Synthesis, Characterization, Antimicrobial and Larvicidal assay of a novel polyamide

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***Abstract*—A new polyamide (MT-1) was synthesized from Melamine (MA) and 2,5-Thiophenedicarboxylic acid (TDCA) using methanol as a solvent in an inert atmosphere at 150°C. The polymer was characterized extensively by UV-visible, FT-IR, NMR spectra. Thermal properties were determined by TG-DTA, DSC techniques. Its morphology was ascertained by SEM-EDAX study. Further, the synthesized polyamide MT-1 was tested for antifungal activity against two pathogens, Aspergillus niger and Candida albicans at four different concentrations (500, 250, 100, and 50 µg/mL) using the Agar well diffusion method. The antibacterial activity was assayed against four pathogens, S. oralis-2696, P. acnes-1951, A. hydrophila-7696 and K. pneumoniae. Additionally and its larvicidal activity was screened. In comparison to the standard drug, the newly synthesized MT-1 exhibited good to significantly moderate activity against all tested bacterial pathogens, good to limited antifungal activity at the highest test concentration against two tested fungal pathogens and an excellent mortality rate against tested larvae.**

***Index Terms*—Polyamide; Melamine moiety; 1,3,5-Triazine copolymer; Spectral. Thermal. Morphology study, Antimicrobial activity; Larvicidal activity.**

I. INTRODUCTION

Polymers are employed in nanomaterial production and they are widely used as antimicrobials. They are generally made from biodegradable polyesters, such as polycaprolactone (PCL) or polylactic acid. They have benefits of being used in infections until their complete absorption [1]. It may be noted that it is quite challenging to get a homogenous structure in the formation of polyester materials. However, polyamide nanofibers are non-degradable synthetic polymers, can be easily synthesised with structural modification and are considered to be more homogenous [2-4]. Further, polyamide materials possess high mechanical resistance and thermal stability. Hence these polymers are employed in food packaging, the manufacture of joint replacements, and wound treatments in medicine [5]. In the attempt to produce medicinally important polyamides, a new family of nitrogen-rich polymers known as melamine-condensed polyamides is created by condensing melamine with dicarboxylic acids or their derivatives. Besides, the polyamides containing s-triazine rings in the polymer chain have special qualities such excellent thermal stability, flame resistance, chemical endurance, and increased mechanical strength. They are very appealing for a variety of sophisticated material applications due to their high nitrogen content and structural stiffness. Melamine-based polyamides have drawn a lot of attention lately due to their prospective applications in filtration membranes, flame-retardant materials, corrosion-resistant composites, and high-performance coatings [6].

Furthermore, their suitability for green and sustainable chemical concepts has created opportunities for eco-friendly materials in packaging, biomedical applications, and building. Their function in creating lightweight engineered polymers, water purification systems, and smart materials is still being investigated [7-30].In order to improve the stability and processibility of heterocyclic polymers while maintaining their thermal stability, a number of published studies have described the synthesis and properties of polymers with an s-triazine ring in the main chain, such as polyesters [31], polyamides [32], polyimides [33], polyureas [34], polyazomethines [35], and so on. The superior solubility and remarkable

thermal stability of polyamides containing s-triazine as a heterocyclic unit in the main chain are widely recognized [36,37]. Several reports have led to the synthesis of polyamides having s-triazine rings in the main chain [38–41]. Extensive literature survey reveals that there are no reports on the synthesis of s-triazine-containing polyamides made from 2,4,6-triamino-1,3,5-triazine (Melamine, MA) and 2,5-thiophene dicarboxylic acid (TDCA).We report the synthesis of MT-1 polymer in one pot synthesis. The polyamide is characterised using UV-visible, FT-IR, NMR spectra to confirm its structural unit. The thermal stability is ascertained by TG-DTA technique. The extensive antibacterial activity of Melamine (MA) scaffold functionalized polyamide (MT-1) is explored. Additionally, the synthetic chemical MT-1's larvicidal ability was evaluated.

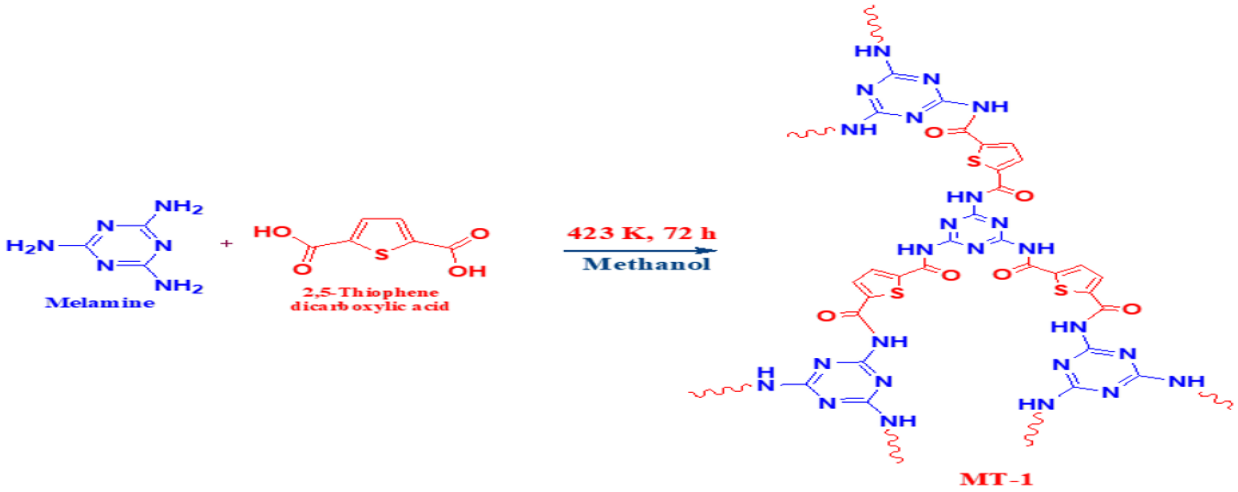
II. METHODS

2.1. Chemicals

2,4,6-Triamino-1,3,5-triazine (Melamine, MA, 99%), 2,5-Thiophene dicarboxylic acid (TDCA, 99%) and methanol were purchased from Sigma-Aldrich, India and they were used as such in the one pot synthesis of MT-1. The solvents used in solubility study were of AnalaR grade samples.

2.2. Synthesis of Melamine-based Polyamide (MT-1)

Melamine-based polyamide (MT-1) was synthesized by one-pot polycondensation in mild reaction conditions [42] in N_2 atmosphere using 1:1 molar ratio of melamine (MA, 1.2612 g) and 2,5-Thiophene dicarboxylic acid (TDCA, 1.7616 g). Methanol was used as the solvent and the synthetic procedure is shown in Scheme 1. The reaction mixture was introduced into 100 mL three necked round bottom flask in nitrogen atmosphere and the reaction mixture was heated to 120°C. The temperature was maintained for 3 hours and the reaction mixture was further heated to 150°C and retained the temperature for 72 hours. The resultant precipitate was filtered, washed several times with water and methanol. Finally, the obtained melamine based condensed polyamide (MT-1, 2.0895 g) white powder, was dried at room temperature for about 24 hours.



