Studies on the effect of partially ultra-violet blocking films on the plant growth, pigmentation and insect and disease controls of some horticultural crops

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Chapter 1

General Introduction

Agriculture has been playing a major contribution utilizing natural resources and biodiversity and plays an important social and environmental role. It is clear that technology and innovation plays a major role in increasing yields and profitable utilization of lands and therefore, reduces the withdrawal of agricultural activities. The contribution of plastic materials to agriculture has been a real revolution to traditional agriculture that has made possible the increases of productive areas of the world with the incorporation of areas with unfavorable climatic conditions. Protected crops especially horticultural production has increased in the last decade with the development of new types of plastic films which have achieved a high degree of specialization with different applications and properties (anti-dripping, ant-fogging, anti-thermal, anti-pest etc.). At present, greenhouses are mainly distributed in two regions of the world; one of them is Asia, especially in China, Korea and Japan, with almost 80% of the total area, and in the Mediterranean region covering about 15% of the world (Espi et al., 2006). This protected production requires the use of 1,000,000 $t.year^{-1}$ of plastic films to cover all the protected crops grown worldwide (Espi et al., 2006). Nowadays, Bangladesh is facing a climate change effect on soil and crop fields. So, it was time demanding to focus on protected cultivation system adopting known comparatively easy to handle techniques like using plastic films; polyethylene, polyolefin (PO), polyester etc as farmers are habituated to use plastic mulching sheet.

The adverse effect of climatic changes in Bangladesh will require farmers to modify their current cropping system and some researches are needed to develop high yielding crop that is adaptable to changing environment, adapted to likely future conditions. Choices are available for farmers as climatic condition changes and have to identify/develop new production technologies (i.e. mulching, water management, plastic tunnels, mini houses and, insect and diseases managements etc.) for crop production in the vulnerable areas of Bangladesh. In this regard, plant responses to light spectral quality can be exploited to deliver a range of agronomically desirable end points in protected crops. The use of ultra-violet blocking (UV-blocking) polythene sheet/film is now widely known to be an effective method in reducing many flying insect pests.

A growing number of vegetable growers indeed adopt such techniques and ultraviolet (UV) absorbing films as cover material is one of them. Production of high-value crops is often performed under protected cultivation. In recent years, various spectral modifications have been made in different types of crops; few studies suggest that solar UV-A has significant effects on growth, morphology and tissue chemistry in a range of species. Although UV-A radiation (315–400 nm) has lower energy per photon than UV-B (280–315 nm), its damaging and inhibiting effects on the growth and photosynthesis of aquatic and terrestrial plants can also be considerable. Few researches suggested that UV-A radiation may also induce increased amounts of UV-absorbing pigments and, UV-exclusion studies conducted on cucumber and, a red-pigmented lettuce indicates that ambient UV-A radiation greatly inhibits leaf enlargement, stem elongation and biomass production. Few of the main reasons to modify the spectral characteristics in protected cultivation in changing climate, to suppress the proliferation of several foliar diseases and to protect crops from insects and insect-borne virus diseases. The study was undertaken to evaluate the effectiveness of partial blocking capable UV films/filters as a means of flying insect pest and disease control, and to find out the pigmentation quality, physiological analysis within horticultural cropping systems.

1.1 Characteristics of UV-blocking films

It is known to all that most of UV radiation does not reach Earth's surface due to its interaction to the atmospheric components. In fact, UV-C radiation might be completely absorbed by the atmospheric gases, UV-B radiation is absorbed by the stratospheric ozone layer, whereas UV-A radiation is hardly absorbed by this layer (Lidon et al., 2012). UV-A radiation (315-400nm) is not absorbed by the ozone layer and is present at much higher intensities in sunlight than UV-B radiation. UV-A can impact plant morphology and pigment formation as well (Paul and Gwynn-Jones, 2003). For several *Brassica* species, responses to UV-exposure as well as influences on herbivores have been studied (Grant-Petersson and Renwick, 1996; Caputo et al., 2006; Foggo et al., 2007). In an experiment with broccoli plants (*Brassica oleracea L.* var. *botrytis*) exposed to ambient UV-A (315-400 nm) and UV-B levels, or grown under reduced levels of UV radiation, only show differences in biomass accumulation when plants experience the different environmental conditions during germination and early growth , that was also supported by Reifenrath et al. (2007).

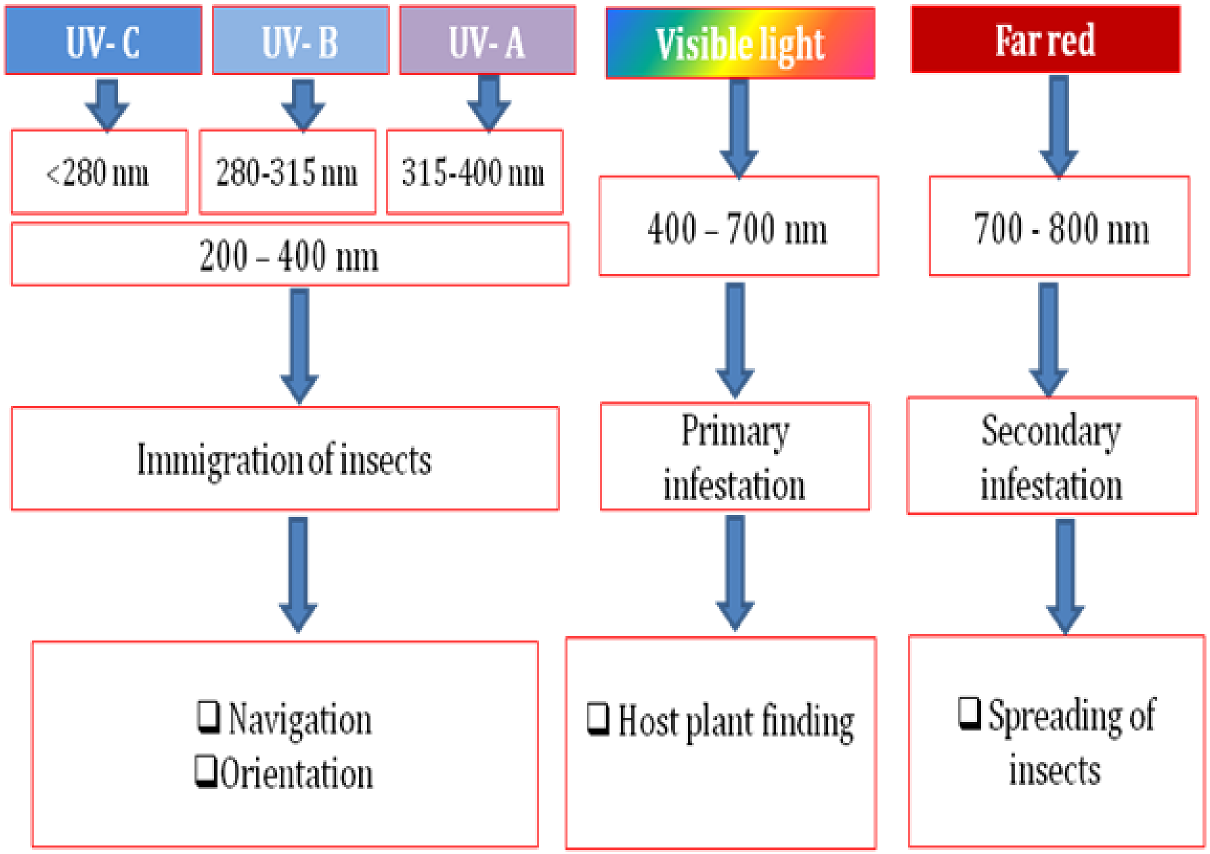
The first additives incorporated into polyethylene or PO films were UV stabilizers to protect them from fast degradation. Later, thermal stabilizers were also incorporated and the introduction of anti-fog and anti-drip agents was applied to minimize the negative effects of the condensation which could reduce the amount of light transmission into the greenhouse and also reduce the risk of fungal diseases (Cemek and Demir, 2005). Nowadays a new family of additives for agricultural films has been developed to manipulate their optical properties. Optical properties include the manipulation of different regions of the light spectrum that are necessary for photosynthesis and in consequence to enhance the process of plant growth and crop yield (Winsel, 2002). The same principle was used in our proposed study to manipulate the natural light spectrum in the UV region to improve pest and disease management. These types of materials act as a photo-selective barrier by blocking the transmission of the UV radiation (280-400 nm) to the interior of the greenhouse (Espi et al., 2006). The lack of UV radiation has a positive effect on plant growth and contributes to reduce the damage due to insect pests and plant diseases.

The international brand “UV-blocking films” includes different plastic films available for the farmers provided by various manufacturers with different capacities to absorb UV-wavelength below 400 nm for reducing the damage caused by insect pests. Different types of UV-blocking plastic films and types of structures have been tested under different climatic conditions and regions of the world mainly against the insect and disease organisms. Also, there are some studies on the impact of these types of films on the natural enemies of insect vectors and other beneficial organisms. To use photo-selective plastics is a quite recently developed method that can block or modify the transmitted light to obtain specific benefits (Catalina et al., 2000). In a study, Nakagaki et al. (1982) reported the first evidence of the inhibitory effect of UV-blocking materials on the invasion of greenhouses by insects. Several studies have been conducted to evaluate the effect of this plastic materials used as films to reduce insect population and disease in protected crops, and these will be discussed in the dissertation.

In addition, changing the light spectrum underneath the film cover may alter plant morphogenesis, and consequently may change herbivores or sucking insects’ response. In the same way, the manipulation of light quality in greenhouses was used as a non-chemical alternative method for growth regulation of vegetable and or ornamental crops (Rajapakse and Kelly, 1995). The crop yield and quality can be increased at the time when pest and disease damage is significantly reduced by changing the characteristics of plastic films. Photo-selective plastic barriers have also shown good efficacy in the control of plant pathogenic fungi (Honda et al., 1977). At the same time these types of barriers have proved positive effects by increasing the persistence and viability of the entomo-pathogenic fungi, *Beauveria bassiana* (Costa et al., 2001) and of viruses (Lasa et al., 2007) used as biological control agents.

1.2 Importance of using UV-blocking films in insect pest management

However, pesticides have a negative impact on farmers, consumers and the environment (Pimentel and Greiner, 1997). Currently the situation is unsustainable and other alternative control measures need to be implemented because many of the active ingredients are being banned in the European Union (DOUCE, 2002) and elsewhere, and also pesticide efficacy is also not enough to control many of the key pests and diseases because resistance build-up to insects and pathogens often occurs (Urech et al., 1997). In addition, consumers are very sensitive to environmentally friendly agricultural systems (organic agriculture, integrated production, etc.), and are demanding pesticide-free high quality and low cost food products. All these demands require the investigation of new control tactics for pest and disease control.



Data source: Diaz and Fereres, 2007. Prepared by AHM Solaiman, 15-09-2015, Yamagata University, Japan.

Figure 1-1. The mode of invasion of insects at different wavelength of radiation.

It is known that habitat manipulation is an insect pest management strategy that provides an unfavorable environment for insect pests and more favorable habitat to their natural enemies. In this way, physical barriers are one of the oldest tactics used to control insect pests and had a significant role in Integrated Pest Management (IPM) Programs in the last few decades (Boiteau and Vernon, 2001). Different kinds of physical barriers have been used in the past to exclude insects, modify their behavior and provide a deleterious environment for their development and population increase. For example, insect screens act as an exclusion physical method between the plants and the pest and have been successfully adopted by many growers around the world (Prokopy and Owens, 1983). Some physical barriers are often based on manipulating insect vision dependent behaviors by using UV-blocking or UV-reflective materials to interfere with host finding, landing and orientation (Antignus, 2000) (Figure 1.1). In this way, Costa et al. (2002) found that UV-absorbing components that block the majority of UV-light at wavelengths below 380 nm had more influence in reducing insect numbers than those that blocked light at wavelengths below 360 nm. These materials have been used commonly in three different forms, such as mulches for open-grown crops, and as plastic sheets or screen/nets for protected crops.

1.3 Effect of UV-blocking plastic films on insect invasion and feeding damage

UV radiation can modulate interactions between plants and their natural enemies. This has been shown by several experiments with UV attenuating filters. The intensity of herbivores is generally associated with UV-induced changes in plant tissue characteristics. However, insects are also capable to respond directly to different UV conditions (Mazza et al., 1999; Mazza et al., 2002). We found that naturally occurring whiteflies (Aleyrodidae) and aphids (Aphididae) favoured high ambient UV-conditions over low UV-conditions, whereas thrips (Thripidae) avoided high UV-conditions (Kuhlmann and Müller, 2009, Chapter 1). A preference of whiteflies and aphids for high UV radiation conditions has also been detected by Antignus et al. (1996); Costa and Robb (1999); Costa et al. (2002); Chyzik et al. (2003) and Díaz et al. (2006). However, contrasting results have been found for thrips behavior in response to different UV-conditions (Antignus et al., 1996; Costa and Robb, 1999; Costa et al., 2002; Díaz et al., 2007). Mazza et al. (1999; 2002) have proven that thrips are able to perceive UV-B radiation and to respond with avoidance behavior, whereas UV-A triggers attraction. In contrast to leaf chewing and cell content feeding insects, phloem-feeding insects only cause tissue damage and only get in contact with plant compounds transferred by phloem sap. Few experiments suggested that feeding behavior of insect vectors change in a way that reduces their transmission ability under UV-blocking plastic film and in consequence a dramatic reduction in virus disease incidence was observed (Antignus et al., 1996).

1.4 Importance of UV-blocking films in different locations of the world

Most of the experiments conducted to evaluate the efficacy of UV-blocking plastic films were done in deserted areas from southern Israel but the latest works on this subject were conducted in other Mediterranean and temperate regions, and also has the importance in tropical and sub-tropical countries in the context of climate change. For this, it is important to consider the higher relative humidity which demands a different design of greenhouses structure and specific management tactics, such as strategies to increase ventilation over the crop canopy. Habitats with significant UV levels can be found at high altitudes, where solar radiation has to penetrate a thinner layer of the atmosphere. Consequently, UV-blocking films will be more adequate to use under these types of high intensity UV-light conditions and in geographical locations closer to the equator, where UV-light differences within the greenhouse and the outside light environment are greater (Doukas and Payne, 2007).

In general, plastic films or nets reduces the efficiency of natural ventilation with the consequent increase of temperature inside the covered structure, although this principle is not applicable to UV-plastic films, because the heat load depends on the overall energy transmittance and not on the different spectral properties of the films (Von Elsner and Xie, 2003). However, the specific external climatic conditions of a given region determines the need for ventilation inside the cover structure as has been described by Kumar and Poehling (2006) in studies conducted in the humid tropics (Thailand). These high humidity external conditions reduced the efficiency of UV-plastic films to mitigate insect immigration because side wall ventilation is a pre-requisite under such climatic conditions. Conversely, the efficiency of UV-absorbing barriers is much higher in dry regions of Germany where crops can be grown under closed tunnels, as shown by Mutwiwa et al. (2005).

1.5 UV-blocking films differed in construction structures

It is important to consider that most works have been conducted in enclosed greenhouses or tunnels obtaining in some cases contradictory results compared with those obtained from open-side greenhouse structures. For example, UV-blocking materials were not able to reduce the population density of *T. vaporariourum* in open-side greenhouses, while a positive effect of the film in reducing insect density was observed in enclosed tunnels (Costa et al. 2002; Mutwiwa et al. 2005; Diaz et al., 2006). Also, Costa et al. (2002) found a significant reduction in the number of aphids and thrips captured on yellow sticky traps (YST) in greenhouses covered by UV-plastic films in a commercial cut-flower green-house. Antignus et al. (2001) found that penetration of the whitefly *B. tabaci* into walk-in tomato tunnels covered with UV-absorbing films was strongly inhibited as well as the attraction of whiteflies to these types of structures. Also, a reduction in the number of aphids captured on YST, a delay in aphid immigration and colonization were recorded in lettuce grown in walk-in tunnels under UV-blocking plastic films (Diaz et al., 2006). Similar results were obtained with *B. tabaci, Cerathothripoides claratis* and *A. gossypii* that showed a reduction in their immigration rate into a tomato greenhouse covered with UV-blocking plastic films (Kumar and Poehling, 2006).

Once the invading insects entry into the protected crop, they must recognize and locate their host plants. As a result, insects begin the second phase of the process of host plant infestation, which is primary infestation. Studies conducted by Antignus et al. (1996) show that *B. tabaci* is attracted by 254-366 nm when exposed to monochromatic UV sources as well as to full-spectrum light, explaining the inability of this whitefly to recognize the host plants under UV-blocking materials. Also, the same effect was observed on the landing rate of, *M. euphorbiae* and *F. occidentalis* on lettuce plants grown under a UV-light deficient environment. The third phase of greenhouse infestation by insects consists in the secondary spread of the insects within the greenhouse, by the movement of insects by walking or flying from plant to plant. UV-blocking materials have a positive effect on the movement of insects within the protected environment, not only by reducing the secondary spread of the pest, but also by reducing the incidence of insect-transmitted virus diseases. In this way a positive effect of the UV-light deficient environment was observed on the population growth and spread of *M. persicae* (Chyzik et al. 2003) and on the movement of *M. euphorbiae* and *F. occidentalis* across lettuce plants, reducing the percentage of lettuce plants affected with Poty virus and TSWV, respectively (Diaz et al., 2006). In addition, a delay of Capsicum chlorosis virus (CaCV) symptoms on tomato plants was observed in a greenhouse covered with UV-blocking plastic films (Kumar and Poehling, 2006).

It is evident that insects have ocular photoreceptors in a bandwidth of UV-A (315-400 nm) and visible or photosynthetically active radiation (PAR, 400-700 nm). Therefore, wavelengths in the UV region have incidence on insect behaviour, such as, orientation, navigation, host finding and feeding (Antignus and Ben-Yakir, 2004) (Figure 1-1). In this context, Meyer Rochow et al. (2002) studied the UV-induced damage in photoreceptors in four species of insects from higher and lower geographic latitudes.

1.6 Effect of UV-irradiation on horticultural crops and their relationship with herbivores

In general, plants can utilize both UV-A and UV-B radiations as a source of information for environmentally affected ecological process (Paul and Gwynn-Jones, 2003). However, long exposure to UV radiation (especially UV-B) induce damages not only for living organisms but also for plants because the UV photons have enough energy to disturb vital functions such as RNA transcript, membrane integrity that eventually induces structural and biochemical changes (Kovács and Keresztes, 2002; Jacobs et al., 2007). In this way, responses to UV attenuation by selective filters include increased growth and yield of an eggplant soilless crop (Kittas et al., 2006). Also, changing in quality, such as pigmentation and taste of lettuce was found when plants were grown under UV-opaque film, which absorbed 50% of UV-A and 95% of UV-B light (Paul et al., 2005).

Plants need UV light for the synthesis of specific pigments such as anthocyanins which are required for coloring of vegetables such as red amaranth. Therefore, UV-blocking films should not be used to protect crops in which anthocyanin pigmentation is a determinant of their quality (Antignus and Ben-Yakir, 2004). Plastic films with different transmission of UV radiation were used to investigate the changes of leaf and flower colors of ornamental plants showing that UV-B causes a decrease of the chlorophyll content in different crops (Hoffman, 1999).

1.7 Effects of UV-blocking films on beneficial predators and pollinators

Photo-selective plastic films should be compatible with natural enemies of pests and other beneficial organisms such as pollinators because biological control is one of the most widely used strategies in vegetable production due to its well known environmental benefits (Viñuela, 2005). The parasitization begins with the host habitat locations by the female parasitoids, and then followed by their host locations. In each of these steps, different cues are perceived from the natural habitat (shape, color, texture) and from the target host itself (Willis, 1997). Some species of parasitoids locate their host through very specific cues making a unique host-parasitoid relationship (Harris and Bautista, 2003). In this way, Chiel et al. (2006) studied the effects of UV-absorbing plastic sheets on the host location ability of parasitoids that are available commercially and commonly released by the growers under greenhouse conditions. These results provide practical information for growers about the release of these parasitoids in greenhouses covered by UV-blocking materials. Chyzik et al. (2003) showed that UV-blocking films suppressed both the propagation and flight activity of predators within walk-in tunnels, without reducing the effects of the host-finding activity and fecundity.

However, no experiment has been carried out to evaluate the effects of the UV-blocking or partially UV-blocking covering materials on the predators which were commonly released for growers. That’s why; we planned to assess the number of predators/parasitoids that were trapped inside the tunnels of broccoli and turnip seedlings and also with red amaranth.

1.8 Impact of UV-radiation on crop diseases

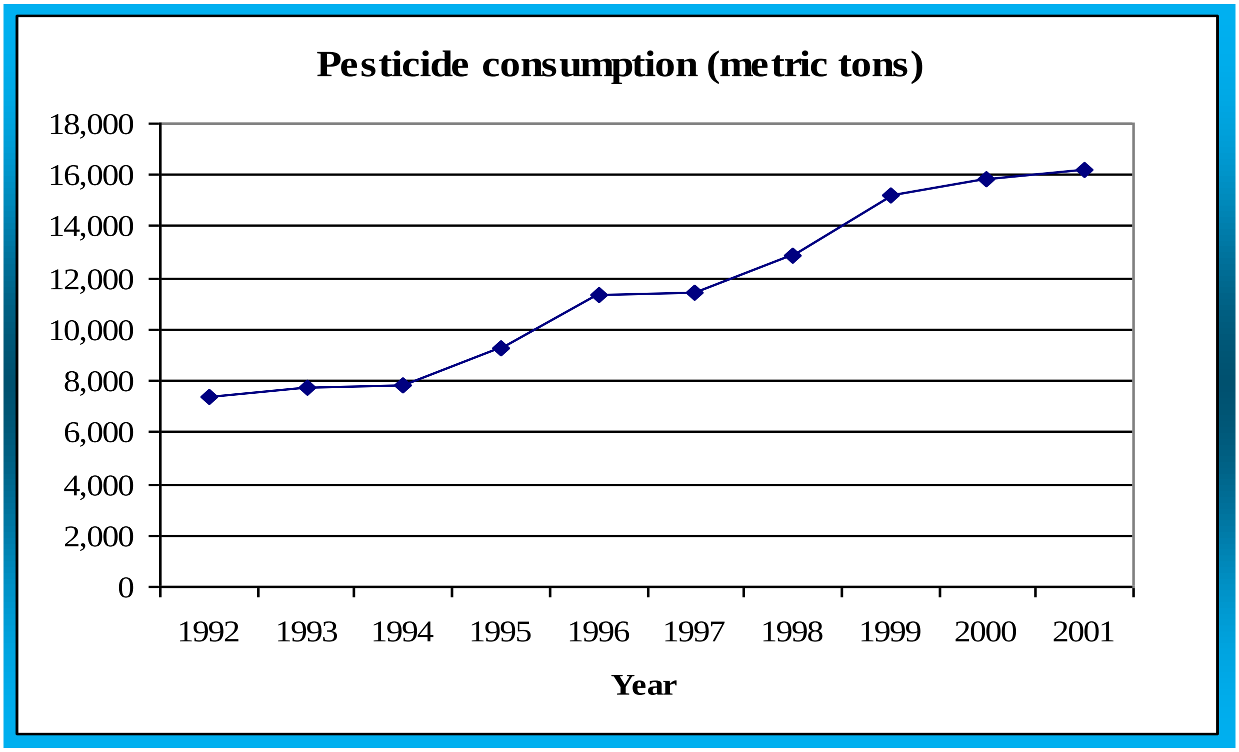
It is obvious that protected agriculture crops suffer important economic damage from insect pests as well as diseases caused by viruses (e.g. CMV, TSWV, TYLCV) and their vectors (e.g. aphids, thrips, whiteflies etc), fungi (e.g. *Botrytis cinerea*, powdery mildew), and bacteria (e.g. *Clavibacter michiganensis* and *Pectobacterium spp*.). The most extended and common practice to control insect pests and plant pathogens is the application of large amount of pesticides.

It is also described in a filed study that the effect of solar UV-radiation alters phyllosphere bacterial community of peanut leaves using plants grown under UV-B transmitting or UV-B excluding plastic filters. Besides, using *C. michiganensis* determined that strains which produce pigments are characterized as UV-tolerant, enhancing the ability of bacterial strains to maintain population size in the phyllosphere (Jacobs and Sundin, 2001; Jacobs et al., 2005).

The low survival and persistence of microbial organisms used for pest control exposed to UV radiation is one of the limitations for its wider use as microbial insecticides in field or greenhouse conditions. Under field conditions, Costa et al. (2001) found that the persistence of viable spores from a commercial formulation of the entomopathogenic fungi like *Beauveria bassiana* significantly increased under a plastic that blocked UV-light wavelengths shorter than 380 nm compared to that with plastics that blocked UV-irradiation wavelengths shorter than 360 nm. The use of microbial insecticides combined with UV-blocking materials could be an effective and very promising strategy to enhance pest control in protected crops. But there is no data on the effect of UV-radiation specifically on partially UV-blocking on the canker disease in the literature so far.

1.9 Importance of UV-blocking films in minimization of chemical pesticide use

In recent years, Bangladesh farmers have used or have been forced to use chemical pesticides for minimizing the insect pest of vegetable crops (Figure 1-2). Combined with the severe agro-climatic conditions (for example, annual flooding), agricultural production will have further difficulty in meeting future demands. As a direct consequence of these difficult conditions, agriculture has been highly susceptible to crop pest attacks and diseases. Conservative estimates of annual crop losses are in the range of 10-15% without any direct intervention. In their defense, farmers have begun to use more toxic chemicals for pest control that have reputations of speed and effectiveness. The Government of Bangladesh also promotes the use of pesticides to expand its agricultural frontiers and increase output per acre of land (Meisner, 2004). According to statistics from the Government of Bangladesh, consumption of pesticides increased from 7,350 metric tons in 1992 to 16,200 metric tons in 2001, more than doubling in the past decade (Figure 1-2). Moreover, generally farmers do not wait long enough to wash off after spraying before harvesting because of their high demand for farm products and low perception of the toxic effects of pesticide residues in agriculture commodities especially for those are consumed fresh causing enormous health hazards. (Figure 1-3; Bhuiyan and Ali, 2010; Hossain et. al., 2013).



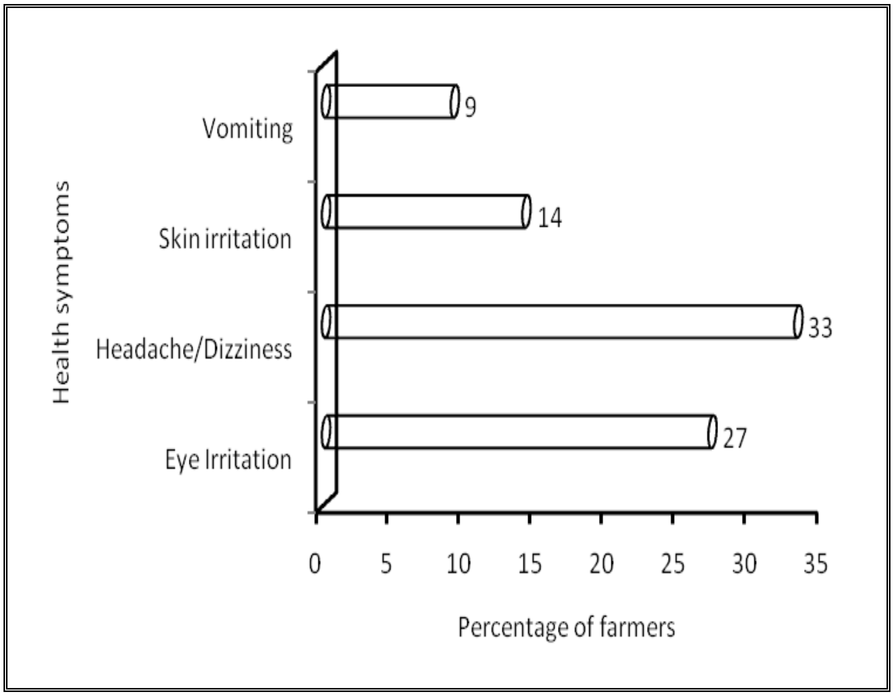
Source: Department of Plant Protection Wing, Department of Agricultural Extension (DAE), Bangladesh. In 2005, it was 25,479 m. t. and 300 g active ingredient/hectare (Rahman, 2007).

Figure 1-2. Pesticide consumption trend in the past decades in Bangladesh

In contrast to the 47% among the general pesticide-using population only 31% of the IPM farmers faced the health effects just after spraying (Plant protection wing, DAE) (Figure 1-3). Considering the uncontrolled use of chemical pesticides and subsequent environmental hazards, the introduction of IPM should be come into light (Lykas et al., 2008, Bhuiya and Ali, 2009). Photo-selective plastics which affect not only the radiation use efficiency but also the mode of insect behavior are often used in vegetable production for obtaining the specific benefit of sunlight (Catalina et al., 2000; Dáder et al. 2015). Among them, UV-blocking plastics have been commercially used mainly for reducing the number of phytophagous insects in greenhouses (Nakagaki et al., 1982; Dáder et al. 2015; Miranda et al. 2015).

