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A 4-(*o*-chlorophenyl)-2-aminothiazole: Microwave assisted synthesis, spectral, thermal, XRD and biological studies

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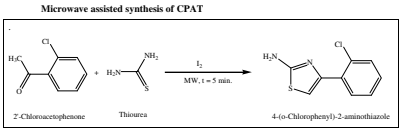
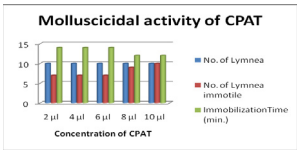
HIGHLIGHTS

- Microwave assisted synthesis is rapid and convenient than convention method.
- CPAT is thermally stable.
- CPAT exhibit good antibacterial activity against *B. subtilis* and *E. coli*.
- CPAT exhibit good antifungal activity against *A. niger* and *C. albicans*.
- CPAT shows nematicidal activity against *Meloidogyne javanica* and molluscicidal activity against *Lymnea auricularis*.

GRAPHICAL ABSTRACT





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ABSTRACT

A 4-(*o*-chlorophenyl)-2-aminothiazole (CPAT) has been synthesized by reacting o-chloroacetophenone, iodine and thiourea under microwave irradiation as a green chemistry approach. The reactions proceed selectively and within a couple of minutes giving high yields of the products. The compound was characterized by elemental, spectral (UV–visible, IR, NMR and GC–MS), XRD and thermal analyses. The TG curve of the compound was analyzed to calculate various kinetic parameters (n, E, Z, ΔS and ΔG) by using Coats–Redfern (C.R.), MacCallum–Tanner (M.T.) and Horowitz–Metzger (H.M.) method. The compound was tested for the evaluation of antibacterial activity against *B. subtilis* and *E. coli* and antifungal activity against *A. niger* and *C. albicans*. The compound was evaluated for their in vitro nematicidal activity on plant parasitic nematode *Meloidogyne javanica* and molluscicidal activity on fresh water helminthiasis vector snail *Lymnea auricularia*. The compound is biologically active in very low concentration. X-ray diffraction study suggests a triclinic crystal system for the compound.

Introduction

Microwave mediated synthesis has played an important role in organic synthesis over the last decade [1,2]. Seipel et al. [3] reported that the microwave assisted reaction times are eighty times faster than the conventional heating reaction times and these reactions are more energy efficient than those which use conventional heating. Microwave heating provides better heating efficiency, high rate of reaction, energy and better quality products and therefore it is of interest to use microwave mediated reactions in organic synthesis.

Thiazoles and their derivatives belong to an important class of heterocyclic compounds having an important position in medicinal chemistry, because of their wide range of bioactivities. Many of them exhibit an excellent antibacterial and antifungal [4,5], anti-HIV [6,7], hypertension [8], anti-inflammatory [9,10], anticancer [11], anti-convulsant [12], analgesic [13] and anti-tubercular activities [14]. Substituted 2-aminothiazoles shows anti quorum sensing [15] activity. Organophosphorous derivatives of thiazoles posses pesticidal and nematicidal [16] activity.

Most vegetable crops are attacked by one or more species of nematodes. The root-knot nematode (*Meloidogyne javanica*)

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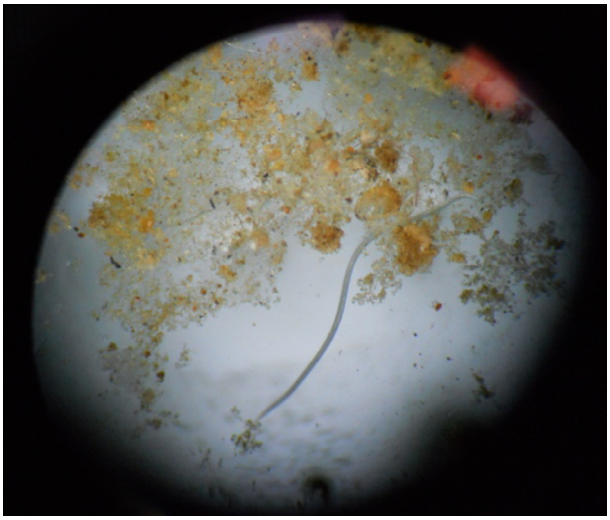


Fig. 1. Root knot nematodes.

