



## PHOTOTOXICITY OF $\alpha$ -TERTHIENYL AND PSORALENE IN ROSE AND MUSTARD APHIDS (*MACROSIPHUM ROSAE* & *LIPAPHIS ERYSIMI*) IN DOON VALLEY

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**Abstract:** Climate change has an impact on agriculture and insect pests that affect the crop production. Aphid pests occur throughout the temperate region of the world and cause direct damage by sucking plants sap which affects growth and yield of the crops. Species of aphids are diverse and ubiquitous but their importance among crops differs. Ultraviolet radiation (UVR) is an electromagnetic radiation and constitutes about 10% of the total light output of sunlight. Ultraviolet-B coming due to ozone depletion is the most photochemically reactive wavelength of solar radiation and an important stressor to organisms particularly in their early stages of life and affects the ecosystem. Psoralene and  $\alpha$ -terthienyl are naturally occurring plant metabolites found in many crops and become photo-reactive in the presence of solar ultraviolet-B radiation.

Measurement of solar ultraviolet radiation (UV-B and UV-E) was performed in the Garhwal region of Uttarakhand. Morphological, behavioural changes, mortality rate and glutathione contents were observed in mustard and rose aphids after ultraviolet-B irradiation with psoralene and  $\alpha$ -terthienyl photo-sensitizers. UV-B and UV-E radiation showed seasonal, diurnal and altitudinal variations.  $\alpha$ -terthienyl and psoralene with ultraviolet-B radiation showed stunted growth, morphological changes, low reproduction and high mortality up to 26% in aphids. Reduction in glutathione (GSH) level in aphids was also observed after UV-B and photosensitizer exposure. Enhanced UV-B was found more toxic with photosensitizers and thus, can be used as a natural insecticide. Dose, intensity and species-dependent changes in toxicity of psoralene and  $\alpha$ -terthienyl were observed in aphids. Results indicate that UV-B radiation with photosensitizers has a toxic effect on aphid population, dispersal and diversity. Psoralene was found more toxic than  $\alpha$ -terthienyl in aphids. Mustard aphid (*Lipaphis erysimi*) was found more sensitive than rose aphid (*Macrosiphum rosae*).

**Key words:** Phototoxicity, Psoralene,  $\alpha$ -terthienyl, Aphids, UV-index, Climate change

### Introduction

Ultraviolet (UV) is an electromagnetic radiation with a wavelength from 200 nm to 400 nm and constitutes about 10% of the total light output of sunlight. Due to ozone depletion UV-B (280-315 nm) is partly absorbed by the ozone layer and reaches the Earth's surface. It has been reported that long-term exposure of humans to UV radiation may be very detrimental, inducing skin cancer, cataracts and weakening of immune functions (Diffey, 1996). Ultraviolet-B radiations coming as an outcome of ozone depletion to the

Earth's surface is the most photochemically reactive wavelength of solar radiation and an important stressor to organisms particularly in their early stages of life. It shows a broad range of effect on the biotic ecosystem. Erythema is the most common effect of an intense overexposure to UV radiation (UNEP, 2003). Nevertheless, UV radiation exposure is also beneficial for the health, for example, it contributes towards human Vitamin D synthesis (Webb et al., 2006). The effects of UV-B on organisms depend on the dose



of the harmful radiation to which an individual organism is exposed. The deleterious effects of UV-B is enhanced and become more toxic, when organisms containing photosensitizers produces oxidative radicals. Different species of aphids (Homoptera: Aphididae) are cosmopolitan in distribution and found abundant in temperate climate and attack a wide variety of plants both cultivated and wild and may feed on roots, stems, leaves, inflorescence, fruits, and seeds (De Barro & Carver, 1997). Temperature directly affects insects by altering their behaviour, metabolic rate and downstream cellular and physiological processes (Baleet al. 2007). Aphids feed on wide range of plants species and have a complex life cycle some of them are host alternating species while others live and multiply on particular host community whether these particular host plant species in anyway are associated with plant taxonomy. Aphids have a complex life cycle and involves many morphologically distinct forms and parthenogenesis generation alternating with asexual generation and in about 10% of species this is associated with host alternation (Footitt et al., 2008). Aphid pests occurs throughout the temperate region of the world, causes direct damage by sucking plants sap which affects growth and yield of the crops (Gulidov & Poehling, 2013).

