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Impact of Ultraviolet Radiation with Alpha-terthienyl on the Aphids Pea Aphid (*Acyrothosiphon pisum*) and Mustard Aphid (*Lipaphis erysimi*)

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ABSTRACT

Background: Due to ozone depletion, the potential threat of rising solar UV radiation levels at the Earth's surface has led to demand for worldwide measurement of solar UV radiation. UV is electromagnetic radiation with a wavelength ranging from 200 nm - 400 nm and constitutes about 10% of the light output of sun. It is the most photochemically reactive wavelength of solar radiation and important critical abiotic stressor for organisms, particularly in their early stages of life. UV radiation directly regulates insect life processes and indirectly changes in insect biochemistry and morphogenesis.

Methods: In this study, measurement of solar UV-B was performed in the Haridwar and Tehri Garhwal, Uttarakhand with the help of radiometer having UV-B sensor. Artificial UV-B provided with UV-B lamp. Mortality rate, Protein content and GSH level were observed in mustard and pea crop aphids species after exposure to solar and artificial UV-B irradiation separately and with alpha-terthienyl photosensitizer.

Result: The data of measurement of UV-B showed that the maximum intensity of UV-B was found in during month of June and minimum in the month of January. Mortality rate of aphids that artificial UV-B radiation with alpha-terthienyl shows a highly toxic effect on aphids population diversity and dispersal. Protein and GSH level decreased in all groups compared to control. Maximum reduction in protein and GSH level was found in after treatment of artificial UV-B with alpha-terthienyl. Exposure of artificial UV-B radiation with alpha-terthienyl shows stunted growth, morphological changes, low reproduction and high mortality in aphids. Artificial UV-B was found to be more toxic than solar UV-B. Mustard aphid (*Lipaphis erysimi*) was found to be more sensitive than pea aphid (*Acyrthosiphon pisum*). Climate change and increase in UV-B levels affect aphid development and population growth.

Key words: Abiotic stressor, Agriculture pest, Climate change, GSH, Photosensitivity, UV-B.

INTRODUCTION

Ultraviolet (UV) is an electromagnetic radiation with a wavelength from 200 nm to 400 nm and constitutes about 10% of the light output of sun. As a result of excessive anthropogenic activity, the ozone layer is gradually depleting, resulting in an increase in UV-B radiation reaching the Earth's surface. This climate change has led to an increase in global temperatures. Aphids show a dynamic of complex associations with increasing level of UV radiation and they show decline respond to long term environmental changes and high intensity of UV radiation (Hance *et al*., 2007). The loss of ozone and rise in earth's surface temperature have led more attention to solar ultraviolet radiation (UV) in recent years. UV- B radiation represents a small but important part of the solar spectrum, it notably affects many biological and photochemical processes and is quite harmful to organisms (Neale *et al*., 2021). Long-term exposure of UV radiation is very harmful to humans, that can cause skin cancer, cataracts and weakening of immune functions (Diffey, 1991).

Ultraviolet radiation, composes a small part of the incoming solar light at the earth surface (Iqbal, 1983) is usually divided into three bands according to its biological effects. UV-C (100-280 nm), completely absorbed by the ozone layer and oxygen before reaching the Earth's surface, UV-B (280-315 nm) partly absorbed by the ozoneand UV-A (315-400 nm), weakly absorbed by the ozone and mostly arrives at the Earth surface (Lee *et al*., 2020). Solar UV-B is the most detrimental band that contributes less than 10% of total UV irradiance at ground level (Diffey, 1991). This effect is due to the high sensitivity of living organisms to

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radiation with a wavelength lower than 315 nm (Serrano *et al.*, 2006) modify the insects physiology and behaviour, this response termed as the phenotypic plasticity (Pigliucci *et al*., 2006) and is controlled by several physiological mechanisms like transcription, translation, enzymeand hormonal regulation that produce local or systemic responses (Whitman and Agrawal, 2009).

