each plant extract and bacterium was prepared from 2.5 ml of plant extract (200 μg/mL with 2 % DMSO) and 2.5 ml of bacterium suspension (2×10^5 CFU/mL distilled water) to obtain 5 ml of mixture with a final concentration of 100 μg/mL (Plant extract) and 1×10^5 CFU/mL (Bacterium). Therefore, 11 treatments were carried out: 1×10^5 CFU/mL (bacterium alone), 5 treatments with 100 μg/mL (for each plant extract), and 5 treatments of mixtures of bacterium with the 5

tested plant extract. Two control groups were used in this experiment: control 1—aphids were dipped in distilled water to correct mortality of bacterium alone; control 2—aphids were dipped in DMSO (1 %) to correct mortality of each plant extract alone and of mixtures. The contact was applied as previously described in the plant extract bioassay. Each treatment or control was repeated 5 times, where each replicate contained 20 aphid individuals. Then, all Petri dishes were investigated after 72 h for mortality. All treatments were carried out under the controlled conditions of 25 ± 2 °C, 65 ± 3 % RH, and 16L: 8D photoperiod.

2.6. Statistical analysis

The median lethal concentration (LC_{50}), 95 % confidence limits of lower and upper values, slope, intercept, and chi-square (χ^2) were estimated using probit analysis of mortality versus concentration by SPSS software program, version 23 (Spss, 2015). Statistical analysis for all experiments was performed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). Meanwhile, two-way ANOVA with Duncan's test was used to compare among corrected mortalities caused by individual treatments of plant extracts and EPB ($p < 0.05$). Moreover, one-way ANOVA was conducted to assess the compatibility of each plant extract and EPB on aphid toxicity.

3. Results

3.1. Toxicity of plant extracts

The contact and residual assays demonstrated that all five plant extracts had variable degrees of toxicity against rose aphid in three solvents (Tables 2, 3). The contact efficacy of the five plant extracts on *M. rosae* mortality was revealed in Table 2. The percent corrected mortality rate of adult aphids was found to be directly related to the plant extract species, concentration, solvent, and exposure time. Among the five tested plant extracts, results indicated that highest mortality (66.3 %) was significantly recorded by *T. erecta* extract; followed by *T. vulgaris* (57.8 %), *R. officinalis* (57.3 %) and *C. colocynthis* (53.7 %) which were not significantly differ; while *W. somnifera* afforded lower mortality (48.5 %). The results also revealed superiority of methanol extracts over acetone and ethanol ones implying none of the acetone or ethanol extracts at five concentrations killed more aphid individuals than methanol plant extracts. Moreover, for all plant extracts, the 800 µg/mL concentration was most effective in achieving maximum toxicity compared to the other four concentrations. Furthermore, the data also indicated that mortality is correlated to prolonged time exposure (Table 2). The mortality rates after 24 and 48 h were 2 % for the control, whereas, it recorded 4 % after 72 h exposure. The maximum corrected mortality rate (98.9 %) was recorded by methanol extract of *T. erecta* at 800 µg/mL and 72 h post treatment, followed by acetone and ethanol extracts with 96.9 % mortality percentage each at the same concentration and exposure time. Moreover, methanol extract of *T. erecta* at low concentrations (200 and 100 µg/mL) afforded extreme mortality after 72 h exposure which was 91.8 % and 89.6 %, respectively. Likely, methanol extract of *R. officinalis* at 72 h exposure and 800 µg/mL induced 94.4 % mortality, whereas, acetone and ethanol extracts induced 92.2 % mortality each at the same duration and concentration, while at 100 µg/mL in the same solvent it gave considerable mortality (55.6 %). The lowest mortality percentage was recorded for extracts of *W. somnifera* at all five concentrations. The methanol and acetone extracts of *W. somnifera* performed slightly better than its ethanol counterpart with mortality rates 53.9 %, 50.5 % and 41.1 %, respectively. At 100 µg/mL concentration, ethanol *W. somnifera* extract produced minimum mean percentage mortality of 17.8 % after 24 and 48 h exposure.

The data in Table 3 reveal that all plant extracts had a highly significant effect on the mortality of *M. rosae* adults ($p < 0.05$) via residual assay test. The results show that the five plants extracts increased mortality of *M. rosae* adults ($p < 0.05$) within the three solvents, five concentrations and three exposure periods comparing with the control treatment, which demonstrated zero mortality at 24 h and 10 % mortality at 48 and 72 h. Among all tested plant extracts, results demonstrated that highest mortality (65.3 %) was significantly observed by *T. erecta*, followed by *T. vulgaris* (52.5 %) and *R. officinalis* (42.6 %), while *C. colocynthis* and *W. somnifera* induced the lowest mortality (34.9 %) and (30.3 %), respectively. Similarly, among the three tested solvent, methanol extract afforded higher *M. rosae* mortality than ethanol and acetone extracts. There were statistically significant differences among the treatments on each concentration of plant extract and day after application. On 1st, 2nd, and 3rd day 800 µg/mL produced significantly higher mortalities than 200 and 100 µg/mL plant extract concentrations (Table 3). At 72 h exposure; maximum mortality was elicited by methanol extract of *T. erecta* with mean mortality percentages of 97.8 %, 93.3 %, 90.6 %, 80 % and 70 % at 800, 600, 400, 200 and 100 µg/mL concentrations, respectively. However, ethanol extract caused aphid mortality rates ranging from 93.3 % to 62.2 %, whereas, percentage mortality dipped to range from 95.6 % to 48 % for acetone *T. erecta* extract at the same concentrations and exposure time, respectively. Analysis of variance revealed that methanol extract did not differ significantly than ethanol extract in *T. erecta*, *R. officinalis* and *T. vulgaris* plants but was significantly different from acetone extract for all three plants. Although ethanol *W. somnifera* extract at 800 µg/mL produced the highest mean percentage mortality (70.8 %) 72 h post treatment, it recorded the lower mortality (8.9 %) at 200 and 100 µg/mL concentrations after 24 and 48 h exposure.

