A COMPREHENSIVE REVIEW OF CHITOSAN'S IMPACT ON ORCHID PRODUCTION AND FLOWER QUALITY

Abstract

Orchids are among the world's most beautiful sought-after cut flowers and ornamental crops. Orchid genera, namely *Dendrobium*, *Cymbidium*, *Paphiopedilum*, *Phalaenopsis*, *Vanda*, *Cattleya* and *Oncidium* are cultivated for cut flower production. In India, these genera are largely cultivated for cut flowers and potted plant production. Due to the intensification of agriculture, there has been a rise in environmental pollution, primarily caused by the excessive and uncontrolled application of synthetic and chemical inputs in farming. This has resulted in research focusing on the use of natural products in tissue culture and production technology of cut flowers. One of the promising and eco-friendly groups of natural biopolymers that significantly enhances the growth of plants is chitosan, which is obtained from natural sources, such as fungi and many marine organisms. Shell waste produced by the seafood industry is a rich source of chitosan. Chitosan is vital for both the tissue culture of plants and the large-scale production of horticultural crops. Many studies have conclusively proven the efficacy of chitosan on plant growth, flower production, and quality in orchids. This review attempts to elucidate the multifaceted roles of chitosan in orchid multiplication and production.

Keywords: orchids, chitosan, bioregulator, growth regulator, plant defense

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I. INTRODUCTION

The second-largest family of flowering plants, the Orchidaceae, is widespread across the planet [1]. Within this family, 28,000 species and 850 genera have been recognized so far [2]. Many different species may be found in the subtropical and tropical regions of Asia, South America and Central America [3]. Many of them are fascinating and have beautiful flowers, which are in high demand worldwide [4]. Orchids are highly recognized for their beauty as cut flowers and as elegant potted plants. Additionally, orchids make for a greater share of the global floriculture trade than any other variety of flowers [5]. Both the floriculture and pharmaceutical industries may make extensive use of orchids for commercial purposes. Orchids contain qualities that make them both visually attractive and therapeutic [6]. Soil health is of the utmost importance in modern agriculture due to the increased usage of chemical inputs. Because of this, using products made from biopolymers is essential for promoting and aiding soil health [7]. Chitosan offers a broad range of applications due to its biodegradability and biocompatibility [8]. By inhibiting hyphal growth, mycelial elongation, spore formation, spore germination, and the production of virulence factors, chitosan is able to effectively prevent the proliferation of phytopathogenic fungi [9]. Additionally, it boosts the plant's immune system so that it can more effectively fight off pathogens [10]. This chapter provides the review of chitosan's origin, solubility characteristics, impact on vegetative development, blooming propensity, floral quality, disease resistance, growth control, and use in *in vitro* research of numerous orchid species.

II. SOURCE OF CHITOSAN

Chitin was discovered in 1811 by Henri Braconnot while studying mushrooms. Chitin may be treated with alkali to produce a chemical that can be dissolved in acids, as Professor C. Rouget discovered in 1859. Hoppe Seiler later gave this chemical the name "Chitosan" [11]. Large amounts of discarded shrimp shells, which include vital components are produced by shrimp processing enterprises. These substances might be effectively used to produce chitosan. This not only makes shrimp waste more useful, but it also helps keep the environment cleaner [12]. Chitin is a biopolymer that was discovered from the cell walls of fungus and the exoskeletons of arthropods. Generated from chitin is the chitosan which is a natural biopolymer. It is the carbon source with the second greatest rate of potential regeneration after lignocellulosic biomass [13]. Additionally, it was discovered in the linings of worms' digestive systems, the beaks of cephalopods, and the eggs of certain species [14]. Chitosan may be found in a wide range of areas, including crustaceans [15], insect cuticles [16], fungi and certain types of algae [17]. The methods for extracting chitosan from shrimp shells include demineralization, deproteinization, and deacetylation [18]. As a natural by product of the seafood industry, numerous prawn and crab shell waste can also be utilized to produce chitin [19] from which chitosan can be extracted.

