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Efficacy assessment of two biopesticides in control of *Aphis gossypii* Glover damaging cucumber (*Cucumis sativus* L.) in Benin

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Abstract

The production of cucumber (*Cucumis sativus* L.) is hampered by several constraints that include pest attacks which negatively affect its production in quantity and quality. Our study aimed to promote the use of biopesticides to control the attack of *Aphis gossypii* Glover through an assessment of the efficacy of two biopesticides on the infestation and yield components in Benin. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Two varieties of cucumber (Nandini 732F1 and Poinsett+) were grown and submitted to three treatments: (i) *Control*: no pesticide was sprayed, (ii) *Neem*: plants were sprayed with 2 L/ha of neem oil at seven day interval sand (iii) *CNSL*: plants were sprayed with 2 L/ha of *CNSL* as prior. Data recorded were the number of infested leaves and female flowers, leaf infestation rate, weight, length and median diameter of fruits, and the yield. Results showed that the *CNSL* treatment was more effective than that of neem oil on leaves and flowers.The yield lost due to the insect were 59.12% and 62.41% for Nandini 732F1 and Poinsett+, respectively. Farmers could valorise the cashew shell considered as agricultural waste to produce the *CNSL* against *A. gossypii*.

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Introduction

Agriculture is part of main sectors of activity source of employment in Benin (Soulé, 2004). It contributes to the socioeconomic development of populations, mainly in rural areas (IFM, 2017). The country agriculture employs approximately 70% of the active population and contributes to 33% of the Gross Domestic Product (MAEP, 2017). Vegetable production is an important sector of Beninese agriculture, since it provides different types of crops (roots, fruits, leafy, etc.) to improve nutrient intake of the local population, whose staple food consists mainly of carbohydrates such as yams, cassava, maize and millet (Yolou *et al.*, 2015). Moreover, incomes generated by this activity help several hundred families to meet their daily needs.

Cucumber (*Cucumis sativus* L.) is among vegetables grown by Beninese farmers. Several varieties of cucumber are available and grown in the country. The production of cucumber is challenged by several constraints including pest infestations. Some of pest attacks relevant to the whitefly (*Bemisia tabaci* Gennadius), weevil (*Hypolixus nubilosis* Boheman) and cotton aphid (*Aphis gossypii* Glover) were reported in West Africa including Benin (James *et al.*, 2010). These pests can highly affect the yield and quality of cucumber fruits.

The cotton aphid, *Aphis gossypii* Glover is a small insect (1-1.5 mm in body length) distributed worldwide. The pest species includes a wide range of host crops that belongs to over 92 plant families (Ebert and Cartwright, 1997).

This includes important food crops, fibre plants and ornamental plants. The pest can directly damage several host plants or transmit plant viruses. Previous research mentioned that *A. gossypii* is a vector of cucumber mosaic virus, potato leafroll virus and yam mosaic virus (Ebert and Cartwright, 1997). Several chemical insecticides are often used by growers to protect plants against attacks of the cotton aphid. Nowadays, resistance is found in the species to chemicals as Cyhalothrin and Demeton (Whalon, 2003). Biological control methods that consist in the use of natural enemies and sustainable methods to control *A. gossypii* were also studied. El Habi *et al.* (1999) showed that *Coccinella septempunctata* reduced *A. Gossypii* populations on cucumber in the greenhouse.

This result was confirmed by Eid *et al.* (2018), who demonstrated that the release of more than 10 individuals/m^{2}of *C. septempunctata* significantly reduced the infestation of *A. gossypii* on cucumber crop in greenhouse. Those previous authors recommended the use of the parasitoid *Aphidius colemani* (more than 4 individuals/m^2) to control infestations of the cotton aphid. Moreover, other researchers found out that the release of both predators *Chrysoperla carnea* (1:5) and *Coccinella undecimpunctata* (1:50) eliminated the cotton aphid in okra (Zaki *et al.*, 1999).

Due to the high cost and technical constraints related to the use of predators, this biocontrol method is not affordable to Beninese growers. In addition, the high price of biopesticides recommended to vegetable crops obliges growers to buy chemical pesticides applied on these plants most of time cultivated in the street, or in neighbouring houses (Mondédji, 2010). However, the inappropriate use of chemical pesticides has several consequences on growers, consumers and environment (Williamson *et al.*, 2008). To avoid this risk in the application of chemicals, it is important to encourage the production and use of biopesticides by farmers.

