# Methods to Enhance Terpene Production

## Introduction

Terpenes are a large and diverse group of natural compounds that constitute the most abundant class of plant secondary metabolites. These volatile organic compounds play critical roles in plant defense against herbivores and pathogens, mediate plant-plant communication, and attract pollinators (1). Beyond their ecological functions, terpenoids have immense commercial value in pharmaceutical, food, cosmetic, and agricultural industries (2). The increasing demand for these compounds in essential oil crops such as mint, citrus, lavender, and other aromatic plants has driven research into methods for enhancing their production. This article reviews scientifically validated approaches to boost terpene biosynthesis in commercially relevant crops.

Mint and orange are two commercially relevant crops where terpene content is strongly related to quality.

# Understanding Terpene Biosynthesis

Before discussing enhancement methods, it is important to understand the fundamental pathways of terpene production. Plants synthesize terpenoids through two independent but interconnected pathways: the cytosolic mevalonate (MVA) pathway and the plastidial methylerythritol phosphate (MEP) pathway (3). The MVA pathway primarily produces sesquiterpenes and triterpenes, while the MEP pathway generates monoterpenes and diterpenes. Both pathways produce the universal precursors isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which serve as building blocks for all terpene structures.

Terpene synthases (TPSs) are the key enzymes responsible for converting these precursors into the diverse array of terpene structures found in nature (3). In aromatic plants like mint and citrus, these compounds are produced and stored in specialized structures called glandular trichomes and oil glands, respectively. The expression of TPS genes and the activity of these enzymes are tightly regulated by environmental factors and developmental signals, making them prime targets for enhancement strategies.

# Controlled Drought Stress Management

Controlled water deficit represents a powerful tool for enhancing terpene production in many aromatic crops. Plants respond to drought by upregulating the biosynthesis of protective secondary metabolites, including terpenoids (4). This response helps plants cope with oxidative stress and signals other plant tissues to activate defensive mechanisms.

Research on medicinal plants has shown that moderate drought stress significantly increases terpenoid content. A study on

Bupleurum chinense demonstrated that drought stress stimulated the terpenoid backbone and triterpenoid biosynthesis pathways, leading to increased saikosaponin accumulation (5). Similarly, work on cumin plants revealed that drought-stressed plants showed significant increases in terpene levels alongside upregulation of key biosynthetic genes including 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and geranyl diphosphate synthase (GPPS) (6).

In basil and other members of the Lamiaceae family, moderate drought conditions enhanced sesquiterpene production while also improving the overall quality of essential oils (7). The optimal level of drought stress varies by species and growing conditions. Excessive water deficit can inhibit photosynthesis and reduce overall biomass, ultimately decreasing total terpene yield despite higher concentrations per unit mass. Growers should aim for moderate stress that maintains plant health while triggering enhanced secondary metabolism.

# Temperature Optimization

Temperature plays a dual role in terpene biosynthesis, affecting both enzyme activity and gene expression. Research on birch and aspen demonstrated that elevated night-time temperatures significantly increased daytime terpenoid emissions. Plants grown with night temperatures of 18 to 22 degrees Celsius showed substantially higher emissions of sesquiterpenes and certain monoterpenes compared to those at lower temperatures (8).

Temperature affects terpene production through multiple mechanisms. Higher temperatures increase the volatility of terpenes, potentially leading to greater emissions from storage structures. More importantly, temperature influences the expression of genes encoding enzymes in both the MEP and MVA pathways (2). However, excessively high temperatures can denature enzymes and degrade already-produced terpenes, so careful monitoring is essential.

For citrus crops, temperature during fruit development significantly affects the terpene profile of essential oils extracted from peels (9). For most aromatic crops, maintaining daytime temperatures between 25 and 30 degrees Celsius with slightly lower night temperatures of 18 to 22 degrees Celsius appears optimal for terpene production. This temperature differential mimics natural conditions and supports robust secondary metabolism without inducing heat stress.

| Crop Type | Optimal Day Temperature | Optimal Night Temperature | Effect on Terpenes |
|---|---|---|---|
| Mint species | 25-30°C | 18-22°C | Enhanced monoterpene production |
| Citrus species | 24-28°C | 16-20°C | Improved essential oil quality |
| Basil and herbs | 26-30°C | 18-22°C | Increased sesquiterpene content |
| Lavender | 22-28°C | 15-18°C | Enhanced linalool production |

# Nutrient Management Strategies

Soil nutrient availability profoundly impacts terpene biosynthesis through its effects on carbon and nitrogen allocation. The carbon-nutrient balance hypothesis suggests that when nitrogen is limiting, plants allocate more carbon to secondary metabolites like terpenes rather than to nitrogen-rich primary compounds such as proteins (10).

Phosphorus and potassium play particularly important roles in terpene production. Phosphorus is essential for the production of the phosphorylated precursors DMAPP and IPP, while potassium affects enzyme activation and osmotic regulation under stress conditions. Moderate nitrogen limitation during the reproductive phase can enhance terpene production by shifting metabolism toward secondary compound synthesis (10).

In mint cultivation, nutrient management significantly affects essential oil yield and composition. Studies have shown that excessive nitrogen application can reduce menthol content while promoting vegetative growth at the expense of oil production (11). Sulfur supplementation deserves special attention as this element is incorporated into certain terpenes and affects the overall terpenoid profile. Research has shown that sulfur-containing amendments can enhance the production of sulfur-bearing terpenes while supporting general secondary metabolism.

# Elicitor Application

Plant hormones and signaling molecules can act as powerful elicitors of terpene biosynthesis. Methyl jasmonate (MeJA) is the most extensively studied elicitor, with numerous studies demonstrating its ability to dramatically increase terpenoid production (2).

MeJA treatment induces the expression of TPS genes and upregulates the entire terpenoid biosynthetic pathway. In Norway spruce, MeJA application increased terpene emissions by more than 100-fold for linalool and over 30-fold for sesquiterpenes (12). The hormone mimics the plant's natural defense response to herbivore damage, triggering a cascade of gene expression changes that result in enhanced secondary metabolism.

Salicylic acid represents another important elicitor that can promote terpenoid biosynthesis. Research has shown that salicylic acid upregulates key enzymes in the terpenoid pathway, including farnesyl pyrophosphate synthase (FPPS) in various species (12). In mint species, jasmonate application has been shown to enhance both the quantity and quality of essential oils, particularly increasing the production of oxygenated monoterpenes like menthol and menthone. The optimal concentration and timing of elicitor application depend on the target species and desired terpene profile.

# Transcription Factor Regulation

Understanding the transcriptional regulation of terpene biosynthesis opens possibilities for targeted enhancement. Several families of transcription factors (TFs) play crucial roles in controlling terpenoid production, including WRKY, MYB, AP2 or ERF, bHLH, and NAC families (13).

These transcription factors respond to environmental signals and developmental cues by binding to specific promoter regions of genes involved in terpene biosynthesis. For example, WRKY transcription factors regulate sesquiterpene artemisinin synthesis in Artemisia annua and diterpene biosynthesis in rice (13). In citrus, the transcription factor MYC5 has been identified as crucial for oil gland development and the biosynthesis of essential oils (14). While direct genetic manipulation of transcription factors requires advanced techniques, understanding their role helps in timing environmental interventions to coincide with periods of high TF activity.

# Metabolic Engineering in Mint Production

Mint species, particularly peppermint and spearmint, represent important commercial sources of monoterpene essential oils. The monoterpenoid biosynthesis pathway in mint is well characterized, making these crops attractive targets for metabolic engineering approaches to enhance oil production (15).

Research has demonstrated that overexpressing genes encoding enzymes in the MEP pathway, particularly 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), can increase essential oil yields in peppermint. The most encouraging results were obtained when multiple genes were manipulated simultaneously. Plants where DXR was overexpressed and menthofuran synthase was down-regulated showed oil yield increases of up to 61% over wild-type controls while reducing undesirable by-products (16).

Another successful strategy involved overexpression of lipid transfer proteins, which increased trichome size and enhanced monoterpenoid production. Plants expressing tobacco lipid transfer protein showed increases in limonene levels of 1.6-fold and dramatic increases in other monoterpenes (15). While metabolic engineering requires sophisticated molecular biology techniques, these advances demonstrate the substantial potential for enhancing terpene production through targeted genetic modifications.

# Agronomic Practices for Enhanced Production

Beyond molecular and environmental approaches, specific agronomic practices can significantly impact terpene yields in essential oil crops. For mint cultivation, planting method, timing, and plant density all influence essential oil production (11).

Ridge planting systems have been shown to provide superior results compared to flat-bed cultivation. Studies on menthol mint demonstrated that plants grown on ridges with optimal spacing of 166,666 plants per hectare yielded maximum essential oil content while reducing water requirements and accelerating crop maturity by approximately 30 days (11). The timing of planting also significantly affects oil yield, with early season planting generally producing higher essential oil content and better quality profiles.

For citrus crops, proper handling during harvest and post-harvest processing critically affects terpene retention. Cold-pressing methods preserve more volatile terpenes compared to heat-based extraction, and storage conditions must be carefully controlled to prevent oxidation and degradation of essential oils (17).

# Harvest Timing Considerations

The timing of harvest critically affects the final terpene content of plant material. Terpene concentrations fluctuate throughout plant development and can vary substantially even over the course of a single day due to circadian regulation (12).

For many aromatic plants, terpene content peaks during specific developmental stages. In mint, essential oil content typically reaches maximum levels just before full flowering. For citrus, the maturity stage of fruit significantly influences both the quantity and composition of peel oils, with different terpene profiles characterizing immature versus fully mature fruit (9).

Harvesting during the morning hours, after dew has evaporated but before peak temperatures, often captures plants at their maximum terpene content before heat-induced volatilization occurs. Post-harvest handling also significantly impacts terpene retention. Rapid drying at moderate temperatures (below 30 degrees Celsius) and protection from light help preserve volatile terpenes. Proper curing in controlled environments allows for the gradual breakdown of chlorophyll while maintaining terpene content.

| Crop | Optimal Harvest Stage | Time of Day | Post-Harvest Consideration |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Peppermint | Just before full bloom | Mid-morning | Rapid drying at 25-30°C |
| Spearmint | Early flowering | Morning hours | Shade drying preferred |
| Citrus peels | Fully mature fruit | Any time | Cold-press immediately |
| Basil | Before flowering | Early morning | Quick drying essential |

# Integrated Enhancement Strategies

The most effective approach to enhancing terpene production often involves combining multiple strategies rather than relying on a single method. Environmental factors interact in complex ways, and their effects on terpene biosynthesis can be synergistic (10).

A practical integrated approach for mint cultivation might include selecting cultivars with naturally high terpene production as the foundation, implementing controlled drought stress in the final weeks before harvest, optimizing the nutrient regime to favor secondary metabolism with moderate nitrogen restriction during flowering, applying elicitors such as methyl jasmonate at strategic developmental stages, using appropriate planting methods and densities, and timing harvest to coincide with peak terpene accumulation.

For citrus production, an integrated strategy would focus on temperature management during fruit development, appropriate irrigation scheduling to avoid excessive vegetative growth, balanced fertilization that does not over-supply nitrogen, and optimization of harvest maturity and processing methods to preserve volatile compounds.

