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# Aqueous botanical formulations from *Annona* Seeds for the management of selected vegetable pests

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## Abstract

**Botanical pesticides are a group of bio-pesticides derived from phytochemicals, which can be used as an alternative to synthetic pesticides, with several advantages. They can be used in pest management as ready to use, self-made, aqueous formulations or commercial formulations. Present study evaluated the insecticidal activity of selected aqueous botanical formulations against cabbage leaf eating caterpillar complex and cowpea aphid, *Aphis craccivora* Koch. Aqueous formulations prepared from the seed extracts of family *Annona* spp. demonstrated significant (*p*<0.05) insecticidal activity against cabbage leaf eating caterpillars, *Plutella xylostella* L. (Lepidoptera:Yponomeutoidea), *Crocidolomia binotalis* (Lepidoptera: Crambidae) and *Chrysodeixis* spp. (Lepidoptera: Chrysodeixidae), and *Aphis craccivora* (Hemiptera: Aphididae). The activity of the Annonaceae seed extracts was comparable to that of the aqueous seed extracts of neem [*Azadirachta indica* (A. Juss.), the commercial neem formulation LakGro Neem 7.5 g $L^{-1}$ EC®, and in most instances superior to Spinosad (Success 25 g $L^{-1}$ SC®). Furthermore, seed extracts of Sugar Apple (*Annona squamosa* L.) and Cherimoya (*A. cherimola* Mill.) were more effective than the rest of the tested species. The study revealed that the aqueous seeds extracts of *Annona* species have a potential to be used in pest management.**

**Keywords: *Annona* seed extracts, Aqueous formulations, Botanical pesticides**

## Introduction

Bio-pesticides refers to naturally occurring substances derived from living organisms (e.g., natural enemies), their products (e.g., microbial products, phytochemicals) or their by-products (e.g., semiochemicals) that can control pests by non-toxic mechanisms. In contrast, synthetic pesticides based on a single active ingredient have rapid knock down activity, however, pose a number of drawbacks due to increase in cost of application, development of insect resistance, occupational hazards, and potential contamination of agricultural produce with residues. As a result, bio-pesticides are being used as an alternative to synthetic pesticides in Integrated Pest Management (IPM) and organic farming.

Botanical pesticides are a group of bio-pesticides derived from plants, which contains phytochemicals. Aqueous formulations of botanicals have traditionally been used for pest management all over the world (Anon, 1975). However, after the introduction of synthetic pesticides with rapid knockdown activity, and their availability at lower price coupled with wide publicity given through mass media, they have become a popular replacement to traditional formulations which have comparatively slower activity. As a result, the knowledge on the traditional preparations has gradually disappeared. However, in recent times, with the advancement of modern extraction and formulation technologies, commercial level production of botanical pesticides has become feasible, enabling them to suit current market requirements and demand competing with commercial synthetic pesticides (e.g., Neem and Pongamia seed extracts). Furthermore, the potential use of self-made ready-to-use botanical pesticides in areas where raw-materials are freely available is feasible, especially in small scale cultivations (Wafaa *et al*., 2017). However, re-introduction of simple botanical formulations in Sri Lanka as recommended by the government Department of Agriculture (DOA) has not widely been achieved. This is mainly due to the lack of scientific evidence on the effectiveness and safety of botanical formulations. Moreover, commercial exploitation of those has not taken place effectively due to the lack of analytical and toxicological data to fulfill registration requirements. The present investigation aimed at exploring the effectiveness of traditionally used botanical formulations to enable DOA to recommend such simple formulation in small scale applications for effective pest management.

## Materials and Methods

Candidate botanicals for the evaluation were selected based on the local and international literature (Anon, 1975; Stoll,1998). Laboratory, planthouse and research field evaluations were carried out at the Horticultural Crops Research and Development Institute (HORDI), at Peradeniya, Sri Lanka from 2011 to 2018. Cabbage leaf eating caterpillar complex, diamond back moth (DBM *Plutella xylostella* L.; Lepidoptera: Yponomeutidae) and cowpea aphid (*Aphis craccivora* Koch.; Hemiptera: Aphididae) were used as the test insects in laboratory evaluations. Effective formulations were further evaluated against the cabbage leaf eating caterpillar complex and cowpea aphids in long bean (*Vigna unguiculata* L.) in Gannoruwa (Kandy district) and Bandarapola (Matale district) to confirm their effectiveness under actual farmer field conditions.

