Asian Journal of Crop Science

ISSN 1994-7879

OPEN ACCESS Asian Journal of Crop Science

ISSN 1994-7879
DOI: 10.3923/ajcs.2019.71.76

Research Article
Efficacy of Bitter Apple (*Solanum incanum*) in Relation to Contact Time for Controlling Cabbage Aphids (*Brevicoryne brassicae*)

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Abstract

Background and Objectives: Southern Africa needs plant pesticidal technologies to control aphids (*Brevicoryne brassicae*) as alternatives to synthetic chemicals. This study sought to determine the efficacy of Bitter Apple (BA) (*Citrullus colosynthis*) fruit solution to control cabbage aphids by considering aphid mortality in case of varied BA contact time. **Materials and Methods:** Treatments were prepared by pounding ripe BA fruits to form a paste, then mixed with distilled water to obtain BA fruit concentrations (30, 60 and 90 mL L^{-1}). The treatments were mixed with 3, 6 and 12 g of sugar, respectively. Negative and positive controls were no BA (distilled water only) and dimethoate 40% EC, respectively. Efficacy of each concentration of BA was evaluated at 6, 24 and 48 h after application. **Results:** Bitter apple was significantly ($p<0.001$) efficacious to cabbage aphid. All BA concentrations increased aphid mortality at all the contact times as the contact time increased. At a concentration of 90 mL L^{-1}, the highest mortality (13.00) was at 6 h and was significantly different from the negative control. The lowest concentration (30 mL L^{-1}) was significantly different from the negative control at 24 and 48 h. **Conclusion:** This study concluded that bitter apple fruit extract should be used at a concentration of 90 mL L^{-1} at 48 h of contact time to obtain highly significant cabbage aphid mortality.

Key words: Bitter apple, synthetic chemicals, cabbage aphids, contact time, aphid mortality

Citation: Culver Mvumi, 2019. Efficacy of bitter apple (*Solanum incanum*) in relation to contact time for controlling cabbage aphids (*Brevicoryne brassicae*). Asian J. Crop Sci., 11: 71-76.

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cabbage (*Brassica oleracea*) is a popular vegetable grown in southern Africa, for its economic and nutritive values, especially in the rural areas. Due to its better resilience than rape in dry regions, cabbage is preferred by most rural people to all the other *Brassica* spp. because it meets the economic and nutritive values they crave for. Cabbage is rich in vitamin A and C and also contains minerals like phosphorus, potassium, calcium, sodium and iron[1]. However, like rape (*Brassica napus*), cabbage is heavily attacked by aphid (*Brevicoryne brassicae*), which is one of the most destructive pests widely distributed in warm and even temperate regions around the world[2]. The aphid attack on cabbage in such conditions leads to low head yield[3,4]. The yield loss may rise up[5] to 70% and in very favorable conditions, aphids can cause total crop loss on brassica leafy vegetables[6]. The status of the cabbage aphid is enhanced by its high reproductive capacity[7]. One mature aphid can give birth to 2-5 live young ones per day which mature in 5-7 days after birth[7]. Aphids feed by sucking the sap from the plants and if in large numbers, remove sufficient sap to cause leaves to wrap or curl inwards[8].

Some smallholder cabbage farmers pre-dominantly use synthetic pesticides to control aphids[4,9,10]. The most widely used chemical to control cabbage aphids in Zimbabwe is dimethoate 40% EC. Smallholder farmers cannot afford the commercial chemicals due to their high costs. They also have no easy access to the synthetic chemical due to its unavailability in local shops. Synthetic pesticides destroy the non-target insects, even though they may be very effective[11]. Synthetic chemicals have been reported to cause environmental hazards[12,10].

Medicinal plants are usually locally available. They are less expensive, easy to use and are environmental friendly[13]. They have little impact on natural enemies of pests[14], which means they can also be pest-specific. Indeed, locally available pesticidal plant products are great technologies. Botanical pesticides can be easily managed, are environmentally benign, for example, the poorest farmers following a demonstrative research can safely use them[15,16]. A study on the efficacy of lantana (*Lantana camara*) against aphids in rape (*Brassica nupas*) over varied periods of time (contact time) was carried out[17]. Contact time is critical in determining the efficacy of a biopesticide as it is the basis of how long a plant is safe from a particular pest attack. Interestingly, the efficacy of organic pesticides and contact time taken vary with the pesticidal plant used and the type or species of pest to control[17,18].

