

Effects of Humic acid, Paclobutrazol and boron on growth of *Tabernaemontana coronaria* (Crape jasmine) plants.

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Abstract

A pot experimental study was carried out during the two successive seasons of 2019/2020 and 2020/2021 to evaluate the effect of paclobutrazol (PP333) at 0.0, 20 and, 40 ppm, Humic acid (H) at 0.0 and 2g l⁻¹ and Boron (B) at 0.0, 2 and 4 mg/l on growth of potted *Tabernaemontana coronaria* plants. The obtained data showed that: All PP333 and B concentrations decreased plant height, especially PP 333 at 40ppm in both seasons. On contrary, all applied treatments of PP 333 and B significantly increased the root size, root and leaves fresh weight to reach its maximum by using humic acid at 2g l⁻¹, boron at 2 mg/l and 40ppm pp333-sprayed plants in the two seasons. Furthermore, PP333 and B combined with H treatments significantly increased total chlorophylls contents as compared with un-sprayed plants in the two seasons.

Key words: *Tabernaemontana*, Paclobutrazol, humic acid, Boron, growth, total chlorophylls.

Introduction

Tabernaemontana coronaria Stapf (Synonym: *Ervatamia coronaria*) is a glabrous, evergreen, dichotomously branched shrub, which belongs to Family Apocynaceae. It grows in upper Gangetic plain, Garhwal, East Bengal, Assam, Karnataka, Kerala and in Burma. It is a spreading, bushy, multi-branched shrub and grows to a height of 15 to 25 cm tall and 150 to 250 cm wide. Its leaves are long with wavy margins; dark green above and pale green beneath (Pushpa *et al.*, 2011).

The terms growth retardants is used for all chemicals that retard cell division and cell elongation in shoot tissues and regulate plant height physiologically without formative effects (PGRSA, 2007). One of the most

widely used growth retardants is paclobutrazol (pp333) [(2RS,3RS) -1-(4-chlorophenyl-4,4 -dimethyl-2-(1H-1,2,4 triazol-1-yl) pentan-3-ol)] is a well-known plant growth retardant (Davis and Andersen, 1989). Paclobutrazol functions by inhibiting cytochrome P-450, which mediates oxidative dimethylation reactions, including those which are necessary for the synthesis of ergosterol and the conversion of kaurene to kaurenoic acid in the gibberellins biosynthetic pathway (Fletcher *et al.*, 2000). From this function, paclobutrazol has long been used to reduce plant height for potted plant production, particularly ornamental plants (Beattie *et al.*, 1990; Fletcher *et al.*, 2000). Paclobutrazol at concentration of 35 mg a.i./pot reduced plant foliage height and flower stem length, without affecting inflorescence length and delaying the production of potted Thai Tulip (Pinto *et al.*, 2006).

Boron (B) is an essential nutrient for normal growth of higher plants; it plays an important role in

physiological and biochemical processes and has a primary function in plant cell wall integrity (Mengel and Kirkby, 2001). It is needed by the crop plants for cell division, nucleic acid synthesis, and uptake of calcium and transport of carbohydrates.

Humic acid (HA) exists in arable soils and is derived from soil humus; it is a microbial metabolized organic matter and constitutes over 60% of soil organic matter. It is used as a plant bio-stimulant (Peña-Méndez *et al.*, 2005 and Muscolo *et al.*, 2013).

The purpose of this study was to investigate the effects of Humic acid, Paclobutrazol and boron on growth and chemical composition of potted *Tabernaemontana coronaria* plants.

Material And Methods

A pot experiment was conducted during 2019-2020 and 2020-2021 seasons to study the effect of humic acid (H), Paclobutrazol (PP₃₃₃) and boron (B) on *Tapernamontana* plants. The design was a randomized complete block, factorial experiment. After one month from repotting process (March 1st during the two seasons), the plants were received three sprays with paclobutrazol at 20, and 40 ppm and boron at 2 and 4mg/l plus tap water as control at one month intervals. The plants were sprayed with a hand pump mister to the point of runoff. A surfactant (Tween 20) at a concentration of 0.01% was added to all tested solutions including the control. Plants were grown in PVC pots of 10kg soil pot⁻¹. Soil of the pots was a mixture of a clay soil: sand: peat moss (at a ratio of 2:1:1 by volume). Table 1 shows the main properties of the clay soil used. Soil analysis was done by methods cited in (Black *et al.*, 1965).