Global warming is the primary challenge faced by ecologists in predicting the impact of variation on the biology of ectothermic organisms. (Tougeron *et al*., 2020). UV-B radiation effect herbivorous insects through host plant interaction. Infestation of aphids in high number can change plant biochemistry, it led to decreased indolyl glucosinolate concentrations in plantand the change depend on the threshold infestation. UV-B radiation not only has impacts on the plant traits but also affect phloem feeding aphids, whereas it forces some plants to generate specific defence responses (Kuhlmann and Müller, 2010). Aphid species variation is higher and is found all over the world in all temperate regions, causing direct damage to plant by sucking its sap which affects growth and yield of the crops (Gulidov and Poehling, 2013). Aphids feed on plant nutritive sap. Plants exhibiting aphid damage shows variety of symptoms, *i.e*., decreased plant growth rates, yellowing, mottled, curled and stunted growth of leaves, wilting, low yields and death. Lack of sap creates lack of vigour in the plantand also aphid saliva is toxic to the plants (Nichols, 2007). Aphids form colonies on the stems, petioles and leaves of their host plants and causes different damages (Christelle, 2007; Eaton, 2009) and this damage is grouped into two categories direct damage due to host sap absorption and indirect damage associated with the transmission of phytopathogenic viruses (Blackman and Eastop, 2000) and the ejection of honeydew causing fumagine formation that affects photosynthetic activity. Under severe infestation, the production of plant productivity is reduced from 70% to 80% (Khattak *et al*., 2002). Aphids frequently transmit disease causing organisms like plant viruses to their hosts (Nichols, 2007). They also migrate over long distances. Aphids are ectothermic organisms, all their physiological processes largely depend on climatic variables (Tougeron *et al*., 2020). Aphids have short lifespan (20 to 40 days) their excessive reproduction rate compensates for their short lifespan and maintains their population. The life cycle of various aphid species varies. Aphids have a complex life cycle some of aphid species are host alternating species while others live and multiply on a particular host. Complex life cycle of aphids involves morphologically distinct forms and parthenogenesis generation alternating with asexual generation and about 10% is associated with host alternation (Foottit *et al.*, 2008). Direct exposure to UV-B radiation and increased temperature formed abiotic stress that alters cellular integrity and damages DNA in most living organisms, which also affect aphid life cycle (Rathore and Tiwari, 2017).

This study was designed to study the impact of increasing UV- B radiation with photosensitive chemicals on aphid species behaviour, fecundity and mortality. Mustard and pea both are commercial crops and aphid reduces the production of these crops. Mustard aphid (*Lipaphis erysimi*) and Pea aphid (*Acyrothosiphon pisum*) were selected for different intensity of UV-B radiation in Garhwal range (300 msl-2000 msl) of Uttarakhand.

MATERIALS AND METHODS

Study area

Altitude range 300 msl (Haridwar) and 2200 msl (Tehri Garhwal) of Uttarakhand situated between 29°-94′ to 31°-28′ north latitude and 78°-16′ to 78°-32′ east longitude was selected for this study. Monitoring of solar ultraviolet radiation (UV-B) was done using the instrument and sensor Kipps and Zonen Radiometer, Netherland having 312 nm spectral sensitivity. Data recorded were stored in Kipps and Zonen data logger (Logbox- SD). Data from these sites keeping in view the various factors such as season, altitude, latitude, weather condition and monthly variations were collected. UV monitoring were performed on clear sunny days at (300 msl and 2200 msl). Altitude and longitude measurements were done with the help of GPS Garmin USA. Aphid samples were collected from both sites according to experimental protocol and cultured in the laboratory using stock cultured method of Hughes and Woolcock, (1965) and as modified by Tisher and Songlake (2001). Mesh netting cages were also used for aphids culture. Experiment was conducted in Haridwar and Tehri. Pea aphid (*Acyrothosiphon pisum*) and Mustard aphid (*Lipaphis erysimi*) were divided into 6 groups with two replicates. Group one was kept as control, group two was exposed to Solar UV-B radiation, group three was exposed to Artificial UV-B radiation, group four was taken as alpha-terthienyl, group five was exposed to alpha-terthienyl +Solar UV-B radiation and group six was exposed to alpha-terthienyl + Artificial UV-B radiation. Artificial UV- B radiation of the suitable intensity was given during experimental period in the months of February - March by Philips UV-B lamps. Experiment was performed for six days with an exposure of 2 hours per day. Alpha-terthienyl (2,2':5',2"-terthiophene) photosensitizes of Sigma Aldrich was used in the experiment. Concentration of photosensitizer used was 10 mg/litre. Morphological, behavioural changes and mortality rate were recorded by the method of (Borowiak-Sobkowiak *et al.,* 2017). Glutathione (GSH) is a small molecule of tri-peptide made up of 3 amino acids *i.e.* glutamic acid, glycine and cysteine, it mostly exists in free form but forms mixed- disulphide bond with co-enzyme A or cysteine. Glutathione is present in all known organism, involved in primary and secondary metabolite. Glutathione redox status indicates the cellular reduction-oxidation state. Total glutathione is determined spectrophotometrically by measuring absorbance at 405 nm (Beutler *et al*., 1963).

