INFLUENCE OF DIFFERENT PLANT GROWTH REGULATORS ON IN VITRO FLOWERING OF *Spilanthes acmella L.* (MURR.)

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Abstract : Efficient findings on influence of plant hormones on in vitro flowering of Spilanthes acmella L. Murr.(Synonymous to Acmella oleracea L. Janson) are reported in BAP (2 mg/l) and BAP (1mg/l) + IBA (1mg/l) than other concentrations. The minimum number of days (18.53) is required for in vitro flowering in the M.S. Media fortified with the BAP (1mg/l) + IBA (1mg/l) with 1.6 numbers of flowers per explants, than other combinations or individual cytokinin concentrations. Flower growth is showing vigorous growth with fully developed single, short, pink bud with healthy appearance. Highest number of flowering (1.9) per explants is occurred in BAP (2 mg/l) in 21.90 days. The flowering morphological response are reported 60 % with Long, healthy pink flower bud with vigorous growth. All cultures are established under 16/8 hr light and dark conditions with 25 ± 2 °C temperature.

IndexTerms **- Spilanthes acmella, in- vitro flowering, BAP, Kinetin, IAA, IBA, etc.**

I. INTRODUCTION

The plants are the resources of all needs of man. They provide food, shelter, cloth, wood, etc. But the most important things are they are the resources of medicinal drugs. *Spilanthes acmella* is known from ancient times (In ayuerveda named as *akarkarabha*) used for premature ejaculation and other health problems. In ayuerveda it is used as Vajikarana (Aphrodisiac, Virilification therapy) and Veeryastambhana (Restoring premature ejaculation) [13]. They has other medicinal properties too such as anti-inflammatory [03, 23, 33], analgesic [03, 06, 07, 09, 24, 28, 36, 41], free radical scavenging properties [42], etc. It is also helpful to increase Testosterone, FSH, and LH etc [34] hence used in sexual medicines like Viagra and some muscle building sterols. It also useful for the control of toothache, rheumatism and fever [8]. But most known medicinal use is the relief on oral and dental gum problems hence referred as Anti-toothache plant. Mostly the plant parts used as flower buds which biologically hub of various medicinal drugs such as Spilanthol [10, 12, 14, 19, 26, 29, 38], Scopoletin [25], Stigmasterols [4] Alkyal amide and other minor secondary metabolites. Some minor derivatives like Myricyl alcohol and pentacyclic triterpene [21, 39] etc, which used in various treatments such as in cancer, thyroid and cardiovascular problems etc. Its various decoctions are prepared and used to treat human and animal diseases. It also has insecticide, fungicide and other antimicrobial activity such as antiimalarial [20], antibacterial and antifungal [11] properties. Other parts like leaves, roots, and seeds also used in various medical treatments hence quantified huge demand in national and international pharmaceutical trade. This trade leads to plant in endangered or threatened category hence over-exploitation is avoided by raising such important pharmaceutical part in in-vitro condition. We do research on in vitro flowering response with respect to various plant growth hormones to identify best suitable plant growth hormones for in vitro flowering response.

The plant is belonging to Asteraceae family, Heliantheace tribe and Ecliptinae sub tribe [27]. It has more than 300 species [2, 15] and in India it has been reported 9 species as threatened species such as *Spilanthes oleracea, Spilanthes radicans* Jacq., *S. mauritiana L. S. calva D.C. S. ciliata* Kunth, *S. paniculata D.C. S. uliginosa* Sw., *S. acmella Murr,. S. acmella L. var. oleraceae Clarke,* [37,40]. The plant is originated from Brazil and Peru but in India it is acquainted by Portuguese through Indian laborers in 1900 [5]. Seed germination is quite well but not effective to propagate in large scale for phytochemical isolation without exploiting from natural resources. In vitro propagation technique will be solution to avoid over exploitation of these plants for ever-increasing demand of pharmaceutical trade.