Table 1: Main properties of soils of the seasons 2019-2020 Soil particle size distribution and texture

	Unit	Season	
		2019-2020	2020-2021
Coarse sand	%	14.15	15.65
Fine sand	%	13.98	13.86
Silt	%	16.65	16.84
Clay	%	55.22	53.65
Texture		Clay	Clay

Chemical properties

Parameter	Unit	Season	
		2019-2020	2020-2021
Organic matter	gkg ⁻¹	15.7	23.3
Available N	mgkg ⁻¹	65	70
Available P	„	9.0	9.0
Available K	„	50	50
CaCo3	gkg ⁻¹	5.5	5.7
Fe	mgkg ⁻¹	12.8	20.0
Zn	„	4.3	4.8
Mn	„	5.6	15.7
Cu	„	2.9	2.5
B	„	2.0	5.0
pH		7.8	7.5

The humic acid (obtained from Agrolink Agricultural Co., Roxy, Cairo, Egypt) was added to the irrigation water at a concentration of 2 gL⁻¹. Boron (B) (used in the form of boric acid; 170 g Bkg⁻¹) as well as Paclobutrazole (PP₃₃₃) was obtained from Sigma Aldrich. Thus there were 18 treatment combinations (2 H X 3 B X 3 PP₃₃₃). Treatments were in 3 replicates. After two months from replanting, the plants were fertilized every month with NPK fertilizer using ammonium sulfate (20.5% N), calcium superphosphate (15.5% P₂O₅) and potassium sulfate (48% K₂O). A mixture of the three fertilizers, with a ratio of 1: 1: 1 (N: P₂O₅: K₂O), was prepared and applied to the pots at the rate of 5 g/pot. Common agricultural practices (irrigation, manual weed control, . . . etc.) were carried out when needed.

Sampling and collecting data:*1- Growth characteristics:*

Growth traits and characteristics were measured at 90 days after transplanting. Five plants from treatments were randomly taken for measurements. The following characteristics were inspected:

- Size of the root system according to Hanson and Churchill (1968). Roots were gently obtained by washing the soil round them under flow using water.
- Plant height (cm)
- Fresh weight of roots g /plant⁻¹.
- Root size (cm³)
- Fresh weight of leaves g/ plant⁻¹.

Samples were then dried in oven at 70° C for 72 hours till weight stability and dry weight was measured.

2-Total chlorophyll content (SPAD value):

Total chlorophyll in leaves was determined at 90 days after planting using analytical apparatus; chlorophyll meter (Model SPAD) Minolta Camera Co. Ltd, Japan.

Results and discussion.

Effect of paclobutrazol, Humic acid and Boron on growth, chemical composition, of Tabernaemontana coronaria plants:

1- Effect on vegetative growth:

Table (1) show that on vegetative growth traits i.e., plant height as affected by paclobutrazol (PP₃₃₃) at 20 and 40ppm, Humic acid(H) and boron(B) at 2and 4mgL⁻¹ are presented in Tables (1). Here, it could be noticed that all PP₃₃₃, H and B treatments were positively affected the previously mentioned vegetative growth traits of Tabernaemontana plants as compared with control in the two seasons. However, plants received the different PP₃₃₃, H and B treatments were shorter than the untreated control plants. Also, of the different PP₃₃₃ and B treatments, the highest concentration of PP₃₃₃ was the most effective one for producing the shortest plants in both seasons. Besides, PP₃₃₃ at 40ppm and B at 4mgL⁻¹ compared with H gave highly significant reduction in the plant height in the two seasons. Of interest, is to note that the abovementioned results when related with the histological features of treated plants and also with their growth aspects. Since, gibberellin is known as a stimulating and individual hormone for longitudinal growth in different plants (Devlin and Witham., 1983 ; Feleafel et al., 2019 and Jeevitha and Vasudevan., 2019) . Hence, reduction of endogenous gibberellins level due to the use of growth retardants

treatments (as will be mentioned later) led to reduction in the length of different cell types and consequently reduction in the plant height especially

when the reduction of both gibberellins and auxins level is considered.

