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Original Contribution

THE EFFECT OF TRIFLURALIN AND COLCHICINE TREATMENTS ON MORPHOLOGICAL CHARACTERISTICS OF JIMSONWEED (*DATURA STRAMONIUM* L.)

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ABSTRACT

Datura is a pharmaceutical plant with a wide range of properties. Polyploidization sometimes causes large-scale genomic reorganizations and is accompanied by a wide variety of phenotypic alterations in vegetative and morphological characteristics. Several polyploid datura plants were developed by trifluralin and colchicine treatments and were compared with their diploid counterpart for vegetative and morphological characteristics. There were doses related effects of the ploidy treatments on quantitative traits resulting in reductions in traits such as plant height, number of leaves per branch, but increases in number of branches, number leaves per plant, leaf dry weight and chlorophyll content. This finding demonstrates the existence of genetic variation for the morphological response to ploidy change in *Datura sramonium L*.

Key words: *Datura stramonium* L., ploidy, colchicine, trifluralin, morphological traits, SPAD value, correlation.

INTRODUCTION

The genus Datura produces a great range of tropane alkaloids (1). In which two of them as called: hyoscyamine and scopolamine, are important for pharmaceutical industry (2). An increased secondary metabolite production is correlated with a low cell division rate in cell suspension cultures (3). Similarly, secondary metabolite production at the stationary phase of growth has been related with tissue organization (4), morphological differentiation (5), and low growth rates (3).

Polyploidy agents such as colchicine and oryzaline and trifluralin often are used to modify the plant shape and to restorer fertility which is valuable tools to get bigger flowers.

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These chemicals were successfully applied in the past decade in many ornamental crops (6) to obtain the new genotypes with variants in size and/or color of flowers and leaves (7). The use of colchicine on shoot meristems has been the primary method of somatic polyploidization in roses (8-10). Colchicine has been widely used for polyploidization in plants since it was first reported in the 1930s to have this property (11). It works by disrupting the polymerization of microtubules which, in turn, disrupts spindle fiber development and mitosis. Cells arrested at metaphase may recover and enter the next mitotic cycle with twice as many chromosomes (12). besides colchicine, some herbicides such as trifluralin, have been shown to have greater affinity for binding to plant microtubules than animal microtubules (13). Compared to colchicine, the efficiency of such herbicides in polyploidization of plants has been variable but promising (12). The amount of chlorophyll produced per gram leaf tissue is affected by environmental conditions and genetic composition of the plant as well (14). As a

matter of fact, in polyploid plants, the photosynthetic rate per cell is correlated with the amount of DNA per cell. Also, the photosynthetic rate per unit leaf area is related to the number of photosynthetic cells per unit area (15).

Number of chloroplasts per guard cell pair, ovary diameter, petal and anther diameter, and leaf length by width ratio are good indicators of plant ploidy characterization (16). Polyploidy can confer individual characteristics, for example, increased quality (17), hardiness (18) and disease resistance (19).

For medicinal plants, polyploids are usually more valuable because they exhibit increased biomass and content of effective compounds (20). In this study we examined the effects of some of ploidy inducer agents such as colchicine and trifluralin on germinated seeds of jimsonweed (*Datura stramonium* L.) to investigate the changes in chlorophyll content and morphological characters in developed plant and on, for example morphological changes in plant height, the number of leaf per plants, number of branches per plant and etc.

MATERIAL AND METHODS

The seeds of Datura Stramonium L. were soaked in water and incubated at 4°c in refrigerator for three days to stimulate seed germination. Emergenced seeds with about 2mm root were placed in ploidy induction media. Two experiments were performed to ploidy induction: 1) trifluralin in four levels (0, 7.5,15 and 22.5) and two time scales (24 and 48). 2) Colchicine in four levels (0, 0.125, 0.25 and 0.5g/l) at two time scales (24 and 48). Both of them were solved in DMSO solution (1%). These experiments were performed by factorial arrangement based on completed randomized design with five replications. 60 germinated seeds was allocated for each treatment (12 seeds per replication) after 24 or 48 the seeds were extracted from ploidy inducing solution and washed with tap water for about 1 h, and then cultured in greenhouse after 45 days formed shoots were harvested, obtain data was recorded for below traits: plant height (H), the number of branches per plant (No.Br), number of leaves per plant (No.Pl), number of leaves per branch (No. LeBr) and leaf dry weight (LDW). Another analysis was carried out using polyploidy derivatives of datura plants. We investigated all possible pair-wise (character pair) correlation coefficients to discover of the relationships among the six characters measured for two ploidy inducer agents; colchicine and trifluralin. Since the leaves of Datura are an important source of tropane alkaloids (21) we decided to investigate the correlation of leaf dry weight and the other traits.

Also chlorophyll content was measured by a chlorophyll meter (Single-Pass Albumin Dialysis, SPAD) (22, 23 and 24). To measurement of leaf dry weight, plant materials were placing in oven at 75°C for 2 days.

Data were collected and analyzed by SAS software based on Duncan multi ranged test. Obtained results for each trait were separately analyzed based on complete randomized design by factorial arrangement and the effect of different concentrations and incubation times of ploidy inducers were studied. Correlation coefficient between LDW and other traits and correlation coefficient among all the traits were determined and the best equilibrium of regression was figured based on different times and concentrations of ploidy inducers.