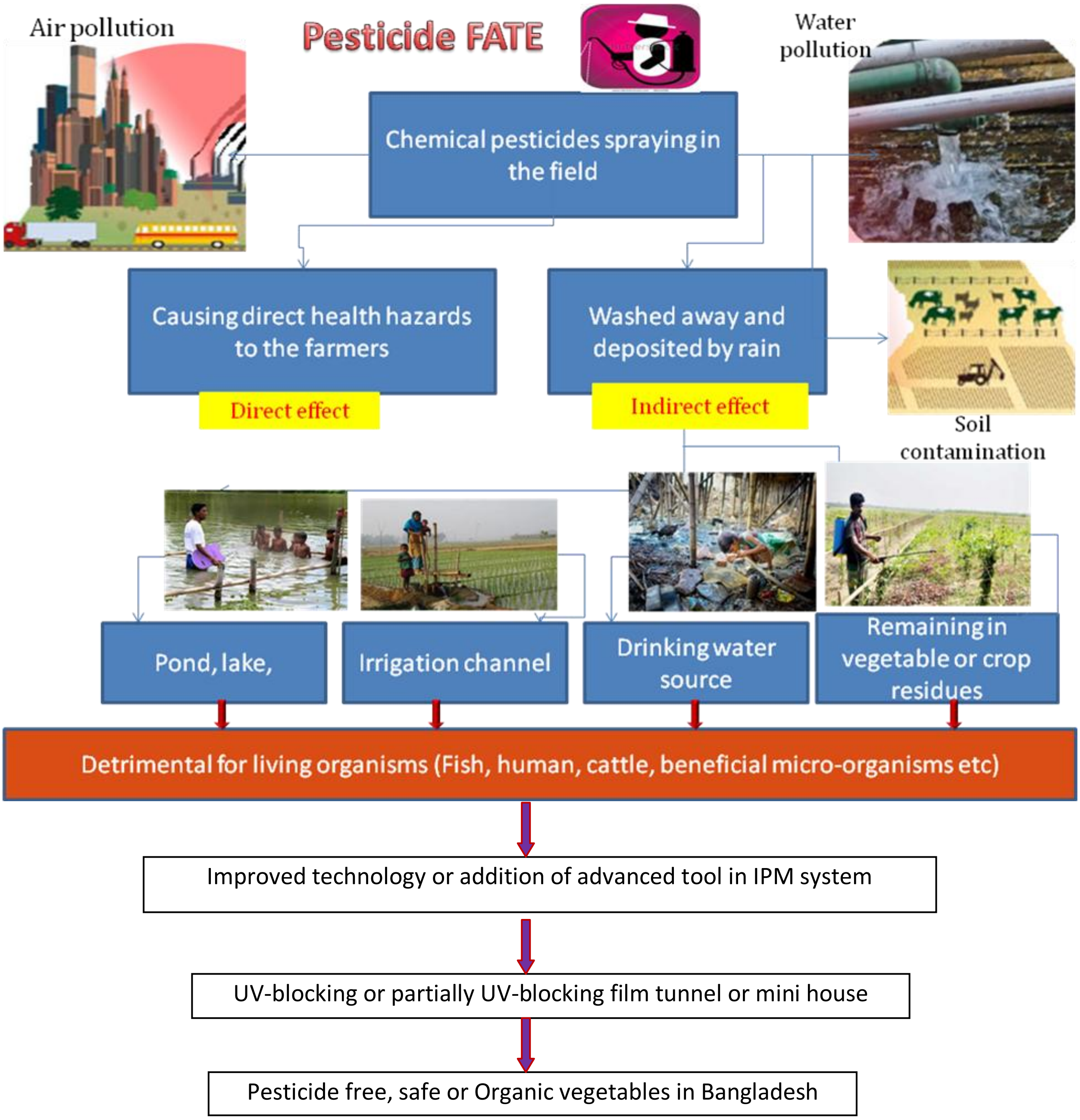
Photo taken by: AHM Solaiman, 2014, Chandina, Comilla, Bangladesh.

Figure 1-3. A Farmer spraying pesticide for flying insect pest in a vegetable crop field in an unsafe manner (common scenario).



Data source: World Bank, 2012, prepared by AHM Solaiman, 11-09-2015, Yamagata University, Japan.

Figure 1- 4. Specific health effects have shown in farmers of Bangladesh. Results have taken for the last one decade.



Flow chart prepared for the thesis by: AHM Solaiman, 2015-09-11, Yamagata University, Japan

Figure 1-5. Impact of pesticide and importance of using UV-blocking film in Bangladesh context.

1.10 Why partially UV-blocking films?

Insecticides have commonly been used in the production of a wide range of vegetables and often results in increased cost of vegetables production and environmental pollution (Figure 1-5). In protected vegetable production, IPM has currently been emphasized to control insect pests (Kogan, 1998), but there are still some difficulties in looking for effective control measures without relying on chemicals. In this sense, the use of UV-blocking plastic film on the roof of greenhouses will be an environmentally-enhancing technique for reducing infestations of insects because the invasion of phytophagous insects into greenhouse is often reduced under UV-blocking conditions (Doukas and Payne, 2007). However, UV-blocking conditions also often disturb the behavior of honey bees because they have a photoreceptor with peak sensitivity in the UV-A (344 nm) (Reisenman and Giurfa, 2008).

In addition, UV irradiation is often involved in regulating certain physiological aspects of crops such as anthocyanin biosynthesis (Gläßgen et al., 1998) and plant growth (Krizek et al., 1998). Therefore, selection of the optimal UV-blocking wavelength where the invasion of phytophagous insects can be effectively excluded but both the behavior of honeybees and the physiological responses of crops are not disturbed is important to develop the practical use of UV-blocking plastic film. But the film of specific wavelength can make sense compare with the UV-blocking films (Nishizawa et al., 2012). In our experiment, broccoli and turnip seedlings and also red amaranth (*Amaranthus tricolor*) were grown under several selectively UV-blocked plastic film tunnels that differed in their ability to transmit UV radiation but did not alter the available PAR and the effects of these altered levels of UV-radiation on the insect and disease control were investigated. The seedlings and crops were grown in field with near commercial conditions in order to make the results relevant to commercial production.

1.11 Importance of using sticky adhesives for capturing insect pest in protected horticultural production system

In protected cultivation system colored sticky traps have been used for controlling insect pests for promoting chemicals free horticultural crop. The design or shape, size of a trap for monitoring or mass trapping insect pests, it is often important that these visual variables be considered. The sticky traps based on color characteristics have been used extensively in the field and greenhouse for a variety of pests (Moffitt, 1964; Kirk, 1984; Brødsgaard, 1989; Gillespie and Vernon, 1990; Matteson and Terry, 1992; Teulon et al., 1999; Blackmer et al., 2004a, 2006; Demirel and Cranshaw, 2006; Blackmer et al., 2008). The benefit of traps which actually employ visual cues that is instantaneously established, and they function independently of air movement, and they are generally effective from a distance and from any direction, provided there are no obstacles between the trap and the insect (Miller and Strickler, 1984). Detection of host plants by phytophagous insects often involves visual recognition. This recognition may include responses to color, size, shape or silhouettes (Prokopy and Owens, 1983).

Sticky traps are glue-based frequently used in pest control to catch and monitor insects and other pests. Most of the sticky traps do not contain any pesticides, although some may be marketed with aromas designed to attract certain pests. Sticky traps are useful for monitoring pest in crop field for walking or flying insect pests all day long and as such are frequently used by researchers to enhance their ability to detect pests in pest control strategies. Sticky traps allow researchers to detect pests that are active at night or other times when farmers are not present. Trapping systems for insects are important components IPM programs. Trapping data can be used to make decisions on the initiation or termination of control measures, as well as to assess efficacy of control approaches that have been implemented. It is known that sticky traps are an effective tool to monitor the presence and absence of beneficial insects and their numbers (Wallis and Shaw, 2008). Traps based on the response of insects to color have been applied widely in various crops like bell pepper, crucifers (Gerling and Horowitz, 1984; Hill and Hooper, 1984; Chandler, 1985; Meyer dirk and Oldfield, 1985).

1.11.1 Types of traps

There are different colored of traps have been used in the field of horticultural research. They are blue, yellow, opaque yellow, orange, green, purple, red, clear, black and white sticky traps for different insect pest (Gerling and Horowitz, 1984; Hill and Hooper, 1984; Chandler, 1985; Meyer dirk and Oldfield, 1985; Blackmer et al., 2008; Nishizawa et al., 2012).

1.11.2 Advantages, drawbacks and cost of using of the traps

Sticky traps are non-toxic, comparatively cheap and needs no technical know how. Sticky traps have been used to control insect pests in garden, urban agriculture systems, and also in the commercial farmers' field. Colored sticky traps could be a simple and low-cost method for determining the relative abundance of harmful and beneficial insects, including pollinators.

Some times, the sticky traps are not cost-effective for large scale production as they need to change frequently where labor may be imposed to the production cost. In the commercial crop field or orchards, it will have to use large number of traps that might be unacceptable than spraying chemicals for controlling pest. But in the protected cultivation system, specifically for the targeted pests for the specific horticulture crop would be cost-effective and safe for the farmers and consumers.

1.11.3 Most commonly used blue and yellow sticky traps (BST and YST)

Among the traps, blue and yellow traps have been used commonly in the horticultural crop cultivation system. Use of yellow sticky traps is a considerable tool in terms of pest management and they have been used commercially to catch some sucking pest such as;

whiteflies, aphids, leafhoppers, thrips, and leaf-miners and some fruit flies (Diptera: Tephritidae) Mediterranean fruit fly, and olive fruit fly, (*Bactrocera oleae*) in parts of the world (Ozdem and Kilincer, 2009; Hazır and Ulusoy, 2012). Additionally, white, blue, red, and green colored traps are commercially available for the control of some insect pests. Colored sticky bases have recently become available and have also been tested whether they are better than the white sticky traps or not. Trap color is measurable as spectral reflectance, is known to affect the attraction of insects that are active in the days. In an experiment, Thomson et al. (2004) confirmed that yellow trap was effective for capturing the Hymenoptera insects.

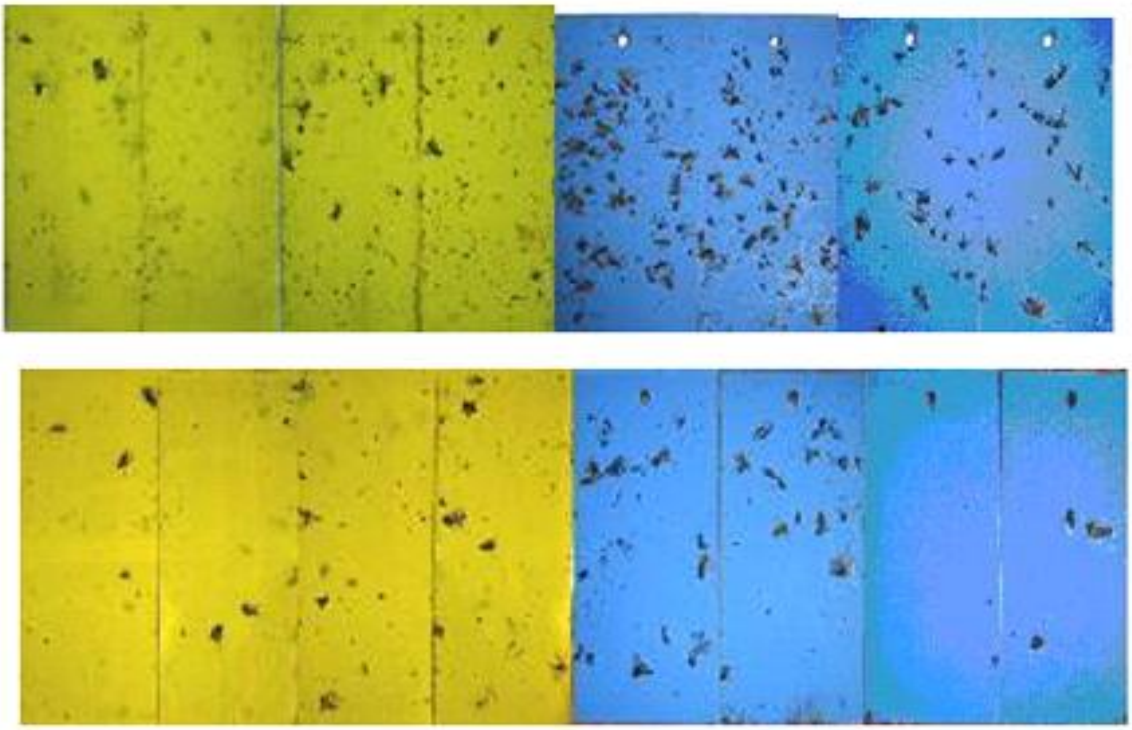


Figure 1-6. Yellow and blue adhesives used in Experiment 1 and 2 (Chapter 2 and 3) for capturing insect pest and predators

1.12 Preparation of the tunnels and mini house with partially UV-blocking films

Plastic tunnels (140 cm × 140 cm × 60 cm) were prepared in the research field of Sher-e-Bangla Agricultural University, Bangladesh and were placed on the beds after the germination (7 days) of the seeds of broccoli turnip and red amaranth and two of each beds were covered with different polyolefin films (0.13 mm thickness) which had ability to block UV-lights shorter than <400, <360, <350, and <340 nm respectively (Mitsubishi Plastics Agri Dream, Tokyo, Japan) (Experiment-1 and 2, described in Chapter-2 and 3). A 10 cm (17 %) from the soil level of each tunnel was remained to open for the ventilation and allowing the invasion of insects. The remaining two beds were not covered by plastic films and used as uncovered control described in Chapter 1 and 2 (Figure 1- 7 A, B).

On the other hand, in the next cropping season, fifteen plastic mini houses of 1.8 m × 1.9 m × 0.6 m (L×B×H) with same thickness were prepared for Experiment-3 (Chapter-4) and remaining three mini houses were also covered with a UV-transmitting PO film. The first 60 cm (33%) from the soil level remained open to control the heat of the tunnels and allow the invasion by insects (Figure 1-8 A, B).

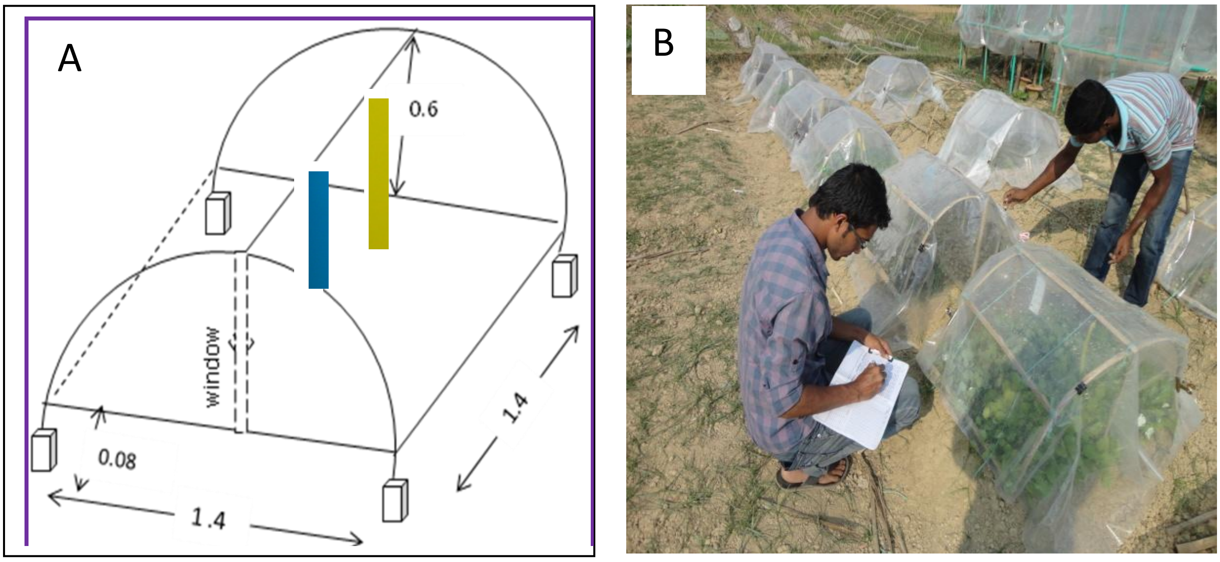


Photo: AHM Solaiman, 2013

Figure 1- 7A, B. Field of experiment 1 A, B (Broccoli and turnip plants inside the small tunnels with yellow and blue sticky traps). A-Measurements of the tunnels are in meters (Experiment 1 and 2 were conducted in tunnels).

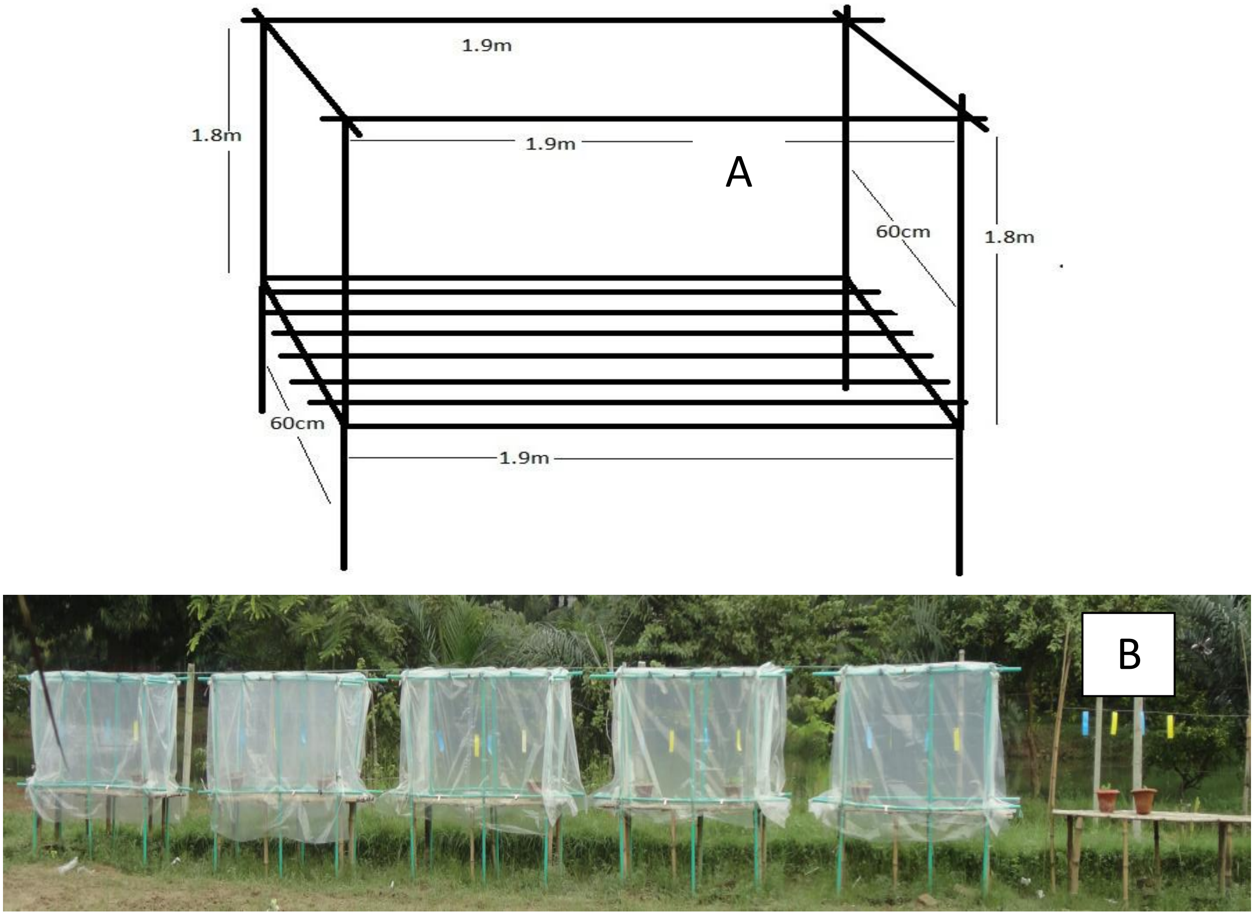


Photo: AHM Solaiman, 2013

Figure 1-8 A, B. Actual measurement (A) of mini house (B) prepared in the field for diagnosing the citrus canker disease (Experiment-3).

1.12 Aims and objectives of the study

The main aim of the proposed research was to evaluate the effectiveness of partially UV-blocking PO filters as a means of flying insect pest control within small-scale organic horticultural cropping systems in Bangladesh conditions. It is known that insufficient qualities and quantities of sunlight in conventional greenhouses can generate plants, which are morphological instable, more vulnerable to insects and endangered to get “sunburned” when exposed in the field. In this proposed study, morphological changes of the crop species broccoli (*Brassica oleracea var. italica* cv. KB superstar) and turnip (*B. rapa var. rapa* cv. Ledia) in response to specific/ partially blocked UV radiation- and/ or herbivore-exposure were also investigated. The study aimed to understand the effect of using innovative greenhouse covering materials, it was possible to investigate the unknown effect of partially UV-blocking on plants and insect and disease invasion. Furthermore, the pigmentation and anthocyanins of red amaranth (*Amaranthus tricolor,* cv. BARI Lal Shak-1) were also examined.

Two types of constructions (small tunnel and mini house) which were covered with either UV-transmitting (+UV) or UV-blocking (-UV) films and the results were compared with the crops that were grown in outdoors in Bangladesh conditions (Figure 1- 7 & 1-8 A, B). The films were utilized to examine partially UV-blocking film effects on different developmental stages of broccoli, turnip and red amaranth. Furthermore, partially UV-blocking effect was also examined on *Xanthomonas axonopoides* pv *citri* that is presently the most devastating disease impacting world citrus production including Bangladesh, and naturally occurring herbivorous and predators were also investigated.

The objective of the research is to update the information available on the use of the partially UV-blocking plastic materials used in protected environments to control insect pests and diseases, with special attention to their impact on insect vectors of plant diseases, their natural enemies and other beneficial organisms. In addition, the use of the UV blocking materials as a tool in IPM programs was also discussed.

In order to standardize the technology of utilizing UV-blocking films under Bangladesh conditions, the proposed study has been undertaken with the following objectives:

I. to grow a range of organic vegetables in field condition covered with UV and non-UV blocking films with specific wavelengths;

II. to use production systems comparable to those employed by other organic growers within Bangladesh;

III. to collect indicative evidence of the effects of partially UV-blocking PO films on the pigmentation and insect control under outdoor field conditions that might be effective for the different protected commercial cultivation systems;

IV. to assess visually the level of pest infestation and predators in sticky traps on the crops grown under the different PO films.

V. to assess the effect of partially UV-blocking films on the microorganism causing diseases in horticultural crops ;

VI. to find out the effectiveness of films in controlling insects invasion in field conditions specially for seedlings and leafy vegetables (short duration crop) in Bangladesh condition;

VII. to study the proportion of beneficial insects that are being trapped along with the insects.

Figure 1-6 A. Red amaranth cultivated under tunnels with partially UV-blocking films and the results were compared with crops grown in outdoors and UV-transmitting film covering tunnels.

Figure 1-6 B. Research supervisor advising on red amaranth crops grown in field condition of Bangladesh (December, 2013).

Chapter 2

Experiment-1

Studies on the effect of partially UV-blocking films on the growth and insect control of turnip and broccoli seedlings

2.1 Abstract of the published paper

The effect of partially UV-blocking films was investigated on the promotion of growth and insect control as well as the herbivores-predators balance in the tunnels of turnip and broccoli seedlings. The experiment was comprised with six treatments, viz., outdoor, UV-transmitting, partially UV-blocking (UVs shorter than 340, 350, 360 and 400 nm respectively, were blocked) with three replications. A tunnel covered with UV-transmitting polyolefin film was also prepared, and the results were compared with the seedlings that were grown in the outdoors. In turnip, plant height, leaf number, and leaf area of increased by UV-blocking, while broccoli was not affected. Insects invaded into the tunnels were trapped using blue and yellow adhesive films, and seven different insects, viz. aphid (*Brevicoryne brassicae*), brown plant hopper (*Nilparvata lugens*), short horn grass hopper (*Melanoplus femurrubrum*), white backed plant hopper (*Sogatella furcifera*), dipteran fly, mirid bug (Heteroptera: Miridae), and mosquitoes were captured up to 35 days after planting. Aphid was found as the most predominant insect followed by mosquito, dipteran fly, and white baked plant hopper. Among the treatments, the largest feeding damage was found in the outdoors for broccoli (26%) and turnip (22.5%). The ratio of the herbivores and predators was also influenced by the films and it was remarkable in both broccoli (35-65%) and turnip (25-75%) seedlings, respectively. The partial UV-blockings effectively reduced the feeding damage compared with the outdoors, irrespective of seedling. Therefore, the sticky traps were found to be effective, and specific UV-

blocking films showed different effects in controlling insect pests. Hence, the partially UV-blocking films can be an effective component for the IPM system rather than fully UV-blocking films.

2.2 Introduction

In Bangladesh, an overwhelming majority (89.3%) of the farmers use medium to high chemical pesticides for vegetable cultivation (Bhuiya and Ali, 2009).The most extended and common practice to control insect pests and plant pathogens is the application of large amount of pesticides (Diaz and Fereres, 2007). Broccoli and turnip seedlings are always attacked by insects easily. Hence, we choose these two species of seedlings in order to minimize the use of chemical pesticides introducing the UV-blocking films. On the other hand, Photo selective plastics can block or modify transmitted light to obtain the specific benefit for crop production (Catalina et al., 2000) and UV-blocking materials can inhibit the invasion and modifies insects' behaviors (Nakagaki et al., 1982). Kuhlmann et al. (2009) studied with broccoli and stated that the responses to UV-B and to insect herbivore were partially similar and UV-induced changes of plant traits did not alter attractiveness to herbivorous insects: thrips, whiteflies, and aphids attacked plants independently of UV-B pretreatment but our investigation lies with specific UV-blocking shorter than 340, 350, 360 and 400 nm wavelength to control the herbivores. Our target was to minimize the attack of insects especially to the *Brassica* seedlings that might encourage farmers to avoid chemical spraying even in the seedlings, turning farmers to organic cultivation.

However, *Brassica* showed highly species-specific and leaf-age dependent effect to the environmental stress like including or excluding UV-A plus UV-B radiation (Reinfenrath, 2007). But we chose the specific UV-blocking films (shorter than 340, 350, 360 and 400nm wavelength) to investigate the effect on *Brassica* seedlings related to growth and insect control.

The effect of the climatic change forces plant growers to modify their current production techniques and systems. Hence, there is a scope of research to develop high yielding crops that might be adaptable in varying climatic or environmental conditions. Such technologies include natural or artificial mulching, plastic tunnels and insect management etc. The use of plastic materials specially polyethylene, polyolefin, polyester films etc, have been a booming revolution to traditional agricultural systems that made possible increasing the production areas of the world in contrast with adverse climatic conditions.

It is almost clear that UV-radiation has a significant influence on plant growth (Krizek et al., 1998; Caldwell et al., 2003) and development of morphology (Teramura and Ziska, 1996; Jansen et al., 1998). Wavelength selective filters, such as polyester, show a range of significant responses across many plant species and locations (Paul et al., 2005). Also, changing growth and quality in some high value crops like lettuce that were grown under UV-opaque film, which absorbed 50% of UV-A and 95% of UV-B light (Paul et al., 2005). In our case, we used tunnels covered by specific UV-blocking films in the open field and our hypothesis is to get the higher seedling growth inside tunnels and insect trapping by sticky strips that can be effective in controlling the insect pests for broccoli and turnip seedlings. Consumers are very sensitive to environmental friendly agricultural systems (organic agriculture, integrated production, etc.), and are demanding pesticide-free high quality and low cost food products (Bhuiyan and Ali, 2009). All these demands require the investigation of new tactics for pest control, while our study was undertaken to introduce new tactic especially in Bangladesh which can be an important tool for IPM system.

The goal of the proposed study was to investigate the effects of specific UV-blocking films on two species (turnip and broccoli) that might be effective for the growth of seedlings and to also to find out the effectiveness of partially UV-blocking films in controlling insects' invasion in field conditions especially for seedlings in Bangladesh condition. It is stated that young leaves are more valuable for the plant than old leaves and also young leaves should build up protection mechanisms against harmful radiation with priority (Reifenrath et al., 2007). Therefore, we expected stronger responses in specific UV-treated young plants comparing with the plants in open conditions in respect to growth and insect control. The present study was undertaken to investigate the effect of specific UV-blocking films on the insect control and along with the growth of turnip and broccoli seedlings. The aim of this research is to update the information available on the use of the UV-blocking plastic materials used in protected environments to control insect pests and other beneficial organisms.

Only a few studies were performed as outdoor experiments using UV-filtering foils to expose plants to natural irradiation including or lacking the UV-portion of the spectrum (Turunen et al., 1999; Kolb et al., 2001; Caputo et al., 2006). Colored traps especially Yellow sticky traps (YSTs) have been used for the decades for the managements of different flying insects and acting as a key role in IPM programme inside the greenhouse (Steiner et al., 1999; Kaas, 2005; Park et al., 2011a; Delia and Irene, 2013). Described above primary studies allow to raise questions on the effect of specific UV-blocking films on seedling growth and insect control of *Brassica* seedlings, that have not been widely discussed in world literature so far.

In this dissertation, it was described about the effect of specific UV-blocking films on the growth and insect control of two *Brassica* namely broccoli and turnip seedlings produced under outdoor field conditions along with specific UV-blocking films covered tunnel.

2.3 Materials and Methods

2.3.1 Plant cultivation and UV-blocking:

Seeds of broccoli (*Brassica oleracea var. italica*, cv. KB superstar) and turnip (*B. rapa var. rapa,* cv. Ledia) were sown in the raised beds of 100 × 100 × 50 cm during October, 2013 to December, 2013 at the experimental field of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. Application of fertilizers and irrigation, and other operations were followed by FRG (2012).

Five plastic tunnels (L × B × H =1.4 × 1.4 × 0.6 m) were prepared after the germination (7 DAS) of the seedlings, and covered with different polyolefin films which can block UV-radiations shorter than 400 (<400), 360 (<360), 350 (<350), and 340 nm (<340), respectively. A tunnel was also covered with UV-transferring polyolefin film. An 8 cm (13.3 %) from the soil level was remained to open for protecting the heating of the tunnels and allowing the invasion of insects.

On 30 DAS, seedlings were harvested, and measured the seedling growth. The results were also compared with those of the seedlings grown in the outdoors. Shaded or yellowish leaves were not taken under consideration (discarded) because specifically green leafy seedlings were acceptable for selling in most of the crops (Rashid, 1999).

2.3.2 Measurement of environmental conditions

Temperature, humidity, illumination intensity, and UV-irradiation were measured daily during the experiment. Both temperature and humidity were recorded at 12:00 pm, 20:00 pm and

24:00 pm, while illumination intensity and UV-radiation were measured at noon time (12:00 pm).

2.3.3 Measurement of invaded insects

On 21 November, two (blue and yellow) sticky traps (18 × 5 cm) were suspended at the middle and center position of the tunnels. All adhesives were changed every week and total captured insects were counted from 21 November to 21 December daily (finally converted to average week basis). It was necessary to find out the types of insects that are generally responsible for attacking in broccoli and turnip seedlings in Bangladesh condition as a tool for IPM program. Parasitoids were also counted as they are beneficial for the environmental balance and Bangladesh farmers have anonymously been spraying chemicals to control insects where they are killing beneficial insects as well. So, it was needed to investigate the effect of specific UV-blocking film on the invasion or accumulation of parasitoid inside the tunnel.

