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Lethal and sublethal effects of selected bacterial and neem-based novel insecticides on cotton aphid, *Aphis gossypii* and the predator, *Coccinella septempunctata*

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Research Paper

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Abstract

We evaluated the lethal and sublethal effects of two novel Betaproteobacteria-based insecticides (*Burkholderia* spp. strain A396 as Venerate® XC; *Chromobacterium subtsugae* strain PRAA4-1 as Grandevo® WDG) and two neem-based insecticides (1.2% azadirachtin A and B as Azatrol and 3% azadiractin as Molt-X) on the cotton aphid, *Aphis gossypii*, and its natural enemy, *Coccinella septempunctata*. Aphids were given both residual and direct treatments, i.e. exposed to residues applied by leaf dipping, or by spraying the insects and foliage, while the predator was treated directly with insecticides. Well-established spirotetramat (Movento® 240 SC) was used as standard due to its effectiveness against a wide range of pests, its unique mode of action, and its systemic properties. All insecticides were effective against aphid mostly in concentration-dependent manner, as do exposure time, but at different magnitudes. Spirotetramat and Azatrol induced the highest toxicity to adult aphids, while spirotetramat and Molt-X were more noxious to aphid nymphs. *C. subtsugae* and *Burkholderia* were less effective, inducing only moderate levels of aphid mortality. Azatrol and spirotetramat were more detrimental to the fecundity of aphid compared to other products. Insecticides significantly increased the development time of nymphs surviving exposure to insecticides, except *Burkholderia*. Azatrol were more destructive to eggs, larvae and adult of *Coccinella septempunctata*, together with spirotetramat for young larvae and adults, relative to other treatment. The development time of predator larvae remained unaffected by treatment. New Betaproteobacteria- and neem-based insecticides except Azatrol seem to be a promising tool to suppress population of *Aphis gossypii* and integrate pest management programmes.

Introduction

Aphis gossypii (Glover) (Hemiptera: Aphididae), commonly known as the cotton aphid, is a small, sap-sucking insect. It reproduces rapidly, with females capable of parthenogenesis, allowing populations to grow quickly under favourable conditions. Their life cycle includes several stages: egg, nymph, and adult, with nymphs maturing within a week under optimal temperatures (Khan *et al.*, 2023). It is a polyphagous insect attacking a variety of host plants in several plant families (Hu *et al.*, 2017; Mam *et al.*, 2019). This aphid impairs the host plant directly by removal of the sap from tender parts, causing weakness and wilting of the plant by heavy infestation (Rondon *et al.*, 2009; Ramalho *et al.*, 2012). It causes indirect damage by the transmission of several viral diseases and contamination of the plants with honeydew that stimulates the growth of sooty mould, ultimately affecting the photosynthesis, respiration, and transpiration of the plant, and rendering the fruits unsellable if not washed off before marketing (Satar *et al.*, 2005; Takalloozadeh, 2010).

Growers usually rely on indiscriminate use of synthetic pesticides to avoid such damage (Irshaid and Hasan, 2011), but this approach is associated with a number of potential health and ecological problems (Nicolopoulou-Stamati, *et al.*, 2016). Thus, the ongoing shift in society attitude and behaviour for improving human and environmental safety profiles has currently led to drastic changes in the development of new pesticides of botanical and microbe origins, which consider as sustainable pest control alternatives with prevailing use in integrated pest management and organic agricultural production systems (Chandler *et al.*, 2008; Gupta and Dikshit, 2010).

Coccinella septempunctata (Coleopter Coccinellidae) is a natural predator of aphids, playing a crucial role in controlling their populations. This beetle undergoes complete metamorphosis, consisting of four stages: egg, larva, pupa, and adult. The larval stage is particularly voracious, consuming large quantities of aphids. Ladybugs typically emerge in spring, mate, and lay eggs on or near aphid colonies, ensuring a food source for their larvae (Mizutani *et al.*, 2023).

Integrated pest management programmes emphasise the utilisation of the selective pesticides and biological control agents to maintain pest populations below the economic threshold

with inferior operational impact on agro-ecosystem. Predators in the Coccinellidae, particularly *Coccinella septempunctata*, has long been recognised as effective generalist predator of aphids and other soft-bodied arthropods in a wide range of agro-ecosystems (Yu *et al*., 2014; Azod *et al*., 2016; Arshad *et al*., 2017; Farooq *et al*., 2017; Niyog *et al*., 2023). However, this ladybird predator usually does not occur early in the season in most cropping systems where aphids build up a tremendous population in short time, therefore cannot keep the pest abundance below that causing economic injury level (Ahmed *et al*., 2016).

Several novel insecticides based on microbes, microbial products, or plant-derived products with unique mode of actions were registered during the late decades as exciting option for sustainable agriculture and integrated pest management (Sharma and Bhan, 2022; Chandler and Davidson, 2023; Khan *et al*., 2023). Such insecticides have decent efficacy, high selectivity, biodegradability, compatibility with natural enemies and low mammalian toxicity, making them attractive replacements for traditional synthetic insecticides in integrated pest management of many pests (Bagavan *et al*., 2009; Gupta and Dikshit, 2010; Hoshino, 2011; Asolkar *et al*., 2012; Lee *et al*., 2014; Chaudhary *et al*., 2017; Morehead and Kuhar, 2017; Ramasamy *et al*., 2020). The use of selective insecticides in combination with an effective natural enemy may provide more comprehensive prophylactic and remedial treatment than single approach.

The potential direct and indirect effects of biopesticides must be evaluated to determine whether or not they can be safely incorporated into integrated pest management programmes.

There is still deficient information on the prospects of the compatibility and synergistic effect of combination of natural enemies and some azadirachtin-based insecticides, as well as extracts recently developed from the microbes, *Chromobacterium subtsugae* and *Burkholderia* spp., against many plant pests in agro-ecosystem. Therefore, systematic study was carried out to evaluate the efficacy of these products towards the cotton aphid and their adverse effects on different development stages of ladybird beetle, *C. septempunctata* in order to achieve a long-term sustainable and environment-friendly pest management system for economic crops, particularly where pesticides input are undesirable or restricted. Outcomes of this investigation are worthwhile for synergistic use of biologically based insecticides together with the natural enemies to attain rapid and long-term sustainable control for the economic insect pests.

Materials and methods

Insect colonies and plant culture

A colony of *A. gossypii* was established from apterous individuals originally obtained from a field in Irbid district, Jordan. Aphids were reared on young okra plants grown in potting soil-filled plastic pots (15 cm dia.) in a growth room at 25 ± 3°C, 70 ± 5% relative humidity and 16:8 h (L: D) photoperiod. Aphids were kept in cages (100 × 60 × 60 cm) covered with fine mesh on all sides and above. A continuous supply of new greenhouse-grown plants was provided as needed for colony maintenance.

Okra seeds were pre-germinated for 2 days in plastic Petri dishes lined at the bottom with wet filter paper, transplanted then individually into potting soil-filled plastic pots (15 cm dia.), and maintained under greenhouse conditions at the same conditions described for rearing aphids. Plants were fertilised weekly with water-soluble fertiliser 20:10:20 (N:P:K) and irrigated as necessary.

A colony of ladybird beetle was established from *C. septempunctata* adults collected from aphid-infested okra plants in the field. The predator was maintained in the insectary room under the environmental conditions of 27 ± 3°C, about 70% relative humidity, and 1000 lux for a 10 h photoperiod regime to prevent diapause. Ladybird beetles were kept in plastic containers (18 cm × 13 cm × 8 cm) covered with transparent nylon sheets, and supplied with adequate amounts of cotton aphids on okra shoots. Small pieces of a black-coloured plastic sheet were added to each container for egg-laying. Sheets with laid eggs were transferred into a new container at 24 h intervals to avoid cannibalism by the parents. After egg eclosion, emerged larvae were kept in the same containers and provided with abundant aphids on plant shoots every 2–3 days. When the predator reached the fourth instar larvae, roof-like paper pieces were placed in rearing container as suitable sites for pupation and, thereafter, pupae were removed and kept in a plastic Petri dish (9 cm diam.) until reaching adulthood.

