Chapter 7

Coumarin-Based Heteroaromatics as Plant Growth Regulators

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Additional information is available at the end of the chapter

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Abstract

Heterocyclic compounds are the largest and most versatile class of organic compounds. Plants produce valuable heterocyclic substances called phytohormones to carry out the growth process. They control the growth or other physiological functions. Both hormones and vitamins are generally referred as plant growth regulators. Aromatic compounds having lactone ring are called coumarin. Coumarin is one of the most important natural substances in plants and is referred to as anti-auxins, as these compounds are considered to play an essential role in plants growth as well as defense.

Keywords: coumarin, inhibitors, phosphorous, heteroarylacetic acids, lactones, glycosides

1. Introduction

Almost all the plants contain some substances which control their growth process and development. These plant growth regulators facilitate the growth processes in a better way to meet the requirements of food supply in general. However, with the increase of food demand and invention of tissue culture in plant science, it was obvious that more and more growth regulators should be designed. A vast majority of growth regulators have been synthesized and tested for their effects on plant growth. In the proceeding paragraphs, the effects of coumarins and derived compounds have been described.

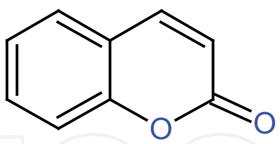
The plant growth regulating activity can be defined as bustle of an active ingredient on the physiological processes of the plant hormones directly responsible for growth reliant on the method to use, development stage on which preferably used and concentration of the active substance used in vitro. The compositions can be prepared by mixing of the active ingredients with some suitable transporters, that is, liquid solvents or solid carriers and optionally surfactants or emulsifiers.

1.1. Coumarins

Coumarins (**1**) are heteroaryl compounds containing lactone ring and are of great medicinal importance. These compounds are supposed to intricate in the various actions comprising plant growth hormones and growth regulators in order to rheostat the respiration, photosynthesis, as well as protection against infection. They have an imperative role in plant biochemistry and physiology, stand-in as antioxidants, enzyme inhibitors and precursors of toxic substance [1].

Coumarins are commonly found in almost every plant family [2]. Plants probably use them as growth inhibitors (anti-auxins) [3, 4] as well as defense compounds mean play an important role in plants' defense system against pests and diseases, including root parasitic nematodes [5]. In some plant families, such as Leguminosae (bean family), Rutaceae (citrus family) [6] and Umbelliferae (Apiaceae) (parsley-fennel family), coumarins are produced and used in larger quantities [7]. The effect of coumarins, umbelliferone (**2**) and xanthotoxins (**3**) is once compared in cucumber, maize and garden pea, and the results are simply thought provoking. Umbelliferone retards root growth less strongly than coumarin and xanthotoxins.

All plants show dissimilar response depending on their Species, size and may be associated with different effects on the process of cellular respiration and enormous changes happening in mitochondria [8].



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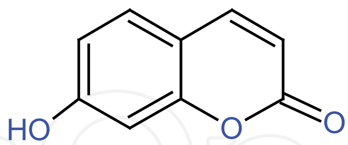

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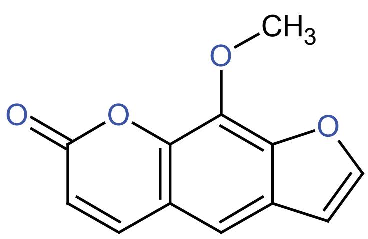

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1.1.1. Effects on seed germination

Abscisic acid (ABA) is a plant growth inhibitor, and indole butyric acid (IBA) is a plant growth activator. At 100 ppm, both ABA and IBA suppress the germination of wheat and sorghum seeds, although this effect is more pronounced in ABA rather than IBA. At 10 ppm, germination percentage is 60–90%, whereas at 1 ppm germination in IBA is >90% in both wheat and sorghum but ABA shows 75–90% germination.

The behavior of coumarin derivatives toward the germination is inhibitory [9, 10]. Different coumarin derivatives show 70–95% germination at 10 and 1 ppm in comparison. 7-Hydroxy-coumarin (**4**) and 7-hydroxy-4-methylcoumarin (**5**) almost completely inhibit the germination in wheat and sorghum seeds at 100 ppm, and percentage germination used to be <50%. All other compounds show 70–95% germination at 10 and 1ppm in comparison to control [11] (**Figure 1**).

