Effect of Some Growth Regulators on the Levels of Endogenous Hormones and Chemical Constituents of Rose Plant

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Abstract: These experiments were done in the screen house of National Research Centre, during two successive seasons to investigate the effect of gibberellic acid (GA₃), kinetin (Ki) and Brassinosteroids (BR₉) as foliar spray on endogenous hormones and chemical composition of Rosa hybrida cv. "Rouge Mielland". The obtained data indicated that foliar application of growth substances GA₃, BR₉, GA₃ + BR₉, Ki + BR₉, and GA₃ + Ki + BR₉ increased the endogenous levels of the growth promoters IAA, gibberelins and cytokinins as compared with control plants. The highest values of chlorophyll (a), chlorophyll (b) content and total carotenoids content were obtained in plants which treated by (40 ppm Ki + 15 ppm BR₉) at the 1st and 3rd spray in both seasons; (40 ppm Ki + 15 ppm BR₉) at the 1st spray and (50 ppm GA₃ + 15 ppm BR₉ + 40 ppm Ki) at the 3rd spray, respectively. Moreover, spraying rose plants with (50 ppm GA₃ + 40 ppm Ki + 15 ppm BR₉) resulted an increase in total carbohydrates and soluble sugars contents.

Key words: Rosa hybrida · growth hormones · gibberellic acid (GA₃) · kinetin (Ki) · Brassinosteroids (BR₉) · chemical constituents

INTRODUCTION

Rose plants (Rosa hybrida) Hybrid tea rose cv. "Rouge Mielland" is one of the most important ornamental flowering shrubby and odorous plants. Its volatile oil utilized in the manufacturing of perfumes. Under Egyptian conditions in winter season there are a remarkable declined flowers yield of rose plants in quantity and quality. It is worthy to mention that, obtained flowers with special specifications needs several treatments. In this respect, growth regulators application may be one of such treatments which aims to affect plant behaviour by stimulating the induction, initiation and development of rose flower buds under different conditions. Additionally, growth regulators may supplement the deficiency of natural endogenous promoting hormones and replacement the low temperatures, which affect on the induction of flowering stage.

Brassinolide (BR) a newly discovered natural plant growth regulator extracted from pollens of Brassica napus [1]. Krizek and Mandava [2,3] noted an increase in fresh and dry weight of leaves and shoots treated with brassinolide.

Many investigators evaluated and recommended this substance for increasing flowering, fruit, crop yield, stress tolerance and diseases resistance [4-6].

Shalaby and Abdel-halim [7] also reported that foliar spray of faba bean plants with brassinosteroids significantly increased plant height, number of branches, number of pods, pods dry weight, number of seeds/plant and seed yield/plant. Also, Helmy et al. [8] reported that foliar application of brassinosteroids significantly promoted the growth and yield characters of broad bean plants.

The aim of this study is to investigate the effect of the plant growth substance i.e. GA₃, kinetin and brassinosteroids of rose plants to overcome the difficulties of winter flowering of rose plants, aiming to increase flowering of Rouge Mielland cv. with higher yield and quality of flowers.

MATERIALS AND METHODS

Two pot experiments were carried out at the experimental screen house of the Botany Department, National Research Centre, Dokki, Giza, Egypt in the two successive seasons to investigate hormonal and chemical constituents of Rosa hybrida cv. "Rouge Mielland" as
affected by foliar spraying with gibberellic acid, kinetin and brassinosteroids.

The plant material was obtained from Ornamental Research Section, Ministry of Agriculture, Giza. One plant was cultivated in clay pot 30 cm diameter filled with 15 kilograms clay loamy soil. The plant was selected for uniformity is shape and size.

Three growth regulators were used as follows: Gibberellic acid at the rate of 50 ppm, Kinetin at the rate of 40 ppm, Brassinosteroids at the rate of 1 ppm and combination between them in addition to the control. Teepol was adding as detergent agent. Seven treatments were arranged in complete randomized design. Plants were sprayed at different physiological stages three times and control were sprayed with distilled water at the same dates.