## Challenges and Future Directions

While significant progress has been made in understanding and manipulating terpene biosynthesis, several challenges remain. The genetic regulation of terpenoid production is extremely complex, involving hundreds of genes that respond to multiple environmental signals (3). Predicting how plants will respond to combined stresses or elicitor treatments remains difficult.

Future research should focus on developing more precise tools for monitoring terpene production in real-time, allowing for adaptive management strategies. Advanced metabolic engineering approaches, including CRISPR-based gene editing of regulatory elements, may eventually allow for the creation of plants with constitutively elevated terpene production without the need for environmental manipulation (18). Understanding the molecular mechanisms controlling oil gland and trichome development will also be crucial for maximizing the sites of terpene biosynthesis and storage (14).

## Conclusion

Enhancing terpene production in commercially important plants requires a multifaceted approach based on sound scientific principles. Controlled drought stress, when carefully managed, can significantly increase terpenoid concentrations through activation of stress response pathways. Temperature optimization, particularly elevated night-time temperatures, enhances terpene biosynthesis and emission. Strategic nutrient management, including moderate nitrogen limitation coupled with adequate phosphorus and potassium, shifts plant metabolism toward secondary compound production.

The application of elicitors such as methyl jasmonate provides a powerful tool for rapidly inducing terpene biosynthesis. Understanding the role of transcription factors in regulating these pathways helps in timing interventions for maximum effectiveness. In crops like mint where the biosynthetic pathways are well characterized, metabolic engineering offers promising opportunities for substantial yield improvements. Appropriate agronomic practices, including planting methods, spacing, and timing, significantly influence essential oil production. Finally, optimizing harvest timing and post-harvest handling ensures that enhanced terpene production translates into improved final product quality.

As our understanding of terpene biosynthesis continues to grow, new enhancement strategies will undoubtedly emerge. Growers who stay informed about the latest research and are willing to experiment with different approaches will be best positioned to maximize the terpene content of their crops. However, success requires careful attention to plant health, as excessive stress can be counterproductive. The goal is to find the optimal balance that stimulates terpene production while maintaining overall plant vigor and yield. The commercial value of essential oils continues to drive innovation in this field, promising continued advances in our ability to enhance these valuable compounds in aromatic and medicinal plants.

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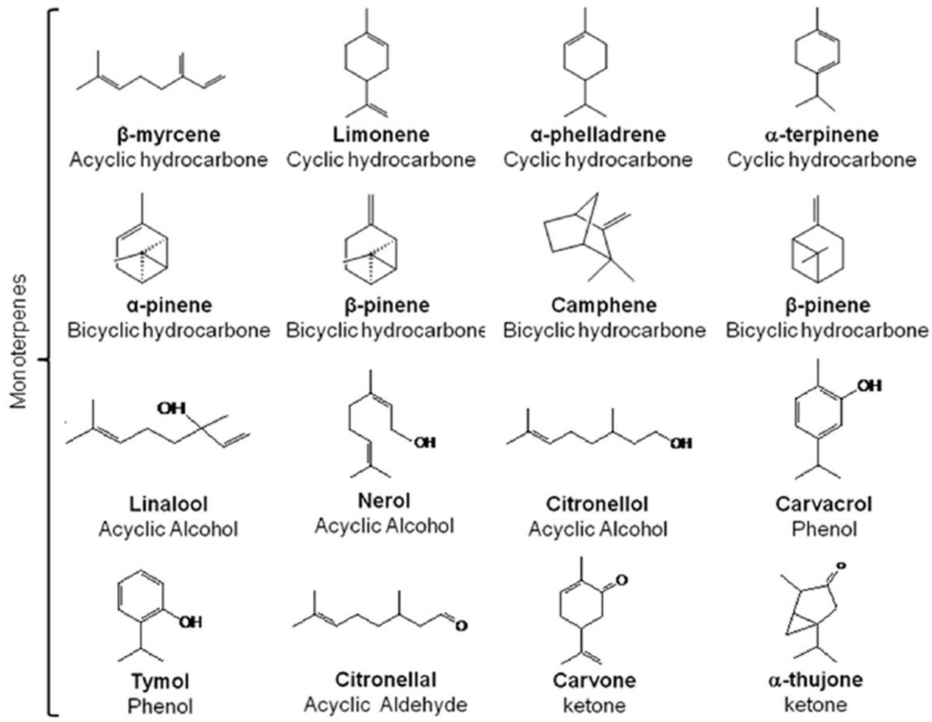
# Exogenous Terpenes in Agriculture: Can External Application Improve Crop Performance?

Terpenes are among the most diverse and abundant secondary metabolites produced by plants. While these compounds are well known for their roles in plant defense and stress responses, recent research has explored whether applying terpenes externally to plants can provide practical benefits in commercial agriculture. This post examines the current scientific understanding of exogenous terpene applications through both foliar sprays and root zone treatments.



Models for a collection of commonly found monoterpenes

# What Are Terpenes and Why Do Plants Produce Them?

Terpenes represent the largest class of plant secondary metabolites, with approximately 55,000 known members across the plant kingdom (1). Plants synthesize these compounds through the methylerythritol phosphate pathway in plastids and the mevalonate pathway in the cytosol. The resulting molecules range from simple monoterpenes containing 10 carbon atoms to complex diterpenes with 20 carbons and beyond.

The primary ecological function of terpenes involves plant protection. These volatile organic compounds help plants defend against pathogens and herbivores, attract beneficial insects and pollinators, and provide protection against environmental stresses such as heat and drought (2). Given these natural protective functions, researchers have investigated whether externally applied terpenes might confer similar benefits to crops.

# Foliar Application of Monoterpenes

The most comprehensive study on foliar terpene application comes from research on tomato plants under water deficit stress. When a mixture of nine monoterpenes was applied as a foliar spray at concentrations ranging from 1.25 to 5 mM, the treated plants showed significant improvements in oxidative stress management (3).

The foliar-applied monoterpenes were readily absorbed by tomato leaves, increasing total foliar monoterpene content by up to 2.5-fold compared to untreated controls. Most importantly, the treatment substantially decreased hydrogen peroxide accumulation and lipid peroxidation in plants exposed to drought stress. At the optimal concentration of 1.25 mM, plants showed a 50% reduction in oxidative damage compared to controls, though this protective effect did not extend to preventing photosynthetic decline (3).

The mechanism appears to involve direct quenching of reactive oxygen species by the terpenes themselves. Interestingly, higher concentrations of 2.5 and 5 mM increased activity of antioxidant enzymes like superoxide dismutase and ascorbate peroxidase, but also induced some oxidative stress, suggesting a threshold effect where lower concentrations may be more beneficial than higher ones.

| Monoterpene Concentration | $H_2O_2$ Reduction (%) | Lipid Peroxidation Reduction (%) | Enzyme Activity Change |
|---|---|---|---|
| 1.25 mM | ~50% | ~45% | No change |

| | | | |
|---|---|---|---|
| 2.5 mM | ~35% | ~30% | Increased |
| 5.0 mM | ~25% | ~20% | Increased |

## Root Zone Applications and Belowground Signaling

While foliar applications have shown promise for stress mitigation, root zone applications of terpenes have been explored primarily for pest management through biological control. The sesquiterpene E-β-caryophyllene serves as a particularly well-studied example of how terpenes function in the rhizosphere.

When maize roots are damaged by western corn rootworm larvae, they naturally emit E-β-caryophyllene, which attracts entomopathogenic nematodes that parasitize and kill the pest insects (4). Field experiments demonstrated that when synthetic E-β-caryophyllene was applied to soil near maize varieties that do not naturally produce this signal, adult beetle emergence decreased by more than 50%, demonstrating the practical potential of this approach.

The effectiveness of soil-applied E-β-caryophyllene depends heavily on soil properties. Research has shown that this sesquiterpene diffuses primarily through the gaseous phase of soil rather than the aqueous phase. In clay soils at 10% water content, diffusion was significantly limited, but increasing moisture to 20% substantially improved signal propagation in clay loam and sandy loam soils (5).

In controlled field trials, maize plants engineered to constitutively emit E-β-caryophyllene and treated with entomopathogenic nematodes suffered 60% less root damage and had significantly fewer adult beetles emerge compared to non-emitting lines (6). This demonstrates that strategic application of specific terpenes to the root zone can enhance biological control efficacy.

## Disease Resistance Through Diterpene Application

Diterpenes have shown particularly strong antimicrobial properties when applied to plants. Two labdane-type diterpenes isolated from tobacco, sclareol and cis-abienol, were tested as exogenous treatments on tobacco, tomato, and Arabidopsis plants. These compounds effectively inhibited bacterial wilt diseases, with microarray analysis revealing that they activated genes encoding components of plant immune responses, including MAP kinase cascades and defense-related biosynthetic pathways (1).

In maize, the diterpene epoxydolabranol demonstrated simultaneous effectiveness against two major fungal pathogens, Fusarium graminearum and Fusarium verticillioides. The diterpene momilactone B showed allelopathic properties, completely inhibiting germination of several weed species at concentrations of 4 to 20 ppm when applied to soil (1).

| Terpene Type | Application Method | Target Organism | Effective Concentration |
|---|---|---|---|
| Monoterpenes (mixed) | Foliar spray | Drought stress | 1.25 mM |
| E-β-caryophyllene | Soil drench | Root pests | 200-20,000 ng |
| Sclareol/cis-abienol | Root application | Bacterial wilt | Not specified |
| Momilactone B | Soil application | Weeds | 4-20 ppm |

## Practical Considerations for

# Application

Several factors influence the effectiveness of exogenous terpene applications. For foliar treatments, the physiochemical properties of individual terpenes significantly affect uptake and translocation. Compounds like α-terpinene and terpinolene show greater solubility and cellular accumulation compared to more volatile molecules like α-pinene and limonene (3).

Timing and application frequency also matter. Foliar sprays applied twice daily showed better results than single applications, suggesting that maintaining adequate concentrations on leaf surfaces requires repeated treatments. For soil applications, the water content and texture of the growing medium critically influence how well terpene signals diffuse through the root zone.

Cost remains a significant consideration. Production of terpenes for agricultural use requires either chemical synthesis or bio-production in heterologous hosts. For structurally complex terpenes, chemical synthesis may be economically prohibitive, making microbial production platforms like engineered Escherichia coli or Saccharomyces cerevisiae more practical options (7).

# Limitations and Future Directions

Current research reveals important limitations. While exogenous monoterpenes effectively reduced oxidative stress in tomato plants, they did not prevent the photosynthetic decline associated with stomatal closure during drought. This suggests that terpene applications may be most useful as supplementary treatments rather than standalone solutions for stress management (3).

The dose-response relationship appears complex, with higher concentrations sometimes producing counterproductive effects. In the tomato study, 5 mM monoterpene applications induced oxidative stress while attempting to protect against it, highlighting the importance of careful concentration optimization for each crop and application method.

Much of the existing research has been conducted under controlled laboratory or greenhouse conditions. Large-scale field trials examining the agronomic and economic viability of exogenous terpene applications remain limited. Questions about the persistence of applied terpenes under field conditions, their environmental fate, and potential non-target effects require further investigation.