RESULT AND DISSCUSSION

1- MORPHOLOGICAL CHARACTERISTICS

1-1. The effects of different incubation times and concentrations of trifluralin

1-1-1. Variance analysis

The effect of trifluralin on plant height at level 5% and on the traits of the number of branches per plant (H), number of leaves per branch (No.Br), number of leaves per plant (No. LePl) and leaf dry weight (LDW) at level 1% was significant. Time durations (24 or 48 h) had significant effects at level 1% on different traits such as the number of branches per plant, number of leaves per branch, number of leaves per plant and leaf dry weight but had no significant effect on plant height. Although time durations did not effect on height, reciprocal interaction of time and applied trifluralin levels was significant for this trait at level 1%. Also the reciprocal interaction between trifluralin and times was significant at level 1% for the other traits **(Table 1).** Besides variance analysis, means comparisons were performed to determinate the effects of different concentrations and incubation times of trifluralin on mentioned traits, which explained bellow:

Table 1*. Results of variance analysis of trifluralin and incubation time on characteristical traits.*

S.O.V	Df.	Mean Square				
		Plant Height (cm)	No. Branches per Plant	No. Leaves per Branch	No. Leaves per Plant	Leaf dry weight(g)
Tri	3	168.483*	5.547**	39.371**	582.780**	29.531**
T	1	43.375 ^{ns}	10.667**	168.704**	700.002**	36.531**
Tri*T	3	385.281**	2.537**	21.383**	92.363**	4.143**

Tri= trifluralin; T= incubation times, the symbols ns, *, ** are non significant, significant at level 5 % and significant at level 1 % based on Duncan's multiple range test, respectively.

Height

Mean comparison based on Duncan multi range test showed that the maximum effect of trifluralin on height was belongs to control treatment while trifluralin at level 22.5µM and 48h has the minimum effect on plant height. Also there was significant difference between control and the other treatments. There were significant differences among applied treatments at level 1% for height, as well. Generally, incubation time of 24h was taller than 48h. The average height of plants is decreased by increasing of trifluralin concentration. With neglect of incubation time, average height decreased with increasing of trifluralin concentration **(Fig. 1a)**.

Number of branches per plant

The maximum number of branches was observed in the treatment containing 15 µM trifluralin and time 48h and then in the treatment containing 22.5 µM and time 48h. The minimum number of branches was related to the control. Also, the treatments of 15µM/24h and 7.5µM/24h were classified in the same statistical group. Generally, the plants exposed to the trifluralin treatments were significantly different with control **(Fig.1b)**.

Number of leaves per branch

The maximum leaf production per branch were observed in the treatment 7.5 µM/24h and then in control. The minimum leaf production per branch was observed in the treatment 22.5 µM/48h, as well. Significant differences were observed among the treatments. Generally, leaf production was better in time duration of 24h with neglect of applied concentrations **(Fig.1c)**

Number of leaves per plant

After comparing the means of treatments based on Duncan's multi range test at level 1%, it observed that the treatment 22.5 µM/48 and then the treatment 15 µM/48h produced the maximum leaves per plant. The minimum leaf production was belongs to the control. There were no significant differences between 22.5µM/24h and 15µM/24h of trifluralin **(Fig. 1d)**.

Leaf dry weight

Based on Duncan multi range test (at level 1%) the maximum leaf dry weight (g) were belong to the treatment 22.5 µM/48h and then the treatment 15 µM/48h. the minimum dry weight was belongs to the control, as well. Leaf dry weight (g) was better in time period of 48h than period of 24h with neglect of applied concentrations. There were significant differences between control and the other treatments and also among the treatments **(Fig. 1e)**.

1-1-2. Relationship of Morphological Characteristics and Different incubation Times and Concentrations of Trifluralin

Plants height was linearly decreased by enhancement trifluralin concentration or incubation time. There was medium-negative correlation between plants height and trifluralin concentration (R2≈0.62). The slope of regression line based on applied different trifluralin was -2.523, and the regression equation was: y = -2.5236 x + 49.763 **(Fig. 2a and Table 2)**. There was rising linearly relation between the number of branches and different times and trifluralin concentrations. Also, There was significantly low- positive correlation between them (R2=0.4). For this trait, the slop of regression line was 0.37. The regression equation was: y = 0.375 x + 5.0411 **(Fig. 2b and Table 2)**.

