

Impact of Altitude on Insect Pest Occurrence in Mandarin (*Citrus reticulata* Blanco) Orchards Within Jajarkot District

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Abstract

An experiment was conducted to assess the pest status and effect of altitude on insect pests of mandarin orchards from April to June 2023 at Kushe rural municipality, Jajarkot, Nepal, located within the Citrus Zone under PMAMP. The experiment comprised five different altitude ranges as treatments: 1400-1500 masl, 1500-1600 masl, 1600-1700 masl, 1700-1800 masl, and 1800-1900 masl. Sample data were collected through visual observation and monitoring using yellow sticky traps. The research employed a randomized complete block design with five treatments and five replications. Data analysis was conducted using R (version 4.3.1) and MS-Excel. The study identified citrus leaf miners (*Phyllocnistis citrella*) and fruit flies (*Bactrocera tau*) as major threats in mandarin orchards in Jajarkot. Likewise, scale insects (*Aonidela auranti*), aphids (*Toxoptera spp*), whiteflies (*Dialeurodes citi*), lemon butterflies (*Papilio demoleus*), citrus tree borer (*Chilodoniium cinctum*), red citrus mites (*Panonychus citri*), red ants (*Oecophylla smaragdina*), citrus green stink bugs (*Rhynhocoris humeralis*), and mealybugs (*Planococcus citri*) were present in minority. The results showed that there is a significant effect of altitude on the percentage of leaf damage caused by citrus leaf miners ($P < 0.001$), and the number of trapped fruit flies ($p < 0.01$), aphids ($P < 0.01$), and whiteflies ($P < 0.01$). However, altitude showed a non-significant effect on scale insect incidence. All pests except scale insects were more abundant at lower altitudes, with the highest occurrence at 1400-1500 masl and the lowest at 1800-1900 masl. Mealybugs, red citrus mites, and citrus tree borer damage were not seen at altitudes above 1800 masl. This study highlights altitude as a key factor in pest occurrence.

Keywords : Citrus, Citrus leaf miner, Fruit fly, Monitoring, Visual observation

Introduction:

Citrus is one of the major fruit crops grown in Nepal, which is considered as a significant source of income for the farmers in hilly areas of the country. The mid-hill region of Nepal (1000 m to 1500 m altitude) has a comparative advantage in the cultivation of citrus fruits.

Because of the climatic suitability for citrus cultivation and increased demand for fresh fruit and processed products, the area under mandarin production is gradually increasing in Nepal (MoALD, 2022). Mandarin (*Citrus reticulata* Blanco) is the most important citrus crop in terms of production and coverage among all citrus

Table 1: Details of treatment

Treatments	Altitude range	Altitude of orchard (masl)	GPS coordinates of the orchard
A	1400 to 1500 masl	1450 masl	28.79098N 82.15657E
B	1500 to 1600 masl	1535 masl	28.79451N 82.15919E
C	1600 to 1700 masl	1643 masl	28.79628N 82.16231E
D	1700 to 1800 masl	1758 masl	28.79789N 82.16732E
E	1800 to 1900 masl	1854 masl	28.79964N 82.16968E

(Note: masl, meters above sea level)

Sampling method

The sampling method for monitoring was based on visual observation and the use of yellow sticky traps. One plant from each treatment replication was selected at random. A total of 10 terminal shoots measuring at least 10 cm were sampled randomly from each plant representing all directions, namely, north, south, east, and west. The sampling interval was 10 days, and data was collected 6 times except for an altitude of 1450 masl, where data were recorded 5 times only due to weather disturbance. The number of damaged leaves from different pests like leaf miners and lemon butterflies was counted and divided by the total number of leaves to express the damage in percentile. Similarly, the number of pests like scale insects, mealy bugs, and other leaf and young shoot damaging pests were also counted. This method of sampling is slightly modified from that of Deka & Chattopadhyay (2016). Aphids, whiteflies, thrips, fruit flies, and psyllids are attracted to yellow color, and hence, yellow sticky traps were used to capture such insects for monitoring purposes (Atakan, 2004; Jacomien, 2017; Stansly et al., 2010). One sticky trap was placed on a randomly selected orange tree at a height of 1.5 meters from the ground level on the edge of the canopy, and the trap was replaced after each reading. One trap was considered as one replication.

