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Physicochemical, in *Vitro* Nematicidal and Molluscicidal study of 4-(2'-fluorophenyl)-2-Aminothiazole

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ABSTRACT: *4-(2'-fluorophenyl)-2-aminothiazole (FPAT) has been synthesized by reacting 2'-fluoroacetophenone, iodine and thiourea under microwave irradiation by a green chemistry approach. The compound was characterized by elemental, spectral, XRD and thermal analyses. The various kinetic parameters (n, E, Z, ΔS and ΔG) were calculated by using TG curve of the compound. The FPAT was evaluated for their in vitro nematicidal and molluscicidal activities on plant parasitic nematode Meloidogyne javanica and freshwater helminthiasis vector snail Lymnea auricularia. The FPAT is biologically active in very low concentration. X-ray diffraction study suggests a triclinic crystal system for the compound.*

KEYWORDS: *Microwave mediated synthesis, aminothiazole, nematicidal, molluscicidal activity.*

1. INTRODUCTION

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 [1,2], anti-HIV [3,4], hypertension [5], anti-inflammatory [6,7], anti-convulsant [8], analgesic [9] activities. Number of revives shows that thiazoles and their substituted derivaties shows anticancer [10] and anti-tubercular activities [11]. Substituted 2-aminothiazoles shows anti quorum sensing [12] activity. Organophosphorous and chloro derivatives of thiazoles posses pesticidal, nematicidal and molluscicidal [13,14] activity.

Most vegetable crops are infected by one or more species of nematodes. The root-knot nematode (*Meloidogyne javanica*) (figure 1) is the most important species associated with tomato and brinjal (*Lycopersicon esculentum*) [15]. 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 FPAT on *M. javanica*.

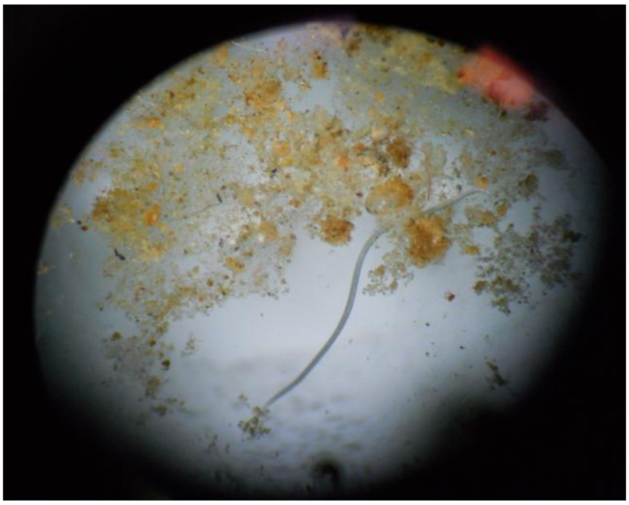


Fig. 1: Root knot nematodes of tomato plant

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 and the uncomplicated Fascioliasis, but also the notorious 'black disease'. The considerations of the family Lymnaeidae and species of snails, which act as intermediate host [16] for *F. hepatica* and *F. Gigantia*. The present investigation was made to study the Molluscicidal activity of FPAT on *Lymnea 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 [17]. The Schiff bases and their metal complexes obtained from thiazole derivatives have good antimicrobial activity [18].

Microwave synthesis of aminothiazoles is of interest in view of green chemistry approach. Kabalka et. al.[19] reported MW promoted synthesis of 2-(N-substituted) aminothiazoles from α-bromoketones. Similarly, Khrustalev et. al. [20] reported synthesis of 2-amino-4-phenylthiazole under microwave irradiation. In the present communication, we are reporting the microwave mediated synthesis of FPAT by reacting 2'-fluoroacetophenones, iodine and thiourea under microwave irradiation. The compound FPAT (Fig.-2) was identified by spectral (Uv-visible, IR, NMR and GC-MS), X-ray diffraction and thermal analysis.