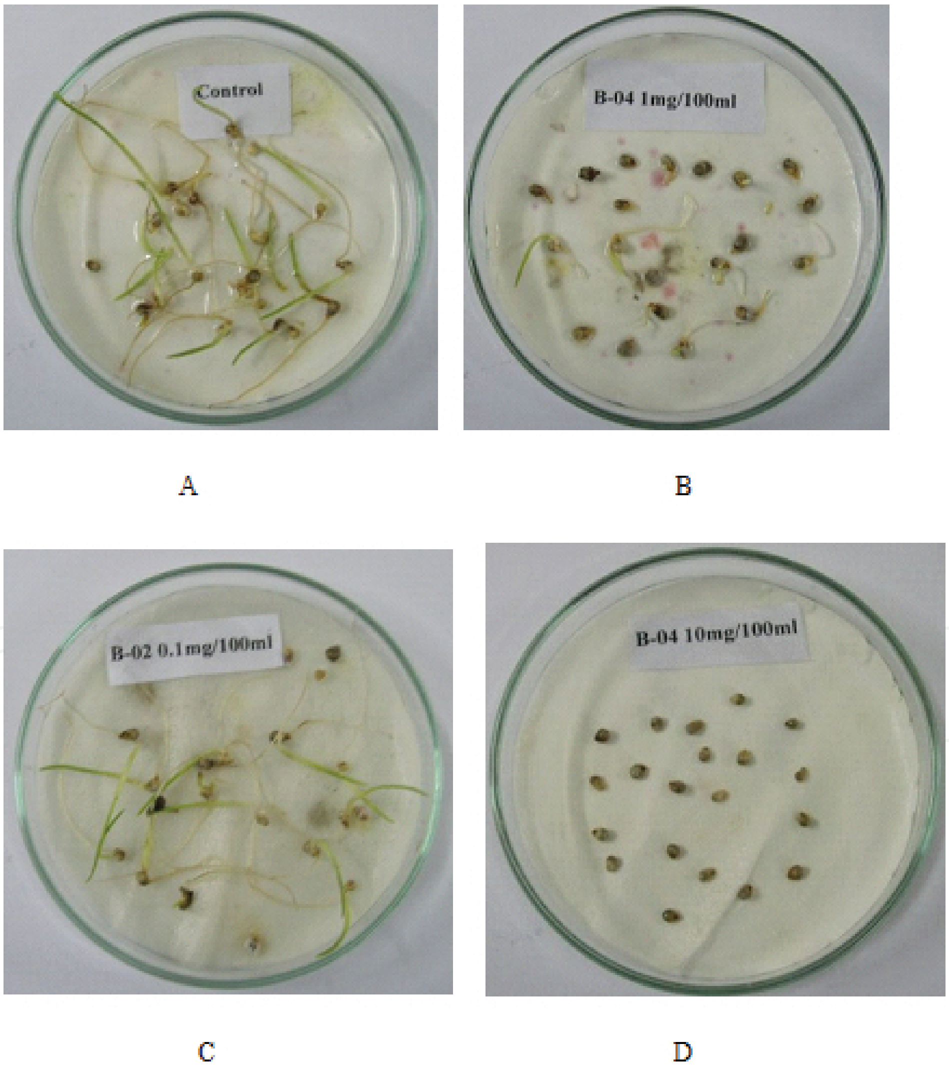
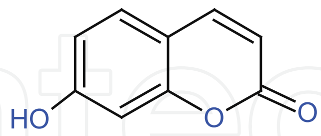


Figure 1. Effect of coumarin derivatives (B = 4, C = 1, D = 5) in comparison with control (A).

Williams et al. [12] have described that hydration and dehydration of radish (*Raphanus sativus* L.) seeds in the presence of coumarin delay the germination and reduce the seedling growth.



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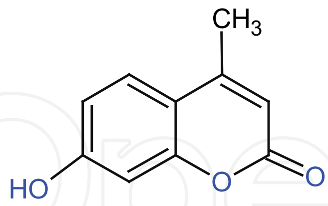

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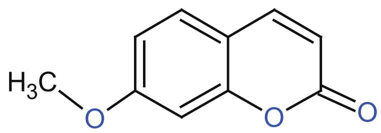
Synthesis of coumarin is of much valuable for the plant scientists as coumarin is supposed to play an important role in the growth regulation.

1.1.2. Effects on shoot length

Reduced shoot growth is discerned with 7-methoxycoumarin (**6**), 7-methylcoumarin (**7**) and 7-hydroxy-4-methylcoumarin (**5**) in comparison with that of untreated or control. At 1 ppm concentration, reduced shoot length is perceived by coumarin (**5**) and 7-methylcoumarin (**7**) and 7-hydroxycoumarin (**12**) at 1 and 10 ppm; 7-methoxycoumarin (**6**) and 7-hydroxy-4-methylcoumarin (**5**) at 100 ppm result in 60–85% reduction in shoot growth [13] (**Figure 2**).



