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Judicious use of Bioregulators to increase productivity and quality in Grapes

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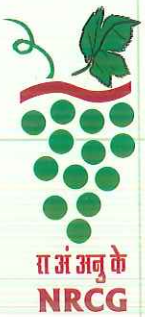

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Abbreviations

2,4-D	:	2, 4 Dichloro Phenoxy Acetic Acid
2,4,5-T	:	2, 4, 5 Trichloro Phenoxy Acetic Acid
6BA	:	6-Benzyl Amino Purine
ai	:	Active Ingradient
ABA	:	Abscissic Acid
CCC	:	Chlormequate Chloride or Cycocel
CIB	:	Central Insecticide Bureau
CK	:	Cytokinines
CPPU	:	N-(2-chloro-4-pyridyl)-N -phenyl urea
DAP	:	Days after Pruning
DNA	:	Deoxyribo Nucleic Acid
GA3	:	Gibberellic Acid
HACCP	:	Hazard Analysis and Critical Control Point
IAA	:	Indole Acetic Acid
IBA	:	Indole-3, Butyric Acid
NAA	:	1-Napthalene Acetic Acid
PGR	:	Plant Growth Regulators
ppm	:	Parts per million
RNA	:	Ribo Nucleic Acid
TDZ	:	Thidiazuron
TSS	:	Total Soluble Solids

PREFACE

Grape is the one of the important commercial fruit crop grown in India. Maharashtra occupies more than 75 per cent of the total area under grape cultivation in the country. In India, the grapes are grown by and large for table purpose and same quality is used for, raisin and wine making. Out of total production, approximately 78 per cent grapes are utilized for domestic consumption and two per cent for export. The reasons for less export are mainly due to non compliance of quality standards of the international markets. To achieve these quality standards, the use of bioregulators is an important tool to enhance the metabolic process which results in better quality in final grape produce.

This technical bulletin covers in detail the use of bioregulators and its role in application for grape cultivation. It also covers the stage of crop, dose of application and method of application for effective results in grapes. All this information has been substantiated based on the research data carried out at global level and partly at this institute and same has been given in tabular form with photographs.

The information given in bulletin will serve as guidelines for the grape growers to know more about the use of bioregulators judiciously to get quality production and also to increase the production per unit area. Further, the bulletin is also handy for the growers as well as for the researchers and the students engaged in the research and developmental activities of grape. I also take this opportunity to acknowledge the help received from our colleagues of this institute in bringing out this bulletin.

Place : Manjri, Pune
Date : March, 2010

P. G. ADSULE
(Director)

Introduction

Grape (*Vitis vinifera* L.) is one of the major important fruit crops of the country. It is cultivated in the sub-tropical regions of India on an area of 80,000 ha. The major grape growing states in India are Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu, Punjab, Uttar Pradesh and Haryana. Among them, Maharashtra ranks 1st in area (approximate 75% with a production of 1415,000 MT (NHD-2009)). In Maharashtra, Nashik, Sangli, Solapur and Pune are the major grape growing regions. The grapes in the country are grown for different purposes like table purpose, wine, juice and raisin. Among these, table grapes produced for export is receiving more importance from the point of view of foreign exchange. The survey has indicated that Thomson Seedless and its clones (Tas-A-Ganesh and Sonaka) and Sharad Seedless, a clone of Kishmish Chorney are commercially being cultivated for table purpose mainly for the domestic consumptions to the extent of 78 per cent. It has also been estimated that approximately 18 to 20% of the total production of the grapes in India is being used for the production of raisins while, a mere 1.5 to 2% is diverted for wine preparations. Grapes cultivated in Maharashtra are mainly exported to Middle East, Europe and West Asian countries which accounts approximately in the range of 1.5 to 2.0 per cent of total grape production. The main competitors for Indian grapes in the international market are Chile, Greece, South Africa, Italy and Spain.

To achieve the export quality standards, the cultivation practices like precision in use of balanced nutrition, water management and bioregulators etc. plays an important role in the growth and development of any crop. In Grapes, nutritional factors are mainly related to the synthesis of proteins and carbohydrates; the utilization of these metabolites depends on the hormonal status of the plants. Use of growth regulators particularly GA_3 has become a common practice among the grape growers in India concerned with export. The phyto-hormones are also being used for root initiation, dormancy termination, flowering, fruit set, delay in abscission and senescence and enhanced growth rate. Plant hormones are extremely important magic chemicals in the integration of several metabolic processes and are also concerned with the response of plants to external physical environment. Grape cultivation is nearly impossible without the use of plant growth regulators.

Since the cost of vineyard establishment and its cultivation is very high as compared to any other fruit crops and therefore better price is the only alternative. Before going for grape cultivation, the importance of this fruit and the quality should be taken into consideration. The production of export quality grapes are as follows.

1. The bunch should weigh 400 – 500 g.
2. The bunch should be uniform in green colour.
3. The berries in a bunch should be of 3.5 to 4.0 g and diameter more than 18 mm.
4. The berries in a bunch should contain 18 °Brix at the time of harvest.
5. The bunch should be well filled loose and free from scars, pest and diseases.
6. The bunch should have maximum shelf life.

In addition to the above general characteristics of a bunch, as per the AGMARK standard fixed, the grapes selected for export should fulfill the following requirement.

Bunch and berries of table grapes shall be

a. Clean, sound, free of any visible foreign matter.

b. Free of pest affecting the general appearance of the produce.

c. Free of damage caused by pest and disease.

d. Free of abnormal external moisture.

e. Free of any foreign smell and/ or taste.

f. Free of all visible trace of moulds.

g. Berries shall be intact, well formed and normally developed.

h. Table grapes shall comply with the residue level of heavy metals, pesticide and other food safety parameters as laid down by the Codex Alimentarius Commission for export.

i. Table grapes shall have minimum soluble solids of 16 °Brix and minimum sugars/acid ratio of 20:1.

Bioregulators

Plant growth regulators (PGR's) are usually defined as organic compounds other than nutrients that affect the physiological process of growth and development in plants when applied in low concentration. For practical purposes plant growth regulators can be defined as either "natural or synthetic compounds" that are applied directly to a target plant to alter its life process or it's structure to improve the quality, increase yield or facilitate the harvest. The response of a plant or a

plant part to plant growth regulators may vary with the variety of plant. Even a single variety may respond differently, depending on its age, environmental conditions, physiological state of development and nutrition.

