Effect of the Blowflies (Diptera: Calliphoridae) on the size and weight of Mango (*Mangifera indica* L.)

Shafqat Saeed, Muhammad Nadir Naqqash, Waqar Jaleel, Qamar Saeed, Fozia Fozia Ghouri

Background: Pollination has a great effect on the yield of fruit trees. Blowflies are considered as an effective pollinator compared to hand pollination in fruit orchards. Therefore, this study was designed to evaluate the effect of different pollination methods in mango orchards. **Methodology:** The impact of pollination on quantity and quality of mango yield by blowflies was estimated by using three treatments, i.e., open pollinated trees, trees were covered by a net in the presence of blowflies for pollination, and trees were covered with a net but without insects. **Results:** The maximum number of flowers was recorded in irregular type of inflorescence, i.e., 434.80flowers/inflorescence. Fruit setting (bud) was higher in open pollinated mango tree (i.e. 37.00/inflorescence) than enclosed pollination by blowflies (i.e. 22.34/inflorescence). The size of the mango fruit was the highest (5.06mm) in open pollinated tree than the pollinated by blowflies (3.93mm) and followed by without any pollinator (3.18mm) at marble stage. We found maximum weight of mango fruit (201.19g) in open pollinated trees. **Discussion**: The results demonstrated that blowflies can be used as effective mango pollinators along with other bees. The blowflies have shown a positive impact on the quality and quantity of mango. This study will be helpful in future and also applicable at farm level to use blow flies as pollinators that are cheap and easy to rear.

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Manuscript Title

Effect of the Blowflies (Diptera: Calliphoridae) on the size and weight of Mango (*Mangifera indica* L.)

Running Title: Pollination in Mango by Blowflies

Authors

***Shafqat Saeed[1], Muhammad Nadir Naqqash[2], Waqar Jaleel[3], Qamar Saeed[4], Fozia Ghouri[5]**

[1]Entomology, Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan

[2]Plant Protection, Faculty of Agricultural Sciences and Technology, Nidge, Turkey

[3]Entomology, South China Agriculture University, Guangzhou, China

[4]Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Multan, Pakistan

[5]Plant Genetics, College Agriculture, South China Agricultural, University, Guangzhou, Guangdong, China

***Corresponding Author:**

Email: bumblebeepak@gmail.com

Abstract

Background: Pollination has a great effect on the yield of fruit trees. Blowflies are considered as an effective pollinator compared to hand pollination in fruit orchards. Therefore, this study was designed to evaluate the effect of different pollination methods in mango orchards.

Methodology: The impact of pollination on quantity and quality of mango yield by blowflies was estimated by using three treatments, i.e., open pollinated trees, trees were covered by a net in the presence of blowflies for pollination, and trees were covered with a net but without insects.

Results: The maximum number of flowers was recorded in irregular type of inflorescence, i.e., 434.80flowers/inflorescence. Fruit setting (bud) was higher in open pollinated mango tree (i.e. 37.00/inflorescence) than enclosed pollination by blowflies (i.e. 22.34/inflorescence). The size of the mango fruit was the highest (5.06mm) in open pollinated tree than the pollinated by blowflies (3.93mm) and followed by without any pollinator (3.18mm) at marble stage. We found maximum weight of mango fruit (201.19g) in open pollinated trees.

Discussion: The results demonstrated that blowflies can be used as effective mango pollinators along with other bees. The blowflies have shown a positive impact on the quality and quantity of mango. This study will be helpful in future and also applicable at farm level to use blow flies as pollinators that are cheap and easy to rear.

Key words: Blowflies, Mango, Pollination

Introduction

Mango, *Mangifera indica* L., is very popular and economically important fruit. It is dicotyledonous plant and widely cultivated in the tropical and subtropical areas of the world (Tjiptono et al., 1984). Although some varieties of mango fruit plant are self-pollinated, adequate pollinators are required to transfer the pollen to female part of the plant (Popenoe, 1917; Singh, 1954; Free & Williams, 1976). Biology of mango pollinators have been studied in India and Israel, and their results demonstrated that insects of the Diptera and Hymenoptera play major roles in the pollination of this important fruit (Singh, 1988; Bhatia et al., 1995; Singh, 1997; Dag & Gazit, 2000). Crop pollination mediated by wild and domesticated animals is a crucial and endangered ecosystem service (Potts et al*.,* 2010; Klein et al*.,* 2007). Recently, the global economic value of pollination from domesticated and wild animals has been estimated at €153 billion, while the consumer surplus loss associated with a total loss of animal pollination service was estimated between €190 and €310 billion (Gallai et al., 2009). About 87 crops, i.e. 70% of the 124 main crops used directly for human consumption in the world, depend on the pollinators (Klein et al., 2007).

