

# Monitoring and spatial distribution of the red scale insect *Aonidiella aurantii* (Hemiptera: Diaspididae) infesting guava trees

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**Abstract:** The red scale insect *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) is a major pest of guava trees in different parts of the world. This study aims to determine the population abundance and spatial distribution pattern of *A. aurantii* during the two successive growing seasons of 2022–2023 and 2023–2024. This pest was surveyed every two weeks in a private guava grove located in the Armant district of the Luxor region of Egypt. The results showed that individuals of *A. aurantii* were found on the leaf surfaces of guava trees at varying densities throughout the year. In the south-eastern site, where population density remained continuously high during the two years of the study, the pest favoured the upper leaf surface in the basal canopy layer. In this context, the spatial distribution pattern of *A. aurantii* at all sites on the guava tree was aggregated using dispersion measures over the two years. To distinguish the estimates of *A. aurantii* individuals in the sixteen coordinates studied, the cluster analysis method was used in conjunction with correlation analysis. Principal component analysis was performed, followed by two-dimensional analysis of sixteen coordinates to establish their correlation. Based on the findings of this study, an integrated pest management strategy can now be developed to help mitigate pest populations of *A. aurantii* found in guava tree orchards.

**Keywords:** guava tree, population abundance, sampling program, scale insect pest, spatial distribution

## INTRODUCTION

The red scale insect, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), is a common pest that feeds on many kinds of plant species (Grafton-Cardwell *et al.*, 2021), especially guava trees (Salman *et al.*, 2022). It causes serious damage to their leaves, stems, fruits, and other tree components (Miller and Davidson, 2005). When this pest infests guava leaves, it feeds by drawing plant sap from parenchyma cells and inserting poisonous saliva (Abd-Rabou, 2009; Abdel-Rahman, 2021). Feeding damage caused by the pest can result in tree malformations such as: branch death, diminishing fresh branches, insufficient blooming, smaller fruits, unusual development in young plants, leaf yellowing, leaf drop, and younger tree mortality (Bakry, 2009; Bakry and Mohamed, 2015; Gaber, Ghanem and Ali, 2024).

Armoured or hard-scale insects, such as the red scale, lay their eggs beneath the protective test (hard covering over the adult female armoured scale), which protects them throughout the winter. These eggs hatch into mobile crawlers in the spring (Hang, 2012). Tree cardinal orientations affect male flight and movement behaviour, with east-west orientations being the most common. Scale insects prefer habitats in warm climates with an average temperature of 25–30°C; however, they can survive in an environmental temperature of 10–35°C, as well as moderate to high humidity levels of 59–71%. They are most active during the period from spring to summer when temperatures and host availability are conducive as noted above. In addition to abiotic environmental conditions, the pest's dispersal behaviour helps to provide effective monitoring tactics and methods (Draz *et al.*, 2011; Karar *et al.*, 2013; Bakry and Abdel-Baky, 2020).

A population's distribution pattern is influenced by its interactions with the surrounding environment. Therefore, recognising this pattern allows for a more thorough and precise identification of the ecospecies. In addition, the type of pest and treatment strategies considered will determine the most appropriate sampling method for collecting specific data. Understanding the distribution status also facilitates informed decision-making by providing vital information into how the pest interacts with its ecosystem (Bancroft, 2005). Although considerable literature exists regarding the spatial distribution of *A. aurantii*, the mechanisms influencing its dispersion, spread, and distribution still need to be examined. According to D'Auria *et al.* (2016), studying the abundance of pest populations across different seasons is essential for successful integrated pest management programmes because they can enable prompt surveillance and application of control techniques regardless of the season.

One of the main defining traits of insect populations is their spatial distribution (Wearing, 1988; Cho *et al.*, 2001). It is a prominent and common ecological feature in many insect populations (Debouzie and Thioulouse, 1986). Field sampling can only be effective once the underlying spatial distribution is fully elucidated and understood (Taylor, 1984). Therefore, understanding the seasonal behaviour of an insect population, along with selecting the most appropriate field monitoring method, can offer a major benefit whether a population is distributed in an aggregated, regular, or random pattern (Binns, Nyrop and Werf van der, 2000).

The way a population interacts with its surroundings determines its distribution pattern. A clearer understanding of population growth and behavioural trends across different seasons enables more accurate and precise identification of the populations under study (Arbabsafti, Fathipour and Ranjbaram, 2021). Therefore, this precision in collecting essential data can be accomplished using modelling. Modelling depends on choosing the best sampling technique or method for obtaining vital information required for the model design. In addition, it is necessary to design appropriate sampling plans to be conducted when determining spatial distribution patterns of a population across seasons (Rajabpour and Yarahmadi, 2024). The sampling technique or method used in pest management agroecosystems is selected based on the type of pest and the management tactics to be employed (Surendra, 2019). Understanding the distribution status of a pest population also helps to comprehend how it interacts with the biotic and abiotic components of its agroecosystem (Verberk, 2011). Additionally, it provides valuable information on spatial variation and supports the design of appropriate sampling methods (Tsai, Wang and Liu, 2002). A key component of successful and cost-effective pest management programme requires the adoption of appropriate sampling techniques or methods. Data generated serve as a foundation for economically sound decision-making (Adam *et al.*, 2010).