2.3.4 Measurement of feeding damage

The leaf areas that were fed by insects measured by software named Image J (Figure 2.3.4.1). This is open access software to measure the area updated by Larry, 2007 (ref: http://imagej.nih.gov /ij). Affected seedlings are not sold in good price or low price in Bangladesh market, so the area was measured and also the total leaf area infested by herbivores were also monitored to determine the rate of damage. The affected leaves were measured after uprooting them from the seedbed at 30 DAS.

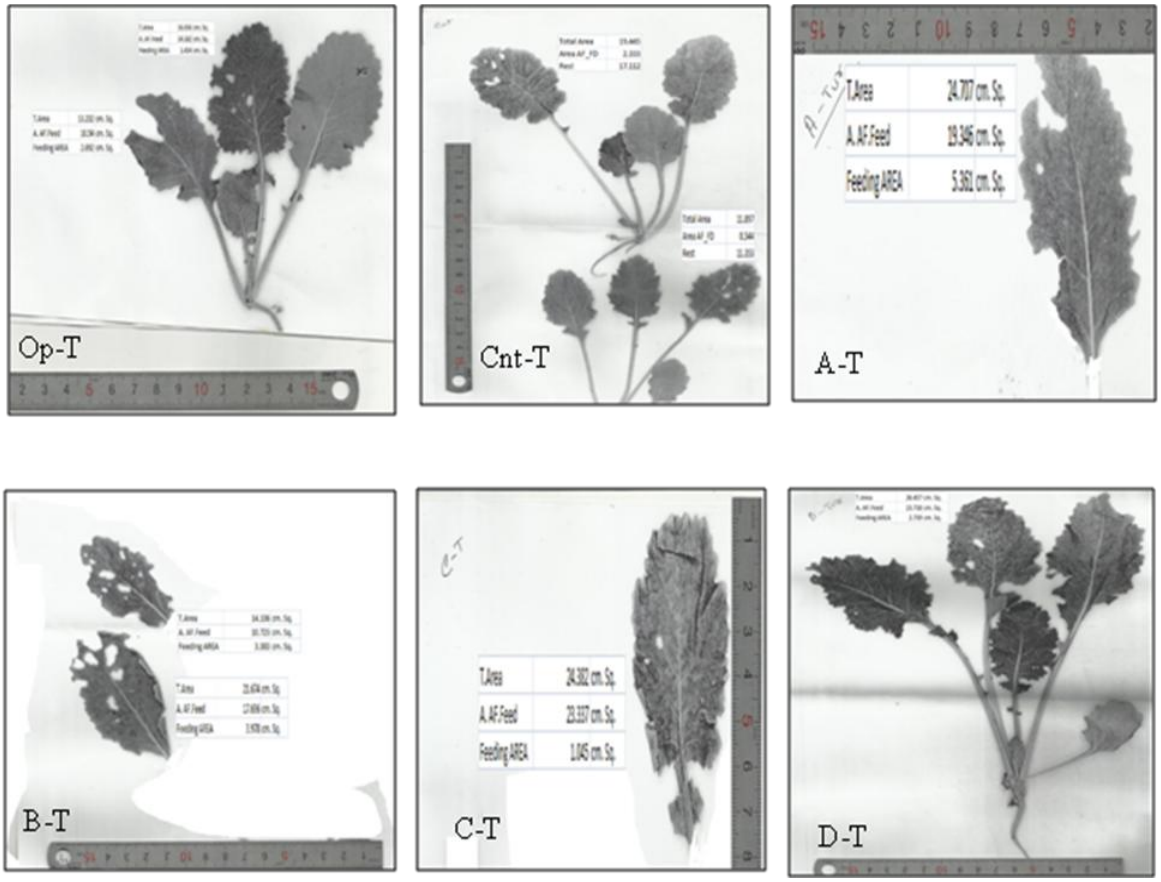


Figure 2.3.4.1. The leaf area fed by insects were measured by Image J (Ref. Larry, 2007 http://imagej.nih.gov/ij). Op-T: Outdoor; Cnt-T: UV-transmitting; A-T: <340 nm; B-T: <350 nm; C-T: <360nm and D-T: <400 nm. These measurements are for turnip seedlings, same technique was followed for broccoli seedlings (T-turnip).

2.3.5 Statistical analysis

The means of all the treatments were calculated and the analysis of variance (ANOVA) for each of the characters was performed by *F*-test. Data were analyzed using MSTAT-C software. Differences at $P \leq 0.05$ level is considered as significant.

All the above-mentioned measurements were recorded outside and inside tunnel one by one within 15 min.

2.4 Results and Discussion

During the experiment, mean temperature in the tunnels at 20:00 and 24:00 was 20.3 - 21.9 °C and there was no significant difference among the treatments (Table 2-1). However, mean temperature at 12:00 was 2- 4 ° C higher than the outdoors. It is natural that films raise the temperature inside tunnels during day and night time. In our experiment, day temperature differed a little but night temperature didn't differ significantly. The experiment was conducted during dry season and the humidity in the tunnels at 12:00 was 53.1%, However, the mean humidity at 20:00 and 24:00 was 87.8% and 91.5%, respectively and the value in the tunnels was often significantly higher than that of the outdoors, especially when UV-blocking rate was high (Table 2-1). Similar findings on non-significant changes in temperature have been reported by Kittas et al. (2006).

Both the illumination intensity and UV radiation under the partial UV-blockings were blocked higher than 60%, but there was no significant difference in the illumination intensity among partial UV-blockings. On the other hand, UV radiation significantly decreased continuously as the UV-blocking rate increased. As the results, penetrating rate of partial UV-blocking films under <400, <360, <350 and <340 nm were 86, 76, 73, 67%, respectively, compared to that of UV-transferring film. In an experiment, Nishizawa et al. (2012) prepared mini houses (L×W×H=200×140×230cm) covered with the same UV-blocking polyolefin film with 40 cm open side from the ground level, and reported that the UV-penetrating rate was 70%, compared to that of UV- transferring film (50%). The difference of the UV-blocking rates of our experiment would be due to the difference of tunnel sizes and UV-penetration through the open side, since we prepared small tunnels of 14 cm height and protected the penetration of UV radiation through the open side of the tunnels by covering the tunnels up to 10 cm from the ground level.

Temperature, visible light intensity and humidity inside the tunnels were not influenced by the different polyolefin films (Table 2-1) that is also supported by Nishizawa et al. (2012) but among abiotic factors, temperature has an impact on the plant growth and development and in the flight behavior of different insect pests (Weber, 1931; Bellows et al., 1988; Blackmer and Byrne, 1993; Liu et al., 1994; Isaacs and Byrne, 1998) and also the sticky traps to an influential mark (Biffi Urteaga, 2009). It is investigated that below 20° C few insects like white fly, *T. vaporarium* and *B. tabaci* restricts or stops flight behavior inside the greenhouse (Liu et al., 1994; Biffi Urteaga, 2009). It is found that humidity and light intensity may have positive correlation with trap catches (Delia and Irene, 2013). However, insects' flight activity coincides with morning hours which depends on light period and influenced by temperature (Delia and Irene, 2013; Liu et al., 1994; Hoffman and Byrne, 1986). In the proposed study temperature ranges mostly in between 20 to 35° C, though at 12.00 and 20.00 pm, it went down to 15° C at the last two weeks (19 to 26 December). These two weeks had the minimal number of insects inside the tunnels may be due to the low temperature, insects restricted their invasion.

The plant height was significantly increased with decreased as the UV-blocking increased (UV-blocking of <340, <350, <360, and <400 nm were 21, 23, 24, 25 cm, respectively for broccoli seedlings at 30 DAS and 28.4, 31.7, 31, and 32.8 cm at 30 DAS respectively for turnip seedlings). The leaf number showed almost similar variations (4 to 5 at 30 DAS) contrast with the seedlings grown in open field and under control film for turnip seedlings (Table 2-2), while, broccoli seedlings showed a remarkable increasing trend in leaf number with the increasing of UV-blocking wavelength (20-24 at 30 DAS, contrast in outdoor that was 18). So, it is clear that UV-blocking of <340, <350, <360 and <400 nm increased their height and leaf numbers for the *brassica* seedlings.In an experiment on flowers (*Lisianthus, Solidago, Chrysanthemum*), <380nm showed minimal growth and development and no remarkable differences were observed (Costa et al., 2002) but in our investigation, plant growth was influenced by the use of UV-blocking films.

The changes in plants morphology induced by UV-B may affect competition for light (Barnes et al., 1988). The negative effects of UV-B radiation resulted in deformed morphological parameters. Exposure to UV-B decreased plant height, leaf area and plant dry weight increased auxiliary branching and leaf curling (Dai et al., 1995; Green-berg et al., 1997; Furness et al., 1999; Dai et al., 1995) reports that after a few weeks of UV-B exposure, leaf area and plant dry weight of rice was significantly reduced. In a study with cucumber seedlings showed 75% higher plant height than tomato seedlings (Nishizawa et al., 2012) under the specific UV-blocking films, though the seedlings showed non-significant variations. In my investigation, it was significant for both of the *Brassica* seedlings whereas, UV-B irradiation influence much than UV-A and reduces the plant growth of many crops (Krizek et al., 1998; Yamasaki et al., 2007). Generally, old leaves are less nutritious than young leaves (Raupp and Denno, 1983; Lambdon et al., 2003) for most of the crops. UV-A plus UV-B radiations were shown highly species-specific and leaf-age dependent in the two investigated *Brassica* (*Sinapis alba, Nasturtium officinale*) studied by Reifenrath et al. (2007) .

The plasticity response to UV might be related to different species that should be adapted to their specific environment and plants have to adjust their defense mechanism against harmful radiation and feeding insects. Increasing costs for chemical production or for reallocation of defense chemicals might accompany this process of adaptation, depending on the abundance and sensitivity of generalist insects and optimal levels of chemical defense decrease with leaf-age (Iwasa et al., 1996).Weigh et al. (1998) stated that enhanced UV-A decreased leaf area per unit plant biomass (leaf area ratio) but increased biomass productivity both per unit leaf area (leaf area productivity) and per unit leaf nitrogen (leaf nitrogen productivity).

The extent of feeding damage by insects was significantly influenced by the partial UV-blocking films for both vegetables (Figure 2-2). The minimum number of plants (5- 10%) were attacked inside the tunnels where partial UV-blockings were applied rather than outdoors and UV-transmitting. So, it is evident from our experiment that less number of insects invaded inside the tunnels which can be an effective tool for minimizing the attack of plants rather than fully UV-blocking. On the other hand, the fed areas (%) showed significant variation among the treatments by which it can be said that if the number of insects maximize, it does not mean that the area of fed would be the maximum (Figure 2-3). Now, the question is among the insects trapped, all of them are detrimental for the plants, may be not, because there must have some beneficial insects which were also trapped by the adhesives inside or outside tunnels. This type of variations may hamper the balance of the ecology and in that case some predators will be killed and the insect pest invasion will be maximized. Insects fed areas were significantly influenced by the types of *Brassica* species, broccoli seedlings were attacked more than turnip seedlings inside the tunnels where both of them were seeded in same time. The maximum leaf area fed by insects found inside the tunnels covered with <340 nm UV-blocking for both the species.

The fed area can be influenced by both of the seedling densities and the area of the leaves; maybe that's why insects attacked mostly in broccoli seedlings (Nishizawa et al., 2012). The leaves area were maximized as maximizing the leaf size with the age of the seedlings because the areas were measured at the end of the experiment and naturally broccoli seedlings beard the bigger leaf than turnip (Reifenrath et al., 2007). Aphids attack normally at the all stages of *Brassica* crop and also act as a vector of mosaic virus (Vernon and Keith, 1998), especially seedlings may be stunted or die because of aphid feeding. Though the feeding may not be done only by flying insects that were trapped but also some other creeping or walking insects can eat. So it cannot be concluded that the number of trapped insects are only responsible for attacking or feeding the leaves of seedlings. Anyway, It is important to protect seedlings from their attack though it can be managed by spraying insecticides (Webb, 2013). But now a days, Bangladeshi farmers are aware of using chemicals in the crop field may be for high price and/or health consciousness, they are turning to organic cultivation. The capturing of insects through plastic films (UV-blocking) might be more effective for controlling dipteran fly and aphids. The plastic films (UV-transmitting) also minimized the invasion of insects than outdoors. Though the other insects invasion was found non-significant may be for the duration of 35 days, the number of insects were not captured as many as we expected, it was clear that the specific UV-blocking films can be useful for controlling pests rather than chemicals; indicating a key component for IPM programme in *Brassica* seedlings production of Bangladesh.

In the case of broccoli seedlings, total number of insects was trapped maximum in outdoors than tunnels and the trapped insects' number were almost similar in respect to partial UV-blocking (Figure 2-1 B). In the yellow traps, insect number was non-significant in UV-transmitting and partial UV-blocking but in blue, it was almost similar in respect to the number of trapped insects. Whereas, in the case of turnip, trapped insect number was decreased with increasing UV-blocking and for yellow and blue traps, insect trapping was affected same as the total number (Figure 2-1 A). From the figure, it can be concluded that partial UV-blocking films can be effective for trapping insects equally than UV-blocking films.

We used the tunnels with low height and a small opening with 10 cm which might interference the flying behavior of different insects. The plant growth was also influenced the infestation of insects as in <400 nm tunnels plants grew vigorously than other tunnels. For some insects, maximum numbers of insects were trapped under <400 nm tunnels than others, whereas, in previous experiment with tomato and cucumber (Nishizawa et al., 2012) trapped number was maximum in <360 nm. Broccoli and turnip seedlings are only the seedlings, no flowering were there and also the leaves and stems were more succulent than other plants inside the <400 nm tunnels.

Furthermore, the insects as predators were distinguished from herbivores/sucking insects. For both broccoli and turnip, 10-20% predators or parasitoids were trapped and significantly controlled the herbivores/sucking insects with the use of partially UV-blocking films (Figure 2-4). Predators are also important in environmental balance and also for control pest or herbivores/sucking insects by the predators or parasitoids that might be an influential agent for bio-control of herbivores/sucking. That’s why; it was tried to know the proportion of predators and herbivores/sucking that was trapped inside or outside tunnels in blue or yellow adhesives. It is evident that UV-blocking films can inhibit the insect invasion but among them, how many beneficial insects were being trapped along with pests that might be issue. In the experiment, it is observed that significant number of beneficial insects have been trapped inside and or outdoors (Figure 2-4). For parasitoid in turnip seedlings, about 25% of the predators were trapped inside tunnels and on the other hand, 32% predators were trapped inside the tunnels of broccoli seedlings (Figure 2-4). Especially for the broccoli seedlings, more than 35% beneficial insects were trapped in the tunnels with partially UV-blocking films. This proportion should be considered whether the blue or yellow adhesives were used for catching insect-pest as a tool for control. In our experiment, partial UV-blocking of <350 or <360nm captured almost same as the fully blocked UV-films rather than outdoors. In that case, it would be much wise and commercially acceptable to use partially UV-blocking films than UV-blocking films to control insect pests (Nishizawa et al., 2012).

In the proposed study, several species of insects were trapped where seven species were identified such as mirid bug (*Cyrtorhinus lividipennis,* Heteroptera: Miridae), aphid (*Brevicoryne brassicae*), brown plant hopper (BPH) (*Nilparvata lugens*), dipteran fly, mosquito (*Toxorhynchites* sp.), short horn grass hopper (SHGH) (*Melanoplus femurrubrum,* Acrididae), and white backed plant hopper (WBPH) (*Sogatella furcifera,* Hemiptera: Delphacidae) (Figure 2-5 A B and 2-6 A B). We also counted the other insects that were trapped by blue and/or yellow adhesive films which were categorized as miscellaneous insects which were failed to classify or found only in few numbers. Therefore, these insects were classified as miscellaneous.

This is expected that sticky traps captured only the winged and sometimes walking insects, but insect pest counts on plants included both adult and immature. However, a lot of other factors could contribute differences and plant height could reduce the invasion density of insects inside the tunnels. On the contrary, though the *Brassica* seedlings did not produce any flowers, the insects invasion may have immigrate inside and outside the tunnel, but the difference showed significant minimization inside the tunnel than outside, where the tunnels were opened at the bottom. Aphid prefers the color of background mainly by the contrast of green (plant) and soil as background to land (Doring and Chittka, 2007). Different secondary compounds of the plants that might influence members of the next trophic level by affecting host plant, feeding and Oviposition behavior of herbivores/sucking insects and their performance in different degree (e.g. McCloud and Berenbaum, 1999; Lindroth et al., 2000; Warren et al., 2002). In addition the duration of plant growth was only 30 days, the invasion numbers could vary with the duration of the experiment. Scientists showed that UV-blocking materials have properties to filter the UV radiation (280-400 nm) interfering with the vision of insects and in consequence, their behavior related with movement, host location ability and their population parameters (Diaz and Fereres, 2007). They also stated that once the invading insects' enter into the protected crop; they must recognize and locate their host plants. As a result, insects begin the second phase of the process of host plant infestation, which is primary infestation. Especially, dipteran fly and aphids showed significant invasion inside the tunnel and for the WBPH it was remarkable in YSTs only whereas, BSTs showed non-significant effect (Figure 2-5 A, B). The dipteran fly invaded 3 times more in outdoor than covered tunnel and it was gradually decreased as the UV-blocking rate increased (<340, <350, <360, and <400 nm were 50, 33, 17 and 17%, respectively). In the case of aphid (*Brevicoryne brassicae*), invasion was 50% in UV-transmitting film than outdoors and it was decreased as 28, 13, 18, and 20% with the increasing rate of UV-blocking (Figure 2- 6 A, B). The YSTs were influential for the control of WBPH where it significantly trapped most number of insect inside the tunnel. The number of insects' invasion was not too much might be due to the smaller size of the tunnels and smaller opening of the tunnels for invading. The trap counts were also correlated with the densities with tomato and cucumber seedlings (Gillespie and Quiring, 1987; Kim et al., 1999; Nishizawa et al., 2012) that supported sticky traps as an influential tool for estimating population densities.

Photo-selective plastic films should be compatible with natural enemies of pests and other beneficial organisms such as pollinators because biological control is one of the most widely used strategies in vegetable production due to its well known environmental benefits (Viñuela, 2005). In an experiment, Chiel et al., (2006) showed the host location ability of few parasitoids (*Aphidius colemani*a parasitoid of *M. persicae, Diglyphus isaea* a parasitoid of *Lyriomyza bryoniae,* and *Eretmocerus mundus*, a parasitoid of *B. tabaci.*) that are available in the market and often released by the growers inside green house affected by the UV-absorbing plastic sheets.

In Bangladesh, new techniques are being introduced now days to cope up with environmental change effect for crop production. Many microbes, plants and animals use UV radiations as a source of information about their environment affecting many ecological processes (Paul and Gwynn-Jones, 2003). A large number of experiments using wavelengths-selective filters, such as polyester, show a range of significant responses across many plant species and locations (Paul et al., 2005) and Cloyd (2009) mentioned that the economic threshold levels (ETs) can be different for different crops and the way of placing the traps. However, Growers can develop their own action thresholds depending on their experience that might help to reduce the use of pesticides (Delia and Irene, 2013). In this case, it is found that UV-blocking films <400 nm yielded the maximum leaves number and gave the highest plant height. It is also studied that UV-blocking films is to increase the growth and yield compare with UV transparent film (Garcia-Macias et al., 2007; Tsormpatsidis et al., 2008).

From the above study, it can be concluded that the partially UV-blocking films (<340, <350, <360 and <400 nm) had significant effect on the growth promotion and can be an important tool in IPM system for *Brassica* seedlings specifically for aphid, dipteran fly and white baked plant hopper. The plant growth was promoted by using partial UV-blocking films, so that it is much wise or commercially acceptable not to use UV-blocking films rather than partially UV-blocking films. In the Experiment-1, it is found that the proportion of Insect-pest and predators/sucking insects was hampered due to the use of UV-blocking films. The effects of UV-blocking materials on predators or parasitoids have never been well established. We tried to find out the effectiveness of using partially UV-blocking films on the pest-predator balance inside the controlled cultivation techniques. It is also evident that the cost effectiveness, timing, and nature of crops might also be the effective points for successful IPM system in Bangladesh.

Table 2-1. Mean values of recorded temperature and RH (daily, at 3 times), light intensity and UV-reading (at 12:00 pm) inside and outside tunnels during the period of measurements

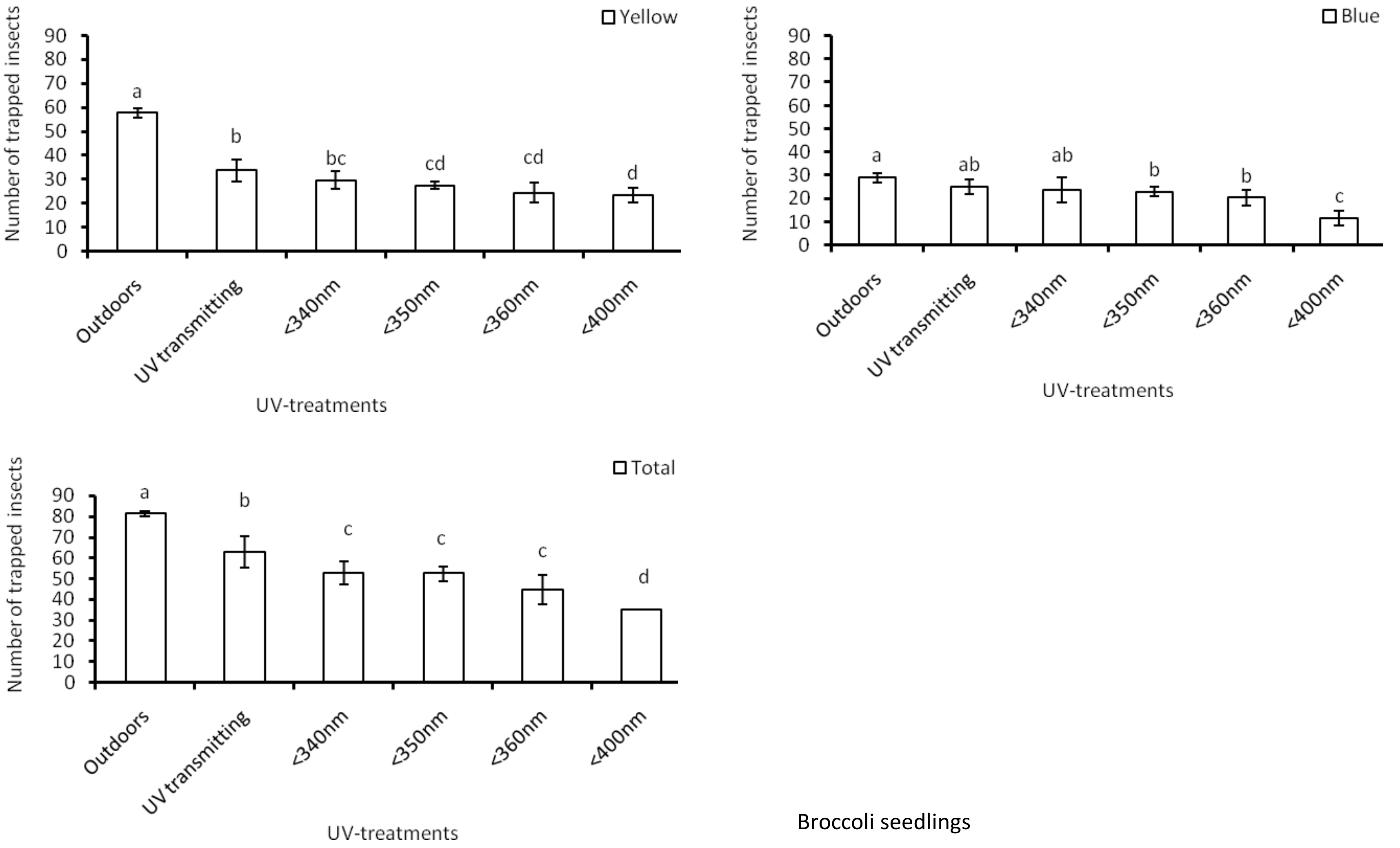
UV-blocking	Temperature (°C)			Relative humidity (%)			Light intensity (W cm^{-2})		UV average (mW cm^{-2})	
	12:00	20:00	24:00	12:00	20:00	24:00	12:00	% of outdoors	12:00	% of outdoors
Outdoors	31.9 c	21.7	20.5	53.6	85.4 b	89.1 c	20.6 a	100	721.6 a	100
UV-transmitting	33.9 b	21.9	20.4	53.5	85.4 b	89.7 bc	17.6 bc	85.5	449.2 b	48.7
Partial UV-blocking										
<340 nm	34.8 ab	21.4	20.3	54.3	88.0 a	90.9 bc	18.2 bc	88.4	309.0 c	33.5
<350 nm	35.8 a	21.5	20.7	53.0	89.1 a	91.6 b	16.1 c	78.4	250.5 c	27.2
<360nm	35.4 ab	21.5	20.9	53.5	89.2 a	93.8 a	17.0 bc	82.6	219.3 cd	23.8
<400 nm	35.4 ab	21.5	20.5	50.8	89.7 a	93.8 a	17.6 bc	85.4	132.1 d	14.3
Significance	**	ns	ns	ns	*	**	**		**	

ns- non significant, *- significant at $P \leq 0.01$; **- significant at $P \leq 0.05$. Different uppercase letters beside the mean value indicate significant at $P \leq 0.05$ or 0.01. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software.

Table 2-2. Mean values of the measured and calculated growth parameters of broccoli and turnip seedlings inside and outside tunnels.

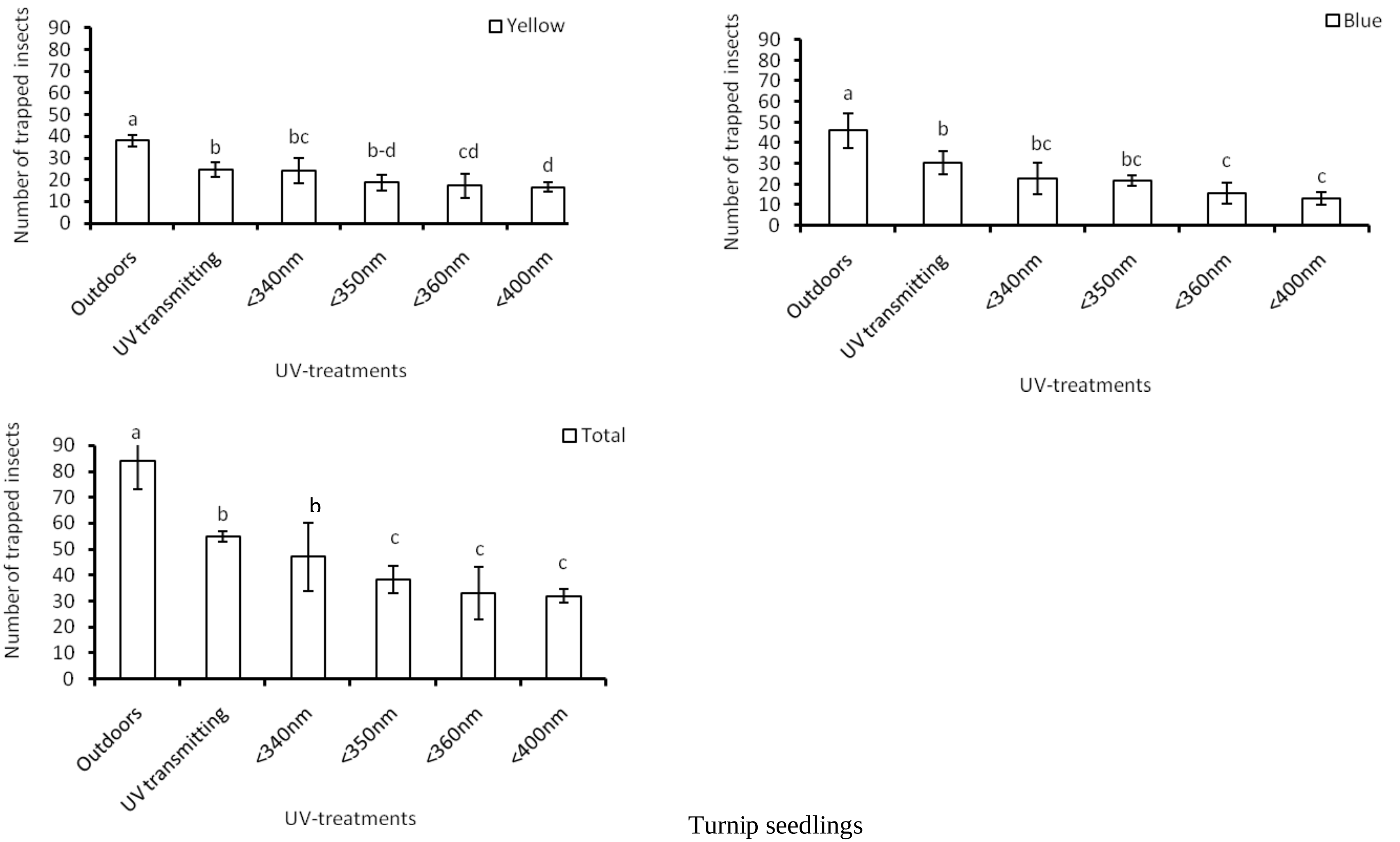
UV-blocking	Turnip					Broccoli				
	Plant height (cm)	Number of leaves	Leaf area (cm^2)	Fresh weight (g)	Dry matter (%)	Plant height (cm)	Number of leaves	Leaf area (cm^2)	Fresh weight (g)	Dry matter (%)
Outdoors	24.5 e	5.1	146.7 c	4.5 d	17.8 a	18.7 c	3.7 b	45.4 b	4.4 ab	23.93 c
UV-transmitting	32.9 ab	5.4	226.3 a	6.9 a	11 b	23.1 a	4.4 a	74.7 a	4.9 a	16.47 d
Partial UV-blocking										
<340 nm	27.0 d	5	162.1 c	5.7 b	16 a	20.8 bc	4.2 ab	57.7 ab	3.4 c	32.87 a
<350 nm	30.7 bc	5	178.9 bc	5.1 c	17.7 a	22.1 ab	4.2 ab	56.1 ab	3.66 c	32.07 ab
<360nm	30.1 c	5.1	170.8 bc	6.2 b	12.3 b	23.6 a	4.4 a	62.8 ab	3.7 bc	25.84 bc
<400 nm	33.0 ab	5.2	198.5 ab	7.3 a	11.5 b	24.0 a	4.5 a	59.6 ab	5.1 a	14.23 d
Significance	**	ns	**	**	*	**	*	**	**	**

ns-non significant, *- significant at $P \le 0.01$; **- significant at $P \le 0.05$. Different uppercase letters beside the mean value indicate significant at $P \le 0.05$ or 0.01. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software.



