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### Parasitoids associated with mango leaf miner, Acrocercops syngramma (Meyrick) Lepidoptera: Gracillariidae in Mango Orchard

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#### Abstract

The experiment was conducted on leaf miner, *Acrocercops syngramma* (M.) infestation and its parasitoids in Saeed Khan Kanher, mango orchard, Khairpur Sindh, Pakistan, during 2015. The results revealed were significant, (P<0.05) highest leaf miner larval population was recorded on Chaunsa (15.806/shoot). The leaf miner remained active during the emergence of new flushes in plants from August to October, with four population peaks. However, two parasitoids, *Chrysocharis nephereus* (W.) and *Sympiesis hyblaeae* (S.) were recorded in leaf miner larvae. The high adult's population of parasitoids; *C. nephereus* and *S. hyblaeae* (4.502/plant and 1.767/plant) recorded on Chaunsa and minimum on Sindhri. While, significantly maximum parasitism percentage of both parasitoids *C. nephereus* and *S. hyblaeae* were recorded on leaf miner larvae on Chuansa. The correlation results of temperature and relative humidity with leaf miner populations on different mango varieties showed non-significant and negative correlation on Chaunsa, Fajiri and Sindhri.

Keywords: Leaf miner, Mango, Parasitoid, Population

#### 1. Introduction

Mango, Mangifera indica (L.) is considered a delicious and pulpy fruit amongst the all fruits grown in tropical and subtropical areas of the world [1]. Mango orchards cultivation has ranked second after citrus and is being grown on the second largest area in Pakistan [2]. Mango fruits are rich in nutritional substance, i.e. proteins, carbohydrates, vitamins, fatty acids and amino acids [3]. It is placed at a top among the all other fruits [4]. The mango orchards were grown in 87 countries all over the world, from which India, Pakistan, Brazil, Philippines, Mexico, China and Thailand are the main producers of the mango crop [5-7]. It is national fruit of Pakistan, India and Philippines, whereas it is the national tree of Bangladesh [8]. Mango orchards is cultivating in some Districts of Punjab and Sindh Pakistan. However, it produces an about 11.20 tones/hectare, 1732 thousand tons fruit yield per year and second main fruit crop of the country [9-11]. The Sindh Province is producing mango 390,486 tones out of which exporting to the world is about 61,632 tones [12]. The mango variety Sindhri commercially cultivated in the large areas of Sindh, it singly contributed 80-85% of total mango production in Sindh [13]. The mango trees become vulnerable to the pests at both vegetative as well as reproductive stages (flower and fruit damagers). The defoliators and borers damage young trees before the fruit bearing stage. The minute caterpillars excavate under the dorsal epidemics of top leaves and feed inside consequently gravish white epidemic lines and patches appear on the leaves [14]. The indirect infestation of leaf miner in newly leaves would be continuing as longer destructive for the plant health through the direct loss of photosynthetic tissue [15]. The larval feeding on the leaves tissues through mining leaving the epidermal layers unattached that creates blister like patches on the leaves between the upper and lower epidermis of newly emerged leaves. While, the damaged leaves turn into wrinkle and zigzag mined spots, dried up foremost to form the large holes on the leaves [16]. The mango leaf miner, Acrocercops syngramma (Meyrick) Lepidoptera: Gracillariidae is a major pest of mango plant, it damages to newly emerged flushes of mango plants during the month of August to November and cost maximum infestation on Chuansa and minimum on Langra varieties [17-18]. However, the [17, 19] reported that the leaf miner were damaged 17.4% to 46.245% to cashew and mango plant leaves. While, [20] mentioned that the A. syngramma appeared in the month of October-November to April-May on newly emerged plant leaves.

The biological control of insects by natural enemies, i.e., predators, parasitoids, parasites, nematodes and different microorganisms can be used to keep them at below economic injury levels. Many beneficial insects belong to family Eulophidae are parasitoids of several leaf miner species belongs to order Lepidoptera [21-22]. The [23] reported that an about 50% leaf miner population was decreased by three Chelonus sp. (Braconidae), Cirrospilus sp. and Sympiesis sp. (Eulophidae) larval parasitoids under field conditions. However, the [24] mentioned that the natural enemy parasitoids are group signally to decrease harmful insect populations. The [25] mentioned that the biological control is an important component of integrated pest management for to reduce insecticide-resistant and minimize the usage of insecticides against harmful insects. However, the [26] reported that over 119 species were belongs to genus Sympiesis are world widely recognized as ectoparasitoids, hyperparasitoids, or larval and pupal parasitoids of different insect orders i.e., Lepidoptera, Coleoptera, and Diptera.

