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Monitoring of Population Dynamics and Fruits Infestation of Tephritid Fruit Flies (Diptera: Tephritidae) in Guava (*Psidium guajava* L.) Orchard.

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ABSTRACT

Guava (*Psidium guajava* L.), is one of the most important commercial fruits and its various parts are used for the development of a variety of industrial and pharmaceutical products. The fruit as well as its juice are freely consumed for their great taste and nutritional benefits. Fruit flies (Diptera: Tephritidae) are considered the key insects of guava fruit production causing yield losses and quality degradation. This paper explains studies made on monitoring of fruit flies through the use of methyl eugenol as a trapping to measure their distribution and abundance, whilst their damage monitored using periodic fruit samplings to assess the percentage infestation in guava orchard. One tephritid species namely the *Bactrocera zonata* (Saunders) was the most frequent, constant and dominant species associated with guava at the experimental site. Fruit fly analysis by traps installation in guava orchard characterized high population frequency of *B. zonata* (116.67-307.58) captured per trap per week in June to August and accounting for 8.05-18.59% fruit infestation values recorded. Pest incidence in field continued till the whole year, but more damage started at the ripening fruiting stage. The fruit infestation intensity exhibited a high link with fruit flies density on the host plant. The results of this study have important implications on the decision-making process for the safe and effective monitoring and management strategies to give information on fruit fly activity in an area to determine whether the number of pests are increasing or decreasing in the locality.

INTRODUCTION

Guava (*Psidium guajava* L.) is one of the most gregarious tropical fruits of the family Myrtaceae and commonly known for its food and nutritional values throughout the world. Quite a significant amount of work on the biological activity, pharmacological and possible application of chemical compounds from whole parts of the plant has been done. Guava is a well known cultivated tree and this plant seemed worthy in folk medicine; extracts of its roots, bark and leaves are used to treat gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums and a number of other conditions [1]. According to the findings of the antibacterial assay, the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus*, which are some of food-borne and spoilage bacteria. On the basis of the findings, guava leaf-extract might be a good candidate in the search for a natural antimicrobial agent and other pharmacological properties [2]. Guava fruit is an oval or pear-shaped berry, white or yellow at maturity with yellow or dark pink flesh having numerous seeds. The fruit as well as its juice are freely consumed for their great taste and nutritional benefits [3]. Guavas are often included among super fruits, being rich in dietary fiber, vitamins A and C, folic acid, dietary minerals, potassium, copper and manganese. Having a generally broad, low-calorie profile of essential nutrients, a single common guava fruit contains about four times the amount of vitamin C than an orange [4]. This is one of the fruits that contains high amount of ascorbic acid [5]. The fruit is also a source of antioxidant dietary fiber. Guavas contain both carotenoids and polyphenols which are the major classes of antioxidant pigments by giving them relatively a high potential antioxidant value among plant foods [6].

Fruit fly pests are members of the Family Tephritidae in the Order Diptera and they feed on hundreds of host plants leading to poor commercialization in domestic markets and quarantine restrictions from importing countries. Tephritidae fruit flies are considered a very destructive group of insects that cause enormous economic losses in agriculture, especially in a wide variety of fruits, vegetables and flowers [7]. The guava is subjected to attack by several kinds of pests and about 80 species of insects have been recorded on guava, but only few of them have been identified as pest of regular occurrence and causing serious damage [8]. Fruit fly infestations often spread quickly in guava trees which produce sweet-smelling with an edible rind and creamy white, yellow or pink flesh. When guava fruits ripe, these emit a pungent, musky odor that attracts fruit flies. Adult female fruit flies have a needle-like ovipositor with which they puncture the skin of fruits to lay their eggs in the flesh. Upon contacting the host, fruit flies lay their eggs beneath the skin of fruit and the hatching maggots feed on the flesh, and the resulting damage causes guavas rotting due to microbial decay of the flesh. When the guava fruit is squeezed, the tiny punctures that leak juice from surface indicate the infestation of fruit flies. At first the oviposition marks are difficult to detect but as within one to two days the eggs hatch, oviposition marks appear as a distinct spot with a brownish patch around puncture site [9, 10, 11].

Fruit flies damage levels to guava varied depending on the guava host availability in the field and oviposition by fruit flies. A damage of 25-50% has been reported to guava fruit by *Dacus zonatus* alone particularly in summer season [12]. The highest level of fruit fly damage and percentage of infestation (92.5%) on guava fruit were observed due to the most abundant species *Bactrocera invadens* [13]. High infestation of guava has resulted in abandoning the production of this popular fruit in Southern Pakistan [14]. In some cases where no control measures are adopted, the infestation becomes so serious that the entire guava crop is damaged [15]. By the reason of a wide dispersal and movements of fruit flies, a large number of hosts, field based individual monitoring and consequently pest management cannot be employed. For that reasons, farmers and integrated pest management trainers need to adopt pest surveillance and monitoring on area-wide basis to accurately scrutinize fruit fly populations to plan and implement location and situation specific management strategies. This paper explains the findings on population dynamics and fruit infestation of tephritid fruit flies in guava orchard.