**Extraction, purification and determination of endogenous hormones:** Samples were taken from the plants of the experiment to determine the endogenous IAA, IAN, ABA, GA₃-like substances and cytokinins. The samples were taken after 10 days from the 3rd spray. Replicated three times of each treatment (100 g fresh weight), from the shoots of plants, except buds and flowers, were used. The plant material was frozen in a liquid air immediately after sampling and stored at -30°C until extraction. The frozen plants were extracted with cold methanol using a blender and the extract was fractionated as described by Fadl et al. [9].

A volume of acidic extract of IAA, ABA and GA₃-like substances equivalent to 10 gm fresh weight of plant material was located onto chromatography paper and developed in an ascending manner in the dark at 25°C ± 2°C using solvent system composed of isopropanol-ammonia: water (10: 1: 1 v/v) as recommended by Aung et al. [10].

The chromatograms were segmented transversely into equal segments (representing of Rf values) and their activity was estimated biologically using the straight growth coleoptile section bioassay test developed by Linser [11, 12] and modified by Youssef et al. [13] for IAA and ABA and the sorghum first leaf bioassay test developed by Bently Mowatt [14] and modified by Abdel-Wahhab [15] for GA₃-like substances.

Cytokinins were separated from aqueous extracts as described by Van Staden [16], chromatographed by paper chromatography using solvent system comprising of isopropanol-ammonia-water (10: 1: 1 v/v) as reported by Hayashi et al. [17] and the activity of cytokinin was estimated by the sunflower cotyledonary leaf bioassay test developed by Esachi and Leopold [18] and modified by Shalaty [19].

Chlorophyll (a) and (b) as well as total carotenoids were determined in the fresh materials as described by Von Wettstein [20]. Total carbohydrates and soluble sugars were determined by using colorimetric method and phenol-sulphuric acid method according to Dubois et al. [21].

**Statistical analysis:** Data obtained were subjected to standard analysis of variance procedure. The values of LSD were obtained whenever F values were significant at 5% level as reported by Snedecor and Cochran [22].

**RESULTS AND DISCUSSION**

A. **Endogenous hormones:**

1- **Effect on endogenous gibberellin-like substances:** The biological activities of endogenous gibberellins in sorghum 1st leaf bioassay test of purified acidic extracts of treated and untreated rose plants are illustrated in Fig. (1). All used treatments increased promotion activities over control plants.

Plants sprayed with 50 ppm GA₃ showed an increase in promotion activities of GA₃ gibberellin with maximum activity at Rf, 0.5 - 0.6. In case of 40 ppm, Ki it showed also one zone of gibberellin activities located at Rf, 0.0 - 0.1.

Extracts of plants treated with 15 ppm BR, showed maximum promotion at Rf, 0.5 - 0.6. On the other hand plants sprayed with 50 ppm GA₃ + 40 ppm Ki exhibited one zone of gibberellin activities at Rf, 0.0 - 0.1 and maximum activities at Rf, 0.6 - 0.7. Plants sprayed with 50 ppm GA₃ + 15 ppm BR, caused a maximum promotion of gibberellin activities at Rf, 0.2 - 0.5 and 0.7 - 0.9.

In case of 40 ppm Ki + 15 ppm BR, the regions of gibberellin activities were clear at Rf, 0.0 - 1.0 and Rf, at 0.7-0.8 giving the highest values of promotion. In case of extracts from plants treated with (GA₃, 50 ppm + Ki 40 ppm + BR, 15 ppm) the maximum promotion was found of Rf, 0.7-0.8.

Regarding untreated plants, very low gibberellin activities were observed at Rf 0.2-1.0 and one inhibition zone was found at Rf 0.0-1.0. The highest biological activity of gibberellin like substances was determined for rose plants treated with 50 ppm GA₃ followed by 50 ppm GA₃ + 15 ppm BR, higher than control plants.

Within the growth regulators treatment, it was clear that spraying rose plants with 40 ppm Ki resulted in very low gibberellin activity.
Fig. 1: The biological activities of endogenous gibberellins like substances in Sorghum first leaf bioassay test of purified extracts of treated and untreated rose plants.
- Load of each chromatogram equivalent to 10 gm of fresh weight
- Shaded zones indicate promotion or inhibition significance. *(t)* designates L.S.D.
Fig. 2: The biological activities of endogenous IAA and ABA in wheat coleoptile section test bioassay of purified extracts of treated and untreated rose plants.

