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Comparison of Droplet Deposition, 28-Homobrassinolide Dosage Efficacy and Working Efficiency of the Unmanned Aerial Vehicle and Knapsack Manual Sprayer in the Maize Field

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Abstract: Brassinolides (BRs) are naturally-occurring phytohormones, which are essentially important to improve the crop adoptive capacity to various stresses. Spray volume (SV) and agrochemical application methods are associated with chemical deposition and field efficiency. The objective of this study was to compare the possible effects of 28-Homobrassinolide (HBL) dosages 18, 22, and 30 mg a.i. ha^{-1} for unmanned aerial vehicle (UAV) sprayers (15 L ha^{-1} and 30 L ha^{-1}) and 22 mg a.i. ha^{-1} for Knapsack manual sprayers (KMS) (450 L ha^{-1}) at maize silking stage on droplets deposition distribution, photosynthetic parameters, grain filling process and yield. The results showed that the droplet deposition of UAV (15, 30 L ha^{-1}) was 47.04%, 8.89% higher than KMS. However, the UAV sprayer had a poor droplet deposition distribution. HBL significantly increased the photosynthetic parameters, grain filling rate, and yield. A UAV spray volume of 15 L ha^{-1} with 22 mg a.i. ha^{-1} significantly increased grains yield by 4.16–5.64%, 7.5–12.09% compared to KMS and CK in both years. Considering the high efficiency of the UAV sprayer and better effects of HBL on final yield, spraying 22~30 mg a.i. ha^{-1} with UAV spray volume 15 L ha^{-1} at the silking stage could be a better strategy.

Keywords: 28-Homobrassinolide; unmanned aerial vehicle (UAV); Knapsack Manual Sprayer (KMS); droplets deposition distribution; grain filling

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1. Introduction

Maize (*Zea mays* L.) is the most important cereal crop in the world. It is widely grown for food, feed and industrial purposes [1]. According to Erenstein et al. [2], the worldwide maize is cultivated in a total area of 197 million ha, which is 27% of the total area of cereals, and production worldwide is 1137 million metric tons, which constitutes a 39% share of total cereals production. Whole maize grains are rich in digestible starch, proteins, fat, oil, vitamin A, B, and other minerals [3,4]. Demand for maize is increasing with an increase in its consumption [5]. In recent decades, abiotic stresses, including extreme weather, temperature, and climate changes have been the main obstacles in crop production [6]. For better crop production, proper agriculture practices should be adopted to increase plant resistance against environmental stress [7].

Brassinolides (BRs) are the novel plant growth promotors that regulate the biological process in plants and are widely used in agriculture to increase production and enhance plant resistance against biotic and abiotic stresses [8–10], moisture stress [11], drought stress [12,13], heavy-metal stress [14,15], salinity stress [16,17], and nitrosative stress [18]. In the current situation of maize high demand and weather concerns, including extreme weather and limited resources, there is a need to increase yield per unit area. Final grains weight is the main component of total grain yield determined by the grains filling rate and duration [19]. Photosynthesis is an essential phenomenon in plant growth and development. BRs play an essential role in the chlorophyll and in plant photosynthesis, while promoting stomatal activity by increasing magnesium (Mg) content in several plant species [20], e.g., *Cucumis* sativus [21,22], *Brassica juncea* [17,23], *Oriza sativa* [24], *Triticum aestivum* [25], *Vigna radiata* [26,27], *Lycopersicon esculentum* [28], and *Glycine max* [29]. It promotes grain filling, sugar synthesis in *Brassica juncea* [30] and maintains photosynthetic parameters to maintain CO_2 assimilation during grains filling, which has resulted in an increased grain filling rate and, thus, has increased the yield of maize [31] and rice [32,33]. Chemical penetration and spraying methods are closely associated; it is important to choose suitable spraying methods to get a better output.

However, an analogue of BRs i.e., 28-Homobrassinolide (HBL) is gaining attention because of its high advantages, such as increasing chlorophyll content and photosynthetic activity, accelerating translocation from source to economic parts. HBL increases the enzyme levels, which are responsible for sugars, proteins and nucleic acid. HBL promotes the proline production, which imparts a greater increase in plants' resistance against adverse weather conditions than other BRs [34]. Edupuganti [35] already showed improvement in maize crop growth due to exogenously applied HBL. However, extensive studies with respect to different dosages and application methods were not properly documented, which has played a key role in decreasing the cost and quantity of HBL.

In China, more than 88% of sprayers are operated manually [36]. Because of rural-urban transfer of population causes high-cost labor, there is a need to replace the traditional sprayers with Unmanned aerial vehicles (UAVs). UAVs cannot be ignored because of their high efficiency, reduced labor cost, low volume spraying, low altitude, quick and uniform functions, and are better for plant protection. In recent decades, UAVs have been rapidly replacing manual sprayers. In October 2019, 55,000 drones covered an area of 300 million hectares in 2019 in China [37,38]. During spraying, there is the risk of chemical exposure. UAVs have more security for farmers health [39–41]. Droplet coverage, density, deposition, and deposition rate are the main components that characterize the droplet distribution for different spray volumes (SV) [42]. UAVs can cover a spraying area of 40–80 times more than KMS [43], 49.1–57.1%, with 98% more deposition [42,44]. Chemical dosage and adjuvants in UAV close to 10 L ha^{-1} formulations can increase the chemical penetration; use of adjuvants with UAV 15 L ha^{-1} spray volume has resulted in better deposition [29,43,45]. Flight speed, SV, altitude, and canopy of plants greatly affect the uniformity of droplet deposition. Pesticides applied by a UAV sprayer with 3.5 m height and 4 m s^{-1} speed have shown better results than KMS [45–47]. UAVs with a spray volume of one nozzle output around 15 L ha^{-1} and a KMS spray volume of 300 L ha^{-1} have shown the same results when at a 20% reduced chemical dosage. UAVs have also shown better results on the same dosage [48]. There are relatively few studies regarding the droplet deposition distribution and field effects of plant growth regulators; in particular, HBL studies have not been reported.

In this study, we compared the spraying effect of different HBL dosages and sprayer volumes of KMS and UAV sprayers on maize crop growth and development. We hypothesized that HBL application by UAV will have a better effect on maize physiology and higher yield than KMS. The objective of this study was to: (1) Investigate the physiological effect of HBL on maize crop, (2) obtain an optimum dosage of HBL for maize, (3) find an efficient spray volume for UAVs and KMS, (4) examine whether the results of KMS and UAV are different when HBL dosage decreased by 18% in UAV as compared to KMS, and (5) evaluate the droplets deposition distribution of UAV and KMS on maize canopy.

2. Materials and Methods

In this experiment, the efficiency of UAV and KMS sprayers were compared by applying HBL dosages on maize. The working efficiency of these two instruments was compared in different aspects, including droplet coverage rate, density, droplet deposition, deposition rate, and droplet uniformity of different maize canopy.

2.1. Spray Equipment

A battery-operated DJI AGRAS MG-IP (UAV) (SZ DJI Technology Co., Ltd. Shenzhen, China) was used in this experiment. This UAV was based on an operating frequency of 5.725–5.850 GHz, a transmission range of 3–5 km; the speed measurement accuracy was 0.03 m s^{-1}; the flying speed was 4 m s^{-1} by containing SV of 15 L and 6 m s^{-1} for SV 30 L, as well as two batteries of 12,000 mAh, having 13 minutes' flight time with a full 10 L tank. It had four nozzles (Model: XR11001VS) in a vertically-downward direction with a spraying capacity of 0.379 L min^{-1}; droplet size was 130–250 µm, depending on the environment and spraying speed, which was automatically adjusted by the nozzles. The flight height was 2 m above the plant canopy; a well-trained engineer operated the system. The comparison system was a KMS Super green 16 Matabi, Goizper Co., Spain. It had a 16 L capacity, adjustable conical standard nozzles with a flow rate of 0.3 L min^{-1}, and a spraying capacity of 0.61 L min^{-1} at three bars. The travelling velocity of the KMS was about 1.2–1.4 km h^{-1}. Before spraying, as for testing speed, water was added in the UAV tank as an equal amount to chemical formulation and performed on another plot of the same size to adjust the acceleration for 3 times. After knowing the exact speed, HBL was added in the UAV tank and practiced in parallel direction to the plot lines in the selected treatment. After finishing one treatment, the UAV tank was washed to avoid the dosage mixture and the next treatment dosage was applied; in this way, the whole spraying session was performed. The same method was used for the application of HBL by the KMS in a parallel direction.

