Article

Evaluating the effectiveness of a sprayer UAV in controlling cotton bollworm

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Received 18 March 2021; *Accepted 5 June 2021*; *Published 1 September 2021*

Abstract
Chemical control is a common way against cotton bollworm (*Helicoverpa armigera*), a key cotton pest, in developing countries. Therefore, the main objective of current study was to compare the effect of three common sprayers including Unmanned Aerial Vehicle (UAV), Boom sprayer and Hand lance tractor mounted sprayer in controlling cotton bollworm. To evaluated two important spray characteristics such as Volume Median Diameter (VMD) and Percent area coverage (AC) on a canopy, labeled Water Sensitive Papers were installed in three different plant positions (top, middle and bottom) and stain of droplets analysed by ImageJ ® software. To Assessment of larval *H. armigera* management, the number of *H. armigera* larvae was recorded (on 10 random plants per strip-plot) after Thiodicarb (Larvin® 75% WP) spraying (before and 1,3,5, 7 and 14 days after spraying). The mortality was transformed by √x + 0.1 and analyzed by SAS® software. The highest and lowest percentage of coverage belonged to lance sprayer (5.5%) and UAV (2.94%), respectively at the top of plants. Although the spraying characteristics (AC and VMD) of the UAV were not good, the larval mortality on the third day after spraying was 61.1% significantly different from the other sprayer types. UAVs are alternative spraying ways in high-dense cotton canopy fields to save plants and larval management at the same time.

Keywords *Helicoverpa armigera*; ImageJ®; UAV; water sensitive paper.

Arthropods
ISSN 2224-4255
URL: http://www.iaees.org/publications/journals/arthropods/online-version.asp
RSS: http://www.iaees.org/publications/journals/arthropods/rss.xml
E-mail: arthropods@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Cotton is one of Iran's most important industrial crops. Fiber production, mill consumption, and import demand have trended upward recently. Pesticides are still being used for crop protection in the most of the developing countries due to continuously increasing demands for quantity and quality of cotton. Larvicides are sprayed routine 6 to 10 times in non-Bt-cotton fields of Iran, China, etc., depending on pest population (Zhang et al., 2018). The quality of sprayers and pesticides has an important role in sprayer efficiency. The majority of the sprayers performed unsatisfactorily, because of poor designed, poor quality materials and mishandled by the farmers (Mulatu, 2018).

Boom sprayers are widely used for wide farm crops due to their high working efficiency and effective spraying. Boom sprayers are the hydraulic equipment (move the liquid to the individual nozzles along with the boom) generally used for pesticide application on field crops such as cotton, soybean etc. However, using tractor mounted boom sprayer leads to several consequences, such as rolling cotton plants, hitting cotton bolls and pulling cotton branches, which reduce the yield and quality of cotton fiber. Another type of sprayer that is used in Iran is hand lance tractor mounted sprayer. This type of sprayer is used when the high of cotton plant doesn't permit the tractor to move in the farm easily. The lance connects to a tank by a long hose and it handles in form by laborers (Damalas and Koutroubas, 2016).

The newest pesticide applications which have been rapidly developed are Unmanned Aerial Vehicles (UAVs). UAVs were equipped with precise variable rate technology for realizing crop type could improve economic justifiability of cotton production by increasing the spraying efficiency and decreasing pesticide concentration and their harmful residues at the same time (Qin et al., 2018). A good example of popularizing pesticide spraying by UAVs is China, which could increase the mechanization level, intellectualization and light simplification of cotton. According to the statistical reports provided by the Ministry of Agriculture and Rural of the People's Republic of China, about 30,000 farmers used UAVs for spraying approximately 17.8 million hectares of crop in 2018 (Xiao et al., 2019). There are not numerous scientific studies about crop protection of UAVs but few types of research on the defoliant spraying of cotton by UAVs have been reported (Ma et al., 2016; Qin et al., 2016; Hu et al., 2018; Wang et al., 2018; Xin et al., 2018).

Assessment of spray distribution usually involves the use of a quantitative method for determining spray deposition and drift. Generally, the choice of a particular method depends on multi-variable such as the availability of human resources, biological and physical characteristics of the target crop and expected accuracy. Literature review of spray sampling methods reveals that existing techniques aren't exactly appropriate for all application scenarios; therefore, the disadvantages of each technique must be well considered before choosing a method for a particular application.