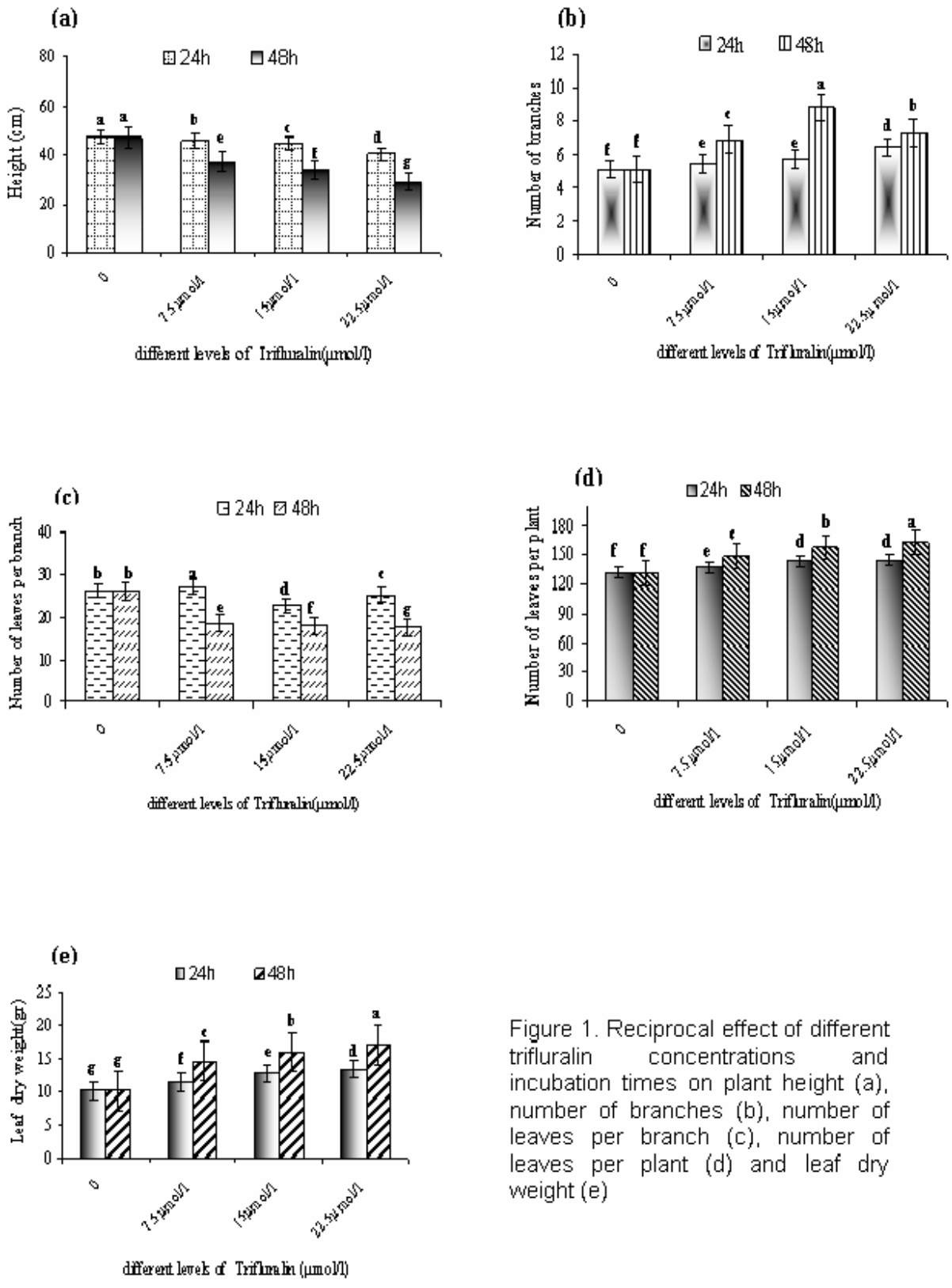

Figure 1. Reciprocal effect of different trifluralin concentrations and incubation times on plant height (a), number of branches (b), number of leaves per branch (c), number of leaves per plant (d) and leaf dry weight (e)

The number of leaves per branch had low-negative relation with different times and trifluralin concentrations (R2=0.33). Obtained regression was linearly. The slop of regression line in this trait was -1.108. The drawn regression line was decreased with different times and concentrations of trifluralin. The number of leaves per branch was negatively related with different times and concentrations of trifluralin. For this trait, the regression equation was: y = -1.1082 x + 26.599 **(Fig. 2c and Table 2)**.

Between the number of leaves per plant and different times and concentrations of trifluralin, there was rising linearly relation. This correlation was strong (R2=0.68). The slope of regression line for the number of leaves per plant and different concentrations of trifluralin was 4.14**(Fig. 2d).**the regression equation was: y = 4.1393x + 130.1 **(Table 2**), as well.

The relationship between leaf dry weight and applied trifluralin concentrations was linearly increased. Leaves dry weight was significantly strong correlated with different incubation times and levels of trifluralin (R2=0.68) **(Fig. 2e)**. The slope of regression line of leaves dry weight and applied trifluralin concentrations was 0.928. The regression equation was: y = 0.9286 x + 9.86, as well **(Table 2)**.