This study aimed to promote the use of biopesticides to control *Aphis gossypii* infestation on two varieties of cucumber in Ketou municipality in Benin (West Africa).

Material and methods

Study area

The study was conducted in the farm ECO-FARM-NIRMALA in the municipality of Ketou in southern Benin (West Africa). This municipality is located in latitudes 7°10 and 7°41'17"Nand longitudes 2°24'24" and 2°47'40"E. It is characterized by a bimodal rainfall regime with the rainy seasons ranging from March to July (major rainy season) and September to November (minor rainy season). The mean annual rainfall of the municipality is about 1073 mm and the average annual temperature is around 25 °C. Soils are mainly ferrallitic.

Plant material and experimental design

This study was conducted from September to December 2020. Two elite varieties of cucumber grown by Beninese farmers, namely Nandini 732 F1 (variety 1) and Poinsett+ (variety 2) were used. Each of the two varieties was subjected to three (03) treatments: *(i) Control*: no pesticide applied, (*ii*) *Neem*: plants were sprayed with neem oil at a dose of 2 l /ha each single seventh day from the fourteenth day after sowing to the end of the plant life cycle; (*iii*) *Cashew Nut Shell Liquid* (CNSL): plants were sprayed with the Cashew Nut Shell Liquid at a dose of 2 l /ha as prior. The oil neem used is produced and traded by the society Biophyto (Benin), while the CNSL used was produced by heating 5 kg of crushed cashew kernels in 1 l of peanut oil for 45 minutes and pressed. Both biopesticides were sprayed with a hand-operated sprayer. The experimental plot was submitted to natural infestation of the aphid *Aphis gossypii*.

Treatments were arranged in a randomized complete block design with three (3) replications. Therefore, the experimental plot was subdivided into 18 subplots (2 varieties x of 3 treatments x 3 repetitions) of 3.6 m^2 (3 x 1.2 m) each. Subplots were separated 1 m from each another. Three cucumber seeds were sown per hole on 20 September 2020. Plantlets were thinned to one per hole at two weeks after sowing. Each subplot consisted of 12 plants distributed on two rows with a row to row spacing of 1 m. onto the same row, plant spacing was 50 cm. The pre-plant fertilization consisted in applying 1.2 kg of rabbit compost per hole. Fifteen days after the emergence, 0.2 kg of the same compost was also applied per hole. Weeds were cleaned and collected from the experimental plot manually.

Data collection and analysis

The parameters recorded during the assessment are:
(1) The number of infested leaves and rate of leaf infestation due to *Aphis gossypii*: the number of leaves infested by *A. gossypii* was counted every seven days by subplot from 18 days after sowing until 46 days after sowing. The rate of leaf infestation was measured based on the presence of galls. The biting of *A. gossypii* induces the appearance of galls, which results in the deformation of the leaves (Lopes, 2007). Fifteen (15) leaves were randomly chosen per subplot, with average of 1 to 2 leaves per plant, while the rate of infestation was recorded using visual observation as follows. Infestations were categorized into three levels: (*i*) Low: when galls cover less than 1/4 of the leaf area; (*ii*) Moderate: when galls cover between 1/4 and 1/2 of the leaf area and (*iii*) Severe: when galls cover more than the 1/2 of the leaf area.

(2) The number of infested female flowers: female flowers were counted every seven days per subplot, from the beginning of the blooming during three (3) weeks.

(3) Fruit characteristics and yield: at the harvest, 10 fruits were randomly chosen per subplots and measured. Doing so, 30 fruits were measured per treatment. The weight was recorded with an electronic scale (accuracy ± 1 g) and the length with a graduated ruler (precision ± 1 mm). The median diameter of fruits was measured with a digital calliper (precision ± 1 mm). The yield was calculated using the total weight of fruits harvested per subplot.

A two-way analysis of variance (ANOVA) was performed to compare the mean value of the number of infested leaves, the number of infested female flowers, the weight of fruits, the median diameter of fruits, the length of fruits and the yield. Percentages were estimated for the rate of infestation by treatment while z-tests were run to compare them.