Quantitative methods including colorimetry (Hoffmann and Salyani, 1996), fluorimetry (Pergher and Gubiani, 1995), spectrometry (Derksen and Gray, 1995), etc despite being more accurate was replaced by Water Sensitive Paper (WSP), alternatively inexpensive technique because of costly and time-consuming for spray deposition assessment. Water-sensitive paper (WSP) card has been available for consecutive years (about 30 years) on the market (Salyani et al., 2013). The cards have been used to visualize and quantify spraying distribution and deposition of ground and aerial applications by researchers, farmers, and commercial purposes. In fact that aqueous droplets could able to leave a blueish-yellow or blue stain on the surface of WSP, The stained cards could be analyzed visually with direct observation (Hall et al., 1987; Thériault et al., 2001; Nuyttens, 2007; Khot et al., 2011), a colorimetric method (Giles et al., 1989) or image processing system (Salyani et al., 1987; Fox et al., 2001; Panneton, 2002; Hoffmann and Hewitt, 2005; Zhu et al., 2011; Cunha et al., 2012).

WSP cards have applied in several laboratory studies including calibration of the droplet density for insecticide application onto leaf targets (Hall et al., 1987), investigation of the deposition efficiency of different droplet sizes (Salyani et al., 1987), comparison of the drift potential of spray tips in a wind tunnel (Wang et al., 2020), spray deposition characteristics of conventional and air-induction nozzles (Guler et al., 2007), or visualizing the droplet distribution on the targets prepared for evaluating fluorescent dye degradation (Khot et al., 2011). In the field settings, the cards have been used to compare spray coverage quality among various sprayers, nozzles, or operating variables in several crops including: citrus (Salyani and Fox, 1999), apples (Holownicki et al., 2002), soybeans (Zhu et al., 2008), greenhouse plants (Derksen et al., 2010), and wheat (Ozcan et al., 2012). WSP has also been used to determine optimal spray volume for conventional nursery sprayers (Zhu et al., 2011), evaluate spray deposition consistency inside ornamental nursery canopies for variable-rate sprayer development (Jeon et al., 2011), adjust sprayer output for optimally pest control (Zhu et al., 2011), or monitor spray distribution patterns of aerial applications (Fritz et al., 2007). In evaluating spray coverage with WSP targets, Thériault et al. (2001) and Fox et al. (2003) compared visual rating with image analysis of the stained cards. Panneton (2002) developed a field portable image analysis system to overcome the problem of changing WSP back ground color due to varying droplet density.

Generally, WSP needs a special image analysis system with a trained operator and fixed-physical factors to convert spot size to droplet size. Hoffmann and Hewitt (2005) compared three image analysis systems using WSP for droplet sizing. Also, Cunha et al. (2012) compared several image analysis systems in assessing spray coverage, droplet density, and droplet size spectrum.

Another aspect of current studies was focused on the controlling efficiency of different methods against the target pest of cotton. Cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), is one of the most important and economic pests of cotton in Iran and other parts of the world. *H. armigera* is a polyphagous pest that has a wide range of crop hosts such as cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), sesame (*Sesamum indicum* L.), hemp (*Cannabis sativa* L.), tomato (*Solanum lycopersicum* L.), pea (*Pisum sativum* L.), sorghum (*Sorghum bicolor* L.), beans (*Phaseolus sp.*), sunflower (*Helianthus* sp.), soybeans (*Glycine max* (L.) Merr.) and peanut (*Arachis hypogaea* L.) in Iran (Fallahnejad-Mojarrad et al., 2017).

In order to use various pesticides to control pests in cotton fields and damage natural enemies of pests in the ecosystem, therefore a combination of control methods is recommended to keep this pest population under the economic threshold (Soleimannejad et al., 2010). The different spraying methods have mainly affect on pest control. Relationship between Droplet deposition and control effect of insecticides sprayed with an unmanned aerial vehicle against planthoppers by Qin et al. (2016). Droplets of 480 g l^{-1} Chlorpyrifos were collected using water-sensitive paper, and the coverage rates of the droplets on the rice canopy and lower layer were statistically analyzed for further affecting insect control (Qin et al., 2016).

