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Field effectiveness of yellow sticky sheets, aluminium foil and malathion for vectored viral disease in cucurbits

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Abstract

Extensive losses in many agricultural and horticultural crops are caused by vector borne viral diseases. The problem is really grave if the nature of transmission is non-persistent and we are left with only a few options to control. The present study was conducted to evaluate the efficacy of aluminium foil mulch, yellow coloured sticky sheets and insecticide for the management of vector borne viral disease in bottle gourd and its effect on yield. The experiment was conducted using RCB design with three replications and four treatments with ten plants in each treatment. Four treatments including the control were applied for the management of vectors i.e. Yellow sheets painted with mobile oil on 3×3 feet board to trap insects whilst Malathion insecticide was applied as second treatment, diamond aluminium foil was used with holes as third treatment and control plots. Based on results, yellow sticky sheet, aluminium foil mulching and insecticide application had significant effect on plant growth and yield. Yellow sticky sheets resulted in lowest disease incidence (10.83%) of viruses with good impact on yield parameters resulting in maximum fruit yield (7.51kg plant^{-1}), highest number of fruits (7 plant^{-1}) and maximum vine length (263.4cm) followed by aluminium foil mulching where disease incidence of virus was (13%), fruit yield was 6.42kg plant^{-1}, fruits number was 5.23 plant^{-1} and vine length was 253.37 plant^{-1}. Highest disease incidence (42.98%), minimum fruit yield (1.5kg plant^{-1}), least number of fruits (4 plants^{-1}) and minimum vine length (216.83cm) were recorded in untreated plants. Current study suggests that yellow sticky sheet is highly effective for virus vector control followed by aluminium foil. It can suppress vector population thereby reducing the virus incidence which ultimately increase fruit yield of bottle gourd.

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Introduction

Cucurbit vegetable crop is badly affected by viral diseases worldwide (Jawdah *et al.,* 2000). Symptoms like mosaic, mottling, enation and puckering of foliage, distortion of produce and plant stunting are caused by viral infections (Damicone, 2007). The quality and quantity is severely affected, especially when early infections occurs during the crop growth resulting in severe stunted plants and symptoms which in turn renders the fruit unmarketable. The viruses are spread by insect vector in different manner and many species of insects play a key role in virus disease epidemics. The important insects that vector viruses are aphids, beetles, planthoppers, mealybugs, whiteflies, thrips and leafhoppers. Cucurbits are susceptible to many diseases and insect pests, but main problem for last few decades has been the composite of aphid-borne viruses, mostly cucumber mosaic virus (CMV), watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV), which vary in infectivity and amount from season to season (Hartz *et al.,* 1996). Also, the silver leaf whitefly (*Bemisia argentifolii*) has been reported as a severe late-season pest and the disease resulting in 13% decrease in cantaloupe cultivation in Fresno County (Jetter *et al.,* 2001). Despite of virus and disease transmission, these insects reduce the plant vigour and make it more weak and vulnerable (Barlow *et al.,* 1977; Buntin *et al.*, 1993).

Although, there is no safe and cost-effective chemical (viricide) for control of virus infected plants, the various methods of plant disease control depend on indirect means, such as vector control, disease resistance and cultural practices of management (Kapoor, 2012). There are limited choices for controlling diseases of cucurbits caused by viruses transmitted non persistently. One of the extensively used control method is the use of insecticide against these pests. Insecticides are ineffective in management of virus disease when the virus is transmitted by aphids in non-persistent manner. The virus gets transmitted, even before the insecticides act to kill them (Stapleton and Summers, 2002). Moreover, insecticides have a bad effect on growers, consumers and the surroundings (Pimentel and Greiner, 1997). When whitefly populations are high, systemic insecticides are even not enough to control virus transmission (Webb *et al.,* 2007). In such cases an integrated approach is the best option exploring other alternative management strategies to be implemented because many of the active ingredients are being banned in the European Union (Douce, 2002) and in other places as well. Because of the resistance build-up in pathogens and insects, the insecticide are not that much effective and sufficient to control many of the important pests and diseases (Nyoike and Liburd, 2008). Moreover, end users are very thoughtful to environment friendly (organic agriculture, integrated production etc.) pesticide-free management and are interested in high quality and low cost food items. Investigation of new control strategies for pest and disease control is required to meet all these demands (Diaz and Fereres, 2007). In case when the virus is transmitted nonpersistently we are left with no choice but to use methods other than pesticides because they will not serve the purpose rather might increase disease and losses. Controlling or diverting the vectors away from the crop will result in no or low and delayed viral disease incidence which will enhance the quality and quantity of yield. In Pakistan, vector transmitted viruses like *Zucchini yellow mosaic virus* (ZYMV), *Papaya ring spot virus* (PRSV), *Cucumber mosaic virus* (CMV) and *Watermelon mosaic virus* (WMV) has already been reported (Ali *et al.,* 2014). These viruses produce mosaic, chlorosis, mottling and leaf deformation symptoms and may infect singly or in combination and can only be differentiated from each other serologically (Khalid and Ahmad, 1997; Ali *et al.,* 2004; Malik *et al.,* 2010). This research work deals with methods aimed at managing vectors so as to interfere with the contact between the vector and vulnerable crop and thus interrupt the transmission process.

