Research Article

In Situ Evaluation of Different Refractive Color Sheets and Reduced-Risk Insecticide Formulation Against *Bactrocera cucurbitae* (Diptera: Tephritidae) on Bitter Gourd (*Momordica charantia* L.)

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Abstract | Bitter gourd or bitter melon (*Momordica charantia* L.) is an important summer vegetable of tropical and subtropical world. Melon fruit fly, *Bactrocera cucurbitae* (Coquillett) is the key pest of *M. charantia* and other cucurbitaceous vegetables incurring 30 to 100% crop loss. This study evaluated the use of refractive color sheets on fruit fly incidence and infestation on bitter gourd plants and compared it with reduced-risk insecticide formulations of spinosad. Refractive sheets of red, orange, yellow, green, blue, black and white were installed in *M. charantia* beds at three different angles (30º, 60º and 90º). Results revealed that control treatment exhibited significantly higher fruit fly infestation (60%) with minimum marketable fruit yield (374g/bed) than all other treatments. Among treatments, yellow colored refractive sheet treatments exhibited maximum fruit fly incidence (i.e. 5 flies/net-sweep and 3-4 maggots/fruit) and fruit infestation (17%) as compared to other color sheets. Minimum fruit fly infestation (6%) and higher marketable yield (855g/bed) was recorded for reduced-risk insecticide spinosad, followed by blue colored refractive sheets. However, there was no significant effect of installation angles on any of the parameters measured. Moreover, maximum and minimum CBR were found for spinosad formulation (1:4.9) and yellow color refractive sheet treatments (1:1.8), respectively. It is concluded that, in combination with biorational insecticides such as spinosad, installation of different colored refractive sheets, except yellow, at 30° or 60° angles would be more effective and economical to control fruit fly incidence on different vegetable crops such as *M. charantia*.

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Introduction

Bitter gourd or bitter melon (*Momordica charantia* L.) belongs to family Cucurbitaceae and is a common summer vegetable grown in Asia and other parts of the tropical and subtropical world including India, Pakistan, Sri Lanka and China (El-Batran et al., 2006; Singh et al., 2006). *M. charantia* like other cucurbit crops are of great nutritional and medicinal importance (Palada and Chang, 2003; Tan et al., 2016). For instance, all parts of bitter gourd such as roots, stems, leaves, green fruits and seeds are used to fight against cancer, diabetes, HIV/AIDS and can also be used as digestible, anthelmintic, appetizer, cures biliousness, bronchitis, urinary discharges, blood diseases, ulcers, anemia, asthma and eye related diseases (Kumar and Bhowmik, 2010).

Fruit flies (Diptera: Tephritidae) are the important pests of fruits and vegetables causing considerable loss both in quality and quantity (Dhillon et al., 2005 a, b; Shooker et al., 2006). Out of 4,000 fruit fly species, about 392 species (10%) have been recorded in Indo-Pak region (Kapoor, 1993; Akhtaruzzaman et al., 2000). Among tephritid pests, melon fruit fly, *Bactrocera cucurbitae* (Coquillett) is the key pest of cucurbitaceous vegetables (Dhillon et el., 2005b; Ekesi and Billah, 2007) and cause 30 to 100% loss to cucurbits crops (Hollingsworth et al., 1997; Dhillon et al., 2005a, b; Shooker et al., 2006). *B. cucurbitae* has been reported to damage 81 host plants and is a major pest of cucurbitaceous vegetables particularly of bitter gourds (*M. charantia*), muskmelons (*Cucumis melo*), Snap melons (*C. melo* var. *momordica*), watermelons (*Citrullus lanatus*), round melons (*Praecitrullus fistulosus*), and snake gourds (*Trichosanthes anguina*) (Dhillon et al., 2005a). These flies directly damage the fruits by ovipositing into the fruits and make small punctures on them by ovipositor. Maggots feed inside on the pulp of fruits and spoil them. The eggs are also laid into unopened flowers, and the larvae successfully mature in the taproots, stems and tender leaf stalks (Weems and Heppner, 2001). Infested fruits and flowers do not develop desired size in which ultimate reduction of yield. Their indirect damage includes spread of certain phyto-pathogens being facilitated by fruit fly damaged fallen rotten fruits in the field (Dhillon et al., 2005b).