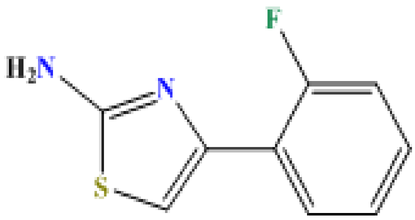


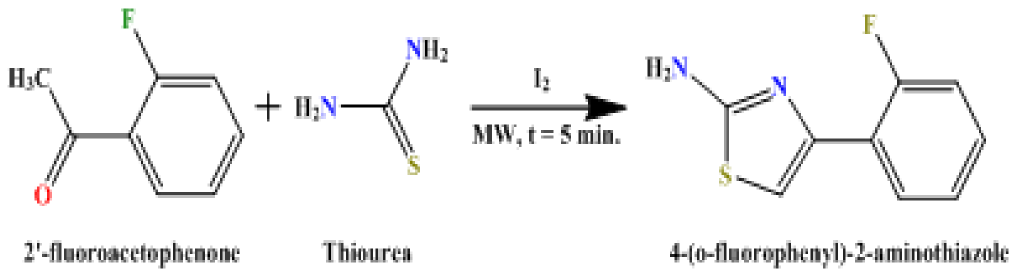
Fig. 2: 4-(o-Fluorophenyl)-2-aminothiazole (FPAT)

II. EXPERIMENTAL

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

2.1 *Microwave mediated synthesis of 4-(o-fluorophenyl)-2-aminothiazoles (FPAT)*

The compound FPAT was synthesized (Reaction1) [20] by reacting 2'-fluoroacetophenone (0.05 mol), iodine (0.1 mol) and thiourea (0.1 mol) as shown in scheme 1. The reactants were mixed well with mortar-pestle and placed in small conical flask at room temperature. The mixture was then exposed to microwave irradiations for five minute with 30 sec. pause at 180 W. Then 100 ml distilled water was added in the mixture and heated in microwave for 5-6 minute at 270 W with 1 minute pause till the precipitate dissolve. The yellow solution was filtered and pH was adjusted to 9.5 using aqueous ammonia. The precipitated product was separated out by filtration and recrystallized with ethyl alcohol followed by diethyl ether and dried under reduced pressure.



Scheme -1: Scheme of synthesis

2.2 *Biological Evaluation*

2.2.1 Nematicidal activity

For the toxicity and efficacy ratio of FPAT on root-knot nematode *M. javanica*, they were isolated from roots of tomato plants (*Lycopersicon esculentum*) for *in vitro* study by using sieve plate method [21]. 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 FPAT 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 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. [22] is used for present study. The test animals are divided in to 9 groups, each group contains 10 phytonemates, with test compound (FPAT) concentration of 2 µl to 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 (FPAT) under compound and stereomicroscope and then determined the percentage of efficacy.

***2.2.2 Molluscicidal* activity**

Snails were collected from natural habitats and reared in the laboratory in glass aquaria and/or plastic containers by following appropriate technique [23]. The fresh water snails *Lymnea* 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. *S*nails of particular species were taken in large petridishes. Snails were submerged in distilled water.

The test animals are divided into 9 groups, each group contains 10 snails (*Lymnea* auricularia), with test compound (FPAT) concentration of 2 µl to 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 (FPAT) under compound and stereomicroscope and then determined the percentage of efficacy.

III. RESULTS AND DISCUSSION

3.1 Chemistry

The microwave irradiated synthesis of FPAT is completed in a couple of minutes (~5 min) giving 85 % yield. The colorless, crystalline solid compound FPAT is having sharp melting points 107 °C and soluble in common organic solvents. The compound FPAT gave satisfactory C, H and N analyses data.