# Conclusions

Exogenous terpene applications represent an emerging area of agricultural research with demonstrated benefits in specific scenarios. Foliar monoterpene sprays can mitigate oxidative stress from drought at appropriate concentrations. Soil-applied sesquiterpenes like E-β-caryophyllene enhance biological pest control by attracting beneficial nematodes. Diterpenes show promise as antimicrobial agents when applied to roots.

However, practical adoption requires further development. Growers interested in this technology should recognize that terpene applications are most likely to succeed as part of integrated management strategies rather than as standalone interventions. The variable responses across different terpene types, concentrations, and application methods mean that each crop system will require careful optimization.

As production costs decrease and application protocols become more refined, exogenous terpenes may find their place in the grower’s toolkit, particularly for organic production systems seeking alternatives to synthetic pesticides. Until then, this remains a promising but still developing technology that warrants continued research attention.

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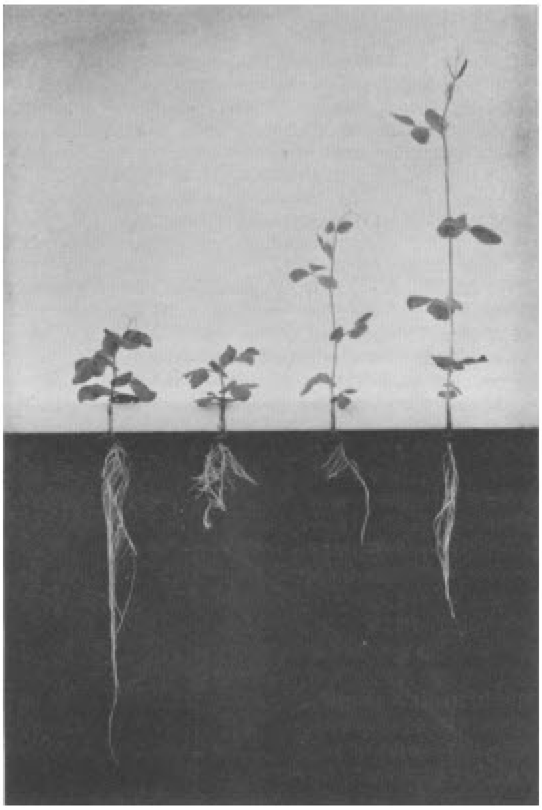
# Kinetin, a powerful hormone for flowering plants

Kinetin was the first cytokinin ever discovered. Scientists have used it extensively to stimulate cell division in tissue culture, as it is a powerful growth hormone. However, there isn’t a clear understanding of the effects of kinetin in large flowering plants, reason why it hasn’t been widely used as an additive in plant culture. In this post, we are going to take a look into the practical application of kinetin. We are going to look into published research and discuss whether kinetin could be used to enhance plant yields. I will refrain from discussing the history and chemical structure of kinetin, for a basic introduction about kinetin and its history, I suggest reading this paper (1). I will also use some information contained in this review (5).



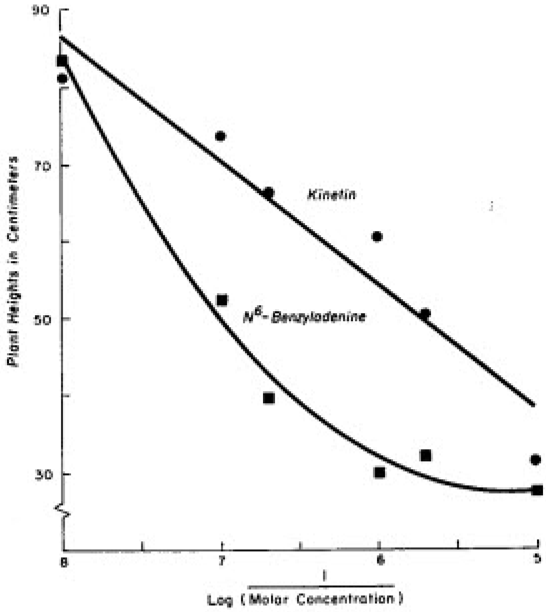



Fig. 4. Effects of kinetin and gibberellin, singly and in combination, in the solution culture root medium on internode elongation of the 'Little Marvel' dwarf pea. Left to right: control (no kinetin), kinetin $10^{-6}$ M, kinetin $10^{-6}$ M + gibberellin $A_3$ $10^{-6}$ M, and gibberellin $10^{-6}$ M. Plants photographed after 10 days' exposure to the chemical stimuli.

Fig. 5. Comparative inhibitory effects of kinetin and $N^6$-benzyladenine on the height of the 'Alaska' pea.

Tomatoes, peas and cucumbers grown in solutions containing kinetin were significantly shorter. Root and flowering changes were also present. Taken from (2).

# The effects of exogenous kinetin

In tissue culture, what kinetin does seems to be clear, it promotes cell division in the presence of auxins. However, for large plants in soilless media, the effect does not seem to be that straightforward. One of the first thorough studies of kinetin in flowering plants was done in the early 1960s (2). In this study, tomatoes, cucumbers, and peats were grown in solutions containing different concentrations of kinetin, going from $10^{-5}$ to $10^{-7}$ molar. The researchers showed that kinetin in solution behaved like a gibberellin inhibitor, directly suppressing plant height as a function of concentration. The plants developed several root abnormalities and changes in their flowering cycle, with kinetin inhibiting flowering in tomatoes, but accelerating it in peas.

You can see in this study that the effective concentration is quite low. The range of kinetin concentrations tested goes from 0.0215mg/L to 2.15 mg/L. These values are quite small compared to the amounts of other hormones, such as IBA or NAA, generally used in plant culture. The concentration of kinetin plays a key role in its effect. A 2008 study on red goosefoot (3) shows the strong impact kinetin concentration can have. These researchers showed that low concentrations of kinetin increased bud formation and increased the height of the apical meristem, while large concentrations inhibited flowering and made the plants shorter.

The entire literature on exogenous kinetin applications is therefore split between apparently contradictory effects. Some studies show effects that are more in line with a gibberellin inhibitor, with shorter plants, while others show stimulation of shoot growth. What you get is dependent on concentration and plant species, making kinetin a hard hormone to use. Use too much and you might compromise flowering and yields, use too little and you might have undesirable elongation effects or simply no effects at all (4, 6).

Kinetin can also have an effect on the sex determination of plants. For example, kinetin induces female flowers in cannabis and can ameliorate the production of male flowers in female plants (12).

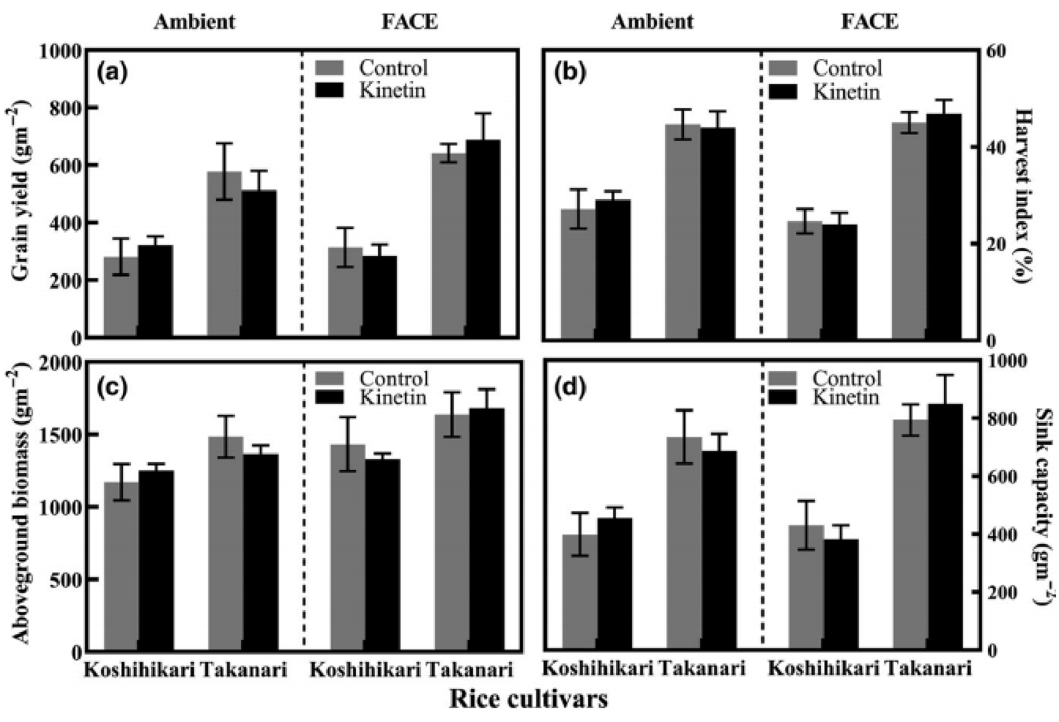
# Kinetin foliar sprays

The mode of application makes a big difference as well. While most of the root studies I read using kinetin kept their application rates below 3mg/L, many foliar studies explore kinetin application rates that are significantly higher. In this study (9), for example, they perform kinetin applications at 100 ppm. From the foliar studies I read, I found this study (7) particularly interesting. In it, kinetin applications at 2.5, 5, and 10 mg/L were done using foliar spraying on tomato, cucumber, and pepper plants.

The researchers found that the cucumbers had an excellent response to the 2.5 mg/L treatment, with taller plants, larger leaf area, and bigger yields, while they showed negative responses to the 10ppm treatment, with lower yields. While tomatoes showed a similar response, peppers gave their best results with the 10 ppm kinetin sprays. This again highlights not only that plants will respond negatively to excessive doses of kinetin, but that this response is significantly species-dependent.

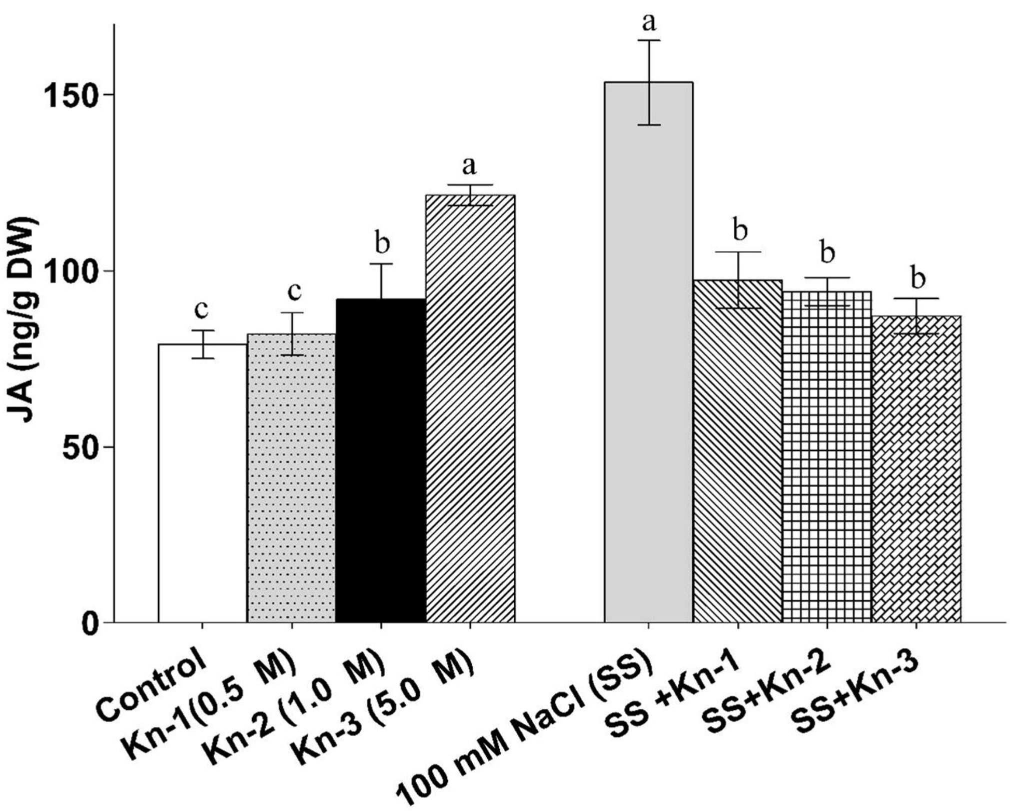
# Environmental conditions

Furthermore, environmental conditions can play a significant role in the effects of kinetin. This study (8) found that kinetin could help rice plants give better yields under carbon dioxide enrichment. However, this worked only for some of the varieties of rice used. For the varieties for which it worked, kinetin applied as a foliar at 10.75 ppm was able to enhance the carbon dioxide fertilization effect.