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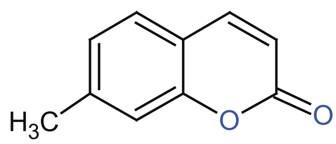

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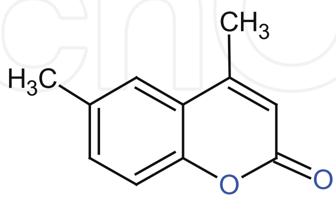

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Shoot length of sorghum seedlings on the fifth day of germination was completely inhibited by 7-methylcoumarin (**7**), and at 10 ppm, 4,6-dimethylcoumarin (**8**) is the most effective inhibitor of shoot length while other compounds show 20–40% inhibition in shoot growth.

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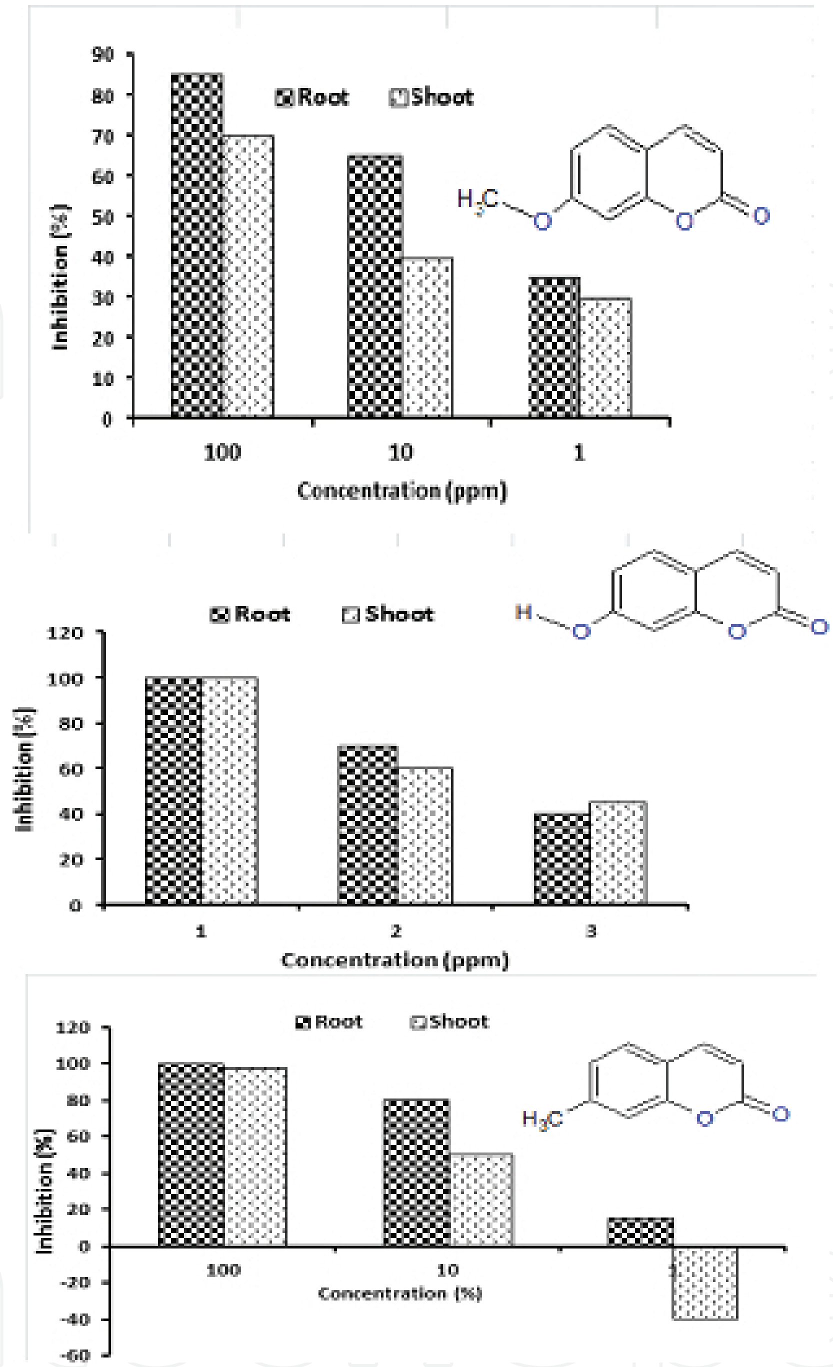


Figure 2. Percentage inhibition of root and shoot growth by 7-methoxycoumarin, 7-hydroxycoumarin and 7-methyl-coumarin at various concentrations. Positive bars are growth inhibitors, whereas negative are growth activators.

