CHAPTER: III

EFFECT OF SOME UV ABSORBERS ON PHOTOSTABILIZATION OF AZADIRACHTIN-A

III.1 ABSTRACT

The effect of photostabilization of azadirachtin-A was studied, when exposed to sunlight and ultraviolet light (UV) in the presence of four structurally different ultraviolet stabilizers namely p-aminobenzoic acid, 2,4-dihydroxybenzophenone, 4,4'-dihydroxybenzophenone and phenyl salicylate, in solid phase.

The percentages of azadirachtin-A recovered from the solutions, after six hours exposed to UV radiation in the presence and absence of UV absorbers indicated that the order of stabilization of azadirachtin-A by these absorbers was similar to that obtained in the solid phase experiments.

The percentages of Aza-A recovered at different time intervals from slides exposed to different light conditions with and without UV stabilizers as well as kinetic studies indicated that the addition of phenyl salicylate in methanolic solution of Aza-A (in 1:1 mole ratio) provides the best photostabilization of Aza-A molecule among the four UV stabilizers studied.

III.2 INTRODUCTION AND OBJECTIVES

The sun emits electronic radiation with wavelengths that range from the X-ray region to the far infrared. The earth's atmosphere, particularly the ozone in the stratosphere, is an excellent absorber for the highly energetic short wavelength of radiation and only light with wavelength > 290 nm reaches the surface of the earth.[1] Although only a relatively small amount of the sunlight energy is in the UV region of 290-400 nm, it is sufficient to induce photochemical degradation of many organic molecules.[1] Many organic compounds

when exposed to sunlight are prone to undergo chemical reactions subsequent to electronic excitation.[2] These molecules may be synthetic, for example fibers, plastics, dyestuffs, pesticides or natural, such as bio-pesticides and living organisms. The protection of these compounds from harmful ultraviolet radiation is of considerable significance, both commercially and medicinally.[3]

Sensitivity to sunlight, limits the use of some botanical pesticides in agriculture. Classical approaches to overcome this problem generally involve chemically modified molecular structures of the pesticides or the use of UV absorbing molecules in the formulations. However, the former method suffers from drawbacks, since any chemical modification may seriously affect the pesticidal activity of the compound and may cause ecological problems.[4] Therefore, the latter method, which is free from the above-mentioned disadvantages is more suitable to extend the environmental life of the pesticides.[5-8]

The mechanism of photostabilization appears to be due to any of the following processes.[4]

Preferential absorption of light by UV absorbers, thereby preventing photo-excitation of the pesticide molecules.

Transfer of the excess energy from the excited pesticide molecules to UV absorbers, through various energy transfer mechanisms.[9,10] It is evident that both Aza-A and UV absorber must have matching UV spectra with near or similar λ_{max}.[7,8]

It should be remembered however, that large quantities of UV absorbers are not desirable to avoid environmental problems.

Azadirachtin-A (Aza-A) is found to be an environment friendly pesticide and has many desirable properties. It is selective with short persistence, toxic to target pests, has minimal toxicity to nontarget

and beneficial organisms and causes less damage to the ecosystem than the conventional insecticides.[11,12]

Azadirachtin-A is photolabile, either breaking down or isomerising in sunlight, probably due to the presence of sensitive functionalities like π-electrons, ester linkages, tigloyl moiety and epoxide ring in the molecule.[7,8,13-19] The tigloyl moiety in the Aza-A molecule is the major site of photochemical degradation.[13] The tigloyl group may undergo photochemical reactions such as dimerization, *cis-trans* isomerization, carbon-carbon double bond migration, rearrangement, dissociation of the bond adjacent to the carbonyl carbon, as well as formation of allylic hydroperoxides, alcohols, ketones and more complicated products.[13,20-24]

Barnby *et al* reported the biological activity of azadirachtin, its derivatives and their ultraviolet irradiation products against tobacco budworm larvae and suggested that the resulting products showed decreased biological activity.[13] Stocks and Redfern have found that the antifeedant potency of azadirachtin exposed to sunlight for 7 days was reduced by more than half as compared to the non-exposed azadirachtin against first instar larvae of *S. frugiperda*, with no activity remaining after 16 days.[15] Saxena's group and Meisner *et al* have found much shorter persistence of activity of neem extracts containing azadirachtin after exposure to sunlight than that reported by Pradhan and Jotwani.[25-27] In accordance to these reports, Ermel *et al* have also reported that exposure of neem extracts to sunlight and UV radiation decreased their content of azadirachtin.[28]

Sundaram and Curry have found that azadirachtin is very labile on conifer and deciduous foliage with a dissipation half life (DT_{50}) of about 20 hrs.[16,29] While Tewari reported that the azadirachtin loses

50 % of its activity after seven days and 100 % after sixteen days on exposure to sunlight in acetone solution.[30]

Recently, Johnson and Dureja studied the photostability of Aza-A, in the presence of non-ionic surfactants. They found that surfactants such as Span-80, Atlox 3400B, Tween-80, Agrimul 52B, and Agrimul N4S enhanced the rate of photodegradation of Aza-A on exposure to UV light, whereas, surfactants such as Triton-X, Emulsol CFA, Tween-20, Emulsol MAS, and Emulsol-N-33 decreased the rate of degradation. Emulsol-N-33 was found to be the most effective surfactant, as half-life of Aza-A in the presence of Emulsol-N-33 was found to be 94.93 min in comparison to 48 min for Aza-A alone under UV light.[31]

Degradation of the insecticide in sunlight is the major limitation encountered in its use in agriculture, because the insecticide should remain intact on the leaves so as to provide the insect enough time to ingest the material causing death of the insect. Therefore Aza-A molecule should be photostabilized by addition of ultraviolet stabilizers for its effective use. It should be noted that the stabilizer itself must not be destroyed during long-term exposure and should have low volatility and chemical inertness to the substrate or additives.[1]

UV stabilizers such as p-aminobenzoic acid, and its esters, substituted hydroxybenzophenones and their derivatives have been used to reduce its photolytic decomposition by ultraviolet radiation.[5,6,32,33]

Sundaram and Curry have obtained marginal photostabilization of Aza-A in the presence an amphipathic surfactant lecithin, when exposed to sunlight and UV light on glass and foliar surfaces.[8]

It should be mentioned here that, the effect of different UV stabilizers on its photodecomposition under sunlight, which is required for the

purpose of field applications was not studied earlier prior to our own reports.[34,35]

Thus, in the present study we describe the effect of four structurally different UV stabilizers namely p-aminobenzoic acid, 2,4-dihydroxybenzophenone, 4,4'-dihydroxybenzophenone and phenyl salicylate (Figure:III.1) on the photostabilization of Aza-A precoated on glass plates. The plates were exposed to ultraviolet radiation and sunlight with and without UV stabilizers. Control experiments were also performed by keeping the precoated plates in dark.

