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Impact of Bioregulators on Phytochemicals and Quality of Black Night Shade *Solanum nigrum*

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Studies on influence of bioregulators (Panchagavya, leaf extracts of mukia, moringa, prosophis and root extract of withania) for yield and quality in *Solanum nigrum* indicated that the total drymatter production was favourably increased due to the application of all the bioregulators when compared to the control. Among these bioregulators, Panchagavya four per cent foliar spray registered highest drymatter production of 23.56 g and single plant yield of 73.10 g followed by moringa five per cent foliar spray recording 20.45 g and 69.98 g respectively. The quality parameters like leaf and fruit solasodine content, ascorbic acid, total soluble solids, total phenolics and solasodine content were maximum enhanced with the application of Panchagavya. The High Performance Thin Layer Chromatography (HPTLC) analysis of various compounds present in hexane extract showed the impact of Panchagavya in production of greater number of phytochemicals.

Key words: Bioregulators, *Solanum nigrum*, Panchagavya, Moringa, Mukia, *Prosophis*, *Withania*, phytochemicals, yield and quality.

The medicinal plants as a whole, occupy significant position in modern medicine, as the industry is showing special interest in synthesizing natural substances as they are found to be more effective in particular applications. India, one among the twelve biodiversity centre in the world, is regarded as the paradise of vegetation due to the rich diversity of agroclimatic and socio-cultural conditions prevailing in the country. According to one estimate, about 7195 plant species are being used in various Indian systems of medicine, particularly 1773 in Ayurvedic, 1122 in Siddha and 75 in Unani medicine (Prajapati, 2003). Nearly 4720 plants are being used in traditional or village medicines. Black nightshade, (Tamil: Manathakkali) is the vernacular name of *Solanum nigrum* L., which belongs to the family Solanaceae. This herb is nowadays gaining importance in pharmaceutical industry due to its amazing drug potential. The active principle in this herb is solasodine, a glucosidal alkaloid. Lack of basic information on crop production techniques is a limiting factor in herbal agriculture. There is therefore a need for studies on genetic, physiological and agronomic manipulations to increase productivity with quality, which leads to future expansion of area under medicinal plants. In recent years, there is growing awareness on use of environment-friendly and easily biodegradable natural organic sources and botanicals which act as a biostimulant and do not accumulate chemical residues in atmosphere, soil flora and fauna (Singh *et al.* 2001). The present investigation was carried out to generate knowledge and technology for improving the productivity and quality of black nightshade, with particular emphasis on sustainable production and prudent use of bioregulators.

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Materials and Methods

The present investigation was carried out during Rabi season at Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore (11°2" N and 76°57" E with 426.76 m above MSL). The main objective was to study the influence of various bioregulators on yield, quality and phyto chemicals of Black nightshade (*Solanum nigrum* L.). The soil of the experiment site is sandy clay loam. The available soil nitrogen, phosphorus and potassium were 106, 20 and 225 kg/ha respectively; Soil pH 7.2; EC 0.24 dSm^{-1}. The experiment was laid out in Randomized Block Design with sixteen treatments and three replications. The different treatments employed were foliar sprays of Panchagavya 2% (T_1), Panchagavya 3% (T_2) and Panchagavya 4% (T_3), mukia 1% (Mukia madraspatna) (T_4), mukia 2% (T_5) and mukia 5% (T_6), moringa 1% (Moringa oleifera) (T_7), moringa 2% (T_8) and moringa 5% (T_9), prosophis 1% (Prosophis juliflora) (T_{10}), prosophis 2% (T_{11}) and prosophis 3% (T_{12}), withania 1% (Withania somnifera) (T_{13}), withania 2% (T_{14}) and withania 3% (T_{15}) and unsprayed control (T_{16}). The plants were planted with a spacing of 45 x 30 cm. The bio-regulators were sprayed at 35th, 50th and 65th day after sowing with a hand sprayer, using teepol as adjuvant @ 1.0 ml l^{-1}. The leaves and stems were