Table1: Effect of Effect of Boric acid, Pacllobutrazol and Humic acid on Plant height (cm plant-1) of *Tabernaemontana coronaria* (Crape jasmine) plants .

H	B	2019-2020				2020-2021					
		P ₀	P ₁	P ₂	Mean	P ₀	P ₁	P ₂	Mean		
B	B ₀	33.50	23.50	19.00	25.33	62.00	65.00	83.50	70.16		
	B ₁	42.00	40.50	24.50	35.67	29.50	30.50	20.00	26.66		
	B ₂	23.50	19.00	21.00	21.17	24.50	22.00	31.50	26.00		
	Mean	33.00	27.67	21.50	27.39	38.66	39.16	45.00	40.94		
H ₁	B ₀	42.50	19.00	17.00	26.17	65.00	69.50	62.50	65.66		
	B ₁	44.50	50.50	29.50	41.50	36.00	42.50	32.50	37.00		
	B ₂	22.50	29.00	20.00	20.83	36.50	41.50	36.50	38.16		
	Mean	36.50	29.83	22.17	29.50	42.25	45.16	44.41	46.94		
G. mean		34.75	28.75	21.83		42.25	45.16	44.41			
Means of B											
	B ₀	38.00	21.25	18.00	25.75	63.50	67.25	73.00	67.91		
	B ₁	43.25	45.50	27.00	38.58	32.75	36.50	26.25	31.83		
	B ₂	23.00	19.50	20.50	21.00	30.50	31.75	34.00	32.08		
LSD 0.05		H:1.93	B:2.37	P:2.37	HB:3.34	LSD 0.05		H: 2.26	B:2.76	P:2.76	HB: 3.91
BP:4.10		HP:NS	HBP:5.81			BP: 4.79		HP: 3.91	HBP:6.78		

Treatment designations: Pacllobutrazol P₀, P₁ and P₂ sprayed as solution of 0, 20 and 40 mg L⁻¹ respectively ; B₀, B₁ and B₂ sprayed boric acid solution of 0, 2 and 4 g L⁻¹ respectively ; H₀ and H₁ humic acid added to soil at 0 and 2 g kg⁻¹ respectively.

As shown in **Table (2)** different applied treatment significantly increased the root size (cm³)/plant, it was noticed that significant increase of this parameter at 60 days after transplanting during two seasons was obtained. Also, in this respect Boron separately or when combined with Humic acid exhibited the less significant increase of this size. While pp₃₃ when applied separately or combined with Humic acid showed the highest significant increase of this parameter. In this respect, the height root size where reached (to 19.00, 15.50, 19.50 and 26.00 cm³ in the first season and 17.50, 16.50, 43.00 and 45.50 cm³/plant in the second season with H0P2B1, H1P0B1 and H1B1P2 respectively. These results are in agreement with those reported by (Motaium et al., 2019 and Faisal et al., 2019).

With regard to the fresh weight of roots, as shown in **Table (3)** it could be noticed that significant increase of this parameter was the dominant result of all applied treatments.

In this respect, pp₃₃ separately or in combinations with Humic acid and B was pronounced its effect. Here, the height values were 19.68 and 33.07 g/plant & 28.63 and 48.53 g/plant with pp₃₃ at 40 ppm +B at 2mgL+H & pp₃₃ at 20 ppm +B at 4mgL+H during two seasons, respectively. These results are in agreement with those reported by (Mahmoud et al., 2020); (Mazen et al., 2019); Bradar-Jokanovic, (2020); Motaium et al. (2019); Sharaf-Eldien et al. (2017).