Statistical inferences were drawn by using Students 't' test (Fisher, 1963).

RESULTS AND DISCUSSION

The results of monitoring of solar UV- B radiation recorded in two sites from different altitudinal range Haridwar (300 msl) and Tehri Garhwal (2200 msl) reveal the highest value of solar UV-B radiation in the month of June and minimum value in the month of January. The results indicate that the highest level of UV-B radiation (0.998 mw/cm^2) is found at high altitude (Tehri) in the month of June and the lowest level of UV-B radiation (0.313 mw/cm^2) is found in the month of January at low altitude (Haridwar) (Fig 1). The natural solar UV- B radiation level was recorded to be minimum at low altitude and highest at high altitude (Mckenzie *et al*., 2011). According to evidences solar UV-B radiation is low at low altitude in winters particularly in the month of January and February due to haze, fog, sun's position, air mass and less exposure to sun and solar ultraviolet UV-B radiation is highest in summers particularly in the month of July, August and September at high altitude due to clear weather, better exposure to sun, air mass and position of sun (Bornman *et al*., 2019). Skin cancer results from 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 may replicate by error prone mechanism to give rise to mutations that drive the formation of cancer (Dakup and Gaddameedhi, 2017).

Result on mortality rate in aphids indicates that mortality rate increases in all groups in comparison to control. Non- significant increase in mortality was observed on exposure to solar UV-B radiation + Alpha- terthienyl photosensitizers (Fig 2-4). Significant increase in mortality was found on exposure to artificial UV-B radiation and solar UV-B radiation + alpha-terthienyl exposure. Highest mortality was reported after exposure to artificial UV-B radiation along with alpha- terthienyl photosensitizers in *Lipaphis erysimi* followed by *Acyrothosiphon pisum*. Alpha-terthienyl was found to be less toxic in absence of light (UV-B) but in presence of increasing intensity of light (solar UV-B or artificial UV-B) it shows high photo reactivity. It generates reactive oxygen species (ROS) and has capacity to inhibit other enzymes (Bin *et al.*, 2018; Kumar and Saini, 2021). Enhanced UV-B intensity was found more toxic thus indicating dose/intensity dependent toxicity in aphids