The members of the family Calliphoridae (Schizophora, Calyptratae, Oestroidea) are commonly known as blowflies, bluebottles, cluster flies or greenbottles. They are worldwide distributed, with over 1,000 species and about 150 genera described (Shewell, 1987; Vargas & Wood, 2010). Diptera was probably among the first important angiosperm pollinators and may have been instrumental in the early angiosperm radiation (Labandeira, 1998; Endress, 2001; Skevington & Dang, 2002), comprised of over 160,000 species and 150 families (Evenhuis et al*.,* 2008). At least, seventy-one families of Diptera contain flower-visiting flies, and flies are pollinators of almost 555 flowering plant species (Larson et al*.,* 2001) and more than 100 cultivated plants, including important crops such as mango, cashew, tea, cacao, onions, strawberries, canola, and sunflower (Heath, 1982; Hansen, 1983; Mitra & Banerjee, 2007; Clement et al*.,* 2007; Heath, 2015). Blowflies thought to be the most dislike fly among all the flies of Dipteran, and it is a carrier of most of diseases and cause myiasis (Zumpt, 1965; Greenberg, 1973). This character has been recognized nearly 1500 years ago that flies are transmitters of diseases (Greenberg, 1973). Because earlier research was done only on the negative aspects of flies but now most of the studies have shown that blowflies species have many beneficial aspects such as surgeons, pollinators, agents of decay, forensic indicators, and recreational uses (Jarlan et al., 1997; Losey & Vaughan, 2006; Klein et al., 2007; Heath, 2015).

Considering the importance of beneficial aspects of blowflies in Pakistan, role of pollinators especially dipterans (blowflies) were never studied in *Mangifera indica*. Therefore, this research was conducted to evaluate the effects of blowflies on the mango pollination and fruit yield. The blowflies are the cheapest source of pollination as compared to other pollinators, such as honey bees, syrphid flies, *xylocopa spp* that are difficult to rear.

Material and methods

Plant material

The impact of pollination by blow fly on mango yield was studied in the orchard of Faculty of Agricultural Science and Technology (FAS&T), Bahauddin Zakariya University Multan. A total of three trees and 10 branches from each tree were selected for recording the data. Following treatments were used: 1) open pollinated trees; 2) fruit trees were covered by net and blowflies were used for pollination; 3) fruit trees were covered by nets and no insect was kept inside the net for pollination. Three replications were used for each treatment.

Rearing of blow fly for mango pollination

Blowfly's adults were collected from the different poultry farms of Multan, Pakistan. Mass culture of blowflies was reared in Bio-Ecology Lab of FAS&T, BZU Multan. Adults were released into the plastic cage (18 cm in diameter and 24 cm in height) with diet (10 percent honey solution), and chicken livers were also placed in the plastic trays for egg laying. The six plastic cages were used for rearing blowflies. Then hatched larvae were separated into the plastic pots (4cm in diameter and 8cm in height) that were half filled with sterilized sand and 50 g chicken liver. In each plastic pot, 20 larvae were released and maximum adults of blowflies were reared in the laboratory for field application.

Installation of cages

Mango trees with a height of 2.1m and width of 2.4m at the emergence of inflorescence were selected for the installation of cages. The cages, made by muslin cloths, were used for the covering of mango trees (3.35×3.35×3.35 meters). A total of 100 adults of blowflies were released for pollination efficacy in the covered mango trees (Figure 1) and control was kept free of blowflies.

Data recording

Total number of flowers and their types of inflorescences were counted in each treatment (Figure b). Data as number of flowers on each type of inflorescence, size (mm) and weight (g) of fruits at marble stage (30 days after the fruit set and have no stone inside the fruit) of mango was recorded by tagging ten twigs in each repeating unit.