As indicated in **Table (4)** leaves are the very important organ of *Tabernaemontana* plant in which they are the source of photosynthates as well as the economic ornamental vision. As for, the fresh weight of leaves, clearly increased significantly. In this respect, pp₃₃ either separately or when combined with B and H showed the highest values of this number. Where, the heaviest fresh weights of leaves/plant were gained by 40ppm PP₃₃- sprayed plants. Also, PP₃₃ at 20ppm and B at 2mgL combined with H gave highly significant increments in these parameters in both seasons. Such results showed similar trend to those obtained by many investigators worked on PP₃₃ and B on other plants. In this concern, (EL- Khwaga et al., (2020) on *Luffa cylindrica* L., Faisal et al., (2019) on *Gazania rigens* L and Noor El-Deen., (2020) on *Ruellia simplex*. In general, some of the other studies has been carried out in which obtained nearly similar results, of these studies are (Ghatas., 2016)) reported that spraying pacllobutrazol increased branches and fresh and dry weights of leaves but it decreased plant height. As for Boron the abovementioned results of B are in harmony with found by EL-Mahmoudy et al., (2019) reported that the highest values were recorded in vegetative growth traits (plant height, leaves fresh weight, and dry weight) in comparison with the control.

Table 2: Effect of humic acid(H) boron(B) and Paclobutrazol(PP₃₃₃) on Root size(cm)³ of *Tabernaemontana coronaria* (Crape jasmine) plants .

H	B	P							
		Root size(cm) ³ 2019-2020				Root size(cm) ³ 2020-2021			
		P ₀	P ₁	P ₂	Mean	P ₀	P ₁	P ₂	Mean
	B ₀	15.50	8.00	3.50	9.00	20.50	37.50	18.50	25.50
	B ₁	6.00	11.00	19.00	12.00	8.50	21.50	17.50	15.83
	B ₂	15.50	6.00	12.00	11.16	12.50	15.50	16.50	14.83
	Mean	12.33	8.33	11.50	10.73	13.83	24.83	17.50	18.72
H ₁	B ₀	13.00	10.50	6.00	9.84	21.00	23.50	40.00	28.16
	B ₁	19.50	21.50	26.00	22.34	32.50	27.50	35.50	31.83
	B ₂	11.50	17.00	13.50	14.00	43.00	42.50	17.00	34.16
	Mean	14.99	16.33	15.16	15.39	32.16	31.16	30.83	31.38
G. mean		13.50	12.33	13.33		23.00	28.00	24.16	
Means of B									
	B ₀	14.25	9.25	4.75	9.41	20.75	30.50	29.25	26.83
	B ₁	12.75	16.25	22.50	17.16	20.50	24.50	26.50	23.83
	B ₂	13.50	11.50	12.75	12.58	27.75	29.00	16.75	24.50
LSD 0.05	H:1.76	B:2.15	P:NS	HB:3.04	BP:3.72	LSD 0.05	H:2.01	B:2.46	P:2.46
	HP:3.04	HBP: 5.27					BP:4.27	HP: 3.48	
							HBP:6.04		

Treatment designations: Paclobutrazol P₀, P₁ and P₂ sprayed as solution of 0, 20 and 40 mg L⁻¹ respectively ; B₀, B₁ and B₂ sprayed boric acid solution of 0, 2 and 4 g L⁻¹ respectively ; H₀ and H₁ humic acid added to soil at 0 and 2 g kg⁻¹ respectively

Table 3:Effect of humic acid(H) boron(B) and Paclobutrazol(PP₃₃₃) on Root fresh weight (g plant⁻¹) of Crape jasmine (*Tabernaemontana coronaria*) plants :