- Load of each chromatogram equivalent to 10 gm of fresh weight
- Shaded zones indicate promotion or inhibition significance, (t) designates L.S.D.
Fig. 3: Biological activities of endogenous cytokinin in Sunflower cotyledonary leaf. Bioassay test of purified extracts of treated and untreated rose plants.

- Load of each chromatogram equivalent to 10 gm of fresh weight
- Shaded zones indicate promotion or inhibition significance, (f) designates L.S.D.
Effect on endogenous IAA, IAN and ABA: The biological activities of endogenous IAA, IAN and ABA in wheat coleoptil section test bioassay of purified extract of rose plants as affected by different growth regulators are illustrated in Fig. 2.

Data revealed promotion zone at RF, 0.6-0.5 the promotion activity in all treatments and untreated plants except for RF, 0.0-0.6 were correlated with authentic IAA. Moreover, this zone in all cases gave purple colour with Erlich reagent, pink colour with salkowski and fluorescent colour, when exposed to UV rays. On the basis of RF value, colour reaction and ultraviolet fluorescence, this growth promoter IAA.

An inhibition zone was evident at RF, 0.5-0.8 except for BR, 0.6-0.8, which is corresponded with RF, of ABA authentic standard.

A promotion zone was also detected at RF of 8-1.0 in all treatments as well as untreated plants. The activity of this acidic promoter was higher in all treatments as compared with untreated plants. In addition this zone of inhibition gave the colour of fluorescence of ABA on TLC plates after spraying with 5% pH 2 H_2SO_4 and examined by UV. The inhibitory level was lower in all treatments compared with untreated plants. The magnitude of inhibition in treated plants declined to reach about quarter the value of that shown by control plants. It is worthy to mention that the highest activities of IAA, IAN and disappearance of ABA appears clear at foliar spraying of rose plants with 15 ppm BR, 40 ppm Ki + 15 ppm BR, 50 ppm GA_3 + 40 ppm Ki + 15 ppm BR, and 40 ppm Ki, respectively.

Effect on endogenous cytokinins: The biological activities of endogenous cytokinin in sunflower cotyledonary leaf bioassay test of purified extracts are illustrated in Fig. 3. It is evident that untreated rose plants showed low activity of cytokinins, whereas all treatments of rose plants showed high activities. These activities were expressed by expanded region at 0.0-1.0 for plants treated with all used growth regulators.

In case of the amount of Zeatin (Z), Zeatin gulcoside (ZG) and Zeatin riboside (ZR) the data shows that the maximum values of Z were found in plants treated with 40 ppm Ki + 15 ppm BR, while the lowest value was found in plants treated with 50 ppm GA_3. The maximum values of ZG were found in plant treated with 50 ppm GA_3 + 15 ppm BR, and the lowest values were resulted for plants treated with 50 ppm GA_3 + 40 ppm Ki. In case of ZR the maximum values were recorded for plants treated with GA_3 + Ki + BR, the lowest amount was found in plants treated with 50 ppm GA_3.

Generally, the highest activities of endogenous cytokinins were obtained from spraying rose plants with Ki + BR, respectively.

