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EFFECT OF PACLOBUTRAZOL AND CYCOCEL ON GROWTH, FLOWERING AND PHYSIOLOGICAL CHARACTERISTICS OF OLIVE cv. PENDULINO

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Abstract

An experiment was laid out during two subsequent years to ascertain the influence of paclobutrazol and cycocel on growth, cropping and physiological characteristics of Olive cv. Pendulino. The trial was undertaken at the Olive Development Centre, Kigus, District Mandi, Himachal Pradesh, India. In this experiment, eight treatments *viz.* PP_{333} at 2 and 4 g *a.i.* $tree^{-1}$ applied once in November or December as soil drench and cycocel at 1000, 2000 and 3000 ppm applied as foliar spray each year, two weeks after flowering to the trees of olive cultivar Pendulino, with three replications in a randomized block design. Growth retardants have a great potential for controlling the growth, water relations and improving cropping parameters in rain fed conditions. During the study, PP_{333} at 4 g *a.i.* $tree^{-1}$ when applied in November as soil drench decreased growth (15.05 cm), length of internodes (1.84 cm), leaf area (4.09 cm^2), stomatal size (13.75 µm), water potential (-17.84 bar), transpiration rate (0.148 m mol $m^{-2}s^{-1}$), endogenous GA_3 (8.81µg/g fresh weight), stomatal conductance (0.93 mol $m^{-2}s^{-1}$) and increased leaf thickness (0.64 cm), leaf chlorophyll content (2.95 mg/g fresh weight), photosynthesis (19.82 µ mol $m^{-2}s^{-1}$), osmoprotectants *i.e.* Proline (2380 µg/g), ABA (61.23 (µg/g fresh weight), flowering intensity (0.58%) and fruit set (6.64%) and thus might be helpful in mitigating water stress under rainfed conditions.

Key words : Olive, osmoprotectants, pendulino, paclobutrazol, cycocel.

Introduction

The olive (*Olea europaea* L.) is an evergreen tree requires chilling for fruiting. In India, olive cultivation is restricted to the states of Jammu and Kashmir, Himachal Pradesh and Uttrakhand. Although, olive tree has been designated as a drought tolerant yet, it requires sufficient soil moisture during certain stages of growth. Soil and foliar application of growth retardants such as paclobutrazol and cycocel 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. They retard sub-apical meristematic activity, generally without similarly 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 has been reported to reduce vegetative flush and increase proportion of flower bearing shoots. Paclobutrazol application at 500-4000 mg/liter reduced shoot growth, number of nodes, and internode length, total leaf area and at the higher concentration caused bending of the main shoots, but increased shoot number and fruit set in olive (Porlingis and Voyiatzis, 1986). Olive trees treated with paclobutrazol showed increased water potential but decreased stomatal resistance (Frakulli and Voyiatzis, 1999). However, paclobutrazol significantly increased the accumulation of osmolytes such as amino acids and soluble sugars compared with untreated plants, which enable plants to sustain under stressful conditions. Foliar application of cycocel has also been tried to increase the productivity of olive trees by increasing the number of fruits per tree and improving physicochemical characteristics and oil content of fruits. Its application also enhanced transplant growth and improved leaf minerals in olive trees. Cycocel application has also been found to increase the size of panicles and percentage of perfect flowers in the olive cultivars Blanquetta, Serrana and Picual (Hegazi and Stino, 1985). Keeping in view of these points, the present studies were undertaken to examine the effect of growth

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retardants on growth, flowering and physiological characteristics of olive cultivar pendulino under the rain-fed conditions of Himachal.