MATERIALS AND METHODS

Experimental site

Studies on the seasonal fly monitoring and fruit infestation estimation were recorded during the seasons 2011 and 2012 made in the guava orchard of the Post-Graduate Agriculture Research Station (PARS) of University of Agriculture, Faisalabad. The scientific research to monitor fruit flies and infestation was analyzed by installation of traps and fruit samplings on a guava orchard variety "Gola". The selected orchards contain guava trees as the main plantation, and in neighboring are mango trees grown along with field crops. The experiment consisted of repeated trials distributed in a completely randomized design in three replications. The data-taking segments of this experiment consisted of the following parts: -

Observation of fruit fly populations

A total of 3 methyl eugenol baited traps were set up in each replicate at 50 m apart from each other to avoid the interference of the traps. Each trap had two holes of 30 mm on both sides and a cotton wad soaked with 0.5 ml methyl eugenol and 1 ml Endosulfan were placed in a loop of iron wire inside each trap. All the traps were serviced with these chemicals at monthly intervals and old blocks traps baited with methyl eugenol collected and buried at a depth of more than 40 cm in the soil. All the traps for fruit fly detection were hung in the orchard at about eye level within a host tree in the shady place at a density of just about 1 trap per acre. At weekly intervals, collected adult flies were identified upto the species level, counted the number of catches and recorded. After counting numbers of trapped flies, fruit flies collected from each trap were pooled for each standard week throughout the study period, and calculated Flies/ Trap/ week by dividing Total number of flies trapped with the Number of trapping weeks x Number of traps. If any of the traps were cloudy, moldy or damaged, changed with a fresh one and after handling the lure hands and all equipments washed with 70% ethyl alcohol to remove traces of methyl eugenol.

Evaluation of fruit fly infestations

For assessing the amount of fruit fly damage to guava fruits, recording of fruit fly oviposition marks on fruits was the first step to carry out study of infestations. For making this estimation, from the various trees a minimum of 100 fruits were randomly observed for fly oviposition punctures. Whenever, this practice was avoidable, in addition to the fruits observed from the tree, only freshly fruits fallen on ground were collected and examined by avoiding old fallen fruits, which might be damaged on the ground by other insect pests. The different species of guava fruits were not mixed together, but all of them kept separately. For assessing infestations each sampled guava fruit was examined in good light conditions and counted how many of these showed oviposition marks of fruit fly. Usually, whenever fresh oviposition punctures were not visible to the naked eye then a magnifying

hand lens was used to aid in detecting the damage. The percentage of fruit fly infestation (%) for all fruits crop at the time of sampling was calculated by counting total number of fruits showing fruit fly oviposition marks divided by total number of fruits examined or collected and multiplying by 100. After dissecting or cut opening fruit to look for larvae and counting the numbers of larvae if any fruit, even with one fruit fly larva in it was considered infested.

Statistical analysis of Data

Fruit flies collected from each trap were pooled for each standard week throughout the study period, using the Statistix 8.1 software. The data means calculated were compared according to ANOVA and subjected to LSD test at $P \leq 5.0$.

RESULTS AND DISCUSSION

The population dynamics of fruit flies and their infestation were studied in guava orchard, and the population of fruit flies in guava remained present from flowering to maturity of the fruits throughout the fruiting season. The species of fruit fly namely, *B. zonata* was recorded through methyl eugenol traps in guava orchard and the results are presented for throughout the year. The fruit maturity factor has some significant contribution towards population fluctuation and infestation percentage of fruit flies. The populations were low during most of the year, but increased when guava fruit reached maturity and the general information regarding research areas is given in Table 1.

Surveillance of fruit fly populations

The incidence of flies was observed throughout the period; however, their maximum density was observed when the fruit host availability density was appreciably high. The relative abundance revealed that one tephritid species namely the *Bactrocera zonata* (Saunders) was the most frequent, constant and dominant species associated with guava at the experimental site. The invasion of *B. zonata* in lure baited traps commenced from January (11.33 flies per trap per week) and attained its peak during July and August (161.21 and 307.58 flies, respectively). In general, the species of *B. zonata* showed lower densities during periods of January, February, March, April and May, and then during October, November and December. All these adults activity (maximum 55.33 and minimum 11.33 flies per trap per week) were found non-significant with one another in abundance. While in June and August adults activity (maximum 116.67 and minimum 58.25 fly catches per trap per week) were found significant with one another in abundance (Table 1).

Table 1: Relative abundance of the fruit flies recovered (Methyl eugenol trap catches) and their Infestation from the Guava (*Psidium guajava* var. Gola) host fruit Orchard.

Month	Fruit fly population per trap per week	Fruit infestation (%)
January	11.33 d	1.86 d
February	12.00 d	0.00 d
March	24.33 d	0.00 d
April	42.33 d	0.00 d
May	55.33 d	2.76 d
June	116.67 bc	8.05 c
July	161.21 b	13.37 b
August	307.58 a	18.59 a
September	58.25 cd	0.00 d
October	46.67 d	0.00 d
November	42.58 d	2.82 d
December	28.67 d	2.53 d
S. Error	29.349	1.482
LSD Value	60.867	3.073

Mean values followed by the same letters in the same column are not significantly different ($P < 0.05$).

