Research Article

Influence of Dormex on the Biochemical Dynamics and Bud Break of Dormant Kiwifruit (*Actinidia deliciosa* Chev.) Vines

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Abstract

The study comprised of seven treatments viz., T_1 (DORMEX 2%), T_2 (DORMEX 2% + Mineral oil 2%), T_3 (DORMEX 4%), T_4 (DORMEX 4% + Mineral oil 2%), T_5 (DORMEX 6%), T_6 (Mineral oil 2%) and T_7 (control-water spray). Dormex was sprayed approximately, 45 days before natural bud break as foliar spray. From two years study, it was elucidated that Dormex 4 per cent along with mineral oil 2 per cent markedly increased bud peroxidase activity and decreased catalase activity as compared to other treatments. This treatment also resulted in highest C/N during different sampling dates. Application of Dormex 4 per cent along with mineral oil 2 per cent resulted in an advancement of bud break by 9-10 days, flower bud emergence by 9 and full bloom by 9-11 days, fruit set was advanced by 11-12 days and resulted in maximum total fruit yield as compared to control.

Keywords: Dormex, kiwifruit, catalase, peroxidase, C/N, bud break, full bloom, fruit set, total fruit yield

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Introduction

The kiwifruit (*Actinidia deliciosa* Chev.) is a deciduous and dioecious vine native to the Yangtze River valley of northern China [1]**.** It has gone enormous popularity in parts of the world including India, owing to its refreshing delicate flavor, pleasing aroma with high nutritive and medicinal value. Although, the kiwifruit was introduced in India about six decades back but the commercial importance has been realized only during last two decades in sub-Himalayan region of the country. With the changing climatic scenario the conditions for temperate fruit cultivation specially, apple, pear and cherry have become marginal in the northern part of India. This has paved the way for some new exotic crops like kiwifruit which has shown tremendous potential for cultivation in the mid-hill region. The research efforts done for commercialization of this important crop at Dr. Y S Parmar University of Horticulture and Forestry, Nauni-Solan (H.P.) have recommended Allison cultivar due to precocity, prolific bearing habit and regularity in bearing. However, the Hayward cultivar has shown shy bearing habit which may be attributed to insufficient chilling.

In order to overcome low chill occurrence, application of dormancy breaking agents like Hydrogen Cyanamide and mineral oil have been a practice in orchards [2-4]. Therefore, the effect of insufficient winter chilling can be reduced by an application of dormancy breaking chemical namely; Dormex (49 % Hydrogen Cyanamide). It has been recognized that the Hayward variety of kiwifruit has the highest winter chilling requirement among commercial varieties of kiwifruit [5]. It has been pointed out that changes in the activities of enzymes especially, peroxidase and polyphenol oxidase (PPO) lead to the sprouting of buds [6].

The chilling is required to cause the transition of both vegetative and floral buds of temperate or semi-deciduous subtropical fruit species from dormant to active state [7]. In addition to increased and highly synchronized bud break, the use of hydrogen cyanamide is also known to increase the number of flowers per shoot and synchronizes the flowering period of pistillate cultivars. Addition of mineral oil to several chemical compounds with a view to break the dormancy has been found to enhance the effect of such chemicals [8]. Thus, the present investigation was carried out to study the influence of Dormex on the biochemical changes associated with the fruit bud differentiation process in Hayward kiwifruit.

Experimental details

Plant materials and experimental procedure

The experiment was conducted in the Kiwifruit Block of Department of Fruit Science, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni-Solan, H.P. (India) during 2015-16, located at $30^0$51'N latitude. Twelve years old vines of Hayward kiwifruit, planted at a distance of 4m × 6m, trained on T-bar system were selected for the study which comprised of seven treatments viz., T_1 (DORMEX 2%), T_2 (DORMEX 2% + Mineral oil 2%), T_3 (DORMEX 4%), T_4 (DORMEX 4% + Mineral oil 2%), T_5 (DORMEX 6%), T_6 (Mineral oil 2%) and T_7 (control-water spray). Dormex was sprayed approximately, 45 days before natural bud break immediately after pruning as a foliar spray.

