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A case study of insect pest complex of citrus and their management at Keren, Eritrea, and a note on their natural enemies

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Abstract
The present study was undertaken to record the infestation of insect pests in citrus orchard during November 2014 to April 2015 at Keren, Eritrea and to control key insect pests with botanicals or chemical and document the natural enemies' situation. The present study recorded the infestation of woolly whitefly (*Aleurothrixus* sp.), cottony cushion scale (*Icerya purchasi*), citrus leaf miner (*Phyllocnistis* sp.), diaspine black scale (*Parlatoria* sp.) and brown scale (*Coccus* sp.). To control the high incidence of *Aleurothrixus* sp. and *I. purchasi*, field evaluation of locally prepared botanicals (5% neem seed kernel extract, 5% balanites kernel extract) and imidacloprid 17.8% SL were carried out. Overall results indicate the effectiveness of these botanicals and chemical insecticide in managing the pests at three weeks post-foliar spray. Surprisingly no natural enemy was observed which could check the growing populations of insect pests.

Keywords: Citrus insect pests, woolly whitefly, cottony cushion scale, management, natural enemies, Eritrea

1. Introduction

Citrus is an exotic plant introduced to Eritrea during the Italian colonial era, produced as a cash crop [1]. In the early 1960's and 70's, citrus especially orange and mandarin along with other fruit crops and vegetables were the main exported crops of Eritrea to the Middle East and Europe; worth of 4-5 million USD annually [2]. However, due to the long war of independence against Ethiopia, the production of citrus fruit reduced sharply and its exportability ceased. After independence citrus cultivation like all other agricultural developments has been rehabilitated and expanded in its area of production in the country except Southern Red Sea region [1]. It is mainly produced in Alla plains, Mai-Habar, Beareza, Adereso, Maiseraw-Gensel, Mai-Ayini, and Mereb. Furthermore, it is also grown in the valleys of Gash, Anseba, Barka and Sawa [1] and the areas such as Elabered, Ghinda, Solomuna, Keren, Tekreret, Binbina and their vicinities are very conducive for citrus plantations [3].

The orange is the second most important tropical fruit in Eritrea in terms of total area of cultivation (520 ha) and fruit production (8,230 t); first being banana (24,012 t of fruit produced from 1,452 ha of cultivated land) whereas other tropical fruits including guava, mango, mandarin, lemon, lime and papaya occupies 792 ha of the cultivated area and contribute 9,223 t of the total fruit production [4]. The total citrus production in the country has decreased from 228 and 221 q/ha (416,678 q to 166,226 q from 751 ha to 1,825 ha), respectively [5]. Some farmers have the potential of producing an average yield of 150-170 q/ha. Whereas the expected potential yield is 200 q/ha which is twice the production of the present cultivation practices and thus the low yield is due to many factors such as abiotic, biotic, and socio-economic factors (i.e. no supply of pesticides, poor/lack of knowledge of management practices by the farmers and there are few plant protection experts to provide advice and give recommendation that help carry out the management practices to the farmers). The losses caused due to insect pests range 50 to 77% in zoba Debub and zoba Anseba together [6]. At present the infested area by several insect pests is estimated at about 5,000 ha and over 2,000 households are affected associated with citrus industry.

The various factors which attribute to low yields of citrus fruits are inadequate capital, inadequate planting materials, poor soil fertility, poor orchard management practices,

infestation of insect pests and diseases, improper weed management, etc. [6]. Of these, insect pests such as scale insects, mealy bugs, whiteflies, psyllids and aphids infest and affect foliage, twigs, and fruit of citrus deteriorating the tree health and fruit quality, and thus impact mature fruiting as well as newly planted groves and resets [7, 8].

The main objectives this study were to explore the insect pest complex infesting the citrus plantation in the Hamelmalo citrus orchard, Keren, Eritrea and identify them using the available literatures, and control of these insect pests with the locally made botanicals and a systemic chemical insecticide. The reason why botanicals were chosen is due to their effectiveness in reducing the percentage of pest infestation causing no hazardous side effects on environment and their safety to natural enemies.

2. Materials and Methods

2.1 Insect pest identification

The record of pest complex of citrus was done by the collection of different stages such as egg, nymph and adults present on the leaves. The insect samples were put in alcohol (70%) for their identification at a later date. The insect pests were identified up to generic level under a stereoscopic light microscope in the laboratory. The illustrated diagnostic keys of Martin [9-11], Martin *et al*. [12] and Gullan [13] were consulted to confirm the identification of whitefly and mealy bug.