Differences in droplet density and spectrum of pesticide deposited on foliage after spray were examined to determine differences in gypsy moth, *Lynumtria dispar* L., larval mortality. The susceptibility of 2nd, 3rd, and 4th instars of the gypsy moth to undiluted *Bacillus thuringiensis* kurstaki was examined under laboratory conditions. Droplets of known size (100, 200, and 300 μm) and density (1, 5, and 10 drops/cm^2) of *B. thuringiensis* were sprayed onto leaf disks of oak foliage and fed to gypsy moth larvae (Maczuga and Mierzejewski, 1995). Comparison of spray deposition, control efficacy on wheat aphids and working efficiency were studied in the wheat field by the unmanned aerial vehicle with boom sprayer and two conventional knapsack sprayers (Wang et al., 2019). The efficacy of aerial electrostatic - charged sprays was evaluated for spray deposit characteristics and season - long control of sweet potato whitefly (SWF), *Bemisia tabaci* Genn. biotype B (aka *B. argentifolii* Bellows & Perring), in an irrigated 24 ha cotton field (Latheef et al., 2009).

The main objective of this study was to compare the effect of UAVs with boom and hand lance sprayer in controlling cotton bollworm. Water sensitive paper was used in characterizing spray droplet distribution and quantitative assessment of spray deposition on the larval population of cotton bollworm in field applications.

2 Materials and Methods

2.1 Instruments and equipment

A UAVs sprayer compared with two common sprayers which are used more than other sprayers at cotton fields in Iran. The specification of each sprayer mentioned as follow:

(a) UAVs sprayer (Fig. 1): the UAV used Global Navigation Satellite System and Real-Time Kinematic (GNSS RTK) navigation technology, with the accuracy of the flying height and flying velocity both controlled to remain within the centimeter level. The UAV was powered by a 12,800 mAh Li-Po battery. The flying time was 15 min with a full tank. The nozzles (Teejet sprayer technology, with a nozzle flow range of 0–1.5 L/min) were placed at intervals of 1.24 m and the installation angle was vertically downward. The chemicals were transferred from the tank to the nozzles by a high-pressure pump. The flight height and flight velocity were controlled by the well-trained operator. The flight height was 2.0 m and the effective spraying width was 3.4 m.

(b) Boom sprayer: major components of the boom sprayer are the barrel, power sprayer pump, and boom, where 25 nozzles are installed at intervals of 50 cm. The nozzle flow range is 1.2 L/min in 8 bar pressure. The capacity of the barrel is 700 L.

(c) Hand lance tractor mounted sprayer: the component of this sprayer was like a boom sprayer but instead of the boom, a lance was replaced with a long hose. The effective spraying width was 13 m. The nozzle flow range is 11.4 L/min in 19 bar pressure. The capacity of the barrel is 700 L.

Fig. 1 UAVs sprayer, DJI Agras MG-1 used Global Navigation Satellite System and Real-Time Kinematic (GNSS RTK) navigation technology.

2.2 Evaluation of spray deposition

To facilitate the evaluation of spray deposition on a canopy of the cotton plant, the plant was divided into three different positions contain top, middle and bottom. The WSP cards were stapled on each position to observe the deposition of the droplets. After spraying, all WSPs were removed and placed in zip-lock bags along with a label describing the treatment, replication and WSP placement information. Samples were placed into light-proof sealed boxes immediately after collection and transported to the laboratory for analysis. A digital image analyzer was used to determine stain diameter and droplet size which analyze these samples after 24 hours of application to ensure that droplets had stopped spreading. A 20×20 mm sample area at the center of each card was used for image analysis. In the laboratory, the WSPs were scanned at a resolution of 600 dpi with a scanner. ImageJ® software (ImageJ® Ver. 1.3 8, National Institutes of Health, Bethesda, MD, USA) was used to extract droplet deposits in the digital image for the analysis of the droplet size, number of spray deposits and the area of coverage. Volume Median Diameter (VMD) is one of the most popular methods of evaluating droplet sizes. VMD is the droplet size at which 50% of the spray volume is in droplets larger than the VMD and 50% of the volume is in droplets smaller than the VMD (adapted from Matthews (2000)). Percent area coverage (AC) establishes the relationship between droplet spot area coverage on WSP and spray deposition on absorbent paper targets. The field capacity of the sprayer was calculated using the following equation:

$$FC=A/T$$

where, FC= Field capacity (ha/hr), A = Total area covered by the sprayer (ha), and T= Total time (hr).

