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ABSTRACT

A series of heterocyclic Schiff bases (SB-1 to SB-3) have been synthesized by reacting 4-(o-flouropheryl)-2-aminothiazole and R-substituted salicylaldehyde (R=H, 3-CH₃, 3-Cl). The ligands (SB-1 to SB-3) were characterized by elemental, spectral and XRD analysis. All the ligands (SB-1 to SB-3) evaluated for their *in vitro* nematicidal and molluscicidal activities. They are active in very low concentration on plant parasitic nematode *Meloidogyne javanica* and freshwater helminthiasis vector snail *Lymnea auricularia*. X-ray diffraction studies suggested a triclinic crystal system for all Schiff bases.

KEY WORDS: *Lymnea auricularia*, Nematicidal activity, Molluscicidal activities, Thiazole Schiff bases

INTRODUCTION

Schiff's base show greater flexibility and diverse structural aspects, a wide range of these compounds have been synthesized and their complexation studied (Syamal, 1989). Thiazoles are well known as biologically active substances and they exhibit a wide spectrum of antitubercular, antibacterial, antifungal, hypotensive and hypodermic, anticancer and quorum sensing activity. Hence the Schiff bases derived from thiazoles are expected to be biologically active compounds. Microwave assisted synthesis of chemical compounds is an efficient and eco-friendly synthetic strategy and has now become a powerful tool for green chemistry. Microwave-assisted organic reaction is a well-established technique for the synthesis of various heterocyclic compounds (Kabalka, 2006; Khrustalev, 2008). So, in view of these facts, and as part of our ongoing studies in developing new anti-microbial agents, it was envisaged to synthesize microwave-assisted synthesis of Schiff's base of 2-aminothiazole and explore its novel functionality as nematicidal and Molluscicidal active molecules as well, which is hitherto un-attempted.

Most vegetable crops are attacked by one or more species of nematodes. The root-knot nematode (*Meloidogyne javanica*) (fig. 1) is the most important species associated with tomato (*Lycopersicon esculentum*) (Christie, 1936). 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 Thiazole Schiff bases (SB-1 to SB-3) 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 (Imani-Baran 2011) for *F. hepatica* and *F. Gigantia*. The present investigation was made to study the Molluscicidal activity of newly synthesized Thiazole Schiff bases (SB-1 to SB-3) on *Lymnea auricularia*.

MATERIALS AND METHODS

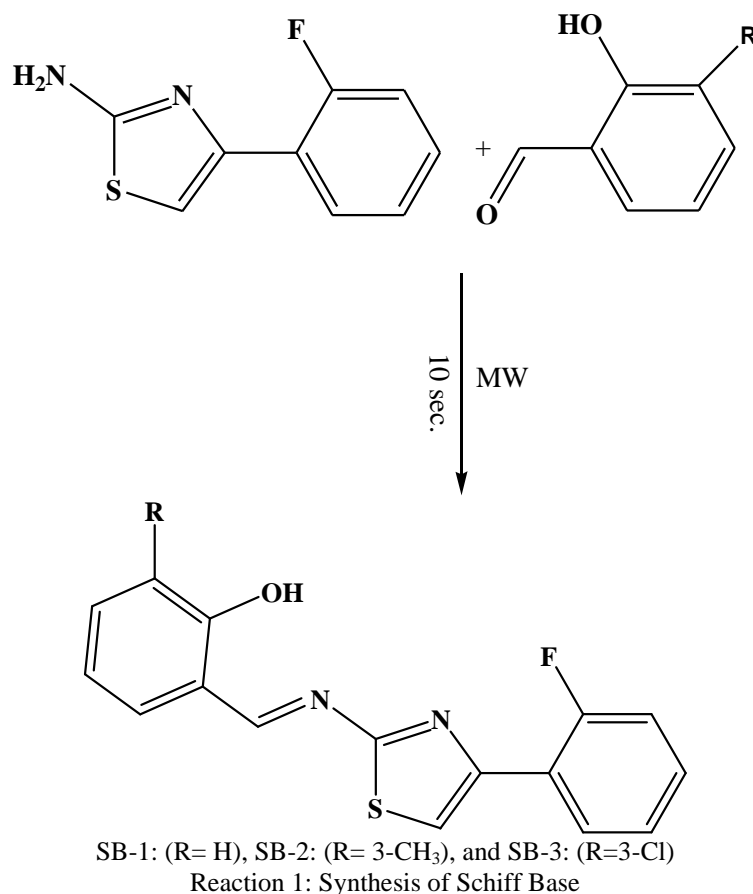
Microwave assisted synthesis of Schiff bases (SB-1 to SB-3)

4-(o-flouropheryl)-2-aminothiazole (1mmole) and o- hydroxyaldehyde (1mmole) were mixed with each other in mortar-pestle and the reaction mixture was placed in small conical flask at room temperature, then 1 ml alcohol was added. The mixture was then exposed to microwave irradiation at 10% power for 10-20 sec. (Reaction 1). Completion of the reaction was tested by TLC. The reaction mixture was then cooled to room temperature. The yellow coloured Schiff base was obtained, which was recrystallized from ethanol and dried under reduced pressure.