As shown in Table 4, probit analysis exposed the LC_{50}, slope value, intercept, Chi-square, probability and confidence interval limits at 95 %. The data indicate that *T. erecta* extract was significantly more efficient against *M. rosae* individuals than other four plant extracts under laboratory conditions. The *T. erecta* extract exhibited lower LC_{50} values of 195.7, 84.6 and 34.7 µg/mL for contact assay, whereas, it recorded 375.1, 98.7 and 81.6 µg/mL for residual efficacy at 24, 48 and 72 h exposure, respectively. For the other four plants, LC_{50} values at 72 h post application ranged from lowest to highest as follows: 77.5 and 87.6; 121.5 and 176.7; 126 and 283.4; and 249.4 and 417.5 µg/mL for *T. vulgaris*, *R. officinalis*, *C. colocynthis* and *W. somnifera* via contact and residual assay, respectively. Table 4 shows that the highest degree of homogeneity in the *M. rosae* individual response was observed after exposure to the *T. vulgaris* extract via contact assay, with a slope value of 0.058 at 48 h post treatment. On the contrary, the other tested plant extract concentrations exhibited low slope values (Table 4), which recognizes the uniformity in the response of *M. rosae* individuals to these concentrations and assay methods.

3.2. GC–MS analysis of T. erecta extract

The chemical compounds of the methanol extract of *T. erecta* flowers with their retention time, molecular formula, and peak area percentage are presented in Table 5. A total of 31 components were identified in the extract of *T. erecta* flowers. The GC–MS analysis results revealed the presence of Hydrogen isocyanate, which is the most abundant compound (26.47 %); followed by Tetrahydro-4H-pyran-4-ol (21.99 %); Glycine, ethyl ester (15.83 %); 1H-Pyrazole, 4,5-dihydro-3-methyl-1-propyl- (8.95 %); 1,3-Dioxolane-4-methanol, 2-pentadecyl-, acetate (5.69 %); l-Norvaline, *N*-ethoxycarbonyl-, pentyl ester (5.59 %); 4-

Table 2
Aphicidal activity of plant extracts on *M. rosae* via contact assay.