Effect of kinetin application in several different rice cultivars with or without carbon dioxide enrichment (8)

Other environmental conditions, such as salinity stress and oxidative stress, can also play a big role in the effect of kinetin. As a strong antioxidant, kinetin can help plants deal with oxidative stress (10). It has also been tested many times as a way to deal with salinity-induced stress, for example, see this article on kinetin applications in soybeans (11). In this last study, you can see how kinetin upregulates the gibberellin biosynthesis pathway when it was actively suppressed by the high salinity. Some effects, such as the production of jasmonic acid, are actually opposite in the control and in the salinity-induced environments as a function of kinetin concentration.




Changes in jasmonic acid content for soybean plants grown with or without salt stress and treated with kinetin. Kinetin increases JA when no salt stress is present and decreases it otherwise.

# Conclusion

Kinetin can be a powerful and versatile hormone in flowering plants. It can be used to achieve a variety of different effects, including making plants shorter, increasing budding sites, increasing yields, or relieving sources of stress. However, the choice of concentration, method, and application time is critical and can lead to completely opposite effects if not done correctly. Low applications tend to increase growth and leaf area, while larger concentrations will show an effect similar to a gibberellin inhibitor. However, the concentrations that work best for a given plant cannot be known before experimentation is done. However, do consider that higher concentrations consistently lead to decreases in yields.

If you want to use kinetin in your crop, start with a foliar dose at around 2ppm and take note of the effects. From there, you will be able to gauge whether you want to have a higher or lower concentration of kinetin. If the dose is too high, you will start to see some negative effects. Also, time your applications so that they are in line with the effects you want to achieve. If you want to feed kinetin through the roots, use an even lower concentration and make sure your applications are properly timed, avoid having permanent exposure of roots to kinetin, as this is likely to be negative.

**Have you ever used kinetin in your crops? What concentrations have you used and what effects have you seen? Let us know in the comments below!**

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# The value of Fulvic Acid in hydroponics

Fulvic and humic acids have been studied for decades and used extensively in the soil and hydroponic growing industries. I previously talked about the use of humic acid in hydroponics and the way in which it can improve crop results. In that post, we talked about how humic acids can improve nutrient chelation and how this can lead to improvements in yields depending on the origin and properties of the humic substances used. In this post, we are going to take a look specifically at fulvic acid substances, which are a smaller family that has potentially more valuable uses in the hydroponic space. We will start by discussing what differentiates fulvic and humic acids and what the current peer-reviewed evidence around fulvic acids tells us.

This is a model of the general type of molecule that makes up fulvic acid. Note that fulvic acid is not a pure substance, but a mixture of many substances with similar chemical properties.

Fulvic acids are not chemically pure substances, but a group of chemicals that result from the decomposition of organic matter. This process generates both humic and fulvic acids. However, fulvic acids are different from humic acids in mainly two ways. The first is that fulvic acids are soluble at both acid and alkaline pH values, and the second, is that fulvic acids generally have much lower molecular weights. Fulvic acids are therefore more soluble and are more easily accessible to plants compared to humic acids, which have much larger molecular weights. But why should we use them in hydroponics and exactly how?

Sadly, not many publications have tackled the use of fulvic acid in crops specifically. One of the few examples of reviews that touch on the matter is this paper, which covers most of the literature around fulvic acids before 2014. I also did a literature review myself, trying to find articles in which the fulvic acid source, application type and rate, and the results against a control without fulvic acid were clearly explained.

The table below shows you the results of my search, I was able to find 10 papers overall, with a mix of root and foliar applications of fulvic acid, with a range of application rates and plant species. Almost all of these papers found positive results from the use of fulvic acid, except two papers that found either no effect or mixed results from their use.

The range of application depends substantially on the application type. Most papers that tackled foliar applications chose application rates in the 1-3g/L range, while papers that tackled root applications generally stayed in the 25-150ppm range. This is normal since foliars are generally much more concentrated than root applications. Both types of applications have different effects. Root applications are going to exercise an additional strong nutrient chelating role, while foliar applications are more likely to exert a hormonal role. A study around genetically modified tomato plants showed that plants engineered to be insensitive to IAA were also unable to respond to fulvic acid, hinting at the fact that fulvic acid has an auxin-like effect in plants.

| Ref | Application Type | Crop | Application (ppm) | Effect |
|---|---|---|---|---|
| 1 | Foliar | Tomato | 800-1100 | yield+ number+ cracking- |
| 2 | Root | Cucumber | 100-300 | growth+ |
| 3 | Foliar | Grapevines | 500 | yield+ growth+ quality+ |
| 4 | Root | Pepper | 25 | quality+ |
| 5 | Foliar | Wheat | 500-1000 | no effect |
| 6 | Root | Impatiens | 40 | yield+ flowering+ |
| 7 | Foliar | Faba Beans | 1500-3000 | yield+ |
| 8 | Root | Tomato | 15-30 | mixed |
| 9 | Root | Okra | 1500-3000 | yield+ quality+ |

| | | | | |
|---|---|---|---|---|
| 10 | Root | Potato | 150 | yield+ |

Literature search of fulvic acid related publications. The websites where you can read the articles are linked in the “Ref” column.

The effects seem to be quite positive overall, with increases in yield, quality, and flower numbers across the board. The studies above that investigated nutrient transport also showed substantial benefits when root applications of fulvic acid were used. Plants grown in a Hoagland solution showed better nutrient transport when fulvic acid substances were used in the nutrient solution. This is possibly both due to their ability to chelate micronutrients and their ability to provide an additional pH buffer at the region of interest in hydroponics (5.5-6.5). This study, shows how fulvic acid substances can have pKa values in this precise region, although their still relatively large molar mass implies that they will contribute marginally to buffering capacity, especially if used only in <100 ppm concentrations.

Fulvic acids also seem to be synergistic with several other biostimulants in the studies showed above. When tests were done with humic acids or other biostimulants, the effect of the combination is usually better than the effect of either part on its own. This means the fulvic acid might not only be a good addition on its own, but it might also contribute significantly to enhance the effect of other biostimulants used.

It is however important to note that fulvic acids do have negative effects when used in excess, reason why their application rates need to be carefully controlled. Using too much can lead to drops in yields and quality along with slower growth. If you want to start using them, it is, therefore, wise to start at the lower range of the application rates shown above and climb up as you gauge the effects. It is also important to note that – as humic acids – different sources of fulvic acid might have different effects, as the actual molecules that make up the substance will change.

A big advantage of the use of fulvic acids in hydroponics is also that their solubility is quite high, so the risk of clogging or damaging equipment is low. This is a significant advantage over humic acids, which have lower solubility and can cause problems because of this in hydroponics culture, especially if there are drops in the pH. In hydroponics, fulvic acids can also lead to additional solution stability, especially in recirculating systems, where the destruction of heavy metal chelates as a function of time can become a bigger risk.

**All in all, fulvic acids represent a relatively cheap addition to a hydroponic regime that has limited risk and a lot of potential upsides**. Literature research shows us that low rate applications, if anything, might just have no effect, so the risk of damage to a hydroponic crop by trying fulvic acid applications is low. The synergistic effects shown by fulvic acid are also interesting since this means that they might make other additives you are currently using even more potent. When looking for fulvic acids, make sure you check for high solubility, solubility in low and high pH, and a source that matches the sources used in the literature results you’re interested in reproducing.

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# The effect of Seaweed/Kelp extracts in plants

Few bio-stimulants are more popularly used than seaweed/kelp extracts. These are used by many growers to increase plant quality and yields, in particular, extracts from the

*Ascophyllum nodosum* species are an all-time favorite of the industry. These extract have also been studied extensively for the past 40 years, with large amounts of evidence gathered about their effects and properties across several different plant species. In this article, I will be talking about what the research says about their use, why these extracts work, how these have usually been applied and what you should be looking for when using this type of bio-stimulant.

Composition of the seaweeds extracts Maxicrop and Algifert (content in mg $kg^{-1}$). The content of dry matter in the liquid extract of Maxicrop is 8.0-8.2%. *Source:* Alternatieve Landbouwmethoden (1977).

| Element | Maxicrop | Algifert |
|---|---|---|
| N | 7 200 | 8 700 |
| P | 9 000 | 1 400 |
| K | 26 000 | 19 000 |
| Mg | 3 500 | 10 600 |
| Fe | 2 200 | 60 |
| Al | 60 | 20 |
| Ca | 3 500 | 11 900 |
| S | 23 000 | 49 600 |
| Cl | 67 000 | 55 400 |
| Si | 1 000 | 1 000 |
| Na | 70 000 | 19 400 |
| I | 900 | 200 |
| Br | 800 | 0.6 |
| Cu | 40 | 0.5 |
| Co | 4 | 2 |
| Ni | 24 | 5 |
| Zn | 100 | 33 |
| Mo | 10 | 0.6 |
| Mn | 40 | 24 |
| B | 1 | 50 |

Composition of some seaweed extracts in 1991 (taken from (1) linked below)

The use of kelp extracts is so common, that there was already enough research done about their use to publish a review on the subject in 1991 (1), a lot of the information below comes from this source. Seaweed has been used by farmers for hundreds of years, as it could be used as an alternative to lime in order to alkalinize acidic peatmoss soils, due to the high basicity of seaweed extracts (as some are very high in calcium carbonate content). Seaweed extracts also contain a lot of micro and macro nutrients – as shown above – in proportions that are useful for their use as fertilizer. They are a significant source of potassium and calcium, although the variability of the composition – as shown in the table above – can be quite important. They also contain micronutrients but their low presence relative to plant needs implies that the positive effects of the extracts are most likely not due to them.

