EFFECT OF 2, 4 D ON FRUIT DROPPING AND BIOCHEMICAL PROPERTIES OF LITCHI CV. Bedana

A THESIS

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JOTY RANI ROY

Student No. 1707114

Thesis Semester: January-June 2024

MASTERS OF SCIENCE (MS)

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DEDICATED

TO MY

BELOVED PARENTS

&

HONOURABLE TEACHERS

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Abstract

The experiment was carried out in a litchi orchard at Shikhderhat, Dinajpur sadar to investigate the dropping of litchi fruits and biochemical nutrients of ripen fruits during April to June, 2023 to investigate the effect of 2, 4 D on fruit dropping and biochemical properties of litchi cv. Bedana. 2, 4 D was applied twice @ 0, 20, 30 and 40 ppm. Parameters like inflorescence number, fruit retention, fruit crack, weight of fruit, seed and pulp, chemical and biochemical properties like ash, acidity, TSS, fibre, ascorbic acid, total sugar, total phenol content, minerals and fruit yield /tree were monitored. Plant growth regulator (2, 4-D) is known to have a great influence on fruit drop and biochemical properties of litchi. The highest of fruit dropping percentage was found in T_0 (38.51) and the lowest percentage value was obtained in T_1 (2.00) respectively. The highest number of litchi fruits was obtained by reducing fruit dropping in the plants spraying with 20 ppm 2, 4-D (T_1) and the lowest number was found in control (T_0). The highest fruit weight value was found in T_1 (24.05 g) and the lowest value was found in T_0 (17.52 g). Litch fruit cv. Bedana contained the highest 82.68 % moisture in T_3 treatment, 0.41 %Ash in T1 and 0.57% fiber in controlled treatment. The highest vitamin C was 25.53 mg 100 g^{-1} found in control (T_0) and the lowest value found in 20 ppm 2, 4-D (T_1) was 13.86 mg 100 g^{-1}. The highest total sugar content was 26.76 mg 100 g^{-1} contained in the fruits collected from the plants spraying with 20 ppm 2, 4-D (T_1) and the lowest total sugar was 19.63 mg 100 g^{-1} found in control (T_0). The highest protein content was 1.64 present in the fruits treated with 20 ppm 2, 4-D (T_1) and the lowest value present in control 1.15. Therefore, spraying of 20 ppm (T_1) 2, 4-D twice might be suitable for reducing fruit dropping and improved biochemical properties of litchi cv. Bedana.

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CHAPTER I

INTRODUCTION

Litchi (*Litchi chinensis L.*) is considered as one of the kings of sub-tropical fruits and popular for its delicious taste in Bangladesh. Litchi are attractive, tasty, highly nutritive (Singh *et al.*, 2012, Srivastava *et al.,* 2015) and worldwide cultivated fruits (Vercammen and Schmitz, 2001, Singh *et al.*, 2019). Litchi is a highly priced, popular and major table fruit. In Bangladesh, the greater Dinajpur, Rangpur, and Pabna districts are known for commercial litchi production, although it is cultivated throughout the country (FAO, 2001, Ahad *et al.*, 2010). Commercial litchi cultivation has become widespread because of its increasing demands for its excellent quality such as juiciness, slightly sour-sweet taste, characteristics pleasant flavor and attractive colorin the local and international markets. In 2, over eighty thousand 08 metric tons of litchi were produced from over 21759 acres area of litchi orchards in Bangladesh (BBS, 2023).

In Bangladesh, there are mostly cultivating high yielding varieties like China- 3, 'Bedana', Bombay, 'Mozaffarpuri' and Madrazi and late variety. From those varieties, *Bedana* variety is juicy and larger in size than other litchi varieties available in the country. *Bedana* litchi cultivar is two types: a) Early *Bedana* this cultivar ripens early and usually have small seeds. This may be a reason for calling this variety 'Early Seedless' in Punjab. It is a medium fruit yielding cultivar and fruits are generally of 15-18g weight having oval or heart shape, rough surface with uranium green skin (Singh and Babita, 2002). The skin is covered with red tubercles at maturity (Kumar, 2011). The pulp of the fruit is white, soft and juicy while the seed is very small, shrunken, shiny and the pulp ranges between 7.2-19. b) Late *Bedana*: The Late *Bedana* or Late Seedless is late ripening cultivar yielding fruit 60- 80kg per tree. This cultivar is sized medium but the pulp content is more. Fruits are of conical shape with vermillion to carmine color along with dark blackish brown tubercles at maturity. The TSS content of pulp is 18 20 B with low acidity. Though the farming of this variety is available in some other parts, the variety grown in the soil of northern part (Dinajpur) of Bangladesh has a special aroma, taste and sweetness. The price of this litchi variety is higher too than other varieties because of it has a special appeal to its consumers.

Fruits are important as food because they have sufficient amount of vitamin and mineral. We should eat 115 grams fruit every day. Fruits increase our digestive power. An intake of fruit everyday keeps us hale and hearty. Litchi is extremely rich in vitamin-C than orange. Litchi

contains 77.83% water, 6.74-20.60% sugar, 0.08-.09% protein, 0.03% fat, mineral specially calcium, phosphorus and 0.7% iron and vitamin 40.2-90 mg/100 g C of fruits (Bose and Mitra, 2017). Litchi makes an excellent canned fruit. A highly flavored squash is also prepared from the litchi fruits, which is used during summers. Various other products such as pickles, preserves and wine are also made from litchi in China. Dried litchi commonly called litchi-nut is very popular among the Chinese.

More than several varieties of sweet edible litchi can be found in Dinajpur district. It is estimated that around 85% people of the mentioned districts are directly or indirectly dependent on litchi cultivation and business (Dhaka Tribune, 2018). Among the main constituents of this fruit, carbohydrates and acid contribute a great deal to the food value of the fruit. High production of litchi has been accompanied by high post-harvest loss of mango because excess fruits in the market during the peak seasons.

Litchi cultivation and its potential yield is affected by many physiological disorders like fruit cracking and splitting, flower and fruit drop, sunburn, retarded fruit development, irregular bearing and black spot etc. Therefore, it is necessary to know the causes and symptoms properly to manage these disorders for increased quality production. The incidence and severity of these physiological disorders vary with locality, season, cultivar and orchard management practices. Fruit drop is one of the widest spread problems of most tropical and subtropical fruits and litchi is not beyond of this problem. Fruit crack of litchi and fruit let dropping are most common constraint in litchi and most of the fruit fall down during initial period (one month after fruit set) (Lal *et al.,* 2019). Although, dropping of fruit can be seen till ripening but the factors associated with different stages of fruit drop are different. The knowledge of stages of fruit drop and associated factors is very important for the grower to effect control of fruit drop.

Out of various growth regulators, foliar application of synthetic auxin 2,4-Dichlorophenoxy acetic acid checks fruit drop (Kaur *et al.,* 2007). Although few reports on the effect of 2,4-D on fruit drop, yield and quality of litchi are there in the literature but the effect of growth regulators on fruit drop, yield and quality of litchi has not yet. Kumar *et al*., (2014) also reported that growth substances like NAA, 2,4-D, PCPA (parachlorophenoxy acetic acid), GA (Gibberellic acid), BA (Benzyle aminopurine) and CCC (Cycocel) proved beneficial in minimizing the drop and cracking and enhancing quality of litchi fruits. Auxin stimulation due to 2,4-D and GA_3 might be the reason for the accumulation of building block at faster

rate and better execution of source-sink relation registering higher fruit setting, retention and less cracking (Kumar *et al.*, 2009). On the basis of above problematic issues of litchi cultivation and production, various aspects related use of PGRs in litchi production has been discussed. The plant growth regulators (PGR) act as messengers and are needed in small quantities at low concentrations (Kassem *et al.*, 2010). Generally, their site of action and biosynthesis are different and thus a single or more PGR may influence several entirely different processes.

Plant growth regulator play important role in the integrated developmental activities of the fruits yield as well as biochemical quality of the fruits. Exogenous application of plant bio regulators in small quantities has become an important component for inhibiting or modifying the physiological processes by replacing or supplementing the endogenous hormones when their levels are below than required to bring a positive change in the course of plant development, thereby regulating different aspects such as fruit drop, quality and yield. Keeping all these factors in consideration, the present literature has been reviewed to clear the concepts and approaches involved with the use of different PGRs in relation to litchi fruit growth, development, yield, quality, shelf life etc. Both Micronutrients and Plant Growth Regulators have been observed to influence yield and qualities of litchi fruit. Boron on the other hand is considered to be necessary for hormone metabolism, photosynthetic activities, cellular differentiation and water absorption in plant's bodies. Physiological changes or abnormalities such as heavy fruit drop or early abscission in litchi might be due to the lack of hormones needed for the embryo and fruit development, lack of pollination and/or fertilization etc. 2, 4 D changes the biochemical properties of litchi in different stages in fruiting times. All these can be dealt with effectively by understanding the concepts behind the use of 2, 4 D PGR being used worldwide in litchi at different stages of growth and development.

Considering the above facts the present investigations was undertaken with the following Objectives:

- To investigate the effect of 2, 4 D on fruit dropping of litchi cv. Bedana
- To analyze the effect of 2, 4 D on biochemical and nutritional elements of litchi cv. Bedana

CHAPTER II
REVIEW OF LITERATURE

There is no doubt that litchi fruits are important as food because they have sufficient amount of vitamin and mineral. Fruits increase our digestive power. Litchi juice has vitamin C and perhaps vitamin A, potassium and calcium, depending on the product. The production of litchi fruit decreases because of fruit dropping which can be reduced by foliar application of plant growth regulator (2,4-D). Application of 2,4-D PGR on litchi fruits improve the biochemical quality of fruits. The purpose of review of literature in any research is to review the past research works, which are related to the present study. The review of literature in any research is necessary as it provides a new dimension for reviewing the stock of knowledge and information relevant to the proposed research. This knowledge gives a guideline in furnishing the future research problem and validating the existing findings. With this end in view, literature and research works in the line with the present study were searched in the relevant libraries, research institutes, offices and websites.

