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Growth, flowering and physiological responses of olive trees to growth retardants under rain-fed conditions of Himachal Pradesh

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ABSTRACT

A field experiment was carried out during two subsequent years to ascertain the influence of paclobutrazol and cycocel on growth, cropping and water relations in olive. Eight treatments, *viz*., PP_{333} at 2.0 and 4.0 g *a.i.* tree^{-1} applied once in November or December as soil drench and cycocel @ 1000, 2000 and 3000 ppm as foliar spray two weeks after flowering to the trees of olive cv. Leccino. Soil application of PP_{333} at 4.0 g *a.i.* tree^{-1} in November decreased growth (15.69 cm), length of internode (1.84 cm), leaf area (4.60 cm^2), stomatal size (13.61 µm), water potential (-15.69 bar), transpiration rate (0.160 m mol m^{-2} s^{-1}), endogenous GA_3 (9.61hg/g fresh weight), stomatal conductance (1.51 mol m^{-2}s^{-1}) and increased leaf thickness (0.62 cm), leaf chlorophyll content (3.05 mg/g fresh weight), photosynthesis (24.62 µ mol m^{-2} s^{-1}), proline (2717 µg/g) and ABA (60.74 (hg/g fresh weight), flowering intensity (0.65 %) and fruit set (7.57%), and thus might be helpful in mitigating water stress under rainfed conditions.

Key words: *Olea europaea*, cycocel, leccino, paclobutrazol.

INTRODUCTION

In India, olive cultivation is restricted to the states of Jammu & Kashmir, Himachal Pradesh and Uttrakhand. The olive (*Olea europaea* L.) is an evergreen tree requires chilling for fruiting. Soil and foliar application of growth retardants have successfully been tried elsewhere to increase productivity of olive trees by enhancing their flowering and fruiting and by ensuring optimum use of available water in the plant system. Growth retardants retard sub-apical meristematic activity without affecting the apical meristem. Use of growth retardants like triazol compounds such as paclobutrazol has also been advocated in drought prone areas to increase the degree of fruitfulness in olive. Paclobutrazol application at 500-4000 mg/liter reduced shoot growth, internodal length, leaf area, and at the higher concentration increased shoot number and fruit set in olive (Porlingis and Voyiatzis, 6). Olive trees treated with paclobutrazol showed increased water potential but decreased stomatal resistance (Frakulli and Voyiatzis, 4). Foliar application of cycocel has also been tried to increase the productivity of olive by improving physico-chemical characteristics, flowering traits and number of fruits per tree. (Hegazi and Stino, 5). Hence, this study was undertaken to examine the effect of growth retardants on growth, flowering and physiological characteristics of olive cv. Leccino under rain-fed conditions of Himachal Pradesh.

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MATERIALS AND METHODS

A field experiment was carried out at the Olive Development Centre, Kigus, Himachal Pradesh (Lat. 32°20' N, Long., 77°E and Lat., 1090 m msl). The climate is sub-temperate with maximum temperature goes up to 35ºC during summer, whereas winters are very cold. The experiment comprises of 8 treatment combinations [T_1: PP_{333} 2.0 g *a.i.*/tree, T_2: PP_{333} 4.0 g *a.i.*/tree, T_3: PP_{333} 2.0 g *a.i.*/tree, T_4: PP_{333} 4.0 g *a.i.*/tree, T_5: CCC 1000 ppm, T_6: CCC 2000 ppm, T_7: CCC 3000 ppm and T_8: control]. Treatment 1 and 2 was applied as soil drench in mid-November and T_3 and T_4 was applied as soil drench in mid-December. Paclobutrazol treatments were given in the first year and not repeated in the following year. Cycocel treatments (T_5, T_6 & T_7) were applied as foliar application two weeks after flowering. In case of cycocel, treatments were given in both the years of study.