It was also thought to conduct a similar study in the solution phase, where the energy transfer between donor and accepter molecules will be highly efficient. Accordingly we have also explored the photo stabilizing effect of these UV absorbers on Aza-A in solution. The methanolic solutions of Aza-A with and without UV absorbers were irradiated individually in the presence of ultraviolet radiation using a high-pressure mercury vapor lamp in an immersion well type of reactor.

III.3 RESULTS AND DISCUSSION

Azadirachtin-A absorbs energy from sunlight in the UV region, ranging from 290 to 390 nm forming electronically excited Aza-A* molecules with specific configurations. They are deactivated either by degradation to form other products or reach the ground level by emitting the excess energy. The rate of degradation of Aza-A* depends upon the intensity and duration of radiation, Aza-A concentration, type and number of chromophores on it.[33]

The possible excitation and emission mechanisms of Aza-A are listed below:[33]

$$\text{Aza-A}\ (S_0) + h\nu \longrightarrow \text{Aza-A}^*\ (S_1)$$

$$\text{Aza-A}^*\ (S_1) \longrightarrow \text{Aza-A} + h\nu \quad \text{(fluroescence)}$$

$$\text{Aza-A}^*\ (S_1) \longrightarrow \text{Aza-A}\ (T_1) \quad \text{(ISC)}$$

$$\text{Aza-A}^*\ (T_1) \longrightarrow \text{Aza-A}\ (S_0) + h\nu \quad \text{(phosphorescence)}$$

(where S_0 refers to ground state and S_1 refers to excited state and ISC refers to intersystem crossing, the energy exchange between states belonging to different spin systems; S and T refer to singlet and triplet electronic energy states).

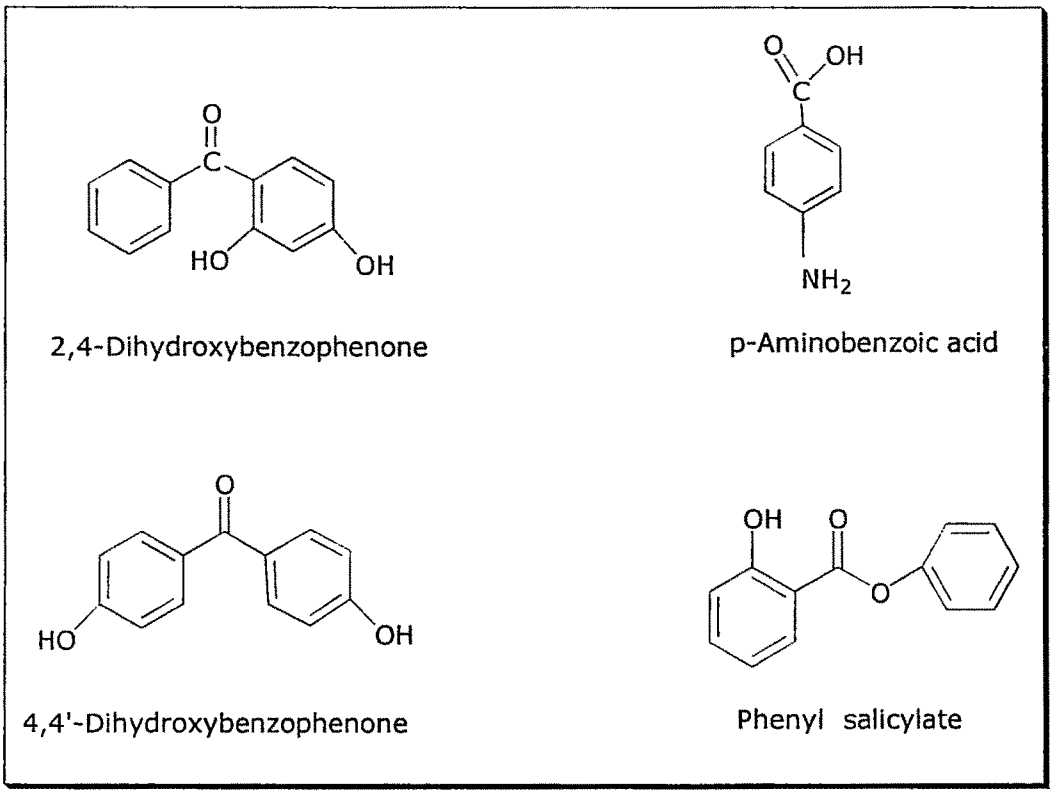


Figure: III.1 Structures of Four UV absorbers.

In the presence of a second chromophore [UV stabilizer (UVS)], the energy may be transferred from Aza-A* to UV stabilizer, the acceptor, ending with Aza-A in its ground state (S_0) and photo stabilizer (UVS) in an excited state, as shown below:

$$\text{Aza-A}^* + \text{UVS} \longrightarrow \text{Aza-A} + \text{UVS}^*$$

For the efficiency of the energy transfer, following condition should be met. The UV spectra of both should have similar excitation wavelengths and proper orientation of the chromophores.[33]

It is known that different UV stabilizers function by different mechanisms. The mechanism by which 2-hydroxybenzophenone derivatives dissipate absorbed energy involves a keto-enol tautomerism as shown in Figure: III.2.

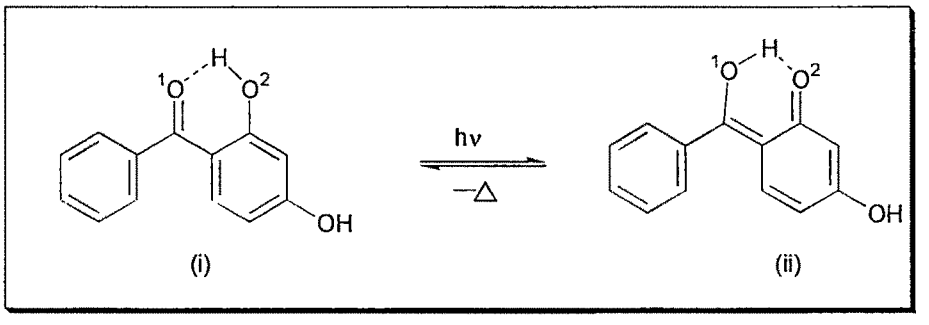


Figure: III.2 Keto-enol tautomerism of 2-hydroxybenzophenones.