2.1 Origin of litchi

Litchi (*Litchi chinensis L.*), which originated in southern China and possibly northern Vietnam belongs to the Sapindaceae family. The Sapindaceae is a relatively large family containing at least 125 genera and 1,000 species, which are widely distributed in the tropics and warm sub-tropics. The most widely cultivated fruit trees in this family other than litchi are rambutan (*Nepheliumlappaceum Linius*.) and longan (*Dimocarpuslongan Linius*.) (Minas and Frank, 2012). Litchi is a juicy fruit and one of the most important evergreen fruit trees. It is also regarded as one of the kings of subtropical fruits and famous for its excellent quality such as slightly sour-sweet taste, pleasant characteristics and for attractive color. Litchi is delicate and highly perishable, with a short storage life, hence, fruit should be sent to consumers on the day of harvest to avoid physiological loss in weight and ensure for maximum fruit quality. Insects, diseases, disorders, 15 nutrition deficiencies injuries litchi due to transit and storage environment may shorten the post-harvest life of litchi. Although the precise knowledge of growth processes is complicated and of little relevance to the litchi growers, but the basic understandings are important and will help us to make wise decisions in the field.

2.2 Historical Background of Litchi Research

Litchi is cultivated as very popular fruit and has a long history of acceptance in China and many parts of Southeast Asia. The cultivated litchis originated in the region between southern China, northern Vietnam and Malaysia. Litchis have a long history in Southeast Asia with unofficial Chinese records going back to about 2000 BC. From about 1600 AD, the species was distributed too much of the tropical land sub-tropical world, but it is currently not widely grown as it does not give flower and crop successfully over a wide range of climates. The main center of origin of litchi is believed to be between latitudes 23° and 27° north in the subtropical parts of southern China, northern Viet Nam, and Malaysia. It seems to have been in cultivation since about 1500 BC by people of Malayan descent and has since been subjected to intense selection. China has a long history of litchi cultivation for more than 2000 years and from China it reached Burma (Myanmar) by the end of 17th century and was introduced in India and Thailand about 100 years later (Zhang *et al.,* 2003). Litchi reached Madagascar and Mauritius around 1870 and was introduced in Hawaii in 1873 by a Chinese trader. It arrived in Florida, from India, between 1870 and 1880 and was introduced in California in 1897. Litchi was probably brought to Australia by Chinese migrants in 1954 and arrived in Israel sometimes between 1930 and 1940. China, Taiwan province of China, Thailand, India, South Africa, Madagascar, Mauritius and Australia are now major litchi producing countries in the world. A native of South China, it reached India by the end of seventh century. India ranked second in the world next to China in litchi production. Most area falls in north Bihar comprising Muzaffarpur, Vaishali, Samastipur, Begusarai, east and west champaran and Bhagalpur districts. Litchi is famous for its excellent quality, pleasant flavor, juicy pulp (aril) with attractive red color (Food and Agricultural Research of United States, 2018)

The important studies which were conducted in the recent past related to the present study are discussed below

2.3 Some research works of 2, 4 D on fruit dropping and biochemical properties of litchi

According to research by Huang *et al.,* (2018)**,** the application of 2,4-D can effectively reduce the rate of fruit drop in litchi. The study found that treated trees exhibited delayed abscission due to enhanced auxin levels, which counteracted the natural decline in auxin associated with fruit maturation. Zhou *et al.,* (2017) demonstrated that 2,4-D treatment alters the hormonal balance in litchi, particularly by increasing auxin and reducing ethylene production. This shift

can prevent premature fruit drop and extend the harvest window. Research by Nagar *et al.*, (2020) indicates that 2,4-D application can enhance the nutritional quality of litchi. The study found increased levels of phenolic compounds and vitamin C in fruits treated with 2,4-D, which are crucial for antioxidant properties and overall fruit quality. Gupta *et al.*, (2021) explored how 2,4-D impacts metabolic pathways in litchi. The researchers noted that treatment with 2,4-D affects the synthesis of key metabolites, including sugars and organic acids, which are critical for flavor development. They observed that treated fruits exhibited higher sugar content, positively influencing taste. Zhang *et al.,* (2019) found that 2,4-D application can delay the ripening process in litchi, affecting the accumulation of desirable ripening markers. This delay can be beneficial for postharvest handling, allowing for longer storage periods without quality deterioration. 2,4,5-TP @67 ppm and 3,5,6-TPA @ 50 ppm have been found to increase the number of fruits per tree, doubled the fruit yield, increased fruit size and enhanced the red color of ripe fruit of Fei Zi Xiao and Hei Ye litchi trees (Stern *et al.,* 2001). According to Kumar *et al.,* (2009), the highest aril percentage individual fruit weight per tree and number of fruits per tree was recorded in litchi trees of cv *Purbi s*prayed with 2,4-D @10 ppm.

O'hare and Turnbull (2004) observed that the concentration of a cytokinin, Zeatin Riboside in potted plants of litchi cv Tai so kept at different temperatures increased to maximum in terminal buds just prior to shoot emergence and its exogenous application to dormant buds could initiate the bud break. According to Mishra *et al.,* (2012), the application of BA (benzyl adenine) @ 40 ppm was the best treatment to delay the fruit maturity, and in terms of increase in ascorbic acid content in litchi cv Rose Scented. Orchard experiments were carried out in Botswana with the objective of evaluating the effect of 2, 4- dichlorophenoxyacetic acid (2,4-D) on reducing premature fruit drop. Different concentration levels of the 2,4-D (8, 16 and 20 mg/L were applied exogenously to mature fruit trees of sweet orange (*Citrus sinensis* L.) in the 2004/2005 season. In the 2005/2006 season the 2,4-D treatments ranged from 20 to 40 mg/L concentration. There appeared a general increase in fruit drop for the month of October in all treatments but a decrease in fruit drop was observed in the fruit trees with 16 and 20 mg/L 2,4-D concentration, that is, from November through February; with the latter showing the least number of fruits that dropped throughout the execution of the experiments. The application of 20 mg/L 2,4-D significantly reduced fruit drop by more than 50% but higher concentration levels of the plant growth regulator significantly increased fruit drop. It was also evident that, small sized fruits were more susceptible to fruit drop than larger fruits.

These findings suggested that, 2,4-D can be an effective tool to control fruit drop by enhancing retention, as well as improving the quality of navel oranges under dry climatic conditions**.**

2.4 Effect of PGR (2, 4 D) on flowering, Crop Regulation, Fruit Set, Fruit Growth and Development

Kumar *et al.,* (2014) examined that different litchi cultivars show variations in their flowering and bearing habits, may accordingly be classified as regular, irregular, shy bearing etc. Litchi bears the flowers mainly staminate, hermaphrodite and pseudo-hermaphrodite. The first flowers to pollen are males, followed by hermaphrodites functioning as females and pseudo hermaphrodites functioning as males. Kumar *et al.,* (2014) mentioned that the two spray of 100 ppm NAA, potassium nitrate (1%) or ethrel (0.05%) during October and November promote flowering and application of paclobutrazol (PP 333) @ 5ml/m canopy diameter at 90 days before the expected date of flowering inhibit the shoot growth and panicle length ultimately increases the fruit set and yield in litchi. Spraying of NAA has been also found to enhance the fruit set, fruit retention and fruit weight Plant growth regulators (PGR's) are known to have a great influence on fruit drop and fruit retention in fruit trees. Potjanapimon *et al.,* (2000) found that the cytokinin was low in litchi cv. 'Hong Huay' in Chiang Mai 6-8 weeks prior to flowering and increased at 4 weeks to reach a maximum two weeks prior to flowering. Gibberellins were high at four and three weeks before flowering and decreased two weeks prior to flowering to below detectable levels at flower emergence. The flushing habit of litchi varieties was intimately connected with irregular bearing. Problem is generally due to failure of flower initiation which puts forth vegetative growth prior to panicle emergence and flowering, thus eliminating the crop completely. However, Negi *et al.,* (2012) obtained early panicle emergence, improved fruit retention and yield in 'Rose Scented' litchi when sprayed with TIBA (1 g/l) at monthly interval from September to December. TIBA is considered a polar auxin transport inhibitor and increases the endogenous cytokinin level in the lateral buds. There is may be a positive relationship between cytokinin level and flower formation which may be due to the positive impact of TIBA. Sing *et al*., (2012) reported that soil drench application of PBZ @7.5 ml proved to be the most effective treatment for suppressing shoot growth, panicle size, male flower percentage fruit drop and sex ratio.