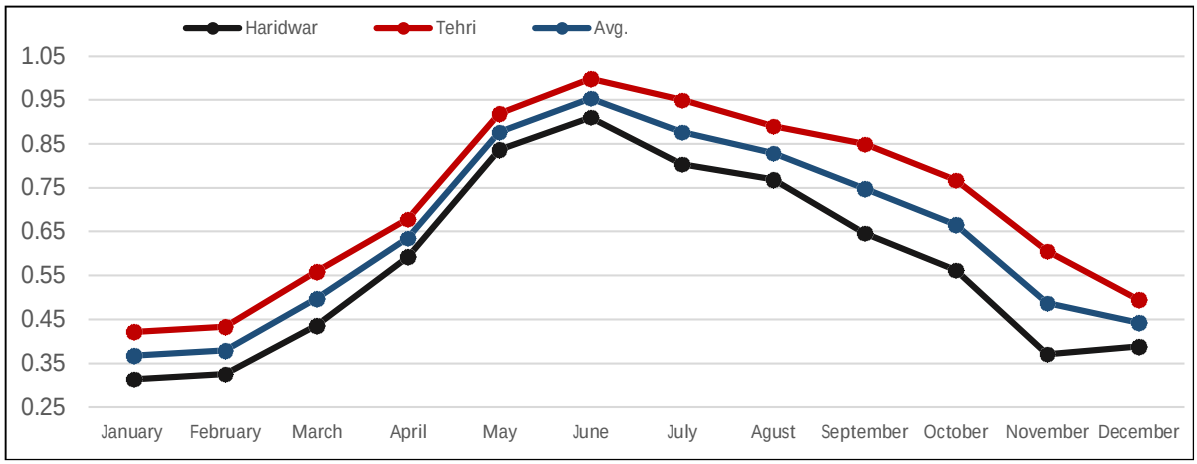


Fig 1: Seasonal variation in solar UV-B radiation level at different altitudinal region of Uttarakhand.



Fig 2: Mustard aphid (*Lipaphis erysimi*) and pea aphid (*Acyrothosiphon pisum*) in natural condition.

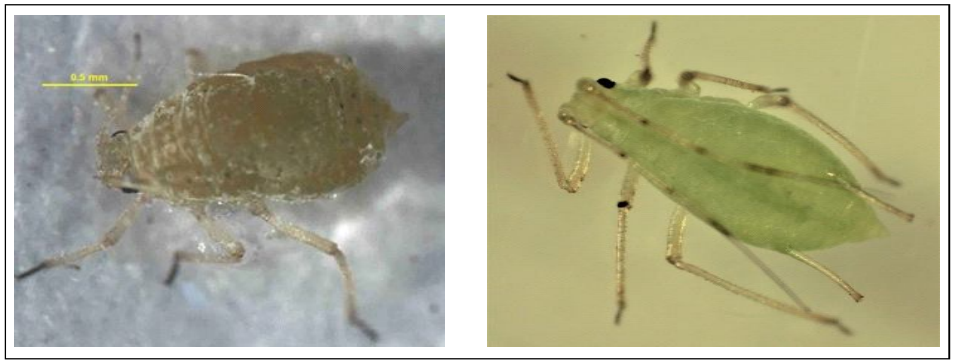


Fig 3: Mustard aphid (*Lipaphis erysimi*) and pea aphid (*Acyrothosiphon pisum*) in before exposure.

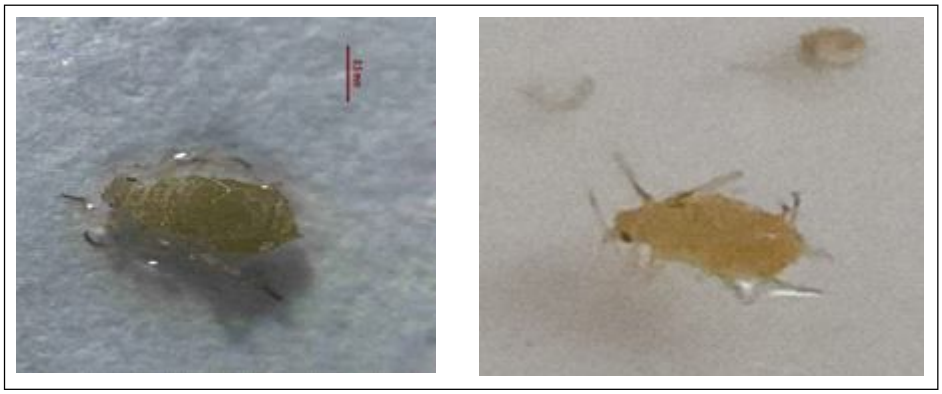


Fig 4: Mustard aphid (*Lipaphis erysimi*) and pea aphid (*Acyrothosiphon pisum*) in after exposure.