In the ground state, the phenolic structure (i) is preferred. Absorption of UV light causes the shift of electron density away from O^2 toward ^1O, which results in the jump of the proton in the same direction. Tautomer (ii) is unstable and rapidly loses its energy in the form of heat reverting back to the ground state structure (i). This

process is responsible for the light stability of 2-hydroxybenzo-phenones.[36] This type of keto-enol tautomerism is not possible in the case of 4,4'-dihydroxybenzophenones.[37]

Aryl esters like phenyl salicylate, do not dissipate absorbed energy by direct absorption of UV light but instead undergo a photo-Fries type rearrangement on prolonged exposure to light, to yield strongly absorbing 2,4'- and 2,2'–dihydroxybenzophenones (Figure: II.3).

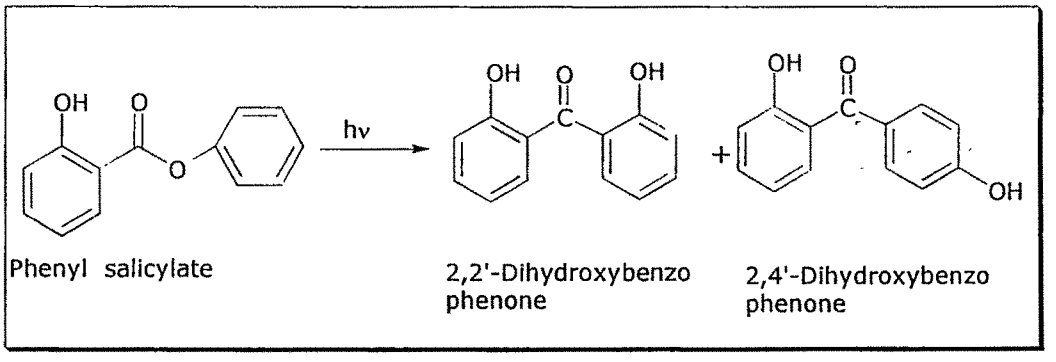


Figure: III.3

These two molecules then dissipate absorbed energy through keto-enol tautomerism as shown in the case of 2,4-dihydroxy benzophenone (Figure: III.2).[36] p-Aminobenzoic acid has a λ_{max} at 290 nm, with a high extinction coefficient of 18,600 and a small absorption peak near 215 nm. Because of this as well as due to its non-toxic nature, it is widely used as a sunscreen agent, where it acts as a filter to block out the ultraviolet radiation from the sun in these regions.[38]

➢ Irradiation experiments in solid phase:

From the foregoing discussion, we chose the four structurally different UV stabilizers as candidates to examine the photostabilizing effect on Aza-A molecules in 1: 1 mole ratio on the glass plates. Thus, standard solutions (100 µL each containing 116 µg of Aza-A) of pure Aza-A with and without each UV stabilizer were applied onto surface of glass slides as a thin film of uniform thickness by using a Hamilton syringe. Slides with UV stabilizers were containing equimolar solutions of Aza-A and the UV stabilizers. The solvent was evaporated at room temperature in a dust free chamber. Ten such precoated slides each with and without UV stabilizers, were kept in a chamber providing ultraviolet radiation (254 nm, Chromline India, low-pressure mercury lamp) from a distance of 21 cm from the source. Another ten such precoated slides each with and without UV stabilizers, kept in a glass-chamber to protect them from dust, were exposed to sunlight incident upon the glass surface during the study period. Remaining ten such precoated slides without any UV stabilizer were kept in a dark chamber under similar experimental conditions, which served as a control. Two glass slides each with and without UV stabilizers were removed from the respective chambers at intervals of 6 hr up to a total period of 30 hrs of exposure to ultraviolet light / sun light. These glass slides were then rinsed with methanol, filtered through a Himedia nylon-66 filter and analyzed for their remaining Aza-A content by analytical HPLC. Control samples were collected at the same intervals and extracted and analyzed similarly.

The percentage remaining of Aza-A recovered at different intervals of time (in hours) for the five solutions (methanolic solutions of Aza-A and its mixture with four UV stabilizers) from different slides exposed

to different light conditions with and without UV stabilizers are shown graphically in Figures III.4 and 5 respectively.

As shown in Figure: III.4, after completion of the experiment (30h, post-irradiation), the percentage residual amounts of Aza-A in the presence of four UV absorbers, namely 4,4'-dihydroxybenzophenone, p-aminobenzoic acid, 2,4-dihydroxybenzophenone and phenyl salicylate on exposure to UV radiation were 45.38, 48.81, 64.84 and 66.13 % respectively, while the same in the absence of any UV absorber was 44.35 %. The percentage recovery of Aza-A on the last day of experiment (30h, post-irradiation) under similar experimental conditions shown in Figure: III.5, exposed to sunlight were 22.54, 24.40, 44.37, 49.32 % respectively, while the same in the absence of any UV absorber was 18.76 %.

Thus, the data presented here suggests that the percentage recovery of Aza-A in the presence of phenyl salicylate was found to be highest as compared to the other three UV absorbers under examination.

Kinetics of Aza-A Dissipation:

The dissipation half-life values (in hours) and rate constant values (in $hours^{-1}$) for degradation of Aza-A under ultraviolet radiation and sunlight were obtained using first order rate equation.[39,40]

$$Y=Y_0e^{-kt} \qquad (1)$$

Where Y represents the Aza-A concentration at time t (d or h, depending on rate), Y_0 represents the initial Aza-A concentration and k is the rate constant. Logarithmic transformation yielded the linear equation.