Statistical analysis

The data were analyzed by using LSD test as Post-ANOVA at 5% levels for estimating the effect of different pollination methods on the number of flowers/inflorescence. Each type of the inflorescence (i.e., conical, pyramid and irregular) was analyzed in treatments. The number of flowers, buds, size and weight of mango fruit were also analyzed by using SAS (SAS, 2011).

Results

The number of flowers/inflorescences was compared among three treatments in each type of inflorescence (conical, pyramid and irregular). We detected highly significant differences in the number of flowers/inflorescence in the open pollinated mango trees on irregular type of inflorescence (434.80) than pyramid (400.90) and conical (327.97) types of inflorescence in open pollinated trees. However, number of flowers/inflorescence was significantly increased than blowfly and closed cage treatments in open pollinated trees (Figure 2).

The number of buds/inflorescence was compared between three different treatments in each type of inflorescence (conical, pyramid and irregular). Maximum number of buds/inflorescences was found in blowfly's cage on irregular inflorescence (4.16) that was significantly higher than pyramid inflorescence (2.96) and conical inflorescence (2.73). However, higher number of buds was found in blowfly's cage for irregular inflorescence than open pollinated and closed cages. After 10 days, the highest number of buds/inflorescence was found in open mango pollinated trees for irregular inflorescence (2.67) compared to pyramid (1.96) and conical inflorescence (1.93). Similar pattern was recorded in blowflies and closed cage. However, the number of buds/inflorescence for each type of inflorescence was significantly higher in blowflies than closed cage treatment (Figure 3b).

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Mango fruit formation at marble stage was compared between three different treatments in each type of inflorescence (conical, pyramid and irregular inflorescence). The maximum numbers of fruits/inflorescences were found in open pollinated mango tree at marble stage for irregular inflorescence (1.36), which was significantly higher than pyramid (1.24) and conical (1.20). The trees covered with blowflies produced significantly higher number of fruits for each type of inflorescence than closed cages (Figure 4).

The size and weight of mango fruits at marble stage was statistically higher in open pollinated trees (5.06mm and 210.20g, respectively) than blowflies cages (3.93mm, 180.80g, respectively) and closed cages (2.88mm, 139.51g, respectively). In open pollinated condition, mango size and weight were highly significant because of a variety of pollinators e.g. *Apis dorsata, Apis florea*, *Episyrphis balteatus*, *E. scutellaris* and *lucilia spp* at the farm. Overall result showed that open pollinated trees have maximum and good quality of fruits. However, blowflies also showed great impact on mango pollination because higher quantity and better quality of fruits were recorded than close cage trees.

Discussion

Pollination is a process in which pollens transferred from the male part of the plant to female reproduction organs and a huge number of economically nutritive plants depend on different types of pollinators for pollination. Pollinator-dependent products are essential part of human diets (Eilers et al., 2011). Modern farming techniques have enabled higher yield of crops (Aizen et al., 2008, 2009), but significant decline have been observed in insect pollinators, primarily due to the isolation from natural habitats (Klein et al., 2006; Garibaldi et al., 2011). In most of the habitats, pollinating flies guarantee or enhance seed and fruit production of many plants such as medicinal, food and ornamental plants, Most of the flies were kept in production seed banks and considered important part/life of Greenland. Due to the large gaps in the knowledge about the Diptera, there is a need to address the role of Diptera in pollination network. Diptera flies have potential to survive in variable ranges of temperature or environment (Ssymank et al., 2008; Munawar et al., 2011, Abrol, 2012). Here, the blowflies were studied as a pollinator of mango fruit, which is considered as the cheapest source of pollination. The results revealed that blowflies have significant effect on the mango yield.

