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Impact of alpha- terthienyl phototoxicity on mustard aphid (*Lipaphis erysimi*) and pea aphid (*Acyrothosiphon pisum*)

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Abstract
Ozone depletion threatens rising solar UV radiation levels, prompting worldwide measurement. UV radiation, a photochemically reactive wavelength, is a critical abiotic stressor for organisms, regulating life processes and indirectly altering insect biochemistry and morphogenesis, especially in early life stages. Aphids are small insect pests that cause problems for a variety of crops, fruits, vegetables, and decorative plants. They feed plant phloem sap, which affects plant growth, flowering, and productivity. Alpha-terthienyl is a naturally occurring plant metabolite that is found in tagget species of Asteraceae family plants and becomes photoreactive in the presence of solar UV-B radiation.
Monitoring of solar UV-B was performed in Dehradun and Tehri Garhwal. Mortality rate, protein, and glutathione contents were observed in mustard aphid (*Lipaphis erysimi*) and pea aphid (*Acyrothosiphon pisum*). After solar and artificial ultraviolet-B irradiation with alpha-terthienyl photosensitizer. UVB radiation showed seasonal, diurnal, and altitude variations. After exposure of alpha-terthienyl with solar and artificial UV-B on aphids showed morphological changes, low reproduction, and increased mortality. A reduction in protein and glutathione (GSH) levels in aphids was also observed after UV-B and photosensitizer exposure. Artificial UV-B was found toxic, but its toxicity increases with photosensitizers and thus can be used as a natural pesticide. Aphids exposed to alpha-terthienyl showed dose intensity, and species-dependent variations in toxicity. Results indicate that UV-B radiation with photosensitizers has a toxic effect on aphid population, dispersal, and diversity.

Keywords: UVB, glutathione, alpha-terthienyl, photosensitive, ozone depletion

Introduction

Ultraviolet radiation is the most photochemically reactive wavelength of solar energy reaching the surface of the earth and has a broad range of effects on organism biogeochemistry, biota, and ecosystems. Anthropogenic impacts on the earth's atmosphere are leading to significant changes in UV radiation exposure in various environments. Aphids are the small insects that belong to the family Aphidae. They show a dynamic of complex associations with increasing UV radiation and they may show decline in response to long-term environmental changes, high temperature, and high intensity of UV radiation (Hance *et al*., 2007) [13]. In recent decades, increased solar UV has received special attention due to thinning of ozone and increasing earth surface temperature. UV radiation, one of the most important small parts of the solar spectrum, is significantly required for many biological and photochemical processes but is quite harmful to organisms (Neale *et al*., 2021). Skin cancer, cataracts, and a potentially weakened immune system are all consequences of prolonged exposure to UV radiation for humans. (Diffey, 1991). Solar ultraviolet radiation is divided into three bands based on its biological effects: UV-C (100–280 nm), which is completely absorbed by the ozone layer and oxygen before reaching the Earth's surface; UV-B (280–315 nm), which is partially absorbed by the ozone; and UV-A (315–400 nm), which is weakly absorbed by the ozone and mostly reaches the Earth surface (Lee *et al*., 2020) [25]. According to Diffey (1991), UVB is the most harmful band, making up less than 10% of the total UV irradiation at ground level. Because living things are extremely sensitive to radiation with a wavelength shorter than 315 nm, this impact results (Serrano *et al*., 2006) [33].

The primary challenge of global warming faced by ecologists is to predict variation that occurs in the biology of ectothermic organisms (Brodeur *et al*., 2013). UV-B radiation impacts herbivorous insects through host plant interaction. Infestation of aphids in high numbers can change plant biochemistry. High numbers of aphid progeny on low UV-B plants led to decreased indolyl glucosinolate concentrations. Induced change in indolyl glucosinolates depends on the threshold infestation. UV-B radiation impacts plant traits and also affects phloem-feeding aphids, whereas high growth of aphid forces some plants to generate specific defence responses (Kuhlmann and Müller, 2010) [20]. Different species of aphids are found all over the world, but the majority are found in temperate regions. Variation of aphid species is higher in temperate regions than tropical regions. They migrate over short to little long distances. Aphids have a short life span. Their excessive reproduction rate compensates for their lifespan and maintains their population. The life cycle of various aphid species varies widely. Reproduction involves asexual as well as sexual reproduction. Aphids are ectothermic organisms; all their physiological processes largely depend on several climatic variables that include temperature (Brodeur *et al*., 2013). Aphid pests occur throughout the temperate region of the world and cause direct damage by sucking plant sap, which affects growth and yield of the crops (Gulidov & Poehling, 2013) [12].