### Experiment 1. Laboratory Evaluation of aqueous seed extracts of *Annona* spp. against the larvae of diamond back moth

### Preparation of aqueous formulations

Seeds of *Annona cherimola* were collected from Gannoruwa, *A.squamosa* and *A.reticulata* from Polonnaruwa and *A.glabra* from Telijjawila and Gannoruwa (Figure 1). Seeds (25 g) were ground using a mortar and pestle and the resultant powder was soaked in water for 24 h. Then the mixture was squeezed through a net (Organdy®) several times by adding portions of water to form 1 L of the extract. Thereafter, the extract was mixed with 3mL of general purpose surfactant (Teepol®) or 3 g of soap to prepare a ready-to-use aqueous formulation. Adding of surfactant was essential to emulsify the non-polar compounds in water to ensure uniform spray and to enhance cuticle penetration.

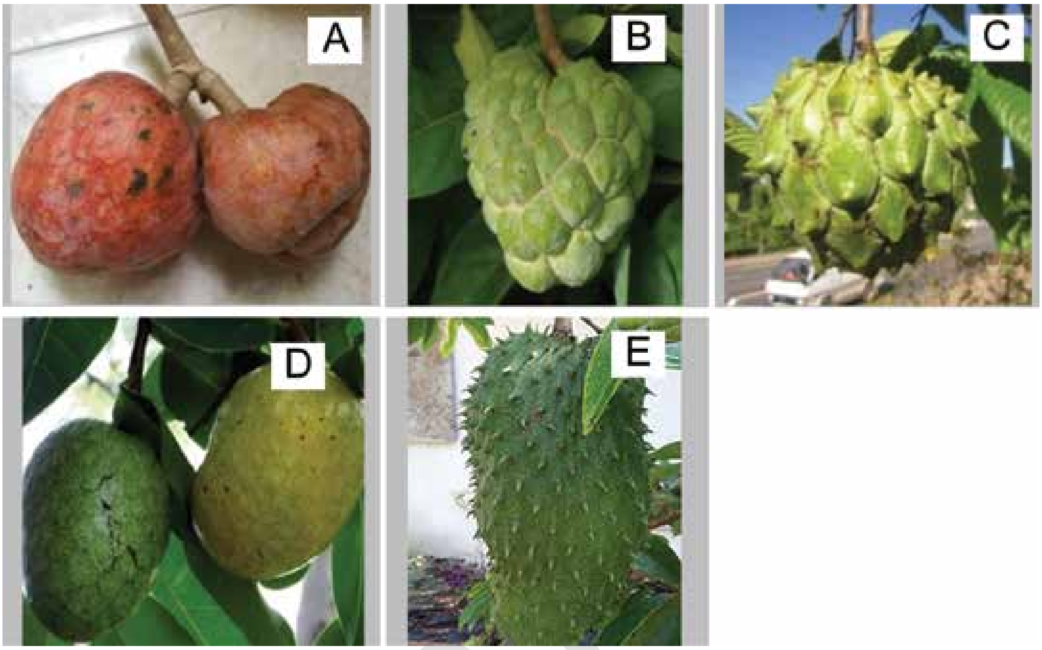


**Figure 1.A -Custard apple (*A.reticulata*), B - Sugar apple (*A. squamosa*), C - Cherimoya (*A. cherimola*), D - Pond annona/*Welatha* (*A. glabra*), and E - Soursop (*A. muricata*)**

## Leaf dip bioassay:

Experiments were conducted in the entomology greenhouse at the HORDI, Gannoruwa using the second instar larvae of DBM in 2012. Leaf discs (30 mm diameter) obtained from the plant house-grown cabbage seedlings (var.*Oxylus*) were dipped in the test formulation and then air dried for 10 minutes. The treated discs were then placed in glass Petri dishes (9 cm diameter) lined with filter paper, where each Petri dish was introduced with10, $2^{nd}$ instar larvae of laboratory-reared DBM. Four test formulations and water mixed with the surfactant was used as the untreated control. The experiment was laid out in a RCBD with four replicates under prevailing weather conditions (Temperature 27-30 ˚C, RH 66-88%). The larval mortality, feeding area and the adult emergence were recorded while feeding them with fresh leaf discs when necessary.