Growth Regulators are chemicals that regulate the plant growth. They are also called as plant growth substances or plant hormones. Plant hormones are chemicals that are produced within the plant and occur in very low concentrations. These chemicals regulate the cellular processes in targeted cells locally and also when moved to other locations. Plant hormones also determine the formation of flowers, stems, leaves, shedding of leaves, development and ripening of fruit. Plant hormones helps in giving the shape to the plant, time of flowering, the sex of flowers, senescence of leaves and fruits. They affect the tissues growing upward and downward (Auxins), leaf formation and stem growth (CCC), fruit development (GA) and ripening (ethylene), plant longevity and even plant death.

Types of Bioregulators

The plant hormones exist naturally or synthesized by humans or other organisms, including chemicals that inhibit plant growth or interrupt physiological processes within the plants.

The five major classes of plant growth regulators are as follows:

1. Abscisic acid (ABA)

Abscisic acid is also called as ABA. It was discovered and presented in two different names before its chemical properties were fully known. It was called dormin and abscisin II. Once it was determined that the two latter named compounds were the same, it was named as abscisic acid. The name "abscisic acid" was given because it was found in high concentrations in newly-abscised or freshly-fallen leaves.

This class of PGR is composed of one chemical compound normally produced in the leaves of plants, originating from chloroplast, especially when plants are under stress. In general, it acts as an inhibitory chemical compound that affects bud growth, seed and bud dormancy. It mediates changes within the apical meristem causing bud dormancy and the alteration of the last set of leaves into protective bud covers. In plant species from temperate parts of the world it plays a role in leaf and seed dormancy by inhibiting growth, but, as it is dissipated from seeds or buds, growth begins. In other plants as ABA levels decrease, the level of

gibberellins increases resulting into the growth of a plant. Since ABA dissipates slowly from the tissues and its effects takes time to be offset by other plant hormones, there is a delay in physiological processes that provide some protection from premature growth.

During the process of fruit maturation, ABA accumulates within the seeds and prevents seed germination within the fruit. The plant undergoes many stresses during its growth and development. Under water stress, ABA plays a role in closing the stomata. Soon after plants are water stressed and the roots are deficient in water, a signal moves up to the leaves causing the formation of ABA precursors then move to the roots. The roots then release ABA which is translocated to the foliage through the vascular system and modulates the potassium and sodium uptake within the guard cells, which then lose turgidity and closes the stomata.

The life of a plant starts with from the seeds. At the initial stage, the seed has high ABA level. However, just before the seed germination and also at early growth stage of seedling, ABA level decreases to the maximum. The ABA level in plant starts increasing with shoot emergence and formation of fully functional leaves. This results into the slowdown of cellular growth in mature leaves of plant. The ABA production in plant is sensitive to any type of stress.

2. Auxins

Auxins are mainly concerned with cell enlargement. It affects the plasticity of the cell walls and induces them to grow. Auxins are compounds that positively influence cell enlargement, bud formation and root initiation. They also promote the production of other hormones and in conjunction with cytokinins. They control the growth of stems, roots, fruits and convert stems into flowers. The production of auxin is related to light. In light, the auxin production decrease and increases under dark. It stimulates cambium cells to divide and in stems cause secondary xylem to differentiate. Auxins act to inhibit the growth of buds also called as apical dominance and also to promote lateral and adventitious root development and growth. Leaf abscission is initiated by the growing point of a plant ceasing to produce auxins. Auxins in seeds regulate specific protein synthesis as they develop within the flower after pollination causing the flower to develop a fruit to contain the developing seeds. The production of auxins in plant should be in appropriate quantity. If the auxins are either produced or applied in large quantity, it is toxic to the plant while they are more toxic to dicots and less toxic in monocots. Because of this property, synthetic auxin herbicides including 2, 4-D and 2, 4, 5-T have been developed for the control of weed in agriculture. Auxins, especially 1- Napthalene acetic acid (NAA) and Indole-3, butyric acid

(IBA) are also commonly applied to stimulate root growth when cuttings from the plants are selected for propagation. The most common auxin found in plants is indole acetic acid or IAA.

Role of Auxins

1. **Apical Dominance :** Removal of apical bud stimulates lateral buds. Auxins inhibit lateral bud formation since they are synthesized in apex. This phenomenon is called apical dominance. e.g. Potato tubers for apical buds forming.
2. **Cell division and cell elongation :** This helps in shoot and root growth of a plant.
3. **Xylem Differentiation :** It helps in establishing contact between vascular tissues of the callus and that of bud and makes it possible for the bud to grow properly in callus.
4. **Cell enlargement :** Nucleic acid activities of IAA increases total RNA by synthesizing specific enzymes leading to cell enlargement.
5. **Other activities :** Plays specific role in seed germination, growth, rooting, flowering, abscission, parthenorcarpy and tissue culture.

Use of Auxins in Horticulture

1. Propagation of plants by hormone treatment of cuttings
2. Prevention of pre harvest drops of fruits.
3. Increasing parthenocarpy.
4. Increasing fruit set.
5. Prevention of sprouting by inhibiting buds.
6. Inhibition of prolonged dormancy.
7. Control of flowering.
8. Prevention of leaf fall or abscission.
9. Thinning of compact fruits.
10. Selective weed killer.

3. Cytokinins

Cytokinins are one of the major classes of plant growth hormones. It helps in promoting the cell division and shoot formation. It is primarily involved in cell growth, cell differentiation and other physiological processes in the plant. It also help in delay the senescence process or the aging of tissues that are responsible for mediating auxin transport throughout the plant. It also affect inter nodal length and leaf growth. The combination of cytokinin and auxin affect major growth during the lifecycle of plant.

There are two types of cytokinins :

a) **Adenine type :** These cytokinins are represented by kinetin, zeatin and 6-benzyl amino purine (6BA). The adenine-type cytokinins are synthesized in stems, leaves and roots, which is the major site cambium and possibly other actively dividing tissues are also sites of cytokinin biosynthesis.

b) **Phenylurea type :** This cytokinins like diphenylurea (CPPU) or thidiazuron (TDZ). There is no evidence that the phenylurea cytokinins occur naturally in plant tissues. Cytokinins are involved in both local and long distance signaling.

Role of cytokinin

The cytokinin application plays an important role in plants during its growth and development. The major role of cytokinin are as follows.

1. Initiation of cell division
2. Delay of senescence
3. Use in tissue culture
4. Counteract apical dominance

Action and application

i) Cell division

ii) Cell enlargement

iii) Morphogenesis

iv) Dormancy

v) Apical dominance

vi) **Mobility :** Immobile obstructs the movement of amino acid, phosphate and various other substances

vii) **Nucleic acid metabolism :** Quick increase in the amount of RNA and decreases DNA

viii) **Protein synthesis :** Increases DNA

ix) **Florigens :** Induction of flowering in short day plants.