Scheme 1. Synthesis of Melamine-based Polyamide (MT-1)

2.3. Characterization Techniques

The synthesized melamine-based polyamide MT-1 was characterized by spectral investigations. UV-Visible spectrum was recorded in Jasco V-750 UV-spectrophotometer using a solution of the polymer in DMSO.FT-IR (Thermo Nicolet 380 FT-IR spectrophotometer) and ^{1}H-NMR and ^{13}C-NMR (Bruker Avance III 400 NMR spectrometer, 400 MHz) spectroscopy were used to establish the structural units present in the polymer chain. The TG-DTA analysis was studied by using NETZSCH-STA 449 F3 JUPITER. The FESEM with EDAX analysis was studied by using CARL ZEISS-SIGMA 300 FESEM with EDAX.

2.4. Antimicrobial study

A freshly inoculated agar plate seeded with test organisms was used to facilitate the diffusion of antimicrobial agents from the sample into the medium. This resulted in a uniform confluent lawn of growth

with clearly defined circular zones of inhibition. The diameters of the inhibition zones were measured in millimeters [43]. Similarly, the antifungal agent in the sample was allowed to diffuse into the medium using plates freshly seeded with the test organisms, producing uniformly circular zones of inhibition, the diameters of which were measured in millimeters [44-46] (Seesupplementary information). Larvicidal activity of the synthesized sample has been examined by using the procedure of WHO with some modification (Seesupplementary information).

III. RESULTS AND DISCUSSION

3.1 Physical Properties of MT-1

3.1.1. Solubility of MT-1

The polymer obtained is a white amorphous substance. Its solubility was determined qualitatively in thirteen solvents of varying polarity. The synthesized polyamide MT-1 is soluble in dimethyl acetamide (DMAC), dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) even at room temperature (Table I). However it is sparingly soluble in hot acetone while it is insoluble in acetone at room temperature. In both cold and hot conditions, the MT-1 is insoluble in ethanol, toluene, benzene, chloroform, ethyl acetate, diethyl ether, n-hexane, methanol and water (Table II).

Table I. Solubility of MT-1

S. No.	Solvent	Conditions	
		Cold	Hot
1	Benzene	Insoluble	Insoluble
2	Chloroform	Insoluble	Insoluble
3	Ethyl acetate	Insoluble	Insoluble
4	Diethyl ether	Insoluble	Insoluble
5	n-Hexane	Insoluble	Insoluble
6	Methanol	Insoluble	Insoluble
7	Water	Insoluble	Insoluble
8	Acetone	Insoluble	Partially soluble
9	Ethanol	Insoluble	Insoluble
10	Toluene	Insoluble	Insoluble
11	Dimethyl acetamide	Soluble	Soluble
12	Dimethyl formamide	Soluble	Soluble
13	Dimethyl sulfoxide	Soluble	Soluble

3.1.2 Density of PTT-1

The density of MT-1 was determined at five different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0%) in DMAc medium and the data are summarized in Table II. It may be noted that DMAc solution of MT-1 is lighter than water the density of MT-1 is increases uniformly with increasing concentrations of MT-1.

Table II. Density of PTT-1 in DMAC

Concentration of PTT-1 solution in DMAC (C, %)	Density of PTT-1 solution (d, g/mL)
0.2	0.9244
0.4	0.9280
0.6	0.9469
0.8	0.9499
1.0	0.9504

3.1.3 Viscosity of PTT-1

The kinematic viscosity of the polyamide MT-1 has been determined at five different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0%) in DMAc solution at 303K nd summarized in Table III . It is observed that kinematic viscosity of MT-1increases with increasing concentration (Fig. 2). The kinematic viscosity is greater than unity confirming the formation of polymer.

Table III. Kinematic Viscosity of PTT-1 in DMAC at 303K

Concentration of PTT-1 solution in DMAC (C, %)	Flow time of PTT-1 solution (sec)				Relative viscosity ($\eta_{rl}=T/T_0$)	Specific viscosity ($\eta_{sp}=\eta_{rl}-1$)	Reduced viscosity ($\eta_{red}=\eta_{sp}/C$	Inherent viscosity $\eta_i=\ln(\eta_{rl})/C$	Absolute viscosity ($\mu=\eta_{rl}$ x 0.92)	Density of PTT-1 solution (d, g/mL)	Kinematic viscosity (v=μ/d)
	T_1	T_2	T_3	T							
0.2	25.1	25.3	25.3	25.4	1.13	0.16	0.87	0.82	1.04	0.94	1.14
0.4	26.4	26.2	26.2	26.2	1.23	0.21	0.53	0.53	1.11	0.95	1.15
0.6	27.2	27.1	27.3	27.3	1.22	0.23	0.42	0.36	1.14	0.98	1.21

0.8	28.1	28.3	28.3	28.3	1.31	0.32	0.33	0.34	1.22	0.98	1.27
1.0	29.5	29.5	29.3	29.4	1.35	0.33	0.36	0.33	1.25	0.94	1.33

*Flow time of solvent (T_o) = 21.54333 sec

3.2 Spectralcharacterization

3.2.1 UV-Visible spectrum

UV-Visible spectrum was recorded using the solution of the polymer in DMSO and the spectrum is given in Fig. 1.The UV-Visible spectrum of MT-1 shows two absorption maxima, one at shorter wavelength, 238 nm. This absorption may be due to strong π→π* transition from the aromatic π-systems and triazine π-systems. Another peak at longer wavelength, 300 nm (Fig. 1) and it is attributed to n→π* transition involving lone pairs on heteroatoms (eitherN or S of thiophene system). It may be noted that it is weaker than the peak due to π→π* transition.

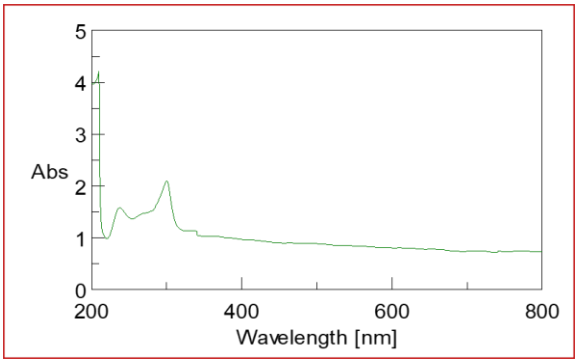


Fig. 1. UV-Visible sspectrum of MT-1

3.2.2 FT-IR study of MT-1

The FT-IR spectrum of the polymer is obtained by incorporating it in a KBr pellet. The vibrational frequency observed at 3107 cm^{-1}, is assigned to aromatic C-H stretching vibration of MT-1 and TDCA moieties (Table I, Fig. 2) [47-55]. The N-H stretching vibration of polyamide linkage between the MT-1and TDCA is observed at 3340 cm^{-1} (Fig. 2).