H	B	P							
		Root F.W 2019-2020				Root F.W(g) 2020-2021			
		P ₀	P ₁	P ₂	Mean	P ₀	P ₁	P ₂	Mean
H ₀	B ₀	5.91	6.16	19.68	10.58	19.60	36.72	28.63	28.32
	B ₁	5.74	14.68	4.47	8.29	7.32	16.77	8.60	10.89
	B ₂	3.31	9.97	10.44	7.91	9.31	17.04	15.18	13.84
	Mean	4.98	10.27	11.53	8.93	12.08	23.51	17.47	17.68
H ₁	B ₀	17.98	32.56	31.45	27.31	22.49	36.96	40.03	33.16
	B ₁	11.07	33.07	15.58	20.00	31.90	35.41	31.68	33.00
	B ₂	10.32	30.12	12.74	17.72	48.53	34.48	15.81	32.94
	Mean	13.10	31.92	20.01	21.68	34.31	35.62	29.17	33.03
G. mean		9.04	21.09	15.77		23.19	29.56	23.32	
Means of B									
	B ₀	11.91	19.36	25.56	18.95	21.05	36.84	34.33	30.74
	B ₁	8.40	23.88	10.16	14.14	19.61	26.09	20.14	21.94
	B ₂	6.82	20.04	11.59	12.81	28.92	25.76	15.49	23.39
LSD 0.05	H:1.54	B:1.89	P:1.89	HB:2.68	BP:3.28	LSD 0.05	H:1.95	B:2.39	P:2.39
	HP:2.68	HBP:4.64					BP:4.15	HP:3.38	
							HBP:5.87		

Treatment designations: H₀ and H₁ humic acid added to soil at 0 and 2 g L⁻¹ irrigation water respectively. B₀, B₁ and B₂ sprayed boric acid solution of 0, 2 and 4 mg B L⁻¹ respectively Paclobutrazol P₀, P₁ and P₂ sprayed as solution of 0, 20 and 40 mg L⁻¹ respectively ;

Table 4 : Effect of humic acid(H) boron (B)and Paclobutrazol (PP₃₃₃) (on Leavest fresh weight (g plant⁻¹) of Crape jasmine (*Tabernaemontana coronaria*) plants :

H	B	P							
		Leaves F.W 2019-2020				Leaves F.W (g) 2020-2021			
		P ₀	P ₁	P ₂	Mean	P ₀	P ₁	P ₂	Mean
H ₀	B ₀	20.01	19.67	22.55	20.74	27.91	32.71	36.21	32.27
	B ₁	7.39	24.76	23.57	18.57	10.47	26.00	24.10	20.19
	B ₂	13.67	10.80	12.61	12.36	25.52	16.72	18.83	20.35
	Mean	13.69	18.41	19.58	17.22	21.30	25.14	26.38	24.27
H ₁	B ₀	24.17	27.42	28.77	26.78	36.17	38.91	29.86	34.98
	B ₁	9.51	10.60	8.91	9.67	24.76	29.44	21.25	25.15
	B ₂	7.00	8.64	10.61	8.75	30.53	23.62	20.27	24.81
	mean	13.56	15.55	16.09	15.07	30.48	30.65	23.79	28.31
G. mean	13.62	16.98	17.83		25.89	27.90	25.08		
Means of B									
	B ₀	22.09	23.54	25.66	23.76	32.04	35.81	33.03	33.62
	B ₁	8.45	17.68	16.24	14.12	17.61	27.72	22.67	22.67
	B ₂	10.33	9.72	11.61	10.55	28.02	20.17	19.55	22.58
LSD 0.05	H:2.37	B:2.90	P:2.90	HB:4.10	BP:5.03	LSD 0.05	H:2.90	B:3.55	P:3.55
HP:NS	HBP:7.11					BP:6.15	HP:5.02		HBP:8.70

See footnotes of Table 2 for treatment designations

Table 5: Effect of humic acid(H) boron(B) and Paclobutrazol(PP₃₃₃) on Total chlorophyll (SPAD)of *Tabernaemontana coronaria* (Crape jasmine) plants .

