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Mitigating chilling stress of pepper plant (*Capsicum annuum* L.) using Dimethyl Ether combustion gas in controlled greenhouse

Jayanta Kumar Basak<sup>1,2</sup>, Waqas Qasim<sup>1</sup>, Frank Gyan Okyere<sup>1</sup>, Fawad Khan<sup>1</sup>, Yong Jin Lee<sup>1</sup>, Jihoon Park<sup>1</sup>, Hyeon Tae Kim<sup>1\*</sup>

<sup>1</sup>Department of Bio-systems Engineering, Gyeongsang National University (Institute of Agriculture & Life Science), Jinju 52828, Korea

<sup>2</sup>Department of Environmental Science and Disaster Management, Noakhali Science and Technology University, Noakhali-3814, Bangladesh

# \*Corresponding author: bioani@gnu.ac.kr

# Abstract

In this study, the possibility of enhancing chilling stress tolerance of pepper plant (*Capsicum annuum* Linnaeus) during early growth stages was investigated using DME combustion gas in controlled greenhouse. The experiment was conducted to determine the performance of DME combustion gas when used as a fuel for DME burner for raising temperature and  $CO_2$  concentration in greenhouse and also to examine its effects on morphology parameters of hot pepper in winter season. To analyze the relationship between the application of DME and morphology parameters of pepper plant, three treatments (DME-1, DME-2 and DME-3) for three controlled greenhouses were assayed. DME-1 and DME-2 treatments consisted of average DME flow quantity in duct were 17.4 m<sup>3</sup>/min and 10.2 m<sup>3</sup>/min, respectively to greenhouse-1 and greenhouse-2 and no DME gas was supplied to greenhouse-3 which was left as control (DME-3). Morphology parameters such as plant height, leaf area and leaf area index (LAI), net assimilation rate (NAR), relative growth rate (RGR), fresh weight and dry weight were measured for eight weeks for each treatment and analyzed using completely randomized designs through analysis of variance with a significance level of P < 0.05. Although DME-1, DME-2 and DME-3 received same crop management practices and controlled environmental factors, the highest changes (p < 0.05) of plant height, leaf area, LAI and fresh weight were found from the DME-1 treatment, followed by DME-2. A comparison of relative growth rates among the treatments indicated more rapid relative growth rate of morphology parameters at vegetative phase of plant implying better yield. Therefore endorsed quantify of DME combustion gas for a specified crop can be applied to greenhouse to improve the plant growth and enhance yield with mitigating chilling stress in winter season.

**Keywords:** DME, Greenhouse, Pepper, Morphology parameters, Vegetative phase. **Abbreviations:** DME\_ Dimethyl Ether; LAI\_ Leaf area index, NAR\_ Net assimilation rate; RGR\_ Relative growth rate.

## Introduction

The optimum temperature for growth of pepper plant is between  $21^{\circ}C$  and  $27^{\circ}C$ , with growth reduction occurring when temperature is below  $12^{\circ}C$  and above  $30^{\circ}C$ . The plants give the best yield and better seed set at the temperature range between  $21^{\circ}C$  to  $27^{\circ}C$  during the day time and  $15^{\circ}C$  to  $20^{\circ}C$  at night (Kahsay, 2017; Aleemullah et al., 2000; Lemma, 1998). Lack of optimum temperature during the critical growth stage of pepper plant may hamper yield (Reddy and Kakani, 2007). Exposure of plants to chilling temperatures during winter season may stunt plant growth, induce wilting, cause necrotic lesions on leaves, and increase susceptibility to diseases and pathogens. This study attempts to measure performance of DME gas to mitigate chilling stress of pepper plant by increasing temperature and  $CO_2$  concentration in controlled greenhouses. In Korea, pepper is usually cultivated in greenhouses during winter season due to the extreme cold temperature and to control other growth variables. Low temperature during winter is reported to be one of the most challenging factors that affect yield significantly (Hong et al., 2018; Schwartz et al., 2006; Wolfe et al., 2005; Xiao et al., 2008; Rosenzweig, 2002). For proper growth of pepper plant, it is essential to maintain warm and high humid climatic conditions, however at maturity stage, it requires dry weather. Like as temperature, different crops also exhibit differential response to  $CO_2$  (Hatfield et al., 2011). Many studies confirmed the significant contribution of  $CO_2$  to the enhancement of plant growth subsequently improving yield (e.g., Kimball and Mauney, 1993; Kimball et al., 2002; Ainsworth and Rogers, 2005; Kimball, 2010; Basak et al., 2010). Allen et al. (2003) mentioned that increasing the variability of different environmental factors such as precipitation, temperature etc. due to climate change, may offset the positive impacts of rising  $CO_2$  on plant growth.