Psoralene and  $\alpha$ -terthienyl are naturally occurring metabolite found in many crop plants which shows adverse impacts on organisms in presence of ultraviolet. Climate change that is predicted to cause a global increase in temperatures and higher UV-B radiation affect the dynamics of complex associations such as aphids and how they may respond to long term environmental changes (Hance et al., 2007). Direct UV-B radiation is another kind of abiotic stress that alters cellular integrity and damages DNA in most living organisms, which also affect aphid life cycle. Work on the potato aphid *Macrosiphum euphorbiae* did not reveal any direct effects of UV-B on reproduction (Nguyen et al., 2017). The primary source of bioactive vitamin D production in human skin upon solar UV-B (280-315nm)

exposure; approximately 90% of circulating levels of vitamin D are attributed to sun light exposure (VoPham et al., 2017). Aphids are very small insects and usually poorly melanized, and hence potentially less protected against radiative stress. This study was designed to monitor UV-B and UV-E radiation in Doon valley, Garhwal region and to assess the impact of  $\alpha$ -terthienyl and psoralene photosensitizers on rose aphid (*Macrosiphum rosae*) and mustard aphid (*Lipaphis erysimi*) at different intensity of UV-B radiation.

### Methodology

**Study Area:** Doon valley situated between 29°-30' to 31°-28' north latitude and 78°-03' to 78°-32' east longitude was selected for the study. Aphid samples were collected from our Doon valley and reared in the laboratory. UV radiation (UV-B and UV-E) monitoring was done using Kipps and Zonen Radiometer, Netherland having 312nm spectral sensitivity. Data recorded were stored in Kipps and Zonen data logger (Logbox- SD). Data from different sites keeping in view of various factors such as season, altitude, latitude, weather condition and monthly variations were collected. UV monitoring was performed on clear sunny days at (600 msl- 1900 msl). Altitude and longitude measurements were done with the help of GPS Garmin USA.

Experimental protocol in aphids was designed using stock culture method (Hughes and Woolcock, 1965) and as modified by Tisher and Songlake (2001). Mesh netting cages were used for culture of aphids. Experiment was conducted in Dehradun. Rose aphid (*Macrosiphum rosae*) and mustard aphid (*Lipaphis erysimi*) were divided into 9 groups with two replicates. Group one was kept control, group two exposed to UV-B low dose, group three exposed with UV-B high dose, group four was given  $\alpha$ -terthienyl, group five was given psoralene, group six exposed to  $\alpha$ -terthienyl + UV-B low dose, group seven exposed to  $\alpha$ -terthienyl + UV-B high dose, group eight exposed to psoralene + UV-B low dose and group nine was exposed to psoralene + UV-B high