2.2. Experimental Condition

The field experiments were conducted at Shandong Academy of Agricultural Sciences research station (SAAS) (36°58′ N, 116°58′ E), Jinan district, Shandong, China in the year 2019 and 2020 (Figure 1). At the time of chemical application, the soil at the research station was sandy clay loam with pH 8.5. A soil depth of 0–40 cm contained 1.05 g kg^{-1} of total N, 40.1 mg kg^{-1} of available P, 130 mg kg^{-1} of available K, and 15 g kg^{-1} of organic matter contents. The weather data of this site was obtained.

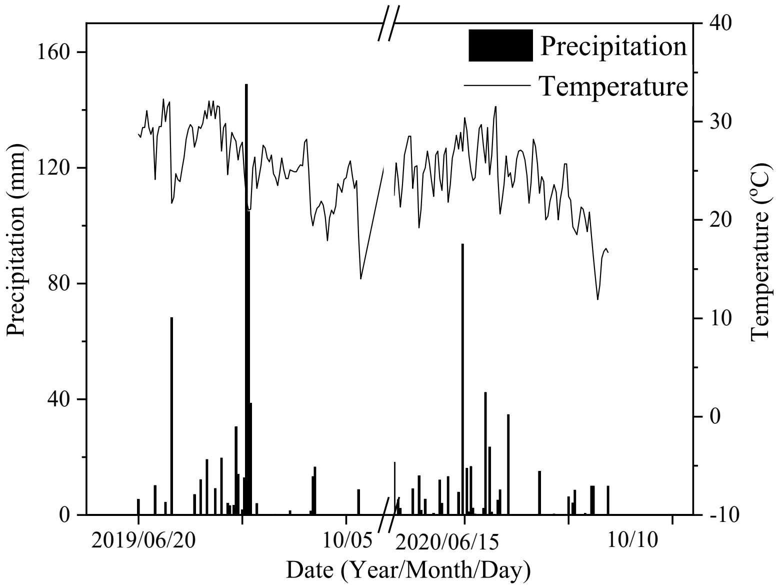

Figure 1. Mean temperature and precipitation during maize growing seasons in 2019 and 2020.

2.3. Experimental Design

Randomized complete block design (RCBD) with three replications was used in this experiment. This study consisted of two factors with 7 treatments and one control treatment. HBL (Jiangxi Windeal Biotechnology Co., Ltd. Nanchang, China) with 3 dosages (18, 22, 30 a.i mg ha^{-1}) was applied by UAVs; each dosage of HBL was applied with two different spraying volumes (15 L ha^{-1} and 30 L ha^{-1}) to check the efficiency of the UAV for better chemical deposition and penetration in plants, which can result in an effective bio-physiochemical processes in the plant body that results in increasing yield production. To increase the HBL penetration applied by UAV, aviation spray adjuvant Beidatong (Hebei Mingshun Agricultural Technology Co., Ltd., Shijiazhuang, China) with the dosage of 15 g L^{-1} was added to the HBL solution. For the KMS, 22 mg a.i. ha^{-1} dosage of HBL with a 450 L ha^{-1} spraying volume was used (Table 1). HBL was applied at the flowering stage, 55 days after sowing (DAS), during two years' experiments. One blank control (CK) was kept to compare the results of all treatments. To control maize weeds, a recommended dosage (1.2 kg ha^{-1}) of nicosulfuron-atrazine was applied at the V4 stage. To control borer moth at the V4 stage, benzoate-cyhalothrin was applied with a recommended dosage of 90 mL ha^{-1}; to control Mythimnaseparata walker in maize, 200 g L^{-1} of chlorobenzamide suspension with a recommended dosage of 225 mL ha^{-1} was applied at the V13 stage.

Table 1. Experiment treatments in 2019 and 2020.

Treatments	Spray Method	HBL Dosage (mg a.i. ha^{-1})	Adjuvant (g ha^{-1})	Spray Volume (L ha^{-1})
UAV1	UAV	18	225	15
UAV2		18	450	30
UAV3		22	225	15
UAV4		22	450	30
UAV5		28	225	15
UAV6		28	450	30
KMS	KMS	22	0	450
CK		0	0	0

One maize cultivar Deng hai 605 (♀DH351 × ♂DH382, produced by Shandong Denghai Seeds Co., Ltd.) with a planting density of 75,000 plants ha^{-1}, was sown on June 20, June 15 and harvested on October 5, October 10 in 2019 and 2020, respectively. A total area of 3.78 ha (37,800 m^2) was used for the experiment. Each treatment area was 2700 m^2 (180 m × 15 m); the buffer area between UAV, KMS and control treatments was 10 m. Samples were taken from the 3 small plots from the middle of the main plot; each small plot was (20 m×15 m) in size; 15 plants were tagged to record phonological data.

2.4. Sampling and Measurements

2.4.1. Measurement of Droplets Deposition Distribution

The droplets coverage rate, droplet deposition density, droplet deposition, deposition rate and droplet distribution uniformity were measured by placing droplets collection cards (DCCs) (4 cm × 6 cm) (Standard & Poor's Office Co., Ltd. Hangzhou, China) on top, middle and bottom leaves; each leaf contained two cards, one on the top and one on the lower middle position of each leaf of maize plants of all UAV and KMS treatments in 2019; each DCC was stuck with sticky material to the plant (Figure 2). Water-soluble food color allura red was mixed with HBL with a quantity of 15 g/L^{-1} to determine spray deposition. It does not affect the chemical formulation and can efficiently trace droplets [49]. A single treatment had 15 sampling positions. DCCs were placed on the top, middle (ear leaf), and bottom leaf of the maize plants. A total of 15 maize plants were selected from sampling points, 6 DCCs on one maize plant and 90 DCCs per treatment. Thirty minutes after spraying, dried DCCs were collected, packed in the zip lock bags and scanned at 600 dpi resolution with a scanner (DCP-1608, Brothers (China) Commercial Co., Ltd.). To determine the droplets coverage rate and droplet density, ImageJ 1.3.8 software (National Institutes of Health, Bethesda, MD, USA) was used [50]. Plant samples were taken two hours after chemical application. In total, 15 plants were removed from a single treatment. Upper, lower and middle leaves were removed; after taking the leaf area, these leaves were put in a zip lock bag. Remaining plant leaves were chopped and packed in a zip lock bag. 0.1 L distilled water was added and shaken for 30 s. Samples were filtered by a 0.22 μm membrane; the value was determined by ultraviolet spectrophotometer (UV2550, Shimadzu Scientific Instruments). Droplet deposition was calculated according to Lou et al. [51]:

$$\beta_{dep} = \frac{\left(\rho_{smpl} - \rho_{blk}\right) F_{cal} V_{dil}}{\rho_{spray} A_{col}} \tag{1}$$

where β_{dep} is the deposition per unit area (μL cm^{-2}), ρ_{smpl} is the reading of the sample, ρ_{blk} is the readings of the blank sample, F_{cal} is the relationship between the absorbance value and the tracer concentration (μg L^{-1}). V_{dil} is the volume of the eluent (unit = mL), ρ_{spray} is the tracer concentration in the spray solution (unit = g L^{-1}), A_{col} is the area of the sampled leaf.