The mostly growers of Pakistan depends on insecticides to minimize pest damage on mango orchards, vegetable, fiber and cereal crops. The [27] reported that misuse of pesticides in many areas of the country, creating pest resistance, increasing residual amount of toxic material in fruits, vegetable and cereal grains as well as increasing environmental pollution and decreasing the activities of beneficial insects and wildlife population. However, the [28] estimated that 27% country stocks of pesticides were used on vegetable and fruit crops in Pakistan. The [29] reported that insecticides have created most severe problems in agriculture and natural ecosystems. In this regard US encourages the minimum usage of pesticide, up to 50% without any crop yield losses or changes, at superficial standards through the adaptation of integrated pest management (IPM) programme for insect pest control. However, the host plant resistance is a major component of integrated pest management (IPM) that developing the Physio-morphological characteristics in plants to deter the pest population as compared to other varieties [30].

Prior to this study, no any research work has been reported on mango host plant resistance and parasitoids, *Chrysocharis nephereus* and *Sympiesis hyblaeae* (Eulophidae: Hymenoptera) of leaf miner *A. syngramma* in Pakistan. The purpose of this study was to find out relative resistance of different mango varieties, parasitoids of leaf miner as to find out an alternate pest control methods. The output of this study would be help full for mango growers of Sindh Province as well as at country level.

#### 2. Materials and Methods

### 2.1 Population abundance of leaf miner, A. syngramma on different mango varieties

The population abundance and trend of leaf miner was recorded on different mango varieties. The field experiment was conducted in Saeed Khan Kanhar mango orchards located at Taluka Kingri, District Khairpur (Mir's) during, 2015. The observation on population fluctuation of the pest was recorded from 20<sup>th</sup> August to 2<sup>nd</sup> November on five commercial and famous mango varieties viz, Sindhri, Langra, Chaunsa, Fajiri, and Siroli. The experiment was laid out in randomized complete block design (RCBD) with three replications. Each replication comprised of one mature mango plant. The observation were taken from randomly selected five flushes twice a week from 20<sup>th</sup> August after harvesting when new flushes appeared on the plant till the stoppage of fresh flushes cycle.

The data on climatic factors (temperature and relative humidity) were collected from the Herbarium, Shah Abdul Latif University, Khairpur during 2015. The correlations between leaf miner population and weather factors were worked out in different varieties.

### 2.2 Population and parasitism of parasitoids on mango leaf miner

The five leaf miner infested flushes were collected twice a week from the different mango varieties i.e., Sindhri, Langra, Chaunsa, Fajiri, and Siroli for counting parasitoids mummies populations. The every infested shoot was kept separately in plastic jars covered with muslin cloth in the laboratory under room temperature of 25–30°C ± 2°C. The moisten soil was placed at bottom of the plastic jars. Every mango shoot was dipped in moisten soil for remaining freshness of leaves. The emerging parasitoids adults mines the leaf miner larvae in the plastic jars were collected by using a pooter and preserved in ≥99.5% (vol.) ethanol before to identification. The emerged parasitoids were counted separately and identified according to [31]. The parasitism percent was calculated with the following formula:

Parasitism percent Total number of larvae x 100

#### 2.3 Abiotic factor correlated with pest population

The data of abiotic factors (temperature and relative humidity) were correlated with pest population to find out the influences of weather factors on pest development by using computer software Statitix 8.1.

#### 2.4 Statistical Analysis

The recorded data were statistically analyzed to compare significant difference in the leaf miner larvae and parasitoid parasitism percentage separated by LSD test of different mango varieties on computer software Statitix 8.1 (Analytical Software, USA).

#### 3. Results

The experiment was conducted to examine the population of leaf miner and parasitism percentage of leaf miner A. syngramma, parasitoids on different mango varieties i.e. Sindhri, Langra, Fajiri, Chaunsa and Siroli during 2015 at Taluka Kingri District Khairpur. Significant differences (P<0.05) were observed in the population of parasitoids, Chrysocharis nephereus, Sympiesis hyblaeae, leaf miner and parasitoids parasitism percentage between mango varieties. The mango fruits were matured and harvested in the month of July. However, plants were started newly flushes cycle in the month of August. The mango leaf miner was status a major pest of mango, it occurs to start damage on newly emerged flushes in the month of August. While, it disappeared with the stoppage of emerging plant flushes cycle in the month of November.