3.1.1 Spectral analysis

Uv-visible spectrum of 2-aminothiazole exhibits λ_{max} at ~275 and compounds having comparable structures exhibits λ_{max} at ~300 nm [24]. Uv-visible spectra of FPAT exhibit λ_{max} at ~310 nm and this value of λ_{max} is in accordance with the earlier reports. IR spectrum of FPAT exhibits ν(NH_2), ν(C=N) and ν(C-S-C) modes at ~3417, ~1601 and ~575 cm^{-1}, respectively and these values match well with the literature reports[25]. ^{1}H NMR spectrum of FPAT shows signals at ($CDCl_3$, TMS, δppm) 5.5 (2H, s, NH_2), 6.9 (1H, s, H-thiazole), 7.2 (2H, m, Ar-H), 7.9 (2H, t, Ar-H). The assignment of the signals is in agreement with the earlier reported results[26]. The mass spectrum of FPAT exhibits M^+ peak at m/z ratio 194 (relative intensity 100 %) corresponding to the molecular weight of FPAT and confirms the molecular formula as $C_9H_7FN_2S$. The molecular ion undergoes rupture of thiazole ring [27] to give fragments at m/z 152 (67.26%). These fragment further loose one fluorine atom to give fragments having m/z 132 (7.08 %). The fragment m/z 132 (7.08 %) then undergoes decomposition to give smaller fragments represented as m/z (relative intensity %):122 (15.04 %), 107 (16.81 %), 97 (4.42 %), 75 (5.31 %), 69 (5.31 %), 45 (7.96 %). The fragmentation pattern and mass spectrum are shown in Fig. 3 and 4, respectively.

