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Monitoring of Population Density and Fruit Infestation Intensity of Tephritid Fruit Flies (Diptera: Tephritidae) in *Citrus reticulata* Blanco Orchard.

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Research Article

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ABSTRACT

Pakistan is a vital global producer and exporter of the fruits and its citrus production is mainly reliant on mandarins. Generally, a few percent of the citrus produced is lost during pre and post-harvest stages primarily due to insect pest attacks specially the fruit flies (Diptera: Tephritidae). Monitoring of fruit flies insect pests for their prevalence and incidence can give a better understanding of the numbers and the species present in the field as well as their degree of economical losses. The study reported in this paper was designed to test the *Citrus reticulata* Blanco var. mandarin with the aim to determine the population density and fruit infestation intensity of fruit flies in orchard. The present groundwork study had shown the presence of peach fruit fly *Bactrocera zonata* (Saunders) year round in citrus fruit orchards of the experimental site with its highest and the least numerical density and fruit injury intensity. Monitoring of fruit fly population in *C. reticulata* orchard indicated that the highest density of *B. zonata* (27.83-79.58 per trap per week) and fruit infestation (3.33-3.70%) were found in October and November. The least numerical density (1.20-1.56%) from May to July may be due to beginning of the fruit settings of the orchards when the oranges were still dark green and small size compared with 1.40% in December as a result of winter season causing poor survival of *B. zonata*. This information will ensure early detection of fruit flies entering in the orchard and timely application of protective sprays to prevent establishment and spread of adult pests.

INTRODUCTION

Citrus is a most common genus of flowering plants in the family Rutaceae of Order Sapindales in Plantae Kingdom. Citrus consists of different edible fruit species including the best-known examples are the oranges, lemons, grapefruit and limes. The differences between the various species of citrus are mainly due to their flavor, shape and size of the fruit. The flavors are primarily controlled by the balance between sour (acid) and sweet (sugar) factors. There is considerable evidence that citrus foods may help to reduce the risk or retard the progression of several serious diseases and disorders. Citrus is most commonly thought as a good source of vitamin C (ascorbic acid). Vitamin C, which is an essential water-soluble vitamin, plays a key role in the formation of collagen that is a primary component of much of the connective tissue in the body. Adequate collagen synthesis is essential for strong ligaments, tendons, dentin, skin, blood vessels and bones, and for wound healing and tissue repair. The weakening of these tissues is a symptom of vitamin C deficiency. Vitamin C is an important aid in the absorption of inorganic iron; and it has also been shown to aid in the treatment of anemia and stress [1; 2]. Characterized by the distinct aroma and delicious taste, citrus fruits have been recognized as an important food and integrated part of our daily diet, playing key roles in supplying energy and nutrients and in health promotion. With low protein and very little fat content, citrus fruits supply mainly carbohydrates, such as sucrose, glucose and fructose. Fresh citrus fruits are also a good source of dietary fiber, which is associated with gastrointestinal

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disease prevention and lowered circulating cholesterol [3]. Citrus fruits are nutrient-dense foods that can be good sources of carbohydrates, including dietary fiber, and many vitamins and minerals. For example, a medium orange contains 60 to 80 kcal; a grapefruit 90 kcal and a tablespoon (15 ml) of lemon juice only 4 kcal. The orange is a juicy fruit of the citrus genus, abundant all over the world and its nutrients are plentiful and diverse. Many papers have been published concerning nutritional requirements furnished by citrus and information is available showing a 225 ml glass of orange juice provides 75 mg of folic acid, and contains approximately 125 mg of vitamin C and 500 mg of potassium. One medium orange can provide approximately 235 mg of potassium and contains about 3.0 g of non-starch polysaccharides, and citrus fruit can make a valuable contribution to meet the daily fiber goal [4]. Furthermore, the peel oils of limes, lemons, mandarins, oranges, and grape fruits, exhibit toxic insecticidal properties, and are known for their antibacterial properties [5]. Most fruit flies are facultative breeders that can lay eggs whenever their host fruits are available and so may have many generations per year depending on host fruit availability. Their damage starts when the female fruit flies puncture the fruit with their long and sharp ovipositor. The fruit skin is breached, and bacteria enter and the fruit starts to decay. The larvae that hatch from the eggs feed on the decaying fruit tissue, and on the yeasts and bacteria that multiply in it. It is believed that some fruit fly females or may be all carry bacteria with them that they inject into the fruit at oviposition so that the fruit decays faster making it more nutritious for the larvae. Fruits with fruit fly larvae in them decay quickly and sometimes it is possible to cut out the damage for home consumption of the remaining part of the fruit, but infested fruits are generally un-saleable, and can certainly not be exported. Crop losses can vary from a few per cent up to 100%, and losses of 90% or over are common in the orchard [6]. Most citrus varieties can be attacked by fruit fly but some varieties are more attractive than others. Citrus may not the most favorable host for fruit fly because of its thick skin and rind oil, which can kill eggs and larvae. Citrus fruits are normally stung when they are silver green, just changing color to fully colored. Fruit damage can be high in situations when there are no other suitable hosts and there are high numbers of flies or fruits have thin skins or are already damaged (splitting, hail damage). In addition to the direct damage, fly can cause to fruit, an infestation that can has serious implications for movement of fruit beyond and within states, especially for export. Fruit infested with fly larvae usually falls from the tree. Damage by larvae tunneling in the fruit varies with the type and maturity of the fruit, the number of larvae in it, and the weather. Frequently citrus fruits, although stung, do not develop larvae, but the stung fruit sometimes fall and larvae can successfully develop in most citrus varieties [7]. Mature attacked fruits may develop a water soaked appearance and young fruits become distorted and usually drop [8].

