**Synthesis and Biological investigations of newly synthesized substituted imidazole[2,1-c] [1,2,4] triazole derivatives from imidacloprid**

**M. B. Deshmukh1, S.R. Yankanchi1, S. M. Deshmukh2 and Bhagawan Patil1\***

1Dept of Agrochemical & Pest Management, Shivaji-University, Vidyanagar, Kolhapur-Maharashtra, INDIA.

2Dept of technology, Shivaji-University, Vidyanagar, Kolhapur-Maharashtra, INDIA.

**\* Email id: bvpatil777@gmail.com**

**ABSTRACT**

New molecules with promising insecticidal properties like imidazo[2,1-c][124] triazole analogs were synthesized, starting with imidacloprid and bio-assayed. The structures of synthesized molecules were confirmed with diverse modern methods like FT-IR, 1H NMR, 13C NMR, and Mass spectrometry. The synthesized molecules are screened to investigate their insecticidal and anti-bacterial activity. The bioassay screening showed that synthesized compounds chloro (3-(4-chlorophenyl)-7-[(6-chloropyridin-3-yl)methyl]-2,5,6,7-tetrahydro-3H-imidazo [2,1-c] [1,2,4] triazole (4b), the 3-(2-chlorophenyl)-7-[(6-chloropyridin-3-yl)methyl]-2,5,6,7-tetrahydro-3H-imidazo[2,1-c] [1,2,4] triazole (4c), nitro(7-[(6-chloropyridin-3-yl)methyl]-3-(4-nitrophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c] [1,2,4] triazole (4d) and 7-[(6-chloropyridin-3-yl)methyl]-3-(2-nitrophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c] [1,2,4]triazole (4e) showed higher bioactivities than imidacloprid against Hubner (*H.armigera)*, Mealybugs (*Planococcus citri*), and Mango hoppers (*Idioscopus clypealis*), and tobacco and tomato bacterial wilt. The results of biological activity were determined. Compounds with electron-withdrawing groups showed potential as vector control agents.

**Keywords:** Substituted Triazole, Synthesis, Characterization, Biological Activity.

**INTRODUCTION**

*H. armigera* (Hübner), Mealybugs (*Planococcus citri*), and Mango hoppers (*Idioscopus clypealis*) are recognized as an insect pest having highly harmful potential for various commercially important crops across the globe like tomatoes, cotton, corn, tobacco, and soybean1. Nicotine is one of the earliest known insecticides with a plant origin and remarkable insecticidal properties. Nicotine quickly kills the insects within an hour, causing tremors, convulsions, and eventually paralysis. The crude extract of tobacco leaves showed insecticidal properties and used to was manage insects before 1746. According to Metcalf, 1.2 million pounds of free nicotine were used in farming in the United States during 19442. Some biological traits, like mobility, polyphagy, and facultative diapauses, can boost pest survival and population growth in agrosystem3. These pests, which attack over 150 different host species, are regarded as the most commercially important insect pests in several countries, including Japan, China, India, and Southeast Asia4. Because of their biological characteristics and higher damage potential, successful control of these pests has become tough work in the prevention and management of *H. armigera5*.Triazole6-16 and imidazole17-19 derivatives showed antibacterial properties. Nevertheless, relying solely on the use of synthetic insecticides to eradicate *H. armigera* has not been much effective and has led to the emergence of pesticide resistance, environmental contamination, disruption of ecological stability, and health risks20.

Insecticides with neonicotinoid active ingredients, such as imidacloprid21, are the newest class of synthetic insecticides to enter the market in the last two decades. As a result, efforts have been made to develop substituted techniques for its management. The discovery of novel insecticides recently highlighted the importance of a heterocyclic moiety, and several modifications in their structure have been reported. The current work creates new insecticidal molecules by incorporating a hydrazone substructural unit into the imidacloprid chemical structure. An imidacloprid derivative with a substituted triazole substructure is designed and synthesized based on this supposition. According to biological tests, the insecticidal activities of the synthesized compound against various insect species are promising.

**Experimental procedure**

**Materials and Methods**

All the reagents and chemicals were purchased from E. Merck chemical company ltd. and used without further purification. Melting points were determined by an open capillary method and are uncorrected. Thin layer chromatography is performed with E. Merck pre-coated silica gel plates with iodine as a developer. FTIR spectra were recorded in KBr pellets on a Perkin-Elmer FTIR 783 spectrometer. 1H NMR (400 MHz) and 13C NMR (100 MHz) spectra were recorded in CDCl3 as a solvent containing tetramethyl silane (TMS) as an internal reference on Bruker Avance II (400 MHz) spectrometer. Elemental analysis was performed on a PerkinElmer 2400 and the mass spectra were obtained by using QP2010 (Shimadzu) spectrometer.

**Synthetic procedure**

**Synthesis of 2-chloro-5-{[-2-hydrazinylideneimadazolidin-1-yl] methyl} pyridine (2):**

1-[(6-Chloropyridin-3-yl) methyl]-N-nitroimidazolidin-2-imine (0.255 gm, 1 mmol) and SnCl2.2H2O (0.113 mg, 0.5 mmol) are taken in 10 ml of absolute ethyl alcohol and heated on a steam bath at 70°C-80°C. After the completion of the reaction, the mixture was allowed to cool and concentrated and poured into an ice water mixture. 5% NaOH was added to make pH alkaline and then extracted with ethyl acetate. The organic layer is thoroughly washed with Braine solution and dried over sodium sulphate to get a yellowish-brown compound dried over sodium sulphate. **Results and Discussion**

As per Scheme-1, 7-[(6-chloropyridin-3-yl) methyl]-3-substituted phenyl-2,5,6,7-tetrahydro-3H-imidazo[2,1-c] [1,2,4] triazole (4a-f) was obtained from 2-chloro-5-({(2E)-2-[(2E)- (substituted phenyl methylidene) hydrazono] imidazolidin-1-yl} methyl) pyridine(3a-f) by intramolecular cyclization by using POCl3. All compounds (3a-f) were prepared by reacting with 2-chloro-5-({-2-hydrazinylidene imadazolidin-1-yl] methyl} pyridine with the different aromatic aldehyde. 2-Chloro-5-({-2-hydrazinylidene imadazolidin-1-yl] methyl} pyridine (2) was obtained by the reduction of imidacloprid, to get desired compound 2. Compound sl. no.3a-f and sl. no. 4a-f also purified and analysed by FT-IR, 1H NMR, 13C NMR and Mass spectral data. (Scheme-1)

SCHEME 1 ****

# Spectral Data of Compound

2-Chloro-5-{[-2-hydrazinylideneimadazolidin-1-yl] methyl} pyridine (2)

Yield: (0.344g.),79%, Melting Point: 148°C.