The efficacy of Bitter Apple (BA) fruits against aphids has not been fully researched. Additionally, insufficient studies have been done to show the effect of contact time on the efficacy of organic pesticides. The current study investigated the efficacy of BA on the control of the cabbage aphid considering the length of contact time taken after application.

MATERIALS AND METHODS

The efficacy of BA in relation to contact time in controlling cabbage aphids was evaluated in the field in 2017.

Site: The trial was carried out in Biriiri, Chimanimani district (coordinates 19°48' 0.00"S, 32°51' 36.00"E), Zimbabwe. The area receives an average annual rainfall of 350-400 mm which is erratic. Biriiri experiences an annual temperature range of 24-32°C in summer (August-March), but can drop to 12°C in winter (April-July). The soil is acid (Table 1) and is shallow sandy to sandy loam, with stony patches which pose difficulties during cultivation.

Biological materials: Certified cabbage (*Brassica oleracea* var. *capitata*) seed was obtained from farm and city in Mutare. Cabbage seeds were sown on 23 July, 2017 and seedlings were raised on a nursery bed. Ripe freshly harvested bitter apple fruits were collected from Biriiri fields in Chimanimani district. Aphids were taken from the host cabbage crop in a nearby cabbage field. Only the ones identified as adults were selected. After a thorough check of the experimental plants that they were clean of aphid infestation, 20 adult aphids were immediately introduced on each plant, 6 weeks after transplanting; the introduction of the aphids was done following the protocol of Mvumi and Maunga[17], with slight modifications. The aphids were introduced on the same day and about the same time, 6 weeks after transplanting. The aphids were physically checked that they were healthy and active as these were the fundamental characteristics of the aphids' ability to infest the plants. The infestation period was 15 min after introducing the adult aphids to the plants, before application

Table 1: Chemical characteristics of the soil used in Biriiri during the 2017-2018 cropping period

Nutrient level in the soil						
pH ($CaCl_2$ scale)	Ca (me %)	Mg (me %)	K (me %)	TEB (me %)	p-value (ppm)	Total N (%)
4.8	5.90	3.85	0.50	10.13	12.5	0.08

of treatments. The shortness of time (15 min) which was allowed for infestation period was for ensuring that the number of aphids (20 aphids per plant) would not change before the BA treatments were applied. To ensure no aphids could have escaped, physical re-examination to determine the consistence of the number of adult aphids (20) previously introduced per plant[19] was done.

Bitter apple extract preparation: The fresh BA fruits were pounded in a mortar to paste. The paste was then thoroughly compressed in a perforated net cloth to extract the concentrated fruit pulp. After the filtering process, each concentration (obtained from 1 L^{-1} distilled water which had been mixed with each of the pounded amounts of Moringa leaves (3, 2 and 1 kg) was then mixed with 5 g of sugar and stored in air tight glass bottles at 4°C before it was used. Sugar was integrated with all treatments to act as a sticking agent and as an attractant. Sugar is used in similar processes in horticultural agronomy[20]. Sugar has sweetness characteristic which attracts pests to a host or retains a pest on a host, implying that it has a strong impact on aphid-host choice[21]. The mixture was left for 24 h at a temperature of 5°C before use.

Treatments and experimental design: Treatments were prepared by mixing BA extract with distilled water to obtain BA fruit concentrations (30, 60 and 90 mL L^{-1}) were then mixed with sugar[20,17] (3, 6 and 12 g of sugar, respectively) and stored in air tight glass bottles at 4°C before the treatments were used. Dimethoate 40% EC5l5l^{-1} of distilled water (v/v) was the positive control, while no BA extract (distilled water only) was the negative control. The treatments were replicated four times and arranged in a randomized complete block (RCBD). The experiment was repeated twice.