Enzyme Assay for Catalase and Peroxidase

The analyses for catalase and peroxidase activities were done at 10 days after the date of Dormex application at weekly interval and determined as per the method suggested by Thimmaiah [9], slightly modified. For catalase, one gram of wood sample was ground with 0.1M phosphate buffer of pH 7.0 in a pre-chilled mortar and pestle. The homogenate was centrifuged at 15,000 rpm for 30 min at 4°C and the supernatant was used as enzyme extract. Three ml of phosphate buffer, 2ml of hydrogen peroxide (H_2O_2) and 1ml of enzyme extract were pipetted out into test tube and then the tubes were incubated at 20°C for one minute. The reaction was then stopped by adding 10ml of 0.7N H_2SO_4. The reaction mixture was titrated against 0.01M $KMnO_4$ to find residual H_2O_2 until faint purple color persist for atleast 15 seconds. The blank was prepared by adding the enzyme extract to acidified solution of reaction at zero time. One unit of catalase was defined as that amount of enzyme which breakdown 1µm mole of H_2O_2 under standard assay condition.

Peroxidase was extracted by homogenizing one gram of sample with 0.1M phosphate buffer of pH 6.0 in a chilled pestle and mortar using sand as abrasive. Homogenate was stained through two fold of muslin cloth and centrifuged 16,000 rpm for 20 minutes at 4°C. The supernatant was used as enzyme extract. One ml of O-dianisidine, 0.5 ml of H_2O_2, 1ml of phosphate buffer and 2.4 ml of distilled water pipetted out in a test tube. For blank, H_2O_2 was excluded and additional volume of water added. The test tubes were incubated at 30°C and reaction was initiated by adding 0.2 ml of enzyme. After 5 minute, 1ml of 2N H_2SO_4 was added to terminate the reaction and absorbance was read at 430 ηm on spectrophotometer. The increase in absorbance was plotted against time and from linear phase; the change in absorbance was read considering one unit of enzyme causing an increase in OD by 1.0 under standard conditions.

Carbohydrate nitrogen ratio

Carbohydrates content

Carbohydrate content of dormant shoots was estimated by Anthrone reagent method as suggested by Thimmaiah [9]. The 100 mg of sample was crushed and taken in the test tube and then hydrolysed by keeping it in boiling water bath for 3 hours with an addition of 5 ml of 2.5 N HC1. The sample solution was cooled to room temperature. The solid sodium carbonate was added until the effervescence ceased. After the neutralization, the volume of contents was made to 100 ml with distilled water and centrifuged. The supernatant was used for estimation of carbohydrate content.

One ml of aliquot was taken in the test tube for analysis and 4 ml of Anthrone reagent was added to it. The mixture was heated for 8 minutes in boiling water bath. The contents were cooled rapidly and the intensity of green to dark green colour was measured at 630 nm on UV-VIS Spectrophotometer. The standard curve was prepared with glucose and the carbohydrates content present in the samples was expressed in per cent. The carbohydrate present in the sample was worked out by the following formula:

$$\text{Amount of corbohdrate}(\%) = \frac{\text{Sugar value from graph}(\text{mg})}{\text{Aliquot sample used}(1\ \text{ml})} \times \frac{\text{Total volume of exract (ml)} \times 100}{\text{Weight of sapmles}(\text{mg})}$$

Digestion and estimation of samples for Nitrogen

One gram well dried and ground wood samples were used for estimation of nitrogen. The samples were digested on automatic digestion system using one gram of digestion mixture and 20 ml of concentrated

sulphuric acid. The digestion mixture was prepared by mixing 400 parts potassium sulphate, 20 part copper sulphate. The boiling of samples was continued till the appearance of light blue color. The samples were cooled and diluted to 100 ml with distilled water. Nitrogen was determined by Kjeltec Auto 2300 Analyzer.

Bud break and blooming characteristics

The time of bud break was recorded by randomly tagging of 10 shoots/ vine when bud break was distinctly visible. The emergence of flower buds was also recorded on these tagged shoots and marked as date of bud break. The date of full bloom was recorded when more than 75 per cent flowers opened and the date of fruit set when all petals dropped after complete fruit setting.