Numerous variables, including environmental parameters, the presence of a suitable habitat and niche, the abundance and availability of food supplies, endemic intrigued competition, and human activity, can affect an insect's distribution (Jactel, Koricheva and Castagnayrol, 2019; Zhao *et al.*, 2023) and rate of spread (Lehmann *et al.*, 2020; Rajabpour and Yarahmadi, 2024). For managing insect populations, whether in agricultural, conservation, or public health contexts, it is essential to understand the above mentioned aspects for their abundance and

prevalence. Principal Component Analysis (PCA) is a sophisticated method used on behalf of dimensionality reduction and multivariate analysis (Greenacre *et al.*, 2022). Notable uses of the method include data compression, image processing, visualisation, exploratory data analysis, pattern identification, and time series prediction (Lv, Yi and Li, 2014). The PCA method reduces the number of features in a dataset while conserving the original information. Principal component analysis is widely used due to its three crucial features. The proposed linear scheme is an optimal method for compressing a collection of high-dimensional vectors into a group of low-dimensional vectors and subsequently rebuilding the original set, as measured by mean squared error. Furthermore, it is possible to calculate the model parameters directly from the data, similar to the method of digitising the sample covariance matrix (Wang and Battiti, 2005). This is achieved by identifying the directions of maximum variation in the data and projecting the data onto a new space with fewer dimensions (Maćkiewicz and Ratajczak, 1993).

The purpose of this study was to examine *A. aurantii* population density and its spatial distribution pattern in various guava tree coordinates, utilising similarity analysis, principal component analysis (PCA), and hierarchical cluster analysis (HCA). The academic literature contains scant information on this topic. Therefore, the present investigation aims to identify appropriate sampling strategies for *A. aurantii* in a guava plantation and to determine the key variables affecting its population density and spatial distribution.

## MATERIAL AND METHODS

### POPULATION ABUNDANCE OF *Aonidiella aurantii* INDIVIDUALS ON GUAVA TREES

**Location of experiment.** The study was conducted on ten guava trees ('Balady' cultivar) in a private grove located in Armant, Luxor region, Egypt (25°37'50"N, 32°29'52"E) between the beginning of June 2022 to the middle of May 2024. The trees were eight years old, approximately five metres tall, and naturally infested with *A. aurantii*. They were grown under uniform farming methods and similarly selected based on their size, height, and phenology. No chemical treatments or pesticides were applied prior to or during the experimental period.

**Methods of sampling.** Ten trees were sampled every two weeks over two consecutive years (2022–2023 and 2023–2024). Four fundamental quadrants (directions) were applied to each group of chosen trees: southeast (1), southwest (2), northeast (3), and northwest (4). Additionally, each tree was divided into two strata: base (trunk) i.e. ≤1.5 m above the soil surface, and the apical parts (leaves) of the tree which are more than 1.5 m above the soil surface based on the tree's height and structure. Five leaves were chosen at random and gathered from each quadrant and strata. Ten replicates of a split-split-plot design were used, with the main plots distributing the orientations (southeast, southwest, northeast, and northwest), the split plots consisting of the tree layers (apical and basal), and the sub-split-plots consisting of the upper and lower leaf surfaces. Therefore, over the two years, all specimens were taken from the terminal shoots of 19,200 leaves (5 leaves × 4 quadrants × 2 levels × 48 trees × 10 trees). There were 9,600 leaves sampled annually. After the leaves

were removed from the trees, they were placed in resealable plastic bags, and transported to the laboratory of Plant Protection Research Department at the El-Mattana Agricultural Research Station, Luxor, Egypt, for leaf examination, so that the scale insects could be examined using a stereo zoom microscope (model: NTB-3A/C, power: 220 V 50HZ, made in China, Novel company, location: China) at a 10× magnification. Professor Dr. Fatima Mahroum from the Agricultural Research Center, Plant Protection Research Institute, Giza, Egypt, classified the pest insect. Both upper and lower leaf surfaces were inspected. Individuals were classified into female stages, which includes both gravid and virgin females, along with the nymphal stage, which included pre-adults and crawlers. The temporal population oscillation of *A. aurantii* was determined by analysing guava leaf data every two weeks.

### ESTIMATING OF THE INITIAL SAMPLING AND DISTRIBUTION INDICES

Several dispersion or aggregation indices were applied to measure the spatial distribution pattern of *A. aurantii* populations per leaf. Each sampling unit was composed of leaves taken in all directional combinations (noted above) from the canopy, vertical strata, and leaf surfaces of guava trees in 2022–2023 and 2023–2024 as described above. The data collected from population counts were collated and various statistical parameters calculated.

#### Initial sampling

Sample means, variances, and relative variance were estimated. The relative variance (RV) (Hillhouse and Pitre, 1974) was used to compare the effectiveness of different sampling methods.

The relative net precision (RNP) was calculated according to Equation (1):

$$RNP = \frac{100}{RV \cdot CS} \quad (1)$$

where: CS = time that was used to collect, identify, and record a sample for sixty minutes, RV percentage = standard error divided by the population mean times 100.

The optimal movement pattern in the field can be determined by comparing any pattern that has the lowest RV and the maximum RNP (Rajabpour and Yarahmadi, 2024).