III. SOLUBILITY OF CHITOSAN

Chitosan is a polysaccharide that contains N-acetyl glucosamine and D-glucosamine molecules [20], [21]. These molecules are linked to one another by beta 1,4 glycosidic bonds [21], [22]. Chitin cannot be dissolved in water without first disintegrating into oligomers with seven different degrees of polymerization [23]. Due to chitin's high molecular weight and restricted solubility, as well as its inability to dissolve in water or mild acids due to the presence of its acetamide group, chitosan was then discovered in the late 1850s [24]. Chitosan, on the other hand, is an appealing substance for a number of applications since it is naturally occurring, cationic, and dissolves in a variety of aqueous solutions that include organic and inorganic acids [25].

IV. EFFECT OF CHITOSAN ON VEGETATIVE GROWTH OF ORCHIDS

It has been shown that chitosan promotes seedling growth in several plants [26]. Numerous studies have shown that chitosan has a variety of benefits, including the promotion of root development, enhancement of plant health, and increase of photosynthetic pigment efficiency [27]. By raising the germination index and the dry weight of the roots and shoots, it improves the growth of ornamental plants [28]. The nitrogen content of chitin and its derivatives is high, ranging from 6.1 to 8.3 percent [29]. By facilitating the plants' better absorption of nutrients, the addition of chitin to soil encourages greater plant growth [30]. When *Paphiopedilum* orchids were sprayed with chitosan at a concentration ranging from 2.5 to 40 mg/L, the length of the leaves was increased [31]. The application of polymeric chitosan at dosages of 10, 20, and 40 mg/L significantly increased the average length of plantlet shoots in hybrid *Dendrobium* orchids [32]. The combination of 100 mg/L of chitosan and 5 g/L of lotus extract resulted in the *Dendrobium* 'Suree Peach' producing more leaves and shoots [33]. The highest fresh weight was found in *Mokara* seedlings given chitosan at a dosage of 20 mg/L, whereas the biggest leaf area were found in seedlings fed chitosan at a concentration of 40 mg/L. Twelve months after being transplanted, the seedlings' total shoot-to-root ratio increased with both treatments. *Phalaenopsis* seedlings that received chitosan at concentrations of 10 and 20 mg/L had higher fresh weight and leaf area. The greatest shoot to root dry matter ratio was obtained by applying chitosan to the leaves at a concentration of 40 mg/L [34]. The results of a study reported in [35] indicated that *Paphiopedilum niveum* plants' vegetative growth was stimulated by chitosan produced from a variety of mushrooms. It has been shown that chitosan treatment enhances the quantity of chlorophyll in plants [36]. Young leaves from plants treated with chitosan at 10 and 50 mg/L had chloroplasts that were noticeably larger in diameter than leaves from plants that were not treated with chitosan [37].