**FTIR (KBr, νmax cm-1):** 3408, 3302(NH str.), 2908 (CH2 str.),2859 (C-H), 1617(C=N str.), 1444 (CH=CH str.), 758(C-Cl str).

**1H NMR (CDCl3, ppm):** δ, 3.15(2H, t, N-CH2), 3.45 (2H, t, NH-CH2), 4.50(2H, s, Ar-CH2), 5.0 (2H, s, NH2), 6.1(2H, s, NH), 7.4 (1H, Py-H), 8.30 (1H, s, Py-H), 8.99 (1H, s, N-H).

**13C NMR (CDCl3, ppm):** δ,159, 150, 149, 139, 130, 124, 100,50, 46

**MS (C9H12N5Cl), (m/z):** 225(M+), 189, 184,183,125, 100, 87, 85, 69.

**Synthesis of 2-Chloro-5-({(2E)-2-[(2E)- (phenyl methylidene) hydrazono] imadazolidin-1-yl} methyl) pyridine (3a):**

The mixture of 2-chloro-5-{[-2-hydrazinylideneimadazolidin-1-yl] -methyl} pyridine (0.225g.,0.05mmol.) and benzaldehyde (0.53 gm, 0.05mmol.) in methanol (10 ml) was refluxed on a water bath for 4 hrs. using a catalytic amount of acetic acid (0.2 ml) and the progress of the reaction was monitored by TLC. After the completion of the reaction, the mixture was cooled and the separated product was filtered, dried, and recrystallized from ethanol to get 2-chloro-5-((2E)-2-[(2E)- (phenyl methylidene) hydrazono] imadazolidin-1-ylmethyl] pyridine.

**Spectral data of Compound (3a)**

Yield-(0.596g.,70 %), melting Point:146°C.

**FTIR (KBr, νmax cm-1):** 3304 (NH str.), 3006 (Ar CH str.), 2909(CH2 str,),1804(NH bending) 1616 (C=N str.), 1481, 1442 (CH=CH str.),757(C-Cl str.)

**1H NMR (CDCl3, ppm)**: δ ,3.4(2H, t, N-CH2), 3.51(1H, t, N-CH), 3.6(1H, t, N-CH), 4.5(2H, s, Py-CH2),7.25(1H,d,Py-H), 7.4(5H,m, Ar-H), 7.65(1H, d, Py-H), 7.8(1H, m, Py-H), 7.95(1H, s, NH), 8.4(1H, s,=CH).

**13C NMR (CDCl3, ppm):** δ, 173, 158, 151, 150, 148, 135, 132, 131, 129, 128, 124, 123, 49, 48, 45.

**MS (C16H16N5Cl), (m/z):** 313(M+), 285, 277, 210, 208, 188, 183, 125, 105, 87, 71.

The compounds 3b-f were synthesized using a similar method described for 3a and characterized.

**2-Chloro-5-{[(2E)-2-{(2E)-[(4-chlorophenyl) methylidene] hydrazono} imidazolidin-1-yl] methyl} pyridine (3b)**

Yield: (0.436g.,)69%, Melting Point:174°C.

**FTIR (KBr, νmax cm-1):** 3304(NH str.), 3008 (Ar CH str.), 2909(CH2 str.), 1616(C=N str.), 1481,1442(CH=CH str.), 757,702(C-Cl str.).

**1H NMR (CDCl3, ppm):** δ ,3.35(1H, t, N-CH), 3.50(2H, t, N-CH), 3.7(1H, t, N-CH), 4.5(2H, s, Py-CH2),7.13(1H,s,Py-H), 7.4(2H,m,Py-H), 7.55(2H,d, Ar-H), 7.63(2H, d, Ar-H), 7.83(1H, m, Py-H), 8.0(1H, s, NH), 8.4(1H, s,=CH)

**13C NMR (CDCl3, ppm):** δ, 172,158, 151, 150, 138, 137, 133,131.7,131.7,130.8,130, 124, 83, 57, 48, 45.

**MS (C16H15N5Cl2) (m/z):** 347(M+), 319,311,222,210,183,165, 137, 125, 87,86, 71, 69.

**2-Chloro-5-{[(2E)-2-{(2E)-[(2-chlorophenyl) methylidene] hydrazono} imidazolidin-1-yl] methyl} pyridine (3c)**

Yield: (0.613g.,)72%, Melting Point:168°C.

**FTIR (KBr, νmax cm-1):**3304(NH str,), 3008 (Ar CH str,), 2909(CH2 str,), 1616(C=N str.), 1481,1442(CH=CH str.), 757,698(C-Cl str.).

**1H NMR (CDCl3, ppm):** δ, 3.33(1H,m,N-CH), 3.5(2H,q,N-CH2), 3.55(1H,m,N-CH), 4.5(2H,s,Ar-CH), 7.1(1H,s,Py-H), 7.3(2H,d,Ar-H), 7.6(2H,d,Ar-H), 7.7(1H,s,Py-H), 8.3(1H,s,Py-H), 8.4(1H,s,=CH)

**13C NMR (CDCl3, ppm):** 173,160, 152,151, 138,135, 132, 134,133, 132,131,130, 124,49,48.9,48.1,42.

**MS (C16H15N5Cl2), (m/z):** 347(M+), 319, 311,265,250, 222, 210, 183, 137, 125, 87,86, 71,69**.**

**2-Chloro-5-{[(2E)-2-{(2E)-[(4-nitrophenyl) methylidene] hydrazono} imidazolidin-1-yl] methyl} pyridine (3d)**

Yield (0.522g.,)64%, Melting Point:152°C.

