Production of Thiophene from Tagetes patula

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Abstract

Thiophene chemistry proved to be very interesting for several industrial branches. The thiophene moiety was found to have larvicidal action on mosquito larvae. Thiophene is present in the leaves of T. patula. It was extracted from leaves by solvent extraction and also by tissue culture. The results were analysed by Spectrophotometer, Fourier Transform Infrared Spectroscopy and larvicidal action.

Keywords: Thiophene; Leaves; Tissue culture

Introduction

Plant derived products are gaining importance in the field of biotechnology [1]. Apart from various advantages compared to synthetic products they provide low cost drugs and vaccines. But commercializing of these products is minimal due to lack of good manufacturing practices to field grown plants.

In the past years the plant kingdom has been of great interest as a source of potential of insecticidal products. In addition natural insecticides can provide core structures from which new insecticidal agents can be synthesized. Although the natural products are very effective against many insects, their synthetic preparation is not that efficacious. Plant insecticides still represent only a small fraction of the insecticidal material used very year. However as a consequence of stricter environmental legislation, increased resistance of pests to synthetic pesticides, growing residue awareness among the consumers, mounting industrial research and the development cost of chemical insecticides, there has been shift towards the interest for the use of natural insecticides. Thiophene is a photosensitive natural insecticide which is really effective and has many applications both in industrial and in pharmaceutical products.

The leaf tissue of T. patula was used to initiate the callus growth and the medium used was the Murashige and Skoog’s with 2-4 dichlorophenoxyacetic acid and kinetin [2]. Maximum thiophene content and maximum biomass accumulation was recorded.

Materials Required

Marigold stem and leaf branches, Distilled water, Methanol, Hexane, 20.20 blade, solvent extractor, Bavistin, 0.1% HgCl₂, Hitastin, Glass Jar (250 ml), Soxhlet apparatus, MS medium.

Procedure

Media preparation

MS Medium (Stock): Medium prepared in varying concentration of hormones. Murashige and Skoog’s medium was prepared using Macronutrients Stock 1 (Table 1), Macronutrients Stock 2 (Table 2), Macronutrients Stock 3 (Table 3), Macronutrients Stock 4 (Table 4) and Vitamins (Table 5). The medium for tissue culture contained macronutrients, micronutrients and vitamins. The components of MS medium (Stock 1) are ammonium nitrate, potassium nitrate, potassium dihydrogen phosphate and magnesium sulphate. Specific amounts of each were measured and the solution was made up to 1 L using distilled water. Stock 2 contained calcium chloride. Stock 3 contained disodium salt of EDTA and ferrous sulphate hydrate. Stock 4 contained Magnesium sulphate tetrahydrate, zinc sulphate, boric acid, Potassium iodide, disodium molybdate, cuprous sulphate pentahydrate and cobalt chloride. Stock 5 contained glycine vitamins nicotinic acid, pyridoxine HCl and thiamine.

Media preparation: Stock solution from 1-5 were mixed in specific amounts. 20 ml of stock I, 20 ml of stock II, 10 ml of stock III, 10 ml of stock IV, 8 ml of stock V were used to prepare the medium with the addition of distilled water to make it 1 liter.

Table 1: Macronutrients (Stock : I).

| Component            | Weight (g/l) |
|----------------------|--------------|
| Ammonium nitrate     | 82.5         |
| Potassium nitrate    | 95           |
| Potassium dihydrogen phosphate | 8.5          |
| Magnesium sulphate   | 18.5         |

Murashige and Skoog’s MS medium. Composition (ml/l).

Table 2: Macronutrients (Stock: II).

| Component         | Weight (g/l) |
|-------------------|--------------|
| Calcium chloride  | 22           |

Table 3: Macronutrients (stock: III 20ml).

| Component                          | Weight (g/l) |
|------------------------------------|--------------|
| Disodium salt of EDTA              | 3.7          |
| Ferrous sulphate hydrate           | 2.8          |

Table 4: Micronutrients (stock: IV 10ml).

| Component                          | Weight (g/l) |
|------------------------------------|--------------|
| Magnesium sulphate tetra hydrate   | 2.23         |
| Zinc sulphate                      | 0.860        |
| Boric acid                         | 0.620        |
| Potassium iodide                   | 0.083        |
| Disodium molybdate                 | 0.02         |
| Cuprous sulphate pentahydrate      | 0.002        |
| Cobalt chloride                    | 0.002        |

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stock IV, 10 ml of stock V added 1% myo-inositol, 3% sucrose and different concentration of hormones: kinetin and 2-4-D (Table 6) were added with pH maintained at 5.8. Four medias of varying hormone concentration were prepared for callus culture.

