

STUDIES ON EFFECTS OF PLANT GROWTH REGULATORS ON PHYSIOLOGY OF LAC INSECT (*KERRIA LACCA* KERR.) INFESTED *FLEMINGIA SEMIALATA* (ROXB.)

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e-mail: [*pintuvsd@gmail.com](mailto:pintuvsd@gmail.com)Key words: Lac insect, *Kerria lacca* Kerr., *Flemingia semialata*, Biochemical constituents**ABSTRACT**

Lac Insect (Kerria lacca Kerr.) produces an important non-wood forest product (NWFPs) i.e. lac resin and its cultivation is boon for communities who depend on the forest resources for their livelihood. Plant growth regulators (PGRs) are considered as new generation of agro-chemicals after fertilizers, pesticides and herbicides which regulate overall growth rate of plants. Here, we examined the effect of PGR on growth, lac yield and leaf biochemical constituents of Lac Insect (Kerria lacca Kerr.) infested Flemingia semialata. For this purpose the plants were sprayed with four different PGR in different concentration, fifteen days before inoculation. The percent increase of plants height, total number of leaves and fresh biomass per plant was highest in NAA 25 ppm (22.77 % for plants height, 54.3 % for number of leaves and 45.61 % for fresh biomass) as compared to control plants. The percent increase in lac yield was highest in NAA 50 ppm (97.73 %) and NAA 25 ppm (86.36 %) compared to control. The percent increase in total sugar was highest in NAA 50 ppm (66.59 %) and NAA 25 ppm (45.81 %) compared to control. However, MH 1000 ppm showed highest (84 %) percent increase in soluble protein followed by MH 500 ppm (61.38 %) and NAA 50 ppm (56.72 %) compared to control. The percent increase in free phenol was highest in MH 500 ppm (61.5%) followed by kinetin 100 ppm (61.38 %) compared to control. The conclusive remarks of this study advocated the NAA spray should be used to obtain high lac yield on host plants Flemingia semialata (Roxb.).

Introduction

Lac Insect (*Kerria lacca* Kerr.) is an economically important sap sucking insect belongs to family Kerriidae (Ahmad *et al.*, 2012; Jan and Andre, 1989). They are mainly cultured for the production of lac resins on various host plants. Lac resins are a type of non-wood forest products (NWFPs) secreted by tiny gregarious lac insect, which have huge commercial importance. In India, conventionally *Butea monosperma* (Palas), *Schleichera oleosa* (Kusum) and *Ziziphus mauritania* (Ber), fruit ber varieties are the major lac host plants (Ghosh *et al.*, 2014, 2016). New plantations of these host plants are very challenging because of slow growing nature. Due to deforestation and adverse climatic changes, number of lac host plants species continuously decreases which directly affect the lac production. Moreover, the global demand for natural product is increasing steadily due to its wide application in several products viz., food, cosmetics, pharmaceuticals, varnishes and paints (Sharma and Ramani, 2011). To meet this ever increasing demand novel ways need to be found to increase the lac production in India. Possible ways are bringing unutilized lac hosts under cultivation or by increasing the production per unit area or by identifying new lac hosts or variants which will augment the lac production.

Keeping in view, a potential fast growing bushy plant species *Flemingia semialata* (Roxb.) has been identified for intensive lac cultivation during winter season. It has been gaining importance as host plant in lac cultivation due to its bushy and quick growing nature. In view of being responsive to high coppicing, manageable stature, bushy type of growth, ease in intercultural operations, it proved an excellent intercrop in any cropping system.

One more possible way is the use of Plant Growth Regulators which can be defined as naturally occurring or synthetic compounds that affect developmental or metabolic processes in higher plants, and also function as chemical messengers for intercellular communication. They are considered as new generation of agro-chemicals after fertilizers, pesticides and herbicides. That is they are concerned in the regulation of overall growth rate of plants and in the correlations of growth activities, by acting as chemicals (Upreti and Sharma, 2016). With this above background, the main aim of this study to know the effect of PGR

on growth, lac yield and leaf biochemical constituents of Lac Insect (*Kerria lacca* Kerr.) infested *Flemingia semialata*.