2.5 Effect of PGR on Controlling of Fruit Drop

Research by Huang *et al.,* (2018) demonstrated that 2,4-D treatment effectively decreased fruit abscission in litchi by maintaining higher auxin levels, counteracting the abscission-promoting effects of ethylene. Gibberellins (GAs) also play a crucial role in fruit retention. Ghosh *et al.,* (2015) found that GA application delayed fruit drop in litchi by promoting cell elongation and maintaining the structural integrity of fruit attachments, ultimately improving fruit size and quality. Cytokinins delay senescence and can help reduce fruit drop. According to Zhou *et al.,* (2020)**,** the application of cytokinins in litchi led to a significant reduction in fruit drop rates by enhancing cell division and promoting fruit retention. Combining different PGRs can enhance effectiveness. Nagar *et al.,* (2019) reported that a combination of 2,4-D and gibberellins led to lower fruit drop rates than individual applications, suggesting a synergistic effect that can improve retention strategies. Kumar et al. (2014) also reported that growth substances like NAA, PCPA (parachlorophenoxy acetic acid), GA, BA and CCC (Cycocel) proved beneficial in minimizing the drop and cracking and enhancing quality of litchi fruits. The minimum fruit drop was recorded with 20 ppm PCPA. Fruit cracking was least with 20 ppm NAA. The maximum fruit weight and highest aril percentage were obtained with 100 ppm GA. Fruits sprayed with 500 ppm CCC had the highest total soluble, total sugars and ascorbic acid contents, but had the least acidity. The fruit weight, blush colour, flesh firmness, soluble solids content, starch pattern index, and Streif index were monitored during the investigation period. It was found that Pomonit Super 50 SL reduced fruit drop by 67% in comparison with the untreated control. The effectiveness of Pomonit Super 050 SL applied in a mixture with Agrostym 480 SL for managing fruit drop was lower. Nevertheless, fruits in this treatment had more intensive blush but significantly lower firmness and starch content. The mixture of Pomonit Super 050 SL and Agrostym 480 SL determined more intensive fruit ripening processes (Nomeda Kvikliene *et al.,* 2010). The use of growth regulators has become an important component of agro-technical procedures for most of the cultivated plants and especially for fruit plants. So far in fruit crops, excessive fruit drop can be controlled by the exogenous application of plant growth regulators. The auxin and gibberellins are widely used to control the fruit drop and to improve the quality of fruit. Ontogenic development from fruit set to fruit ripening and final reach to customer, several agents are responsible for elimination of some fruits from fruit set to final maturity. In this review, we focus on the major functions of plant growth regulators in fruit production (Suman *et al.,* 2017).

2.6 Effect of PGR on Fruit Yield and Quality

Singh *et al.,* (2012) noted that soil drenching of PBZ @ 7.5 ml/tree respond better for increasing number of hermaphrodite flowers, fruit set, fruit retention and sugar content whereas PBZ @ 5.0 ml/tree was responsible of increasing fruit breadth and weight. They also found that the foliar application of CCC @ 2000 ppm in mid-September to mid-November resulted in maximum pulp weight, pulp/stone ratio, total soluble solids and minimum acidity.

Ethylene is widely applied in litchi production, which can promote fruit ripening, improves anthocyanin levels in peel, and mean-while decreases the chlorophyll content. Dixit *et al.*, (2013) reported that GA_3 @ 10 ppm was found effective treatment to increase fruit set, fruit retention and size of fruits. GA_3 @ 20 ppm produce maximum number of fruits/trees. Least fruit cracking was also noted with GA_3 @ 20 ppm treated fruits. Auxin stimulation due to 2,4-D and GA_3 might be the reason for the accumulation of building block at faster rate and better execution of source-sink relation registering higher fruit setting, retention and less cracking (Kumar *et al.,* 2009).

Puchooa *et al.,* (2005) indicated that GA reduced cracking by lowering the activity of cellulose. Aastha *et al.,* (2011) found that ethephon sprays at 10 mg/l reduced cracking from 12% (control) to 6% in Early Large Red. Stern *et al.,* (2001) suggested that synthetic auxins like 2,4,5-TP (as Tipimon) @ 67 ppm and 3,5,6-TPA (as Maxim) @ 50 ppm have very positive effect on yield, fruit size and fruit color in several litchi cultivars in China.

As an alternative to air-layering, micro propagation offers an attractive method for vegetative propagation of lychee. It requires only very small amounts of propagating material and has the potential of providing very large numbers of cloned plants. The exogenous application of plant growth regulators also an important aid to stimulate rooting potential during in-vitro explant culture. Poochua *et al.,* (2005) Studied in-vitro plantlet regeneration of litchi (Litchi chinensis cv 'Tai So'). Litchi plantlets were successfully regenerated in-vitro using young leaf explants. They examined that 2,4-D @ 1.5 mg/L) in combination with cytokinin (BAP @ 2.0 mg/L) was more efficient in callus initiation while BAP, in combination with IAA @ 3.0 mg/L was more appropriate for the differentiation of shoots from callus. IBA @ 2.0 mg/L) promoted rooting of the shoots. Although this method of propagation has been improved by the use of younger branches, small earth balls and 1, 4-indole-3-butyric acid (IBA), the process is still slow and inefficient.

In crosses involving plants which have tendency to produce these fruits as the female parent, many of the most valuable progeny are lost prior to harvest. Singh *et al.,* (2012) found that PBZ @ 7.5 g per tree was the most effective treatment for suppressing shoot growth, panicle size, male flower percentage, fruit drop and increased the hermaphrodite percentage, sugars, fruit size and fruit yield per tree in litchi cv Calcuttia. Singh *et al.,* (2017) applied PBZ @ 1, 2, 3 and 4 g ai. per meter tree canopy as soil drenching along with combination of two levels of potassium nitrate @ 100 and 200 mg/l respectively as triple application starting from 3 months after harvesting of the fruit at 15 days interval on trees of litchi cv China and observed the significant reduction in vegetative winter flushing and more floral shoots as compared to control. Also he found that the trees treated with PBZ @ 4 mg/L canopy of tree proved to be the most significant as the number of vegetative flushing in winter was significantly reduced with 66 per cent emergence of floral branches. Hung and Nghi (2006) observed that in litchi cv Binhkhe PBZ applied @ 20 mg/L tree in late Aug-early Sept at mature bud stage resulted in the inhibition of winter bud emergence, reduced inflorescence size but increased female flowers, fruit set and yield by 61.5-85.2 per cent than control. Growth Regulators for Yield and Quality Enhancement in Litchi (*Litchi chinensis* L).

Aklimuzzaman *et al.,* (2011) revealed that, the fruit quality attributes of three commercially important litchi varieties of Bangladesh, namely 'Bombai', 'Bedana' and 'China 3'. Physico-chemical parameters such as peel color, pericarp browning, weight loss, dry matter content and pulp pH to increase with the duration of storage, whereas moisture content and vitamin C content decreased with the progress of storage. Among the varieties, changes in the above parameters were slower in *'Bedana*' as compared with 'China 3' and 'Bombai'. On the other hand, pulp to peel ratio and TSS increased initially but declined afterwards in all varieties. The level of disease incidence and severity increased proportionally during the storage period. Fungal pathogens like *Aspergillus spp, Rhizopus spp* and *Penicillium spp* were identified from the infected fruits. Significant difference in respect of shelf life was also observed among the varieties. The longest shelf life was observed in 'Bedana' (3.75 days) as compared to those of 'China 3' (3.07 days) and 'Bombai' (2.08 days) varieties

2.7 Effect of 2, 4 D on biochemical properties

The application of 2,4-Dichlorophenoxyacetic acid (2,4-D) significantly affects the biochemical properties of various plants, including litchi. Here's a discussion supported by research findings. Nagar *et al.,* (2020) demonstrated that 2,4-D application increased the levels of vitamin C and phenolic compounds in litchi. These compounds are crucial for

antioxidant properties, enhancing the nutritional quality of the fruit. Gupta *et al.,* (2021) study highlighted that 2,4-D treatment modifies key metabolic pathways, particularly those involved in sugar and organic acid synthesis. The results indicated that treated fruits exhibited higher sugar content, which can improve flavor and overall fruit quality. Zhang *et al.,* (2019) found that 2,4-D application delayed the ripening process in litchi, affecting the accumulation of ripening markers. This delay can be beneficial for postharvest storage, as it helps maintain fruit quality over a longer period. Huang *et al.,* (2018) indicated that 2,4-D treatment reduced ethylene production in litchi. Since ethylene promotes ripening and senescence, its inhibition can help extend the shelf life and improve the overall marketability of the fruit. The use of 2,4-D significantly influences the biochemical properties of litchi, enhancing nutritional content, altering metabolic pathways, delaying ripening, and reducing ethylene production. These effects can be strategically utilized to improve fruit quality and extend shelf life. Investigations were undertaken to explore the possibility of improving setting, retention and weight of fruits in 'Early Seedless' and 'Calcuttia' cultivars of lichi (*Litchi chinensis*) by means of growth regulators. Indole acetic acid (IAA) at 20, 40 and 80 mg l^{-1}, 2,4-dichlorophenoxy acetic acid (2,4-D) at 2,4 and 8 mg l^{-1} and gibberellic acid (GA_3) at 50, 100 and 150 mg l^{-1} were sprayed on panicles in the first fortnight of April, when 50–100% flowers had opened. All 3 growth regulators caused a favorable effect on fruit setting, fruit retention and weight of individual fruits, but IAA at 20 mg l^{-1} proved the best for enhancing setting, GA_3 at 50 mg l^{-1} for increasing retention and GA_3 at 100 mg l^{-1} for improving fruit weight. IAA and GA_3 should, therefore, be used in combination. Between the 2 cultivars tested, 'Calcutta' proved superior to 'Early Seedless' in fruit setting, fruit retention and weight of individual fruits.