$$2.303 \log_{10} (Y/Y_0)= -kt \qquad (2)$$

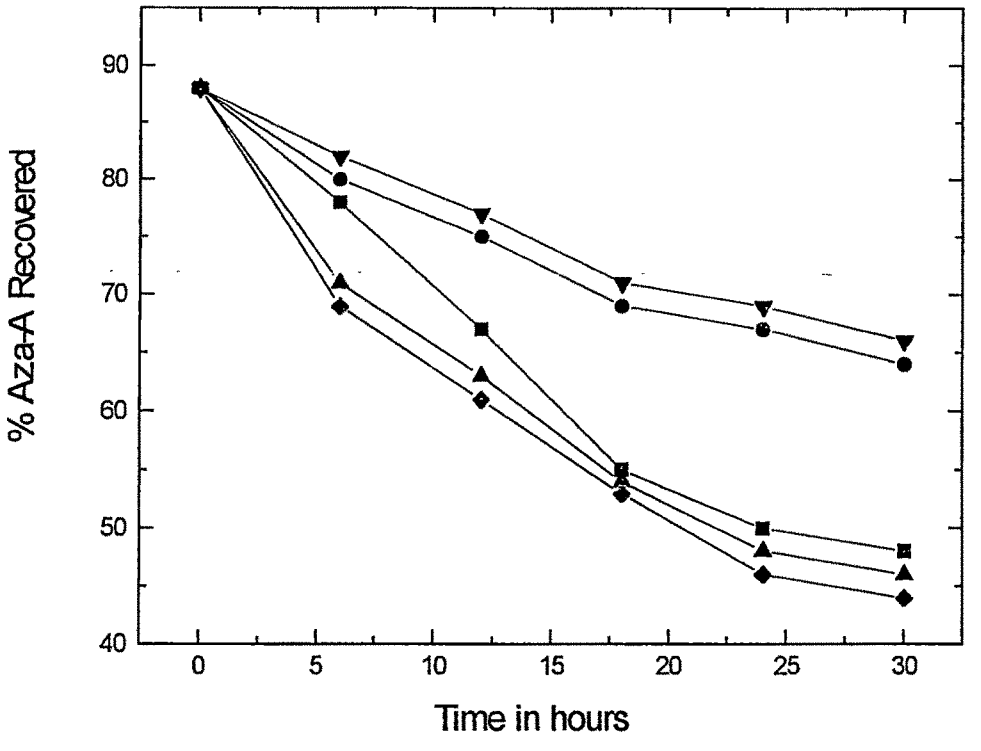
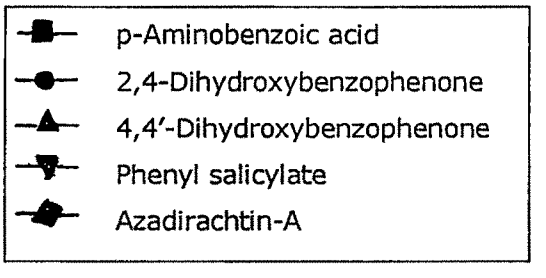


Figure: III.4 Recovery of Aza-A after irradiation of the mixture of Aza-A and different UV stabilizers under ultraviolet radiation.



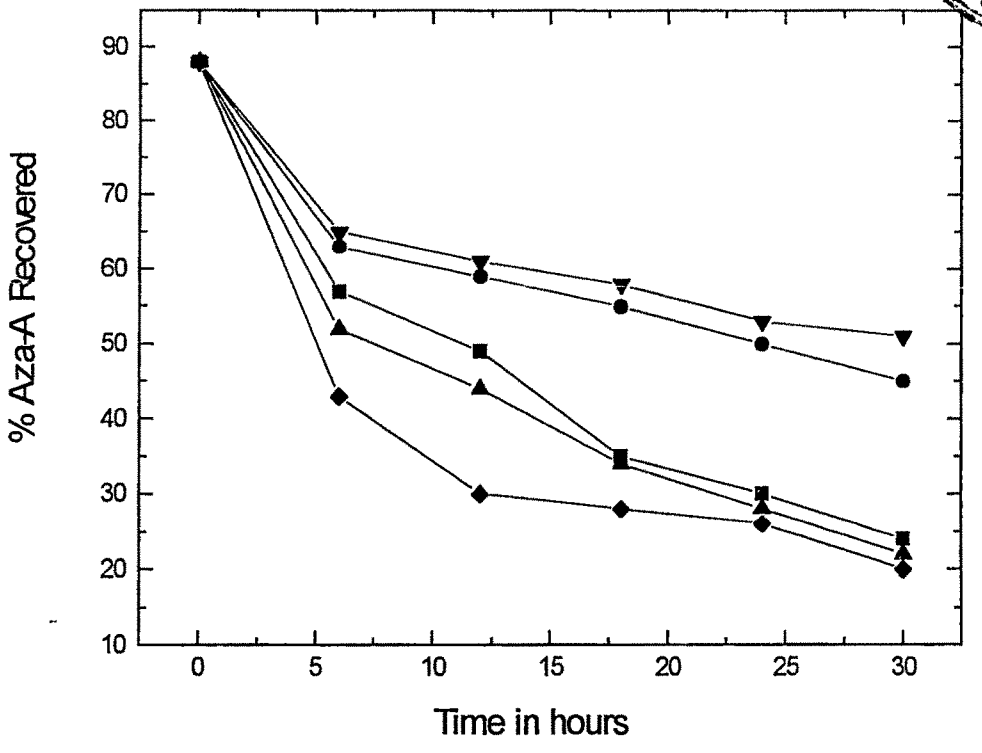
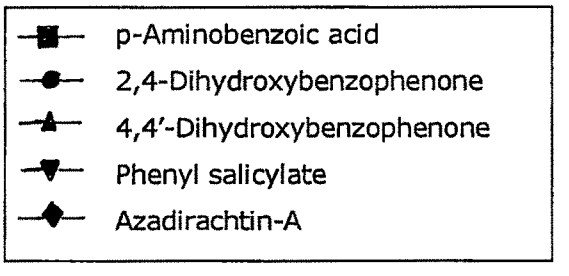


Figure: III.5 Recovery of Aza-A after irradiation of the mixture of Aza-A and different UV Stabilizers under sunlight.



As the concentration dissipated to 50 % of the initial amount, the DT_{50} (time required for 50 % of the initial concentration to dissipate) value could be determined from equation (3) for each experiment.

$$DT_{50} = (2.303 \log_{10}{}^{2})/k$$

or

$$t_{1/2} = 0.693/k \qquad (3)$$

The degradation data obeyed the exponential equation (1) indicating that the reaction followed first order kinetics. The values of dissipation half-life (DT_{50}) and rate constant (K) of Aza-A with and without four UV stabilizers are given in Tables: III:1 and 2 respectively.

DT_{50} values (in hours) for Aza-A in the presence of four UV stabilizers under ultraviolet radiation and sunlight were found to be higher as compared to those obtained for Aza-A alone (Table:III.1). For example, in Aza-A the DT_{50} values found on glass slides in presence of 4,4'-dihydroxybenzophenone, p-aminobenzoic acid, 2,4-dihydroxybenzophenone and phenyl salicylate, when exposed to UV radiation were 24.75, 30.13, 50.21 and 58.72 h, respectively, compared to the DT_{50} value of 22.64 h obtained without addition of UV stabilizers.