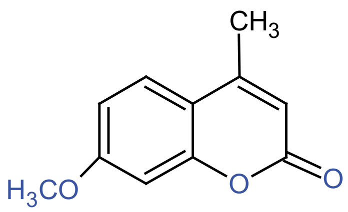
1.1.3. Effects on root length

Coumarin inhibits the radicle, seminal and nodal root lengths by 50% in solutions of 6, 1 and 0.25 mM [14]. Coumarin decreases the number of lateral roots. The branching density is usually affected more in the seminal than in the radicle roots. The order of sensitivity to coumarin observed to be as: nodal > seminal > radicle roots.

Few reports have been published on the relation between the structure and activity of coumarin and its derivatives. Goodwin and Taves [15] have shown that coumarin is the most powerful root growth inhibitor but some of its derivatives are almost as active as coumarin itself, namely 7,8-dihydroxy coumarin, 7,8-dihydroxy-4-methylcoumarin, 8-methyl coumarin and coumar-in-3-carboxylic acid. However, 3-methyl substitution greatly diminishes the inhibitory action on root growth.

Root growth inhibition is about 70-90% by 7-methoxycoumarin at different concentration (1, 10, 100 ppm) and 4,6-dimethylcoumarin and 7-hydroxy-4-methylcoumarin possess the least root growth inhibition activity at 100 ppm. At 10 ppm, 70–90% root length is inhibited by 7-methoxycoumarin and 7-methylcoumarin (**Figure 2**). 6-Hydroxycoumarin, 6-methylcoumarin and 4,6-dimethylcoumarin demonstrate no effect on root length and are seen closer to the control value.

7-Methoxycoumarin is the most effective inhibitors of root growth at 100 ppm compared with that of control. 7-Hydroxy-4-methylcoumarin shows accelerated root growth at 100 ppm. While at 10 ppm, 7-methoxycoumarin, 7-hydroxycoumarin and 7-methylcoumarin inhibit root growth but 6-hydroxycoumarin, 4,6-dimethylcoumarin and 7-methoxy-4-methylcoumarin (**9**) show more growth of seedlings than the control. At 1 ppm, 6-methylcoumarin (**10**), 7-methoxy-4-methylcoumarin and 6-hydroxy-4-methylcoumarin (**11**) are active than that of control.



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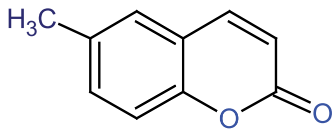

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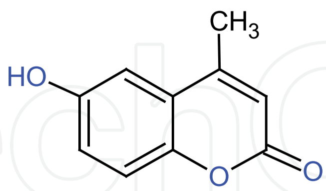

11

1.2. Phosphorous-containing coumarins:

The plant growth regulating activity of phosphorus derivatives of coumarin is of discernible in comparison to parent coumarin as it inhibits the stem growth of pea plants. The elongation of wheat coleoptile segments is also affected by coumarin derivatives. Almost all compounds at concentrations 0.1 and 0.01mM causes stimulation of the pea plant root fresh weight and the stimulating effect reaches 40–49% in ethyl-7-(diethylamino)-2-ethoxy-2*H*-1,2-benzoxa-

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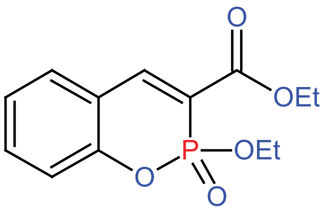
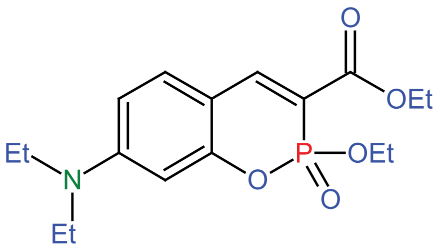
phosphinine-3-carboxylate-2-oxide (compounds 1**2**) and 2-ethoxy-2*H*-1,2-benzoxaphosphinine-3-carboxylate-2-oxide (compounds **13**) while reaches 62% for chloro derivatives (compound **14**).

The influence of the compounds on the growth of cucumber and wheat seedlings is apparent that all phosphorous-containing derivatives inhibit the cucumber root and hypocotyl growth with more than 95% at 1mM, but the effect on wheat roots is weaker and growth reduction is about 80%. Inhibitory activity declines with the decrease of the concentration applied. At concentrations 1 and 0.1 mM, all the compounds exceed the effect of the standard coumarin. Derivatives containing phosphorus in the ring possess strong inhibitory activity at lower concentrations [16].