Total fruit yield

The total fruit yield was determined on the basis of total weight of fruits harvested from the vine under each treatment and average yield per vine was calculated. This is a long term project so the yield was recorded in the first year of experiment. The yield was expressed in kilogram per vine (kg/vine).

Statistical analysis of data

The data obtained from the investigation were statistically analyzed according to Randomized Block Design and the differences exhibited by different treatments were tested for their significance as per the procedure described by SPSS [10].

Results and Discussion

Effect on Dormex on enzyme activity

The activities of catalase and peroxidase enzymes were significantly affected by the application of Dormex and Mineral oil, sprayed immediately after pruning. The activity of CAT was highest in control vines during both the years of study. From the pooled data illustrated in **Figure 1**, it was inferred that the minimum catalase activity (43.17 Ug^{-1}) was observed with the treatment of Dormex 4 per cent + mineral oil 2 per cent (T_4). This treatment resulted significantly higher than all other treatments. It was observed that the catalase activity reduced progressively towards the date of bud break from date of Dormex application. The rate of catalase activity after 17, 24 and 31 days after application remain minimum with treatment T_4, recording 39.67, 34.67 and 23.00 Ug^{-1} catalase activity and followed a declining trend towards date of bud break. Similar trends for catalase activity were noticed during 2015 and 2016.

The maximum peroxidase activity was observed when vines were treated with Dormex 4 per cent along with mineral oil 2 percent before 45 days of anticipated date of bud break. This treatment recorded 57.83, 62.67, 68.17 and 73.17 Ug^{-1} peroxidase activity after 10, 17, 24, and 31 days of application, respectively during 2015-16. The peroxidase activity exhibited an increasing trend towards bud break. This treatment was closely followed the sole application of Dormex treatments i.e. Dormex 6% (T_5) and Dormex at 4 per cent (T_3) during both the years. The peroxidase activity followed a declining trend with the passage of time towards bud break (**Figure 2**).

Dormex (Hydrogen cyanamide) penetrates in the bud scale better and gets absorbed in the buds which initiate the process leading to bud break [11]. Dormex is rapidly metabolized in plants and helps in the synthesis of amino acids in catalase activity [6, 12]. Catalase plays a very important role in the plants because it detoxifies hydrogen peroxide by catalysing its breakdown to water and oxygen. When the action of catalase is inhibited by Dormex, the plant detoxifies hydrogen peroxide via a sequence of reactions which are eventually coupled with the oxidative pentose phosphate pathway (PPP). Dormex stimulates these reactions, which in turn leads to an increase in the rate of turnover of the PPP. Due to stimulation of the PPP, a range of substances responsible for new growth are produced at higher rates. In kiwifruit, it has been reported that Dormex can advance the date of bud break by 10 to 15 days [13].

The results pertaining to enzyme activity obtained in the present investigation are in conformity with Haggag et al. [14], who conducted an experiment at Alexandria University on 6-year-old Banati pomegranate trees. These trees were sprayed with 0, 0.5, 1, 2 or 4 per cent Dormex (Hydrogen Cyanamide) in January or February. They found that Dormex at 4 per cent markedly increased bud peroxidase activity and decreased catalase activity. Mohamed et al. [15]

investigated the effect of hydrogen cyanamide (HC) at 2% on dormancy release and carbohydrates metabolism in the bud and underlying internode tissues of Superior Seedless grapevines.