Perhaps one of the most important factors surrounding seaweeds is their content of bioactive molecules. These extracts contain an important array of cytokinins, which are plant hormones that will significantly affect plant growth. Auxins, gibberillin-like substances and ethylene precursors like aminocyclopropanecarboxylic acid, have also been detected in seaweed extracts. The cytokinins are usually present in concentrations of around 2-20 ppm in the concentrated extracts, which are enough to cause effects, even if the final diluted versions will be at much lower concentrations. The application of seaweed extracts is usually done through an entire crop cycle and is usually cumulative in nature.

Application rate, frequency, seaweed species and extract processing methods can substantially affect results, with many contradictory results showing up in the literature, with some people showing increases in growth and yields while others show no effects at all. The review quoted above describes many examples of positive results, including examples showing weight gains, yield gains and increases in certain nutrients, like P and N. The review also talks about the ability of seaweed extracts to increase resistance to pests and improve crop quality. A more recent review from 2014 (2) further expands on a lot of these positive effects, citing extensive literature showing increases in yields, dry weights and quality for a wide variety of plant species. *In total, more than 30 different papers showing increases in yields due to the use of kelp extracts are cited in this review.* There are also more than 20 articles cited describing increases in disease resistance or other mechanisms of defense elicitation due to the use of the seaweed extracts.

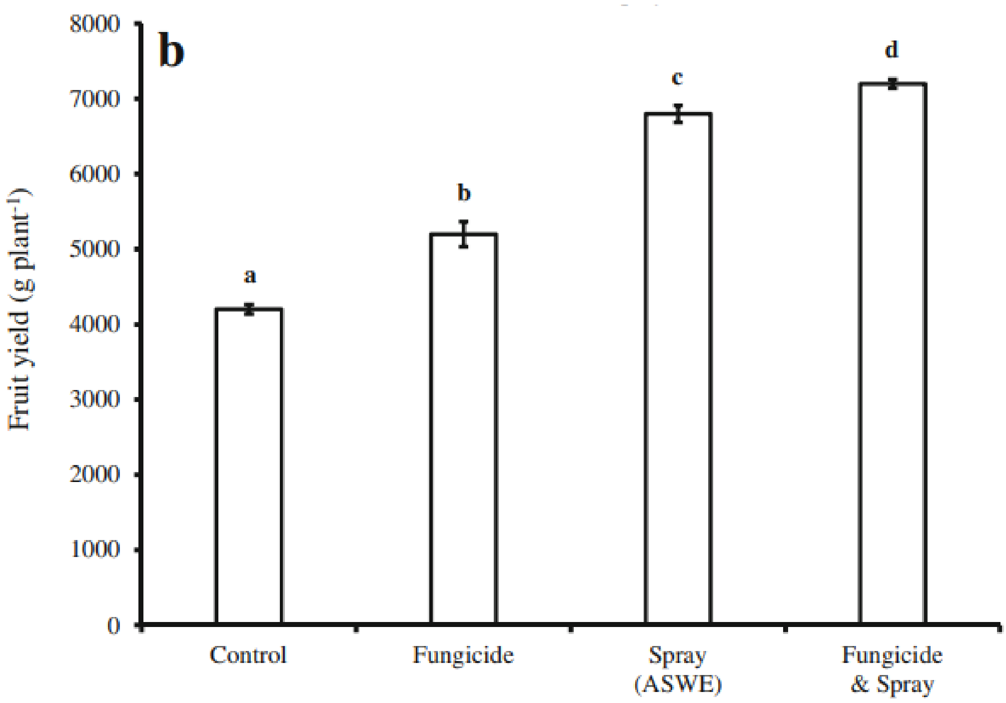


**Fig. 2** Fruit yield of field-grown tomato plants from **a** field experiment 1, 90 days after transplantation with eight treatments including seaweed extract made from *A. nodosum* (ASWE) at a concentration of 0.2 % and **b** field experiment 2, 120 days after transplantation with four treatments, including ASWE at a concentration of 0.5 %. Yields are g plant$^{-1}$ of fresh weight accumulated over several harvests. Data are means±SE ($n$=30 plants); *different letters* according to Fisher's Least Significant Difference (LSD) test ($P$=0.05); LSD is 372.3 and 306.1 for **a** and **b**, respectively

Results of a seaweed extract application in tomatoes (taken from (3))

Foliar applications of seaweed can be carried out at varied levels of frequency and concentration. Applications at a 0.2-0.5% w/v of dry extracts are most common, although higher or lower concentrations have also been found to be effective. As a root drench applications will tend to be on the lower side, as the seaweed contains a substantial amount of NaCl, which can be damaging to plants. Timing of applications can also be quite critical, some growers apply the extract equally spaced through the entire growing periods, while others attempt to time the application with a specific growth phase. Success is reported in both cases, although papers that describe different timing of single applications often find significant differences. To arrive at the optimal usage for a plant species it will be necessary to carry out tests with single applications at different intervals, although single weekly applications are likely to be successful if a less involved approach is desired.

*Although the use of seaweed extracts can be very positive, it is also worth mentioning that it is very dependent on the quality and consistency of the extract being produced*. Since we know that most of the positive effects of these seaweeds are related to their plant hormone content, their use can sometimes be replaced with specific applications of plant hormones, if the effects are properly understood. The discussion in (2) cited before points to the fact that kinetin applications have been able to match the effects of kelp extracts, at a fraction of the cost and the environmental impact at least in a few cases.

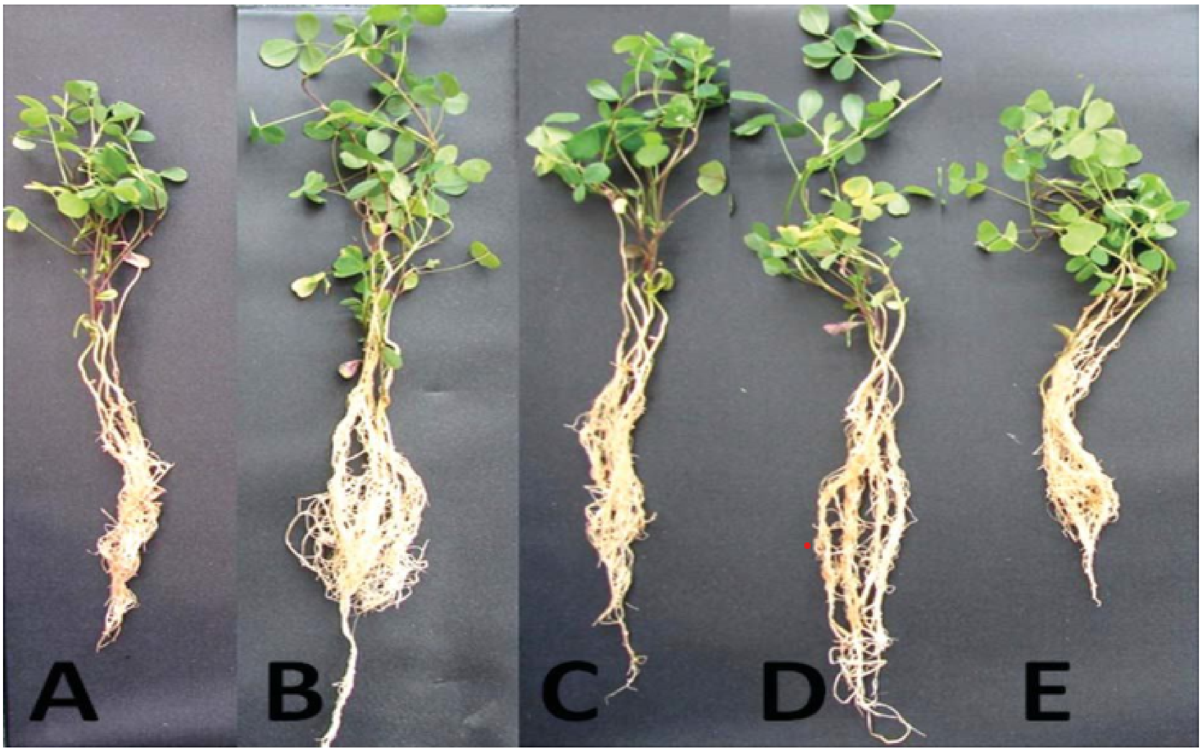


**Fig 1:** Effects of 1 g L”1, *Ascophyllum nodosum* extract (ANE) and its organic sub-fractions on root nodulation growth and development of alfalfa plants 6 weeks after the treatment: (a) control, (b) *Ascophyllum nodosum* extract (ANE) (c) methanol extract, (d) chloroform, and (e) ethyl acetate. (Khan *et al*. 2013).

Photographs showing the effect of kelp extract on root nodulation in alfalfa. Taken from this review (4)

With all the above said, it is quite evident that kelp/seaweed extracts have been widely confirmed to have positive effects in the growing of plants, beyond any reasonable doubt. This effect is mostly related with the hormones they contain and is therefore dependent on the seaweed species, where it is grown and how the seaweed powder is generated. Although root and foliar applications of kelp can both be used to improve results, the use of foliar applications is often favored in order to avoid the introduction of some undesired ions into the growing media. **If you’re not using kelp, go ahead, it’s bound to help!**

# The use of phosphites in plant culture

Plants normally get most or all of their phosphorous from inorganic phosphorus sources. Most commonly these sources are monobasic or dibasic phosphate ions ($H_2PO_4^-$ and $HPO_4^{-2}$), which are naturally formed from any other phosphate species at the pH values generally used in hydroponics (5.5-6.5). However these are not the only sources of inorganic phosphorous that exist. Phosphite ions – which come from phosphorous acid $H_3PO_3$ – can also be used in plant culture. Today we are going to talk about what phosphite does when used in hydroponics and why it behaves so differently when compared with regular phosphate sources. In research P from phosphate is generally called Pi, so I will follow this same convention through the rest of this post. A good review on this entire subject can be found here.

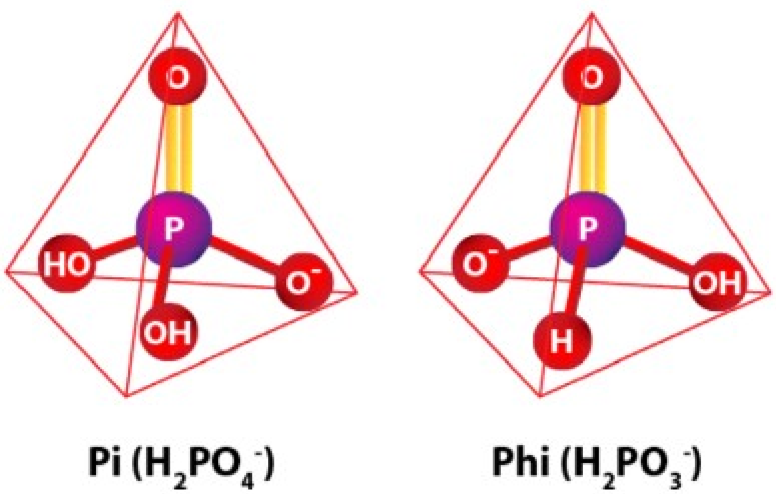


**Fig. 1.** Three-dimensional chemical structures of phosphate ($H_2PO_4^-$; Pi) and phosphite ($H_2PO_3^-$; Phi) forming tetrahedral structures.