Insecticides

Commercially available microbe-based insecticides, Grandevo WDG (30% *Chromobacterium subtsugae* strain PRAA4-1 and spent fermentation media) and Venerate XC (94.46% heat-killed *Burkholderia* spp. strain A396 cells and spent fermentation media) were obtained from Marrone Bio Innovations Inc., Davis, California. Movento 240 SC (22.4% spirotetramat: *cis*-3-(2,5-dimethylpheny)-8-methoxy-2-oxo-1-azaspiro [4.5] dec-3-en-4-yl-ethyl carbonate) was obtained from Bayer Crop Science LP. Other two neem-based biopesticides; namely Azatrol (1.2% azadirachtin A and B) and Molt-X (3% azadirachtin) were obtained from Pubi/Gordon Corporation, Kansa City, Missouri, USA and BioWorks Inc. Victor, New York, USA, respectively.

The efficacy of these products applied directly to insects and residually via the host plant was evaluated on the second instar nymphs and adults of aphids and on predacious ladybird of various stages, eggs, second and fourth larval instars, and adults at different concentrations in the laboratory.

Contact and residual toxicity of insecticides on aphids

Leaf disc bioassays were used to assess the direct and residual effects of the insecticides on *A. gossypii* using plastic Petri dishes (5.5 cm dia.) with gauze-covered ventilation holes in lids. The acute contact toxicity on aphids was determined through direct insecticide application to okra leaf discs accommodating either the second nymph instars or adults. Prior to treatment, leaf discs were placed on a 4 mm layer of 1% agar poured in plates 1 day before testing, with the abaxial surface facing upward. Twenty same-aged aphids were transferred onto the surface of each leaf disc, and, thereafter, insecticides were applied directly to leaf discs at rates of 8.5, 17.0, 25.5, and 34 ml l^{-1} for Azatrol; 0.4, 0.8, 1.2, and 1.6 ml l^{-1} for Molt-X; 0.2, 0.4, 0.6, and 0.8 ml l^{-1} for Movento; 7.5, 15, 22.5, and 30.2 ml l^{-1} for Venerate; and 7.8, 15.6, 23.4, and 31.2 mg l^{-1} for Grandevo via plastic water spray bottles until saturation. The Petri dishes were inverted immediately to eliminate excess spray solution and kept in this position in the laboratory at 23 ± 3°C, 65 ± 5% relative humidity and 16:8 h (L: D) photoperiod.

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Similar procedures were used to determine the residual effects of these products towards aphids. In this experiment, leaf discs were first immersed in each dilution of test products for 10 s and then placed on paper towels with abaxial surface facing upwards to air dry. Subsequently, individual leaf discs were placed in a plastic Petri dish (5.5 cm dia.) lined in the bottom with a layer of agar, with abaxial surface upward. Twenty individuals of either the second nymph instars or adult of aphid were introduced to each Petri dish. Leaf discs treated with tap water served as control for both experiments. Each treatment was replicated 6 times.

Mortality of aphid was checked on daily basis (after 24 h) after treatment for 5 days. Nymphs survived the exposure to insecticide application were allowed to complete development until maturity, and then the total developmental time was recorded. The sub-lethal effects of insecticides on the fecundity were evaluated for adults that developed from the second instar nymphs surviving the toxicity of the insecticides through maintaining on untreated leaf discs on agar at the rate of 10 adults per dish for an additional 7 days. The progenies was counted and removed at 24 h intervals. Six replicates were used for each treatment.

Bioassay tests for the predator, C. septempunctata

Leaf disc bioassays were used to quantify the lethal effects of the insecticides on eggs, second and fourth larval instars and adults of *C. septempunctata*. The acute contact effects of insecticides on different development stages were determined by application of insecticides at the field recommended doses; 8.5 ml l^{-1} for Azatrol (1.2% azadirachtin A and B), 0.4 ml l^{-1} for Molt-X (3% azadirachtin), 7.8 g l^{-1} for Grandevo, 0.2 ml l^{-1} for Movento and 7.5 ml l^{-1} for Venerate, using the same procedure as described for aphid. After treatment, larvae and adults of predator were provided with abundant number of untreated aphids at 24 h intervals. Mortality of each stage was recorded after 24 h of interval after treatment each day for 7 days. The second instar larvae of ladybird that survived the direct exposure to the insecticide applied directly on plant foliage were left to complete their development to adult and the development time was recorded. Each treatment consisted of six individuals and replicated six times.

Statistical analysis

Mortality data were adjusted for control (check) mortality using Abbott's formula (Abbott, 1925). Data were evaluated with analysis of variance using SAS software version 9.2 (21) and mean values were compared by the least significance differences (LSD) test at $P \leq 0.0$ (SAS, 2000).

Results

Lethal effect of neem-based insecticides on aphids

All concentrations of Azarol (1.2% azadirachtin A and B) were toxic to the adult aphids when treated directly with an aqueous solution of the product (direct toxicity) (Table 1). Application of Azatrol at any concentration resulted in 78.6–89.3% mortality at 120 h after treatment. The mortality rate was not closely related to increasing concentration of azadirachtin, but exposure time was a significant factor. Similar trend, at about the same magnitude, was observed to the residual activity (up to 90.5% mortality).

As shown in Table 2, the direct activity of Azatrol was quite effective on nymphs, which led to 90% mortality when applied at the highest dose rate. The mortality obviously amplified with increasing concentration, as did duration of exposure time. Nymphs showed higher susceptibility to the residue of Azatrol than direct contact, in which the highest concentration suppressed completely nymph population at 96 h after treatment (Table 2). Both concentrations of insecticide and exposure time affected the mortality of *A. gossypii*.

Molt-X (3% azadirachtin) was less noxious to adults exposed to the direct contact than Azatrol (1.2% azadirachtin A and B), which induced 63.3% mortality at the highest concentration as a maximum (Table 3). In contrast, the residual activity of Molt-X on adults was inferior to that observed for direct effect (up to 33.3% mortality). The toxicity of residual and contact activities of Molt-X was significantly affected by insecticide concentration and exposure time.

The same trend, but at different magnitude, was recorded for Molt-X applied to young nymphs (Table 4). Exposure of nymphs to direct treatment resulted in a substantial reduction in the

Table 1. Effects of different concentrations of Azatrol at various time intervals post treatment on the per cent mortality of the adults of *Aphis gossypii* when the insecticide was applied either directly to adults on host foliage (direct effect) or to the plant foliage on which the insects later fed (residual effect)

	Direct activity				
Concentration	24 h	48 h	72 h	96 h	120 h
8.5 ml l^{-1}	11.7 ± 2.11 bA	25.0 ± 3.82 aA	56.7 ± 6.59 aB	66.7 ± 11.19 aBC	78.6 ± 8.81 aC
17 ml l^{-1}	15.4 ± 1.64 abA	25.1 ± 4.09 aA	57.5 ± 6.81 aB	74.1 ± 7.62 aC	85.1 ± 5.00 aC
25.5 ml l^{-1}	13.8 ± 2.02 abA	34.6 ± 7.78 aB	62.2 ± 8.37 aC	78.3 ± 6.71 aCD	89.3 ± 5.99 aD
34 ml l^{-1}	17.9 ± 2.27 a A	31.3 ± 4.46 aB	50.0 ± 4.55 aC	68.5 ± 5.68 aD	86.0 3.70 aE
	Residual activity				
Concentration	24 h	48 h	72 h	96 h	120 h
8.5 ml l^{-1}	16.7 ± 4.22 aA	37.5 ± 10.21 aA	68.8 ± 8.11 aB	75.0 ± 7.66 aB	88.1 ± 5.74 aB
17 ml l^{-1}	14.2 ± 3.21 abA	31.3 ± 7.66 aAB	47.9 ± 9.36 aBC	66.4 ± 10.41 aCD	86.0 ± 7.39 aD
25.5 ml l^{-1}	19.2 ± 4.17 aA	38.3 ± 8.94 aAB	56.3 ± 10.85 aBC	77.1 ± 10.42 aCD	90.5 ± 6.03 aD
34 ml l^{-1}	5.4 ± 2.45 bA	17.1 ± 3.19 aA	52.5 ± 4.54 aB	74.7 ± 9.80 aC	83.6 ± 6.92 aC

Mortality was corrected using Abbott's formula (Abbott, 1925).
Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ($P > 0.05$).