Nematicidal activity

For the toxicity and efficacy ratio of thiazole Schiff base (SB-1 to SB-3) 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

(McSorley, 1982). 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 thiazole Schiff bases (SB-1 to SB-3) were 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 (J₂, J₃). For in vitro nematicidal activity, the method described by Dama (1999) is used for present study. The test animals are divided in to 9 groups, each group contains 10 phytonematodes, with test compound concentration of 2 μ l to 10 μ l. The flask that contained distilled water and DMSO serves as control for first group (Mane, 2000; Dama, 2002). Each treatment was replicated for three times. Data on Juvenile mortality was recorded after 6h, 12h and 24h exposure of test compounds 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 (Fetterer, 1989). 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. Snails of particular species were taken in large petridishes. Snails were submerged in distilled water.

The test animals are divided in to 9 groups, each group contains 10 snails (*Lymnea auricularia*), with test compound concentration of 2 μ l to 10 μ l. 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 6h, 12h and 24h exposure of test compounds under compound and stereomicroscope and then determined the percentage of efficacy.

RESULTS AND DISCUSSION

Chemistry

The Schiff bases (SB-1 to SB-3) are yellow crystalline solids having sharp melting points. They are soluble in common organic solvents and gives satisfactory elemental (C, H and N) analyses.

Spectral analysis

UV-visible spectra of the Schiff bases (SB-1 to SB-3) in chloroform exhibit an intense band at ~400nm. Infrared spectra of the Schiff bases (SB-1 to SB-3) in KBr pellets exhibit $\nu(\text{O-H})$, $\nu(\text{C=N})$, $\nu(\text{C-O})$ and $\nu(\text{C-S-C})$ modes at ~ 3400, ~1630, ~ 1280 and ~665 cm^{-1} respectively. These values are in accordance with the earlier reported values. ^1H NMR data of the Schiff bases is represented below. The assignments of NMR signals show close resemblance with the earlier results (Silverstein, 1991).

Table 1. Nematicidal activity of SB-2 on root-knot nematode *Meloidogyne* spp.

| Concentrations of SB-2 | 2 μl | 4 μl | 6 μl | 8 μl | 10 μl |
|----------------------------|-----------------|-----------------|-----------------|-----------------|------------------|
| No. of nematodes | 10 | 10 | 10 | 10 | 10 |
| No. of nematodes immotile | 8 | 6 | 8 | 8 | 10 |
| Immobilization Time (Sec.) | 60 | 60 | 60 | 60 | 60 |



Figure 1. Root knot nematodes

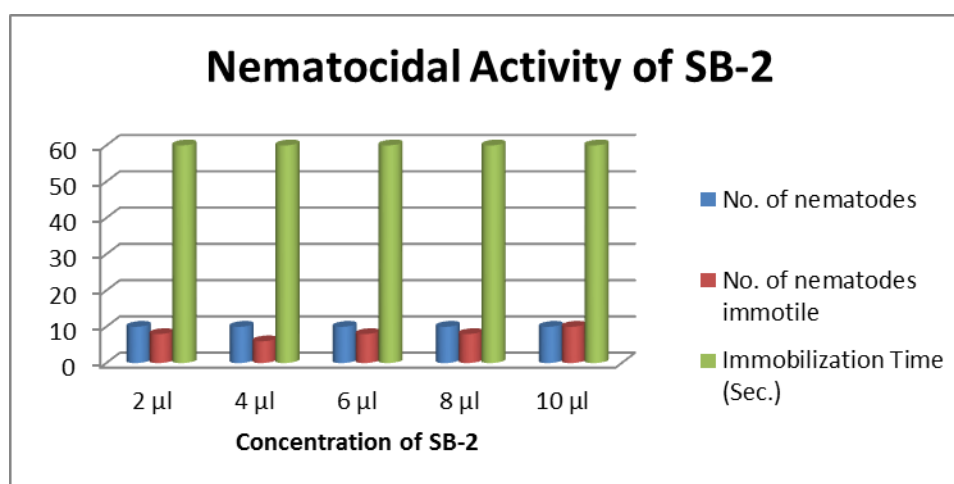


Figure 2. Nematicidal activity of SB-2 on root-knot nematode *Meloidogyne* spp.