2.2 Population study plan

2.2.1 Inclusion criteria: Due to the heavy infestation of two major insect pests, woolly whitefly and cottony cushion scale on citrus plantation in the citrus orchard, we prepared four treatments with each of them having seven replications.

2.2.2 Exclusion criteria: The rest of the populations were excluded because counting of the infested area would take more time than the available time for the research.

2.3 Preparation of botanical extracts

The locally crude extracts of botanicals such as neem and balanites were prepared and used in the experiments. The mature desert date (*Balanites aegyptiaca*) fruits, locally known as *mekie* or *agulum*, were collected from naturally grown trees during November 2014 and March 2015, whereas neem (*Azadirachta indica*) seeds were collected during July-August 2014. The kernels from seeds were released from such fruits using a mortar and a pistil. The crude seed kernel extracts was obtained through water soaking method for the two botanicals (neem seed kernel extract, NSKE; balanites kernel extract, BKE). Finely ground powder of the previous parts was prepared one day before the start of each experiment. Accordingly, 5 g powder needed for each sample was weighed on an electronic balance and then added to 50 ml of tap water and thoroughly mixed with a glass rod, and allowed to stand overnight. Thereafter, the mixture was agitated manually for 10-15 seconds before filtration using a fine mesh. The final volume of the extract was made up to 100 ml to get the required concentration of 5% (w/v).

2.4 Laboratory evaluation

The laboratory bioassay of two botanicals and one neonicotinoid systemic insecticide imidacloprid 17.8% SL (Brand Victor) was carried out against woolly whitefly and cottony cushion scale before the field evaluation. The crude kernel extract of the selected local botanicals were prepared as described previously. The crude extract was sprayed with hand-held pneumatic sprayer on woolly whitefly- or cottony cushion scale-infested citrus leaves. The petioles of leaves were tightened with wet cotton swab to prevent dryness of the leaves and kept at ambient temperature (25 ± 4 ºC) in the laboratory. The insect mortality was observed at 48 and 96 h post-spray. There were four treatments: NSKE 5%, BKE 5%, imidacloprid 17.8% SL and control (tap water alone). The application of one treatment on each leaf infested by either woolly whitefly or cotton cushion scale was considered as one replication. There were three replications in each treatment.

2.5 Field evaluation

The locally made crude botanicals (NSKE and BKE) and imidacloprid were prepared as described above. There were four treatments: NSKE, BKE, imidacloprid and control (tap water). The application of one treatment on each leaf infested by woolly whitefly or cotton cushion scale was considered as one replication. There were seven replications in each treatment. The spraying on citrus plantation was done three times during February and April 2015 with hand sprayers and later sprayed leaves were tagged. The date of spraying was 20/2/15, 6/3/15 and 23/3/15 for woolly whitefly and 25/2/15, 11/3/15 and 3/4/15 for cottony cushion scale. The observation on treatment effect on insects was checked one week of after-spray by taking photographs of tagged leaves. The snaps were taken by a digital camera, Canon PowerShot SX40 HS of 12.1 MP resolution and the insect population was counted on each leaf.

2.6 Record of natural enemies

In order to document natural enemies of pest complex, the citrus leaves infested by major insect pests such as whitefly, mealy bugs, scale insects were plucked and put in polythene bags and plugged with non-absorbent cotton for aeration of incubating bioagents, if any. The leaf samples were checked twice a week. After two weeks of incubation, the samples were thoroughly checked under the microscope and careful observation was taken to record the presence of natural enemy. The process of incubation and observation of insect-infested leaf samples were repeated during the period November 2014 to April 2015.

2.7 Statistical analysis

The effectiveness of botanicals and chemical insecticide was analysed by comparing the insect populations before and after spray and finding the differences between them. For the field evaluation of treatments, the insect populations were counted on each photographed leaf using Adobe Photoshop® CS3 Extended version 10 Software in Grid View Mode by the formula $[[(P_o - P_f) / P_o] \times 100]$, where P_o stands for initial mean population count (n = 7) before spray, P_f as final mean population count (n = 7) after spray of one week and %D is the percent decrease in insect population after one week of spray (Table 3). One-way ANOVA analysis was carried out separately for woolly whitefly and cottony cushion scale by running the software programme GenStat Release 10.3 [14]. The means were separated using LSD and the differences among means were considered significant at $P < 0.05$.

3. Results and discussion

3.1 Insect pest complex of citrus

During the study period of six months (November 2014 to April 2015), we encountered five most significant insect pests

infesting the citrus plantation. The recorded insect pests were identified at generic-level as woolly whitefly (*Aleurothrixus* sp.), cottony cushion scale (*Icerya purchasi*), citrus leaf miner (*Phyllocnistis* sp.), diaspine black scale (*Parlatoria* sp.) and brown scale (*Coccus* sp.) (Table 1). Species-level identification was not done due to unavailability of materials needed to study the specimens at higher magnification as well as limited availability of literature resources.