dried in oven at 80°C for 72 h and the total drymatter production (TDMP) was measured as g plant^{-1}. The ascorbic acid content was extracted from fruits and leaves with oxalic acid and estimated by titrimetric method using 2, 6-dichlorophenol indophenol dye (A.O.A.C., 1975) and expressed in mg 100g^{-1}. Total soluble solids (TSS) of fruits were determined by using Carl-Zeiss hand refractometer and the results were expressed in degree brix (°Brix). Total phenolic content in leaves was extracted using hot ethanol and assayed according to Mallick and Singh (1980) and expressed as mg g^{-1}. The solasodine content was estimated by the method suggested by Bakshi and Hamied (1972) and later modified by Kaul and Zutshi (1982). The statistical analysis was done by adopting the standard procedures of Gomez and Gomez (1984).

Plant extracts

The leaves from the plants Mukia (*Mukia madraspatena*), Moringa (*Moringa oleifera*) and Prosophis (*Prosophis juliflora*) were collected and their extract was prepared with hot water in a ratio of 1:8 (w/v). Similarly Withania (*Withania somnifera*) root powder extract was also prepared with hot water at 1:32 ratio (w/v).

Panchagavya

The following ingredients were used to prepare approximately 20 litres of Panchagavya stock solution. Biogas slurry/ cow dung (5 kg), cow's urine (3 litres), cow's milk (2 litres), cow's curd (2 litres) and cow's clarified butter /ghee (1 litre). In addition sugarcane juice (3 litres), tender coconut water (3 litres) and ripened banana (1 kg) were added to accelerate the fermentation process (Natarajan, 2002). All the materials were added to a wide mouthed mud pot and kept open under shade. The contents were stirred twice a day for about 20 minutes both in the morning and evening to facilitate aerobic microbial activity. After 15 days, concentrations were prepared @ two, three and four per cent from this stock solution and used for spraying.

Solvent extraction

Solvent extraction is a method to extract a soluble fraction of phytochemicals from a solid medium. Four different solvents *viz.,* Hexane, Chloroform, Methanol and Water were used to perform the extraction. Sample weighing about 5.0 g was taken in a flask of known weight and about 250 ml of different solvents were added for extraction through Soxhlet. The extraction was completed when the solution in the Soxhlet chamber has the same color as that of pure solvent. After that, it was evaporated to dryness using a rotary evaporator at a temperature of 45 5°C. After all the visible solvent was removed by the rotary evaporator, the flask was placed in a vacuum oven at 40 ± 1°C for 24 ±1 hour. Then the extract was used for the separation of phytochemicals (Moore and Johnson, 1967).

Separation of phytochemicals by Thin Layer Chromatography (TLC)

For phytochemical separation, commercially available analytical TLC plate of 0.2 mm thickness of silica sorbent was used. Separation was carried out by the application of the various extract as a spot on to the sorbent. Then it was placed in a tank with sufficient suitable solvent mobile phase (Hexane: ethyl acetate, 9:7 ratio) to just wet the lower edge of the plate. The solvent front then migrates up the plate through the solvent by capillary action. Relative front of various phytochemical (R_f) was calculated by the following formula.

$$R_f = \frac{\text{Compound distance from origin}}{\text{Solvent front distance from origin}}$$

Results and Discussion

The differential effect of varied concentration of bioregulators on TDMP of black nightshade is shown in Table1. Maximum TDMP was observed in T_3 (Panchagavya @4%). For withania an increase in TDMP was observed in T_{14}. Higher concentration of Panchagavya (T_3) recorded the highest yield followed by moringa 5% foliar spray. Even the lower concentration of prosophis foliar spray (T_{10}) also increased the yield to a tune of 2.96 per cent over control. The major yield components of black nightshade are leaves, stem and fruit. These components contribute to total drymatter production of shoots, which have commercial value. The fruit and leaves are considered major yield components since they contain more amount of solasodine, a phytochemical having therapeutical value. The increased yield was mainly due to efficient partitioning of assimilates which was differentially