Table-I. FT-IR stretching vibrations of MT-1

S. No.	Vibrational Frequency (KBr, ν in cm^{-1})	Assignments
1	3340	N-H stretching
2	3107	C-H stretching
3	1658	C=O stretching
4	1572	C-O stretching, C=C stretching & deformation of N-H
5	1520	C=N stretching
6	1372	C-N stretching & C-C stretching
7	1331	C-O stretching & C-C stretching
8	1230	C-O stretching & C-C stretching
9	1108	C-O stretching & C-C stretching
10	1033	C-O stretching & C-C stretching
11	957	deformation of C-H
12	910	deformation of C-H
13	804	deformation of C-H
14	750	deformation of C-H
15	695	C-S stretching
16	678	C-S stretching
17	555	C-S stretching
18	464	C-S stretching

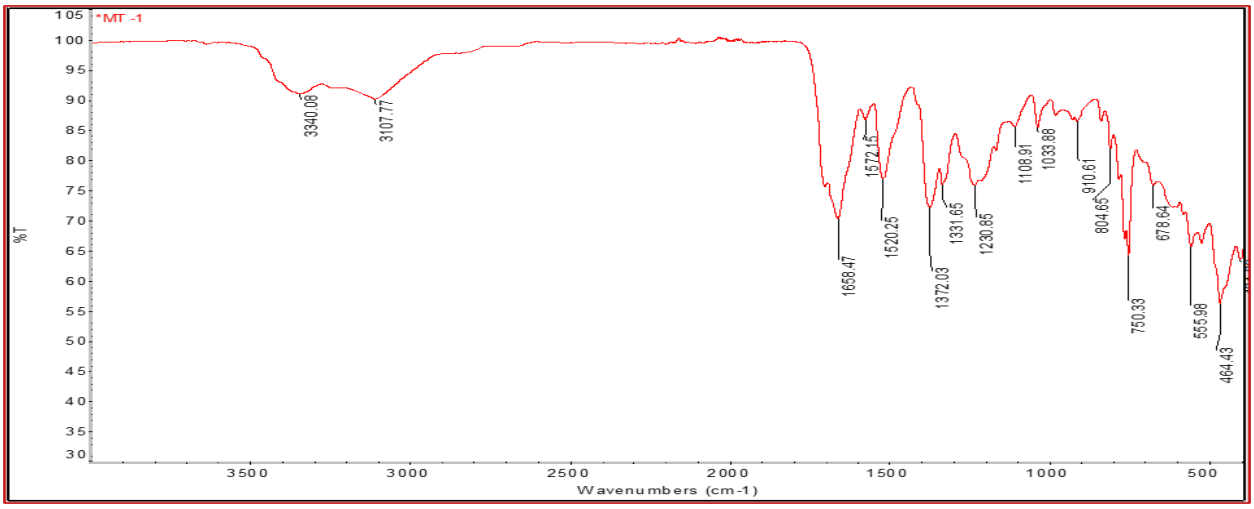


Fig. 2. FT-IR spectrum of MT-1

The vibrational band at 1520 cm^{-1} is due to C=N stretching frequency [47-55]. In addition, the C-N stretching vibration and the C-C vibration are observed at 1372 cm^{-1}. The stretching vibration of C=O is observed at 1658 cm^{-1} (Table I, Fig. 2). The C-O, C=C stretching and deformation of N-H vibrations are shown at 1572 cm^{-1} [52-54]. The vibrational frequency is observed at 1572 cm^{-1} and the vibrational frequency at 695, 678, 555, 464 cm^{-1} are assigned to C=C stretching and C-S stretching vibrations respectively. The deformation of C-H is observed at 957, 910, 804 and 750 cm^{-1} (Table I, Fig. 2). Amide (C=O): A very strong and sharp peak at 1658 cm^{-1}, primarily indicating the newly formed carbonyl group. Amide (N-H bending & C-N stretching): A prominent peak around 1572 cm^{-1}.C-N stretching: A band of medium intensity appearing between 1200 cm^{-1} to 1400 cm^{-1}.

3.2.3. NMR study of MT-1

The ^{1}H-NMR spectrum of MT-1contains three absorptions at δ = 7.59, 7.58 ppm as doublet and another three protons are assigned at 7.16-7.02 ppm as multiplet. These are indicating the protons of thiophene moiety in the polymer. The characteristic polyamide proton peak is observed at δ = 4.41 ppm as broad singlet peak which is attributed to the amide (-NH-CO-) protons (Fig. 3). The absence of free carboxylic acid peak in the ^{1}H-NMR spectrum of MT-1, confirms the formation of polyamide linkage between the melamine (MA) and 2,5-Thiophenedicarboxylic acid (TDCA, Fig. 3). In the ^{13}C-NMR spectrum of MT-1, the aromatic azomethine carbons of the melamine moiety are showed at δ = 164.39 ppm (Fig. 4) The polyamide carbonyl carbon is observed at δ = 163.51 ppm. The aromatic carbons of the 2,5-Thiophenedicarboxylic acid (TDCA) are retained at δ = 142.38 and δ = 132.36 ppm.