A mango panicle contains around 200-4,000 flowers and a mature tree may has approximately 600-1,000 panicles (Manning, 1995). With a huge number of flowers, these flowers are attractive to insect pollinators. About 46 kinds of pollinators belonging to three orders, Coleoptera, Diptera, and Hymenoptera are capable of pollinating mangoes (Singh, 1988; Bhatia et al., 1995; Singh, 1997; Dag & Gazit, 2000). In native areas, many plants/trees have various pollinators but they visit variety of flowers (Waser et al., 1996). Plants have a generalized community mostly visit similar pollinators for pollination (Waser et al., 1996; Bluthgen et al., 2006). Mango flowers are of different kinds, and various insects are an important source of pollination for this fruit (Heard, 1999). These pollinators are very crucial for successful fruit set in mango (Free & Williams, 1976; Anderson et al., 1982; Richards, 2001; Carvalheiro et al., 2010). They are not only sensitive to change in their natural habitat and/or niche, but are also sensitive to pesticides (De Siqueira et al., 2008).

One of the main components of ecosystem is pollinator, like say main component of global biodiversity (Garibaldi et al., 2013). Pollinator has two types as one is domesticated and other is wild pollinators, and both are very important for the pollination of plants. Our result showed that open trees produced maximum yield, followed by covered trees with blowflies and without insects. These results are consistent with the previous study, who revealed that insects increase the yield of fruits by amplifying pollination (Mingjian et al., 2003). Previous studies also demonstrated that diversity of pollinators has greater impact on the yield of fruit trees and environmental hazards have declined the different types of pollinators (Jones & Emsweller, 1934; Fajardo et al., 2009). Our results showed that blowflies have great potential for the pollination and to increase the yield of mango. This study explains the impacts of blowflies on pollination of mango fruits.

Conclusion

The results revealed that weight and size of the mango fruit was significantly reduced in the trees covered without insects than the mango trees covered with the blowflies. However, we detected fruits with maximum weight and size in the open pollinated mango trees where more number of pollinators visit the trees for pollination and resulted in the better quality and quantity of mango fruit. We concluded that blowflies have the potential for pollination in *M. indica*. So this research will be helpful in future and applicable at farm level where honey keeping in the orchard is difficult for pollination because of environment and high cost. We speculated that blowflies are the best, cheaper source of pollination as a replacement of honey bees and other pollinators which are difficult to purchase and maintain in the orchards for pollination. This study also showed that irregular type of inflorescence have maximum number of flowers, buds and fruits, so the breeders could focus on to develop the variety of *M. indica* having more number of irregular types of inflorescence..

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Table 1. Total number of inflorescences and their types in different treatments

Treatments	**Trees**	**Number and types of mango inflorescence**			
		Conical	**Pyramid**	**Irregular**	**Total**
Closed	Tree 1	13.00	18.00	22.00	53.00
	Tree 3	15.00	34.00	20.00	69.00
	Tree 5	34.00	19.00	33.00	86.00
	Mean	20.67	23.67	25.00	69.33
Blowflies	Tree 1	19.00	21.00	21.00	61.00
	Tree 3	11.00	13.00	12.00	36.00
	Tree 5	20.00	34.00	20.00	74.00
	Mean	16.67	22.67	17.67	57.00
Open	Tree 1	45.00	50.00	30.00	125.00
	Tree 3	27.00	23.00	26.00	76.00
	Tree 5	23.00	43.00	20.00	86.00
	Mean	31.67	38.67	25.33	95.67

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Figure Legends

1. Mango tree was covered with muslin cloth
2. Effect of different pollination methods on the different types of mango flowers
3. Effect of different pollination methods on the bud formation/inflorescence

(a) 15 days after the treatments (b) 10 days after the treatments

4. Effect of different pollination methods on the number of fruits at marble stage
5. Effect of different pollination methods on the fruit size and weight at marble stage