All the Analyses were performed using the software SigmaStat 3.5 at a significance level of 5% while the graphs were drawn using Excel 2007.

Results

Number of infested leaves and leaf rate infestation

Except during the first week of assessment, statistical differences were found between treatments along days of assessment (figure 1). The number of infested leaves was overall low on planted sprayed with the CNLS followed by those sprayed with the neem oil. Similarly, leaves of the V1 were less infested from day 32 to day 46. Plants treated with the CNSL overall showed the lowest rate leaf infestation independently of the varieties (figure 2). The percentages of infested leaves were 87%, 74% and 58% on the Control, neem oil and CNSL, respectively. Treatments applied were statistically different. A significant difference was also found between the two varieties used (V1= 69% *vs*. V2= 77%; Z=3.3; $p < 0.001$).

Table 1. Results of comparison of fruits characteristics and yield.

Treatments	Fruit length (cm)	Fruit weight (g)	Fruit median diameter (mm)	Yield (T/ha)
V1Neem	21.12 ±0.94b	461.67 ±35.54b	59.37 ±0.75b	9.46 ±0.80b
V1CNSL	22.99 ±0.18a	555.83 ±41.42a	63.13 ±2.25a	15.14± 0.37a
V1Ctrl	19.56 ±0.60c	380.433 ±33.98c	56.14 ±0.93cd	6.19 ±1.05c
V2Neem	18.06 ±0.66d	333.13 ±42.14cd	57.77 ±1.48bc	5.55 ±0.19c
V2CNSL	20.39 ±0.34bc	478.06 ±3.75b	63.12 ±0.79a	8.94 ±0.68b
V2Ctrl	16.75 ±0.74e	297.36 ±8.12d	53.76 ±1.69d	3.36 ±0.16d

* Values within columns with different letters are significantly different at $p < 0.05$.

The number of infested female flowers

There is a statistical difference between treatments (df = 2; F = 9.56; $p < 0.001$). Application of biopesticides induced a lower number of infested flowers on plants sprayed in CNSL and the neem oil treatments on both two varieties (figure 3). A significant difference was found between the two varieties (df = 1; F = 4.21; $p = 0.046$) with 3.63 ± 1.38 and 5.44 ± 1.37 as mean number of infested female flowers found on V1 and V2, respectively.

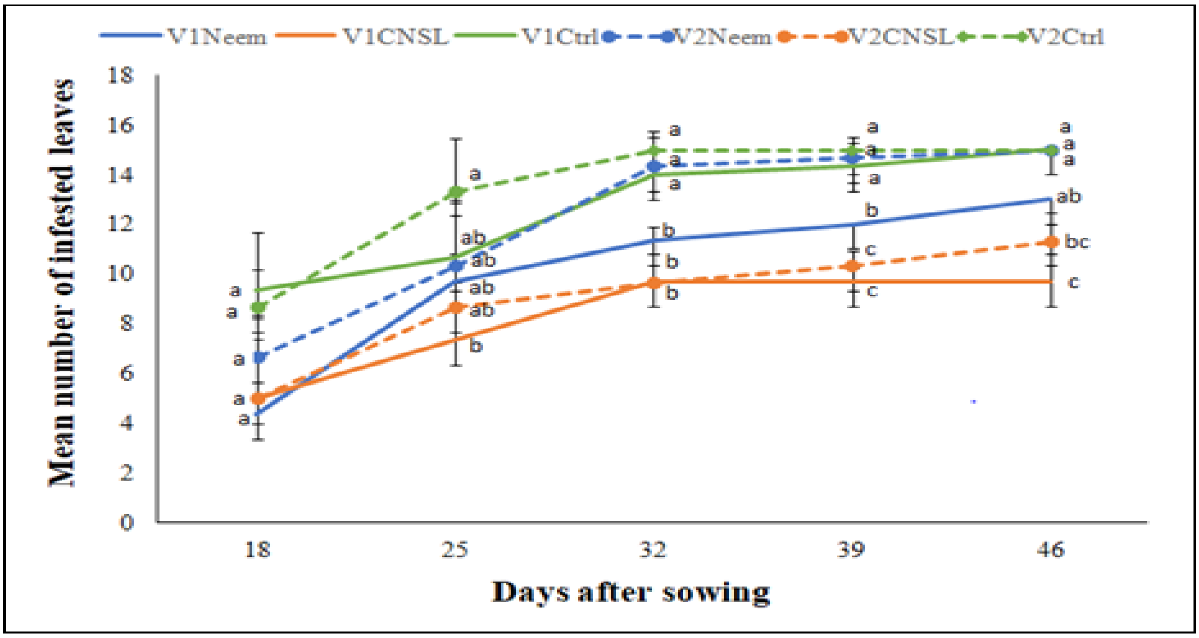

Fig. 1. Mean number of infested leaves per treatment.