The productivity of citrus orchards is still below the desired level because this sector is exposed to several constraints such as the of pest problems and among these, the fruit flies are the most important pest. Therefore, the renewed attempts are needed to elaborate an Integrated Pest Management (IPM) strategy in the country for citrus orchards. The objectives of this study were to quantify the population density and fruit infestation intensity of fruit flies entering in citrus orchard for timely apcplication of protective measure to prevent establishment and spread of adult flies. The use of current and comprehensive information on the pests and their incidence, in combination with available pest control methods by the most economical means, can be exercised to manage pest damage with the least possible hazard to people, property and the environment.

MATERIALS AND METHODS

Trials were laid in citrus fields located in the north of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. The experimental tests in fields were performed from January to December 2011-12 and study was conducted in orchards grown with *Citrus reticulata* Blanco var. mandarin. The population density of *Bactrocera* pests was calculated per week in each trap at a year round. Fly arthropods captured were identified to assess the selectivity degree of traps and attractants. Pest's infestation was calculated through randomly selected trees and fruits in each orchard per week at a year round.

Inspection of fruit fly populations

For pest monitoring for presence of fruit flies, Para-pheromone traps were used to observe fly populations in citrus orchards to increase attractiveness. The trapping systems were consisted of the trap and a lure (methyl eugenol) which contained a mixture of the Para-pheromone or male attractant and an insecticide. The insecticide purchased from the local agricultural supplier and used in traps included Chlorpyrifos. The lure in the traps attracted only the male fruit fly, which were then killed by the insecticide and these chemicals were effective for about 3 months then traps charged again with these chemicals. A minimum of three traps were used with an average of one trap per acre in a block. Traps were installed in trees at eye-level parts of the trees and properly placed out of reaching direct sunlight in the east or north

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east side of the tree. Traps were inspected daily until the first fly was caught, and then examined at weekly intervals for detecting fruit flies pests.

Observation of fruit fly infestations

The orchards were selected randomly and observations recorded on 100 fruits randomly from samples taken directly from the tree or from the fresh fallen fruits on the ground for weekly counting of the fruit fly damage. From each selected orchard, randomly 5 trees were selected, and on each selected trees, 20 fruits were observed randomly for recording observation on pest's infestation. For any external symptom of fruit fly infestation, fruits were separated into two categories like how many are possibly infested as indicated by the presence of puncture mark or other relevant signs, and none infested as indicated by the how many are not infested. From each sample of the possibly infested category of fruits, these were then dissected to confirm the presence of fly larvae inside. From the possibly infested record category, the numbers of fruits were confirmed to have fly larvae inside, then counted the percentage of infested fruits using formula:- number of confirmed infested fruits/ 100 samples x 100%.

Analysis of data

Fruit flies pest monitoring by each trap and observation recording on pest's infestation were pooled for each standard week all over the study period, using the Statistix 8.1 software. The average data variables calculated were subjected to ANOVA and compared according to LSD test at P≤ 5.0 level. Data are presented in table as means with standard errors and Least Significant Difference (LSD) test values.

RESULTS AND DISCUSSION

The citrus orchards showed numerous diversities of pest population and incidence having considerable values among the basic sector of horticulture economy. The peach fruit fly, *B. zonata*, was one of the most devastating pests of citrus fruits and considered as the most invasive of all members of the Tephritidae at study site.

Population density of *Bactrocera zonata*

Table 1: The population density of *Bactrocera zonata* (adults caught by traps) and percent fruit infested in *Citrus reticulata* var. mandarin orchards.

Month	Fruit fly population per trap per week	Fruit infestation (%)
January	4.16 c	0.00 f
February	3.00 c	0.00 f
March	3.46 c	0.00 f
April	4.26 c	0.00 f
May	4.40 c	1.20 e
June	4.90 c	1.13 e
July	5.46 c	1.56 d
August	7.66 c	2.40 c
September	9.58 c	2.56 c
October	27.83 b	3.33 b
November	79.58 a	3.70 a
December	11.83 c	1.40 de
S. Error	4.281	0.149
LSD Value	8.879	0.310

Within rows, comparisons made between mean values followed by the same letter are not significantly different by ANOVA at *P* = 0.05.