Materials and methods

Efficacy of different management strategies were evaluated for the control of vector transmitted viruses infecting cucurbit crops at Agricultural Research Institute, Tarnab-Peshawar.

Treatments and control experiment

Field research was conducted during cropping season 2016 following the standard agronomic practices to examine the effect of different treatments like yellow sticky sheets (yellow sheets painted with used mobile oil), aluminium foil (Diamond aluminium foil) and insecticide (Rout by Kanzo, 57EC). For control plots no treatment was applied. Trails were laid out in randomize complete block design (RCBD) with 3 replications and four treatments.

For each treatment seeds were sown with a plant to plant distance of 1.5 feet and row to row distance 3.75 feet and reducing the final number of plant to ten by roughing out infected (seed transmitted virus infection) or extra plants. For yellow sticky sheets a 3×3 feet board fixed with yellow sticky sheets were installed to trap the insects, the sheets were painted with mobile oil. Insecticide Malathion (Rout by Kanzo, 57EC) was used as second treatment to control insects. Aluminium foil (Diamond aluminium foil) was spread by making holes in it for the emerged plants. Field was maintained using standard land preparation and routine agronomic practices.

Data collection

Plants were kept under observation for incidence of viral symptoms regularly. Leaf samples were collected in a plastic bag and were brought to the laboratory of Department of Plant Pathology, the University of Agriculture, Peshawar for further analysis. The samples were subjected to the Double Antibody Sandwiched Enzyme Linked Immuno Sorbent Assay (DAS-ELISA) to confirm the presence of insect transmitted viruses likecmV, PRSV, ZYMV and WMV. Fruits from each treatment were harvested regularly on maturity and fresh weight was determined. Data taken on growth and yield of the plants were analysed using analysis of variance technique.

Serological Detection of Virus

DAS-ELISA kits were purchased from Adgen, UK to confirm the presence of virus in infected samples. The test procedure used for DAS-ELISA in the study was as per recommendations of the manufacturer. The main steps are summarized below:

1. Coating (PAb) antibodies were diluted (1:1000v/v) in coating buffer and 100µl of diluted antibodies were aliquoted to each well of the plate.
2. Plates were wrapped tightly in aluminium foil and incubated at 37˚C for 2-4 hours.
3. Plates were rinsed with washing buffer three times by aliquoting 100 µl of the washing buffer in each well and throughing it out.
4. Antigen was extracted by grinding 1g of leaf or root hair with 5ml extraction buffer in a pestle and mortar. The samples were filtered with muslin cloth to avoid debris.
5. 100µl of each sample was added to separate well along with positive and negative control as provided with kits by the manufacturer (ADGEN, UK). Plates were wrapped again and incubated at 4˚C overnight (16 hours).
6. Plates were again washed three times with PBST
7. Conjugate antibodies were diluted in conjugate buffer (1:2000 v/v) and 100 µl were loaded to each well and incubated at 37˚C for 1 hour.
8. Plates were washed three times to remove all unbound conjugate from the wells.
9. Substrate was prepared by dissolving two tablets in 10ml of substrate buffer. 100µl of substrate was added to each test well. Micro titer plates were again wrapped and incubated in the dark for 1 hour at room temperature.
10. Reactions were recorded after 30, 45 and 60 minutes by visual inspection.