#### Distribution indices

To estimate spatial distribution indices for *A. aurantii*, different dispersal indices have been used, such as variation to the mean (Patil and Stiteler, 1974), Lewis index, Cassie index (Cassie, 1962), mean crowding (Lloyd, 1967), mean clumping (David and Moore, 1954), and patchiness index (Lloyd, 1967).

**Variation to mean.** This parameter is calculated using Equation (2).

$$\text{Variation to mean} = \frac{S^2}{\bar{X}} \quad (2)$$

where:  $S^2$  = population variation,  $\bar{X}$  = mean value of population.

The values lower than 1 indicate uniform distribution, values equal to one indicate random distribution, and values >1 indicate aggregated distribution.

**Lewis index ( $I_L$ ).** When using the  $I_L$ , the resultant values <1 indicate uniform distribution; values equal to 1 indicate random distribution, and values >1 indicate aggregated distribution.

$$I_L = \sqrt{\frac{S^2}{\bar{X}}} \quad (3)$$

**Cassie index ( $Ca$ ).** It is a measure of dispersion that helps determine whether individuals are clustered together or distributed randomly, or uniformly. The values of  $Ca$  and mean clumping ( $I_{DM}$ ) help determine whether the distribution is regular (<0), random (= 0), or clustered (>0).

$$Ca = \frac{S^2 - \bar{X}}{\bar{X}^2} \quad (4)$$

$$I_{DM} = \frac{S^2}{\bar{X}} - 1 \quad (5)$$

**The  $K$  value** of the negative binomial distribution is commonly used to quantify the degree of aggregation in insect populations exhibiting a clumped or aggregated spatial pattern. When  $K$  values are low and positive,  $K = (0; 2>$ , they indicate a highly aggregated population;  $K$  values ranging from 2 to 8 indicate moderate aggregation; and  $K > 8$  indicate a random population (Southwood, 1995). The  $K$  values were calculated using the method of moments (Costa *et al.*, 2010), and given by:

$$K = \frac{\bar{X}^2}{S^2 - \bar{X}} \quad (6)$$

Departure from a random distribution can be tested by calculating the  $I_D$ :

$$I_D = \frac{(n-1)S^2}{\bar{X}} \quad (7)$$

where:  $n$  = number of samples and  $I_D$  is approximately distributed as  $\chi^2$  with  $n-1$  degrees of freedom; values of  $I_D$  that fall outside a confidence interval bounded with  $n-1$  degrees of freedom and selected probability levels of 0.95 and 0.05, for instance, would indicate a significant departure from a random distribution.

This index can be tested with the  $Z$  value as follows:

$$Z = \sqrt{2I_D} - \sqrt{(2\nu - 1)} \quad (8)$$

where:  $\nu = n - 1$ .

If  $Z$  is between 1.96 and -1.96, the spatial distribution would be random, but if  $Z < -1.96$  or  $Z > 1.96$ , it would be uniform or aggregated, respectively (Patil and Stiteler, 1974).

**Mean crowding ( $\bar{X}^*$ )** (Lloyd, 1967):

$$\bar{X}^* = \bar{X} + \left( \frac{S^2}{\bar{X}} - 1 \right) \quad (9)$$

**Patchiness index ( $I_p$ ):**  $I_p < 1$  indicate uniform distribution,  $I_p = 1$  indicate a random distribution, and  $I_p > 1$  indicate an aggregated distribution.

$$I_P = \frac{\bar{X}^*}{\bar{X}} \quad (10)$$

Green's index (GI):

$$GI = \frac{(S^2/\bar{X}) - 1}{n - 1} \quad (11)$$

This index is a form of the  $n$ -independent cluster size index (Green, 1966). Aggregation dispersion is indicated by  $GI > 0$ , uniformity or regular dispersion is indicated by  $GI < 0$ , and randomness is indicated by  $GI = 0$ .

Using an **aggregation index** ( $1/K$ ), the study assessed fluctuations in the distribution of the population estimates across the examined years (Southwood and Henderson, 2000). The equation that follows was used to determine the aggregation index:

$$1/K = \frac{\bar{X}^*}{\bar{X}} - 1 \quad (12)$$

where:  $\bar{X}^*$  = Lloyd's patchiness index,  $1/K$  = aggregation index (Cassie's index); according to Feng and Nowierski (1992), the values of  $1/K < 0$ ,  $1/K = 0$ , and  $1/K > 0$  signify the population's regularity, random, and aggregation in the spatial pattern, respectively.

**Examination of aggregation causes.** In order to analyse the potential phenomena for the insect population's aggregated state, the population aggregations mean ( $\lambda$ ) (Blackith, 1961) was computed as follows:

$$\lambda = \frac{m}{2K} \gamma \quad (13)$$

where:  $m$  = mean of population, when the degree of freedom value is  $2K$ ,  $\gamma$  equals  $X^2_{0.5}$ . When  $\lambda < 2$ , the environment causes the aggregation of insect individuals; if  $\lambda > 2$ , the phenomenon is generated by the aggregation behaviour or the aggregation behaviour in conjunction with the environment (Li *et al.*, 2017).