Table 1. Effect of bioregulators on TDMP and yield in *Solanum nigrum*

	TDMP (g plant^{-1})			
Treatment	50 DAS	65 DAS	80 DAS	Yield (g plant^{-1})
T_1	4.620	9.281	19.506	66.88
T_2	4.636	10.106	20.002	71.49
T_3	5.983	11.932	23.569	73.10
T_4	4.477	7.983	17.394	60.40
T_5	3.660	7.457	16.695	57.80
T_6	3.738	7.500	16.486	52.88
T_7	4.147	8.705	17.738	62.36
T_8	4.439	9.655	18.546	66.72
T_9	5.762	10.883	20.455	69.98
T_{10}	4.687	7.344	16.541	50.43
T_{11}	5.270	8.001	16.694	51.74
T_{12}	5.380	8.107	17.338	54.06
T_{13}	4.219	7.129	16.689	50.23
T_{14}	5.212	8.144	19.527	57.20
T_{15}	5.167	7.533	18.162	18.162
T_{16}	3.369	7.014	14.556	14.556
Mean	4.722	8.633	18.281	18.281
CD (P=0.05)	0.025	0.048	0.033	0.071

modified due to different bioregulators. The foliar spray of bioregulators significantly increased the quality parameters *viz.,* plant ascorbic acid content, total phenolics, TSS and solasodine content (Table 2). Ascorbic acid content was recorded maximum in T_2, T_3, T_8, T_9 and T_{15}. The total phenol content increased consistently with increased concentration of bioregulators except mukia and withania foliar sprays. Among the treatments, Panchagavya @ 4% recorded the highest value and the percent increase over control was 24.69. The TSS content of the fruit was significantly increased by means of application of bioregulators when compared to the control. Among all the treatments, T_2 and T_3 recorded the maximum value of 9.2°Brix followed by T_8 and T_9 recording 8.8° Brix. Irrespective of the concentration, solasodine content was maximum for Panchagavya treated plants, which was followed by moringa (1.87) and mukia (1.5). The quality of raw materials of Black nightshade is assessed based on solasodine content, ascorbic acid content and total phenolics content of leaves and in fruits, in addition to the former two quality parameters, total soluble solids are to be assessed. Panchagavya @ 4% concentration and moringa 5% concentration had better influence on increasing the quality parameters. Regarding

Table 2. Effect of bioregulators on quality parameters in *Solanum nigrum*

Treatment	Ascorbic acid content (mg 100 g^{-1})	Total phenolics (mg g^{-1})	Fruit	Leaf	Ascorbic acid content (mg 100 g^{-1})	TSS (°brix)
T_1	24	0.626	1.78	0.65	32.0	9.0
T_2	26	0.683	1.90	0.60	32.0	9.2
T_3	26	0.697	2.03	0.57	34.0	9.2
T_4	22	0.669	1.55	0.48	26.0	7.8
T_5	22	0.644	1.51	0.43	24.0	7.8
T_6	22	0.646	1.47	0.41	26.0	7.8
T_7	24	0.659	1.92	0.61	28.0	8.6
T_8	26	0.678	1.88	0.53	28.0	8.8
T_9	26	0.685	1.92	0.45	28.0	8.8
T_{10}	20	0.567	1.50	0.46	24.0	7.6
T_{11}	22	0.574	1.36	0.38	26.0	7.8
T_{12}	22	0.582	1.22	0.36	26.0	7.8
T_{13}	24	0.601	1.22	0.52	28.0	7.8
T_{14}	24	0.612	1.50	0.47	28.0	8.0
T_{15}	26	0.604	1.36	0.58	28.0	8.0
T_{16}	18	0.559	1.12	0.22	20.0	7.6
Mean	23	0.634	1.59	0.487	27.61	8.29
CD (P=0.05)	0.8	0.003	0.10	0.04	1.113	0.19

the concentrations of different bioregulators the fruit solasodine content was increased with increase in concentration only for Panchagavya. The reverse trends i.e., negative association between concentrations and fruit solasodine content was noticed for mukia, moringa and prosophis. It is interesting to note that as against all other parameters the leaf solasodine content was decreased when the concentration was increased for all the bioregulators except withania, in which lower per cent of leaf solasodine was recorded at T_{14}. The multiple effect of Panchagavya and moringa favours the yield and quality of black night shade due to the presence of either nutrients or biostimulants or plant growth hormones or combination of all these. This is in accordance with the observations found in chilli, onion and lemon (Sridhar *et al.,* 2001), annual moringa (Beaulah, 2001) and greengram (Somasundaram *et al.* 2003). The yield and yield components were also improved