2.3 Assessment of *H. armigera* management

The location of the study area was selected Hashem-Abad cotton research station with latitude: 36.890819 N, longitude: 54.345674 E; MASL= 7 m). The study was carried out on Goleatan®, a commercial cotton cultivar, in a Randomized Complete Design with 4 replications and 3 spraying treatments. Experimental plots included 10-meter planting lines with an 80×20 cm planting system. A typical larvicide, Thiodicarb (Larvin® 75% WP) was sprayed with sprayer treatments included: hand lance tractor, unmanned aerial vehicles (UAVs) and Boom sprayer. For sampling schedule, before and 1, 3, 5, 7 and 14 days after foliar application, 10 cotton plants were selected from each plot and recorded the number of immature stages (larval) of the *H. armigera* per plant. All data were analyzed using the Kolmogorov–Smirnov normality test. Subsequently, the data were transformed by $\sqrt{x} + 0.1$ and submitted for statistical analysis. The efficiency of each treatment in controlling cotton bollworm was modified by Henderson-Tilton formula (Henderson and Tilton, 1955), the mean of the treatments was compared with The one-way analysis of variance (ANOVA) with Duncan's multiple range test by SAS® software (Bewick et al., 2004).

3 Results and Discussion

VMD and AC parameters are important characteristics by which the quality of spraying operations could be evaluated. This research was carried out on a strip plot design in a completely randomized design with three replications. The main treatments were the sprayer type in three levels: boomed sprayer, lance sprayer and UAV sprayer; and the sub-treatments included plant high in three levels: Top, middle and bottom. The analysis of variance of VMD is presented in Table 1. The results showed that the treatment of different levels of plant high was significant at level of 1% and the sprayer treatments on the average of VMD were not significant. Also, sprayer-plant high interaction between treatments was significant.

Table 1 ANOVA of VMD and Percent area coverage (AC). ^{ns} non-significance and ** significance difference between treatments on sampling days based on Tukey HSD test is given at 1% level.

	df	VMD	AC
Sprayer (A)	2	14448.23^{ns}	18.38^{**}
Plant high (B)	2	55723.87^{**}	27.36^{**}
A*B	4	19255.69^{*}	5.36^{**}
Error	18	5923.19	1.10
C.V		25.42	42.14

Comparison of the mean effect of sprayer type and height levels on the volume median diameter (VMD) was shown in Fig. 2. According to Fig. 2, there is no significant difference in different levels of plant high in UAV. In the boom sprayer, the top of plant has the highest VMD, which is significantly different from the middle and bottom of plant high. In lance sprayer, the maximum of VMD was indicated in the middle of plant high. VMD in this sprayer in top and bottom of plant high are in the same statistical group. In the middle of the plant, there was a difference between the boom and the lance sprayer, while there was no significant difference between them and the UAV. The results of Fiaz et al. (2020) showed that the VMD of the UAV spray at a height of 2 meters and speed of 2 m/s was 448.75 µm, which is close to the results of current research on the top of plant. The results of De Lima Junior et al. (2018) showed that the VMD were not statistically different in the middle and bottom plant high; while the results of the present study showed that there was no significant difference in the UAV spray at different plant high levels.