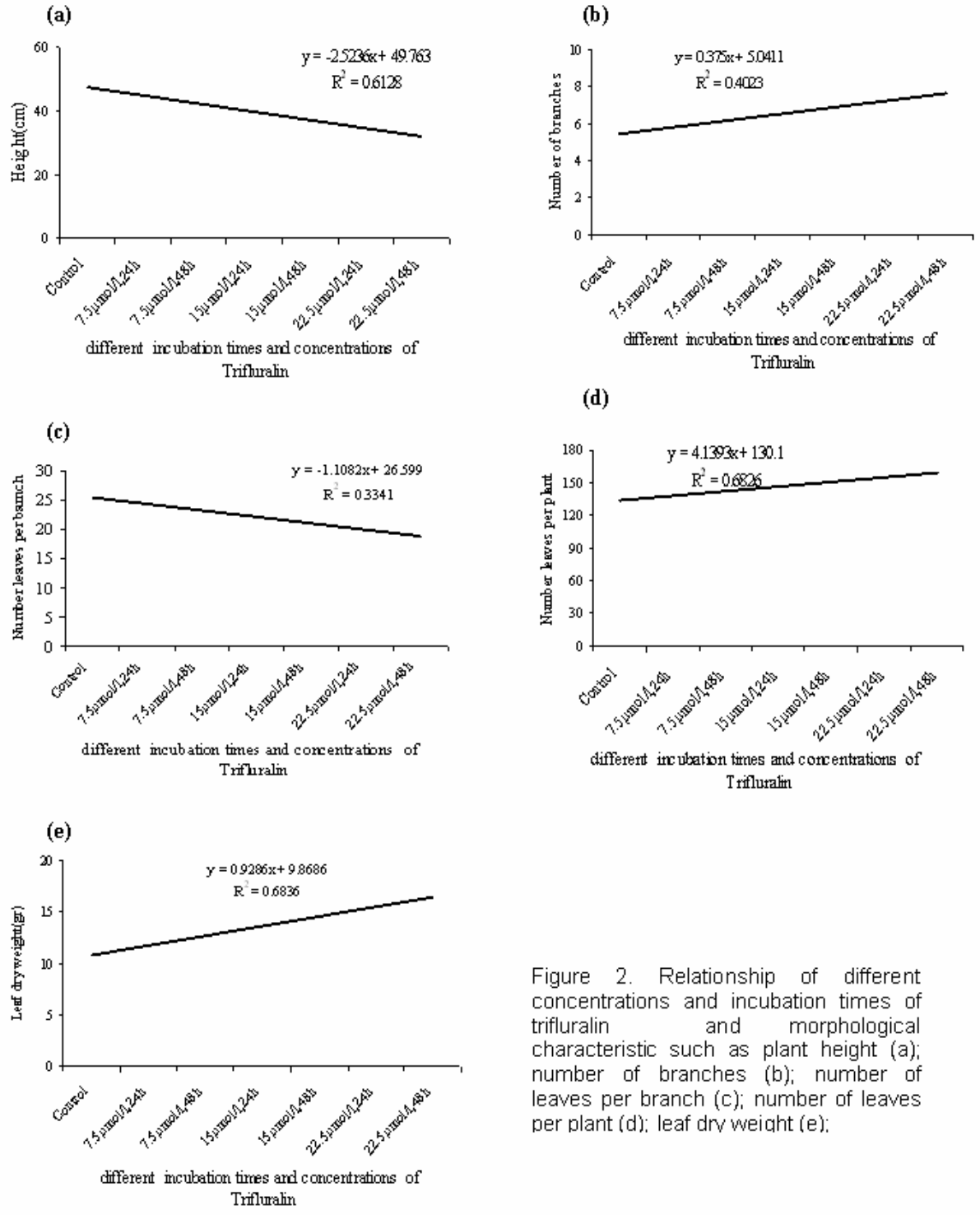

Figure 2. Relationship of different concentrations and incubation times of trifluralin and morphological characteristic such as plant height (a); number of branches (b); number of leaves per branch (c); number of leaves per plant (d); leaf dry weight (e);

Table 2. *Linear regression between traits and different incubation times and concentrations of trifluralin*

traits	Regression equation	R2
H (cm)	y = -2.5236x + 49.763	0.62
No. Br	y = 0.375x + 5.0411	0.4
No. LeBr	y = -1.1082x + 26.599	0.33
No. LePl	y = 4.1393x + 130.1	0.68
LDW (g)	y = 0.9286x + 9.86	0.68

The symbols ns, *, ** are non significant, significant at level 5 % and significant at level 1 % based on Duncan's multiple range test

1.1.3-Correlation analysis

Many of the characters were highly correlated with each other, demonstrated by the fact that 11 of the 15 correlation coefficients were 0.5 or higher. There were positive correlations between leave dry weight (LDW) and below trait; Plant height and the number of leaves per plant at level 1%, and with number of leaves per branch at level 5%. There was no correlation between LDW and the number of branches per plant **(Table 3)**. By neglect of positive or negative status, LDW had the maximum and the minimum correlations with NoLePl and No.Br, respectively. Therefore, LDW increases by increment in plant height and the number of leaves per plant.

Table 3.*Correlation coefficient between leaf dry weight (LDW) and other traits in trifluralin different treatments.*

Traits	H	No. Br	No. LePl	No. LeBr	LDW (g)
H	1				
No.Br	0.548*	1			
No.LePl	0.689**	0.803**	1		
No.LeBr	-0.037	0.426*	0.823**	1	
LDW	**0.634****	**-0.281**	**0.734****	**0.475***	**1**

The symbols * , ** are representative for significant at levels 5 and 1% respectively and the data without symbol are nonsignificant.

1.2. The effects of different concentrations and incubation times of colchicine

1.2.1. Variance analysis

The effect of colchicine was significant on plant height at level 5% and on the traits of the number of branches per plant, number of leaves per branch, number of leaves per plant and leaf dry weight at level 1%. The effect of times was not significant on plant height, but was significant on the other traits. The reciprocal interaction between colchicine and incubation times was significant for all the traits at level 1% **(Table 4).**

Table 4. *Results of variance analysis of colchicine and, incubation time on characteristical traits*

S.o.V	df	Mean Square				
		Plant Height (cm)	No. Branches per Plant	No. Leaves per Branch	No. Leaves per Plant	Leaf dry weight(g)
col	3	350.463*	1.149**	23.231**	233.413**	14.686**
T	1	669.504 ns	1.86**	79.10**	267.734**	32.81**
col*T	3	90.1350**	0.207**	17.008**	39.563**	3.887**

Col= colchicine treatments; T= incubation times
The symbols ns, *, ** are non significant, significant at level 5 % and significant at level 1% based on Duncan's multiple range test

Height
As shown in Fig. 3, the maximum plants height was observed in the controls and in the treatment 0.125g l^{-1}/24h of colchicine. The minimum plant height was belongs to the treatment 0.5g l^{-1}/48h, as well. There were significant differences between the applied treatments. Generally, in the time period of 48h average heights of plants were less than 24h at level 1%. As matter of fact, plant height decreased with increment of incubation times and concentrations of colchicines **(Fig. 3a)**.

Number of branches per plant
The maximum number of branches was observed in the treatment containing 0.5g l^{-1}/48h and then in the treatment containing 0.25gl^{-1}/48h. The minimum number of branches was belongs to the control, as well. The treatments were classified in seven statistical groups. Generally, the number of branch in incubation time of 48h was more than 24h **(Fig. 3b)**.