**FTIR (KBr, νmax cm-1):**3304(NH str.), 3008(ArCH str.),2909(CH2 str.), 1616(C=N str.), 1572,1528(NO2 str.),1481,1442(CH=CH str.), 757(C-Cl str.).

**1H NMR (CDCl3, ppm):** δ, 3.33(1H,m,N-CH), 3.5(2H,s,N-CH2), 3.55(1H,s,N-CH), 4.5(2H,s,Py-CH2), 7.1(1H,s,Py-H), 7.25(2H,d,Ar-H), 7.5(1H,s,Py-H), 7.8(2H,d,Ar-H), 8.0(1H,s,Py-H), 8.3(1H,s,=CH)

**13C NMR (CDCl3, ppm):** 173,158, 151,148.5, 148, 140,139, 138,132,131, 126, 125, 49, 48, 41

**MS (C16H15N6O2Cl), (m/z):** 358(M+), 330, 325,311,307, 283,251,210, 208, 183, 150, 148, 125, 97, 87, 80.

**2-Chloro-5-{[(2E)-2-{(2E)-[(2-nitrophenyl) methylidene] hydrazono} imidazolidin-1-yl] methyl} pyridine (3e)**

Yield: (0.492g.,)70%, Melting Point:129°C.

**FTIR (KBr, νmax cm-1):** 3304(NH str.), 3008 (Ar CH str.), 2909(CH2 str), 1616 (C=N str.),1576, (NO2 str.), 1442(CH=CH str.), 57(C-Cl str.),

**1H NMR (CDCl3, ppm):** δ, 3.3(2H,s,N-CH2), 3.5(2H,q,N-CH2), 4.5(2H,s,Py-CH2), 7.1(2H,m,Ar-H), 7.25(2H,m,Ar-H), 7.5(1H,m,Py-H), 7.55(1H,m,Py-H), 7.95(1H,s,Py-H), 8.3(1H,s,=CH).

**13C NMR (CDCl3, ppm):** 173,158,151, 148,139,138,134, 132,130, 127, 126, 123, 50,49,

**MS (C16H15N6O2Cl), (m/z):** 358(M+), 330, 322, 312,307,283, 277,210, 208, 183, 150, 145, 125, 97, 87, 80.

**2-Chloro-5-{[(2E)-2-{(2E)-[(4-methoxyphenyl) methylidene] hydrazono} imidazolidin-1-yl] methyl} pyridine (3f)**

Yield:(0.512g.,)71%, Melting Point: 92°C.

**FTIR (KBr, νmax cm-1):**3304(NH str.), 3006 (Ar CH str.),2909(CH2 str.), 2897 (CH3 str.), 1616(C=N str.), 1481,1442 (CH=CH str.),1102(COC str.), 757(C-Cl str.),

**1H NMR (CDCl3, ppm):** δ, 3.5(1H,s,N-CH2), 3.5(2H,m, N-CH2), 3.62(1H,m,N-CH), 3.7(3H,s,OCH3), 4.5(2H,s,Py-CH2), 6.4(2H,d,Ar-H), 6.65(2H,d,Ar-H), 7.1(1H,s,Py-H), 7.3(1H,d,Py-H), 7.7(1H,d,Py-H), 7.9(1H,s,Py-H), 8.3(1H,s,=CH).

**13C NMR (CDCl3, ppm):** δ, 173, 162, 158, 151, 148,146,141,138, 132, 130, 126,124, 115, 110,55, 49, 48, 45.

**MS (C17H18N5OCl), (m/z):** 343(M+), 335,315, 307,286,250, 218, 210, 208, 183, 135, 125, 87, 82,

**Synthesis of 7-[(6-Chloropyridin-3-yl) methyl]-3-phenyl-2,5,6,7-tetrahydro-3H-imidazo[2,1-c] [1,2,4] triazole (4a)**

In a rounded bottom flask fitted with a reflux condenser and calcium chloride guard tube, above 3a (1.2 gm, 0.049 mmol) (10 ml, 0.107 mmol) in POCl3 (10 ml) was taken and the mixture heated on an oil bath at 130 - 140°C for 5 hrs. and excess of POCl3 (10 ml) was removed under reduced pressure in an oil bath (80 -100 mm Hg)/50-60°C. The residue was slowly poured on a well-stirred mixture of 25 ml. of conc. ammonia solution, containing 50 gm. of ice, and 50 ml. of chloroform. Then conc. ammonia solution was added until the solution become basic and kept overnight. The organic layer was separated and an aqueous layer was extracted with an additional 20 ml of chloroform. The final mixture was left overnight. The organic layer was dried using CaCl2 and the separated product was crystallized from ethanol.

**7-[(6-Chloropyridin-3-yl) methyl]-3-phenyl-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4] triazole (4a)**

Yield (1.54g.,74%),Melting point: 158°C.

**FTIR (KBr, νmax cm-1):** 3261(NH str.), 3002 (Ar CH str.), 2901,2897(CH2 str), 1619(C=N str.), 1480,1446 (CH=CH str.), 756(C-Cl str.),

**1H NMR (CDCl3, ppm):** δ, 3.55(2H,m,CH2),3.71(2H,m,N-CH2) 4.22 (2H, s,Py-CH2), 4.25 (1H,s,CH), 6.4(1H,d,-NH), 7.1-7.6(5H,m,Ar-H), 8.1(1H,s,Py-H), 8.15(1H,d,Py-H), 8.25(1H,s,Py-H)

**13C NMR (CDCl3, ppm):** 161,155, 151, 150.5,150 137, 132, 129, 123, 119,110, 76, 69,51,49,45.

**MS(C16H16N5Cl), (m/z):** 313(M+), 285, 277, 250, 210, 208, 188, 183, 125, 105, 87.

Compounds Sl.No. 4b-f was synthesized by a similar procedure as described for the preparation of 4a.

**7-[(6-Chloropyridin-3-yl)methyl]-3-(4-chlorophenyl)-2,5,6,7-tetrahydro-3H-imidazo [2,1-c] [1,2,4] triazole (4b)**

Yield:(0.890g.,)57%, Melting point: 181°C.