## PRINCIPAL COMPONENT AND CLUSTER ANALYSIS

Principal component analysis (PCA) was used to simplify and summarise important information from the dataset by linearly transforming the original variables into orthogonal principal components, with the former capturing maximum variance (Jolliffe, 2005; Bro and Smilde, 2014). This process helps reduce dimensionality and identify underlying patterns. A two-dimensional PCA plot was extracted to visualise the multiple dimensions of *A. aurantii* estimates at different guava tree coordinates in a scatter plot using R software (R Core Team, 2022).

Based on the similarity matrix, a dendrogram was constructed showing the relationships between *A. aurantii* estimates at the different tree coordinates. Hierarchical clustering analysis (HCA) was performed using the unweighted pair group method with arithmetic average (UPGMA) based on the Euclidean distance between the groups. The analysis was conducted using the PAST software package (Hammer, Harper and Ryan, 2001).

## RESULTS AND DISCUSSION

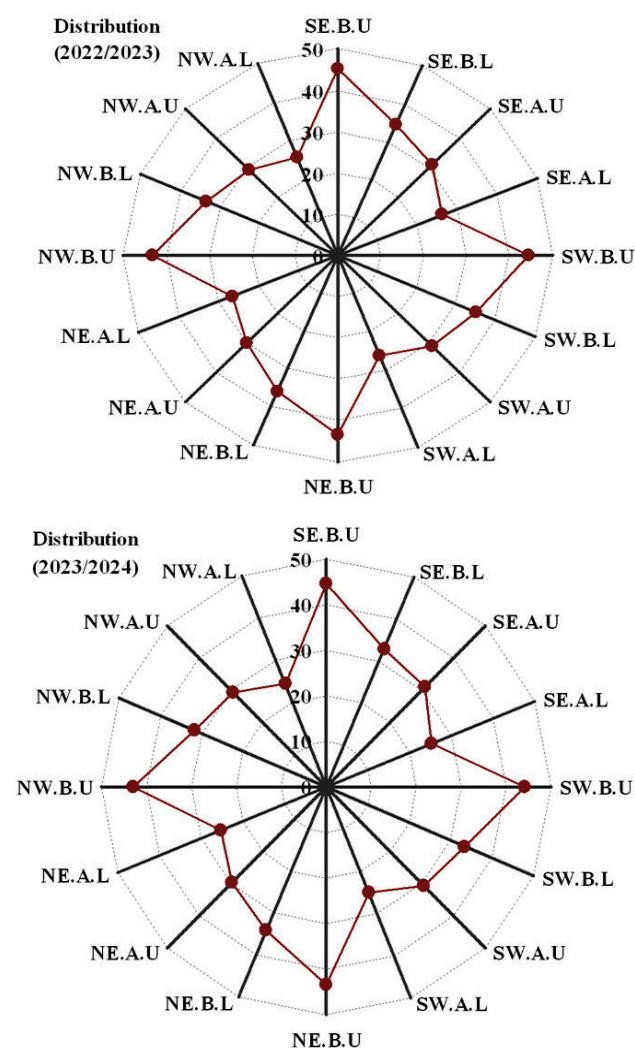
### ABUNDANCE AND SAMPLING PROGRAM OF *Aonidiella aurantii* ON GUAVA TREES

#### Seasonal abundance

This study examined the population density and dispersion indices of *A. aurantii* on guava tree leaf and bark surfaces and their interaction over two consecutive years (2022–2023 and 2023–2024), as illustrated in Figure 1.

The population of *A. aurantii* was found on guava trees year-round in all quadrants, strata, and surfaces of leaves (Fig. 1). Concomitantly, the pooled effects of tree canopy areas, layers, and leaf surface on *A. aurantii* abundance revealed that in the south-eastern region (coordinates: 1.1.1), individuals of the species preferred the upper surface over the basal layer leaves, with an average of 45.15 and 44.69 individuals per leaf, respectively, over the two years.

The upper leaf layer in the north-western region (coordinates: NW.A.L) exhibited the lowest infestation levels, with 25.54



**Fig. 1.** Mean estimates of *Aonidiella aurantii* per leaf in sampling units formed by sixteen combinations of canopy quadrants (SE = southeast, SW = southwest, NE = northeast, and NW = northwest), vertical strata (B = basal, and A = apical) and leaf surfaces (U = upper, and L = lower of guava trees; source: own study)

and 24.48 individuals per leaf over two years, respectively (Fig. 1). According to this study, the combination of wind direction and sunshine exposure increased the likelihood of an *A. aurantii* infestation in the southeast quadrant of guava trees. Bakry (2022) observed that pest crawlers are driven south-eastward for feeding and growth development by the northwest wind direction.

In addition, the combined effects of wind direction and the duration of leaf exposure to environmental factors may account for these variations in pest distribution. Specifically, the prevailing wind direction from north to south may facilitate the dispersal of freshly born crawlers, increasing the likelihood of their settlement and subsequent oviposition by adult females on leaves located in the downwind direction (Bakry and Abdel-Baky, 2020).

At the same time, abiotic environmental factors such as sunlight exposure and wind direction contribute to variations in the dispersal patterns of pests on tree layers. The basal leaves offer greater protection for scale insects, serving as a more secure refuge for feeding, development, and growth. However, the apical leaves of guava plants are less susceptible to pest infestation. Existing literature indicates that *A. aurantii* shows a preference for the lower canopy leaves of guava trees. Although conducted on different host plants and insect species, Mohammed (2020) from the El-Behaira Governorate in Egypt observed similar results.

