

Effect of Triacontanol on photosynthesis, alkaloid content and growth in opium poppy (*Papaver Somniferum* L.)*

N.K. SRIVASTAVA** & SRIKANT SHARMA

Central Institute of Medicinal and Aromatic Plants, Post Bag No. 1, P.O. RSM Nagar,
Lucknow-226 016 India. **Author for correspondence

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Abstract. The influence of different foliar applications of Triacontanol (Tria.) on growth, CO₂ exchange, capsule development and alkaloid accumulation in opium poppy was studied in glasshouse conditions. Plant height, capsule number and weight, morphine content, CO₂ exchange rate, total chlorophyll and fresh and dry weight of the shoot were significantly maximum at 0.01 mg/l Tria. At the highest concentration (4 mg/l) total chlorophyll, CO₂ exchange rate and plant height were significantly inhibited. Thebaine and codeine contents remained unaffected at all the concentrations. The concentration of Fe, Mn, Cu in shoots were maximum at .01 and Zn at 0.1 mg/l Tria. Increase in shoot weight, leaf area ratio and chlorophyll content were significantly correlated with morphine content.

Key words: alkaloids, CO₂ exchange, growth regulator, opium poppy, *Papaver somniferum* L., Triacontanol

1. Introduction

Growth promoting effects of Triacontanol (Tria.) on vegetables [18], cereals [19, 5] and horticultural crops [8] are well documented. These are associated with increased protein content, water uptake [10], uptake of elements [17] and photosynthetic CO₂ fixation [8]. Most previous studies on Tria. have concentrated on physiological aspects of crops leading to effects on components of yield.

The opium poppy (*Papaver somniferum* L.) is a rich source of opium alkaloids used frequently as analgesic, antitissive and antispasmodic agents in modern medicine. It is also grown as a source of edible seeds and oil in tropics and subtropical countries. The capsule is the most important plant organ being the major deposition site of latex which is the raw material for subsequent biosynthesis. However, poppy straw has also been used for the extraction of alkaloids [13].

The influence of growth regulators on alkaloid biosynthesis and accumulation has received little attention. Nevertheless, the effect of some

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growth regulators including chlormequat chloride, dimethyl sulphoxide [6], maleic hydrazide [22] and ethephon [16] on alkaloid constituents in poppy have been attributed to changes in membrane permeability, affects on seed development due to suppression of embryogenesis and enhancement of crude opium yield.

Since Tria. has been reported to increase dry matter production in several species, it was decided to investigate its possible affect on the inter-relationship between primary and secondary metabolism in opium poppy. This is an area of active research as evidenced by recent publications on crops having secondary metabolites of economic value [9, 4, 21, 7, 20]. The present study examines the effect of Tria. on alkaloid biosynthesis in opium poppy and the relationship between alkaloid production (capsule and constituents) and physiological parameters related to productivity including CO₂ exchange rate, chlorophyll production, leaf area ratio, biomass production and ion uptake using a range of concentrations under controlled conditions in a glasshouse.

2. Material and methods

Opium poppy plants (*Papaver somniferum* L. Cv. Shyama) were raised in pots filled with purified acid-washed silica sand by the method of Hewitt [11] modified by Agarwala and Sharma [1]. Plants were given between 200 and 500 ml of complete Hogland solution [12] periodically and were maintained in a glasshouse at ambient temperature (30–35 °C) and light intensity (800–1000 $\mu\text{E}/\text{m}^2/\text{sec}$), the latter measured by a LICOR light meter (Model 188B). Sprays of Tria. at 0.001, 0.01, 0.1, 1.0, 2.0, and 4.0 mg/l were applied on three occasions at intervals of four weeks using a hand held sprayer. Tween 20 (0.1% w/v) was added as a surfactant. Care was taken to wet both leaf surfaces to ensure maximum application. Solutions were applied to groups of six plants in three replicates with one set serving as control (no spray).