Materials and Methods

The study was carried out on *Flemingia semialata* grown at institute research farm (IRF) of ICAR-Indian Institute of Natural Resins and Gums, Namkum, Ranchi (Jharkhand). The plants were sprayed with plant growth regulators of different concentrations (NAA 25 ppm, NAA 50 ppm, Kinetin 100 ppm, Kinetin 200 ppm, Mepiquat Chloride 100 ppm, Maleic Hydrazide 500 ppm and Maleic Hydrazide 1000 ppm) fifteen days before inoculation. Plants were inoculated with *kusmi* strain of lac insect (*Kerria lacca* Kerr.). Plants with no spraying along with lac insect served as control. The standard cultural/ package of practices for *kusmi* lac cultivation were followed. The Morphological data such as plant height, number of leaves per plants, fresh biomass per plant and lac yield was recorded after PGR treatments on lac insect infested *F. semialata* along with control. Fresh healthy leaves of *F. semialata* were taken for the biochemical profiling that was immediately extracted and assayed according to the previously described method with minor modification. Total Sugar was estimated by phenol method as described by Dubois *et al.*, (1951) and Buysse and Merck, (1993). Estimation of Total (Free) Phenols was carried out by Folin-Ciocalteu method as described by Bray and Thorpe (1954). Estimation of total soluble protein was done as per method described by Lowry *et al.*, (1951). Estimation of Hydrogen peroxide (H₂O₂) was done by using the protocol of Alexieva *et al.*, (2001). Superoxide radicals were estimated as per Chaitanya and Naithani (1994). Standard statistical package was followed to analyze the data.

Results**Effect of Plant Growth Regulators on vegetative growth**

All vegetative growth parameters were significantly affected by the variously applied PGRs on lac insect infested *F. semialata* (Table1). The results revealed that NAA treatment (25 and 100 ppm) significantly increased plant height, number of leaves and fresh biomass in lac insect infested *F. semialata* compared to control (plants with lac insect infestation with no PGR spraying). Treatment NAA 25 ppm recorded highest plant height (155.6 cm) as compared to control (124.32 cm) and the percent increase was 22.77 % (Fig.1). Similar trend was found in number of leaves per plants and fresh biomass where treatment NAA 25 ppm recorded highest number of leaves (228) and fresh biomass per plant (1.66

kg) as compared to control (147 & 1.14 kg). NAA 50 ppm is also at par with NAA 25 ppm in terms of fresh biomass (1.65 kg). The percent increase in number of leaves was 54.3 % and in biomass it was 45.61 % over control (Fig.1).

Lac yield

In our study PGRs also significantly influence lac yield per plant in *F. semialata* (Table 2). Treatment NAA 50 ppm (0.87 kg) and NAA 25 ppm (0.82 kg) showed the highest broodlac yield per plant as compared to control (0.44 kg). Significantly lower broodlac yield per plant was recorded in Mepiquat Chloride 100 ppm treatment (0.46 kg). The percent increase in lac yield in NAA 50 ppm was 97.73 % and NAA 25 ppm was 86.36 % over control (Fig.1).

Effect of Plant Growth Regulators on leaf biochemical constituents

The data indicate the significant influence of PGR on leaf biochemical constituents viz., total sugar, soluble protein, free phenol, hydrogen peroxide and superoxide radicals. Among the treatments NAA 50 ppm recorded the highest total sugar (38 mg/g fr. wt.) as compared to control (23 mg/g fr. Wt.) and the percent increase was 66.59 % (Table 2 & Fig. 2). Treatment Malic Hydrazide (MH) 1000 ppm recorded the highest soluble protein (99 mg/g fr. wt.) as compared to control (54 mg/g fr. wt.) with 84 % increase (Table 2 & Fig.2). Treatment MH 500 ppm recorded the highest free phenol (26.3 mg/g fr. wt.) as compared to control (16.2 mg/g fr. wt.) and the percent increase was 61.5% (Table 2 & Fig. 2). Treatment Malic Hydrazide 500 ppm recorded the highest H_2O_2 (7077 mmol/g fr. wt.) as compared to control (6774 mmol/g fr. wt.) (Table 2). Treatment Malic Hydrazide 1000 ppm recorded the highest superoxide radicals (32.7 $\Delta A_{540}/\text{min}/\text{g}$ fr. wt.) as compared to control (24.8 $\Delta A_{540}/\text{min}/\text{g}$ fr. wt.) (Table 2). Treatments NAA, Kinetin showed decrease in superoxide radicals and H_2O_2 , whereas other treatments showed increased content (Fig. 2).