Based on the findings described above, we advise guava growers to examine the upper surfaces of leaves located in the tree basal layer, particularly in the south-eastern canopy quadrants when scouting for *A. aurantia* infestations.

### Sampling program

As shown in Tables S1 and S2, the percentage of relative variation in the initial sampling data of *A. aurantii* across different coordinates of guava tree leaves showed that the pest population estimates – based on combinations of quadrants, canopy layers, leaf surfaces, and their interactions – ranged from 4.02 to 6.87% in 2022–2023 and from 3.31 to 5.58% during 2023–2024 season. These results indicate that the estimates of relative variation were consistent and the sampling programme for *A. aurantii* was suitably designed.

In this model, sampling units with  $RV > 25\%$  – indicating minimal precision – were excluded (Southwood and Henderson, 2000; Silva *et al.*, 2019). The absolute (whole-tree) densities and relative densities of the remaining units ( $RV \leq 25\%$ ) were subsequently analysed (Lopes *et al.*, 2019; Silva *et al.*, 2019). In this context, the *RNP* of *A. aurantii* sampling data at different coordinates of guava tree leaves showed that the estimates of pest numbers in different quadrants-layers-leaf surfaces and their interactions ranged from 4.77 to 18.90% in 2022–2023 and from 3.24 to 11.50% during 2023–2024. These findings indicate that the *RNP* were higher than the *RV*, suggesting better movement of the pests within the guava orchard. Bakry (2018) reported that the proportionate variance of the basic sample data for the total population estimates of the wax scale insect (*Waxiella mimosae* (Signoret)) (Coccinellidae: Coccidae) infesting sunt trees, varied from 8.52 to 19.79% throughout the year. According to Bakry (2020), the overall population estimates based on the relative difference for the elementary sample data of olive scale (*Parlatoria oleae* (Colvée)) (Hemiptera: Diaspididae) damaging mango trees were 2.41, 2.35, and 1.73% during the first, second, and pooled years, respectively. According to Bakry and Arbab

(2020), the proportionate variance and overall population estimates of the seychelles scale *Icerya seychellarum* (Westwood, 1855) (Hemiptera: Margarodidae) infesting guava trees were 4.07, 5.62, and 3.55% for the sample data over the first, second, and pooled years, respectively.

### SPATIAL DISTRIBUTION PATTERN

Twelve analyses identified the distribution indices among the sample units (Tabs. S1, S2). The distribution results utilising the variance of the *A. aurantii* individuals in the different quadrants-strata-leaf surfaces and their interactions on guava trees exceeded the average of the population counts, and thus the  $\frac{S^2}{\bar{X}} > 1$  in population estimates. As a result, the spatial distribution of *A. aurantii* numbers at all sampling coordinates on the guava trees exhibited an aggregative distribution pattern during the two years (2022–2023 and 2023–2024).

The  $I_L$  of the *A. aurantii* individuals was much higher than one at all coordinates on the guava trees, indicating highly dispersed distribution. Since the distribution of *A. aurantii* individuals was greater than zero, this indicated that the estimates had an aggregated spatial pattern.

The negative binomial dispersion ( $K$ ) of the *A. aurantii* individuals at the different coordinates on the guava trees is presented in Table S1. The  $K$  values ranged from 3 to 8 over the first year (2022–2023), implying moderate level of aggregation. In contrast,  $K$  values in the second year (2023–2024) exceeded eight, indicating a random aggregation (Tab. S2).

The  $I_{DM}$  values for *A. aurantii* individuals at the various coordinates on the guava trees were positive for the negative binomial (Tabs. S1, S2). The  $Z$ -test coefficients were greater than 1.96. The  $GI$  was greater than zero, and the  $IP$  was greater than one.

Over the two years, all of these indices revealed an aggregated distribution of *A. aurantii* population estimates. Temporal fluctuations in the distribution were observed each year, as indicated by values of  $1/k$  (aggregation index). The values remained greater than zero at different coordinates on the guava trees, indicating a cumulative pattern that disperses over time (Tabs. S1, S2).

The aggregation values ( $\lambda$ ) were less than two for *A. aurantii* individuals at all coordinates on the guava trees over the two years, indicating that the population of *A. aurantii* individuals was regulated by environmental variables (Tabs. S1, S2).

The aforementioned results of dispersion revealed an aggregated pattern of the pest infestation at all coordinates on the guava trees for both years. A similar pattern was observed by Meats and Wheeler (2011) on citrus trees; they concluded that *A. aurantii* also exhibited a clumpy distribution.

Furthermore, no published literature currently addressed the spatial distribution pattern of *A. aurantii*. However, studies on different insect pests support the presence of aggregation in population distribution. For example, Chellappan *et al.* (2013) observed that the value of mean crowding increased as the mean population density of the papaya mealybug (*Paracoccus marginatus* Williams and Granara de Willink) increased with different pest species and different host plants. According to Li *et al.* (2017), the aggregation index, the  $Ca$ , and the  $K$  value of the negative binomial distribution for the carambid moth (*Parapoynx crisonalis* Walker, 1859) (Lepidoptera: Crambidae) on water



chestnuts were all higher than zero in May, suggesting a larval aggregation pattern. Bala and Kumar (2018) also found that the bean bug (*Chauliops fallax* Sweet & Schaeffer) (Hemiptera: Malcidae) estimates for soybeans had  $I_L$  values higher than one for all sampling intervals, suggesting that the population distribution was aggregated.