The field studies indicated that the fruit fly *B. zonata* population density in orchards under study differed between the year around (Table 1). It reached the highest peak of 27.83-79.58 flies per trap per week in orchards found in October and November. It had other peak in the study orchards reaching 11.83 insect/ trap per week through December during the maturity period of citrus fruits. The significant differences were achieved between fly captures by trapping in the orchards and months for study year. The

population density of *B. zonata* in orchards had been very low throughout the year from January to September and ranged 4.16- 9.58 insect/ trap per week under conditions of the region.

Fruit infestation intensity of *Bactrocera zonata*

Fruit infestation by *B. zonata* reached its highest numerical mass in citrus orchards (3.33-3.70%) found during October and November of study year. Data presented in Table 1, indicated that the *B. zonata* caused losses in citrus fruit production and damage in orchards reached to 1.20-3.70% from May to December of study year. The losses intensity due to pest was the highest in November (3.70%) tracked by 3.33% in October than compared with other months due to maturity period of citrus fruit. The pest caused lower economic losses in citrus during August and September which reached to 2.40% and 2.56% of the fruits, respectively. The least numerical density (1.20-1.56%) from May to July may be due to beginning of the fruit settings of the orchards when the oranges were still dark green and small size compared with 1.40% in December as a result of winter season causing poor survival of *B. zonata*. The results revealed that the percentage of punctured fruit was significantly different between months for study year in the fields. The present study had shown that the fruit fly *B. zonata* has a year round presence in citrus fruit orchards of study region. The monitoring of the captured fly showed that the pest generations most importantly coincided with the maturity of citrus. Further observations showed that oranges were attacked severely when they were at ripening stage and the puncturing rate reached at peak at early October and November. This behavior of *B. zonata* was probably favored by the fact that when the other citrus fruits were harvested at early December, the only host available was the mandarin orange. This phenomenon was described as the return on the host explained by the strong trend of females of the fly to lay their eggs in the host plant in which they made their larval development [9]. The result obtained in newly studies for citrus is close to other findings that fly control could be improved by an early installation of traps, before the ripening of fruits [10]. Current evaluations of fruit damage were limited to fruit stings or presence of larvae in host. Since citrus somewhat acts to stop eggs hatching and not to stop female flies from stinging fruits, it is possible that the larval population in the fruits decreased [11]. Thus, future trials could include incubation of damaged fruits to count pupation and adult emergence to detect these effects.

Field trials using traps baited with the lure performed well in reduction of fly population and decrease of fruit damage. These data further confirmed the reduction of fruit punctures by fly pest. Similar field trials had been conducted and currently being used over larger areas to control fruit flies. It was pointed out that the perimeter trapping strategy has obtained satisfactory results to avoid fruit fly intrusions in medium to large orchards, and this strategy depends on the efficacy of traps and lures [12]. The results of such published research as reported earlier underlined the crucial role of the mass trapping technique on the reduction of fly populations in citrus orchards [13]. However, it is suggested that both types of baits for capturing the males and females fruit flies can mutually decrease the numbers of the pest. This statement can be supporting from many previous data reports indicating effectiveness of male and female targeted baits. It was advisable to use both male and female targeted baits in separate and distant traps and not jointly in the same trap because the efficacy of detection or monitoring trials could be compromised [14]. It is reported that it is better to use traps that achieve higher captures and better female proportions [15]. Many previous studies have demonstrated that the efficacy of trapping using female targeted attractant depends on trap types. Indeed, it is indicated that trap type modifies the proportion of females caught [16]. Thus, the proportion of female catches is obviously influenced by attractant type. The results obtained had been suggesting that the mass trapping technique based on the use of the female targeted lure could be involved as effective strategy [17; 18]. Therefore, research and development of effective control methods as alternative to chemical control are needed especially [19]. These methods could include the use of traps baited with the male targeted and female targeted lures as an appropriate strategy for the control of the fly without including spraying treatment to avoid leaving of pesticide residues on fruits.

Fruit flies are serious pests for fruit production industries and their fruit hosts may vary between various territories and states. The traps are effective and convenient apparatus for detection of fruit fly but they are also an important tool for monitoring the efficacy of bait and spray programs. This technique could be integrated with some of other methods in integrated pest management approaches because it is a effective treatment without leaving pesticide residues on fruit. Its efficiency to protect fruits until the harvest, increases significantly providing that the farmers participation are involved in the operation. This is very important step because it is an effective and environmentally sensitive approach that relies on a combination of common-sense practices. In conclusion, since citrus fruits are valuable and important export commodity from Pakistan, the use of male lure along with female fly food bait lure is of particular interest for monitoring, detecting and controlling programs to minimize significant economic losses. Our

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study can further support the conclusion that maintaining of good orchard hygiene parctices are associated with a modest reduction in fruit flies damage. Orchard sanitation is an important part of fruit flies management, which include the removal of unwanted fruits from trees and around houses, sheds and along boundary hurdles, control of fruit flies in all other host plants, and removal of all late hanging fruits missed during harvesting. A trade zone or in other words the fruit fly exclusion zone can be established to maximize access to export markets by maintaining freedom from fruit flies within that zone. On a centralized Net Work server, digitization of such data might enable the pest administrator to analyze the pest activity with no loss of time and pass on information to facilitate the pest monitoring and the pest control guidelines.

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