**Experiment 2. Field evaluation of aqueous botanical formulations against cabbage leaf eating caterpillars**

Field effectiveness of selected formulations of *Annona* species (25 g $L^{-1}$) and sweet flag+garlic+ginger each at 20 g $L^{-1}$ were compared with aqueous seed extract of neem (ASEN) (30 g $L^{-1}$) as the standard botanical insecticide (Anon, 2009) and water with Teepol® as the untreated control against cabbage caterpillars in a RCBD with three replicates at HORDI field in 2012/13 *Maha* season (October to February). Cabbage variety *Oxylus* was planted in 2.0 m ×3.0 m plots and agronomic practices were carried out according to the DOA recommendations. Two weeks after transplanting, 10 plants were randomly labeled from each plot and the number of larvae of the leaf eating caterpillar belonging to different species in those plants was recorded. Then, the treatments were imposed on the respective plots using a knapsack sprayer (hollow cone nozzle under 1-2 bars pressure with 1.4 L $min^{-1}$ flow rate). The treatments were repeated six times in seven-day intervals after counting the number of larvae in labeled plants. Leaf damage caused by the caterpillars was recorded in a 0 to 4 scale (0=0-5%, 1= 6-25%, 2=26-50%, 3= 51-75%, 4= >75% area damage) at 40 days after transplanting. Total and marketable yields, after removing all the damaged leaves, were recorded at harvest. Yield loss (%) was estimated using the Equation 1.

$$\%\ \text{Yield loss} = \frac{(\text{Weight of the Total Yield} - \text{Weight of the damaged leaves} \times 100)}{\text{Total yield}} \qquad \text{Equation 1}$$



**Experiment 3. Field evaluation of aqueous botanical formulations with recommended insecticides against cabbage leaf eating caterpillars under organic farming conditions**

The treatments of the Experiment 2 was repeated with a DOA recommended insecticide, Spinosad 2.5 SC 1 mL $L^{-1}$ as the control at the organic research field in HORDI, in the 2013/14 *Maha* season to compare the effectiveness of selected botanical formulations.

## Experiment 4. Farmer field evaluation of selected aqueous botanical formulations against cabbage caterpillar complex

A non-replicated pilot scale farmer field evaluation was carried out in 2013/14 *Maha* season at Bandarapola in Matale, Sri Lanka to evaluate the performances of selected botanical formulations under farmer field conditions against the leaf eating caterpillar complex of cabbage. Cabbage variety Oxylus was planted in 8 m × 6 m plots and other agronomic practices, except pest management, were carried out by the farmer according to their conventional practices.

The pest management practices were carried out by the farmer following the instructions given by the research team. Aqueous formulations of *A.cherimola* (30 g $L^{-1}$), ASEN (40 g $L^{-1}$), DOA recommend standard insecticide Spinosad (Success® 2.5 SC) 1 mL $L^{-1}$ and farmers practice (alternate spraying of insecticides profenophos (Calcron®); etofenprox (Tribon®); indoxicarb (Avaunt®) at seven-day intervals) were evaluated. Spraying of treatments were initiated two weeks after transplanting and repeated in fortnight intervals.Pest counts were taken before each application, leaf damage at 40 day after transplanting and before harvests (0-4 scale) and data on yield loss were recorded by the research team as described in Experiment 2.