Plant extract	Solvent	Exposure time (h)	[a] Corrected Mortality (%)					Solvent Means	Plant Extract Means
			Concentration (µg/mL)						
			800	600	400	200	100		
C. colocynthis	Acetone	24	[b]51 ± 1.9	47 ± 2	44 ± 4	35.6 ± 1.4	27.8 ± 2.5	56.1b	53.7c
		48	66.7 ± 2.5	62.2 ± 3.2	60 ± 4.1	48 ± 3.7	40 ± 0		
		72	85.6 ± 1.4	81.1 ± 1.4	77.8 ± 2.5	68.9 ± 1.4	45.6 ± 5.7		
	Ethanol	24	48 ± 2.5	45 ± 2.2	42 ± 3.7	35.6 ± 5.4	20 ± 3.2	44.4c	
		48	57.8 ± 3.8	51.1 ± 4.4	46 ± 5.1	44.4 ± 3.5	33.3 ± 4.9		
		72	61.1 ± 4.9	52.2 ± 4.2	48.9 ± 4.4	47.8 ± 5.9	33.3 ± 4.9		
	Methanol	24	62.2 ± 2.1	60 ± 3.2	54.4 ± 3.7	46 ± 2.4	34.4 ± 4.4	60.5 a	
		48	66 ± 2.9	64 ± 2.4	56.7 ± 2.1	50 ± 3.2	45.6 ± 5.7		
		72	92.2 ± 3.3	88.9 ± 3	83.3 ± 7.5	57.8 ± 4.2	46.7 ± 2.8		
Concentration Means			65.6 a	61.3 ab	57b	48.2c	36.3 d		
T. erecta	Acetone	24	57.8 ± 3.8	45.9 ± 5	42.9 ± 6.9	38.8 ± 4.6	28.6 ± 3.2	66.2 ab	66.3 a
		48	76.5 ± 2.6	73.5 ± 2.9	69.4 ± 4.6	69.4 ± 3.2	59.2 ± 4.6		
		72	96.9 ± 2.1	94.4 ± 1.8	93.8 ± 2.6	83.3 ± 5.3	62.5 ± 7.1		
	Ethanol	24	60 ± 4.1	57.8 ± 3.8	44.9 ± 2.9	31 ± 8.4	20.4 ± 3.8	63.7b	
		48	76.5 ± 3.1	72.4 ± 3.8	69.4 ± 4.6	59.2 ± 4.6	48.9 ± 7.2		
		72	96.9 ± 2.1	92.7 ± 3.1	83.3 ± 7.1	72.9 ± 4.2	69.6 ± 7.2		
	Methanol	24	76.5 ± 2.6	57.8 ± 3.8	48.9 ± 3.6	38.8 ± 4.6	28.6 ± 3.2	68.9 a	
		48	91.8 ± 5	88.8 ± 4.7	83.7 ± 6.9	63.2 ± 6.9	48.9 ± 7.2		
		72	98.9 ± 1	95.8 ± 1.9	92.7 ± 3.1	91.8 ± 5	89.6 ± 3.3		
Concentration Means			81.3 a	75.5b	69.9 bc	60.9c	50.7 d		
R. officinalis	Acetone	24	69 ± 4.8	54 ± 6.2	50 ± 7.1	46 ± 5.1	38 ± 3.7	58.6b	57.3b
		48	75.6 ± 1.4	66.7 ± 1.8	58.9 ± 3.3	51.1 ± 4.8	40 ± 2.7		
		72	92.2 ± 6.5	70 ± 5.2	63.3 ± 3.3	55.6 ± 2.5	48.9 ± 2.7		
	Ethanol	24	56 ± 2.9	46 ± 2.9	40 ± 3.2	40 ± 3.2	29 ± 4.8	53.1c	
		48	73.3 ± 2.1	64.4 ± 3.8	54.4 ± 2.7	44.4 ± 7.9	33.3 ± 3.5		
		72	92.2 ± 3.8	65.6 ± 5.1	57.8 ± 6.5	53.3 ± 4.2	46.7 ± 4.2		
	Methanol	24	63.3 ± 2.2	54 ± 3.3	47 ± 3	44 ± 2.4	40 ± 6.3	60.1 a	
		48	66 ± 2.9	57.8 ± 2.2	53.3 ± 4.2	51.1 ± 2.7	40 ± 2.7		
		72	94.4 ± 1.8	86.7 ± 2.8	83.3 ± 1.8	64.4 ± 6.7	55.6 ± 3.5		
Concentration Means			75.8 a	62.8b	56.4 bc	49.9c	41.3 d		
T. vulgaris	Acetone	24	67 ± 1.2	56 ± 1	47 ± 2	40 ± 2.7	10 ± 3.2	57.3b	57.8b
		48	76.7 ± 3.2	73.3 ± 2.1	71.1 ± 2.7	57.8 ± 4.2	11.1 ± 3.5		
		72	86.7 ± 1.4	81.1 ± 2.2	75.6 ± 3.8	62.2 ± 5.7	44.4 ± 4.9		
	Ethanol	24	71.1 ± 2.1	60 ± 1.6	50 ± 3.2	44.4 ± 7.9	22.2 ± 4.6	56.3 bc	
		48	73 ± 1.2	64.4 ± 4.2	57.8 ± 6.5	46 ± 4	30 ± 3.2		
		72	73.3 ± 3.2	72.2 ± 2.5	66.7 ± 3.5	57.8 ± 5.4	55.6 ± 3.5		
	Methanol	24	65 ± 2.7	54 ± 3.7	51 ± 3.3	43 ± 3.7	33.3 ± 3.5	59.7 a	
		48	72.2 ± 3.5	57.8 ± 4.8	55.6 ± 3.5	44.4 ± 7.9	40 ± 3.2		
		72	86.7 ± 2.8	81.1 ± 3.3	77.8 ± 6.1	68.9 ± 4.2	64.4 ± 5.4		
Concentration Means			74.6 a	66.7 ab	61.4b	51.6c	34.6 d		
W. somnifera	Acetone	24	57 ± 3.7	55 ± 4.5	50 ± 7.1	28 ± 3.7	26 ± 5.1	50.5b	48.5 d
		48	72.2 ± 0	55.6 ± 0	50 ± 4.3	50 ± 2.5	33.3 ± 3.5		
		72	74.4 ± 1.4	62.2 ± 2.1	56.7 ± 1.1	54.4 ± 2.1	33.3 ± 3.5		
	Ethanol	24	46 ± 4	43 ± 5.4	37 ± 5.4	24 ± 8.1	17.8 ± 5.7	41.1c	
		48	60 ± 2.7	46.7 ± 1.4	38.9 ± 2.5	36.7 ± 2.2	17.8 ± 5.7		
		72	70 ± 1.4	60 ± 2.1	52.2 ± 2.8	42.2 ± 2.8	24 ± 8.1		
	Methanol	24	64 ± 4.8	55.6 ± 3.5	48.9 ± 5.4	46.7 ± 4.2	34 ± 4	53.9 a	
		48	70 ± 2.8	58 ± 4.1	51.1 ± 5.7	48 ± 5.8	42.2 ± 4.5		
		72	76.7 ± 1.1	63.3 ± 1.4	52.2 ± 3.3	52 ± 5.8	45.6 ± 3.7		
Concentration Means			65.6 a	55.5b	48.6c	42.4 cd	30.4 d		

[a] Each treatment was represented by five replicates, each containing 20 adult insects.
[b] The numbers in each column indicate corrected mortality ± standard error. Within the same column or row, means with different letters differ significantly ($p < 0.05$ using Duncan's Multiple Range Test).

Methoxycarbonyl-4-butanolide (4.05); [(2-Ethyl-5-methylfuran-3,4- (1.67 %); Propanethioic acid, S-propyl ester (1.21 %); beta-Amyrin (1.19 %); and a small quantity of other components.

3.3. Virulence of EPB

The data in Fig. 1 represent the toxicity of entomopathogenic bacteria *X. budapestensis* DSM 16,342 at six concentrations against *M. rosae* adults via contact and residual assay at three exposure times under laboratory conditions. The EPB cells significantly affected aphid mortality ($p < 0.05$) in the two method of evaluation. The percentage of adult mortality increased significantly with bacterial cell concentration and exposure duration ($p < 0.05$). The interactive effect of bacterial cell suspension concentrations, and exposure time on individual infection via contact assay was not significant ($p = 0.9765$). The highest mortality percentage (97.9 %) was recorded in the plates where the adults were exposed to 10^8 CFU/mL distilled water after 72 h of application, followed by 10^7 CFU/mL (93.7 %) without significant differences between these two concentrations. On the other hand, the lowest (44.9 %) was recorded when the individuals were exposed to 10^3 CFU/mL of *X. budapestensis* 24 h post treatment (Fig. 1A). Similarly, the residual efficacy of EPB on *M. rosae* adults recorded the same results. The data indicate that the mortality rate had a direct relationship with the exposure time and bacterial CFU concentration ($p < 0.05$). The regression analysis of the data shows that the mortality of *M. rosae* adults significantly increased with increasing bacterial concentration ($R^2 = 0.9852$; $p < 0.05$). Maximum (92.2 %) and minimum

Table 3
Aphicidal activity of plant extracts on *M. rosae* via residual assay.