Broccoli seedlings

Figure 2-1 A. Total number of insects trapped by adhesives (blue and yellow) inside the tunnels during 30 days of the broccoli seedlings. Yellow- Insect trapped in yellow adhesives, Blue- Insect trapped in blue adhesives and Total- Total number of insects trapped inside and outside tunnels during the 35 days. Different lowercase letters above the vertical error bar lines indicate significant ($P \leq 0.05$). The error bars represent the standard deviation of the values. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software.



Turnip seedlings

Figure 2-1 B. Total number of insects trapped by adhesives (blue and yellow) inside the tunnels during 30 days of the turnip seedlings. Yellow- Insect trapped in yellow adhesives, Blue- Insect trapped in blue adhesives and Total- Total number of insects trapped inside and outside tunnels during the 30 days. Different lowercase letters above the vertical error bar lines indicate significant ($P \leq 0.05$). The error bars represent the standard deviation of the values. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software.

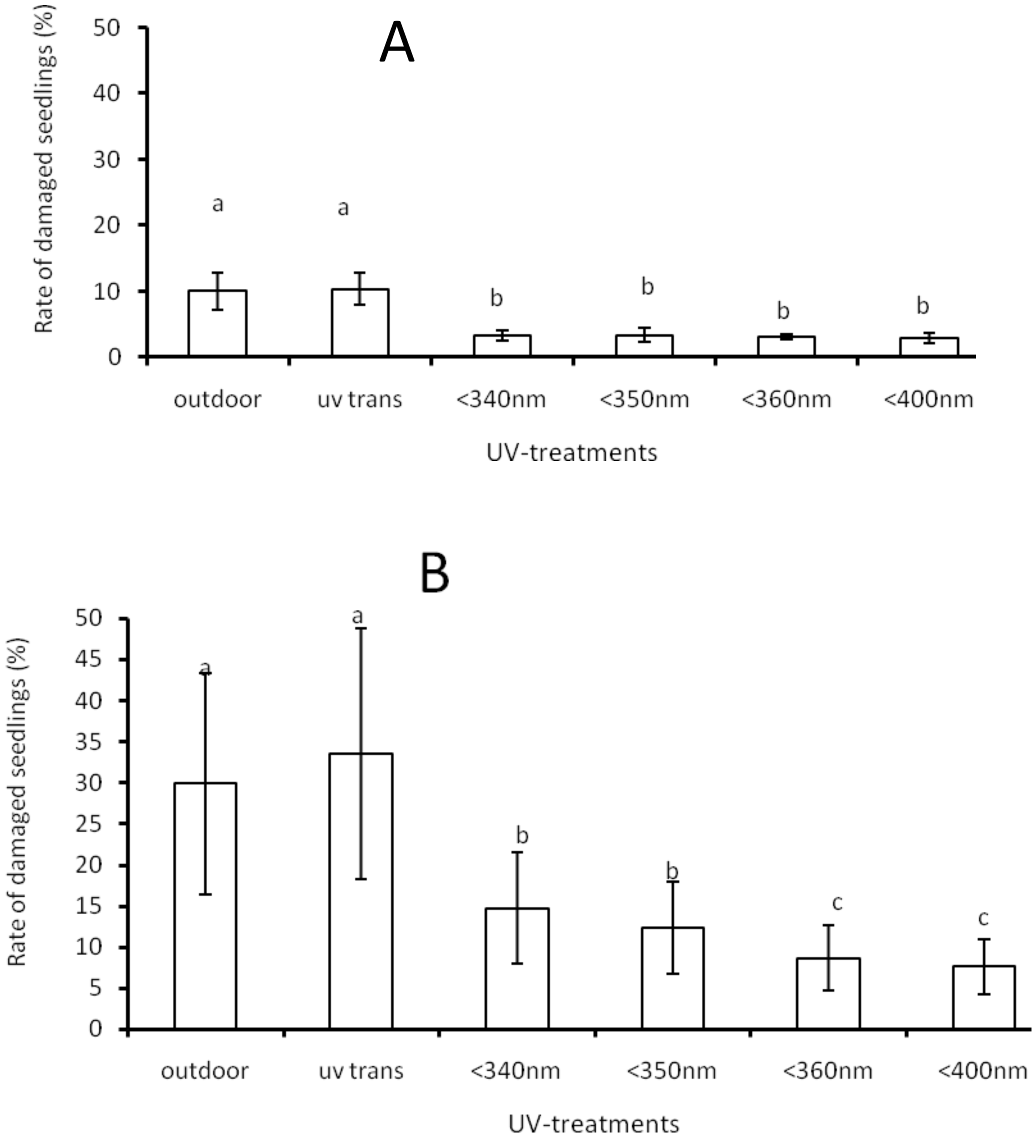


Figure 2-2. Feed damage (%, number of attacked seedlings) by insects in each plots as influenced by partially UV-blocking films (A-broccoli, B-turnip seedlings). Lowercase letters above the vertical error bar lines indicate significant ($P \leq 0.05$). The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. The error bars represent the standard deviation of the values.

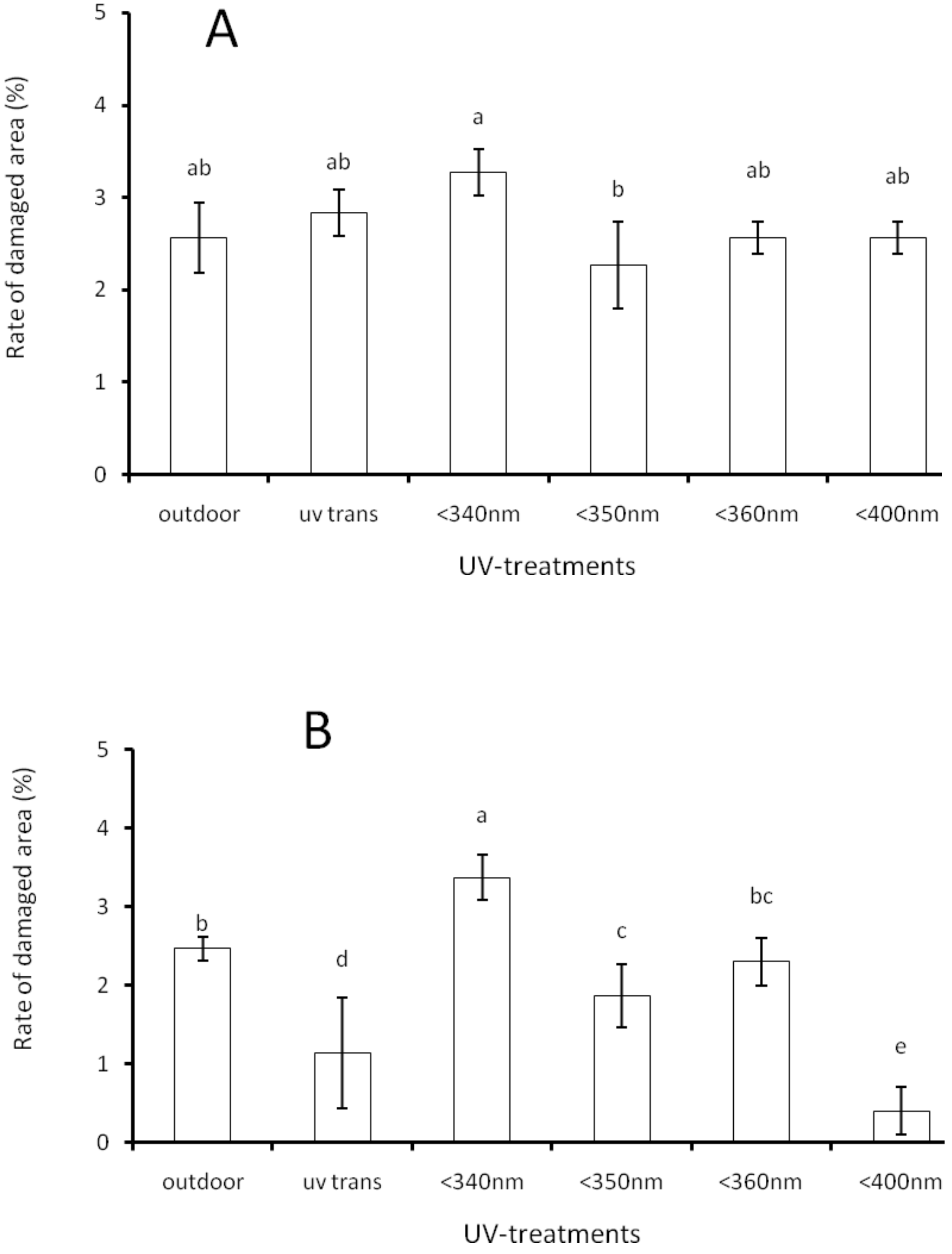


Figure 2-3. Mean value of leaf fed area (%) by insects at harvest as influenced by partially UV blocking films (A-broccoli, B-turnip seedlings). Lowercase letters above the vertical error bar lines indicate significant ($P \leq 0.05$). The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. The error bars represent the standard deviation of the values.

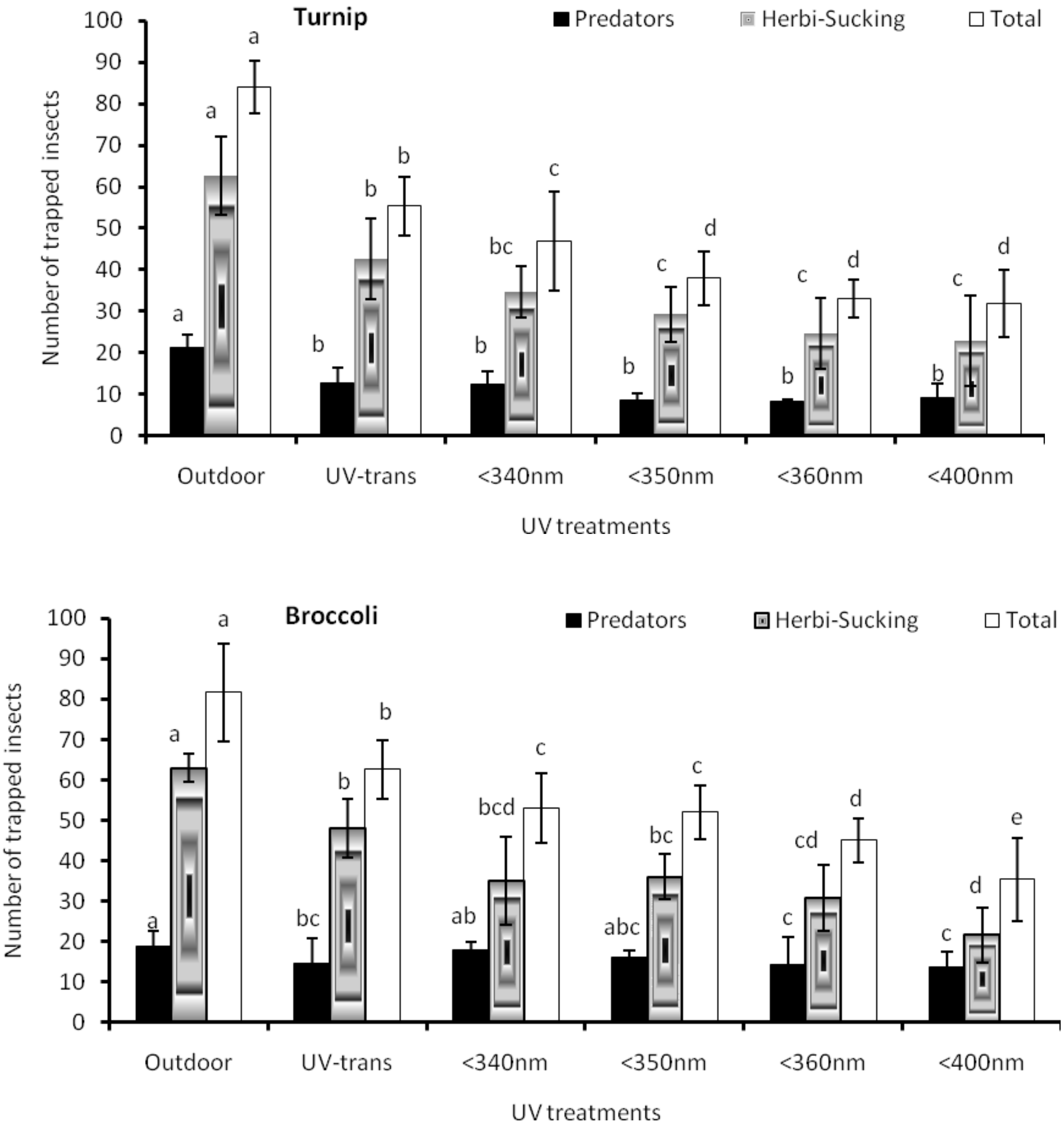


Figure 2- 4. Cumulative numbers of predators and herbivores/sucking insects that were trapped by adhesive films during the experiment. Here, Herbi-Sucking- Herbivores or Sucking insects. Predator: Mirid bug; herbivores or sucking insects: Aphid, BPH, Dipteran fly, Mosquito, SHGH, WBPH and miscellaneous insects. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. Different lowercase letters above the vertical bar lines indicate significant ($P \leq 0.05$). The error bars represent the standard deviation of the values.

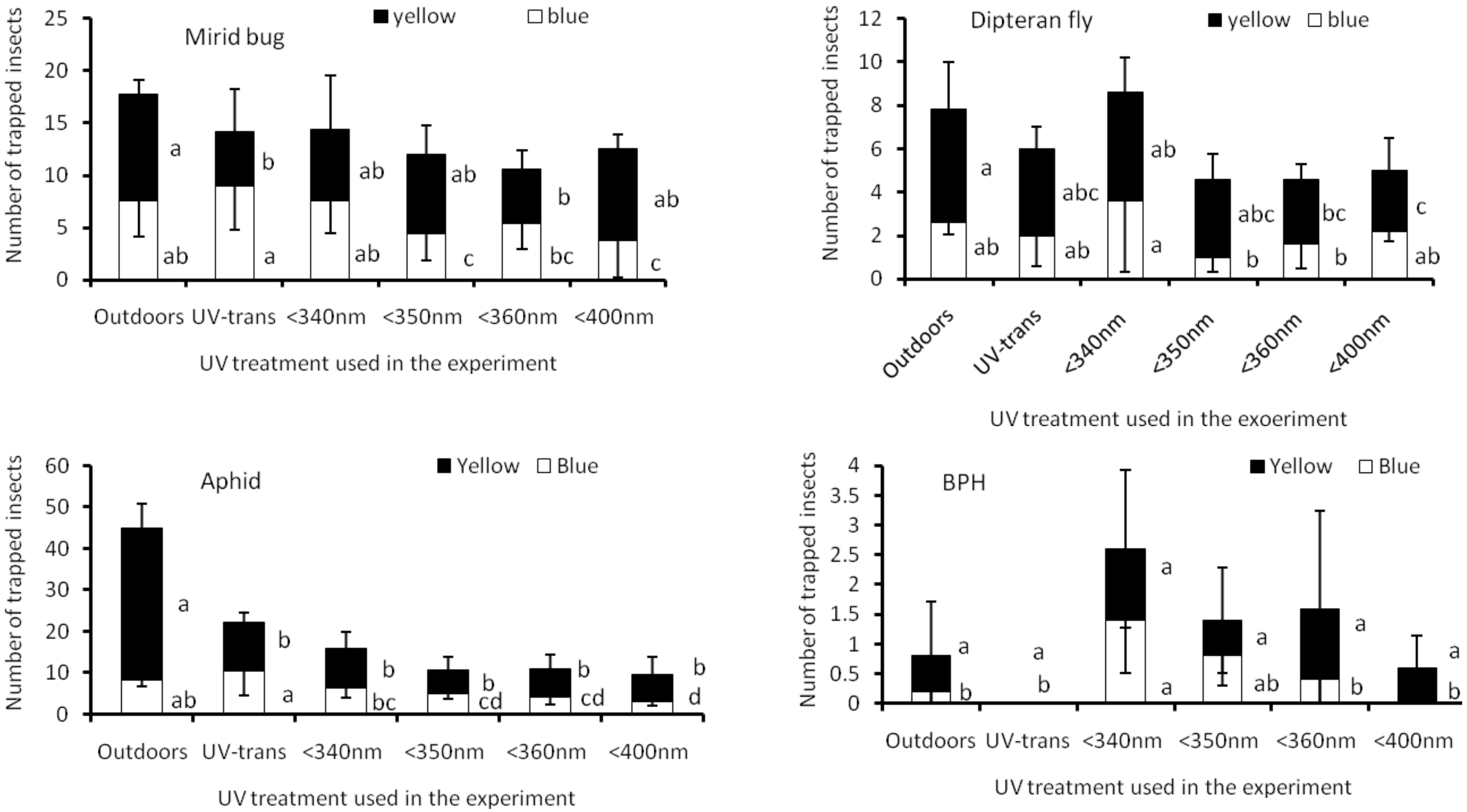


Figure 2-5 A. Mean numbers of major insect pest trapped in adhesives (Blue, yellow) inside and outside tunnels during the experiments (Broccoli seedlings). Here, BPH- Brown plant hopper. Mirid bug- classified as predator and Dipteran fly, Aphid and BPH- classified as herbivores or sucking insects. Different lowercase letters above the vertical bar lines indicate significant ($P \leq 0.05$). The error bars represent the standard deviation of the values.

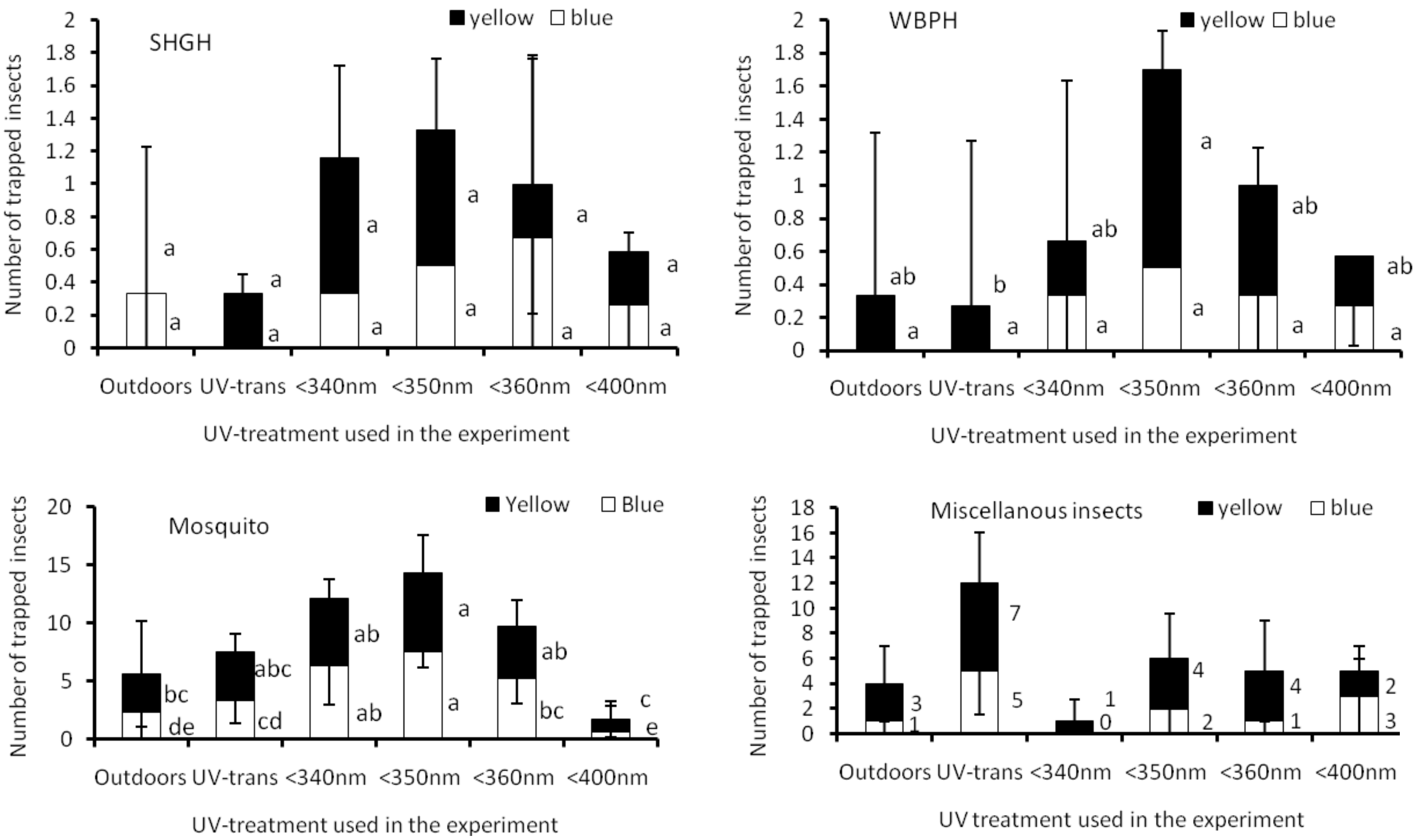


Figure 2-5 B. Mean numbers of major insect pest trapped in adhesives (Blue, yellow) inside and outside tunnels during the experiments (Broccoli seedlings). Here, - SHGH - Short horn grass hopper, WBPH- White backed plant hopper. Herbivores or Sucking insects- SHGH, WBPH, Mosquito and miscellaneous insects- unclassified and only in few numbers. Different lowercase letters above the vertical bar lines indicate significant ($P \leq 0.05$). The error bars represent the standard deviation of the values.

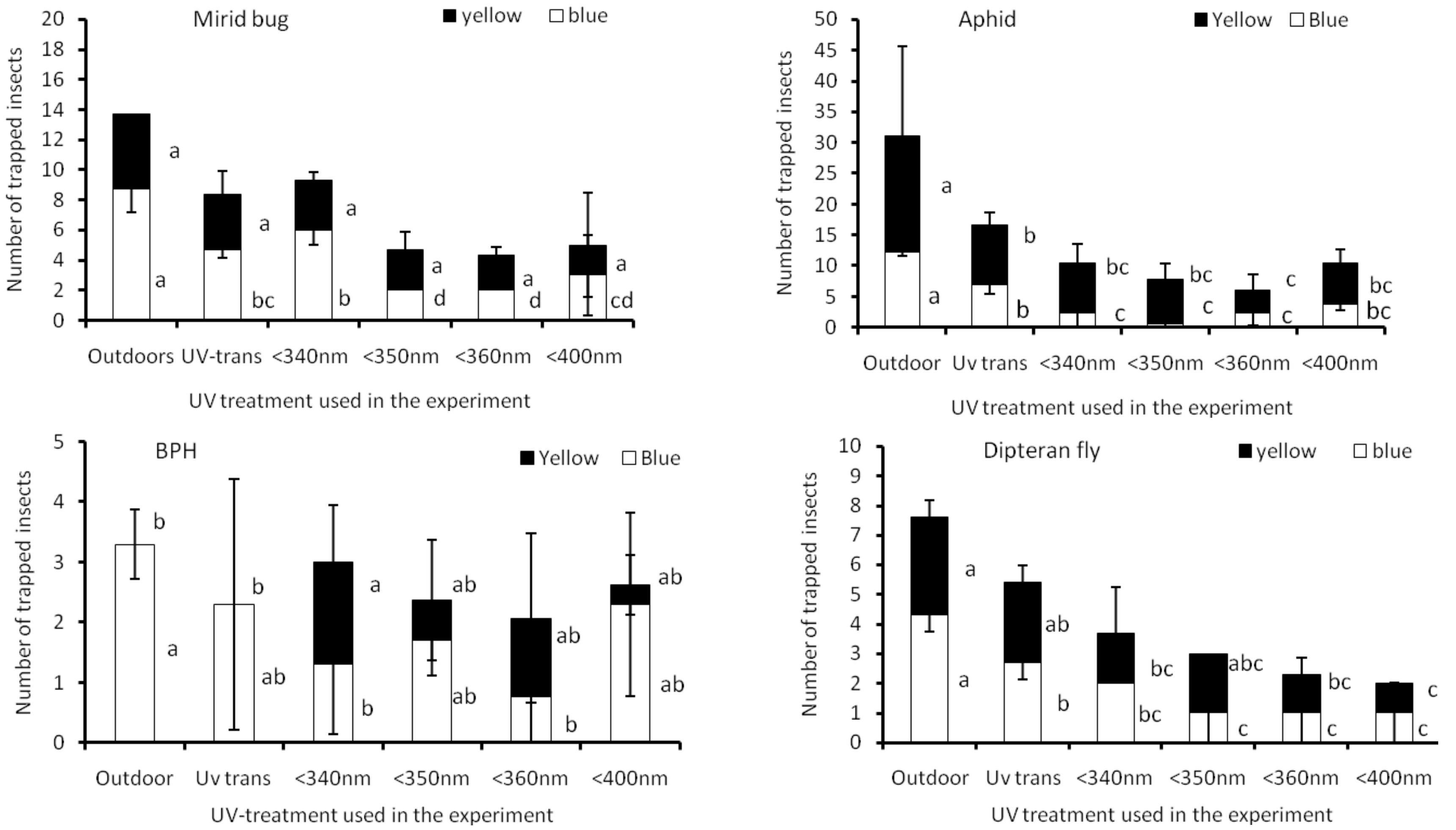


Figure 2-6 A. Mean numbers of major insect pest trapped in adhesives (Blue, yellow) inside and outside tunnels during the experiments (Turnip seedlings). Here, BPH - Brown plant hopper, Predators - Mirid bug; Herbivores or Sucking insects- Aphid, BPH, Dipteran fly. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. Different lowercase letters above the vertical bar lines indicate significant ($P \leq 0.05$).

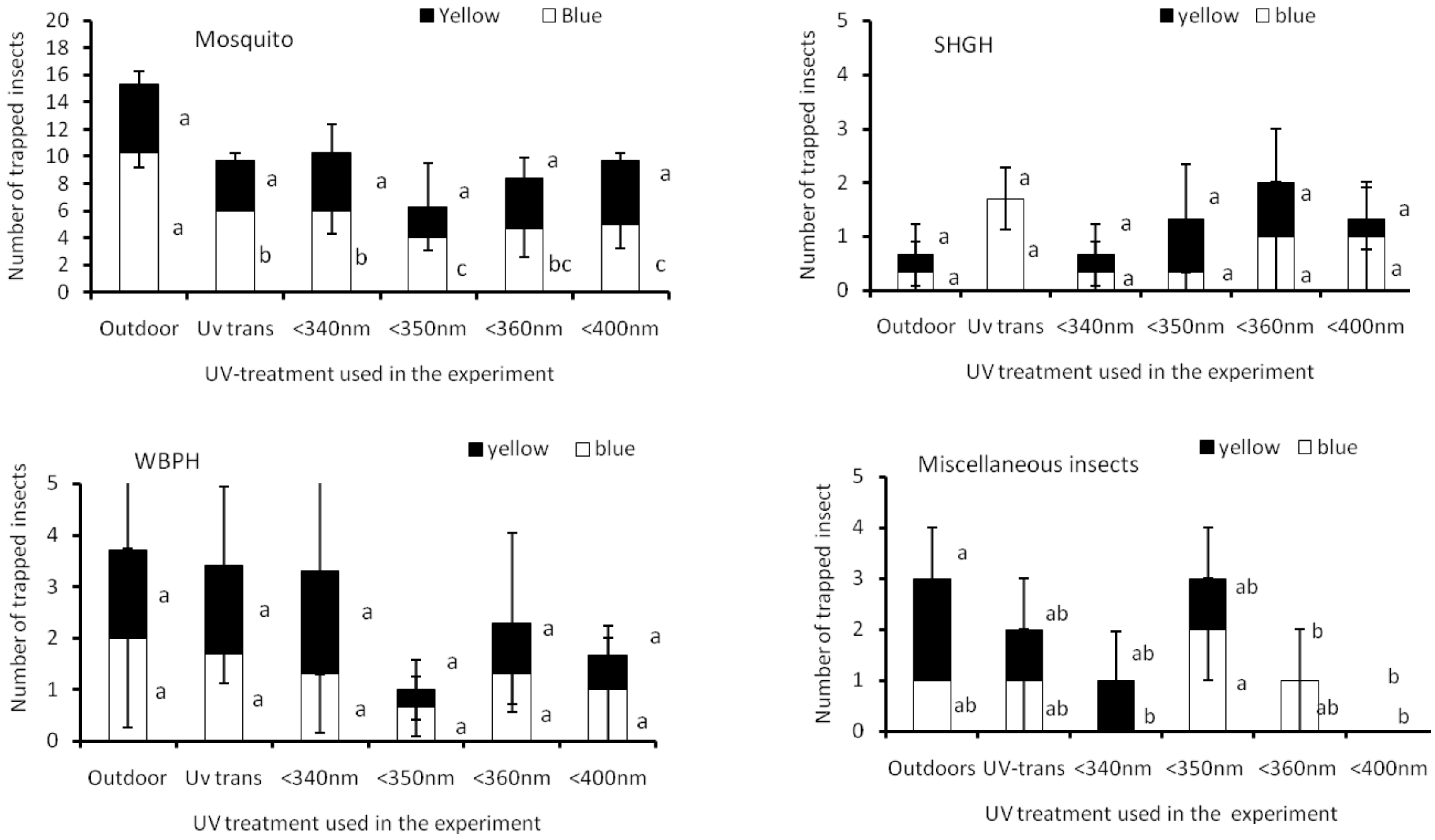


Figure 2-6 B. Mean numbers of major insect pest trapped in adhesives (Blue, yellow) inside and outside tunnels during the experiments (Turnip seedlings). Here, WBPH - White backed plant hopper, SHGH - Short horn grass hopper. Also, predators- Mirid bug, herbivores/sucking insects- Aphid, BPH, Dipteran fly, Mosquito, SHGH, WBPH and miscellaneous insects- unclassified and only in few numbers. Different lowercase letters above the vertical bar lines indicate significant (*P* ≤ 0.05).