Figure 1. Mango tree was covered with muslin cloth

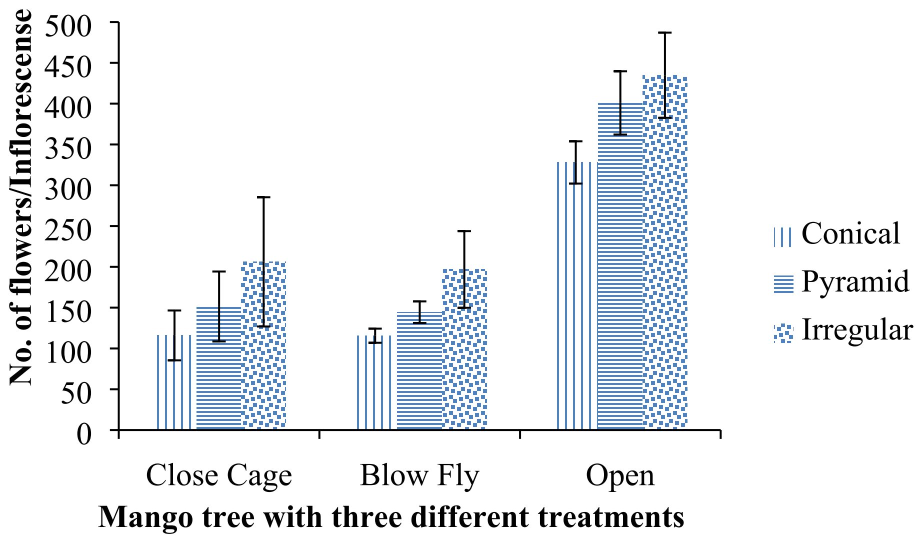

Figure 2. Effect of different pollination methods on the different types of mango flowers Mean values sharing similar letters did not differ significantly with in the treatments (P≤0.05). Bars indicate the standard deviation (SD) of the observation

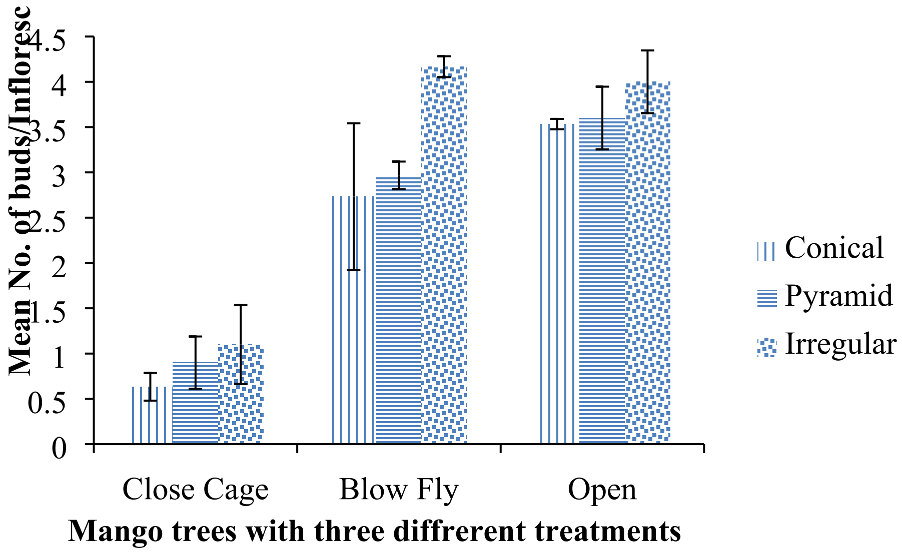

Figure 3a

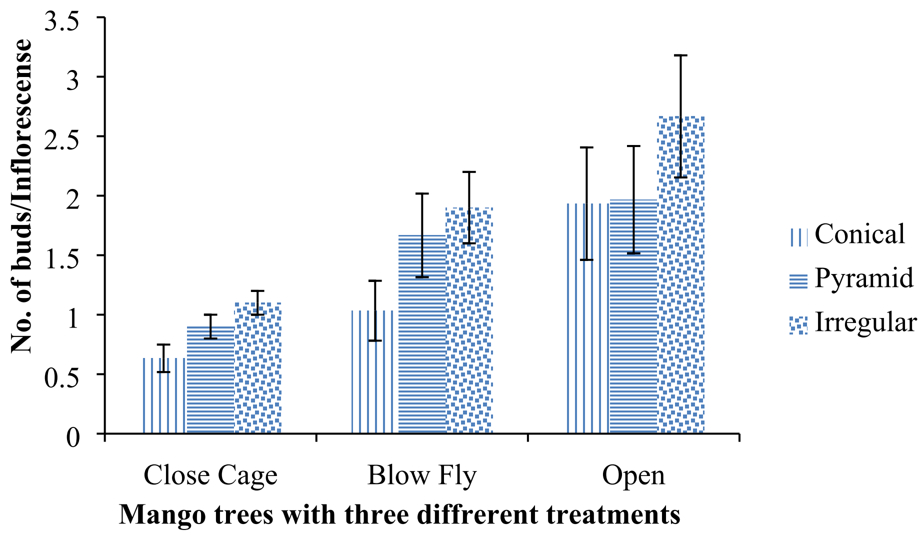

Figure 3b

Figure 3. Effect of different pollination methods on the bud formation/inflorescence a). 15 days after the treatments (b). 10 days after the treatments Mean values sharing similar letters did not differ significantly with in the treatments ($P \leq 0.05$). Bars indicate the standard deviation (SD) of the observation