The outcome of these results obviously show that all treatments increased the level of endogenous IAA, gibberellin and cytokinins and decreased the level of ABA. These results are in agreement with Khajigay and Moussa [23], they obtained an increasing content of endogenous gibberellin in cotton seeds and cotyledons as a result of application of GA_3. Mahgoub [24] reported that spraying rose plants with GA_3 at the concentration 50, 100, 200 ppm increased the level of IAA, IAN and decreased the level of ABA, these results agreed with Lau and Yang [25], they obtained an increment in the level of endogenous auxins and attributed these changes to the effect of cytokinins on enhancing IAA uptake and suppressing the conversion of IAN and IAA to indole-3-acetic acid. In case of benzyl adenine treatment the results were in agreement with those reported by Chin and Beevers [26], they found that suppression of ABA inhibition on leaves of nasturtium due to kinetin treatment also, Susumu et al. [27] indicated that application of benzyl adenine for breaking dormancy of Allium wakgei bulblets caused sharp decrease in ABA content and significantly increased IAA content. Mahgoub [24] found that an increases in IAN, IAA and decreases in ABA in rose plants, treated with BA at 250, 500 and 750 ppm also who found an increasing in Z, Zr and ZG in plants treated with BA and GA_3 at all concentration used. Concerning the effect of brassinosteroids treatment Shalaby and Abdel-Halim [7] showed that spraying faba bean with zink (300 ppm), soluble potassium at (100 ppm) and BR_3 at (30 ppm) increased the amount of growth promotion (mainly IAN, IAA ) and gibberellins like substances and markedly decreased in the amount of growth inhibitors (mainly ABA) as compared with control. On the other hand Helmy et al. [8] found that spraying board bean plants by BR_3 at ( 15, 30 and 60 mg L^{-1}) increased IAA, IAN and gibberellins like substances and decreased ABA as compared with control plants.

In this respect Shalaby and Talaat [28] found that spraying BR_3 at 50 and 100 ppm on Calendula officinalis L. plants resulted in significant increases in the amount of auxins, gibberellins like substances and cytokinins, with the amount of ABA was markedly decreased. The hormonal picture indicated that the growth regulator alone or in combinations caused an increase in IAA, IAN and GA_3 like substances BR, may be regarded as a new group of plant hormones[4,29] with regulatory function in
cell elongation and division. Moreover, they also have a role on protein synthesis, which in turn increase the endogenous hormones. In addition, the effect of BRₙ has a specific role on endogenous hormones levels. Also, protein synthesis, i.e. amino tryptophan as a precursor to IAA [30].

Consequently application of exogenous growth substances (GA₃, Kᵈ, BRₙ, GA₃ + Kᵈ, GA₃ + BRₙ, Kᵈ + BRₙ, and GA₃ + Kᵈ + BRₙ) increased the endogenous levels of the growth promoters IAA, gibberellin and cytokinin as compared with control treatment. Nag et al. [31] on rooting of mung bean cutting (Vigna radiata cv. 105) mentioned that treating cuttings with putrescine (10⁻⁴ M) increased the level of IAA and decreased of ABA compared to untreated cuttings. Zaghloul [32] found that using polyamine (sperrmidine) on Mung bean (Vigna radiata cv. Giza 1) at the concentrations of 10 and 20 mg L⁻¹ increased endogenous IAA level and decreased the content of endogenous ABA during seedling stage. Also, Belketa et al. [33] found that thyme plant (Thymus serpillum L.) with stigmasterol at the rate of 50 or 100 ppm increased the level of IAA and GA₃ and decreased the level of endogenous ABA compared with untreated plants. In addition, Bais and Ravishnaka [34] dealing with Cichorium intybus L. plants found that treating hairy roots with putrescine at concentration of 1.5 mM led to maximum level of endogenous IAA as compared to the control. Youssaf et al. [35] indicated that foliar application of 250 mg L⁻¹ putrescine on Matthiola incana L. increased the level of GA₃ and cytokinin and decreased the level of ABA in the treated plants. Recently, Talaat et al. [36] indicated that spraying periwinkle plants (Catharanthus roseus L.) with putrescine at the concentration of 10⁻⁵, 10⁻⁴ M or 10⁻³ M caused obvious changes in the levels of IAA, GA₃, cytokinin and ABA compared to the untreated plants. In addition the role of BRₙ having specific hormones in plants and promotion of protein, i.e. amino tryptophan as a precursor to IAA [30].

Mahgoub et al. [37] noticed that application of stigmasterol and / or putrescine at all treatments used (50, 100 ppm stigmasterol and 200, 400 ppm putrescine) on carnation (Dianthus caryophyllus) plants an increased the content of endogenous natural promoters (IAA, GA₃ and cytokinins) in comparison with those obtained from untreated plants. On the other hand application of the same treatments caused dramatic reduction in the levels of endogenous natural inhibitor (ABA) as compared to the control plants.