For the surveillance of major pests in situ counts was done in selected plants. For minor pests, binary data on whether a plant had been damaged or not or whether the pests were present or not was recorded by mass inspection of the tree. This method is comparable to the method used by Paudel et al. (2021). The samples of each insect collected were taken in plastic bags for further identification purposes. The identification was done by observing their morphological features with the help of a hand lens and by taking and comparing photographs.

Collection of weather parameters

The data on weather parameters was collected from the Jajarkot Weather Station, which is the nearest weather station to Kushe. This precipitation-type station is stationed in the periphery of the District Administration Office, Khalanga, situated at an altitude just above 1300 masl. All the data retrieved were daily temperature data recorded in the evening (5:45 PM).

Statistical analysis

Statistical analysis was conducted by first entering the observed data in Microsoft Excel (version 16.34), followed by analysis in R (version 4.3.1). The experimental design was a randomized complete block design (RCBD) with five treatments and five replication blocks. Data were subjected to analysis of variance (ANOVA) to assess statistical significance. Square root transformation was applied when necessary to meet the assumptions of linearity. Mean separation and comparisons were performed using Duncan's Multiple Range Test (DMRT) at a 5% significance level, implemented through the "agricolae" package (version 1.3-6) in R.

Results:

Visual observation of trees

A total of 145 trees were inspected across six time periods from April 12 to June 3, 2023, to assess the presence of insect pests or their damage symptoms. During the whole period of study, 11 different types of citrus pests were identified (Table 2). Among them, eight types of pests were observed from visual observation. During visual observation, citrus leaf miner (CLM) damage and scale insect infestation were observed in all sampled trees, while other pests were absent in some observations (Table 3). Based on the pest (or damage) observed, citrus leaf miner damage was the most common, followed by scale insects. The insect occurrence showed a decreasing trend with the increasing altitude (Figure 2).

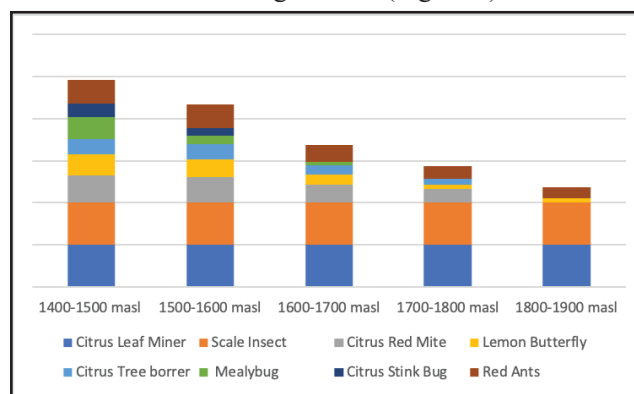


Figure 2. Graph showing pattern of occurrence of pests across altitudes (one vertical unit represents 100% presence of pest).

Table 2: List of identified pests with their identifying features from April to July 2023 in Kushe-5, Jajarkot