Results

Effect of different treatments on virus disease (s) incidence

Based on ELISA results, yellow sticky sheets were found more effective than other treatments (aluminium foil mulching and insecticide application) for reducing virus disease. However, all the three treatments were effective in decreasing disease incidence compared to control plants on which maximum disease was observed. We were not able to detect WMV and PRSV in any of the sample tested. In all the tested samples we could detectcmV, ZYMV or both of these viruses. For the yield parameters we selected only those plants which were doubly infected with both the viruses. Plants with symptoms typical of CGMMV or tested positive for CGMMV using DAS ELISA were roughed out right at the beginning of the experiment to exclude virus transmitted through seeds or sources other than vectors. In case of yellow sticky sheets the disease incidence was 10.83% while in aluminium foil mulching treatment 13.00% disease incidence was observed. In insecticides treated plants the disease incidence was 37.11%. Maximum disease incidence of 42.98% was observed in control plants. The graphical representation of the data in Fig. 1 shows that the percent infection was considerably reduced in the plants where yellow sticky sheets were installed, followed by Aluminium foil mulching. The insecticide however was not able to control the infection to a considerable rate. The maximum infestation was observed in plants where no treatment was applied.

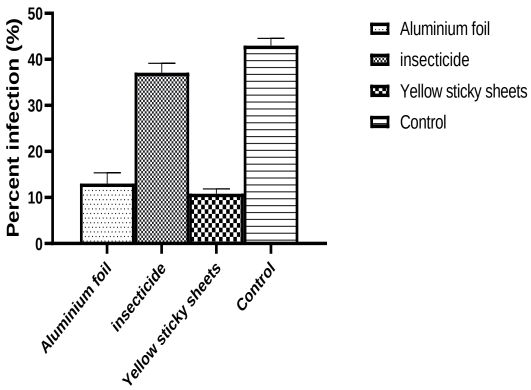

Fig. 1. Effect on percent infection of different control strategies for vector transmitted viruses in Bottelgourd.

Number of fruits plant⁻¹

All the treatments were a significant effect on number of fruits plant^{-1}. Maximum number of fruits (7 plant^{-1}) was obtained from plants treated with yellow sticky sheet. Aluminium foil mulching and insecticide application yielded similar number of fruits (5plant^{-1}). Least number of fruits (4 plant^{-1}) was obtained from untreated plants (Table-1). Results showed that the treated plants produced more fruits as compared to the untreated plants (Fig. 2). Virus infection affects the growth of the plants by producing symptoms like mosaic, chlorosis and therefore the plants were weak and produce less number of fruits.

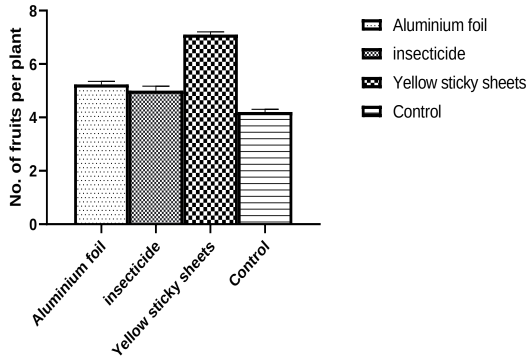

Fig. 2. Effect on number of fruits per plant of different control strategies for vector transmitted viruses in Bottelgourd.

Fruit weight plant^{-1}

Fruit weight was significantly affected by all the treatments. Maximum fruit weight was obtained by yellow sticky sheets (7.51kg plant^{-1}), followed by Aluminium foil (6.42kg plant^{-1}) and insecticide (5.96kg plant^{-1}). Fruit weight yielded by aluminium foil and insecticide was statistically at par and therefore were grouped together. Results showed in Fig.3 revealed that the treated plants yielded more fruit weight as compared to control. The untreated plants produced only 4.49kg fruit plant^{-1}. Virus infection reduced the yield as the growth of the plant is highly affected by viruses.