Similarly, in the sunlight the DT_{50} values obtained for Aza-A in the presence of UV stabilizers were 11.82, 13.07, 21.00 and 23.10 h respectively, compared to 9.24 h obtained in the absence of UV stabilizers.

Similarly, rate constant values (in $hours^{-1}$) for Aza-A in the presence of four UV stabilizers under ultraviolet radiation and sunlight were found to be lower as compared to those obtained for Aza-A alone (Table: III.2). This is indicative of the rate of disappearance of Aza-A in the presence of four UV stabilizers is delayed. The DT_{50} values for

Aza-A mixed with phenyl salicylate were found to increase approximately 2.6 and 2.5 folds as compared to Aza-A alone under ultraviolet radiation and sunlight respectively. The addition of 2,4-dihydroxybenzophenone, increased its photostability nearly twofold, while 4,4'-dihydroxybenzophenone and p-aminobenzoic acid provided moderate degree of photostabilization to Aza-A in ultraviolet radiation and sunlight. Photostabilization of Aza-A by different stabilizers appears to be due to competitive energy absorption of UV photons by the stabilizer molecules, which cause degradation of Aza-A molecules.

➢ Irradiation experiments in solution:

It has already been shown by Sundaram and Curry earlier, that the photostabilization could be due to either intermolecular energy transfer or competitive absorption of UV photons by the absorber.[7,8] Hence, it was thought to conduct similar study in solution, where the energy transfer between donor and acceptor molecules will be highly efficient. Thus, the standard solutions of pure Aza-A (2.5 mg/5 mL) were prepared separately with and without UV absorbers in methanol. These were then placed in a quartz immersion-well type of a photochemical reactor (Figure:III.6) and were irradiated individually using a high-pressure mercury vapour lamp (HPMV, 250W, Bajaj India) for six hours. The irradiated solutions were then filtered through a Himedia nylon-66 filter and diluted with methanol (25 mL) and analyzed for the remaining Aza-A content by analytical HPLC. Control sample was also irradiated and analyzed similarly.

The percentages remaining of Aza-A recovered from the solutions after six hours of exposure to UV radiation are shown in Table: III.3. After completion of the experiment (6 hr) the residual amounts of

Aza-A in the presence of three UV absorbers namely p-aminobenzoic acid, 2,4-dihydroxybenzophenone and phenyl salicylate on exposure to UV radiation were found to be 1.50, 1.88, and 2.53 % respectively, while in the case of 4,4'-dihydroxybenzophenone, Aza-A was not recovered at all. In case of pure Aza-A (no UV absorber) following six hours of exposure to UV radiation, HPLC chromatogram (Figure:III.7) showed complete disappearance of the peak corresponding to Aza-A and appearance of a number of unidentified peaks. The comparison of percentages of Aza-A recovered from the solutions after six hours of exposure to UV radiation in the presence and absence of different UV absorbers indicated that the addition of phenyl salicylate in Aza-A (in 1:1 mole ratio) provides best photostabilization of Aza-A molecule in solution, among the four candidates studied.

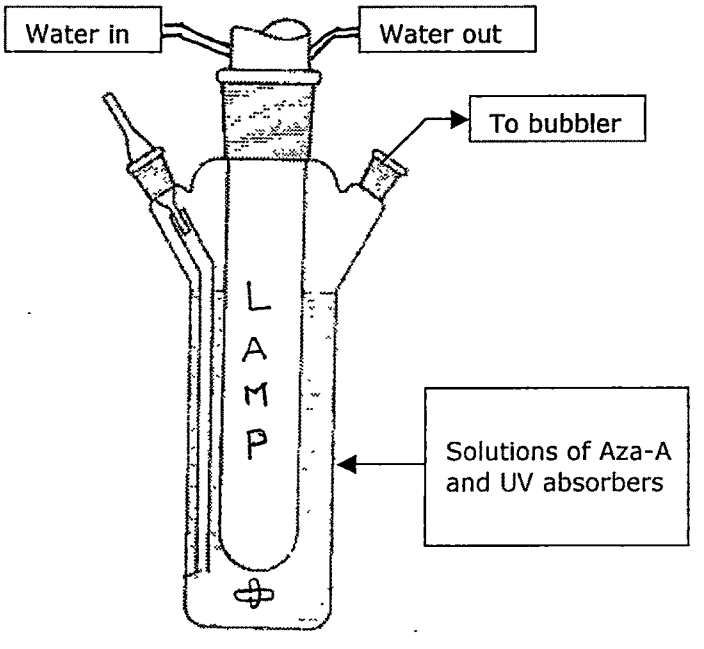


Figure: III.6 Immersion-well type of photo reactor

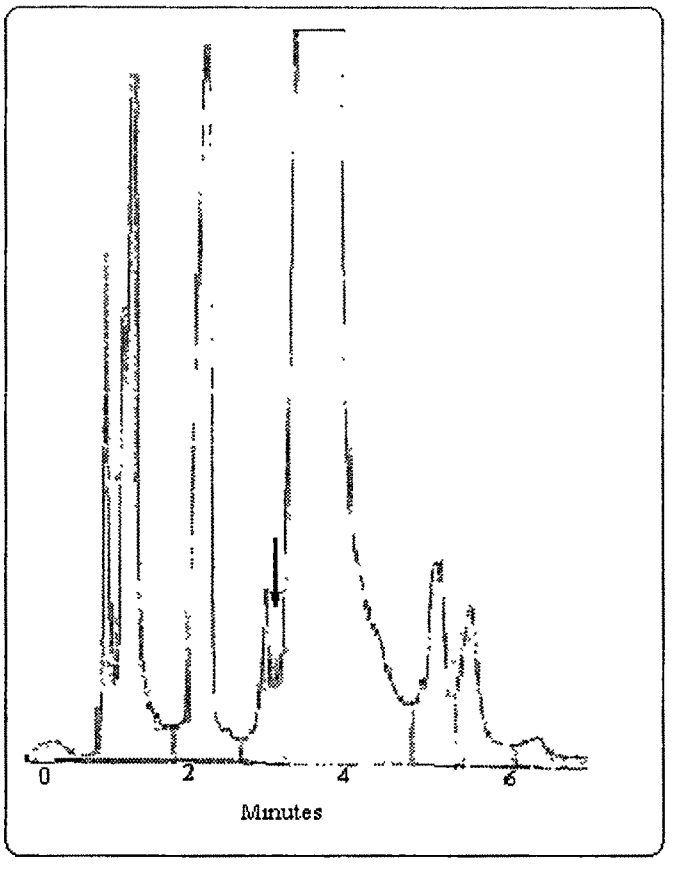


Figure: III.7 Analytical HPLC chromatogram of Aza-A in methanolic solution after exposure to UV radiation for six hours. Arrow indicates peak position of non-irradiated Aza-A.