4. Ethylene

Ethylene is a gas that forms through the Yang cycle from the breakdown of methionine which is available in all the cells. It has limited solubility in water and does not accumulate within the cell but diffuses out of the cell and escapes out of the plant. Its effectiveness as a plant hormone is dependent on its rate of production versus its rate of escaping into the atmosphere. Ethylene is produced at a faster rate in rapidly growing and dividing cells especially in darkness. New growth and newly germinated seedlings produce more ethylene than can escape the plant which leads to elevated amounts of ethylene inhibiting leaf expansion.

Role of Ethylene

1. **Abscission :** Ethylene is an accelerator of abscission. It is capable of promoting changes associated with pre abscission and aging of leaves, petioles, flowers and fruits.
2. **De-greening in fruits :** It occurs after ethylene treated fruit is exposed to air that accelerates maturity and induces uniform ripening in fruits.
3. **Inducing fruit ripening :** Ethylene is a natural product of ripening fruit. Ethylene is a gas at temperatures under which a plant can live. It helps in fruit ripening.
4. **Increase in female flowers :** Auxins increase ethylene level in plants and auxin actions are attributed through ethylene such as increased percentage of female flowers and apical dominance.

5. Gibberellins

Gibberellins (GA's) are plant hormones that regulate growth and influence various developmental processes including stem elongation, germination, dormancy, flowering, sex expression and leaf and fruit senescence.

Gibberellins include a large range of chemicals that are produced naturally within plants and also by fungi. They were first discovered when Japanese researchers including Eiichi Kurosawa noticed a chemical produced by a fungus called *Gibberella fujikuroi* that produced abnormal growth in rice plants. Gibberellins are

important in seed germination, affecting enzyme production which mobilizes food production used for growth of new cells. This is done by modulating chromosomal transcription.

Gibberellins are involved in the natural process of breaking dormancy and various other aspects of germination. Before the photosynthetic apparatus develops sufficiently in the early stages of germination, the stored energy reserves of starch nourish the seedling. Usually in germination, the breakdown of starch to glucose in the endosperm begins shortly after the seed is exposed to water. It is believed that gibberellins in the seed embryo signal starch hydrolysis through inducing the synthesis of enzyme-amylase in the aleurone cells. The production of GA is more when the plant is exposed to cold temperatures. They stimulate cell elongation, breaking dormancy and budding, seedless fruits and seed germination

Role of Endogenous Gibberellins

1. Apical bud dormancy
2. Role in sub apical meristem
3. Cell elongation
4. Fruit growth
5. Flowering
6. Metabolization of food in seed storage cells.

Practical Applications of Gibberellins

a) **Germination :** The use of gibberellins increases length of hypocotyl and cotyledenary leaf area.

b) **Root growth :** Inhibits the root growth.

c) **Leaf expansion :** Leaves become broader and enlarged (grapes, cabbage, and sweet corn).

d) **Hyponasty of leaves :** GA treated leaves of some of the flowering plants like chrysanthemum holds their leaves more erect.

e) **Flowering :** The use of gibberellins induces flowering in long day plants and in plants requiring cold induction. It also promotes formation of male flowers.

f) **Parthenocarpy :** The production of seedlessness in major fruit and vegetable crops is achieved with the help of gibberellins (brinjal, guava and grapes).

g) **Fruit setting :** Increased fruit setting in some of the fruit plants like Phalsa, Sweet lime, Grapes.

h) **Fruit drop :** Not much effective.

i) **Breaking dormancy :** In temperate plants, buds become dormant in later summer and do not sprout even when exposed to sufficient moisture, temperature and oxygen. They require low temperatures or long days or red light. GA overcomes this dormancy. Enhanced cell elongation push through the endosperm (seed coat).

j) **Other uses :** Thompson seedless grape bunches if sprayed with GA, causes elongation of bunch, thus becoming less tightly packed and less susceptible to fungi.

Use of Bioregulators in grapes

Grapes are by and large propagated through cuttings, layering, grafting and budding. However, it is also propagated through seeds in the breeding program to produce the seeds for further multiplications.

Grape flowers and berries react in diverse ways to growth substances, depending on the time of application. Pre- anthesis treatment with auxins causes premature growth of the ovule, while gibberellins inhibit seed growth. Gibberellins applied during or after anthesis promote pericarp growth mainly through cell enlargement. Seeds prevent this response. Auxins and ethylene applied during the slow-growth phase delay the onset of ripening, but ABA hastens it. Certain varieties of many species of plants develop their fruits parthenocarpically or without fertilization. In some varieties of grapes, parthenocarpy is of great importance. Because of this naturally occurring phenomena, seedlessness in other fruit crops has various synthetic growth regulating substances are given due importance. The growth regulators used in different crops and in relation to grapes is given as below.

Propagation

In the propagation of fruit plants by aseptic methods, synthetic cytokinins and auxins are essential components of culture media for axillary shoot proliferation and adventitious root formation and micro propagation has also become a widely-used procedure in the horticultural crops. Similarly, plant growth regulators are essential components of systems for plant regeneration in vitro by organogenesis and somatic embryogenesis. The use of these compounds is fundamental to the application of biotechnology to the genetic improvement of fruit crops.

The use of auxin-like compounds for promoting adventitious root formation in hardwood and softwood cuttings is one of the earliest and most successful practical applications of plant growth regulators in grapes. The treatment of cuttings with preparations containing indole 3, butyric acid (IBA) alone or in combination with naphthalene acetic acid has been a routine procedure for many years.

Seed germination

In grapes, the seed is used only when the research program is carried out to breed the varieties resistant to biotic and abiotic stresses. However, it is hypothesized that :

(a) The hormonal control of the first rapid growth of seed pericarp resides in interactions between auxins, cytokinins, gibberellins and abscisins and the relative importance of each change as development progresses from cell division to cell enlargement.

(b) The seed is the chief source of these hormones.

(c) The second rapid growth phase is associated with ABA and sugar accumulation, initially in the skin.

For a period after anthesis the ovary appears to exert no control over the flow of organic nutrients to it. As a result, its abscission and early development is dependant on the overall supply of nutrients in the vine. Later, the berry develops an ability to attract nutrients and different limitations to accumulation.

Seed dormancy breaking

Seed dormancy is nature's way of setting a time clock that allows seeds to initiate germination when conditions are normally favorable for germination and survival of the seedlings. Viable seeds that do not germinate are said to be dormant. Dormancy can be regulated by the environment or by the seed itself. If a seed is not exposed to sufficient moisture, proper temperature, oxygen and for some species light, the seed will not germinate. In this case, the seed dormancy is due to unfavorable environmental conditions. On the other hand, some seeds may not germinate because of some inhibitory factor of the seed itself. Gibberellins are said to be more useful in breaking the seed dormancy in fruit and vegetable crops. Gibberellins treatment helps in breaking dormancy in seed potatoes resulting in uniform crop emergence.