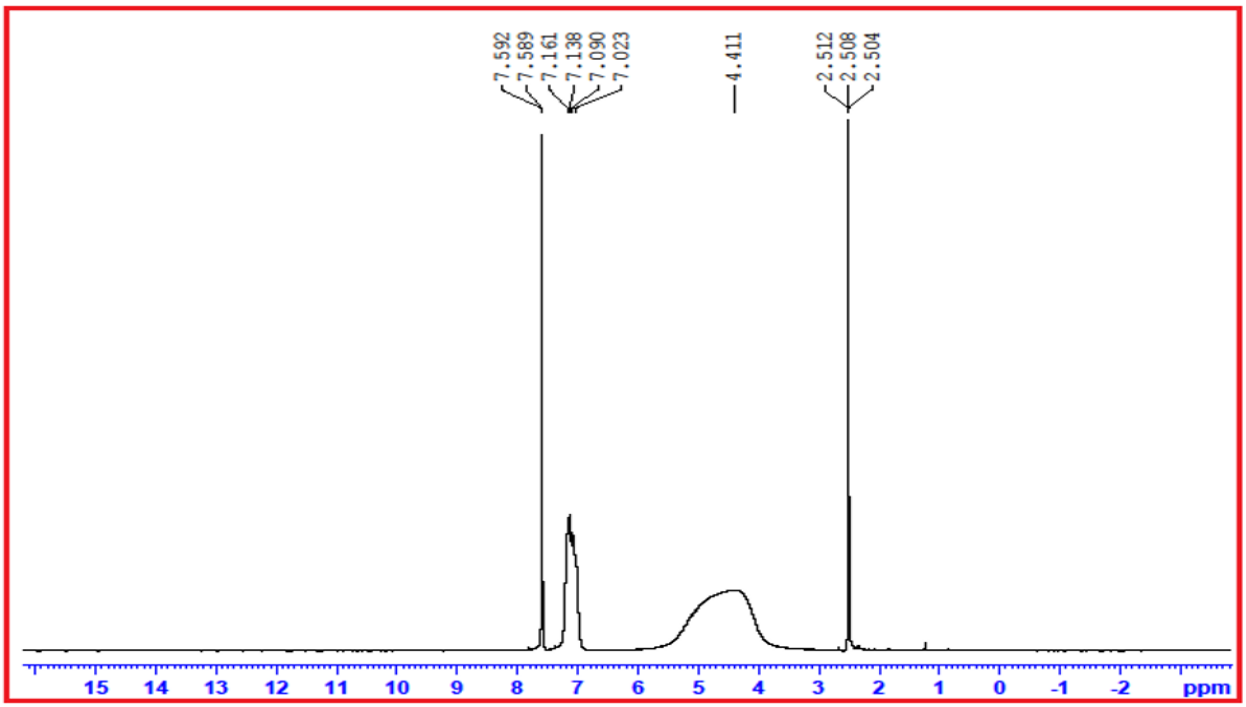


Fig. 3. ^{1}H-NMR spectrum of MT-1

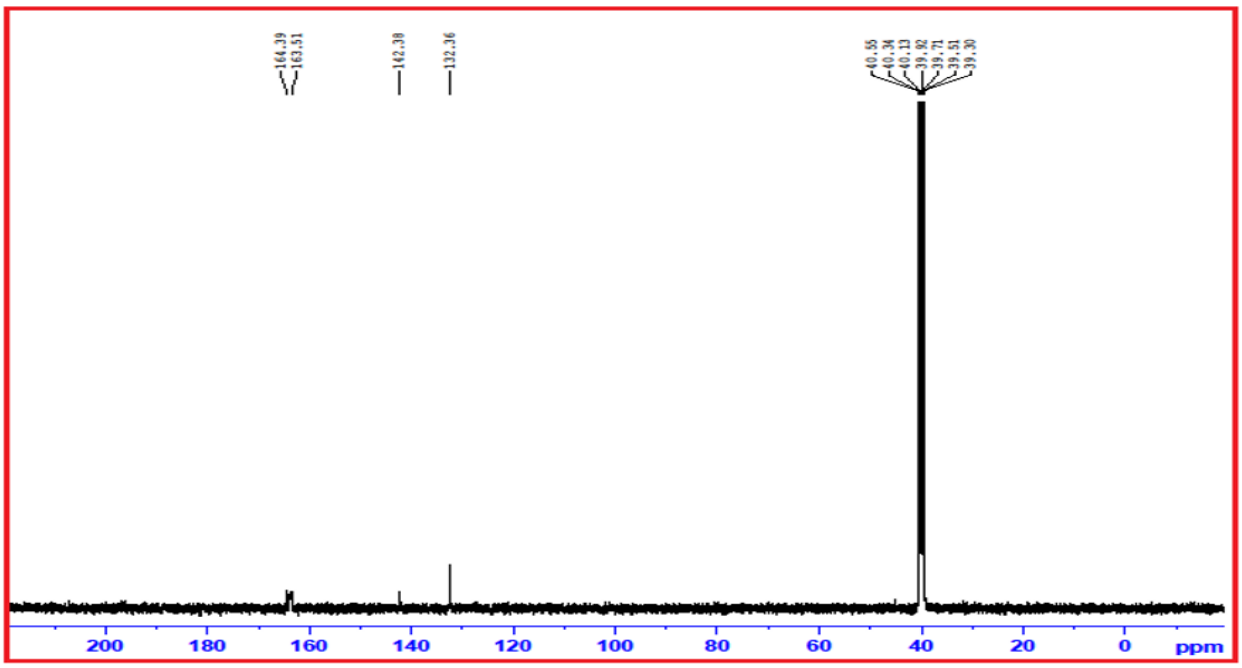


Fig. 4. ^{13}C-NMR spectrum of MT-1

3.3 Thermal Properties of MT-1

To ascertain thermal stability of MT-I and to determine thermal transition temperatures, TG and DTA thermograms were recorded. It is found that the polymer has Tg value of 129.3^{0}C. The endotherm observed at 213˚C in DTA thermogram (Fig.5) of the polymer is due to melting and this shows that the polymer is thermally stable uptoabout 213˚C (Fig.6) and processing of this polymer is possible below this temperature. In order to determine the thermal transition temperatures accurately DSC thermogram was recorded and it also confirmed the same T_g and melting temperatures as deduced in TG-DTA curves.