The length of annual shoot growth was measured in mid-March and in late August. The internodal length of current season's shoots measured at the end of summer flush growth in late August. Leaf area was determined by using leaf area meter, LI-COR Model-3100. The leaf thickness was measured with the Digimatic Calipers. Microscopic studies of anatomical characteristics made with the help of LICA DMLB compound microscope, using LICA imaging and analysis software. Leaf water potential was recorded with the help of portable 'Plant Water Status Console' in May and June. Ten mature leaves were selected from all over the periphery of the tree at random in

the month of June to record stomatal conductance, transpiration rate and photosynthetic rate with the help of LI-COR 6200 Portable Photosynthetic Meter between 10:00 and 11:00 hours. Waters HPLC was used for the estimation of GA_3 and ABA in plant tissue. In order to estimate the leaf nutrient status, leaves with petioles were sampled in mid-August from middle portion of the current season's shoot situated all around the periphery of the tree. Potassium, calcium and magnesium in the leaf extract were estimated on Perkins Elmen Atomic Absorption Spectrophotometer. The trial was laid out in randomized block design with three replications and the data recorded during both the years of field trial were pooled and analyzed.

RESULTS AND DISCUSSION

In the present investigation, different treatments of paclobutrazol and cycocel reduced shoot growth, internodal length and leaf area (Table 1). PP_{333} @ 4.0 g *a.i.* tree^{-1} when applied in November as soil drench was most effective in decreasing the shoot growth (15.69 cm), internodal length (1.84 cm). In this study, lower levels of endogenous gibberellin like substances and higher levels of ABA in PP_{333} treated trees might have accounted for the retardation of shoot growth, possibly by reducing the cell elongation. The results confirm the findings of Frakulli and Voyiatzis (4). Leaf thickness (0.62 mm) was significantly more in the trees treated with PP_{333} @ 4.0 g *a.i.* tree^{-1} applied in November or December (Table1). However, significant decrease in leaf area was observed following the application of these compounds at higher concentration (PP_{333} @ 4.0 g *a.i.* tree^{-1} in November or December (4.60 cm^2) and CCC at 3000 ppm (4.64 cm^2).

Trees treated with PP_{333} @ 4.0 g *a.i.* tree^{-1} in November (3.05 mg/g fresh weight) or December (3.03 mg/g fresh weight) had significantly higher leaf chlorophyll contents compared to control (Table 2). The higher concentrations of both growth retardants showed greater influence on the flowering attributes. The significant increase in the flower intensity (0.65%), number of perfect flowers (54.19%), fruit set (7.57%) and lower fruit drop (45.78%) observed when plants were treated with PP_{333} @ 4.0 g *a.i.* tree^{-1} in November, which was statistically at par with the December application of PP_{333} @ 4.0 g *a.i.* tree^{-1}, whereas minimum percentage of perfect flowers (40.99%), fruit set (4.74%) and lower fruit drop (62.80 %) were observed in control. This might be attributed to interference of growth retardants with GA biosynthesis (Fletcher *et al.,* 3), which in turn creates favorable conditions for flower bud differentiation and subsequent development of floral organs. Thus, it seems that higher production of perfect flowers in paclobutrazol treated trees might have promoted fruit set and decreased fruit drop.

Paclobutrazol @ 4.0 g *a.i.* tree^{-1} in November (35.63/0.04 mm^2) or December (35.23/0.04 mm^2) caused greater increase in stomatal density, whereas, significantly lower stomatal density (31.31/0.04 mm^2) was observed in control (Table 3). The stomatal size (length and breadth) in olive was however, decreased considerably by paclobutrazol treatments over the control. Stomatal length and breadth was significantly lower in PP_{333} 4.0 g *a.i.* tree^{-1} application in November (13.61 µm) and in December (9.37 µm). The increased stomatal density in paclobutrazol and cycocel treated trees can be attributed to the reduced leaf area. It was also evident that the significant decrease in number of primary xylem vessels (133.9) and secondary xylem (43.45 µm) were recorded in the trees treated with PP_{333} @ 4.0 g *a.i.* tree^{-1} in November month when compared with rest of the treatments. The present findings are

Table 1. Effect of growth retardants on growth characteristics of olive cv. Leccino.