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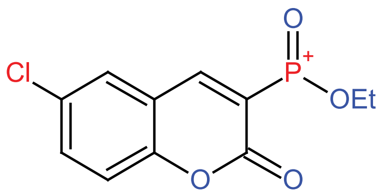

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1.3. Coumaryl-β-D-glucopyranuronic acid

The effect of coumarin on germination and growth has been studied widely, but very little is known about the natural derivatives of coumarin which occur in plants. It is assumed that in plants they occur in the form of glycosides, and are frequently physiologically inactive even when present in high concentrations [17].

This principle illustrated in an experiment on the effect of various concentrations of o-coumaryl-β-D-glucopyranuronic (CouGN **15**) acid on shoot formation. When o-coumaryl-β-D-glucopyranuronic acid is cleaved by the action of β-glucuronidase (**16**), o-coumaric acid (**17**) is released. o-Coumaric acid is spontaneously converted to coumarin (**1**). This involves the elimination of an acid group, and thereby an increase of pH to a level at which the activity of the native plant β-glucuronidase is presumed to be reduced (**Figure 3**). o-Coumaryl-β-D-glucopyranuronic acid in the growth medium inhibits shoot regeneration induced by BA_3GN sodium salt but not by BA. Best results are observed by using an o-coumaryl-β-D-glucopyranuronic acid at concentration about 3-4 mM. Several mechanisms are involved in the reduction of shoot formation induced by BA3GN, including an increased pH due to the release of o-coumaric acid, leading to a lower frequency of hydrolysis of BA_3GN and a reduced transport of BA3GN into the cells. It is believed that an increased pH is likely to have been at least partially responsible. It indicates that the selectivity of the positive selection system may be improved by using the introduced β-glucuronidase gene to establish a self-regulating mechanism which can significantly reduce the effect of any background enzyme [18].

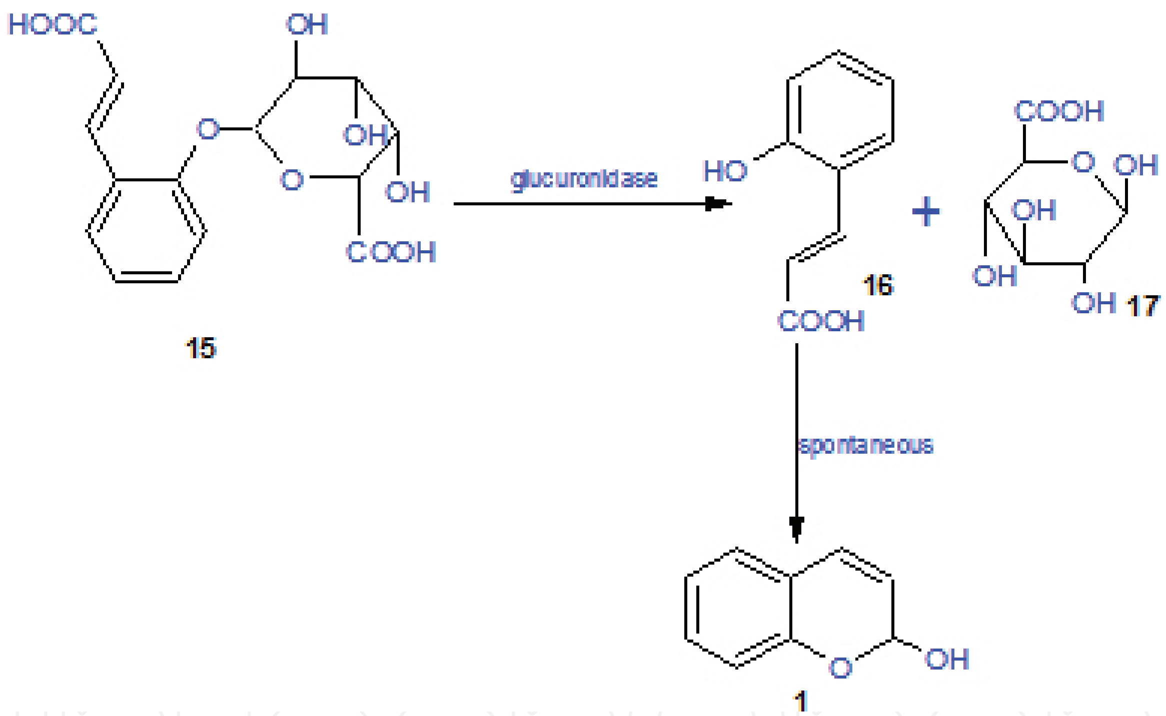
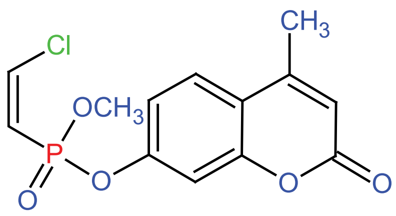


Figure 3. Mechanism of o-coumaryl-β-D-glucopyramuronic (CouGN 15) acid cleavage to coumarins in the plant cell.