**Tissue Culture**

**Sterilization**

The shoots of the Marigold plant were sterilized using disinfectant Bavistin. Shoots were cut at 2 internodes interval. Branches were placed in separate glass jars of 250 ml capacity. Used autoclaved sterile water and kept swirling continuously to remove any remaining dirt. Then drain the water and fill the jar till half with mercuric chloride (0.1%) and water and keep swirling for 5 minutes. Again drain out the liquid mixture and add sterile water and swirled for 5 minutes twice.

**Initiation**

Switch on the UV for 20 minutes before entering the lab. Surface sterilized the laminar hood (top and table) with ethanol. Sterilized all equipment to be used in the steripot (SHIMAS). The leaves were cut a bit away from the node [3] and put the leaf in various bottles containing full MS medium with same hormonal composition.

**Subculture**

The calluses obtained in different bottles were collected. Glass jars of 250 ml capacity were filled with about 100 ml of MS medium of varying hormonal concentration. The callus were then inserted a bit into different test media (1,2,3 and 4) with mouth facing the HEPA filter to avoid contamination. This procedure was repeated for two weeks [4] (Figure 1) shows the callus grown in medium, (Figures 2-4) show the callus growth observed on the fourth, eighth and twelfth day.

**Solvent extraction**

Fresh leaves of Marigold were taken and sterilized with distilled water. 500 ml conical flask was filled with about 150 ml of hexane. The soxhlet apparatus was set and water connection to top and bottom of the condenser was given. Temperature was set at about 65°C. Extraction was continued for about 6 hours. Light pale yellow color extract was obtained in the conical flask (Figure 5). Stored the extract in dark bottles as thiophene is photoactive. After the initial extract was concentrated, it was again redissolved in hexane. The above procedure was repeated for callus of *T. patula*. Fourier Transform Infrared Analysis was done analysis for detection of thiophene.

**Results and Discussion**

The callus growth was observed in Media 1, 2, 3 and 4 (Figures 6-9). The figures show the size of the callus grown on four Medias. It was observed that the callus biomass was the highest in the media 4 containing 0.4 mg kinetin and 0.075 mg 2-4 D.

**Spectrophotometric analysis (Uv)**

Peak was observed in the range 200-250 nm for both extract of *T. patula* callus as well as *T. patula* leaves. The peak in the range of 200-250 among other peaks was similar to the standard data for thiophene [5].

| Components | Weight (mg/l) |
|------------|--------------|
| Glycine    | 200          |
| Nicotinic acid | 50          |
| Pyridoxine HCl | 50          |
| Thymine HCl | 10           |

**Table 5:** Vitamins (stock: V 10ml).

| Media   | Kinetin | 2,4-D  |
|---------|---------|--------|
| Media 1 | 0.1 mg  | 0.2 mg |
| Media 2 | 0.2 mg  | 0.4 mg |
| Media 3 | 0.3 mg  | 0.6 mg |
| Media 4 | 0.4 mg  | 0.075 mg |

**Table 6:** Concentration of hormones.
Ft-Ir Spectrometric analysis

The various derivatives of thiophene were observed at different wave numbers [6].

Extract 1

Callus culture of *T. patula* extracted with hexane as solvent was subjected to FT-IR spectroscopy. The result was as obtained in the table 7. The derivatives of hexane were observed at wave numbers in the range of 2969-2965/cm, 2929-2912/cm, 2884-2883/cm, 2861-2849/cm, the compound observed was n-alkalene. At 1466-1468/cm n-alkanes was observed.

Thiophene derivatives were present from 1375-688/cm. At 1375-1360/cm, 1282-1275/cm, 1280-1240/cm, 1172-1165/cm, 1145-1125/cm, 1070-1040/cm, 1010-990/cm, 884.59/cm, 818/cm, 760-650/cm and 730-720/cm the compound observed was n-alkalene. At 1466-1468/cm n-alkanes was observed.