Materials and Methods

A field experiment was carried out at the 'Olive Development Centre', Kigus, District Mandi, Himachal Pradesh, India (Lat. 32°20' N, long., 77°E and Lat., 1090 m amsl). The climate is sub-temperate with maximum temperature goes up to 35°C during the summer, whereas winters are very cold and receives an annual average rainfall of 80-90 cm, which mostly occurs from July to September months. 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 analyzed. The experiment comprises of four paclobutazol (Cultar) treatments, three cycocel (Vam C) tratmetns and one control. Thus, there were 8 treatment combinations [$\mathbf{T_1}$: PP_{333} 2 g *a.i.*/tree, $\mathbf{T_2}$: PP_{333} 4 g *a.i.*/tree, $\mathbf{T_3}$: PP_{333}2 g *a.i.*/tree, $\mathbf{T_4}$: PP_{333} 4 g *a.i.*/tree, $\mathbf{T_5}$: CCC 1000 ppm, $\mathbf{T_6}$: CCC2000 ppm, $\mathbf{T_7}$: CCC 3000 ppm and $\mathbf{T_8}$: Control. Treatment 1 & 2 applied as soil drenching in the month of Mid November and $\mathbf{T_3}$ & $\mathbf{T_4}$ also as soil drench in the month of Mid December. Paclobutrazol treatments were given during 1st year and not repeated in the following year, instead of their residual effects were studied. Cycocel applied as foliar application two weeks after flowering in treatment $\mathbf{T_5}$, $\mathbf{T_6}$ & $\mathbf{T_7}$. In case of cycocel, treatments were given in the 1st years and 2nd year.

The length of each annual shoot was measured in mid-March and in late August. The internodal length of ten randomly selected 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 Calliper. The observations on the leaf yellowing, leaf rolling, shoot tip bending and cropping parameters were recorded from five randomly selected branches of each experimental tree. The stomata size was recorded with the help of Leica Stereoscopic Microscope. Microscopic studies of anatomical characteristics namely, number of primary xylem vessels and development of secondary xylem were made with the help of LICA DMLB compound microscope, using LICA imaging and analysis software. Soil moisture readings were taken from the four randomly installed access tubes of AquaPro portable soil moisture probe in the experimental orchard at 30 and 60 cm of soil depth, at fortnightly intervals during the growing season. InfraPro thermometer was used to record canopy temperature from all the four sides of tree canopy from a distance of five meter. Leaf water potential was recorded with the help of portable 'Plant Water Status Console' in May and June. Water potential readings were recorded between 10:00 to 12:00 hours by placing freshly detached leaf in pressure chamber. 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. The leaf proline content was estimated according to the method described by Bates *et al*. (1973). Free amino acids were estimated using ninhydrin method. Waters HPLC was used for the estimation of GA_3, Cytokinin and ABA in plant tissue.

Results and Discussion

In the present investigation, different treatments of paclobutrazol and cycocel reduced shoot growth, internodal length, leaf area, leaf yellowing, leaf rolling and shoot tip bending (table 1). Treatment of PP_{333} at 4 g *a.i.* tree^{-1} when applied in November as soil drench was most effective in decreasing the shoot growth (15.05 cm), internodal length (1.84 cm), leaf rolling (3.59%), leaf yellowing (3.05%) and shoot tip bending (4.11%) in cultivar Pendulino. In this study, lower levels of endogenous gibberellin like substances (table 2) 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. Present findings are in line with those of Frakulli and Voyiatzis (1999), who observed a significant decrease in water stress symptoms such as leaf yellowing and rolling and shoot tip bending in olive trees treated with paclobutrazol. Olive trees treated with paclobutrazol and cycocel produced smaller and thicker leaves (table 1). Leaf thickness (0.64 mm) was significantly more in the trees treated with PP_{333} at 4 g. *a.i.* tree^{-1} applied in November or December. However, significant decrease in leaf area was observed following the application of these compounds at higher concentration (PP_{333} at 4 g. *a.i.* tree^{-1} applied in November or December (4.09 cm^2) & CCC at 3000 ppm (4.43 cm^2). These findings are in line with the earlier reports Eliwa and Ashour (2004). Experimental results in table 1 indicate that the trees treated with PP_{333} at 4 g *a.i* tree^{-1} in November (2.95 mg/g fresh weight) or December (2.94 mg/g fresh weight) had significantly higher leaf chlorophyll contents when compared with control and the trees under other treatments. The flower intensity (0.58%) and proportion of perfect flowers (51.70%) in table 1 were significantly increased with the application of paclobutrazol at 4 g *a.i* tree^{-1} in November, followed by Paclobutrazol application at 4 g *a.i.* tree^{-1} in the month of December. Gibberellin in fruit plants is inhibitory to flowering and thus in this study, paclobutrazol might have increased flowering by maintaining lower level of GA in olive plants (table 2), due to its effect on the inhibition of GA biosynthesis (Fletcher *et al*., 2000). Present studies have also revealed a significant influence of paclobutrazol and cycocel on the proportion of perfect flowers, fruit Set, low fruit drop in olive cultivar. However, the higher concentrations of both growth retardants showed greater influence on this flowering attribute. The significant increase in the number of perfect flowers (51.70%), fruit set (6.64%) and lower fruit drop (52.64%) was observed when plants treated with PP_{333} at 4 g *a.i*. tree^{-1} in November, which was statistically at par with the December application of PP_{333} at 4 g *a.i*. tree^{-1}, whereas minimum percentage of perfect flowers (36.97%), fruit set (4.12%) and lower fruit drop (65.10%) was reported in control trees with no chemical treatment. The application of growth retardants might be attributed to their interference with GA biosynthesis, which in turn creates favorable conditions for flower bud differentiation and subsequent development of floral organs. Hegazi and Stino (1985) also observed increase in the proportion of perfect flowers in olive following the application of chlormequat. Thus, it seems that higher production of perfect flowers in paclobutrazol treated trees might have promoted fruit set. During the course of study, the fruit drop was decreased with paclobutrazol applications (table 1).