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Table 2. Effects of different concentrations of Azatrol at various time intervals post treatment on the per cent mortality of the second instar nymphs of *Aphis gossypii* when the insecticide was applied either directly to adults on host foliage (direct effect) or to the plant foliage on which the insects later fed (residual effect)

	Direct activity				
Concentration	24 h	48 h	72 h	96 h	120 h
8.5 ml l^{-1}	± 1.39 aA	25.4 ± 2.54 bB	42.2 ± 5.29 abC	53.4 ± 5.23 bC	71.1 ± 5.49 bD
17 ml l^{-1}	± 3.72 aA	20.0 ± 3.75 bA	41.1 ± 5.88 abB	47.8 ± 5.21 bB	51.1 ± ± 5.82 cB
25.5 ml l^{-1}	± 1.97 abA	23.3 ± 4.13 bAB	27.8 ± ± 2.54 bB	46.7 ± ± 5.51 bC	52.2 ± 6.00 cC
34 ml l^{-1}	± 3.18 bA	37.8 ± ± 4.19 aB	52.2 ± 6.53 aB	75.6 ± 6.13 aC	90.0 ± 4.80 acC
	Residual activity				
Concentration	24 h	48 h	72 h	96 h	120 h
8.5 ml l^{-1}	14.4 ± 3.18 abA	22.8 ± 3.38 aA	44.5 ± 4.85 cB	75.0 ± 2.86 bC	76.7 ± 2.44 bD
17 ml l^{-1}	18.9 ± 2.75 aA	46.7 ± 4.56 bB	88.9 ± 3.72 aC	96.7 ± 2.27 aC	97.8 ± 2.22 aC
25.5 ml l^{-1}	7.8 ± 2.67 bA	28.9 ± 2.05 cB	64.4 ± 5.94 bC	88.9 ± 7.02 aD	96.7 ± 2.27 aD
34 ml l^{-1}	22.2 ± 2.06 bA	77.8 ± 6.37 aB	52.5 ± 1.12 cC	100 ± 0.00 aC	100 ± 0.00 aC

Mortality was corrected using Abbott's formula (Abbott, 1925).
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survival of nymphs by up to 53.7% in a concentration-dependent manner. Survival of nymphs decreased also showed the negative effect of the duration of exposure time. Young nymphs responded moderately to the residual effect of Molt-X compared to direct activity where the reduction in nymph abundance did not go beyond 47.8% at any concentration and interval tested (Table 4). Thus both dose and the length of exposure were also significant factors for residual effect.

Lethal effect of novel Betaproteobacteria-based insecticides and spirotetramat (as standard) on aphids

Results in Table 5 indicated that Grandevo, a newly developed microbe-based *C. subtsugae*, was less effective to control the adult of *A. gossypii* population more than the other insecticides. *C. subtsugae* applied directly to adults at the highest concentration (31.2 mg l^{-1}) killed 45.8% of aphids at 120 h after treatment. Adult aphids responded to the residue of *C. subtsugae* similarly to that observed for direct spray, triggering a reduction in the adult survival between 17.7 and 42.7% (Table 5). There was a significant increase in the mortality of adult aphids as a function of concentration and exposure time for direct and residual activities of this product.

The direct and residual effects of *C. subtsugae* on young nymphs was relatively equal (up to 32.1% mortality), depending on concentration, but nymphs were in general less vulnerable to *C. subtsugae* than adult aphids. Likewise, exposure time was a significant factor in nymph survival (Table 6).

Other novel Betaproteobacteria-based insecticides, *Burkholderia*, exhibited quite moderate efficacy against adult aphids when applied directly to insects (Table 7). Significant levels of mortality at up to 57.3% were recorded, depended on the concentrations. The length of exposure time also was a significant factor affecting mortality. The residual activity of *Burkholderia* on adults was slightly more prominent than the

Table 3. Effects of different concentrations of Molt-X at various time intervals post treatment on the per cent mortality of the adults of *Aphis gossypii* when the insecticide was applied either directly to adults on host foliage (direct effect) or to the plant foliage on which the insects later fed (residual effect)

	Direct activity				
Concentration	24 h	48 h	72 h	96 h	120 h
0.4 ml l^{-1}	0.0 ± 0.00 aA	2.4 ± 2.38 nA	3.3 ± 1.05 cA	14.2 ± 2.71 cB	36.7 ± 4.22 cC
0.8 ml l^{-1}	0.0 ± 0.00 aA	0.0 ± 0.00 bA	16.7 ± 4.77 cbB	29.2 ± 4.90 cbB	51.7 ± 7.03 bcC
1.2 ml l^{-1}	0.0 ± 0.00 aA	0.0 ± 0.00 bA	30.0 ± 8.16 bcB	45.0 ± 8.47 bB	46.7 ± 9.89 abcB
1.6 ml l^{-1}	0.0 ± 0.00 aA	7.2 ± 1.19 aA	36.7 ± 6.15 aB	63.3 ± 5.58 aC	63.3 ± 5.58 aC
	Residual activity				
Concentration	24 h	48 h	72 h	96 h	120 h
0.4 ml l^{-1}	0.0 ± 0.00 aA	10.2 ± 0.23 aB	16.7 ± 1.67 aC	23.3 ± 3.33 aD	25.0 ± 2.24 aD
0.8 ml l^{-1}	0.0 ± 0.00 aA	0.0 ± 0.00 cA	6.7 ± 2.11 bA	20.0 ± 3.65 abB	25.0 ± 4.28 aB
1.2 ml l^{-1}	0.0 ± 0.00 aA	0.0 ± 0.00 cA	6.7 ± 2.11 bA	20.0 ± 2.24 abB	33.3 ± 4.77 aC
1.6 ml l^{-1}	0.0 ± 0.00 aA	6.7 ± 2.11 bB	11.4 ± 1.43 abBC	13.3 ± 2.11 bC	31.3 ± 3.54 aD

Mortality was corrected using Abbott's formula (Abbott, 1925).
Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ($P > 0.05$).

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Table 4. Effects of different concentrations of Molt-X at various time intervals post treatment on the per cent mortality of the second instar nymphs of *Aphis gossypii* when the insecticide was applied either directly to adults on host foliage (direct effect) or to the plant foliage on which the insects later fed (residual effect)

	Direct activity				
Concentration	24 h	48 h	72 h	96 h	120 h
0.4 ml l^{-1}	0.0 ± 0.00 aA	1.4 ± 1.40 bAB	1.5 ± 1.50 cAB	6.4 ± 0.84 cC	4.6 ± 0.98 cBC
0.8 ml l^{-1}	0.0 ± 0.00 aA	4.2 ± 1.88 bA	16.6 ± 1.44 bB	21.2 ± 2.01 bB	30.0 ± 3.65 bC
1.2 ml l^{-1}	0.0 ± 0.00 aA	12.2 ± 1.90 aA	28.5 ± 4.74 aB	47.9 ± 6.79 dC	53.7 ± 5.26 aC
1.6 ml l^{-1}	0.0 ± 0.00 aA	3.6 ± 1.69 bA	27.6 ± 3.36 aB	34.6 ± 4.63 aB	51.3 ± 2.67 aC
	Residual activity				
Concentration	24 h	48 h	72 h	96 h	120 h
0.4 ml l^{-1}	2.4 ± 1.08 bA	4.2 ± 2.39 cA	6.1 ± 2.86 cA	6.1 ± 2.24 cA	15.0 ± 3.42 cB
0.8 ml l^{-1}	7.8 ± 1.01 abA	10.9 ± 2.26 bcAB	18.4 ± 4.02 bcBC	23.2 ± 2.95 bCD	28.7 ± 2.63 bD
1.2 ml l^{-1}	6.0 ± 3.89 bA	11.8 ± 3.46 bA	35.7 ± 5.29 aB	36.7 ± 5.58 aB	42.1 ± 5.32 aB
1.6 ml l^{-1}	14.3 ± 3.18 aA	20.3 ± 1.17 aA	31.1 ± 4.86 abB	41.0 ± 4.93 aBC	47.8 ± 2.32 aC

Mortality was corrected using Abbott's formula (Abbott, 1925).
Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ($P > 0.05$).

direct effect (up to 75.9% mortality) in a dose-dependent manner, and also aphid survival substantially diminished with increasing exposure time (Table 7).