Different management strategies are used for the control of fruit flies such as chemical, mechanical and cultural control methods, and use of pheromones (e.g. methyl eugenol) and cue-lure traps and baits (Khoo and Tan, 2000; Neupane, 2000; Dhillon et al., 2005c; McQuate et el., 2005; Sarwar, 2015). Conventional management and control of fruit flies have been practiced using bait applications in which some attractant like hydrolyzed protein is mixed with some killing agent (Nascimento and Carvalho, 2000). Similarly, several attract and kill devices are employed against fruit flies using different refractive color sheets and bio-rational insecticides (Wu et al., 2007). Many reduced-risk insecticides are being used against cucurbit fruit flies and these insecticides usually impose a reduced risk to human health and to the environment and other non-target species. Therefore, there is need to use reduced risk insecticides such as spinosad (derived from a natural product of soil bacterium; Bret et al., 1997), particularly against fruit flies (Muhammad et al., 2007).

However, the capture of fruit flies by different attract-and-kill techniques depends on the color of the surface, fluorescence, adhesive material and food-source (Davis et al., 1984; Wu et al., 2007). The colored surfaces having broad spectrum wavelength are least attractive to fruit flies. The most attractive colors for fruit flies are yellow, green, and red, orange and blue which are actually species-specific (Davis et al., 1984; Wu et al., 2007). Nevertheless, there is a paucity of literature regarding the visual response of melon fruit fly *B. cucurbitae* to various colors. The present study was therefore designed to evaluate the impact of integration of reduced-risk insecticides and colored refraction sheets on the incidence of melon fruit fly and yield of bitter gourd under field conditions.

Materials and Methods

The study was done in the experimental area of the Department of Entomology, University of Agriculture, Faisalabad (Punjab, Pakistan). Size of main plot was 100 ft in length and 70 ft in width. The main plot was further prepared to have 72 planting beds. Size of each bed was 3.5 ft wide and 13 ft long. Bitter gourd (var. FSD-Long) was sown with bed to bed and plant to plant distance of 60 and 30 cm, respectively.

Refractive sheets of seven colors including red, black, orange, blue, yellow, green and white were purchased from the local market. The sheets were installed in the experimental field with the help of wooden sticks implanted deep in soil at three different angles (30, 60 and 90 degree). All these sheets were installed about one week before the onset of flowering and fruiting in bitter gourd plants. Any damaged sheet was replaced with the new one if needed during the experiment.

Reduced-risk (R-R) insecticide formulation i.e. spinosad formulation GF-120™ NF Naturalyte fruit fly bait designed by Dow® AgroSciences LLC (Indianapolis, USA) and two other biorational insecticides i.e. spinosad and lufenuron were acquired from an authenticated local pesticide dealer. These three insecticides were sprayed on crop as per their recommended doses. Their application scheme in integrated treatments involved the board-spray of GF-120 followed by one alternate spray of spinosad and lufenuron when required; whereas in standard treatment, their alternative spray was applied at fortnightly interval.

In order to determine the incidence of melon fruit fly infestation on bitter gourd beds treated with various colored refractive sheets integrated with R-R-insecticides, net-sweeping was practiced with a three days interval and the flies trapped in sweep-nets were sex-wise enumerated. For the collection of data regarding fruits quality and quantity, the collected fruits were brought into IPM laboratory and weighed. Ten fruits were picked randomly from the collected fruits and observed under microscope. The infested fruits were separated, counted and weighted to calculate % fruit infestation and yield loss. The density of maggots inside the infested fruits was also assessed. The non-infested fruits were counted and weighed to determine the marketable yield. At the end of last picking, total yield, marketable yield, yield loss and fruits infestation were counted. The input invested in plants protection and the revenue from bitter gourd yield were calculated for all treatments to determine the cost-benefit ratio.

Data regarding fruit fly adult captures, fruit infestation, marketable fruit yield, yield loss, and cost-benefit ratio were subjected to ANOVA and the means of significant results were compared by Tukey's HSD test.