Number of leaves per branch
There were negative relation between colchicine concentration and the number of leaves per branch. So, it decreased with increment of colchicine concentration or incubation time. The maximum number of leaves was belonging to control and 0.125g l^{-1}/24h. With the exception of the control all the treatments were significantly different. The treatments were grouped in seven statistical classes **(Fig. 3c)**.

Number of leaves per plant
The maximum number of leaves per branch was observed in the treatment containing 0.5g/l in time period of 48 h. there were significant differences among all the treatments. Also, with neglect of colchicine concentration, the number of leaves per plant in time period of 24h was relatively less than time period of 48h. The minimum number of leaves was belongs to the control. Generally, the number of leaves increased by enhancement of colchicines concentration or incubation time **(Fig. 3d)**.

Leaf dry weight
The mean comparison of the treatments showed that the effect of incubation time was significant on leaf dry weight, since leaf dry weight for 0.125g l^{-1}/48h was more than 0.25g l^{-1}/24h and for 0.25g l^{-1}/48h was more than 0.5g l^{-1}/24h of colchicine, as well. But in same incubation time leaf dry weight was increased with increment of colchicine concentration. All the treatments were significantly different at level 1% based on Duncan multi range test **(Fig. 3e)**.

1.2-2. Relationship of morphological characteristics and different incubation time and concentrations of colchicin
Regression line for the effects of different cholchicine treatments on plant height was shown in **Fig.4a**. Plants height was linearly decreased by enhancement of cholchicine concentrations or incubation time. Plants height and cholchicine concentrations was negative strongly correlated (R2≈ 0.63). Regression equation and the slop of regression line were y = -2.1875x + 49.746 and -2.187 **(Table 5)**, respectively.

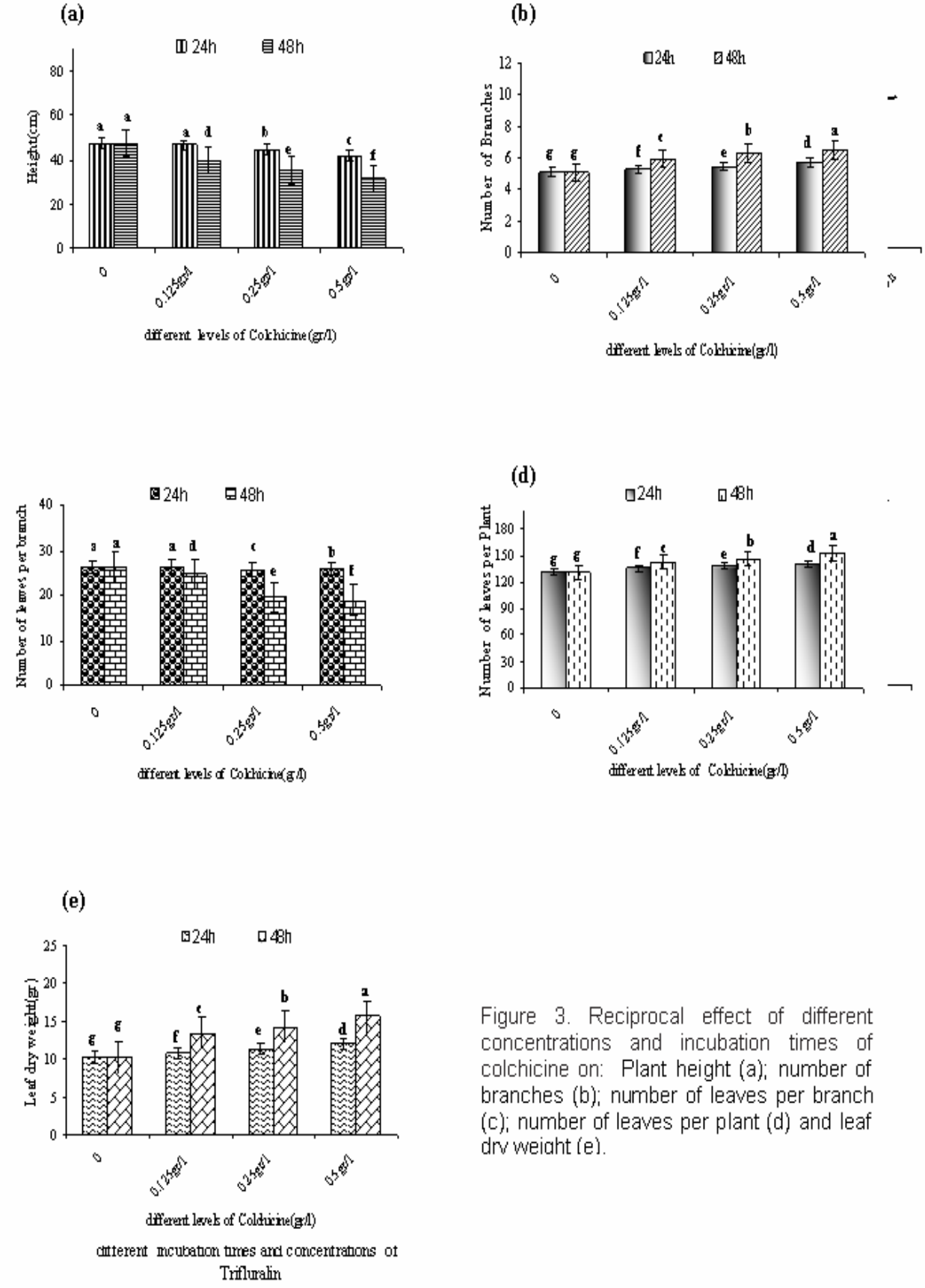

Figure 3. Reciprocal effect of different concentrations and incubation times of colchicine on: Plant height (a); number of branches (b); number of leaves per branch (c); number of leaves per plant (d) and leaf dry weight (e).