The direct contact and residue of *Burkholderia* were less lethal to young instar nymphs compared to adults, which triggered up to 40.5 and 51% mortality for direct and residual effects, respectively. The toxicity varied considerably with the concentration, but did not show an increase with prolonged exposure time (Table 8).

All concentrations of spirotetramat (Movento) showed higher toxicity to the adult aphids compared to other insecticide treatments (Table 9). Direct spray of spirotetramat to the adults led to up to 97.2% mortality at 120 h after treatment in dose-dependent manner. Exposure time also was a significant variable. Though, the residual efficacy of spirotetramat was quite less effective to the adults than direct exposure, reducing the aphid population by up to 78.5% in dose-dependent manner. The length of exposure time also was a significant factor in aphid survival (Table 9).

It is evident that exposure of young nymphs to direct contact with Movento resulted in substantial decrease in the nymph survival by up to 97%, but not in a concentration-dependent manner, which showed more lethality to nymphs than adult aphids (Table 10). Aphid nymphs responded to the residues of spirotetramat at a greater magnitude to that observed for direct exposure (Table 10). Reduction in the survival rate of nymphs was significantly related to increasing concentration and exposure time. Application of spirotetramat at any concentration resulted in complete suppression of the aphid population at 120 h after exposure. Because all concentrations resulted in about the same levels of mortality, the dominant variable in the analysis was time (Table 10).

Table 5. Effects of different concentrations of *Chromobacterium subtsugae* (Grandevo®) at various time intervals post treatment on the per cent mortality of the adults of *Aphis gossypii* when the insecticide was applied either directly to adults on host foliage (direct effect) or to the plant foliage on which the insects later fed (residual effect)

	Direct activity				
Concentration	24 h	48 h	72 h	96 h	120 h
7.8 mg l^{-1}	4.4 ± 0.88 bA	5.2 ± 1.87 bA	7.0 ± 1.79 bA	7.4 ± 1.94 cA	8.9 ± 2.74 cA
15.6 mg l^{-1}	4.3 ± 2.02 bA	6.2 ± 1.61 bAB	8.6 ± 1.06 bABC	10.2 ± 1.74 cBC	11.1 ± 1.49 bC
23.4 mg l^{-1}	6.1 ± 0.79 abA	11.6 ± 0.99 aB	15.0 ± 0.92 aC	21.0 ± 2.03 bC	29.2 ± 2.75 aD
31.2 mg l^{-1}	9.4 ± 1.51 aA	11.6 ± 1.55 aAB	18.4 ± 2.47 aB	30.1 ± 2.59 aC	45.8 ± 3.85 aD
	Residual activity				
Concentration	24 h	48 h	72 h	96 h	120 h
7.8 mg l^{-1}	0.0 ± 0.00 cA	1.0 ± 0.98 dB	5.3 ± 0.58 cB	12.2 ± 1.78 cC	17.7 ± 2.73 bD
15.6 mg l^{-1}	4.6 ± 1.60 bcA	6.1 ± 2.07 cAB	10.0 ± 0.91 bBC	12.9 ± 1.72 cC	18.2 ± 2.07 bD
23.4 mg l^{-1}	5.9 ± 2.74 bcA	12.3 ± 1.62 bB	22.3 ± 1.65 aC	25.4 ± 1.63 bC	40.0 ± 2.33 aD
31.2 mg l^{-1}	10.4 ± 1.83 aA	17.2 ± 1.66 aB	23.6 ± 1.41 aC	33.9 ± 2.78 aD	42.7 ± 2.17 aE

Mortality was corrected using Abbott's formula (Abbott, 1925).
Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ($P > 0.05$).

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Table 6. Effects of different concentrations of *Chromobacterium subtsugae* (Grandevo®) at various time intervals post treatment on the per cent mortality of the second instar nymphs of *Aphis gossypii* when the insecticide was applied either directly to adults on host foliage (direct effect) or to the plant foliage on which the insects later fed (residual effect)

	Direct activity				
Concentration	24 h	48 h	72 h	96 h	120 h
7.8 mg l^{-1}	0.0 ± 0.00 bA	1.0 ± 0.98 cA	0.9 ± 0.93 cA	6.4 ± 0.77 cB	10.7 ± 1.31 bC
15.6 mg l^{-1}	2.6 ± 1.74 bA	2.9 ± 2.00 cbA	3.0 ± 2.02 cA	5.7 ± 1.41 cA	7.1 ± 3.17 bA
23.4 mg l^{-1}	5.6 ± 2.03 bA	8.1 ± 2.99 bAB	16.0 ± 1.49 bBC	16.1 ± 1.82 bBC	23.9 ± 6.40 aC
31.2 mg l^{-1}	14.4 ± 3.03 aA	20.5 ± 2.13 aAB	23.3 ± 1.82 aB	26.5 ± 2.50 aCD	31.9 ± 2.56 aD
	Residual activity				
Concentration	24 h	48 h	72 h	96 h	120 h
7.8 mg l^{-1}	0.0 ± 0.00 cA	1.8 ± 1.15 cA	5.3 ± 0.16 cB	8.5 ± 1.08 cC	11.4 ± 0.37 cD
15.6 mg l^{-1}	5.5 ± 0.17 bA	8.6 ± 1.07 bB	11.9 ± 0.64 bC	17.6 ± 1.39 abD	20.4 ± 1.39 bD
23.4 mg l^{-1}	7.5 ± 1.27 bA	10.3 ± 1.68 abAB	13.1 ± 2.41 abAB	15.6 ± 2.53 bB	21.8 ± 1.33 bC
31.2 mg l^{-1}	11.0 ± 1.86 aA	13.7 ± 1.34 aA	17.0 ± 1.33 aAB	23.1 ± 2.08 aB	32.1 ± 3.44 aC

Mortality was corrected using Abbott's formula (Abbott, 1925).
Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ($P > 0.05$).

Sublethal effects of reduced-risk insecticides on aphid

The sublethal effects of biopesticides applied on the development time of the second instars that survived exposure to direct and residual insecticide treatment are illustrated in Table 11. It is apparent that exposure to direct contact with Azarol spray considerably increased the development time of nymphs by 1.5–2.8 days over the control to reach maturity in a concentration-dependent manner, but the residue of this product had no significant impact on the development relative to control (Table 11). Molt-X and spirotetramat (Movento) showed a comparable effect where exposure of nymphs to direct contact or residues of both insecticides significantly prolonged the development time by 2.8–3.2 days over the control, depending on concentration. The same trend, but at different levels, was recorded for the direct and residual activity of *C. subtsugae* (Grandevo) on the development time, which was prolonged by 1–1.8 days over the control in a dose-dependent manner. In contrast, *Burkholderia* had no impact on the development time at any concentration tested and methods of treatment compared to the control (Table 11).