Chemical composition:
Chlorophyll (a) content: Data in Table 1 revealed that spraying rose plants with GA₃ (50 ppm) did not show any increase in chlorophyll (a) content at the three sprays in both seasons as compared with control plants. The content of chlorophyll (a) was significantly increased by using 40 ppm Kᵈ in the 1st spray at the two seasons and the 2nd and 3rd spray at the second season. BRₙ treatment, no significant effect had been achieved as a result of application.

Generally, using growth regulators showed only their effect early at the beginning of their application, while after that the effects were not detected clearly. Kinetin was the most effective on synthesis of chlorophyll (a) followed by GA₃ + Kᵈ + BRₙ, then GA₃ + BRₙ, and Kᵈ + BRₙ.

Chlorophyll (b) content: The obtained data in Table 1 show the effect of different growth regulators on chlorophyll (b) content. The presence of GA₃ showed no effect on increasing chlorophyll (b) content in most cases for the two seasons. Either Kᵈ or BRₙ had only significant effect on chlorophyll (b) content in the absence of GA₃. This means that synthesis of chlorophyll (b) may be inhibited by using GA₃ for spraying the plants. The effect was clear in the 1st two sprays, while in the 3rd one the effect was not significant in the 1st season.

Total carotenoids: Data presented in Table 1 reveals that spraying GA₃, Kᵈ and BRₙ, produced a significant increase in the two experimental seasons. The recorded data were higher in all cases than that obtained from control plants. Generally, the values were the highest for the 1st spray, followed by the 2nd and finally for the 3rd spray. The presence of GA₃ seems to effect positively on the synthesis of carotenoids, when it was alone or combination with other growth regulators. These results were agreement with Han et al. [38] who obtained an increase in the leaf chlorophylls content by using epi brassinolide on tobacco. Mahgoub [24], also concluded that treating rose plant by GA₃, BA at various concentration increased chlorophyll (a), (b) and total carotenoids. Talaat and Youssaf [39] on Hibiscus sabdariffa L. reported that using BA and BRₙ resulted in an increase in chlorophyll content might be due to promotion of synthesis [40] or retardation of breakdown [41] by using growth regulators. Bekheta et al. [33] recorded that foliar application of brassinosteroid and stigmasterol on Thymus serpyllum plants increase significantly chlorophyll (a), chlorophyll (b) and total carotenoids.
Table 1: Chlorophylls contents (mg/gm d.wt.) of rose plants as affected by GA3, KI and BRs treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>GA3</th>
<th>KI</th>
<th>BRs</th>
<th>GA3+KI</th>
<th>GA3+BRs</th>
<th>KI+BRs</th>
<th>GA3+KI+BRs</th>
<th>L.S.D. at 5% level</th>
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<td>Time of spray</td>
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<td>40 ppm</td>
<td>15 ppm</td>
<td>50+40 ppm</td>
<td>50+15 ppm</td>
<td>50+40+15 ppm</td>
<td>50+40+15 ppm</td>
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<td>7.71*</td>
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<td>6.72</td>
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<td>3.85</td>
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<td>4.42</td>
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*: Significant compared to the control.

Table 2: Carbohydrates contents (% d.wt.) of rose plant as affected by GA3, KI and BRs treatments

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<th>Treatments</th>
<th>Conc.</th>
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<th>GA3</th>
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<th>BRs</th>
<th>GA3+KI</th>
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<td>50+40+15 ppm</td>
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<tr>
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<td>16.78*</td>
<td>17.69*</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td>Total soluble sugars</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First season</td>
<td>1.85</td>
<td>1.94</td>
<td>2.28</td>
<td>2.35</td>
<td>2.25</td>
<td>2.17</td>
<td>2.28</td>
<td>3.46*</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>second season</td>
<td>1.98</td>
<td>2.05</td>
<td>3.10*</td>
<td>3.44*</td>
<td>2.51</td>
<td>2.31</td>
<td>2.55</td>
<td>3.54*</td>
<td>1.01</td>
<td></td>
</tr>
</tbody>
</table>

*: Significant compared to the control.

content of the leaves. Mahgoub et al. [37] show that foliar application of stigmasterol at 50 mg L⁻¹ or putrescine at 200 mg L⁻¹ on carnation plants increased the content of chlorophyll (a), chlorophyll (b) and carotenoids as compared to the control plants. The increment in chlorophyll content as a result of putrescine, application at 200 mg L⁻¹ could be attributed to the influence of high Mg²⁺ content of the shoots where Mg²⁺ is known as essential element for chlorophyll synthesis [42].