CHAPTER III

MATERIALS AND METHODS

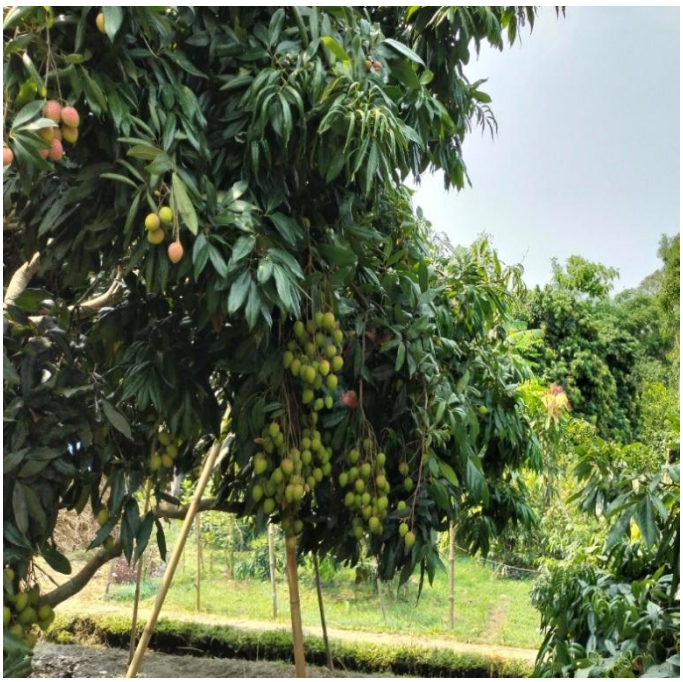
This chapter deals with the materials and methods used to carry out the present research work. The materials and methods used to investigate the effect of 2, 4 D on fruit dropping and biochemical properties of litchi cv. Bedana.

3.1 Experimental Site

The research was conducted under field conditions at Shikhderhat litchi orchard, Dinajpur and ripen litchis at laboratory of the Department of Agricultural chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur during April to June, 2023 to investigate the effect of 2, 4 D on fruit dropping and biochemical properties of litchi cv. Bedana.

3.2 Test sample

A. Litchi variety**:** Bedana



Picure 3.1: Experimental litchi orchard

B. Plant growth regulator: 2,4-D
C. Replication: 03
D. Treatment: 04

T_0: 0 ppm
T_1: 20 ppm
T_2: 30 ppm
T_3: 40 ppm

3.3 Plant selection and experimental design

The experiment was conducted under field conditions at Shikhderhat litchi orchard, Dinajpur. The orchard was about 20 years old with air layered trees attaining an average height of 7.1 m. The litchi tree was 7.62 m apart in both directions i.e., between the rows and within the plants. Eight (8) fully matured navel litchi trees were randomly selected for use in the investigations. Trees were divided into three replicate groups basing on the four treatments that were applied. A split plot approach was adopted for the experimental design. The main plot being the concentration of the 2,4-D applied to the trees. This was done in order to eliminate the effect of the sunlight on fruit drop. During the experiment, the normal cultural practices with regard to nutrition, weed control pest and disease control were adopted.

3.4 Preparation of 2,4-D solution and sprayer calibration

A stock solution of 4.8 g/L was prepared from the concentrated solution of 2,4-D (480 g/L) and the prepared solution was refrigerated at a temperature of about 4 - 7°C. A 6 L knapsack sprayer was calibrated, each of the 8 fruit trees was sprayed to a point of run-off. It was observed that, about 5 L was required to spray one tree to a point of runoff in 10 min at an approximate pressure of 28 kg/cm. The results from the calibration of the sprayer enabled an accurate determination of the volume of 2,4-D solution and pressure required to spray a single experimental tree. The compound 2,4-D was applied to 8 trees in various concentrations (0, 20, 30 and 40 ppm) with each treatment replicated thrice. The three control trees were used in the investigation had distilled water applied to them. In order to be able to determine the threshold level of 2,4-D efficacy, a repeat experiment but with higher concentrations of 2,4-D (0, 20, 30 and 40 ppm) with the same control was carried out in the following season. Each treatment was applied by spraying a whole tree evenly with 5 L of 2,4-D solution, but in the case of treatment zero (control) distilled water was sprayed onto the

trees. Trees were first foliar sprayed with the chemical in April, thereafter; application of treatment was carried out after a period of 30 days for the second and the third treatment, as according to AOAC (2000).

3.5 Determination of fruit drop

Three medium sized branches were selected towards the edge of the tree branches, on opposite sides of the fruit trees. One was selected on the eastern orientation of the tree and the other on the western side. The fruits on the branches were counted using a manual counter, prior to the application of the PGR. Fruit counting resumed a month after application of treatment, on weekly interval. All fruits dropped prior to the application of the chemical were counted on weekly bases and after counting the fruits were removed from under the tree. The same practice was carried out even after the application of the treatments. Observations on fruit drop were recorded during the months of April, May & June 2023.

3.6 Size of the fruits that dropped

The area beneath the canopy of the tree was divided into four quarters. Thereafter, two quarters from opposite sides were selected from each tree. The diameter of dropped fruits in those quarters was measured using Vernier calipers and recorded once every week, that is, prior and after the application of the treatments and after treatments application.

3.7 Sample collection

Fresh and mature Litchi was collected from research orchard during the harvesting season (2023). Litchi was sorted based on firmness and variety. Litchi is extremely rich in vitamin C and thus has positive effects on protective the body from free radicals that cause atherosclerosis, bronchitis and heart diseases associated with diabetes, asthmatic attacks, osteoarthritis and rheumatoid arthritis and damage bowel cells which may lead to cancer.

Picture 3.2: Sample collection of *Bedena* variety

3.8 Sample preparation and preparation of experimental materials

The experiment of biochemical analysis was conducted at laboratory of the Department of Agricultural chemistry of Hajee Mohammad Danesh Science and Technology University, April to June, 2023 to investigate the effect of 2,4-D on biochemical properties of litchi cv. *Bedana*. Fully matured and disease-free ripe litchi was collected from research orchard. Analytical food grade reagents and chemicals were obtained from Agricultural Chemistry laboratories at HSTU. Sugar, plastic basin, muslin cloth and sieves were all purchased from HSTU market, Dinajpur.

Picture 3.3: Sample preparation of litchi

3.9 Tools and Equipment's used

Knife, muslin cloth, wooden spoon, tea spoon, glass jar, glass bottles, butter paper, plastic jar, tasting salt, palm oil, saucepan, thermometer, weighing machine, mixer grinder, pH meter, refract meter, burette, and oven were used in the experiment.

3.10 Litchi sample preparation ingredients

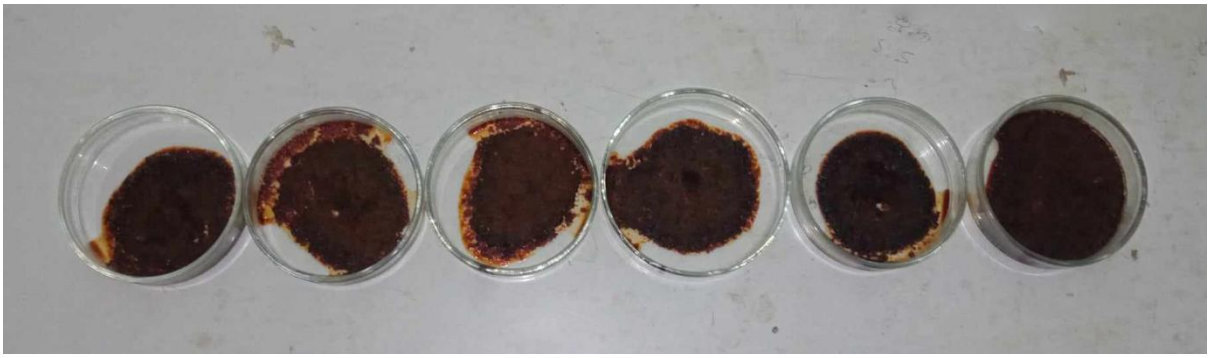
The ingredients are below:

1. Litchi juice
2. Sugar
3. Agar agar
4. Water
5. Citric acid
6. Sodium benzoit

3.11 Litchi sample preparation

a) The juice is first extracted and the required quantity of sugar is added and cooked.
b) A citric acid concentration of 7g/kg of sugar is used and then mixed with water at the time of cooking.
c) When the endpoint reach sat 105 to 107 ^{o}C, cooking is stopped and the product is filled in glass jars.

d) Good jelly formation requires a high ratio of juice to sugar and thus Litchi jelly made with the juice of 18% soluble solids, combined with sugar in a 55:45 ratio.
e) The pH of the juice should be adjusted with phosphoric acid to 3.7 and subsequently to pH 3.2 with citric acid.
f) It was found that the addition of 1% commercial slow set pectin to litchi jelly is sufficient for good gel formation.



Picture 3.4: Sample preparation for bio-chemical analysis of litchi

3.12 Biochemical analysis of litchi

3.12.1 Determination of pH

Digital pH (HANNA, pH-211) meter was used to determine the pH value of the sample by performing two-point calibrations (with buffer 7.0 and buffer 4.0) before measuring the samples pH value by K. J. H. McGowan (2018).

3.12.2 Total Soluble Solid (TSS)

Brix value (Brix) or TSS for all samples has been determined using table-top refract meter (Abbe, *Germany*) and the lens is adjusted to be midway between the bright and dark side lines by Horwitz, W. (2010)..

3.12.3 Determination of titrable acidity

Method suggested by AOAC (2000) was followed for the estimation of titrable acidity. The following reagent was used for the determination of titrable acidity.

- Standard NaOH solution (0.1 N).
- Phenolphthalein indicator

Preparation of sample

10 g of mango pulp was taken in a 250 mL beaker and 25 mL distilled water was added to it and it was blending with a blender then it was filtered with thin cloth then it was transferred in a 100ml volumetric flask and volume made up to the mark with distilled water.

Picture 3.5: Acidity test

Procedure

10 mL of sample was taken in a conical flask. 2-3 drops of phenolphthalein indicator was added to it and titrated with standard NaOH solution from burette. At the end point pink color will be appeared.

$$\% \text{ Titrable acidity } = \frac{T \times N \times V_1 \times E \times 100}{V_2 \times W \times 1000}$$

Where,

T= Titrable value

N= Normality of NaOH

V_1= Volume made up

V_2= Volume of extract (used)

E= Equivalent weight of alkali

W= Weight of sample

3.12.4 Determination of vitamin C

L ascorbic acid was determined by following the method of Rangana (1979)

Chemical required:

1. **Metaphosphoric acid solution:** 30 g meta phosphoric acid dissolved in 80 ml glacial acetic acid then this solution transferred in a 1000 ml volumetric flask volume up to the mark with distilled water.
2. **Dye solution:** 260 mg 2,6 di-chlorophenol Indophenol and 210 mg $NaHCO_3$ dissolved in 1000 mL distilled water.
3. **Standard vitamin C solution**: 100 g vitamin C (L-ascorbic acid) dissolved in 1000 ml metaphosphoric acid solution.