With the growing concern of controlling two major environmental important factors, temperature and  $CO_2$  in greenhouse, DME gas used by a burner is emerging as an alternative fuel (Semelsberger et al., 2005). DME is produced from natural gas through the synthesis gas which is clean and considered to be economically an alternative fuel. Ogawa et al. (2003) found the similar properties of DME and liquefied petroleum (LP) gas. Nowadays, DME is used widely in various fields as a fuel such as power generation, transportation, home heating, cooking, etc. (Bhattacharya et al., 2013; Olah et al., 2009; Arcoumanis et al., 2008; Kim et al., 2008; Marchionna et al., 2008). In this study, DME gas was used as a fuel for DME burner to increase temperature and  $CO_2$  concentration in greenhouse for pepper cultivation.

Primarily synthesis gas is produced by transforming organic waste, natural gas and sometimes from biomass and then synthesis gas is converted into DME through following two-step synthesis reactions (EBTP, 2011; Yuan and Eden, 2016). DME gas also known as unpolluted fuel can be used for burning to produce heat in agricultural facilities. Moreover, it also provides sufficient  $CO_2$  without any high concentration of toxic gases.

 $2H_2 + CO \le CH_3OH (\Delta H^0 = -90.56 \text{ kJ mol}^{-1})$ 

2CH<sub>3</sub>OH <=> CH<sub>3</sub>OCH<sub>3</sub> + H<sub>2</sub>O ( $\Delta$ H<sup>0</sup> = -49.43 kJ mol<sup>-1</sup>) CO+H<sub>2</sub>O <=> CO<sub>2</sub>+H<sub>2</sub> ( $\Delta$ H<sup>0</sup> = -41.12 kJ mol<sup>-1</sup>)

To identify the optimal amount of DME combustion gas supply to greenhouse, quantification of complex cropclimate and phenology analysis is needed. The mismatch between what the amount of DME gas application and the crop can be cultivated in greenhouse could potential lead to inaccuracies when considering the beneficial impact of DME on morphology parameters of plant as a consequence of yield. Research has been conducted to evaluate the process and application of DME in different fields such transportation, power generation, cooking, room heating etc. (Zhao et al., 2011; Zhang et al., 2016; Liu et al., 2013; Sun et al., 2014). However, in agricultural field, very few number of researches have been conducted to evaluate the impacts of DME gas on crop yield, especially in greenhouse where crops in winter season face low temperature stress (Basak et al., 2018; Qasim et al., 2018). Qasim et al. (2018) reported that chlorophyll content of lettuce and Chinese cabbage plant increased up to 15% when DME gas was applied at a rate of 17.4 m<sup>3</sup>/min in greenhouse.

The dependence of crop production on temperature and  $CO_2$  concentration is precarious concern for increasing food production in limited resources as well as changing the climate variability. The objectives of this study were to (i) evaluate the changing pattern of temperature and  $CO_2$ 

concentration applying DME in a controlled greenhouse system; (ii) determine the impacts of DME gas on morphology parameters and soil moisture content and (iii) compare among the three different treatments (DME-1, DME-2 and DME-3) for their effects on plant, the study find out the appropriate rate of DME gas in greenhouse for pepper cultivation. The results of this study provide a guideline to pepper growers for getting optimum yield in winter season with mitigating chilling stress.