**FTIR (KBr, νmax cm-1):** 3371(NH str.), 3006 (Ar CH str.), 2905,2888(CH2 str.), 1617(C=N str), 1480,1442(CH=CH str), 757,701(C-Cl str),

**1H NMR (CDCl3, ppm):** δ,3.5-3.75(4H, m, 2xN-CH2), 4.25(1H, s, N-CH),4.55(1H, d, CH), 6.15 (1H, d, NH), 6.8-7.4(5H, m, Ar-H), 7.7(1H, s, Py-H), 8.15-8.2(2H,m,Py-H).

**13C NMR (CDCl3, ppm):** 161,156, 151, 150, 143,137, 133, 129, 128, 125, 123, 119,69,51,49.4,45.

**MS (C16H15N5Cl2) (m/z):** 347(M+), 319, 311, 287,261,250, 237, 222, 208, 139, 125, 96, 86, 51.

**7-[(6-Chloropyridin-3-yl)methyl]-3-(2-chlorophenyl)-2,5,6,7-tetrahydro-3H-imidazo [2,1-c] [1,2,4] triazole (4c)**

Yield:(1.12g.,)65%, Melting point: 171°C.

**FTIR (KBr, νmax cm-1):**3369(NH str.), 3005 (Ar CH str.), 2905,2887(CH2 str.), 1617(C=N str.), 1481,1447(CH=CH str.), 756,698(C-Cl str.).

**1H NMR (CDCl3, ppm):** δ, 3.5-3.75(4H, m, 2xN-CH2), 4.5(2H, s, Py-CH), 4.55(1H,d,CH), 6.75 (1H, d, NH), 6.70(1H, s, Ar-H), 7.2-7.4(3H, m, Ar-H), 7.7(1H, s, Py-H), 8.2(1H,d,Py-H), 8.25(1H,s,Py-H).

**13C NMR (CDCl3, ppm):** 161,156, 151, 150,143, 137, 133, 128, 127, 123,118,110, 66, 52, 49, 45.

**MS (C16H15N5Cl2), (m/z):** 347(M+), 319, 311, 287, 237, 222, 208, 190,139, 125, 96, 87, 51.

**7-[(6-Chloropyridin-3-yl) methyl]-3-(4-nitrophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c] [1,2,4] triazole (4d)**

Yield:(1.55g.,)70%, Melting point: 156°C.

**FTIR (KBr, νmax cm-1):** 3371(NH str.), 3007(Ar CH str.),2908,2893(CH2 str.), 1619 (C=N str.),1575,1526(NO2 str.),1483,1445(CH=CH str.),756(C-Cl str.)

**1H NMR (CDCl3, ppm):** δ, 3.15(2H,d,N-CH2), 3.2(2H,d,N-CH2), 4.5(2H,s,Py-CH2), 4.6(1H,d,-CH), 6.25(1H,d,NH), 7.1-7.6(4H,m,Ar-H), 8.13(2H,d,Py-H), 8.15(1H,s,Py-H)

**13C NMR (CDCl3, ppm):** 156, 151, 150, 147, 137, 136,133, 131,127,126, 124, 123, 69, 58,51, 45

**MS (C16H15N6O2Cl), (m/z):** 358(M+), 330, 322, 311,237,233, 208, 150, 125, 97, 87, 51

**7-[(6-Chloropyridin-3-yl) methyl]-3-(2-nitrophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c] [1,2,4] triazole(4e)**

Yield:(1.616g.,)77%, Melting point: 137°C.

**FTIR (KBr, νmax cm-1) :**3370(NH str.), 3007 (Ar CH str.), 2906,2898(CH2 str.), 1617 (C=N str.), 1581,1533(NO2 str.),1481,1443 (CH=CH str.), 757 (C-Cl str.),

**1H NMR (CDCl3, ppm):** δ, 3.5(2H,t,N-CH2), 3.8(2H,t,N-CH2), 4.5(2H,s,Py-CH2), 4.75(1H,d,-CH), 6.5(1H,d,NH), 7.25-7.6(4H,m,Ar-H), 8.10(2H,m ,Py-H), 8.25 (1H,s,Py-H).

**13C NMR (CDCl3, ppm):**156,151,146,141,137,136,135,132,127,126, 125,123,67, 51, 49,45.

**MS (C16H15N6O2Cl), (m/z):** 358(M+), 330, 322, 311,237,233, 208, 150, 125, 96, 87, 52.

**7-[(6-Chloropyridin-3-yl) methyl]-3-(4-methoxyphenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c] [1,2,4] triazole (4f)**

Yield: (1.25g.,71%), Melting point: 97°C.

**FTIR (KBr, νmax cm-1) :**3374(NH str.), 3007 (Ar CH str.), 2911,2891(CH2 str.), 2896 (CH3 str.),1617 (C=N str.),1483,1439 (CH=CH str.),1098(COC str.), 757 (C-Cl str.).

**1H NMR (CDCl3, ppm):** δ,3.6(2H,t,N-CH2), 3.7(2H,tN-CH2), 3.78(3H,s,OCH3), 4.7(2H,s,Py-CH), 4.9(1H,d,CH), 6.3(1H,d,NH), 6.6-7.4(4H,m,Ar-H), 8.2(2H,d,Py-H), 8.3(1H,s,Py-H)

**13C NMR (CDCl3, ppm):** 160, 156, 151, 141,137, 133, 129, 123, 122, 115,113,76, 67, 55, 51, 49,45.

**MS (C17H18N5OCl) (m/z):**343(M+), 342, 315, 311, 307, 237, 218, 208, 192, 125, 87, 82, 52.

**Biological Screening**

**A) Insecticidal activity**

The standard solutions of synthesized compounds were prepared by dissolving them in acetone (1%) and DMF (1%) with Tween-20 (0.1%) solution, to get 300, 600, and 800 ppm concentrations. The treatments of these compounds were done through the oral route, by dipping the fresh tobacco leaves in differently concentrated solutions and then fed to Hübner *(H. armigera*), Mealybugs (*Planococcus citri*), and Mango hoppers nymphs (*Idioscopus clypealis*). The mortality data were collected, after 24, 48, and 72 hrs. of treatment and presented in **Table-1-3.**