Delayed and un-uniform bud break is a major problem for grapevines in warm climates, leading to highly vegetative unfruitful vineyards. Hydrogen cyanamide is one of those chemicals which was found most potent for breaking grapevine dormancy and even at higher efficiency than in many other species.

Cuttings

In earlier days, the grapes were grown on their own roots. However, with the onset of different problems of soil and irrigation water, the yield of the grape vines of own roots was started decline. This has resulted into the use of rootstock in grape cultivation. Production of desired quantity of rooted cuttings in a season became mandatory for nurserymen. Considering the need of the planting material, the research work on use of growth regulators in multiplication of rooted plants through hardwood cuttings has been started. Various treatments like callusing, soaking in water and use of bioregulators (IBA) can be given to the cuttings before planting them in the nursery. It has been found that soaking of cuttings in plain water for 24-48 hours improves the rooting irrespective of the nature of the cutting. This helps in leaching out of rooting inhibitors available on the cuttings of rootstocks. The treatment of IBA @ 1000 to 2000 ppm has been found to be very effective in increasing the rooting of hardwood cuttings while, in softwood cuttings, the best results are obtained at 750 to 1500 ppm.

Fig. 1 : Cuttings treated with IBA

Grafting

In grapes, wedge grafting is usually followed in Maharashtra and other parts of the country. For successful graft union, plant hormone like 6-BA plays an important role as other conditions likes humidity and temperature. For better callusing, the cuttings are dipped in 6- BA solution of 15 - 20 ppm just before grafting where the fresh cut is taken. The scion and stock are placed together with their tongue interlocking. The graft can be tied firmly with a waxed stains or polyethylene strip. The use of bioregulators under this situation helps in easy and faster callus formation.

Fig. 2 : Grafted plant

Growth and development of a vine in different grape varieties/type

Bud Breaking

The buds of temperate woody plants including grapevine, undergo a dormancy cycle during the winter induced by decreasing photoperiod and/or temperatures.

In warm-winter regions, prolonged dormancy is a major obstacle for commercial production of temperate fruits including grapes which are widely distributed in subtropical regions.

Hydrogen cyanamide is the most useful for bud dormancy-breaking compound for grapevine. Although the mechanism by which hydrogen cyanamide exerts its dormancy-breaking effect is not clear, its application provides a uniform and effective response.

In case of table grapes, the use of hydrogen cyanamide is common for bud breaking. In addition to the use of hydrogen cyanamide, leaf removal during fruit pruning is also followed. This helps in early bud swelling that results in to the early bud break.

Fig. 3 : Bud sprouting by hydrogen cynamide

However, without use of hydrogen cyanamide, bud breaking in time and regular sprouting is not possible. The concentration used for bud break varies with the varieties, cane diameter, time of pruning and also the weather condition available during the period of pruning. The dose of hydrogen cyanamide varies from 25 ml to even 50 ml per liter of water under cold condition.

Shoot development

Inflorescences and tendrils in grapevines are derived from meristematic structures called primordial which develop from shoot meristems and are found opposite two of every three leaves. The primordial are formed on actively growing shoots that develop into tendrils whereas those in latent buds develop into inflorescences. The tendrils are generally regarded as vegetative appendages which provide support for climbing plants but the tendrils of the grapevine are morphologically homologous to inflorescences and can be regarded as tendrils and inflorescences both arise from Anlagen- meristematic protuberances formed by terminal and axillary bud meristems (Srinivasan and Mullins, 1976). Anlagen may be induced to form either tendril primordia or inflorescence primordia. The grapevine tendrils may be interpreted as weakly differentiated inflorescences and it was shown earlier that the gibberellins causes stem elongation and leaf expansion.

Under the Indian condition particularly in Maharashtra, the grapevine is pruned twice in a year. The shoot growth and development is required for effective fruit bud differentiation after back pruning and bunch development after fruit pruning in grapes. To promote the shoot growth in grapevine, application of nitrogenous fertilizers is considered as one of the major tools. However, the application of gibberellins at proper stage after fruit pruning helps in better way for shoot development. Application of GA_3 @ 10 – 15 ppm helps the vine to impart more vegetative growth in addition to the elongation of a bunch at pre-bloom stage.

Fruit bud formation

Fruit bud in the grapevine involves three main steps : 1) Formation of Anlagen or uncommitted primordia 2) Differentiation of Anlagen to form inflorescence primordia and 3) Differentiation of flowers.

Growth regulators play an important role in formation of fruit buds in grapes. The growth regulators also act as growth retardants to regulate the shoot vigour since the excess shoot vigour is detrimental at the time of fruit bud formation. The excess shoot vigour can be reduced with the use of growth regulating chemicals. The growth regulating chemicals activates the physiological processes by

increasing the cytokinin/gibberllins (CK/GA) ratio and also the RNA/DNAratio in the buds, thus helping the vine for fruit bud differentiation.

Fruit bud formation is the transformation of vegetative primordium into reproductive primordium in a bud. This transformation takes place in three stages. Among these stages, formation of inflorescence primordium is the most sensitive stage. The factors like light, cytokinins or ribonucleic acid (RNA) plays an important role in fruitfulness. On the other hand, if the gibberellins and other growth promoting substances are in excess and the soil and environmental conditions are conducive for vegetative growth, the anlagen do not proliferate resulting in the formation of either tendril primordia or shoot primordia. Formation of inflorescence primordium would make the anlagen to differentiate into either tendril or shoot. Sometimes, a partly differentiated inflorescence primordium can revert back & convert into tendril primordial, which is referred to as a filage.

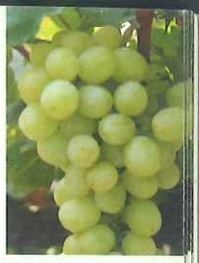
The fruitfulness in grapes depends mainly on the climatic condition during the fruit bud differentiation stage and also the cultural practices followed during that period. The pruning in March is advisable, because the fruit bud differentiation would have occurred by third week of May when there is no dearth of sunlight. Secondly, avoidance of application of nitrogen and start of application of potash at the beginning of the grand growth period was found to check the shoot growth and it resulted in increased shoot thickness consequently the reduction in shoot length leads to increase the angle of shoots in bower trained vines in Thompson Seedless. Mild water stress during 40-60 days after back pruning was found to reduce the shoot growth in Thompson Seedless. This facilities increase the exposure of bud to sunlight. The practices to be followed to increase fruitfulness are given in table 1.

Table 1 : Practices to be followed to increase fruitfulness.