The number of branches had significantly positive-strong correlation with different incubation times and concentrations of trifluralin (R2=0.68). In **Fig 4b**, regression was linearly, the slope of this regression was 0.198, the regression equation was y = 0.1986x + 4.9324 **(Table 5)**.

Table 5. *Linear regression between traits and different incubation times and concentrations of colchicine*

traits	Regression equation	R2
H(cm)	y = -2.1875x + 49.746	0.63
No.Br	y = 0.1986x + 4.9324	0.68
No.LePl	y = 2.675x + 130.26	0.71
No.LeBr	y = -0.9982x + 27.954	0.45
LDW(g)	y = 0.7218x + 9.5943	0.6

The symbols ns, *, ** are non significant, significant at level 5 % and significant at level 1 % based on Duncan's multiple range test

As shown in **Fig. 4c**. There were negative relation between the number of leaves per branch and applied colchicine treatments. Also their correlation was medium (R2≈0.45).resultant regression equation was linear and was as: y = -0.9982x + 27.954. Line slop was -0.99 for this equation **(Table 5).**

The number of leaves per plant increased by enhancement of colchicine concentrations and incubation times. This trait had linearly relation and very strong correlation with different incubation times and colchicine concentrations (R2=0.71). Regression equation and the line slop of regression were y = 2.675x + 130.26 and 2.67, respectively **(Fig. 4d and Table 5)**.

There was rising linearly relation between leaf dry weight and different times and concentrations of colchicine. This correlation was strong (R2≈0.6) with the regression line slope of 0.72. The regression equation was y = 0.7218x + 9.5943, as well **(Fig. 4e and Table 5)**.

1.2.3-Correlation analysis

The highly correlated were among traits, affirmed by the fact that 11 of the 15 correlations were 0.5 or higher (as the result of trifluralin applied treatments). The result of correlation analysis showed that there were positive correlation between leaf dry weight (LDW) and plant height and the number of leaves per branch at level 5%, also there were positive correlation between LDW and the number of leaves per plant at level 1%. Moreover, there was negative correlation (-0.37) between LDW and the number of branches per plant at level 1%. By neglect of positive or negative status, LDW had the maximum and the minimum correlations with No.Le.Pl and No.Br, respectively. these results indicates that LDW increases with increment of plant height, number of leaves per branch and number of leaves per plant, whereas it decreases with increment of the number of branches per plant **(Table 6)**.

2. *CHLOROPHYLL CONTENT (SPAD VALUE)*

2.1. The effect of applied treatments of trifluralin on chlorophyll content

Analysis variance of different trifluralin concentrations and incubation times has been shown in the **Table 6.** The effect of trifluralin, incubation time and reciprocal effect of them are significant at level 1% based on Duncan multi range test. It indicates that both trifluralin and incubation time impacts the leaf chlorophyll content **(Table 7)**.