### 3.1 Population of mango leaf miner on different mango varieties

The population of mango leaf miner was varied (F=683.28; df= 4; P= < 0.0001) significantly on mango varieties. The data are presented in Fig. 1 revealed that maximum leaf miner larval population was calculated on mango variety Chaunsa (15.806 leaf miner/shoot) and found highly susceptible, while, Langra was comparatively susceptible with mean number of (10.615 leaf miner/shoot) respectively in 2015.

### 3.2 Population fluctuation of leaf miner in mango orchards

The mango leaf miner considered as a minor pest of mango plants, but recently its status reached as a major pest at Taluka Kingri District Khairpur Sindh Pakistan. The leaf miner larvae severely damaged the newly emerged flushes; the experiment was conducted to furnished population abundance of mango leaf miner for the relative resistance of different mango varieties under field conditions. The leaf miner populations appeared on different mango varieties during 3<sup>rd</sup> week of August and fluctuated till the 1<sup>st</sup> week of November. There were four peaks of population of the leaf miner were observed fluctuated with the new flushes cycle in 2<sup>nd</sup> and 3<sup>rd</sup> week of September and 1<sup>st</sup> and 2<sup>nd</sup> week of October respectively, in the experimental field of mango orchards in 2015 (Fig. 2).

### 3.3 Adult population of natural enemy *C. nephereus* on different mango varieties

The adults parasitoids *C. nephereus* were emerged from mango leaf miner was observed (F=88.05; df= 4; P= < 0.0001) significantly differences on different mango varieties. The results in Fig. 3 revealed that the highest adult population were calculated on mango variety Chuansa (4.502/plant) followed by Fajiri (3.519/plant), Siroli (3.386/plant), Sindhri (2.529/plant) and Langra (2.495/plant) respectively in 2015.

### 3.4 Adult Population of natural enemy S. hyblaeae on different mango varieties

The adult population of mango leaf miner parasitoid, *S. hyblaeae* was observed (F=444.75; df= 4; P= < 0.0001) significantly varied on different mango varieties. The data are depicted in Fig. 3 showed that the maximum adult population were recorded on mango variety Chuansa (1.767/plant) followed by Siroli (1.398/plant), Fajiri (1.293/plant), Sindhri (1.151 parasitoids/plant) and lowest on Langra (0.688/plant) respectively in 2015.

## 3.5 Parasitism percentage of leaf miner by parasitoids, *C. nephereus* and *S. hyblaeae* on different mango varieties

The parasitism percentage of mango leaf miner by parasitoids, C. nephereus and S. hyblaeae were (F=42.37; df= 4; P= <0.0001) significantly varied on different mango varieties. The data are presented in Fig. 5 revealed that the maximum leaf miner larvae were parasitoids by C. nephereus and S. hyblaeae on mango variety Chuansa (54.118/plant) followed by Siroli (40.088/plant), while, minimum on Sindhri (20.922/plant), Langra (21.607/plant) and Fajiri (22.635/plant) respectively in 2015. The parasitoids leaf miner larval mummies' pictorial in Fig. 7 to 10.

# 3.6 Parasitism percentage of parasitoid, *C. nephereus* and *S. hyblaeae* in different observation dates on mango varieties

The status of natural enemy parasitoids, *C. nephereus* and *S. hyblaeae* of mango leaf miner were monitored in mango orchard at Taluka Kingri District Khairpur Sindh Pakistan. The parasitoids parasitism percentage of *C. nephereus* and *S. hyblaeae* Fig. 6 on mango leaf miner was found significantly difference (F=3.30; df=18; P= < 0.0001) in different observation dates. The parasitoids caused maximum leaf miner parasitism on mango variety Siroli (97.336/shoot) and Chuansa (79.664/shoot) on 3<sup>rd</sup> week of October. However, minimum parasitism was observed on mango variety Langra (6.977/shoot) on 1<sup>st</sup> observation date during 3<sup>rd</sup> week of

August in the experimental field of mango orchard.