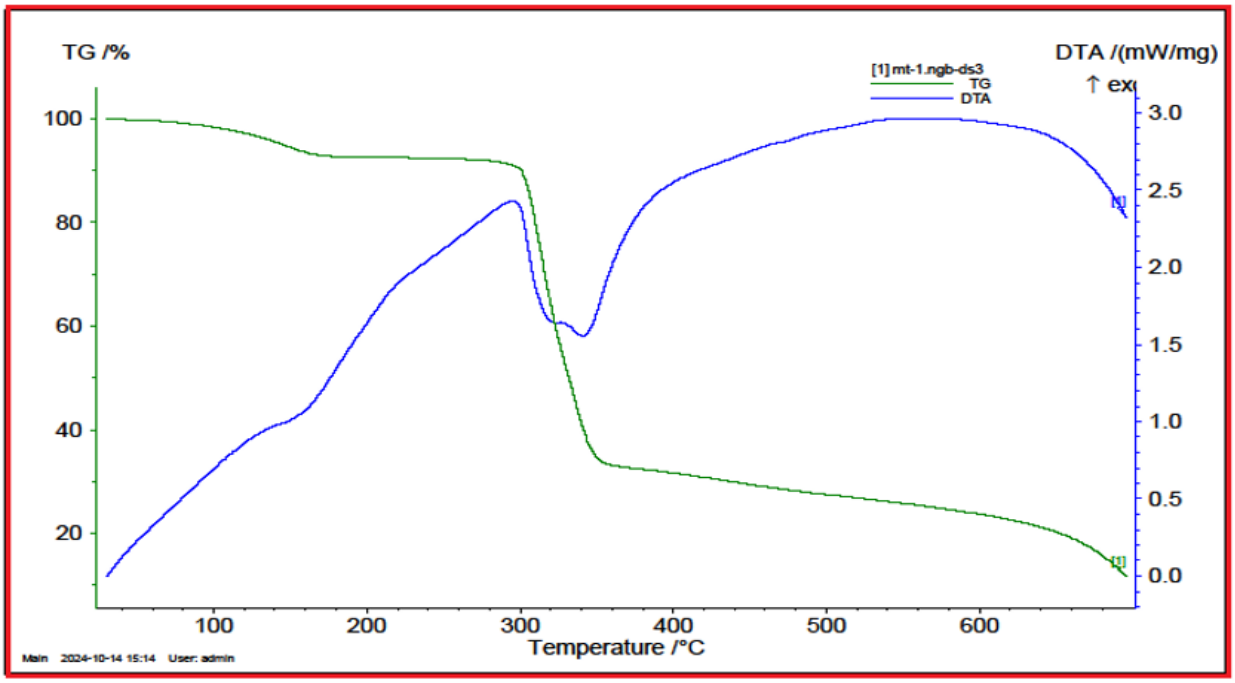


Fig. 5. TG-DTAthermogram of MT-1

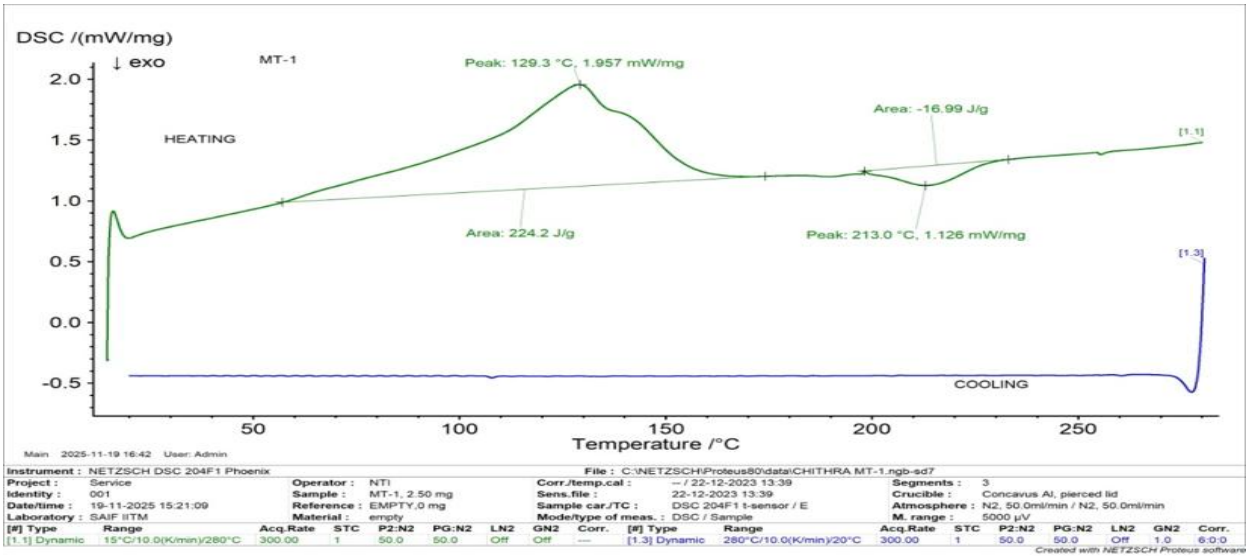


Fig.6. DSC thermogram of MT-1

3.4. SEM-EDAX Study of MT-1

In order to ascertain the morphology of the polymer SEM images were taken (Fig. 7). SEM image reveals that the synthesized s-Triazine condensed polyamide MT-1 is of in rod-shaped homogeneous morphology (Fig.7 (a)) [55]. All SEM images of polyamide MT-1 show that there is a moderate agglomeration present in between polyamide (Fig. 7(b)). The SEM-EDAX shows that the weight and atomic percentage of the elements (CNOS) present in the synthesized polyamide MT-1 (Fig. 7(c)). The results of the weight and atomic percentage of the carbon, nitrogen, oxygen, sulphur were obtained as 36.08 and 42.05%, 32.35 and 39.32%, 18.03 and 15.77%, 6.54 and 2.86% respectively. These values establish the structure of repeating units in the polymer chain.