Chapter-3

Experiment-2

Effect of partially UV-blocking films on the growth, yield, pigmentation and insect control of popular Bangladeshi crop red amaranth (*Amaranthus tricolor*).

3.1 Abstract of the published paper

Red amaranth (Amaranthus tricolor) was grown in tunnels covered with partially UV-blocking films (<340, <350, <360, and <400 nm) for five weeks after sowing, and plant growth, anthocyanin concentration, and insect burden were compared with those of UV-transmitting films and outdoors. The values of plant height, stem cell length, leaf area, and fresh weight were higher in the plants grown under higher UV-blocking conditions, while the plant dry weight was greater under lower UV-blocking conditions due to the lower dry matter percentage under higher UV-blocking conditions. The red color of the upper leaves became lighter as the UV-blocking rate increased, while there was no such distinct difference in either the lower leaves or stem. Anthocyanin concentrations of both the stem and leaves of the plant apex were significantly lower under UV-blockings than under UV-transmitting conditions and outdoors, but there was almost no significant difference among different UV-blocking rates. Yellow and blue sticky traps were also suspended at the center of the tunnels, and twelve insects were trapped during the experimental period. The maximum invasion by insects and feeding damage of the seedlings were observed outdoors, followed by plants grown under UV-transmitting conditions. Partially UV-blocking conditions significantly reduced invasion by insects and feeding damage of the seedlings compared to outdoors and under UV-transmitting conditions, but there was no significant difference among different UV-blocking rates. The

number of herbivores tended to decrease as the UV-blocking rate increased, while that of predators was little affected by the UV-blocking rate. The results showed that partially UV-blocking conditions were as effective as fully UV-blocking conditions, especially for controlling some herbivores such as aphids and white fly, and also for maintaining an advantageous balance between herbivores and predators. On the other hand, anthocyanin pigmentation of higher plant parts was often inhibited under UV-blocking conditions.

3.2 Introduction

Nowadays, the most alarming issue is the uncontrolled use of pesticides which is easily available, relatively cheap and farmers think the application is also easier. Moreover, generally farmers do not wait long enough to wash off after spraying before harvesting because of their high demand for farm products and low perception of the toxic effects of pesticide residues in agriculture commodities especially for those are consumed fresh causing enormous health hazards. (Bhuiyan and Ali, 2010; Hossain et al., 2013).

The use of UV and partially UV-blocking polythene is now widely known to be an effective method for reducing many flying insect pests. In recent years various spectral modifications have been made in different types of crops, few studies suggest that solar UV-A has significant effects on growth, morphology and tissue chemistry in a range of species (Tsormpatsidis et al., 2008). Few researches suggested that UV-A radiation may also induce increased amounts of UV-absorbing pigments and, UV-exclusion studies conducted on cucumber and, a red-pigmented lettuce indicates that ambient UV-A radiation greatly inhibits leaf enlargement, stem elongation and biomass production (Tsormpatsidis et al., 2008; Nishizawa et al., 2012). Few of the main reasons of modify the spectral characteristics in protected cultivation in changing climate is to suppress the proliferation of several foliar diseases and to protect crops from insects and insect-borne virus diseases in order to get the targeted yield.

Red amaranth (*Amaranthus tricolor* cv. BARI Lal shak-1, Amaranthaceae) is the most popular leafy vegetables and can be found mostly in Bangladesh and India that are harvested at 25-35 days after sowing (DAS) (Rashid et al., 1999; Mondal et al., 2011). It can be grown year-round and mostly attacked by phytophagous insects like whiteflies and aphids. So, the target was to control the insect-pests and observe the growth parameters by using the partially UV-blocking films in Bangladeshi condition.

The use of photo selective plastic materials have been used in traditional agriculture systems that made possible increasing the productive areas of the world in contrast with adverse climatic conditions. It is almost clear that UV-radiation has a significant influence on plant growth (Catalina et al., 2000; Caldwell et al., 2003; Kittas et al., 2006; Tsormpatsidis et al., 2008), physiology as anthocyanin biosynthesis (Antignus and Ben-Yakir, 2004) and has inhibitory effect on the invasion of insects (Nakagaki et al., 1982; Dáder et al., 2015; Miranda et al., 2015). Besides, wavelength selective filters show a range of significant responses across many plant species and locations (Paul et al., 2005). Also, changing in quality, pigmentation and taste of lettuce was found remarkable when plants were grown under UV-opaque film, which absorbed 50% of UV-A (Paul et al., 2005).

In general, the sticky traps are used to predict insect densities that cause the crop damage or yield reduction or commodity losses so that any control action can be taken. Traps have been widely used with several advantages for growth such as low cost and low training demands, and in controlled condition, pests are normally guided by adult densities on traps (Kaas, 2005; Delia et al., 2013). In this case, the target of the proposed study was to minimize the attack of insects especially to the leafy vegetable like red amaranth that might encourage farmers to avoid chemical spraying. Generally, old leaves were found to be less nutritious than young leaves and chemical defense were found to decrease with leaf age that may make the young plants more prone in biomass removing to insects (Lambdon et al., 2003; Boege and Maquis, 2005; Barton and Hanley, 2013). In addition, the greenhouse design and the amount of unfiltered light that enters the system appear to be an important component in determining the level of protection provided by UV-blocking films (Costa et al., 2002).

Described above primary studies allow to raise questions on the effect of partially UV- blocking films on growth and insect control of red amaranth that have not been widely discussed in the world literature so far. So, the effect of partial UV-blocking on the growth, coloration, anthocyanins, and insect control under outdoor field conditions was discussed that might be effective for commercial horticulture production and also to control insect pests and other beneficial organisms especially in Bangladesh condition.

3.3 Materials and Methods

3.3.1 Plant cultivation and UV-blocking

The seeds of red amaranth (*Amaranthus tricolor* cv. BARI Lal shak-1) were collected from Bangladesh Agricultural Research Institute (BARI) and were sown in the raised beds of 100 × 100 × 50 cm (L×B×H) during November to December, 2013 at the experimental field of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

Application of fertilizers and irrigation, and other operations were followed by FRG (2012).

In this proposed study, fifteen plastic tunnels (Length × Breadth × Height =140 cm × 140 cm × 60 m) were prepared after the germination (7 DAS) of the seedlings, and covered with different polyolefin films which can block UV-radiations shorter than 400 (<400), 360 (<360), 350 (<350), and 340 nm (<340), respectively. A tunnel was also covered with UV-transmitting PO film following three replications. In the study, a 15 cm (20 %) from the soil level was remained to open for protecting the heating of the tunnels and allowing the invasion of insects. After 30 days of sowing (DAS), the seedlings were harvested and measured the plant growth.

3.3.2 Measurement of environmental conditions

Temperature, humidity, and visible and UV-light irradiations were measured daily during the experiment. Both temperature and humidity were recorded at 12:00, 20:00 and 24:00, while visible and UV-light irradiations were measured at 12:00.

3.3.3 Measurement of growth

At harvesting stage (35 DAS) the plant height (cm), leaf number, leaf area (cm^2), fresh weight (FW, g), dry weight (DW, g), stem diameter (cm, at 5 and 15cm from the ground), were measured. The following formula was used for determining dry matter (% DM) content:

$$DM\ (\%) = \frac{\text{dry weight}}{\text{fresh weight}} \times 100$$

The chlorophyll reading was observed just before harvesting of the crops with a chlorophyll meter, (SPAD 502 plus, Konika Minolta, Japan).

3.3.4 Determination of total soluble solids (TSS, °brix).

TSS was measured by portable hand refractometer (ERMA, Tokyo, Japan) with leaf juice of five plants of each tunnel. Mean was collected for each treatment.

3.3.5 Color measurements

Color was measured with a color spectrophotometer (NF333, Nippon Densyoku, Tokyo, Japan) using the CIE Lab L*, a* and b* color scale. The 'L*'value is the lightness parameter indicating degree of lightness of the sample; it varies from 0 = black (dark) to 100 = white (light). The 'a*' which is the chromatic redness parameter whose value means tending to red color when positive (+a*) and green color when negative (–a*). The 'b*' is yellowness chromatic parameter corresponding to yellow color when it is positive (+b*) and blue color when it is negative (–b*). Each sample consisted of 10 leaves, each of which was measured thrice. Chroma = $\sqrt{a^{*2} + b^{*2}}$ was calculated and higher numbers of chromaticity indicate a more vivid color, whereas lower numbers correspond to dull colors. Color measurement was done just before harvesting of amaranthus leaves and stems separately.

3.3.6 Total anthocyanin measurement

The pigment (anthocyanin, at 500 and 900nm) of the leaf and stem was investigated with a UV-VIS spectrophotometer (PD-303S, Apel, Saitama, Japan). Ten equivalent aged leaves from each tunnel were collected early in the morning (one from each of the 10 plants). In total 30 leaf discs were collected from these leaves (three per leaf) using a 1.5 cm cork borer. All leaf discs were collected from the distal ends (4cm, exposed to sunlight) of the leaf. Each sample was extracted with 15 ml of metahanol: HCl (99:1) and placed in a vial. Then the procedure was followed according to Tsormpatsidis et al. (2008) and then the results were expressed as mg·$100g^{-1}$ fresh weight (FW). The absorbance measurement was done within 20-50 min of preparation.

The anthocyanin pigment concentration expressed as cyaniding-3-glucoside equivalent, as follows:

Anthocyanin pigment (cyaniding-3-glucoside equivalents, mg·$100g^{-1}$ FW)

$$= \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1}$$

Where, $A = (A_{500nm} - A_{900nm})$ pH 1.0 – $(A_{500nm} - A_{900nm})$ pH 4.5; MW (molecular weight) = 449.2 $g.mol^{-1}$ for cyaniding-3-glucoside; DF = dilution factor; 1 = path length in cm; ε = 26, 900 molar extinction coefficient, in $L \times mol^{-1} \times cm^{-1}$, for cyaniding-3-glucoside and 1000 = factor for conversion from g to mg.

3.3.7 Cell number count

Thin sections of stem bark (cell number and cell size measurement) were collected from the standing plant by using transparent nail paint and observed under a microscope fitted with digital camera (Motic images plus 2.0) and attached with a computer for observing the cell number or size (Figure 3-2).

3.3.8 Measurement of invaded insects and feeding damage

In second week of November, two (blue and yellow) sticky trap films (18 × 5 cm) were suspended at the middle position of the tunnels for capturing invaded insects. The adhesives were replaced every week, and the kind and the number of captured insects were counted daily until 24 December.

During the 30 days of cultivation under different UV-blocking conditions, leaves of some seedlings showed feeding damages by herbivores. Therefore, the feeding damages of the seedlings were evaluated by two different ways as follows:

1) Rate of damaged seedlings (%) = $\frac{\text{Number of damaged seedlings per plot}}{\text{Total number of seedlings per plot}} \times 100$

2) Rate of damaged leaf area (%) = $\frac{\text{Damaged area}}{\text{Total leaf area}} \times 100$

Rate of damaged leaf area was calculated using an open access software (Image J, NIH, MD, USA).

3.3.9 Statistical analysis

Data were subjected to analysis of variance, and significant differences of the means among treatments were analyzed using MSTAT-C software (East Lansing, MI, USA). The differences among treatment means were evaluated by Duncan’s multiple range test (DMRT).

3.4 Results and Discussion

Throughout the duration (30 days) of the investigation, the maximum day temperature (12:00 pm, mid day) (Table 3-1) did not exceed 35.8 °C and did not go down 34.1 °C inside the tunnels. The average temperature at 8:00 pm was 21.6 and at 24:00 am it was 20.2 °C and the relative humidity (RH %) was highest (51.8%) and the lowest was 46.8% during the experimental period inside and outside the tunnels. The light intensity was blocked around 70% by UV-blocking films whereas, 77% was blocked in UV-transmitting films respect of outdoors. The UV was blocked 48% in the tunnels that were covered with UV-transmitting films and was decreased with the increasing of specific UV-blocking up to 13% in <400 nm tunnels (Table 3-1)

The highest plant was recorded inside the tunnels that were covered with <400 nm wavelength PO film and the lowest was in the plots outdoor (Table 3-2) but the highest mean number of leaves (11) were recorded from the UV-transmitting tunnels at harvest (35 DAS) as well as in <400 nm which showed non-significant effect on leaf number. The leaf area was significantly influenced ($P \leq 0.05$) by the treatments and the maximum area (119.1 cm2) was found in the tunnels covered with <400 nm film than in outdoor (59.7 cm2) which was almost doubled (Table 3.2). The changes in plants morphology induced by UV-radiation may affect competition for light (Barnes et al., 1988).

The negative effects of UV radiation resulted in deformed morphological parameters and exposure to UV-radiation decreased plant height, leaf area and plant dry weight increased auxiliary branching and leaf curling (Dai et al., 1995; Green-berg et al., 1997; Furness et al., 1999). Dai et al. (1995) reports that after a few weeks of UV-radiation exposure, leaf area and plant dry weight of crops of Graminie family were significantly reduced. In an experiment, the highest plant height was observed in poultry manure + synthetic fertilizer treated plots (37.5 cm) and the lowest (17.1 cm) was and higher leaf number (18) was also obtained from the same treatments (Adhikary et al., 2013) but in this experiment standard chemical fertilizers were applied and got good result with inclusion of specific UV-blocking films .

The plant height was increased but the question was whether the cell number maximized or the cell sizes were broadened. To find out this answer, the skin of the bark examined and found that the cell size was enlarged rather than the cell number count (Table 3-2). Weigh et al. (1998) stated that enhanced UV-A decreased leaf area per unit plant biomass (leaf area ratio) but increased biomass productivity both per unit leaf area (leaf area productivity) and per unit leaf nitrogen (leaf nitrogen productivity). The stem diameter (at 5 and 15cm from ground level) also differed significantly with the varying specific UV-blocking films (Table 3-2). The stem diameter (at 5 cm) was maximum (1.2 cm) in the plants grown in the tunnels with <400 nm UV-blocking films than in outdoor (0.65 cm) and the diameter showed decreasing trend with decreasing of partially UV-blocking (Table 3-2). The FW of RA showed variations among the treatments. The FW of the plants that were grown inside the tunnels of <400 nm gave 200g more yield than those grown in outdoors and UV-transmitting films (Table 3-2). Also, Diaz et al. (2006) found a reduction in fresh weight of lettuce under UV-transmitted condition.

The dry weight (DW) was maximum (19.6 %) in crops that were grown under partially UV-blocking films and the lowest was also inside the tunnels with specific UV-blocking (Table 3-2). In an experiment conducted in 2005 and 2006, Tsormpatsidis et al.

(2008) also found highest leaf number and dry weight under <400 nm films which also supported the proposed study. He also pointed out that lettuce plant grown under complete UV-blocking film (UV400) produced 40% and 122% more above ground DW than UV-transparent, and<320 nm produced 10% and 34% more above ground DW than <280 nm in 2005 and 2006, respectively.

In addition, a large number of experiments where solar UV-A has been attenuated using wavelengths-selective filters, such as polyester, show a range of significant responses across many plant species and locations (Paul et al., 2005). In a study it's also showed that total above ground dry weight is positively correlated with the degree of UV-radiation cut off transmitted by the films (Tsormpatsidis et al., 2008). He also concluded that plants in the presence of <280 nm appeared not to be stressed and they accumulate secondary products which effectively protect the photosynthetic apparatus and at the end he suggested the <280 nm films for the commercial use (Tsormpatsidis et al., 2008).

The leaf brix (°brix) was double than those of stem. The leaf brix showed highest in outdoors and the lowest was recorded in the crops that were grown with <350 nm UV-blocking films (Table 3-2) while, in the case of stem it was also highest in outdoor (Table 3-2). The mean value of chlorophyll reading in leaf (alternate leaf) was found significant ($P \leq 0.01$) at harvesting stage (35 DAS, standing plant) in the plants of <400 nm.

The pigments (total anthocyanins) were investigated both for stem and leaf of red amaranth at the wavelength of 500 and 900 nm (Figure 3.3). In the case of leaf and stem, the calculated values were reduced inside the tunnels with partially UV-blockings. In an experiment, Tsormpatsidis et al., 2010) showed that total anthocyanin content was also increased under UV-transmitting films than UV-blocking films in green and red lettuce. In another experiment, Tsormpatsidis et al. (2008) found higher anthocyanin under <280 nm and <320 nm than in plants under <350 nm, <370 nm, <380 nm and <400 nm films which also in agreement with the proposed study. He also found the lowest anthocyanin content which was eight times lower than UV-transmitting and <280 nm film studied in 2006 in Lollo Rosso lettuce.

In addition, since growth reduction under UV environment was accompanied by accumulation of anthocyanins, it may be that the plant diverts energy produced by photosynthesis to synthesize these compounds to protect itself from UV damage. Scientists suggested that further studies are needed in which photo-synthetic rate will be measured directly to test whether growth inhibition is due to a high cost of photo-protection. It is said that accumulation of anthocyanins in the leaf epidermis may create internal shading which can lead to a reduction of light available to chlorophyll (Steyn et al., 2002; Neill and Gould, 2003) and also this possible light reduction may have also contributed to growth reduction (Tsormpatsidis et al., 2008).

No significant differences were observed in L* and a* measured parameters in the leaf (lower leaves) and stem colour, while the significant differences were found L*, a*, b* (upper leaf), a*, b*, in stem colour and b* (lower leaf) and in the calculated chroma for all parameters as well (Table 3-3). In case of colour, lowest L* value was found in red amaranth with photoperiod conducted by Ali et al. (2009) whereas, in this experiment, significant L* value was recorded in upper portion of the leaf than lower portion and also in stem (Table 3-3). The higher L* value indicates the lighter color but in case of red amaranth dark color is acceptable that’s why specifically UV-blocking can be an interesting tool for the growth of this crop. The chroma values for all the parameters were non-significant in respect to all treatments. The redness value (+ a*) was highest in the upper leaves than lower leaves and also in the stem. Similarly Ali et al. (2009) found deeper color (higher redness value and lower yellowness value) with lower lightness value in some red-fleshed leafy vegetables worked in Gifu, Japan.

The cell number showed significant differences among the treatments and the highest plants that were found in the tunnels with <400 nm UV-blocking films showed the lowest number of cells, while outdoor plants had the maximum number of cells (Table 3-2).

The maximum damged plant (29%) was recorded in outdoors and 25% in <340 nm and the other partial UV-blocking films showed lower number of damage inside the tunnels (Figure 3-4) and it was gone down (5%) upto the tunnels covered with fully blocked films of < 400nm (Figure 3-4). The maximum fed areas (more than 2%) was found in outdoor and decreased with increasing UV-blocking (Figure 3-5). It is obvious that feeding of leaves may not occurred by only flying insects but may also be fed by other creeping or walking insects. It was necessary to find out the types of insects that are generally responsible for attacking leafy vegetables (red amaranth) in Bangladesh condition as a tool for IPM program. Affected plants are not sold in good price or low price in Bangladesh market, so the area was measured and also the total leaf area infested by herbivores were also monitored to determine the rate of damage. The affected leaves were measured after uprooting them from the tunnels at 35 DAS (BARI, 2010).

In the experiment, the cumulative number of insects trapped almost 300 in outdoor and it was reduced in the tunnels with partially UV-blocking films in one third than outdoors (Figure 3-6). In addition, YSTs also trapped more flying and walking insects than BSTs. In this experiment, it is found that the partially UV-blocking film has the capability of reducing insect pest as the fully blocking films did. The twelve numbers of insects were trapped using yellow and blue adhesive. Among them, aphid, mirid bug (MB), dipteran fly (DF), brown plant hopper (BPH), mosquito, lady bird beetle (LBB), white fly (WF), field cricket (FC), flee beetle (FB) showed significant trapping but white backed plant hopper (WBPH), carabid beetle (CB) and red ant showed less number of trapping (Figure 3-7). The most number of insects trapped in outdoors are aphid (137 in total) where 104 in YSTs followed by mosquito (50), MB (35) and WF (28) and the number of trapped insects decreased as the UV-blocking increased (Figure 3-7). Aphid, mosquito and white fly (WF), invaded in maximum numbers inside and outdoor of the tunnels (Figure 3-6). Though few insects like leaf beetle (LB), White backed plant hopper (WBPH), carabid beetle (CB) showed non-significant result.

On the contrary, the insect trapping with YSTs and BSTs inside the tunnel covered by specific UV-blocking films (<340, <350, <360 and <400 nm) showed a variety of influence (Figure 3-7). The DF, aphid, WF and WBPH showed significant difference and rest of them showed insignificant trapping. The immigration of aphid was significant inside the tunnel. The highest number of aphids were trapped outside, while the lowest was inside <350 nm UV-blocking tunnel in YSTs and BSTs, respectively. The WBPH was significantly trapped by YSTs where as BSTs showed non-significant effect. The maximum number of WBPH was trapped inside control and in <350 nm but the lowest

(2.0) was found in <360 nm UV blocked tunnels. The numbers were not many may be because of the duration of the crop harvest or the open area kept was not optimum for the invasion. The white fly uses red amaranth as their host plants and the numbers of aphid and mosquito trapped in maximum numbers and it was also identified that UV-blocking conditions (<344 nm) frequently agitate behavior of some beneficial insects like honey bee (Reisenman and Giurfa, 2008). Photo selective plastic films should be compatible with natural enemies of pests and other beneficial organisms such as pollinators because biological control is one of the most widely used strategies in vegetable production due to its well known environmental benefits (Viñuela, 2005; Diaz and Fereres, 2007). In fact, white flies and aphids can attack plants at all stages of development and aphids feed on plant foliage and white fly uses red amaranth as their host plant (Webb, 2013). The attacked plants may be stunted or die as a result of aphid feeding. In addition, foliage may be contaminated with aphid bodies, cast skins, and honeydew (Webb, 2013). The kind of UV-blocking plastic used in a structure determines the level of protection and can affect the population levels of some insect species. In this way, Costa et al. (2002) found that UV-absorbing components that block the majority of UV-light at wavelengths below 380 nm had more influence in reducing insect numbers than those that blocked light at wavelengths of <360 nm.

In an experiment, Antignus et al. (2001) found a positive correlation between the level of protection and the capacity of the sheets to absorb UV light (<400 nm). Also, Costa et al. (2002) found a significant reduction in the number of aphids and thrips captured on yellow sticky traps (YSTs) in greenhouses covered by UV-plastic films in a commercial cut-flower green-house. Besides, Antignus et al. (2001) also showed that penetration of the whitefly into walk-in tomato tunnels covered with UV-absorbing films was strongly inhibited as well as the attraction of whiteflies to these types of structures. Also, a reduction in the number of aphids captured on YSTs, a delay in aphid immigration and colonization was recorded in lettuce grown in walk-in tunnels under UV-blocking plastic films (Diaz et al., 2006). Also, they found that UV-absorbing plastic films were effective in reducing the population density of *F. occidentalis* (Pergande) and the spread of TSWV as well as the population density of the lepidopteran pest, *A. gamma* (L.). *T. vaporariorum* showed a distinctive preference to penetrate and disperse in UV-rich tunnels, both without and with tobacco plants (Mutwiwa et al., 2005). Similar results were obtained with *B. tabaci*, *Cerathothripoides claratis* and *A. gossypii* that showed a reduction in their immigration rate into a tomato greenhouse covered with UV-blocking plastic films (Kumar and Poehling, 2006).

The number of predators were separated from herbivores/sucking insects and found that about 46% predators were trapped inside the tunnels of <360 nm UV-blocking and minimum numbers (14%) were trapped in outdoors (Figure 3-8). A ration of 30% predators were trapped inside the tunnels with fully UV-blocked of <400 nm and the following percentage were 27 (<350 nm), 26 (UV-transmitting) and 25% inside tunnels of <340 nm (Figure 3-8). The ration was investigated to find out the proportion of predators as they are beneficial for agriculture and ecosystem, while Bangladesh farmers have anonymously been spraying chemicals to control insects and anonymously they are killing beneficial insects as well. So, it was needed to investigate the effect of specific UV-blocking film on the invasion or accumulation of predators inside the tunnel.

It is established that many microbes and plants use UV-A radiation as a source of information about their environment affecting many eco-logical processes (Paul and Gwynn-Jones, 2003). In this way, Chiel et al. (2006) studied the effects of UV-absorbing plastic sheets on the host location ability of few insects specially predators that are available commercially and commonly released by the growers under green house conditions, such us, *Aphidius colemani* a predators of *M. persicae, Diglyphus isaea* a parasitoid of *Lyriomyza bryoniae,* and *Eretmocerus mundus*, a parasitoid of *B. tabaci.* Aphid landing preference is determined by the colour of the background, mainly by the degree of contrast between a green target (the plant) and the colour of the soil (background colour) (Doring and Chittka, 2007).

In the experiment- 2, the UV was blocked 48% in the tunnels that were covered with UV-transmitting films and was decreased with the increasing of partially UV-blocking up to 13% in <400 nm tunnels. In the study, the plant height, leaf area, stem diameter, and fresh weight showed maximum values at <400 nm, and the values tended to decrease as the UV-blocking rates decreased. However, both the dry weight and percentage of dry matter were maximal in outdoors, and the values tended to decrease as UV-blocking rates increased. These results indicate that growth promotion under UV-blocking conditions is not necessarily correlated with biomass production. Additionally, the increased mutual shading under UV-blocking conditions, the difference of visible-light transmittance rates among the treatments might also affect carbon fixation, because the lowest leaf TSS value was observed at <350 nm, which also showed the lowest visible light transmittance. The leaf color of the plants grown outdoors was fully red, while that of the plants grown under PO films became a little lighter. Since the partially UV-blocking films used in the experiment can block all UV-radiation shorter than the specific UV-A wavelength as well as the fully UV-blocking film, the presence of almost no significance differences in anthocyanin concentrations among UV-blocking films suggests that UV-B may be more important than UV-A for anthocyanin biosynthesis. Feeding damage by herbivores/sucking insects largely reduces the commercial value of red amaranth. Therefore, the feeding damage at 30 DAS was shown as two indices: the rate of damaged plants and the rate of damaged area.

The results showed that the number of herbivores/sucking insects decreased gradually as the UV-blocking rate increased, while that of predators was little influenced by the partially UV-blocking conditions. Therefore, partially UV-blocking films will be advantageous for IPM programme from the aspect of maintaining a favorable balance between herbivores/sucking insects and predators.

Table 3-1. Effect of partially UV-blocking films on the recorded temperature and RH (daily, 3 times), light intensity, and UV reading (at 12 pm) inside and outside tunnels during the period of measurement.

UV-blocking	Temperature (˚C)			Relative humidity (%)			Visible light intensity (W.cm^{-2})		UV intensity (mW·cm^{-2})	
	8:00	12:00	24:00	8:00	12:00	24:00	12:00	% of outdoors	12:00	% of outdoors
Outdoors	21.7 a	31.9 c	20.5 a	84.1 ab	53.6 a	89.1 c	23.3 a	100.0	729.4 a	100.0
UV-transmitting	21.9 a	33.9 b	20.3 a	82.7 b	53.5 a	89.7 bc	19.6 b	76.9	483.2 b	47.9
Partially UV-blocking										
<340 nm	21.4 a	34.8 ab	20.1 a	85.2 ab	54.3 a	90.9 bc	18.5 bc	73.8	306.2 c	30.4
<350 nm	21.5 a	35.8 a	20.3 a	86.5 ab	53.0 a	91.6 b	16.8 d	61.9	257.5 cd	25.5
<360nm	21.5 a	35.4 ab	20.2 a	87.1 a	53.5 a	93.8 a	18.3 bc	70.0	219.3 d	21.8
<400 nm	21.5 a	35.4 ab	20.1 a	87.3 a	50.8 a	93.8 a	18.1 c	70.9	127.4 e	12.6
Significance	ns	**	ns	**	ns	**	**		**	

ns-non significant, *- significant at $P \leq 0.05$, **- significant at $P \leq 0.01$. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. Different lowercase letters beside the mean value indicate significant at $P \leq 0.05$ or 0.01.

Table 3-2. Effect of partially UV-blocking films on different growth parameters of red amaranth.

Treatments	Plant height	Leaf number	Leaf area (cm2)	Stem (cm)	diameter	SPAD value	Fresh weight (gm) ^{X}	Dry weight (gm) ^{Y}	Dry matter (%)	Cell length (µm) ^{Z}	TSS (°brix)	
				P	Q						Leaf	Stem
Outdoor	26.7 d	9.0 c	59.7 c	0.65 d	0.43 c	34.1 ab	537.1 cd	27.9 e	5.2 c	101.3 b	6.0 a	3.5 a
UV-transmitting	36.5 c	11.1 a	73.5 bc	0.79 cd	0.55 b	32.7 ab	568.2 c	43.2 a	7.6 a	100.5 b	5.2 b	3.2 a
< 340 nm	39.9 bc	10.9 ab	85.6 b	0.79 cd	0.54 b	29.5 b	548.6 cd	41.0 b	7.5 a	112.0 b	5.2 b	3.2 a
< 350 nm	36.8 c	9.9 abc	83.9 b	0.80 c	0.55 b	29.4 b	516.8 d	32.3 d	6.3 b	111.6 b	4.4 c	2.2 b
< 360 nm	42.3 b	9.4 bc	75.7 bc	0.84 b	0.63 a	32.5 ab	661.9 b	32.7 d	5.0 c	127.2 ab	5.2 b	2.0 b
< 400 nm	47.0 a	10.6 abc	119.1 a	1.1 a	0.66 a	38.3 a	737.0 a	36.2 c	4.9 c	145.0 a	5.0 b	2.3 b
Significance	**	*	**	**	**	**	**	**	**	**	**	*

^{X} - Fresh weight of 100 plants, ^{Y}- dry weight of 100 gm fresh weight basis, P and Q- Stem diameter measured at 5 and 15 cm above ground level, respectively. ^{Z} - Cell length measured from the average area of 158 ×118µm2 (0.0186 mm^{2}) under microscope. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. ns - non significant, *- significant at P≤0.05, **- significance at P≤0.01. Different lowercase letters beside the mean value indicate significant at *P* ≤ 0.05 or 0.01.