Common name	Scientific name	Family	Identifying features
Citrus leaf miner	<i>Phyllocnistis citrella</i>	Phyllocnistidae	Serpentine mine with silvery appearance (length of 50-100 mm) usually on underside of young leaves (Heppner, 1993).
Whitefly	<i>Dialeurodes citri</i>	Aleyrodidae	Adult body is creamy-yellow with 2 pairs of white wings appearing as whitish bloom in yellow sticky traps which tends to disappear or becomes orange after few days(CABI, 2021a).
Aphid	<i>Toxoptera spp</i>	Aphididae	Small-medium sized dark aphids, siphunculi are short, dark and slightly longer than cauda. Pear shaped body with long & thin legs and antennae, wings are longer than the body (CABI, 2021b).
Fruit fly	<i>Zeugodacus (Bactrocera) tau</i>	Tephritidae	Abdomen is orange brown with dark colored transvers bands in tergite 3 and rectangular mark on T4. Presence of Median longitudinal stripe on T3-T5 and coastal band expanded into a spot at wing apex (Latha & Sathyanarayana, 2015).
Scale insects	<i>Aonidela auranti</i>	Diaspididae	Adult females brownish or orange red and oval to circular about 1.5-2 mm long while nymphs are shiny light green with a conspicuous black, irregular U-shaped internal marking (Makale et al., 2020).
Lemon butterfly	<i>Papileo demoleus</i>	Papilionidae	Larva are dark brown to green developing irregular white markings and resembling bird's drop(CABI, 2022).
Red citrus mite	<i>Panonychus citri</i>	Tetranychidae	Females are deep red to purplish colored while males are smaller with light red and sometimes orange colored, causing tiny grey or silvery spots on leaves and fruit (stippling). They contain 4 pairs of legs and the front legs are shorter than the body (Vacante, 2010).
Citrus Mealy bug	<i>Planococcus citri</i>	Pseudococcidae	Nymphs are yellowish covered with white waxy particles while adults are wingless and coated with white wax and bearing short waxy filaments around the margin. Filaments at the rear end are slightly longer (Makale et al., 2020).
Citrus tree borer	<i>Cheledonium cinctum</i>	Cerambycidae	The adult is dull metallic green to dark violet with a yellow band across the middle of the elytra. Grubs bore hole into the stem and feed by tunnelling the internal tissue.
Citrus green stink bug	<i>Rhynchosoris humerslis</i>	Pentatomidae	Shield shaped body and green in color. Nymph possess pattern of black spots
Red Ants	<i>Oecophylla smaragdina</i>	Formicidae	Small to medium sized ants with light red color.

Table 3: Percentage of sampled trees with the presence of pests across different altitudes

in April to July 2023 in Kushe-5, Jajarkot

Altitude (masl)	Number of sampled trees	Percentage of trees with presence of pest							
		Citrus leaf miner	Scale insects	Lemon Butterfly	Red ant	Mite	Citrus tree borer	Mealy bug	Citrus green stink bug
1400-1500	25	100	100	52	56	64	36	52	32
1500-1600	30	100	100	43.33	56.67	60	36.67	20	16.67
1600-1700	30	100	100	23.33	40	43.33	23.33	6.67	0
1700-1800	30	100	100	10	30	33.33	13.33	0	0
1800-1900	30	100	100	10	26.67	0	0	0	0

(Note: masl, meters above sea level)

Table 4: ANOVA table for regression analysis of insect catch and average maximum daily temperature (independent variable)

Dependent Variable	Source	DF	Sum of Squares (SS)	Mean Square (MS)	F-value	p-value	r	r ²
White Flies	Regression	1	7227.41	7227.41	2.49	0.2126	-0.6735	0.4536
	Residual	3	8705.79	2901.93				
Aphids	Regression	1	65187.67	65187.67	37.97	0.0086**	0.9627	0.9268
	Residual	3	5151.13	1717.04				
Fruit Flies	Regression	1	43271.08	43271.08	4.31	0.1295	0.7679	0.5896
	Residual	3	30118.92	10039.64				

(Note: Independent variable, Average maximum daily temperature; DF, Degree of Freedom; r, Correlation Coefficient; r², Coefficient of Determination; **, Significant at 1% level of significance)

Effect of altitude on occurrence of Citrus leaf miner and scale insects

The average number of scale insect nymphs per shoot (more than 10 cm long) along the altitudinal gradient was subjected to analysis of variance (Table 5). The average number of scales insects was the highest at the elevation of 1800-1900 masl (2), followed by 1700-1800 masl (2), 1600-1700 masl (2), 1400-1500 masl (2) and 1500-1600 masl (2). The results showed that altitude had a non-significant effect on scale insect infestation.