## Experiment 5. Evaluation of aqueous formulations of *Annona* seed extracts against cowpea aphid

This experiment was carried out in the entomology plant house at HORDI in 2012 as a RCBD with four replicates to evaluate the insecticidal activity of aqueous formulations of *Annona* seed extracts against cowpea aphid. The formulations were prepared as described in Experiment 1. Seven day old cowpea seedlings in clay pots (two plants per pot) were introduced with five, day-old apterous adult aphids obtained from the laboratory culture and they were removed after 24 h. Five days after the hatching, the nymphs which were developed into the final instars stage or into adult stage were counted and the plants were sprayed with different treatments using a hand sprayer. The number of live adults in each treated plant was recorded 24 h after spraying.

## Experiment 6. Field evaluation of the efficacy of aqueous botanical formulations against cowpea aphid in long bean

Formulations prepared from three species of *Annona* seeds were evaluated against aphids of long bean in the research field at HORDI in 2014/15 *Maha* season. Long bean variety Polon mae was planted in 5.4 m × 6 m plots and the experiment was laid out in a RCBD with four replicates. Plants were allowed for the natural infestation of aphids and when the pest population was built up to sufficient level, five infested pods were selected and labeled from each plot. The number of aphids (adults and nymphs) in each labeled pods were counted in 1 to 4 scale (1= 1-10 aphids, 2= 11-50, 3= 51-200, 4= over 200) and plants were sprayed with botanical formulations of *A.cherimola* 30 g $L^{-1}$, garlic 25 g+kerosene 2.5 mL $L^{-1}$, and ASEN as the standard botanical formulation with untreated control using a hand sprayer. Botanical formulations were prepared following the same procedure as described in Experiment 1. The DOA procedure was followed when preparing ASEN (Anon, 2009).The aphid population in labeled pods of each plot was re-estimated two days after treatment.

## Experiment 7. Pilot scale evaluation of botanical formulations against cowpea aphid in long bean in an organically cultivated field

This field evaluation was carried out in already infested long bean crop and the variety was Polon mae planted in 6.0 m × 1.6 m plots established in organic farming research field at HORDI, which was maintained according to the principles of organic agriculture. Formulations of *Annona* seed extracts were prepared as described in Experiment 1. The garlic with kerosene formulation was selected from the literature and prepared as explained by Stoll (1998). Garlic (20 g) was ground to form a paste and mixed with 2 mL of kerosene oil. This paste was kept in a closed container for 24 h and then mixed thoroughly with sufficient amount of water, mixed with a surfactant (3 mL of Teepol®) and filtered through a net to make 1 L of spray formulation. The procedure described in experiment six was followed when imposing treatments and recording data. The DOA-recommended commercial neem formulation, LakGro Neem 7.5 EC® was used as the standard botanical insecticide, and water was used as the control. Data were analyzed using ANOVA and mean separation by LSD using SAS

## Results and Discussion

The results of the experiment 1 revealed that the botanical formulations prepared from aqueous extracts of sugar apple and cherimoya,followed by custard apple,caused significantly higher larval mortality, feeding inhibition and lower adult emergence of DBM larvae than the untreated control ($p$<0.05; Table 1). No adults emerged from the sugar apple and cherimoya treatments. However, soursop showed a comparatively lower activity. Insecticidal activity of the seed extracts of sugar apple has been well recognized where annonin (squamocin) and anoveldihydroxy-bistetrahydro furan fatty acid lactone (acetogenin) have been identified as active compounds. These two compounds are reported to be toxic to eggs, larvae and adults of fruit fly (Kawazu *et al*., 1989). A strong insecticidal activity of the seed extracts of *A. squmosa* has also been reported mainly due to acetogenins (anonins or anonacins: asimicin, annonastatin, bullatacin, bullatacinone and squamocin) (Secoy and Smith, 1983; Pinto et al., 2005). Methanolic extract of *A. squmosa* was reported as toxic to DBM and simple formulations were recommended in Indonesia for controlling the pests (Leatemia and Isman, 2007; Mondal *et al*., 2018).