# **Results and Discussion**

# Temperature and CO<sub>2</sub> pattern

At the beginning of this experiment, it was examined the changing pattern of temperature and CO<sub>2</sub> concentration that occurred due to the different levels of DME gas application. It was observed that the higher levels of DME gas application accompanied a substantially increment in CO<sub>2</sub> concentrations which subsequently increased the temperature in a given period (Figure 4a). Average 24 hour data on temperature and CO<sub>2</sub> during the four months experimental period showed that CO<sub>2</sub> concentration increased up to 290% and 205% treated with DME at 17.4 m<sup>3</sup>/min and 10.2 m<sup>3</sup>/min respectively compared to control condition in each morning between time intervals at 6 to 8 o'clock. Due to the increase the level of CO<sub>2</sub> concentration in greenhouses, temperature increased up to 5.5°C and  $3.5^{\circ}$ C in greenhouse 1 and 2 respectively in the same time. Qasim et al. (2018) found a close relationship between CO<sub>2</sub> concentration, temperature and DME gas application. The study reported that CO<sub>2</sub> concentration and temperature sharply increased as DME burner was operated at a rate of 17 m<sup>3</sup>/min and 10 m<sup>3</sup>/min in greenhouses. As was measured in the study, the raises of temperature occurred due to high concentration CO<sub>2</sub>, was closely related to apply DME combustion gas. Shakun et al. (2012) reported that there is a close link between CO<sub>2</sub> concentration and temperature changes. In greenhouse-3, CO<sub>2</sub> did not change extensively with time, however due to the presence of sunlight, temperature changed in day time (Figure 4b). On the basis of these results, the effects of the variability of CO<sub>2</sub> and temperature on plant morphology were examined and analyzed.

# Plant height

The evaluation of plant height, root and shoot length with time under different application rate of DME (DME-1, DME-2 and DME-3) in the experimental period is presented in Figure 5a-c. Plant height was significantly different among the three treatments with time. Compared to the other two treatments, plant height for DME-1 was maximum. Mean ( $\pm$ SD) plants height (mm) at vegetative phase of pepper plant in the 8<sup>th</sup> week of the experiment were 272 $\pm$ 3.83, 264 $\pm$ 5.19 and 255 $\pm$ 7.64 for DME-1, DME-2 and DME-3 respectively. The differences in plant height for DME-1, DME-2 and DME-3 were statically significant with p < 0.05 (Figure 6). Moreover, due to growing time, plant height increased at a substantial rate in one week to another week

Table 1. Agronomy practices of pepper plant in three greenhouses during the experimental period.

Parameter	Practices			
Plantation area	Greenhouse			
Planting method	Seedling			
Date of seedling	2 February, 2018			
Plant population per pot	1			
Row spacing at seedling stage	3 cm			
Seedling depth	1 cm			
Transplant of seedling	No			
Fertilizer (N-P-K) application ratio				
<ul> <li>25 days after germination</li> </ul>	5-5-5			
<ul> <li>45 days after germination</li> </ul>	5-10-10			
Application of irrigation	5 min/day by sprinkler irrigation system			
Application of pesticides	No			
Application of herbicides	No			
N: Nitrogen; P: phosphorus; K: potassium.				



Fig 1. Schematic diagram of Greenhouse. Greenhouses were equipped with automatic controlled system recording ambient parameters.

Table 2. The specification of the DME gas burner.

Items	Specification		
Quantity of energy (kcal / h)	40000		
Rotational frequency of motor (rpm)	1495		
Output of motor (W)	200		
Quantity of flow (m <sup>3</sup> / min, CFM)	1060		
Diameter of outlet (mm)	400		
Length of burner (mm)	1360		



Fig 2. Experimental design of DME supply to greenhouses. DME gas was supplied to greenhouse-1 and greenhouse-2 and no DME gas was supplied to greenhouse-3 which was left as control.

<b>Table 3.</b> Concentration of gases after compussion of DIVIE ga
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Table 5. concentration of gases after combustion of DME gas.				
Gases (unit)	Amount			
CO <sub>2</sub> µmol/mol (ppm)	3316.1			
H <sub>2</sub> μmol/mol (ppm)	0.7			
CO µmol/mol (ppm)	11.6			
CH <sub>4</sub> µmol/mol (ppm)	1.7			
O <sub>2</sub> cmol/mol (%)	21.3			
N <sub>2</sub> cmol/mol (%)	77 8			

\*Samples were collected in front of DME burner.



**Fig 3.** Images of DME supply to greenhouses. DME gas was burned using DME burner and the combustion gas was supplied through connected polyvinyl chloride (PVC) pipe between DME burner and greenhouse-1 and greenhouse-2.