**Table-1:** Mortality data of treated compounds against sucking insect pests.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sr.  No. | Compound Name | Concentrations ppm | Mortality after 24 hrs. of treatment | | |
| *(H. armigera)* (Hubner)\* | Mealybugs *(Planococcus citri*) \* | Mango hoppers *(Idioscopus clypealis*) \* |
| **1** | **7-[(6-Chloropyridin-3-yl)methyl]-3-phenyl-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4a)** | 300 | 58 | 53 | 82 |
| 600 | **94** | 82 | 88 |
| **800** | **96** | **87** | **90** |
| **2** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(4-chlorophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4b)** | 300 | 72 | 59 | 83 |
| 600 | **91** | 83 | **90** |
| **800** | **97** | **93** | **95** |
| **3** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(2-chlorophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4c)** | 300 | 75 | 58 | 83 |
| 600 | **91** | 85 | **91** |
| **800** | **98** | **96** | **99** |
| **4** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(4-nitrophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4d)** | 300 | 62 | 58 | 83 |
| 600 | **95** | 85 | **90** |
| **800** | **97** | **91** | **98** |
| **5** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(2-nitrophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4e)** | 300 | 60 | 59 | 84 |
| 600 | **95** | 85 | **90** |
| **800** | **96** | **93** | **97** |
| **6** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(4-methoxyphenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4f)** | 300 | 50 | 44 | 78 |
| 600 | **91** | 80 | 81 |
| **800** | **92** | **84** | **83** |
| **7** | **Imidacloprid** | 300 | 52 | 46 | 90 |
| 600 | 100 | 100 | 100 |
| 800 | 100 | 100 | 100 |
| **8** | **Control (Solvent)** {acetone (1%) and DMF (1%) with Tween-20 (0.1%) solution} | -- | 5 | 4 | 8 |

\*Means of six replication

**Table-2:** Mortality data of treated compounds against sucking insect pests.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sr.  No. | Compound Name | Concentrations  ppm | Mortality after 48 hrs. of treatment | | |
| *(H. armigera)* (Hubner)\* | Mealybugs *(Planococcus citri*) \* | Mango hoppers *(Idioscopus clypealis*) \* |
| **1** | **7-[(6-Chloropyridin-3-yl)methyl]-3-phenyl-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4a)** | 300 | 59 | 54 | 82 |
| 600 | **94** | 82 | 89 |
| **800** | **96** | **88** | **90** |
| **2** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(4-chlorophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4b)** | 300 | 73 | 61 | 83 |
| 600 | **91** | 83 | **91** |
| **800** | **98** | **95** | **98** |
| **3** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(2-chlorophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4c)** | 300 | 75 | 58 | 84 |
| 600 | **92** | 86 | **91** |
| **800** | **98** | **96** | **99** |
| **4** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(4-nitrophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4d)** | 300 | 62 | 59 | 84 |
| 600 | **96** | 85 | **91** |
| **800** | **97** | **91** | **98** |
| **5** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(2-nitrophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4e)** | 300 | 60 | 59 | 84 |
| 600 | **96** | 85 | **91** |
| **800** | **96** | **93** | **97** |
| **6** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(4-methoxyphenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4f)** | 300 | 51 | 44 | 78 |
| 600 | **91** | 81 | 81 |
| **800** | **92** | **84** | **83** |
| **7** | **Imidacloprid** | 300 | 52 | 46 | 90 |
| 600 | 100 | 100 | 100 |
| 800 | 100 | 100 | 100 |
| **8** | **Control (Solvent)** {acetone (1%) and DMF(1%)with Tween-20(0.1%) solution} | -- | 5 | 4 | 8 |

\*Means of six replications

**Table-3:** Mortality data of treated compounds against sucking insect pests.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sr.  No. | Compound Name | Concentrations ppm | Mortality after 72 hrs. of treatment | | |
| *(H. armigera)* (Hubner)\* | Mealybugs *(Planococcus citri*) \* | Mango hoppers *(Idioscopus clypealis*) \* |
| **1** | **7-[(6-Chloropyridin-3-yl)methyl]-3-phenyl-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4a)** | 300 | 61 | 55 | 83 |
| 600 | **94** | 83 | 89 |
| **800** | **96** | **89** | **91** |
| **2** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(4-chlorophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4b)** | 300 | 75 | 63 | 85 |
| 600 | **95** | 83 | **93** |
| **800** | **100** | **98** | **99** |
| **3** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(2-chlorophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4c)** | 300 | 77 | 65 | 84 |
| 600 | **94** | 84 | **94** |
| **800** | **100** | **99** | **99** |
| **4** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(4-nitrophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4d)** | 300 | 63 | 60 | 84 |
| 600 | **96** | 85 | **93** |
| **800** | **97** | **95** | **98** |
| **5** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(2-nitrophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4e)** | 300 | 64 | 61 | 85 |
| 600 | **96** | 86 | **94** |
| **800** | **98** | **97** | **98** |
| **6** | **7-[(6-Chloropyridin-3-yl)methyl]-3-(4-methoxyphenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4f)** | 300 | 53 | 45 | 79 |
| 600 | **91** | 86 | 81 |
| **800** | **93** | **86** | **86** |
| **7** | **Imidacloprid** | 300 | 59 | 54 | 82 |
| 600 | 100 | 100 | 100 |
| 800 | 100 | 100 | 100 |
| **8** | **Control (Solvent)** {acetone (1%) and DMF (1%)with Tween-20(0.1%) solution} | -- | 5 | 4 | 8 |

\*Means of six replications.

The mortality rate of *H. armigera* (Hub), Mealybugs (*Planococcus citri),* and Mango hoppers [ *Idioscopus clypealis*] against synthesized novel neonicotinoid derivatives are shown in Table-1. The death rate of all insects at 800 ppm concentration solution was found to be higher than the death rate for the rest of the concentrations. The biological assay revealed that most of the synthesized compounds have excellent insecticidal properties against various insect species.