II Materials and Methods:

Healthy plants are raised in the shade house of Department of Botany, in S. M. Dnyandeo Mohekar College Kalamb with all prerequisite cultural practices and care. The various explants are choosing as leaf, nodal segment and meristem. This explants are rinsed with running tap water for 30 minutes then five minutes washing with labolin solution and frequent three times washing with distilled water each for 5 minutes. This explants then surface sterilized with using bavistin (1 %), streptomycin (0.03 %) both for 10

minutes and then rinsed with two times of distilled water (each 5 minutes) to remove its content. These explants then again surface sterilized with 0.1 % $HgCl_2$ for 4 minutes after 30 seconds washing with 70 % ethanol treatment. The excess ethanol and $HgCl_2$ are removed by three times washing with distilled water for 10 minutes each and repeated if needs more. All operations are carried in pretreated UV rays Laminar Air flow platform. The explants (1-2 cm) are then excised and exposed with pre-fortified autoclaved M.S. Media and predetermined hormone concentrations.

The MS media with Cytokinin, Kinetin and BAP (each at 1 and 2 mg/l) and combinations of MS media with Cytokinin (BAP and Kinetin at 1, and 2 mg/l) and Auxins (IAA and IBA, at 1 and 2 mg/l) are tested effectively with different types of explants. All prepared media initially get autoclaved for 121 0 C at 15 lb pressure for 20 minutes to avoid contamination. Inoculated bottles are kept in tissue culture lab with providing 16/8 hr of light/ dark photoperiodic condition along with 25 ± 2 0 C temperature. When there is proper root formation this cultures are then shifted to carry bags filled with (2:1) Soil and cocopeat media and their hardening response are checked for 15 days.

III Statistical analysis.

All observations are recorded manually after 4 weeks and statistically analyzed with one way ANOVA and Duncan Multiple Range Test (DMRT) using Statistical Package for Social Science (SPSS, version 11.5) software at 5% level ($p<0.05$). The treatments are repeated with three times and each treatment has 20 bottles. The results are calibrated initially with their mean ± SE.

IV Results:

In-vitro flowering response results are showing the effectiveness of various plant growth regulators at different concentrations. The M.S. Media fortified with the combination of IBA (1 mg/l) + BAP (1 mg/l) takes lowest number of days (18.53) with 60 % flowering response for in vitro flowering followed by IAA (1 mg/l) + Kin. (1mg/l) in 18.77 days with 50 % flowering response (Table no 1 and Graph no 1,). On individual basis the BAP (2 mg/l) are most significant than Kinetin for the in vitro flowering with minimum no days (21.90) are required for flowering along with 60 % flowering response than other BAP (1 mg/l) concentration and Kinetin (1mg/l, and 2 mg/l).

Highest significant number of flowers per explants (1.9) are reported in BAP (2 mg/l) followed by 1.7 number of flowers per explants in BAP (1mg/l) [Table and graph no 1]. The flowers are single, long, pink and healthy with vigorous fully developed bud growth. The treatment combination of IBA and BAP are most effective than Kinetin and IAA with different treatment combinations. The IBA (1 mg/l) + BAP (1 m/l) are most significant than other treatment combinations for maximum number of flowers (1.5) and flowering response (60 %) than others. Morphologically the flowers are short with pink and healthy characters (Table No: 01 and Graph No: 1).

V Discussion:

The role of cytokinin is reported biologically active where it stimulates the hormonal balance of the plant for in vitro flowering. In our experimental analysis flower inductions start early when the use of auxins and cytokinin combinations. The IBA (1 mg/l) along with BAP (1 mg/l) takes lowest number of days (18.53) with 60 % flowering response for in vitro flowering which determines presence of auxins with cytokinin induce flower early (Photo no 1.b). Highest number of flowers (1.9) is reported in BAP (2 mg/l) shows BAP is the most important and effective compound for in vitro flowering (Photo no 1.a). Most important role of BAP in in-vitro flowering are noticed in *Withania somnifera* [31], *Rauvolfia tetraphylla* [1] and *Anethem graveolens* [17] . Similar role of Kinetin also reported in Nicotiana tabacum [22].

The role of auxins in in-vitro flowering take part as a floral inhibitor [32]. There is also similar output reported in vitro flowering *Lycopersicon exculentum* at high levels of endogenous auxins [35]. Similar role of IBA in in-vitro flowering of *Hyptis suaveolens* is reported [18]. Off course presence of sucrose in M.S. media always plays major role along with cytokinins for in-vitro flowering as they interact with each other as noticed in *Sinapis alba* [16] and Arabidopsis thaliana [30] by moving interaction between shoot and root.