Fruit characteristics and yield

Table 1 shows the mean values of several parameters recorded on fruits as the length of fruit, the diameter fruit, the weight of fruit and the yield. Significant differences were obtained between the length of fruits of the two varieties (df =1; F = 121.27; $p < 0.001$) and between treatments (df = 2; F = 60.01; $p < 0.001$). Fruits of V1 = 21.20 ± 0.99 cm were longer than those of V2 = 18.41 ± 1.06 cm. Fruits harvested on CNSL and the neem oil treatments were longer than those harvested on control plants, with 21.69 ± 1.29, 19.59 ± 1.53 and 18.16 ± 1.40 for CNSL, neem oil and Control treatments, respectively. There was a significant difference between treatments for the mean diameter of fruits (df =2; F = 51.27; $p < 0.001$). Widest fruits were obtained on plots sprayed with biopesticides with 63.12 ± 0.005, 58.56 ± 0.85 and 554.995 ±1.19 or CNSL, neem oil and Control treatments, respectively. The widest fruits were also obtained on V1 (58.22 ± 2.02 mm) against 59.62 ± 2.71.mm on V2. Results showed a significant difference was between treatments for the weight of fruit (df = 2; F = 57.29; $p < 0.001$) and between varieties as well (df = 1; F = 52.81; $p < 0.001$). Fruits of V1 have heavy weight (470 ± 50.68g) than those of V2 (370 ± 55.25 g). Fruits harvested on plots sprayed with the CNSL were heavier than those harvested from plots sprayed with the neem oil, which were heavier than those harvested from controlled ones.

The highest yield was recorded on V1 (10.26 ± 2.61 tons/ha) while the lowest was recorded on V2 (5.95 ± 1.62 tons/ha). Treatments increased the plant yield with 12.04 ± 3.09 tons/ha, 7.50 ± 1.95 tons/ha and 4.77 ± 1.41 tons/ha recorded on CNSL, neem oil and control treatments, respectively.