No. of Days after foundation pruning	Practice to be followed	Purpose
30 - 40	Nitrogen application to be stopped in case of vigorous vines*	To reduce the shoot growth
40 - 60	Reduce irrigation level to one-third to that of the vegetative phase**	To reduce the shoot growth

*Petiole – Nutrient guide has to be followed

**This period generally coincides with the onset of rainy season. Irrigation may or may not be required.



Rachis elongation or panicle growth

The main axis of cluster is called rachis, from which branches arise at irregular intervals and divide to farm pedicels and the flower stalks. The main rachis may divide to farm secondary and tertiary branches in some varieties. The length of main rachis, its branching and the number of flowers per unit length of the rachis is dependant on endogenous levels of gibberllins and shoot vigour. In Thompson Seedless grapes, gibberellic acid is given externally to have more rachis growth, interns avoids the bunch compactness. The application of GA_3 to be followed for rachis elongation in case of table grapes is as follows.

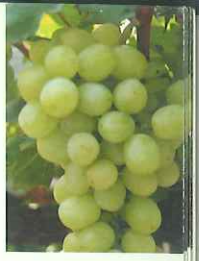
Fig. 4 : Rachis elongation achieved through growth regulator (GA_3)

Table 2 : Formation of rachis elongation by GA_3 treatment.

Sr.	Stage	Concentration used	Purpose	Spray/Dip
1.	Parrot green stage of panicle	GA_3 @ 10 ppm	Rachis elongation	Spray
2.	4-5 days after above spray	GA_3 @ 15 ppm	Rachis elongation	Spray
3.	4-5 days after above spray	GA_3 @ 20 ppm	Rachis elongation	Spray or dip depends on rachis growth

Flowering

Flower clusters appears soon after the emergence of young shoots. The time required for first flower to open and the time from the opening of first blossom till the large proportion of clusters bloomed seems to vary according to temperature conditions. In case of table grapes limited berries has is be retained. For this purpose application of GA_3 @ 40 ppm at 50% flowering becomes essential. As a



Rachis elongation or panicle growth

The main axis of cluster is called rachis, from which branches arise at irregular intervals and divide to farm pedicels and the flower stalks. The main rachis may divide to farm secondary and tertiary branches in some varieties. The length of main rachis, its branching and the number of flowers per unit length of the rachis is dependant on endogenous levels of gibberllins and shoot vigour. In Thompson Seedless grapes, gibberellic acid is given externally to have more rachis growth, interns avoids the bunch compactness. The application of GA_3 to be followed for rachis elongation in case of table grapes is as follows.

Fig. 4 : Rachis elongation achieved through growth regulator (GA_3)

Table 2 : Formation of rachis elongation by GA_3 treatment.

Sr.	Stage	Concentration used	Purpose	Spray/Dip
1.	Parrot green stage of panicle	GA_3 @ 10 ppm	Rachis elongation	Spray
2.	4-5 days after above spray	GA_3 @ 15 ppm	Rachis elongation	Spray
3.	4-5 days after above spray	GA_3 @ 20 ppm	Rachis elongation	Spray or dip depends on rachis growth

Flowering

Flower clusters appears soon after the emergence of young shoots. The time required for first flower to open and the time from the opening of first blossom till the large proportion of clusters bloomed seems to vary according to temperature conditions. In case of table grapes limited berries has is be retained. For this purpose application of GA_3 @ 40 ppm at 50% flowering becomes essential. As a

result, 50% beries only set and avoids the compactness of the bunch. If the rachis is well elongated, the use of GA_3 my be avoided.

Table 3 : Formation of elongated berries by GA_3 treatments during flowering particularly for Sonaka and black varieties.

Sr.	Stage	Concentration of GA_3	Purpose
1.	10% Flowing	GA_3 @ 10 ppm	Berry elongation
2.	50% Flowing	GA_3 @ 15 ppm	Berry elongation
3.	90% Flowing	GA_3 @ 20 ppm	Berry elongation

Increasing berry set

Plant growth regulators are commonly used in grapes for different purposes. Pollination and fertilization must takes place for ovule development, seed formation and development in normal seeded grapes. Plant growth regulators are also effective for fertilization and embryo development. Gibberellins which initiate cell enlargement and berry expansion.

The application of GA_3 becomes important to increase the berry set. Therefore, the berry number decreases while their weight and volume increases with the increase in GA_3 concentration. The effect of GA_3 on fruit set varies with the concentration, stage of development and also with the cultivars. With the seedless

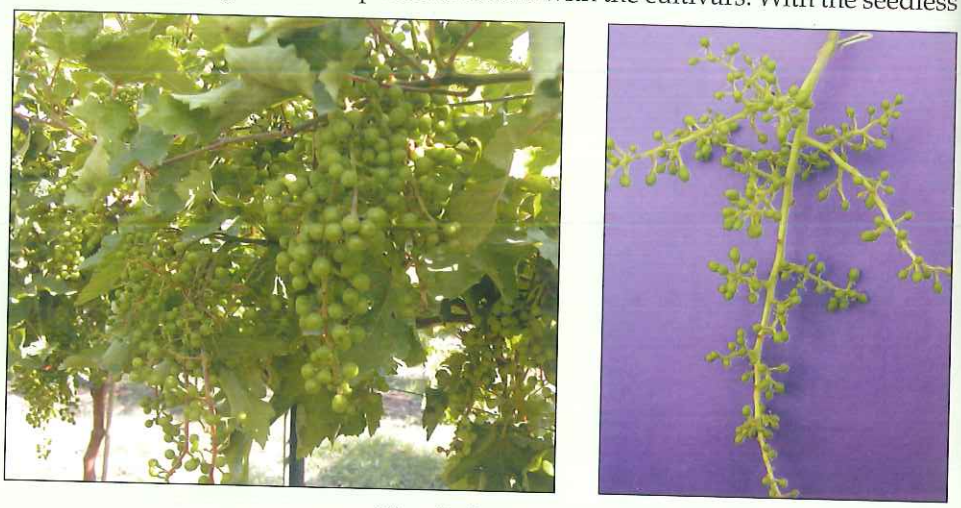
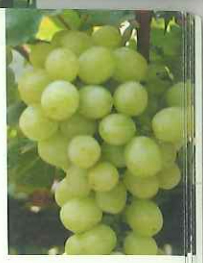


Fig. 5 : Berry set



cultivars, the GA_3 reduces the number of seeded berries but increase the number of seedless berries.

In order to improve berry set, some techniques such as gibberellic acid application, girdling and shoot pinching are applied. GA_3 is a hormone which starts cell growth and enlargement and shows a rapid growth in the first growth period just after berry set and reaches the highest level towards the middle of this period. Among gibberellic acids, GA_3 is widely used in the enlargement of grape berries in seedless grape varieties (Mander, L.N.A., 1994).