Standardization of dye solution:

5 ml of standard vitamin C solution was taken in a 100 mL conical flask and titrated with dye solution from burette.

$$\text{Dye factor} = \frac{0.5}{\text{Titrated value}}$$

Preparation of sample:

1.0 g of fruit sample blended with about 50 mL metaphosphoric acid solution then it was filtered with white thin cloth then it was transferred in a 100 mL volumetric flask and then volume made up to the mark with metaphosphoric acid solution.

Titration with unknown solution

10 mL of fruit sample was taken in a conical flask and titrated with dye solution from burette.

$$\%\ \text{vitamin C} = \frac{\text{Titrate value} \times \text{Dye factor} \times \text{Volume of sample made up} \times 100}{\text{Volume of sample used} \times \text{Weight of sample} \times 1000}$$

Determination of beta carotene

Lycopene content was determined with a slightly modified method described by Nagata and Yamashita (1992).

Chemicals:

1. Acetone.
2. n-hexane

Preparation of solvent: Acetone: n-hexane (4:6).

Preparation of sample solution

Accurately weighted 1.0 g of sliced fruit part was homogenized with 10.0 mL of acetone hexane solution. Homogenized solution was centrifuged at 3600 rpm for 10 minutes and filtrated the solution.

Spectrophotometer

A little amount of supernatant sample was taken in curette and placed in spectrophotometer. The absorbance of the prepared supernatant solution was measured at 663 nm, 505 nm and 453 nm.

Calculation

β- Carotene content was calculated by the following formula

β- Carotene (mg/100 mL) = $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{45}$

Determination of starch

The starch content was assessed using the Lane and Eynon (1923) method, with certain modifications. 1.0 g of oven dried and desiccated powdered sample was taken in an Erlenmeyer flask and 50 mL of cold water was added. The content of flask allowed standing for one hour with occasional stirring. It was then filtered and residue was washed with 50 mL of distilled water. The sample was hydrolyzed with 10% HCl for 2.5 hours under reflux.

The hydro lysate was neutralized with dilute NaOH solution and filtered. The filtrate was collected in a 100 ml volumetric flask and volume made up to 100 mL.

The reducing sugar in the filtrate was determined by Fehling's titration method and the amount of glucose was calculated

$$\% \text{ Starch} = \% \text{ invert sugar} \times 0.9$$

3.12.5 Determination of total sugar

Method suggested by AOAC (2000) was followed for the estimation of total sugar.

Chemical required:

Fehling A: 69.28 g copper sulphate was dissolved in distilled water. Diluted to 1000 mL filter and stored in amber colored bottle.

Fehling B: 346 g Rochelle salt (potassium sodium tarterate) and 100 g NaOH was dissolved in distilled water. Diluted to 1000 mL filtered and stored in amber colored bottle.

Potassium oxalate solution: 10% potassium oxalate solution was prepared. This reagent was used to remove the excess lead used in clarification.

Lead acetate solution: 20% neutral lead acetate solution was prepared.

Methylene blue Indicator: 1% of methylene blue solution was prepared in distilled water.

3.12.6Estimation of non-reducing sugars

& Non-reducing sugar = % total sugar - % reducing sugar

3.12.7 Estimation of total sugars

An aliquot of 50 mL of the clarified, de-leaded filtrate was pipetted to a 100 mL volumetric flask. 5 mL conc. HCl and allowed to stand at room temperature for 24 hours. It was neutralized with concentrated NaOH solution followed by 0.1 N NaOH solution. The volume was made up to the mark and transferred to 50 mL burette having an offset tip and performed the titration on Fehling's solution similar to the procedure described in the determination of reducing sugar (AOAC, 2000).

$$\% \text{ Total sugar } = \frac{\text{Fehling factor } \times \text{Dilution } \times 100}{\text{Weight of sample } \times \text{Titre}}$$

3.12.8 Estimation of reducing sugars

It was determined according to the method described by (Haq and Rab, 2012) and Santini *et al.*, (2014) with slight modification. Twenty gram of the mango pulp was crushed in a mortar and pestle then transferred in a 200 mL volumetric flask. The volume was adjusted to 150 mL by adding purified water. After a few minutes to allow the sugar dissolution, 10 mL of lead acetate solution and the minimum amount of potassium oxalate solution were added. The

volume of the resulting solution was adjusted to 200 mL, and the solution shacked, filtered and transferred in a burette for the titration. 5mL of Fehling solution A, 5 mL of Fehling solution B and 40 mL of purified water were transferred in a flask. The solution was heated up to boiling point and the solution was added drop by drop till the nearly complete de-coloration of the Fehling reagent. Two drops of methylene blue were added, and the boiling continued for 3 minutes. The solution from the burette was added till the blue coloration of the indicator disappeared and the solution turned into a red color. Reducing sugar was calculated using the following equation:

$$\% \text{ Reducing sugar } = \frac{\text{Fehling factor} \times \text{ Dilution } \times 100}{\text{Titre } \times \text{Weight or volume of sample}}$$

3.12.9 Determination of moisture content

The moisture content of the samples was determined in accordance to moisture measurement by AOAC (2001) method.

Procedure

About 5g sample was taken in a previously dried crucible. Then the crucible was dried in an oven at 100-105 ^{o}C for 24 hours or more till constant weight. After drying the crucible was removed from the oven and cooled in desiccators. The crucible was removed from the desiccators and weighed soon after reaching room temperature.

$$\% \text{ Moisture } = \frac{\text{Loss of weight}}{\text{Weight of sample}} \times 100$$

3.12.10 Determination of ash content

AOAC (2000) method was used to determine the total ash content.

Procedure

5 mL sample was taken in a dry clean porcelain dish and weighted accurately. Hot air oven method was applied to remove the moisture. Then the sample was burned using an electric heater. This was done to avoid the loss of sample in the muffle furnace at higher temperature of 550 °C and ignited until light gray ash was appeared. The sample was then cooled in desiccators and weighed. The ash content is expressed

$$\% \text{ ash} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

3.12.11 Proximate analysis of mineral content

Picture 3.6: Before digestion

Picture 3.7: After digestion

3.12.13 Determination of calcium content

Calcium was estimated by complex metric method of titration using Na_2-EDTA as a Complexing Agent.

Procedure

5 mL of plant extract solution was taken in a 250 mL conical flask. 20 mL distilled water was added into the conical flask and shaken thoroughly.10 drop of each masking agent was added a) Hydroxylamine hydrogen chloride, b) Potassium ferocyanide and c) Triethanolamine (TEA). 5 mL NaOH buffer solution was added and shaken thoroughly and then added 5-6 drops calcon indicator into the conical flask and shaken the flask thoroughly. Then it was titrated against 0.01 M Na_2-EDTA solution. The titration was continued until pink color of the solution completely turns to pure blue color. The titration was repeated at least 3 times to minimize the error. A blank titration was also conducted by taking all reagent as above except calcium stock solution. 5 mL more distilled water was taken into conical flask instead of calcium solution. Tabulated the data and calculated the amount of calcium by the following formula:

1 mL 1M EDTA ≡ 1mL 1M Ca = 40.08 mg Ca

3.12.14 Determination of Vitamin A

Vitamin A content of the samples was determined in accordance to moisture measurement by AOAC (2001) method.

Preparation of solvent:

Acetone: n-Hexane (4:6). Procedure: one (1) g sample was taken in a test tube with stopper and 15 mL solvent was added to it. It was heated for 15 minutes, then it was filtered and measured absorbance at 663 nm, 505 nm and 453 nm. Beta carotene content was estimated in mg/100 mL by using the following equation: β-Carotene = 0.216 × A663 - 0.304 × A505 + 0.452 × A453 mg/100 mL.

3.12.15 Estimation of Magnesium content

The Magnesium content of the samples was determined in accordance to moisture measurement by AOAC (2001) method.

Chemicals required

1. Disodium EDTA
2. Ammonia ammonium buffer
3. EBT Indicator
4. Methanol.
5. Masking agent:
    a) Hydroxylamine hydrogen chloride
    b) b)Potassium ferocyanide
    c) Triethanolamine(TEA)
    d) Sodium tungstate.

Procedure

Exactly 5 mL of plant extract solution was taken in 250 mL conical flask. 20 mL distilled water was added into the conical flask and shake thoroughly.10 drops of each masking agent of (a) Hydroxylamine hydrogen chloride (b) Potassium ferocyanide (c) Triethanolamine (TEA) (d) Sodium tungstate was added. Ammonia ammonium buffer solution and shake thoroughly. Add 5-6 drops EBT indicator into the conical flask (depending on the concentration of the indicator solution) and shake the flask thoroughly. Then titrate against 0.01 M Na_2-EDTA solution from burette. Continue the titration until pink color of the solution completely turns to pure blue color. Repeat the experiment at least 3 times. Conduct

a blank experiment also by taking all the reagents as above except plant extract solution. Take 5 ml more distilled water into conical flask instead of extract solution. Tabulate the data and calculate the amount of calcium present in the prepared solution.

Formula of Calculation:

1 mL 1 M EDTA ≡ 1mL 1 M Mg = 24.305 mg Mg

Determination of Phosphorus

The Phosphorus content of the samples was determined in accordance to moisture measurement by AOAC (2001) method.