V. EFFECT OF CHITOSAN ON EARLY FLOWER INDUCTION AND QUALITY PARAMETERS IN ORCHIDS

Chitosan has shown to have favourable impacts on plant growth and development [38]. The effects of different concentrations, levels of deacetylation, and levels of polymerization of chitosan on the growth and development of *Dendrobium* Sonia Jo 'Eiskul', a well-known cut flower orchid in Thailand, were specifically evaluated during the deflasking stage. However, the oligomeric and polymeric forms of chitosan with 70, 80, and 90 percent deacetylation at concentrations of 1, 10, 50, and 100 mg/L had no effect on vegetative growth, as revealed by the findings. Chitosan caused early flowering in *Dendrobium* Sonia Jo 'Eiskul'. [39]. Additionally, it has been shown that chitosan oligosaccharides may shorten flowering times and increase the overall yield of flowers [40]. Chitosan application to the leaves of *Dendrobium* orchid plants did not promote the growth of their foliage, but it did aid in boosting early flowering, the development of flower buds, and the production of high-quality flowers [41], [37]. *Dendrobium* Sensational Purple had more flower shoots overall and tended to provide superior yields when treated with chitosan [41]. Chitosan was applied to *Dendrobium* orchids in concentrations ranging from one part per million to one hundred parts per million. This resulted in early flowering and increased inflorescence production from each plant. Regardless of the dosage used in the experiment, chitosan induced *Dendrobium* 'Eiskul' to bloom sooner and increased the overall number of inflorescences the plant produced. Additionally, the 'Miss Siam,' 'Bom17K,' and 'Khaw Sanan' *Dendrobium* orchids yield was enhanced. Additionally, the proper application of chitosan may improve the floral diameter, and extend the shelf life of cut *Dendrobium* orchid flowers [37]. *Dendrobium* Sonia 'No. 17' was sprayed with chitosan at concentrations of 0 (water), 200, 400, or 600 mg/L on a weekly basis. The findings showed that the fresh weights of the florets that had been sprayed with 600 mg/L of chitosan were higher. Before harvest, the sepals may be sprayed with chitosan at a 600 mg/L concentration, which increased their width [42]. Three-and-a-half-year-old *Dendrobium* orchid plants were shown to have longer inflorescences after being sprayed with chitosan. In contrast, there was no noticeable difference in the size of the florets or the growth of new shoots. The quantitative and qualitative inflorescence yields in *Dendrobium* 'Earsakul' were significantly increased by the application of chitosan with a molecular weight of 45 kDa and a degree of deacetylation of more than 90% [37]. Chitosan treatment on *Dendrobium* orchid plants increased the length of inflorescences and the number of open florets, but there was no noticeable difference in the orchids' vase life [43]. It has been shown that orchids treated with chitosan exhibit longer internodes, longer spikes, more spikes per square metre, larger flowers, and more florets per spike [44]. The number of florets per spike increased as a result of this treatment. The plants were sprayed with chitosan, which increased the width of the sepals at harvest [42]. It was shown that adding chitosan at a concentration of 40 mg/L with either the recommended quantity of fertilizer or half the recommended application dosage might lengthen the vase life [45].

VI. EFFECT OF CHITOSAN ON DISEASES OF ORCHIDS

Numerous antimicrobial research studies have been conducted on chitosan [46], [47], and [48]. Antiviral, antibacterial, and antifungal properties have been shown for chitin and chitosan [49]. The first investigation into chitosan's antipathogenic properties was published in 1979 by Allan and Hadwiger. They determined that chitosan had fungicidal effects on fungi with various cell wall compositions. The polycationic nature of chitosan, which exhibits natural antifungal activity without the need for any chemical treatment [50]. A number of plant species have been demonstrated to respond more aggressively when exposed to chitosan [51]. Chitosan is used to treat infections both before and after harvest and has been shown to strengthen a plant's inherent defense mechanisms. Thus chitosan is a potent bio-fungicides, bio-bactericides, and bio-virucides [52], [53], [54]. It has been shown that chitosan treatment may affect the expression of a variety of genes in plants, including those involved in activating signalling pathways for plant defense. As a result, phytoalexins are produced and pathogenesis-related (PR) protein production is elevated [55]. The results of using chitosan showed that it may increase plant immunity and have antibacterial effects against diseases and microparasitic fungi [56], [57], [58]. The activity of defense enzymes including peroxide (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and beta-1,3-gluconase significantly increased after the application of chitosan, which resulted in an exceptionally heightened defensive reaction [59].

The use of synthetic fungicides may be harmful to human health, and their widespread usage may have an impact on the development of infections that are resistant to these compounds. On the other hand, chitosan provides a fascinating and may be natural alternative for disease management. It has been shown that it has a broad-spectrum fungicidal effect against a range of phytopathogenic fungi, which in turn prevents the growth of these fungi at different stages of their life cycle. Chitosan treatments have been demonstrated to be beneficial in lessening the severity of the leaf spot disease that affected *Dendrobium* 'Missteen' [43].