Discussion

The production per unit area can be increased by management of lac host plants using plant growth regulators. It is known that these plant growth regulators are known to modify the source-sink relationship and increase the translocation and photosynthetic efficiency. Plant height is an important morphological character in *F. semialata* which provides seat for nodes and internodes from where side branches emerge, and thus play an important role in determining the morphological frame work relating to productivity (Patil, 1989). The increase in plant height in *F. semialata* is mainly attributed to biological activities of auxins (NAA) viz., stimulation of stem elongation, cell elongation and promotion of cell division. NAA application increases plant height in turn lateral branches leading to increase in number of leaves which increase photosynthesis rate in plant. This result in fresh biomass per plants also increases. This is in agreement with Gare *et al.*, (2017) and Meena *et al.*, (2017) who reported that the application of NAA increased plant height in chili and okra respectively. Significantly lower plant height was recorded when sprayed with Maleic Hydrazide and Mepiquat Chloride in *F. semialata* as compared to control. Maleic Hydrazide and Mepiquat Chloride interfere with GA synthesis that results in compact structured and short statured plants by inhibiting cell elongation and reducing length of the internodes (Rademacher, 2000, Tung *et al.*, 2018). In our study treatment NAA recorded the highest broodlac yield per plant as compared to control. This is due to the fact that NAA promotes plant height, leaf number and total fresh biomass of the plant in our study. Increase in plant height provides sufficient space for lac insect settlement and high leaf number enables plant to synthesize

more photosynthates in turn fulfills the heavy demand of sucrose by lac insect.

Lac insect (*K. lacca* Kerr.) which is a phloem sap feeder like aphids, whiteflies, leafhoppers and cicadas exclusively feed on phloem sap of stem and sometimes leaf petioles (Ahmad *et al.*, 2012). In the present study treatment NAA (25 & 50 ppm) recorded the highest total sugar as compared to control. These treatments also increases plant height and number of leaves and produce high total fresh biomass. This in turn increases the lac yield of the plant. Agusti *et al.*, (2001) reported that the application of the synthetic auxin 3,5,6-TPA at the cell enlargement stage increased hexoses in developing fruit of Satsuma mandarin, cv. 'Okitsu' (*Citrus unshiu* Marc.). The carbohydrate accumulation induced by 3,5,6-TPA indicates that its stimulatory effect on fruit growth might operate via promotion of sink strength. Protein plays an important role as a macromolecule in biological system, made up of different types of amino acids forming the building block, working as signal molecule in various pathways. In the present study treatment Maleic Hydrazide (1000 ppm) recorded the highest soluble protein as compared to control. The increase in soluble protein was reported by Sable and Mane (2006) in onion when sprayed with Maleic Hydrazide 100 ppm. Phenolics are the class of secondary products that contain a phenol group—a hydroxyl functional group on an aromatic ring. Many serve as defense compounds against herbivores and pathogens (Taiz and Zeiger, 2002). In the present study treatment Maleic Hydrazide 500 ppm recorded the highest free phenol as compared to control. The accumulation of polyphenols in onion was reported by Sable and Mane (2006) when sprayed with Maleic Hydrazide 100 ppm. However NAA and kinetin produces lower free phenol content indicates that they are able to suppress the phenol production and helps in reducing the plant resistance towards lac insect development.

Oxidative radicals play an important role in plants during various stresses, including the biotic stress such as insect infestation. In our earlier study it was found that superoxide radical and hydrogen peroxide level increases in the host plant when infested with lac insect as compared to host plant with no lac infestation. Our results indicate that a decrease in the expression of the reactive oxygen species (ROS) scavenging enzyme may enable an increase in the concentration of ROS and H_2O_2 , which are directly toxic to insects. In present study growth retardants like Maleic Hydrazide produces the high superoxide radicals and H_2O_2 whereas NAA and kinetin spray lowered the free radicals as compared to control. Dubey *et al.* (2013) also observed down regulation of scavenging enzymes for reactive oxygen species during the infestation by sap-sucking insects. This suggests that growth promoters like NAA and Kinetin suppresses the production of free radicals and helps in insect development.