Plant extract	Solvent	Exposure time (h)	[a] Corrected Mortality (%)					Solvent Means	Plant Extract Means
			Concentration (µg/mL)						
			800	600	400	200	100		
C. colocynthis	Acetone	24	[b]28.6 ± 1.6	24.5 ± 1.9	22.4 ± 2.5	20.4 ± 3.8	12.2 ± 2.5	28.9 bc	34.9 d
		48	36.7 ± 2.6	33.7 ± 2.3	32.7 ± 2.5	22.4 ± 2.5	13.1 ± 5.6		
		72	51 ± 3.1	43.8 ± 5.3	39.6 ± 6.1	27.1 ± 3.3	25 ± 6.1		
	Ethanol	24	26.5 ± 2.6	23.5 ± 2.3	20.4 ± 3.8	12.2 ± 2.5	8.9 ± 4.1	32.6b	
		48	46.9 ± 3	42.7 ± 4.4	40.6 ± 4.5	28.6 ± 3.2	18.4 ± 3.2		
		72	56.1 ± 7.8	52 ± 9.9	48.9 ± 3.7	31.3 ± 2.6	31.3 ± 2.6		
	Methanol	24	28.6 ± 1.6	24.5 ± 1.9	20.4 ± 3.8	20.4 ± 3.8	12.2 ± 2.5	43.2 a	
		48	42.9 ± 7.1	37.8 ± 7.9	33.7 ± 9.1	28.6 ± 3.2	22.4 ± 4.1		
		72	90.6 ± 3.5	84.4 ± 4.7	79.2 ± 7.4	75 ± 9.7	47.9 ± 7.4		
Concentration Means			45.3 a	40.8 ab	37.5b	29.6 bc	21.3c		
T. erecta	Acetone	24	61.1 ± 2.5	56 ± 1.9	52 ± 3.7	50 ± 4.5	44.4 ± 3.7	61.1b	65.3 a
		48	72 ± 2.5	56.7 ± 3.7	55.6 ± 3.5	53.3 ± 4.2	48 ± 3.7		
		72	95.6 ± 2.1	87.8 ± 4.1	80 ± 4.2	55.6 ± 3.5	48 ± 3.7		
	Ethanol	24	67.8 ± 3.7	58 ± 1.2	58 ± 3.7	55 ± 2.2	48 ± 3.7	67.1 a	
		48	68 ± 2.5	65.6 ± 2.7	68.9 ± 4.2	68.9 ± 4.2	56.7 ± 4.4		
		72	93.3 ± 2.1	92.2 ± 4.2	80 ± 3.8	63.3 ± 2.2	62.2 ± 5.7		
	Methanol	24	71 ± 1.9	62 ± 3	54 ± 2.4	50 ± 3.2	38 ± 3.7	67.8 a	
		48	75.6 ± 2.8	73.3 ± 2.1	64.4 ± 2.2	53.3 ± 4.2	44.4 ± 3.5		
		72	97.8 ± 1.4	93.3 ± 2.1	90.6 ± 3.5	80 ± 4.2	70 ± 2.2		
Concentration Means			78 a	71.7b	67.1c	58.8 cd	51.1 d		
R. officinalis	Acetone	24	24.5 ± 2.9	20.4 ± 2.6	18.4 ± 3.2	18.4 ± 3.2	8.9 ± 4.1	39.2b	42.6c
		48	57.1 ± 2.6	55.1 ± 2.5	53.1 ± 4.1	38.8 ± 4.6	22.4 ± 5.2		
		72	64.6 ± 3	62.5 ± 2.6	58.3 ± 3.3	47.9 ± 7.4	37.5 ± 7.4		
	Ethanol	24	50 ± 5.7	45.9 ± 5	42.9 ± 13.1	18.4 ± 3.2	12.7 ± 3.7	44 a	
		48	52 ± 8.2	46.9 ± 3.2	42.9 ± 6.9	28.6 ± 4.6	20.4 ± 3.8		
		72	75 ± 1.9	70.8 ± 2.7	68.8 ± 3.3	47.9 ± 7.4	37.5 ± 3.3		
	Methanol	24	30.6 ± 4.7	26.5 ± 6.2	24.5 ± 6.9	22.4 ± 2.5	22.4 ± 4.1	44.7 a	
		48	58.2 ± 3.4	52 ± 3.8	42.9 ± 8.3	28.6 ± 3.2	28.6 ± 3.2		
		72	79.2 ± 1.6	76 ± 2.1	72.9 ± 4.2	68.8 ± 4.7	37.5 ± 7.4		
Concentration Means			54.6 a	50.7 ab	47.2b	35.5c	25.3 d		
T. vulgaris	Acetone	24	31.6 ± 2	25.5 ± 2	20.4 ± 3.8	20.4 ± 3.8	12.2 ± 2.5	32.7b	52.5b
		48	33.7 ± 2.3	26.5 ± 1.2	22.4 ± 2.5	20.4 ± 3.8	20.4 ± 3.8		
		72	63.5 ± 2.9	60.4 ± 2.7	58.3 ± 3.3	37.5 ± 7.4	37.5 ± 3.3		
	Ethanol	24	56.1 ± 3.8	52 ± 5.5	48.9 ± 7.2	42.9 ± 6.9	20.4 ± 3.8	61.8 a	
		48	73.5 ± 5.4	71.4 ± 5.9	69.4 ± 7.2	59.2 ± 4.6	38.8 ± 4.6		
		72	92.7 ± 3.5	89.6 ± 2.9	87.5 ± 3.9	72.9 ± 4.2	52.1 ± 2.6		
	Methanol	24	36.7 ± 2.6	33.7 ± 3.2	28.6 ± 3.2	28.6 ± 3.2	20.4 ± 3.8	63.1 a	
		48	85.7 ± 5.4	81.6 ± 5.9	79.6 ± 7.2	69.4 ± 3.2	69.4 ± 4.6		
		72	93.8 ± 2.6	92.7 ± 3.5	80 ± 3.8	76 ± 2.1	70.8 ± 8.9		
Concentration Means			63 a	59.3 a	55 ab	47.5b	38c		
W. somnifera	Acetone	24	34.7 ± 1.9	26.5 ± 2.6	22.4 ± 2.5	14.3 ± 2.5	8.9 ± 4.1	28.7 bc	30.3 e
		48	42.9 ± 2.5	36.7 ± 2.6	32.7 ± 5.2	24.5 ± 6.9	16.7 ± 6.6		
		72	44.8 ± 2.7	41.7 ± 1.9	37.5 ± 3.3	27.1 ± 3.3	18.4 ± 3.2		
	Ethanol	24	24.5 ± 1.9	17.3 ± 2.5	12.2 ± 2.5	8.9 ± 4.1	8.9 ± 4.1	29.3b	
		48	34.7 ± 1.9	30.6 ± 4.1	28.6 ± 4.6	8.9 ± 4.1	8.9 ± 4.1		
		72	70.8 ± 8.9	64.6 ± 4.1	62.5 ± 7.2	41.7 ± 7.1	16.7 ± 3.3		
	Methanol	24	28.6 ± 1.6	24.5 ± 1.9	22.4 ± 2.5	12.2 ± 2.5	12.2 ± 2.5	32.8 a	
		48	36.7 ± 2.6	32.7 ± 2.5	28.6 ± 3.2	18.4 ± 4.6	18.4 ± 3.2		
		72	67.7 ± 8.5	63.5 ± 6.9	61.5 ± 5.4	37.5 ± 6.6	27.1 ± 4.7		
Concentration Means			42.8 a	37.6 ab	34.2b	21.5c	15.1 d		