Table 5 reveals that the altitude has a significant effect (F-probability <0.001) on the number and percentage of leaves damaged by citrus leaf miners. The percentage of CLM damaged leaves was highest at 1400-1500 masl altitude (24.63 %), followed by 1500-1600 masl (24.08%), 1600-1700 masl (18.94%), 1700-1800 masl (14.69%), 1800-1900 masl (11.2 %) respectively. Percentage damage at altitudes of 1400-1500 masl was found statistically similar to altitudes of 1500-1600 masl

and 1600-1700 masl but was significantly different from the remaining two altitudes. The lowest percentage of damage was at an altitude of 1800-1900 masl, which was statistically at par with 1700-1800 masl altitude. Similarly, percentage damage was statistically similar at altitude ranges of 1600-1700 masl and 1700-1800 masl. The total number of leaves mined by CLM was also significantly different among different altitudes (table 5). The highest number of mined leaves was sampled from the altitude of 1400-1500 masl (158), which was significantly at par with 1500-1600 masl (156). All other altitudes were found to be statistically different from each other. The lowest number of mined leaves was seen at altitudes 1800-1900 masl (68), followed by 1700-1800 masl (89) and 1600-1700 masl (115), respectively.

Monitoring with yellow sticky traps

Among many insects trapped in sticky traps, fruit fly (*Zeugodacus tau*), black citrus aphids (*Toxoptera citricida*), and whiteflies (*Dialeurodes citri*) were caught

Table 5: Effect of altitude on the percentage leaf damage of Citrus leaf miner, total number of CLM damaged leaves, and number of scale insects per twig in April to July 2023 in Kushe-5, Jajarkot

Treatments	Percentage of CLM damaged leaves	Total number of CLM damaged leaves sampled	Number of scale insect nymph per twig
1400-1500 masl	24.63 ^a (4.96)	157.85 ^a (12.56)	1.69 (1.30)
1500-1600 masl	24.08 ^a (4.91)	156.34 ^a (12.5)	1.64 (1.28)
1600-1700 masl	18.94 ^{ab} (4.35)	115.17 ^b (10.73)	1.72 (1.31)
1700-1800 masl	14.69 ^{bc} (3.83)	88.78 ^c (9.42)	1.82 (1.35)
1800-1900masl	11.20 ^c (3.35)	68.10 ^d (8.25)	1.9 (1.38)
LSD	0.62	1.15	NS
SE _m (±)	0.31	0.85	0.02
F-probability	<0.001	<0.001	0.98
CV%	10.75	8.05	20.46
Grand mean	4.28	10.69	1.32

(Note: masl, meters above sea level; CLM, citrus leaf miner; NS, Non-Significant; SE_m, Standard Error of Mean; LSD, Least Significant Difference; CV, Coefficient of Variation; Same letter(s) within column indicate no significant difference between the treatments based on Duncan's Multiple Range Test (DMRT) at 0.05 level of significance, figures in the parenthesis indicate square root transformed values)

in higher numbers, which are considered pests in citrus groves (Parajuli et al., 2023). Results showed that the average total number of these insects trapped varied significantly ($P < 0.001$) along the elevational gradient (Table 6). The highest number of pests was trapped at altitudes 1400-1500 masl (83), and the lowest was found at the altitude of 1800-1900 masl (30), which was statistically lower than all other altitudes. Altitudes of 1500-1600 masl (68) showed a statistically similar level of pests trapped with 1400-1500 masl. Similarly, the number of pests trapped was statistically at par in elevation of 1600-1700 masl (54) and 1700-1800 masl (45). The highest number of fruit flies was trapped at altitude altitudes of 1400-1500 masl (29), which was statistically at par with altitudes of 1500-1600 masl (24). The lowest number of pests was observed in the highest elevation, i.e., 1800-1900 masl (10), which was statistically similar with elevation ranges of 1600-1700 masl (17) and 1700-1800 masl (12). Similarly, the trapped fruit flies' number was found statistically similar at altitudes of 1500-1600 masl and 1600-1700 masl.