1.4. 2-Chloro-ethyl-phosphonic acid-O-methyl-O-(4~methyl-coumarin-7-yl)-ester

In biological tests the change of the height of plants can be prominent feature to observe a noticeable change when used plant growth regulators. When compared with the untreated or control 2-ch1oro-ethyl-phosphonic acid-O-methyl-O-(4-methylcoumarin-7-yl)-ester (**18**) is supposed to reduce the growth of sunflower at different tested doses. The plant growth is intensively inhibited by 2-chloro-ethyl-phosphonic acid, whereas 7-hydroxy-4-methylcoumarin does not affect the growth of sunflower and double of the same compound slightly inhibited its growth [19].



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A mixture of 2-chloro-ethyl-phosphonic acid and 7-hydroxy-4-methyl, that is 2-chloro-ethyl-phosphonic acid-O-methyl-(4′-methyl-coumarin-7′-yl)-ester, significantly increases the growth of the tomato compared to the untreated or control. It can be seen further that the growth stimulating activity is more intensive than that of 7-hydroxy-4-methyl-coumarin and at higher doses the growth inhibiting activity is lower than that of 2-chloro-ethyl-phosphonic acid.

When using 2-chloro-ethyl-phosphonic acid-O-methyl-O-(4′-methyl-coumarin-7′-yl)-ester in soybeans, all doses reduced the height of soybeans differently from the results observed for tomato. The extent of the reduction of the height is lower than the values measured for 2-chloro-ethyl-phosphonic acid with similar rates. 7-Hydroxy-4-methyl-coumarin stimulated at lower concentration whereas four times of the same compound inhibited the growth. The depressant activity of 2-chloro-ethyl-phosphonic acid is moderated by the 7-hydroxy-4-methyl-coumarin at various concentrations [20].

2. Coumarinacetic acids

Coumarins are naturally occurring compounds. A lot of coumarins have been identified from natural sources, especially green plants. The pharmacological and biochemical properties and therapeutic applications of coumarins depend upon the pattern of substitution [21]. Coumarins have attracted intense interest in recent years because of their diverse pharmacological activities [22–24]. Among coumarin derivatives, Coumarin acetic acids are scarcely reported in the literature.

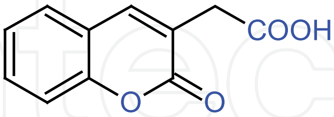
2.1. Effects of coumarin-3-acetic acids on seed growth

Coumarin-3-acetic acid and its different derivatives were synthesized and tested for their seed germination and plant growth regulating activity. Data show that different derivatives had different effects on seed germination and early growth of plants.

2.2. Effects on seed germination

Coumarin-3-acetic acid (**19**) and 6-aminocoumarin-3-acetic acid (**20**) exhibit 80–95% germination at 100 ppm. Wheat and sorghum seeds possess germination inhibitory activity by the certain coumarin-3-acetic acid derivatives. 5-Hydroxy-4,7-dimethylcoumarin-3-acetic acid

(**21**), 4,6-dimethylcoumarin-3-acetic acid (**22**), 4,7-dimethylcoumarin-3-acetic acid (**23**), 7-methoxy-4-methylcoumarin-3-acetic acid (**24**) and 7-chloro-4-methylcoumarin-3-acetic acid (**25**) at 100 ppm solution show no germination That may be due to reduced water intake that results no imbibition hence seems to possess hindrance in germination [25].



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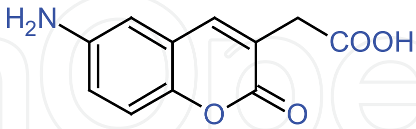

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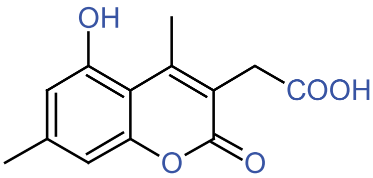

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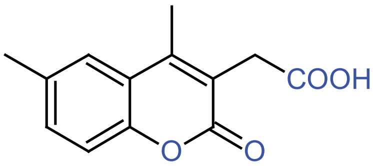

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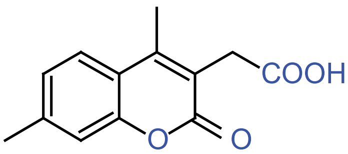

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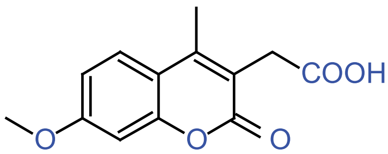