Aphids consume the phloem sap of plants, and infested plants display damage with a range of symptoms, including reduced growth rates, mottled leaves, yellowing, curled leaves, wilting, low yields, and mortality. Aphid saliva is harmful to plants, and lack of phloem sap causes a plant to

become weaker. Aphids also transmit pathogenic organisms, such as disease-causing bacteria and viruses, to their host plant. (Nichols, 2007) [29]. The intensity of solar light (UV) and temperature are directly affecting small insects like aphids, drosophila, and mosquitoes by altering their behaviour, metabolic rate, and downstream cellular and physiological processes (Bale *et al*. 2007). Aphids have a complex life cycle, feeding on various plant species, with some being host-alternating and others multiplying on specific hosts. Foottit *et al*., 2008) [11]. Aphids form colonies on plant stems, petioles, and leaves, causing direct damage due to sap absorption (Christelle, 2007; Eaton, 2009) [7] and indirect damage from phytopathogenic virus transmission (Blackman & Eastop, 2000) [4] and the ejection of honeydew, causing fumagine formation that affects photosynthetic activity. In cases of severe infestation, aphids reduce the plant productivity up to 70–80% (Khattak *et al*., 2002) [18]. The continuous rise in atmospheric CO2 and global air temperature is directly affecting the interactions between insects and plants. Elevated CO2 and temperature immediately alter plant characteristics that are vital to insects, such as nitrogen content, whereas insects are only directly impacted by temperature. Direct UV-B radiation and increased temperature form abiotic stress that alters cellular integrity and damages DNA in most living organisms, which also affects the aphid life cycle (Rathore and Tiwari, 2017) [32].

This study was designed on the monitoring of solar UVB intensity in Dehradun and Tehri Garhwal and to assess the impact of the Mustard aphid (*Lipaphis erysimi*) and Pea aphid (*Acyrothosiphon pisum*) behaviour, fecundity and mortality rate with solar and artificial UVB radiation individually and with light reactive chemical alpha-terthienyl.

Study area and Methodology

Dehradun and Tehri are two sites we selected for the monitoring of UVB in the regions of Uttarakhand situated between 29 -30' to 31°-28' north latitude and 78 -16 to 78°-32' east longitude. Monitoring of UV radiation was done by using the Kipps and Zonen Radiometer, Netherlands, having 312 nm spectral sensitivity. Data recorded were stored in Kipps and Zonen data loggers (Logbox-SD). Data from these sites, keeping in view of various environmental factors such as season, altitude, latitude, weather condition and monthly variations, was collected.

Aphid samples were collected from these sites and reared in the laboratory. For the experimental protocol and study of the impact of solar and artificial UV-B radiation with alpha-terthienyl on aphid mortality or fecundity rate, morphological and behavioural changes were designed using the stock culture method for established aphid colonies on potted plants (Hughes and Woolcock, 1965) [14] and as modified by Tisher and Songlake (2001) [36]. For observed changes in aphids in a different time interval, leaf cages and leaf clip cages were also used. Aphids were cultivated in mesh netting cages.

Mustard aphid (*Lipaphis erysimi*) and pea aphid (*Acyrothosiphon pisum*) were divided into six groups with replicates. Group 1 was kept control, group 2 and 3 were exposed to solar UV-B and artificial UV-B, group 4 was exposed with individual exposure of alpha-terthienyl photosensitizer, and group 5 and 6 were exposed to solar UV-B and artificial UV-B with alpha-terthienyl. During the experimental period artificial UV-B radiation was given by Philips UV-B lamps. The concentration of photosensitizer used was 10 mg/liter. The experiment was performed for 7 days. Morphological, behavioural changes, and mortality rate were recorded by the method of Wilkaniec *et al*., 2017. For the level of protein concentration measured in aphid homogenates, the method of Bradford (1976) was used. As a protein standard, in this method bovine serum albumin (BSA) was used. Spectrophotometric measurement of absorbance at 405 nm yields the total glutathione level. (Beutler *et al*., 1963) [2]. The status of glutathione redox represents the cellular oxidation-reduction state and it is capable to preventing damage to cellular component from ROS, and peroxides. Mostly glutathione exists in free form in the cell, but it creates a mixed-disulphide bond with co-enzyme A or cysteine. Statistical inferences were drawn by using the students't' test (Fisher, 1963) [10].