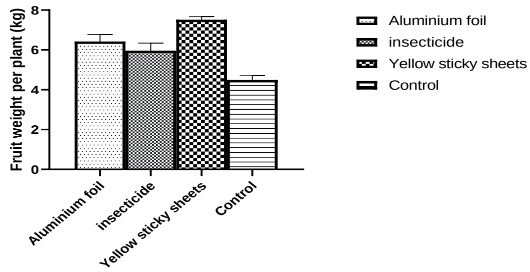

Fig. 3. Effect on weight of fruits per plant of different control strategies for vector transmitted viruses in Bottelgourd.

Vine Length

According to data presented in Fig. 04 vine length of bottle gourd was significantly affected by all treatments. Maximum vine length (263.4cm) was produced by plants treated with yellow plastic sheets, followed by mulching with Aluminium foil (253.37cm). The plants treated with insecticide showed 226.59cm vine length. However, least vine length (216.83cm) was recorded for the untreated plants. Results further revealed that all the treatments have controlled insects and hindered virus entry to the plants. This has a positive impact on vegetative growth. Plants infected with viruses have less growth and produce a range of symptoms by utilizing the plant energy pathway for their own replication and spread and therefore affected the normal growth. That is why the infected plants were weak, stunted with small leaves having short veins and showed less vegetative growth (Fig.5).

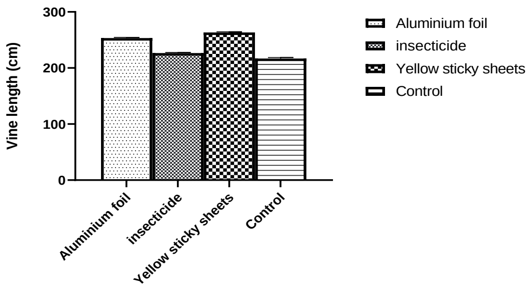

Fig. 4. Effect on weight of fruits per plant of different control strategies for vector transmitted viruses in Bottelgourd.

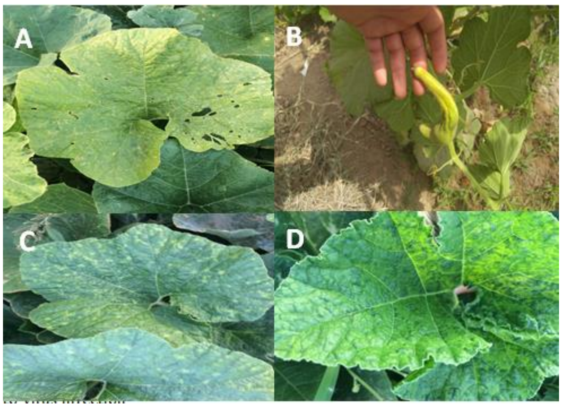

Fig. 5. Bottle gourd leaves showing symptoms caused by A: insect attack B: deformation of fruits due to virus infection C: leaves showing yellow spots due to virus infection and D: Mosaic due to virus infection.

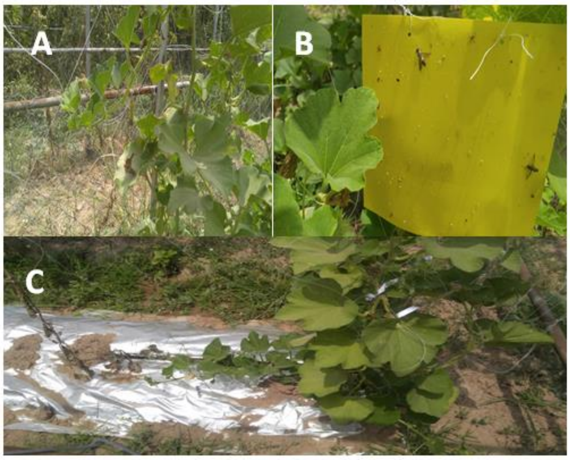

Fig. 6. A. bottle gourd plants treated with insecticide, B. yellow sticky sheet showing trapped insects, C. aluminium foil mulching showing healthy bottle gourd plant.