Principle

In presence of molybdenum ion (Mo^{6+}) and stannous chloride ($SnCl_2.2H_2O$), the orthophosphate ($H_2PO_4^-$) forms a blue color of the molybdo phosphoric blue complex which shows an optimum absorption at 660 nm wavelengths. These heterophony complexes are thought to be formed by the co-ordination of molybdenum ions with phosphorus as the central coordinating atom, the oxygen of the molybdate radicals being substituted for the molybdate radicals being substituted for that of phosphate ions ($PO4^{\equiv}$).

The hypothetical reaction is as follows.

$H_3PO_4 + 12H_2MoO_4$ ----------> $H_3P(Mo_3O_{10})_4 + 12H_2O$

Preparation of Different Reagents

a) Preparation of primary standard phosphors solution (50 ppm P): 0.2195 g of potassium dihydrogen phosphate ($KH_2PO_4^-$, AR grade) was Dissolve exactly and oven dried at 40^0C in about 400 ml distilled water was taken in a volumetric flask.25mL of 7 N H_2SO_4 was added and shaken the flask thoroughly. Distilled water was taken and then make the volume up to the mark. The concentration of that solution was 50 ppm P.

b) Preparation of secondary standard phosphorus solution (20 ppm and 2 ppm P): For 20 ppm P standard solution, 200 mL of 50 ppm P solution to 500 mL solution was diluted with distilled water. Next, 50 mL of 20 ppm P solution to 500 mL was diluted with distilled water into the volumetric flask.

c) Preparation of phosphorus standard series solution: Phosphorus standard solutions was prepared which containing 0.05 ppm P, 0.1 ppm P, 0.2 ppm P, 0.3 ppm P, 0.4 ppm P, 0.5 ppm

P and 0.6 ppm P from 2 ppm P standard solution in seven different 100 ml volumetric flask by pipetting 2.5 ml, 5 ml, 10 ml, 15 ml, 20 ml, 25 ml and 30 ml, respectively. The volume was made up to the mark with distilled water in each flask. Then, added 4ml sulphomolybdic acid followed by 5-6 drops stannous chloride solution.

d) Preparation of sulphomolybdic acid solution (2.5%): 25 g of ammonium molybdate tetra hydrate (AR grade) was dissolved exactly in 200 mL distilled water and warm it at 60 0C until the solution become clear. Then, diluted exactly 275 mL of concentrated H_2SO_4 (P and as free, AR grade) to 750 mL with distilled water. After cooling, ammonium molybdate solution was added slowly with constant stirring to H_2SO_4 solution. Making the volume of mixed solution up to 1000 ml mark with distilled water.

e) Preparation of stannous chloride solution: Approximately 5.0 g of stannous chloride was dissolved in 10 ml of concentrated HCl with warming if necessary to dissolve. Diluted the solution to 100 ml with hot distilled water giving about 0.2 M Sn^{2+} ion.

Preparation of triple super phosphate (TSP) working solution

Exactly l.0 g of plant sample was powdered in a mortar in a 100 ml beaker. Added 30-40 ml distilled water and then stirring the contents with a glass rod. The contents were heated on a hot plate with magnetic stirrer until it dissolves. Then, cool the solution and filter through filter paper (Whatman No.1) in a 100 ml volumetric flask. The volume was made up to the mark with distilled water. That was the plant extract solution.

Procedure:

a. Development of molybdophosphoric blue color:

5 mL of plant extract solution was taken in a 100 mL volumetric flask. 4 mL of sulphomolybdic acid solution was added to it followed by the addition of distilled water up to 2/3rd volume of the flask. The solution was mixed thoroughly after the addition of 5-6 drops of stannous chloride solution. The volume was made up to the mark with distilled water. The full color was developed intensity within 3-4 minutes and then, read the colored solution instantly in a spectrophotometer at 660 nm wavelength. A blank solution also prepared by taking all the reagents as described except phosphorus solution.

b. Preparation of standard or calibration curve:

A standard or calibration curve was prepared by plotting the absorbance (optical density) of light in Y axis and concentrations of the solutions in X axis in a graph paper. By plotting the spectrophotometer reading on the standard curve, the concentration of test sample (plant extract solution) was easily obtained. X ppm P = X µg P/mL.

Statistical analysis

The data was collected on different parameters under the experiment were statistically analyzed to compare treatment means using the Statistix10 computer software developed. If the treatments were significant the differences between pairs of means were compared by LSD followed by Duncan's Multiple Range Test (DMRT).

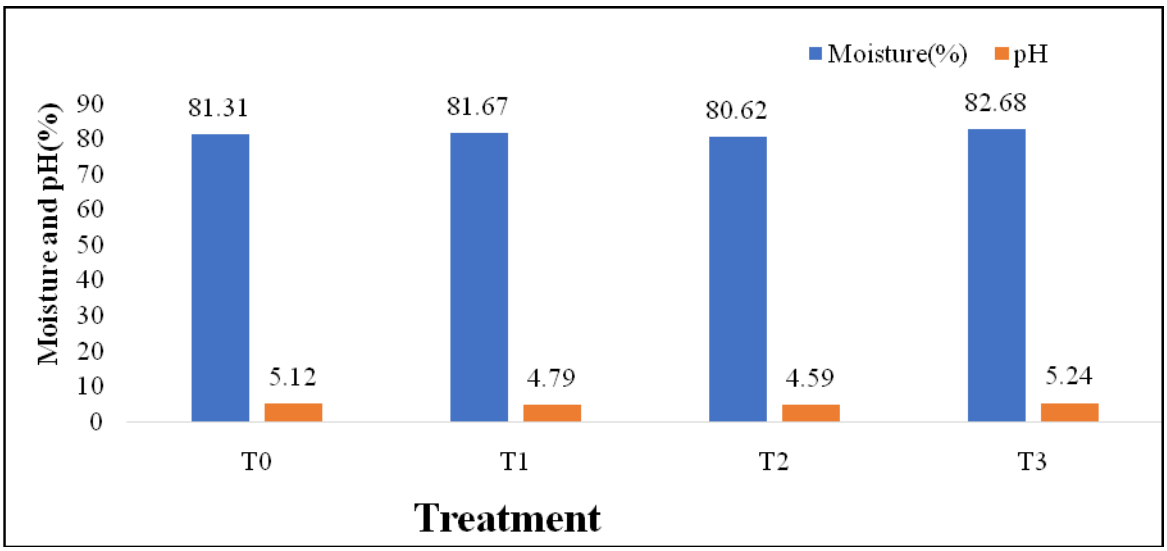
CHAPTER IV

RESULTS AND DISCUSSION

The current study on "the effect of 2, 4 D on fruit dropping and biochemical properties of litchi cv. Bedana" was carried out in the year of April to June, 2023. The result which has found is tabulated and discussed in this section.

4.1.1 Moisture

Changes in moisture in litchi cv. Bedana shown in Figure 4.1. A significant ($p<0.05$) differences in moisture among litchi samples were found. Figure 4.1 showed that the highest moisture value was found in T_3 (82.68) and the lowest value was found in T_0 (81.31). The present finding was in well agreed with the result of Nisha and Radhamany (2019). The moisture content of the food is normally used as indicator of its shelf life (Fellows, 2000). The moisture content of other variety like Bombai was reported by Jahan *et al.*, (2024).



Note: T_0= 0 ppm (control), T_1 = 20 ppm, T_2 = 30 ppm, T_3= 40 ppm

Fig.4.1: Moisture (%) and pH Change in different treatment

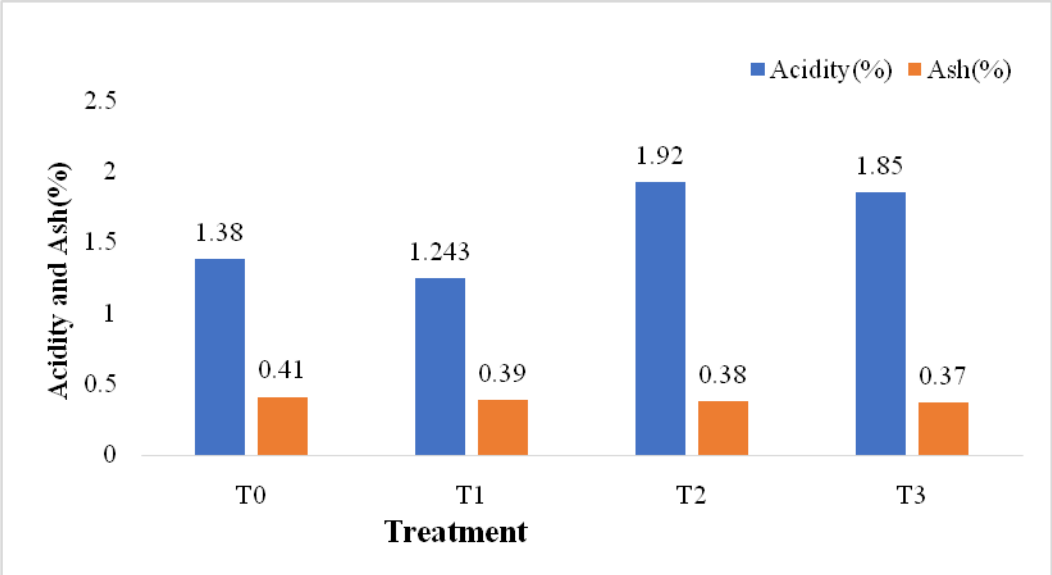
4.1.2 pH

In the Figure 4.1, A significant difference in pH among different levels of 2,4-D treatments was found. Results of this research exposed that pH varied from treatment to treatment. The highest pH value was 5.24 found in T_3 and the lowest value was 4.59 found in T_2. The pH of

litchi pulp juice was 4.10 reported by Talukder *et al.,* (2020) which was slightly deviated from the present findings.