(Fig. 1) is the most important species associated with tomato (*Lycopersicon esculentum*) [17]. This phytonematode species causes chlorosis, premature leaf drop and stunting. The disease is becoming one of the most serious calamities for the successful cultivation of tomato crop. These nematodes cause up to 70–90% yield losses in tomatoes and brinjal. In India, yield loss of tomato due to root-knot nematodes (*Meloidogyne* spp.) ranges from 39.7% to 46.0%. The present investigation was made to study the nematicidal activity of newly synthesized CPAT on *M. javanica*.

The fresh water snails *Lymnea auricularia* family Lymnaeidae are familiar members of the fauna of ponds, lakes, ditches and other kind of standing waters throughout the World. It is an intermediate host of liver fluke. The *Fasciola* spp. causes great damage to live stock throughout the world. It is responsible not only for liver rot, the uncomplicated Fascioliasis, but also the notorious 'black disease'. The considerations of the family Lymnaeidae and of species of snails which act as intermediate host [18] for *F. hepatica* and *F. Gigantia*. The present investigation was made to study the molluscicidal activity of newly synthesized CPAT on *L. auricularia*. Among thiazoles, 2-aminothiazoles have attracted the attention of researchers because they form Schiff bases with aldehydes. Schiff bases possess strong ability to form metal complexes [19,20].

Microwave synthesis of aminothiazoles is of interest in view of green chemistry approach. Kabalka et al. [21] reported MW promoted synthesis of 2-(N-substituted) aminothiazoles from α-bromoketones. Khrustalev et al. [22] reported synthesis of 2-amino-4-phenylthiazole under microwave irradiation. In the present communication, we are reporting the microwave mediated synthesis of CPAT by reacting 2′-chloroacetophenone, iodine and thiourea under microwave irradiation. The compound CPAT (Fig. 2) was identified by spectral (UV–visible, IR, NMR and GC–MS), X-ray diffraction and thermal analysis.

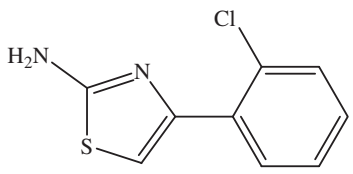


Fig. 2. 4-(*o*-Chlorophenyl)-2-aminothiazole (CPAT).

Experimental

Instrumentation

UV–visible spectra was recorded in ethanol on Shimadzu A 600 UV–visible spectrometer. IR spectra was recorded in KBr pellets on Shimadzu FT-IR 8400 spectrometer. ^{1}H NMR spectra was recorded in $CDCl_3$ using TMS as the standard on Varian 300 MHz spectrometer. GC–MS was recorded on Shimadzu GC–MS QP 5050 mass spectrometer. Thermogram was recorded on V2 4F TA thermal analyzer at the heating rate 1 °C per minute in nitrogen atmosphere. Microwave mediated reaction was carried out in conventional 25 DLX microwave oven. X-ray diffractogram was run in the range 10–90° using a Philips PW-1710 diffractometer attached to a digitized computer along with graphical assembly where Cu Kα radiation source connected with a tube of Cu–NF 2 kV/20 mA was used. The carbon, hydrogen and nitrogen contents were determined on Perkin–Elmer (2400) CHNS analyzer.

All reagents used such as 2′-chloroacetophenones, thiourea and iodine were pure AR grade. Solvents such as ethanol and diethyl ether were purified prior to use as per standard procedure.