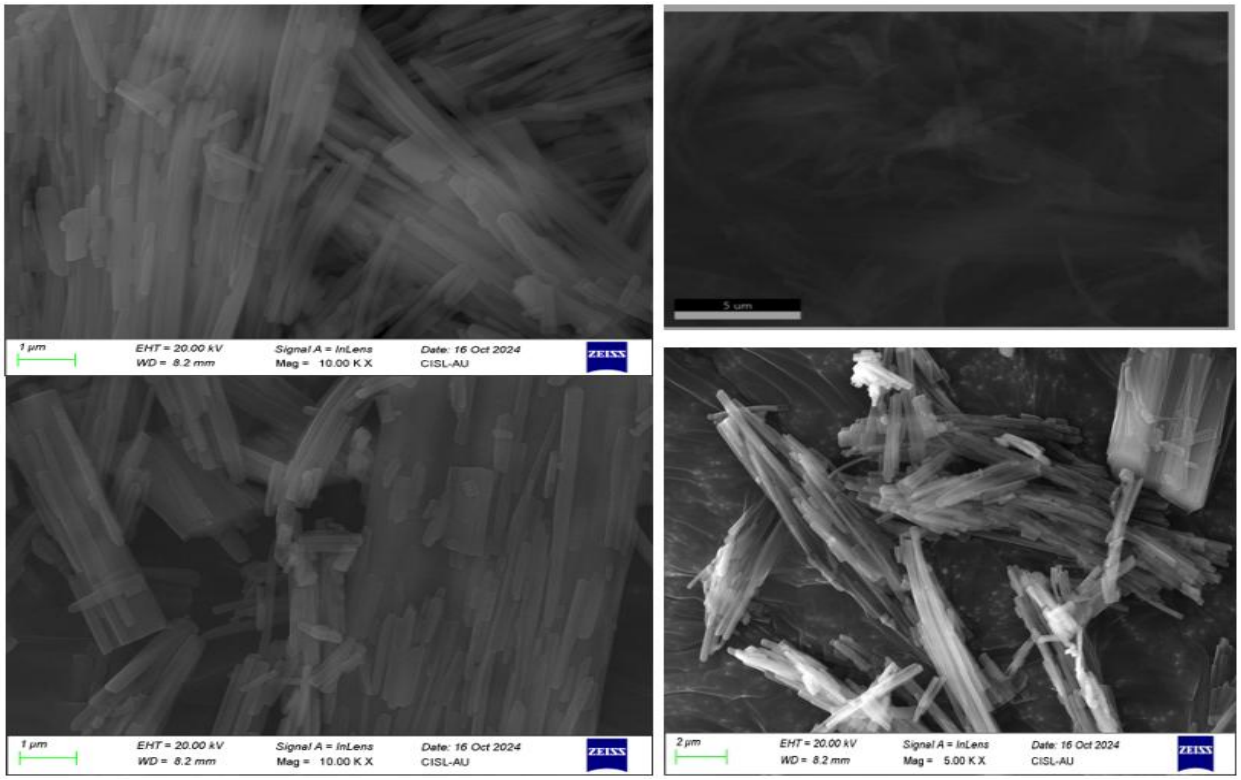


Fig. 7(a). SEM images of MT-1

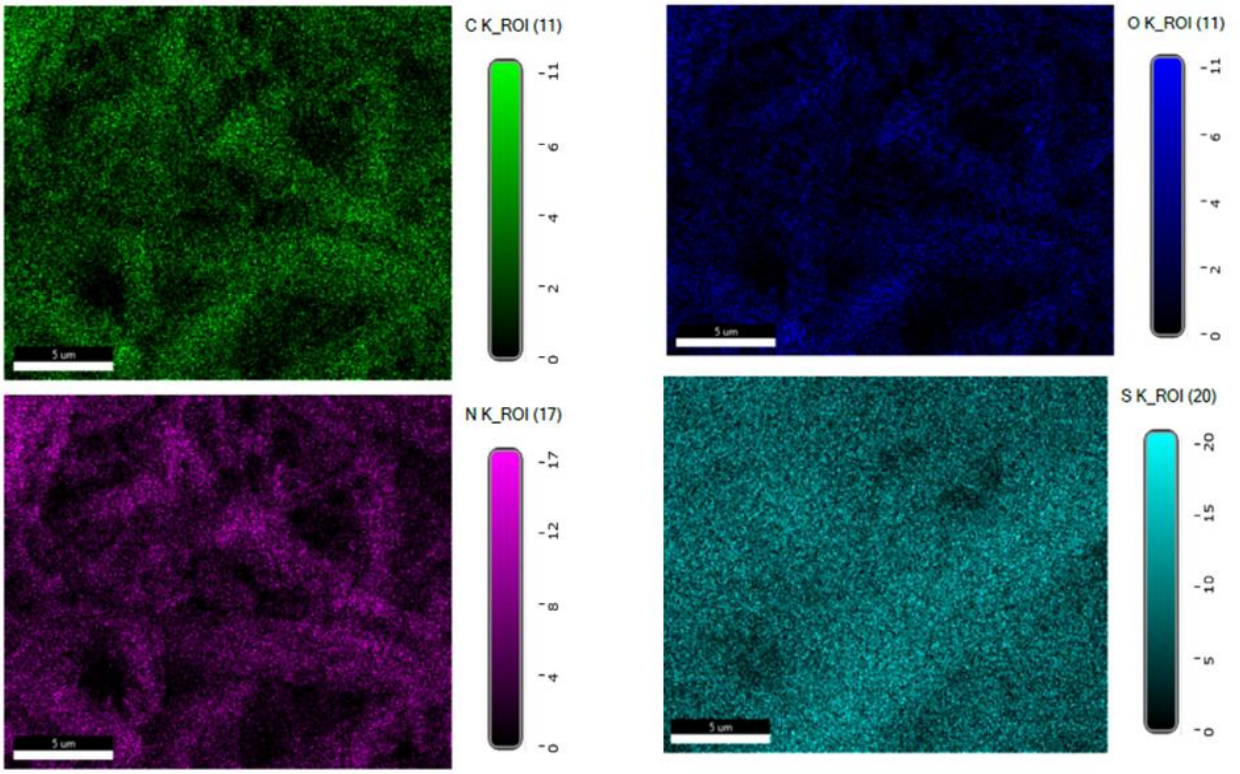
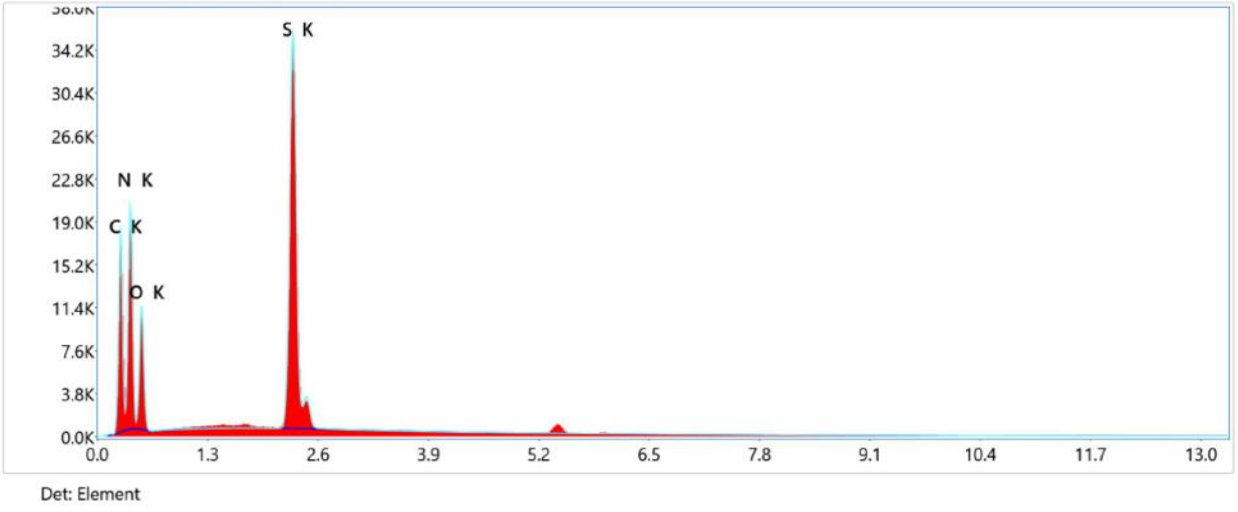


Fig. 7 (b). SEM-EDAX of MT-1



Quant Results

Element	Weight %	Atomic %
C K	36.08	42.05
N K	39.35	39.32
O K	18.03	15.77
S K	6.54	2.86

Fig.7 (c). SEM-EDAX Quant results of MT-1

3.5. Biological Evaluation of MT-1

3.5.1. Antibacterial activity of MT-1

The synthesized polyamide MT-1 did not show any accepted bacterial inhibition against all the tested bacterial pathogens at the concentration of 50 µg/mL and 100 µg/mL (Table III, Figs. 8-11). The MT-1 exhibited very good activity against S.oralis compared to standard drug (16.75±0.35) and the zone of inhibition is acquired as 10.25±0.35 mm and 12.0±0.70 mm at the concentration of 250 µg/mL and 250 µg/mL respectively (Table III, Fig. 8). The MT-1 exhibited moderate inhibition activity against P.acnes compared to standard drug (22.75±0.35) and the zone of inhibition is acquired 10.5±0 mm and 12.25±0.35 at the concentration of 250 µg/mL and 250 µg/mL respectively (Table III, Fig. 8). The MT-1 exhibited an excellent inhibition activity against A.hydrophila compared to standard drug (15±1.41) and the zone of inhibition is acquired 12.75±0.35 mm and 13.5±0.70 at the concentration of 250 µg/mL and 250 µg/mL respectively (Table III, Fig. 9). The MT-1 exhibited moderate inhibition activity against K.pneumoniae compared to standard drug (20.75±1.06) and the zone of inhibition is acquired 11.25±0.35 mm and 12±0 at the concentration of 250 µg/mL and 250 µg/mL respectively (Table III, Fig. 10).