### 3.7 Correlation of temperature and humidity with leaf miner population

The data are presented in Tables 1.0 and 2.0 indicated a non-significant and negative correlation of leaf miner populations with 9.0 am and 12.0 pm temperature and relative humidity with Chaunsa, Fajiri and Sindhri mango varieties, while, non-significantly and positively correlated with temperature and relative humidity on Langra and Siroli varieties respectively. However, data in Table 3.0 revealed that leaf miner population was non-significantly and positively correlated with 6.0 pm temperature and relative humidity on all mango varieties except relative humidity, non-significant and negatively correlated with leaf miner population with a Chaunsa mango variety during 2015.

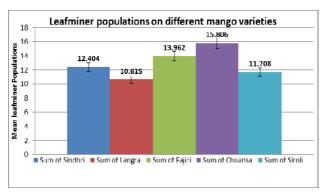


Fig 1: Mean population of leaf miner in different mango varieties

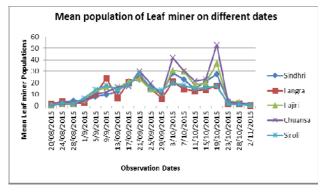
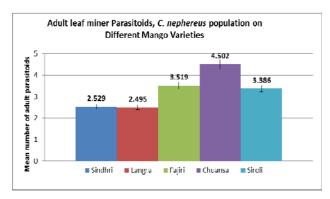


Fig 2: Mean Population of leaf miner in different observation dates on mango varieties



**Fig 3:** Mean adult population of leaf miner parasitoid, *C. nephereus* on different mango varieties

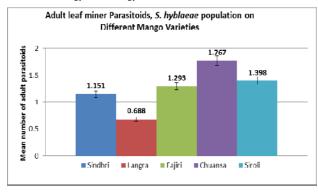


Fig 4: Mean adult population of leaf miner parasitoid, *S. hyblaea* on different mango varieties

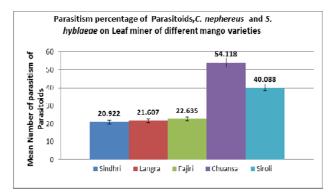
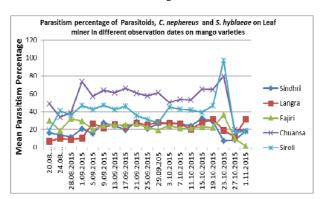


Fig 5: Mean parasitism percentage of leaf miner by parasitoids in different mango varieties



**Fig 6:** Mean Parasitism Percentage of Parasitoid, *C. nephereus* and *S. hyblaeae* on different mango varieties in different observation dates

**Table 1:** Correlations (Pearson) of mango leaf miner population on different mango varieties with temperature and humidity at 9.0 am during-2015

	Temp	Humid	Chaunsa	Fajiri	Langra	Sindhri
Humid	0.4919					
P-VALUE	0.0324					
Chaunsa	-0.1282	-0.3460				
	0.6008	0.1467				
Fajiri	-0.0568	-0.2375	0.9660			
	0.8172	0.3276	0.0000			
Langra	0.1280	0.0351	0.7437	0.8297		
	0.6015	0.8867	0.0003	0.0000		
Sindhri	-0.0846	-0.1920	0.9401	0.9614	0.8290	
	0.7305	0.4311	0.0000	0.0000	0.0000	
Siroli	0.1451	0.1500	0.7754	0.8578	0.9232	0.8673
	0.5533	0.5399	0.0001	0.0000	0.0000	0.0000

**Table 2:** Correlations (Pearson) of mango leaf miner population on different mango varieties with temperature and humidity at 12.0 pm during-2015

	Temp.	Humid.	Chaunsa	Fajiri	Langra	Sindhri
Humid	0.0956					
P- VALUE	0.6971					
Chaunsa	- 0.1146	-0.2608				
	0.6404	0.2808				
Fajiri	0.0589	-0.1743	0.9660			
	0.8107	0.4755	0.0000			
Langra	0.1648	0.0405	0.7437	0.8297		
	0.5003	0.8691	0.0003	0.0000		
Sindhri	0.0370	-0.2004	0.9401	0.9614	0.8290	
	0.8804	0.4107	0.0000	0.0000	0.0000	
Siroli	0.1956	0.0810	0.7754	0.8578	0.9232	0.8673
	0.4224	0.7417	0.0001	0.0000	0.0000	0.0000

**Table 3:** Correlations (Pearson) of mango leaf miner population on different mango varieties with temperature and humidity at 6.0 pm during-2015