The role that phosphite (Phi) plays in plant nutrition and development has now been well established. Initially several people claimed that Phi was a better P fertilizer than Pi so researchers wanted to look into this to see if Phi could actually be used as an improvement over Pi fertilization. However research was heavily disappointing, studies on lettuce (here) , spinach (here), komatsuna (here) as well as several other plants showed that Phi fertilization provides absolutely no value in terms of P nutrition, meaning that although plants do absorb and process the Phi it does not end up being used in plant tissue to supplement or cover P deficiency in any way. Furthermore there are some negative effects when Phi is used in larger concentrations (as those required for Pi) so it quickly became clear that Phi is not a good fertilizer at all.

Why should anyone use Phi then? Well, research started to show that some of the earlier positive results of Phi fertilization were not because it was covering Pi deficiencies but mainly because it was offering a protective effect against some pathogens. Research on tomatoes and peppers and other plants (here and here) showed that phosphites had some ability to protect plants against fungi with plants subjected to Phi applications showing less vulnerability to the pathogens. However the evidence about this is also not terribly strong and a few papers have contested these claims.

**Table 1**
Beneficial effects of phosphite (Phi) as a biostimulator in vegetable crops.

| Crop | Phosphite source (dosage) | Method of application | Improved trait/s | Reference |
|---|---|---|---|---|
| Celery | Phosphorous acid | Foliar spray | Yield | Rickard (2000) |
| Lettuce | Phosphorous acid (50% of total P as Phi) | Nutrient solution in hydroponics | Biomass dry weight, foliar area and P content in the whole plant | Bertsch et al. (2009) |
| Onion | Phosphorous acid | Foliar spray and soil application | Percentage of jumbo size onions | Rickard (2000) |
| Potato | Phosphorous acid | Foliar spray | Size and yield of US No. 1 grade potatoes | Rickard (2000) |
| Potato | Potassium phosphite | Foliar application | Phytoalexin and chitinase content, and yield maintenance | Lobato et al. (2011) |
| Potato | Potassium phosphite | Sprays applied to seed tubers and foliage | Reinforcement of the cell wall and defense response | Olivieri et al. (2012) |
| Potato | Potassium phosphite | Liquid solution applied to tubers | Emergence, early growth and mycorrhizal colonization | Tambascio et al. (2014) |
| Potato | Potassium phosphite | Foliar spray | Chlorophyll content, protection against UV-B light and activation of the antioxidant system | Oyarburo et al. (2015) |
| Sweet pepper | Phosphorous acid | Drip irrigation and foliar spray | Size and yield of US No. 1 grade peppers | Rickard (2000) |
| Tomato | Phosphorous acid (50% of total P as Phi) | Nutrient solution in hydroponics | Biomass dry weight, foliar area and P content in the whole plant | Bertsch et al. (2009) |

*Note*: Most studies were based on the application of commercial Phi-containing products without clear indication on the labels of their precise Phi content. Therefore, Phi dosage in the table is only indicated when precise data are available in the cited articles.

**Table 2**
Beneficial effects of phosphite (Phi) as a biostimulator in fruit crops.

| Crop | Phosphite source (dosage) | Method of application | Improved trait/s | Reference |
|---|---|---|---|---|
| Avocado | Phosphorous acid | Foliar spray | Yield of commercially valuable sized fruit | Lovatt (2013) |
| Banana | Phosphorous acid (50% P as $HPO_4^{2-}$ and 50% as $H_2PO_3^-$) | Nutrient solution in hydroponics | Biomass dry weight, foliar area and P content in the whole plant | Bertsch et al. (2009) |
| Citrus | Phosphorous acid | Foliar spray | Yield and acid content in fruits | Lovatt (1998, 1999) |
| Citrus | Phosphorous acid | Foliar spray | Yield | Albrigo (1999) |
| Citrus | Phosphorous acid | Foliar spray | Yield | Rickard (2000) |
| Peach | Phosphorous acid | Foliar spray | Sugar and soluble solids content | Rickard (2000) |
| Raspberry | Phosphorous acid | Foliar spray | Fruit firmness | Rickard (2000) |
| Strawberry | Potassium phosphite | Plants soaked and irrigated | Fruit acidity, ascorbic acid and anthocyanin content | Moor et al. (2009) |
| Strawberry | Potassium phosphite (6.7% of total P as Phi) | Root application through a controlled watering system | Growth of roots and shoots | Glinicki et al. (2010) |
| Strawberry | Phosphorous acid (30% of total P as Phi) | Nutrient solution applied to the roots | Concentrations of chlorophylls, amino acids and proteins in leaves | Estrada-Ortiz et al. (2011) |
| Strawberry | Phosphorous acid (20% of total P as Phi) | Nutrient solution applied to the roots | Sugar concentration and firmness of fruits | Estrada-Ortiz et al. (2012) |
| Strawberry | Phosphorous acid (20–30% of total P as Phi) | Nutrient solution applied to the roots | pH, EC and anthocyanin concentration in fruits | Estrada-Ortiz et al. (2013) |

*Note*: Most studies were based on the application of commercial Phi-containing products without clear indication on the labels of their precise Phi content. Therefore, Phi dosage in the table is only indicated when precise data are available in the cited articles.

Those who say that Phi is not mainly a fungicide claim that positive results are mainly the effect of Phi acting as a biostimulant (here). These groups have shown through research across several different plant species, including potatoes, onions, lettuce, tomatoes, wheat, oilseed rape, sugar beet and ryegrass that foliar or sometimes root applications of phosphites consistently yield some positive effects, meaning that there is a strong biostimulant effect from the Phi that is not related to either P nutrition or a fungicidal effect. A recent review looking at the overall biostimulant effects of

Phi (here) shows how researchers have obtained evidence of biostimulation in potatoes, sweet peppers, tomatoes and several other species (the images in this post were taken from this review). The different studies mentioned in the review show increases in quality and even yields across these different plant species (see tables above).

While we know that Phi is not a good source of P nutrition and we know it can help as a fungicide in some cases it is clear now that under enough Pi nutrition Phi can provide some important biostimulating effects. Negative effects from Phi seem to be eliminated when enough Pi nutrition is present so rather than be thought of as a way to replace or supplement P nutrition it should be thought of as an additive that has a biostimulating effect. Phi may become a powerful new tool in the search for higher yields and higher quality, while not serving as a replacement for traditional Pi fertilization.

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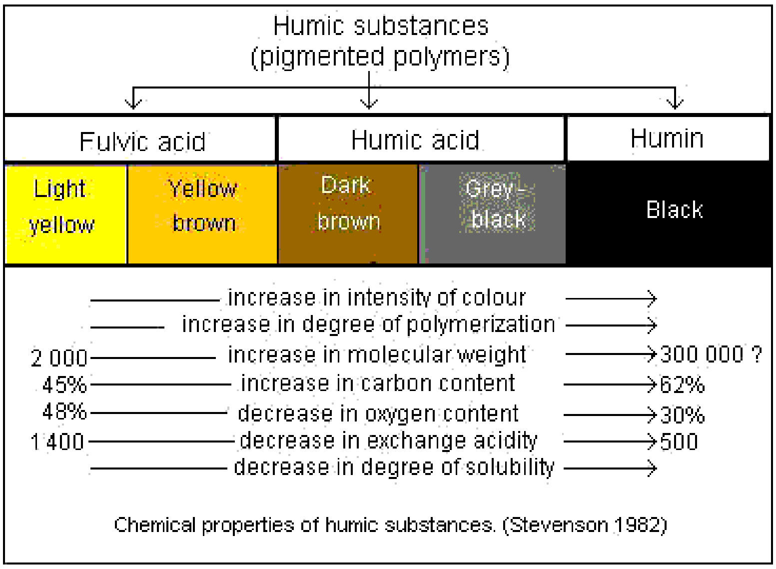
# Humic acids in hydroponics: What is their effect?

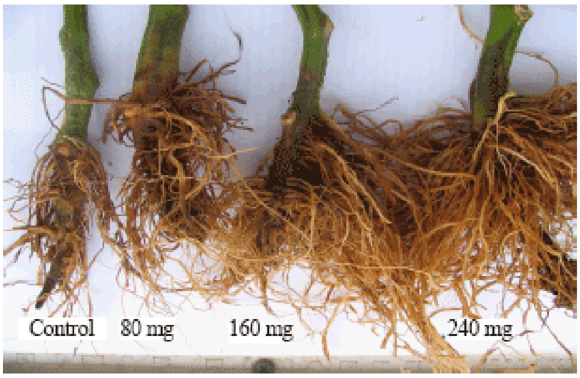
Plants and microorganisms affect the substrates in which they grow in many ways. If you start growing plants in an inert substrate – with nutrient applications of course – you will notice that the substrate's chemical composition will start to change with time and it will start to get enriched in carbon containing substances. As plants and microorganisms grow, thrive and die, some of the chemicals that made up their cells end up enriching the substrate they grow on. This process – whereby organic materials from living organisms become part of a substrate – is what generates the soils around us. One of the most prevalent class of components in this organic material, is what we call humic acids.




Humic substance chemical properties.

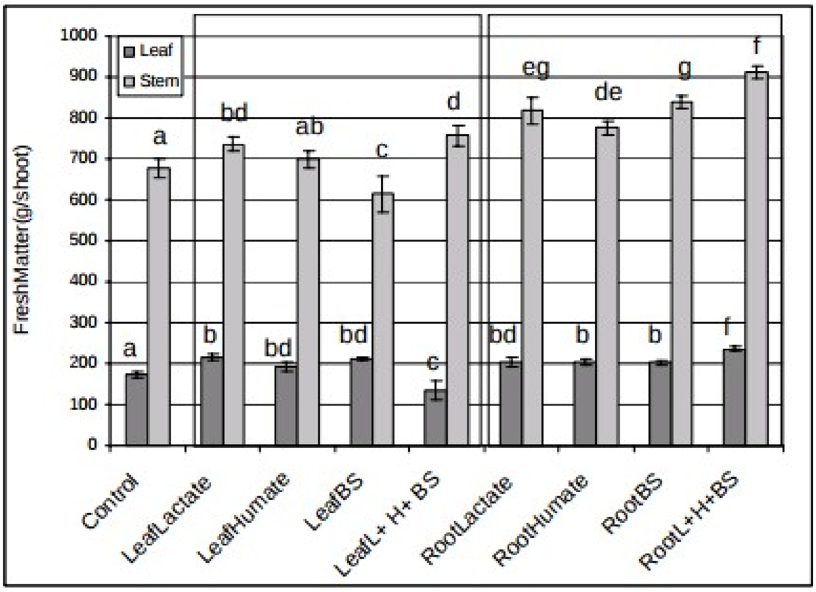
Humic acid is not a single substance but a wide range of substances that are created as a product of plant and microorganism decomposition. This is why you often hear people talk about "humic acids" instead of simply "humic acid". They are called "acids" because the humic substances contain molecules that have groups that resemble those found in phenol and vinegar. They are also differentiated from fulvic acids in the fact that they are only soluble at basic pH values while fulvic acids are generally small enough molecules to be soluble across most of the pH spectrum. Since humic acids are a very important component of enriched soils and can be used in soiless culture, people have started using them as supplements in soiless and pure hydroponic culture.