**Table 1. Bio efficacy of aqueous extract of *Annona* spp. seeds against the larvae of diamond back moth, *Plutella xylostella* in the laboratory**

| | Treatment | Concentration g L$^{-1}$ | Larval mortality (%) | | | Leaf area consumed (mm$^2$/10 larvae) 72 HAT | Adult emergence % 6 DAT |
|---|---|---|---|---|---|---|---|
| | | | 24 HAT | 72 HAT | 6 DAT | | |
| 1 | Soursop, *Annona muricata* | 25.0 | 0 b | 5.0 $^c$ | 10.0 $^b$ | 84.5 $^b$ | 55 $^b$ |
| 2 | Sugar Apple, *Annona squamosa* | 25.0 | 38.7 $^a$ | 93.7 $^a$ | 93.7 $^a$ | 4.4 $^d$ | 0.0 $^c$ |
| 3 | Cherimoya, *Annona cherimola* | 25.0 | 31.6 $^a$ | 70.0 $^a$ | 90.0 $^a$ | 3.1 $^d$ | 0.0 $^c$ |
| 4 | Custard Apple, *Annona reticulate* | 25.0 | 5 $^{ab}$ | 20.0 $^b$ | 50.0 $^a$ | 41.9 $^c$ | 40.0 $^b$ |
| 5 | Untreated Control | - | 0.0 $^b$ | 0.0 $^c$ | 0.0 $^b$ | 228.5 $^a$ | 100 $^a$ |
| | CV% | | 34.3 | 24.3 | 29.2 | 24.6 | 11.8 |
| | LSD | | 2.0 | 1.62 | 1.25 | 27.54 | 0.44 |

*Within a column, means followed by the same letter are not significantly different at p=0.05; HAT- hours after treatment, DAT- days after treatment*

Comparatively poor insecticidal activity of the methanolic extract of *A. muricata* has been reported when compared with *A. squmosa* against *Spodoptera litura* (Leatemia and Isman, 2004). However, the insecticidal activity of *A. muricata* against many other insect pests has been reported (Moghadamtousi *et al*., 2015;Amalia and Yusa, 2018). The present study confirmed the comparatively poor insecticidal activity of aqueous seed extract of *A.muricata* against DBM larvae. Anti-feedant activity of *A.squamosa* on DBM has been reported (Leatemia and Isman, 2004). Where similar results were reported *A. squmosa* and *A.cherimola* against DBM larvae, in the present study.

According to the results of experiment 2, all the tested botanical formulations has caused significant ($p$<0.05) reduction in the presence of DBM and *C. binotalis* caterpillar pests (Table 2). However, tested formulations failed to show significant reduction ($p$>0.05) of *S.litura* population in this experiment and this may be due to the high variability of their presence among replicates. *A. cherimola* and sweet flag, garlic and ginger (20 g each $L^{-1}$) mixture failed to show a significant reduction ($p$>0.05) of the population of *Chrysodeixis* spp. when compared with the untreated control.

**Table 2. Bio efficacy of different aqueous botanical formulations against cabbage leaf eating caterpillars and their damage under field conditions (2012/13 *Maha* season)**

| | Treatment | Average number of caterpillars in 10 plants | | | | Leaf damage (0-4 scale) | % yield loss |
|---|---|---|---|---|---|---|---|
| | | *P. xylostella* | *S. litura* | *Chrysodeixis* spp. | *C. binotalis* | | |
| 1 | ASEN+Teepol® (standard treatment) | $1.70^{ab}$ | 2.80 | $1.53^{b}$ | $6.10^{b}$ | $2.23^{b}$ | $31.56^{b}$ |
| 2 | *Annonacherimola* + Teepol | $1.46^{b}$ | 3.66 | $4.20^{a}$ | $2.53^{b}$ | $2.33^{b}$ | $34.54^{b}$ |
| 3 | Sweet flag+Garlic+ Ginger+Teepol | $1.13^{b}$ | 7.26 | $4.10^{a}$ | $9.73^{b}$ | $3.03^{b}$ | $42.11^{b}$ |
| 4 | Untreated control | $2.40^{a}$ | 9.90 | $3.90^{ab}$ | $39.53^{a}$ | $4.83^{a}$ | $64.4^{a}$ |
| | LSD | 0.91 | Ns | 2.40 | 13.47 | 1.43 | 19.18 |
| | CV% | 21.15 | 98.47 | 34.9 | 46.55 | 15.8 | 22.25 |