➢ Absorption spectra of Aza-A and UV absorbers:

Figure: III.8 (A to E) shows the absorption spectra of pure Aza-A and four UV absorbers in methanol. An examination of the UV spectra of pure Aza-A and phenyl salicylate (Figure: III.8 A and E) shows that both the molecules absorb strongly near 215 nm and 214.20 nm respectively. Better photostabilization of Aza-A by phenyl salicylate molecule appears to be due to competitive energy absorption of UV photons, which cause degradation of Aza-A molecule by the absorber molecule. The $\ddot{e}_{max}$ of 2,4–dihydroxybenzophenone (Figure:III.8 B, 211 nm) is somewhat different from that of Aza-A, which causes relatively less efficient stabilization of Aza-A molecule. While, p-aminobenzoic acid and 4,4 -dihydroxybenzophenone showed only marginal effect in stabilizing Aza-A as they showed $\ddot{e}_{max}$ at 290 nm and 296 nm (Figure:III.8 C and D) respectively, and small absorption peak near 215 nm. Evidently their ability to capture the photons at 215 nm is low and hence decrease in their capacity to stabilize UV-labile Aza-A.

Although, it was also suggested earlier by Sundaram and Curry[7,8] in similar experiments, that the photostabilization could be due to either intermolecular energy transfer or competitive absorbtion of UV photons by the absorber, however in our opinion, the later possibility seems more likely. In our previous experiments, we suggested that the addition of phenyl salicylate in (1:1 mole ratio) provides excellent photostabilization of Aza-A molecule in the solid phase among the four absorbers studied. Our results of solution phase obtained as above indicated that the order of stabilization of Aza-A by these absorbers were similar to that obtained in solid phase in accordance with our previous observations.

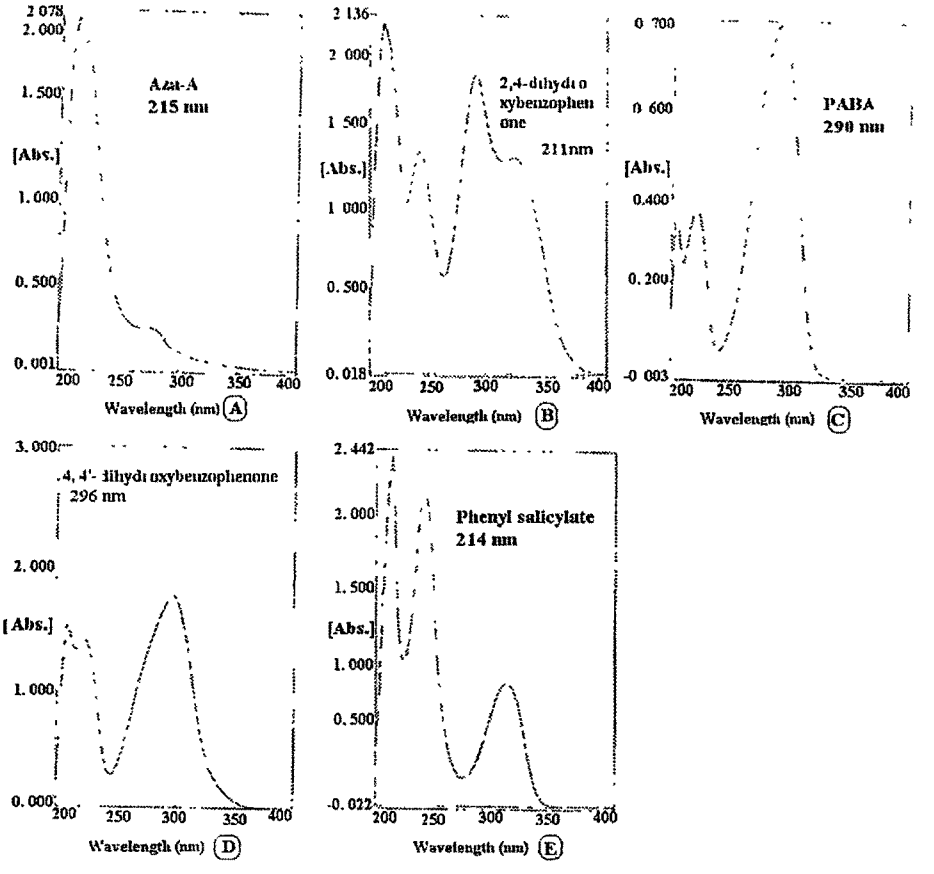


Figure: III.8 UV-Visible spectra of pure Aza-A and the four UV Absorbers.

➢ Relative merits of Four UV absorbers:

The order of photostabilization of Aza-A by four UV stabilizers both in solid phase and in solutions has been found to be as follows.

(1) Phenyl salicylate (2) 2, 4-Dihydroxybenzophenone (3) p-Amino benzoic acid (4) 4, 4'-Dihydroxybenzophenone.

The above order of providing photostabilization to Aza-A may be attributed to following factors.

Phenyl salicylate on photolysis undergoes a photo-Fries type rearrangement to form 2,2'- and 2,4'-dihydroxybenzophenones (Figure:III.3). Both of these products then absorb light and dissipate the absorbed energy through keto-enol tautomerism as showed earlier in Figure: III.2. The use of phenyl salicylate thus effectively amounts to produce two UV absorbers, each of which is able to undergo activation- deactivation cycle through absorption of light and emission of heat.

Obviously, 2,4-dihydroxybenzophenone is able to photostabilize Aza-A to a lesser extent than phenyl salicylate. But it is still more efficient than p-aminobenzoic acid and 4,4'-dihydroxybenzophenone, which are not able to dissipate the absorbed energy through keto-enol tautomerism as shown in Figure III.2.

p-Amino benzoic acid and 4,4'-dihydroxybenzophenone which have small absorption peaks in the region of 215 nm, appear to capture the photons in this region although to a small extent. Thus, they provide photostabilization moderately. Further, energy dissipation through keto-enol tautomarism as shown in Figure: III.2 is not possible in these two molecules.

Thus, the addition of phenyl salicylate in Aza-A molecules provides the best photostabilizatioin of Aza-A molecules in solid phase as well as in solution among the four UV absorbers studied.