Microwave mediated synthesis of 4-(o-chlorophenyl)-2-aminothiazole (CPAT)

CPAT was synthesized (Reaction 1) [22] by reacting 2′-chloroacetophenone (0.05 mol), iodine (0.1 mol) and thiourea (0.1 mol). They were mixed well with mortar-pestle and placed in small conical flask at room temperature. The mixture was then exposed to microwave irradiations for 5 min with 30 s pause at 180 W. Then 100 ml distilled water was added to the mixture and heated in microwave for 5–6 min at 270 W with 1 min pause till the precipitate dissolve. Filter the yellow solution and alkaline it with ammonia solution. Separate out the product and recrystallize with ethyl alcohol followed by diethyl ether and dried under reduced pressure.

Biological evaluation

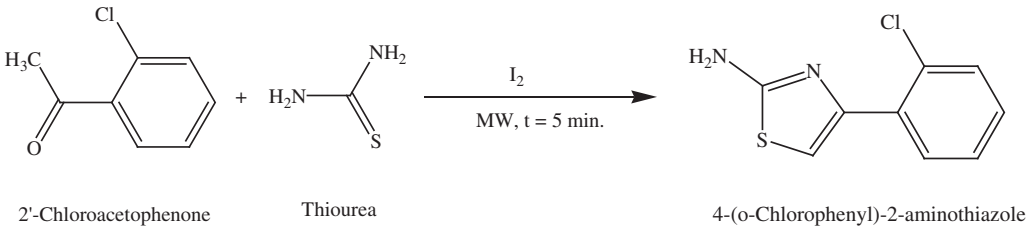
Antibacterial and antifungal activity

Antibacterial and antifungal activity of CPAT was tested by serial dilution technique [23]. Eight test tubes containing 5 ml of sterile nutrient/sabouraud broth were inoculated with 0.02 ml of 24 h old culture of bacteria *S. aureus* and *K. pneumoniae* and fungi *A. niger* and *C. albicans* respectively. Different amounts of CPAT in ethanol were aseptically added with the help of sterile pipettes from the stock solution 200 μg/ml to 5 ml quantities of respective media so as to reach the concentration from 1 μg/ml to 50 μg/ml. All test tubes were inoculated at 37 °C and at room temperature for bacteria and fungi respectively. Test tubes inoculated with organism were observed for presence of turbidity after 24 h and 48 h, respectively. The lowest concentration of CPAT inhibiting the growth of organism was determined as MIC value.

Nematicidal activity

For the toxicity and efficacy ratio of CPAT on root-knot nematode *M. javanica*, they were isolated from roots of tomato plants (*L. esculentum*) for in vitro study by using sieve plate method [24]. More eggs were recovered by repeated sieving and rinsing. The number of nematodes in an aqueous suspension was determined by using a counting dish.

The newly synthesized CPAT was tested in vitro nematicidal activity against root-knot nematodes *M. javanica* isolated from roots of tomato plants. The infected roots were macerated in 2% sodium hypochloride solution for 5 min to extract eggs and



Reaction 1. Scheme of synthesis.

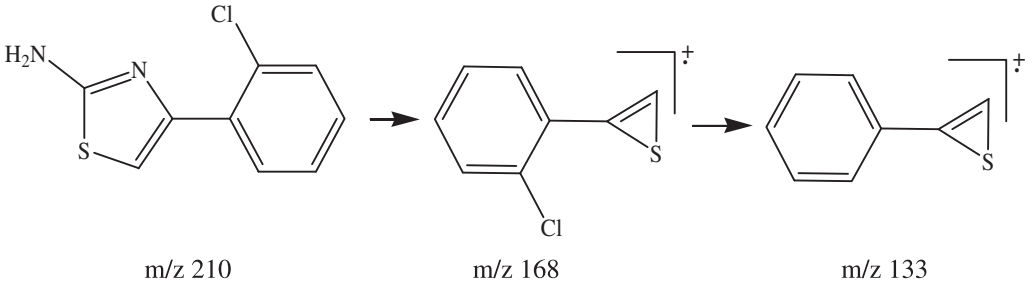


Fig. 3. Fragmentation pattern of CPAT.