Table 3. Phytochemical investigation on hexane extract of Panchagavya treated (T) leaves Vs control (C)

R_L value	Control	Treated	Remarks
0.11	+	+	Present in both T and C
0.12	+	+	Present in both T and C
0.14	-	+	Present in T
0.15	-	+	Present in T
0.18	+	+	Present in both T and C
0.18	+	-	Present in C
0.22	-	+	Present in T
0.23	-	+	Present in T
0.25	+	+	Present in both T and C
0.26	+	-	Present in C
0.27	+	-	Present in C
0.28	+	+	Present in both T and C
0.29	-	+	Present in T
0.32	-	+	Present in T
0.33	+	+	Present in both T and C
0.34	+	-	Present in C
0.35	-	+	Present in T
0.36	+	+	Present in both C and T
0.38	+	-	Present in C
0.39	-	+	Present in T
0.40	-	+	Present in T
0.41	+	+	Present in both C and T
0.41	-	+	Present in T
0.43	+	-	Present in C
0.46	-	+	Present in T
0.47	-	+	Present in T
0.48	+	-	Present in C
0.48	-	+	Present in T
0.49	+	-	Present in C
0.50	-	+	Present in T
0.50	+	-	Present in C
0.51	-	+	Present in T
0.55	-	+	Present in T
0.60	-	+	Present in T
0.61	+	-	Present in C
0.62	+	+	Present in both C and T
0.64	+	+	Present in both C and T
0.64	-	+	Present in T
0.69	-	+	Present in T
0.71	-	+	Present in T
0.74	+	-	Present in C
0.76	+	+	Present in both C and T

+ = Present - = Absent

by soil application of different tree leaves (Padmaja and Narayanan, 2001) and various growth regulators (Angamuthu, 1991; Belakbir, 1998; Brahmachari and Rubirani, 2000; Maibangsa *et al.,* 2000; Sivakumar *et al.,* 2001; Sujatha and Prakash Rao, 2001). The separation of phytochemicals and their quantification in TLC was carried out and analyzed in High Performance Thin Layer Chromatography (HPTLC). The HPTLC analysis data revealed that totally 42 phytochemicals, having different R_f values were recorded, in both Panchagavya treated and control. The data revealed that 11 phytochemicals with various R_f values were present only in hexane extract of control plants. Similarly 11 phytochemicals of varying Rf values were noticed in both treated and control plant hexane extracts. It is interesting to note that 20 phytochemical compounds were recorded only for hexane extract of the treated plants (Table 3). The marginal benefit of withania, mukia and prosophis might be due to the influence of various phytochemicals, which had stimulant activity on various physiological parameters and also acted as antioxidants of certain phytochemicals. The isolation of phytochemical compounds through HPTLC brings out the fact that certain compounds have been synthesized in higher amounts due to the application of Panchagavya when compared to the control. These phytochemicals might act as a biostimulant for activation of various physiological processes, and enhance the growth and yield attributes. More compounds (20 phytochemicals) were found in the extract of Panchagavya treated plants. This shows that Panchagavya treated plants synthesized more number of secondary metabolite compounds which might have favoured the physiological processes and resulted better growth performance coupled with yield and quality parameters of black nightshade. In-depth analysis indicated that certain phytochemicals can be newly synthesized due to Panchagavya treated plants, which might act as biostimulant for influencing growth, yield and quality attributes of black nightshade. Similarly, some compounds available under untreated control plants are absent due to Panchagavya treatment. This implies that these phytochemicals might influence the yield and quality characters of black nightshade. Purification and characterization of these photochemical may lead to further understanding of the quality of these phytochemicals and their possible usage in other agricultural crops.

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