It is also interesting to note that the photodecomposition of Aza-A in the sunlight was much more pronounced than that in the presence of UV light. Sunlight consists of photons mostly between 300-1100 nm and thus Aza-A molecules are exposed to a wide range of radiation and hence degrade rapidly. While in case of UV irradiation the lamp emitted radiation mostly at 254 nm (more than 90 %) thus, exposing Aza-A molecules to a narrow range of radiation resulting in relatively less amount of photodecomposition.

III.4 SUMMARY

The percentages of Aza-A recovered at different intervals of time from glass slides exposed to different light conditions with and without UV stabilizers as well as kinetic studies indicated that the addition of phenyl salicylate in methanolic solution of Aza-A (1:1 mole ratio) provides the best photostabilization of Aza-A molecule among the four UV stabilizers studied.

The order of photostabilization by different UV absorbers in the solutions was found to be similar to that in the solid phase.

Thus, the data presented here on percentage recovery of Aza-A after irradiation under different light conditions in solid phase as well as in solutions, suggest that the addition of phenyl salicylate (in 1:1 mole ratio) can provide best photostabilization of azadirachtin molecule in neem based agro formulation.

Interest is mounting world wide to use botanical insecticides for pest control. However, one of the major limitations is their susceptibility to UV degradation, resulting in reduced efficacy. Use of selective UV absorbers, as seen in this study, can enhance their performance.

III.5 EXPERIMENTAL

Materials

Isolation of Aza-A from neem seed kernels by preparative HPLC method and determination of its purity by analytical HPLC method were done by following the procedure described in chapter II (section: II.6)

All the UV stabilizers shown in Figure: III.1 purchased from Lancaster, England and HPLC-grade solvents purchased from Glaxo (Qualigens) India Ltd. were used without any purification.

HPLC Instrumentation

The HPLC used in the study was Waters (LC-4000) analytical cum preparative HPLC equipped with a variable wavelength detector (190 - 600 nm, Model 486), flow controller and Millennium 2010 software. The instrument employed an automatic degassing system; dual solvent system and dual pump heads with common drive, which gave stable and reproducible flows. The Millennium 2010 provided the chromatogram, percent area, and retention time (R_t), for each peak. Detailed descriptions of HPLC procedures are given in chapter II.

Spectroscopic Measurements:

UV/Visible absorption spectra (200-400 nm) of methanolic solutions of analytical grade Aza-A and the four UV absorbers were measured using a Perkin-Elmer Lambda spectrophotometer and are reproduced in Figure: III.8 (A to E).

➢ Irradiation experiments on Glass surfaces:

Standard Solutions

Solutions of 91 % pure Aza-A sample (5.8 mg / 5 mL) were prepared along with the four UV stabilizers in the mole ratio of 1: 1 (Aza-A: UV stabilizer) and 1: 0 (no UV stabilizer) in ethyl acetate. The solutions were stored in amber-coloured bottles between 0 to 4 °C and the Aza-A content in each was determined by HPLC.

Irradiation Experiments

Standard solutions (100 μl each containing 116 μg of Aza-A) of 91 % pure Aza-A with and without each UV stabilizers were applied onto surface of glass slides (each of 75 × 25 mm^2, 1 mm thick) as a thin film of uniform thickness by using a Hamilton syringe. Slides with each UV stabilizer were containing equimolar solutions of Aza-A and the UV stabilizer. The solvent was evaporated at room temperature. Ten such precoated slides each with and without UV stabilizer, were kept in a chamber providing ultraviolet radiation (254 nm, Chromline India, low-pressure mercury lamp) from a distance of 21 cm from the source. Another ten such precoated slides each with and without UV stabilizers, kept in a glass-chamber to protect them from dust, were exposed to sunlight (~300-1100 nm, open sky in May-June 1999, noon with no cloud cover over the study area, grid reference 22° 18′ N; 73° 13′ E, 37 ~ 40 ± 2 °C) incident upon the glass surface during the study period. Remaining ten such precoated slides without any UV stabilizer were kept in a dark chamber under similar experimental conditions, which served as a control.

Extraction and Analysis

Two glass slides each with and without UV stabilizers were removed from the respective chambers at intervals of 6 hr up to a total period

of 30 hrs of exposure to ultraviolet light / sun light. These glass slides were then rinsed with methanol (2 × 2 mL), filtered through a Himedia nylon-66 filter (0.45 µm removal rating, 0.13 mm diameter) and analyzed for their remaining Aza-A content by analytical HPLC. Control samples were collected at the same intervals and extracted and analyzed similarly.

➢ Irradiation experiments in solutions:

Standard Solutions:

Standard solutions of 91 % pure Aza-A sample (2.5 mg /5 mL) were prepared along with the four individual UV absorbers the mole ratio of 1:1 (Aza-A: UV absorber) and 1:0 (no UV absorber) in methanol. The solutions were stored in amber-coloured bottles between 0-4 C and the Aza-A content in each was determined by analytical HPLC.

Irradiation Experiments:

The standard solutions (5 mL diluted to 25 mL with methanol) of pure Aza-A prepared as above with and without individual UV absorbers, placed in a quartz immersion-well type of a photochemical reactor were irradiated individually using a high-pressure mercury vapour lamp (HPMV, 250 W, Bajaj India) for six hours. The irradiated solutions were then filtered through a nylon-66 filter media (0.45 µm removal rating, 0.13 mm diameter) and diluted with methanol (25 mL) and analyzed for the remaining Aza-A content by analytical HPLC. Control sample was irradiated and analyzed similarly.