**Antibacterial activity**

An antimicrobial assay was conducted to evaluate the effectiveness of different concentrations of antimicrobial agents against *Bacillus megaterium* and *Pseudomonas solanacearum* (tomato & tobacco bacterial wilts) species. Nutrient agar media plates were prepared and inoculated with *Bacillus megaterium* and *Pseudomonas solanacearum*. After the drying of the plates, 6mm holes were created using a cork borer. A series of sample solutions (A, B, C, D, E, and F) were prepared at the concentrations of 300 ppm, 600 ppm, and 800 ppm. Each sample solution was poured into its respective hole on the plates. The plates were then incubated at 35 degrees Celsius for 3 days, and the zones of inhibition were measured. The data obtained from this assay provides valuable insights into the antimicrobial activity of the tested agents *Bacillus megaterium* and *Pseudomonas solanacearum*.

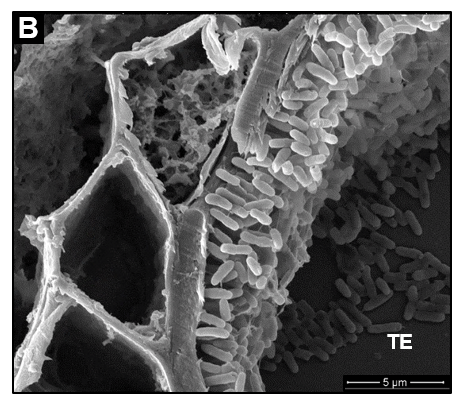
**Introduction**

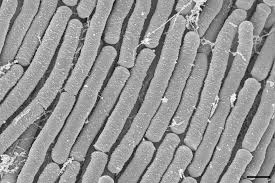
***Ralstonia solanacearum*** formerly called [*Pseudomonas*](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/pseudomonas) is an [aerobic](https://en.wikipedia.org/wiki/Aerobic_organism) non-spore-forming, [Gram-negative](https://en.wikipedia.org/wiki/Gram-negative), [plant-pathogenic](https://en.wikipedia.org/wiki/Plant_pathogen) bacterium. *R. solanacearum* is soil-borne and [motile](https://en.wikipedia.org/wiki/Motile) with a [polar flagellar tuft](https://en.wikipedia.org/wiki/Polar_flagellar_tuft). It colonizes the [xylem](https://en.wikipedia.org/wiki/Xylem), causing bacterial [wilt](https://en.wikipedia.org/wiki/Wilting) in a very wide range of potential in host plants. It is known as [Granville wilt](https://en.wikipedia.org/wiki/List_of_tobacco_diseases) when it occurs in [tobacco](https://en.wikipedia.org/wiki/Tobacco). Bacterial wilts of tomato, pepper, eggplant, and Irish potato are caused by *R. solanacearum*, particularly in the humid [lowlands](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/lowlands). Tomato bacterial wilt commonly occurs in humid conditions at a relatively high temperature. The bacterium moves systemically through the plant xylem, inducing affected plants' terminal leaves to wilt abruptly without leaf yellowing. This is followed by a sudden and permanent wilt of the plant within a short period. They turn brown and sometimes become water-soaked with hollow [veins](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/vein) on the stems. The bacterium survives in the field soils and gets ingressed into the roots of young plants through wounds made by [transplanting](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/transplanting), cultivation, insects, or certain nematodes. It is spread through irrigation water, soil, and infected transplant movement.

***Bacillus cereus*** is an aerobic spore-forming bacterium that is commonly found in soil, on vegetables, and in many raw and processed foods. *B. cereus* food poisoning may occur when foods are prepared and held without adequate refrigeration for several hours before serving, with *B. cereus* reaching >106 cells/g. Foods incriminated in past outbreaks include cooked meat and vegetables, boiled or fried rice, vanilla sauce, custards, soups, and raw vegetable sprouts. Two types of illness have been attributed to the consumption of foods contaminated with *B. cereus*. The first and better known is characterized by abdominal pain and non-bloody diarrhea. it has an incubation period of 4-16 hrs. following ingestion with symptoms that last for 12-24 hrs. Secondly, an acute attack of nausea and vomiting occurs within 1-5 hrs. after the consumption of contaminated food.

***Bacillus megaterium*** is a gram-positive, spore-forming bacterium commonly found in soil and other natural environments. It is known to cause spoilage in various industries, making it essential to assess the efficacy of antimicrobial agents against this organism. In this study, an antimicrobial assay was performed to determine the zones of inhibition produced by different concentrations of the antimicrobial agents on nutrient agar plates inoculated with *Bacillus megaterium* and *Pseudomonas solanacearum*.

**Selected bacteria species for antibacterial activity**

 **. . (A) (B)**



**(C)**

**Fig.No.: 3.1. Photographs of A) *Pseudomonas solanacearum, B) Bacillus megaterium and C) Bacillus Cereus.***

**Scientific Classification**

***Pseudomonas solanacearum Bacillus megaterium Bacillus Cereus***

Kingdom: Bacteria Kingdom: Bacteria Kingdom: Bacteria

# Phylum: Pseudomonadota Phylum: Bacillota Phylum: Bacillota

Class: [Betaproteobacteria](https://en.wikipedia.org/wiki/Betaproteobacteria) Class: [Bacilli](https://en.wikipedia.org/wiki/Betaproteobacteria) Class: [Bacilli](https://en.wikipedia.org/wiki/Betaproteobacteria)

Order: Burkholderiales Order: Bacillales Order: Bacillales

Family: Burkholderiaceae Family: Bacillaceae Family: Bacillaceae

Genus: *Ralstonia*  Genus: *Bacillus* Genus: *Bacillus*

Species: *solanacearum*  Species: *megaterium* Species: *cereus*

**Materials and Methods:**

**Preparation of Nutrient Agar Media**: Nutrient agar media were prepared following standard protocols.

## **Composition of Nutrient Agar**

|  |  |
| --- | --- |
| Ingredients\* | Amount (gm/L) |
| Beef extract | 3.0 gm |
| Peptone | 5.0 gm |
| Sodium chloride | 5.0 gm |
| Agar | 15.0 gm |
| Distilled water | 1000 mL |

\*Formula adjusted, standardized to suit performance parameters

1)Dissolve the above ingredients in the appropriate volume of distilled water i.e., 28 gm dehydrated nutrient agar in 1000 mL distilled water.