When talking about the effects of humic acids it is worth mentioning that since we're talking about a group of molecules – not a single substance – effects are generally dependent on the source of the humic acid used. For example you can find a study on tomatoes here where two different sources of humic acids – from peat and leonardite – were used to grow tomatoes. The study shows a clear difference between both with the first only stimulating root growth while the second stimulated both roots and shoots. However in both cases there was an increased iron availability to plants, although the mechanism for this was not established.




Tomato plants inoculated with root rot at different humic acid application rates

In plants like gerberas humic acids applied at 1000 ppm can offer increases in harvested flowers of up to 52% (see here), somewhat positive effects can also be seen in tomatoes across the literature with most studies showing increases in yields and mineral contents (see here), reports of positive effects on gladiolus have also been published (here). Since the 1990s there has been a somewhat established understanding of some general beneficial effects for humic acid applications, it is well established that they can prevent and eliminate micro nutrient deficiencies due to their abilities to increase their availability(see here). The literature is also quite consistent in that the largest effects are often seen on root growth rather than on shoot growth or mass. There are however some types of humic acids that have showed higher increases of shoot mass, for example in an article studying humic substances derived from municipal waste on barley this was the observed effect. For some plants however – despite these beneficial effects – increases in yields in hydroponic culture are not evident (see here and here). A look at the effect of a humic acid source on several different plant species can be found here.




Effect of humic acid, bacteria and lactate applications on tomato plants.

It is worth noting that humic acid applications are also not limited to the root zone. Since humic acids can enhance the absorption of some nutrients they can also be applied in foliar sprays. Experiments on strawberries ([here](#)) showed that an application of 1.5-3ppm of humic acids led to an increase in the quantitative and qualitative properties of the fruits. Combinations of humic acids with other biostimulants are also common. For example a combinations of lactate, humate and beneficial bacteria was tested on tomatoes ([here](#)) but the experiments showed that the effect could be stimulating or inhibiting depending on the particular conditions, even though most combinations were beneficial.

With the high variability between humic substance origins, application rates and effects it is very hard to say whether humic acid applications will definitely help your crops in terms of yields. For almost all humic acid sources it is probably warranted that micronutrient absorption will be somewhat augmented due to their ability to chelate these nutrients, but only if the nutrients are not efficiently chelated already. This sole ability might lead to crop improvements if deficiencies are present but improvements in yields will strongly depend on humic acid substance origin and particular properties. However humic acids do seem to lead to general product quality improvements and since negative effects are rare there seems to be no harm in carrying out field tests to determine if their use is worth it for your particular crop.

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# Using triacontanol to increase yields in hydroponics

Usually additives used in hydroponics need to be added in rather large quantities to obtain palpable results. Molecules like salicylic acid – which we have discussed before – need to be used in concentrations in the order of $10^{-4}$ to $10^{-2}$ M to obtain a significant effect. This means that you need to use quantities in the order of 20-150ppm of most additives in order to see a significant result. However there is a molecule called 1-triacontanol that can generate very significant results with only a fraction of that concentration. Today we will talk about this substance, what it does, how to use it and why it's such a desirable tool in your hydroponic additive arsenal. Many of the things I will talk about in this article are derived from this 2011 review on triacontanol (make sure you read that for a deeper insight into why this molecule works).

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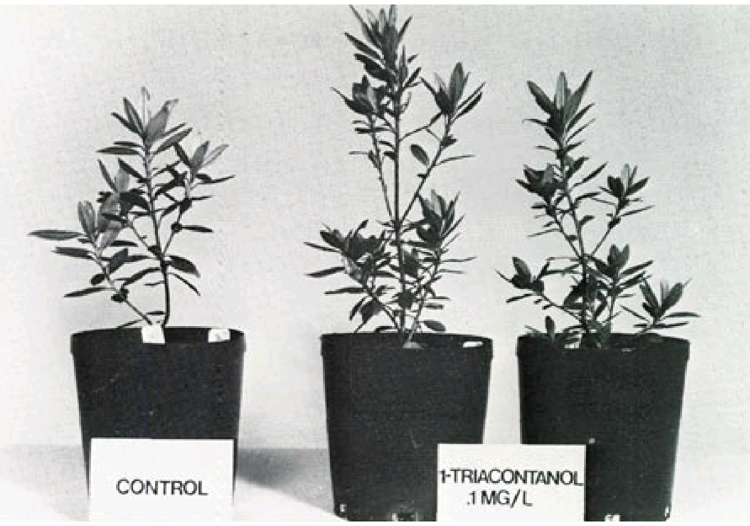


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Triacontanol is a very long fatty alcohol. Each molecule has 30 carbon atoms linked in a linear structure which makes this molecule extremely hydrophobic and hence very hard to dissolve in something like water. Using triacontanol therefore involves dissolving this molecule in something other than water –  for example Tween 20, chloroform, methanol – before adding water in order to prepare an emulsion for use in either root applications or foliar feeding. Most research using triacontanol has used foliar feeding as this is the easiest way to control the application of the molecule and also how it seems to have the largest effect.

The effects of this molecule are not short of miraculous. Triacontanol is usually applied in concentrations on the order of $10^{-7}$ to $10^{-9}$ M, which means it is used from around 0.01 to 1 ppm. This means that we use about 1000 times less triacontanol than other additives in order to obtain a meaningful result. The table below shows some of the effects that triacontanol has showed in peer reviewed studies, with plant height, weight and yields increasing across a variety of different species, from tomatoes to japanese mint. Papers on other plants besides those on the chart have also been published, for example triacontanol has showed to significantly increase yields in lettuce crops (here).  Some studies have also found that the effect of triacontanol can also be enhanced through the use of magnesium or in conjunction with other hormones ([here](here)).

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Table 1 Positive response of various plant species to foliar application of triacontanol.

| Name of plant | Botanical name | Family name | Growth attributes | Yield attributes | Biochemical attributes | Quality attributes | Reference citation |
|---|---|---|---|---|---|---|---|
| Opium poppy | *Papaver somniferum* L. | Papaveraceae | Plant height, dry weight and number of branches | Number of capsules, seed yield per plant, and crude opium yield per plant | Chl *a*, Chl *b*, and total content | Morphine content and morphine yield per plant | Khan et al. (2007) |
| Tomato | *Solanum lycopersicum* L. | Solanaceae | Height per plant, number of leaves and plant fresh and dry weights | Number of fruits per plant, weight per fruit and fruit yield per plant | Total chl and carotenoids content, leaf-N, -P, and -K contents | Fruit ascorbic acid and lycopene contents | Khan et al. (2009) |
| Hyacinth bean | *Lablab purpureus* L. | Fabaceae | Plant fresh and dry weights, leaf-area per plant, number and dry weights of nodules | Number of pods per plant, number of seeds per pod, 100-seed weight and seed-yield per plant | Photosynthetic rate ($P_N$), stomatal conductance (gs) and transpiration rate, total chl and carotenoid content, NR and CA activities, leaf-N, -P, -K, and -Ca content, nodule-N and leghemoglobin contents | Seed-protein content, total carbohydrate content, and tyrosinase activity | Naeem and Khan (2005), Naeem et al. (2009) |
| Artemisia | *Artemisia annua* L. | Asteraceae | Shoot and root lengths, plant fresh and dry weights | Artemisinin yield | $P_N$, gs and transpiration rate, total chl and carotenoid content, NR and CA activities, leaf-N, -P, and -K content | Essential oil content, artemisinin content | Aftab et al. (2010) |
| Coriander | *Coriandrum sativum* L. | Umbelliferae | Shoot and root lengths, plant fresh and dry weights | | Total chl and carotenoids content, NR and CA activities, leaf-N, -P, and -K content | Essential oil content | Idrees et al. (2010) |
| Coffee senna | *Senna occidentalis* L. | Fabaceae | Plant fresh and dry weights | Number of pods per plant, number of seeds per pod, 100-seed weight and seed yield per plant | $P_N$, gs and transpiration rate, total chl and carotenoid content, NR and CA activities, leaf-N, -P, -K, and -Ca content | Total anthraquinone and sennoside contents, and seed-protein content | Naeem et al. (2010) |
| Sweet basil | *Ocimum basilicum* L. | Labiatae | Shoot and root lengths, number of spikes per plant, total leaf area, plant fresh and dry weights | Essential oil yield | Chl *a*, Chl *b*, total Chl, and carotenoid contents, activities of NR and CA, leaf-N, -P, and -K contents | Leaf-protein and carbohydrate contents, essential oil content, linalool, methyl eugenol, and eugenol contents | Hashmi et al. (2011) |
| Japanese mint | *Mentha arvensis* L. | Lamiaceae | Plant height, leaf-area, leaf-yield, and plant fresh and dry weights | Herbage yield, essential oil yield | Total chl and carotenoid contents, activities of NR and CA, leaf-N, -P, and -K contents, total phenol | Essential oil content, menthol, L-menthone, isomenthone, and menthyl aceteate contents | Naeem et al. (2011) |

Abbreviation: Chl, Chlorophyll; NR, Nitrate reductase; CA, Carbonic anhydrase.



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With such an impressive array of effects and such a low expected toxicity – due to its very low solubility – it's definitely one of the best additives to use to get production gains in hydroponic crops. This also makes it one of the most commonly used substances in commercially available grow enhancers. Nonetheless since it's used in such a small quantity it's very easy for someone to buy a small amount of triacontanol and use it for years before running out. You can buy small amounts of triacontanol as a powder (there are several reputable sellers on ebay) and you can then prepare your own concentrated triacontanol solution in Tween 20 – not water – that will last you for ages. A liter of 2000ppm solution of triacontanol will last you for 1000-2000 liters of foliar spray. You cannot get more economical than that.

The optimum application rate and frequency for triacontanol varies across different species but if you want to take an initial guess use a foliar application of a 0.5 ppm solution every week. There is usually a sweet spot for concentration – after that you start to see a decrease in results compared to the highest point – so you want to start below a 1 ppm application rate. For some crops repeated applications might be unnecessary – with just one or two applications giving most of the effect through the entire crop cycle – while for others you do want to apply every week. How you initially dissolve the triacontanol to make your concentrated solution is also important with Tween 20 being the most ecologically friendly – although not the easiest – option.

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# Salicylic acid and its positive effect in hydroponics

When looking for ways to increase crop yields we usually want something that is safe for the environment, safe for us and able to give us a substantial bang for our buck. From the multitude of additives that have been researched during the past 30 years one simple organic molecule seems to fit all the requirements very well: salicylic acid. Today we are going to talk about why this additive is so interesting for use in hydroponic culture, the results it has shown in peer reviewed publications and how we can use it to increase our crop yields. For those of you interested in this molecule I would also recommend reading this 2010 review which contains a much more detailed look into the scientific literature surrounding salicylic acid research in higher plants.