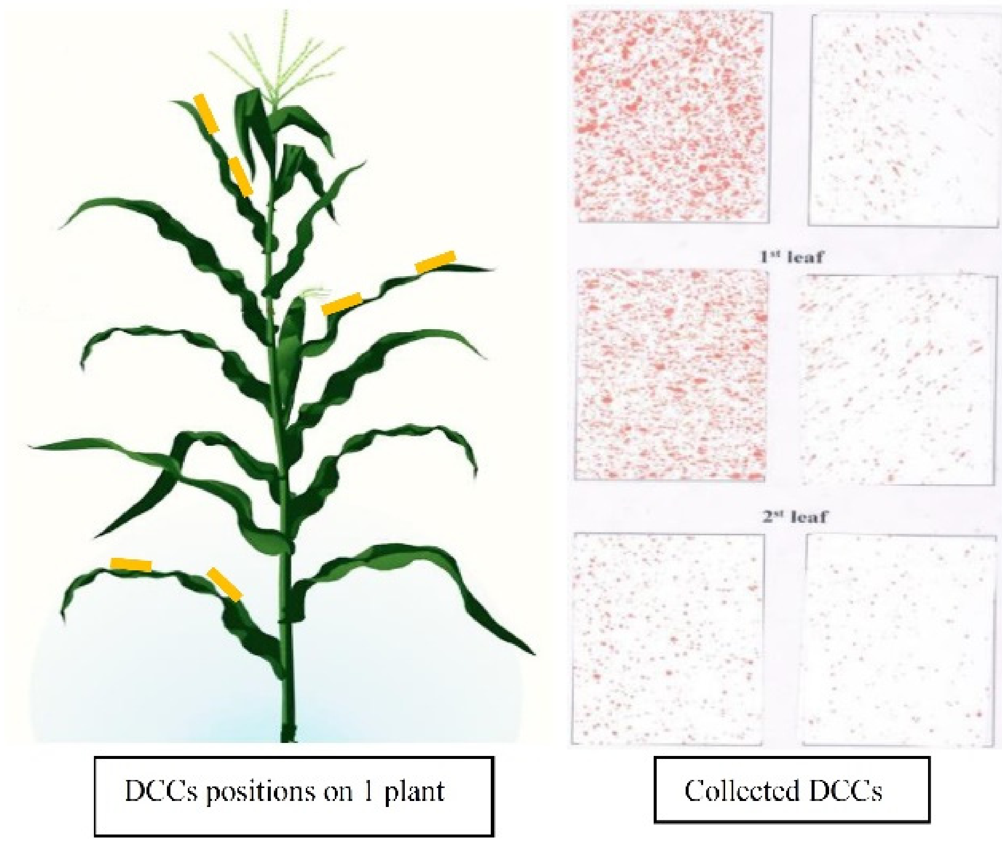

Figure 2. DCCs placing positions on plant and collected cards after spraying.

Droplet deposition rate (%) was calculated according to Lou et al. [51] with some modifications, such as:

$$Droplet\ deposition\ rate(\%) = \frac{F_{cal} D_{plant}\left[\left(\rho_{upper}+\rho_{middle}+\rho_{lower} - 3\rho_{blk}\right) V_{dil1}+\left(\rho_{remain} - \rho_{blk}\right) V_{dil2}\right]}{\rho_{spray} V_{spray}} \times 100 \tag{2}$$

where D_{plant} is the total number of plants per hectare, ρ_{upper}, ρ_{middle}, and ρ_{lower} is the absorbance of upper, middle and lower leaf, V_{dil1} is the volume of eluent (0.1 L), V_{dil2} is the volume of remaining eluent (0.4 L), ρ_{remain} is the volume of eluent of remaining leaves of the plant, and V_{spray} is the spray volume per hectare.

2.4.2. Uniformity of Droplet Deposition Distribution

To illustrate the consistency of droplet deposition of spray width, mean droplet deposition, mean deposition density, and coefficient of variance (CV) was calculated according to Xiao et al. and Gao et al. [45,52];

$$CV = \frac{S}{\overline{X}} \times 100\% \tag{3}$$

$$S = \sqrt{\sum_{i=1}^{n} \left(X_i - \overline{X}\right)^2 / (n-1)} \tag{4}$$

S refers to the standard deviation; X_i represents the droplet deposition density ($\mu L\ cm^{-2}$) of each sampling point, $\overline{X}$ is the mean deposition density ($\mu L\ cm^{-2}$) of every sampling unit, and n is the total number of DCCs.

2.4.3. Chlorophyll Content

Chlorophyll content SPAD of ear leaf was measured by chlorophyll meter SPAD-502 Plus (Konica Minolta Sensing Inc., Sakai, Japan) from 15-tagged plants of 3 replications from each plot on 0, 20 and 40 days after application (DAA) of HBL in 2019 and 2020.

2.4.4. Gas Exchange Attributes

Net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 (Ci), and transpiration rate (Tr) of ear leaf were measured on sunny days at 1200–1500 h by using IRGA (Li-6400XT Portable Photosynthesis System Lincoln, New York, USA). Leaf gas exchange parameters were measured from three replications by selecting 15-tagged plants of each treatment at 20 and 40 DAA in 2019 and 2020. The PAR during measurement of maize was 1200 $\mu mol\ m^{-2}\ s^{-1}$, and the atmospheric temperature was 25 $\pm$ 1 °C.

2.4.5. Grain Filling Dynamics

To measure the grain dry matter accumulation, 50 plants tasseled on the same day, same height, and ear diameter were tagged from each treatment. After HBL application, 5 ears from representative plants were taken by 5-day interval until 20 days. After 20 days, samples were taken by 10-day interval. 300 grains were obtained from each selected ear top and middle portions. After the mixture of grains of each portion, 100 grains were selected from the middle and top portions separately. These grains were first oven-dried at 105 °C for half an hour, then 70 °C until the constant weight. The grain filling rate was determined according to Gao et al. [52]. It was determined by fitting logistic growth equation: $W = A/1 + Be^{-Ct}$), where W is the kernels weight, t refers to the days after pollination, day of pollination is ($t_0 = 0$), A is the final weight of grain at physiological maturity, B refers to the primary parameter, and C refers to the growth rate parameters, which are to be determined. The physiology phase and filling phase calculated by the equation was different. The whole process of grain filling was divided into three periods according to the shape of the logistic curve: the (i) gradual increase period, the (ii) rapid increase period, and the (iii) slight increase period. The ending time of filling period of early-stage was calculated as: (t_1) = (lnB − 1.317)/C, the grain weight $w_1 = A/(1 + Be^{-Ct1})$; the ending time of grain filling of middle stage was (t_2) = (lnB+1.317)/C; the grain weight was $w_2 = A/(1 + Be^{-Ct2})$; the ending time of grain filling of late-stage was (t_3) = (lnB+4.59512)/C; w_3 is weight of grains when it reached 99%. Duration of early, middle and late filling periods was calculated as; $T_1 = t_1$, $T_2 = t_2 - t_1$, and $T_3 = t_3 - t_2$, and the increment in grains dry weight was: $W_1 = w_1$, $W_2 = w_2 - w_1$, $W_3 = w_3 - w_2$, where w_1, w_2, and w_3 were the difference in values of dry grains weight taken at 3 different times.

2.4.6. Yield and Yield Components

Maize yield was measured at physiological maturity. 5-m double-row were selected from one replication, and six rows from each maize plot were hand-harvested on October 5 and October 10 in 2019 and 2020. The harvested sample was used to measure thousand kernels weight (TKW) after drying at 70 °C of constant weight, the number of grains per ear, ear number m^{-2}; meanwhile, final grains yield was determined.

2.4.7. Statistical Analysis

Data were analyzed statistically using the software SPSS.10 (SPSS, Chicago, IL, USA). The one-way analysis of variance (ANOVA) was performed to estimate the difference in results obtained from all treatments used. To estimate the difference in the results of UAV treatments (SV and HD effects) individually and to check the interaction between SV and HD of UAV treatments, two-way ANOVA (factorial ANOVA) was performed followed by least significant difference (LSD) test. All treatments value means were compared using LSD test at the probability level of $p < 0.05$. The standard error was also calculated. All linear fitting and other equations were fitted by Origin 2019 software (OriginLab Co., Northampton, MA, USA).