Larvicidal test

The solvent extract of *T. patula* callus and the leaves were used for

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**Table 7: FT-IR analysis of *T. patula* leaf callus.**

| WAVE NUMBER (FREQUENCY/Cm) | VIBRATION | COMPOUND |
|-----------------------------|-----------|----------|
| 2969-2965                   | Anti symmetric | n-alkalene |
| 2929-2912                   | Antisymmetric CH\_stretch | n-alkalene |
| 2884-2883                   | Symmetric CH\_stretch | n-alkalene |
| 2861-2849                   | Symmetric CH\_stretch | n-alkalene |
| 1466-1468                   | CH\_ deformation | n-alkanes |
| 1375-1360                   | Symmetric NO\_2 stretch | Secondary nitro alkanes |
| 1282-1275                   | Symmetric NO\_2 stretch | Alkyl nitrates |
| 1280-1240                   | Ring stretch | Epoxide derivatives |
| 1172-1165                   | Symmetric SO\_2 stretch | Alkyl sulfonates |
| 1145-1125                   | Symmetric SO\_2 stretch | Dialkyl sulfones |
| 1070-1040                   | S=O stretch(1 or 2 bands) | Aliphatic sulfides |
| 1040-990                    | Ring vibrations | Pyrazoles |
| 884.59                      | Weak vibration | Thiophene |
| 818                         | Ring breathing | Tetra hydro pyran |
| 760-650                     | Symmetrical skeletal stretching | Tert-butyl group |
| 730-720                     | CCl stretch PC conformation | Primary chloroalkanes |
| 688                         | Ring breathing | Tetra hydro thiophene |

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**Table 8: FT-IR analysis of *T. patula* leaves.**

| WAVE NUMBER (FREQUENCY/Cm) | VIBRATION | COMPOUND |
|-----------------------------|-----------|----------|
| 2969-2965                   | Anti symmetric | n-alkalene |
| 2929-2912                   | Antisymmetric CH\_stretch | n-alkalene |
| 2884-2883                   | Symmetric CH\_stretch | n-alkalene |
| 2861-2849                   | Symmetric CH\_stretch | n-alkalene |
| 1466-1468                   | CH\_ deformation | n-alkanes |
| 1375-1360                   | Symmetric NO\_2 stretch | Secondary nitro alkanes |
| 1282-1275                   | Symmetric NO\_2 stretch | Alkyl nitrates |
| 1280-1240                   | Ring stretch | Epoxide derivatives |
| 1145-1125                   | Symmetric SO\_2 stretch | Dialkyl sulfones |
| 1070-1040                   | S=O stretch(1 or 2 bands) | Aliphatic sulfides |
| 1040-990                    | Ring vibrations | Pyrazoles |
| 1010-990                    | Triagonal ring breathing | Mono meta and 1,3,5 substituted benzene |
| 984.30                      | Ring breathing | Benzene |
| 951.65                      | S bond CH\_ rocking vibration | Methyl Sulphonate |
| 844.59                      | Weak vibration | Thiophene |
| 818                         | Ring breathing | Tetra hydro pyran |
| 760-650                     | Symmetrical skeletal stretching | Tert-butyl group |
| 730-720                     | CCl stretch PC conformation | Primary chloroalkanes |

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the larvicidal test. The mortality rate of mosquito larvae was observed to be 80% for callus and 60% for *T. patula* leaves.

**Conclusion**

From the above results we came to the conclusion that media 4 containing higher amounts of kinetin and lower amounts of 2-4 D produced callus of large biomass as compared to media 1,2 and 3. The amount of thiophene present in the callus is directly proportional to the biomass. Hence maximum thiophene content was present in the Media 4 calluses observed on the 12th day.

The UV spectrophotometric analysis showed the presence of thiophene and its derivatives from 200-250 nm in both hexane extract of callus and leaves as compared to standard data.

The results of FT-IR analysis showed that thiophene and their derivative were present in both the leaf calluses of *T. patula* and in the leaves of *T. patula*. From the FT-IR analysis showed the presence of thiophene at 884.59/cm in both the callus and leaves extract.

Higher percentage of larvicidal action was observed in callus compared to that of leaves. The results of FT-IR analysis showed that thiophene and their derivatives were present in both the leaf callus of *T. patula* and in the leaves of *T. patula*. Thiophene acts specifically on superoxide dismutase enzyme present in the gut of mosquito and thereby leading to its death. The larvicidal action was observed to be 80% for leaf calluses and 60% for leaves of *T. patula*. The prominence of thiophene in callus than in the leaves was observed. The larvicidal action would help in repressing diseases such as malaria, dengue, chikungunya caused by mosquito as vector.

Hence thiophene presence was confirmed in callus and leaves by UV spectrophotometer analysis, FT-IR analysis and Larvicidal test.

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