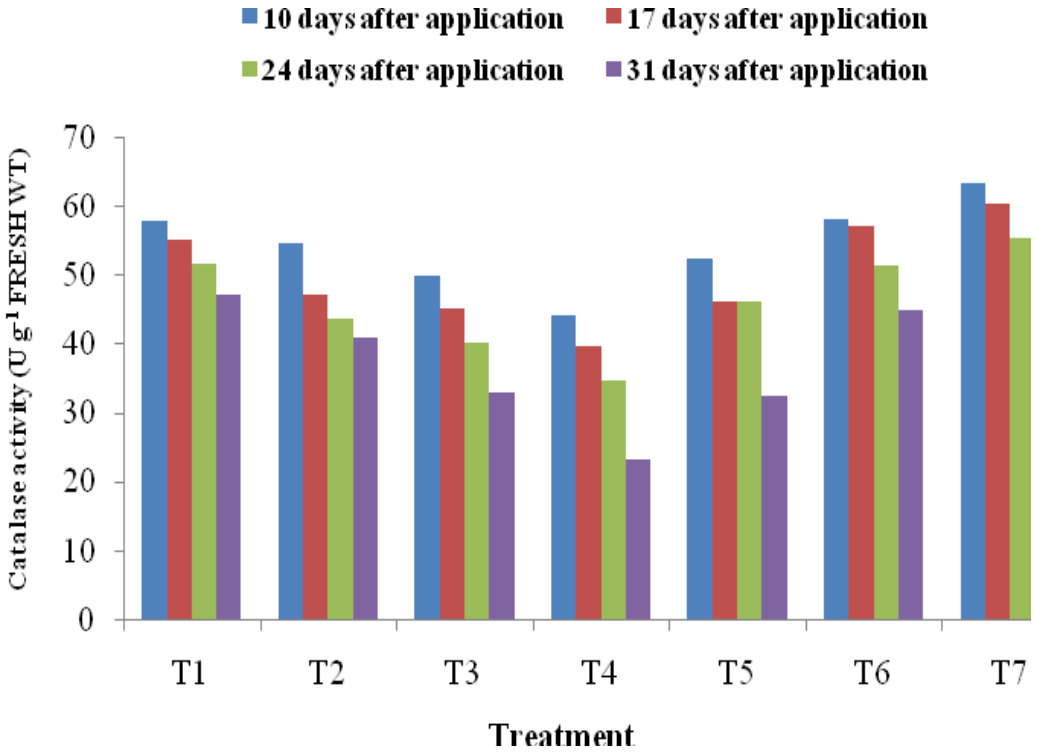

Figure 1 Effect of Dormex on the time course changes in Catalase activity of dormant kiwifruit vines cv. Hayward during 2015-16

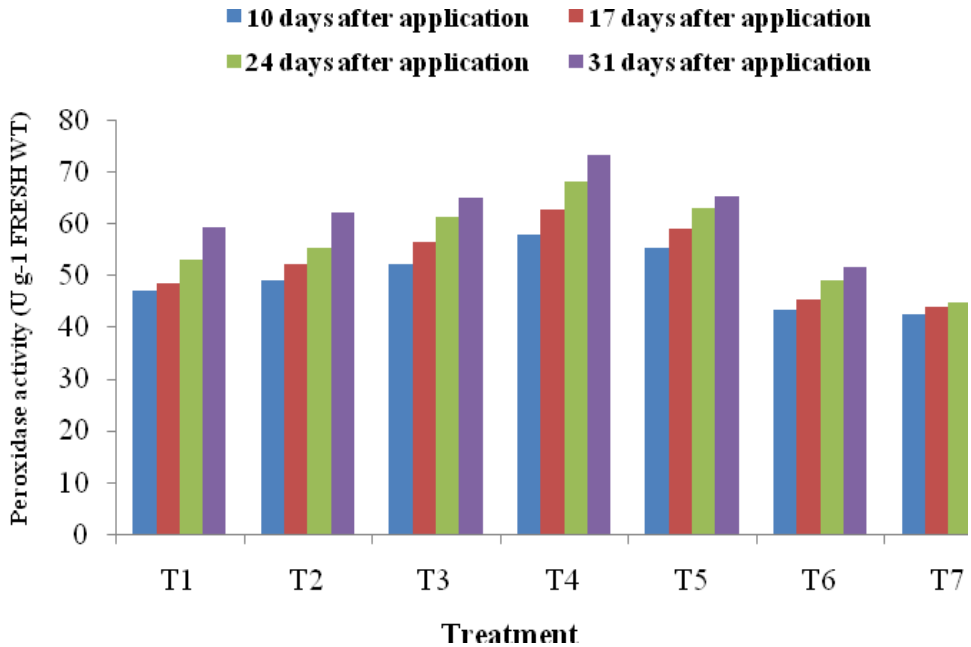

Figure 2 Effect of Dormex on the time course changes in Peroxidase activity of dormant kiwifruit vines cv. Hayward during 2015-16