	Temp.	Humid.	Chaunsa	Fajiri	Langra	Sindhri
Humid	0.2871					
P- VALUE	0.2333					
Chaunsa	0.0087	-0.0233				
	0.9719	0.9244				
Fajiri	0.0871	0.0384	0.9660			
	0.7229	0.8761	0.0000			
Langra	0.2538	0.2137	0.7437	0.8297		
	0.2944	0.3797	0.0003	0.0000		
Sindhri	0.1189	0.0437	0.9401	0.9614	0.8290	
	0.6277	0.8590	0.0000	0.0000	0.0000	
Siroli	0.2687	0.2835	0.7754	0.8578	0.9232	0.8673
	0.2659	0.2395	0.0001	0.0000	0.0000	0.0000



Fig 7: Parasitoids larvae of mango leaf miner, A. syngramma in infested mango leaves

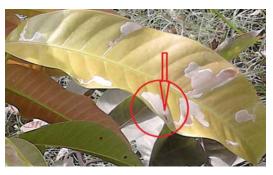


Fig 8: Parasitoids larvae of mango leaf miner, A. syngramma in infested mango leaves



Fig 9: Parasitoids larvae of mango leaf miner, A. syngramma in infested mango leaves



Fig 10: Parasitoids larvae of mango leaf miner, A. syngramma in infested mango leaves

#### 4. Discussion

Present studies on population of leaf miner, A. syngramma on different mango varieties indicates that the lowest larval population of pest on mango varieties i.e., Langra (10.615/shoot) and Siroli (11.708/shoot), whereas, maximum population was recorded on Chuanca (15.806/shoot) followed by Fajiri (13.962/shoot) and Sindhri (12.404/shoot) during the study period. The present results are fully agreement with those of Vanitha et al. [20] who were recorded 15-45 leaf miners, A. syngramma larvae in a single leaf of cashew plant. Agboton et al. [32] recorded 262 insect species on cashew plants, amongst them, the leaf miner, Eteoryctis gemoniella Stainton, was showed as the major insect pest and creates damage to the leaves of cashew trees in Benin. However, Kannan and Rao [33] reported that the leaf miner, A. syngramma was caused 12.11% damage to leaves of 0-5 year's old mango plants and 10.52% to 15 and above years old trees. A. heirocosma was created heavy damage (17.69 percent) to leaves of Litchi plants Ahmed [34]. Jacob [35] reported that the infestation of leaf miner, Conopomorpha syngramma (Acrocercops syngramma) on the leaves of cashew, Anacardium occidentale caused photosynthetic loss in the trees. Butani [36] reported that the leaf miner, A. syngramma was a major pest of mango, cashew and Jamun plants. The incidence of leaf miner on newly emerged leaves in District Khairpur, Sindh Pakistan is an alarming situation to the mango growers, due to its infestation plant loss the newly emerged leaves; the leaves are the food factory for plants, leaves synthesize xylem after the photosynthetic process to phloem which is essential food for plant health. It is, therefore, necessary to develop control approaches for to keep population below the economic threshold level.

The present results on adult population of parasitoids *C. nephereus* and *S. hyblaeae* and their parasitism percentage of leaf miner were found highly significant difference in each observation dates and on mango varieties. The adult

population of parasitoid, *C. nephereus* was found more than *S. hyblaeae* on leaf miner. Both parasitoids were caused parasitism to 2<sup>nd</sup> and 3<sup>rd</sup> instar leaf miner larvae on different mango varieties. However, highest parasitisms were occurred on Siroli (97.337%) and Chuansa (79.664%) during 3<sup>rd</sup> week of October. The maximum leaf miner parasitism of parasitoid, *C. nephereus* and *S. hyblaeae* were calculated on mango variety Chuansa (54.118%) and minimum on Sindhri (20.922%). The results are fully agreements with those of Vanitha <sup>[24]</sup> recorded an about 50% leaf miner population was decreased by three eulophid larval parasitoids out of them *C. nephereus* caused 90% parasitoids of cashew leaf miner larvae under field conditions. Whereas, Beevi *et al.* <sup>[37]</sup> were recorded parasitoid, *S. hyblaeae* in the larvae of cashew leaf miner, *Conopomorpha syngramma*.