Average number of aphids also showed significant differences in different altitudes ($P < 0.01$) (Table 6). The lowest number of aphids were trapped in elevations 1800-1900 masl (15), which is statistically not different than at the altitudes of 1700-1800 masl (19) and 1600-1700 masl (23). The highest aphid population was observed at the altitude of 1400-1500 masl (32), which was statistically similar to the altitude range of 1500-1600 masl (26) and 1600-1700 masl (23). Similarly, the number of trapped aphids in elevations of 1600-1700 masl was statistically

at par with the altitude ranges of 1500-1600 masl and 1700-1800 masl.

A similar trend was seen in the case of whiteflies as well. The highest number of white flies were trapped in 1400-1500 masl altitude (22), followed by 1500-1600 masl (19), 1600-1700 masl (14), 1700-1800 masl (14), and > 1800 masl (5) respectively. The result of the analysis showed the significant effect of altitude on the abundance of white flies ($p < 0.01$). All other treatments had statistically similar levels of white flies trapped except for the elevation 1800-1900 masl.

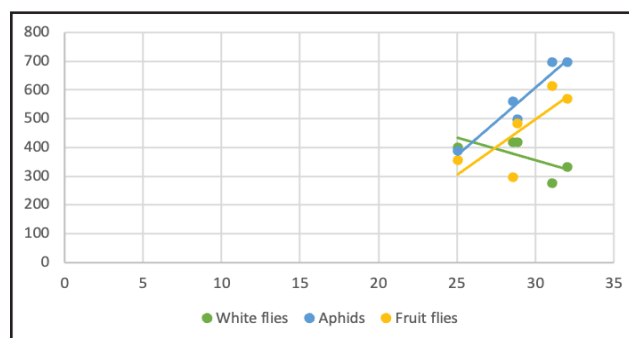


Figure 3. Scatterplot representation correlation of Average maximum daily temperature (in degrees Celsius) during the 10 days interval and number of pests trapped in each sampling in April to July 2023 in Kushe-5, Jajarkot

Temperature and insect catch

The correlation analysis between pest counts on yellow sticky traps and the average maximum daily temperature over the 10-day trap placement period revealed distinct

Table 6: Effect of altitude on the average number of citrus fruit flies, citrus aphids, whiteflies, and the total number of pests trapped from April to July 2023 in Kushe-5, Jajarkot

Treatments	Citrus fruit fly	Citrus black aphids	White flies	Total number of insect pest (Fruitfly + Aphids + White flies)
1400- 1500 masl	29.08 ^a	31.92 ^a	21.56 ^a	82.56 ^a (9.06)
1500-1600 masl	24 ^{ab}	25.64 ^{ab}	18.76 ^a	68.40 ^a (8.25)
1600-1700 masl	17.2 ^{bc}	22.68 ^{bc}	14.32 ^a	54.20 ^b (7.35)
1700-1800 masl	12.2 ^c	18.60 ^{bc}	14.16 ^a	44.96 ^b (6.65)
1800-1900 masl	10.32 ^c	14.72 ^c	4.92 ^b	29.96 ^c (5.45)
LSD	8.43	8.41	7.23	0.84
SE _m (±)	3.54	2.95	2.83	0.63
F-probability	<0.01	<0.01	<0.01	<0.001
CV%	33.87	27.62	36.57	8.57
Grand mean	18.56	22.71	14.74	7.35

