Agrotechniques to maximize productivity of Hydroalcoholic extract from medicinal garden herb Calendula

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Abstract

Among the various agrotechniques, optimum nutrient management is one of the most important factors, which affect the growth and overall accumulation of active ingredients in the different organs of the plants as the capacity to produce dry matter per unit area is dependent on nutrient management. Two years field experiment was carried in winter season during 2000-2002 at Horticulture Research Farm, BCKV, Kalyani, Nadia (West Bengal) on clay loam soil taking 27 treatment combinations comprised of three levels (0,10, 20g/m²) of each of N, P₂O₅ and K₂O under 3 replications to work out the optimum nutrient management for obtaining higher hydroalcoholic extract (HAE) from different parts of Calendula. The highest amount of HAE was noticed in the flower followed by leaf, shoot and to a lesser extent in root. The treatment combination of nitrogen, phosphorus and potassium in the ratio of 20:10:20 g/m² was observed to provide optimum nutrition for maximum productivity of flower as well as percentage of HAE producing 2308.70 g/m ² flower and 16.2, 14.4, 9.6 and 6.0% of HAE in flower, leaf, shoot and root, respectively on clay loam soil.

Keywords: Calendula, Marigold, Calendula officinalis, Hydroalcoholic extracts, Nutritional variability.

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Introduction

Calendula or Marigold, Calendula officinalis Linn. (Family – Asteraceae) is not only an attractive garden plant but also a medicinal herb having great demand in pharmaceutical preparations. The plant extract is a better medicine for wound healing in the tropical route of administration when prepared as an ointment by extracting flower heads. It is a traditional medicine where an alcoholic extract of flower infusion is used as bactericidal and antichloristic medicines. The crude aqueous extract was also antipyretic and analgesic (Shahnaz et al, 2000). The plant is used to treat inflammations of skin and mucosa. The
leaves. The chemical composition includes compounds such as flavonol glycosides, essential oil, triterpene glycosides, triterpene alcohols, polysaccharides, and fatty oil. Calendula extract is anti-inflammatory (Qamar et al., 1998), antimicrobial (Tomas et al., 1991), analgesic (Akihisa et al., 1996), and anti-diarrhoeal drug for the treatment of diarrhoea (Vargas et al., 1998). Flavonol glycosides, essential oil, terpenes, and fatty oils are present.

Among the various agrotechniques, optimum nutrient management is one of the most important factors, which affect the growth and overall accumulation of active ingredients in different parts of the plants as capacity to produce dry matter per unit area is dependent on nutrient management. Hence, the present study was undertaken to work out optimum nutrient management for obtaining higher hydroalcoholic extract (HAE) from different plant parts of Calendula.

**Materials and Methods**

A field experiment was conducted during two winter seasons of 2000-2002 at Horticulture Research Farm, Kalyani, Nadia (West Bengal), India (23°N, 89°E and 9.75 m altitude). The soil was clay loam having pH 7.4, 0.61% organic carbon, 0.062% total N, 28.0 kg/ha available P₂O₅ and 120.0 kg/ha available K₂O. The climate in this region is subtropical humid with mean highest and lowest temperatures of 34.78°C and 9.09°C in April and January, respectively. The experiment was laid out in Factorial Randomized Block Design with 27 treatments fewer than 3 replications. The treatment combinations comprised of 3 levels (0,10,20, g/m²) of each of N, P and K denoted as N₀, N₁, N₂; P₀, P₁, P₂; and K₀, K₁, K₂ to study the individual and combined effect of the elements. Seedlings of 21 days age were transplanted at a spacing of 20 cm × 20 cm. Full doses of P and K and ½ dose of N were applied as basal treatment along with 5 kg/m² of well rotten FYM. The remaining half dose of N was applied into two equal split doses at 20 and 40 days after transplanting.

Plants representing treatments were sampled from the replications, parts separated, chopped and dried separately at 35°C for 72 hours. Dried samples were ground and 5g of each sample was taken into conical flasks separately containing 100ml of 60% hydroalcoholic solvent which were kept at ordinary room temperature for 48 hours sealing the mouths of the flasks with cotton plug. The aliquots were taken out of the flasks using Whatman No.1 filter paper into other pre-weighed conical flasks and dried at a temperature of 70°C for 36 hours. Flasks containing dried extract was taken out from the oven, cooled and weighed. The differences of the weights of empty flasks and those containing dried extract were worked out and expressed in percentage of fresh weight.