3. Results

3.1. Droplet Deposition Distribution Analysis

Droplet distribution of three positions of maize leaves was measured by DCCs. The coverage rate of KMS was significantly higher than all UAV treatments. There was no significant difference observed between the average values of UAV spray volumes. KMS coverage rate was 22.73 times higher than UAV 15 L ha^{-1} and 7.33 times higher than UAV 30 L ha^{-1}. Distribution of droplets coverage on top, middle and bottom leaf varied by different SV. The middle leaf coverage rate was 12.15% and 3.95% higher than the top and bottom leaf positions in KMS. The top leaf showed a higher coverage rate than the middle and bottom leaf positions in both spray volumes of UAV (Figure 3A). Droplet deposition density increased along with SV. The average droplet deposition density of KMS was significantly higher in the top, middle, bottom leaf positions than in UAV 15 L ha^{-1} and UAV 30 L ha^{-1} treatments. At the same time, UAV 30 L ha^{-1} displayed a higher droplet deposition density than UAV 15 L ha^{-1}. Deposition density at different leaf positions varied with SV. Droplet deposition density of KMS and UAV 15 L ha^{-1} was higher on the top leaf, but, for UAV 30 L ha^{-1}, it was higher on the bottom leaf (Figure 3B). Droplet deposition is an important factor indicating the chemical penetration per unit area. Compared with KMS and UAV 30 L ha^{-1}, the droplet deposition of UAV 15 L ha^{-1} was higher in the top, middle leaf positions and lower than UAV 30 L ha^{-1} in the bottom leaf. The average value of droplet deposition of UAV 15 L ha^{-1} was higher than KMS and UAV 30 L ha^{-1}, but two UAV treatments were not statistically different (Figure 3C). The droplet deposition rate of UAV 15 L ha^{-1} and UAV 30 L ha^{-1} was significantly higher than KMS. No significant effect was noticed between the two UAV treatments (Figure 3D).

3.2. Uniformity of Droplet Deposition Distribution

Uniformity of droplets distribution was determined for UAV treatments and KMS (Figure 4). Average droplet coverage CV (%) of KMS was very poor compared to UAV treatments and statistically there was no significant difference between UAV treatments. Average CV of droplet deposition density followed the same order as in coverage, UAV 30 L ha^{-1} > UAV 15 L ha^{-1} > KMS. While on the bottom leaf, UAV 15 L ha^{-1} treatment was better than UAV 30 L ha^{-1} and KMS. There was no significant difference between average CV of droplet deposition; all treatments except for CV of KMS and UAV 30 L ha^{-1} were recorded less on the top and bottom leaf, while 15 L ha^{-1} CV was only less on the middle leaf of maize.

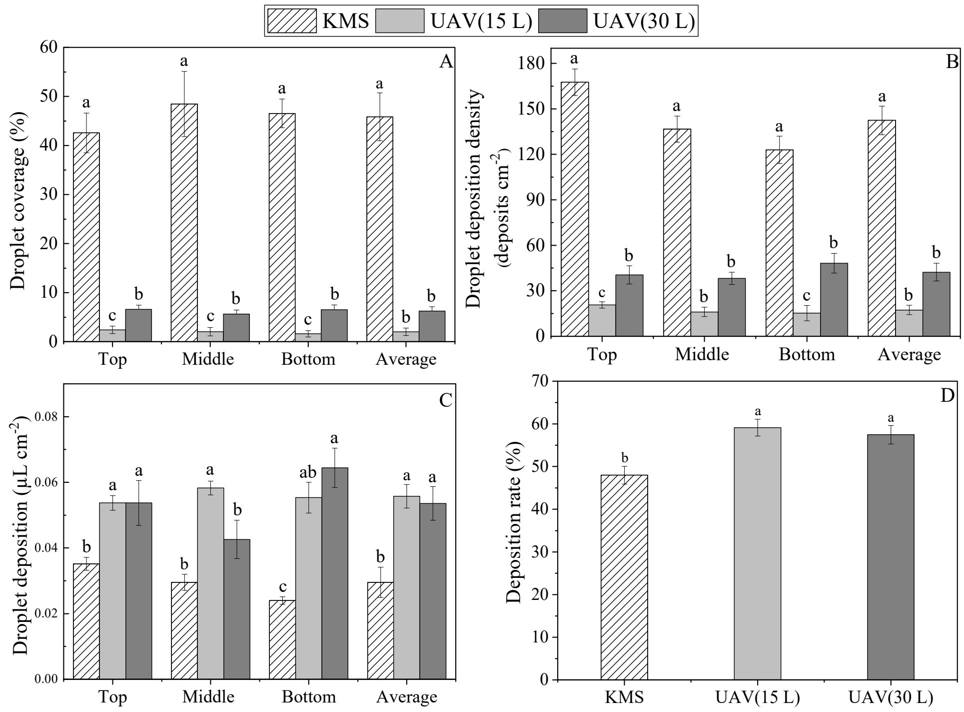

Figure 3. The droplet distribution of different SV on maize plant canopy: (**A**) Droplet coverage rate; (**B**) The droplet deposition density; (**C**) The droplet deposition; (**D**) The droplet deposition rate on top, middle, and bottom and the average leaf of maize plants with a spray volume of KMS (450 L ha^{-1}), UAV (15 L ha^{-1}), and UAV (30 L ha^{-1}). One-way ANOVA was performed determined by LSD test at $p < 0.05$, Bars with different letters above the lines show significant differences in results among treatments.

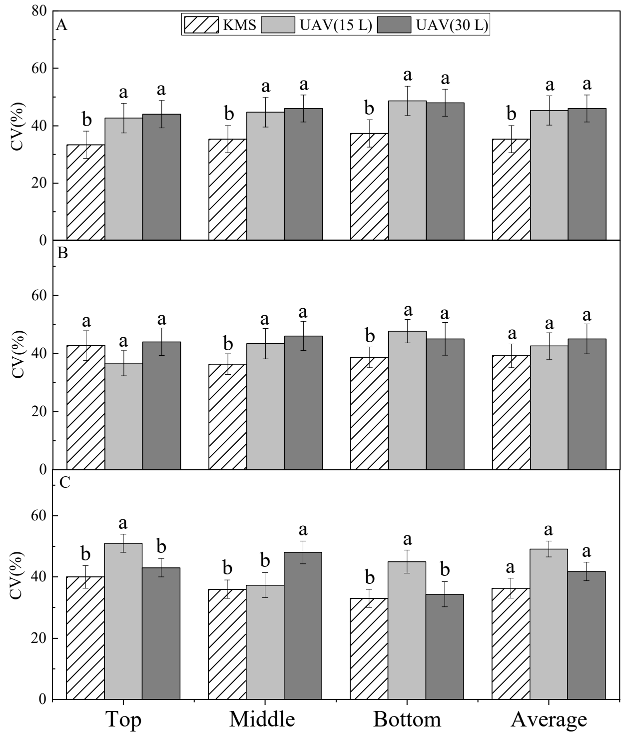

Figure 4. Coefficient of variation (CV) of droplet coverage (**A**); Droplet deposition density (**B**); droplet deposition (**C**). One-way ANOVA was performed determined by LSD test at $p < 0.05$, Bars with different letters above the lines show significant differences in results among treatments.

3.3. Chlorophyll SPAD Values

SPAD values increased significantly along with different chemical dosages and SV. As compared to CK, UAV5, UAV3 increased 8.39% and 6.30% in 2019 and, later on, 11.68% and 11.09% in 2020 on 20 DAA. While KMS, UAV1, UAV2 have no significant effect on chlorophyll SPAD values in 2 years. The gradual decrease was noticed in SPAD values of different treatments on 40 DAA. While KMS, UAV1 and UAV2 treatments were not significantly different (Figure 5). Mean SPAD values of different chemical dosages and spray volumes of UAV were significantly different on 20 DAA in 2 years.