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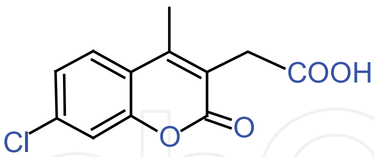

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2.3. Effects on shoot length

Shoot length (mm) of developing seedlings measured on the fifth day of germination shows inhibition trend. At 100 ppm, 6-nitrocoumarin-3-acetic acid (**26**) completely inhibits wheat seed germination and hence shoot or root growth is usually not observed. Reduced shoot growth is perceived with coumarin-3-acetic acid, and 6-aminocoumarin-3-acetic acid exhibits shoot growth comparable with that of control. At 10 ppm, reduced shoot growth is experiential in 7-hydroxy-4-methylcoumarin-3-acetic acid (**27**) and 7-chloro-4-methylcoumarin-3-acetic acid (**Figure 4**).

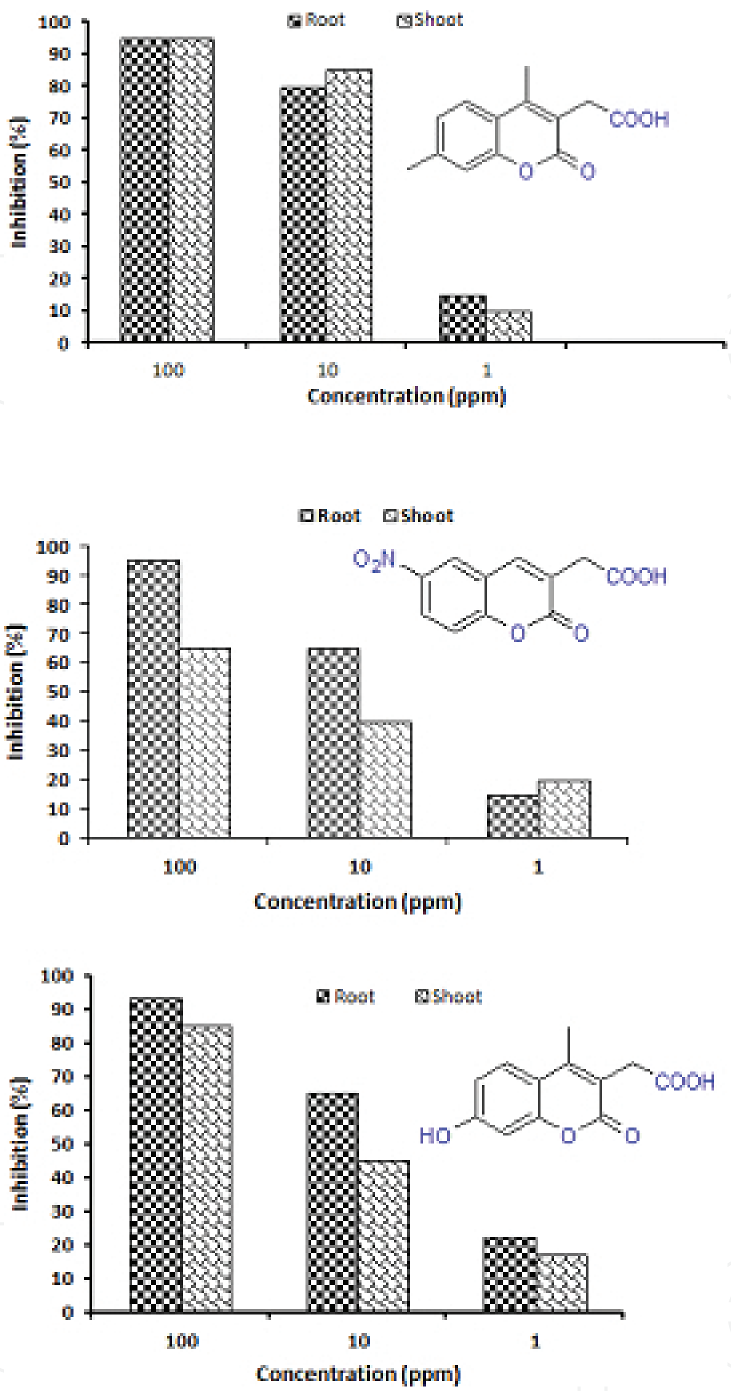
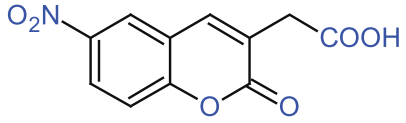


Figure 4. Percentage inhibition of root and shoot growth of seed by action of 4, 6-dimethylcoumarin-3-actic acid **(23)**, 6-nitrocoumarin-3-acetic acid **(26)** and 6-nitrocoumarin-3-acetic acid **(26)** and 6-hydroxy-4-methylcoumarin-3-acetic acid **(27)**.