Table 3-3. Mean values of measured and calculated chromatic characteristics of the red amaranth leaf and stem inside and outside tunnel during the experiment.

Treatments	Stem color				Upper leaf color				Lower leaf color			
	L *	a *	b *	Chroma	L *	a *	b *	Chroma	L *	a *	b *	Chroma
Outdoor	34.4 a	15.4 b	-9.0 b	19.4 b	32.9 b	5.7 a	4.7 b	7.5 b	42.9 a	10.2 a	0.2 ab	10.2 a
UV-transmitting	28.6 a	28.7 ab	-2.4 a	28.8 ab	34.7 b	3.3 ab	6.1 b	7.4 b	40.7 a	8.1 a	1.5 ab	8.3 ab
Partially UV-blocking												
<340 nm	33.9 a	26.1 ab	-3.0 ab	26.3 ab	39.1 a	-0.8 c	11.9 a	12.1 a	42.1 a	3.9 a	3.4 a	7.9 ab
<350 nm	33.9 a	34.1 a	-5.2 ab	34.5 a	33.9 b	2.2 bc	5.7 b	6.1 b	39.3 a	9.5 a	0.2 ab	9.5 ab
<360nm	30.5 a	22.8 ab	-5.0 ab	23.7 ab	35.8 ab	0.9 bc	7.5 b	7.7 b	41.3 a	5.9 a	2.1 ab	6.6 b
<400 nm	30.4 a	29.3 ab	-2.2 a	29.5 ab	33.0 b	2.7 ab	7.7 b	8.2 ab	39.3 a	8.7 a	-0.8 b	8.7 ab
Significance	**	**	**	*	*	**	*	ns	ns	ns	**	**

The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. ns - non significant, *- significant at $P \leq 0.05$, **- significant at $P \leq 0.01$. Different lowercase letters beside the mean value indicate significant at $P \leq 0.05$ or 0.01.

Figure 3-1. Photographs of harvested red amaranth plants on 30 days after sowing. Here, Plants grown under different tunnels; A-outdoor, B- UV-transmitting, C- <340 nm, D- <350 nm, E- <360 nm, F- <400 nm.

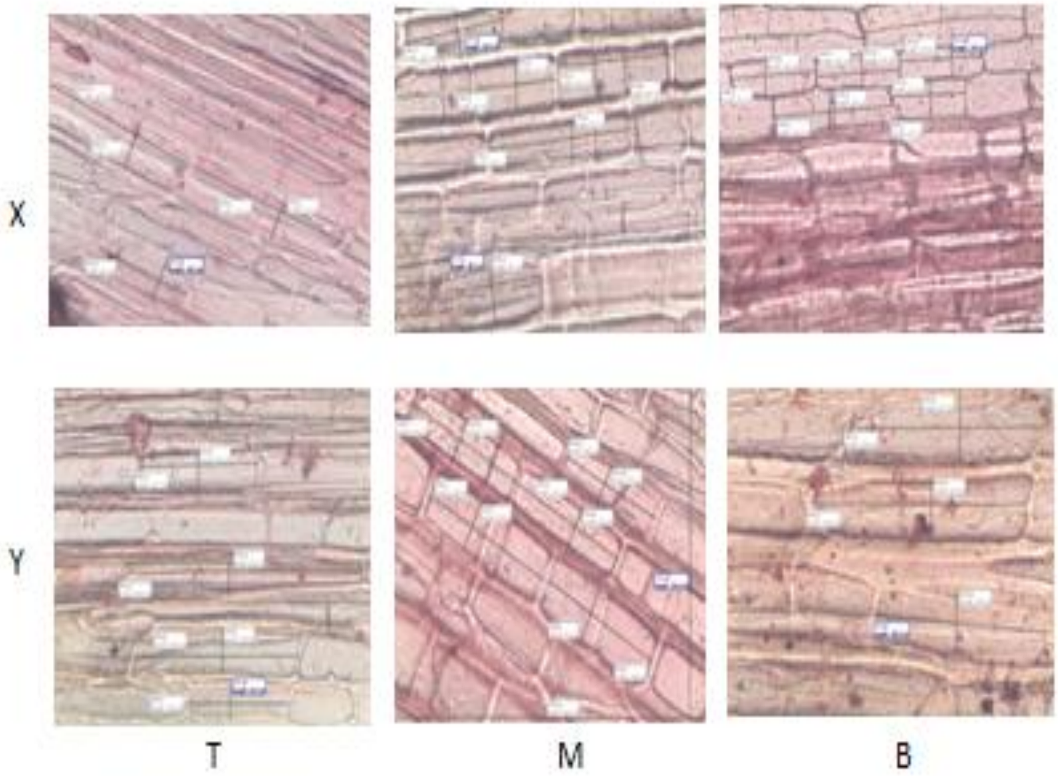


Figure 3-2. Photographs showing of cells that were taken from the skin of the stem of red amaranth under microscope. Here, X – indicates the cell taken from the plants that were grown in outdoor and Y – indicates plants inside tunnels of < 400 nm and also, T- Top, M- middle and B- bottom portion of the stem selected for the investigation under microscope and counted the cell number. Cell number counted from the average area of 158 ×118 μm^2 by motic images plus 2.0.

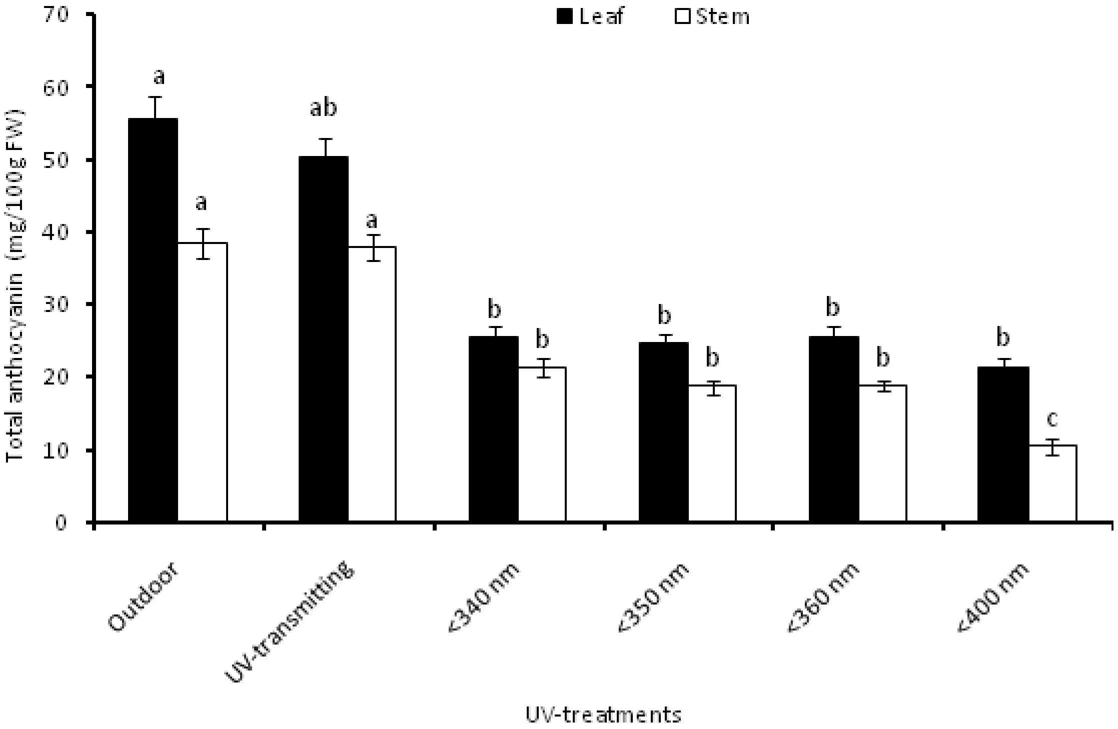


Figure 3-3. Total anthocyanin of red amaranth leaf and stem grown inside and outside tunnels. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. Different lowercase letters above the vertical error bars represent significant at $P \leq 0.05$.

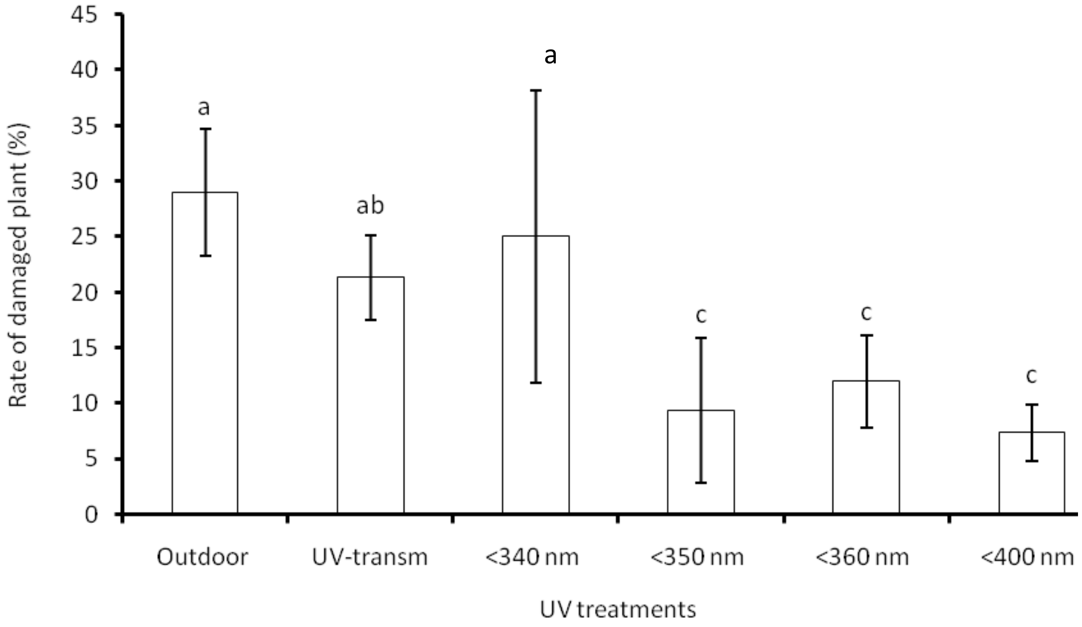


Figure 3-4. Rate of damaged plant (%) by insects as influenced by different partially UV-blocking films inside tunnels and outdoor during the experiment. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. Different lowercase letters above the vertical error bars represent significant at $P \leq 0.05$.

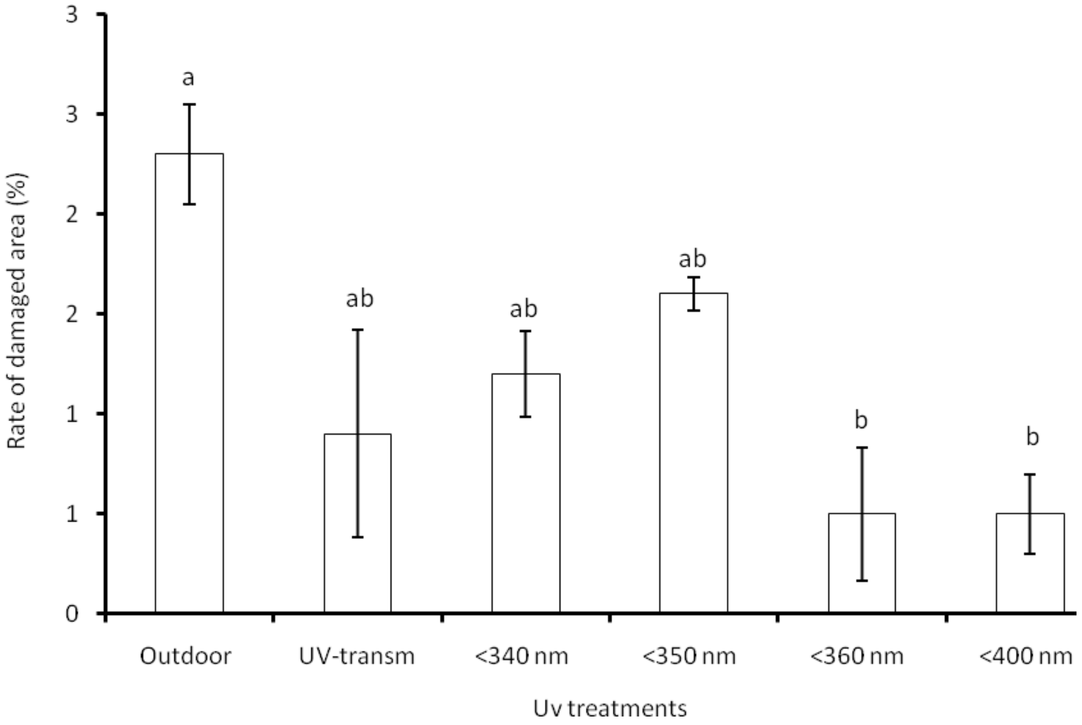


Figure 3-5. Rate of feeding area (%) in leaf fed by insects as influenced by different partially UV-blocking films inside tunnels and in outdoor during experiment. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. Different lowercase letters above the vertical error bars represent significant at $P \leq 0.05$.

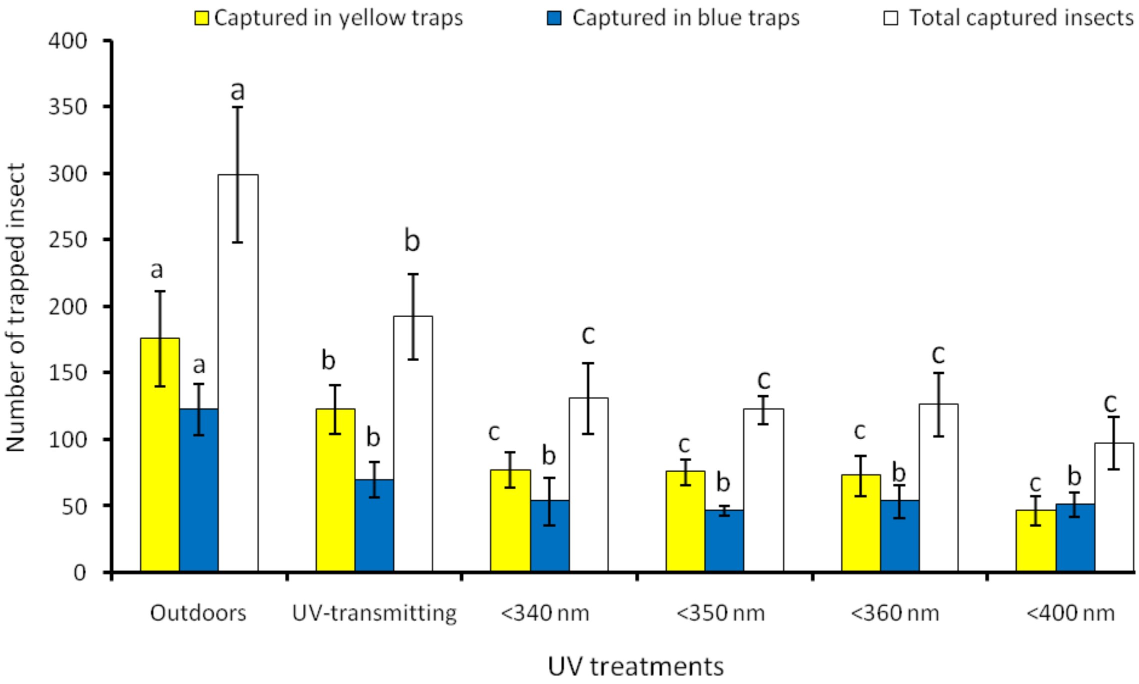


Figure 3-6. Mean total number of insects that were trapped in yellow and blue adhesives during the experiment. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. Different lowercase letters above the vertical error bars represent significant at $P \leq 0.05$.

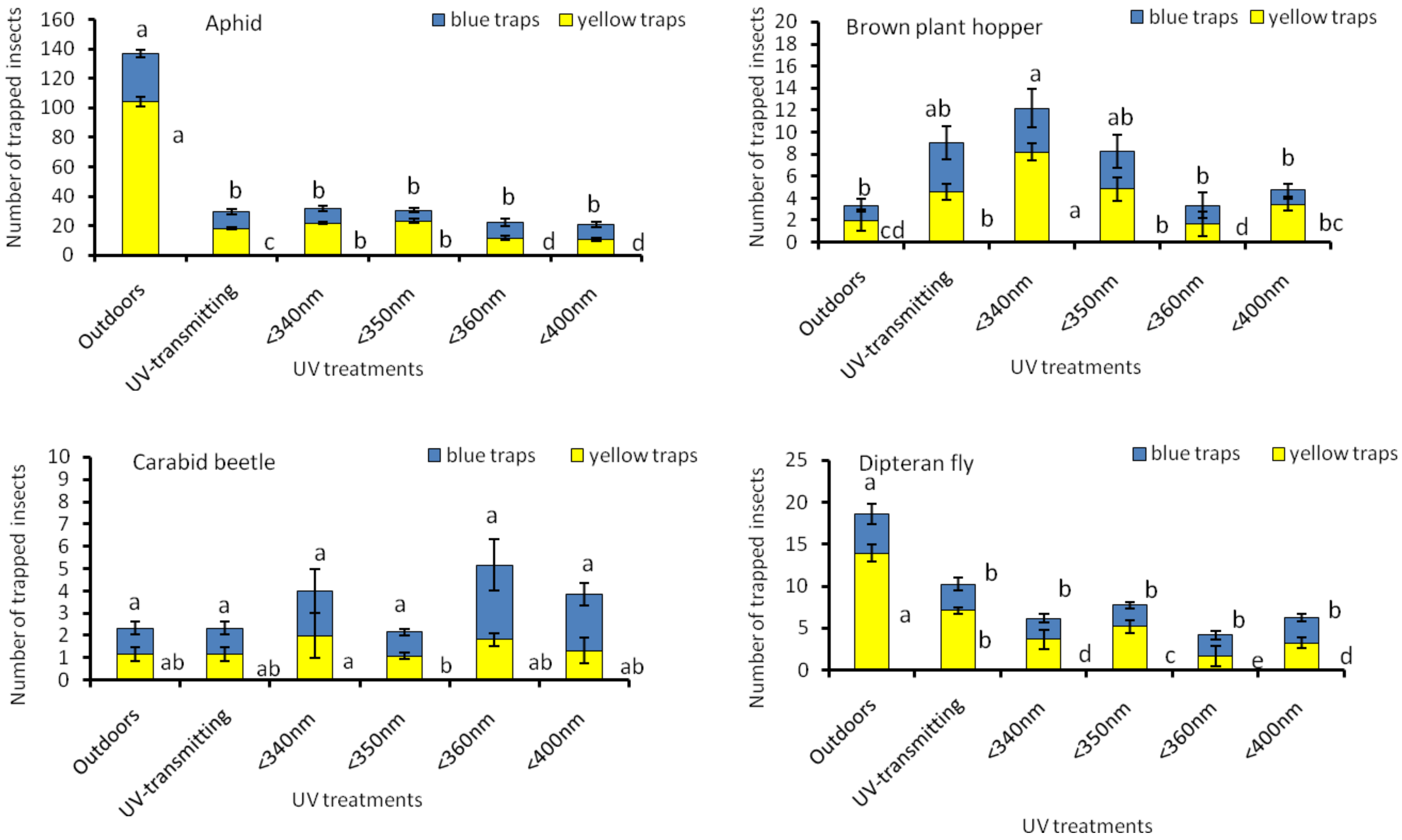


Figure 3-7 A. Mean numbers of individual insect pests that were trapped in adhesives (blue, yellow) inside and outside tunnels during the experiment. Blue colored bars indicate-blue traps and yellow colored bars indicate - yellow traps that were used in the experiment. Here, Different lowercase letters above the vertical error bars represent significant at $P \leq 0.05$.

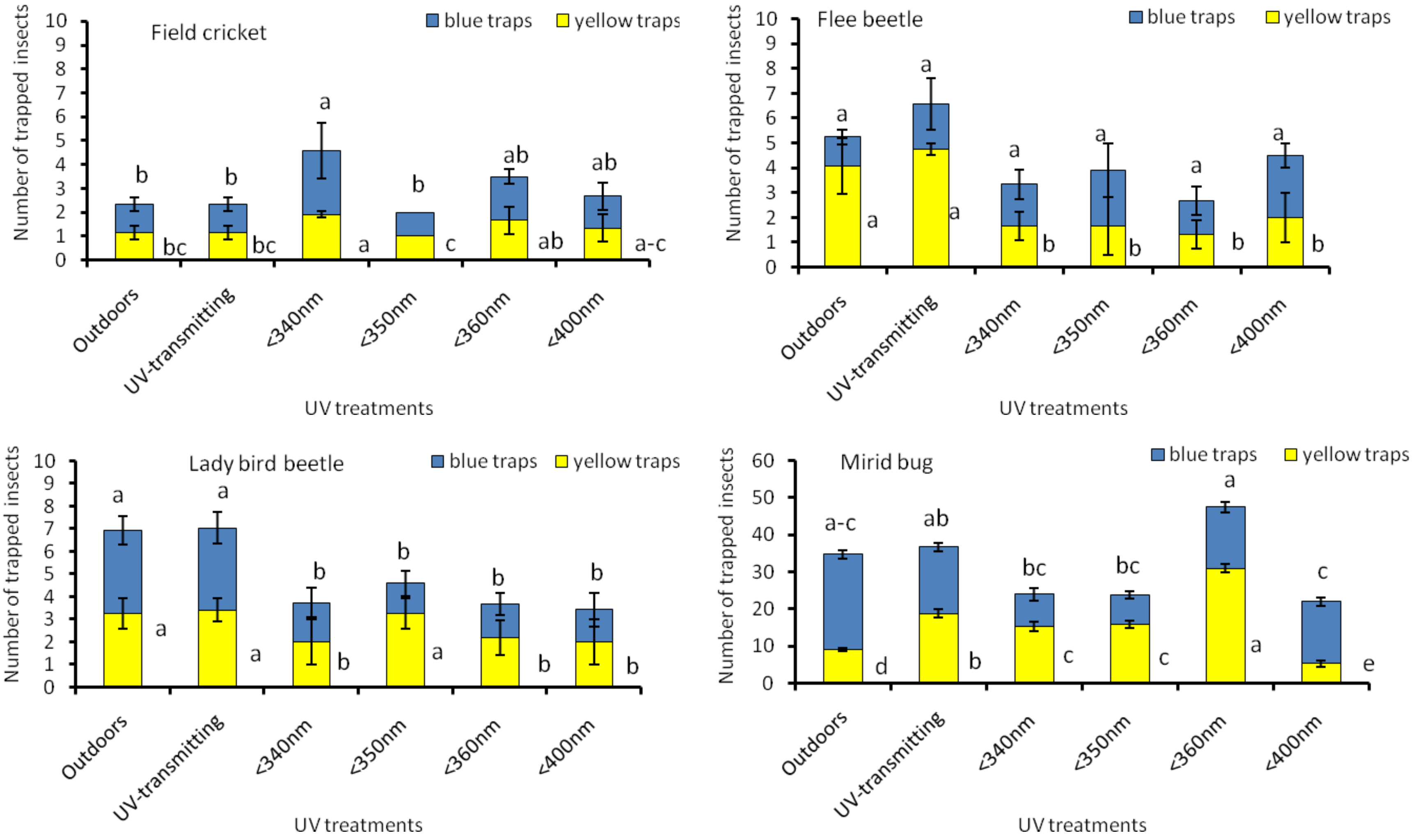


Figure 3-7 B. Mean numbers of individual insect pests that were trapped in adhesives (blue, yellow) inside and outside tunnels during the experiment. Blue colored bars indicate-blue traps and yellow colored bars indicate - yellow traps that were used in the experiment. Here, Different lowercase letters above the vertical error bars represent significant at $P \leq 0.05$.

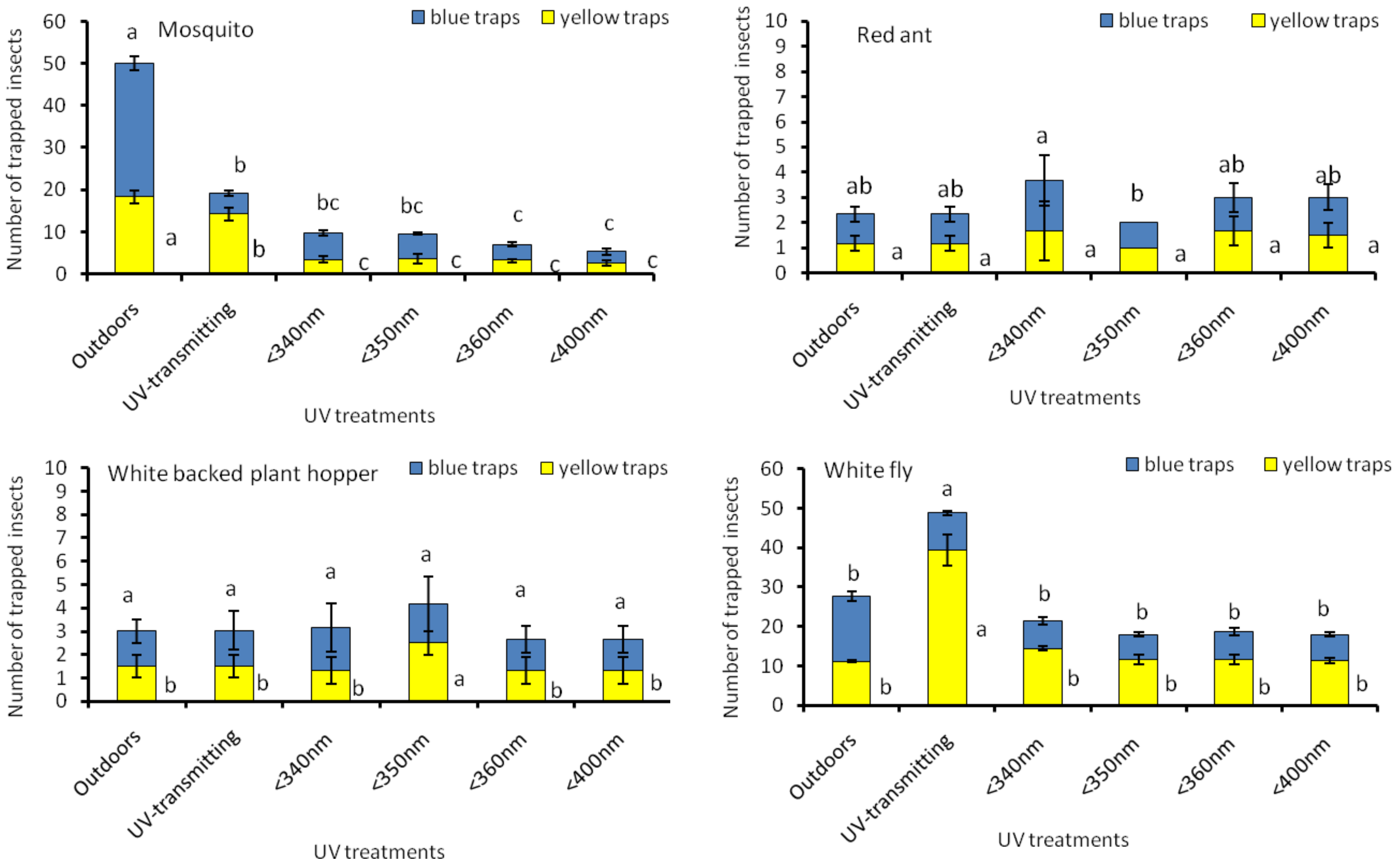


Figure 3-7 C. Mean numbers of individual insect pests that were trapped in adhesives (blue, yellow) inside and outside tunnels during the experiment. Blue colored bar-blue traps, yellow colored bar- yellow traps used. Here, Different lowercase letters above the vertical error bars represent significant at $P \leq 0.05$.

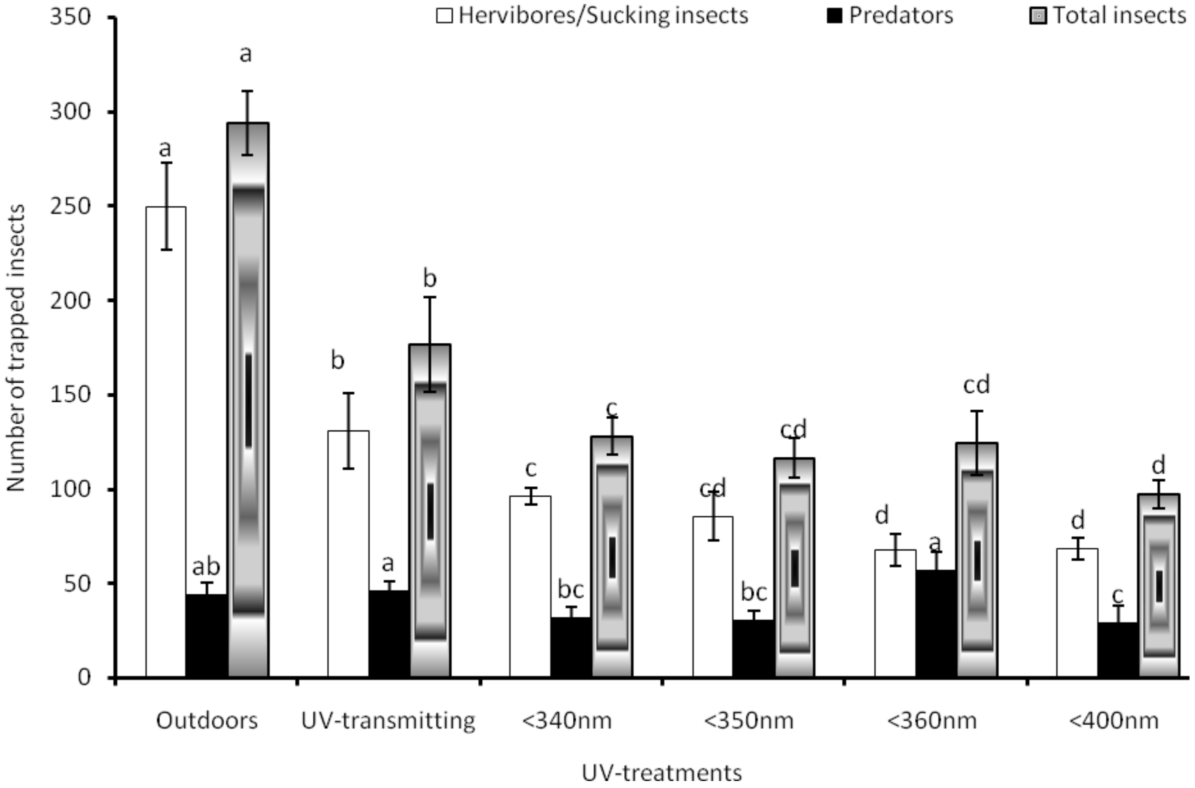


Figure 3-8. Mean values of herbivores/sucking insects and predators that were captured inside and outside tunnels during the experiments. Here, Predators- mirid bug, carabid beetle, lady bird beetle, red ant and herbivores/sucking insects- aphid, diteran fly, mosquito, brown plant hopper, white backed plant hopper, white fly, field cricket, flee beetle. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. Different lowercase letters above the vertical error bars represent significant at $P \leq 0.05$.