4.1.3 Acidity

There was a marked difference in acidity as shown in Figure 4.2 for different treatments. The highest acidity value was found in T_2 (1.92%) and the lowest value was obtained in T_1 (1.24%). The changes in titrable acidity was significantly affected by the different levels of 2,4-D. Singh *et al.,* (2012) noted that the foliar application of CCC @ 2000 ppm in mid-September to mid-November resulted in maximum pulp weight, pulp/stone ratio, total soluble solids and minimum acidity.



Note: T_0= 0 ppm (control), T_1 = 20 ppm, T_2 = 30 ppm, T_3= 40 ppm

Figure 4.2: Acidity and ash (%) Change in different treatment

4.1.4 Ash

Ash content in litchi fruit was varied due to different levels of 2,4-D (Figure 4.2). The maximum ash content was 0.41% found in control treatment but the minimum value of ash was found T_3 (0.37%). The present finding was slightly differed with the result of Bangar *et al*., (2021) where ash content was 0.45-0.62 % but it might be due to varietal difference.

Table 4.1: Effect on protein, fiber, fat component of litchi under different treatment of 2, 4 D

Treatment	%Protein	%Fiber	%Fat
T_0	1.35b	0.50a	4.22b
T_1	1.15c	0.57a	3.12c
T_2	1.64a	0.27b	5.09a
T_3	1.25bc	0.29b	4.18b
CV (%)	3.31	3.31	4.58
LSD0.05	0.12	0.07	0.53
Level of significance	**	**	**

Different letters in columns means significantly different at 5% level of significance (*P<0.05)*

4.1.5 Protein

Changes in protein are shown in Table 4.1 for different treatment. The highest value was found in T_1 (1.64 %) and the lowest value was found in T_2 (1.15 %). Naz *et al.,* (2014) reported that the protein contents was almost similar with the present findings.

4.1.6 Fiber

Fiber content in litchi fruit was varied significantly due to difference in 2,4-D treatments shown in Table 4.1. From Table 4.1, the maximum fiber content was found in T_1 (0.57%) which was statistically similar to T_0 but the minimum fiber content was found in T_2 (0.27%) which was statistically similar to T_3 treatment. The present finding was similar to the result of Bangar *et al*., (2021) where fiber content was 0.45-0.62 (%).

4.1.7 Fat

There was a significant difference in fat content of litchi fruit pulp among the different levels of 2,4-D treatments. Table 4.1 showed that the maximum fat content was 5.09 % found in T_2 and the minimum fat content was 3.11% found T_1. This finding was quite similar to Cabral *et al.,* (2014) that the value of fat content was 1.47-1.34 %.

Table 4.2: Effect on total sugar, reducing sugar, non-reducing sugar component of litchi under different treatment of 2, 4-D

Treatment	Total sugar (mg 100 g^{-1})	Reducing Sugar (mg 100 g^{-1})	Non-reducing sugar (mg 100 g^{-1})
T_0	19.63ns	17.32ns	2.31ns
T_1	26.76ns	23.32ns	3.44ns
T_2	26.36ns	23.17ns	3.19ns
T_3	25.79ns	22.26ns	3.53ns
CV (%)	12.35	12.53	28.04
$LSD_{(0.05)}$	8.6	7.62	2.47

Different letters in columns means significantly different at 5% level of significance (*P<0.05)*

4.1.8 Total Sugar

A marked difference in fat content of litchi fruit pulp was observed among the different levels of 2,4-D treatments. In Table 4.2, results of variation in the total sugar content among the treatment due to 2, 4 D plant growth regulator application. The highest total sugar was found in T_1 (26.7 mg 100 g^{-1}) and the lowest value found in T_0 (19.63 mg 100 g^{-1}). Applying moderate doses (10-20 mg/L) during critical growth phases (e.g., flowering or early fruit development) tends to yield the highest sugar content. These doses can effectively support fruit development and enhance metabolic processes related to sugar accumulation (Sharma *et al.,* 2016).

4.1.9 Reducing Sugar

In Table 4.2, results of variation in the total sugar content among the treatment due to 2, 4 D plant growth regulator application. The highest reducing sugar was found in T_1 (23.32 mg 100 g^{-1}) and the lowest value found in T_0 (19.63 mg 100 g^{-1}). Applying moderate doses (10-20 mg/L) during critical growth phases (e.g., flowering or early fruit development) tends to yield the highest sugar content. These doses can effectively support fruit development and enhance metabolic processes related to sugar accumulation (Sharma *et al.*, 2016).

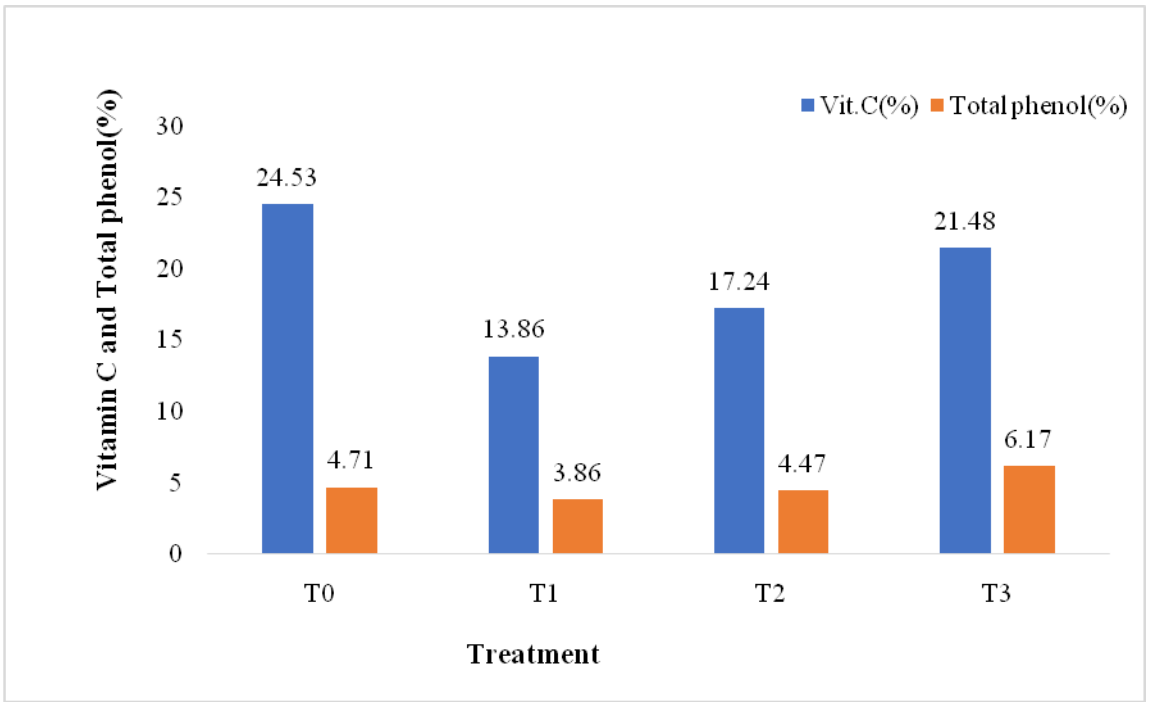
4.1.10 Non reducing sugar

A marked difference in fat content of litchi fruit pulp was observed among the different levels of 2, 4-D treatments. In Table 4.2, results of variation in the total sugar content among the

treatment due to 2, 4 D plant growth regulator application. The highest total sugar was found in T_3 (3.53 mg 100 g^{-1}) and the lowest value found in T_0 (2.31 mg 100 g^{-1}). Applying moderate doses (10-20 mg L^{-1}) during critical growth phases (e.g., flowering or early fruit development) tends to yield the highest sugar content.

4.1.9 Vitamin C

Figure 4.3 indicated that highest vitamin-C value was found in T_0 (24.53 mg 100 g^{-1}) and the lowest value was found in T_1 (13.86 mg 100 g^{-1}) of litchi cv Bedana. Different levels of 2,4-D affect the metabolism process of litchi which reduce the content percentage of Vitamin C. Different results were found by Singh *et al*., (2016) and Islam *et al*., (2003). They found that the vitamin-C content of litchi varieties varied from 6.79-7.38. This difference in Vitamin C might be due to varietal as well as environmental factors.



Note: T_0= 0 ppm (control), T_1 = 20 ppm, T_2 = 30 ppm, T_3= 40 ppm

Fig. 4.3: Vitamin C and Total phenol (mg 100 g^{-1}) of litchi in different treatment