**Table 4.** Pairwise comparison of DME application and changes of morphology parameters between and among the treatments in three greenhouses in the 8<sup>th</sup> week data. Experimental data were tested with a post-hoc Tukey's HSD.

Parameters	Group	df	Mean Square	F-value	P-value
Shoot length	Between groups	2	423.61	32.25	0.000
	Within groups	57	13.132		
Root length	Between groups	2	334.71	24.264	0.000
	Within groups	57	13.795		
Plant height	Between groups	2	1506.05	45.193	0.000
	Within groups	57	33.325		
Fresh weight	Between groups	2	0.070	10.625	0.000
	Within groups	57	0.007		
Dry weight	Between groups	2	0.044	33.585	0.000
	Within groups	57	0.001		
Leaf area	Between groups	2	6.248	11.405	0.000
	Within groups	57	0.548		
LAI	Between groups	2	0.024	11.405	0.000
	Within groups	57	0.002		
RGR	Between groups	2	0.000	0.627	0.538
	Within groups	57	0.000		
NAR	Between groups	2	0.000	1.374	0.261
	Within groups	57	0.000		
SMC	Between groups	2	3.821	1.139	0.327
	Within groups	57	3.356		

\*The mean difference is significant at the 0.05 level.



**Fig 4.** Changing pattern of  $CO_2$  concentration and temperature. The data of the figures show the mean values of the two parameters during experimental period in three greenhouses.



**Fig 5.** Trend of changes of plant height, root length, shoot length and leaf area index in each week in different treatments of DME of three greenhouses. Values were represented as mean ± SD.



**Fig 6.** Mean (±SD) number of plant height, root length and shoot length in the 8<sup>th</sup> week in three treatments of DME in three greenhouses are presented (n=20). Different asterisks above the error bars denote significant differences of plant height, root length and shoot among treatments at p < 0.05 based on Tukey's HSD post-hoc test (\*\*\*: p < 0.001 as compared with control and #: p < 0.05 and ###: p < 0.001 as compared with DME-2).



**Fig 7.** Mean (±SD) number of leaf area and leaf area index in the 8<sup>th</sup> week in three treatments of DME in three greenhouses are presented (n=20). Different asterisks above the error bars denote significant differences of leaf area and leaf area index among treatments at p < 0.05 based on Tukey's HSD post-hoc test (\*: p < 0.05; \*\*: p < 0.01 and \*\*\*: p < 0.001 as compared with control and NS: not significant).



Fig 8. Trend of changes of fresh weight, dry weight, RGR and NAR of pepper in each week in three treatments of DME of all three greenhouses. Values were represented as mean ± SD.



**Fig 9.** Mean (±SD) number of fresh and dry weight in 8 weeks in three treatments of DME in three greenhouses are presented (n=20). Different asterisks above the error bars denote significant differences of leaf area and leaf area index among treatments at p < 0.05 based on Tukey's HSD post-hoc test (\*: p < 0.05; \*\*: p < 0.01 and \*\*\*: p < 0.001 as compared with control and ##: p < 0.01 as compared with DME-2 and NS: not significant).

in all treatments. The root and shoot length of plants which directly related to plant height were also examined. The current experimental findings show that the effect of DME on the length of root and shoot did not keep up with the same rate within this period. With a DME gas application of 17.4 m<sup>3</sup>/min in greenhouse-1 and 10.2 m<sup>3</sup>/min in greenhouse-2, shoot increased up to 10.12% and 4.3%, and root length increased up to 5.84% and 3.4% respectively compared to the control greenhouse in eight week. Likewise plant height, shoot and root length were increased along with time. The current experimental findings are in agreement with the results of Jang et al. (2013) and Lia et al. (2000). Sharangi and Kumar (2011) reported that plant height of pepper plant was correlated significantly with growing period and nutrient schedule.