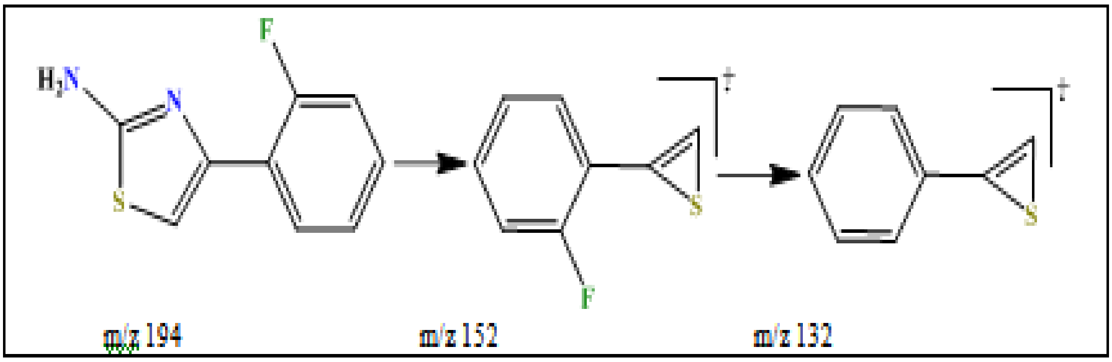


Fig. 3: Fragmentation pattern of FPAT

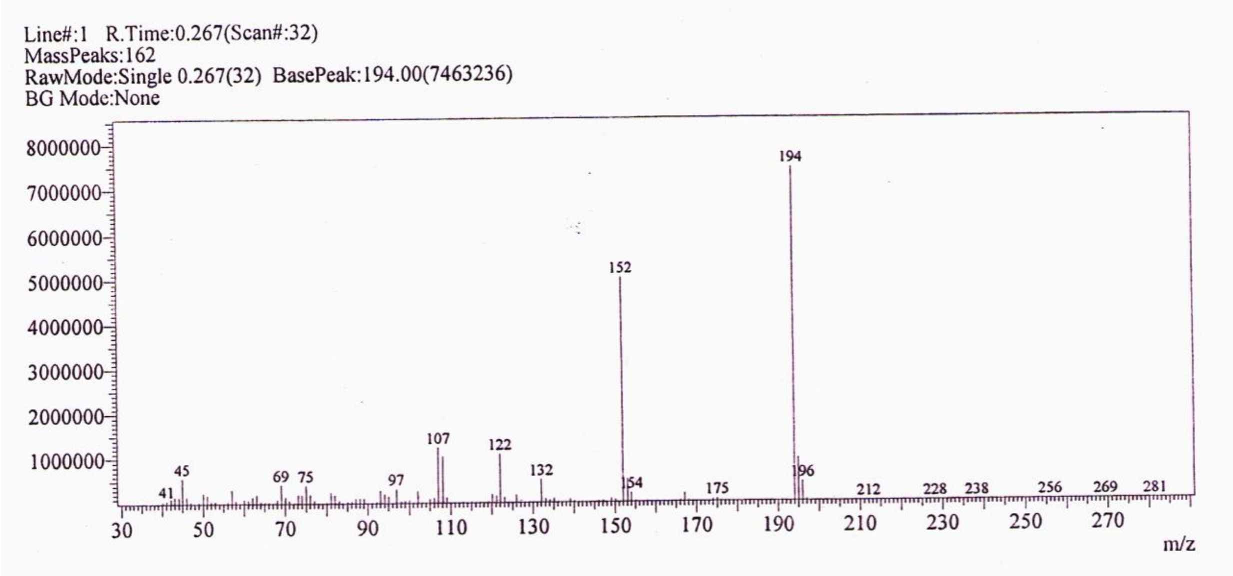


Fig. 4: GC-MS of FPAT

3.1.2 Thermal Analysis

The TG curve of FPAT (Fig.-5) is critically analysed in order to evaluate various kinetic parameters such as n- order of reaction, E- energy of activation, Z- pre-exponential factor, ΔS- entropy change and ΔG- free energy change by using Coats – Redfern (C.R.)[28], MacCallum-Tanner (M.T.)[29] and Horowitz-Metzger (H.M.) [30] methods as follows.

Coats-Redfern method

$$\log \frac{1-(1-\propto)^{1-n}}{(1-n)T^2} = \log \frac{ZR}{Eq} - \frac{E}{2.303R} \times \frac{1}{T} \qquad \text{...... 1}$$

MacCallum- Tanner Method

$$\log \left(\frac{1\text{-}(1\text{-}\ \alpha\ )^{1\text{-}n}}{(1\text{-}\ n)} \right) = \log \frac{ZE}{Rq} - 0.485E^{\,0.435} - \frac{0.449 + 0.217E}{T} \cdot 10^3 \qquad \text{......2}$$

Horowitz – Metzger Method

$$\log \left(\frac{1\text{-}(1\text{-}\ \alpha\ )^{1\text{-}n}}{(1\text{-}\ n)} \right) = \log \frac{ZRT_S^{\,2}}{Eq} - \frac{E}{2.303\ RT_s} + \frac{E\ \theta}{2.303\ RT_s^{\,2}} \qquad \text{......3}$$

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 equations 4 and 5.

ΔS = 2.303 x Log [(Z x h) / (Ts x k)] 4

ΔG = E-(ΔS x Ts) 5

The compound FPAT undergo decomposition in single stage. AT-7 decomposes in the range 140^0C to 236^0C (96.25% 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 23 – 28 Kcal mol^{-1}) and ΔG (in the range 30 -37 Kcal mol^{-1}) are sufficiently high indicating that FPAT is thermally stable. The ΔS values are negative.