** Log transformed values were used for analysis. Means with the same letter in each column are not significantly different at p=0.05; Mean separation by LSD*

Soursop leaf extract was effective against *P. xylostella*, a leaf eating caterpillar in Delima rose apples (Amalia and Yusa, 2018). Methanolic seed extracts of *A. squamosa* and *A. atemoya* were toxic to the third instar *Trichoplusia ni* larvae and reduced leaf consumption and larval growth (Seffrin *et al*., 2010). However, all three tested botanical formulations in the present study reduced the leaf damage and yield loss caused by caterpillar pests and increased marketable yield. Insecticidal properties of ASEN has been proven and already been recommended as a simple botanical pesticide by the Department of Agriculture, Sri Lanka (Anon, 2009). Insecticidal activity of garlic and ginger oils on *S. littoralis* have also been reported (Hamada *et al*., 2018).The present study showed that the aqueous formulations of *A. cherimola* seeds and simple formulations made from sweet flag, garlic and ginger (20 g $L^{-1}$) were equally effective for the controlling cabbage caterpillar pests.

Experiment three indicated that the ASEN, followed by the recommended commercial insecticide Spinosad (Success 2.5 SC®) and the aqueous formulations prepared from *A.cherimola* seeds and sweet flag, garlic and ginger (20 g each) mixtures significantly reduced ($p$<0.05) leaf damage and yield loss caused by leaf eating caterpillars (Table 3). The results further showed that the effectiveness of ASEN was superior to spinosad and the rest of the botanicals used in this evaluation.

**Table 3. Comparative evaluation of aqueous botanical formulations against cabbage leaf eating caterpillars under organic cultivation conditions (2013/14 *Maha* season)**

| Treatment | Average number of caterpillars in ten plants | | | | Leaf da mage at harvest (0-4 scale) | yield loss % |
|---|---|---|---|---|---|---|
| | DBM | S. *litura* | *Chrysodeixis* spp. | *C. binotalis* | | |
| Sweet flag+Garlic+ Ginger+Teepol | $0.3^{ab}$ | $20.8^{b}$ | $4.2^{ab}$ | $0.16^{b}$ | $1.48^{b}$ | 15.85 b |
| A.cherimola+ Teepol+water | $0.0^{b}$ | $6.8^{c}$ | $1.2^{c}$ | $0.0^{b}$ | $1.38^{b}$ | $15.12^{b}$ |
| AENS(DOA) recommendation) | $0.0^{b}$ | $0.0^{c}$ | $0.2^{c}$ | $0.0^{b}$ | $0.22^{c}$ | $2.47^{a}$ |
| Spinosad (DOA recommendation) | $0.0^{b}$ | $46.5^{a}$ | $3.0^{b}$ | $0.0^{b}$ | $0.94^{b}$ | $8.75^{ab}$ |
| Untreated Control | $1.0^{a}$ | $37.8^{ab}$ | $6.3^{a}$ | $4.0^{a}$ | $1.95^{a}$ | $24.96^{c}$ |
| LSD | 0.56 | 12.4 | 2.0 | 2.6 | 0.42 | 7.127 |
| CV% | 32,8 | 48.2 | 34.2 | 38.5 | 28.5 | 28.17 |

*Within a column, means followed by the same letter are not significantly different at p=0.05*

Toxicity and anti-feedant activity of essential oils of sweet flag, *A. calamus* against *S. litura* has been reported (Melani *et al*.,2016). Insecticidal activities of oils from garlic, cloves, *A. sativum* and ginger rhizomes were evaluated on *S. littoralis*. The treatments significantly prolonged ($p$<0.05) the durations of larvae and pupae, increased pupal weight, and decreased egg hatchability reducing the emergence of adults when compared with the untreated control (Hamada *et al*., 2018).

Farmer field evaluation of selected aqueous botanical formulations against cabbage caterpillar complex showed that the simple formulation of ASEN showed the highest activity, i.e. the lowest number of caterpillars, lowest leaf damage and yield loss (Table 4). Its activity was superior to the farmer practice, which included weekly spraying of three insecticides and Spinosad for controlling the caterpillar pests. Spinosad and the simple formulations prepared from aqueous seed extract of *A. cherimola* showed moderate effectiveness against the caterpillar pests. This further confirms the insecticidal activity of ASEN, and indicated the moderate effectiveness of seed extract of *A. cherimola* against the leaf eating caterpillar pests of cabbage.