**Results and Discussion**

The pooled analysis of two years' data showed that flower yield and HAE in each plant part of flower head, leaf, shoot, and root improved significantly by the application of N, P and K. The highest level (20g/m²) of each of N, P and K combination i.e. N₂P₂K₂ produced 2410.70 g/m² flower which was significantly superior to other treatments excepting N₀P₀K₀ (20:10:20 g/m² N: P: K) which produced 2308.70 g/m² flower, while N₀P₀K₀ (control) produced the lowest yield 359.65 g/m². The flower yield of the treatment combination N₂P₂K₂ was statistically at par with that N₂P₁K₁ (20:10:20g/m²). Present findings were also supported by earlier reports on Dahlia (Rahaman & Mitra, 1974), Chrysanthemum (Joiner & Smith, 1962) and Calendula (Vieira et al., 1999) and on some medicinal plants (Lieres et al., 1994).

The experiment showed that the flower had maximum extract followed by leaf, shoot and root under all treatment combinations (Table 1). Different levels of potassium application were found to influence the extract in calendula plant organs significantly. Higher doses of nitrogen i.e. N₂P₀K₀ (20g N/m²) was seen to project the highest percentage of extract (14.0%) as compared to 10g N/m² i.e. N₁P₀K₀ (11.4%) and control i.e. N₀P₀K₀ (8.4%) in flowers (Fig.1). Same trend of effects of N were found in the leaves producing 10.8, 8.2 and 5.2%, shoots having 7.0, 4.8 and 2.2% and in the roots 4.2, 2.8 and 1.6% under N₂P₀K₀, N₁P₀K₀ and N₀P₀K₀ treatments, respectively. Present observations corroborate the findings of Robbelen and Theobald (1991). However, the effect of phosphorus was not so contrasting, where the application of 20g P₂O₅/m² (i.e. N₀P₂K₀) showed 10.0, 6.8 and 2.4% of extract in flower, leaf, shoot and root, respectively, which were statistically at par with the treatment N₀P₁K₀ (10gP₂O₅/m²) having 9.6, 4, 2.9 and 2.2% of extracts in the respective plant parts. But those were significantly superior over their respective control (Table 1). Similar trend of observations had been found under different levels of potassium application.
where N, P, K (20 g K₂O/m²) showed 10.4, 7.0, 3.6 and 2.4% of extract in flower, leaf, shoot and root, respectively, were significantly superior to the results obtained from the control but at par with N, P, K (10 g K₂O/m²) producing 9.8, 6.6, 3.2 and 2.4% in flower, leaf, shoot and root, respectively.

The most predominating effect of nitrogen over both phosphorus and potassium had been found under different combinations (Table 1). In case of combined application of the elements the observations of most of the treatments varied significantly where distinct fluctuation was noticed in flower, leaf and shoot but less in root. The highest percentage of extract was recorded from all parts of the plant under the treatment combination N₂P₂K₂ (20:20:20 g/m²) which was significantly superior to other treatments but was at par with the records of the treatment combination N₂P₁K₂ (20:10:20 g/m²) that produced 16.2, 14.4, 9.6 and 6.0% of extract in flower, leaf, shoot and root, respectively. The worst performance was observed under control (Table 1 & Fig.1).

In addition to the doses of the applied nitrogen (Robbelen & Theobald, 1991) there were other factors also, viz. perfection, uniform plant population (Dijk et al, 1992) and plant growth conducive to higher plant height, blooming period, management practices, harvesting technology (Junghanns, 2000) which influence the accumulation of active ingredient.

Table 1: Effect of N × P × K on flower and HAE yield of Calendula

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Flower yield (g/m²)</th>
<th>Hydroalcoholic extract (%)</th>
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<tbody>
<tr>
<td></td>
<td>Flower</td>
<td>Leaf</td>
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<tr>
<td>T₁</td>
<td>N₀P₀K₀</td>
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</tr>
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<td>T₂₇</td>
<td>N₂P₂K₂</td>
<td>2410.70*</td>
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*Highest Value
Conclusion

From the present experiment distinct effect of major nutrients on productivity of the extract was observed in flower, leaf and shoot but minor effect in root. The flower had highest percentage of HAE followed by leaf and shoot and to a lesser extent in root. Finally it may be concluded that the treatment combination of nitrogen, phosphorus and potassium in the ratio of 200:100:200 kg/ha was observed to be the optimum nutrition for the maximum productivity of flower as well as percentage of HAE on clay loam soil.