[a] In this experiment, each treatment was represented by five replicates, each with 20 adult insects.
[b] Numbers in each column indicate corrected mortality ± standard error. Means with different letters within the same column or row differ significantly ($p < 0.05$ using Duncan's Multiple Range Test).

(42 %) mortality rates were achieved when the adults were treated with 10^8 CFU/mL at 72 h exposure and 10^3 CFU/mL at 24 h, respectively (Fig. 1B).

Likewise, the data in Table 6 reveal that the contact assay was more effective against *M. rosae* adults than the residual one, with LC_{50} values at 24, 48 and 72 h after treatment of 3.98×10^3, 9.33×10^2 and 1.41×10^2 CFU/mL, respectively, for the former method and 1×10^4, 2×10^3 and 7.94×10^2 CFU/mL, respectively, for the latter.

3.4. Compatible activity of plant extracts and EPB

Fig. 2 show the corrected mortality rates after 72 h of treatment for both individual and combination applications. Control groups 1 and 2 had a mortality rate of 4 % after 3 days, as stated in the "Methods" section. The mortality rates produced by *T. erecta* methanol extract treatment (82.3 %) were clearly different from the other four plant extracts, but not significantly different from *X. budapestensis* (83.3 %). After 72 h of application, however, the combination of each plant extract and *X. budapestensis* was significantly different from the individual treatments for either of them. Maximum mortality (97.9 %) was recorded by concomitant application of *T. erecta* extract and *X. budapestensis* cell suspension, whereas, minimum mortality was recorded via single application of *W. somnifera* extract which was 20.8 %.

4. Discussion

Because of the environmental impacts associated with the use of synthetic insecticides for pest control and the development of

Table 4
Probit analysis of the effects of plant extracts on *M. rosae*.

Plant extract	Bioassay	Exposure time (h)	LC_{50} (μg/mL)	95 % confidence limits		Slope ± SE	Intercept	X^2	*p*-Value
				Lower	Upper				
C. colocynthis	Contact	24	609.5	480.2	858	0.034 ± 0.008	29.2	2.81	0.023
		48	332.9	128.6	471.1	0.032 ± 0.004	39.3	0.72	0.005
		72	126	44.2	227.3	0.049 ± 0.011	44.3	4.22	0.022
	Residual	24	4213.7	1792.8	7476.7	0.022 ± 0.003	11.3	0.26	0.008
		48	1366.6	813.3	4911.5	0.032 ± 0.006	18.5	0.27	0.014
		72	283.4	199	382.4	0.042 ± 0.007	34.5	0.09	0.008
T. erecta	Contact	24	195.7	71.8	306.6	0.042 ± 0.003	23.7	0.60	0.001
		48	84.6	35.4	130.6	0.039 ± 0.008	53.6	2.40	0.019
		72	34.7	11.5	60.5	0.032 ± 0.006	74.3	1.14	0.011
	Residual	24	375.1	414.9	894.9	0.029 ± 0.004	42.9	0.90	0.006
		48	98.7	15.6	175.1	0.028 ± 0.005	50.1	0.52	0.011
		72	81.6	50.5	110.4	0.053 ± 0.007	57.2	3.78	0.006
R. officinalis	Contact	24	558.5	268.4	701.8	0.034 ± 0.005	33.4	2.29	0.006
		48	254.6	153.2	306.8	0.044 ± 0.005	36.7	0.95	0.003
		72	121.5	60.1	246.7	0.056 ± 0.006	45.1	10.7	0.002
	Residual	24	2640.7	1257.1	4307.2	0.028 ± 0.004	13.9	0.15	0.007
		48	550.2	418.2	832	0.045 ± 0.007	22.8	0.22	0.009
		72	176.7	114.8	233.3	0.045 ± 0.013	41.3	1.08	0.040
T. vulgaris	Contact	24	403.4	301.9	474.5	0.057 ± 0.011	23.8	2.40	0.015
		48	230	199.3	312.3	0.058 ± 0.015	30.9	1.94	0.028
		72	77.5	28.5	124.3	0.038 ± 0.006	54.4	0.14	0.008
	Residual	24	1639.5	884.3	3728.9	0.029 ± 0.007	19.9	1.35	0.030
		48	204.1	82.6	315.6	0.029 ± 0.004	42.7	0.07	0.007
		72	87.6	41.4	130.4	0.042 ± 0.008	53.2	0.30	0.013
W. somnifera	Contact	24	614	446.5	1061.3	0.043 ± 0.006	24.3	0.15	0.005
		48	360.8	265	506.1	0.044 ± 0.008	30.2	3.6	0.012
		72	249.4	179.1	323.1	0.049 ± 0.008	33.9	2.54	0.007
	Residual	24	6617	2071.8	9478.7	0.028 ± 0.001	7	0.73	0.001
		48	1788.6	1034.4	3440.5	0.035 ± 0.005	12.1	0.76	0.006
		72	417.5	337.2	535.6	0.055 ± 0.013	22.6	1.36	0.026

LC_{50}—lethal concentration that kills 50% of insects; X^2—chi-square value; SE standard error; *p*-value—probability.