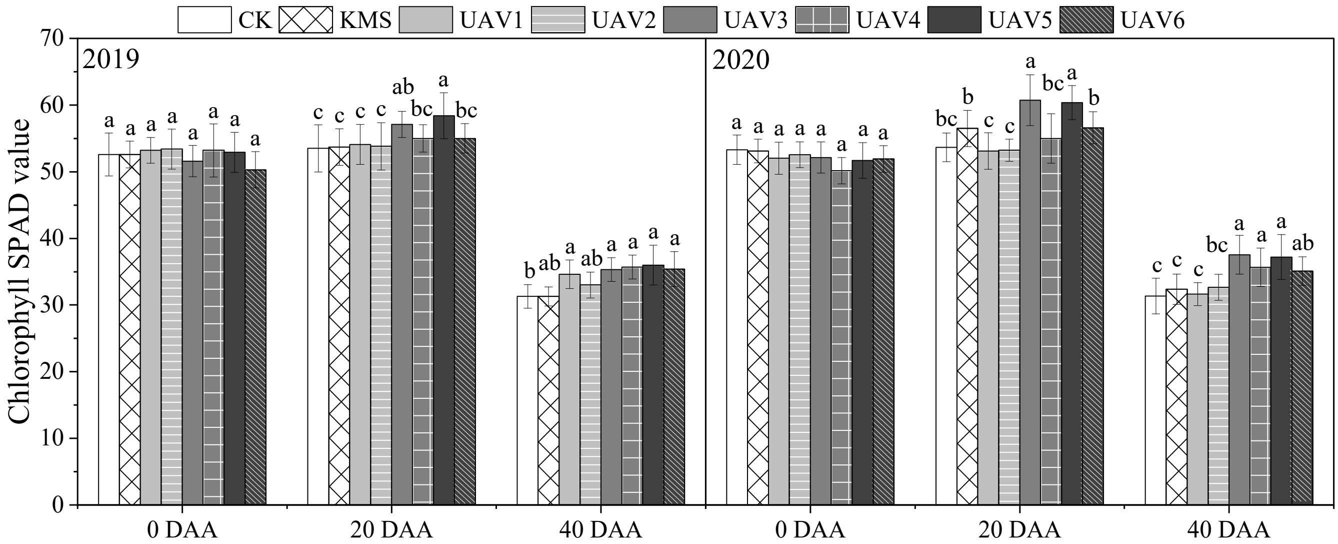

Figure 5. Chlorophyll SPAD in ear leaf of maize in 2019 and 2020. DAA: days after HBL application. One-way ANOVA was performed and determined by LSD test at $p < 0.05$, Bars with different letters above the lines show significant differences in results among treatments.

3.4. Leaf Gas Exchange Parameters

Different HBL dosages affected leaf gas exchange parameters significantly. As shown in Figure 6A, 20 DAA Pn increased by increasing HBL dosage at an optimum level. Compared to CK, Pn of UAV3 and UAV5 was increased by 14.58%, 11.82% in 2019 and 22.42%, 22.42% in 2020, respectively. There was no significant difference between the results obtained from UAV1, UAV2 and CK in 2 years. On 40 DAA, a gradual decrease in Pn was recorded. The trend for two years was similar. The difference in mean comparison between Pn values for different HBL dosages (18, 22, and 30 mg a.i. ha^{-1}) was highly significant on 20 DAA and 40 DAA in 2019 and 2020. The Tr of maize leaves was significantly affected by HBL treatments; the trend of two years' results was similar (Figure 6B). Compared to CK, Tr of UAV3 and KMS was increased by 32.72% and 24.48% on 20 DAA. Non-significant difference was recorded between UAV1, UAV2, and CK treatments on 40 DAA. Gs increased with medium and high dosage of HBL (Figure 6A). There was no significant difference between CK and low dosage treatments, such as UAV1 and UAV2. Gs of UAV3 and KMS increased by 38.23% and 27.52% over CK. On 40 DAA, UAV3 and UAV5 decreased slowly, while other treatments decreased by a greater percentage in two years. Ci was higher in UAV3 and UAV5 on 20 DAA and decreased with a lower ratio on 40 DAA than other treatments (Figure 6B, 2020). A non-significant difference was noticed between CK, UAV1, UAV2, and KMS in 2 years. On 40 DAA, a gradual decrease was recorded in all treatments; however, the decreasing percentage of UAV3 and UAV5 was much less than other treatments in 2 years. The mean comparison of different chemical dosage values was significantly different, but non-significant difference was noticed between different UAV spray volumes. Two years have same trend for all parameters.

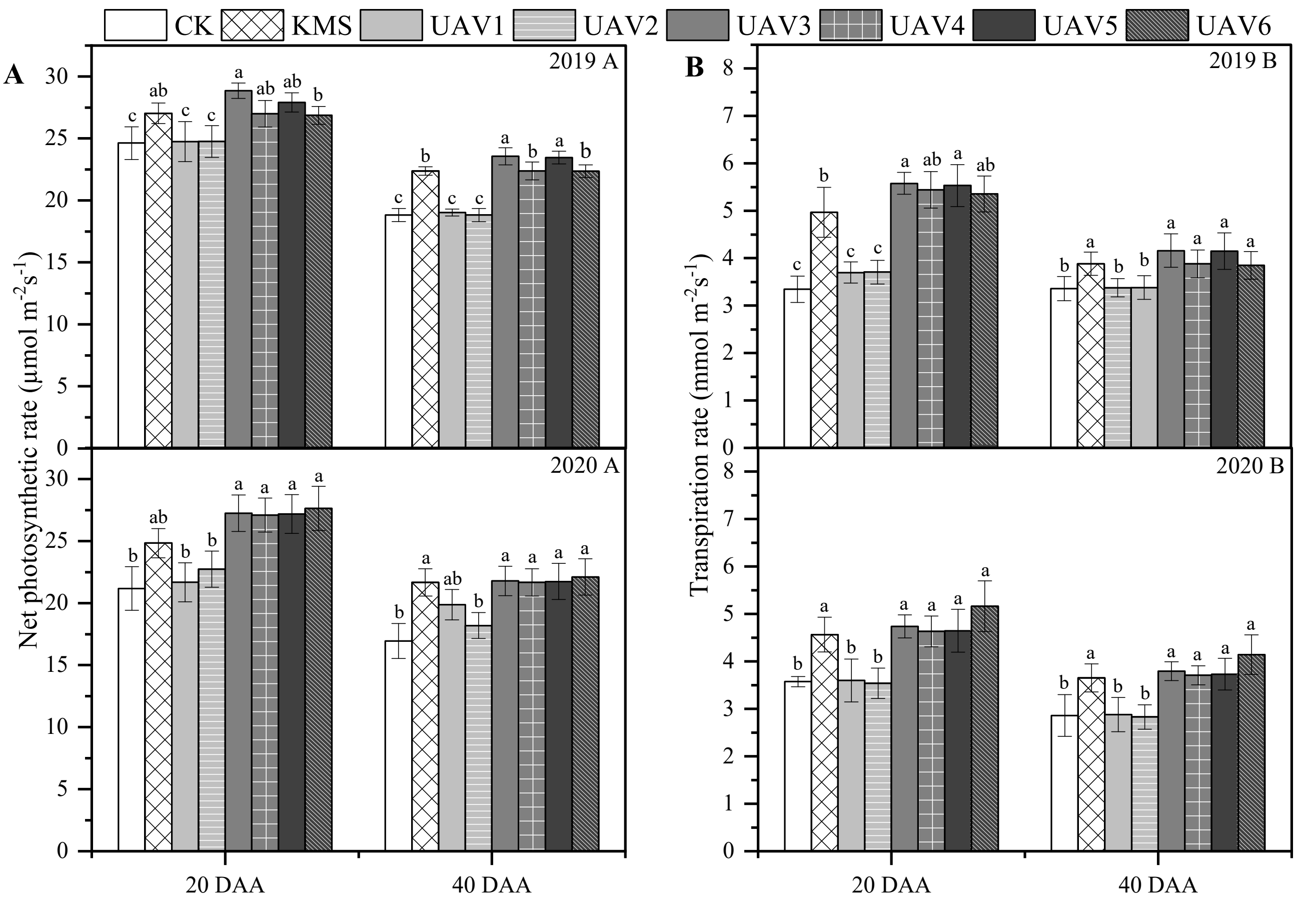

Figure 6. Effect HBL and different SV on net photosynthetic rate and transpiration rate in ear leaves of maize in 2019 and 2020. **A** (Net photosynthesis rate (Pn)); **B** (Transpiration rate (Tr)). DAA: days after HBL application. One-way ANOVA was performed determined by LSD test at $p < 0.05$, Bars with different letters above the lines show significant differences in results among treatments.