**Total carbohydrates content:** Data in Table (2) show that the total carbohydrate contents were significantly increased as a response to foliar spray with growth regulators. Plants treated with different growth regulators contained higher values of total carbohydrates content than control plants. The highest values 12.88 and 17.69 (% d.w.) were found in plants treated with GA3 + KI + BRs at the used concentrations, i.e. 50 + 40 + 15 ppm while the lowest value, 8.90 and 11.56 (% d.w.) were resulted from plants sprayed with 50 ppm GA3, 40 ppm KI, for the 1st and 2nd season, respectively, the differences were significant in most cases.

**Soluble sugars content:** Data in Table 2 show that soluble sugars of rose plant were significantly affected by foliar application of growth regulators in both seasons. The obtained values were higher in all
treatments than the untreated rose plants, which contained 1.85 and 1.98 (% d.w.) in the 1st and 2nd season, respectively. The highest values for treated plants were 3.46 and 3.54% resulted from the treatment of \( GA_3 + Ki + BR \) while the lower values (1.94 and 2.05%) were recorded for plants treated with \( GA_3 \) for the 1st and 2nd season, respectively.

With regard to of the total carbohydrates and soluble sugars contents in rose plants, the application of growth regulators increased these components, this might be explained that these growth regulators affected the plant metabolism processes. These results are in agreement with HajSam [43] on Vines leaves and Kandil [44] on strawberries they reported that \( GA_3 \) increased total carbohydrates and total soluble sugar content. On the other hand, Mahgoub [24] mentioned that spraying rose plants with \( GA_3 \) and BA caused an increase in total carbohydrates and soluble sugars content in all treatment. Youssef and Tallat [45] on \textit{Lavandula officinalis} found that application of \( BR_3 \), \( Ki \) and combination between them caused an increase in total carbohydrates and soluble sugars contents. Similar effect was found by Talaat and Youssef [39] on \textit{Hibiscus sabdariffa}, they reported that using \( BA 40 \text{mg L}^{-1} \) and \( BR_3 (30 \text{mg L}^{-1}) \) caused an increase in total carbohydrates. Petzold et al. [46] suggested that the promotion of sucrose uptake as a result of \( BR \) treatment was probably due to modulation of H⁺-ATPase activity. Jai et al. [47] found that application of 0.01 mg L⁻¹ epibrassinolide foliar spray increased the activity of nitrate reductase in roots and leaves of \textit{Cicer arietinum} plants. On the other hand, Sairam [48] reported that application of homobrassinolide had a positive effect on total soluble protein content who concluded that homobrassinolide induced promotion of metabolic activity where this idea mediated through increasing enzymes protein synthesis, as well as, uptake of water resulting in enhanced relative water content under moisture stress. Talaat [49] shows that foliar application of \( BR \) (15 mg L⁻¹) on \textit{Nigella sativa} L. plants caused a significant increase in total carbohydrate and soluble sugars content of the seeds, these effects might be attributed to the enhancement of most metabolism processes such as protein phosphate RNA and riposom formation [50]. Abd-El wahed and Ali, [51] and Bakheta et al. [33] they found that foliar application of stigmasterol on \textit{Beta vulgaris} L. and thyme plants increased total carbohydrate contents. Mahgoub et al., [37] show that application of stigmasterols at (50, 100 ppm) and putrescine at (200, 400 ppm) alone or in combination increased total carbohydrate contents. The highest values were obtained from the application of stigmasterol at 50 mg L⁻¹, putrescine at 200 mg L⁻¹ and their combined treatment (50 + 200 mg L⁻¹) as compared with control may be attributed to increase in photosynthetic process efficiency which led to increase net assimilation of leaf \( CO_2 \) which is known as the basic unit of carbohydrate. In addition the role of \( BR_3 \) having specific hormones in plants and promotion of protein, i.e. amino tryptophan as a precursoer to \( IAA \) [30].

REFERENCES