Chapter 4

Experiment-3

Effect of partially UV-blocking films on *Xanthomonas axonopoides* pv. *citri* causing citrus (*Citrus aurantifolia*) canker.

4.1 Abstract of the published paper

Citrus canker caused by *Xanthomonas axonopoides* pv *citri* is currently the most devastating disease impacting world citrus production. The proposed experiment was conducted to testify the hypothesis that partially UV-blocking film has the ability to suppress the development of canker disease of citrus. The quantified variables were latent period of the pathogen, temperature, relative humidity, partially UV-blocking conditions, disease incidence and disease severity. The mini houses were constructed and covered with four types of PO films that have the ability to block solar UV-irradiation shorter than UV-A: <400 nm, <360 nm, <350 nm, and <340 nm, and the results were compared with that of UV-transmitting and outdoors. The result showed that the leaves under <400 nm and <360 nm UV-blockings took less time (8 days) to express the symptom than outdoors (13 days). The lowest incidence (66.7%) was recorded in outdoors and highest incidence (100%) was recorded under <400 nm at 45 days after inoculation. Similarly, lowest severity (10%) was recorded in outdoors, and the highest severity (43.3%) was recorded under <400 nm at 45 days after inoculation. Disease incidence and severity gradually decreased as the UV blocking rates decreased. Solar radiation with contains combination of different UV light may be detrimental for multiplication of the bacteria *Xanthomonas axonopoides* pv *citri*.

4. 2 Introduction

Citrus (*Citrus aurantifolia*) belonging to the family Rutaceae is one of the most important nutritious fruit crops of the world as well as Bangladesh. In Bangladesh, the total acreage under citrus cultivation is about 5, 995 ha while the total production is around 136,756 mt (BBS, 2012). Various factors are responsible for lower citrus production in Bangladesh. Among them, plant disease is one of the major influential factors. Different species of citrus grown in the world suffers from more than 100 diseases (Klotz, 1973). In Bangladesh, twelve diseases are known to occur in different species of citrus where citrus canker is considered as most important disease.

The export of citrus from Bangladesh is seriously hampered due to this disease. Citrus canker is distributed over thirty countries of the world (Das, 2003). This disease caused by *Xanthomonus axonopodis* pv. *citri*, which is a rod-shaped gram-negative bacterium (Graham et al., 2004). Plants infected with citrus canker have characteristic lesions on leaves, stems, and fruit with raised, brown, water-soaked margins, usually with a yellow halo or ring effect around the lesion. Older lesions have a corky appearance, still in many cases retaining the halo effect (Gottwald, 2002). Temperatures between 15 to 20 °C and 35 to 40 °C are conducive for infection and development of citrus canker disease, respectively (Pria et al., 2006). Relative humidity between 75 to 85 % is also favorable for infection and development of this disease (Rashid et al., 2014).

In most of the countries of the world, farmers commonly use chemical control measures like spraying copper compounds for controlling citrus canker (Kuhara, 1978; Stall et al., 1982; Leite and Mohan, 1990; Das, 2003; Graham and Leite, 2004). However, continuous use of copper compounds leads to soil contamination (Kollar, 1998), as well as to the emergence of copper-tolerant phytobacterial strains (Louws, 2001), which in turn results in reduced the efficiency of copper bactericides. It is also mentioned in different literature that the major drawback of using chemical control measures is that phytopathogenic bacteria frequently develop a resistance to those compounds (Sigee, 1993)

Widespread appearance of copper-tolerant *X. axonopodis* pv. *citris* trains and subsequent loss of disease control have already been reported in different part of the world (Canteros, 2002; Canteros, 2004). Furthermore, mixing maneb or mancozeb fungicides with copper bactericides (copper mancozeb) increases the bactericidal efficacy (Louws, 2001; Canteros, 2002; Canteros, 2004); however, full control of canker is not possible to attain, if weather conditions favor disease development.

Management practice of this devastating disease is still limited. However, chemical compounds are very much hazardous for human health and for the environment and moreover, hazardous chemicals are not allowed for organic and safe food production systems (Hossain et al., 2013). Therefore, public concern is focused on alternative methods for pest and disease control (Sutton, 1996). Ultraviolet radiation (UV-R) is an important factor for bacterial communities and may be an effective way to reduce this disease (Moran and Zepp, 2000). UV light is the component of solar radiation. UV-R can be divided into three spectral regions, viz. UV-C (100–280 nm), UV-B (280–320 nm), and UV-A (320–400 nm). On a photon basis, UV-A radiation contains less energy than UV-B radiation; however, the fraction of solar UV-R in the UV-A region (95%) is far greater than that in the UV-B region (5%). Thus, UV-A can potentially cause substantial biological damage (Frederick and Lubin, 1988; Moran et al., 1999).

The effects of UV-A radiation are considered to be mostly indirect, that is, mediated by reactive oxygen species (ROS) formed via photodynamic reactions involving intracellular or extracellular photo-sensitizers (Santos et al., 2013). These ROS can react with cellular constituents, most notably proteins and lipids, leading to altered membrane permeability and/or disruption of trans-membrane ion gradients that can eventually cause cell death. The growth of *Penecillium digitatum* was reduced in citrus fruit under UV irradiation (Arcas et al., 2000). UV-irradiation can contribute for the reduction of postharvest losses caused by citrus black spot and reduce the use or doses of fungicides on disease control (Maria et al., 2011). The effect of partially UV-blocking shorter than UV-A on citrus canker is still unknown. Therefore, the present study was undertaken to investigate the effect of UV-A on citrus canker disease.

4. 3 Materials and Methods

4.3.1 Experimental site

Effect of partially UV-blocking shorter than UV-A on citrus canker was observed during 10 August to 30 September in 2014. Laboratory experiments were conducted in Plant Disease Diagnostic Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh and field experiments were conducted in the Horticultural farm, Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka Bangladesh.

4.3.2 Isolation and identification of the causal organism

Leaves were collected from infected citrus (*Kagji lebu*; *C. aurantifolia*) field and surface sterilized by 0.1% $HgCl_2$ (Mercuric chloride) solution for 30 seconds and then thoroughly washed with distilled water thrice to remove any trace amounts of $HgCl_2$ (Behlau, 2007). The excess moisture was removed by placing these pieces in between two folds of sterilized blotter paper. Then the causal bacterium, *Xanthomonas axonopodis* pv. *citri* was isolated using the techniques described by Goszczynska and Serfontein (1998). Colonies of bacteria were purified on NA and SX media plate. The causal organism of citrus canker, *Xanthomonas axonopodis* pv. *citri* was identified by following gram's staining reaction (Gerhardt, 1981), potassium hydroxide (3% KOH) test (Suslow et al., 1982), starch hydrolysis test (Cowan, 1974), catalase test (Schaad, 1992) and oxidase test (Kovacs, 1956).

4.3.3 Pathogenicity test

A bacterial suspension (5 µl, OD: 0.5) containing 10^8 colony forming units per ml (CFU/ml) was inoculated into the lower surface of citrus leaf with a sterile syringe by injection method and observed for 15 days (Patel and Padhya, 1964) (Plate 1. C). Visual symptoms were recorded and examined. To confirm Koch's postulates, bacteria re-isolated from diseased leaves were streaked on NA plate and re-identified using the methods outlined by Lin et al. (2008).

4. 3.4 Treatments used in the experiment

Six mini houses were constructed and covered with four types of PO films that has the ability to block solar UV-irradiation shorter than UV- A: <400 nm (T1), <360 nm (T2), <350 nm(T3), and <340 nm (T4), and the results were compared with that of UV-transmitting (T5) and outdoors(T6).

4. 3.5 Preparation of UV mini house

Fifteen plastic mini houses of 1.8 m × 1.9 m × 60 cm (L×B×H) with different PO films (0.13-mm thickness) were prepared for experimentation (Figure 1-7, A, B). Mini houses were covered with different polyolefin films (Figure 1-7, B), which can block UV-radiations shorter than 400 nm (<400 nm), 360 nm (<360 nm), 350 nm (<350 nm), and 340 nm (<340 nm), respectively (PO film collected from Mitsubishi Plastics Agri Dream, Tokyo, Japan). Three mini houses were covered with UV-transmitting polyolefin film. A bamboo bench was used above three feet of the soil level for each tunnel to protect soil heat. The first 60 cm (33%) of the tunnel from the soil level remained open to control the heat of the tunnels and allow the invasion by insects (Figure 1-6). Earthen pots with citrus plants (one year old) were transferred in those mini houses and three plants were put under each tunnel. Three pots were placed in outdoors where no film was used.

4. 3.6 Cultivation of citrus seedling and inoculation with bacteria

Citrus seedling (1 year old) collected from Krishibid Nursery, Bangladesh were used for this assay. The seedlings were planted in 25 cm × 25 cm earthen pots (one plant.pot^{-1}). Sterile loamy soil and sand (2:1) were used as potting media. Seedlings were irrigated with tap water once a day. No additional nutrients were supplied during the study period. Seedlings were inoculated with bacterial suspension (5 μl, OD: 0.5) containing 10^8 colony forming units per ml (CFU.ml^{-1}) by injection method using 1 ml Dispo van single use syringe (Hindustan Syringe & Medical Devices Ltd.) after 15 days of transplantation. Five leaves of each plant were inoculated. Plants were covered with polythene bag after inoculation for 24 hours to maintain suitable moisture condition. Then the plants were transferred under the UV film to observe the post inoculation effect of UV-A on citrus canker.

4. 3.7 Measurement of environmental conditions

Temperature, humidity, visible and UV-light irradiations were measured in daily basis during the experiment. Both temperature and humidity were recorded at 8:00, 12:00 and 24:00, while visible and UV-light irradiations were measured at 12.00.

4. 3.8 Data recording

Data on disease incidence and severity were collected after 15, 30 and 45 days of inoculation (DAI). Assessment of disease incidence and severity was calculated using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased leaf among the inoculated leaf in each plant}}{\text{Number of total inoculated leaf in each plant}}$$

$$\text{Disease severity (\%)} = \frac{\text{Amount of disease in the inoculated leaf in each plant}}{\text{Amount of total disease in the inoculated leaf in each plant}}$$

4. 3.9 Experimental design

The experiment was laid out following randomized complete block design (RCBD) with three replications. Six treatments were used in the experiment including UV-transmitting and outdoors.

4. 3.10 Statistical analysis

Data were subjected to analysis of variance, and significant differences of the means among treatments were analyzed using MSTAT-C software (East Lansing, MI, USA).

4. 4. Results and Discussion

4. 4.1 Isolation and identification of the causal organism

The isolated bacteria identified as *Xanthomonas axonopodis* pv. *citri* according to morphological, biochemical characters of the bacterium as per standard microbiological procedures (Figure 4-4). Typical, yellow, convex, mucoid, colonies of *Xanthomonas axonopodis* pv. *citri* was found on NA plates after 48 hours of incubation at 30±1 ºC (Figure 4-4, A). Chand and Kishun (1991) reported that *Xanthomonas* produce mucoid, circular, convex, yellow, round, glistening and raised colonies on nutrient agar medium. The bacterium was rod shaped with rounded ends, cells appeared singly and also in pairs, gram negative (red colour) and capsulated under the compound microscope at 100 times magnification with oil immersion. It produced a mucoid thread when lifted with the loop (Figure 4-4, D), showed catalase activity, bubbles were formed after adding 3% H_2O_2 (Figure 4-4, B), formed dark purple colour on oxidase disk (Figure 4-4, E), gelatin was liquefied (Figure 4-4, F), starch was hydrolyzed (Figure 4-4, C). The findings are summarized in Table 4-1.

4. 4.2 Symptom expression

After inoculation of bacteria the plants were periodically observed for symptom expression. The latent period (time between inoculation and symptom expression) of the bacteria varied from treatment to treatment. The symptoms of citrus canker disease were first expressed eight days after of inoculation in treatments T1 and T2. Both the treatments T3 and T5 required ten days to produce symptoms. The plants under the film of UV transmitting and outdoor treatments needed thirteen days to express symptoms (Figure 4-1). Initially the symptoms were looked like water soaked small yellow in color which in later became corky appearance with broad yellow hallow zone (Figure 4-3 A & B).

4. 4.3 Incidence and severity of the organism under different treatments

Disease incidence and severity of citrus canker were observed at different days after inoculation under different treatments (Table 4-2; Figure 4-2 and 4-3). Incidence of canker of citrus varied significantly under different UV intensities ranged from 58.3 to 86 % at 15 and 60 DAI (Table 4-2). The highest incidence (100 %) was recorded in treatment T1 at 45 DAI and lowest incidence (58.3%) was recorded in treatment T6 (outdoor) at 15 DAI. Statistically similar incidence (66.7, 83.3 and 83.3 %) at 15, 45 and 60 DAI was recorded in T3 (<350 nm) and T4 (<340 nm). Statistical significant differences were recorded among the other treatments.

Severity of citrus canker also varied significantly under different UV intensities varied from 1.8 to 43.3% at 15 and 60 DAI (Table 4-2). The highest severity (43.3%) was recorded in treatment T1 (<400 nm) at 45 DAI and lowest incidence (1.8%) was recorded in treatment T6 (outdoor) at 15 DAI. Statistically similar severity (3.2, 9.3 and 13.8 %) at 15, 45 and 60 DAI was recorded in treatment T3 (<350 nm) and T4 (<340 nm).

4. 4. 4 Effect of environmental conditions under different treatments

Temperature, relative humidity and UV intensity varied significantly under different treatments. Statistically similar temperatures, 33.9, 33.6, 32.7 and 32.7 °C were found in treatment T1 (<400 nm), T2 (<360nm) and T3 (<350 nm), T4 (<340 nm), respectively. Statistical significant difference was found in treatment T5 (UV transmitting) and T6 (outdoor). Statistically similar humidity (82.70, 82.30, 80.50 and 79.90 %) were found in treatment T_1 (<400 nm), T2 (<360 nm) and T4 (<340 nm), T5 (UV-transmitting). However, statistical significant difference were found in treatment T3 (<350 nm) and T6 (outdoor). Statistical significant difference in UV intensity was recorded among all the treatments (Table 4-3).

4.4.5 Effect of temperature, relative humidity and UV intensity (W-cm^{-2}) on disease incidence and severity of citrus canker

Correlation coefficient and regression equation were calculated to find out the Effect of temperature, relative humidity and UV intensity (W. cm^{-2}) on disease incidence and severity of citrus canker (Table 4-4). Plants treated with different wavelengths of UV radiation showed gradually decreasing disease incidence and severity with decreased temperature and relative humidity. Negative correlations coefficient (r^2) = -3.35, -16.6 and -11.4 were found among temperature, humidity, UV intensity and disease incidence (Figure 4-2 A, C and E). Again negative correlations coefficient (r^2) = -0.97, -1.08 and -0.42 were found among temperature, humidity, UV intensity and disease severity (Figure 4-2 B, D and F).

Citrus production and export is being threatened in Bangladesh due to devastating outbreak of citrus canker disease. Still now no effective chemical control measure is available for this disease. Nowadays chemical control is discouraging due to its residual effects and environmental hazardousness. Solar radiations that contain different UV radiation (UV-A, UV-B) have profound effect on different microorganisms (Perez and Sommaruga, 2007; Paul et al., 2011; Moran and Zepp, 2000; Sundin and Jacobs, 1999; Coohill and Sagripanti, 2008). This study focused on the effect of UV-A on citrus canker disease development. The experiment revealed that the *Xanthomonas* bacterium is sensitive to UV light. Disease incidence and severity were higher in treatment T1 (<400 nm film) where only visible light were passed and all kinds of UV radiation were blocked by the film. On the other hand lowest disease incidence and severity were measured in treatment outdoor in which no UV protecting film was used and plants got the combined solar radiation (UV-A+UV-B). As different wavelengths of UV-A was blocked gradually by the treatment T1 to T4 resulted gradual decrease of disease incidence and severity.

The result also revealed that outdoor and UV transmitting treatments required higher time to express symptom after inoculation which gradually decreased to treatment T1. This finding indicates the multiplication rate of the bacteria in host plant may be interrupted by UV light. The multiplication rate of bacteria may be increased when the bacteria is not imposed under UV light and vice versa. UV-A may cause indirect damage by producing ROS at cellular level and UV-B may cause direct damage by breaking DNA of bacteria while the UV- C may cause both direct and indirect damage of bacteria. The lower incidence and severity of disease in T6 and T5 treatment may be due to the combined effect of this three UV radiation that may slow down the multiplication rate of bacteria.

From the result, it can be stated that treatments T2, T3 and T4 have lower incidence and severity than T1 but higher incidence and severity than T6. That indicates UV-A may have less influence on the multiplication rate of *Xanthomonas* than outdoor which got full solar radiation but may have higher influence than visible light. Lower incidence and severity in outdoor indicates the lower multiplication rate of bacteria inside the plant tissue. Outdoor treatment got full solar radiation that contained different wavelength of UV-A and UV-B. The combined effect of this UV light may reduce the multiplication rate of bacteria in outdoor treatment. Similarly, the T2, T3 and T4 treatments got only different wavelength of UV-A which may be the reason of lower influence on the multiplication rate of *Xanthomonas*. UV-A may have higher effectiveness than visible light against *Xanthomonas* but when it is combined with UV-B, the effectiveness may increase which may be the cause of lower incidence and severity.

The study also confirms that temperature and relative humidity in combined with UV intensity may have potential role in disease development. In treatment T1 both the temperature and relative humidity were higher than outdoor treatment that may have positive role for disease development. Moreover, in treatment T2 and T1 both the temperature and humidity were non significant but UV intensity, disease incidence and severity were statistically significant. These findings indicate that, higher UV intensity has potential role to reduce disease incidence and severity of citrus canker. Furthermore, temperature and humidity showed positive correlation and UV intensity showed negative correlation with disease incidence and severity. So it can be concluded that disease incidence and severity increase with high temperature, humidity and low UV intensity and vice-versa. From the findings of this study it may be suggested that tropical and sub-tropical regions are comparatively more suitable to cultivate citrus against the canker disease due to having more UV radiation. However, further similar investigations are needed for UV-B and UV-C to find out their individual effects against the disease.

Table 4-1. Biochemical tests to identify *Xanthomonas axonopodis* pv. *citri*

Name of tests	Reaction
Gram Staining	-
KOH solubility test	+
Starch hydrolysis test	+
Catalase test	-
Oxidase test	+
Motility indole urease agar (MIU) test	+
Gelatine liquefaction test	+
Tobacco hypersensitivity test	+

- = yes, + = No

Table 4-2. Incidence and Severity of citrus canker under different UV intensity.

Treatment	Disease incidence (%)			Disease severity (%)		
	15 DAI	30 DAI	45 DAI	15 DAI	30 DAI	45 DAI
<400 nm	91.7 a	100.0 a	100.0 a	4.1 a	16.7 a	43.3 a
<360nm	83.3 ab	91.7 ab	91.7 ab	3.5 a	11.5 b	18.3 b
<350 nm	66.7 ab	83.3 abc	83.3 abc	3.2 ab	9.6 bc	14.2 bc
<340 nm	66.7 ab	83.3 abc	83.3 abc	3.2 ab	9.3 bc	13.8 bc
UV-transmitting	58.3 b	75.0 bc	75.0 bc	2.1 bc	8.1 c	10.8 c
Outdoor	58.3 b	66.7 c	66.7 c	1.8 c	7.0 c	10.0 c
Significance	*	*	*	**	**	**

DAI- Days after inoculation; The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. ns-non significant, *- significant at $P \leq 0.05$, **- significant at $P \leq 0.01$. Different lowercase letters beside the mean value indicate significant at $P \leq 0.05$ or 0.01.

Table 4-3. Effect of Partially UV-blocking films on temperature, relative humidity (RH%) and UV intensity during the experiment.

UV Treatment	Temperature (^{0}C)			Relative humidity (RH %)			Visible light intensity (W.cm^{-2})		UV intensity (mW-cm^{-2})	
	8:00	12:00	24:00	8:00	12:00	24:00		% of outdoors		% of outdoors
<400 nm	24.7 a	30.8 d	22.4 a	71.8 a	79.2 d	81.5 a	20.1 c	78.3	76.8 f	8.2
<360nm	24.3 a	31.9 c	21.9 a	70.7 a	79.9 c	80.3 a	20.3 bc	79.2	101.3 e	10.8
<350 nm	23.8 a	32.7 b	22.3 a	71.7 a	80.5 c	80.3 b	18.8 d	73.3	128.5 d	13.7
<340 nm	23.3 a	32.7 b	21.7 a	70.5 a	81.3 b	80.5 c	20.5 bc	80.0	151.9 c	16.3
UV-transmitting	24.1 a	33.6 a	22.1 a	71.2 a	82.3 a	79.9 c	21.6 b	84.2	340.5 b	36.4
Outdoor	23.9 a	33.9 a	21.9 a	70.1 a	82.7 a	79.2 d	25.3 a	100	935.1 a	100
Significance	ns	**	ns	**	**	**	**		**	

The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. ns- non significant, * - significant at $P \le 0.05$, **- significant at $P \le 0.01$. Different lowercase letters beside the mean value indicate significant at $P \le 0.05$ or 0.01.

Table 4-4. Linear correlation analysis on the effect of temperature, relative humidity and UV intensity on the incidence and severity of citrus canker.

Climatic factors	Slope (b)		Correlation co-efficient (r^2)		Probability (p)		Intercept	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Temperature	0.09	0.06	0.98	0.72	0.001	0.08	24.70	31.39
Relative humidity	0.11	0.08	0.97	0.77	0.007	0.05	71.58	79.43
UV	-28.26	-14.52	-0.81	-0.44	0.03	0.36	2677.97	589.58

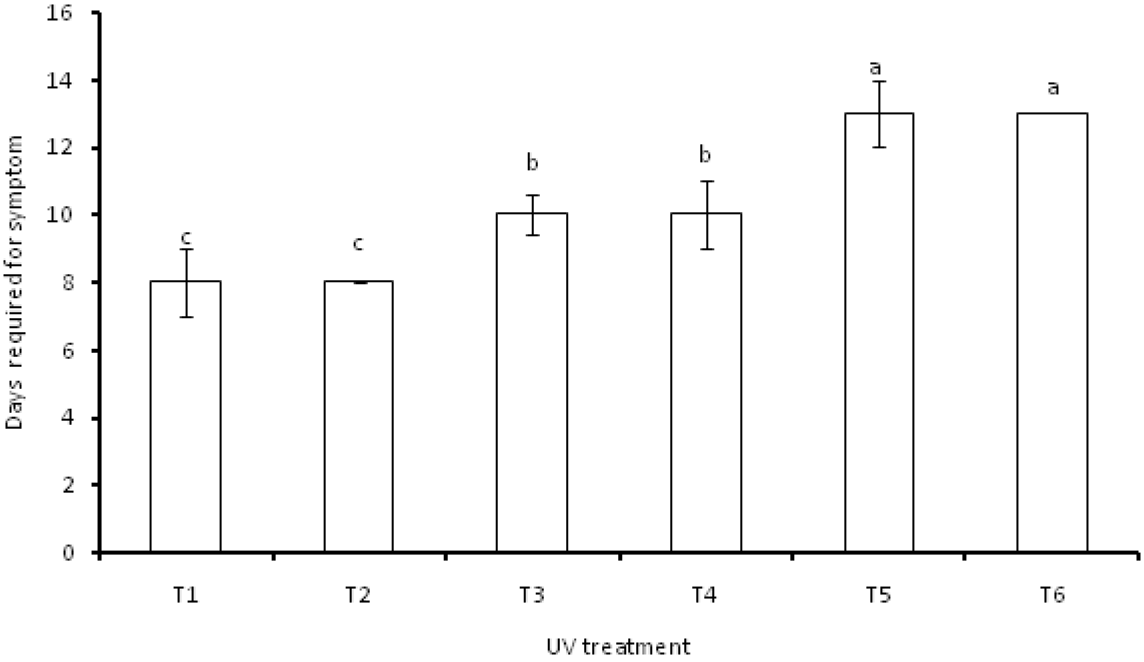


Figure 4-1. Days required for symptom expression in citrus plant after inoculation.

T1- <400 nm, T2- <360 nm, T3- <350 nm, T4- <340 nm, T5- UV-transmitting, T6-Outdoors. The ANOVA for each of the characters was performed by *F*-test and data were analyzed using MSTAT-C software. Different lowercase letters above the vertical bars indicate significance at $P \leq 0.05$.

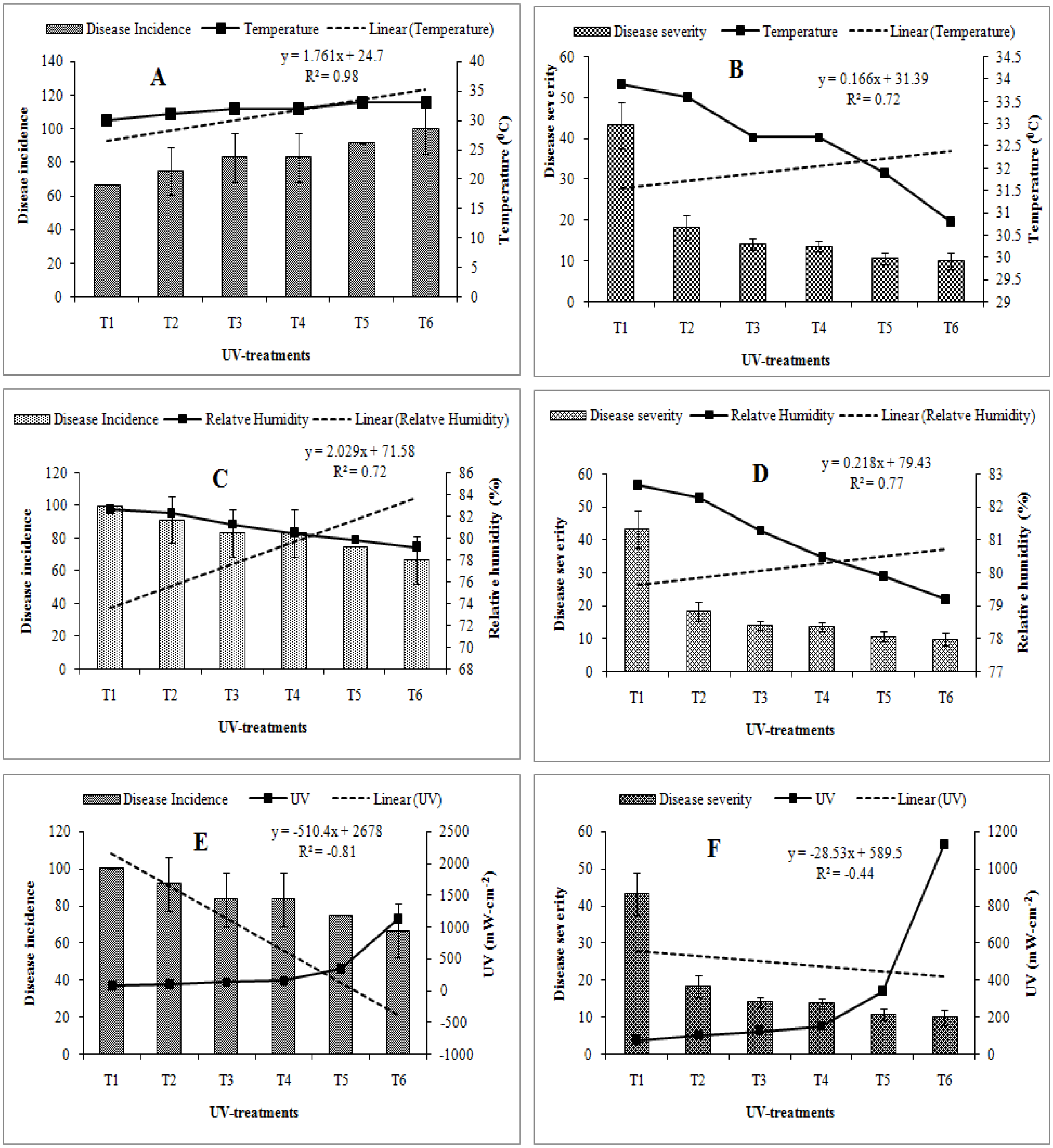


Figure 4-2. Correlation of temperature, relative humidity and UV intensity with disease incidence and severity. A-correlation between temperature and incidence; B- correlation between temperature and severity; C- correlation between humidity and incidence; D-correlation between humidity and severity; E- correlation between UV intensity and incidence; and F-correlation between UV intensity and severity. T1- <400 nm, T2 - <360 nm, T3 - <350 nm, T4 - <340 nm, T5 - UV-transmitting, T6 - Outdoors.

Photo: AHM Solaiman, 2014.

Figure 4-3. Series of figures representing proposed research study on bamboo shelf (A-D).