4.1.10 Total Phenol

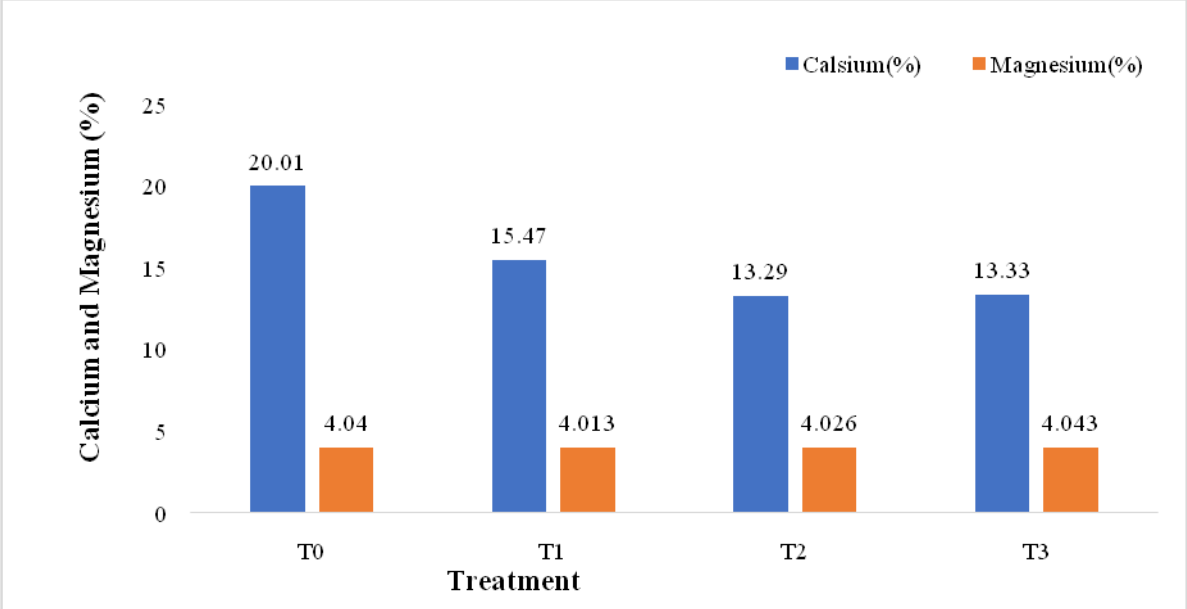
There was a marked variation was in total phenol content among the different levels of 2,4-D treatments (Figure 4.3). The highest value was found in T_3 (6.17 mg 100 g^{-1}) and the lowest value found in T_1 (3.86 mg 100 g^{-1}). A study by Nagar *et al.*, (2021) showed that moderate doses of 2, 4-D (e.g., 10-20 mg/L) resulted in a significant increase in total phenolic compounds compared to control samples.

4.1.11 Calcium (Ca)

Fiber content in litchi fruit was varied significantly due to difference in 2, 4-D treatments shown in Table 4.1. The highest Ca was found in T_0 (20.01 mg 100 g^{-1}) and the lowest Ca was found in T_2 (13.29 mg 100 g^{-1}). This finding was not conformed with Cabral *et al.*, (2014) who reported that Ca content differed due to varietal variation.

4.1.13 Magnesium (Mg)

There was a marked variation was in total phenol content among the different levels of 2,4-D treatments (Figure 4.3). The highest Mg was (4.04 mg 100 g^{-1}) found in T_3 and the lowest Mg was (4.01 mg 100 g^{-1}) found in T_1. The findings from Gupta *et al.*, (2020) suggested that an optimal concentration of 15 mg/L of 2, 4-D was particularly effective in enhancing magnesium content.



Note: T_0= 0 ppm (control), T_1 = 20 ppm, T_2 = 30 ppm, T_3= 40 ppm

Fig. 4.4: Calcium and Magnesium (mg 100 g^{-1}) of litchi in different treatment

4.1.14 Iron (Fe)

In Table 4.2, results of variation in the Iron content among the treatment due to 2, 4 D plant growth regulator application. The highest Iron (Fe) was (0.08 mg 100 g^{-1}) found in T_3 and the lowest value was found in T_0 (0.07 mg 100 g^{-1}). Fe content was the highest in cv. MD-2 cultivar (0.61 mg 100 g^{-1}), revealed by Nisha and Radhamany (2019), which was not similar to the present study.

4.1.15 Zinc (Zn)

Zinc content in litchi fruit was varied significantly due to difference in 2, 4-D treatments shown in Table 4.1. The highest Zinc was (10.88 mg 100 g^{-1}) found in T_1 and the lowest value was (10.79 mg 100 g^{-1}) found in T_0. PGR application increase the amount of Zn in litchi fruits by inhabited the metabolism process. Zinc is useful for protein synthesis, normal body development and recovery from illness (Muhammad *et al.*, 2011).

Table 4.3: Effect on iron, zinc component of litchi under different treatment of 2, 4 D

Treatment	Iron (Fe)	Zinc (Zn)
T_0	0.07b	10.88a
T_1	0.08a	10.79a
T_2	0.07b	10.84a
T_3	0.08a	10.87a
CV (%)	3.81	1.97
LSD 0.05	8.16	0.6
Level of significance	**	Ns

Different letters in columns means significantly different at 5% level of significance (*P<0.05)*

4.2.1 Fruit weight

In Table 4.2, results of variation in the fruit weight among the treatment due to 2, 4 D plant growth regulator application. The highest fruit weight value was (24.05g) found in T_1 and the lowest value was (17.52) found in T_0. The application of 2, 4-D on litchi plants increased the size and weight of their fruits, probably due to the action of this PGR in stimulating the cell division and elongation (Ghosh *et al.,* 2009 Roa *et al.,* 2015).

Table 4.4: Effect of 2, 4 D on Fruit weight, Diameter and Number of fruits per branch

Treatment	Weight (g)	Diameter (mm)	Number of fruits per branch		Fruit drop (%)
			Before	After	
T_0	17.52d	25.88c	17.89b	11.00 a	38.51a
T_1	24.05a	31.44a	17.00b	16.66 ab	2.00d
T_2	22.58b	28.66b	23.11ab	19.77 ab	14.45c
T_3	21.16c	28.11b	26.33a	19.88 b	24.49b
CV (%)	0.8	1.29	30.83	36.37	17.31
LSD 0.05	0.48	1.04	6.32	5.95	3.47
Level of significance	**	**	**	**	**

Different letters in columns means significantly different at 5% level of significance (*P<0.05)*

4.2.2 Fruit diameter

The diameter of fruit varied from treatment to treatment. The highest diameter value was (31.44mm) found in T_1 and the lowest value was (25.88mm) obtained in T_0. Increased fruit diameter is in corroboration with the findings of (Singh, 2008) and (Hoang, 2003) who reported that application of 6BAP and 2,4-DPA increased total fruit weight, pulp, seed and peel weight of litchi.

4.2.3 Fruit dropping

Fruit dropping in litchi fruit was varied significantly due to difference in 2, 4-D treatments shown in Table 4.1. The highest of fruit dropping percentage was (38.51%) found in T_0 and the lowest percentage value was (2.00%) obtained in T_1 respectively. Kumar *et al.,* (2009) observed that the highest aril percentage individual fruit weight per tree and number of fruits per tree was recorded in litchi trees of cv Purbi sprayed with 2,4-D @20 ppm. Application of 2, 4-dichlorophenoxyactic acid and 2, 4-D + GA_3 delayed fruit ripening, reduced production of ethylene, increased fruit size and reduced fruit drop.

CHAPTER V

SUMMARY AND CONCLUSION

Being an agro-based country, different agricultural product provides economic return to Bangladesh. Litchi is enjoyed exceptionally much as a table natural product all over the world. The experiment was conducted in Farmers orchard, Sikdarhat, Dinajpur Sadar upazila. The research work was conducted during litchi growing season (February to June, 2023) which is also known as Rabi and Kharif-1 season.

The soil of the experimental plot was silty loam. The air temperature was relatively low at the beginning of the season and was increasing with advancement of the season towards Kharif-1 with hot weather. Low to moderate rainfall was detected during experimental period in 2023. Air temperature sometimes reached 40^{o}C during maturity stage. For the experimental design, one factor design (randomized complete block design) using 2,4-DPA (2,4-Dichlorophenoxyacetic acid) at four levels - 0, 20, 30 and 40 ppm was sprayed three times.

This study was to investigate some morpho-physiological, yield characteristics and biochemical substances in fruit, responses to 2, 4-D and to determine the effects of 2, 4-D to reduce fruitlet dropping at the fruit maturity stage of litchi cv. Bedana. The results revealed that the plant biostimulants-2, 4 DPA significantly reduced fruit dropping. 2, 4-D improved litchi fruit quality and food nutrient values. This study investigates of 2, 4-D on fruit dropping and biochemical properties of litchi. There are four treatments of different rate of 2, 4-D plant growth regulator. The highest of fruit dropping percentage was found in T_0 (38.51) treatment and the lowest percentage value was obtained in T_1 (2.00) treatment respectively. The highest amount of fruit gets by reducing fruit dropping was found in 20 ppm 2, 4-D (T_1) application and the lowest value found in 0 ppm 2, 4-D (T_0) treatment. The highest diameter value of litchi fruits was found in T_1 (31.44mm) treatment and the lowest value (25.88mm) was obtained in T_0 (0 ppm) treatment. The highest fruit weight value was found in T_1 (24.05g) treatment and the lowest value was found in T_0 (17.52g) treatment. The highest vitamin C (25.53%) was found in T_0 (0 ppm) 2, 4-D treatment and the lowest value (13.86%) found in 20 ppm of 2, 4-D (T_1) treatment. The highest total sugar present in 20 ppm 2, 4 D (T_1) is (26.76 mg 100 g^{-1}) and the lowest value present in 0 ppm 2, 4 D (T_0) is (19.63 mg 100 g^{-1}). The highest protein value present in 20 ppm 2, 4 D (T_1) is (1.64%) and the lowest value present in 0 ppm 2, 4-D (T_2) is (1.15%). The highest value of fiber present in 20 ppm 2, 4-D (T_1) is (0.57%) and the lowest value present in 0 ppm 2, 4-D (T_2) is (0.27%).

According to the above data we see that the highest fruit getting, fruit diameter, highest vitamin C and other nutrient content in 20 ppm 2, 4-D (T_1) application and lowest value present in 0 ppm 2, 4-D (T_0). So, according to above data 20 ppm 2, 4-D (T_1) is better than other treatment. So, 20 ppm of 2, 4-D treatment is best for biochemical properties and reducing fruit dropping of litchi cv. Bedana.

Future Research Recommendations

Future studies should be

- Investigate the impact of varying 2, 4-D concentrations and application frequencies at different growth stages to optimize growth and yield.
- Conducted by setting more treatments of 2, 4-D to study the maximum growth and yield of litchi cv. Bedana at different places of Bangladesh.

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