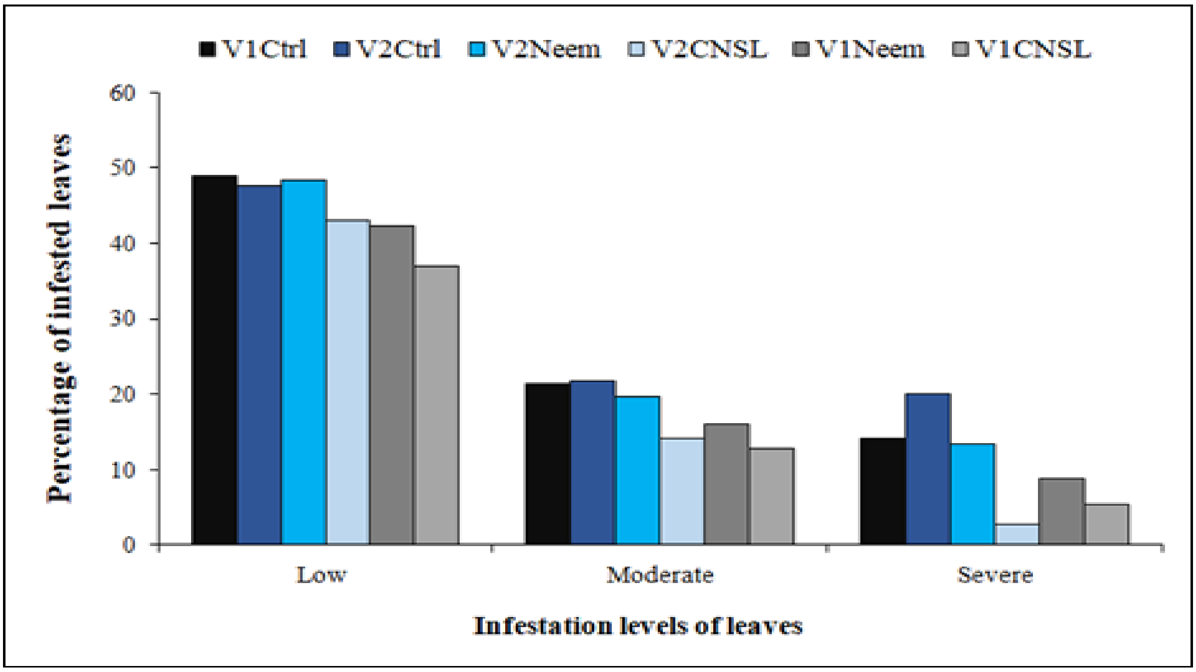

Fig. 2. Rates of infestation on leaves per treatment.

Discussion

This study demonstrated that the neem oil and the cashew nut shell liquid (CNSL) reduced the populations of *Aphis gossypii* on cucumber plants. The percentage of infested leaves and flowers significantly decreased under the effect of these biopesticides. The effect of the neem oil on the aphid *A. gossypii* is undoubtedly due to its composition in azadirachtin, which is the primary active ingredient of most neem-based pesticides (Shannag *et al.*, 2015). It is considered as the key responsible for its toxic effects in insects (Mordue and Nisbet, 2000). Previous studies showed that this component adversely affects different physiological events of a wide range of insects (Morgan, 2009). More specifically, dos Santos *et al.* (2004) showed that aqueous extract of neem seeds at different concentrations caused nymph mortality and reduced the survival period and fecundity of *A. gossypii*. The response of *A. gossypii* to the cashew nut shell liquid could be related to its toxic components. Indeed, the CNSL extracted via the heating process contained anacardic acids, cardanols and cardols (Trevisan *et al.*, 2006).It was revealed that the aforementioned constituents of the CNSL as well as the CNSL had lethal effects on the eggs and larvae of *Musca domestica* L., *Chrysomya megacephala* Fabricius and *Spodoptera frugiperda* J.E. Smith (de Carvalho *et al.*, 2019). The combine effect of these different toxic constituents of CNSL may explain the best effect of this biopesticide on the aphid *A. gossypii* when applied at the same dose. Despite the good performance of CNLS on the infestation of cucumber plants, it is necessary to investigate its optimal dosage to guarantee a good benefit for cucumber growers.

The adverse effect of the two biopesticides on the aphid infestation levels resulted in the improvement of fruits characteristics and accordingly to a yield increase.