Table 1: Mortality rate in different aphid species after solar and artificial UV-B exposure with photosensitizers.

Groups	*Acyrothosiphon pisum*	*Lipapis erysimi*
Control	5.2±1.14	6.2±1.14
Solar UV-B	13.4±1.64^{NS}	14.6±1.34 ^{NS}
Artificial UV-B	19.8±1.64*	20.4±1.34*
Alpha- terthienyl	8..8±1.48 ^{NS}	9.2±1.58 ^{NS}
Solar UV-B ± Alpha-terthienyl	23±1.58*	24.6±1.14**
Artificial UV-B ± Alpha-terthienyl	24.2±1.67**	26.8±1.30**

Results are mean ± S.E. of 5 observations in each group.

Table 2: Protein level and reduced glutathione (GSH) level) in different aphid species after solar and artificial UV-B exposure with photosensitizers.

Groups	Protein level (gm/dl)		Reduced glutathione (GSH) level (mg/g of tissue)	
	Acyrothosiphon pisum	*Lipapis erysimi*	*Acyrothosiphon pisum*	*Lipapis erysimi*
Control	6.23±0.61	6.16±0.42	982.8±2.68	986.5±2.23
Solar UV-B	5.41±0.54^{NS}	5.37±0.25 ^{NS}	980±1.86 ^{NS}	984.2±1.98 ^{NS}
Artificial UV-B	5.12±0.40 ^{NS}	5.07±0.23 ^{NS}	957±4.02*	954.23±4.59*
Alpha-terthienyl	5.43±0.56 ^{NS}	5.16±0.68 ^{NS}	981±1.09 ^{NS}	981.18±2.14 ^{NS}
Solar UV-B + Alpha-terthienyl	3.95±0.68*	3.39±0.54*	909.87±2.52**	910.6±2.08**
Artificial UV-B + Alpha-terthienyl	2.93±1.34**	2.88±1.54**	852.9±2.12**	853.86±1.94**

(Table 1). Protin level was found to reduce consistently in all groups compared to control. A non-significant reduction in the level of protein was found on exposure to alpha- terthienyl photosensitizers, solar and artificial UV-B radiation. Exposure to alpha-terthienyl photosensitizers along with solar and artificial UV-B radiation show significant decrease in protein level in aphids.

Reduction in glutathione content GSH was observed in all the groups. Non-significant reduction in GSH was observed after exposure to solar UV-B radiation and photosensitizers individually. Significant decrease in the level of GSH was found on exposure to artificial UV-B radiation. Maximum reduction in glutathione was observed after alpha - terthienyl + artificial UV- B radiation in *Lipaphis erysimy* (Table 2). Decline glutathione (GSH) content is the indicator of reduction in antioxidative potential of aphids (Kumar and Saini, 2020).

Aphid pests are found in all temperate regions of the world and directly affect plants by sucking their nutritive substance (sap), which reduces the crop growth and productivity of crop. UV- deficient environments reduce winged aphids flight and reproduction ability, thereby reducing aphid propagation and dispersal rate within greenhouses (Kumar and Saini, 2020). Stages of aphids life were not counted separately because aphids density rather than stages and structure of the aphid colony is the main factor influencing egg production and oviposition (Jia and Liu, 2018). Solar radiation as well as temperature are important abiotic stresses to aphid populations (Kumar and Saini, 2021). Plant biochemical components can be substituted by UV-B radiation, which alters how herbivorous insects and other animals consume plant tissues (Thines *et al*., 2008).

UV radiation influences organisms' migration patterns, varying seasonally and independently of exposure. Photo enhanced toxicity occur through photo modification and photosensitization. Photo modification 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 (Kumar *et al*., 2016).