Table 5
The chemical composition of *T. erecta* methanol extract.

No.	R.T.	Compound Name	Molecular Formula	Area (%)
1	6.1012	Tetrahydro-4H-pyran-4-ol	$C_5H_{10}O_2$	21.99
2	6.6189	4-Methoxycarbonyl-4-butanolide	$C_6H_8O_4$	4.05
3	6.7734	1H-Pyrazole, 4,5-dihydro-3-methyl-1-propyl-	$C_7H_{14}N_2$	8.95
4	7.0905	Hydrogen isocyanate	CHNO	26.47
5	7.1326	Glycine, ethyl ester	$C_4H_9NO_2$	15.83
6	7.2164	4-Benzyloxy-3-methoxy-2-nitrobenzoic acid	$C_{15}H_{13}NO_6$	0.48
7	8.8763	1,3-Dioxolane-4-methanol, 2-pentadecyl-, acetate,	$C_{21}H_{40}O_4$	5.69
8	8.9501	l-Norvaline, *N*-ethoxycarbonyl-, pentyl ester	$C_{13}H_{25}NO_4$	5.59
9	9.0027	Propanethioic acid, S-propyl ester	$C_6H_{12}OS$	1.21
10	9.1526	Benzene, 1,2-difluoro-	$C_6H_4F_2$	0.37
11	12.0871	Dioxoethylenebis (iminosulfur pentafluoride)	$C_2H_2F_{10}N_2O_2S_2$	0.08
12	12.5619	2-Thiobarbituric acid, tris(*tert*-butyldimethylsilyl) deriv	$C_{22}H_{46}N_2O_2SSi$	0.77
13	12.6616	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	0.97
14	12.8194	3-Trifluoromethylbenzylamine, N,*N*-diundecyl	$C_{30}H_{52}F_3N$	0.04
15	13.3310	Cholestan-3-one, dimethylhydrazone, (5alpha)-	$C_{29}H_{52}N_2$	0.01
16	13.5587	Ethylphosphonic acid	$C_2H_7O_3P$	0.63
17	15.4391	Heptyl methyl ethylphosphonate	$C_{10}H_{23}O_3P$	0.24
18	15.8932	Pentanedioic acid, (2,4-di-*t*-butylphenyl) mono-ester	$C_{19}H_{28}O_4$	0.15
19	16.0149	Furan, 2,5-dibutyl-	$C_{12}H_{20}O$	0.19
20	16.9014	Trioxide, bis(trifluoromethyl)	$C_2F_6O_3$	0.45
21	24.1026	Trimethylphosphine oxide	C_3H_9OP	0.01
22	24.1507	Syringylacetone	$C_{11}H_{14}O_4$	0.85
23	24.1527	(Phenylthio)acetic acid, propyl ester	$C_{11}H_{14}O_2S$	0.27
24	28.0613	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	0.68
25	29.0088	2-Butynenitrile, 4,4,4-trifluoro-	C_4F_3N	0.01
26	30.1970	D-Alanine, *N*-propargyloxycarbonyl-, dodecyl ester	$C_{19}H_{33}NO_4$	0.13
27	32.1660	5-(Pent-3-en-1-yn-1-yl)-2,2′-bithiophene	$C_{13}H_{10}S_2$	0.42
28	41.9315	[(2-Ethyl-5-methylfuran-3,4-	$C_{13}H_{26}O_3Si_2$	1.67
29	48.6880	2-(2′,4′-Dimethoxyphenyl)-6-methoxy-benzofuran	$C_{17}H_{16}O_4$	0.11
30	56.0952	alpha-Tocopherol-.beta.-D-mannoside	$C_{35}H_{60}O_7$	0.51
31	60.0878	beta-Amyrin	$C_{30}H_{50}O$	1.19

R.T., Retention time (min).

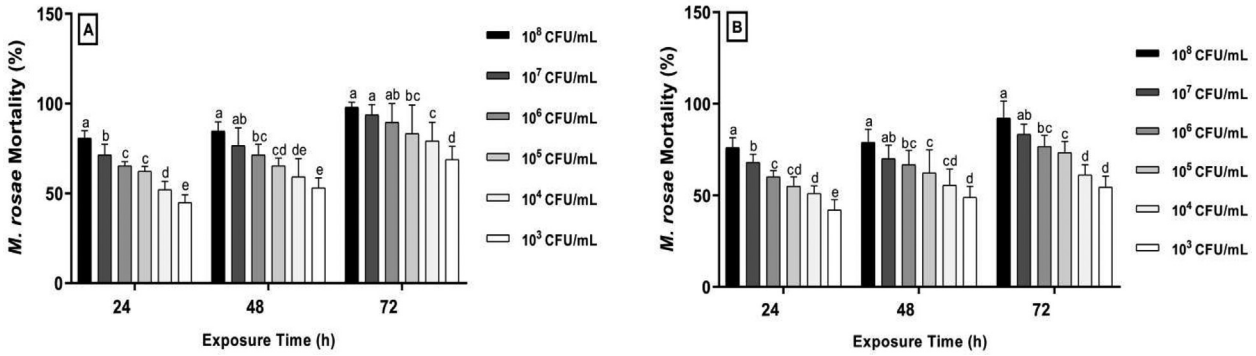

Fig. 1. Corrected mortality percentage (mean ± SE) of the rose aphid.