References

Synthetic seeds — A potential tool to conserve plants

The definition of synthetic seed refers to the encapsulation of somatic embryos that functionally mimic the behaviours of true seeds and sprout into seedlings under suitable conditions. Synthetic seed production technology is considered to be one of the potential alternatives to produce commercially important agronomic, horticultural crops and elite plant species. Transgenic plants produced through genetic engineering, somatic and cytoplasmic hybrids obtained by protoplast fusion as well as sterile and unstable genotypes might be able to multiply through this technology. In near future, synthetic seed production technology might become a valuable tool to study the germination of true seeds and various unfamiliar events in germination process.

The concept of artificial seed has been developed from somatic embryoids, which are formed adventitiously from cultured somatic tissue in the laboratory. Somatic embryos are the embryos, which are developed from nonsexual cells of plants called somatic cells by the process of somatic embryogenesis. Scientists with the help of tissue culture technique have developed somatic embryoids from various plant parts and encapsulated them by a protective gel-like substance so that embryoids could survive and not desiccate even after planting into soil. Such encapsulated embryoids are termed as synthetic or artificial seeds by them.

Seed coat (testa) and endosperm that provide protection and nutrition for zygotic embryos in developing seeds are absent in somatic embryoids. If these somatic embryoids are sown directly in the field soil they are not able to survive due to microbial contamination and desiccation. While preparing synthetic seeds nutrients and growth regulators are added to the encapsulation matrix, which serves as artificial endosperm. They increase the efficiency of germination and viability of encapsulated embryoids. Addition of nutrients, fungicides, antibiotics and microorganisms into encapsulation matrix prevents the embryo from desiccation and mechanical injury. Phytohormones and pesticide fortification can also be done to enhance sprouting, germinating capacity and control various insect and nematode born diseases of seeds.

Method of Encapsulation

Encapsulation can be done by two ways one of them is molding and other is hydrogel encapsulation via a dropping procedure.

1. Molding is temperature dependent gel encapsulation in which polymers like gelrite are heated to molten form and are distributed in wells of microtitre plate containing embryos. Gel beads are formed in wells encapsulating embryo by cooling the temperature.

2. In hydrogel encapsulation, embryos are encapsulated in water soluble polymers, generally sodium alginate, at low temperatures. The somatic embryos are mixed in 1-5% sodium alginate solution. These are then added drop wise in the solution of complexing agents like calcium chloride or potassium chloride. Somatic embryos are encapsulated in alginate gel matrix/beads by the polymerization.

The hydrogel encapsulation method is more preferable than molding because molding may harm embryo during encapsulation. Several hydrogels like agar, alginate, carboxy methyl cellulose, carrageenan, gelrite, guargum, sodium pectate, tragacanth gum, etc. were tested for synthetic seed production. Among all these, alginate encapsulation was found to be more suitable for synthetic seed production. As compared to agar it is better for long-term storage and enhances formation of capsule and rigid beads, which are able to protect the enclosed somatic embryos against mechanical injury.

Method of synthetic seed production

The first essential requirement to produce synthetic seed of particular plant is to obtain somatic embryos. This can be done by somatic embryogenesis. Somatic embryogenesis may be initiated in two different ways such as direct embryogenesis and indirect embryogenesis. In some cultures, somatic
embryogenesis occurs directly from the somatic cells of small plant part like leaves, stem, root, etc. called explant without intervening an unorganized mass of cells called callus. The cells of the explant undergo direct embryogenesis from proembryonic-determined cells in absence of callus proliferation. In the second type of somatic embryogenesis, cells of the explant first undergo callus proliferation and embryoids develop within the callus tissue from the induced embryogenic cells. Somatic embryogenesis has been reported in Carrot, ornamental Camellia, Indian Valerian, Banana, Chlorophytum, Spruce, Mulberry, Sweet potato, Eucalyptus, Orchids, Basil, Apple, Tea, etc.

The explant is obtained by taking a small piece of carrot and making it germ free in laboratory. The callus is induced by keeping this part on specific medium containing nutrients and hormones under appropriate laboratory conditions. Somatic embryogenesis in carrot and in many other species can be induced by placing callus on hormones like auxin containing medium. After a short "auxin pulse" the cells are placed on hormone free medium for embryo development. Carrot cell clusters 50-100 µm in diameter containing 10-20 small, highly cytoplasmic merestematic cells which have a high potential for embryogenic development. The process also requires a reduced form of nitrogen which can be provided by glycine, glutamine, yeast extract, ammonium. Nitrogen solely in the form of NO₃⁻ seldom gives rise to embryos. Multiplication of plants from cell cultures by somatic embryogenesis has the potential for the highest rates of plant production. Thousands of somatic embryos can be produced in a single flask. These embryos after encapsulating in hydrogels are utilized as synthetic seeds. These formed synthetic seeds can either be sown directly in field after testing germinating efficiency of embryo or greenhouse or can be stored in refrigerator till used.