Table: III.1 Dissipation half-life (in hours) of Aza-A in the presence of different UV stabilizer (Aza-A / UV stabilizers 1:1)

Sr. No	Light	Aza-A alone (no UV stabilizer)	UV stabilizers			
			4,4' –dihydroxy benzophenone	p-amino benzoic acid	2,4-dihydroxy benzophenone	phenyl salicylate
1	UV	22.64	24.75	30.13	50.21	58.72
2	Sunlight	9.24	11.82	13.07	21.00	23.10

Table: III.2 Rate constant values (in hours^{-1}) of Aza-A in the presence of different UV stabilizers (Aza-A / UV stabilizer 1:1)

Sr. No	Light	Aza-A alone (no UV stabilizer)	UV stabilizers			
			4,4' –dihydroxy benzophenone	p-amino benzoic acid	2,4-dihydroxy benzophenone	phenyl salicylate
1	UV	0.030	0.028	0.023	0.013	0.011
2	Sunlight	0.015	0.014	0.012	0.009	0.007

Table: III.3 Percentage recovery of Aza-A in presence and absence of UV absence of UV absorbers on exposure to UV radiation after 6 hours (Aza-A: UV absorber, 1: 1 mole ratio)

Sr. No	Samples	% Recovery
1	Aza-A (no UV Absorbers)	-
2	4,4'-dihydroxybenzophenone	-
3	2,4-dihydroxybenzophenone	1.88
4	p-amino benzoic acid	1.50
5	Phenyl salicylate	2.53

III.6 REFERENCES

1. Kirk-Othmer, Encyclopedia of Chemical Technology, 3rd Edition, Vol: **23**, Wiley (Inter science), New York, pp. 615-627, (1984) and references cited there in.
2. H. J. Heller, Eur. Poly. J., 105, (1969).
3. R. Pater, J. Heterocyl. Chem., **7**, 1113, (1970).
4. K. Banerjee and P. Dureja; Pestic. Sci., **43**, 333, (1995).
5. M. Hussain, H. Perschke and R. Kutscher, J. Pestic. Sci., **28**, 345, (1990).
6. J. Gan, M. Hussain, H. Perschke and M. N. Rather, Chemosphere, **21**(4-5), 589, (1990).
7. K. M. S. Sundaram and J. Curry, Chemosphere, **32**(4), 649, (1996).
8. K. M. S. Sundaram and J. Curry, J. Environ. Sci. Health, B**31** (5), 1041, (1996).
9. D. L. Dexter, J. Chem. Phys., **21**, 836, (1953).
10. G. J. Kavarnos and N. J. Turro, Chem. Rev., **86**, 401, (1986).
11. J. D. Stark and J. F. Walter, J. Agric. Food Chem., **43**, 507, (1995).
12. K. R. S. Ascher, Arch. Insect Biochem. Physiol., **22**, 433, (1993).
13. M. A. Barnby, R. B. Yamasaki and J. A. Klocke, J. Econ. Entomol., **85**, 58, (1989).
14. R. O. Larson, " The Commercialization of Neem" in Focus of Phytochemical Pesticides, The Neem Tree, in: M. Jacobson (ed.). Vol: I, CRC Press Inc., Boca Raton, FL, pp. 155-168, (1988).

15. J. B. Stokes and J. E. Redfern, J. Environ. Sci. Health, A**17** (1), 57, (1982).
16. K. M. S. Sundaram and J. Curry, Pestic. Sci., **41**, 129, (1994).
17. S. R. Yakkundi, R. Thejavathi and B. Ravindranath, J. Agric. Food Chem., **43**, 2517, (1995).
18. L. Ermel, E. Pahlich and H. Schmutterer, Comparison of the azadirachtin content of neem seeds from ecotypes of Asian and African origin, Proc. 2nd Int. Neem Conf., pp: 91-94, (1984).
19. A. J. Mordue (Luntz), and A. Blackwell, J. Insect. Physiol., **32**, 903, (1993).
20. R. O. Kan, Organic Photochemistry, McGraw Hill, New York (1966).
21. D. R. Kearns, Chem. Rev., **71**, 395, (1971).
22. J. March, Advanced Organic Chemistry, Reactions, mechanisms and structures, 2nd edition, McGraw Hill publishers, New York, pp. 644-647, (1977).
23. S. Johnson, E. D. Morgan, I. D. Wilson, M. Spraul and M. Hofmann, J. Chem. Soc., Perkin. Trans., Vol: **I**, 1499, (1994).
24. H. P. Huang and E. D. Morgan, J. Chromato., **519**, 137, (1990).
25. R. C. Saxena, G. P. Waldbauer, N. J. Liquido and B.C. Puma, Effect of Neem Seed Oil on the Rice Leaf Folder, in: Natural Pesticides from Neem Tree. In: H. Schmutterer, K. R. S. Ascher and H. Rembold (eds.). Proc. 1st Int. Neem Conf., Rottach-Egern, Germany, pp. 189, (1980).
26. J. Meisner, K. R. S. Ascher and R. Aly, The Residual Effect of Some Products of Neem on larvae of *Spodoptera litura* in laboratory field trials, in: Natural Pesticides from the Neem Tree. In: H. Schmutterer, K. R. S. Ascher and H. Rembold

(eds.). Proc. 1st Int. Neem Conf., Rottach-Egern, Germany, pp. 157-170, (1980).

27. S. Pradhan and M. G. Jotwani, Chem. Age India, **19**, 756, (1968).
28. K. Ermel, E. Pahlich and H. Schmutterer, azdirachtin content of Neem kernels from all Over the world and its dependence on temperature, air, humidity and light, in: H. Schmutterer and K. R. S. Ascher, Proc. 3rd int. Neem Conf., Nairobi, pp.25-30, (1986).
29. K. M. S. Sundaram and J. Curry, J. Liq. Chromato., **16** (15), 3275, (1993).
30. D. N. Tewari, Monograph on Neem, International Book Distributors, Dehardun, India, pp. 160-161, (1992).
31. S. Johnson and P. Dureja, J. Environ. Sci. Health, B**37**, (1), 75-80, (2002).
32. S.V. Ley, A. A. Denholm and A. Wood, Natural Product Reports, 109, (1993).
33. K. M. S. Sundaram, J. Environ. Sci. Health., B**31** (4), 913, (1996).
34. P.T. Deota, P. R. Upadhyay, K. B. Patel, K. J. Mehta, A.K. Varshney and M. H. Mehta, Natural Product Letters, **16**, 329, (2002).
35. P. T. Deota, P. R. Upadhyay and V. B. Valodkar, Natural Product Letters, Natural Product Research, **17** (1), 21, 2003.
36. (a) J. F. McKeller and N. S. Allen, Photochemistry of Man-made Polymers, Applied Science Publishers, London, pp. 216-255, (1979), (b) Ref No: 1, pp. 615-627.
37. (a) J. A. Otterstedt and R. J. Pater, Heterocycl. Chem., **9**, 225, (1972), (b) Ref No. 3.

38. K. P. C. Vollhardt, Organic Chemistry, W. H. Freeman and Company, New York, pp. 865-867, (1987).

39. K. M. S. Sundaram, L. Sloane and J. Curry, J. Liq. Chromatogr., **18** (2), 363-376 (1995).

40. T. Wang, T. Kadlac and R. Lenahan, R. Bull. Environ. Cotam. Toxicol., **42**, 389, (1989).