2)Heat it with frequent agitation and boil for 1 minute to dissolve completely the powder.

3)Sterilize the medium by [autoclaving (121°C for 15 min.)](https://microbeonline.com/autoclave-principle-procedure-types-and-uses/)

4)Once the nutrient agar has been autoclaved, allow it to cool but not solidify

5)Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified.

**Inoculation of microorganisms**: *Bacillus megaterium, Bacillus cereus* and *Pseudomonas solanacearum* were spread by spread plate method evenly onto the surface of the nutrient agar respective plates.

**Creation of Holes**: Once the plates dried completely, 6mm holes were made using a cork borer, with three holes per plate.

**Preparation of Sample Solutions:** Sample solutions A, B, C, D, E, and F were prepared at concentrations of 300 ppm, 600 ppm, and 800 ppm.

**Pouring of Sample Solutions**: 0.1 ml of each sample solution was poured into their respective labeled holes on the nutrient agar plates.

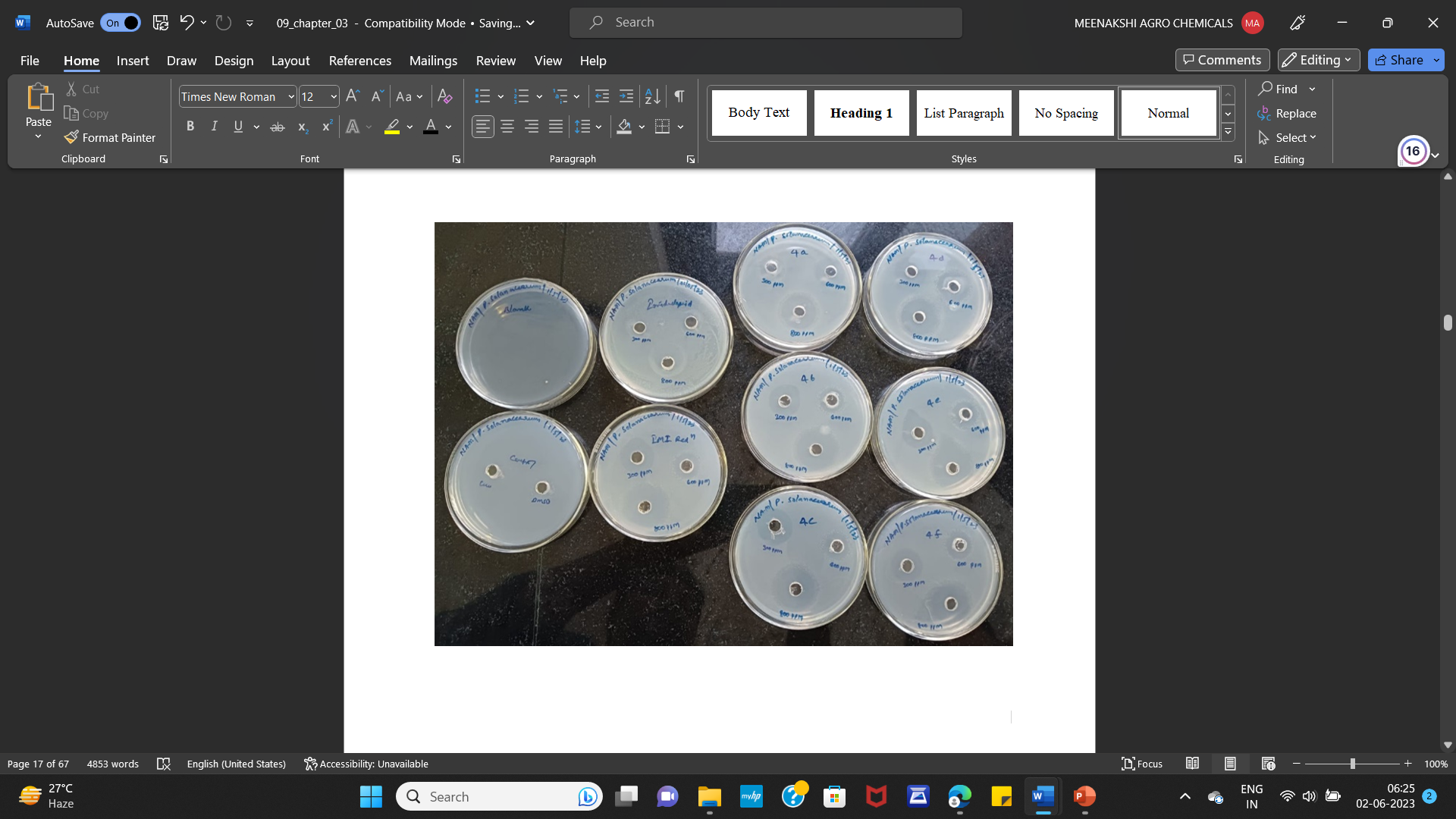
**Incubation:** The plates were incubated at 35°C for 3 days to allow the growth of *Bacillus megaterium, Bacillus cereus* and *Pseudomonas solanacearum* respectively.

**Measurement of Zones of Inhibition:** After the incubation period, the zone of inhibition was observed after 3 days surrounding each hole. These were measured using a measuring scale. In the blank as well as the control sample, we observed that there is no bacterial growth.