Berry growth

The grape berry is a simple fruit, consisting of two seed cavities (locules) surrounded by ovary wall called as pericarp. In seeded varieties there may be as many as four seeds. In case of stenospermocarpic varieties, the locules contain seed traces resulting from the abortion of the ovules early in their development. The fleshy pericarp consists of an exocarp called as skin and a mesocarp, the pulp. In most seedless varieties, the mesocarp accounts for 85 to 90 percent of the berry's fresh weight. Increases in berry weight, volume or diameter during development are typically characterized by a double sigmoid curve resulting from two consecutive stages of growth separated by a phase of slow or no growth. The berry development is found in three different stages, they are as follows.

Stage I : A period of rapid berry growth comes immediately after bloom. During this time, berries grow both through cell division and cell enlargement. Berry texture is firm, while its color is green due to the presence of chlorophyll. The sugar content of the berry remains low, while organic acids accumulate. This stage lasts between 3 and 4 weeks in table grapes.

Stage II : Berry growth slows markedly during the second period while the berries organic acid concentration reaches its highest level. The stage is also called as lag phase. At this stage, the berries remain firm, but begin to lose chlorophyll. The lag phase normally lasts between 2 and 3 weeks depending upon the climatic conditions of that region and grapevine variety.

Stage III : In this stage of berry development, the initiation of ripening commences with the beginning of this stage. The berry starts softening (veraison) which characterizes the initial stages of color development. Berries soften and lose chlorophyll, while in colored varieties red pigments begin to accumulate in the skin. Sugar also begins to accumulate and the concentration of organic acids declines. Aroma and flavor components accumulate in the fruit.

Growth regulator plays an important role in berry development. To increase berry size, use of GA_3 and CPPU is essential. The doses of growth regulators have been standardized for coloured table seedless grape varieties like Sharad Seedless and Flame Seedless and Thompson Seedless. For Flame Seedless and Sharad Seedless CPPU @ 0.5 ppm and GA_3 @ 40 ppm at 3-4 mm and 6-7 mm berry stage is sufficient to achieve the appropriate quality of grapes. For Thompson Seedless, the doses of GA_3 & CPPU have also been standardized and the details are given in table below.

Table 4 : Doses of bioregulators and berry growth stages for Thompson Seedless grapes.

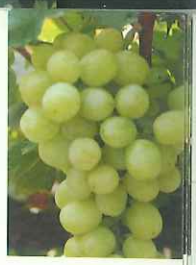
Sr.	Berry stage	GA_3 and other chemicals	Purpose
1.	3-4 mm	GA_3 @ 40 ppm + CPPU @ 2 ppm (pH 6.0) OR	To increase berry size
2.	6-7 mm	GA_3 @ 40 ppm + 1-2 ppm CPPU (pH 6.0)	To increase berry size

Note : This can be done for Sonaka and Sharad Seedless varieties if elongated berries is the requirement.

Berry elongation

In table grapes, after berry set, the growth is either controlled or stimulated with the use of growth regulators. Gibberellic acid is especially used in viticulture. It affects grape berry by means of different ways. Some of these effects include formation of flower cluster, berry set, berry enlargement, cluster length elongation, berry and cluster thinning, prevention of berry cracking, etc. The role of gibberellic acid as pollinicide in grapes is also well known.

The elongation in grape berries is achieved by different means. Cultural practices after fruit pruning and the weather condition available during the period of berry development plays an important role. The role of growth regulators is considered important in achieving the export quality grapes. The elongated berry obtained through use of growth regulators provides more room for the berries to grow larger and makes a loose bunch. A loose bunch allows for better air circulation which in turn reduces disease incidence and improves berry growth and maturation. The spray of gibberellic acid at flowering stage helps to thin or reduce the number of berries in a bunch. This helps to reduce the competition among the berries for nutrients required for berry development after berry set. Thus, the



application of gibberellic acid helps in achieving the berry elongation. The end result of the gibberellic acid is a large, loose cluster, with large and uniformly sized berries that mature evenly.

The growth regulators used for berry elongation in white seedless grapes is given in table 5.

Table 5 : Doses of growth regulators used for berry elongation and development.

Sr.	Berry stage	GA_3 and other chemicals	Purpose
1.	3-4 mm	GA_3 @ 40 ppm + CPPU @ 2 ppm (pH 6.0) OR	To achieve round berries
		GA_3 @ 50 ppm + CPPU @ 1 ppm (pH 6.0)	To achieve elongated berries
3.	6-7 mm	GA_3 @ 40 ppm + 1-2 ppm CPPU (pH 6.0)	To increase berry size

Bunch looseness

For the production of export quality bunch, the application of proper growth regulators and the stage and its concentration needs to be given due importance. This helps in achieving the loose bunch after fruit pruning. This can be achieved by berry and rachis thinning. However, this practice is followed after berry set. Application of growth regulators during pre-bloom stage helps to achieve loose bunch in a greater extent.

Gibberellic acid (GA_3 formulation) sprays are used at bloom to thin berries and increase berry size. The spray or dip of gibberellic acid along with 6- BA and CPPU is generally followed to achieve bunch elongation at pre-bloom stage and also for increasing the berry size.

The results obtained by application of growth regulators are as below.

1. Increase in length between two rachis
2. Increase in length of individual rachis

Fig. 6 : Loose bunch

3. Increase in length of bunch as a whole so as to achieve the cylindrical shaped bunch as preferred in the international market.
4. This ultimately increases the berry diameter above 18 mm.

Berry pedicel thickness and shelf life

The firmness of grape berries is one of the major factors that determine its eating quality. The consumer prefers grape that has firm and soft flesh. Ideally, in table grapes white flesh with high consistency develops towards ripening. Different markets has different requirement. The factors that promote the development of firm berry flesh are only speculations. As results, the growers have no definite practices to be followed so that the firm berries can be ensured to the consumers. The shelf life of berries depends on the availability of the firmness and the pedicel thickness of that bunch.

Fig. 7 : Pedicel thickness of a bunch

The exoskeleton of the plant cell is the cell wall. The cell wall determines the shape and turgor pressure of the cell (Taiz and Zeiger, 1998). The cell wall structure appears to play a role in the firmness of table grapes. Calcium determines the structure of cell wall (Grant et al, 1973).

Calcium application to bunches and calcium fertilization as well as gibberellic acid and CPPU application is used to enhance the berry firmness. However, the mode of action is not yet been understood. Application of calcium to berries helps in enhancing the berry firmness and also the increase in shelf life. When high concentration of calcium is maintained in fruit tissues, during its development, the process of fruit ripening slows down. The softening of berries will also slow down. Application of GA_3 enhances the division and expansion of the parenchyma cell in the pericarp of berry. With the application of CPPU in grape berries, cell division takes place and also increase in shelf life of berries is experienced.