3.5. The Grain Filling

The curve of kernel weight had a similar logistic model similar for all the treatments. The grain filling rate was equal in gradual increase and slight increase stages, while dry matter accumulation of grains was much faster in the rapid increase stage. Grain weight was significantly affected by different HBL dosages. UAV treatments with medium and high dosage were maximum in the top and middle portion in 2 years. Low dosage treatments such as UAV1, UAV2 results (grain weight) were not different from CK. Dry weight of all the treatments in the top and middle portions was not differ until 20 DAA. After 20 DAA, grains dry weight of the middle portion of maize ear increased rapidly compared to the top portion in 2 years (Figure 7). Compared to CK, the maximum grain filling rate was recorded at rapid increase stage in the ear middle portion in UAV3, UAV5, and KMS treatments in 2019 and UAV4, UAV5, and UAV6 treatments increased in 2020. The top and middle portion filling rates were similar in 2019, while, in 2020, the top portion grains weight was less than the middle portion. Grain filling rate at gradual increase stage and slight increase stage of top and middle portions were same in 2 years. Time (d) of grain filling at gradual increase stage, and the rapid increase stage was the same in the middle portion of maize ear; however, it was higher in the slight increase stage in two years. While in top portion, time (d) for the gradual and rapid increase stage was the same but less than the slight increase stage in 2019, and time (d) of the slight increase stage > rapid increase stage > gradual increase stage in 2020 (Table 2).

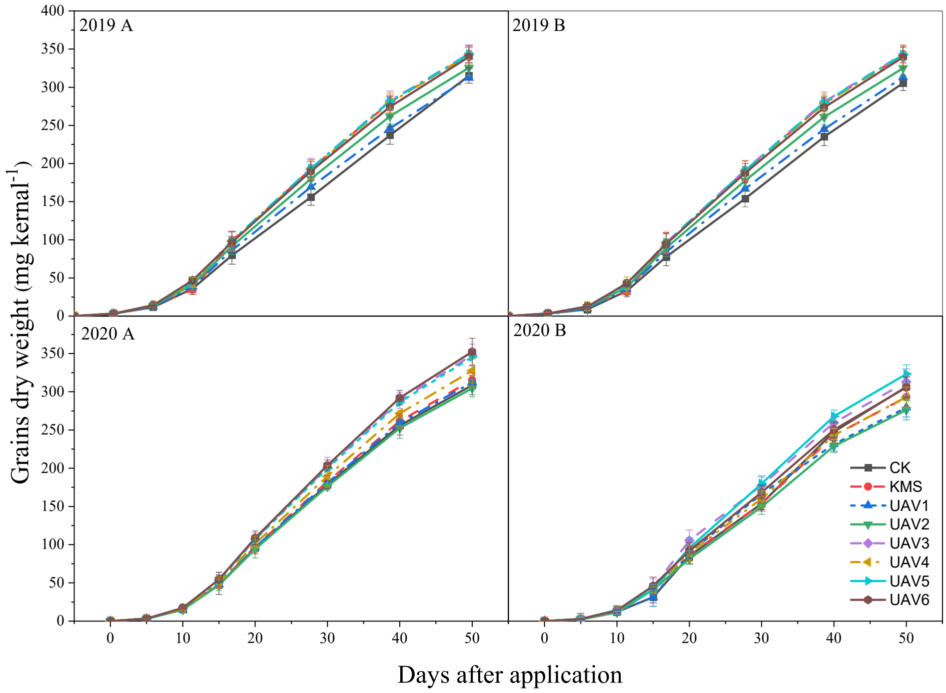

Figure 7. Grain filling dynamics as affected by HBL dosage and different SV. (**A**) represent middle and (**B**) represent the top ear portion in 2019 and 2020. Bars represent the standard deviation of replications.

Table 2. Effect of HBL dosages and SV on grain filling dynamic in maize.

Year	Treatment	SV	HD	Middle Grain						Top Grain					
				Gradual Increase		Rapid Increase		Slight Increase		Gradual Increase		Rapid Increase		Slight Increase	
2019				T1	R1	T2	R2	T3	R3	T1	R1	T2	R2	T3	R3
	CK	0	0	21.5 a	0.34 b	23.4 a	0.88 b	29.2 a	0.25 b	21.9 a	0.34 c	23.1 a	0.89 b	28.7 a	0.26 ab
	KMS	450	22	19.3 b	0.38 ab	18.8 b	1.07 a	23.5 bc	0.31 a	19.6 b	0.37 ab	18.7 bc	1.08 a	23.3 b	0.31 a
	UAV1	15	18	20.2 ab	0.36 b	21.5 a	0.92 ab	26.8 ab	0.27 ab	20.5 ab	0.35 bc	21.2 ab	0.94 ab	26.4 a	0.27 ab
	UAV2	30	18	19.3 b	0.37 ab	19.9 ab	0.98 a	24.8 b	0.28 ab	19.6 b	0.36 ab	19.7 b	0.99 ab	24.6 ab	0.29 a
	UAV3	15	22	19.0 b	0.39 a	19.4 ab	1.05 a	24.2 b	0.31 a	19.3 b	0.38 a	19.3 b	1.06 a	24.0 ab	0.31 a
	UAV4	30	22	19.1 b	0.39 a	19.6 ab	1.04 a	24.4 b	0.31 a	19.4 b	0.38 a	19.4 b	1.05 a	24.1 ab	0.31 a
	UAV5	15	30	19.0 b	0.39 a	19.4 ab	1.05 a	24.2 b	0.31 a	19.3 b	0.38 a	19.3 b	1.06 a	23.1 b	0.31 a
	UAV6	30	30	19.0 b	0.39 a	19.9 ab	1.02 a	24.9 b	0.31 a	19.4 b	0.38 a	19.7 b	1.03 a	24.6 ab	0.30 a
	HD														
	18			19.7 a	0.36 b	20.7 a	0.95 b	25.6 a	0.27 b	20.0 a	0.35 b	20.5 a	0.96 b	25.5 a	0.28 b
	22			19.0 b	0.39 a	19.5 a	1.04 a	24.3 b	0.33 a	19.3b	0.38 a	19.3 b	1.05 a	24.1 b	0.31 a
	30			19.0 b	0.39 a	25.7 a	1.03 a	24.5 b	0.32 a	19.3 b	0.38 a	19.5 b	1.04 a	24.3 a	0.30 a
	SV														
	15			15.2 a	0.38 a	20.1 a	1.01 a	25.0 a	0.29 a	19.7 a	0.37 b	20.1 a	1.01 a	25.0 a	0.29 a
	30			19.1 a	0.38 a	19.8 a	1.01 a	24.7 a	0.30 a	19.4 b	0.37 b	19.5 b	1.03 a	24.2 a	0.31 a
	ANOVA														
	SV			NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS	*
	HD			**	***	**	***	**	***	***	NS	**	**	**	*
	SV*D			NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	*
2020															
	CK	0	0	18.1 b	0.37 ab	19.7 a	0.92 b	24.6 a	0.27 b	19.1 a	0.34 b	21.3 ab	0.83 ab	26.6 ab	0.24 a
	KMS	450	22	18.3 b	0.37 ab	19.0 b	0.97 ab	23.7 b	0.28 ab	18.8 ab	0.36 ab	22.9 a	0.81 ab	28.4 a	0.23 a
	UAV1	15	18	18.7 a	0.36 b	19.0 b	0.97 ab	23.6 b	0.28 ab	17.7 b	0.34 b	20.0 b	0.83 ab	24.9 b	0.24 a
	UAV2	30	18	18.1 b	0.36 b	19.6 a	0.93 b	24.4 ab	0.27 b	18.6 ab	0.34 b	22.4 a	0.77 bc	27.8 a	0.22 a
	UAV3	15	22	18.1 b	0.36 b	19.8 a	0.91 b	24.6 a	0.26 b	17.5 b	0.41 a	22.2 a	0.86 a	27.7 a	0.25 a
	UAV4	30	22	18.2 b	0.39 a	19.7 a	0.98 a	24.6 a	0.29 a	18.4 ab	0.36 ab	22.3 a	0.82 ab	27.7 a	0.24 a
	UAV5	15	30	18.2 b	0.41 a	19.7 a	1.04 a	24.6 a	0.30 a	18.5 ab	0.39 a	21.4 ab	0.93 a	26.6 ab	0.27 a
	UAV6	30	30	18.2 b	0.42 a	19.7 a	1.06 a	24.6 a	0.31 a	16.7 bc	0.36 ab	21.6 ab	0.77 bc	26.9 ab	0.22 a
	HD														
	18			18.5 a	0.36 b	19.3 b	0.95 b	24.0 b	0.27 b	18.2 a	0.34 b	21.2 b	0.78 b	26.4 b	0.24 a
	22			18.1 b	0.37 b	19.7 a	0.94 b	24.6 a	0.27 b	17.9 b	0.39 a	22.3 a	0.84 a	27.7 a	0.26 a
	30			18.1 a	0.44 a	19.7 a	1.05 a	24.6 a	0.32 a	17.6 b	0.37 a	21.5 ab	0.81 a	26.7 ab	0.24 a
	SV														
	15			18.3 a	0.39 a	19.5 a	0.97 a	24.3a	0.28 a	17.9 a	0.38 a	21.2 b	0.83 a	26.4 b	0.25 a
	30			18.1 a	0.39 a	19.7 a	0.99 a	25.6 a	0.29 a	17.9 a	0.35 b	22.1 a	0.78 a	27.5 a	0.24 a
	ANOVA														
	SV			*	NS	NS	**	NS	NS	**	*	**	NS	NS	NS
	HD			**	**	**	***	**	***	***	**	***	**	**	NS
	SV*D			NS	NS	NS	***	NS	NS	**	NS	NS	NS	NS	NS