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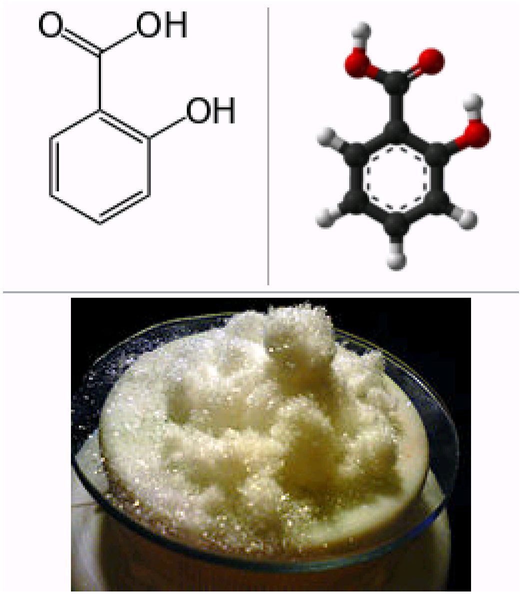


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Salicylic acid is a simple organic molecule with the structure showed above. We have known for a long time that plants produce it and we knew almost right from the start that it played a key role in plants’ response to diseases and stress (see here for some early insights from Tobacco cultivation). Salicylic acid is used as a signaling molecule in plants (a.k.a hormone), moving from stressed organs to non-stressed ones as the plant is attacked. However its role is much more complex, having functions related with chloroplast creation, inhibition of fruit ripening and many other important processes.

After learning that this was an interesting molecule it wasn’t long before people started studying whether exogenous applications provided any benefit. We have learned that it enhances dry mass and leaf area in corn and soybean (here), that it can enhance germination in wheat (here), the oil content in basil (here), the carbohydrate content in maize, etc. There are also several studies pointing to improvement in root development – even from foliar applications (here) – suggesting that this hormone is able to increase plant productivity through several different mechanisms. The incidence of diseases can also be reduced dramatically by salicylic acid applications (here).

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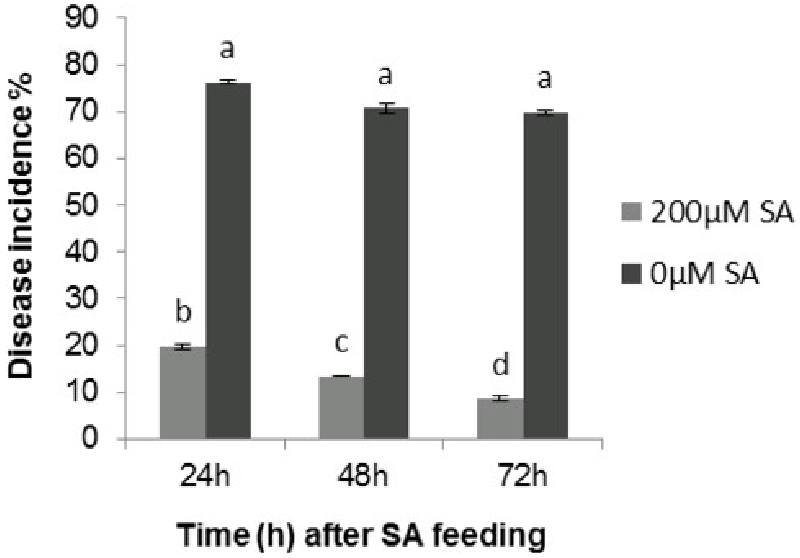


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We also know this molecule has important effects on the flowering process. It can induce earlier flowering in plants and can often cause larger fruit settings in some plants (like papaya (here)). Most importantly foliar spraying of tomato and cucumber plants with salicylic acid has showed important increases in yields (here). It is therefore clear that exogenous applications of salicylic acid can have many important benefits in crop production and this is therefore an important candidate to consider for enhancing crop production.

But how do we apply it? Most commonly this molecule is applied in foliar feeding regimes, although in some cases it is also applied directly in hydroponic solutions. Most commonly concentrations in the order of $10^{-5}$ -$10^{-4}$ M are used since it has been showed across a few studies that negative effects start to show up when the concentration level reaches 1mM.

This means that regular doses will be around 1-100 ppm with the lowest spectrum of dosage being preferred if the effect on the particular plant is unknown. The solubility of salicylic acid is 2.48g/L at 25°C so concentrated solutions of up to around 20-30x can be prepared without issues to make it easier to apply on plants. The preparation of more concentrated solutions requires some tricks but it certainly can be done.

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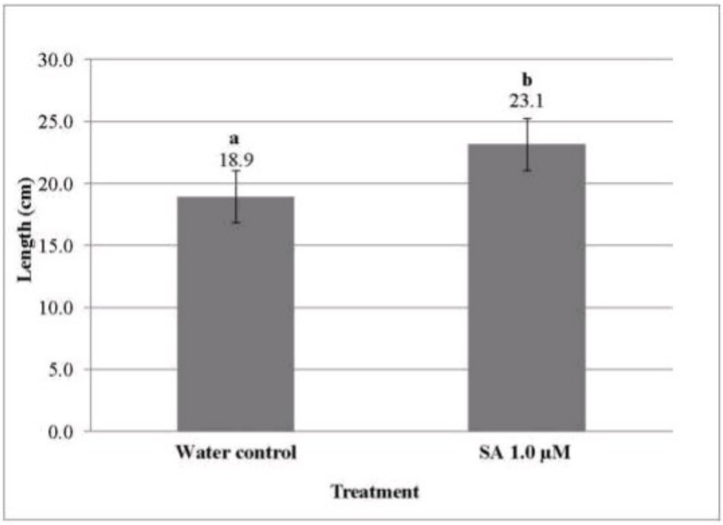


Figure 2. Effect of the spray application of 1µM of Salicylic Acid on the length of *Brosimum alicastrum* seedling stems. The average of 6 repetitions ± standard error is shown. Similar letters indicate no significant difference (Fisher, $p \leq 0.05$).

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Salicylic acid also has the advantage of being a very safe molecule so it can be applied without a lot of worry in order to experiment with its effects. For testing on new plants foliar applications of 20-30ppm would be most common, with applications usually carried out once every 5-10 days. The frequency of application as well as the best concentration to use will of course depend on the particular plant you're growing. There are also several other molecules that can be used with salicylic acid to enhance its effect on some plants, but this will be the focus of a future post.

Finally it is also worth noting that ***salicylic acid is not aspirin*** (aspirin is acetylsalicylic acid, a related yet different molecule) so if you want to experiment with this additive you should buy salicylic acid instead of just “dumping some aspirin” into your foliar or hydroponic nutrient solution.

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# Using titanium to increase crop yields

There are many additives that can be used to enhance the yield of flowering crops. Some have been covered in this blog – like silicon – while others haven’t been mentioned here. Today we are going to talk about a rarely discussed additive that is infrequently used in plant culture these days: Titanium. I want to talk about this additive in light of a literature review that came up recently (April 2017) about the use of Titanium in crop production. The magazine where this review came from (Frontiers in Plant Science) is a magazine that often has good content in the field of innovative crop enhancing techniques.

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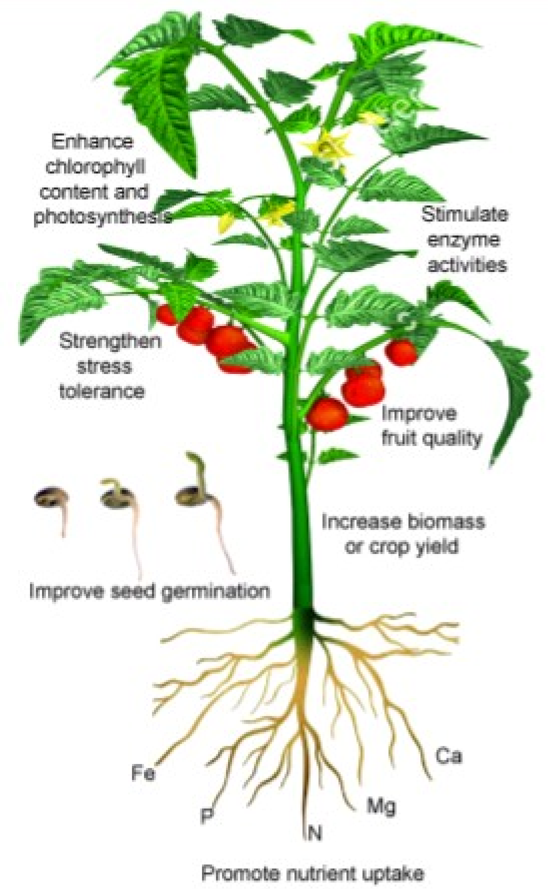


**FIGURE 1 | A schematic illustration of Ti effects on crop performance.** Ti applied via roots or leaves at appropriately low concentrations has been shown to promote seed germination, enhance root uptake of other nutrient elements, stimulate the activity of some enzymes, increase chlorophyll biosynthesis and photosynthesis, strengthen stress tolerance, and improve crop quality and yield.

Titanium use in plant culture is not new. From the early 1980s people started to experiment with titanium as techniques were developed in order to produce titanium chelates that could be used in foliar applications. Basically all reports of yield increases – that show wonderful increases up to even 95.3% in yields – come from a paper on the biological importance of titanium by Dr. István Pais in 1983 and then another publication in 1991 by the same person (here). Other authors have also showed increased yields (here and here) although in some cases in conjunction with other additives (like Si) with results often much less dramatic than the initial 1983 papers. Titanium nanoparticles have also been tested and their effect has mostly been negative with decreases in plant growth and often DNA damage. For this reason when using titanium you want to go with a soluble chelate and not nanoparticle sources.

Creating aqueous stable Ti is not a cake walk. There is currently only one product that carries water soluble Ti (called Tytanit) and as far as I can tell no other commercial products for the application of Ti exist at this moment. This tytanit product is most probably titanium ascorbate – the most popular chelate used – but other organic chelates, like Ti citrate, might be usable as well. Preparing Ti ascorbate is not so easy to get as well – you cannot just buy it on ebay/alibaba as it's not stable as a solid – so you need to prepare it from scratch. Titanium chemistry in solution is sadly very complicated.

However there is probably a route to the easy preparation of such complexes using a simple method involving titanium dioxide and ascorbic acid. We know from dissolution studies of titanium dioxide that it can be dissolved significantly by ascorbic acid but the final concentration of these solutions is not very high with a final concentration of around 0.025M of Ti possible in solution using this method, with a surrounding concentration of 0.15M of ascorbic acid. More acid does not help dissolve more titanium dioxide as this seems to be the solubility limit of the titanium complex. This gives you around 1.2g/L of Ti which you need to dissolve 500-1000x to arrive at the recommended application rate of 1-2 ppm. This will give a final ascorbic acid concentration of 26ppm which is acceptable as an additive as well.

Obviously there are some further formulation steps necessary to get the above to work correctly but this outlines the basics to develop a concentrated titanium ascorbate product that can be used for the creation of a Titanium supplement. Industrally this can be achieved much more efficiently with the use of titanyl sulfate which is a readily soluble and easy to get industrially – but hard to get for your home – form of titanium. You can see this patent for examples of how a fertilizer using titanyl sulfate can be prepared.

Evidence about titanium – applied as titanium ascorbate in a foliar spray – being positive for crops is significant. Various positive effects have been shown across a significant variety of plants across several different plant types – tomatoes, beans, peppers – by different authors. The effect on yields is not so clear – probably in reality not as large as shown in the original studies, but probably significant enough to warrant further studying. The development of low-cost processes for the manufacturing of titanium fertilizers will further enhance their use and increase our knowledge about their true capabilities. More studies with ascorbic/ascorbate controls will also show us clear evidence of whether we are seeing effects related with the ascorbate or the actual Ti chelate.