Berry attachment to the pedicel and its attachment to the rachis accounts for berry adherence. Shrivelling and loss of freshness in grapes is due to loss of water from the berries during storage and transit. Grapes shrivel with 1 to 2% loss of water. Thick pedicels can be achieved with the application of CPPU at 3-4 & 6-7 mm berry stage. The cluster dipping with calcium nitrate @ 0.5 -1% at 75 to 105 days after fruit pruning increases the shelf life. Treating the bunches with NAA (napthalene acetic acid) @ 50-100 ppm 8-10 days prior to harvest enhances the shelf life in grapes (Table 6).

Table 6 : Use of Bioregulators to enhance the shelf life in grapes

Growth regulator	Concentration to be used	Stage of application	Purpose
NAA (Naphthaline acetic acid)	50 - 100 ppm	10 days prior to harvest	To reduce wet drop
GA_3 + CPPU	40-50 ppm GA_3 and 1 - 2 ppm CPPU	3-4 and 6-7 mm berry stages	To reduce dry drop
Calcium nitrate or Calcium chloride	0.5 - 1.0%	75 or 90 or 105 days after pruning	To increase the cell wall turgidity

* If chloride content in the soil is high don't give calcium chloride dip

Berry colour development

In the consumers market, the quality of grapes depends to a large extent on grape ripening, the time of harvest, its completeness and whether the various berry components i.e., skin, flesh and seeds reaches the maturity at the same time. The climacteric fruit continue to ripen even after harvest such as tomatoes, mango, bananas, the ripening can be controlled by ripening hormone (ethephon). Grapes, do not behave in the same way.

Fig. 8 : Berry colour development

Grape berry is classified as a non climacteric fruit which accumulates abscisic acid (ABA) at the beginning of ripening. It seems that this phytohormone is an activating signal of ripening. The exogenous application of ABA results in an advancement of the change of colour by faster decreasing chlorophyll levels. The hormonal profile is modified during colour change period. The ABA levels are enhanced and indole acetic acid (IAA) levels are decreased in the berry during the period of ripening. The application of growth regulators helps in advancing the process of ripening.

Colour is attributed to the diurnal variation in temperature, particularly low night temperature at the time of berry ripening. The use of CPPU at 3-4 and 6-7 mm

berry size stage is also useful to retain uniform green colour of the berries at harvest. It is said that a transient increase of endogenous ethylene production in grapes occurs just before veraison (the start of ripening). To retain green colour of berries for export, 6 BA @ 10 ppm can be used at berry softening stage.

Berry ripening

The ripening of fleshy fruit is proceeded by a shift in metabolism which leads to characteristic changes in the fruit composition, texture and the colour of the fruit. In grapes, this shift is observed at the end of slow growth period of berry development. The changes included berry softening an increase in reducing sugars, a fall in acidity, a loss of green colour and the appearance of anthocyanin pigment. Ethylene accelerates the ripening of all fruits as it is a fruit ripening hormone.

Fig. 9 : Ripe berries

Fruit ripening is a unique plant developmental process with direct implications for our food supply, nutrition and health. In contrast to climacteric fruit where ethylene is pivotal, the hormonal control of ripening in non-climacteric fruit, such as grape (*Vitis vinifera*) is poorly understood. Brassinosteroids (BRs) are steroidal hormones essential for normal plant growth and development. The increases in endogenous BR are associated with ripening in grapes. The application of BR to grape berries significantly promotes ripening while brassinazole, an inhibitor of BR biosynthesis, significantly delayed fruit ripening (Gregory et al., 2006).

However, recent evidence of a transient increase in endogenous ethylene levels prior to veraison suggests that ethylene may play some role during grape berry development (Chervin et al., 2004). Other classical plant hormones, such as auxin (indole-3-acetic acid (IAA) and abscisic acid (ABA) have also been implicated in the control of ripening of grape berries (Seymour et al., 1993; Davies et al., 1997).

The use of ethephon should be restricted to the conditions available in the vineyards. Ethephon should not be used in vineyards that are weak, stressed for water, or partially defoliated. Such vineyards will not respond with improved fruit ripening and applications may prompt further loss of leaves. In majority of

the vineyards during the period of fruit pruning, ethephon is used for leaf fall that results in to early bud sprout and appearance of more bunches in addition to the advancement of early bunch development.

Defoliation

The use of plant growth regulators in grape cultivation is well known. The growth regulators like ethephon not only being used for berry colour development but it also helps in defoliation of leaves. During fruit pruning, the leaf is removed so that the bud gets exposed to sunlight and the food material is accumulated in the bud to be sprouted after fruit pruning. Leaf removal manually is tedious work and also time consuming. Hence, the use of growth regulators like ethephon helps in early leaf fall and uniform bud sprout. This also helps in appearance of more number of bunches per vine in addition to the early bud sprout.

Fig. 10 : Defoliation

Preparation of growth regulator solutions

Most of the bioregulators are used in ppm (parts per million) concentrations, which means a part in a million. To get 1-ppm solutions of any bioregulator, we need to dissolve 1mg of a substance in 1 ltr of water. Most of the pure chemicals of the bioregulators are insoluble in water, and are soluble in ethanol/ methanol/dilute solutions of alkali and acids (Table 7). Hence, for preparing the stock solutions of bioregulators, care has to be exercised in dissolving these chemicals first in any of the above solvents in which they completely dissolve with a care not to exceed 1% of that solvent and then they have to be diluted to required concentration for spray.

Table 7 : Bioregulators, their trade names, chemical formulae, dissolving solvents and their active ingredients.

Sr.	Name of plant growth regulator	Chemical formulae	Solvents used	Active ingradients
1.	Auxins (IAA, IBA)	$C_{10}H_3NO_2$	Alcohol, acetone & ether	Indol
2.	Gibberrellins	$C_{19}H_{12}O_6$	Methanol, ethenol & acetone	Gibbrrellin
3.	Cytokinin	$C_{14}H_7N_5O$	Dil. HCI & NaOH	Adenine
4.	Ethylene	C_2H_4	Methanol & ethenol	Methionine
5.	Abscissic acid	$C_{15}H_{20}O_4$	Sodium bicarbonate, chloroform, acetone & ether	Carotine acids & malenic acid

The Commercial formulations of the bioregulators which are commonly available in the market dissolving water and some have to be sprayed in ppm concentrations and some in percent solutions. To prepare 1% solution, we need to dissolve 1 g of a chemical in the solvent (water) and make up the volume to 100 ml., which gives the strength of 1% solution. Normally, in vineyards, we require 400 to 600 litre of a solution for an acre. Considering the total quantity of the solutions to be sprayed and the concentrations, we need to calculate the amount of the regulator required. The following table (Table-8 & 9) gives an example of how to prepare the solutions of bioregulators.