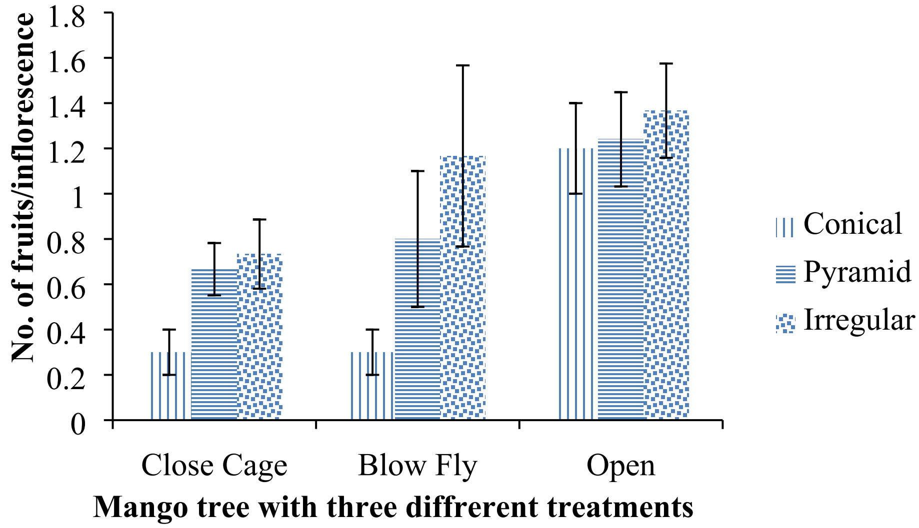

Figure 4. Effect of different pollination methods on the number of fruits at marble stage
Mean values sharing similar letters did not differ significantly with in the treatments ($P \leq 0.05$). Bars indicate the standard deviation (SD) of the observation

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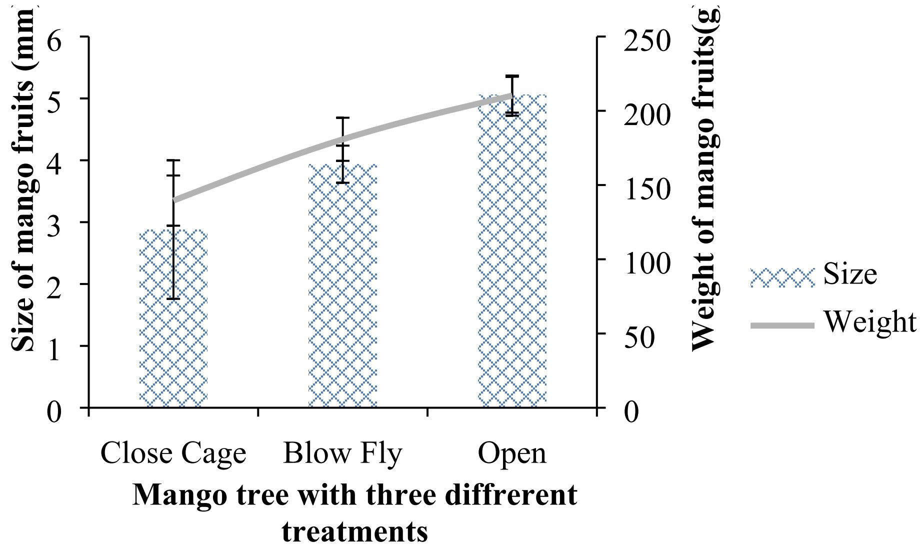

Figure 5. Effect of different pollination methods on the fruit size and weight at marble stage Mean values sharing similar letters did not differ significantly with in the treatments (P≤0.05). Bars indicate the standard deviation (SD) of the observation