centrifuge at 1000 rpm for 4 min. The eggs were laid on wet filter paper over water in pans for 3–4 days to hatch second stage and third stages of juveniles (J_2, J_3). For in vitro nematicidal activity, the method described by Dama et al. [25] is used for present study. The test animals are divided into 9 groups, each group contains 10 phytonemates, with test compound (CPAT) concentration of 2–10 μl. The flask that contained distilled water and DMSO serves as control for first group. Each treatment was replicated for three times. Data on Juvenile mortality was recorded after 6 h, 12 h and 24 h exposure of test compound (CPAT) under compound and stereomicroscope and then determined the percentage of efficacy.

Molluscicidal activity

Snails were collected from natural habitats and reared in the laboratory in glass aquaria and/or plastic containers by following appropriate technique [26]. The fresh water snails *L. auricularia* were taken from laboratory culture maintained in enamel bowls filled with dechlorinated water at room temperature 28 ± 2 °C and relative humidity more than 70%. Adults (more than 12-mm) were used for the toxicity studies. Snails of particular species were taken in large petri dishes. Snails were submerged in distilled water.

The test animals are divided into 9 groups, each group contains 10 snails (*L. auricularia*), with test compound (CPAT) concentration of 2–10 μl. The flask that contained distilled water and DMSO serves as control for first group. Each treatment was replicated for three times. Data on Juvenile mortality was recorded after 6 h, 12 h and 24 h exposure of test compound (CPAT) under compound and stereomicroscope and then determined the percentage of efficacy.

Results and discussion

The microwave irradiated synthesis of CPAT is completed in a couple of minutes (~5 min) giving 75% yield. The compound CPAT is colorless crystalline solid having sharp melting point 136 °C and soluble in common organic solvents. The compound CPAT gave satisfactory C, H and N analyses data. The observed and calculated % of C, H and N in the CPAT were found that C – 55.67 (55.65), H – 3.61 (3.61) and N – 14.43 (14.42).

Spectral analysis

UV–visible spectrum of 2-aminothiazole exhibits λ_{max} at ~275 and compounds having comparable structures exhibits λ_{max} at ~300 nm [27]. UV–visible spectra of CPAT exhibit λ_{max} at ~310 nm and this value of λ_{max} is in accordance with the earlier reports. IR spectrum of CPAT exhibits ν(NH_2)— ~3437 cm^{-1}, ν(C—N)— ~1609 cm^{-1} and ν(C—S—C)— ~580 cm^{-1} and these values match well with the earlier reports [28]. ^{1}H NMR spectrum of CPAT shows signals at ($CDCl_3$, TMS, δppm) 5.1 (2H, s, NH_2), 7.3 (2H, m, Ar—H), 7.8 (2H, t, Ar—H), 7.1 (1H, s, H-thiazole). The assignment of the signals is in agreement with the earlier reported results [29]. The mass spectrum of CPAT exhibits M^+ peak at *m*/*z* ratio 210/212* (relative intensity 100/34.78*%) corresponding to the molecular weight of CPAT and confirms the molecular formula as $C_9H_7ClN_2S$. The molecular ion undergoes rupture of thiazole ring [30] to give fragments at *m*/*z* 168/170* (47.83/18.26*). The peaks marked with (*) are isotopic peaks. These fragment further lose one chlorine atom to give fragments having *m*/*z* 133 (21.74). The fragment *m*/*z* 133 (21.74%) then undergoes decomposition to give smaller fragments represented as *m*/*z* (relative intensity%): 102 (6.09), 89 (25.22), 75 (8.7), 63 (7.83), 44 (36.52), 41 (65.22). The fragmentation pattern and mass spectrum are shown in Figs. 3 and 4, respectively.