Table 1: Record of insect pest complex of citrus and their level of incidence in citrus orchard of Hamelmalo Agricultural College, Keren, Eritrea.

S.N.	Common name	Scientific name* (Order: Family)	Level of incidence
1	Woolly whitefly	*Aleurothrixus floccosus* (Hemiptera: Aleyrodidae)	High
2	Cottony cushion scale	*Icerya purchasi* (Hemiptera: Monophlebidae)	High
3	Diaspine black scale	*Parlatoria ziziphi* (Hemiptera: Diaspididae)	High
4	Brown soft scale	*Coccus hesperidum* (Hemiptera: Coccidae)	Medium
5	Citrus leaf miner	*Phyllocnistis citrella* (Lepidoptera: Gracillariidae)	Low

* The species-level nomenclature of the insects was based on the IPM Team Task Force [6].

In Eritrea, woolly whitefly was introduced and recorded for the first time in Alla valley where it was established and spread out to other areas such as Mai-Ayini, Mai-Habar, Adereso, Ghinda, and Durfo [1]. We recorded this pest from Hamelmalo Agricultural College, Keren orchard, Keren. The source of spread may be through wind or infested food items. With regard to cottony cushion scale, its infestation was low till 2012 in citrus orchard but it has gained a major status due to favourable environmental conditions and now is found in most of the citrus plantation of the college orchard. Other sucking insect pests encountered during our research are black scale and brown scale. As no management practices are employed for its caretaking, high infestation rate is obvious. Generally these pests are kept under control by the natural enemies; however, absence of bio-agents could result in high population build up.

3.2 Laboratory evaluation

Significant results were obtained with regard to laboratory bioassays. More than 90% mortality was achieved with all the three treatments namely, NSKE 5%, BKE 5% and imidacloprid 17.8 SL at 48 h of post-spray. Absolute mortality was recorded at 96 h post-treatment (Table 2).

Table 2: Laboratory bioassays of botanicals and chemical insecticide against citrus insect pests.

Treatments	Percent mortality of insects (hours after treatment)					
	Woolly whitefly			Cottony cushion scale		
	0	48	96	0	48	96
NSKE 5% w/v	87.3	51.7	0	34.0	18.3	0
BKE 5% w/v	69.7	33.7	0	45.3	14.7	0
Imidacloprid 17.8% SL	60	35	0	31.3	18.3	0
Water (control)	34.7	29.3	18.3	15.3	11.3	8.67
Mean	62.9	37.4	4.58	31.5	15.7	2.17
LSD at 5%	NS	NS	0.01	NS	NS	4.2
CV	21	48	56	64.3	41.6	56

3.3 Field evaluation

To control two key insect pests namely woolly whitefly and cottony cushion scale, field evaluation of locally prepared botanicals, NSKE and BKE, and imidacloprid were compared with water as a control treatment. Overall results indicate the effectiveness of NSKE 5%, BKE 5% and imidacloprid 17.8% SL, which were on par compared with control treatment in controlling these two pests at three weeks of foliar spray (Table 3).

One week after the spray on woolly whitefly-infested leaves in citrus plantation, the treatment of BKE 5% was significantly superior to NSKE 5% and imidacloprid 17.8% SL which were on par compared with control treatment (Table 3). However, after the second spray (third week of first spray), performance of NSKE, BKE and imidacloprid were on par compared to control. However, in case of cottony cushion scale, after one week of spray, the performance of BKE and NSKE was on par with each other but superior than chemical treatment; however after the second spray (third week of spray), the performance of NSKE and BKE was significantly superior to imidacloprid which were on par compared with control treatment. After the third spray in cottony cushion scale-infested leaves, all the three treatments (BKE, NSKE and imidacloprid) were on par compared with control treatment in suppressing the insect pest population. It is worth to mention that when destructive observation of woolly whitefly- and cottony cushion scale-infested leaves were checked under microscope, we found only empty exuviae except a few dead nymphs lying within the shed skin.

3.3.1 Observations after first spray: The population of the insect pest post-spray was relatively low compared to pre-spray population on the infected leaf samples. The mortality rate of the whitefly and scales on the leaves was very high (80%) treated with BKE as compared to NSKE and imidacloprid. This probably was due to the presence of active ingredients saponin in the balanites seeds [15].

Table 3: Mean population count before and after the treatment of woolly whitefly and cottony cushion scale.