Measurements of plant height and fresh and dry matter were recorded three weeks after the last spray. Total Chlorophyll (Chl.) content was measured according to the method of Arnon [3] using 80% acetone with absorption measured on a Pye Unicam Spectrophotometer model SP 550 and computed for Chl. a and b. CO₂ exchange rate (CER) was measured by the CO₂ depletion technique using the third leaf and was analysed by a LICOR portable photosynthesis system model LI 6000 at a natural light intensity of 1500 $\mu\text{E}/\text{m}^2/\text{sec}$. CER was computed as mg CO₂/m²/sec and converted to mg CO₂/dm²/hr. Leaf area ratio (LAR) was determined according to the method of Radford [15] as L/A where L is the total leaf area and A, the dry matter of shoot. Leaves were separated and the cumulative

area measured on a LICOR model LI 3000 area meter. Shoot samples were dried at 60–70 °C, milled and digested in HNO₃-HClO₄ mixture [14] for determination of elemental content. Fe, Mn, Cu, and Zn were determined on a Pye Unicam model SP 2900 atomic absorption spectrophotometer. Data on capsule parameters which included number/plant and weight were recorded at the ripe stage. The alkaloid constituents morphine, codeine and thebaine were extracted from capsules and analysed on a Waters HPLC [2]. The individual peaks were identified by coinjection of authentic compounds.

The experiment was carried out using the layout of a randomised complete block design of three replications. Mean values of each character were statistically analysed for significance by means of a Least Significant Difference (LSD) test. Linear correlation coefficients were computed to determine the inter-relationships among various characters.

3. Results and discussion

Foliar applications of Tria. at lower concentrations (0.01 and 0.1 mg/l) stimulated overall growth and significantly enhanced morphine content and capsule weight but was inhibitory at the highest concentration (4 mg/l). Plant height and shoot fresh and dry weight were significantly increased at all concentrations of Tria. application (Figure 1), the association among

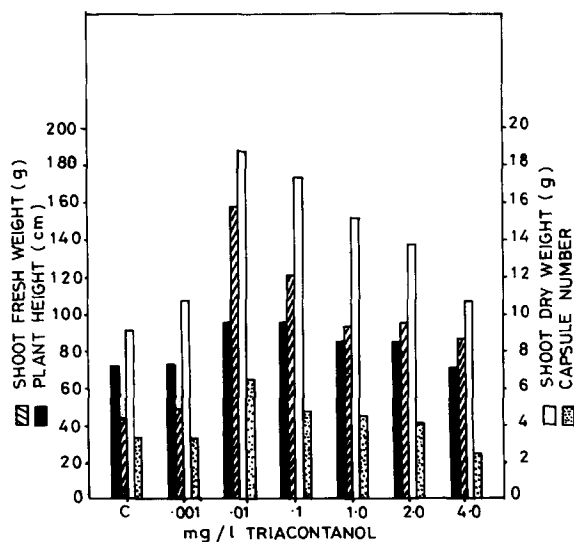


Fig. 1. Effect of Tria. on plant height, shoot fresh wt., shoot dry wt., and capsule number. LSD values at 5% and 1% are 4.8, 6.7 (plant height); 21.0, 29.5 (fresh wt.); 4.3, 5.9 (dry wt.); 1.1, 1.8 (capsule number), respectively.

these characters being significantly positive. However, at concentrations higher than 0.1 mg/l these characters exhibited a decreasing trend.

Among the physiological characters, total Chl., LAR and CER increased significantly till 0.01 mg/l and then decreased. However, Chl. a/b ratio showed an inverse relation indicating the greater influence of Tria. On Chl. a (Figure 2). Both CER and LAR were positively correlated with shoot fresh weight ($r = .816, .674$) and dry weight ($r = .945, .690$). This provides a physiological basis for overall enhancement in growth by Tria. application.

Elemental contents of Fe, Mn, Cu were significantly maximum at 0.1 mg/l and Zn at 0.01 mg/l (Figure 3). Above these Tria. concentrations Fe and Mn contents decreased while that of Cu and Zn remained unaffected. This indicated the differential affects of Tria. on uptake and distribution of micronutrients in poppy.