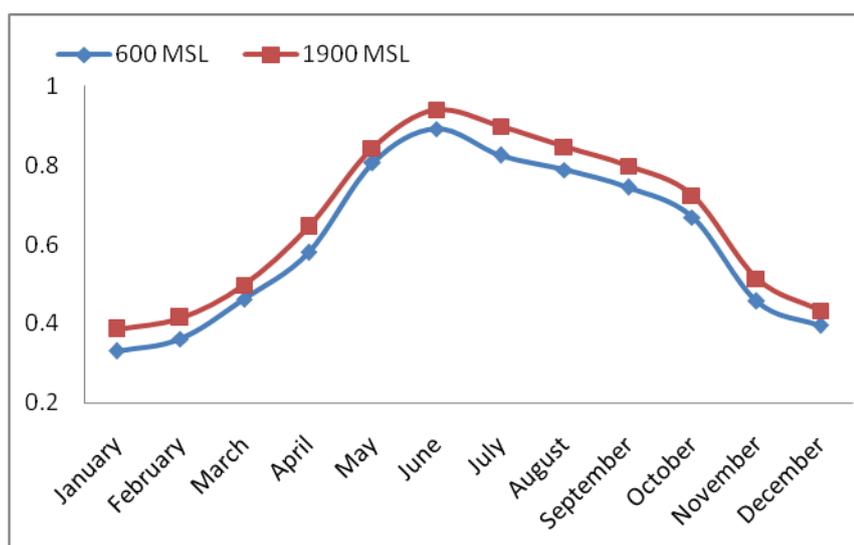


dose. UV-B radiation of low intensity  $0.690 \text{ mw/cm}^2$  and high intensity  $900 \text{ mw/cm}^2$  was given during experimental period in the months of February - March by Philips UV-B lamps. UV-B radiation was measured using Kipps and Zonen Radiometer. Experiment was performed for six days with daily exposure of 2 hour per day.  $\alpha$ -terthienyl (2,2':5',2''-terthiophene). Psoralene (7H-furo[3,2-g]chromen-7-one) photosensitizes of Sigma Aldrich was used in the experiment. Concentration of photosensitizer used was  $10 \text{ mg/litre}$ . Morphological, behavioural changes and mortality rate were recorded by the method (Wilkaniec et. al., 2017). Glutathione (GSH) is a small molecule, a tri-peptide made up of glutamic acid, glycine and cysteine and present in all known organism ranging from bacteria to humans. Glutathione redox status indicates the cellular reduction- oxidation state. It exists in free form

mostly but it forms mixed- disulphide bond with co-enzyme A or cysteine. Total glutathione is determined spectrophotometrically by measuring absorbance at  $405 \text{ nm}$  (Beutler et. al., 1963). Statistical inferences were drawn by using Students' t test (Fisher, 1963).

## Results and Discussion

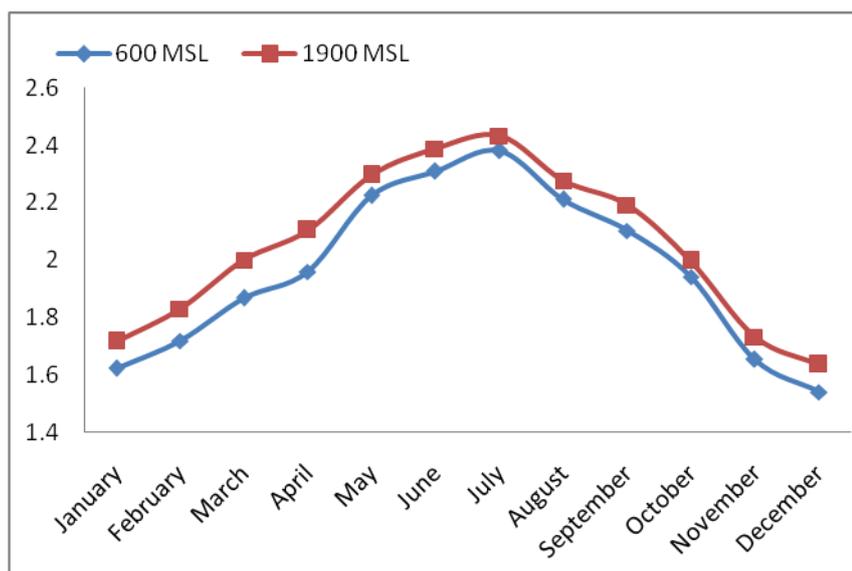
The result on monitoring of solar UV-B radiation recorded in Garhwal region showed the highest value in month of June and minimum value in the month of January. The natural solar UV-B radiation level was recorded minimum at low altitude and increases with high altitude. The value of UV-B seen to increase from morning up to noon and then again seen to decrease gradually up to evening (Fig.-1).



**Figure 1:** Seasonal Variation in Solar UV-B level at different altitudes in Garhwal region.

Solar UV-E radiation also measured every month depending on weather conditions UV-E intensity was found maximum at high altitudes and minimum at low altitudes in Garhwal region. The

maximum value of UV-E was recorded in July and the minimum value was recorded in December. Slight altitudinal variation was observed in solar UV-E intensity (Fig.-2).



**Figure 2:** Seasonal variation in solar UV- E level at different altitudes in Garhwal region

Result on mortality rate in aphids indicates that increase in mortality in all the groups in comparison to control. Non- significant increase in mortality was observed after low intensity of UV-B or with individual exposure of photosensitizers. Highest mortality was reported

after psoralene + UV-B high dose in *Lipaphis erysimi* followed by *Macrosiphum rosae*.  $\alpha$ -terthienyl was found less phototoxicity than psoralene. Enhanced UV-B intensity was found more toxic thus indicating dose/ intensity dependent toxicity in aphids (Table- 1).