SV: UAV Spray volumes (L ha^{-1}); HD: HBL dosage (mg a.i. ha^{-1}); T1, T2, and T3 represent duration (d) and R1, R2, and R3 delegate the grain filling rate (mg d^{-1} $kernel^{-1}$) at early, middle and late-stage, respectively. *, ** and *** indicate significance at the 0.05, 0.01, and 0.001 probability levels, respectively. NS: not significant. Different letters in front of the values shows significant difference based on standard deviation between three replications.

3.6. Grain Yield and Yield Components

The effect of HBL dosages and SV on grain yield was significant in two years. HBL with medium and high dosage performed better in 2019 than in 2020. The increased percentage of yield in the two growing years was different. Compared to CK, grain yield of UAV3, UAV5, KMS increased by 12.09%, 12.09%, 6.83% in 2019 and 7.5%, 5.93%, 3.47% in 2020, although UAV1, UAV2 increased in 2019, but was not significantly different from CK. TKW and kernel number per ear were increased in two years, but the difference between two years was not significant. TKW and kernel number per ear of UAV3, UAV5, UAV6 was better than KMS and other treatments. The difference between Ear number m^{-2} was not different in two years. Pairwise comparison of UAV treatments followed by LSD test showed a significant difference between UAV dosage levels for kernel number m^{-2}, TKW (g) and grains yield (t ha^{-1}). UAV dosages showed no significant difference for all the grain yield and yield components except in kernel number m^{-2}, and a significant difference was noticed between two years in grain yield (Table 3).

Table 3. Effect of HBL dosage and spray volume on yield and yield components of maize.

Treatment	SV (L ha^{-1})	HD (mg a.i. ha^{-1})	Ear Number (m^{-2})		Kernel Number (ear^{-1})		TKW (g)		Grain Yield (t ha^{-1})	
			2019	2020	2019	2020	2019	2020	2019	2020
CK	0	0	7.4 a	7.4 a	556 b	564 ab	317 c	309 c	10.9 c	11.1 b
KMS	450	22	7.4 a	7.4 a	572 ab	568 ab	331 b	315 bc	11.7 ab	11.5 ab
UAV1	15	18	7.4 a	7.3 a	571 ab	564 ab	324 c	312 bc	11.0 bc	11.0 b
UAV2	30	18	7.3 a	7.4 a	558 b	541 b	325 bc	304 c	11.1 bc	10.9 b
UAV3	15	22	7.5 a	7.4 a	573 ab	575 a	343 a	348 a	12.4 a	12.0 a
UAV4	30	22	7.3 a	7.4 a	561 ab	567 ab	341 a	327 b	11.9 a	11.7 a
UAV5	15	30	7.4 a	7.4 a	599 a	578 a	343 a	345 a	12.4 a	11.8 a
UAV6	30	30	7.4 a	7.3 a	577 ab	579 a	340 ab	351 a	12.3 a	11.8 a
SV										
15			7.48 a	7.45 a	581 a	572 a	336.7 a	335.1 a	11.9 a	11.6 a
30			7.38 a	7.44 a	565 b	562 a	335.4 a	327.2 a	11.8 a	11.5 a
HD										
18			7.48 a	7.42 a	564 b	552 b	324.8 b	307.9 b	11.1 b	11.0 b
22			7.42 a	7.46 a	567 b	571 a	341.6 a	337.4 a	12.1 a	11.9 a
30			7.41 a	7.43 a	588 a	578 a	341.8 a	348.2 a	12.4 a	11.8 a
ANOVA										
Year			NS		NS		NS		*	
SV			NS		**		NS		NS	
HD			NS		**		***		***	
SV*HD			NS		NS		NS		NS	

SV: Different spray volume (L ha^{-1}) used in UAV; HD: HBL dosage (mg a.i. ha^{-1}); TKW: thousand kernel weight (g). Different letters to the right of the value indicate significant differences among different treatments of same year at $p < 0.05$ as determined by the LSD test. *, ** and *** indicate significance at the 0.05, 0.01, and 0.001 probability levels, respectively. NS indicates not significant.

4. Discussion

Brassinolide, a potent plant growth stimulator, has been widely used for different crop growth regulations [8–10]. To increase the chemical effectiveness, optimum SV, coverage rate, deposition density and high deposition rate are the key factors [42]. As shown in (Figure 3A,B), our research indicated that higher SV (KMS) shows a higher coverage rate and droplets density but lower deposition rate than UAV 30 L ha^{-1} and UAV 15 L ha^{-1}. Because of low SV, the lower plants canopy received fewer droplets, which resulted in less penetration. Our results are similar to Xiao et al. [45], who reported that the average impacts of defoliant by UAV on the cotton crop was less on lower canopy and lower droplets density in the lower canopy at 15 L ha^{-1} SV [53], As shown in Figure 3C,D, the droplets deposition and droplet deposition rate of UAV treatments was better than KMS. Droplet deposition of 15 L ha^{-1} was 47.04%, 8.89% more than KMS on an upper and middle leaf, which is similar to Zhou et al. [44]. Droplet deposition of UAV 30 L ha^{-1} was higher on the lower leaf than UAV 15 L ha^{-1}, and penetration of chemical on lower plant canopy was also higher but, on this growth stage, the penetration of chemical on the lower leaf of plant is not important. In our study, as shown in Figure 4, the uniformity of droplets coverage, droplets density and deposition of UAV 15 L ha^{-1} and UAV 30 L ha^{-1} treatments, was less, which is also reported in [42,50].