Effect of Dormex on carbohydrate nitrogen ratio

The change in concentration of carbohydrate and nitrogen ratio (C/N) as consequence of Dormex coupled with Mineral oil application was also observed in the vines under study. It was revealed from the data that the synergetic effect of Dormex (4%) and Mineral oil (2%) resulted, a rapid increase in C/N and greatly contributed to the early bud break (**Figure 3**). The influence of Dormex (4%) along with Mineral oil (2%) application resulted in highest C/N in kiwifruit vines after 10th, 17th, 24th and 31st days of Dormex application during both the years under study.

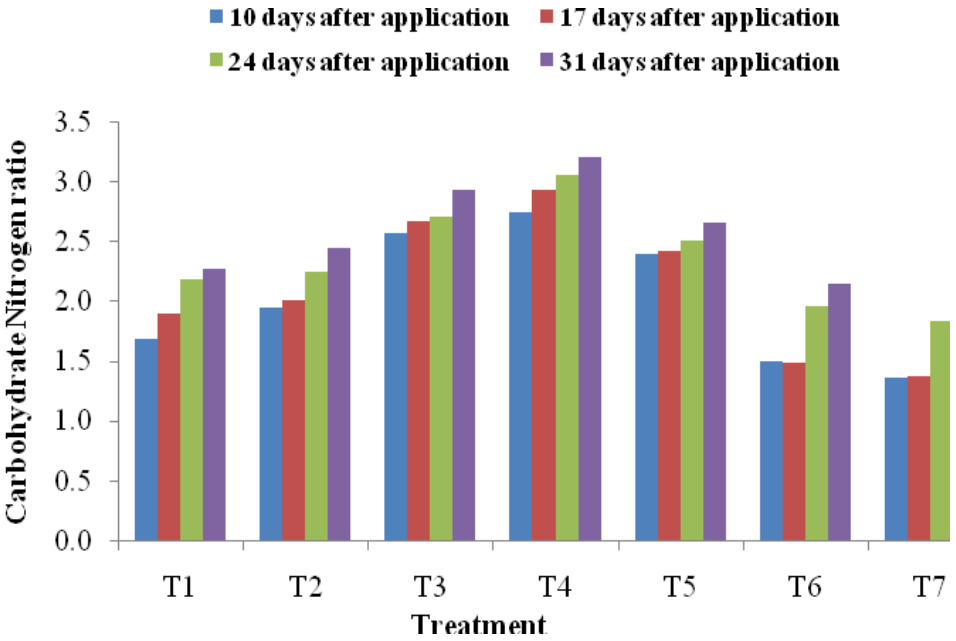

Figure 3 Effect of Dormex on the time course changes in carbohydrate nitrogen ratio of dormant kiwifruit vines cv. Hayward during 2015-16

The high content of carbohydrates accumulation in the treated vines might be due to synthesis of growth substances which might lead to the early bud break. El-Sabrout [16] reported that grape vines sprayed with Dormex at 6.0 per cent had significantly higher leaf N, P, Ca, Mg, Fe contents, bud total carbohydrates and total amino acids compared to the other treatments sprayed on 21 January. This treatment also significantly increased bud IAA and gibberellic acid contents, and significantly reduced bud ABA contents as compared to the control treatment.

Effect of Dormex on bud break

The application of Dormax and mineral oil lead to the early bud break of Hayward kiwifruit. During both the years of study, it was observed that the maximum advancement in bud break was noticed when Dormex (4%) along with Mineral oil (2%) was applied about 7 weeks before anticipated date of bud break. The date of bud break during 2015 and 2016 were observed on 11th March and13th March in vines, treated with Dromex 4% with mineral oil 2%. This indicated an advancement of 9 and 10 days than the vines under control where bud burst was recorded on 20th and 23rd March, respectively, during 2015 and 2016 (**Table 1**). This treatment was closely followed by sole application of Dormex 4%.