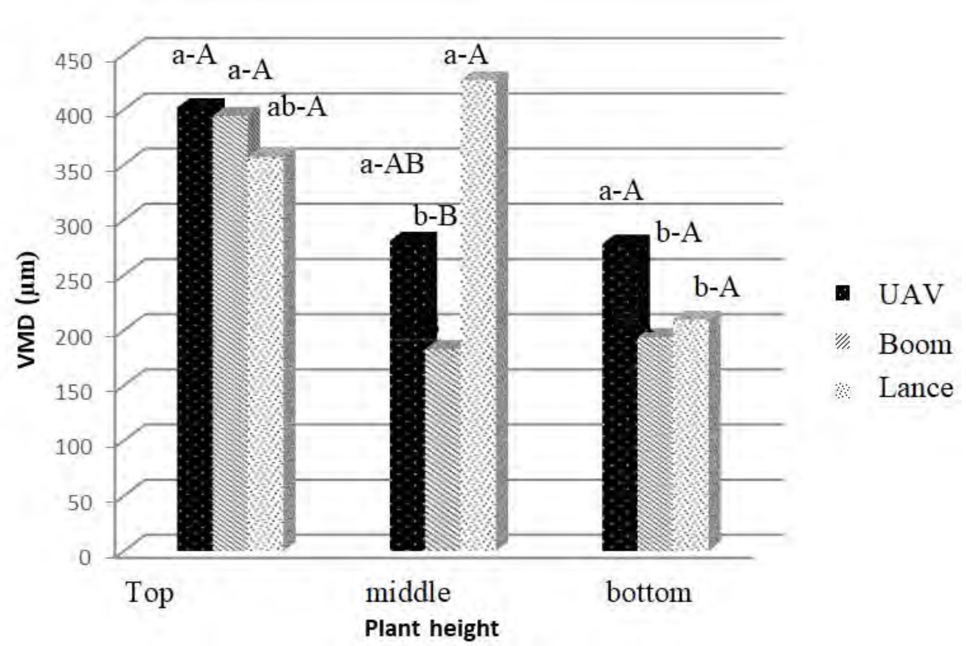

Fig. 2 Comparison of VMD in different sprayers and plant heights (Capital letters compare VMD in the sprayers in the same plant height -Lowercase letters compare VMD in the sprayers in different plant height).

The analysis of variance of the area coverage percentage (AC) data showed in Table 1. According to Table 1, sprayer types and different plant levels were significant on the area coverage percentage. The interaction effect of two treatments was significant at the level of 1%. According to Fig. 3, the highest percentage of area coverage (5.5%) was in the lance sprayer at the top of the plant. Based on results of the lance spray in field, it showed an expected pattern. Also, the highest percentage of area coverage in the upper and middle third of the plant is related to lance sprayer which can be due to spraying pressure and nozzle output. The lowest AC at the top of the plant belongs to the UAV sprayer with a rate of about 2.94%. The smaller size and number of droplets in this type of sprayer is one of the reasons for this percentage of area coverage. In the boom sprayer and UAV sprayer, percentage of area coverage in the middle and bottom of plant high were in the same group, while both levels are significantly different from the top of the plant. In the lance sprayer, percentage of area coverage in the upper and middle of plant high were in the same group, while both levels are significantly different from the bottom of plants. In the top, middle and bottom part of the plant, in terms of the percentage of area coverage, UAV and booms are in the same group (Fig. 3).

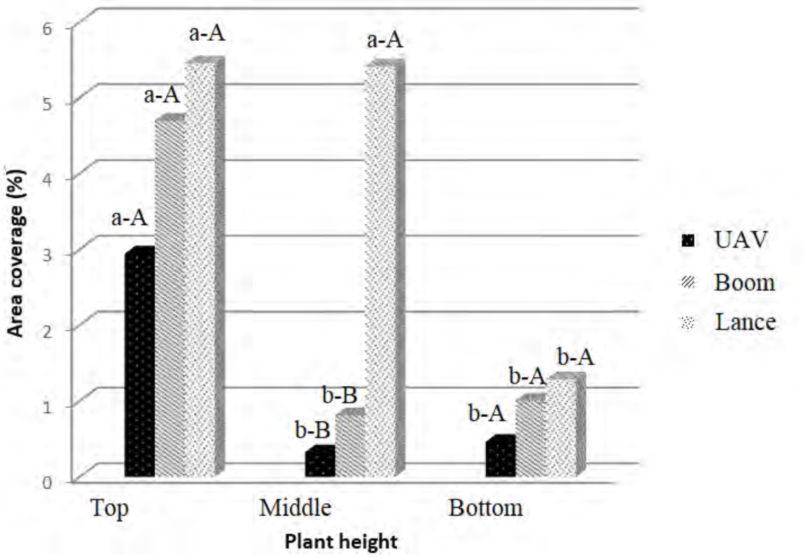

Fig. 3 Comparison of percent area coverage in different sprayers and plant heights (Capital letters compare AC in the sprayers in the same plant height -Lowercase letters compare AC in the sprayers in different plant height).

The results of the present study on area coverage percentage showed that UAV sprayer leads to poor penetration especially in the middle and bottom of plant height. The results of the present study are consistent with the results of Meng et al. (2019) who showed the distribution of droplet coverage in the top of cotton canopy is significantly different from the bottom of canopy. Also, De Lima Junior et al. (2018) and Fiaz et al. (2020) reported that poor penetration of UAV spraying in different plant highs.

The analysis variance of larval mortality of cotton bollworm in treatments (three sprayers including: hand lance, UAV and boom sprayer) at repeated sampling periods showed that a significant difference between the treatments during sampling interval days after spraying (Table 2). As shown in Table 2, there was no statistically significant difference between the treatments on the first day after spraying. This phenomenon declared that the sprayers had not significantly different or the larvicide still didn't show its effect despite reached the target.

Table 2 Analysis of variance (One-Way ANOVA) different sprayers on the larval stage of cotton bollworm. ^{ns} non-significance and ** significance difference between treatments on sampling days based on Tukey test is given at 1% level.