Table 6
Probit analysis of the EPB impacts on *M. rosae*.

Bioassay	Exposure time (h)	LC_{50} (CFU/mL)	95 % confidence limits		Slope ± SE	Intercept	X^2	*p*-Value
			Lower	Upper				
Contact	24	3.98×10^3	7.94×10^2	1.26×10^4	6.9 ± 0.43	25.1	1.50	0.001
	48	9.33×10^2	1.26×10^2	3.47×10^3	6.2 ± 0.17	34.4	1.80	0.001
	72	1.41×10^2	3.31×10^1	4.27×10^2	5.6 ± 0.48	54.7	2.45	0.001
Residual	24	1×10^4	2×10^3	3.16×10^4	6.5 ± 0.37	23.2	1.66	0.001
	48	2×10^3	2×10^2	7.94×10^3	5.6 ± 0.34	32.7	1.06	0.001
	72	7.94×10^2	2×10^2	2.51×10^3	7.4 ± 0.48	32.8	3.31	0.001

LC_{50}—lethal concentration that kills 50% of insects; X^2—chi-square value; SE standard error; *p*-value—probability.

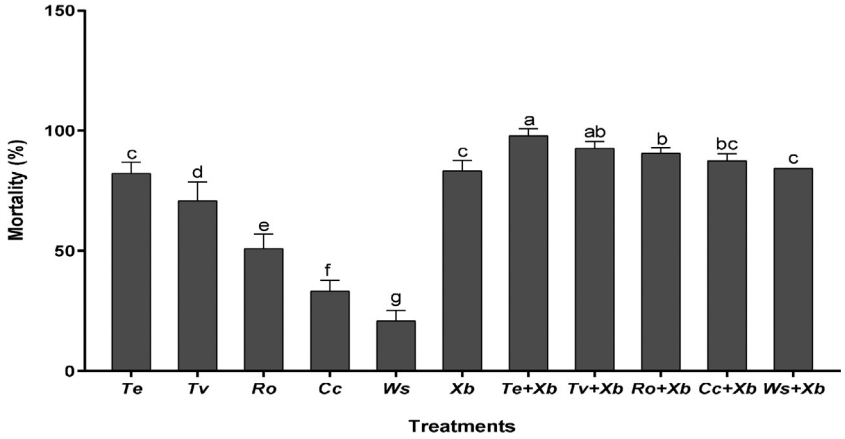

Fig. 2. Corrected mortality rates (% ± SE) of *M. rosae* as affected by five plant extracts and EPB alone and in combination after 72 h of application.

resistant aphid strains, natural products, particularly those derived from plants, have been developed as a potential alternative to chemical pesticides. Extracts of plant origin are often utilized for plant protection measures because of their efficiency against various life stages of insect pests (Chermenskaya et al., 2012). Botanical pesticides have the distinct benefit of being easily accessible, environmentally acceptable, and cost-effective, with minimum residual effects, and widespread public recognition (Yousuf et al., 2021). The present investigation established that extracts of five different medicinal plants were toxic to the rose aphid, *M. rosae*. On their immediate application, all treatment concentrations effectively killed rose aphids, although the three solvent extracts had different toxicity effects. The toxicity produced by studied plant extracts against *M. rosae* can be sorted in the following descending order based on LC_{50} values: *T. erecta* > *T. vulgaris* > *R. officinalis* > *C. colocynthis* > *W. somnifera*. The phytochemical analysis of *T. erecta* revealed the presence of 41 compounds including hexadecanoic acid; phenylthio acetic acid; alpha-tocopherol-beta-D-mannoside; Furan, 2,5-dibutyl; and 5-(Pent-3-en-1-yn-1-yl)-2,2′-bithiophene in addition to a variety of other metabolites were responsible for the toxicity of this plant. In confirming this result, several species of marigold (*Tagetes* spp.) have been found to contain phytochemicals with pesticidal potential. Numerous investigations reveald that *T. erecta* L. has been proven to have insecticidal efficacy against aphids (Ravikumar, 2010), mosquitoes (Sharma and Saxena, 1994), sand flies (Dinesh et al., 2014), termites (Elango et al., 2012), and several caterpillars (Aldana-Llanos et al., 2012). The main biocidal compounds of volatile oils extracted from *Tagetes* spp. aerial plant parts include monoterpenoids, carotenoids, and flavonoids (Ravikumar, 2010). Thiophenes, which are mostly found in roots and flowers, are biologically active against a wide range of insect species (Gil et al., 2002). Our results is in accordance with previous findings (Najafabadi et al., 2018), where *T. vulgaris* and *R. officinalis* essential oils had contact toxicity