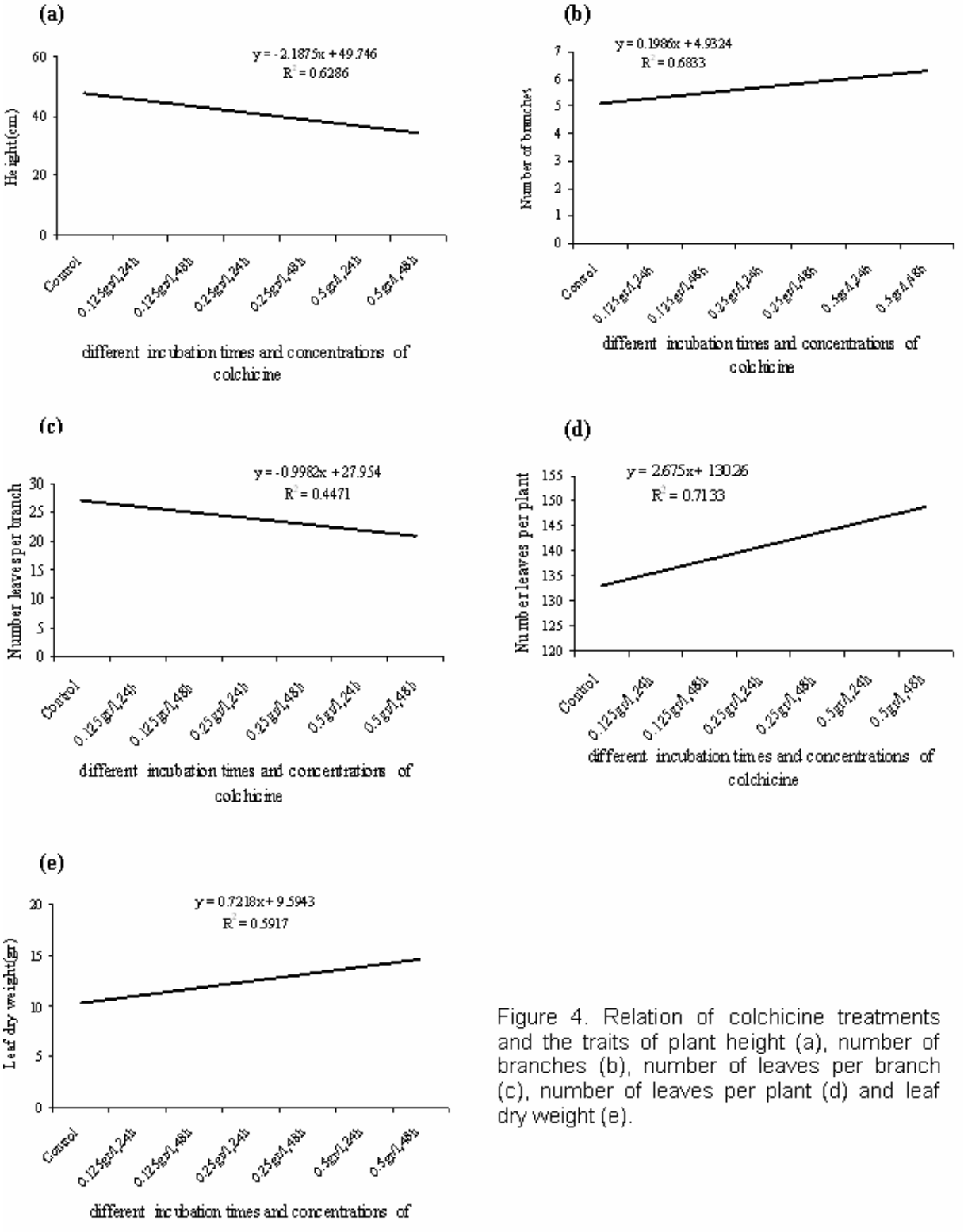

Figure 4. Relation of colchicine treatments and the traits of plant height (a), number of branches (b), number of leaves per branch (c), number of leaves per plant (d) and leaf dry weight (e).

Based on **Table 8** all the treatments were classified in separated group .Data obtained from mean comparison showed that the treatment 22.5 µM/48h had the maximum effect on leaf chlorophyll content. Besides 22.5µM/48h, the treatment of 15µM/48h, 22.5µM/24h, 15µM/24h, 7.5µM/48h and 7.5µM/24h had the most effects on chlorophyll content, respectively. The minimum chlorophyll content was belongs to the controls (24h or 48h).

The more construction of chlorophyll in the treatments 22.5 µM/2h and 15µM/24h in comparing to 7.5 µM/24h represent the importance of trifluralin concentration in this trait. Also, chlorophyll content in the treatment 22.5 µM/48 h was more than it in the treatment 22.5 µM/24h. It showed that incubation time can impacts the chlorophyll content as well.

Table 6.*Correlation coefficient between leaf dry weight (LDW) and other traits in colchicine different treatments.*

Traits	H	NoBr	NoLePl	NoLeBr	LDW
H	1				
No.Br	0.65**	1			
No.LePl	0.73**	0.886**	1		
No.LeBr	0.11	-0.21	0.905**	1	
LDW	**0.433***	**-0.37***	**0.701****	**0.501***	**1**

The symbols * and ** are representative for significants at levels 5 and1% respectively and the data without symbol are nonsignificant.

Table 7. *Analysis variance of both of ploidy inducer such as colchicine, trifluralin and their incubation time on SPAD*

S.O.V	df	Mean Square
Col	3	181.748**
T	1	39.155**
*Col*T*	3	4.190**

S.O.V	df	Mean Square
Tri	3	477.915**
T	1	208.270**
*Tri*T*	3	42.465**

Col= colchicine treatments, Tri= trifluralin treatments= incubation times

*** is significant at level 1% based on Duncan's multiple range test.*

Table 8*. Mean comparison of reciprocal effects of different trifluralin concentrations and incubation times on SPAD.*

Treatments	SPAD	
	24h	48h
control	32.6 g	32.97 g
7.5µmol/l	35.77 f	38.37 e
15µmol/l	42 d	50.77 b
22.5µmol/l	46.6 c	58.43 a

The means with same letter are not significantly different at level 5% (based on Duncan's multiple range test)

2.2. The effect of colchicine applied treatments on chlorophyll content

Base on **Table 7** the results of analysis variance of colchicine showed that the effect of colchicine, incubation time and reciprocal effect between them were significant at level 1% based on Duncan multi range test.

The effects of different colchicine treatments on chlorophyll content of leaves were compared in **Table 9.** The treatments were classified in six statistical classes. The maximum chlorophyll content was belongs to the treatment $0.5gl^{-1}$/48h of colchicine, also there was no significant difference between the treatments 0.25g l^{-1}/48h and 0.5g l^{-1}/24h. The controls had the minimum chlorophyll content.

Table 9. *Mean comparison of different colchicine concentrations and incubation times on SPAD*

Treatments	SPAD	
	24h	48h
control	32.6 [f]	32.97 [f]
0.125g/l	35.7 [e]	37.92 [d]
0.25g/l	39.47 [c]	42.9 [b]
0.5g/l	43.43 [b]	47.63 [a]

The means with same letter are not significantly different at level 5% (Duncan's multiple range test) significant range test)

Chlorophyll content in the treatment 0.25g l^{-1}/24h was more than it in the treatment 0.125g l^{-1}/48h.It confirms the significance of the effect of colchicine concentration mentioned in Table 8. Also, chlorophyll content in the treatment 0.125g l^{-1}/48h was more than it in the treatment 0.125g l^{-1}/24h. It indicates that the effect of incubation time is significant, as shown in the Table 7, as well.

2.3. Relationship of SPAD and Different incubation Times and Concentrations of Polyploidy Inducer

The effect of different levels and incubation times of trifluralin has been shown in **Fig. 5a**. Chlorophyll content (SPAD value) increased linearly. Very high significant correlation (R2=0.90) was between SPAD value and applied treatments. Regression equation was y = 3.9839 x+27.57, which the slop of regression line was 3.98.