Paclobutrazol treatment (table 2) when applied at 4 g *a.i*. tree^{-1} in November (31.13/0.04 mm^2) or December (30.85/0.04 mm^2) caused greater increase in stomatal density, whereas, significantly lower stomatal density (27.07/0.04 mm^2) was observed in control. The stomatal size (length and breadth) in olive was however, decreased considerably by paclobutrazol treatments (table 2) over the control. Stomatal length and breadth was significantly lesser in PP_{333} 4 g *a.i*. tree^{-1} in November (13.75 µm) and (9.33 µm) respectively in comparison to all other treatments. However, maximum length and breadth of stomata was observed under control. The present findings are in agreement with those of Blanco *et al*. (1997), who reported that application of paclobutrazol caused a reduction in size and increase in density of stomata. In this study, paclobutrazol treatments caused higher accumulation of ABA in shoots (table 2), which might be responsible for the reduction in stomata size. The increased stomatal density in paclobutrazol and cycocel treated trees can be attributed to the reduced leaf area. It was evident from table 2 that the significant decrease in number of primary xylem vessels (131.1) and development of secondary xylem (42.21 µm) were recorded in the trees treated with PP_{333} at 4 g *a.i*. tree^{-1} in November month when compared with rest of the treatments. However, the number of primary xylem vessels (148.1) and development of secondary xylem (52.59 µm) was significantly highest in control, which was though statistically higher then rest of the treatments. Number of primary xylem and development of secondary xylem were decreased to the greater extent with the application of or December. The present findings are in conformity with those Antognozzi *et al*. (1989), who observed that application of paclobutrazol at the start of vegetative growth decreased cell size and the development of secondary xylem in olive cultivar Pendulino. Leaf water potential (table 2) developed significantly more negative (-17.84) in the trees treated with the PP_{333} at 4 g *a.i*. tree^{-1} in November, compared to the trees under the remaining treatments. However, the highest leaf water potential (-14.65) was found under control. There was a significant difference in each treatment. The decrease in leaf water potential after application of paclobutrazol and cycocel might be due to osmotic adjustments in the plant system brought up by the higher accumulation of osmoprotectants like proline, free amino acids and ABA. Similar finding was reported by Dichio *et al*. (2003), who observed the development of more negative water potential with accumulation of osmoprotectant like Mannitol in olive cv. Coratina. Significant increase in photosynthetic rate (19.82 µ mol m^{-2} s^{-1}) was recorded highest in trees treated with PP_{333} at 4 g *a.i*. tree^{-1} in November, followed by those treated with PP_{333} at 4 g *a.i* tree^{-1} in December. Similarly, Xia *et al*. (1994) found increased photosynthesis in PP_{333} treated trees. In contrast to photosynthetic rate, the stomatal conductance (0.93 m mol m^{-2} s^{-1}) and the rate of transpiration (0.148 m mol m^{-2} s^{-1}) decreased significantly with paclobutrazol application with PP_{333} at 4 g *a.i*. tree^{-1} in November during the investigation. Higher ABA accumulation (table 2) in paclobutrazol treated trees might have decreased stomatal size (table 2), which may have led to decreased stomatal conductance and consequently decreased transpiration rate. There was a marked influence of growth retardants on leaf proline and free amino acid contents of olive cultivar Pendulino. The trees treated with PP_{333} at 4.0 g *a.i*. tree^{-1} in November or December had higher proline (2380 µg/g) and free amino acid (25.17 µg /g) content in their leaves in comparison to the control and the trees under the remaining treatments. Cycocel treatment at 3000 ppm also caused markedly increases in the leaf proline and free amino acid content. These results are in agreement with the earlier findings (Xia *et al*., 1994; Sharma and Sharma, 2009) that paclobutrazol application increased proline accumulation