Table III. Means ± SDof zone of inhibition obtained by sample MT-1 against Test organisms

S. No	Name of the test organism	Zone of inhibition (mm) Mean±SD				
		500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	S.oralis	12.0±0.70	10.25±0.35	0	0	16.75±0.35
2.	P.acnes	12.25±0.35	10.5±0	0	0	22.75±0.35
3.	A.hydrophila	13.5±0.70	12.75±0.35	0	0	15±1.41
4.	K.pneumoniae	12±0	11.25±0.35	0	0	20.75±1.06

SD–Standard Deviation, *Significance - p< 0.05

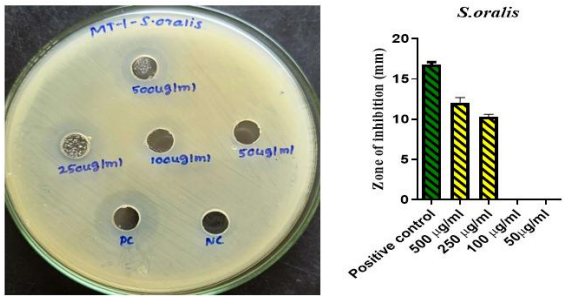


Fig.8. Antibacterial effect of sample MT-1 against S.oralis.

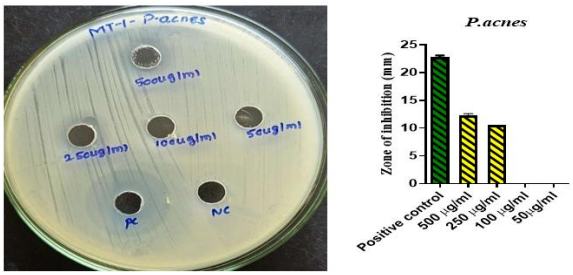


Fig. 9. Antibacterial effect of sample MT-1 against P.acnes.

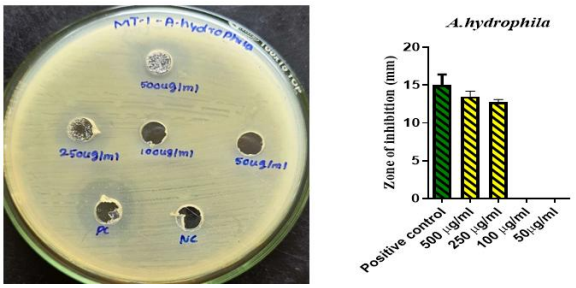


Fig. 10. Antibacterial effect of sample MT-1 against A.hydrophila.

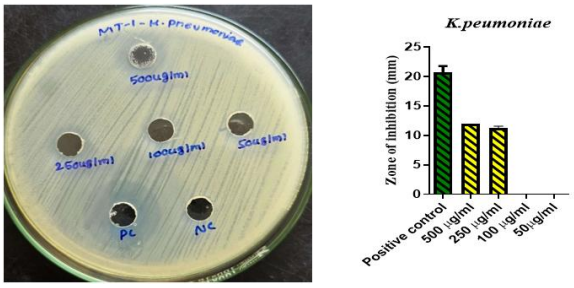


Fig. 11. Antibacterial effect of sample MT-1 against K.pneumoniae.

3.5.2. Antifungal activity of MT-1

The synthesized polyamide MT-1 is showing good to limited fungal inhibition against tested two fungal pathogens (Table IV, Fig. 12& 13). The MT-1 is

showing (Table IV, Fig. 12), very good fungal inhibition against the pathogen Aspergillus niger at the concentration of 500 µg/mL only (12.5±0.70 mm) and other tested concentrations did not show any satisfactory inhibition compared to standard drug (14.75±0.35). At the concentration of 500 µg/mL only (11.25±0.35 mm) and other tested concentrations did not show any satisfactory inhibition compared to standard drug (19.25±1.06) against the pathogen Candida albicans (Table IV, Fig. 13).

Table IV. SD± Means of zone of inhibition obtained by sample MT-1 against Candida albicans, Aspergillusniger.

S. No.	Name of the test organism	Zone of inhibition (mm) SD ± Mean				
		500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	Candida albicans	11.25±0.35	0	0	0	19.25±1.06
2.	Aspergillus niger	12.5±0.70	0	0	0	14.75±0.35

SD Standard Deviation, *Significance - p< 0.05

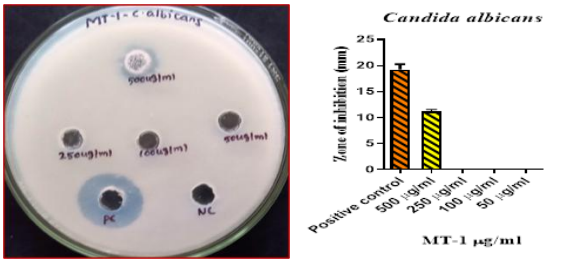


Fig. 11. Antifungal effect of MT-1against Candida albicans

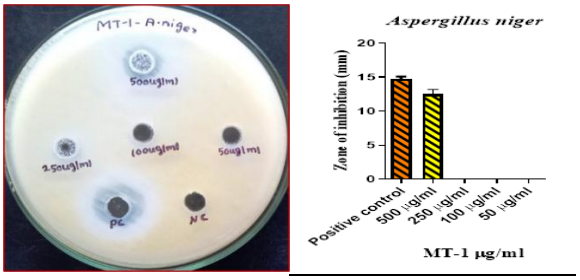


Fig. 12. Antifungal Effect of MT-1against Aspergillus niger

3.5.3. Larvicidal Activity of MT-1

The lavicidal activity and percentage of mortality are demonstrated in Tables V & VI, and Figs 13 & 14. The larvicidal activity of MT-1 is increasing with increase in concentration (Table 5). The live larva is reduced as well as the larva mortality is also increased (Tables V & VI, Fig. 13& 14). At the concentration of 10 µg/mL, 50 µg/mL and 100 µg/mL, the MT-1 is showing no potential larvicidal activity (D: 0, L: 4) compared to standard drug (D: 4, L: Nil). In the concentration of 250 µg/mL, the MT-1 is showing moderate larvicidal activity (D: 1, L: 3) compared to standard drug (D: 4, L: Nil). The MT-1 is showing a good larvicidal activity (D: 2, L: 2) compared to standard drug (D: 4, L: Nil).