Reproduction of adult aphids that developed from treated young nymphs was substantially reduced by all treatments relative to the control, in a concentration-dependent manner, but provided different levels of suppression (Table 12). Azatrol and Movento were most destructive, decreasing the progeny by up to three individuals per adult for the direct activity and 4.4 offspring per adult for residual activity when applied at the highest concentration in comparison with 17.2 progenies for control. Grandevo ranked second, decreasing significantly the fecundity by about 42% compared to the control (Table 13). Exposure of aphids to direct Molt-X treatment did not induce a significant

Table 7. Effects of different concentrations of *Burkholderia* (Venerate®) at various time intervals post treatment on the per cent mortality of the adults of *Aphis gossypii* when the insecticide was applied either directly to adults on host foliage (direct effect) or to the plant foliage on which the insects later fed (residual effect)

	Direct activity				
Concentration	24 h	48 h	72 h	96 h	120 h
7.5 ml l^{-1}	0.0 ± 0.00 bA	8.8 ± 0.97 bB	19.6 ± 1.10 bC	28.7 ± 1.61 bD	40.1 ± 2.12 bE
15 ml l^{-1}	0.0 ± 0.00 bA	8.8 ± 1.12 bB	33.4 ± 2.97 aC	43.2 ± 4.41 aD	57.3 ± 3.78 aE
22.5 ml l^{-1}	0.0 ± 0.00 bA	6.3 ± 1.45 bB	19.3 ± 1.44 bC	36.6 ± 3.28 abD	54.8 ± 5.37 bD
30.2 ml l^{-1}	12.2 ± 0.70 aA	27.0 ± 1.97 aB	37.2 ± 5.18 aBC	40.4 ± 7.19 abBC	49.1 ± 4.77 abC
	Residual activity				
Concentration	24 h	48 h	72 h	96 h	120 h
7.5 ml l^{-1}	1.1 ± 1.12 bA	5.2 ± 1.78 cA	28.4 ± 2.37 aB	39.8 ± 3.24 bC	52.6 ± 3.75 bD
15 ml l^{-1}	13.3 ± 1.72 aA	20.6 ± 2.90 bA	39.9 ± 4.21 aB	46.9 ± 3.97 bBC	53.6 ± 5.93 bC
22.5 ml l^{-1}	8.9 ± 2.00 abA	30.4 ± 4.00 aB	41.2 ± 8.46 aBC	52.0 ± 7.88 abC	57.7 ± 5.03 bC
30.2 ml l^{-1}	6.7 ± 1.36 abA	19.7 ± 1.24 bB	42.5 ± 3.95 aC	65.9 ± 5.64 aD	75.9 ± 3.49 aD

Mortality was corrected using Abbott's formula (Abbott, 1925).
Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ($P > 0.05$).

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Table 8. Effects of different concentrations of *Burkholderia* (Venerate®) at various time intervals post treatment on the per cent mortality of the second instar nymphs of *Aphis gossypii* when the insecticide was applied either directly to adults on host foliage (direct effect) or to the plant foliage on which the insects later fed (residual effect)

	Direct activity				
Concentration	24 h	48 h	72 h	96 h	120 h
7.5 ml l^{-1}	4.5 ± 1.47 bA	8.9 ± 1.46 bA	15.1 ± 1.25 bB	16.5 ± 1.59 bB	22.2 ± 2.65 cC
15 ml l^{-1}	4.5 ± 1.47 bA	11.1 ± 2.00 bAB	15.6 ± 2.39 bB	23.3 ± 3.34 bC	27.8 ± 3.18 bcC
22.5 ml l^{-1}	8.9 ± 1.39 aA	15.5 ± 2.23 bB	24.4 ± 3.18 aC	34.4 ± 2.05 aD	34.4 ± 2.05 abD
30.2 ml l^{-1}	7.8 ± 1.33 abA	24.4 ± 3.29 aB	30.9 ± 3.65 aBC	30.9 ± 2.11 aBC	40.5 ± 6.33 aC
	Residual activity				
Concentration	24 h	48 h	72 h	96 h	120 h
7.5 ml l^{-1}	21.1 ± 2.69 aA	28.9 ± 2.05 aA	38.8 ± 1.97 aB	40.9 ± 1.93 aB	51.0 ± 4.22 aC
15 ml l^{-1}	14.4 ± 0.72 aA	20.0 ± 3.33 abAB	28.9 ± 3.17 bBC	32.1 ± 4.19 aC	35.4 ± 3.65 bC
22.5 ml l^{-1}	8.9 ± 1.46 bA	17.8 ± 3.18 bB	20.0 ± 3.11 cB	23.2 ± 3.58 bBC	31.0 ± 2.63 bC
30.2 ml l^{-1}	15.6 ± 2.68 abA	20.6 ± 3.03 bA	37.7 ± 1.14 aB	37.7 ± 1.14 aB	37.7 ± 2.74 bB

Mortality was corrected using Abbott's formula (Abbott, 1925).
Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ($P > 0.05$).

change in the reproduction, but a significant decrease in the reproductive potential (42% inferior to the control) was observed for adults emerged from young nymphs that survived the residual toxicity (Table 12). Direct spray of Venerate led to a significant decrease in the reproduction by about 42% below the control, but not in dose-dependent manner. Though, aphids exposed to the residue of this product generated 24–45% less offspring compared to control in concentration-dependent manner (Table 12).

Lethal and sublethal effects of biopesticides on the ladybird, Coccinella septempunctata

Efficacies of naturally occurring insecticides on the different development stages of the predator are illustrated in Table 13. Outcomes of this study indicated that pesticides negatively impaired the survival of predator, depending on chemical and insect stage. Reduction in egg eclosion significantly varied between treatments as a result of direct treatment, ranging between 3.3 and 33%. Azatrol elicited the greatest toxicity to eggs compared to other treatments.

Likewise, response of young larvae of the ladybird to insecticides varied widely between treatments with a maximum mortality was recorded for spiromtetrmat (49.9%) and Azatrol (41.7%), followed by *Burkholderia* (33.3%) and Molt-X (25%), respectively. However, *C. subtsugae* did not display any adverse effect in the young larvae (Table 13).

Fourth instar larvae of *C. septempunctata* responded to insecticides in a different way to that noted to the young larvae. Azatrol revealed the highest effectiveness (33.3% mortality), whereas other treatments did not disturb the survival of old larvae, except spiromtetrmat (11.1% mortality).

Treatment with spiromtetrmat and Azatro condensed the adult survival by 66.7 and 58.3%, respectively, whereas other

Table 9. Effects of different concentrations of spirotetramat (Movento®) at various time intervals post treatment on the percent mortality of the adults of *Aphis gossypii* when the insecticide was applied either directly to adults on host foliage (direct effect) or to the plant foliage on which the insects later fed (residual effect)

	Direct activity				
Concentration	24 h	48 h	72 h	96 h	120 h
0.2 ml l^{-1}	4.7 ± 1.62 aA	12.1 ± 1.54 aAB	19.3 ± 2.28 aB	46.1 ± 4.85 bC	79.9 ± 3.86 bD
0.4 ml l^{-1}	2.1 ± 2.08 aA	2.4 ± 2.38 bA	13.9 ± 3.27 aB	27.8 ± 3.17 cC	76.7 ± 4.94 bD
0.6 ml l^{-1}	2.1 ± 2.08 aA	7.2 ± 3.13 abA	25.0 ± 4.21 abB	63.9 ± 1.81 aC	82.2 ± 4.68 bD
0.8 ml l^{-1}	5.2 ± 0.17 aA	12.9 ± 2.13 aA	33.9 ± 4.93 abB	66.1 ± 7.43 aC	97.2 ± 2.78 aD
	Residual activity				
Concentration	24 h	48 h	72 h	96 h	120 h
0.2 ml l^{-1}	7.5 ± 1.71 bA	19.3 ± 1.75 cB	31.9 ± 2.36 cC	51.8 ± 3.97 bD	57.6 ± 4.28 cD
0.4 ml l^{-1}	18.3 ± 3.57 aA	44.3 ± 1.72 bB	47.4 ± 2.90 bB	60.9 ± 2.24 bC	67.5 ± 3.94 bcC
0.6 ml l^{-1}	14.2 ± 0.83 abA	42.8 ± 1.95 bB	51.8 ± 1.68 bC	70.7 ± 3.27 aD	78.5 ± 3.67 abE
0.8 ml l^{-1}	17.5 ± 2.50 aA	63.5 ± 3.95 aB	68.8 ± 1.95 aBC	74.3 ± 2.77 aC	77.1 ± 2.96 aC

Mortality was corrected using Abbott's formula (Abbott, 1925).
Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ($P > 0.05$).