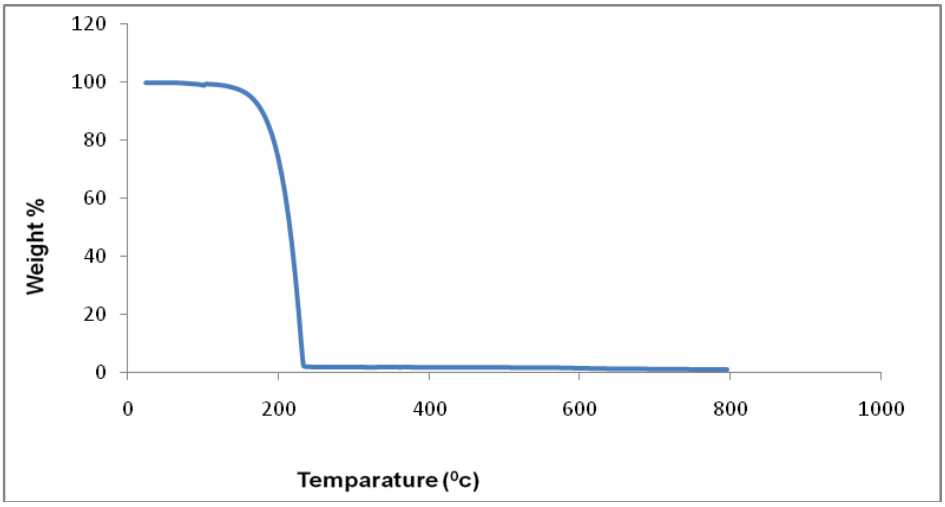


Fig.5: TG curve of FPAT

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

Kinetic parameters	C.R.	M.T.	H.M.
n	0.43	0.43	0.55
E	23.47	27.60	27.79
Z	8.169×10^{6}	8.246×10^{4}	1.204×10^{9}
ΔS	-14.03	-18.63	-9.04
ΔG	30.29	36.65	32.18

Units: E-kcal mol^{-1}, Z-S^{-1}, ΔS- $JK^{-1}mol^{-1}$, ΔG- kcal mol^{-1}

3.1.3 X-ray Diffraction Study

FPAT 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° to 80.00°, with maximum at 2θ = 25.76° and d =3.4556 Å. The cell parameter calculated are mentioned in parenthesis (a=10.5573A0, b=3.8478A0, c=6.7869A0, α=99.0700, β=92.0820 and γ = 95.0510). These cell parameter values are found to be in agreement with those required for a triclinic crystal system where a ≠ b ≠ c and α ≠ β ≠ γ. Therefore it may be concluded that the crystal system of the FPAT is triclinic [31]. The volume of unit cell is 270.84 $Å^3$.

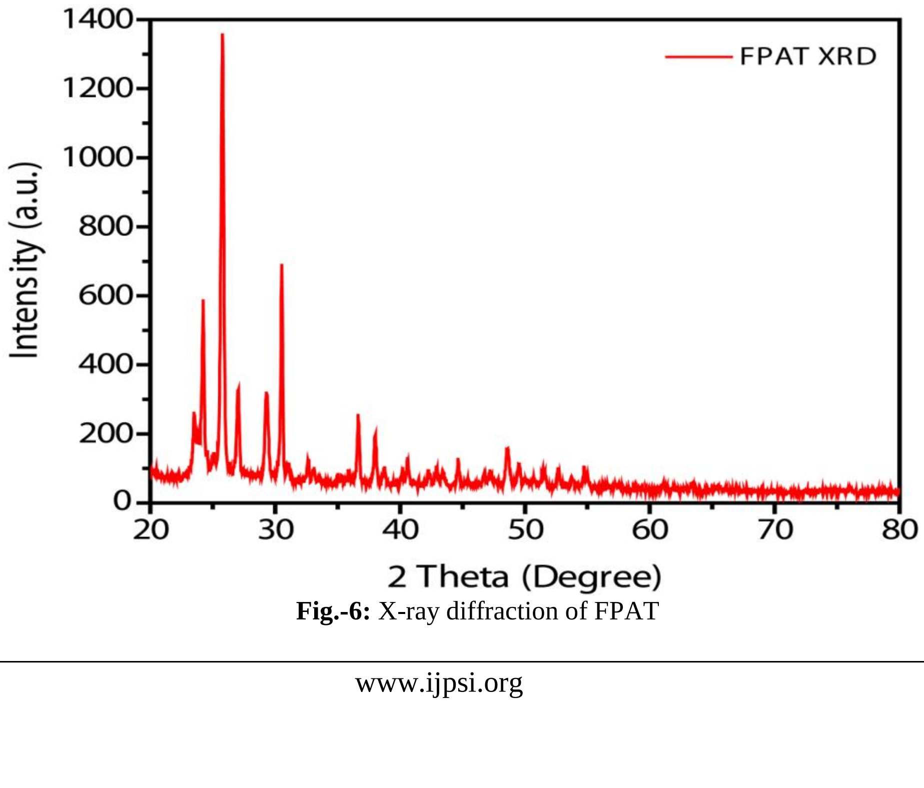


Fig.-6: X-ray diffraction of FPAT

3.2 Biology

3.2.1 Nematicidal Activity

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

Table 2. **N**ematicidal activity of FPAT on root-knot nematode *Meloidogyne javanica*.