Tria. application at 0.01 mg/l increased capsule number (Figure 1), weight and morphine content (Figure 4) significantly, whereas thebaine and codeine contents remained unaffected at different concentrations. The positive correlation between capsule number and weight ($r = .794$) suggested that increasing number of capsules is not a limiting factor for capsule growth. This is possibly because of significant positive association of both these capsule characters with CER ($r = .881, .868$) and total Chl. ($r = .931, .821$)

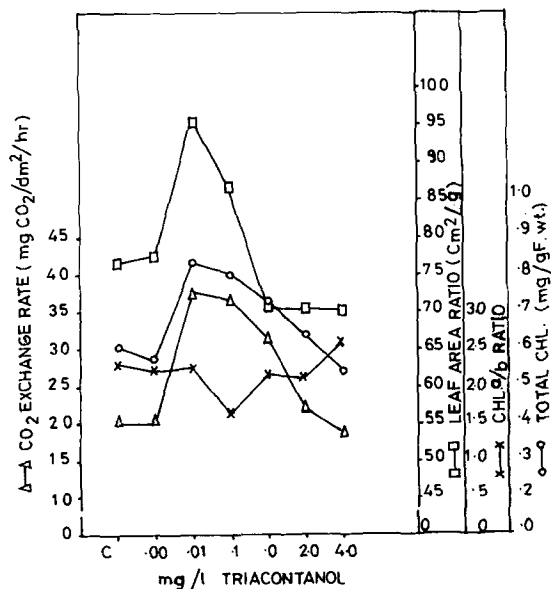


Fig. 2. Effect of Tria. on CER, total Chl., LAR and Chl. a/b ratio. LSD values at 5% and 1% are 4.8, 6.7 (CER); 0.15, 0.21 (total Chl.); 28.4, 39.8 (LAR); 0.6, 0.8 (Chl. a/b ratio) respectively.

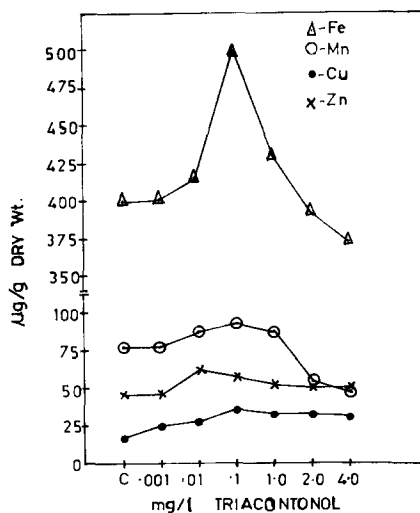


Fig. 3. Influence of Tria. on total elemental concentrations in shoots. LSD values at 5% and 1% are 19.3, 27.1 (Fe); 4.2, 5.9 (Mn); 4.4, 6.2 (Cu); 6.3, 8.9 (Zn) respectively.

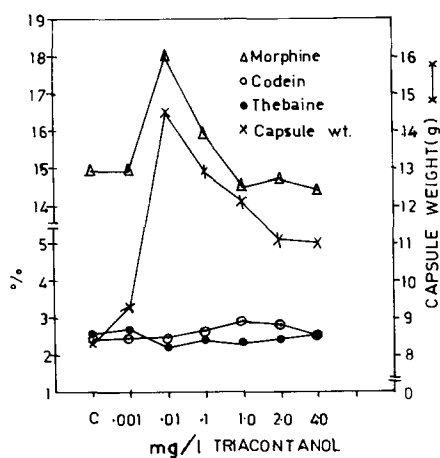


Fig. 4. Influence of Tria. on alkaloid constituents and capsule wt. LSD values at 5% and 1% are 0.8, 1.1 (Morphine); 0.5, 0.7 (Codeine); 0.3, 0.5 (Thebaine); 0.7, 1.0 (capsule wt.) respectively.

which contribute to the sink (capsule). The positive association of morphine content with LAR ($r = .963$), CER ($r = .740$), total Chl. ($r = .792$) and shoot fresh weight ($r = .767$) and dry weight ($r = .740$) suggest the increased supply of latex from shoot to capsule where it is then biosynthetically transformed.

The present investigation reveals that Tria. at concentrations upto 0.1 mg/l significantly enhances various processes related to production physiology in opium poppy. The primary processes in turn contribute significantly in increasing overall yield of straw, capsule and morphine content.

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