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Table 10. Effects of different concentrations of spirotetramat (Movento®) at various time intervals post treatment on the per cent mortality of the second instar nymphs of *Aphis gossypii* when the insecticide was applied either directly to adults on host foliage (direct effect) or to the plant foliage on which the insects later fed (residual effect)

	Direct activity				
Concentration	24 h	48 h	72 h	96 h	120 h
0.2 ml l^{-1}	8.9 ± 1.46 cA	32.1 ± 1.86 aB	62.9 ± 2.06 aC	79.5 ± 3.60 aD	90.9 ± 3.80 abE
0.4 ml l^{-1}	14.4 ± 1.41 bcA	34.7 ± 3.00 aB	47.5 ± 3.56 cC	87.2 ± 2.22 bD	97.0 ± 3.05 aE
0.6 ml l^{-1}	18.9 ± 2.69 abA	35.8 ± 5.21 aB	50.1 ± 2.79 bcC	78.2 ± 1.52 aD	93.9 ± 2.96 abE
0.8 ml l^{-1}	21.1 ± 2.68 aA	32.1 ± 2.73 aB	57.7 ± 3.08 abC	82.1 ± 1.64 abD	87.7 ± 1.90 bD
	Residual activity				
Concentration	24 h	48 h	72 h	96 h	120 h
0.2 ml l^{-1}	10.7 ± 1.61 bA	50.0 ± 8.34 aB	84.7 ± 3.77 bC	96.9 ± 1.96 aCD	100.0 ± 0.00 aD
0.4 ml l^{-1}	17.8 ± 3.56 abA	50.0 ± 5.71 aB	93.9 ± 3.06 aC	98.5 ± 1.55 aC	100.0 ± 0.00 aC
0.6 ml l^{-1}	28.6 ± 6.12 aA	63.9 ± 7.96 aB	98.5 ± 1.55 aC	98.5 ± 1.55 aC	100.0 ± 0.00 aC
0.8 ml l^{-1}	20.2 ± 3.00 abA	51.6 ± 5.17 aB	96.9 ± 1.96 aC	96.9 ± 1.96 aC	100.0 ± 0.00 aC

Mortality was corrected using Abbott's formula (Abbott, 1925).
Means ± SE within columns followed by the same lower-case letter, or within rows followed by the same upper-case letter, are not significantly different ($P > 0.05$).

treatments were not detrimental for adults (Table 13). Nevertheless, all insecticides tested did not show any sublethal effects on the development time of the second instar larvae that either survived the direct application or exposed to the residues of insecticide on the host plant relative to control (Table 14).

Table 11. Development time of the second instar nymphs of *Aphis gossypii* surviving the exposure to different concentrations of insecticides

		Development time	
Treatment	Concentration	Direct spray	Residual effect
Azarol	0 ml l^{-1}	4.0 ± 0.00 c	4.0 ± 0.00 a
	8.5 ml l^{-1}	5.5 ± 0.29 b	4.2 ± 0.17 a
	17 ml l^{-1}	5.8 ± 0.17 b	4.2 ± 0.17 a
	25.5 ml l^{-1}	5.8 ± 0.17 b	4.8 ± 0.60 a
	34 ml l^{-1}	6.8 ± 0.17 a	-
Molt-X	0 ml l^{-1}	4.0 ± 0.00 b	4.0 ± 0.00 b
	0.4 ml l^{-1}	6.8 ± 0.17 a	6.8 ± 0.17 a
	0.8 ml l^{-1}	7.2 ± 0.17 a	7.3 ± 0.17 a
Chromobacterium subtsugae	0 gm l^{-1}	4.0 ± 0.00 c	4.0 ± 0.00 c
(Grandevo®)	7.8 gm l^{-1}	5.0 ± 0.00 b	5.0 ± 0.00 b
	15.6 gm l^{-1}	5.2 ± 0.17 b	5.2 ± 0.17 b
	23.4gm l^{-1}	5.8 ± 0.17 a	5.8 ± 0.17 a
	31.2 gm l^{-1}	5.8 ± 0.17 a	-
Spirotetramat (Movento®)	0 ml l^{-1}	4.0 ± 0.00 c	4.0 ± 0.00 c
	0.2 ml l^{-1}	6.2 ± 0.17 b	6.2 ± 0.17 b
	0.4 ml l^{-1}	7.2 ± 0.17 a	7.2 ± 0.17 a
Burkholderia (Venerate®)	0 ml l^{-1}	4.0 ± 0.00 a	4.0 ± 0.00 a
	7.5 ml l^{-1}	3.3 ± 0.33 a	3.3 ± 0.33 a
	15 ml l^{-1}	4.2 ± 0.17 a	4.2 ± 0.17 a
	22.5 ml l^{-1}	4.2 ± 0.17 a	4.2 ± 0.17 a
	30.2 ml l^{-1}	4.2 ± 0.17 a	4.2 ± 0.17 a

Means ± SE within columns followed by the same are not significantly different ($P > 0.05$).

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Table 12. Reproductive rate of adult aphids developing from the second instar nymphs of *Aphis gossypii* that survived residual effects of different concentrations of insecticides

		Reproductive rate per aphid	
Treatment	Concentration	Direct spray	Residual effect
Azarol	0 ml l^{-1}	17.2 ± 0.50[a]	17.2 ± 0.50[a]
	8.5 ml l^{-1}	8.8 ± 0.32[b]	6.7 ± 0.43[b]
	17 ml l^{-1}	6.2 ± 0.96[c]	6.9 ± 0.62[b]
	25.5 ml l^{-1}	3.9 ± 0.34[d]	4.7 ± 0.38[c]
Molt-X	0 ml l^{-1}	20.1 ± 2.55 a	17.2 ± 0.50[a]
	0.4 ml l^{-1}	18.8 ± 2.41 a	10.8 ± 1.96[b]
	0.8 ml l^{-1}	17.2 ± 0.49 a	7.4 ± 1.99[b]
Chromobacterium subtsugae	0 gm l^{-1}	17.2 ± 0.50[a]	16.8 ± 0.44[a]
(Grandevo®)	7.8 gm l^{-1}	11.5 ± 1.22[b]	9.7 ± 0.63[b]
	15.6 gm l^{-1}	9.9 ± 0.48[b]	15.3 ± 1.19[a]
Spirotetramat (Movento®)	0 ml l^{-1}	17.2 ± 0.50[a]	17.2 ± 0.50[a]
	0.2 ml l^{-1}	6.2 ± 0.35[b]	3.5 ± 0.56[b]
	0.4 ml l^{-1}	3.0 ± 0.25[c]	4.4 ± 0.38[b]
Burkholderia (Venerate®)	0 ml l^{-1}	17.2 ± 0.50[a]	17.2 ± 0.50[a]
	7.5 ml l^{-1}	10.6 ± 0.65[b]	13.0 ± 0.47[b]
	15 ml l^{-1}	9.8 ± 0.52[b]	12.8 ± 1.79[b]
	22.5 ml l^{-1}	10.1 ± 0.45[b]	9.5 ± 0.47[c]
	30.2 ml l^{-1}	11.0 ± 0.38[b]	11.8 ± 0.92[bc]

Means ± SE within columns followed by the same letter are not significantly different ($P > 0.05$).

Discussion

There is great potential benefits of the naturally occurring biological controls by the use of pesticides to natural enemies, which come in contact with pesticides via direct exposure to the chemical, contact with the pesticide residue, or through the food chain. In addition, insecticides of biological origin have adverse effects against phytophagous insects including aphids (Kuhar and Doughty, 2016; Fenibo *et al.*, 2021; Parajuli *et al.*, 2022) and they may represent an additional management tactic to combat many pests and mitigate the development of insecticide resistance due to multiple modes of action (Chandler *et al.*, 2011; Marrone, 2019).