Potential applications of synthetic seeds

1. Natural seeds are produced by sexual reproduction, which depends on the life cycle of the particular plant. Period required for seed production vary with plant species. Therefore, we need to wait for getting seeds till the end of reproductive phase of particular plant. Synthetic seeds on the other hand can be produced within a month.

2. Some plants produce seed only in a particular season on the other hand synthetic seed can be produced in any season of a year.

3. Many plants are sterile and do not set seed. Synthetic seeds would in many instances be preferable to making cuttings as a means to propagate these plants.

4. Some tropical species produce recalcitrant seeds that can not be dried. Therefore, long-term storage of these species in seed banks is not currently possible. Synthetic seeds might be an alternative that would enable long-term storage of these species.

5. Synthetic seeds do not have dormancy period and ready for propagation at any time. Using these seeds life cycle of plant can also be reduced.

6. Meiotically unstable elite genotype can be protected using this method.

7. Synthetic seeds would be useful for large-scale monocultures as well as mixed genotype plantations.

8. Synthetic seed coating have capacity to hold growth promoting microorganisms, growth control agents, pesticides, etc.

9. They are helpful to study the role of endosperm and seed coat formation.

10. The pathogen free propagules are possible to transport across the international borders through synthetic seed technology avoiding bulk transportation of plants, quarantine and spread of diseases.

At present, there are many challenges before synthetic seed technology such as long-time storage, maintenance of low temperature conditions, intensive labour, high cost, time, embryogenic potency of particular plant, etc. The exact application of synthetic seeds may vary from species to species. In self-pollinated crops which currently have good seed production systems, synthetic seeds will not have any practical applications, but in cross pollinating species, especially those where seed production is difficult and expensive, synthetic seeds offer many advantages and opportunities (Contributed by Satish V Patil*, Bipinchandra K Salunke and Javeid A Bhat, School of Life Sciences, North Maharashtra University, Jalgaon 425 001 Maharashtra, India; *Correspondent author, E-mail: svpatil5@rediffmail.com).
Urticaria or hives, known as **Sharaa** in Unani medicine is one of the commonest allergic reactions. They are itchy, elevated, red blotches of varying size. It usually affects the throat, arms, legs and trunk. Rashes often disappear quickly but usually gone within 48 hours, although new ones may continue to appear for days or weeks. When the rashes last within six weeks it is called acute urticaria. But if new rashes keep occurring for more than six weeks it is called chronic urticaria. Chronic urticaria is not serious but can be a sign of an underlying disease process.

Unani or Greeco-Arabic medicine is based on the concept of balancing body humours. They either fell out of balance, which might yield diseases. Unani medicine involves four elements – earth, air, water and fire, along with four natures – cold, hot, wet and dry and four humours – blood or Sanguis (hot and wet), phlegm (cold and wet), yellow bile or Choler (hot and dry) and black bile or Melancholer (cold and dry). In case of urticaria, there is an excess of yellow bile or Choler humour, but it may be due to imbalance of other humours also. Unani system of medicine has described two types of the Urticaria: One is due to predominance of Sanguis humour called **Sanguis Urticaria (Sharaa-e-Damvi)**. The signs and symptoms are much more severe in Sanguis urticaria than Phlegmatic urticaria.

### Causes

Exact cause is not known in about 80% cases of chronic urticaria. However, the common causes of urticaria include medication such as antibiotics, which is most common cause in present day practice, codeine, anticonvulsant drugs, aspirin, etc. Foods such as shellfish, nuts, peanuts, eggs, barriers and food additives and inhalants such as animal dander, pollens and moulds, etc. can also cause urticaria. Contact allergens like plant substances, skin creams, perfumes, chemical hair dyes and insect bites such as bee sting are very common which cause a high intensity urticaria.

There are a number of distinct physical causes also such as exposure to cold water and air. Tight fitting clothes or jewellery (especially artificial) can cause rashes to the skin. Cholinergic urticaria is a rare disorder, which is due to heat, exercise or emotional stress. Another rare type of urticaria is Aquagenic urticaria where rashes appears due to contact with sweat or water. Sun exposure sometimes results in hives known as Solar urticaria. Bacterial, viral, fungal infections and intestinal worms have also been found to cause of hives.