toward the rose aphid and *T. vulgaris* essential oil had higher insecticidal activity than *R. officinalis* essential oil. The toxic effect of plant extracts is attributed to a variety of complex chemical components found in plants, including fatty acids, flavonoids, phenols, saponins, terpenoids, and alkaloids (Yaniv et al., 1999). These phyto components either prevent or disrupt insect feeding by making treated leaf surfaces unattractive or disagreeable to insects (Rajashekar et al., 2012; Talukder, 2006). These plant extracts may also modify insect feeding behavior or disturb hormonal balance, turning their food indigestible. In addition, several plant-derived essential oils, such as thyme, rosemary, lavandula, and ziziphora, have insecticidal characteristics that act against a broad range of soft-bodied arthropod pests (Alexenizer and Dorn, 2007). This is due to a variety of mechanisms of action, including antifeedant and repellent activities, molting and respiration inhibition, growth and fecundity suppression, cuticle disruption, and central nervous system activation on the octopamine pathway (Chaubey, 2007). The toxicity of the methanol plant extracts was higher than that of the acetone and ethanol counterparts, which is consistent with the findings of (Shehawy et al., 2019), who found that the methanolic extract of *C. colocynthis* caused the maximum mortality in *Aphis craccivora*, followed by ethyl acetate and petroleum ether extract. The stronger aphicidal action of methanol plant extracts could be attributed to the higher solubility of many volatile metabolites/secondary components of plants in this solvent, and thus the extracts' potency. This observation is also similar to the findings of (Maqsood et al., 2020), who found that methanol extracts exceeded ethanol and chloroform extracts in regards of cabbage aphid, *Brevicoryne brassicae* L. mortality. It was obvious from our investigation that as the concentration of plant extracts increased, the lethal activity increased as well. Thus, the most effective toxic concentration of plant extracts was 800 µg/mL, followed by 600 µg/mL, while the least efficient toxic concentration was 100 µg/mL. This is somewhat in agreement with Hori (1998) who evaluated essential oils of five plants (rosemary, thyme, peppermint, lavender and spearmint) against *Myzus persicae* and concluded that the rosemary oil at a dose of 10 µl and the thyme oil at a dose of 1 µl, caused 70 % mortality in the population of the aphid. In this study, the toxicity of the EPB, *X. budapestensis* via contact or residual assay increased with the concentration and post application periods, where the highest mortality was achieved with the higher concentration (10^8 CFU/mL) at 72 h post treatment. This finding is in line with previous investigation by our team (Alotaibi et al., 2021), who tested the three bacterial species on the carob moth, *Ectomyelois ceratoniae* and found that *X. budapestensis* had the highest significant virulence. Moreover, chemical composition of this bacterium revealed the presence of 21 compounds. Several secondary metabolites with effective bioactivities such as benzylideneacetone (antibacterial molecule), iodinine, phenethylamides, indole analogues, xenorhabdins, xenorxides, and xenocoumacins (antibiotics), as well as primary metabolites like alkaline protease have all been reported to be produced and secreted by *Xenorhabdus* sp. (Morgan et al., 2001; Caldas et al., 2002; Ji et al., 2004; Mohamed, 2007; Bode, 2009), therefore, all of which are optional to play roles as insecticidal and immunosuppressive compounds. Our results also showed that contact assay of EPB was more effective than residual one on rose aphid. This investigation was confirmed by (Iqbal et al., 2020) who recorded the high mortality rate of cotton aphids (*Aphis gossypii*) induced by crude cell extract, bacterial culture, and methanol extract of EPB, *Xenorhabdus* spp. and demonstrate that toxic metabolites can pass horizontally (most likely through direct contact) between infected and uninfected aphids. In the present study, EPB cell suspension required 3 days only to kill 97.9 % of *M. rosae*, whereas, Mahar et al. (Mahar et al., 2005), reported that *X. nematophila* cell suspension needed up to 6 days to kill 93 % *G. mellonella* larvae. The only disadvantage of EPBs from this genus is their slow action, however this can be overcome by combining them with other compounds in various methods. Recent approaches have shown that using two different components together can result in higher pest mortality than using them separately (Reddy and Chowdary, 2021). Synergism is the term used to describe how combining two products can enhance pest control effectiveness. The use of a plant extract with insecticidal property in combination with an entomopathogen is an unique way to reduce pollution risks while also minimizing the amount of pesticide used and the development of pest resistance (Srivastava et al., 2011). These botanical biopesticide combinations provide effective control, equivalent to synthetic insecticides. For the first time, we attempted to assess the compatible toxicity of selected plant extracts with EPB against *M. rosae*. Thus, the current findings revealed that combining each plant extract with EPB resulted in much higher rose aphid mortality than separate treatments after 72 h of application. It was also clear that the combination between *T. erecta* extract at low concentration and *X. budapestensis* caused a greater *M. rosae* adults mortality than separate treatments of both. This suggests that such plants may be able to sustain EPB's virulence, when they are combined together. Previous reports on the successful synergistic interactions between botanicals and EPB have verified our findings (Mhalla et al., 2018; Konecka et al., 2019; Noureldeen et al., 2019; Konecka et al., 2020). On the contrary, Halder et al. (Halder et al., 2017) claimed that *Bt* combined with neem oil had an incompatible interaction in managing *Epilachna deodecastigma* beetle.

5. Conclusions

Interestingly, our results indicate that the studied extracts, especially *T. erecta*, had strong negative effects on the rose aphid *M. rosae* through its contact and residual toxicity. However, all solvents extract caused significant mortality of *M. rosae*. In addition, we verified the aphicidal contact or residual activity of EPB *X. budapestensis* cells against *M. rosae* adults. Based on our findings, the combination of EPB with each plant extract resulted in a significantly higher rate of aphid mortality than either EPB or plant extracts alone. This indicated that all of the five tested plant extracts were compatible with EPB but with varying degrees. Moreover, these findings are promising for future research as they have the potential to be validated on a commercial scale and considered an important alternative to chemical pesticides to control aphids. Further studies are currently required on these plant extracts to assess their compatibility with other biocontrol agents on insect pests and associated beneficial insects in the field for IPM program.

6. Institutional Review Board Statement

Not applicable.

7. Informed Consent Statement

Not applicable.

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CRediT authorship contribution statement

Ahmed Noureldeen: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Uttam Kumar:** Writing – original draft. **Muhammad Asad:** Writing – review & editing. **Hadeer Darwish:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **Sarah Alharthi:** Data curation, Resources. **Mustafa A. Fawzy:** Formal analysis, Project administration. **Amal M. Al-Barty:** Formal analysis, Methodology. **Saqer S. Alotaibi:** Formal analysis, Resources. **Ahmed Fallatah:** Methodology. **Akram Alghamdi:** Investigation, Writing – review & editing. **Bander Albogami:** Resources, Validation. **Najla Alkashgry:** Investigation, Methodology, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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