Source	df	Mean square					
		Sampling interval after spraying (day)					Total
		1	3	5	7	14	
Treatment	3	955.48 ^{ns}	2460.53**	1091.57**	459.24^{ns}	623.21^{ns}	680.07**
Error	12	309.34	186.02	41.44	25.49	27.33	24.26
C.V. (%)	-	27.55	18.74	7.25	5.33	6.91	5.87

The efficiency of mortality percentage of different spraying methods on the larval stage of cotton bollworm was modified by Henderson-Tillon (1955) equation's (Table 3). The highest mortality percentage in initial effect on larval of *H. armigera* was obtained in UAV method and hand lance sprayer, while non-significant difference between the spraying method and the boom sprayer (Table 2 and 3). Due to the fact turbulent air-flow of UAV, micro-droplets could reach different parts of plants and larvae death by contact effect. The drone was able to precisely targeted the ecological niche of *H. armigera* (by up to down spraying direction), which are fresh, topper leaves, buds of terminal meristem. In hand lance sprayer, due to using human ability completely spraying convergence was showed.

Table 3 Effect of sprayers against larvae of cotton bollworm (mortality rate modified by Henderson and Tilton (1955) formula). Means followed by the same letter are not significantly different (P=0.05) and ns was non-significant different.

Sprayers	Percentage of initial mortality (Days 1)	Percentage of mortality due to residual insecticides in treatments				Average reduction	Average reduction due to residual (%)
		Day 3	Day 5	Day 7	Day 14		
Boom	38.34 ^{ns}	40.1 ^{b}	100 ^{a}	100 ^{ns}	100 ^{ns}	85.02	75.68
Hand lance	42.50 ^{ns}	59.61 ^{a}	70.4 ^{ab}	100 ^{ns}	100 ^{ns}	82.5	74.5
UAV	50.52 ^{ns}	61.1 ^{a}	80.21 ^{a}	100 ^{ns}	100 ^{ns}	85.32	78.36

The mean mortality percentage of different treatments on larval cotton bollworm showed that a significant difference between the sprayers in comparison with control on third days after spraying (Table 3). As the results showed, from the third day after spraying, the difference in mortality rate between the sprayers used in current research was noticeable. The difference between the highest and lowest mortality (boom sprayer and UAV sprayer, respectively) was 21% in statistical groups. But in the next readout (five days), with increasing mortality, differences in the effect of treatments, were recorded in statistical groups. The mortality rate caused by sprayers showed statistically significantly different compare with the control. Hand lance sprayer and UAV were the same statistical group, but the boom sprayer demonstrated a high mortality rate after five-day (Table 3). A full efficiency of the boom sprayer (100%) was an agreement to Volumetric Mean Diameter (VMD) and Percent area coverage (AC) of this study. Boom sprayer had appropriate features (VMD and AC) sprayed a smaller and more uniform volume diameter in all three areas of the plant which leads to better penetration in the whole canopy and surface coverage uniformly in the middle and lower thirds (Fig. 1 and 2). On the seventh and fourteenth day after spraying, did not show a significant difference between sprayers. All sprayers showed 100% mortality but they were statistically different from the control treatment. Therefore, it can be concluded that 100% larval mortality on the seventh day is probably related to the properties and high durability of larvicide (Thiodicarb), which did not matter by spraying method. The current result was in agreement with the middle-term effect of Wang et al. (2019) and Lou et al. (2018) result's which mortality effect of UAV sprayer in compare with the boom to control cotton and wheat aphids. Based on the current results as compared to the other two sprayers, UAV sprayers have the lowest percentage of surface coverage in different plant high. The highest percentage of coverage belonged to lance sprayer with 5.5% at the top of the plant. Also, the lowest percentage of surface coverage with a rate of about 2.94% was indicated in UAV sprayer on top of plant.Although the important characteristics (percentage of coverage and VMD) of the UAV sprayer were not good quality, the larval mortality rate of cotton bollworm on the third day after spraying was 61.1%. However, the boom sprayer recorded 100% larval mortality on the fifth day after spraying, which was significantly different from the other sprayer types.

Acknowledgements

We thank Dr. Habib Kashiri, manager of Hashem-Abad cotton research station for supplying experimental requirements and sharing his experience. This research was scientific cooperation within Golestan University of Agricultural Sciences and Natural Resources, Iran and Cotton Research Institute of Iran.

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