The Sundararaju [38] reported that the 59.1% larvae of leaf miner, A. syngramma were parasitized by singly Sympiesis sp. The beneficial insect parasitoids have had a vital role in decreasing the insect pests in natural, urban, and agricultural ecosystems Butler et al. [39]. Mafi and Ohbayashi [40] observed that the parasitoid, Chrysocharis pentheus were oviposited eggs in 3rd instar larvae of Phyllocnistis citrella Stainton. However, Grabenweger [41] was observed parasitoid, C. nephereus from leaf miner larvae. Grabenweger and Lethmayer [42] who reported that Eulophidae parasitoid, Pnigalio agraules positively correlated with the population of chestnut leaf miner. The natural host plant resistance and natural enemies' parasitoids were significantly lessening factors to pest population below economic injury levels. The ectoparasitoid, Sympiesis striatipes is a major bio-control agent of the gracillariid leaf miners, Acrocercops sp. and Phyllonorycter sp. in many Asian countries Schauff et al. [43]. Heinz [44] reported that the ectoparasitoid, Chrysocharis nephereus caused parasitism on various sizes of leaf miner larvae.

The weather factors play very important role in insect reproduction capability the present results of correlation of abiotic factor with mango leaf miner populations was found non-significant negative and positive with temperature, relative humidity in different mango varieties during the study periods. A results are partial agreement with those of Devi and Sharma [45] worked on weather conditions, i.e. temperature (°C) and relative humidity and their effects on citrus psylla (Diaphorina citri) Kuwayama on Kinnow mandarin. The effect of weather factors, i.e., temperature was found significant and positively correlated with nymphs and adult populations, while, relative humidity was observed negative correlations. Ahmed et al. [46] carried out studies on the relationships of citrus leaf miner population in citrus nurseries and orchards and correlated their population with temperatures (weekly average, maximum and minimum). The population of citrus leaf miner citrus nurseries and orchards was found a significant and positive correlation with temperatures (weekly average, maximum and minimum). However, relative humidity was found negatively correlated. The relative humidity was positively correlated with CLM abundance and incidence. Sharma et al. [47] worked out on influencing weather factors on the tomato leaf miner. However, the leaf miner population was non-significantly and positively correlated with maximum temperature. While, relative humidity was negatively correlated with leaf miner populations. Sharma et al. [48] evaluated leaf miner population and their correlation with abiotic factor on tomato crop. However, the leaf miner population was significant and positive correlations with maximum temperature (r=0.522),

whereas, non-significant and positive correlated with minimum temperature (r=0.125). The relative humidity, rainfall was found negatively correlated with leaf miner. Lomelí-Flores et al. [48] carried out studies to evaluate the impact of ambient temperature, on seasonal abundance of coffee leaf miner Leucoptera coffeella (Guérin-Ménèville). Patel et al. [49] conducted experiments to evaluate the effects of weather factors on population of citrus leaf miner (Phyllocnistis citerlla Stainton) on lime Citrus aurantifolia (Christm) Swingle. However, the maximum larval population of leaf-miner was observed during the month of August-September. The minimum temperature was found positive correlation. While the leaf miner were preferred 18 °C, for their reproduction. Mahmood et al. [50] carried out studies to evaluate the effects of weather factors on leaf miner, Phytomyza horticola G. on pea crop. The minimum temperature and relative humidity were positively correlated with leaf miner larval populations. Whereas, maximum temperature had negative effects on leaf miner larvae, while all weather factors was found non-significant and positive or negative correlations with leaf miner larval populations.

#### 5. Conclusion

During the study it was observed that the mango leaf miner infested to newly emerged flushes of mango plants after fruit harvesting. The host plant resistance and natural enemies parasitoids are the main factors for decline in pest population and kept it in check. For encouragement to conserve the parasite population avoid synthetic chemical pesticide applications. However, the abiotic factors, temperature and humidity had slightly affected the pest. The growers should avoid application of flood irrigation, they should apply irrigation in channel system and different mango varieties are suggested to be cultivated in separate plots for to maintain temperature and humidity through irrigation applications scheduled.

#### 6. Author Contributions

Dr. Fateh Muhammad Kanher had designed experiments and technical guidance was contributed by main author, Khalil Ahmed Kanhar who had conducted experiments and wrote the manuscript, present research is a part of his M.S thesis. Dr. Abdul Manan Shaikh, Rehana Panhwar and Saeed Ahmed Tunio edited and analyzed the data of manuscript. Raja Riaz Hassan Awan had identified the leaf miner and parasitoids adults.

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