VII. CHITOSAN AS A PLANT GROWTH REGULATOR FOR ORCHIDS

Gibberellins, a plant hormone that promotes plant growth and development, are produced in response to chitosan [60]. When compared to conventional plant growth regulators, chitosan had a more favourable effect on shoot multiplication. Additionally, it led to an increase in the plant's production of important secondary metabolites, PAL enzyme activity, and antioxidant activity. An increase in PAL enzyme activity may also operate as a biochemical indicator of the orchid's resistance to biotic stress since PAL is a crucial enzyme in the process of secondary metabolite formation [61]. Chitosan's potential to cause effects similar to those of 6-benzylaminopurine and jasmonic acid has been shown in orchid cultures [62]. By enhancing the delivery of nitrogen to the functioning leaves and stimulating the growth and development of the plant, the application of chitosan increased the activity of critical enzymes involved in the metabolism of nitrogen [63].

VIII. CHITOSAN IN TISSUE CULTURE OF ORCHIDS

Clonal propagation of orchids by plant tissue culture technologies has evolved into a crucial component of the industry since it allows for the rapid production of orchid plantlets in large numbers [32]. In a short span of time, plant tissue culture has been used to create plantlets on a huge scale. As a result, it has developed into a fantastic tool for protecting many plant genera and preventing extinction. It has gained steadily more popularity over the last several years, especially in the fields of forestry and agriculture [64]. The mechanisms of seed germination and micropropagation are crucial for both orchid conservation and commercial orchid production [65]. By adding natural substances to the medium, it is feasible to encourage *in vitro* seed germination [66], which will also stimulate growth and result in genetic changes [32]. Several reliable publications [67, [68] state that chitosan is a polysaccharide that controls plant development. Chitosan has been shown to be a crucial element for promoting the growth of orchids in a controlled environment [69]. A number of studies have shown that the addition of chitosan to the media affects the germination of orchid seedlings [70]. For orchids of the genus *Dendrobium formosum*, the use of chitosan increased the rate of seed germination [71].

When chitosan was added to the solid media, the conversion of seed explants into protocorm-like bodies (PLBs) was drastically accelerated, and as a result, their growth was increased. Another intriguing discovery was the growth promotion of the hybrid *Paphiopedilum bellatulum*, *Paphiopedilum angthong* plant by chitosan. Previous studies indicated that the fresh weight of PLBs, the quantity of PLBs, and the number of plantlets were all enhanced to their maximum values when the Vacin and Went medium was supplemented with 15 mg/L chitosan [72]. After twenty weeks of culture, it was shown that a chitosan concentration of 10 mg/L caused the pseudobulbs of the plant *Phalaenopsis gigantea* to gain higher fresh weight [73]. *Cymbidium aloifolium* protocorm development was aided by adding 0.05 mg/L of chitosan to the tissue culture media. Highest number of shoots, longest shoots, more leaves, the highest fresh weight, and the greatest dry weight were produced as a consequence [74]. When chitosan concentrations of 15 mg/L were employed, *Dendrobium phalaenopsis* had the greatest PLB growth from meristematic buds [67]. In *Grammatophyllum speciosum*, chitosan generated the most new PLBs, shoots, and leaves per explant [75], in addition to increasing the PLBs' diameter. *Grammatophyllum scriptum* exhibited increased plant height and leaf length as a direct result of the efficient addition of chitosan at concentrations ranging from 0.5-0.75 g/L [76]. A chitosan concentration of 10 or 20 mg/L was shown to be the most suitable quantity of chitosan throughout the process of generating PLB shoots, and that concentration also produced greater PLB production. The addition of 10 mg/L of either O-80 or P-80 chitosan produced the best results in terms of both the quantity and quality of plant regeneration. An increase in the survival rate and development rate of plantlets one month after exflasking was achieved by supplementing the exflasking procedure with P-70 chitosan at a dosage of 20 mg/L [32]. The ability of cells to differentiate further is enhanced and increased by chitosan, which leads to a rise in the number of plantlets [77].