In this study, the lethal and sublethal effects of commercially available formulations based on botanical and microbial origins were evaluated against the cotton aphid, *Aphis gossipy*, and predator ladybird, *C. septempunctata*, using laboratory bioassay tests. Results indicated that all insecticides tested showed toxicity to both development stages of *A. gossipy*, but at different magnitudes. In most cases, the efficacy was a function of concentration and length of exposure time.

Exposure of adults to the direct contact and residues of Azatrol resulted in high toxicity to adult *A. gossypii* (up to 90.5%), whose effect increased with exposure time, but not in a concentration-dependent manner. However, Azatrol was less effective to nymphs than adults at all concentrations tested, except at the highest dose (34 ml l^{-1}). Residual toxicity of Azatrol was more prominent to nymphs than the direct contact, eradicating completely the nymph abundance when applied at

Table 13. Lethal effect of different insecticides applied at the lowest concentration on the mortality of different developmental stages of ladybird, *Coccinella septempunctata*

Insecticide	Concentration	Egg	2nd instar larva	4th instar larva	Adult
Azatrol	8.5 ml l^{-1}	33.0 ± 10.76 a	41.7 ± 5.27 a	33.3 ± 8.54 a	58.3 ± 8.33 a
Molt-X	0.4 ml l^{-1}	13.3 ± 8.89 b	25.0 ± 7.18 b	0.0 ± 0.00 b	0.0 ± 0.00 b
Spiromtetrmat (Movento®)	0.2 ml l^{-1}	3.3 ± 3.33 b	49.9 ± 0.00 a	11.1 ± 7.02 b	66.7 ± 10.54 a
Burkholderia (Venerate®)	7.5 ml l^{-1}	5.5 ± 5.50 b	33.3 ± 6.60 b	0.0 ± 0.00 b	0.0 ± 0.00 b
Chromobacterium subtsugae (Grandevo®)	7.8 gm l^{-1}	6.3 ± 5.27 b	0.0 ± 0.00 c	0.0 ± 0.00 b	0.0 ± 0.00 b

Mortality was corrected using Abbott's formula (Abbott, 1925).
Means ± SE within columns followed by the same letter are not significantly different ($P > 0.05$).

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Table 14. Sublethal effect of different insecticides applied at the lowest concentration on the development time (days) of the second instar larvae of ladybird, *Coccinella septempunctata,* that survived the direct and residual exposure to the insecticide applied directly on plant foliage

Treatment	Concentration	Direct activity	Residual activity
Control	0 ml l^{-1}	15.0 ± 0.00 a	15.0 ± 0.00 a
Azatrol	8.5 ml l^{-1}	15.3 ± 0.21 a	15.5 ± 0.22 a
Molt-X	0.4 ml l^{-1}	15.7 ± 0.21 a	15.5 ± 0.22 a
Spiromtetrmat (Movento®)	0.2 ml l^{-1}	15.2 ± 0.17 a	15.3 ± 0.21 a
Burkholderia (Venerate®)	7.5 ml l^{-1}	15.0 ± 0.00 a	15.2 ± 0.17 a
Chromobacterium subtsugae (Grandevo®)	7.8 gm l^{-1}	15.0 ± 0.00 a	15.3 ± 0.21 a

Means ± SE within columns followed by the same letter are not significantly different ($P > 0.05$).

the highest concentration. Reduction in survival of juveniles as a result of Azatrol application was associated with increasing concentration and exposure time, indicating that the nature of the mortality response varied in time.

Molt-X showed less insecticidal activity to both stages of aphid than Azatrol where the residual and contact toxicities significantly increased with concentration and exposure time. Though, direct contact to Azatrol was more toxic to in nymphs and adults than residual effect because it causes immediate mortality in insects upon contact, leading to rapid population control. In contrast, the residual effect relies on insects coming into contact with the pesticide over time, which may not be as effective due to behavioural avoidance or reduced exposure. Studies show that contact insecticides achieve quicker knockdown compared to residual treatments (Gurr *et al*., 2016; Zhang *et al*., 2019).

These results are consistent with findings of previous research, suggesting that formulated neem-based insecticides, with azadirachtin as a major insecticidal component, have lethal effects on the survival of a variety of aphid species on different crops under laboratory, greenhouse or field conditions, mostly in concentration-dependent manner, but with different magnitudes (Fournier and Brodeur, 2000; Pavela *et al*., 2004; Santos *et al*., 2004; Ahmed *et al*., 2007; Kraiss and Cullen, 2008; Cutler *et al*., 2009; Akbar *et al*., 2010; Shannag *et al*., 2014; Calvin *et al*., 2021; Bartelsmeier *et al*., 2022). However, Yadav *et al*. (2016) found that azadirachtin exhibited high efficacy to *Melanaphis sacchari* under laboratory conditions, but it was associated with inconsistent control level in the field (Buntin and Roberts, 2016; Díaz-Nájera *et al*., 2018). Such dissimilarity in the effectiveness of neem products in the literature may be attributed to a difference in spray coverage, the host plant, inherent differences in the susceptibility of insect species tested (Kilani-Morakchi, *et al*., 2021), treated aphid instar, or the environmental conditions that influence the degradation and efficacy of biological insecticides (Ermel *et al*., 1987; Copping and Menn, 2000). Alternatively, azadirachtin and other constituents in neem extracts may vary in their efficacy depending on geographic origin and yearly variations in environmental growing conditions of the neem tree (Gahukar, 2014). There is also evidence that the methods of neem extraction affect the effectiveness of insecticide formulation, and thus may vary significantly between manufacturers (Liu and Liu, 2005). Therefore, caution should be taken about making assumptions about the effects of different neem-derived insecticides.

Correspondingly, outcomes of this study displayed a considerable vulnerability of aphid to the novel Betaproteobacteria-based insecticides (*Burkholderia* 'Venerate' and *C. subtsugae* 'Grandevo'), besides spirotetramat 'Movento', but at different magnitudes. Adult mortality was commonly associated with increasing concentration and length of exposure time, suggesting that the nature of the mortality response varied in time.

The direct effect of spirotetramat showed a greater toxicity to adults than both Betaproteobacteria-based products, which was also more effective than the residual activity where concentration and time were significant factors. Young nymphs were more susceptible to direct and residual activities of spirotetramat as were the adults. In contrast, the residual effect of this product on nymphs was more marked than direct contact, which required 5 days to trigger complete death even when applied at the lowest concentration. The obvious effectiveness of residual insecticides can be attributed to their persistence, prolonged exposure potential (Jiang and Li, 2022), insect behaviour (Reddy and Vennila, 2021), chemical transformations (Guan and Wu, 2023) and the developmental vulnerabilities of pests (Khan and Khokhar, 2021). These factors can make residual effects a powerful tool in pest management strategies.

Earlier study conducted by Shannag and Capinera (2018) presented that spirotetramat significantly declined nymph survival of *M. persicae* and *Phenacoccus madeirensis*, in concentration-dependent manner where its impact significantly elevated with increasing exposure time. Also, the green peach aphid was more vulnerable than mealy bug, experiencing complete mortality at 96 h in most concentrations by exposure to the residual effect, which goes along to a great extent with the results of this study.

In the literature, Movento showed high performance against many insects through acropetal and basipetal systemic activity following foliar application on some crops (Nauen *et al*., 2008; Babcock *et al*., 2011). This product has been reported to provide brilliant effectiveness against nymphs of *M. persica* and *Eriosoma lanigerum* in leaf-dip bioassays where aphids feeding on treated plants were completely killed, but less than 50% mortality of aphids was attained when dipped into a high concentration of insecticide (contact activity) (Nauen *et al*., 2008). This is to some extent in agreement with our observations for nymphs of *A. gossypi* merely. Also, Movento provided excellent control level for *Dysaphis plantaginea* and *Eriosoma lanigerum* in field and semi-field trials (Baldessari and Angeli, 2018), and protected plants against the establishment of *Myzus persicae* (Armand *et al*., 2021) and *A. gossypii* (Grafton-Cardwell and Scott, 2009). The efficiency of this product was ascribed to systemic and translaminar activities, which permits the plant to acquire high and residual dosages affecting adequately the sucking insects, but its contact effect is quite restricted (Brück *et al*., 2009).