**Table 1:** Mortality rate in Aphids with psoralene,  $\alpha$ -tertheinyl at low and high dose of UV-B radiation exposure

S. No	Treatment	<i>Macrosiphum rosae</i>	<i>Lipaphis erysimi</i>
1.	Control	4 ± 0.28	4 ± 0.29
2.	UV-B (low dose)	6 ± 0.45 <sup>NS</sup>	7 ± 0.43 <sup>NS</sup>
3.	UV-B (high dose)	8 ± 0.41 <sup>NS</sup>	9 ± 0.49*
4.	$\alpha$ - tertheinyl	7 ± 0.46 <sup>NS</sup>	8 ± 0.47 <sup>NS</sup>
5.	Psoralene	8 ± 0.75 <sup>NS</sup>	9 ± 0.85*
6.	$\alpha$ -tertheinyl + UV-B (low dose)	13 ± 0.77*	15 ± 0.87*
7.	$\alpha$ - tertheinyl + UV-B (high dose)	16 ± 1.23*	19 ± 1.89**
8.	Psoralene + UV-B (low dose)	17 ± 1.22**	20 ± 1.88**
9.	Psoralene + UV-B (high dose)	22 ± 1.56**	26 ± 2.25**

Results are mean ± S. E. of 5 observations in each group. P- Value < \*0.1, \*\* 0.01,NS- non significant

Reduction in glutathione content GSH was observed in all the groups. Non- significant reduction in GSH was observed after individual exposure of UV-B and photosensitizers. UV-B

and photosensitizer combined exposure cause photo toxicity and reduced the glutathione level significantly. Maximum radiation in glutathione was observed after psoralene + UV- B high dose in *Lipaphis erysimy* (Table- 2).



**Table 2:** Reduced glutathione (GSH mg/g of tissue) in aphids at low and high dose of UV-B radiation exposure with  $\alpha$ -terthienyl and psoralene photosensitizers

S. No	Treatment	<i>Macrosiphum rosae</i>	<i>Lipaphis erysimi</i>
1.	Control	910 $\pm$ 0.83	916 $\pm$ 0.83
2.	UV-B (low dose)	885 $\pm$ 0.95 <sup>NS</sup>	880 $\pm$ 0.94 <sup>NS</sup>
3.	UV-B (high dose)	838 $\pm$ 2.46 <sup>NS</sup>	808 $\pm$ 2.45*
4.	$\alpha$ -terthienyl	905 $\pm$ 2.46 <sup>NS</sup>	890 $\pm$ 2.44 <sup>NS</sup>
5.	Psoralene	895 $\pm$ 2.35 <sup>NS</sup>	875 $\pm$ 2.34*
6.	$\alpha$ -terthienyl + UV-B (low dose)	822 $\pm$ 2.77*	816 $\pm$ 2.67*
7.	$\alpha$ -terthienyl + UV-B (high dose)	814 $\pm$ 2.23*	795 $\pm$ 3.19*
8.	Psoralene + UV-B (low dose)	790 $\pm$ 3.22*	782 $\pm$ 2.18*
9.	Psoralene + UV-B (high dose)	749 $\pm$ 2.56**	732 $\pm$ 3.54**

Results are mean  $\pm$  S. E. of 5 observations in each group. P- Value < \*0.1, \*\* 0.01, NS- non significant

Morphological and behavioural changes were observed in aphids after photosensitizers and UV-B exposure (Fig.3-5).



Rose aphid (*Macrosiphum rosae*)



Mustard aphid (*Lipaphis erysimi*)

**Figure 3:** Rose aphid (*Macrosiphum rosae*) and mustard aphid (*Lipaphis erysimi*) in natural condition.



*Macrosiphum rosae*



*Lipaphis erysimi*

**Figure 4:** Rose aphid (*Macrosiphum rosae*) and mustard aphid (*Lipaphis erysimi*) before exposure.

*Macrosiphum rosae**Lipaphis erysimi***Figure 5:** Rose aphid (*Macrosiphum rosae*) and mustard aphid (*Lipaphis erysimi*) after exposure