Shoot lengths of sorghum seedlings on the fifth day of germination are absolutely repressed by **25** and severely inhibited by **24** at 100 ppm. At 10 ppm, 7-hydroxy-4-methylcoumarin-3-acetic acid is the most effective inhibitor of shoot length while other compounds show 20–40% inhibition in shoot growth [25].



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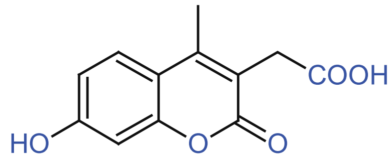

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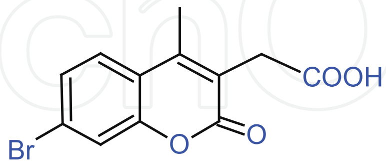

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2.4. Effects on root length

Changes in root length (mm) monitored on the fifth day of wheat seed germination show inhibition activity. 6-Nitrocoumarin-3-acetic acid completely subdued root growth at 100 ppm. Root growth is impeded between 70 and 90% by coumarin-3-acetic acid and 6-aminocoumarin-3-acetic acid at these concentrations, and 7-bromo-4-methylcoumarin-3-acetic acid (**28**) has the least effect on root growth. At 10 ppm, 70–90% root length remains inhibited by 6-nitrocoumarin-3-acetic acid, 7-hydroxy-4-methylcoumarin-3-acetic acid, 7-methoxy-4-methylcoumarin-3-acetic acid and 7-chloro-4-methylcoumarin-3-acetic acid (**Figure 4**).

Changes in root length (mm) in sorghum seedlings monitored on the fifth day of germination stay constrained by coumarin-3-acetic acid, 6-nitrocoumarin-3-acetic acid and 6-aminocoumarin-3-acetic acid at 100 ppm compared with the control. However, root growth induction is by 20-40% by 7-bromo-4-methylcoumarin-3-acetic acid (**Figure 5**) at 10 ppm [25].

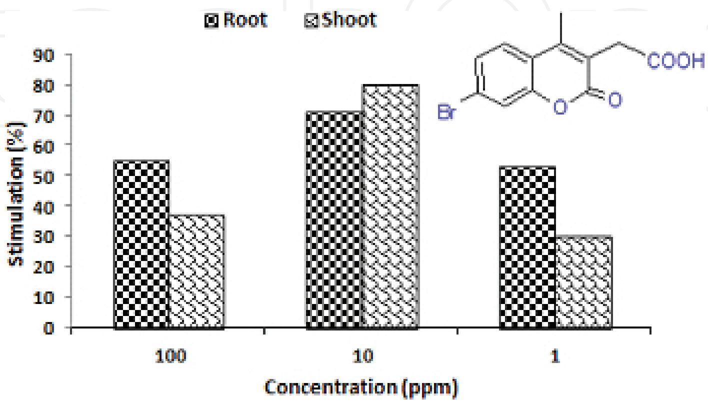

Figure 5. Percentage stimulation of root and shoot growth of seed by the action of 7-bromo-4-methylcoumarin-3-acetic acid **(28)**.

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3. Percent inhibition/stimulation of growth

Percentage inhibition/stimulation of coumarin-3-acetic acids clarifies the difference of its effect in comparison to control. Most of the compounds possess root growth but less inhibitory effects or somewhat stimulation with respect to the control. In wheat, compounds 6-aminocoumarin-3-acetic acid, 5-hydroxy-4,7-dimethylcoumarin-3-acetic acid, 4,6-dimethylcoumarin-3-acetic acid, 4,7-dimethylcoumarin-3-acetic acid, 7-methoxy-4-methylcoumarin-3-acetic acid and 7-chloro-4-methylcoumarin-3-acetic acid are growth inhibitors. 6-Nitrocoumarin-3-acetic acid, 7-hydroxy-4-methylcoumarin-3-acetic acid, 7-methoxy-4-methylcoumarin-3-acetic acid and 7-chloro-4-methylcoumarin-3-acetic acid at lower concentration (10 and 1 ppm) show very good inhibition of both shoot and root growth.

In sorghum, coumarin-3-acetic acid, 6-nitrocoumarin-3-acetic acid, 6-aminocoumarin-3-acetic acid, 7-hydroxy-4-methylcoumarin-3-acetic acid, 4,7-dimethylcoumarin-3-acetic acid and 7-chloro-4-methylcoumarin-3-acetic acid are clearly substantiated to be growth inhibitors of root, whereas 7-bromo-4-methylcoumarin-3-acetic acid ascertain to be growth stimulator of both root and shoot contrary to its effect in coumarin[25].

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