Result and Discussion

The result indicates the monitoring of solar UV-B radiation recoded in the Dehradun and Tehri Garhwal regions of Uttarakhand state. The value of solar UV-B radiation recorded highest in the month of May–June (0.955) in Tehri Garhwal and minimum value in the month of January–February (0.361) in Dehradun. The value of the natural solar UV-B radiation level was found to be minimum in Dehradun as compared to Tehri Garhwal (Fig.-1). Intensity of solar UV-B is low at low altitude, and it increases with increasing altitude; it is also seen to increase from morning up to noon and then again seen to decrease gradually up to evening.

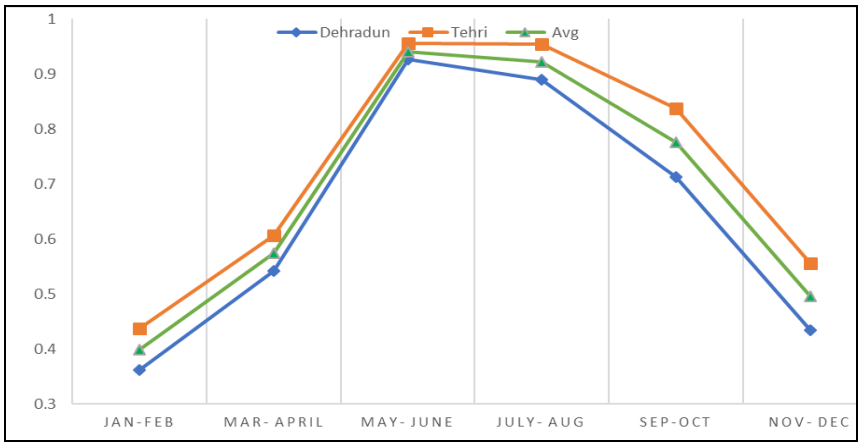

Fig 1: Seasonal Variation of solar UV- B level (mw/cm^2) in Dehradun and Tehri in 2021

The result on mortality rate in aphids indicates an increase in mortality in all the groups in comparison to control. After exposure to solar UV-B, a non-significant increase in mortality was observed. A significant mortality rate was observed in aphids after artificial UVB and individual alpha-terthienyl exposure. The highest mortality was reported after artificial UVB + alpha-terthienyl exposure. Alpha-terthienyl is a photoreactive chemical substance that is found naturally (Table 1).

Table 1: Mortality rate in aphids with alpha- terthienyl with solar and artificial UV-B

S. No.	Groups	*Acyrothosiphon pisum*	*Lipaphis erysimi*
1.	Control	6.2 ± 1.14	5.2 ± 1.14
2.	Solar UVB	14.6 ± 1.34^{NS}	13.4 ± 1.64^{NS}
3.	Artificial UVB	19.4 ± 1.34*	19.2 ± 1.64*
4.	Alpha-terthienyl	20.2 ± 1.58*	19.8 ± 1.48*
5.	Solar UVB + Alpha-terthienyl	24.6 ± 1.14**	23 ± 1.28*
6	Artificial UVB + Alpha-terthienyl	26.1 ± 1.30**	24.2 ± 1.67**

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

The levels of protein consistently decreased in all experimental groups, with significant changes observed after individual exposure to alpha-terthienyl and the maximum decrease in protein level in aphids observed after artificial UV-B with alpha-terthienyl (Table 2).

Table 2: Protein level (g/dl) in mustard aphid and pea aphid after solar and artificial UV-B exposure with alpha-terthienyl photosensitizer

S. No.	Groups	*Lipaphis erysimi*	*Acyrothosiphon pisum*
1.	Control	6.16 ± 0.42	6.23 ± 0.61
2.	Solar UV- B	5.37 ± 0.25 ^{NS}	5.41 ± 0.54^{NS}
3.	Artificial UV- B	4.98 ± 0.23*	5.12 ± 0.40 ^{NS}
4.	Alpha-terthienyl	4.16 ± 0.68*	4.53 ± 0.56*
5.	Solar UV- B + Alpha-terthienyl	3.39 ± 0.54*	3.95 ± 0.68*
6.	Artificial UV-B + Alpha-terthienyl	2.88 ± 1.54**	2.93 ± 1.34**

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

A reduction in glutathione content of GSH was observed in all the groups. A non-significant reduction in GSH was observed after individual exposure to UV-B and photosensitizers. UV-B and photosensitizer combined exposure cause phototoxicity and reduce the glutathione level significantly. Maximum reduction in glutathione level was observed after alpha-terthienyl+ artificial UV-B in both aphids (Table 3).