Indigestion, constipation, heavy meals, teething in children, menstrual irregularities, excessive alcohol intake, ingestion of spicy foods and metabolic disorders have also been described several other causes of urticaria in Unani System of Medicine. It is more prevalent in females then males. It is more common in young people, but can occur at any age.

### Symptoms

Onset of the skin rashes is very sudden in most of the cases but sometimes it may be gradual. Red or skin coloured warts that resemble mosquito bites appear on the skin, which changes quickly in size, shape and location. Itching on the affected part is the most common problem, which compels the patient to visit the doctor.

Swelling of lips, face and tongue (**Angioedema**) may also occur, which increases the severity of the illness.

### Home Remedies

Following preparations are useful in the treatment of Urticaria:

**Preparation No. 1**

Take concoction of the fruits of **Tamar Hindi** (*Tamarindus indica* Linn.) and fruit of **Alu Bukhara** (*Prunus domestica* Linn.) in equal quantity and add sugar. 50ml of this solution can be taken twice daily.
Preparation No. 2

Boil heartwood of *Sandal Surkh* (*Pterocarpus santalinus* Linn.) 3g, fruit of *Unnab* (*Zizyphus sativa* Gaertn.) 5nos., *Saunf* (*Foeniculum vulgare* Mill.) 6g, flower of *Mundi* (*Sphaeranthus indicus* Linn.) 6g, and deseeded fruit of *Maveez munaqqa* (*Vitis vinifera* Linn.) 7nos. in water and filter it. After adding desirable quantity of sugar it can be taken twice daily.

Preparation No. 3

Seed of *Kalizeeri* (*Centratherum anthelminticum* Linn.) 3g and fruit of *Abhal* (*Juniperus communis* Linn.) 3g, boiled in water and filtered can be used once in morning.

Preparation No. 4

Paste of unripe fruit of *Shahtoot* (*Morus alba* Linn.) in vinegar is suggested to apply locally on the affected parts daily.

Preparation No. 5

Extract of *Rasaut* (*Berberis aristata* DC.) 3g, heartwood of *Sandal Safaid* (*Santalum album* Linn.) 3g, and extract of *Kafoor* (*Cinnamomum camphora* Linn.) 1g, are powdered and mixed in distilled concentrate of *Arq-e-Gulab* (*Rosa damascena* Mill.). This paste may be used locally twice daily.

Preparation No. 6

Vinegar and distilled concentrate of *Gulab* (*Rosa damascena* Mill.), mixed in equal parts is suggested for local application twice daily.

DO's

- Keep the temperature of the skin cool.
- Apply cold compresses to an itchy area.
- Wear cool, loose, light and cotton clothes.
- Use hygienic food and drinks.
- Take lukewarm baths using herbal soap and rinsing thoroughly.
- Steam bath at frequent intervals.
- Take easily digestible meals without over eating.
- Mild to moderate exercise.
- Keep fingernails short to avoid skin damage from any inadvertent scratching.
- Engage yourself in different activities to divert the attention from scratching.
- Take up an enjoyable hobby that distracts from the itching during the day and makes you tired enough to sleep at night.

DONT's

- Exposure to excessive heat and humidity for a long time.
- Scratch or rub the itchy areas.
- Take alcohol.
- Use tight fitting clothes.
- Use artificial jewellery.
- Take medicine especially pain relieving drugs without a doctor's advice.
- Eat food responsible for hives.
- Wear rough clothing, particularly wool, over an itchy area.

5. *Arq-e-Ushba* (Syrup) – 20-50 ml daily in divided dosages.
8. *Majoorn Ushba* (Semisolid Preparation) – 10g once daily.
9. *Majoorn Chobchini* (Semisolid Preparation) – 10g once daily.
10. *Jawarish Tamar Hindi* (Semisolid Preparation) – 10g twice daily after meal.
11. *Jawarish Amla* (Semisolid Preparation) – 10g twice daily after meal.
12. *Jawarish Anarain* (Semisolid Preparation) – 10g twice daily after meal.

**Pharmacopoeial Medicines**

1. Sharbat-e-Unnab (Syrup) – 20-50 ml daily in divided dosages.
2. Sharbat-e-Murakkab (Syrup) – 20-50 ml daily in divided dosages.

**Pharmacopoeial Medicines**

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