VI Hardening and acclimatization:

All successful rooted plants with their flower buds are shifted in polythene bag (2:1 proportion of soil and cocopeat) and their survival rate recorded 80 % after removal from cultured bottles. This carry bag is initially supplied with ½ strength M.S. Media and later on tap water with drenching of 1 % bavistin at 15 days interval.

VII Conclusions:

The present investigation on in vitro flowering of *Spilanthes acmella* with different auxins and cytokinin either individual or in combinations find the production of pharmaceutical flower buds for its valuable medicinal compounds. The in vitro production of flowers helps to conserve Spilanthes species without exploitation in field. BAP at 2 mg/l and BAP (1mg/l) and IBA (1mg/l) are the most effective for in vitro flower production with similar morphological characters of the plants are reported.

VIII Acknowledgments:

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Photo 1. (a) M S + BAP (2 mg/l) in vitro flowering response.

Photo 1. (b) Photo 1. M S + B BAP (1 mg/l) in vitro flowering response.

Table No: 1: Effect of BAP and Kinetin on In Vitro Flowering

Conc. (mg/ l)	No of Days for Flower	% of	No of Flower	Flower Characters	Flower

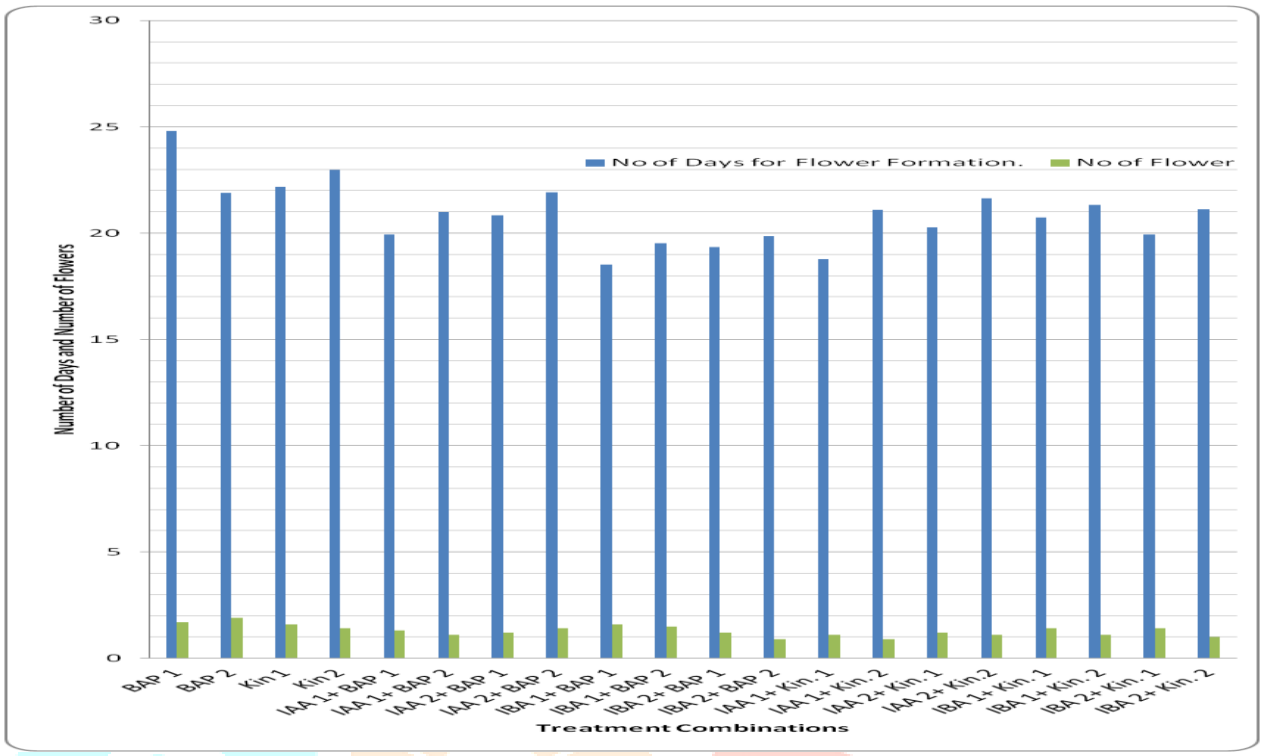
	Formation. (Mean ± SE)	Flower Formation.	(Mean ± SE)		Growth
BAP 1	24.80 ± 1.07	40	1.7 ±0.21	Single, Long, Pink and Healthy	F^{+++}
BAP 2	21.90 ± 0.90	60	1.9 ±0.23	Single, Long, Pink and Healthy	F^{+++}
Kin 1	22.17 ± 0.54	30	1.6 ±0.22	Bunchy, Long, Pink and Healthy	F^{+++}
Kin 2	22.97 ± 0.40	40	1.4 ±0.27	Single, Long, Pink and Healthy	F^{+++}
IAA 1+ BAP 1	19.93 ± 1.11	50	1.3 ±0.26	Single, Short, Pink and Healthy	F^{++}
IAA 1+ BAP 2	21.00 ± 1.16	40	1.1 ±0.28	Single, Long, Pink and Healthy	F^{+++}