Thermal analysis

The TG curve of CPAT (Fig. 5) is critically analyzed in order to evaluate various kinetic parameters such as *n* – order of reaction, *E* – energy of activation, *Z* – pre-exponential factor, Δ*S* – entropy change and Δ*G* – free energy change by using Coats–Redfern (C.R.) [31], MacCallum–Tanner (M.T.) [32] and Horowitz–Metzger (H.M.) [33] methods as follows.

Coats–Redfern method

$$\log\frac{1-(1-\alpha)^{1-n}}{(1-n)T^2} = \log\frac{ZR}{Eq} - \frac{E}{2.303R}\times\frac{1}{T} \quad (1)$$

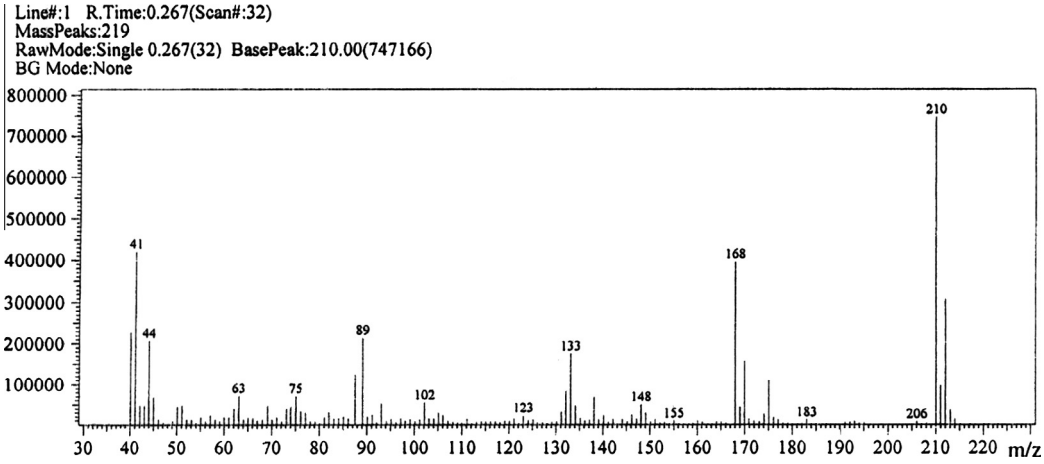


Fig. 4. GC–MS of CPAT.

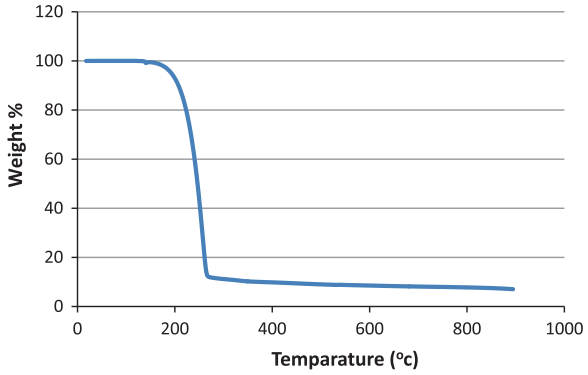


Fig. 5. TG curve of CPAT.

MacCallum–Tanner method

$$\log\left(\frac{1-(1-\propto)^{1-n}}{(1-n}\right) = \log\frac{ZE}{Rq} - 0.485E^{0.435} - \frac{0.449+0.217E}{T} \times 10^3 \quad (2)$$

Horowitz–Metzger method

$$\log\left(\frac{1-(1-\propto)^{1-n}}{(1-n)}\right) = \log\frac{ZRT_s^2}{Eq} - \frac{E}{2.303RT_s} + \frac{E\theta}{2.303RT_s^2} \quad (3)$$

In all three equations: α is fraction of weight loss at particular temperature, T_s is temperature at half weight loss, q is rate of heating, θ is difference of particular temperature and temperature at half weight loss ($T - T_s$). From the calculated values of E and Z, the values of ΔS and G were determined by using the following equations:

$$\Delta S = 2.303 \times \log[(Z \times h)/(Ts \times k)] \quad (4)$$

$$\Delta G = E - (\Delta S \times Ts) \quad (5)$$

CPAT undergoes decomposition in single stage in the range 156–270 °C (87.28% weight loss). The values of kinetic parameters (n, E, Z, ΔS and ΔG) calculated by Coats–Redfern (C.R.), MacCallum–Tanner (M.T.) and Horowitz–Metzger (H.M.) method are given in Table 1. The values of E (in the range 22–27 kcal mol^{-1}) and ΔG (in the range 31–37 kcal mol^{-1}) are sufficiently high indicating that CPAT is thermally stable. The ΔS values are negative.