In his investigation of the spatial pattern of *W. mimosae* on acacia trees, Bakry (2018) used fourteen distribution metrics and found that all estimates exhibited pooled dispersion and conformed to a negative binomial distribution trend for all stage estimates of the pest throughout the studies seasons. Similarly, Bakry (2020) used 21 distribution metrics to examine the spatial pattern of *Parlatoria oleae* infesting mango trees. The results showed that all distribution measures exhibited significant aggregated behaviour annually, with the exception of the  $K$  values of the negative binomial distribution for the overall estimates of pest that ranged between 15 and 17 for every year throughout a two-year period. This suggested a random distribution. Bakry and Abdel-Bakry (2020) reported that distribution parameters of the white mango scale insect (*Aulacaspis tubercularis* (Hemiptera: Diaspididae)) on different mango varieties indicated an aggregative distribution over the two consecutive years (2017–2018 and 2018–2019). Likewise, Bakry and Arbab (2020), after investigating the spatial pattern of *I. seychellarum* infestation of guava trees using distribution parameters, revealed a cumulative distribution pattern throughout the entire year. In another study, Bakry and Shakal (2020) noticed that in all of the wheat genotypes they examined over the course of every planting season, all distribution metrics exhibited significant aggregated behaviour. Bakry, Badawy, and Mohamed (2023) reported that all *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) prevalence indices had a significant clustering behaviour over the two seasons. Bakry, Shehata, and Tolba (2024) reported that all dispersal measures for different stages of *Ferrisia virgata* (Cockerell, 1893) (Hemiptera: Pseudococcidae) showed a significant tendency towards aggregation over the two years.

These strategies can help mitigate the financial losses brought on by this insect and maintaining high yields of the guava crop by lowering the use of synthetic chemical insecticides, thereby safeguarding the environment (Fidelis *et al.*, 2022).

### PRINCIPAL COMPONENT ANALYSIS (PCA)

The scree plot is a useful visual tool for the interpretation of the findings of principal component analysis (Fig. 2). The eigenvalues of the principal components are plotted against their respective indices. Eigenvalues quantify the percentage of variance that is accounted for by each principal component. The first few principal components (PCs) exhibit a rapid decrease in eigenvalues, as shown in Figure 2. This observation suggests that these components effectively capture a significant amount of the variability present in the data. The first principal component (Dimension 1) accounts for a significant proportion of the variance, specifically capturing 87.9% of the overall variation in the data. These findings indicate that just one dimension has the potential to capture a significant amount of the information contained in the dataset.

A PCA was performed, followed by among biplot analysis to examine the associations *A. aurantii* population estimates across different coordinates of the guava tree. Other similar studies have

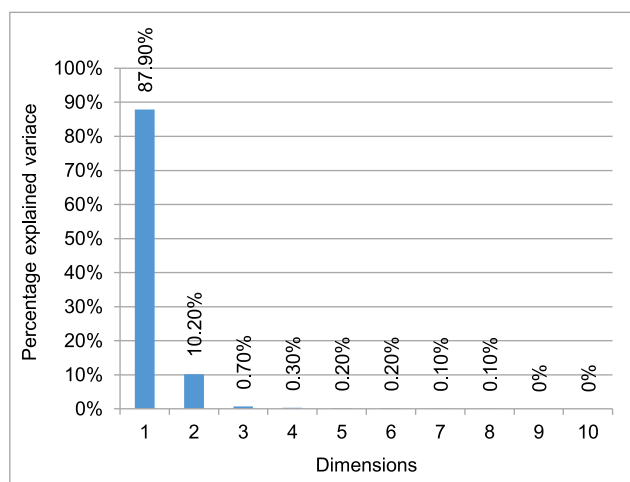


Fig. 2. Scree test for principal component analysis; source: own study

demonstrated the effectiveness of multivariate analyses not only in genetic classification but also in studying the trend among variables within experimental groups (Mancini *et al.*, 2018; Mattioli *et al.*, 2019).

Biplot analysis has been widely applied by researchers to compare samples across different criteria. According to Ullah *et al.* (2019), data were considered in each component with eigen  $F$  value  $>1$  which determined at least 10% of the variation. The higher eigenvalues were considered as the best representative of system attributes in the principal components. Only two PCs showed more than 1 eigenvalue and exhibited about 87.95% cumulative variability (Fig. 3). Thus, these two PCs can be used for further interpretation of the variability. Chunthaburee *et al.* (2016) emphasised that the first principal component ( $PC_1$ ) and the second principal component ( $PC_2$ ) often carry the most meaningful information, while subsequent components contribute minimally to explaining dataset variation.