During both the years under study, the maximum advancement in bud break was noticed on when vines were treated with Dromex 4% with mineral oil 2% exhibited an advancements of 9 and 10 days than the control vines. The results are in line with the finding of Manzi et al., [17] who reported that an early application of mineral oil at 2 per cent induce early reproductive sprouting and advanced harvest date by 13 days in 'Royal Gala' apples. Similarly, Singh and Mann [18] observed in 20 year old pear cv. Pathernakh that the time of flowering, full bloom and fruit set were advanced by 10-12 days with Dormex application.

Table 1 Effect of Dormex on the bud break of kiwifruit cv. Hayward

Treatment Details	Time of bud break	
	2015	2016
T_1: DORMEX 2%	14/03	16/03
T_2: DORMEX 2% + Mineral oil 2%	13/03	15/03
T_3: DORMEX 4%	12/03	14/03
T_4: DORMEX 4% + Mineral oil 2%	11/03	13/03
T_5: DORMEX 6%	16/03	18/03
T_6: Mineral oil 2%	19/03	21/03
T_7: Control (water spray)	20/03	23/03

Effect of Dormex on the blooming characteristics, fruit set and total fruit yield

Application of Dormex and Mineral oil also exerted almost similar trend on the blooming characteristics. The date of flower buds emergence was recorded on 14th and 15thApril in vines sprayed with 4 per cent Dormex along with mineral oil 2 per cent during 2015 and 2016, respectively. This treatment advanced emergence of flower buds by 9 days in both the years and was closely followed by sole Dormex application at 4 per cent. However, the dates of flower bud emergence in control vines were observed on 23rd and 24th April during 2015 and 2016, respectively (**Table 2**). Similarly, earliest date of full bloom was noticed on 24thApril during both the years under study with the treatment of Dormex (4%) + Mineral oil (2%), advancing the full bloom date by 9 days during both the years. This treatment was closely followed by sole application of Dormex (4%) noticing, full bloom on 25th and 26th April during 2015 and 2016, respectively. This treatment advanced the date of full bloom by 6 days in comparison to control.

Table 2 Influence of Dormex on the blooming characteristics and fruit set of Hayward kiwifruit

Treatment	Time of flower bud emergence		Time of Full Bloom		Time of Fruit Set	
	2015	2016	2015	2016	2015	2016
T_1: DORMEX 2%	17/04	19/04	28/04	29/04	07/05	08/05
T_2: DORMEX 2% + Mineral oil 2%	16/04	18/04	26/04	28/04	04/05	06/05
T_3: DORMEX 4%	15/04	17/04	25/04	26/04	02/05	03/05
T_4: DORMEX 4% + Mineral oil 2%	14/04	15/04	24/04	24/04	01/05	02/05
T_5: DORMEX 6%	19/04	21/04	29/04	30/04	07/05	08/05
T_6: Mineral oil 2%	22/04	23/04	02/05	03/05	11/05	13/05
T_7: Control (water spray)	23/04	24/04	03/05	05/05	12/05	14/05

During both the years, the vines sprayed with Dormex at 4 per cent in combination with 2 per cent mineral oil resulted in earliest fruit set i.e. on 1st May in 2015 and 2nd May in 2016 which advanced the fruit set by 11 and 12 days. This treatment was closely followed by the vines sprayed with Dormex at 4 per cent resulting fruit set on 2nd May and 3rd May during 2015 and 2016, exhibiting advancement of 10 and 11 days, respectively over control (Table 2). A perusal of the data revealed that total fruit yield per vine was significantly increased by the different concentrations of Dormex alone and in combination with mineral oil. The highest total fruit yield (38.00 and 41.00 Kg) was recorded with the treatment T_4 in comparison to other treatments. However, lowest fruit yield was recorded in control vines during the year 2015 and 2016, respectively (**Figure 4**).