Table 1
Kinetic parameters estimated by Coats–Redfern (CR.), MacCallum–Tanner (M.T.) and Horowitz–Metzger (H.M.) method.

Kinetic parameters	C.R.	M.T.	H.M.
n	0.38	0.37	0.56
E	21.46	25.60	26.45
Z	2.3519×10^5	2.894×10^3	5.343×10^7
ΔS	−17.64	−22.04	−12.21
ΔG	30.56	36.98	32.75

Units: E – kcal mol^{-1}, Z – s^{-1}, ΔS – J K^{-1}mol^{-1}, ΔG – kcal mol^{-1}

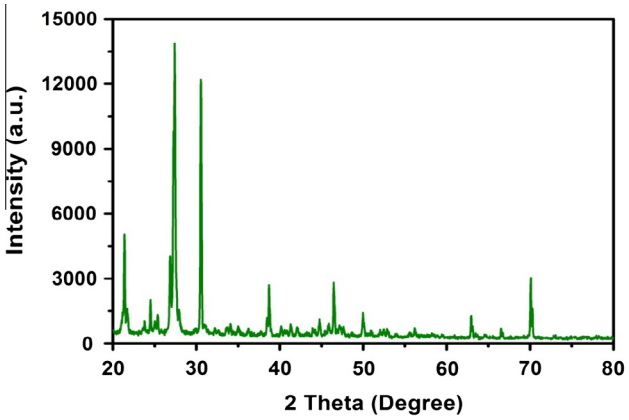


Fig. 6. X-ray diffraction of CPAT.

Table 2
Nematicidal activity of CPAT on root-knot nematode *Meloidogyne javanica*.

Concentrations of CPAT	2 µl	4 µl	6 µl	8 µl	10 µl
No. of nematodes	10	10	10	10	10
No. of nematodes immotile	8	7	8	9	10
Immobilization time (s)	60	60	50	45	40

X-ray diffraction study

The compound CPAT has been characterized by powder X-ray diffraction studies to predict the crystal system. The diffractogram is depicted in Fig. 6 which shows 23 reflection (2θ) between 20.00° and 80.00° with maximum at 2θ = 27.38° and d = 3.2547 Å. The cell parameter calculated are mentioned in parenthesis (a = 11.5602 Å,

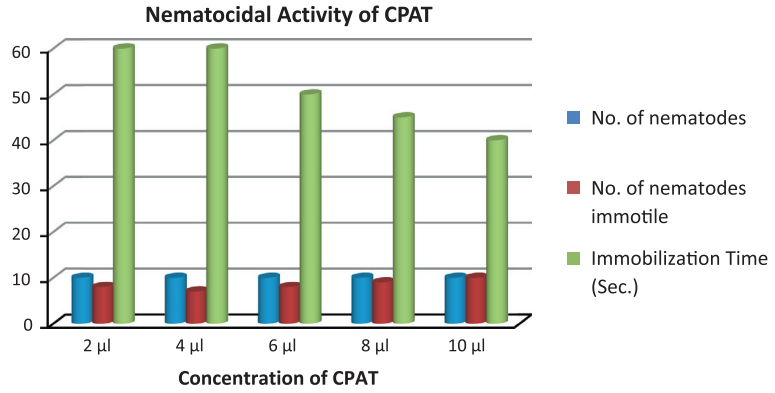


Fig. 7. Nematocidal activity of CPAT on root-knot nematode *Meloidogyne javanica*.