Conclusion

The use of new generation agrochemicals like plant growth regulators significantly influence morphological characters of *F. semialata* such as plant height, number of leaves and total biomass thereby enhancing the lac yield. Among various growth regulators, the growth promoters like NAA and kinetin responded better than any other treatments. Thus, the present study paved the way use of PGRs in enhancing the lac production.

References:

- Agusti, M., Zaragoza, S., Iglesias D. J., Almela, V., Primo-Millo, E. and Talon M. (2001). The synthetic auxin 3,5,6-TPA stimulates carbohydrate accumulation and growth in citrus fruit. *Plant Growth Regulation*, 00: 1–7

- Ahmad, A., Kaushik, S., Ramamurthy, V.V., Lakhanpaul, S., Ramani, R. and Sharma, K.K. (2012). Mouthparts and stylet penetration of the lac insect *Kerria lacca* (Kerr) (Hemiptera:Tachardiidae). *Arthropod Structure & Development*, 41: 435-441.
- Alexieva, V., Sergiev, Mapelli, I. S. and Karanov, E.(2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell and Environment*, 24:1337–1344.
- Bray, H.G. and Thorpe, W.V. (1954). Analysis of Phenolic compounds of Interest in Metabolism. *J Meth Biochem Anal*, 1:27-52.
- Buysse, J. and Merckx, R. (1993). An improved colorimetric method to quantify sugar content of plant tissue. *Journal of Experimental Botany*, 44: 1627-1629.
- Chaitanya, K.S.K. and Naithani, S.C. (1994). Role of superoxide, lipid peroxidation and superoxide dismutase in membrane perturbation during loss of viability in seeds of *Shorea rubusta* Gartn.f. *New Phytologist*, 126: 623-627.
- Dubey, N. K., Goe, R., Ranjan, A., Idris, A., Sing, S. K., Bag, S. K., Chandrashekar, K., Pandey K. D., Singh, P. K. and Sawant, S. V. (2013). Comparative transcriptome analysis of *Gossypium hirsutum* L. in response to sap sucking insects: aphid and whitefly. *BMC Genomics*, 14:241-260.
- Dubois, N., Gilles, K., Hamilton, J.K., Robers, P.A. and Smith, F. (1956). A colorimetric method for determination of sugars. *Analytical chemistry*, 28:350–356.
- Gare, B.N., Raundal, P.U. and Burli, A.V. (2017). Effect of Plant Growth Regulators on Growth, Yield and Yield Attributing Characters of Rainfed Chilli (*Capsicum annum* L.). *Advanced Agricultural Research & Technology Journal*. 1: 195-197.
- Ghosh J, Lohot V. D., Singhal V., Ghosal S., Sharma K. K and Ramani R.(2014). Plant-insect-environment interaction for kusmi lac production in ber (*Ziziphus mauritiana*) varieties, *The Ecoscan*, Special issue, Vol. VI: 407-411
- Ghosh J., Lohot V.D., Singhal V., Ghosal S. and Sharma K.K. (2016) Morphological-biochemical-physiological traits assisted selection for kusmi lac production on ber (*Ziziphus mauritiana* Lam.) varieties. *Indian J. Hort.* 73(1): 19-24
- Jan, W. and Andre V. (1989). The coccid insect dyes: HPLC and computerized diode-array analysis of dyed yarns. *Studies in Conservation* 34: 189–200.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193: 265-275.
- Meena, V.K., Dubey, A.K., Jain, V.K., Tiwari, A. and NEGI, P. (2017). Effect of plant growth promoters on flowering and fruiting attributes of okra [*Abelmoschus esculentus* (L.) Moench]. *Crops Research*, 52: 37-40.
- Patil, S.A. (1989). Genetics of yield, yield related physiological and morphological characters and their breeding implications in cotton (*G. hirsutum* L.). Ph. D. Thesis, University of Agricultural Sciences, Dharwad.
- Rademacher, W. (2000). Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. *Annual review of plant biology*, 51: 501–531.
- Sabale, A.B. and Mane, A.A. (2006). Biochemical parameters in *Allium cepa* L. varieties influenced by maleic hydrazide. *Agricultural Science Digest*, 26:123 – 125.
- Sharma and Ramani (eds.) 2011. Recent Advances in Lac Culture, IINRG, Ranchi.
- Taiz, L. and Zeiger, E. (2002). *Plant Physiology*, Sinauer Associates; 3 edition.
- Tung, S.A., Huang, Y., Hafeez, A., Ali, S., Khan, A., Souliyanonh, B., Song, X., Liu, A. and Yang, G. (2018). Mepiquat chloride effects on cotton yield and biomass accumulation under late sowing and high density. *Field Crops Research*, 215: 59–65.
- Upreti, K. K. and Sharma M. (2016). Role of Plant Growth Regulators in Abiotic Stress Tolerance. *Abiotic Stress Physiology of Horticultural Crops*, N.K.S. Rao et al. (eds.), doi 10.1007/978-81-322-2725-0_2.