According to evidences solar UV-B is low at low altitude in winter season particularly in the month of January and February due to haze, fog, sun position, air mass and low intensity of sun exposure and solar ultraviolet UV-B highest in summer season in the month of July, August and September at high altitude due to clear weather, better sun exposure, air mass and position of sun (Bornman et. al., 2019). Solar radiation is important abiotic stressor for aphid population. UV-B radiation can replace biochemical compounds in plants, which results in changes in the consumption of plants tissues by herbivorous insects and other animals. Aphid pests occur throughout the temperate region of the world, causes direct damage by sucking plants sap which affects growth and yield of the crops. UV-deficient environments reduce winged aphids flight and reproduction ability, thereby reducing aphid propagation and dispersal rate within greenhouses (Legarra et. al. 2012). Aphids stages were not counted separately because aphids density rather than stages structure of the aphid colony is the main factor influencing egg production and oviposition.

UV may interact with other dynamic drivers such as predation pressure to produce pattern in migration that vary seasonally and independently of UV exposure level. Photoenhanced toxicity may occur through two processes, photomodification and photosensitization. Photomodification is the structural modification of chemicals in water to more toxic/ reactive compounds. In photosensitization, the

bioaccumulated chemical transfer light energy to other molecules causing cells damage observed in *cyclops* (Kumar et. al. 2016). Monitoring of solar ultraviolet-B radiation shows seasonal, diurnal and altitudinal variations, which continue to both the positive and negative effects of plants. For example, reduction in herbivore number was reported in UV-B- exposed willows. However, the latter result did not lead to increased herbivory. A review of numerous reports concluded that herbivory is generally reduced by UV-B radiation and it was also suggested that enhanced UV-B radiation may influence the predators of insect herbivores (UNEP, 2005). When exposed to near ultraviolet light, such as in sunlight,  $\alpha$ -terthienyl generates the toxic singlet oxygen.  $\alpha$ -terthienyl results in damage to the respiratory, digestive and nervous system of larvae, resulting in 100% death rates in higher concentrations (Nivsarkar et. al., 2001). They also have use in photodynamic therapy, where the toxin is activated by intense light to destroy cancer cells.

Solar erythemal ultraviolet radiation (EUV) incident at the earth's surface is in the very low wavelength range, which is characterized by high photon energy and indicator UV- index. Excessive level of EUV is harmful to human, animals and other living organisms. In general, EUV is mainly controlled by stratospheric ozone, cloud cover and aerosols. High doses of EUV typical of tropical regions may have adverse effects on human health leading to increases in the risk of skin cancer, cataracts and suppression of the immune system. In addition, high exposure EUV has harmful



effects on plant productivity, marine-ecosystems, biogeochemical cycles, air quality and degrades polymeric materials. High intensity UV induced changes and mortality in insects (arthropods) and *cyclops* reported (Kumar & Kumari, 2011). Insect metabolism and physiology display a high degree of sensitivity to air temperature photoperiodism. Temperature most directly affects insects by altering behaviour and metabolic rate and downstream cellular and physiological processes (Bale et al. 2007). Impact of temperature and CO<sub>2</sub> was observed in aphids morphology and behaviour (Rao et. al., 2016). Anti-oxidative responses of lepidopteron ovarian Tn5B1-4 and Sf-21 cells to photoactivated  $\alpha$ -terthienyl (PAT) are investigated (Huang et al, 2017). Skin cancer results the accumulation of mutations in genomic DNA that are generated primarily by UV radiation. If DNA damaged cell is not properly redirected to apoptosis, the damaged bases can be replicated by error prone mechanism to give rise to mutations that drive the formation cancer (Dakup & Gaddameedhi, 2017).  $\alpha$ -terthienyl occurs in the plant of *Asteraceae* family or found in abundance in the roots of *Tagetes* species and it is toxic to a number of insect species. It generates reactive oxygen species and has capacity to inhibit several enzymes. They become photoreactive in presence of Ultraviolet radiation (Bin et al. 2018). Non-significant decrease in reduced glutathione level was observed after exposure to UV-B low and high dose, Reduction in glutathione level occurs as an indication of decrease in anti-oxidative potential.  $\alpha$ -terthienyl, a thiophene compound present in many plant species including marigolds can be developed as effective photoactivated insecticide (Luan et. al., 2018). Psoralene was found more phototoxic than  $\alpha$ -terthienyl for aphids. Mustard aphid was more sensitive than rose aphid as shown by high mortality. UV radiation with photosensitizer can be used in control of plant aphid pests.

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