SPAD varies with the leaf and age of maize plants. BRs helps to increase and maintain chlorophyll SPAD and gas exchange parameters [20,30]. Exogenous application of HBL on maize plants ensures the distinct increase in photosynthesis parameters and chlorophyll SPAD values till 30 days after silking. Chlorophyll SPAD maintains the leaf area and greenness of the plant leaves. These plant hormones hamper the chlorophyll breakdown during the grain filling stage and provide the source supplies to increase the grains filling. Photosynthesis and SPAD decrease gradually after 30 days of silking (Figure 8) [12,52]. Enough carbohydrates ensure the number of grains [54]; source strength, photosynthesis, and high chlorophyll levels were observed in the plants leaves with exogenous application of BRs [27]. The results showed that BRs affect photosynthesis and photosynthetic pigments [22]. Our results showed that chlorophyll SPAD value increased with HBL treatments on 20 DAA (Figure 5); medium and high dosage of HBL showed better results over CK (Figure 6A,B and Figure 8A,B). This experiment suggested that exogenous HBL maintained the photosynthesis and inhibited chlorophyll breakdown, ensuring a carbon supply to grain filling.

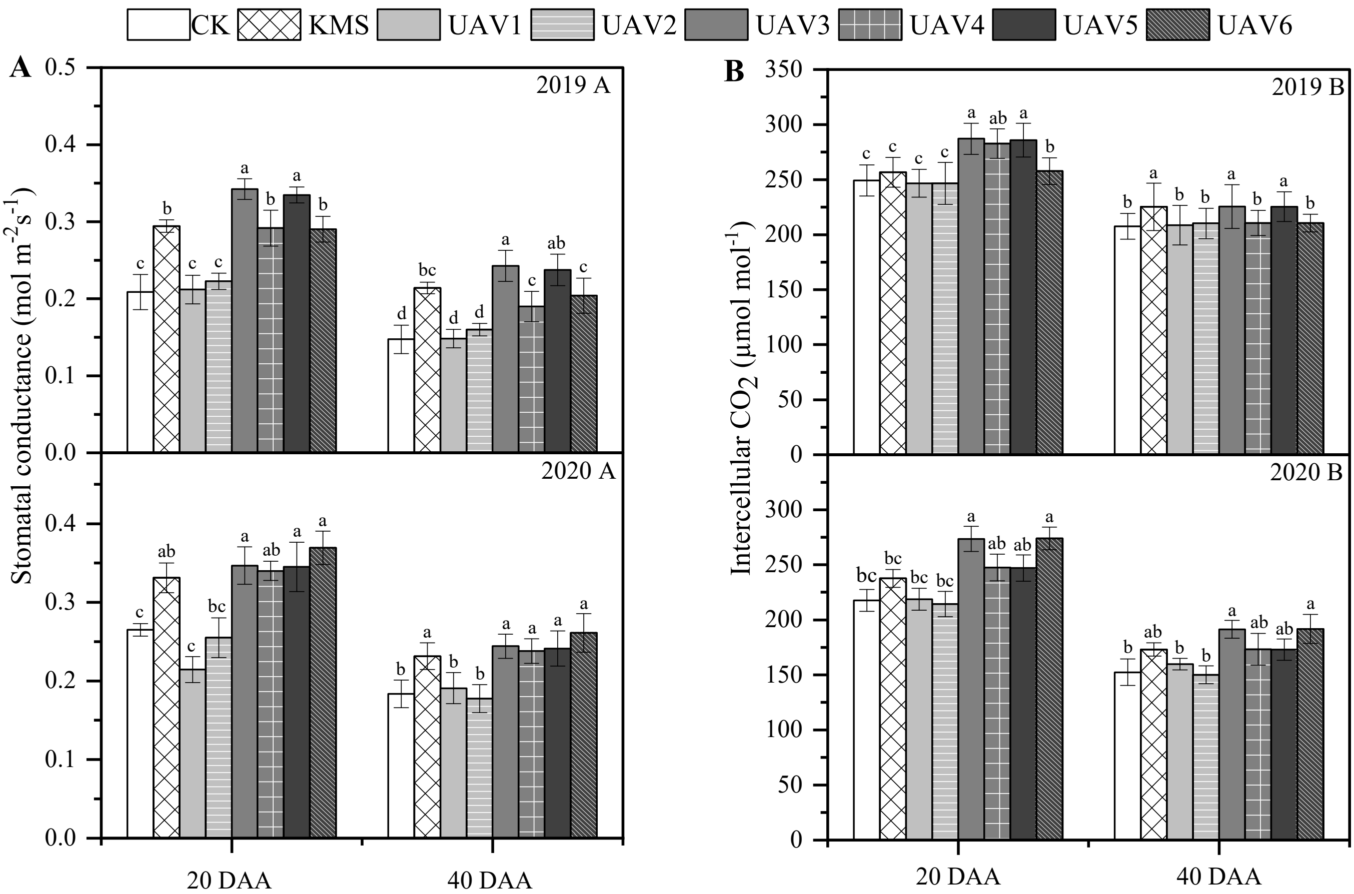

Figure 8. Effect of HBL and different SV on stomatal conductance (**A**) and intercellular CO_2 (**B**) in ear leaves of maize in 2019 and 2020. DAA: days after HBL application. One-way ANOVA was performed determined by LSD test at $p < 0.05$, Bars with different letters above the lines show significant differences in results among treatments.

Furthermore, the link between source and sink caused by feedback between sink activity and photosynthesis includes the changes of carbon assimilation during the grain filling period [31–33,52]. Increasing sink capacity could regulate carbon assimilation, and more carbohydrates could improve the grain filling and final grains yield. Previous studies revealed that optimum dosages of chemicals applied by UAV have better results in wheat yield [38] and rice yield [44] than KMS. Non-significant results were achieved when chemical dosage decreased by 20% in UAV compared to KMS [54]. In the present study, comparing the application of HBL by UAV and KMS, the results of final grain yield were the same when 18% HBL dosage was decreased, while, at same dosage of 22 mg a.i. ha^{-1} UAV have better results (Table 3). This is because downward air pressure by UAV rotors promote droplets to the lower canopy of the plant and increases the droplet deposition rate [44,50]. In this study, the effect of low dosage of HBL (18 mg a.i. ha^{-1}) with two SV was slightly less than the medium and high dosage of HBL. Medium (22 mg a.i. ha^{-1}) and high dosage (30 mg a.i. ha^{-1}) of HBL with UAV (15, 30 L ha^{-1}) performed better in kernel number per ear, TKW and final grains yield as compared to CK and KMS. In this experiment, it is concluded that applying an HBL dosage of 22 mg a.i. ha^{-1} with 15 L ha^{-1} SV can give better results than KMS. In the future, agriculture drones will replace KMS because of their high efficiency, security to farmer's health, environmental protection, wastage reduction, water saving efficiency, lower cost, a wide range of applications and less harm to crops. The difference of increased maize yield between two years was relatively less because weather adversity in two years was not serious, the increase in output of HBL will be more if weather adversity occurs at the reproductive stage in maize.

5. Conclusions

In this experiment, droplet deposition of UAV (15, 30 L ha^{-1}) was 47.04%, 8.89% higher than KMS. However, the UAV sprayer had a poor droplet coverage rate, droplet density, and droplet deposition uniformity. HBL significantly affected chlorophyll SPAD and photosynthesis parameters. This hampered the chlorophyll breakdown, maintaining the greenness of leaves, and prompted grain filling, grains number, grains weight, and final yield. HBL medium dosage (22 mg a.i. ha^{-1}) with spray volume 15 L ha^{-1} increased grains yield of 4.16–5.64% over KMS and 2.54–5.64% higher than CK in both years. While results of UAV and KMS were the same when the HBL dosage was reduced 18% compared to KMS. Considering the high efficiency of UAV and the better effects of HBL on final yield, applying an HBL dosage of 22~30 mg a.i. ha^{-1} by UAV spray volume of 15 L ha^{-1} could be a better strategy to spray on maize crop at silking stage to attain high yield.

Author Contributions: W.T. and M.H. conceived and designed the experiments; M.H., Z.W. and Y.M. performed the field experiment and data investigation; M.H., G.H. and Y.M. analyzed the data; M.H. and W.T. wrote the paper; Z.W., R.K., L.D. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The authors declare that data supporting the findings of this study are available on request from the corresponding author.

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