In this study, the results of the PCA exhibited that total variation exhibited by  $PC_1$  and  $PC_2$  were 87.95 and 10.25%, respectively (Fig. 3).  $PC_1$  was negatively associated with the following coordinates: the lower surface of the apical layer leaves in the southwest quadrant (coordinate: SW.A.L), the lower surface of the apical layer leaves in the northeast quadrant (coordinate: NE.A.L), the lower surface of the apical layer leaves in the northwest quadrant (coordinate: NW.A.L), the lower surface of the basal layer leaves in the southwest quadrant (coordinate: SW.B.L), the lower surface of the basal layer leaves in the southeast quadrant (coordinate: SE.B.L), the lower surface of the basal layer leaves in the northwest quadrant (coordinate: NW.B.L), and the lower surface of the basal layer leaves in the northeast quadrant (coordinate: NE.B.L) (Fig. 3). While, the  $PC_2$  showed 10.25% of the variation (total variation) and was positively correlated with the following coordinates: the upper surface of the basal layer leaves in the southeast quadrant (coordinate: SE.B.U), the upper surface of the basal layer leaves in the northwest quadrant (coordinate: NW.B.U), the upper surface of the basal layer leaves in the northeast quadrant (coordinate: NE.B.U), the upper surface of the basal layer leaves in the southwest quadrant (coordinate: SW.B.U), the upper surface of the apical layer leaves in the southeast quadrant (coordinate: SE.A.U), the upper surface of the apical layer leaves in the southwest quadrant (coordinate: SW.A.U), the upper surface of the apical

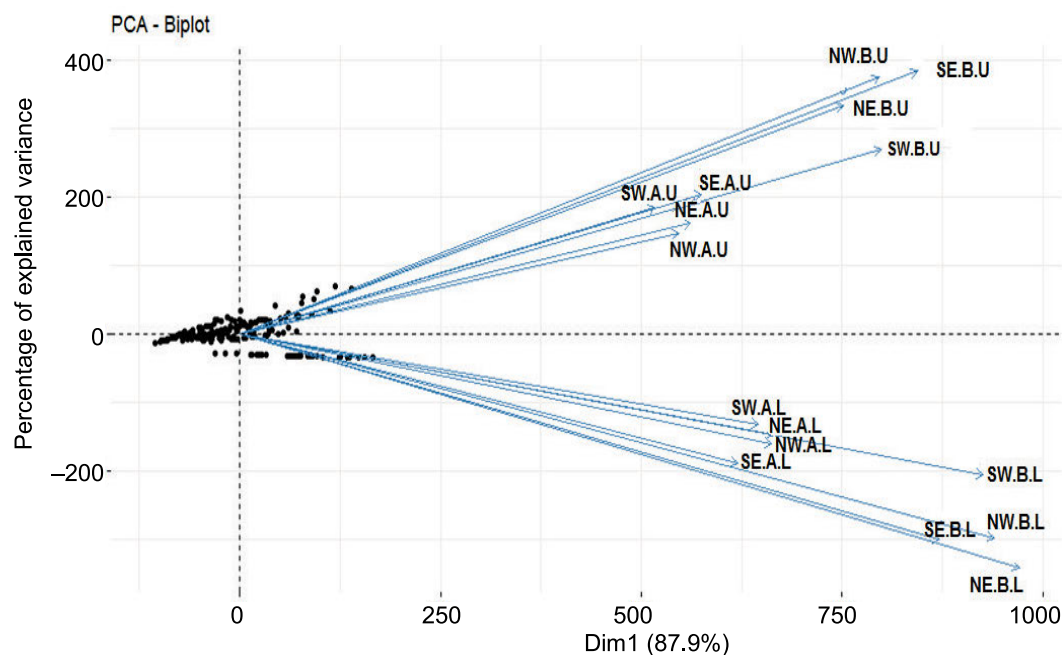


Fig. 3. Principal component analysis of *Aonidiella aurantii* population estimates in the different coordinates of the guava tree over the pooled years; source: own study

layer leaves in the northeast quadrant (coordinate: NE.A.U), and the upper surface of the apical layer leaves in the northwest quadrant (coordinate: NW.A.U), as illustrated in Figure 3.

#### HIERARCHICAL CLUSTER ANALYSIS (HCA)

The dendrogram generated from the hierarchical cluster analysis (HCA) offers valuable insights for identifying similarities and differences in *A. aurantii* population estimates across 16 guava tree coordinates. Furthermore, the findings revealed variations in the composition's quantitative and qualitative aspects, reflecting resemblance noted in the data diversity described by Annemer *et al.* (2022).

Based on the Euclidean distance between groups, the HCA revealed the presence of two distinct population groups (Fig. 4), each characterised by unique population estimates. The initial group, distinguished by a difference of four coordinates, was further divided into four subgroups as follows: the upper surface

of the basal layer leaves in the southeast quadrant (coordinate: SE.B.U), the upper surface of the basal layer leaves in the southwest quadrant (coordinate: SW.B.U), the upper surface of the basal layer leaves in the northeast quadrant (coordinate: NE.B.U), and the upper surface of the basal layer leaves in the northwest quadrant (coordinate: NW.B.U) in Figure 4.