Table 3: Reduced glutathione (GSH) level (mg/g of tissue) in mustard aphid and pea aphid after solar and artificial UV-B exposure with alpha-terthienyl photosensitizer

S. No.	Groups	*Lipaphis erysimi*	*Acyrothosiphon pisum*
1.	Control	982.8 ± 2.68	986.5 ± 2.23
2.	Solar UV- B	980 ± 1.86 ^{NS}	984.2 ± 1.98 ^{NS}
3.	Artificial UV- B	977 ± 4.02*	977.23 ± 4.59*
4.	Alpha-terthienyl	941 ± 1.09**	941.8 ± 2.14*
5.	Solar UV-B + Alpha terthienyl	909.87 ± 2.52**	910.6 ± 2.08**
6.	Artificial UV-B + Alpha-terthienyl	852.9 ± 2.12**	853.86 ± 1.94**

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

Evidence proved that due to sun position, air mass, fog and low intensity of sun exposure, solar UV-B is lowest at low altitude in the winter season and highest in the summer and rainy season at the high altitude due to clear weather, high intensity of sun exposure, and sun position (Bornman *et al*., 2019) [5]. Solar UV radiation is an important abiotic stressor for the aphid population. UV-B radiation can alter the biochemical composition of plants, leading to changes in the consumption of plant tissues by herbivorous insects and other animals.

Aphid pests, found in temperate regions, cause direct damage by sucking plant sap, impacting crop growth and yield (Legarrea *et al*. 2012). The aphid's density, not its stage structure, is the primary factor influencing egg production and oviposition, so aphid stages were not counted separately. UV can interact with other dynamic drivers like predation pressure, resulting in seasonal and independent migration patterns. Photo enhanced toxicity occurs through photo modification (structural modification of chemicals) and photosensitization, where bioaccumulated chemicals in water transform into toxic compounds, causing cell damage in organisms (Kumar *et al*. 2016) [24]. Solar ultraviolet-B radiation, which is monitored seasonally, diurnally, and altitudinal, has both positive and negative effects on plants. It generally reduces herbivory and may influence insect herbivore predators. (UNEP, 2005) [38].

When exposed to near ultraviolet light, such as in sunlight, alpha-terthienyl is a naturally occurring photochemical that generates the toxic free radicals of oxygen when exposed to ultraviolet radiation or solar light. Additionally, it is used in photodynamic therapy of cancer treatment, in which photochemicals are activated by intense light to destroy cancer cells. Alpha-terthienyl results in damage to the insect larvae digestive system, respiratory system and nervous system, resulting in highest death rates in higher concentrations (Nivsarkar *et al*., 2001) [30]. Insect

metabolism and physiology are highly sensitive to air temperature photoperiodism. Temperature and solar UV have a direct impact on insects by affecting their behavior, metabolic rate, and physiological and cellular processes (Bale *et al*., 2007; Rao *et al*., 2016). Many investigations have proven that lepidopteron ovarian Tn5B1-4 and Sf-21 cells' antioxidative reactions to photoactivated alpha-terthienyl (PAT). (Huang *et al*., 2017). Skin cancer is caused by DNA mutations primarily generated by UV radiation, which can be replicated by error-prone mechanisms, leading to cancer formation when the damaged DNA is not properly redirected to apoptosis (Dakup & Gaddameedhi, 2017) [8]. Alpha-terthienyl, a thiophene compound, is toxic to a number of insect species and is found in abundance in the roots of Tagetes plant species of the Asteraceae family. It has the capacity to inhibit several enzymes and produce free radicles of reactive oxygen species (ROS) that become photoreactive in the presence of UV radiation (Bin *et al.* 2018) [3]. Reduction in glutathione and protein levels indicates a decrease in anti-oxidative potential. Ultraviolet radiation is toxic, but artificial UVB radiation was found to be more toxic than solar UV. Mustard aphid was more sensitive than pea aphid, as shown by high mortality. Pests that feed on plants can be controlled with UV radiation and photosensitizers, which can lessen the need for pesticides in general pest management procedures.

Fig 2: Pea aphid in control group

Fig 3: Mustard aphid in control group

Fig 4: Pea aphid after exposure of artificial UV-B radiation with alpha- terthienyl

Fig 5: Mustard aphid after exposure of artificial UV-B radiation with alpha- terthienyl

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