**Table 4. Effectiveness of simple aqueous botanical formulations against cabbage leaf caterpillars under farmer field conditions (2013/14 *Maha* season)**

| **Treatment** | **Number of caterpillars in ten plants** | | | **Leaf damage (0-4 scale)** | **% yield loss** |
|---|---|---|---|---|---|
| | ***S. litura*** | ***Chrysodeixis* spp.** | ***C. binotali*** | | |
| *A. cherimola*+ Teepol+water | 76 | 6 | 1 | 2.02 | 22.12 |
| NSWE(DOA recommendation) | 55 | 1 | 0 | 0.69 | 10.3 |
| Spinosad (Success 2.5 SC®) | 206 | 13 | 1 | 2.16 | 19.0 |
| Farmers practice | 29 | 21 | 0 | 1.21 | 12.1 |

In the experiment five, all the tested aqueous formulations of *A.murucata*, *A. cherimola*, and *A.reticulate* showed significant ($p$<0.05) mortality of cowpea aphids when compared with the untreated control at 24 hours after treatment (Table 5). Potential use of water extract of *A. murucata* against *A. glossipi* in chilli and mites in green house cucumber has previously been reported (Sina, 2003). Insecticidal activity of seed ethanol extract of custard apple, *A. squmosa* against *A. glossipi* and mites in green house cucumber has been reported and its effectiveness was superior to azadirachtin and spintoram (Saleem *et al*., 2019).

All the botanical formulations caused significant reductions ($p$<0.05) in aphid populations when compared with the untreated control in experiment 6. *Annona cherimola* seed extract showed the highest activity and its activity was superior to the standard botanical formulation ASEN (Table 6).

**Table 5. Aphidicidal activity of seed extracts of different *Annona* spp. against cowpea aphid, *Aphis craccivora* in plant house**

| Treatment | % mortality after 24 hours |
|---|---|
| *A. murucata* 25 g + 3 mL Teepol ® $L^{-1}$ | 94.2 $^{a}$ |
| *A. cherimola* 25 g + 3 mL Teepol ® $L^{-1}$ | 91.4 $^{a}$ |
| *A. reticulate* 25 g + 3 mL Teepol ® $L^{-1}$ | 95.7 $^{a}$ |
| Untreated control | 0.0 $^{b}$ |
| LSD | 10.79 |
| CV% | 9.59 |

*Within the column, means followed by the same letter are not significantly different at p=0.05*

**Table 6. Comparative effectiveness of different botanical formulations against cowpea aphid**

| Treatment | Average number of live aphids in the colony | |
|---|---|---|
| | Pre-count* | Post count (48 HAT) |
| Garlic+kerosene | 4 | $3^{b}$ |
| *A. cherimola* seed extract+teepol | 4 | $1^{c}$ |
| ASEN | 4 | $3^{b}$ |
| Untreated control | 4 | $4^{a}$ |
| CV% | 17.27 | 37.0 |
| LSD | ns | 1.78 |

**scale 1-4: 1=0-10, 2=11-50, 3=51-200, 4=>200; Within a column, means followed by the same letter are not significantly different at p=0.05; HAT= hours after treatment*

These results demonstrated the slow insecticidal activity of ASEN which take 4-5 days to show maximum activity. Various preparations based on mineral oil (e.g., kerosene) have been traditionally used for pest control by the farmers in different counties (Van der Werf, 1985). It has been used as an ingredient of commercial insecticide formulations. Insecticidal activity of garlic against various pests has been reported (Prowse *et al*., 2006; Hamada *et al*., 2018). Therefore, simple formulations of *A. cherimola* seed extract can be used as an alternative to already recommended ASEN for controlling aphid pests in home gardens and small-scale commercial vegetable cultivations.