Subsequently, the second group, which varied by eight coordinates, was further divided into five subgroups: the lower surface of the basal layer leaves in the southeast quadrant (coordinate: SE.B.L), the lower surface of the basal layer leaves in the southwest quadrant (coordinate: SW.B.L), the lower surface of the basal layer leaves in the northeast quadrant (coordinate: NE.B.L), lower surface of the basal layer leaves in the northwest quadrant (coordinate: NW.B.L), the upper surface of the apical layer leaves in the southeast quadrant (coordinate: SE.A.U), the upper surface of the apical layer leaves in the southwest quadrant (coordinate: SW.A.U), the upper surface of the apical layer leaves in the northeast quadrant (coordinate: NE.A.U), the upper surface of the apical layer leaves in the northwest quadrant (coordinate: NW.A.U), the lower surface of the apical layer leaves in the southeast quadrant (coordinate: SE.A.L), the lower surface of the apical layer leaves in the southwest quadrant (coordinate: SW.A.L), and the lower surface of the apical layer leaves in the northeast quadrant (coordinate: NE.A.L), as presented in Figure 4.

Significantly, the populations in the subgroups [upper surface of the basal layer leaves in the southeast quadrant (coordinate: SE.B.U), and the upper surface of the basal layer leaves in the southwest quadrant (coordinate: SW.B.U)], [upper surface of the basal layer leaves in the northeast quadrant (coordinate: NE.B.U), and the upper surface of the basal layer leaves in the northwest quadrant (coordinate: NW.B.U)], [the lower surface of the basal layer leaves in the southeast quadrant (coordinate: SE.B.L), and the lower surface of the basal layer leaves in the southwest quadrant (coordinate: SW.B.L)], [the

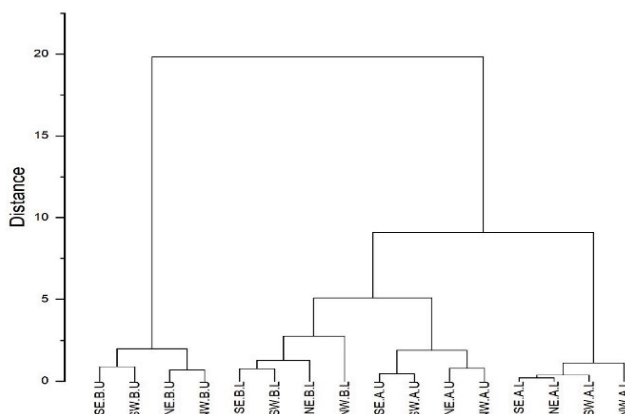


Fig. 4. Dendrogram obtained via hierarchical cluster analysis based on the Euclidean distance between the groups of *Aonidiella aurantii* population estimates in the different coordinates of the guava tree over the pooled years; source: own study

upper surface of the apical layer leaves in the southeast quadrant (coordinate: SE.A.U), and the upper surface of the apical layer leaves in the southwest quadrant (coordinate: SW.A.U)], and [the upper surface of the apical layer leaves in the northeast quadrant (coordinate: NE.A.U), the upper surface of the apical layer leaves in the northwest quadrant (coordinate: NW.A.U)] were clearly identified and formed separate groups in the PCA. This finding also revealed a notable dichotomy in the HCA (Fig. 4).

## CONCLUSIONS

This experiment aimed to determine the distribution of the red scale insect, *Aonidiella aurantii*, on guava trees throughout the two successive years (2022–2023 and 2023–2024). Results indicated that *A. aurantii* population was present on guava trees throughout the year, with the upper surface being preferred over the basal layer leaves in the southeast quadrant. Environmental abiotic factors such as temperature and humidity, and biotic factors, including the presence of natural predators can influence the spread and distribution of red scale insects. These findings underscore the practical application of a pest scouting strategy, which can help promote integrated pest management programmes and effective *A. aurantii* control. In this context, the spatial distribution pattern of *A. aurantii* at all sites on the guava tree was aggregated during the course of the two years, according to the assessment of the data using dispersion measurements.

In order to distinguish the population estimates of *A. aurantii* in the sixteen coordinates, the correlation analysis was applied. Principal component analysis was also performed, followed by a two-dimensional analysis to determine the correlation between them. The optimal sampling plan was implemented to estimate the population counts of *A. aurantii*. The aforementioned results provide comprehensive support for a pest scouting method, which explains patterns observed.

These studies may prove to be highly advantageous in the formulation of successful integrated pest management programmes and the development of efficient control techniques for *A. aurantii*. These strategies can help limit the financial losses caused by this insect, while maintaining the high yield of the guava crop. This also supports a reduced use of synthetic chemical insecticides and protects the environment (Fidelis *et al.*, 2022). The process entails revising the pesticide application schedule to focus on the most affected tree sites. Overall, this study establishes conditions for future research on the behaviour and control of the red scale insect, which may enhance the guava crop's sustainability and profitability. However, further experiments need to be conducted in order to test these hypotheses.

## CONTRIBUTION OF THE AUTHORS

**Moustafa M. S. Bakry:** study design, data collection, statistical analysis, data interpretation, manuscript preparation, and literature search. Statistical analysis, data interpretation, manuscript preparation, and literature search by **Yaghoub Fathipour**. **Pasco B. Avery:** manuscript preparation, and literature search. All co-authors have read and approved of the manuscript before submission.

## SUPPLEMENTARY MATERIAL

Supplementary material to this article can be found online at [https://www.jwld.pl/files/Supplementary\\_material\\_65\\_Bakry.pdf](https://www.jwld.pl/files/Supplementary_material_65_Bakry.pdf).

## CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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