Results and Discussion

Advancements in integrated pest management programs have encouraged the use of environmental friendly techniques. It would necessitate the development of such management and control strategies that would be economical feasible, ecofriendly and compatible with nature having least undesirable impacts. Fruit flies, particularly cucurbit fruit fly *B. cucurbitae* and oriental fruit fly *B. dorsalis*, are the major limiting factors in sustainable production of fruits and vegetables in tropical and subtropical regions (Marwat and Rabbani, 1991). As no solo practice is efficient enough to control fruit fly incidence and infestation, there is a need to search and evaluate novel practices that cope with new challenges in this era of organic farming. This study evaluated the use of refractive color sheets on fruit fly incidence and infestation on bitter gourd plants and compared it with reduced-risk or biorational insecticide formulations.

Fruit fly infestation and fruit yield parameters

Results regarding fruit fly infestation (Table 1) showed that approximately 2 times less fruit fly density per net-sweep (i.e. 5 fruit flies/net-sweep) were observed in treatments where yellow color refractive sheet was installed at 30°, 60° and 90° angle as compared to control (i.e. 11.33 fruit flies/net-sweep). However, 3 to 5 times less fruit flies density (2-3 fruit flies/net-sweep) were captured in treatments when red, black, orange, blue, green and white colored refractive sheets were installed at 30°,60° and 90° angles as compared to control (11.33 fruit flies/net-sweep) (Table 1). Similarly, approximately 5 times less fruit flies were captured when reduced risk insecticide was applied as compared to control. Similarly, treatments with yellow colored refractive sheets exhibited one time less maggot density (3-4 maggot/fruit) as compared to control (6.33 maggot/fruit) regardless of installation angle. However, 2-3 times less maggot density (2-3 maggot/fruit) was observed in all other treatments at 30°, 60° and 90° angles and R_R treatment showed 5 times less maggot density than control (Table 1).

Approximately 2 times less fruit infestation (12-17%) was observed in treatments with yellow color refractive sheet whatsoever the installation angle as compared to control treatment (i.e. 65.66%). However, 3 to 5 times less infestation percent (10-11%) was observed in treatments when Red, Black. Orange, Blue, Green and white colored refractive sheets were installed at 30°, 60° and 90° angles as compared to control and 5 times less fruit infestation was observed for reduced risk insecticide as compared to control (65.66%).

Data regarding bitter gourd fruit yield showed that approximately 1 time more total yield (525-575g/bed) were observed in treatments where yellow color refractive sheet was installed at 30°, 60° and 90° angle as compared to control (i.e. 377.33 g/bed). However, 2 and 3 times more marketable yield was observed for all other color refractive sheets and for reduced risk insecticides, respectively, as compared to control (Table 1). Similarly, yellow color refractive sheet treatments at all angles gave 2 times less yield loss (225-250 g/bed) as compared to control (511.67 g/bed). Other color sheets gave approximately 2-3 times less yield loss as compared to control while R_R insecticide treatments caused 3 times less yield loss as compared to control treatments (Table 1). However, there was no significant effect of installation angles on any parameter measured.

Table 1: *Impact of colored refractive sheets and their positions on fruit fly infestation and yield parameters of bitter gourd.*