## Leaf area and Leaf area index

The different rates of DME application with growing period significantly affected the leaf area and LAIs. Leaf area and LAIs values were 2.52 cm<sup>2</sup> and 0.16 in the 1<sup>st</sup> week which later increased to 17.75 cm<sup>2</sup> and 1.11 respectively in the 8<sup>th</sup> week for DME-1 treatment (Figure 5d). For DME-2 treatments, leaf areas and LAIs values increased gradually 2.45 cm<sup>2</sup> and 0.155 in the 1<sup>st</sup> week to 17.24 cm<sup>2</sup> and 1.08 in the 8<sup>th</sup> week respectively. Leaf areas and LAIs for DME-3 treatment were 2.44 cm<sup>2</sup> and 0.15 to 15.05 cm<sup>2</sup> and 1.04 respectively from 1<sup>st</sup> week and 8<sup>th</sup> week. Thus higher leaf area and LAIs were obtained for DME-1 and DME-2, however the minimum leaf area and LAIs was observed for DME-3 in each week. Statistical analysis of the experimental data shows that the leaf area and LAIs significantly increased (p < 0.05) with application of DME gas (Figure 7).

However the changes of leaf area and LAIs were not statistically significant (p > 0.05) between DME-1 and DME-2. In this study, it was also observed that leaf area increased from 2.56% to 6.71% due to application DME at a rate of 17.4 m<sup>3</sup>/min and 1.17% to 3.61% for 10.2 m<sup>3</sup>/min compared to DME-3 during the experimental period. Another experiment was conducted by Sezen et al. in 2014 for evolution of the LAIs with time under drip and furrow irrigations. The result of the study showed that the highest LAIs of 3.0 and 3.7 were observed in drip treatments consisted of full irrigation at the late flowering stage of pepper plant in 2010 and 2011 respectively. Moreno et al. (2003) reported a maximum LAIs of pepper plant was 3.4 in full irrigation and 2.7 in deficit irrigation in Spain.

## Fresh and dry weight of plant

Fresh and dry weight of pepper is extremely important at vegetative phase of plant due to their influencing control on yield. Fresh and dry weights of plant during the experimental period for each DME treatment with time were shown in Figure 8a-b. DME application levels had a significant (p < 0.05) effect on fresh weight for DME-1. However the differences were not statistically significant (p > 0.05) between DME-1 and DME-2 (Figure 9). The mean values of fresh weight of a plant were recorded 3.80 gm, 3.75 gm and 3.69 gm for DME-1, DME-2 and DME-3 respectively at the end of 8<sup>th</sup> week. For high yields, adequate fresh weight of plant is essential during the growing period. Reduction of fresh weight during the vegetative stage in general has an adverse effect on yield. Moreover in this study it was also found high significant change of dry weight due to DME application in different

treatments (p < 0.05) as well as growing period. The mean values of dry weight were obtained 0.571 gm, 0.532 gm and 0.478 gm for DME-1, DME-2 and DME-3 respectively. Rahman and Inden (2012) also reported that plant dry weights of sweet pepper significantly varied by nigari (an effluent of salt industries) rate, cultivars and their interactions. Haghighi and Barzegar (2017) studied the effect of amino acid and mycorrhiza inoculation on sweet pepper growth under greenhouse conditions and found that mycorrhiza inoculation and mixture of amino acid increased shoot and root fresh weights.

## Relative growth rate (RGR) and net assimilation rate (NAR)

Plant growth is of paramount ecological importance, as their survival, reproduction and competitive interactions depend on individual size (Li et al., 2016). Figure 8 (c-d) shows the changes of RGR and NAR for different treatments of DME during the experimental period. RGR and NAR values were not significantly influenced by DME application (p > 0.05). RGR values ranged from 0.022 gm/plant/day to 0.143 gm/plant/day for the application of 17.4 m<sup>3</sup>/min DME gas in a day. Moreover RGR values varied from 0.0197 gm/plant/day to 0.139 gm/plant/day resulted in the application of DME gas at a rate of 10.2 m<sup>3</sup>/min and it was from 0.0155 gm/plant/day to 0.13 gm/plant/day for DME-3. Experimental result also shows that the value of RGR was the maximum level in initial stage of the vegetative phase of pepper plant. Likewise RGR, NAR values were not significantly (p > 0.05) changed by the different treatments of DME. In this experiment it was examined that NAR values ranged from 0.00067 to 0.0023  $gmcm^{-2}day^{-1}$  in DME-1, 0.0004 to 0.0023 gmcm<sup>-2</sup>day<sup>-1</sup> in DME-2 and 0.00052 to  $0.0024 \text{ gmcm}^{-2} \text{day}^{-1}$  in DME-3 (Figure 8c and 8d).