In the experiment seven, botanical formulations; ASEN, seed extract of *A.cherimola*, and garlic + kerosene mixture controlled aphids in long bean under organically cultivated systems (Table 7). Results further confirmed that the simple formulation of *A. cherimola* seed extract, and garlic and kerosene oil mixture are suitable alternative botanical or natural pesticides for the control of legume aphids in organic cultivations and home gardens.

The overall results indicated that the aqueous formulations made out of seed extracts of different *Annona* species are effective against a wide range of pests, and in someinstances their activity was comparable to ASEN and neem-based commercial pesticide formulation LakGro Neem 7.5 EC®. Family Annonaceae consists of plants with diversified medicinal and pesticidal properties. Apart from being consumed as fruits, they have been traditionally used in herbal medicines because of their anti-microbial, cytotoxic, antioxidant, anti-tumor, insecticidal and anthelmintic activities.

**Table 7. Comparative aphidicidal activity of botanical formulations preparations under organically grown conditions**

| Treatment | Pre count* (1-4 Scale) | Post count (1-4 scale)* | |
|---|---|---|---|
| | | 48 HAT | 5 DAT |
| ASEN (DOA recommendation) | 4 | 3 [a] | 1 |
| Garlic+Kerosene mixture | 4 | 2 [ab] | 1 |
| *Annonacherimola* (Cherimoya) | 4 | 1 [b] | 1 |

**Scale 1-4: 1=0-10, 2=11-50, 3=51-200, 4= >200; Within a column, means followed by the same letter are not significantly different at p=0.05; HAT = hours after treatment; DAT= days after treatment*

Seed extracts of family Annonaceae have widely been used for controlling a number of plant pests and human head lice (Anon, 1975). Phytochemical investigations have showed that these extracts contain annonaseous acetogenins, amajor secondary metabolite that confer theirinsecticidal proprieties. More than 400 annonaceous acetogenins and their stereo isomers have been discovered and they are basically a series of C-35/C-37 natural products derived from C-32/ C-34 fatty acids that are combined with a 2-propanol unit and kill the target organism by ATP deprivation (Mondal *et al*., 2018). This unique mode of action of ATP deprivation facilitates its use in insecticide resistant management. Two botanical insecticides (Anosom® and Bio Rakshak®) have already been commercialized in Indiathat have been developed based on the seed extracts of *A. squamosal* and *A. reticulate*, containing1% squamocin as the active ingredient. Family Annonaceae is rich in potential as a source of therapeutic agent for the development of novel pharmaceutical drugs. Several species belonging to the genus Annonaceae have been well studied and have provided numerous therapeutic substances which able to use for the treatment of many illness (Aziz *et al*., 2016). These evidences support that low concentrations of *Annona* seed extract isnon-phytotoxic and non-toxic to higher animals and has a potential to be used in pest management of agriculture. Several *Annona* species are available in Sri Lanka and can easily be cultivated under the prevailing tropical climate in the country. Therefore, family Annonaceae is a potential source of botanical pesticides and their seeds can easily be formulated to botanical pesticides by the farmer to be used in small-scale cultivations.Therefore,it provides a suitable botanical insecticidealternative to synthetic pesticide for the local farmers. As a simple formulation, a mixture of sweet flag, garlic and ginger showed a moderate activity against the leaf eating caterpillars of cabbage. Garlic and kerosene oil mixture was effective against aphid pests.

## Conclusion

There is a potential to use aqueous formulations of four *Annona* spp., mixture of sweet flag,garlic and ginger (20 g each), and garlic mixed with kerosene as sustainable and safer alternatives to synthetic insecticides in small-scale applications against cabbage leafeating caterpillars and legume aphids. As *Annona* spp. can be grown across the country and seeds are available throughout the year, it provide a locally available renewable resource for the preparation of botanical pesticides, which can be used as an alternative to synthetic pesticides for farmer. Furthermore, simple aqueous formulations made out of sweet flag, garlic and ginger (20 g each) with 3 mL Teepol® $L^{-1}$, and garlic 25 g and kerosene 2.5 ml $L^{-1}$ with a surfactant (Teepol®) also have potential pesticidal properties.

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