Table 2. Molluscicidal activity of SB-2 on helminthiasis vector snail, *Lymnea auricularia*

| Concentrations of SB-2 | 2 μ l | 4 μ l | 6 μ l | 8 μ l | 10 μ l |
|---------------------------|-----------|-----------|-----------|-----------|------------|
| No. of Lymnea | 10 | 10 | 10 | 10 | 10 |
| No. of Lymnea immotile | 7 | 7 | 7 | 9 | 10 |
| ImmobilizationTime (min.) | 14 | 14 | 14 | 12 | 13 |

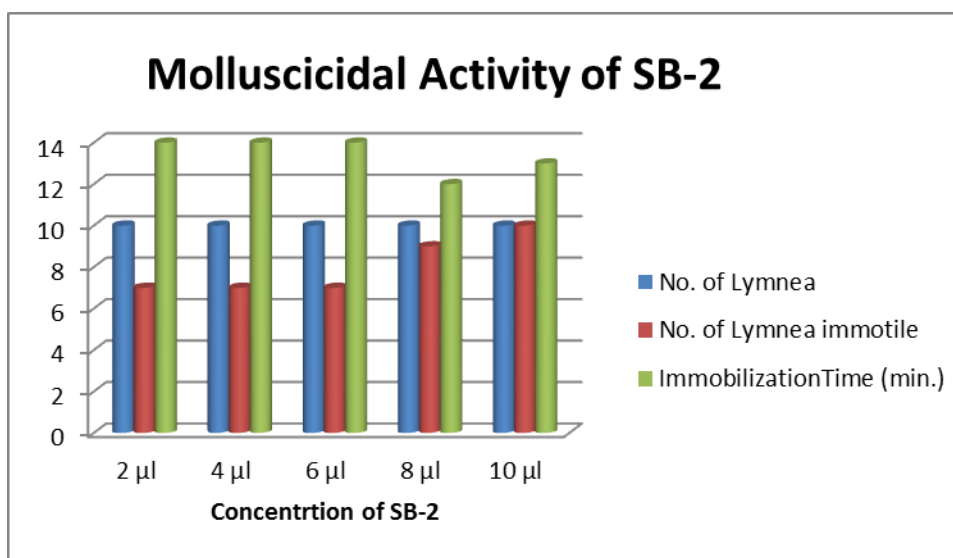


Figure 3. Molluscicidal activity of SB-2 on helminthiasis vector snail, *Lymnea auriculari*

^1H NMR signals (δ ppm): **SB-1**: 12.247 (1H, s, Ar-OH), 9.32 (1H, s, benzylidenimin), 6.976-8.293 (9H, m, thiazole and Ar-H); **SB-2**: 2.33 (3H, s, Ar-CH₃), 12.505 (1H, s, Ar-OH), 9.296 (1H, s, benzylidenimin), 6.849-8.293 (8H, m, thiazole and Ar-H); **SB-3**: 12.947 (1H, s, Ar-OH), 9.329 (1H, s, benzylidenimin), 6.903-8.263 (8H, m, thiazole and Ar-H);

XRD- analysis

A representative ligand SB-2 is chosen for XRD studies. The powder x-ray diffractogram of ligand is depicted 25 reflection (2θ) between 20.82° to 50.96° with maximum at $2\theta = 21.46^\circ$ and $d = 4.1373\text{\AA}$. The cell parameter calculated are mentioned in parenthesis ($a=13.8414\text{\AA}$, $b= 4.7817\text{\AA}$, $c= 4.5918\text{\AA}$, $\alpha = 116.765^\circ$, $\beta = 93.955^\circ$, $\gamma = 91.019^\circ$) 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 ligand SB-2 is triclinic (Woolfson, 1980). The volume of unit cell is 270.30\AA^3 .

Biological Activity

Nematicidal Activity

Direct contact toxicity of newly synthesized thiazole Schiff bases (SB-1 to SB-3) at different dose were analyzed by exposing 100 freshly hatched J₂ and J₃ of *M. javanica* for 24 h. The result indicates that, thiazole Schiff bases (SB-1 to SB-3) are very effective to controlling *M. javanica*. They show highest percentage efficiency in the range of 8 μ l to 10 μ l. representatively the percentage efficiency of SB-2 is shown in Table 1 and Figure 2.

Molluscicidal Activity

Thiazole Schiff bases (SB-1 to SB-3) at different dose were analyzed by exposing 100 fresh water snails *Lymnea auricularia*. The result indicates that, thiazole Schiff bases (SB-1 to SB-3) are very effective to controlling *Lymnea auricularia*. They show highest percentage efficiency in the range of 8 μ l to 10 μ l. representatively the percentage efficiency of SB-2 is shown in Table 2 and Figure 3.



CONCLUSION

All the Schiff bases (SB-1 to SB-3) are yellow crystalline solids having sharp melting points. The spectral analyses (UV-Visible, IR and ^1H NMR) data confirms the structure proposed for the Schiff bases. X-ray diffraction studies suggested a triclinic crystal system for all Schiff bases.

Although observations suggested that the newly synthesized thiazole Schiff bases (SB-1 to SB-3) exerts their biological effects by common mode of action, these ligands are active compounds for nematocidal and molluscicidal activity with low concentration. Schiff bases compounds are promising alternative compounds to the hazardous pesticides now used in agriculture for the controlling nematodes.

ACKNOWLEDGMENT

Authors are thankful to the authorities of Solapur University, Solapur (Maharashtra –India) for providing facilities. One of the authors SVR is thankful to UGC-WRO, Pune for funding through MRP.

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