Color and Installation angle of refractive sheet	MD/F	TY/B	MY/B	YL/B	TFF/NS	FI
Red 30°	3.00^{B}	933.00^{A}	750.67^{A}	165.67^{B}	2.33^{D}	6.66^{B}
Black 30°	2.00^{B}	874.33^{A}	773.00^{A}	96.67^{B}	3.33^{BCD}	8.00^{B}
Orange 30°	2.33^{B}	890.67^{A}	794.67^{A}	131.00^{B}	3.00^{CD}	7.00^{B}
Blue 30°	2.66^{B}	914.00^{A}	707.00^{A}	146.67^{B}	2.33^{D}	7.00^{B}
Yellow 30°	4.33^{AB}	750.67^{A}	525.67^{A}	225.00^{B}	5.33^{BC}	11.66^{B}
Green 30°	3.33^{B}	934.00^{A}	757.67^{A}	172.67^{B}	3.33^{BCD}	7.66^{B}
White 30°	2.66^{B}	909.00^{A}	697.67^{A}	146.00^{B}	3.33^{BCD}	6.00^{B}
Red 60°	2.33^{B}	888.67^{A}	792.33^{A}	123.67^{B}	3.33^{BCD}	6.66^{B}
Black 60°	2.33^{B}	883.67^{A}	792.33^{A}	119.33^{B}	3.00^{CD}	8.00^{B}
Orange 60°	3.66^{B}	936.00^{A}	758.33^{A}	175.66^{B}	3.33^{BCD}	9.00^{B}
Blue 60°	3.33^{B}	933.00^{A}	755.00^{A}	172.00^{B}	2.66^{D}	8.66^{B}
Yellow 60°	3.30^{B}	765.67^{A}	515.67^{A}	250.00^{B}	5.33^{BC}	14.00^{B}
Green 60°	3.00^{B}	931.33^{A}	727.67^{A}	162.67^{B}	3.33^{BCD}	7.33^{B}
White 60°	2.00^{B}	849.67^{A}	771.00^{A}	90.33^{B}	3.33^{BCD}	8.33^{B}
Red 90°	2.33^{B}	882.67^{A}	776.33^{A}	114.33^{B}	3.00^{CD}	6.66^{B}
Black 90°	3.00^{B}	926.33^{A}	724.33^{A}	162.00^{B}	2.66^{D}	7.00^{B}
Orange 90°	2.66^{B}	903.33^{A}	760.67^{A}	142.67^{B}	3.66^{BCD}	8.33^{B}
Blue 90°	2.66^{B}	902.00^{A}	809.00^{A}	142.67^{B}	3.00^{CD}	6.66^{B}
Yellow 90°	4.00^{AB}	736.00^{A}	575.33^{A}	161.67^{B}	5.66^{B}	16.33^{B}
Green 90°	2.66^{B}	901.67^{A}	805.00^{A}	141.00^{B}	3.00^{CD}	7.66^{B}
White 90°	2.66^{B}	896.67^{A}	804.67^{A}	139.00^{B}	3.66^{BCD}	8.33^{B}
R_R	2.33^{B}	967.33^{A}	855.00^{A}	118.33^{B}	2.33^{D}	6.00^{B}
Control	6.33^{A}	886.00^{A}	374.33^{B}	511.67^{A}	11.33^{A}	65.66^{A}
S.E	0.68	39.6	43.864	42.860	2.57	4.88
C.V	2.61	152.54	168.67	164.81	0.66	1.26

MD/F: *maggot density per fruit;* **TY/B:** *total yield (g) /bed;* **MY/B:** *marketable yield/bed;* **YL (kg) /B:** *yield loss/bed;* **TFF/NS:** *total fruit flies/net-sweep;* **FI:** *fruit infestation (%);* **R_R:** *reduced risk insecticide;* **S.E:** *standard error;* **C.V:** *coefficient of variance.*

According to results, all treatments demonstrated significantly lower fruit fly incidence and fruit infestation and higher yield than control treatment. Among treatments, bitter gourd beds treated with yellow colored refractive sheets exhibited higher fruit fly incidence, fruit infestation both in terms of maggots per fruit and percent infestation and lower marketable fruit yield, followed by other refractive sheet colors. Reduced risk insecticidal formulation having spinosad as active ingredient exhibited the least fruit fly infestation and highest fruit yield. Spinosad has been found very effective against a wide range of insect pests of fruits and vegetables including fruit flies. For example, Vargas et al. (2008) and Vayssieres et al. (2009) tested spray-able attract-and-kill dispensers with spinosad and male-specific lure methyl eugenol and cue-lure for area-wide suppression of oriental fruit fly, *Bactrocera dorsalis* (Hendel) and melon fly, *B. cucurbitae* (Coquillet), respectively. The results of this study are in line with those of many previous works demonstrating field efficacy of spinosad against fruit flies (Mangan et al., 2006; Gazit et al., 2013; Muriithi et al., 2016).

These results indicate that melon fruit fly (*B. cucurbitae*) prefer yellow color among all the colors. These results are in agreement with those of Robacker et al. (1990) who reported that attractiveness of Mexicans fruit fly was more towards yellow, green and orange color traps than others. Fruit fly attraction towards yellow color was may be due to their specific wavelength as light wavelengths would have a differential impact on insect behavior (Zhang et al., 2016; Zhou et al., 2016). However, these results seem not in agreement with those of Lopez-Guillen et al. (2009) who documented the peak of attraction of male and female *Anastrepha obliqua* (Macquart) occurring between 380 and 570 nm color. Similarly, green color having spectrum between 490 ~ 560 nm showed the highest attraction for oriental fruit fly than other (Wu et al., 2007). This differential behavior of melon fruit fly could be due to differential and species-specific response of different tephritid flies towards different colors as demonstrated by Davis et al. (1984) and Wu et al. (2007).