H	B	P							
		Total chlorophyll(SPAD) 2019-2020				Total chlorophyll (SPAD)2 2020-2021			
		P ₀	P ₁	P ₂	Mean	P ₀	P ₁	P ₂	mean
H ₀	B ₀	29.85	45.85	44.30	40.00	34.45	36.30	47.80	39.51
	B ₁	39.35	50.90	61.55	50.60	43.05	58.65	68.55	56.75
	B ₂	50.25	52.95	66.75	56.65	71.85	63.55	67.35	67.58
	mean	39.81	49.90	57.53	49.08	49.78	52.83	61.23	54.61
H ₁	B ₀	33.65	34.00	36.45	34.70	37.05	35.45	50.20	40.90
	B ₁	38.30	40.55	52.45	43.76	49.45	46.85	60.80	52.36
	B ₂	53.95	70.50	67.65	64.03	64.25	62.55	66.50	64.43
	mean	41.96	48.35	52.18	47.50	50.25	48.28	59.16	52.56
G. mean	40.89	49.12	54.85		50.01	50.55	60.20		
Means of B									
	B ₀	31.75	39.92	40.37	37.35	35.75	35.87	49.00	40.20
	B ₁	38.82	45.72	57.00	47.18	46.25	52.75	64.67	54.55
	B ₂	52.10	61.72	67.20	60.34	68.05	63.05	66.92	66.00
LSD 0.05	H: NS	B:7.90	P:7.90	HB:11.17	BP:NS	LSD 0.05	H:NS	B:10.04	P:10.04
HP: NS	HBP:NS					HB:NS	BP:NS	HP: NS	
						HBP:NS			

2-1 Chlorophyll contents:

As shown in **Table (5)** different applied treatments increased the total amount of chlorophylls in leaves of treated plants .In this respect, pp₃₃₃ at 40ppm was more pronounced treatment to increase total chlorophylls in the stage of plant growth (i.e., at 60,75 and 90 days after transplanting).

The increment, of total chlorophylls in leaves of this ornamental plant beside the crowndness of leaves (i.e., rosette leaves) with dark green color could rise up the economic value of this public plant.

These results of Humic acid are in agreement with those reported by (El-Hoseiny et al ., 2020; Noor El-Deen and El-Ashwah., 2019 and El-Helaly .,2018). Also, effects of Boron application on photosynthetic pigments could be explained by (Khaleel and Fouad ., 2019) showed that using Boron at 2 gl on *Vigna unguiculata* t showed a significant increase in plant growth and leaf content of total chlorophyll . As for paclobutrazol the abovementioned results of paclobutrazol are in harmony with those attained by (Abd El-Aal and Eid .,2017; Sharaf-Eldien et al., 2017 and Noor El-Deen .,2020) reported that

Paclobutrazol increased leaf chlorophylls (a and b), carotenoids and total phenols of *Ruellia simplex* plant.

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تأثير الهيومك اسيد والباكلوبوترازول والبورون على نمو نبات التابرنامونتانا

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اجريت تجربة اصص خلال موسم 2021/2020-2020/2019 على نبات التابرنامونتانا. التجربه عاملية مكونه من ثلاث عوامل وهم: الهيومك اسيد مضافا للتربه بتركيز 2جم/لتر والباكلوبوترازول بتركيزات 0,20,40 جزء فى المليون والبورون بتركيزات 0,2,4 ملجم/لتر رشا على الاوراق. وبذلك تضمنت التجربه 18 معامله (2 هيومك *3 باكلوبوترازول*3 بورون) ولقد لوحظ انخفاض فى طول النباتات المعامله مقارنه بالكنترول وذلك باستخدام المعاملات الباكلوبوترازول 20 و 40 جزء فى المليون منفردا او مجتمعا مع البورون بتركيز 4ملجم/لتر وذلك فى وجود الهيومك اسيد. وعلى العكس من ذلك كل المعاملات المستخدمه ادت الى زياده فى كل القياسات الخضريه ومنها حجم الجذور والوزن الطازج لكل من الجذور والاوراق.بالاضافه الى الزيادة المعنوية فى محتوى الاوراق من صبغة الكلوروفيل وذلك مقارنه بالنباتات الغير معاملة خلال الموسمين.

الكلمات الاساسية: نبات التابرنامونتانا - لباكلوبوترازول - البورون - الهيومك اسيد - النمو - الكلوروفيل الكلى.