Table V. Larvicidal activity of MT-1

Name of the sample	500 µg/ml		250 µg/ml		100 µg/ml		50 µg/ml		10 µg/ml		Control	
	D	L	D	L	D	L	D	L	D	L	D	L
MT-1	2	2	1	3	0	4	0	4	0	4	4	0

Table VI. Percentage of mortality of MT-1

Name of the sample	500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	10 µg/ml	Control
MT-1	50	25	0	0	0	0

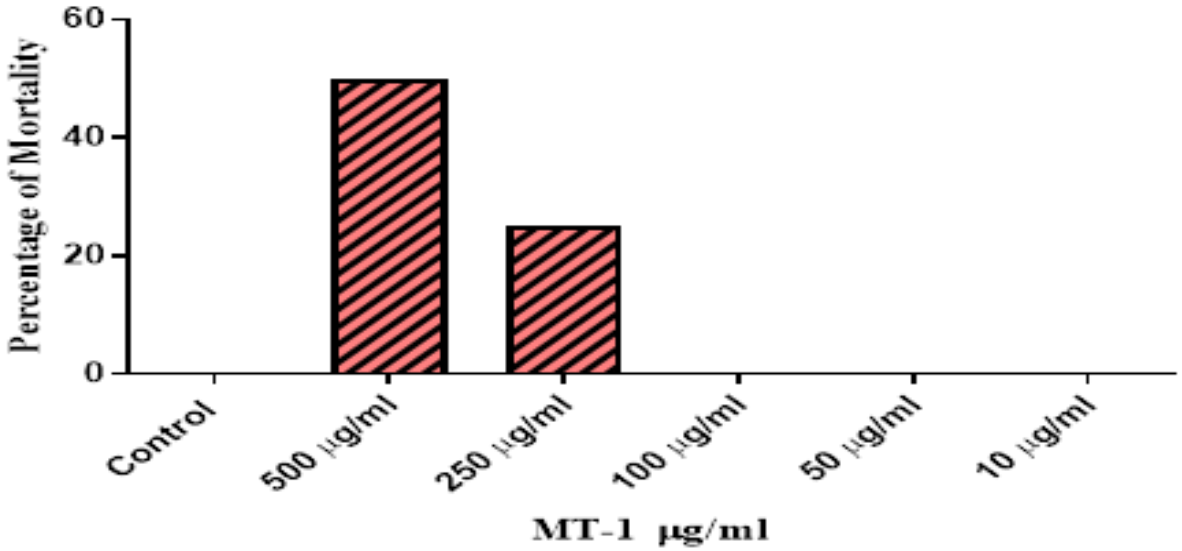


Fig. 13. Percentage of Mortality of MT-1

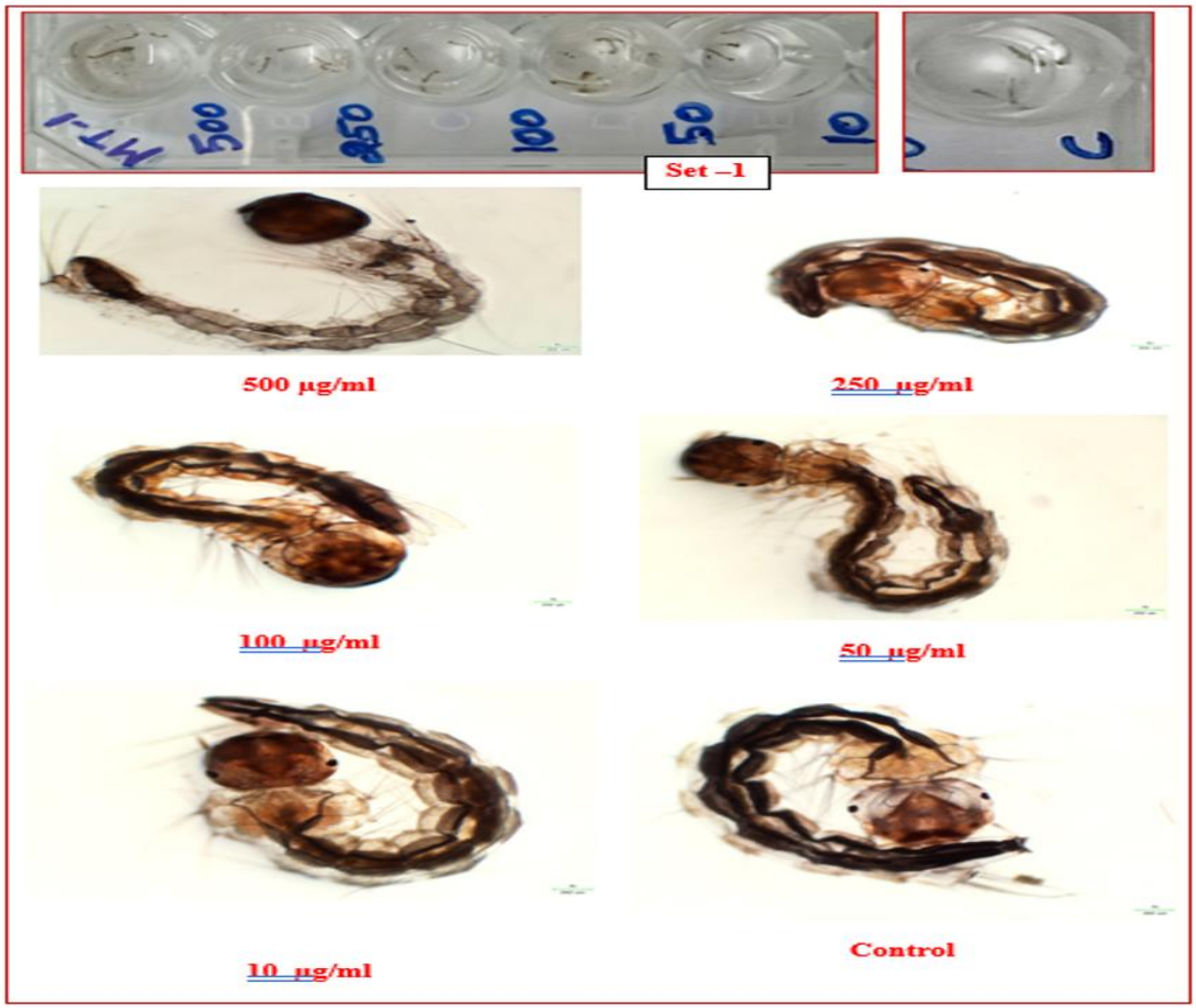


Fig. 14.Lavicidal activity and Mortality of MT-1

IV. CONCLUSION

This paper deals with one pot synthesis of a new polyamide containing melamine, s-triazine and thiophene moieties. It was prepared from Melamine (MA) and 2,5-Thiophene dicarboxylic acid (TDCA) using methanol as a solvent in an inert atmosphere at 150°C. The DMA and DMSO solvents at room temperature, sparingly soluble in acetone, but insoluble in other common organic solvents. The polymer had a T_g value of 129.3° C and melting temperature of 213°C. Thus MT-1 polymer is thermally stable above 200°C. Since the polymer contains nitrogen heterocyclic and sulphur heterocyclic rings it may posseess antimicrobial properties. The MT-1 polyamide exhibited very good activity against the pathogen S.oralis compared to standard drug. The synthesized polyamide has been found to have very good fungal inhibition against tested two fungal pathogens. The lavicidal activity and percentage of mortality are also demonstrated and it is found that the larvicidal activity of MT-1 is increasies with increase in concentration. The live larva is reduced as well as the larva mortality is also increased at specific concentrations.

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