Planting: The seedlings were transplanted to the field at 4 weeks after seed emergence. Soil was pressed around the plant to close air pockets and light irrigation was done. The amounts of fertilizer used were 80 kg N ha^{-1}, 170 kg P_2O_5 ha^{-1} and 110 kg K_2O ha^{-1}. AN = 34.5% N, SSP = 18% P_2O_5, sulphate of potash = 50% K_2O_5. The plant spacing used was 90 cm inter-row × 30 cm in-row.

Treatment application: Spraying was done 2 h after the introduction of cabbage aphids. The control treatment was covered using plastic spray-shields to prevent movement of aphids to other treatments and to prevent chemical drifts[22]. The other 4 treatments were applied at the same time, each having their own applicator. One spray was done followed by 3 recordings which were taken at 6, 24 and 48 h after spraying. An amount of 34.5 kg N ha^{-1} was applied as top dressing fertilizer, 2 times at 3 week interval, starting from 2 weeks after transplanting.

A knapsack was used to apply the treatments[19,22] at a rate of 115 m^{-2}. At contact times of 6, 24 and 48 h after BA extract applications; physical counting of dead aphids was done on each plant sample. White papers were strategically placed underneath the plants for easy identification of the dead aphids; paper-position and mortality were strictly monitored. To check the lethality of the BA, the number of dead aphids was checked after every 1 h and recorded; the numbers were then added up to get the total for each of the periods (6, 24 and 48 h) earmarked as the contact times.

Statistical analysis: One-way analysis of variance (ANOVA) was carried out by using GenStat 14th Edition (VSN International, Hemel Hempstead, UK) to detect significant differences between treatments means. Separation of treatment means all treatments (including positive and negative controls) were then employed using the Turkey's HSD test ($p<0.05$).

RESULTS

Aphid mortality response to bitter apple treatment at 6 h: The BA treatment significantly ($p<0.001$) increased the number of dead aphids. At the highest concentration (90 mL L^{-1}), the highest number of dead aphids (13.00) was at 6 h (Table 2). However, the lowest BA concentration (30 mL L^{-1}) was not significantly ($p>0.05$) different from the negative control (no BA). The positive control (dimethoate 40% EC) showed higher aphid mortality (18.00) than the BA concentration (90 mL L^{-1}).

Table 2: Deleterious effect of the different treatment concentrations on cabbage aphid at 6 h

Bitter apple treatment concentrations (mL L^{-1})	No. of dead aphids at given contact time (h) from treatment time (6 h)
30	1.00^{d}
60	3.00^{c}
90	13.00^{b}
Positive control (dimethoate 40% EC)	18.00^{a}
Negative control (no bitter apple)	0.00^{d}
p-value	<0.001
$LSD_{(0.05)}$	1.779
CV (%)	9

Means within a column are compared and separated using Tukey's HSD test ($p<0.05$) and different alphabetic letters within a column indicate significant differences

Table 3: Deleterious effect of the different treatment concentrations on cabbage aphid at 24 h

Bitter apple treatment concentrations (mL L^{-1})	No. of dead aphids at given contact time (h) from treatment time (24 h)
30	3.25[c]
60	5.00[b]
90	18.50[a]
Positive control (dimethoate 40% EC)	20.00[a]
Negative control (no bitter apple)	0.00[d]
p-value	<0.001
$LSD_{(0.05)}$	1.664
CV (%)	6.6

Means within a column are compared and separated using Tukey's HSD test (p<0.05) and different alphabetic letters within a column indicate significant differences

Table 4: Deleterious effect of the different treatment concentrations on cabbage aphid at 48 h

Bitter apple treatment concentrations (mL L^{-1})	No. of dead aphids at given contact time (h) from treatment time (48 h)
30	3.25[c]
60	6.00[b]
90	20.00[a]
+ve control (dimethoate 40% EC)	20.00[a]
-ve control (no bitter apple)	0.00[d]
p-value	<0.001
$LSD_{(0.05)}$	2.096
CV (%)	7.7

Means within a column are compared and separated using Tukey's HSD test (p<0.05) and different alphabetic letters within a column indicate significant differences

Aphid mortality response to bitter apple treatment at 24 h: The BA treatment significantly (p<0.001) increased aphid mortality. Of all the BA treatments, 90 mL L^{-1} gave the highest number (18.50) of dead aphids which was not significantly different from the positive control. The highest BA concentration at 24 h had comparably higher aphid mortality than at 6 h (Table 3). The lowest BA concentration (30 mL L^{-1}) gave significantly (p<0.05) higher aphid death than the negative control.