Fruit fly infestations

As shown in Table 1, the abundance of fruit fly was observed throughout the year, with two peaks in summer from May to August and during winter from November to January coinciding with availability of guava fruits. The maximum fruit damage (18.59%) occurred in August, and second peak with 13.37% damage observed during period of July. The fruit fly damage gradually decreased from June (8.05%) and reached to a fall down of 2.76% in May. Later, the damage declined reached a lower level of 2.82% in November and it remained more or less at the same level in January (1.86%). Thereafter, a declining or zero level trends were observed during February to April and September to October having a significant positive correspondence with availability of fruits.

Studies pertaining to fruit fly dynamics in guava orchards were carried out with aim to investigate existing fruit fly fauna and how other factors play a role in their population fluctuating. The evidences obtained in this study suggest that, the population of fruit fly *B. zonata* was recorded throughout the year and the host availability factor has influence on the distribution of the flies in the study region. Similar studies were carried out earlier^[16], which underlines that the host availability and abundance of cultivated fruits are important factors which determine population fluctuations and fruit species. The influence of abiotic factors is closely related with fly population dynamics^[17] as well as their distribution^[18]. These results are in agreement with the report of wild hosts and trees that have been determined to be highly important in the re-infestation of orchards by fly pest during the flowering and fruit setting stages. Accordingly, these wild hosts and trees must be completely integrated in any fruit flies control program in a zone. Thus, monitoring of field conditions is the foundation of modern agricultural pests management in order to improve the efficiency of the data collection procedure. It is necessary for monitoring of fruit flies and their relation with biotic and abiotic factors in order to determinate the pattern of Tephritid fruit flies fluctuations. The damage levels on the guava host in the present study field had the fruit fly oviposition marks, and damage by fruit flies which was observed 18.59%. The present results are agree with studies in other countries which indicate that the primary or preferred hosts of *Bactrocera* may vary according to the region, type and host availability^[19]. The guava was the very preferred and damaged host fruit, and this has already been cited as fruit flies most infested host. Earlier damage assessments done have revealed highest percentage of damage on guava (37.5%)^[20] and damaged fruits (32.9%)^[21].

Surveillance of fruit fly populations was undertaken by installation of Steiner traps baited with Methyl eugenol attractant (lure) hung in the tree. The results revealed that two peaks of fruit fly population were recorded during the beginning of May to August and November to January for both periods of fruit settings of the study year. Fruit fly density was positively linked with level of fruit infestation. Further, however fruit fly population was positively related to maturing fruit, or sometime fly infested mostly mature to overripe fruits due to the different level of population prevailing in these orchards. The present results are in line with the findings of fruit flies depositing their eggs on host fruits when they are physiologically ripe^[22]. The peak activity of these fruit flies from August to October which coincided with the maturity period of guava has been observed^[23]. It has been reported that fruit flies exhibited un-modal patterns of population abundance with peak incidence from June to September in guava^[24]. The research in the development of efficient trapping system affords several new opportunities in the efforts to control the fruit flies. Since adult fruit flies use visual and olfactory stimuli to locate hosts, traps that combine visual and olfactory cues proved to be most efficient for capturing fruit flies. The responses of the fruit flies to visual stimuli are dependent on color, shape and size of the stimulus^[25]. These findings have opened new avenues in increasing efficiency of traps by altering the design and choosing the most suitable shape, size and color of trapping devices.

Surveillance of fruit fly populations carried out by installation of Methyl eugenol baited traps, is a direct tool in the control of fruit flies by using traps instead of cover sprays employed directly on orchards, and reduction of trace of insecticides in the field are some of the benefits of using trapping system. This method is known as Male Annihilation Technique (MAT), which can be used to minimize the numbers of male flies in an area. The population of flies will decrease if there are fewer males available to mate with females. The results from the fruit flies collected during this study suggest that as fruit flies attack only maturing fruit or sometimes flies infest mostly mature to overripe fruits, so, harvesting of guavas before they ripen fully is necessary. Due to abundant availability of this fruit during the season of production, its marketing becomes a serious problem and delayed harvesting makes guava vulnerable to the attack of fruit flies, so, early harvesting can prevent certain level of infestation. Picking up of fallen guava fruits before they ripen on the ground and attract the pests is necessary. For monitoring fruits for pest infestation, from the tiny punctures that leak juice when the fruit is squeezed indicate infestation of host. Cultural control can be aided in control operation by gathering all fallen and infested fruits and destroying them. Efforts are needed to keep a watch on development of these insect pests and to follow strict quarantine regulation to check the further spread of introduced pests. By the implementation of fruit flies exclusion, it is necessary to apply monitoring measures at entrance and control points of imported agricultural products as they are possible fruit fly hosts. The monitoring and detection processes will have to be implemented in international airports and at the borders of the state. Moreover, it is urgent to train farmers in the use of sustainable control measures to approach integrated pest management strategy.

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