Table 1 : Effect of growth retardants on growth, flowering and fruiting characteristics of olive cultivar Pendulino growing under rain fed conditions of Himachal Pradesh.

Cultivars	Growth (cm)	Length of internodes (cm)	Leaf area (cm^2)	Leaf thickness (mm)	Leaf chlorophyll content (mg/g fresh weight)	Leaf yellowing (%)	Leaf rolling (%)	Shoot tip bending (%)	Flowering intensity (%)	Perfect flower (%)	Fruit set (%)	Fruit drop (%)
T_1 : PP_{333} 2 g *a.i*/tree (SA)	16.43	2.04	4.64	0.60	2.80	3.26	4.32	5.15	0.53	46.50	5.80	56.20
T_2 : PP_{333} 4 g *a.i*/tree (SA)	15.05	1.84	4.09	0.64	2.95	3.05	3.59	4.11	0.58	51.70	6.64	52.64
T_3 : PP_{333} 2 g *a.i*/tree (SA)	16.57	2.07	4.71	0.59	2.79	3.55	4.57	5.29	0.52	45.53	5.67	57.64
T_4 : PP_{333} 4 g *a.i*/tree (SA)	15.42	1.87	4.29	0.62	2.94	3.12	3.88	4.46	0.57	50.43	6.83	54.14
T_5 : CCC 1000 ppm (FA)	16.83	2.26	4.74	0.57	2.67	3.78	4.95	5.53	0.47	41.48	5.03	63.79
T_6 : CCC 2000 ppm (FA)	16.02	2.12	4.64	0.60	2.77	3.69	4.67	5.97	0.49	43.82	5.80	58.09
T_7 : CCC 3000 ppm (FA)	15.38	2.00	4.43	0.62	2.91	3.69	3.87	4.58	0.51	48.82	6.36	54.89
T_8 : Control	19.06	2.44	4.87	0.55	2.63	4.17	5.58	6.51	0.44	36.97	4.12	65.10
CD=0.05	0.33	0.09	0.06	NS	0.03	0.12	0.10	0.07	0.01	1.15	0.12	1.34

Table 2 : Effect of growth retardants on physiological characteristics of olive cultivar pendulino growing under rain fed conditions of Himachal Pradesh.