The meristematic tissue of the orchid *Dendrobium Phalaenopsis*, grew and developed more rapidly with the addition of shrimp chitosan with a molecular weight of 1 kDa. After six weeks of culture, it was shown that shrimp oligomer chitosan at 1 and 10 kDa was less effective at promoting the growth of orchid PLBs than fungal chitosan (10 kDa) at 15 mg/L [32]. Chitosan was shown to considerably increase the length of the shoots of hybrid *Dendrobium* and *Cattleya* plants at a concentration of 20 mg/L [78]. *Dendrobium* AW 179 plantlets generated significantly more shoots, leaves, and roots when exposed to chitosan [79]. Overall, plantlet growth increased as a result of this. Six different types of chitosan were examined for their ability to stimulate growth in an *in vitro* culture of *Dendrobium* 'Eiskul' orchid [32]. This included evaluating the effectiveness of stimulating the growth of plantlets, shoot regeneration, protocorm-like body multiplication, and plantlet acclimation. Plantlets that were at the transplanting stage and sprayed with chitosan in any and all polymeric forms survived 100% of the time throughout the acclimation stage, with the exception of the highest dose (80 mg/L). In addition to increasing the percentage of survival, chitosan of the proper kind and concentration may enhance the vegetative growth of *ex vitro* acclimated plants [32]. The effects of the chitosan treatments on the morphological characteristics showed a qualitative shift, with the adaxial leaf side showing fewer but larger stomata than the control. In comparison to the other treatments, chitosan at a dosage of 10 mg/L had the most chloroplasts [80]. The amount of chitosan present on the abaxial leaf side had no effect on stomatal quantity or size.

It was found that the chitosan treatment had no discernible impact on plant survival when plants were transplanted for *ex vitro* acclimatisation using the Dynamic Root Floating Technique (DRFT) hydroponic culture with two levels of nutrient ionic strength of the King Mongkut's Institute of Technology Ladkrabang (KMITL) formula [81]. It was discovered that the plants that had been propagated in media containing oil waste and chitosan had more leaves than those that had been grown in other media while acclimatising *Dendrobium* Shavin White seedlings that had been tissue cultured and grown in a net house with various media that included chitosan. This finding was observed when the seedlings adjusted to their new environment [82]. Several measurements, including the width of the leaf, the height of the plantlet, and the stomatal index, were somewhat affected by chitosan; however, the length of the leaves and the number of leaves did not alter much. When the chitosan level was more than 0.75 g/L, a negative effect was seen on the acclimatisation of *Grammatophyllum scriptum* plantlets [76].

IX. CONCLUSION

Chitosan is a naturally occurring compound that may be found in many different places and has a broad range of applications in the growth of orchids. The trash that is generated by the seafood industry in India may be used to make chitosan, which will help to lessen environmental pollution and develop valuable products and riches from the waste that is produced. Chitosan aids in the production of high-quality cut flowers for the orchid industry because it promotes the vegetative growth, blooming, and post-harvest quality of orchid flowers. The orchid business benefits from chitosan as well. Additionally, it protects orchid plants against diseases that reduce the amount of flowers they produce. A major advantage for the orchid industry is that it may serve as a supplement and harden plants produced through tissue culture. Chitosan may be used successfully in the cut flower business for orchids to enhance production and quality standards for both planting material and cut flowers as well as to lengthen the vase life, which is a crucial quality feature of this unique family of blooming plants. This is made feasible by the fact that chitosan has several useful characteristics.

X. ABBREVIATIONS

1. %- percent
2. mg- milligram
3. g- gram
4. L- litre
5. ppm- parts per million
6. PR- Pathogenesis Related
7. PO- Peroxide
8. PPO- Polyphenol Oxidase
9. PAL- Phenylalanine Ammonium Lyase
10. PLB- Protocorm Like Bodies
11. kDa- Kilo Daltons

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