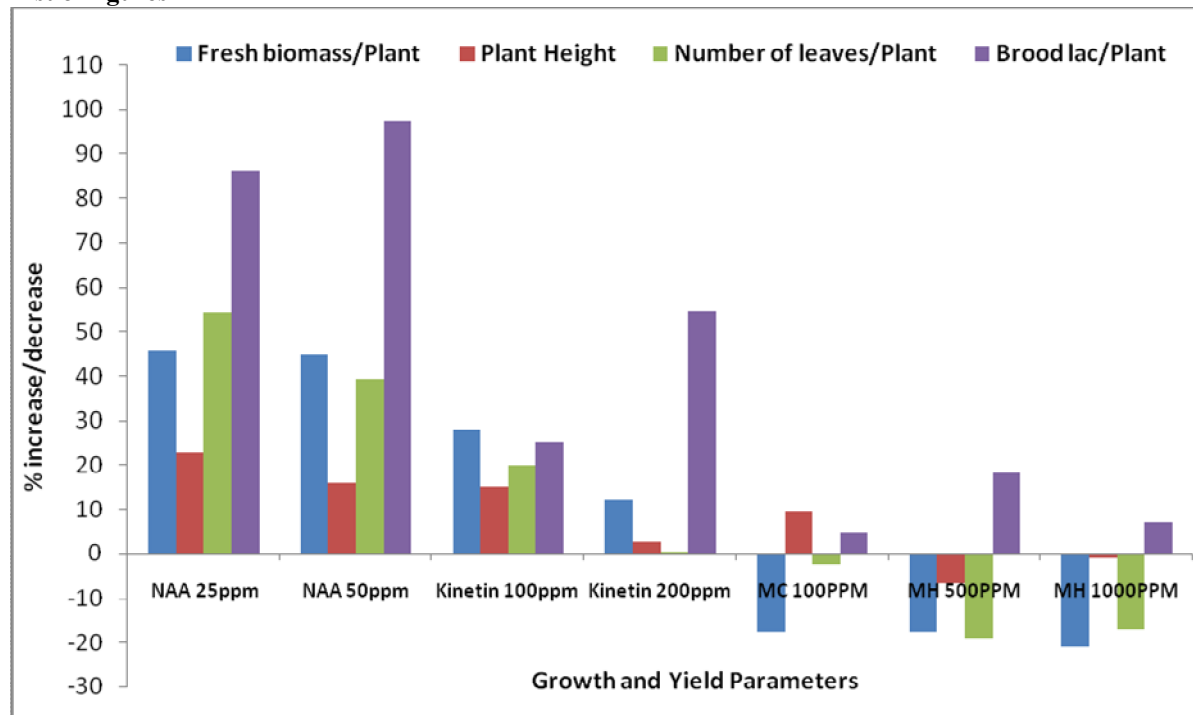
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Table 1. Effect of PGRs spraying on vegetative growth and lac yield of *Flemingia semialata*Table 3. Effect of PGRs spraying on leaf biochemical constituents of *Flemingia semialata*