Cost-benefit ratio for various treatments

The extrapolation of data to calculate cost-benefit ratio (CBR) revealed that application of reduced (R_R) risk insecticides exhibited the highest CBR of about 1:4.9 (Table 2), whereas installation of yellow-colored refractive sheets at 30°, 60° and 90° angles gave the minimum CBR of 1:1.7–1:1.9. While application of refractive sheets of other different sheet colors at different installation angles demonstrated a CBR of 1:3.3–1:4.1. However, refractive sheets of red, black, orange and blue at 30° angles, and the blue, white, green and red at 60° angles, and red, orange, blue, green and white at 90° angles demonstrated comparatively higher CBRs (1:3.6–1:4.1) as compared to control and other treatments (Table 2).

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Table 2: *Cost-benefit ratio on per acre basis for various treatments.*

Treatments	Gross MY/B (kg) (sum of 7 pickings)	MY/B (due to treatment)	Total revenue @ Rs.70 /kg /bed	Expected profit (Rs.) /acre	Expected cost (Rs.) /acre	CBR
Red 30º	5.07	2.19	153.3	56488	14030	1:4.02
Black 30º	5.03	2.15	150.5	55200	14030	1:3.93
Orange 30º	5.06	2.18	152.6	56166	14030	1:4.00
Blue 30º	5.07	2.19	153.3	56488	14030	1:4.00
Yellow 30º	4.07	1.19	83.3	24288	14030	1:1.73
Green 30º	4.84	1.96	137.2	49082	14030	1:3.49
White 30º	4.93	2.05	143.5	51980	14030	1:3.70
Red 60º	4.93	2.05	143.5	51980	14030	1:3.70
Black 60º	4.81	1.93	135.1	48116	14030	1:3.42
Orange 60º	4.84	1.96	137.2	49082	14030	1:3.49
Blue 60º	5.09	2.21	154.7	57132	14030	1:4.07
Yellow 60º	4.14	1.26	88.2	26542	14030	1:1.89
Green 60º	4.94	2.06	144.2	52302	14030	1:3.72
White 60º	4.87	1.99	139.3	50048	14030	1:3.56
Red 90º	5.00	2.12	148.4	54234	14030	1:3.86
Black 90º	4.75	1.87	130.9	46184	14030	1:3.29
Orange 90º	4.90	2.02	141.4	51014	14030	1:3.63
Blue 90º	5.05	2.17	151.9	55844	14030	1:3.98
Yellow 90º	4.06	1.18	82.6	23966	14030	1:1.70
Green 90º	5.10	2.22	155.4	57454	14030	1:4.09
White 90º	5.05	2.17	151.9	55844	14030	1:3.98
R_R	6.48	3.6	216	99360	20700	1:4.86
Control	2.88	-	-	-	-	-

MY/B: *Marketable yield(kg)/bed;* **CBR:** *Cost-benefit ratio;* **R_R:** *Reduced risk insecticide.*

Fruit infestation caused by melon fruit fly was less than 17% for the treatments having yellow color refractive sheets at all installation angles as compared to control treatment where fruit infestation was recorded about 50-70%. Bitter gourd fruit infestation in untreated plots remained up to 70% in this study which is in line with those reported by Gogi et al. (2009) and Rana and Kanwar (2014) who documented 60-90% fruit infestation when no control measures were applied and 5-20% when IPM practices were applied against melon fruit fly *B. cucurbitae*. Similarly, Dhillon et al. (2005c) also documented that melon fruit fly can cause 17-20% and 90-95% fruit infestation in bitter gourd when chemical control measure (insecticides) and no control measures were used respectively.

Author's Contribution

AB, SA and MZM conceived and designed the experimental protocols. AB, GA and MJ performed experiments. SA and GA provided technical assistance in experimentation. AB and MZM performed statistical analyses and prepared the manuscript.

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