### Soil moisture content

Water management of pepper is extremely important at all stages of plant growth due to it has a significantly different effect on morphology parameters. For high yields, an adequate water supply is required during the growing period. Therefore, soil water should be maintained between 65% and 80% of filed capacity (Jones et al., 2000). In this experiment, moisture content of soil was measured using gravimetric wet method at 80°C in 48 hour. To maintain the same level of soil moisture content in all of the three greenhouses, water was applied to the plant at a rate of 5 min/day by sprinkler irrigation during the whole experimental. It was observed that moisture content of soil were not influenced (p > 0.05) by DME application. Moisture content ranged from 76.5% to 81.78% in greenhouse-1; 76.6 to 81.97% in greenhouse-2 and 76.6% to 81.28% in greenhouse-3. The experimental results indicated that moisture content of soil might not be affected on plant growth in different greenhouses due to the same level of water application making moisture contents of soil in all pots were similar during the period.

### **Materials and Methods**

#### **Experimental site**

The experiment was conducted with and without DME at Gyeongsang National University from 15 January to 15 April, 2018. Three greenhouses with same dimensions of width 3m, length 4m, height 2.5m were used. The greenhouses were equipped with automatic control system recording ambient parameters such as temperature, humidity and CO<sub>2</sub>.

### Experimental design

In this experiment, three treatments for the three greenhouses were assayed. Greenhouse 1 and 2 were supplied with DME gas at a given rate and labeled as DME-1 and DME-2 respectively. Greenhouse 3 was used as the control where no DME gas was supplied and it was labeled as DME-3. For DME-1 and 2, DME gas was supplied through a duct at a rate of 17.4 m<sup>3</sup>/min and 10.2 m<sup>3</sup>/min respectively for 1 hour per day in every morning at 6 o'clock (Figure 2). For this process, DME gas was burned using DME burner and the combustion gas was supplied through connected polyvinyl chloride (PVC) to greenhouse-1 and 2 (Figure 3). According to the treatments, gas was controlled by a gas regulator (C835-New Industrial VIPR, Rotarex Headquarters, 24 rue de Diekirch, L-7440 Lintgen, Luxembourg) of the two greenhouses. In all 3 greenhouses there were 2 beds containing pepper seeds. The windows of greenhouse for fresh air were opened daily from 10 am to 6 pm for keeping optimum temperature in the greenhouses. There were a total of 360 pepper plants in each of the three greenhouse.

### Agronomy practices

Seedlings of *Capsicum annuum* Linnaeus, a variety of pepper widely used in South Korea, were gently sowed in same size of pot on 2 February, 2018. In most ways, and perhaps in virtually, all crop management practices were provided to all the plants in each greenhouse at a same time in a fixed rate. Every seed was sowed into a single pot then moved them to the greenhouse after germination in seed-starting trays. Fertilizer and irrigation applications were based on crop growing stages and soil nutrients, however, all treatments received same amount. In each of the growing stage of pepper plant, different doses of fertilizer were applied. Water was provided to plant 5 min/day (1.2 liter per day for 144 plants) by sprinkler irrigation system during this experimental period. Agronomy practices for pepper plant are given in Table 1.

#### DME properties

With growing concerns of environmental pollution using fossils fuel, Dimethyl Ether (DME) gas is emerged as one alternative fuel to minimize this menace (Semelsberger et al., 2005). DME can be produced from coal, biomass and natural gas. The physical properties of DME and LPG are almost similar due to this DME has been specifically identified as a substitute fuel for LPG (Arya et al., 2016). DME gas is known as clean fuel by burning it in agricultural facility, it will not only provide heating but also provide sufficient  $CO_2$  without any high concentration of toxic gases (Qasim et al., 2018).

The specification of DME burner is described in Table 2. For testing, the concentration of gases, samples was collected in air plastic bags of 20 I during operation of DME combustion. Samples were taken to Korean Testing laboratory on the same day and ISO 6974-6 standard method was employed for determination of gases concentration. The testing environment were  $20.0\pm5^{\circ}C$  temperature and  $50\pm20\%$  relative humidity. Table 3 shows the concentration of gases during the combustion of DME.