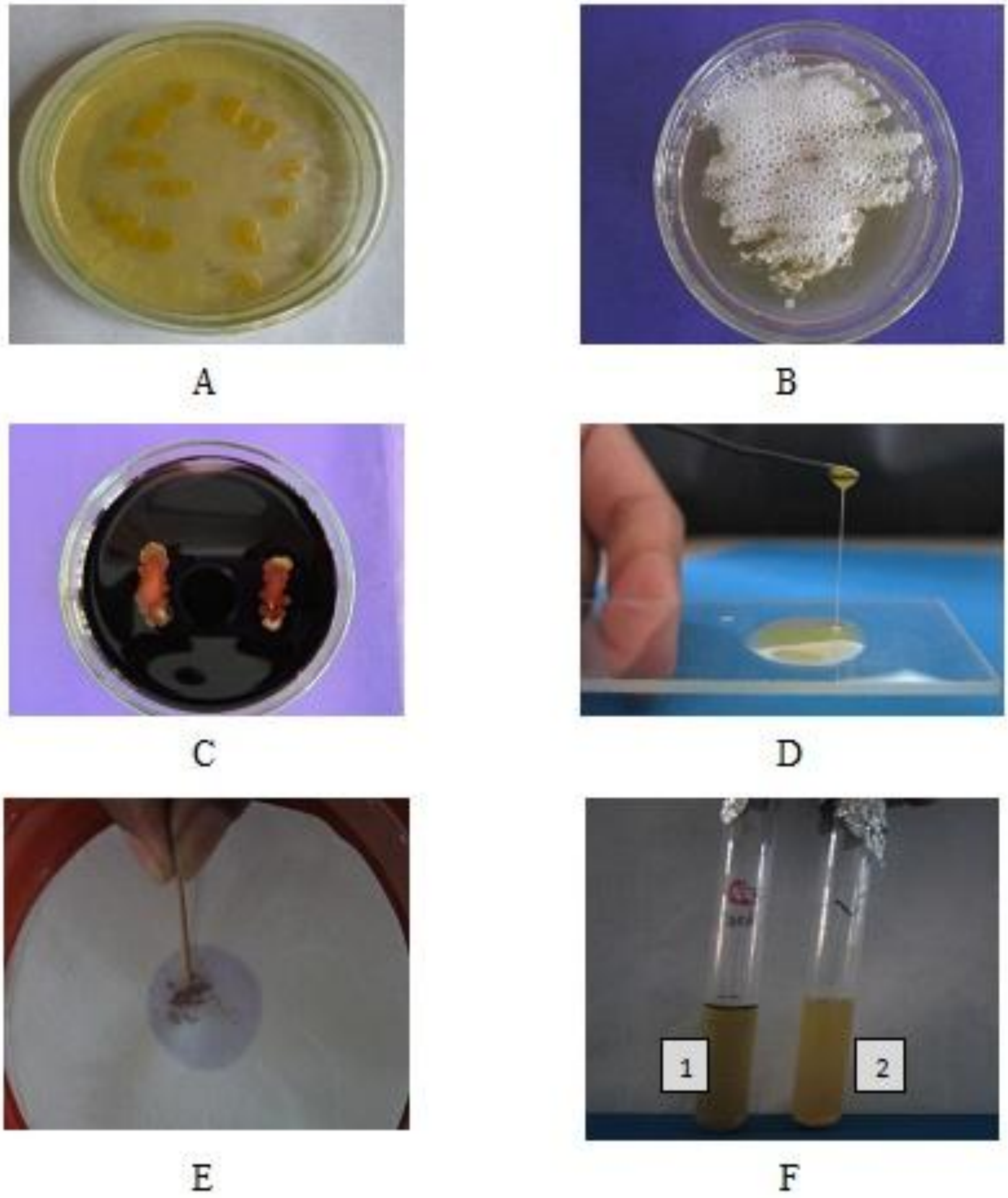


Photo: AHM Solaiman, 2014.

Figure 4-4. Biochemical test of *Xanthomonus.* A-colony of bacteria; B- Catalase test; C-Starch hydrolysis; D - KOH test; E- Oxidise test; and F-Gelatin liquefaction test (1-positive, 2-negative) (A-F).

Chapter 5

General Discussion

UV-blocking plastic materials are a very effective tool to reduce the incidence of pests and diseases, especially under organic and integrated production. But due to the fact that all the strategies to be developed are based on films or nets made out of polyethylene, it is important to have a technology for the correct disposal of such material to avoid its accumulation in the environment. In this way, biodegradable and photodegradable plastics will become more important in the near future. On the other hand, more studies must be carried out for a better understanding of the effects of partially UV-blocking materials on plant diseases, especially on several physiological aspects of fungal pathogens infecting vegetable crops, their effects on fungal antagonists and their influence on disease development. Also, more studies are needed to understand the biological effects of different solar wave-length radiation on microbial and insect ecology, especially on plant pathogenic bacteria.

Three experiments with partially UV-blocking PO films was conducted in Sher-e-Bangla Agricultural University, Dhaka Bangladesh in order to assess the importance of using those films in two winter vegetable crops and in one fruit crop for controlling the canker disease caused by *Xanthomonas axonopoides* pv. *citri.* The weather conditions and light intensity and UV-irradiations were measured daily at 8:00, 12:00, 20:00 and 24:00, while visible- and UV-light irradiations were measured at 12:00 pm.

In Experiment-1 (described in Chapter-2) the tunnels were placed with partially UV-blocking films after the germination of the seedlings (after 7 days) of broccoli and turnip. After 30 DAS, the seedlings were harvested and measured the plant growth and insect-predators invasion inside the tunnels. The plant growth of both turnip and broccoli increased by 20-30% in the <400 nm tunnels than in outdoors, as well as in UV-transmitting tunnels (Table 2-2). It is found that partially UV-blocking also promoted the growth significantly, which might be prescribed for the farmers to use partially UV-blocking films rather than fully UV-blocking films. In the contrary to our results, <380 nm showed the minimum growth or no remarkable difference for some flowers (*Lisianthus, Solidago, Chrysanthemum*) showed by Costa et al., 2002. In a study with cucumber and tomato seedlings, partially UV-A blocking films did not affect the plant growth (Nishizawa et al., 2012). Therefore, effect of partial UV blocking on plant growth would be highly species-specific as shown in *Sinapis alba* and *Nasturtium officinale* (Reifenrath et al., 2007). For broccoli seedlings, maximum dry matter (%) was recorded inside the tunnels of <340 nm but for turnip it was highest in outdoor plants (Table 2-2). The accumulation of dry matter increased with continuous growing of plants under UV-blocking films which was also in the agreement with Tsormpatsidis et al. (2010). In the case of broccoli seedlings, total number of insects were trapped maximum in outdoors and the trapped insects' number were almost similar in respect to partial UV-blocking (Figure 2-1). But in the case of turnip, trapped insect number decreased with increasing UV-blocking and for yellow and blue traps, insect trapping was affected same as the total number (Figure 2-1). From the figure, it is suggested that partial UV-blocking films could be effective for trapping insects equally than UV-blocking films.

In Experiment-1, it is stated that during the 30 days of cultivation under different UV-blocking conditions, leaves of some seedlings showed feeding damages by herbivores/sucking insects. The feeding damage by insects was significantly influenced by the partial UV-blocking films for both vegetables (Figure 2-2). The 5- 10% plants were attacked inside the tunnels where partial UV-blockings were applied rather than outdoors and UV-transmitting. So, it is evident from the experiment that less number of insects invaded inside the tunnels which could be an effective tool for minimizing the attack of plants rather than fully UV-blocking. Besides, it can be said that if the number of insects maximizes, it does not mean that the area of fed would be the maximum (Figure 2- 3). It is obvious that there must have some beneficial insects which were also trapped by the adhesives inside or outside tunnels. This type of variations may hamper the balance of the ecology and in that case some predators will be killed and the insect pest invasion will be maximized. Broccoli seedlings were attacked more than turnip seedlings inside the tunnels where both of them were seeded in the same time. Though, the fed area or the feeding damage may be done by other creeping or walking insects. It is known that the fed area can be influenced by both the seedling density and the leaf area; maybe that's why insects attacked mostly in broccoli seedlings (Nishizawa et al., 2012).

In the Experiment-2, Different types of insects like mirid bug, aphid, BPH, DF, mosquito, SHGH, WBPH. The other insects were counted that were trapped by blue and/or yellow adhesive films which were categorized as miscellaneous insects that were failed to classify or found only in few numbers.

Furthermore, in the tunnels of both seedlings, 10-20% predators or parasitoids were trapped and significantly controlled the herbivores with the use of partially UV-blocking films (Figure 2-4). The proportion of predators were also counted that are also important in environmental balance and also for control the pest or herbivores/sucking insects by the predators or parasitoids that might be an influential agent for bio-control of herbivores/sucking insects. In the experiment-1, it is observed that significant number of beneficial insects have been trapped inside tunnels and or in outdoors (Figure 2-4). In turnip seedlings, about one fourth predators of the total insect were trapped inside tunnels and on the other hand, maximum predators were trapped in broccoli seedlings (Figure 2-4). This proportion should be considered whether the blue or yellow adhesives were used for catching insect-pest as a tool for control. In the experiment-1, partial UV-blocking captured almost same as the fully blocked UV-films rather than outdoors. In that case, it would be much wiser and commercially acceptable to use partially UV-blocking films than fully UV-blocking films to control insect pests (Nishizawa et al., 2012).

It is already known that sticky traps captured only the winged and sometimes walking insects, but insect pest counted on plants included both adult and immature. However, a lot of other factors could contribute differences and plant height could reduce the invasion density of insects inside the tunnels. Aphid prefers the color of background mainly by the contrast of green (plant) and soil as background to land (Doring and Chittka, 2007). It is evident that different secondary compounds of the plants influence members of the next trophic level by affecting host plant, feeding damages and their performance in different degree (McCloud and Berenbaum, 1999; Lindroth et al., 2000; Warren et al., 2002). In addition the duration of plant growth was only 30 days and the invasion numbers could vary with the duration of the experiment. Scientists showed that UV-blocking materials have properties to filter the UV radiation (280-400 nm) interfering with the vision of insects and in consequence, their behavior related with movement, host location ability and their population parameters (Diaz and Fereres, 2007). They also stated that the invaded insects into the protected crops must recognize and locate their host plants. As a result, insects begin the second phase of the process of host plant infestation, which is primary infestation (Figure 1-1).

The insects such as, dipteran fly and aphids showed significant invasion inside the tunnel and WBPH showed remarkable presence only in YSTs though BSTs showed non-significant effect (Figure 2- 7). The dipteran fly found in the outdoors was three times higher than that found in the tunnels, and it was gradually decreased as the UV-blocking rate increased (50, 33, 17 and 17%, respectively). Aphid invasion was 50% in UV-transmitting film than outdoors and it was decreased as 28, 13, 18, and 20% with the increasing rate of UV-blocking (Figure 2-8).

The number of insects' invasion was not too many might be due to the smaller size of the tunnels and smaller opening of the tunnels for invasion. The trap counts were also correlated with the densities with tomato and cucumber seedlings (Gillespie and Quiring, 1987; Kim et al., 1999; Nishizawa et al., 2012) that supported sticky traps as an influential tool for estimating population densities.

Only a few studies were performed in outdoor condition using specific UV-blocking PO films to expose plants to natural irradiation including or lacking the UV-irradiation of the spectrum (Turunen et al., 1999; Kolb et al., 2001; Caputo et al., 2006). Described above primary studies allow to raise questions on the effect of specific UV- blocking films on growth and insect control of leafy vegetables i.e., proposed red amaranth, that have not been widely discussed in the world literature so far. Here, the effect of specific UV radiation on the growth, coloration, TSS (°brix), anthocyanins, and insect control of red amaranth under outdoor field conditions was described in Experiment-2, which might be effective for commercial horticulture production. The goal of Experiment-2 was to study effects of ambient visible and UV radiation on the growth pigmentation and insect-pest control taking into account the fresh young leaves of red amaranth. As young leaves are more valuable for the plant than old leaves, young leaves should build up protection mechanisms against harmful radiation with priority. Therefore, a stronger response was expected in young leaves than in old. Naturally the leafy vegetables especially red amaranth is attacked by different kind of insects; they damage the fresh leaves in the field that also reduce the price in the market.

In the Experiment-2 (described in Chapter-3), the crop red amaranth cultivar BARI Lal shak-1 was cultivated during November to December, 2013 at the experimental field of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. Application of fertilizers and irrigation, and other operations were followed by FRG (2012). Same tunnels were used as broccoli and turnip. In the beds, A 15-cm (20 %) opening from the soil level for protecting the heating of the tunnels and allowing the invasion of insects. The crops were harvested destructively and examined the growth. Shaded or yellowish leaves were avoided because only fresh red colored plants are acceptable for selling in most of the leafy crops (Rashid, 1999). At 30 DAS (harvesting stage), growth parameters, total soluble solids and anthocyanins were measured. In addition, the chlorophyll was also observed just before harvesting and leaf and stem colours (L*, a*, b*, and chroma) were recorded according to Kittas et al. (2006). Affected plants are not sold in good price or very low price in Bangladesh market, so the total leaf area damaged by herbivores/ sucking insects were also monitored to determine the rate of damage. The affected leaves were measured after uprooting them from the bed at 30 DAS.

The longest red amaranth plant was recorded inside the tunnels that were covered with <400 nm wavelength PO film and the lowest was in the plots outdoor (Table 3-2). On the other hand, highest mean number of leaves (11) were recorded from the UV-transmitting tunnels at harvest but in <400 nm showed non-significant effect on leaf number. The maximum leaf area (119.1 cm^2) was found in the tunnels covered with <400 nm film than in outdoor (59.7 cm^2) which was almost doubled (Table 3-2). The stem diameter (at 5 cm) was maximum (1.2 cm) in the plants grown in the tunnels with <400 nm than in outdoor (0.65 cm) and the diameter showed decreasing trend with decreasing of specific UV-blocking (Table 3-2). Furthermore, the whole red amaranth plants weight (FW) gave 200 g more yield in <400 nm than in outdoors (Table 3-2) and biomass production was maximum in the crops that were grown under partially UV-blocking films and the lowest was also inside the tunnels with UV-blocking (Table 3-2). In case of TSS, outdoor plants gave the highest (6 and 3.5 °brix in leaf and stem, respectively) and the lowest was found in tunnels with <350 nm wavelength and the mean value of chlorophyll reading in alternate leaf was found significant ($P \leq 0.01$) at harvesting stage of 30 DAS (one day before harvest) (Table 3-2). The leaf color of the plants that were grown outdoors was fully red, but in the plants grown in tunnels with UV-blocking PO films became a little lighter (Figure 3-1). However, such a difference in red color among the treatments was not clearly shown in a* values, especially in the stem and lower leaves (Table 3-3). This might be occurred because of the effect of mutual shading, since the stem color in our experiment was measured at the mid-point which was subjected to higher levels of mutual shading as well as lower leaves.

Thin stem bark sections were examined under a microscope fitted with digital camera (Motic images plus 2.0) and attached with a computer for observing the cell number or size, whether the plant height is influenced by number or size of the cell. The cell number showed significant differences among the treatments and the highest plants were found in <400 nm tunnels. UV-blocking films showed comparatively bigger cell size but lowest in number of cells (Figure 3.2), while outdoor plants had smaller size but maximum number of cell (Table 3-2 and Figure 3-3).

The highest rate of the damaged plant (29%) was recorded in the outdoors followed by UV-transmitting (22%) and <340 nm (20%), while the damaged plants under the other partial UV-blocking films were less than 10% (Figure 3-3). The maximum fed area (higher than 2%) was also found in the outdoors and decreased with increasing UV-blocking rates, reaching lower than 1% at <360 and <400 nm (Figure 3-4).

It is also important to identify the types of insect that are generally responsible for attacking red amaranth in Bangladesh to promote an IPM program. In the experiment-2, the cumulative number of trapped insects was almost 300 in outdoors, while it decreased to almost one third of that of outdoors under the partially UV-blocking conditions (Figure 4-5). In addition, YSTs trapped more insects than BSTs, except for at <400 nm.

During the experiment, twelve types of insect were trapped. Among them, aphid, dipteran fly (DF), brown plant hopper (BPH), mirid bug (MB), mosquito, and white fly (WF) were the major insects, while ladybird beetle (LB), red ant (RA), field cricket (FC), white backed plant hopper (WBPH), carabid beetle (CB), and flee beetle (FB) were minor insects (Figure 4-6). Among the treatments, aphid, DF, and mosquito were captured the most frequently outdoors, and an apparent repellent effect was found between the outdoors and plastic films rather than between UV-transmitting and UV-blocking conditions.

On the other hand, LB, FB, and WF were captured the most under UV-transmitting conditions, and an apparent repellent effect was found between UV-transmitting and UV-blocking conditions. The remaining insects were found the most frequently under partially UV-blocking conditions, but there was no clear trend between the insect population and UV-blocking rates. Among partially UV-blocking conditions, numbers of aphids, DF, LB, mosquitoes and WF decreased as the UV-blocking rate increased, but the repellent effect among the partially UV-blocking conditions was not apparent compared to outdoors and plastic films or UV-transmitting and UV-blocking conditions.

Fully UV- or partially UV-blocking conditions often significantly reduce the number of aphids (Nishizawa et al., 2012), thrips and whitefly (Doukas and Payne, 2007) or aphid, thrips and diptera (Chiel et al., 2006) compared with UV-transmitting conditions, as shown by the results of the experiment-2. However, the effect of UV-blocking films as a physical barrier to control insect pests does not necessarily produce the same result. Rather, the effect widely changes depending on the conditions, such as the season and site of the experiment (Chiel et al., 2006; Doukas and Payne, 2007; Delia et al., 2013) and amount of UV-radiation in the test area (Nakagaki et al., 1982). Therefore, it is important to understand the environmental characteristics in the test area when fully- or partially UV-blocking films are to be commercially utilized. It was also needed to consider that some insects do not live on plants but in the soil, in many situations in the traps are also trapped insects that come from outside of the greenhouses.

In addition, an ecological balance of insects closely associated with their colonization and reproduction will also affect the number of trapped insects, because herbivores/sucking insects and predators invade the tunnels. Therefore, total numbers of the captured herbivores/sucking insects and predators were recounted (Figure 3-7). The results showed that the number of herbivores decreased gradually as the UV-blocking rate increased, while that of predators was little influenced by the partially UV-blocking conditions. Therefore, partially UV-blocking films will be advantageous for IPM from the aspect of maintaining a favorable balance between herbivores/sucking insects and predators. Many microbes, plants and animals use UV-B and UV-A radiation as a source of information about their environment affecting many ecological processes (Paul and Gwynn-Jones, 2003). Long exposure to UV radiation may damage plants and other living organisms because the UV photons have enough energy to destroy chemical bounds causing photochemical reaction, that induces structural and biochemical changes (Kovács and Keresztes, 2002; Jacobs et al., 2007). A large number of experiments where solar UV-B has been attenuated using wavelengths-selective filters, such as polyester, show a range of significant responses across many plant species and locations (Paul et al., 2005).

Again, it is important to note that the UV-blocking materials may have a direct or indirect effect on plant pathogenic bacteria, affecting the degree of susceptibility of plants to these organisms. The situation may be complex because there is a series of factors that may interfere with the effect of light on microbial development. At present, few works on the effects of UV-blocking films on natural enemies of insect vectors have been con-ducted. Future works should be focused on the effects of UV-light on the behavior of commercial parasitoids and predators. The effects of UV-blocking materials on predators and parasitoids have never been well established. In the same way, the compatibility of UV-blocking materials with other control tools such as microbial or botanical insecticides needs further investigation.

Nowadays, in Bangladesh, almost twelve diseases are known to occur in different species of citrus plants. Among them citrus canker is the most devastating. Citrus canker presently occurs in over thirty countries in Asia, the Pacific and Indian Ocean islands, South America, and the Southeastern part of the world (Das, 2003). It is caused by *Xanthomonus axonopodis* pv. *citri* a rod-shaped gram-negative bacterium (Graham et al., 2004). Plants infected with citrus canker have characteristic lesions on leaves, stems, and fruit with raised, brown, water-soaked margins, usually with a yellow halo or ring effect around the lesion. In recent years, chemical control is not inspiring due to its environmental hazardousness. Solar irradiations that contain different wavelengths have profound effect on different microorganism (Perez and Sommaruga, 2007; Paul, 2011).

The Experiment-3 (Chapter-4) was conducted on the effect of partially UV-blocking films on citrus canker during 10 August 2014 to 30 September 2014 at the Horticultural Farm, Sher-e-Bangla Agricultural University, Dhaka and also the laboratory experiments were conducted in disease diagnostic laboratory at the department of Plant Pathology of the same university. Citrus seedlings cv. Kagji lebu (*C. aurantifolia*) of 1 year old provided by Krishibid

Nursery, Bangladesh were used in this proposed study. The seedlings were planted in 25 × 25 cm earthen pot (one $plant.pot^{-1}$). Data on incidence and severity were collected after 15, 30 and 45 DAI.

Incidence of canker of citrus varied significantly under different UV intensity ranged from 58.3 to 100% at 15 and 60 DAI (Table 4-2). The highest incidence was recorded in treatment T6 at 45 DAI and lowest was recorded in treatment T1 (58.3%) at 15 DAI. Statistically similar incidence (66.7, 83.3 and 83.3 %) at 15, 45 and 60 DAI was recorded in treatment T3 and T4. Severity of citrus canker also varied significantly under different UV intensity ranged from 1.8 to 43.3% at 15 and 60 DAI (Table 4-2). The highest (43.3%) severity was recorded in treatment T6 at 45 DAI and lowest incidence was recorded in treatment T1 (1.8 %) at 15 DAI. Statistically similar severity (3.2, 9.3 and 13.8 %) at 15, 45 and 60 DAI was also recorded in treatment T3 and T4.

In the Experiment-3, citrus plants kept with different wavelength of UV radiation showed increased disease incidence and severity with increased temperature and relative humidity. A negative correlation (r^2) = -3.35 and - 4.79 were found among temperature, humidity and disease incidence (Figure 4-4 A, C). A positive correlation (r^2) = 0.81 and 0.87 were found among temperature, humidity and disease severity (Fig 4-2, B, D). Again a negative correlation (r^2) of -3.61 were found between UV intensity and disease incidence (Figure 4-2, E). A positive correlation (r^2) of 0.47 was found between UV intensity and disease severity (Fig 4-2, F). In Experiment-3, it was focused on the effect of partially UV-blocking films on citrus canker disease development in Bangladesh condition. The experiment revealed that the *Xanthomonas* bacteria were sensitive to UV light. The result showed that disease incidence and severity were higher under <400 nm in which only visible light were passed and all kinds of UV-irradiations were blocked by the film.

On the other hand lowest disease incidence and severity were measured in the outdoors. The specific wavelength of UV were blocked in the treatment from <340 to <400 nm, disease incidence and severity were also gradually increased inside the mini houses. The result also revealed that outdoor and UV transmitting treatments required higher time to express symptom after inoculation which gradually decreased under <400 nm. This result indicates the multiplication rate of the bacteria in host plant may be interrupted by UV radiation. The multiplication rate of bacteria may be increased when the bacteria are not imposed under UV radiation and vice versa. UV-A may cause indirect damage by producing ROS at cellular level and UV-B may cause direct damage by breaking DNA of bacteria while the UV-C may cause both direct and indirect damage of bacteria. The lower incidence and severity of disease in outdoor and UV-transmitting may be due to the combined effect of these three UV radiations that may slow down the multiplication rate of bacteria. The result also revealed that temperature and relative humidity in combined with UV intensity may have potential role in disease development. In fully blocked <400 nm mini houses, both the temperature and relative humidity were higher than outdoor that may have positive role for disease development. Under <360 and <400 nm both the temperature and humidity were non-significant but UV intensity, disease incidence and severity varied significantly. This indicates that higher UV intensity has a potential role to reduce disease incidence and severity.

5.1 Concluding Remarks

The key findings of this study are:

1. Partially UV-blocking films are effective for controlling insect pest.
2. For the cultivation of broccoli and turnip seedlings without pesticide, the partially UV-blocking films (<340, <350, <360 and <400 nm) may be effective for the growth

promotion and can be an important tool in IPM system for *Brassica* seedlings specifically for aphid, dipteran fly and white baked plant hopper. In the experiment, it is found that the proportion of insect-pest and parasitoids or predators balance was hampered due to the use of UV-blocking films. The effectiveness of using partially UV-blocking films on the pest-predator proportion inside the controlled cultivation techniques was also found out. It is clear that the cost effectiveness, timing, and nature of crops might also be the effective points to consider for successful IPM system in Bangladesh.

3. In red amaranth, the stem diameter (at 5 cm from the ground) was maximum (1.2 cm) in the plants grown in the tunnels with <400 nm UV-blocking films than in outdoor (0.65 cm) and the diameter showed decreasing trend with decreasing of partially UV-blocking. The cell number was decreased with the increasing of partial UV-blocking inside stem that were observed under microscope. The damaged plant and the leaf feeding by insects were minimized by partially UV-blockings. The insect trapping was minimized or controlled with the application of partially UV-blockings may be suggested for commercial use as one of the IPM tools in horticultural production.
4. The findings of citrus canker disease diagnosis indicate that higher UV intensity has potential role to reduce disease incidence and severity of citrus canker. Besides, disease incidence and severity increase with high temperature, humidity and low UV intensity and vice versa. From the findings of this study, it can conclude that higher UV-A radiations are suitable to avoid canker disease of citrus in tropical and subtropical regions. However, further similar investigations are needed to clarify the individual effect of UV-A or B against the disease.

5.2 Outcomes of the study

I. The level of pests, both flying and non-flying, was noticeably less within the tunnels covered with UV blocking film.

II. The speed of growth and eventual final size of the crops appeared to be greater within the tunnels covered in the partially UV blocking film.

III. The disease multiplication was less with decreasing partially UV-blockings in the mini house especially for the citrus canker.

5.3 Recommendations for future study

I. The trials must be repeated for the next growing season. Some crop varieties could be changed, but the overall cropping programme should remain very similar.

II. Specific growth measurements must be taken from the crops. This should be a measure of crop height, spread and weight. For other fruit crops like tomatoes and cucumbers an accurate figure for yield (weight) must be recorded.

III. More scientific basis should be given to the counting and recording of the flying insects (both pests and beneficial) within the tunnels.

IV. The individual tunnels must be monitored for climatic differences (e.g. temperature and humidity), which will provide data relative to the crop growth and development.

V. The level of non-flying insect infestation and impact should be monitored.

VI. The longer the tunnels are in production (i.e. number of growing seasons) the higher will be the latent pest population. This should be continued year-on-year for successful interference.

Through these experiments, the use of partially UV-blocking films will become an additional tool in IPM technology in controlling insect pests, and will encourage farmers not to overuse pesticides in the horticultural production of Bangladesh.

Chapter 6

Overall summary

The different experiments were under taken in Sher-e-Bangla Agricultural University to find out the effect of partially UV-blocking films on the growth and insect control and disease colonization of some horticultural crops. The experiments were laid out in tunnels and in mini houses covered with four different polyolefin films which can block UV-radiations shorter than 340, 350, 360 and 400 nm wavelengths. The results were compared with the crops that were grown in the outdoors. On 30 days after sowing, plant height, leaf number, and leaf area of turnip seedlings increased as the rate of UV-blocking increased, while those of broccoli seedlings were not affected by partial UV-blocking. Insects invaded into the tunnels were trapped using blue and yellow adhesive films, and seven different insects: aphid, (*Brevicoryne brassicae*), brown plant hopper (*Nilparvata lugens*), short horn grass hopper (*Melanoplus femurrubrum*), white backed plant hopper (*Sogatella furcifera*) dipteran fly and mirid bug (Heteroptera: Miridae) were captured during 30 days of plant cultivation. Among them, the most predominant insect was aphid, followed by dipteran fly and white baked plant hopper, irrespective of seedling. Percentages of feeding damages were also measured on 30 days after sowing. Among the treatments, largest feeding damage was found in the outdoors for broccoli (19%) and turnip (29%) which was significantly larger than that in the other treatments. Partially UV-blockings effectively reduced the feeding damage compared with the outdoors, irrespective of seedling, but the damage tended to decrease as the UV-blocking rates increased. The sticky traps were found effective and specific UV-blocking films showed different effect in controlling insect pest. The specific UV-blocking films can be effect as an important tool for the integrated pest management system.

Besides, red amaranth crops were grown from seed under UV-transmitting and partially UV-blocking films and destructively harvested after 5 weeks after sowing. Twelve insects were trapped in the yellow and blue sticky traps that were suspended at the centers of the tunnels. The fresh weight of the plants that were grown inside the tunnels covered with partially UV-blocking films shorter than 400 nm gave 200g more yield than those grown in outdoors. The dry matter was 8% more in crops that were grown under partially UV-blocking films and the lowest was also inside the tunnels with partially UV-blockings. The deeper color (higher redness value and higher yellowness value) with lower lightness value in leaf observed inside tunnels covered with partially UV-blocking films. The highest plants were recorded in the tunnels that were covered with partially UV-blockings shorter than 400 nm but plants in outdoor had the maximum number of cell. The total anthocyanin in leaf and stem were reduced inside the tunnels with partially UV-blockings. The invasion of insects was observed maximum in outdoor and it was gone down (4%) upto the tunnels covered with UV blockings shorter than 360 nm. Among the insects, aphids and white fly were in a higher number inside and outside the tunnels. These findings are of particular importance as the potential of partially UV-blocking films to decrease the invasion of insects may offer the opportunity to produce vegetables commercially to those grown under conventional films.

Furthermore, the effect of partially UV-blocking films on citrus canker caused by *Xanthomonas axonopoides* pv *citri* which is currently become the most devastating disease happening in world citrus production. The hypothesis had to be testified that partially UV-blocking film had the ability to suppress the development of canker disease of citrus. The quantified variables were latent period of the pathogen, temperature, relative humidity, partially UV-blocking conditions, disease incidence and disease severity. The mini houses were constructed and covered with four types of polyolefin films that had the ability to block solar

UV-irradiation shorter than UV-A of 400, 360, 350 and 340 nm, and the results were compared with that of UV-transmitting and outdoors. The results showed that the leaves under 400 and 360 nm UV-blockings took less time (8 days) to express the symptom than outdoors (13 days). The lowest incidence (66.7%) was recorded in outdoors and highest incidence (100%) was recorded under <400 nm at 45 days after inoculation. Similarly, lowest severity (10%) was recorded in outdoors, and the highest severity (43.3%) was recorded under 400 nm at 45 days after inoculation. Disease incidence and severity gradually decreased as the UV blocking rates decreased. Solar radiation with contains combination of different UV light may be detrimental for multiplication of the bacteria *Xanthomonas axonopoides* pv *citri*.

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