Discussion

It is difficult to manage plant virus diseases especially when it is done make environment nontoxic and pesticides free. Indirect methods of disease control should be identified and used (Antignus, 2012) to have effective control of viruses with no impact on the environment. Recently, non-pesticidal, control practices are being used to minimize the loss due to these virus diseases by interrupting the disease cycle (Antignus, 2000). Current studies explicated that all the treated plants were clearly superior to the untreated plants reducing the disease incidence and thereby increasing the growth and yield of the plants. Minimum disease incidence was recorded in plants where yellow sticky sheets were used followed by aluminium foil mulching. The untreated plants soon became infected and invaded by the insects (Fig1-3). Budnik *et al.* (1996) also compared the untreated plants with plants treated with mulches and yellow sticky traps and found that the incidence of PVY in peppers was significantly reduced in treated plants. Similar effects on the incidence of PVY,cmV and TSWV in solanaceous crops had been observed by Greenough *et al.* (1990). Natwick *et al.* (2007) also found that yellow sticky traps can trap a large number of whiteflies, aphids and thrips. Aluminium mulch was also reported to be effective in preventing whiteflies and reducing infection of tomato by tomato mottle virus (Csizinski *et al.*, 1995).

Moreau and Isman (2011) observed that yellow sticky traps have the ability of subduing adult populations alone or in combination with other control methods such as biological control. The problem with aluminium foil in crops like cucurbits is getting covered soon due to rapid and vigorous vegetative growth of the plants. It is effective at the beginning but once it is covered by the plants its impact is lost.

Insecticide may provide a sort of control but it has far more adverse effects than its benefits. Insecticides may actually increase the spread of non-persistently transmitted aphid-borne viruses by increasing the probing and vector movement (Elbert and Nauen, 2000). Many insecticides act on the aphid's nervous system, making it to travel from plant to plant very quickly, resulting in the infection of more plants than would normally occur in cases where non-intoxicated aphid are feeding, which settles down and feeds on one plant before travelling to another one. Also virus spread occurs before the insecticide has a toxic effect on the aphid (Prabhaker *et al.,* 1998). Nonetheless, whitefly infestations can be controlled by imidacloprid, a systemic insecticide. However, resistance build up, to imidacloprid and other insecticides, in whiteflies is a major concern (CMRAB, 2003).

The increase in vine length was observed in plants treated with yellow sticky sheet and reflective aluminium foil mulch. Similarly, highest number of fruits and yield were recorded in plants treated with yellow sticky traps followed by mulching with aluminium foil. Plants grown over reflective mulch produced significantly higher yields of marketable fruit than plants grown on uncovered soil (Brown *et al*., 1993; Summers *et al*., 1995). Yellow sticky traps were also found effective in vector control of viruses (Antignus, 2000). Other benefits of mulches include retention of soil moisture and modifications of temperature which may result in increased crop growth and yield, providing greater benefit and cost effectiveness to the grower (Stapleton *et al.,* 1993). Budnik *et al.* (1996) observed significant increase in yield by using yellow sticky traps. One of the major challenges of the present era is to find means to increase agriculture produce without affecting the sustainability of the environment. The inevitable need to limit pesticides usage will accelerate the development of other non-insecticidal methods, while efforts to combat plant viruses continue to rely on 'indirect' means of control. Vertical sticky traps made up of blue and yellow plastic strips (Poly-traps) are used routinely in Israeli greenhouses (Antignus, 2000).

The coloured films are positioned around the lower part of the inner walls of greenhouses to trap invading insects. It is reported that reflective film mulches of white or silver colour have been effective in providing partial disease control by delaying the onset of virus epidemics (Green, 1991). A limitation of reflective films in cucurbits is that plant growth is rapidly covered by the mulch and thereby lessens reflectivity. The application of row covers to summer squash until flowering, was not effective in reducing virus disease, and caused some yield reduction (Damicone *et al.,* 2007). Different individual cultural practices or their combination showed promising management of the disease. Such integrated practice has been adopted and used for management of virus diseases of different crops (Makkouk and Kumari, 2009; Fajinmi and Fajinmi, 2010; Fajinmi and Odebode, 2010).

We conclude that yellow sticky sheets and reflective mulches can be used alone or in combination for effective control of viral diseases that are spread by vectors or at least to delay and keep the disease and its impact under check thereby reducing the amount of losses and improving the quality of the produce. For organic and integrated production these applications will give promising results. It is imperative that for environmental safety, all the materials developed for such applications should be biodegradable. Also further studies are suggested to investigate the compatibility of these treatments with other combinations like biocontrol etc.

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