Concentrations of FPAT	2 µl	4 µl	6 µl	8 µl	10 µl
No. of nematodes	10	10	10	10	10
No. of nematodes immotile	8	8	9	10	10
Immobilization Time (Sec.)	60	60	50	45	40

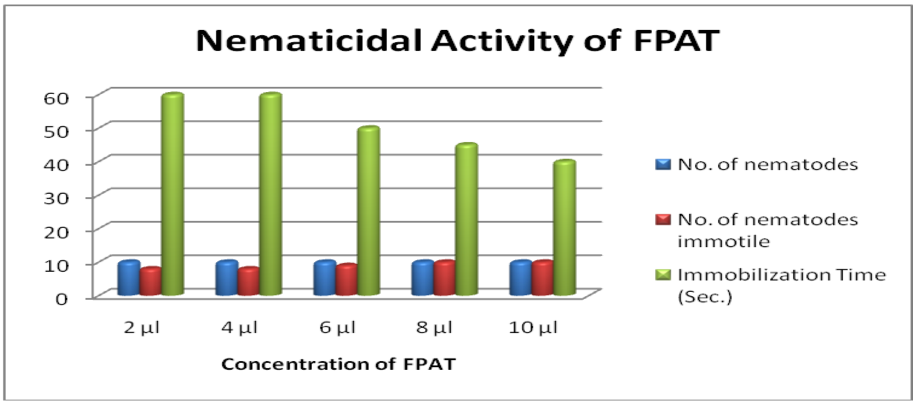


Fig. 7. Nematicidal activity of FPAT on root-knot nematode *Meloidogyne javanica*.

3.2.2 Molluscicidal Activity

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

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

Concentrations of FPAT	2 µl	4 µl	6 µl	8 µl	10 µl
No. of Lymnea	10	10	10	10	10
No. of Lymnea immotile	8	8	9	10	10
ImmobilizationTime (min.)	14	14	14	12	10

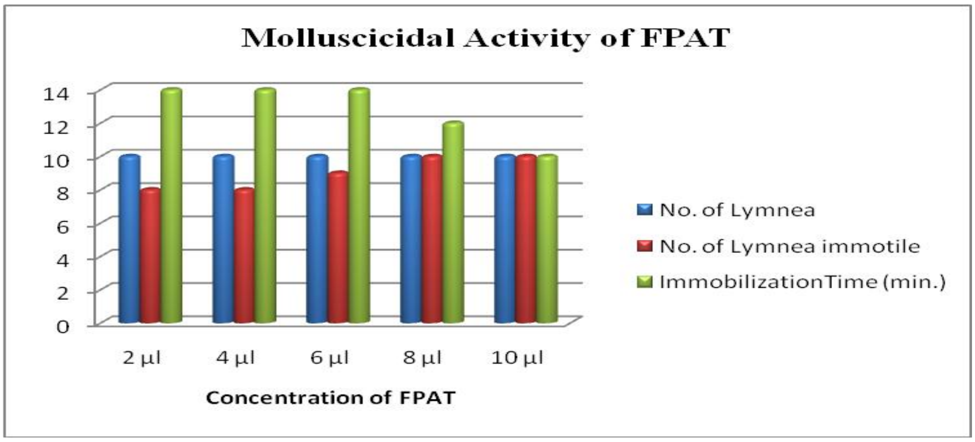


Fig. 8: Molluscicidal activity of FPAT on helminthiasis vector snail, *Lymnea auriculari*

IV. CONCLUSION

Microwave mediated synthesis of FPAT 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 [32] of synthesis of 2-aminothiazole, which requires 20 hours heating on water bath. The compound FPAT is thermally stable. X-ray diffraction study suggests a triclinic crystal system for the compound. FPAT exhibit good nematicidal activity against root-knot nematode *Meloidogyne javanica* and molluscicidal activity against vector snail, *Lymnea auriculari.*

V. ACKNOWLEDGMENT

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