(Note: NS, Non-Significant; SE_m, Standard Error of Mean; LSD, Least Significant Difference; CV, Coefficient of Variation; Same letter(s) within column indicate no significant difference between the treatments based on Duncan's Multiple Range Test (DMRT) at 0.05 level of significance, figures in the parenthesis indicate square root transformed values)

patterns among pest species. Specifically, average maximum temperature showed a positive correlation with aphid and fruit fly counts but a negative correlation with white fly counts (Figure 3). The ANOVA results (Table 4) indicate that maximum temperature significantly predicts the aphid counts, explaining 92.68% of the variance ($P = 0.0086$, $r^2 = 0.9268$), suggesting a strong association between temperature and aphid populations. In contrast, temperature does not significantly predict white fly or fruit fly counts. The negative correlation ($r = -0.6735$) for white flies indicates a possible inverse relationship with maximum temperature, although not statistically significant, while the moderate positive correlation ($r = 0.7679$) for fruit flies similarly lacks statistical significance.

Discussion:

Visual observations of trees

The findings showed that with the increase in altitude, the presence and abundance of pests were less and less severe. This type of result is generally found true in the case of many insect species that are following the general prediction of less insect herbivore damage in higher altitudes (Hodkinson, 2005; Pellissier et al., 2014). This may be due to the low insect survivability, poor development, longer lifecycle, and increased plant defense at higher altitudes compared to a lower altitude (Buckley et al., 2019; Hodkinson, 2005; Pellissier et al., 2014; Rasmann et al., 2014).

Effect of altitude on occurrence of Citrus leaf miner and Scale insects

Variation in the distribution of scale insects along altitudinal gradient was found to be insignificant (Table

5). Hodkinson (2005) and Moreira et al. (2018) also reported similar findings. This is also supported by the results obtained by Nakato et al. (2023) and Paudel et al. (2020) in their respective experiments. However, this result is opposite to the general consideration of decreasing insects' prevalence with increasing altitude gradient (Hodkinson, 2005; Williams & Rankin, 2019). This may be due to the lower abundance of the insect predators of scales in higher altitudes, which is also found true in the case of some natural enemies (wasps and beetles), as concluded by Banko (2002) and Gilbert & Grégoire (2003).

The percentage of leaves damaged by CLM decreased with increasing altitude (Table 5). This result may be attributed to the less CLM insect survivability, poor development, longer lifecycle, and increased plant defense in higher altitudes compared to lower altitudes (Hodkinson, 2005; Pellissier et al., 2014; Rasmann et al., 2014; Buckley et al., 2019). A comparable result was obtained by Tantowijoyo & Hoffmann (2010), in which the distribution of *Liriomyza sativae*, a leaf miner pest, decreased in higher altitudes. However, contrasting results were obtained in the coffee stink bug (Azrag et al., 2018). Similarly, Paudel et al. (2021) found that in tomato leaf miners, the insect damage and insects caught in traps increased with increasing elevation, highest at 1389 masl. Not only positive or negative but neutral effects of altitude on insect herbivory have been reported (Moreira et al., 2018). Nakato et al. (2023) also reported that there is no significant effect of altitudes in the distribution of key banana pests. These contradicting results signify the phenomenon of the asymmetric response of insects to climate warming (Paudel et al., 2020). Thus, the insect pest population, plant resistance, and herbivory may

be a complex effect of variation of abiotic and biotic factors along the altitudinal gradient, either combined or individual (Hodkinson, 2005; Moreira et al., 2018; Paudel et al., 2020).

High levels of plant damage in 1400-1600 masl altitude may be due to the availability of suitable climates in the elevation for the survival of the insect. Mid-elevation areas (1000-1500 masl) are considered suitable for herbivores, resulting in higher herbivory damage in such areas. This argument is also supported by the findings of McCoy (1990) and Williams & Rankin (2019). The different trends in the variation of percentage leaf damage by CLM and total number of leaves damaged by the CLM along the altitudinal gradient may be due to the lower number of leaves per new growing flushes, which may have resulted in less variation among different elevation in percentage leaf damage compared to number of damaged leaves. This can be compared with the findings of Cai et al. (2012), who found decreasing growth and yield of *Plukenetia volubilis* with increasing elevation.