IAA 2+ BAP 1	20.83 ± 0.84	50	1.2 ±0.25	Single, Short , Light Pink and Healthy	F^{+++}
IAA 2+ BAP 2	21.93 ± 0.90	40	1.4 ±0.16	Single, Long, Pink and Healthy	F^{++}
IBA 1+ BAP 1	18.53 ± 1.04	60	1.6 ±0.16	Single, Short, Pink and Healthy	F^{+++}
IBA 1+ BAP 2	19.53 ± 0.94	50	1.5 ±0.22	Bunchy, Long , Pink and Healthy	F^{+++}
IBA 2+ BAP 1	19.33 ± 0.88	60	1.2 ±0.13	Single, Short, Light Pink and Healthy	F^{++}
IBA 2+ BAP 2	19.87 ± 1.13	50	0.9 ±0.10	Single, Short , Light Green and Healthy	F^{+}
IAA 1+ Kin. 1	18.77 ± 1.06	50	1.1 ±0.18	Single, Long, Pink and Healthy	F^{+++}
IAA 1+ Kin. 2	21.10 ± 1.04	40	0.9 ±0.10	Single, Short, Light Pink	F^{++}
IAA 2+ Kin. 1	20.27 ± 0.96	50	1.2 ±0.13	Single, Long , Pink and Healthy	F^{+++}
IAA 2+ Kin.2	21.63 ± 0.88	40	1.1 ±0.18	Single, Short , Light Pink	F^{+}
IBA 1+ Kin. 1	20.73 ± 0.88	50	1.4 ±0.16	Single, Long , Pink and Healthy	F^{+++}
IBA 1+ Kin. 2	21.33 ± 0.88	40	1.1 ±0.23	Single, Short, Light Pink and Healthy	F^{++}
IBA 2+ Kin. 1	19.93 ± 1.04	60	1.4 ±0.27	Single, Long, Pink and Healthy	F^{+++}
IBA 2+ Kin. 2	21.13 ± 0.71	40	1 ±0.26	Single, short and Light Whitish Green	F^{+}

Growth Reponses of Flower Formation:

(-) Underdeveloped, (F^{+}) Normal Growth, (F^{++}) Moderate Growth, (F^{+++}) Vigorous Growth.

Data are based on 20 explants per treatment (With three repetitions) and data record after 4 weeks.

Graph No: 1. Effect of BAP and Kinetin and their combination with IAA and IBA for in vitro flowering


30
25
20
15
10
5
0
No of Days for Flower Formation.
No of Flower
Number of Days and Number of Flowers
BAP 1
BAP 2
Kin 1
Kin 2
IAA 1+ BAP 1
IAA 1+ BAP 2
IAA 2+ BAP 1
IAA 2+ BAP 2
IBA 1+ BAP 1
IBA 1+ BAP 2
IBA 2+ BAP 1
IBA 2+ BAP 2
IAA 1+ Kin. 1
IAA 1+ Kin. 2
IAA 2+ Kin. 1
IAA 2+ Kin. 2
IBA 1+ Kin. 1
IBA 1+ Kin. 2
IBA 2+ Kin. 1
IBA 2+ Kin. 2
Treatment Combinations