Treatment	Growth (cm)	Internode length (cm)	Leaf area (cm^2)	Leaf thickness (mm)	Chlorophyll (mg/g FW)
T_1: PP_{333} 2 g a.i/tree	17.55	2.07	4.82	0.57	2.90
T_2: PP_{333} 4 g a.i/tree	15.69	1.84	4.60	0.62	3.05
T_3: PP_{333} 2 g a.i/tree	17.09	2.15	4.89	0.56	2.90
T_4: PP_{333} 4 g a.i/tree	16.22	1.92	4.63	0.61	3.03
T_5 :CCC 1000 ppm	17.60	2.51	4.84	0.54	2.78
T_6: CCC 2000 ppm	17.18	2.35	4.74	0.56	2.86
T_7 : CCC 3000 ppm	16.26	2.10	4.64	0.60	2.96
$T_{8:}$:Control	20.03	2.67	5.20	0.53	2.69
$CD_{0.05}$	0.33	0.09	0.06	NS	0.03

Table 2. Effect of growth retardants on flowering characteristics of olive cv. Leccino.

Treatment	Flowering intensity (%)	Perfect flower (%)	Fruit set (%)	Fruit drop (%)
T_1: PP_{333} 2 g a.i/tree	0.59	49.89	6.98	51.24
T_2: PP_{333} 4 g a.i/tree	0.65	54.19	7.57	45.78
T_3: PP_{333} 2 g a.i/tree	0.57	48.18	6.58	52.07
T_4: PP_{333} 4 g a.i/tree	0.63	52.85	7.48	46.93
T_5 :CCC 1000 ppm	0.53	44.54	5.80	58.44
T_6: CCC 2000 ppm	0.55	47.48	6.92	52.12
T_7 : CCC 3000 ppm	0.57	50.74	7.25	48.33
T_8:Control	0.51	40.99	4.74	62.80
$CD_{0.05}$	0.01	1.15	0.12	1.34

in conformity with the findings of Antognozzi *et al.* (2) who observed that application of paclobutrazol decreased cell size and development of secondary xylem in olive cv. Leccino.

Leaf water potential was significantly more negative (-15.69) in the trees treated with the PP_{333} @ 4.0 g *a.i.* tree^{-1} in November, compared to other treatments. However, the highest leaf water potential (-17.86) was found under control. The decrease in leaf water potential might be due to osmotic adjustments in the plant system brought up by the higher accumulation of proline and ABA. Significant increase in photosynthetic rate (24.62 μ mol m^{-2} s^{-1}) was recorded in trees treated with PP_{333} @ 4.0 g *a.i.* tree^{-1} in November, followed by those treated with PP_{333} @ 4.0 g *a.i.* tree^{-1} in December.

In contrast to photosynthetic rate, the rate of transpiration (0.160 m mol m^{-2} s^{-1}) decreased significantly with PP_{333} @ 4.0 g *a.i.* tree^{-1} in November. Higher ABA accumulation in paclobutrazol treated trees might have decreased stomatal size (Table 3), which may have led to decreased stomatal conductance and consequently decreased transpiration rate. The trees treated with PP_{333} @ 4.0 g *a.i.* tree^{-1} in November had higher proline (2717 μg/g) content in comparison to the control and other treatments. These results are in agreement with the earlier findings of Sharma and Sharma (7).

Paclobutrazol and cycocel treated trees showed marked changes in the endogenous hormonal levels (Table 4). The contents of GA_3 was significantly lower in the trees treated with PP_{333} @ 4.0 g *a.i.* tree^{-1} in November (9.61 hg/g) or December (9.82 hg/g) when compared to other treatments. In this study, lower levels of GA like substances and higher levels of ABA in PP_{333} treated trees could be attributed to paclobutrazol induced changes in their metabolic pathway (Fletcher *et al.,* 3).