**Table-4: The anti-bacterial screening of synthesized molecules and imidacloprid**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sr.  No. | Compound Name | Conc. in  ppm | Diameters of Zone of Inhibition MIC after 72 hrs. in mm | | |
| *P.Solanacearum Bacterial wilt* (*Tobacco*) | *Bacillus cereus* | *Bacillus megaterium* |
| 1 | 7-[(6-Chloropyridin-3-yl)methyl]-3-phenyl-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4a) | 300 | 22 | 22 | 23 |
| 600 | 28 | 25 | 27 |
| 800 | 35 | 37.5 | 35 |
| 2 | 7-[(6-Chloropyridin-3-yl)methyl]-3-(4-chlorophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4b) | 300 | 28 | 22 | 21 |
| 600 | 29.5 | 24 | 22 |
| 800 | 40.5 | 33 | 37 |
| 3 | 7-[(6-Chloropyridin-3-yl)methyl]-3-(2-chlorophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4c) | 300 | 24 | 23 | 25 |
| 600 | 30 | 26 | 24 |
| 800 | 39.5 | 36 | 39.5 |
| 4 | 7-[(6-Chloropyridin-3-yl)methyl]-3-(4-nitrophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4d) | 300 | 25 | 25 | 23 |
| 600 | 30 | 28 | 28 |
| 800 | 36 | 38 | 34 |
| 5 | 7-[(6-Chloropyridin-3-yl)methyl]-3-(2-nitrophenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4e) | 300 | 24 | 22 | 21 |
| 600 | 26 | 23 | 23.5 |
| 800 | 35.5 | 37 | 38.5 |
| 6 | 7-[(6-Chloropyridin-3-yl)methyl]-3-(4-methoxyphenyl)-2,5,6,7-tetrahydro-3H-imidazo[2,1-c][1,2,4]triazole (4f) | 300 | 21. | 23.5 | 24 |
| 600 | 24 | 27 | 25 |
| 800 | 38 | 35.5 | 38.5 |
| 7 | Imidacloprid | 300 | 20 | 18 | 18 |
| 600 | 22 | 22.5 | 22 |
| 800 | 30.5 | 28.5 | 28 |
| 8 | Control (Solvent) {acetone (1% )and DMSO (1%) with Tween-20 (0.1%) solution} | 200 | 00 | 00 | 00 |

\*Means of three replications



**Fig. 3.2.:** Photograph of antibacterial activity of synthesized compounds against *Pseudomonas solanacearum*tomato bacterial wilt.



**Fig.3.3:** Photograph of antibacterial activity of synthesized compounds against *Bacillus Cereus*



**Fig.3.4:** Photograph of antibacterial activity of synthesized compounds against *Bacillus megaterium*

The synthesized molecule was evaluated for their antibacterial activity by the Disk diffusion method against *Bacillus megaterium, Bacillus cereus* and *Pseudomonas solanacearum* bacterial wilts of plants tobacco as well as tomato. The synthesized compounds showed moderate activity however 800 ppm of the compounds with sl. no.4a,4b,4c,4d,4e, and 4f showed the promising antibacterial activity.

**Conclusion**

The title compounds were synthesized and characterized by using FT-IR, 1H NMR, 13C NMR, Mass spectrometry, and elemental analyses. The title compound exhibits promising insecticidal activities against Hubner (*H. armigera)*, Mealybugs (*Planococcus citri*), and Mango hoppers (*Idioscopus clypealis*) at 300,600 ppm and 800 ppm. Furthermore, at 800 ppm, the synthesized compound and imidacloprid demonstrated comparatively promising antibacterial activity with *Pseudomonas solanacearum* (e.g., bacterial-wilt tobacco and bacterial-wilt tomato). The results are encouraging, which validated that this work is helpful for the discovery of new chemical entities such as pesticides.

**Acknowledgment**

The authors express they’re thanks to Agrochemicals and Pest Management Department, *Shivaji- University, Kolhapur* for their help and encouragement in the study.

**Conflict Of Interest**

Regarding the publishing of this work, the authors declare that there are no conflicts of interest.

# References

1. Dai H., Yu H. B., Liu J.B., Li Y. Q., Qin X., Zhang X., Qin Z. F., Wang T. T. and Fang J. X., General Papers ARKIVOC., 7, 126-142 (2009).
2. Iwasa T., Motoyama N., Ambrose J. T. and Roe R. M., Crop Protection., 23, 371– 378 (2004).
3. Latli B., D'Amou K. and Casida, J. Med. Chem., 42, 2227-2234 (1999).
4. Tian Z., Shao X., Li Z., Qian X. and Huang Q., J. Agric. Food Chem., 55, 2288- 2292 (2007).
5. Kindemba V., [http://www.beyondpesticides.org/ pollinators/ Neonicotinoid](http://www.beyondpesticides.org/%20pollinators/%20Neonicotinoid). insecticides report-1.pdf. (2009).
6. European Food Safety Authority, Modification of the existing MRLs for thiacloprid in table olives, olives for oil production, poppy seeds, and various root vegetables EFSA Journal 7: 1410-1438 (2009).
7. Saimandir J., Gopal M. and Walia S., Pest Manag Sci., 65, 210–215 (2009).
8. Dai Y. J., Ji W. W., Chen T., Zhang W. J., Liu Z. H., Ge F., and Yuan, J. Agric. Food Chem., 58, 2419–2425 (2010).
9. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J., J. Biol.Chem., 193, 265- 275 (1951).
10. 10. Hartree E. E., Anal. Biochem., 48, 422-427 (1972).
11. Wilson K. and Walker J., “Practical Biochemistry: Principles and Techniques”, Cambridge University Press (2000).
12. Chabrol E. and Charonnat R., Presse Med., 45, 1713 (1937).
13. Joseph A. K., Anderson S. and Rawle J. M., Clinical Chemistry., 18, 199-202 (1972).
14. Frings C. S., Fendley T. W., Dunn R. T. and Queen., Clinical Chemistry., 18, 673- 674 (1972).
15. Morris D. L., Quantitative Science New Series., 107,254-255 (1948).
16. Dinesh K. P., SantaRam A. and Shivanna M. B., Res. J. Agric. & Biol. Sci., 6, 449- 452 (2010).
17. Heinrichs E. A., Chelliah S., Valencia S. L., Arceo M. B., Fabellar L. T., Aquino G.B. and Pickin S., , International rice research institute, Los Banos, Laguna, Manila, Philippines, pp 73-80 (1981).
18. Domard A. and Vasseur V., Int. J. Biol. Macromol., 13, 366-368 (1991)
19. Lipke H., Grainger M. M. and Siakotos A. N., J. Biol. Chem., 240, 594-600 (1965).
20. Legocki J. and Polec I., Pestycydy., (1-2), 143-159 (2008).