The results pertaining to blooming characteristics are in conformity with the findings of Chauliaras et al. [19], who observed that the application of Hydrogen cyanamide (Dormex), 45 days before the bud break advanced blooming and fruit set by 12 to 14 days in kiwifruit cv. Hayward. Similarly, Pandey [20] in grapes, Powell [21] in kiwifruit and Cheng [22] in pear reported an advancement of flowering by 12-13 days and fruit set by 10 to 12 days with 4 per cent Dormex application. Hydrogen cyanamide (1 to 2%) coupled with mineral oil (4%) accelerated budburst, the flowering and reduced the flowering period, reducing considerably the symptoms of delayed foliation of mature 'Golden Delicious' apple trees. Budburst increased with Dormex concentration and was calculated 45 per cent more at the 1 and 2 per cent rate on one-year-old wood Sagredo et al. [23]. Results obtained on the total fruit yield are in agreement with Cheng [22] who, reported that hydrogen cyanamide was effective in increasing the yield of 8 year

old kiwifruit cv. Bruno and yield enhancement was attributed to the per cent increase in bud break and synchronization of flowering.

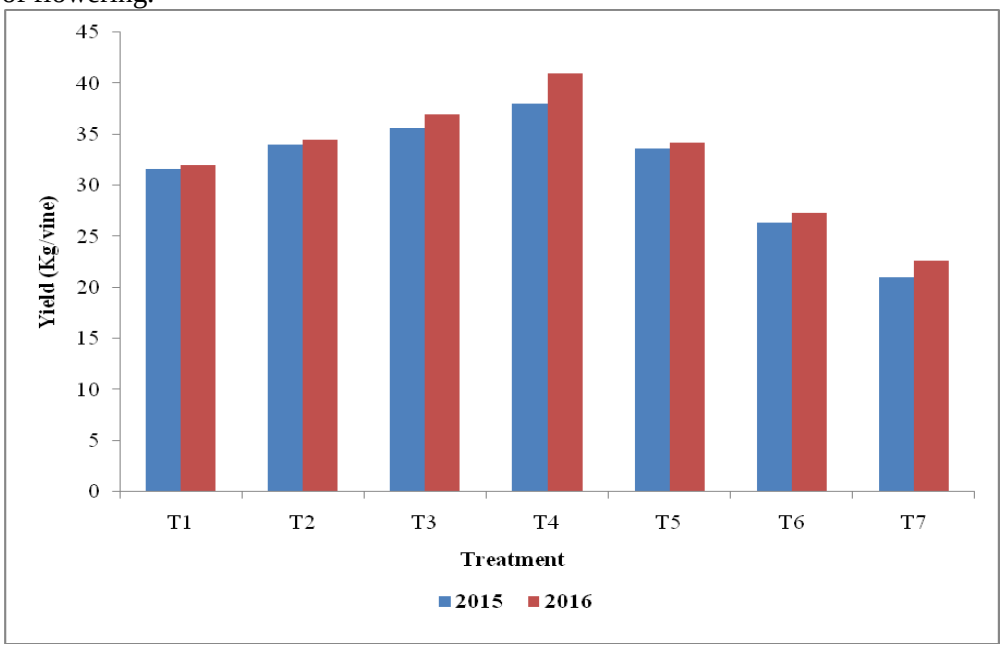

Figure 4 Effect of Dormex on the total fruit yield of kiwifruit cv. Hayward

Conclusions

In the present study, Dormex 4 (%) along with mineral oil 2 (%) was applied 45 days before the anticipated date of bud break, exhibited synergetic effect and markedly decreased catalase (CAT) activity and increased bud peroxidase (POD) activity. It was also concluded that the vines resulted in early bud break and contained highest C/N as compared to untreated kiwifruit vines. An advancement of 8 to 9 days in bud break was observed when Dormex (4%) along with mineral oil 2 (%) and this treatment was adjudged to be the best among all treatments. The time of flower bud emergence, full bloom and fruit set also observed more or less similar pattern as that of bud break during both the years. It was also inferred that advancement in full bloom due to Dormex application lead to synchronization of flowering with staminate cultivars, executing higher fruit set and fruit yield under mid-Himalayan conditions.

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