Significant differences in nutrient level were also observed among different treatments (Table 5). The highest leaf N (2.07%), P (0.148%), K (1.89%), Ca (1.80%) and Mg (0.284%) contents were recorded in trees treated with PP_{333} @ 4.0 g *a.i.* per tree applied in November, which was significantly higher when compared with rest of the treatments. However, minimum leaf N (1.98%), P (0.109%), K (1.53%), Ca (1.56%) and Mg (0.227%) were recorded in control. These findings are in line with those of Abou- Raya *et al.* (1). Higher accumulation of Ca in PP_{333} treated plants might be due to strong growth retardation effect of PP_{333} resulting in lesser use of Ca.

It is concluded from the present investigation that PP_{333} @ 4.0 g *a.i.* tree^{-1} applied in the month of November as soil drench was helpful in mitigating water stress under rain-fed conditions to increase flowering and fruiting in olive.

Table 3. Effect of growth retardants on stomatal characteristics of olive cv. Leccino.

Treatment	Stomata density (0.04 mm^2)	Stomata length (μm)	Stomata breadth (μm)	No. of primary xylem vessels	Development of secondary xylem (μm)	Stomatal conductance (mol m^{-2}s^{-1})
T_1: PP_{333} 2 g a.i/tree	34.02	13.86	9.58	139.0	46.58	1.25
T_2: PP_{333} 4 g a.i/tree	35.63	13.61	9.37	133.9	43.45	1.51
T_3: PP_{333} 2 g a.i/tree	33.98	14.04	9.68	142.3	48.79	1.27
T_4: PP_{333} 4 g a.i/tree	35.23	13.75	9.46	134.8	43.97	1.22
T_5 :CCC 1000 ppm	32.41	14.17	9.87	147.7	50.98	1.34
T_6: CCC 2000 ppm	33.38	14.33	9.84	142.8	48.40	1.28
T_7 : CCC 3000 ppm	34.35	14.14	9.69	135.4	45.30	1.24
T_8:Control	31.31	14.60	9.97	152.2	54.27	1.38
$CD_{0.05}$	0.66	0.08	0.04	0.89	0.69	0.02

Table 4. Effect of growth retardants on physiological characteristics of olive cv. Leccino.

Treatment	Leaf water potential (-bar)	Transpiration rate (m mol m^{-2} s^{-1})	Photosynthetic rate (µ mo m^{-2} s^{-1})	Proline (µg/g)	GA_3 level (hg/g FW)	ABA level (hg/g FW)
T_1: PP_{333} 2 g a.i/tree	14.72	0.215	23.78	2519	10.62	58.20
T_2: PP_{333} 4 g a.i/tree	15.69	0.160	24.62	2717	9.61	60.74
T_3: PP_{333} 2 g a.i/tree	14.35	0.230	21.98	2457	10.99	57.90
T_4: PP_{333} 4 g a.i/tree	15.45	0.177	24.32	2648	9.82	60.47
T_5 :CCC 1000 ppm	12.70	0.323	19.66	2235	11.17	56.43
T_6: CCC 2000 ppm	14.47	0.237	21.78	2458	10.33	57.56
T_7 : CCC 3000 ppm	15.26	0.193	23.65	2547	9.98	59.14
$T_{8:}$:Control	11.55	0.380	17.86	2056	12.66	54.53
$CD_{0.05}$	0.18	0.006	0.43	37.39	0.25	0.49

Table 5. Effect of growth retardants on leaf nutrient status (% dry weight) of olive cv. Leccino.

Treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)
T_1: PP_{333} 2 g a.i/tree	1.98	0.139	1.77	1.69	0.267
T_2: PP_{333} 4 g a.i/tree	2.07	0.148	1.89	1.80	0.284
T_3: PP_{333} 2 g a.i/tree	1.96	0.136	1.76	1.69	0.264
T_4: PP_{333} 4 g a.i/tree	2.04	0.148	1.88	1.78	0.280
T_5 :CCC 1000 ppm	1.93	0.120	1.68	1.67	0.250
T_6: CCC 2000 ppm	1.96	0.133	1.73	1.69	0.261
T_7 : CCC 3000 ppm	2.03	0.144	1.84	1.76	0.265
$T_{8:}$:Control	1.89	0.109	1.53	1.56	0.227
$CD_{0.05}$	0.02	0.02	0.02	0.03	0.005

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