### Data collection and analysis

The plants were periodically examined after the germination stage to observe the changing pattern of morphology parameters due to DME treatment. Twenty plants from each greenhouse were collected randomly after 20 days of germination. The root and shoot length, leaf area and LAI and moisture content of soil for each treatment were measured each week. Root and shoot length of each sampled plant were measured using metric ruler, while the fresh and dry weight of the plant was estimated using digital balance (model-FX-300iWP, A&D Company Limited, Tokyo, Japan) and drying oven (Shelves for 5E-DHG6310: 2 Layers, Changsha Kaiyuan Instruments Co., Ltd, Changsha 410100, P. R., China). Number of researches were followed to get the standard temperature range and time for measuring the dry weight of plant (e.g., Arshadullah and Zaidi, 2007; Cho et al., 2007; Karimi et al., 2009). In this experiment, where different treatments and weekly sampled plants were studied, dry weight of plant was measured at a temperature at 80°C for 24 hour and for soil moisture content, it was maintained 80°C in 48 hour. To estimate the leaf area that was essential for determining the LAI, was measured with the aid of a Leaf Area Meter (Model Portable Laser CI-202, CID Bio-science, 1554 NE 3rd Avenue Camas, WA United States of America) (Dalorima et al., 2018). LAI, RGR and NAR were calculated from leaf area, dry weight plant within a fixed time interval using equations from Breda (2003) and Pope and Treitz (2013), Pommerening and Muszta (2016) and Hoffmann and Poorter (2002). Standard statistical methods were used for data evaluation including analysis of variance to practice with a significance level of P < 0.05. The significance differences between mean values of experimental data were tested with a posthoc Tukey's HSD test in the eight week data. All statistical calculations were performed with Statistix10 (Analytical Software, 2105 Miller Landing Rd, Tallahassee FL 32317, United States of America) and Statistical Package for the Social Sciences (SPSS Version: 22.0.0.0, IBM SPSS Statistics 22.0.0.0, New York, United States of America). Moreover, the daily temperature and CO2 concentrations were measured using sensors (Lutron MCH-383SD, Electro Chemical Engineering, Melbourne, Australia) located in

three different places with fixed three distinct heights in each greenhouse. Lutron MCH-383SD electrochemical sensors were set at three different height of greenhouses to record the date, time interval and it stores this data in its memory. Data were checked at 10-min intervals and recorded data were averaged for further analysis and interpretation.

LAI=(Area of leaf coverage per plant)/(Area of soil covered per plant)

RGR=(InW2-InW1)/(t2-t1)

Where,

W2=Weight of dry matter (g) at time t2

W1=Weight of dry matter (g) at time t1

t2-t1=The time interval in days

NAR=(W2-W1)/(t2-t1)×((InA2-InA1)/(A2-A1))

Where,

A1 and A2 are the leaf areas recorded in  $cm^2$  and W1 and W2 are the total dry matter in gram at time t1 and t2

# Conclusion

The current study demonstrates the effects of DME on plant height, leaf area and LAI, RGR, NAR, fresh weight and dry weight. All these parameters are significantly important in order to obtain higher yield of pepper plant under the cold climatic conditions. The application rate of DME had significant effect on plant height, root and shoot length, leaf area and LAI, fresh weight and dry weight of the plants. A maximum plant height of 27.2 cm in greenhouse-1 was obtained from DME-1 (17.4 m<sup>3</sup>/min) treatment. Moreover, DME treatments resulted in greater leaf area and LAI compared to control greenhouse (DME-3). However, RGR and NAR did not find any statically significant change for DME application. It is suggested that high rate of DME application did not greatly affect with RGR and NAR of pepper plant. Moreover, significant linear relationships were found between the fresh weight and dry weight of plant for each DME treatment. The strong correlation between fresh and dry weight with DME application is an indication of potential suitability for winter season crops in greenhouse and can thus be integrated into crop model. The experimental result also revealed that there was no association between the soil moisture content and DME application. Values of soil moisture content in pots of each greenhouse were almost same in every week during the experimental period. To improve the yield, the response of DME application to morphology parameters, is important in developing new decision making to be used by farmers and researchers for applying DME under extreme cold condition in greenhouse. Moreover, this study creates a scope for further experiment using DME gas burner in greenhouses to measure growth performance of different crops. In addition, it is essential to quantify the accurate rate of DME combustion gas for a specified crop, which can be helpful to improve the plant growth and enhance yield.

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