Treatments	Fresh Weight/Plant (Kg)	Plant Height (cm)	Number of leaves/Plant	Brood lac/Plant (Kg)
NAA 25ppm	1.66*	152.6*	227.9*	0.82*
NAA 50ppm	1.65*	143.9*	205.8*	0.87*
Kinetin 100ppm	1.46*	143.0*	177.2*	0.55*
Kinetin 200ppm	1.28	127.4	148.3	0.68*
MC 100PPM	0.94	136.1*	144.0	0.46
MH 500PPM	0.94	115.9	119.6	0.52
MH 1000PPM	0.90	122.8	122.4	0.47
Control (with lac + no spray)	1.14	124.3	147.7	0.44
CD (5%)	0.22	5.08	27.31	0.1

Treatments	Leaf Biochemical Constituents					
	Total Sugar (mg/g fr wt)	Soluble protein (mg/g fr wt)	Free Phenol (mg/g fr wt)	Proline (mg/g fr wt)	Super Oxide Radical ($\Delta A_{540}/\text{min/g fr.wt}$)	H ₂ O ₂ (mmol/g fr wt)
NAA (25 ppm)	34*	77*	21.3*	0.7	14.6	6374
NAA (50 ppm)	38*	84*	21.8*	1.2	15.9	6078
Kinetin (100 ppm)	31*	73*	23.4*	1.1	24.4	5696
Kinetin (200 ppm)	30*	68	19.2*	0.5	25.0	5565
MC (100 ppm)	28*	32	10.9	0.8	26.5	7001
MH (500 ppm)	31*	87*	26.3*	1.3	27.0	7077*
MH (1000 ppm)	28*	99*	21.1*	0.6	32.7*	6846
Control (With Lac + No Spray)	23	54	16.2	1.3	24.8	6774
CD (5%)	1.38	4.32	1.68	0.07	2.75	260.78

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Fig. 1. Effect of PGRs spraying on percent increase/ decrease in vegetative growth and lac yield of *Flemingia semialata*

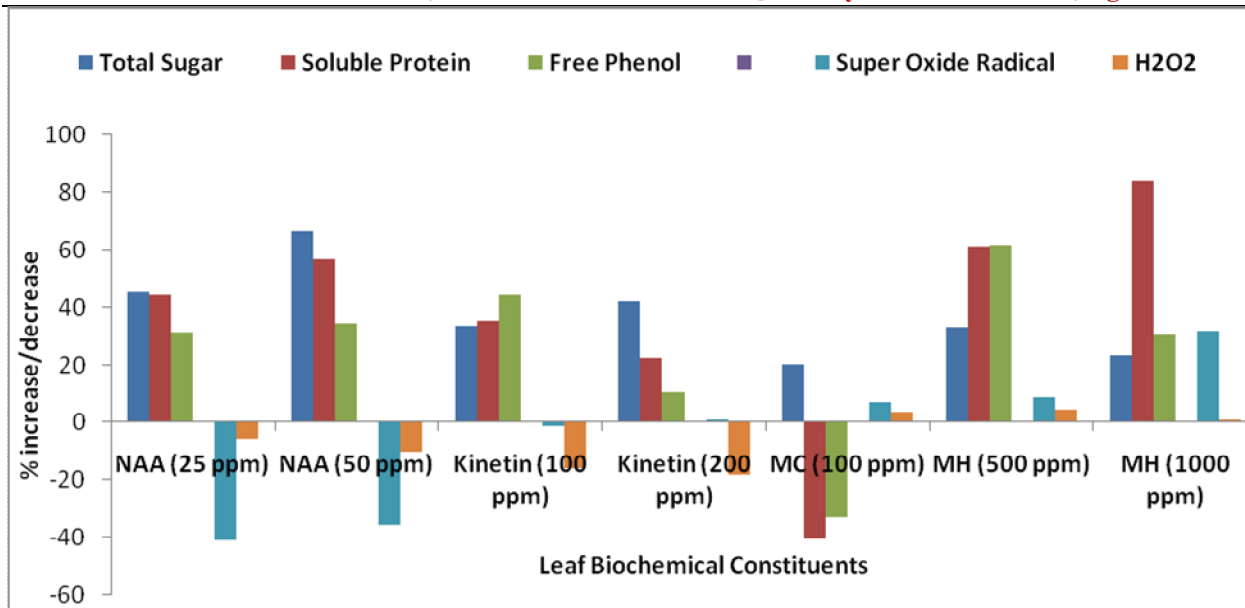


Fig. 2. Effect of PGRs spraying on percent increase/ decrease in leaf biochemical constituents