Treatments*	Spray 1			Spray 2			Spray 3		
	P_o	P_f	%D	P_o	P_f	%D	P_o	P_f	%D
Woolly whitefly									
NSKE 5% w/v	72	34.1b	52.6	30.7	10.4a	66.1	3.45	1.15	66.7
BKE 5% w/v	85	16.6a	80.5	10.6	2.9a	72.6	2.14	0.71	66.8
Imidacloprid 17.8% SL	84	36.4b	56.7	15.1	5.7a	62.3	3.29	1.14	65.3
Control	228	73.0c	68.0	42.9	16.6b	61.3	5.71	2.00	64.9
MEAN	117	40.0	65.8	24.8	8.9	64.1	3.65	1.25	65.7
LSD at 5%	NS	37.58		NS	8.26		NS	NS	
CV%	100	83.6		113.8	82		89.3	103	
Cottony cushion scale									
NSKE 5% w/v	19.9	15a	24.6	11.6	5.6a	51.7	3.6a	2.4a	33.3
BKE 5% w/v	33.4	14a	58	10.4	6.3a	39.4	3.1a	2.3a	25.8
Imidacloprid 17.8% SL	42.3	31.4c	25.7	26.7	19.3b	27.7	8.4a	6a	28.6
Control	33.9	22.9b	32.4	27.4	24c	3.4	18.6b	13.3b	28.5
MEAN	34.4	20.8	39.5	19.0	13.8	27.3	8.4	6.0	28.6
LSD at 5%	NS	11.41		NS	12.82		8.99	6.37	
CV%	53.2	48.8		74.9	82.8		93.9	93.9	

* P_o is initial mean population (n = 7) before spray; P_f is mean population count (n = 7) after spray

3.3.2 Observations after second spray: The data recorded in the second observation of the post-spray was found significant with regard to the population of insect. The highest mortality of 73% was observed with BKE indicating effective treatment due to active ingredient contained as said earlier.

3.3.3 Observations after third spray: The data recorded in the third observation was found to be statistically non-significant. The third observation recorded for the insect pest population was similar to that of the first and second observation but the reason for their non-significant was that population at initially (pre-spray) reached at minimum level. Therefore, it is concluded that the maximum insect-infected leaves population was reduced due to second treatment (P at < 5%) and the spraying of BKE and NSKE of 5% concentration give equal mortality with chemicals.

The major pest, i.e. woolly whitefly was identified by comparing their pictures and characters such as taxonomic diagnostic characteristic description, biology, life cycle and damaging potential. It was concluded that the pest attacking our orchard is *Aleurothrixus* sp. based on the available literature sources of Gullan [13] and Martin [9- 11], Martin *et al.* [12] and also different information gathered from Ministry of Agriculture, Asmara. The survey conducted [1] indicate that the woolly whitefly (exotic pest) at Alla was introduced by an Italian man in year 2000. Even though infestation of leaf minor, *P. citrella*, was at medium level we have not entertained it under our case study because we wanted to concentrate on two key citrus insect pests, i.e. *A. floccosus*, and *I. purchasi*, and shortage of time availability for the research work. It is believed with the current infestation rate, unless sustainable intervention measures are taken against these pests, they will expand and cover other citrus growing areas of the country [6], thus the citrus production of the country is at greater risk. Therefore, a thorough extensive research is warranted to consolidate the data on level of infestation of citrus pests, and adoption of suitable management options.

3.4 Record of natural enemies

It was our surprise that we observed no natural enemy from the insect pest complex of citrus. Absence of natural enemies from citrus ecosystem indicates a disastrous condition to prevail in near future. The current situation warrants deployment of appropriate control measures otherwise further infestation and spread of these pests to other areas will lead to citrus production at greater risk in the country.

4. Conclusion

Citrus is the second most important horticultural crop grown in Eritrea. However, there are a number of insect pests that are plaguing citrus plantation leading to economic loss. Out of these, major insect pests are woolly whitefly, cottony cushion scale and diaspine black scale apart from other minor pests such as brown scale and leaf miner. Their populations could be controlled by following package and practices such as use of high yielding varieties, proper plantation management (including clean cultivation by pruning trees time to time and removal of weeds, which serve as alternate hosts of pest inocula during off season), providing fertilizers supplement, efficient irrigation system, and integrated pest and disease management. As there is scarcity of chemical insecticides in the market, farmers could make use of locally prepared botanicals such as NSKE and BKE whose performance is at par with chemical insecticides. Moreover, the government should take initiative to organize orientation programmes in different zobas to educate the citrus growers and farmers to maximize citrus production.

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