Monitoring with yellow sticky traps

There is a significant effect of altitudinal gradients on fruit flies trapped, with lower elevations showing a greater amount of insect catch. This result is similar to the findings of Salazar-Mendoza et al. (2021), who found that the guava fruit fly abundance and species richness were greater at lower altitudes. A similar result was obtained by Puche et al. (2005), where Mediterranean fruit fly abundance was highest in 1273 masl compared to 1443 masl and 1613 masl. Also, Karki et al. (2023) reported that larval mortality of Chinese citrus fruit flies is higher in high altitudes. They also concluded that in higher altitudes, adult emergence is delayed compared to lower altitude areas, which can be influential in the population buildup of fruit flies.

The variation in aphids along the altitude showed the general prediction of insects being less abundant in higher altitudes, as reported by Hodkinson (2005). Similar findings were obtained by Le Cesne et al. (2015) in Hemipteran insects. They reported the low species richness and insect abundance of Auchenoryncha, which is closely related to the aphid suborder Sternoryncha, in higher altitudes.

The population buildup of citrus whiteflies may be temperature dependent, as in cotton whiteflies, whose optimum maximum temperature is 32°C (Chandi et al., 2021; Ghosal, 2022). So, these observed results in this study may be due to the effect of comparatively low temperatures and other adverse abiotic conditions in higher altitudes (Dong et al., 2015; Linacre, 1982; Wang et al., 2014)

Temperature and insect catch

The results suggest that while temperature is a significant

predictor for Aphids, other environmental factors may more strongly influence the populations of White Flies and Fruit Flies (Table 4). The increasing number of aphids with the increase in temperature may be attributed to the effect of temperature on insect growth, development, and activity. Gilbert & Raworth (1996) reported that growth and development are slower in lower temperatures, which may be correlated with the findings in this study. Also, lower temperature is known to reduce the activity of insects (Mellanby, 1939). According to Dixon et al. (2009), insect survival is negatively affected outside the range of minimum and maximum temperature thresholds. This may explain the low populations of whiteflies in higher temperatures, where the daily temperature range may have exceeded the temperature thresholds of whiteflies. However, the results in this study are not sufficient to confirm this phenomenon, having a lesser number of samples.

Conclusion:

Many of the common pests of citrus plants were present in mandarin orchards of Kushe, Jajarkot. Among them, the incidence of citrus leaf miners and fruit flies posed a matter of great concern for farmers. Scale insects, aphids, and whiteflies were also present in high abundance, though their occurrence was relatively less severe. Some of the trees within the sampled orchards showed the incidence of lemon butterflies, citrus tree borer, citrus red mites, citrus green stink bugs, mealybugs, and red ants. Altitude was a significant factor in influencing the occurrence of citrus pests, except for scale insects. The insect abundance was found to be decreasing with the increasing altitude. Citrus leaf miners, Fruit flies, Whiteflies, and aphids all were significantly more abundant at lower altitudes of 1400 masl to 1500 masl compared to the higher altitudes. Similarly, an abundance of all types of insects was significantly reduced at altitudes above 1700 masl except scale insects. However, it is evident from the findings of this research that the impact of altitude on the occurrence of pests is a pest-specific phenomenon, differing from one pest species to another.

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Declaration of conflict of interest and ethical approval:

R. Ghimire designed the experiment, conducted fieldwork, analyzed the data, and prepared the manuscript. A. Dhakal contributed to designing the experiment and conducting fieldwork. SS. Karkee supervised and monitored the experiment and finalized the manuscript. S. Acharya assisted in writing the manuscript. All the authors have read the manuscript before submission and declare no conflict of interest.

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