Table 8 : Quantity of bioregulators, total volume to be prepared and concentration obtained.

Sr.	Quantity of bioregulator	Total volume of the spray solutions in solvents (litres)	Concentration of Spray solution (ppm)
1.	1.0 mg	1.0 litre	1.0 ppm
2.	10 mg	1.0 litre	10 ppm
3.	1.0 g	100 litre	10 ppm
4.	6.0 g	600 litre	10 ppm
5.	9.0 g	600 litre	15 ppm
6.	12.0 g	600 litre	20 ppm
7.	18.0 g	600 litre	30 ppm
8.	24.0 g	600 litre	40 ppm
9.	30.0 g	600 litre	50 ppm

Sometimes, the bioregulators are available in the form of active ingredients; under such circumstances the following formula can be used for the calculation of the quantity of chemical required for the specific concentration.

$X = 0.1/C \times R$

where, X= unknown quantity of solution (ml) to be used.
C= Active ingredient (a.i.) of these chemicals
R= Required concentration of the solution (ppm)

For example, 1000 ppm of concentration of ethrel is required for the defoliation in grape and the active ingredient of ethrel is 39%, then the quantity of ethrel required is 2.56 ml/l of water

$X = 0.1/39 \times 1000$ ppm = 2.56 ml/litre

Likewise for 10 l, it is 25.6 ml
100 l, it is 256.6 ml

Table 9 : Preparation of Per cent solutions of bioregulators.

Sr.	Quantity of bioregulator	Total volume of the spray solutions (ml)	Concentration of Spray solution (%)
1.	1.0 g	100 ml	1.0
2.	2.0 g	100 ml	2.0
3.	3.0 g	100 ml	3.0
4.	4.0 g	100 ml	4.0
5.	5.0 g	100 ml	5.0
6.	6000 g (6.0 kg)	600 litre	1.0
7.	12000 g (12 kg)	600 litre	2.0
8.	18000 g (18 kg)	600 litre	3.0
9.	24000 g (24 kg)	600 litre	4.0
10.	30000 g (30 kg)	600 litre	5.0

Table 10 : Preparation of spray solutions of bioregulators which are not 100% pure.

Sr.	Name of bioregulator	The volume of the unknown solution (ml or g) for preparation of 1 liter solution	Concentration of Spray solution (ppm)/ per cent
1.	Ethrel (a.i.-39 %)	0.77	300
2.	Ethrel (a.i.-39 %)	0.25	100
3.	Ethrel (a.i.-39 %)	0.64	250
4.	Ethrel (a.i.-39%)	2.5	1000
5.	Ethrel (a.i.-39%)	7.7	3000
6.	Hydrogen cyanamide (ai-50%)	30	15000 or 1.5%
7.	Hydrogen cyanamide (ai-50%)	40	20000 or 2%
8.	Naphthalene acetic acid (a.i. 25%)	0.08	20
9.	Naphthalene acetic acid	0.2	50
10.	Naphthalene acetic acid	0.4	100

Precautions and safety measures

I. Safety measures to be followed while working with bioregulators in the field.

- Wear only ANSI Z87-approved safety goggles, type G or H during spraying and dipping approved by US Consumer Products Safety Commission's file 1984.
- Gloves to be used are made up of a material known to resist penetration by the chemical being handled and that have been checked for pin holes, tears, and the absence of interior contamination while doing the application of hydrogen cyanamide in the field.
- Wear a laboratory coat or apron during preparation of stock or standard solutions of bioregulators.
- Wear footwear that protects the feet from chemicals like hydrogen cyanamide while working.
- The gloves, face shield or goggles and contaminated clothing should be washed every time to avoid corrosion during subsequent use.

II. Storage of bioregulators

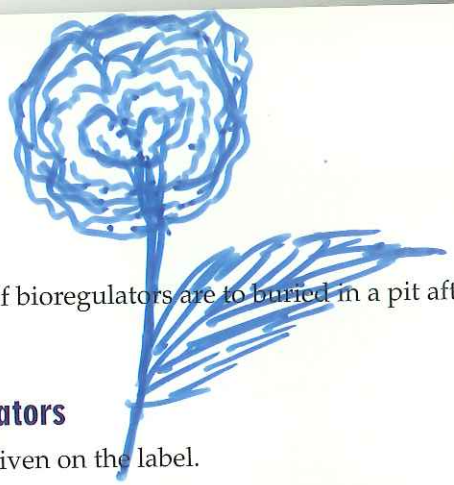
i) Bioregulators should be stored on the shelves and not on the floor. Never use stacked boxes in lieu of shelves.

ii) Store the bioregulators in the closed, original container in a cool and well-ventilated area.

iii) Use only an explosion-proof refrigerator for storage of bioregulators.

III. Purchase, Use, and Disposal of Bioregulators

- Keep the inventory list up-to-date as chemicals are consumed and replacement chemicals are received.
- If possible, limit the purchase of bioregulators in quantities that will be consumed within one season and are packed in small pack.
- Label all the bioregulators that are to be stored with date of receipt.
- Do not purchase or store large quantities of flammable or solidifying liquids but purchase seasonally.



- The empty packets, bottles or tins of bioregulators are to buried in a pit after use.

IV. Guidelines for use of bioregulators

- Read and follow the directions as given on the label.
- Ensure spray tank or pump is clean and free of residues of other formulations prior to use.
- Calibrate the sprayer prior to application to ensure that target application rate is being achieved.

V. Other regulatory information

Use of the products of bioregulators according to the registered label of the manufacturer and HACCP Procedures.

a) Impact on the environment

Ensure that all the bioregulators are regulated under the CIB Act, so that they do not pose an unacceptable risk to human health and the environment.

b) Impact on human health

i) **EYE :** May cause pain and slight transient (temporary) eye irritation.

ii) **SKIN :** Short single exposure not likely to cause significant skin irritation. A single prolonged exposure is not likely to result in the material being absorbed through skin in harmful amounts.

iii) **INGESTION :** swallowing larger amounts may cause injury.

iv) **INHALATION :** Vapors are unlikely due to physical properties. Mists may cause irritation of upper respiratory tract.

VI. First aid measurers

Contact a doctor immediately during emergency and follow the advice given.

SKIN CONTACT : Remove all contaminated clothing immediately. Wash affected areas with plenty of water for at least 15 minutes. Obtain medical advice as above.

EYE CONTACT : Rinse eyes immediately with plenty of clean water including under eyelids for at least 15 minutes. Obtain prompt medical attention preferably from an eye specialist.

Suggested Readings

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