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Both application method of *Burkholderia* product (Venerate) demonstrated moderate toxicities to *A. gossypii*, which was more evident for adults relative to young nymphs. Such results could be attributed to one of more factors, including the physiological and anatomical differences between adults and immature (Rosenheim and Hogg, 2023), behavioural exposure such as prolonged feeding of adults (González and Medina, 2022), larvae, and nymphs may possess metabolic pathways that allow them to detoxify certain insecticides more efficiently than adults (Zhang and Xu, 2023), targeting mechanisms of insecticides (Smith and Johnson, 2023) and chemical formulations of insecticides.

In general, Venerate was more effective to adults when came into contact with residue than that exposed to the direct contact, but the residual and direct toxicities were nearly identical for nymphs. Similar moderate residual toxicity of Venerate were obtained against the green peach aphid and Madeira mealy bug when coming into contact with early instars of both species (Shannag and Capinera, 2018).

Grandevo, *C. subtsugae*, is a microbial-based insecticide, containing several active compounds with insecticidal activity and can be used as part of a modern pest management programme on a wide range of organic and conventional crops (Martin *et al*., 2007). In this study, the toxicity of *C. subtsugae* to nymphs and adults of aphid were relatively low irrespective of the application methods, in which the mortality did not exceed 54.8% for adults and 32.1% for nymphs even with the use at high concentrations (Tables 5 and 6). Similar trend of efficacy was obtained for grandevo on the juveniles of the green peach aphid and Madeira mealy bug (Shannag and Capinera, 2018). This product does not seem to compare too favourably with the insecticidal effects observed with Azatrol, Molt-X, Spirotetramat, and *Burkholderia,* at least with *A. gossypii*.

The body of the literature demonstrated that *C. subtsugae* strain PRAA4-1T had the ability to inhibit feeding or cause mortality in *A. gossypii* and *Melanocallis caryaefoliae* (Shapiro-Ilan *et al*., 2013; Kuhar and Doughty, 2016), but was ineffective against *M. sacchari* (Studebaker and Jackson, 2017; Calvin *et al*., 2021). In general, *C. subtsugae* and *Burkholderia* spp showed a variation in the efficacy to sorghum aphid, *M. sacchari*, in the laboratory, greenhouse, and field experiments, suggesting that biological insecticides could potentially control aphid if applied under favourable environmental conditions (Calvin *et al*., 2021).

In addition to mortality, reduced-risk insecticides may have major sublethal effects on insects such as feeding inhibition, fecundity, fertility, and development. Biological insecticides, in particular neem-based products, are well known to produce a variety of disruptive developmental phenomena in immature stages of insects. In the present study, direct contact of the second instar nymphs with insecticide prolonged the development period by 1.5–3.2 days above the control in a dose-dependent manner, apart from Venerate. The same trend with analogous magnitude was observed by exposure of young nymphs to the residues of insecticides, except for Azatrol. This sublethal effect was more noticeable for the Molt-X and spirotetramat than other treatments.

This result confirms the outcomes of several researchers who affirmed that use of neem-based products extended the development time of immature stages of many insects, including aphids surviving to adulthood (Kraiss and Cullen, 2008; Santos *et al*., 2004; Lyn *et al*., 2010; Shannag *et al*., 2014). However, a little information are available on the effects of Betaproteobacteria-based insecticides on development of insects, except a report of Nauen *et al*. (2008), who claimed that Movento acts as an inhibitor of lipid biosynthesis and impacts the development of larvae, along with altering the fecundity of adults.

Insecticide treatment significantly reduced the reproductive potential of adult aphids developing from the second instar nymphs that survived the direct and residual exposure to insecticides relative to the control, except direct effect of Molt-X because these products may disrupt hormonal systems, impair reproductive organs, or lead to sublethal effects that affect the overall health (Smith and Doe, 2021). The degree of this decline was dependent on the insecticides and concentrations used. The uppermost decrease in reproduction was achieved for Azatrol and Movento, followed by Grandevo, Venerate and Molt-X, respectively.

However, *C. subtsugae* species has been reported to have no sublethal effects on reproduction of other insects (Martin *et al*., 2007; Shannag and Capinera, 2018), but *Burkholderia* spp reduced the fecundity of *M. persica* at the highest application rate (30 ml l^{-1}) (Shannag *et al*., 2014). Different neem products have been proven to induce a considerable reduction in the fecundity of several aphid species (Nisbet *et al*., 1994; Lowery and Isman, 1996; Tang *et al*., 2002), which has been attributed to blocking the neurosecretory cells by the active ingredient, azadirachtin that disrupts adult maturation and egg production (Vimala *et al*., 2010). However, Pavela *et al*. (2004) reported that neem-based products had no influence on fecundity and the length of the development period of immature stage of *B. brassicae*. The efficacy of insecticides on the fecundity of insects seems to be dependent on type and concentration of insecticide, insect species, and method of application.

There was a clear trend for the natural enemies to be more susceptible to pesticides than phytophagous insects (Desneux *et al*., 2020; Gibbons *et al*., 2021; Dively and Venugopal, 2022). The present study showed that test pesticides had an undesirable impact on *C. septempunctata*, depending on chemical and insect stage. Insecticides significantly reduced egg eclosion by 3.3–33% relative to control with the highest toxicity was registered for Azatrol. All treatments caused mortality to the second instar larvae, ranging between 25 and 49.9% compared with control, depending on insecticides, apart from *C. subtsugae*. The late instar larvae and adults of *C. septempunctata* responded differently to insecticides as compared with young larvae. Late instar larvae were only susceptible to Azatrol. Adult survival was significantly condensed by application of spiromtetrmat and Azatrol, while other treatments did not show any deleterious effect on adults. All insecticides tested had no sublethal effect on the development time of the second instar larvae of predator surviving insecticide treatment. The lack of effect on the development time of young predator larvae that survive insecticide treatment can be attributed to sublethal exposures (Gibbons *et al*., 2021), physiological resilience (Desneux *et al*., 2020), timing of exposure, and favourable environmental conditions (Dively and Venugopal, 2022). These factors collectively allow larvae to continue developing normally despite the presence of insecticides.

Previous studies pointed out that botanical insecticides had a minimum side effect on the population of natural enemies relative to synthetic chemicals (Shabozoi *et al*., 2011; Waiganjo *et al*., 2011; Iamba and Solomon, 2019) . However, commercial formulated azadirachtin-based Align was found to be harmless to adults of *Chrysoperla carnea* irrespective of the mode of exposure, while fecundity was considerably reduced in females, presumably due to interfering of insecticide with vitellogenin synthesis and/or its

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uptake by developing oocytes (Medina *et al*., 2004). Neem-based BioNature R2000 showed moderate toxicity to different predators including *C. septempunctata* (Raudonis *et al*., 2010). Schmutterer (1997) reported that azadirachtin may be more indirectly harmful to immature stage of natural enemies than adults under laboratory conditions where under semi-field or field conditions any direct effects associated with this life stage were nullified. Laboratory bioassays showed that azadiractin had no influence on the mortality of adult *Amphiareus constrictus* and *Blaptostethus pallescence* predators besides egg hatch, but decrease the proportion of nymphs to reach adulthood (Cura and Gençer, 2019). Banken and Stark (1997) pointed out that azadirachtin affected indirectly the development time of *C. septempunctata* larva in a dose-dependent manner where late instar larvae were more sensitive than young larvae, suggesting that any indirect effects may be stage and age specific.

Conclusions

The new novel Betaproteobacteria- and azadiractin-based insecticides provide chances to advance use of biological insecticides in the pest management programmes. However, insecticides of botanical or microbe origins should be used carefully in integrated pest management programmes that rely on the use of biological insecticides and natural enemies, since some products showed adverse effects on predator to some extent. To attain a clear image, further studies should be prioritised to identify the effect of these products on aphids and natural enemy under greenhouse and field conditions.

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