Monitoring of solar UV-B shows seasonal, diurnal and altitudinal variations, shows both positive and negative effects on plants *i.e*., reduction in herbivore number was reported in UV-B exposed willows (Julkunen *et al*., 2005). After a thorough analysis of several studies, it was proved that generally UV-B radiation reduces herbivory and it was suggested that increased UV-B radiation may have an effect on the predators of insect herbivores (UNEP, 2005). When exposed to near ultraviolet light, such as in sunlight α-terthienyl (photosensitive chemical) generates a toxic singlet oxygen and results in damage to the respiratory, digestive and nervous system of arthropods larvae, resulting in 100% death rates in higher concentrations (Nivsarkar *et al*., 2001). α-terthienyl also have use in photodynamic therapy, where the toxin is activated by intense light to destroy cancer cells (Kubrak *et al*., 2022).

Enhance intensity of ultraviolet radiation induced changes and mortality in insects (arthropods) and cyclops (Kumar and Kumari, 2011). Insect metabolism and physiology display a high degree of sensitivity to atmospheric temperature, solar light intensity and photoperiodism (Miki, 2020). Solar ultraviolet radiation directly affects insects life by altering their behaviour and metabolic rate and downstream cellular and physiological processes (Ballare *et al*., 2011). Alpha-terthienyl occurs in the plant of Asteraceae family or found in abundance in the roots of Tagetes species and it is toxic to number of insect species. It generates reactive oxygen species and has capacity to inhibit several enzymes and it become photoreactive in presence of Ultraviolet radiation (Bin *et al*., 2018). Anti-oxidative responses of lepidopteron ovarian Tn5B1-4 and Sf-21 cells to photoactivated alpha-terthienyl (PAT) are investigated (Huang *et al*., 2017). 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) and was found more phototoxic than alpha-terthienyl for aphids. Mustard aphid was more sensitive than Pea aphid as shown by high mortality. Ultraviolet radiation with alpha- terthienyl photosensitizer can be used to control plant aphid pests to prevent the loss of productivity of economical beneficial crop.

CONCLUSION

Due to increasing human activities in the environment and the thinning of the ozone layer, more UV-B radiation reaches the earth surface. Intensity of UV-B increases with the increased altitudinal range. The present study is based on monitoring of UV-B radiation in two different sites of Uttarakhand *i.e*., Haridwar and Tehri, that vary in altitude one at 300 msl and another at 2000 msl. The highest values of UV-B radiation were found in the months of June (0.899 and 0.968 mw/cm^2) and the minimum in the months of January. UV-B radiation acts as a critical abiotic stressor and effects the insect's physiology and behaviour, that directly regulate aphid species life processes and indirectly affect the changes in their biochemistry and morphogenesis. All physiological processes of ectothermic insect (aphids) largely depend on climatic variables, *i.e*., light (UV radiation), humidity and temperature. UV-B radiation with alpha-terthienyl photosensitizer cause abiotic stress that alters cellular integrity and damages cell functioning. The lifespan of aphid species is only about 20 to 40 days. Impact of alpha-terthienyl with UV-B had a significant effect on the longevity and fecundity of different aphid species. The longest longevity period of aphids was recorded at solar UV-B exposure and the shortest was found when exposed to artificial UV-B along with photosensitizer. Their excessive reproduction rate compensates for their short lifespan and maintains their population. All immature stages of mortality in aphids are highest exposed to alpha-terthienyl with artificial UV-B and lowest when exposed to alpha-terthienyl, solar and artificial UV-B. As compared to pea aphids, mustard aphids are more sensitive to UV-B radiation. Artificial UV radiation with photochemicals can be used to control the aphid population for preventing the crop lose and excessive use of

insecticide. Aphids can be used as a model for phototoxicity and climate change studies. Artificial UV radiation with photochemicals can be used to control the aphid population for preventing the crop lose and excessive use of insecticide. Aphids can be used as a model for phototoxicity and climate change studies.

Conflict of interest

All authors declare that they have no conflicts of interest.

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