The relationship between Chlorophyll content (SPAD value) and applied colchicine concentrations was linearly increased. SPAD value was very high correlated with different times and level of colchicine (R2=0.98). The regression equation was: y = 2.3404 x +30.589. The slope of this regression line was 2.3404 **(Fig.5b)**.

Generally, it can be deduced that both of concentration and incubation time of colchicin and trifluralin influence the chlorophyll content.

DISCUSSION

The increases in dimensions and area were probably due to the fact that cells with a larger complement of chromosomes grow larger to maintain a constant ratio of cytoplasmic to nuclear volume, and express more proteins with the presence of more genes. This increase in size may translate to an increase in plant and its organs (25).

Polyploidy usually leads to thicker leaves, a deeper green color, increased width-to-length ratio of leaves, larger and heavily textured flowers, and a more compact growth habit (20, 26, 27 and 28).

Derived tetraploid plantlets have fewer large stomata and larger leaves in compare with main diploid plants (29). In other hand, polyploid plants usually have thicker roots and stems (30-32 and 25). In this study plants under ploidy inducer agents differed from diploid plants (controls plant) in growth rate and morphology with broader leaves, more branches and etc. Similar phenotypic variations, such as larger plant organs in polyploid compared with diploid plants, were reported in plants of alfalfa and potato (33) and barley (34).

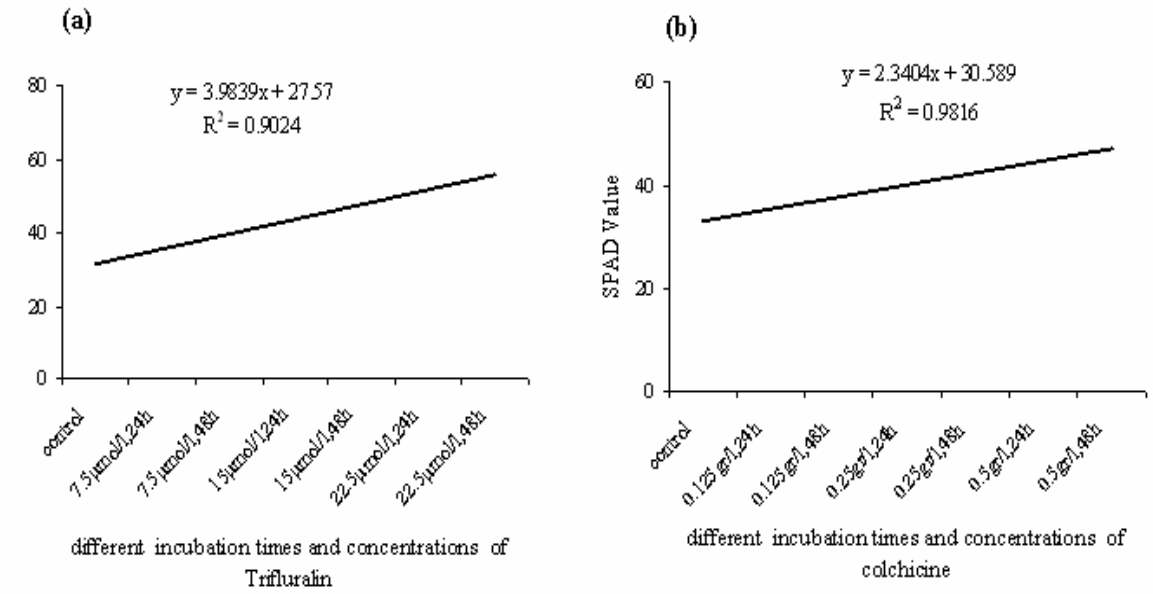

Fig 5. Relationships between SPAD value and Trifluralin applied treatments (a), Colchicine applied treatments (b).

Also, the characteristic slowed growth, altered phenology, and prolonged flowering of polyploids may, in part, result from slowed mitotic divisions and cell divisions of larger cells with more chromosomes (35-36). Total plant height was lower in both tetraploid lines compared to the three diploids, and this reduced stature was partly due to shorter internodal distances (37-39 and 25) reported that colchicine treatment had decreased the plant height. it is agreed with the results of our study.

Liu et al.(2007) stated that the number of branches was also reduced in the tetraploid plants and the average branch length was shorter while Increased branching (bushy habit) has been reported with colchicines treatment in other studies (11 and 40-41), Mensah et al.(2007) reports that colchicine treatments enhanced the number of branches in sesame (Sesame indicum L), as well.In this study, we observed the number of branches increased by increment of incubation times and concentrations of ploidy inducer agents.

Kirhara (1951) also reported that the leaves of tetraploid plants were large, thick and dark green than the diploid plants. Similarly, broader leaves in *Citrus* (43), higher ratio of leaf width to length in *Alocasia* (44), and broader and thicker leaves in *B. globosa* (32) have been reported in tetraploid genotypes. Variation in the leaf size was observed among controls and plant under ploidy inducer agents plants under the same growing conditions. As their result, we were observed leaf dry weight increased in plants under ploidy inducer agents. It can be deduced that increased leaf dry weight cause by colchicine or trifluralin is due to increment of leaf size and thickness.

Ploypoid plants may have darker green leaves than diploids (26-28). Jaskani et al. (2005) reported the chlorophyll content under greenhouse conditions varied in leaves of both ploidy plants. We observed that chlorophyll content was increased at higher concentration of both trifluralin and colchicine. Our finding is in agreed with the results of Mensah et al. (2007) for sesame (Sesame indicum L.), as well.

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