Aphid mortality response to bitter apple treatment at 48 h: The BA treatment significantly (p<0.001) increased aphid mortality. The extract showed its highly efficacious effect by having even the lowest concentration (30 mL L^{-1}) having significantly (p<0.05) higher aphid death than the negative control where no BA was applied. This highest BA concentration had the highest number of dead aphids of all the other BA treatments at 48 h. There was no significant (p>0.05) difference between 90 mL L^{-1} treatment and the positive control (Table 4).

Means within a column are compared and separated using Tukey's HSD test (p<0.05) and different alphabetic letters within a column indicated significant differences.

DISCUSSION

The lowest concentration (30 mL L^{-1}) showed a slight repellent effect. This phenomenon happened might be because of the low concentration and the shortness of time aphids were exposed to BA. Higher concentrations were effective. Other workers have found these results in neem (*Azandirachta indica*) oil and nicotine[19]. The performance of neem oil as seed treatment against aphid (*Aphis gossypii*) on okra (*Abelmoschus esculentus*) crop and was tested by Gandhi *et al.*[23] who found excellent results. No environmental hazards after applying the biopesticides were reported. Studies showed that the biopesticides can be used in control of aphids as they are environmentally safe and have low toxicity to animals (including humans), fish and pollinators[24,25]. Studies done by Abdel-Rahman *et al.*[26] also confirmed these findings. The synthetic pesticide (dimethoate 40% EC) showed higher aphid mortality than the highest BA concentration's mortality[12]. This was also observed by Shiberu and Negeri[27], when neem seed, hop bush (*Dodonaea viscosa*) fresh leaf and lemon grass (*Cymbopogon citrates*) showed less effective control on cabbage aphid as compared to tested insecticides.

A high toxicity of hexane, diethyl ether, ethyl acetate, acetone and ethanol extracts of BA against adults of the cotton aphid was reported[28]. This clearly reflects that BA is highly efficacious in aphid control, although the synthetic chemical kills more aphids within a relatively shorter time in comparison to plant extracts applied at the same time length[3,17]. At 48 h after treatment, aphid mortality (at a treatment of 90 mL L^{-1}) was the highest of all the other treatments. In a different study, an overall performance of two consecutive sprays in two different locations against the population of cabbage aphid by insecticides gave as an average effect after a 3rd day of application[27]. However, the current work further showed that there was relationship between the efficacy of BA concentration and its contact time. Similar results were obtained by Mvumi and Maunga[17], who investigated the efficacy of lantana extract against aphids in rape (*Brassica nupas*) over varied periods of time and found that the extract was most efficacious at the longest contact time. This also agreed with the study on the efficacy of BA on bird cherry-oat aphid *Rhopalosiphum padi* done by other researchers[29]. Based on these results, it can be acknowledged that BA is lethal to cabbage aphid. Therefore, for BA, farmers should use 90 ml L^{-1} for 48 h-contact time in order to achieve the highest aphid mortality.

CONCLUSION

Considering the fruit concentrations tests of efficacy at 6, 24 and 48 h contact times, the fruit concentrations revealed highly significant cabbage aphid mortality at the longest contact time (48 h). Therefore, this can be considered the best contact time to control cabbage aphid when using BA fruit extract.

SIGNIFICANCE STATEMENT

This study discovered the biopesticide (bitter apple) extract that can be beneficial for aphid control in cabbage whereby, among the three different bitter apple fruit solution concentrations used, the 90 mL L^{-1} at 48 h proved to be highly effective against the aphids, in comparison to the other shorter times (6; 24 h). This study will help the researchers to uncover the critical areas concerning plants which are pesticidal and the length of contact time to cause lethality. Flora distribution is biome-specific, such that; many researchers were not able to explore the pesticidal potential of bitter apple. Thus a new theory on research-based identifications of local plants being potentially pesticidal to common pests in the area may be arrived at.

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