Table 3
Molluscicidal activity of CPAT on helminthiasis vector snail, *Lymnea auricularia*.

Concentrations of CPAT	2 µl	4 µl	6 µl	8 µl	10 µl
No. of Lymnea	10	10	10	10	10
No. of Lymnea immotile	7	7	7	9	10
Immobilization time (min.)	14	14	14	12	12

$b = 4.5556$ Å, $c = 3.9615$ Å, $\alpha = 112.978°$, $\beta = 91.977°$, $\gamma = 96.646°$) and these values are found to be in agreement with those required for a triclinic crystal system where $a \neq b \neq c$ and $\alpha \neq \beta \neq \gamma$. Therefore it may be concluded that the crystal system of the CPAT is triclinic [34]. The volume of unit cell is 190.06 Å^3.

Antibacterial and antifungal activities

The compound CPAT has been tested for the evaluation of antibacterial activity against *B. subtilis* and *E. coli* and antifungal activity against *A. niger* and *C. Albicans*. The MIC values for the compound CPAT lie in the range 14–16 µg/ml for antibacterial activity and 8–12 µg/ml for antifungal activity. The compound CPAT exhibits prominent antifungal activity than antibacterial activity.

Nematicidal activity

Direct contact toxicity of newly synthesized CPAT at different doses was analyzed by exposing 100 freshly hatched J_2 and J_3 of *M. javanica* for 24 h. The result indicates that, CPAT is very effective to controlling *M. javanica*. It shows highest percentage efficiency in the range of 8–10 µl. The percentage efficiency of CPAT is shown in Table 2 and Fig. 7.

Molluscicidal activity

The molluscicidal activity of CPAT was analyzed at different doses by exposing 100 fresh water snails *L. auricularia*. The result indicates that, CPAT is very effective to controlling *L. auricularia*. It shows highest percentage efficiency in the range of 8–10 µl. The percentage efficiency of CPAT is shown in Table 3 and Fig. 8.

Conclusion

Microwave mediated synthesis of CPAT is a convenient and rapid process resulting in good yield of the expected product. The reaction rate is 200 times faster than the rate of conventional method [35] of synthesis of 2-aminothiazole, which requires 20 h heating on water bath. The compound CPAT is thermally stable with high energy of activation and free energy change. The compound CPAT exhibit good antibacterial activity against *B. subtilis* and *E. coli* and antifungal activity against *A. niger* and *C. albicans*. It shows better antifungal activity than antibacterial activity. CPAT exhibit good nematicidal activity against root-knot nematode *M. javanica* and molluscicidal activity against vector snail, *Lymnea auricular*i with low concentration.

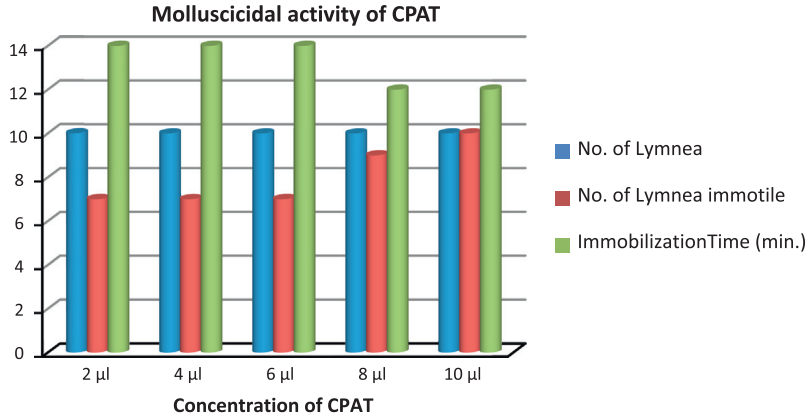


Fig. 8. Molluscicidal activity of CPAT on helminthiasis vector snail, *Lymnea auricular*i.

Acknowledgments

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