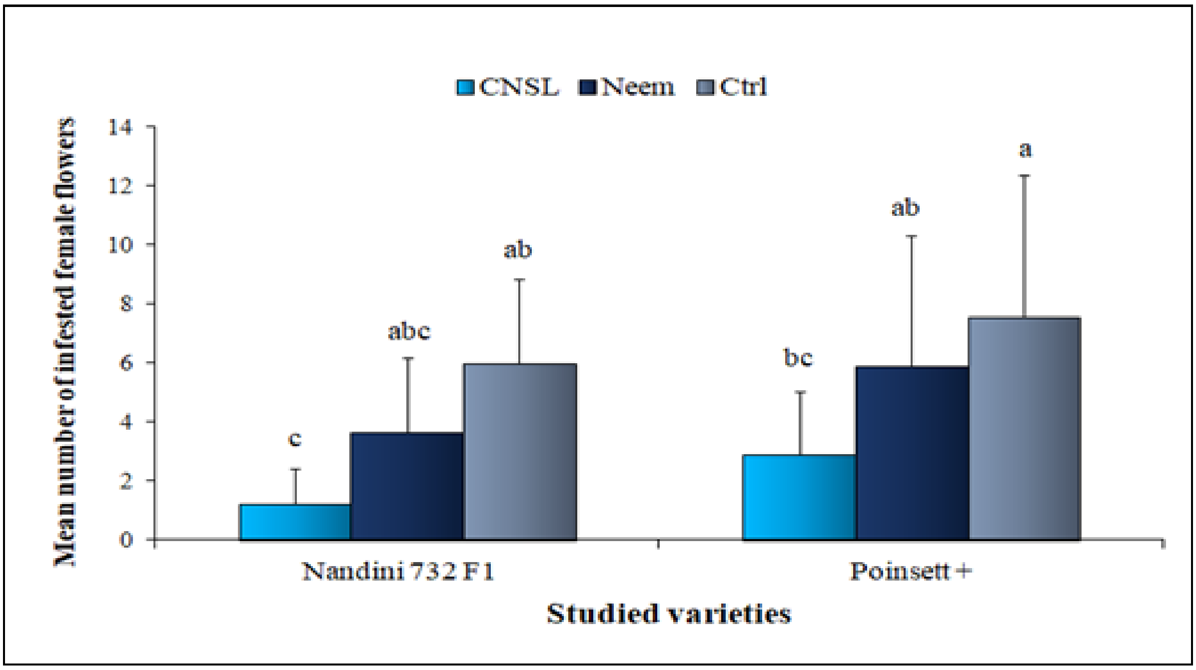

Fig. 3. Mean number of infested female flowers per variety and per treatment.

The weight, length and diameter of fruits sprayed with these products were significantly greater than those harvested on control plants. Additionally, more spoiled fruits were reported on control subplots. *Aphis gossypii* decreases plant growth by sucking the snap, transmitting viruses and producing honeydew on leaves, which can contaminate fruits in the case of a moderate or high density of the aphid (Razmjou *et al.*, 2012). In this case, plants with less infested leaves and female flowers and a lower rate of infestation perform better than those with a high population of aphids. Despite the efficacy of the CNSL and the neem oil, it is necessary to provide sufficient nutrients (*eg.* vermicompost) to cucumber to improve the plant growth and develop resistant varieties to pathogens and insects (Razmjou *et al.*, 2012). This study revealed that V1 had the lowest number of infested leaves and flowers, and lower effect of aphid was recorded on yield. The yield reduction in V1 was estimated at 59.12% while that in V2 was 62.41%.This result suggests that V1 is less susceptible to the aphid than V2. Besides, it is reported that *A. gossypii* is a serious biotic constraint to cucumber production in several countries including Benin. Many growers from the studied area also considered this aphid as a major constraint to cucumber production in this location. The difference between varieties on the number of infested leaves and female flowers could be due to the variation in the amount of cucurbitacin C (a toxic substance) among varieties (Gould, 1978; Horie *et al.*, 2007). Ahmed (1994) also mentioned that the low protein and amino acid contents of leaves in certain varieties could be the reason of their resistance to some pests such as *A. gossypii* and *Bemisia tabaci*. Those chemical contents also affected the reproductive biology, the total longevity of

A.gossypii, and reduced its population on certain varieties (Abdel-Hafiz, 2008). Some aphid resistant cucumber lines were identified, and studied to understand the resistance process, and further exploit them to develop breeding resistant varieties (Liang *et al.*, 2015; Liang *et al.*, 2016).

Fruit quality characteristics as the weight, length, median diameter under control were less affected inV1 than that recorded in V2. However, the highest score reported in V1 on traits above mentioned cannot exhibit a resistance to *A. gossypii* because V2 is known for its smaller fruits. Varietal description data reported that fruits from V1 measure 22 cm in average while these from V2 measure 20 cm in average. In a nutshell, this study recommends the cultivation of V1 to Beninese growers, because of its weak susceptibility to *A. gossypii* and its higher yield. The CNSL can be considered as an effective natural product able to control infestation of the aphid.

This study encourages to increase the production and transformation of cashew nut to have as much as possible the CNSL available in Benin. To cover the country needs in CNSL, it needs to reinforce its own production, despite being among the ten ranked producers of cashew nuts in the world during the period 2009-2019 (FAOSTAT, 2021). Other research can focus on specific components effective to control *A. gosssypii* at different stages. Studies above suggested will help to identify the most active components in the CNSL useful against *A. gossypii.*

Conclusion

At the end of this study, it appears that both V1 and V2 are susceptible to *Aphis gossypii*. The first one was less attacked and showed a lower reduction in yield. The neem oil and CNSL significantly reduced populations of *A. gossypii* on cucumber plants. The application of CNSL was more efficient than that of neem oil. However, both of them are good alternatives to the chemical control of this aphid species. Further studies may be implemented to determine suitable dosages before releasingend products towards cucumber growers.

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