Cultivars	Density of stomata (No/0.04 mm^2)	Length of stomata (µm)	Breadth of stomata (µm)	No. of primary xylem vessels	Development of secondary xylem (µm)	Stomatal conductance (mol m^{-2} s^{-1})	Leaf water potential (-bar)	Transpiration rate (m mol m^{-2} s^{-1})	Photosynthetic rate (µ mo m^{-2} s^{-1})	Proline (µg/g)	Free amino acids (µg /g)	GA_3 level (µg/g fresh weight	Cytokinin (Zeatin) level (pg/g fresh weight)	ABA level (µg/g fresh weight)
T_1 : PP_{333} 2 g *a.i*/tree (SA)	29.93	14.06	9.49	137.4	46.02	0.96	16.23	0.183	17.94	2104	21.05	9.08	85.71	59.33
T_2 : PP_{333} 4 g *a.i*/tree (SA)	31.13	13.75	9.33	131.1	42.21	0.93	17.84	0.148	19.82	2380	23.29	8.81	90.84	61.23
T_3 : PP_{333} 2 g *a.i*/tree (SA)	29.78	14.21	9.61	138.3	46.66	0.97	16.04	0.195	17.33	2103	20.58	9.65	86.46	58.96
T_4 : PP_{333} 4 g *a.i*/tree (SA)	30.85	13.83	9.41	132.0	43.97	0.95	17.36	0.160	19.28	2286	22.45	8.81	90.48	60.88
T_5 : CCC 1000 ppm (FA)	28.09	14.45	9.78	142.6	50.04	1.01	15.75	0.238	15.20	1966	18.79	10.33	85.13	56.86
T_6 : CCC 2000 ppm (FA)	29.44	14.27	9.80	139.2	45.59	0.98	16.39	0.202	17.19	2042	20.28	9.72	85.94	58.32
T_7 : CCC 3000 ppm (FA)	30.20	14.34	9.67	132.7	43.33	0.96	17.09	0.168	18.19	2243	22.52	9.06	87.25	59.97
T_8 : Control	27.07	14.66	9.90	148.1	52.59	1.03	14.65	0.292	14.63	1793	17.71	10.69	84.10	55.32
CD=0.05	0.66	0.08	0.04	0.89	0.69	0.02	0.18	0.006	0.43	37.39	0.33	0.25	1.18	0.49

in the leaves of the treated fruit trees. Anti-gibberellin properties of these growth retardants coupled with increase in ABA accumulation in the PP_{333} and CCC treated trees might have leads to more synthesis of free amino acids in the plant. As evident from the table 2, paclobutrazol and cycocel treated trees showed marked changes in the endogenous hormonal levels as the concentrations of GA_3, ABA and cyotkinin significantly differ among different treatments. The contents of GA_3 (8.81 µg/g) was significantly lower in the trees treated with PP_{333} at 4.0 g *a.i.* tree^{-1} in November or December when compared to rest of the treatments. Increase in the levels of Cytokinin (90.84 µg /g) and ABA (61.23 µg /g) concentration after PP_{333} at 4.0 g *a.i.* tree^{-1} application in November, which was statistically at par with PP_{333} at 4.0 g *a.i.* tree^{-1} of December application. Minimum levels of Cytokinin (84.10 µg/g) and ABA (55.32 µg/g) was observed under control. 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 disturbances in their metabolic pathway (Fletcher *et al.*, 2000). Paclobutrazol inhibit the oxidation of ent-Kaurene to ent-Kaurinoic acid through inactivating cytochrome P450-dependent monooxygenases and thereby blocks the GA biosynthesis. Paclobutrazol also block the catabolism of ABA to phaseic acid and di-phasic acid and other conjugates and thus might be responsible for the buildup of ABA levels in plants (Fletcher *et al.*, 2000). Chlormequat inhibit the conversion of geranylgeranylpyrophosphate to ent-kaurene and thereby may have decreased the endogenous gibberellins in this study. Present results also revealed that cytokinin level increased with the paclobutrazol treatments. Similar observation was made by Zhu *et al.* (2004), who found increase in the levels of cytokinin in paclobutrazol, treated apple and mango trees, respectively.

Therefore, it can be concluded from the field investigation that growth retardants have a great potential in controlling growth, influencing water relations and improving cropping parameters under rain fed conditions. Specifically when growth retardants like PP_{333} at 4 g *a.i.* tree^{-1} applied in the month of November as a soil drench, decreased growth, stomata size, stomatal conductance, transpiration rate and endogenous GA_3 and increased photosynthesis, leaf Cytokinin level, osmoprotectants like leaf proline, and free amino acids, leaf ABA level, flowering intensity and fruit set and thus might be helpful under drought/rain fed conditions.

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